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Study of essential oils and phenolic compounds; their changes and anticancer activity in some species belonging to Asteraceae and Lamiaceae families.

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I dedicate this research work

To

My Beloved Father and Loving Mother For Their Love And Support

Most Precious For Me In This World, My Sisters

My Family

All My Teachers
All My Friends And Colleagues

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Abbreviations

IC_{50}	The half maximal inhibitory concentration
HeE	Hexane extract
ChE	Chloroform extract
MeE	Methanol Extract
EtO_2	diethyl ether
Na_2SO_4	Anhydrous sodium sulphate
GC-MS	Gas chromatography/mass spectrometry
m/z	stands for mass and / stands for charge number of ions.
HOE	Human ovarian epithelial cells
CBPT	Carboplatin
FDA	Food and Drug Administration
TPC	Total phenolic content
B.C	before Christ (used to indicate that a date is before the Christian Era)
EO	Essential oil
mean	mean average
SD	Standard deviation
SRB	Sulforhodamine B colorimetric assay for cytotoxicity screening.
WHO	World Health Organisation.
NCI	The National Cancer Institute.
OC	Ovarian cancer.
RPMI	Roswell Park Memorial Institute.
FBS	Foetal bovine serum .
PBS	Phosphate buffered saline.
DMSO	Dimethyl sulfoxide.
FIGO	International Federation of Gynaecology and Obstetrics.
UK	Unated kingdom.
CA125	cancer antigen 125.

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Introduction

Introduction

In order to live normally in the natural environment, human being had to develop lifesaving mechanisms to fight the disease. In this fight, human body may need a help and the scientific interest has been shifted to the naturally occurring compounds in this regard due to their safety, biocompatibility and cost-effectiveness. To defeat the disease, the main alleys for human in the healing process are the plants, which are considered very safe due to their minimal side effects. About 120 drugs obtained from plants are in use with a large number of therapeutic activities. These include anticancer agents. Plants have a long history with cancer treatment (Mans *et al.*, 2000).

More than 50 anti-cancer agent have been derived from natural products within 1981-2010 and approved for clinical use. These are either un-modified, semi-synthetic, or synthetic compounds, where five of them (eribulin, romidepsin, mifamurtide, vinflunine and cabazitaxel) introduce to market in 2010. This highlights the significance of plant sources for production of novel chemotherapeutic drugs (Newman and Cragg, 2012).

Cancer is a complicated genetic disease defined as uncontrolled growth, invasion, angiogenesis and metastasis, and one of the main leading causes of death worldwide (Moura *et al.*, 2016). According to the American Cancer Society (ACS), deaths resulting from cancer comprise 2–3% of yearly deaths worldwide.

Ovarian cancer is the 5th most common form of cancer among women, accounting for 4% of all new cases of cancer, with approximately over 250,000 cases around the world annually (Abdullah *et al.*, 2017). The incidence rates progressively rising over the last 20 years, mainly in those aged 65 and above (Tortorella *et al.*, 2017). It is regarded as the second most common gynecological malignant carcinoma, and unfortunately, it results in higher fatalities than any other gynecological form of cancer (Kurman, 2013). It is responsible for about 152,000 worldwide deaths each year (Christophers, 2017). The standard treatment of ovarian cancer involves cytoreduction surgery followed by platinum/taxan combination therapy (Abed *et al.*, 2016). Although the response rates to chemotherapy are approximately 80%, the majority of patients relapse with chemoresistant disease (Ledermann *et al.*, 2012). The emergence of chemo-resistance is considered as a major obstacle to long-lasting treatment and only 30-40 % of ovarian cancer patients survive 5 years after the initial diagnosis with advanced disease (Matondo *et al.*, 2017) which urges the need for new therapeutic agents or treatment strategies.

In that, therein, plants are an important source of new natural compounds that could be used as

chemotherapeutic drugs.

One of the most widely used chemotherapeutic agent currently is Taxol which is extracted from the bark of the pacific yew tree, *Taxus brevifolia*. However, the search for novel chemotherapeutic agents collide with the resistance to these drugs and their toxic side effects which represent a serious problems. Adversities following administration of anticancer drugs include: cardiotoxicity, neurotoxicity, haemotoxicity, mylosuppression, nephrotoxiciy, gastrointestinal toxicity, edema, diarrhoea, ulceration, nausea, alopecia, hypersensitivity and anaemia.

Additionally, plant extracts and traditional medicines have largely been recognized for their potential use in the identification of new drugs for the treatment of cancer (Uche *et al.*, 2017).

Research on new anticancer agents and techniques are currently rigorously pursued, mainly due to the unacceptable toxicities associated with the conventional cancer chemotherapeutic agents, so as to identify selective drugs with less adverse reactions that will mainly kill cancer cells while having fewer side effects (Aung *et al.*, 2018).

Analysis of natural products as a source of novel drugs indicates that over 67% of the effective anti-cancer drugs may be traced to natural origin (Safarzadeh *et al.*, 2014).

These agents are usually having small therapeutic windows, with minimal variation between the required dose for their anti-tumour activity and their unwanted toxic dose in healthy tissues. Hence, drugs that especially toxic to the cancer cells but leaving normal cells unaffected may possibly be used as anticancer agents (Morley *et al.*, 2007).

The use of herbal medicinal products for the treatment and prevention of diseases has a long tradition worldwide. Nowadays, it still plays an important role in the health care of numerous divergent societies ranging from developing countries in Asia and Africa to western developed nations (WHO, 2002-2005).

Algeria has a predominantly rich and varied flora with 3139 species belonging to several botanical families, where 15% of them are endemic (Quézel and Santa, 1963). Algeria, with its biodiversity richness, has a long and productive herbal medical tradition practiced both by professional herbalists and healers based at urban centres (Scherrer *et al.*, 2005), where phytotherapy is an integral part of local culture, that is largely continues to be transmitted orally between generations and has not been written down (Bouasla and Bouasla, 2017).

In Algeria, Herbal medicine radically came from Arabic-Islamic medicine, which combines Galenic

humoral medicine with Prophetic one (Greenwood, 1981). Biomedicine is also increasingly available and used in Algeria since colonial times (WHO, 2015).

Pharmaceutical product extracted from any part of a plant, including leaves, flowers, bark, roots, and seeds or from the whole plant is called herbal medicine, which can be sold as either concentrates of active compounds or as a raw herbs (Fadil *et al.*, 2018). Herbal remedies can contain only one plant or a mixture of pharmacologically active plants that have synergetic effect. Both the concentrate and the crude plant contain mixture of organic chemicals, that may include flavonoids, alkaloids, terpenes, fatty acids, sterols, glycosides, saponins, and tannins (Rotblatt and Ziment, 2002).

Polyketides, terpenoids, phenyl propanoids and alkaloids are among the most important natural products that have been reported for their cytotoxic effects (Li and Barz, 2006).

In this study, five medicinal plants are highlighted with their cytotoxic effect of their total phenolic extracts. Moreover, GC-MS analysis of polyphenols and essential oils were carried out. Four of those plants which belong to Asteraceae (Compositae) family are: *Centaurea sphaerocephala* L., *Bellis sylvestris* L., *Asteriscus maritimus* (L.) less. and *Artemisia campestris* L. Also *Lavandula stoechas* L., which belongs to Lamiaceae family.

Review of literature

Chapter 1

Cancer disease

1. Cancer disease

1.1 Introduction to cancer

Cancers are caused by multiple genetic and non-genetic alterations, that happen as a result of several environmental factors that stimulate inappropriate activation or inactivation of some genes leading to abnormal cell growth or cancerous transformations (You and Jones, 2012). In the early stages of cancer development, the information about main cellular events in addition to internal cues and environmental factors that trigger these changes are unclear. Advances in molecular epidemiology made it possible for the scientists to predict the possibility of simultaneously spotting multiple modifications affecting the genome and extra-genomic nature of normal, precursor and tumour cells along with their link to the environment (Schulte and Perera, 1998). By identifying such changes, it is now possible to identify potentially high-risk persons and specifically design an efficient strategy to prevent cancer.

1.2 History of cancer

Cancer is regarded as the second leading cause of death worldwide after cardiovascular diseases (Organization *et al.*, 2018). The word cancer originally came from a Greek words karkinos to describe carcinomas by Hippocrates (460-370 B.C), however, he was not the first to discover the disease. An early evidence of human bone cancer was found in ancient Egyptian mummies and in described in some manuscripts in about 1600 B.C. The human's earliest recorded case of breast cancer was from ancient Egypt in 1500 B.C, where it was mentioned that there is no therapy for the cancer but only palliative treatment. According to some old inscriptions, skin and surface tumors were removed surgically in the same manner as they are removed nowadays (Sudhakar, 2009).

1.3 Cause of cancer

Cancer generates when normal cells in some parts of the body start to grow in an uncontrolled way. There are different types of cancers; in all cancer cells continue to grow, multiply and re-divide to form new abnormal cells instead of dying. In some types of cancer, cells may migrate to other parts of the body through lymphatic or blood vessels, where they begin to grow (metastasis) (Chambers

et al., 2002). For instance, when a breast cancer cell travel through blood stream to the liver, the cancer is still referred to as breast cancer, not a liver cancer (Gupta and Massagué, 2006).

In general, cancer cells arise from normal cells when there is damage to the DNA of the cells. In most cases, whenever DNA is damaged, the body can repair it, however, in cancer cells, the damaged DNA is not repaired.

Additionally, when a person inherits damaged DNA from parents, this can account for inherited cancers. Environmental factors like smoking and radiation may also be responsible for DNA damage and cancer development (Clancy, 2008).

Cancer is often presented in a form of solid tumor. Conversely, other cancers like leukaemia do not form tumours; it involves the blood and the organs that form blood grow into other tissues when they circulate through it.

Furthermore, not all tumours are cancerous, some of them are benign, which do not grow and are less life threatening. The risk of developing several types of cancers may be reduced if the lifestyle is changed and this can be done by eating low fat diet and quitting smoking.

Moreover, when cancer is identified in early stages of the disease, it can be easily treated and may have better outcomes for extending patient's life (Steyn *et al.*, 2006).

1.4 Old theories of cancer development

According to Sudhakar (2009) there are;

1.4.1 Humoral theory

Hippocrates believed that any imbalance in body fluids, like blood, yellow bile, black bile and phlegm may lead to disease and increase of black bile in specific tissues can probably cause cancer. Humoral theory of cancer was standard through the Middle Ages for more than 1300 years, however, because of prohibition of autopsies during this period due to religious reasons, limited knowledge about cancer was obtained.

1.4.2 Lymph theory

It is hypothesized that cancer formation is mainly happening with a fluid called lymph. Previously, life was believed to consist of continuous movement of the body fluids such as lymph and blood.

In the 17th century, this theory was supported that tumors grow from lymph continuously removed out by the blood.

1.4.3 Blastoma theory

In 1838, it has been stated that cancer is made up of cells with no lymph. According to this theory, it has also been demonstrated that all cells, including cancer cells are developed from other cells.

1.4.3.1 The theory of chronic irritation

It has been proposed that chronic irritation is the major cause of cancer development. Later it was shown that cancers can be metastasized as a result of malignant cells spread, but not by some unidentified fluid.

1.4.4 Trauma theory

From the late 1800s until the 1920s, trauma theory proposed that cancers are caused by trauma.

1.4.5 Parasite theory

Until the 18th century, researchers thought that cancer is contagious disease and can be spread through parasite.

1.5 The Role of oncogenes and tumor suppressor genes in cancer

In the middle of the 20th century, researchers began to solve the complex problems of biology and chemistry of cancer. Watson and Crick had received the Nobel Prize in 1962 for the discovery of the helical structure of DNA. Later scientists found how genes are working and how they could be altered by mutations. They identified that cancer could be caused by radiation, chemicals (carcinogens), viruses and also inherited from ancestors. The majority of carcinogens cause damage to the DNA, which lead to abnormal cell growth. Cancer cells with damaged DNA do not undergo apoptosis, whereas normal cells with damaged DNA do.

During the 1970s, researchers discovered two important families of genes, the oncogenes and tumour suppressor genes (Sudhakar, 2009).

1.5.1 Oncogenes

These genes are responsible for rendering normal cells to grow in an uncontrolled way and become cancerous. They are developed by several mutations of certain normal genes called proto-oncogenes (those genes that normally responsible for controlling how often a cell divides and differentiates) (de Leon, 1994).

1.5.2 Tumor suppressor genes

Tumor suppressor genes are normal genes which control cell division, DNA repair and inform cells when to die. Whenever a defect happens in a tumor suppressor gene, cells can grow out of control, and may lead to the development of cancer. Scientists identified tumor suppressor genes and oncogenes that are damaged by radiation or chemicals. For instance, the discovery of breast cancers genes BRCA1 and BRCA2. Many other genes have been discovered that are subsequently responsible for cancers that run in families, like colon, ovary, kidney, thyroid, rectum, pancreas and skin cancers (de Leon, 1994).

1.6 Cancer screening and early detection

Checking for abnormal cells that may develop cancer in people who are present with no symptoms is called screening. Screening can help clinicians to manage several types of cancer as early as possible, before they become life threatening. Early detection is crucial because when abnormal tissue is diagnosed with early stage, it may be easier to be treated. By the time symptoms appear, cancer may invade the body and become resistant to treatment (Curry *et al.*, 2003).
Early detection of cervix, breast, colon, rectum, endometrium, prostate, thyroid, oral cavity, skin, lymph nodes, testes, and ovaries cancers were identified and practiced in the clinic by many methods such as Colonoscopy, sigmoidoscopy, and high-sensitivity fecal occult blood tests (FOBTs), Low-dose helical computed tomography, Mammography, Pap test and human papillomavirus (HPV) testing, Alpha-fetoprotein blood test, Breast MRI, CA-125 test, Clinical breast exams and regular breast self-exams, PSA test, Skin exams, Transvaginal ultrasound and Virtual colonoscopy (Byers *et al.*, 1997).

1.7 Cancer therapeutics

Cancer differs from all other human diseases in that it can develop in any body organ and at any stage in life. Additionally, no two cases of cancer behave exactly alike, some may grow slowly or may remain dormant for years and others may grow rapidly and become highly aggressive. Other types show high cure rates, while in others the cure rates are potentially low and require improved techniques of detection and treatment (Kaplan, 2013).

The availability of different therapeutic options for and other associated services for cancer reveal the biological diversity of this disease. The stage of cancer at diagnosis, the treatment options and the rate of progression, vary significantly with the type of cancer in each patient It is estimated that around 80% of cancer cases are due to lifestyle or environment, and therefore can be potentially preventable (Doll and Peto, 1981).

The risk factors for development of some cancers have been clearly identified, however for others further knowledge is needed. Depending on present studies, at least 30% of future cancers can be prevented by careful and comprehensive considered action, taken now (Organization, 2002).

The treatment of cancer may be determined by the type and location of the cancer, the stage of cancer at diagnosis, the standard treatment guidelines and medical practices in the patient's country, in addition to the ability of cancer patients to afford for treatment cost.

For most solid cancers, if it is rather at an early stage, surgery is the gold standard and most effective form of initial cancer treatment. This option is usually combined with radiation therapy to the tumour bed as well as systemic anticancer therapy as the target is curative more than palliative at this stage. As cancers progress to late stages, treatments typically include radiation, followed by chemotherapy (Paleri *et al.*, 2010).

Recently, Targeted therapy is becoming highly recommended in the majority of cases. Multiple metastases (in different locations) and the sever tumour load eventually limit effective surgical removal and the usefulness of anti-cancer agents. In recurrent cases, when the cancer spread beyond the initial site of the disease, systemic treatment is crucial and the goal of the treatment at this stage is no longer curative, but mostly palliative.

Chemotherapy is the most available form of systemic treatment for cancer, due to its ability to reach, target destroy cancer cells in the body; although the blood-brain barrier, in most cases, limits effectiveness of therapy in the case of brain metastases. Chemotherapy can be used alone as a single agent or in combination with other forms like radiation therapy to specific metastatic regions.

Hormone-regulated tumours, as in case of certain breast, ovarian and prostate cancers utilize the body's natural hormones to develop and grow, and can be more responsive to hormone-based treatments than other forms of cancer. And this is highly important in the case of tumours that develop resistance to standard chemotherapies.
Certain types of cancer can be resistant to systemic options at the time of diagnosis and this is referred to as primary or innate resistance. Others become resistant over a period of months or years after using anticancer agents. In general, 30% to 80% of cancers can become refractory (Van der Schueren *et al.*, 2000).

1.8 Use of medicinal plants in cancer treatment

Since thousands of years natural products, especially plants and vegetables, have been used in traditional medicine for the treatment of various diseases. and continue to play an important role in health care (Pezzuto, 1997). These plants called medicinal plants.
About 80% of the population in the world live in developing countries and mostly depend on plant products for their health care systeme. For the remaining 20% inhabitants, almost 25% of their pharmaceuticals have been derived from plant products (Farnsworth *et al.*, 1985).
In general, there are about 250,000 known higher plant species in the wide world, only 5-15% have been studied for biological usefulness (bio-activity) (Cragg *et al.*, 1997). About 200,000 natural products have been reported in plants (Tulp and Bohlin, 2005). Some of the important phytochemical constituents, found in plants, include alkaloids (atropine, quinine, etc.), flavonoids, tannins, terpenes, terpenoids, steroids, glycosides, saponins, phenolics, and quinones.
The bio-activities of medicinal plants have been linked to the presence of one or more of the various classes of phytochemicals, with isoprenoids, phenolic compounds and alkaloids being the most commonly biosynthesised (Kinghorn *et al.*, 2011).
Investigations into the anticancer property of plants have been for about 60 years and are fairly recent, with even fewer plants being screened (Cragg *et al.*, 2009).
About 120 drugs obtained from plants are in use with a large number of therapeutic activities. These include anticancer agents (Mans *et al.*, 2000).
Plants have a long history with cancer treatment (Mans *et al.*, 2000). Within 1981-2010, about 50 natural products derived anti-cancer drugs were approved, either as un-modified compounds, semisynthetic analogues, or synthetic compounds based on natural product leads, with 5 drugs

namely: romidepsin, cabazitaxel, eribulin, mifamurtide and vinflunine developed in 2010 alone. This underlines the importance of plants as sources of new cancer chemotherapeutic agents (Newman and Cragg, 2012).

While numerous claims of efficacy of some plants in the treatment of different diseases should be viewed with some skeptical attitude; in fact cancer, is prone to be inadequately defined in terms of traditional medicine and folklore, as a particular disease entity (Snader and McCloud, 1994). Medicinal plants involved in the modern management of cancer either by providing the active substances or a specific template for producing synthetic modifications, that lead to more potent anticancer drugs (Cragg and Newman, 2005).

1.9 Plant derived anticancer agents

About 35,000 plant samples from 20 countries have been collected by The National Cancer Institute (NCI) and around 114,000 extracts for anticancer activity have been screened. 60%, Of the 92 anticancer drugs approved worldwide between 1983 and 1994 and commercially available prior to 1983, are of natural origin (Cragg *et al.*, 1997). In that case, natural origin can be defined as natural products, their derivatives as semi-synthetic or synthetic pharmaceuticals agents based on natural product models (Jaspars and Lawton, 1998).

Also, The literature on 5 plant extracts and 96 natural products isolated from higher plants and microorganisms before 2001 and with potential anticancer activity against ovarian neoplasia was reviewed (Silva *et al.*, 2003).

Due to the advanced stage of the cancer when it is diagnosticated and due to the appearance of resistances a small proportion of patients respond to the antitumor agents. Accordingly, plants consideres as an important source to obtain novel compounds that could be used as chemotherapeutic drugs.

In the last decades, plant-derived substances have been entered in to many clinical trials as anticancer agents (Mouhid *et al.*, 2017), and they found to be useful in modulating numerous molecular pathways involved in the development and progression of tumor.

In this senario, the predictable intervention for any plant-derived extract as anticancer agent should exert a reliable cytotoxic effect in cancer cells, without affecting the viability of normal cells.

1.10 Ovarian cancer

1.10.1 Introduction to ovarian cancer

Ovarian cancer (OC) involves a growth of the cancerous cells emerging from cells in and around the ovary. Ovarian cancer leads to high death rates and its treatment is difficult, prolonged, complex and multimodal. As a disease, it is frequently characterized by recurring after treatment and repeated rounds of chemotherapy exhibit decreasing benefit due to the development of drug resistance. For these reasons the disease places a real and substantial load on patients and their families (Butow *et al.*, 2014). Early diagnosis of OC is associated with improved survival, so increasing awareness of disease symptoms among health professionals, women and patients is crucial. Improved treatment strategies that reduce drug resistance also have the potential to improve survival (Rooth, 2013).

1.10.2 Epidemiology and incidence

Ovarian cancer is the 5th most common form of cancer among women, accounting for 4% of all new cases of cancer, with approximately 7,000 new cases across the United Kingdom and over 200,000 cases annually around the world. The incidence rates progressively rising over the past 20 years, mainly in those aged 65 and above (Rooth, 2013). It is regarded as the second most common gynecological malignant carcinoma, and unfortunately, it results in higher fatalities and is considered as more deadly than any other gynecological form of cancer. It is responsible for about 4300 annual deaths in UK with approximately 140,000 worldwide deaths each year (Rooth, 2013). The incidence of ovarian cancer is low in young women and increases with age (**Figure 1.1**). Additionally, the incidence rates of OC are highest in countries from Northern Europe, North America, and Western Europe; whereas rates are lower in most parts of Africa and Eastern Asia by up to 10-folds. Within each country, the incidence rates may differ among ethnic groups (Lukanova and Kaaks, 2005).

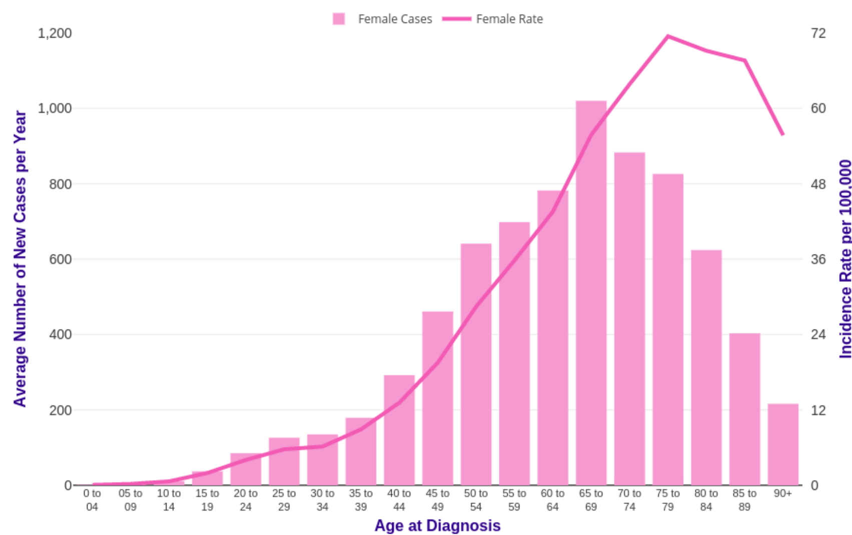


FIGURE 1.1: Average number of new cases each year and age-specific incidence rates of ovarian cancer per 100,000 population, females, UK. 2008-2010. (Cancer Research UK, 2013).

1.10.3 Aetiology of ovarian cancer

Ovarian cancer is a rapidly progressive heterogeneous disease; its heterogeneity can be divided into two wide classes of carcinogenesis, the low-grade tumours which develop through a gradual process of mutation, grow more slowly, are less responsive to chemotherapy, and share molecular features with low malignant potential neoplasms. In contrast, high-grade carcinomas reveal higher genetic instability, rapid metastasis, chemosensitivity and without a distinct precursor lesion (Schorge *et al.*, 2010).

Many factors may contribute to the initiation and subsequent progression of the disease, such as increased age, being overweight, not having children or not breast feeding, never taken the contraceptive pills and early menarche or menopause started later than average. Taking fertility drugs and hormone replacement therapy increases the risk of developing OC (Pearce *et al.*, 2009). The risk of developing OC is increased three- to four-folds in women who have a close relative diagnosed with this disease when compared with those with no family history of the disease (Gayther and Pharoah, 2010). Even though only about 10% of women with a family history of ovarian cancer develop the disease, it has been found that the presence of inherited mutations in the breast and ovarian cancer susceptibility genes (BRCA1 and BRCA2) can increase the risk of OC. The risk of OC is approximately 40% by 70 years of age in case of mutations in BRCA1, with risk is only 10% in BRCA2 carriers (Sunil *et al.*, 2004).

1.10.4 Types of ovarian cancer

Ovarian cancer is presented in several types (as shown in Figure), mainly categorized by the types of cells and tissue from which they originate (Scully, 1987) (**Figure 1.2**).

Over 90% of ovarian malignancies arise when ovarian surface epithelium (which is composed of one layer of cells and oftentimes is described as modified peritoneal mesothelium) undergoes a cancerous conversion. The remaining malignancies come from germ cells that produce the ovary, which characterized by its rapid growing ability and this form of cancer comprises < 5% of all ovarian neoplasms (Koshy *et al.*, 2005), or ovarian sex cord-stromal tumors, which are rare and constituting only 1.2% of all primary ovarian cancers. It starts from structural tissue cells that hold the ovary together and produce the female ovarian hormones (Quirk and Natarajan, 2005).

Another type of the disease is the primary peritoneal cancer, which is characterized by its ability to spread widely inside the peritoneal cavity and in most cases it involves the omentum.

Histologically, it is indistinguishable from primary epithelial ovarian cancer, so it is often described as a type of OC and it is usually treated in the same way too (Roh *et al.*, 2007).

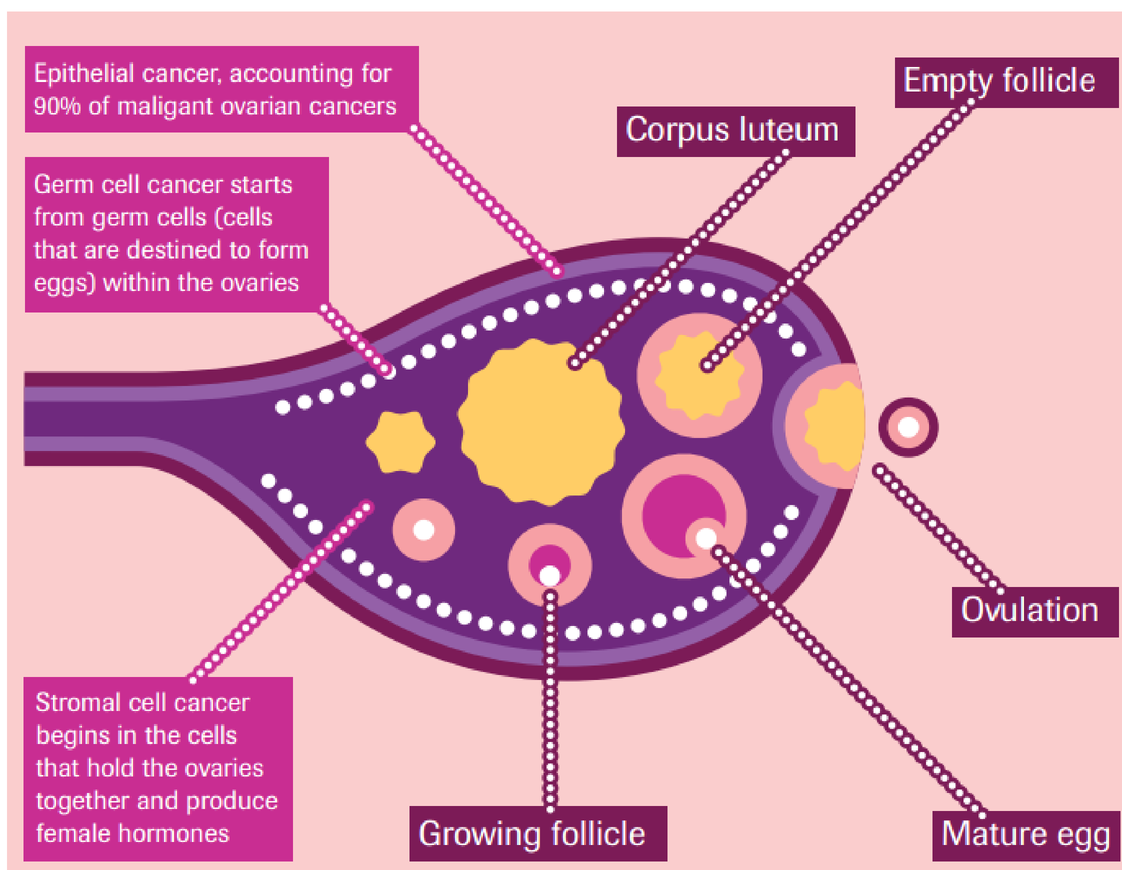


FIGURE 1.2: Types of ovarian cancer (Rosen *et al.*, 2009).

The epithelial neoplasms are sub-classified into ovarian serous carcinoma (30–70%), ovarian endometrial carcinoma (10–20%), ovarian mucinous carcinoma (5–20%), clear cell carcinoma (3–10%), undifferentiated (1%) (Rosen *et al.*, 2009), and transitional cell (Brenner) carcinoma (2%) (Satoshi et al., 2012).

The conventional view of OC has been that ovarian surface epithelium (mesothelium) is the main source for various different tumors and that the development of the different cell types (serous, endometrioid, clear cell, mucinous and transitional cell [Brenner]) occurs by subsequent metaplastic changes, which morphologically resemble the epithelium of the endometrium, gastrointestinal tract, fallopian tube, or endocervix and urinary bladder (Kurman and Shih, 2010).

1.10.5 Symptoms of ovarian cancer

Patients with OC are often diagnosed late in the course of the disease because the symptoms are subtle and women frequently remain unaware of the disease until it reaches an advanced stage (Risch, 1998); the disease continues to be a "silent killer" (Fathalla, 2013).

However, some non-specific and somewhat vague symptoms may be present like persistent pain in the pelvis or abdomen, increased abdominal size with persistent bloating, feeling full quickly after eating and urinary symptoms (Al-Naggar *et al.*, 2013). These symptoms are often misdiagnosed as irritable bowel disease or changes that are associated normally with past pregnancies, aging and menopause (Bankhead *et al.*, 2008).

However, in the United Kingdom, recent guidance urges clinicians to look for OC in patients with the above symptoms, especially if they are of recent onset and persistent (Hamilton *et al.*, 2005).

Ovarian cancer symptoms, the frequency reported to the GP before diagnosis and positive predictive values in primary care are summarized in **(table 1.1)**.

TABLE 1.1: Main symptoms of ovarian cancer. (Hamilton *et al.*, 2009)

Symptoms	Frequency	Positive predictive value
Abdominal pain	53.00%	0.30%
Fatigue	39.00%	Unknown
Abdominal distension	36.00%	2.50%
Diarrhoea	27.00%	Unknown
Bloating	17.00%	0.3

Pelvic pain	16.00%	Unknown
Increased urinary frequency	14.00%	0.2
Abnormal vaginal bleeding	13.00%	0.5
Weight loss	10.00%	Unknown

Positive predictive values for most symptoms are low because the incidence of ovarian cancer is relatively low.

1.10.6 Staging and diagnosis

Most patients with ovarian neoplasm are diagnosed at a progressive stage where they may develop extra ovarian disease (Taylor and Gercel-Taylor, 2008). While more than 90% patients with stage I OC survive for 5 years, only about 21% of patients with progressive stage OC survive 5 years after diagnosis (**Figure 1.3**), so early diagnosis gives rise to best chance for further perfection in OC survival (Taylor and Gercel-Taylor, 2008).

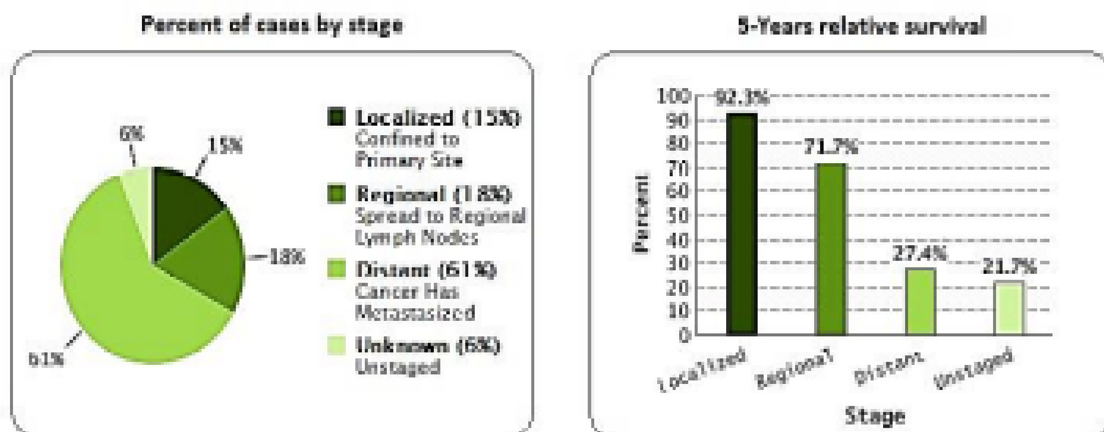


FIGURE 1.3: Percent of cases and 5-years relative survival by stage at diagnosis. National Cancer Institute. Surveillance, Epidemiology, and End Results Program (SEER).

1.10.6.1 Staging of ovarian cancer

Two diagnostic challenges arise when an ovarian mass is detected. The first one is the detection of ovarian malignancy and the second is an assessment of tumor spread, so accurate diagnosis is significant to avoid unnecessary surgery - most of ovarian masses are non-cancerous cysts.

Moreover, the decision of which type of surgery to be done (laparoscopy or laparotomy) is influenced by ovarian mass information (Nam *et al.*, 2010).

The International Federation of Gynaecology and Obstetrics (FIGO) issued a recommendation for staging of ovarian cancer staging in accordance with laparotomy (Tempany *et al.*, 2000) as shown in (**table 1.2**).

TABLE 1.2: FIGO staging and prognosis of ovarian cancer (Jelovac and Armstrong, 2011)

Staging	
I	Growth limited to the ovaries.
Ia	Growth limited to one ovary; no ascites present containing malignant cells. No tumor on the external surface; capsule intact.
Ib	Growth limited to both ovaries; no ascites present containing malignant cells. No tumor on the external surfaces; capsules intact.
Ic	Tumor either stage Ia or Ib, but with tumor on the surface of one or both ovaries, or with capsule ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings.
II	Growth involving one or both ovaries with pelvic extension.
IIa	Extension and/or metastases to the uterus and/or tubes.
IIb	Extension to other pelvic tissues.
IIc	Tumor either stage IIa or IIb, but with tumor on surface of one or both ovaries, or with capsule(s) ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings.
III	Tumor involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis and/or positive regional lymph nodes. Superficial liver metastases equals stage III. The tumor is limited to the true pelvis, but with histologically proven malignant extension to small bowel or omentum.
IIIa	Tumor grossly limited to the true pelvis, with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces, or histologic proven extension to small bowel or mesentery.

IIIb	Tumor of one or both ovaries with histologically confirmed implants, peritoneal metastasis of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative.
IIIc	Peritoneal metastasis beyond the pelvis >2 cm in diameter and/or positive regional lymph nodes.
IV	Growth involving one or both ovaries with distant metastases. If pleural effusion is present, there must be positive cytology to allot a case to stage IV. Parenchymal liver metastasis equals stage IV.

Earlier identification of OC symptoms can improve prognosis and in this regard Hamilton and colleagues reported that symptoms of OC have diagnostic predictive importance. In addition, patient age and family history may be helpful in estimate the danger of the tumour malignant nature (Sobiczewski *et al.*, 2012).

1.10.6.2 Methods of ovarian cancer diagnosis

Adenexal mass palpitation during examination of the patient's pelvis is routinely used to initiate a diagnostic assessment of ovarian cancer (Jelovac and Armstrong, 2011). An ultrasound examination is commonly used as a diagnostic technique to assess the disease and to make a suitable decision about the planned surgery; it can be helpful in the differentiation between a borderline ovarian tumour and OC. It is rapid and useful non-invasive tool for evaluation of multiple parameters of vascular supply and tumor structure (Sobiczewski *et al.*, 2012).

In patients with advanced OC, cancer antigen 125 (CA125) level in serum is elevated by 80% or more, however, this biomarker has a controversial role in diagnosis because it is poorly effective for the detection of early-stage disease. Additionally, CA125 is not specific for OC and it can be elevated in conditions such as pelvic inflammatory disease, endometriosis in addition to other malignancies such as pancreatic and endometrial carcinoma. So, in many medical disorders, false positive results have been noted (Jelovac and Armstrong, 2011). Other studies proposed that the sensitivity and specificity of CA 125 in the diagnosis of OC are poor, because CA125 is only raised by approximately 50% in stage I of the disease and in 75-90% in patients with advanced OC, so it is agreed as a tool for evaluation of response to therapy (Pignata *et al.*, 2011).

Many prediction models have been used to try to improve precision of OC diagnosis and to clarify whether ovarian disease is benign or malignant. In this regard, the risk of malignancy index (RMI) has been developed which is an index including evidence of serum CA 125 concentration, menopausal status of the patient and ultrasonography findings; it has been concluded that the RMI was the best available test to triage patients with ovarian tumours for referral to tertiary oncology units (Kaijser *et al.*, 2013).

New diagnostic techniques are now available that use exosomes which are actively released by tumours into the peripheral circulation. It was demonstrated that there is an association between microRNAs and circulating tumour derived exosomes, so Taylor and Gercel suggested that microRNA profiling of circulating tumour exosomes could be used as substitute diagnostic markers for biopsy profiling, possibly extending to screening asymptomatic patients (Taylor and Gercel-Taylor, 2008). Another new tool of diagnosis is positron emission tomography/computed tomography (PET/CT. This is superior to and more accurate than Doppler US of pelvis and abdomino-pelvic CT scan or pelvic MRI in diagnosis and differentiation between different cases of benign and marginal ovarian malignancies. In addition, stage IV disease and co-existing neoplasms can be diagnosed by PET/CT herewith informing management and prognosis (Nam *et al.*, 2010).

1.10.7 Therapy of ovarian cancer

In patients with advanced disease, the main objective of therapy is prolongation of progression free survival and overall survival (Foster *et al.*, 2009). Standard treatment of OC involves cytoreductive surgery followed by platinum/taxane chemotherapy, which causes response in 70% of patients. Unfortunately, most will relapse within 18 months with recurrence and chemoresistance. Thus, chemosensitization strategies and advanced targeted therapies are crucial to overcome the mortality of ovarian neoplasms (Zhang *et al.*, 2008). Recurring of OC shifts the goal of therapy from curative to palliative. The treatment of recurrent disease involves the addition of other drugs that are free of cross resistance to the main agents (Ozols, 2002).

1.10.7.1 Surgery

In spite of significant evolution in chemotherapy and biologic treatment of OC, surgery remains an important intervention in the treatment of this disease. It aims to obtain a pathological diagnosis, precisely determine the stage of disease and to produce OC cytoreduction (Ramirez *et al.*, 2011).

Debulking or cytoreduction surgery always followed by washing of the peritoneal cavity with normal saline to remove as many cancer cells as possible. Generally, patients have a rather bilateral oophorectomy (removal of both ovaries) and/or a hysterectomy (removal of the uterus) (Gubbels *et al.*, 2010). Surgery alone may be sufficient only in patients diagnosed with early disease, but in late stage disease, debulking surgery must be followed by chemotherapy. Surgery is of great importance in determining the staging of the disease and without examining the surgical extent of metastasis it is not possible to know the stage of cancer (Gubbels *et al.*, 2010).

1.10.7.2 Chemotherapy

The current gold standard for chemotherapy targeting OC is a combination of carboplatin with paclitaxel, an antimitotic drug. Studies have revealed that such combination accompanied by an increase in survival rates (Piccart *et al.*, 2000).

However, a majority of patients that showed initial response to this treatment will eventually suffer from relapse with the emergence of drug resistance that is observed in a majority of these cases, implying an overall poor prognosis (Jain and Meyer-Hermann, 2010).

Platinum-based chemotherapy is essential for better prognosis of women with advanced OC.

However, with time, cancer cells in well-established tumours gradually start to divide more slowly, losing its sensitivity to chemotherapy and becomes more resistant to treatment (Pinato *et al.*, 2013).

Additionally, in early stage patients it is considered that the adverse outcomes of chemotherapy drastically outweigh beneficial effects and use of conventional chemotherapy is not advised in patients with stage I disease (Trimbos *et al.*, 2010). The use of chemotherapy in early stages is generally confined to situations where staging of the disease is uncertain.

Chemotherapy may also be used for recurrent OC, although low chance of response and resistance to platinum compounds developeds in most patients whose OC reoccurs within 6 months after the last dose of chemotherapy (Parmar *et al.*, 2003).

1.10.7.3 Platinum chemotherapy

The platinum-based drugs cisplatin, carboplatin, and the new members (oxaliplatin, satraplatin and picoplatin) are frequently used for the treatment of lung, ovarian, colorectal, breast, testicular head, neck, and bladder cancers. In 1978, cisplatin received approval for use in the treatment of both testicular and ovarian cancer. The second generation platinum drug carboplatin was approved

in 1989 for use in OC treatment. Carboplatin is more stable equivalent to cisplatin, with similar activity in some types of cancer and less toxicity. It considered as a part of the first line therapy for OC, especially when combined with taxane.

In 2002, a third-generation platinum-based drug, oxaliplatin, was approved by FDA for use in colorectal cancer (McWhinney *et al.*, 2009).

The platinum-based compounds are very efficient and successfully used as a cancer chemotherapeutics. However, some limitations encountered with these drugs include development of cell resistance (Cruet-Hennequart *et al.*, 2008). Their benefit further is compromised by severe adverse effects including bone marrow suppression, anemia, neutropenia, neurotoxicity, nausea and neurotoxicity (Rajeswaran *et al.*, 2008).

Platinum-based drugs are sometimes referred to as "alkylating agents" due to similar effects as alkylating anticancer drugs, even though they do not contain an alkyl group. Platinum-based antineoplastic agents induce cross-linking and DNA damage, primarily at guanine residues, generating monoadducts and DNA protein crosslinks that could be intrastrand cross-links or interstrand crosslinks (ICL). The major DNA adduct of the drug results from covalent linkage of two adjacent guanine bases to platinum to form the intrastrand crosslink that prevents strand separation, inhibiting transcription of gene and inhibit DNA repair and/or DNA synthesis in cancer cells.

Carboplatin causes cell cycle arrest in the G2 phase and induces apoptosis if the DNA damage is not properly repaired. The ability to form ICLs correlates with the cytotoxic effect of the particular drug (Jensen *et al.*, 2013).

1.10.7.4 Taxanes

Taxanes are an important family of drugs that are used in the treatment of different types of cancers, they are introduced to the oncology therapy field in the early 1990s (Zhang *et al.*, 2014). The commonly available taxanes, paclitaxel (Taxol®) and docetaxel (Taxotere®), have become widely accepted as very active chemotherapeutic agents with established beneficial effect in the initial therapy of earlier stages of cancer in addition to their unique effectiveness in alleviating the symptoms of many kinds of cancers, including ovary, breast, head, neck, bladder, lung and esophagus carcinomas (McGrogan *et al.*, 2008).

Taxanes produce their cytotoxic effect by inhibition of cell division, chromatid separation, growth

and finally cause apoptotic cell death. They are usually referred to as mitotic inhibitors or microtubule inhibitors because they stabilize microtubules and reduce their dynamic polymerization, inhibiting the normal formation of mitotic spindles, arrest mitosis and lead to cell death, thus they are also called as mitotic poisons (Risinger *et al.*, 2014).

Taxanes also work by decreasing tumor angiogenesis and cell migration, whilst stimulating the body immune system against cancers (Sweeney and Soutar, 2001). This effect is mediated by increasing the levels of tumor necrosis factor alpha (TNF-α) in macrophages (Bogdan and Ding, 1992). TNF-α is a membrane-integrated cytokine (mTNF-α) usually formed in activated macrophages and monocytes, then released in a soluble form (sTNF-α) by the action of the metalloproteinase ADAM-17. Releasing of sTNF-α from cells can then cause either cell death or a cell-survival response, depending on the receptor to which it binds (MacEwan, 2002).

Recently, it has been reported that taxanes can induce a dose-dependent sTNF-α creation in cancer cells at clinically relevant concentrations, which can contribute to their cytotoxic effect.

Disorder in the TNF cytotoxicity pathway or induction of TNF-dependent NF-kappaB survival genes may, in contrast, have an impact on the taxanes resistance in cancer cells (Sprowl *et al.*, 2012).

Unfortunately, therapy with taxanes is accompanied by side effects such as nausea, vomiting, diarrhoea, bradycardia, hypotension and bone marrow suppression. Peripheral neuropathy, myelosuppression, arthralgia and myalgia are usually observed in patients receiving taxanes where this toxicity accumulates throughout the course of treatment, can be a dose-limiting, and may result in dose reduction or termination of therapy. Side effects of taxanes may be increased by disorders that affect drug metabolism and excretion, such as hepatic or renal failure, by increasing serum levels of drugs. Additionally, drugs that affect the metabolism and excretion of taxanes can increase their toxicity if they are used simultaneously drugs (Sarafraz and Ahmadi, 2008).

Patients quality of life may be adversely affected by taxanes induced cutaneous toxicity and skin reactions that includes nail pigmentation, erythema and desquamation, involving primarily the hands (Minisini *et al.*, 2003).

1.10.8 Resistance to chemotherapy

Although there have been substantial advances in current chemotherapeutic strategies, clinical drug resistance remains a major obstacle to successful cancer treatment and is still a limiting factor in

patients survival (Broxterman *et al.*, 2009). This problem is particularly obvious in the treatment of OC where around 80% of patients initially respond to primary chemotherapy, the majority will relapse and eventually develop resistance to currently available treatment options (Zeller and Brown, 2010).

1.11 Natural products in cancer treatment

Phytochemical study of plants, which have a potential traditional use to manage conditions related with cancer symptomatology, has actually often resulted in the isolation of active principles with anticancer activity.

1.11.1 Natural products in cancer treatment of ovarian cancer

1.11.1.1 Phytochemicals approved for the treatment of ovarian cancer

There is two principal phytochemicals approved for the treatment of Ovarian cancer were: Camptothecin (**Figure 1.4**) which was isolated from the Chinese tree *Camptotheca acuminate* (family Cornaceae by Dr. Monroe E. Wall and Dr. Mansukh C. Wani of Research Triangle Institute (Wall *et al.*, 1966). And Paclitaxel (**Figure 1.4**) which was originally discovered from the bark of the Pacific yew tree, *Taxus brevifolia* Nutt. (family Taxaceae) also by Dr. Monroe E. Wall and Dr. Mansukh C. Wani (Wani *et al.*, 1971).

1.11.1.2 Phytochemicals in clinical study for the treatment of ovarian cancer

Food and Drug Administration (FDA) has approved no new drug for ovarian cancer since 2006 (Ambrosio *et al.*, 2014). Currently, several plant-derived products are in clinical trials.

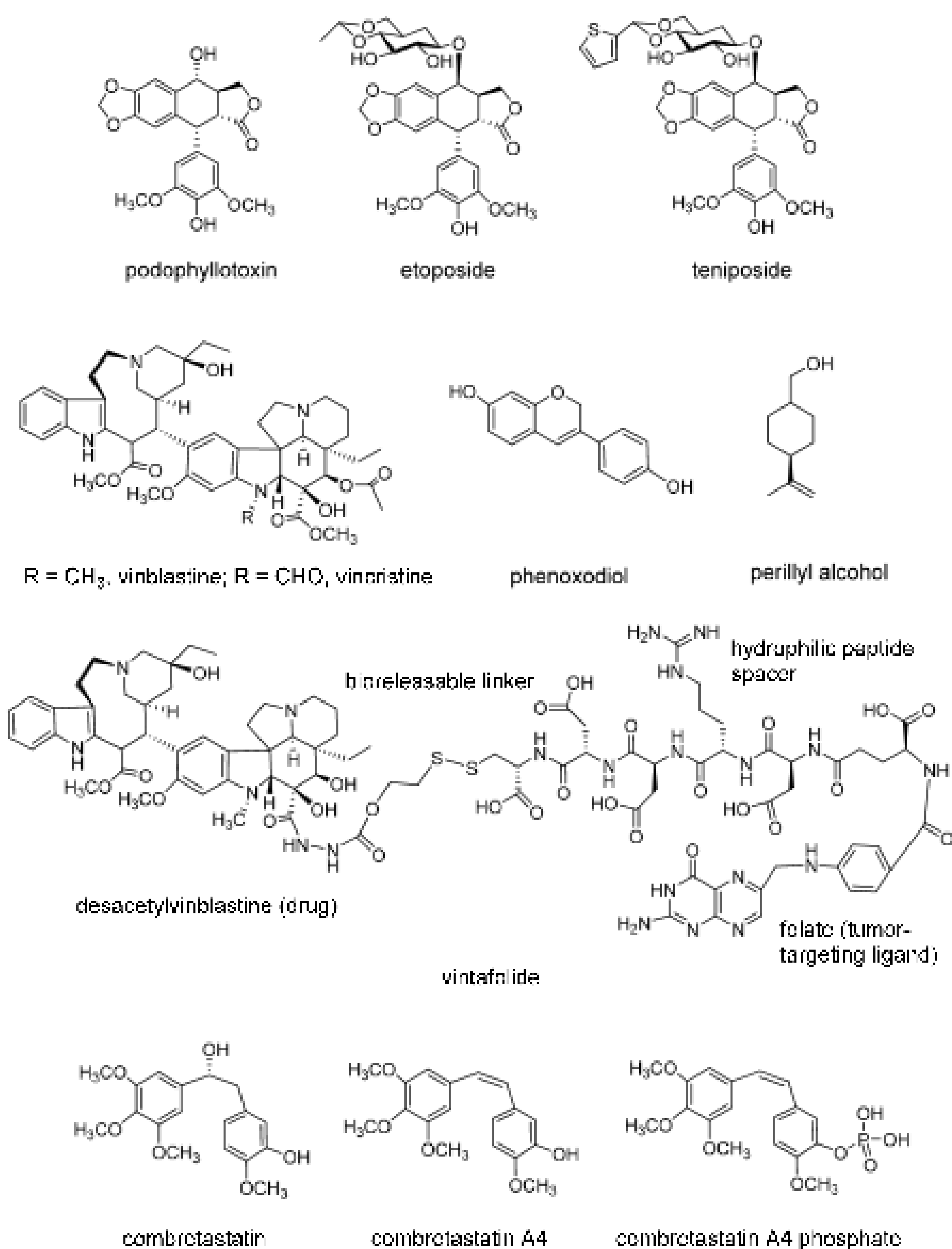


FIGURE 1.4: Phytochemicals and their derivatives currently in clinical trials for the treatment of ovarian cancer (Li *et al.*, 2016).

1.11.1.3 Phytochemicals in preclinical study for the treatment of ovarian cancer

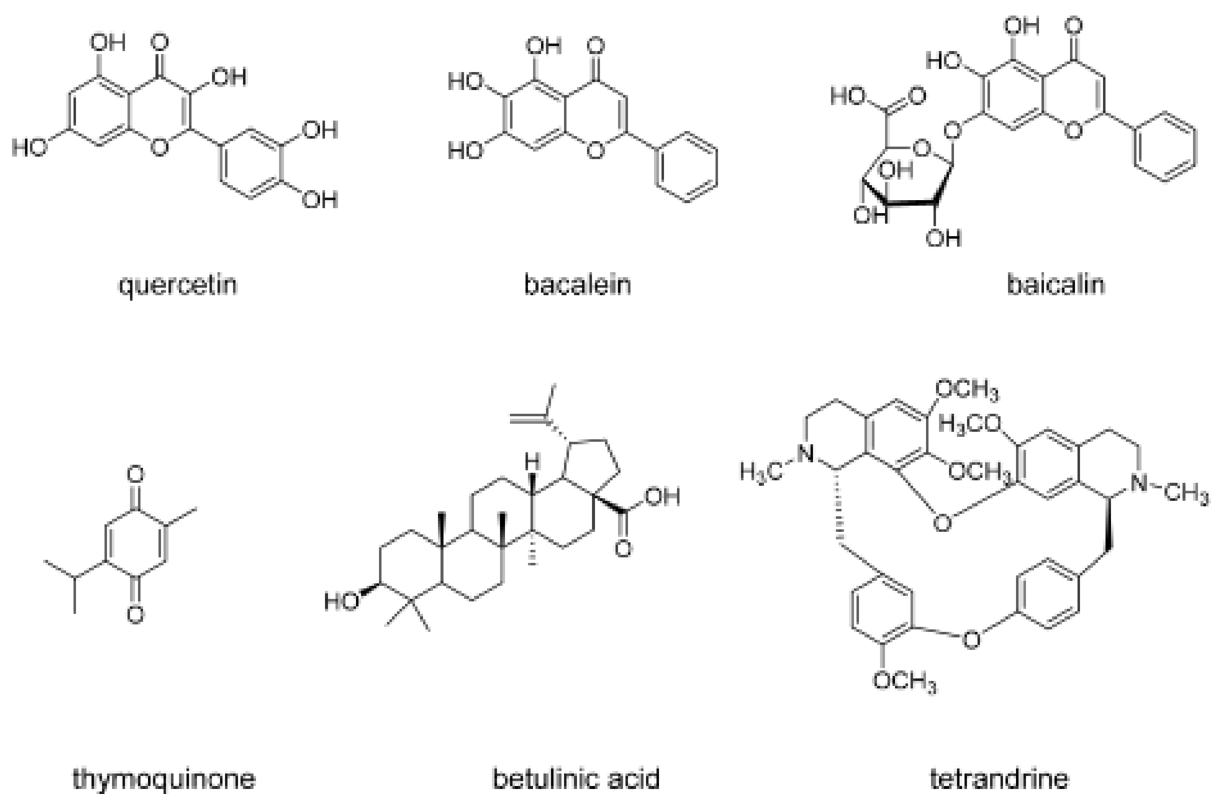


FIGURE 1.5: Phytochemicals currently in preclinical investigation for the treatment of ovarian cancer (Li *et al.*, 2016).

Chapter 2

Species under investigation

2. Species under investigation

2.1 Medicinal plants

Medicinal plants are classified to date as a promising source of natural substances, innovative and original compositions. The latest researches have shown that these natural compounds have served and will serve for the discovery of new natural-based drugs. These natural products and their crude extracts have been used for a long time in the pharmaceutical industries and have played a key role in health care. More than 80% of the world's populations rely on traditional medicine for their primary health care (Kumari and Sharma, 2013). These natural products are classified according to different families, for example, terpenoids, alkaloids, flavonoids, tannins and saponins. These secondary metabolites, usually produced by plants for their defense mechanisms, are involved in the therapeutic properties of most medicinal plants (Znati *et al.*, 2014).

Several Algerian published studies report the dominance of Asteraceae and Lamiaceae families belong to 42 botanical families, which are used by members of the community in their daily lives with 18 and 15%, respectively (Miara *et al.*, 2018).

Asteraceae and Lamiaceae families are the most represented in the Algerian flora with 557 and 183 taxa respectively) (Dobignard and Chatelain, 2010), as well as the Mediterranean flora overall (Médail and Quézel, 2003). Phytochemically, the predominance of this two families may be justified by their organoleptic properties, which are clues to the high content of phenolic and flavonoids compounds responsible for several biological activities (Khaled-Khodja *et al.*, 2014).

2.2 Asteraceae family

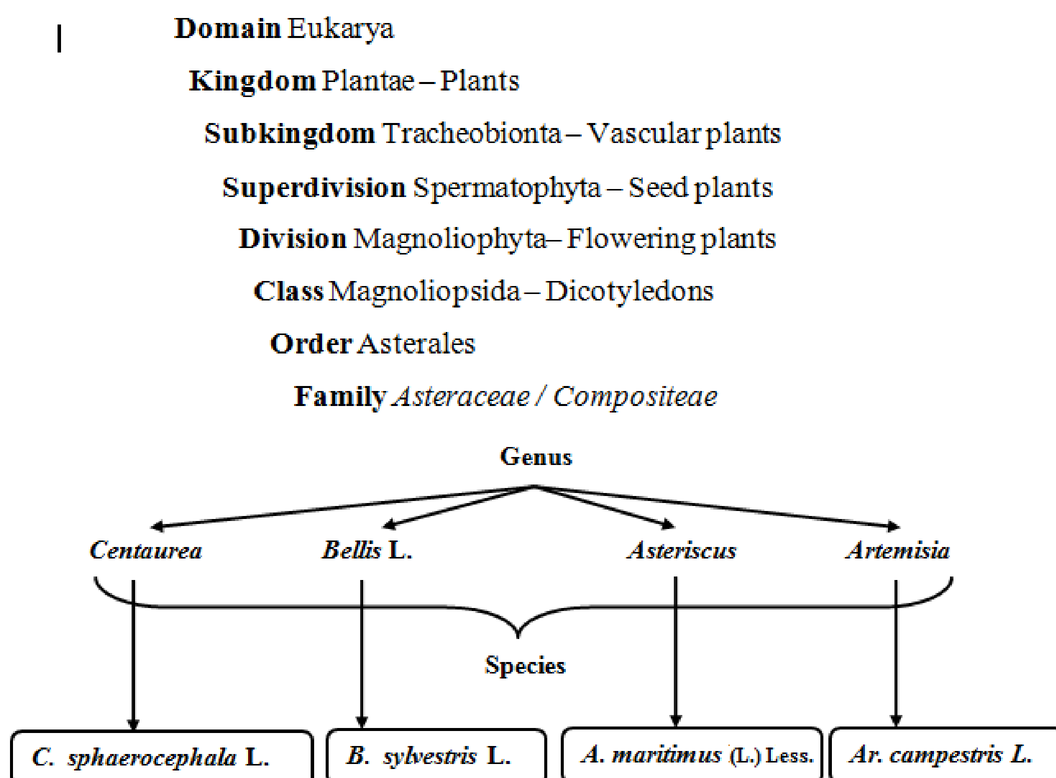
Asteraceae, formerly known as Compositae, is one of the largest families of angiosperms, it represents approximately 10% of all world flora, it contain 5 subfamilies, 30 tribes, 82 subtribes, 1 620 genera and 23 600 species cosmopolitan (Kadereit and Jeffrey, 2007), which are distributed in most ecosystems except for Antarctica (Oberprieler *et al.*, 2009).

Asteraceae family contains a great number of aromatic, spicy, medicinal, and other plants, which contain chemical compounds exhibiting divers biological properties (Hammer *et al.*, 1999), it is a source of valuable drugs and essential oils (EOs) because of its intricate chemical composition comprising several chemotypes. Plant oils and extracts have been used for many purposes, including

raw and processed food preservation, pharmaceuticals, alternative medicines, and natural therapies. Some are popular ornamental plants.

2.2.1 Classification of the studied Asteraceae species

Schema 1 shows the classification of Asteraceae family species.



Schema 1: Classification of the studied Asteraceae species (Morat *et al.*, 2011).

2.2.2 The genus *Centaurea* L. (Centaurée)

2.2.2.1 Morphological description and geographic distribution

The genus *Centaurea* is a large polymorphous genus, includes about 500 species (Quézel and Santa, 1963). It comprises grassy plants, from annual, biennial to perennial, rarely suffruticose. The genus lacks a satisfactory interpretative model and, tentatively, *Centaurea* spp. are divided into groups according to the form of the scales (Kadereit and Jeffrey, 2007).

Centaurea species widespread all around the world, which are predominately distributed around

the Mediterranean region and in West Asia (Djeddi *et al.*, 2008).

In the Algerian flora the genus *Centaurea* is represented by 45 species, including 7 are localised in the Sahara (Quezel *et al.*, 1962).

2.2.2.2 Ethnomedicinal uses of *Centaurea* L. species

Many species of the genus *Centaurea* have traditionally been used for the treatment of various ailments (diabetes, diarrhea, rheumatism, malaria, hypertension) (Sarker *et al.*, 1997) and reported in the literature to be used in folk medicine such as antidandruff, antidiarrhoic, antirhevmatic, anti-inflammatory, and antibacterial (Köse *et al.*, 2007; Csupor *et al.*, 2010; Zengin *et al.*, 2010). Though there are some references of *Centaurea* species usage, most frequently for the treatment of ophthalmia (*C. calcitrapa, C. cyanus*), fever (*C. cyanus, C. jacea, C. solstitialis*), gynaecological problems (*C. cyanus*), digestive complaints (*C. calcitrapa, C. cyanus*), wounds and dermatological complaints (*C. calcitrapa, C. cyanus, C. jacea*) (Hänsel *et al.*, 1992).

2.2.2.3 Phytochemistry of *Centaurea* L. species

The genus has been subjected to several phytochemicals studies leading to the isolation of sesquiterpenes lactones and flavonoids (Yesilada *et al.*, 2004; Koukoulitsa *et al.*, 2002; Negrete *et al.*, 1993 ; Akkal *et al.*, 2003; Karamenderes *et al.*, 2007) also sesquiterpenes (Vanhaelen-Fastré and Vanhaelen, 1976; Gonzalez *et al.*, 1978; Barrero *et al.*, 1995; Bruno *et al.*, 1998) and flavones (Bruno and Herz, 1988; Bruno *et al.*, 1994) as the main secondary metabolites of its species. Also flavanoids, alkaloids and lignans (Shoeb *et al.*, 2005; Karamenderes *et al.*, 2007) and sesquiterpenes lactones, which are guaiane, germacrane, elemane and eudesmane skeletons (Gonzalez *et al.*, 1978; Gurbüz and Yesilada, 2007; Koca *et al.*, 2009).

Several investigations (Buttery *et al.*, 1986; Binder *et al.*, 1990a; Binder *et al.*, 1990b; Lazari *et al.*, 2000; Flamini *et al.*, 2002; Senatore and Bruno, 2003; Senatore *et al.*, 2003; Ertugrul *et al.*, 2003; Dural *et al.*, 2003; Yayli *et al.*, 2005; Asadipour *et al.*, 2005; Başer *et al.*, 2006; Flamini *et al.*, 2006) concerning the composition of the EOs isolated from different parts of world on various *Centaurea* genus showed that germacrene D (21.7–61.0%) and hexadecanoic acid (6.5–30.7%) characterized most species.

The most important metabolites in genus *Centaurea* are acetylenic compounds, flavonoids and terpenoids, mainly sesquiterpenes with guaiane, germacrane, elemane and eudesmane skeletons.

Often, among the sesquiterpenes, the most abundant one is the germacranolide cnicin , sometimes isolated in grams quantity and whose antibacterial cytotoxic and antifungal properties have been investigated (Vanhaelen-Fastré and Vanhaelen, 1976; Gonzalez *et al.*, 1978; Barrero *et al.*, 1995).

2.2.2.4 Pharmacological properties of *Centaurea* L. species

Centaurea species have been used for the treatment of various ailments and in some cases a scientific evidence of their activities has been pointed out, such as antibacterial and hypoglycemic (SUCHY and HEROUT, 1962; Kery *et al.*, 1985), antimicrobial (SUCHY and HEROUT, 1962; Kumarasamy *et al.*, 2003; Yesilada *et al.*, 2004; Güven *et al.*, 2005; Karamenderes *et al.*, 2006; Formisano *et al.*, 2008), antifungal (Panagouleas *et al.*, 2003; Karamenderes *et al.*, 2006), antiprotozoal (Karamenderes *et al.*, 2006), cytotoxic and phytotoxic (Stevens and Merrill, 1985; Koukoulitsa *et al.*, 2002; Medjroubi *et al.*, 2005; Shoeb *et al.*, 2007b; Seghiri *et al.*, 2009;) and anti-inflammatory (Negrete *et al.*, 1993; Garbacki *et al.*, 1999), anti-ulcerogenic (Yesilada *et al.*, 1999); antioxidant (Pieroni *et al.*, 2002; Ugur *et al.*, 2009), antiplasmodial (Medjroubi *et al.*, 2005), antiviral (Rusak *et al.*, 1997).

Aqueous extract of *C. aspera* shows hypoglycaemic effect in diabetic rats (Masso and Adzet, 1976). Antifungal activities were reported in constituents of *C. thessala* and *C. attica* (Lazari *et al.*, 2000), while chloroform extract from *C. musimomum* showed antiplasmodial effects (Medjroubi *et al.*, 2005) and methanolic extract from *C. diffusa* demonstrated antibacterial activities (Skliar *et al.*, 2005).

2.2.2.5 *Centaurea sphaerocephala* L.

Centaurea sphaerocephala an Algerian endemic plant, belonging to the tribe Cynarea of the Asteraceae family, is widespread in the entire Mediterranean region. is a perennial grassy plant with scattered leaves, 50 cm tall, that blooms from June to September.

2.2.2.6 Phytochemistry of *C. sphaerocephala* L.

A literature search reports that studies of analysis of the volatile components of these plants are limited, although some studies have previously referred to a sesquiterpene lactones in the aerial parts (Bruno *et al.*, 1998).

2.2.3 The genus *Bellis* L.

2.2.3.1 Morphological description and geographic distribution

The genus *Bellis* includes 82 scientific plant names of species rank, of these 10 are accepted species names (*B. annua* L.; *B. azorica* Hochst.; *B. bernardii* Boiss. & Reut.; *B. caerulescens* (Cosson) Coss. ex Ball; *B. dubia* Spreng.; *B. hyrcanica* Woronow; *B. longifolia* Boiss. & Heldr.; *B. perennis* L. ; *B. rotundifolia* (Desf.) Boiss. & Reut.; *B. sylvestris* Cirillo), all of which are small annual or perennial herbs, grow from 5–20 cm (2–8 in) tall (Calvo *et al.*, 2012). They have simple erect stems, and most species have basal leaves. They have radiate flower heads that are produced one per stem (of North America Editorial Committee, 1993). The genus is native to Europe, the Mediterranean and Northern Africa. One species has been introduced into North America and others into other parts of the world (Di Foggia, 2014). Most *Bellis* species are endemic to restricted regions and only *B. perennis* (the common daisy), *B. annua* and *B. sylvestris* (the southern daisy) are widespread in their distribution.

In Algeria the genus includes 3 species,e.i, *B. sylvestris* L.; *B. annua* L.; *B. repens* Lamk.

The genus *Bellis* has an important role as a source of medicinal plants. At the same time, these species have commercial importance as an ornamental plant (Morikawa *et al.*, 2008).

2.2.3.2 Phytochemistry and pharmacological properties of *Bellis* L. species

While *B. perennis* L. has been used for centuries as a medicinal plant, no information is available on the medical use of other species of the genus. Most species of *Bellis* genus are characterized by saponins, in preliminary investigations, several deacylated triterpenoid saponins were obtained in yields of about 4‰ from the perennial species *B. perennis* (Hiller *et al.*, 1988), *B. sylvestris* (Schöpke *et al.*, 1994), and *B. bernardii* (Schöpke *et al.*, 1995), while *B. annua* contains only 0.3‰ (Schöpke *et al.*, 1996a). Also the composition of EOs from various species of *Bellis* especially *B. perennis* has been investigated (Scognamiglio *et al.*, 2012) .

2.2.3.3 Pharmacological properties of *Bellis* L. species

The MeOH extract of *B. longifolia* was the most phytotoxic against *Lactuca sativa* L. and *Agrostis stolonifera* L., comparetely with CH_2Cl_2 and H_2O extracts. Bioassay-guided fractionation revealed that a fraction consisting mainly of saponins was the most effective and investigation of the

active fraction led to the isolation and structure elucidation of three previously undescribed triterpene saponins, 3-*O*-β-D-fucopyranosyl polygalacic acid, 28-*O*-α-L-rhamnopyranosyl-(1 / 2)-β-D-fucopyranosyl polygalacic acid and 3-*O*-β-D-fucopyranosyl- 2α,3β,23-trihydroxyolean-12-en-28-oic acid, which were present as the main phytotoxic compounds of the methanol extract. Two triterpenes, polygalacic acid and bellisonic acid and four kaempferol glucosides, as well as chlorogenic acid were also isolated. 3-*O*-β-D-fucopyranosyl polygalacic acid and 3-*O*-β-D-fucopyranosyl-2α,3β,23-trihydroxyolean-12-en-28-oic acid had phytotoxic activity similar to some commercial herbicides (IC_{50} values of ca. 25 mM) against duckweed (*Lemna paucicostata*) (Stavropoulou *et al.*, 2017) .

2.2.3.4 *Bellis sylvestris* L.

B. sylvestris the southern daisy, is an official and edible plant native to the Mediterranean region, flowering begins in autumn and reaches a maximum in winter (Fraga *et al.*, 2007). These species have spathulate or oblanceolate leaves, campanulate involucrum, 1-2 seriate phyllaries, conical receptaculum, 1 seriate ray flowers, white or pink ligules, yellow disc flowers and obovate achenes (Özdemir and Sepet, 2011).

B. sylvestris use is highly super-imposable to that of another plant of the same genus, *B. perennis*. Young leaves are eaten as a salad, while leaf and flowers are known for their diuretic, purgative and diaphoretic characteristics (Calvo *et al.*, 2012; Karakaş *et al.*, 2012). They also have anti-inflammatory and astringent properties and have been utilized to cure common cold and infections of the upper respiratory tract in folk medicine (Cakilcioglu *et al.*, 2010).

2.2.3.4.1 Phytochemistry of *Bellis sylvestris* L.

Previous studies on *B. sylvestris*, led to, the isolation of triterpenoid saponins (Scognamiglio *et al.*, 2012), the isolation of 28 secondary metabolites from leaves, belonging to different classes, one of flavonoids phenolic acids were reported for the first time, and their bacterial strains showed variable degrees of susceptibility to the compounds (Scognamiglio *et al.*, 2016).

2.2.4 The genus *Asteriscus* Moench

In 1997, taxonomic study realized by Greuter demonstrated that the genus *Asteriscus* replaces *Nauplius* CASS (Greuter, 1997). This genus containes 8 species, grow widly in a variety of

coastal and desert habitats throughout the Middle East and Mediterranean areas (Anderberg and Eldenäs, 1994), two of them are very widespread, namely in the Mediterranean (*A. aquaticus* and *A. graveolens*). The other six species have very restricted distributions (Wiklund, 1987).
In Algeria this genus includes 5 species,i.e., *A. maritimus* (L.) Less.; *A. pygmaeus* Coss et CRAL) ; *A. spinosus* (L.) G.G; *A. graveolens* (FORSSK.) DC. and *A. aquaticus* (L.) LESS. (Quézel and Santa, 1963).

2.2.4.1 Phytochemistry and pharmacological properties of *Asteriscus* Moench species

As mentioned in literature, *Asteriscus* species are characterized by a high content of flavonoids, sesquiterpenes, bisabolone and hydroperoxides (Akssira *et al.*, 2006). Also contains EOs (Medimagh *et al.*, 2012; Palá-Paúl *et al.*, 2014; Chaib *et al.*, 2017).
A considerable attention has been given to the genus *Asteriscus* from which mainly sesquiterpenes have been reported. Asteriscunolides A-D were previously isolated from the aerial parts of some *Asteriscus* species such as *A. aquaticus* (San Feliciano *et al.*, 1989) and *A. vogelii* (Rauter *et al.*, 2001). Other sesquiterpene lactones such as Asteriscanolide and Aquatolide were obtained from the hexane extract of *A. aquaticus* (San Feliciano *et al.*, 1984; San Feliciano *et al.*, 1989).
Flavonoids (Ahmed *et al.*, 1991; Youssef *et al.*, 1995), bisabolone hydroperoxides (Sarg *et al.*, 1994) as well as farnesol and thymol derivatives (Ahmed *et al.*, 1991) were also described as constituents of extracts from *Asteriscus* plants and reported to possess antimicrobial and hypoglycemic activities (Ahmed *et al.*, 1991; Youssef *et al.*, 1995), whereas Asteriscunolides C and D have shown phytotoxic activities and the last one also exhibited cytotoxic effects (Rauter *et al.*, 2001).
Extracts of organic solvents from *A. imbricatus* DC. showed antifungal activity and anticorrosion inhibition (Senhaji *et al.*, 2013). However, most of the studies have been focused only on one species, *A. graveolens* (Forssk.) Less., describing their flavonoids (Ahmed *et al.*, 1991), new derivatives (El Dahmy *et al.*, 1985; Sarg *et al.*, 1994), polyphenols, flavonoids, tannins (Ramdane *et al.*, 2017) and EO activities (Znini *et al.*, 2011; Znini *et al.*, 2012; Cristofari *et al.*, 2012). The EO of this species was characterized by a high content of 6-oxocyclonerolidol (66.7%) and 6-hydroxycyclonerolidol (8.8%) which exhibited fungicidal properties towards *Alternaria* sp. (Znini *et al.*, 2011). Also antioxidant, antibacterian antilishmanial and cytotoxicity activities (Ramdane *et al.*, 2017). EOs of some *Asteriscus* species exhibited inhibition of mild steel corrosion (Znini *et al.*, 2012).

Other report suggests that these compounds could be considered as chemical markers of this genus that characterize the stem and leaf oil, while cis-8-acetoxychrysanthenyl acetate, which has been isolated for the first time, was the major compound of the flower oil (Cristofari *et al.*, 2012).
The EOs extracted from *Pallenis spinosa* (L.) Cass (=*A. spinosus* (L.) Chultz Bip) showed germacra-1- (10),5-dien-3,4-diol (18.4%), α-cadinol (14.1%), 3-acetoxygermacra- 1(10),5-dien-4-ol (13.0%), t-cadinol (8.2%) and δ-cadinene (5.8%) as main constituents (Senatore and Bruno, 2003).

2.2.4.2 *Asteriscus maritimus (L.) Less.* (= *Pallenis maritima (L.) Greuter)*

2.2.4.2.1 Morphological description and geographic distribution of *A. maritimus* (L.) Less.

Asteriscus maritimus is a perennial herbaceous plant, native halophyte of lands surrounding the Mediterranean Sea, known since long time to be highly mycotrophic (Mason, 1928). It is perfectly adapted to the semi-arid climate and grows mainly on coastal cliffs and very close to coastal areas (Valdés *et al.*, 1987), being a species perfectly adapted to water stress and salinity. *A. maritimus* is common in rocky areas of the Western portion of the Mediterranean basin, in Southern Portugal as well as in Western and Southern Greece (Fraternale *et al.*, 2001).
In Algeria, *A. maritimus* (L.) Less., found in the Mediterranean region and is excellent for the coverage of large surfaces, in combination with other species. It usually lives in rocky areas near the coast and in scrubland on rocky or well drained sandy soils, it is able to support high levels of insolation and summer droughts. It blooms from April to August, it is papillose, fragrant with petiolate leaves ranging from elongate to spatulate. It has a showy flowering (yellow florets, which are tubular in shape with 5 lobes, hermaphrodites), which is maintained almost throughout the entire year; achenes 1-5 mm, papus 1-1.5 mm.

2.2.4.2.2 Phytochemistry and pharmacological properties of *Asteriscus maritimus* (L.) Less.

Chemical analysis showed that the EO extrcacted from the aerial part of the Italian *A. maritimus* contained myrtenyl acetate (44.2%) as the major component as well as terpinen-4-ol (4.5%), terphenyl and (17.5%) and (Z)-β-farnesene (12.9%) they were reported to be associated with the insecticidal activity of the oil against flesh fly larvae and insects (Fraternale *et al.*, 2001). Other studies have shown that the EOs exhibited insecticidal, antimicrobial and anti-acetylcholinesterase activities (Fraternale *et al.*, 2001; Medimagh *et al.*, 2013). Root oil extrcted from *A. maritimus*

growing in Tunisia, characterized by a high proportion of oxygenated compounds (65.0%) and exhibited antifungal activity against *Aspergillus flavus*, *A. niger*, *Botrytis cinerea* and *Penicillium* sp. (Medimagh *et al.*, 2013).

2.2.5 The genus *Artemisia* L. (Armoise)

Artemisia L. is among the most widely distributed and largest genera of the Asteraceae family (Oberprieler *et al.*, 2009). This genus contains about 500 species of herbs and shrubs (Martín *et al.*, 2003) distributed in Northern Africa, the Mediterranean region, Western Asia and Southwestern Europe, and in Arabian Peninsula (Al-Snafi, 2015). Species of this genus are especially found in the temperate sectors of Northern hemisphere, but restricted numbers of species are also found in the Southern hemisphere of the world (Oberprieler *et al.*, 2009).

Artemisia is represented by 9 species in the Algerian flora,i.e., *A. herba-alba* Asso; *A. AbsinthilJm* L.; *A. arborescens* L.; *A. judaica* L.; *A. atlantica* Coss. et Dur.; *A. alba* Turra; *A. campestris* L.; *A. Verlotorum* Lamotte; *A. vulgaris* L. (Quézel and Santa, 1963)

2.2.5.1 Ethnomedicinal uses of *Artemisia* L. species

Ethnobotany, economic botany, medicinal importance and phytotherapy of *Artemisia* have been reviewed (Parada *et al.*, 2009) and an extensive work on this genus unfolded different traditional and medicinal uses of its species (Wright, 2002).

Since ancient times, some species of *Artemisia* are aromatic and often used worldwide as spices, herbs and folk medicines (Hose, 2002) with various and well-known therapeutic applications (stomachache, colds and coughs, diarrhoea, angina, parasitism, intestinal and bronchial infections, wounds, pimples) (Tan *et al.*, 1998; Benli *et al.*, 2007).

2.2.5.2 Phytochemistry of *Artemisia* L. species

Chemical studies on *Artemisia* species indicate that all classes of compounds are present in the genus with specific reference to flavonoids and terpenoids. These essential metabolites may also include EOs, saponins, phenols, cyanogenic glycosides, tannins, phenolic glycosides, unsaturated lactones, and glucosinolates (Al-Zubairi *et al.*, 2011).

The rich accumulation of EOs and other terpenoids in the genus is responsible for the use of various members for flavouring foods or liqueurs (Wright, 2002).

2.2.5.3 Pharmacological properties of *Artemisia* L. species

Pharmacologically, *Artemisia* is one of the crucial polymorphic genera comprises important medicinal plants which are currently the subject of phytochemical attention due to their biological and chemical diversity. These diverse biological activities are manifested by a lot of secondary metabolites which occurs naturally, and may be important in pharmacology.
Some species of *Artemisia* exhibit antitumor, anti-inflammatory, antimalarial, antioxidant, antispasmodic, antimicrobial, insecticidal, antifungal and antioxidant activities (Tan *et al.*, 1998). There are also several reports concerning the cytotoxic, antidiabetic, antipyretic and analgesic activities of different *Artemisia* species (Wright, 2002; Steinhoff, 1997; Tan *et al.*, 1998; Korkmaz and Gürdal, 2002).

2.2.5.4 *Artemisia campestris* L.

2.2.5.4.1 Morphological description of *Artemisia campestris* L.

A. campestris L. is one of the common species of this genus, commonly known as field sagewort, field wormwood, field sagebrush, beach wormwood, prairie sagewort, tall wormwood, locally named as "T'gouft" (Megdiche-Ksouri *et al.*, 2015). It is a biennial or short-lived perennial undershrub, that may reach 30– 150 cm in height, with branched and ascending stems that form a panicle shape; it is usually brownish-red and glabrous (Quezel *et al.*, 1962). The leaves are green, sericeous when young, often glabrescent when mature (Chalchat *et al.*, 2003).
The plant has a composed inflorescence: the capitulum ovoid and heterogameous, containing 8 to 12 flowers (Ouyahya, 1990). The fruit is an ovoid achene lacking pappus. The central stem and ascending lateral stems are light green to dark red and terete (Quezel *et al.*, 1962; Chalchat *et al.*, 2003).

2.2.5.4.2 Geographic distribution of *Artemisia campestris* L.

Geographically, *A. campestris* L. was native in Asia and now is growing widly in North America (Yun *et al.*, 2007); and predominates in the arid regions of North African countries (Noumi *et al.*, 2010) like Algeria (Rebbas and Bounar, 2014), Morocco (Jamila and Mostafa, 2014), Tunisia (Islem *et al.*, 2014), and Libya (El-Mokasabi, 2014).

In Algeria, *A. campestris* growing widly in the arid and semi-arid regions (Quézel and Santa, 1963; Baba Aissa, 1991).

2.2.5.4.3 Ethnomedicinal uses of *Artemisia campestris* L.

A. campestris L. is largely used as decoction for its pharmacoligical activities (Megdiche-Ksouri *et al.*, 2015), it is commonly used as an anthelmintic and a treatment for cutaneous, respiratory and digestive problems in many countries like Algeria, Tunisia, Morocco, Libya, Turkey, Italy, Spain, Serbia, India, Argentina, Canada and United States (Sassi *et al.*, 2007; Boulanouar *et al.*, 2013; De Natale and Pollio, 2012; HassaniM *et al.*, 2013; Jamila and Mostafa, 2014; Gast, 1989; Hammiche and Maiza, 2006; Kujawska and Hilgert, 2014; Leporatti and Ghedira, 2009; Popović *et al.*, 2012; Shemluck, 1982; Tlili *et al.*, 2013). It is used as an antidiabetic and antihypertensive in Algeria, Morocco and Japan (Aniya *et al.*, 2000; Sassi *et al.*, 2007; Bnouham *et al.*, 2002; Boudjelal *et al.*, 2013; Djidel *et al.*, 2014), and is used to treat kidney, urinary and liver disorders in Algeria, Tunisia and Japan (Aniya *et al.*, 2000; Sassi *et al.*, 2007; Benchelah *et al.*, 2004; Ferchichi *et al.*, 2006; Minami *et al.*, 2010). It has an extensive use as an emmenagogue and as a circulationregulator, especially in post-partum care, in Algeria (Boulanouar *et al.*, 2013; Hammiche and Maiza, 2006) and Serbia (Popović *et al.*, 2012).
Furthermore, it is used as a febrifuge in Algeria (Boulanouar *et al.*, 2013), Tunisia (Sassi *et al.*, 2007) and Italy (Guarino *et al.*, 2008) and is utilized as an anti-venom in Tunisia (Sassi *et al.*, 2007) and India (Bahekar *et al.*, 2016). It possesses other traditional actions, such as anti-inflammatory and tonic (Sassi *et al.*, 2007; Popović *et al.*, 2012), as an analeptic (Hammiche and Maiza, 2006) and it is also recommended in the treatment of eye diseases (HassaniM *et al.*, 2013).
A. campestris flowers were used as hypoglycemic, cholagogue, choleretic, digestive, depurative, antilithiasic, and for the treatment of obesity and to decrease cholesterol. It was used as a decoction as antivenin, anti-inflammatory, anti-rrheumatic and antimicrobial (Hmamouchi, 1999; Bnouham *et al.*, 2002).
The aerial part of the plant was used by the popular medicine as antihelminthic, desinfectant, cholagogue, tonic, hypotensif and antivenom (Akrout *et al.*, 2010). In Algeria, it was used for the treatment of stomach and for the menstrual pain (Dob *et al.*, 2005; Djeridane *et al.*, 2006).

2.2.5.4.4 Phytochemistry of *Artemisia campestris* L.

Chemical analysis revealed that the fresh parts of *A. campestris* contained flavonoids, alkaloids, terpenes and saponins (Al-Snafi, 2015). However, it appeared that oil constituents were vary according to plant source and variety, Table 1 showed a previous phytochemistry studies of *A. campestris* oil.

In Algeria, little variability has been found in EOs obtained from *A. campestris* L. growing in two different regions, which were rich in monoterpenes (84.5–91.7%), while the total of sesquiterpenes were estimated as (5.1%–7.2%). The major components of *A. campestris* L. have been identified as β-pinene (25.6%), α-Terpenyl acetate (18.8%), α-pinene (18.4%), sabinene (17%), (Z, E)-farnesol (10.3%), camphor (9.2%), camphene (7.7%), limonene (6.6%), cedrol (5.4%), borneol (5.2%), *p*-cymene (4.1%), and verbenone (3.8%) (Belhattab *et al.*, 2011).

2.2.5.4.5 Pharmacological properties of *Artemisia campestris* L.

A. campestris, showed several pharmacological activities such as cytotoxic, antimicrobial, antioxidant, insecticidal, anti venomous and many other pharmacological effects (Al-Snafi, 2015).

2.3 Lamiaceae family

Lamiaceae (syn. Labiataea), is a large herb family consists of more than 252 genus and 7 200 species of worldwide distribution (Belhattab *et al.*, 2011) growing under great variety of soils and climates but more abundant in Mediterranean and mountainous region (Arfan *et al.*, 1996).

Many members of Lamiaceae are cultivated in dry, mild and cold districts of Asia, Europe and North Africa due to their aromatic qualities and ease of cultivation (Sonmezdag *et al.*, 2017). This family contains thousands of flowering species widely used in landscapes and butterfly gardens (Wu *et al.*, 2016). Most species of this family produce EOs secreted by glandular hairs on aerial vegetative and some reproductive organs (Dinç and Doğu, 2013).

Lamiaceae family is the most diverse and extensive herbal family in the field of pharmaceutical studies and their pharmaceutical importance is due to the EO available in them, also several genera of this family contain biologically active compounds (Sharafzadeh and Zare, 2011).

2.3.1 Classification of the studied Lamiaceae species

Schema 2 shows the classification of Lamiaceae family species.

Domain Eukarya
Kingdom Plantae – Plants
Subkingdom Tracheobionta – Vascular plants
Superdivision Spermatophyta – Seed plants
Division Magnoliophyta – Flowering plants
Class Magnoliopsida – Dicotyledons
Order Lamiales
Family Lamiaceae / Labiateae
Genus ***Lavandula***
Species *L. stoechas* L.

Schema 2: Classification of the studied Lamiaceae species (Morat *et al.*, 2011).

2.3.2 The genus *Lavandula* L. (Lavender)

2.3.2.1 Morphological description and geographic distribution of *Lavandula* L. species

The genus *Lavandula* is one of the most well-known EO crop in the world with its 39 species, several hybrids and 400 officially registered varieties (Benabdelkader *et al.*, 2011). The geographic distribution of lavender ranges from the Canary Islands to Capo Verde Island including the Mediterranean area, North Africa, the Arab peninsula, the centre and the Southeast of India (Lis-Balchin, 2002). This genus is represented in the Algerian Flora by 6 species, i.e., *L. stoechas* L., *L. multifida* L., *L. coronopifolia* Poiret., *L. pubescens* Dec., *L. dentata* L., and the more recently added *L. antineae* Maire (Quézel and Santa, 1963; Upson and Andrews, 2004).

2.3.2.2 Phytochemistry and pharmacological properties of *Lavandula* L. species

When we consider the rich chemical content and all the pharmacological properties, *lavandula* EO is a significant product and encourage the cultivation of this plant as an industrial crop for EO production (Stanev *et al.*, 2016). EOs distilled from *Lavandula* species have been used for

cosmetics and therapeutic purposes for centuries. It is widely employed in all types of soaps, lotions and perfumes, with the most commonly used species being *L. stoechas*, *L. angustifolia*, *L. latifolia* and *L. x intermedia* (Aburjai and Natsheh, 2003). Thus, *Lavandula* oil promotes healing symptoms for anxiety, stress, exhaustion, migraines, insomnia and depression (Fismer and Pilkington, 2012; Koulivand *et al.*, 2013; Danh *et al.*, 2013; Koulivand *et al.*, 2013; Rafie *et al.*, 2016). The main active constituents of *Lavandula* EO; linalool, linalyl acetate, 1,8-cineole, cis and trans-ocimene, terpinen-4-ol and camphor, has been reported to have antioxidant, antimicrobial and anticholinesterase activities (Cavanagh and Wilkinson, 2002; Hanamanthagouda *et al.*, 2010; Costa *et al.*, 2012; Gonçalves and Romano, 2013).

2.3.2.3 *Lavandula stoechas* L.

2.3.2.3.1 Morphological description and geographic distribution of *L. stoechas* L.

L. stoechas L. ssp. stoechas, is an evergreen, perennial shrub, is widely found in the Mediterranean region characterized by dry, hot and sunny conditions in alkaline soils. In Algeria, *L. stoechas* L. (syn. *Stoechas officinarium* Moench) is known as "Helhal" and is widely distributed across all the Northern fringes of the country, It has been reported that this plant is a predominant aromatic plant species abundant in the Mediterranean region, where it can be a common component of low-growing shrub vegetations on acidic soils (heath) (Upson and Andrews, 2004). The leaves are small with petioles, hairy and long, have white greyish green appearance. The plant grows from root with few branches that are very similar rosemary. The fruits are light brown in color, flowers are the economic parts of the plant, that may reach 30–100 cm (Lis-Balchin, 2002).
Due to its rich aromatic content, the cultivated forms of the plant have been introduced throughout the world. There is an increasing demand of the plant in the fields of cosmetics and perfumery industry and also in pharmaceuticals (Carrasco *et al.*, 2015).

2.3.2.3.2 Phytochemistry of *L. stoechas* L.

The EO composition of wild and cultivated *L. stoechas* collected in several Mediterranean countries has been studied. About 60 chemical constituents have been described. These mostly include mono- and sesquiterpenes. The most commonly reported chemotype is a fenchone/camphor chemotype, though a fenchone/1,8-cineole and a pulegone chemotype have also been reported (Kokkalou, 1988;

Garcia Vallejo, 1992; Valentini *et al.*, 1993; Skoula *et al.*, 1996; Baldovini *et al.*, 1998; Ristorcelli *et al.*, 1998; Gören *et al.*, 2002; Zrira and Benjilali, 2003; Dadalioglu and Evrendilek, 2004; Bouzouita *et al.*, 2005; Arabaci and Bayram, 2006; Dob *et al.*, 2006; Giray *et al.*, 2008). Additional compounds have been detected in non-EO extracts of *L. stoechas*. These include flavonoids (Upson and Andrews, 2004, longipinene derivatives (Ulubelen *et al.*, 1988) and triterpenoids (Topcu *et al.*, 2001).

2.3.2.3.3 Uses in traditional medicine and reported activities of *L. stoechas* L.

This species is one of the most explored lavenders in the world and the reported medicinal properties of this species are also very diverse, since *L. stoechas* has traditionally been used as expectorant, carminative, stimulant, wound healing adjuvant (Gören *et al.*, 2002; Giray *et al.*, 2008), antispasmodic, sedative, diuretic, therapy for rheumatic diseases (Angioni *et al.*, 2006), analgesic and antiseptic (Gülçin *et al.*, 2004a) and anticonvulsant (Gilani *et al.*, 2000). Most of these properties are due to the EO fraction of the plant.

Several recent studies have revealed that EO and other extracts of *L. stochas* have antimicrobial (Gören *et al.*, 2002; Angioni *et al.*, 2006; Dadalioglu and Evrendilek, 2004; Bouzouita *et al.*, 2005; Moon *et al.*, 2007), insecticidal (Traboulsi *et al.*, 2002) and antioxidant (Gülçin *et al.*, 2004a) properties. Some other studies have considered the antibacterial (Dadalioglu and Evrendilek, 2004; Benabdelkader *et al.*, 2011), antifungal (Angioni *et al.*, 2006; Benabdelkader *et al.*, 2011) and antioxidant (Benabdelkader *et al.*, 2011; Messaoud *et al.*, 2012) properties of the lavender EO.

Additionally, the plant has been reported to be useful against urinary infections, together with its wound healing properties as well (Baytop, 1999).

In the Algerian folk medicine, the aerial parts of *L. stoechas*, especially the inflorescences, are used as an antiseptic and stimulant agent (Mahmoudi, 1990), in the Algerian cuisine, they are also used as culinary herb to prepare the most popular couscous. EO of *L. stoechas* have been used to treat depression, diabetes, epilepsy, headaches, it posses sedative properties and anti-inflammatory, antimicrobial, antifungal, and antioxidant activities (Saadi *et al.*, 2016).

Experimental work

Chapter 3

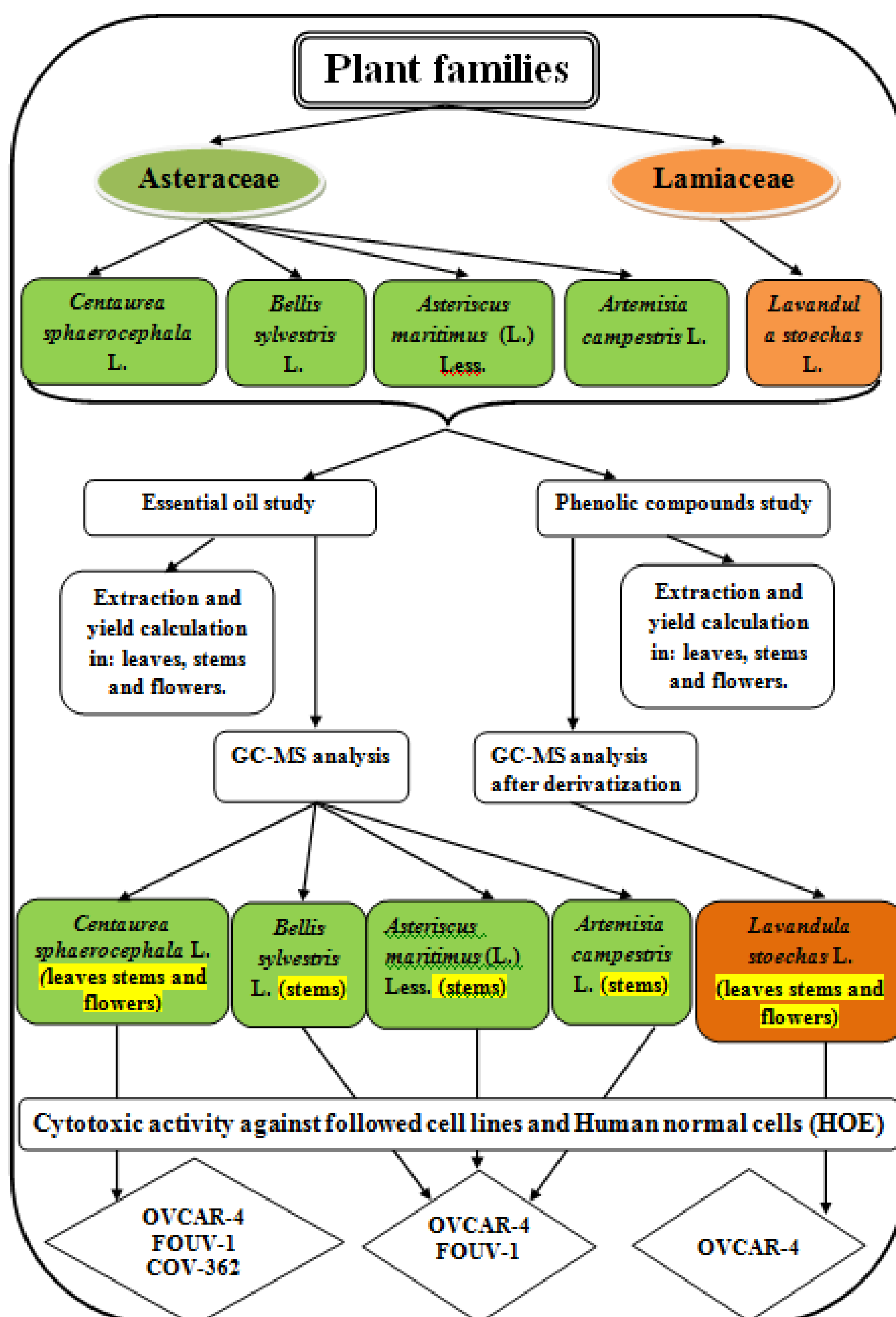
Material and Methods

3. Material and Methods

3.1 Collection of plant material

Asteraceae and Lamiaceae plants were selected on the basis of intensive review and ethnopharmacologic information. studied species were collected at the flowering season during 2012, 2013 and 2014, from two different regions, some of them from wild populations in National Parc Belezma, Batna region, North east of Algeria (latitude 35° - 34' N; longitude 6° - 01' 38' E and altitude 200 m above mean sea level) in May, 2012 and 2013. Whereas the other species were assayed from wild populations in National Parc ElKala, Elkala region North east of Algeria (latitude 36o 53' 44" N; longitude 8o 26' 35' E and altitude 1202 m above mean sea level) in May, 2012, 2013 and 2014. Plant materials were further identified and authenticated on the basis of Quézel and Santa (1963), voucher specimens were deposited in the Bimolecules and plant breeding laboratory , life science and nature department, faculty of exact science and life science and nature, university of "Larbi Ben M'hidi", Oum Elbouaghi 04000 Algeria.

Schema 3 shows the general steps of the experimental protocol.



Schema 3: General schema of the experimental protocol.

3.2 Essential oil study

3.2.1 Extraction of plant materials

The plant material were crushed and ground in a pestle and mortar in order to rupture, maximum, the cell walls of oil sacs. The ground material (100 g) were subjected to extraction bye the process of hydrodistillation using a clevenger type apparatus for 3 hours (Parvez *et al.*, 1992), the plant material immediately charged to the flask fitted with a water cooling condenser, in order to avoid the loss of EO by evaporation. The steam from generator was passed through the material employing a bulb tube with holes made on it to ensure the uniform circulation of steam through the material. The steam along collected in a receiver which was kept in ice cooled water in order to prevent the evaporation of low boiling constituents of the essential oils.

This was kept for or while so as to allow the maximum amount of the essential oil to come at the top in the form of an oily layer. The oil was separated from the aqueous layer with the help of a separating funnel. The aqueous layer was further extracted with diethyl either (EtO_2) in order to recover the partially miscible oxygenated fractions of the oil. The ethereal extracts were dried over anhydrous sodium sulphate (Na_2SO_4). The solvent was removed, under reduced pressure. The oil was recovered and packed in amber-coloured sample bottle at -18 ºC for further studies.

3.2.2 Essential oil yield calculation

The yield of each extraction is calculated by the ratio between the mass of the essential oil extracted and the mass of the treated plant material. The yield expressed as a percentage is calculated by the following formula:

$$\frac{MHE}{MVE} \times 100 \tag{3.1}$$

Y: Yield.

MHE: Mass of essential oil extracted in (g).

MVE: Mass of dried plant material.

3.2.2.1 Statistical analysis

All tests were performed in triplicate and results were expressed on the basis of dry matter weight. Data were expressed as mean ± SD. The means were compared by using the two-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at $p < 0.05$. All analyses were performed by using the SPSS v. 9.1.3 program

3.2.3 Essential oil analysis using gas chromatography - mass spectrometry (GC-MS)

Qualification of the essential oil diluted in diethyl ether (EtO_2) (1ml) was analyzed by GC-MS, using on a Finnigan- MAT 8200 Mass Spectrometer coupled with a Perkin Elmer Clarus 500 GC by using a fused HP-5MS capillary column (30 m $\times$ 0.25 mm i.d., film thickness 0.25 μm). The carrier gas was helium with a flow rate of 1ml/min. The GC oven temperature was programmed at (40°C for 2 min, then 3°C/min to 100°C for 1 min, and then 4°C/min left at 270°C for 1 min). The injection port temperature was 240°C and that of the detector was 230°C (split ratio of 60). The amount of injection was 1 μL. The carrier gas was delivered at a constant pressure of 5 kg/cm^2. The MS conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50 to 550 m/z.

3.2.4 Compounds identification

The identification of the compounds was based on comparison of their mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00) (Miladi *et al.*, 2013) as well as on comparison of their Retention indices according to the Van Den Dool method (Van den Dool, 1963), with those of internal (computer) library, NIST libraries (Massada, 1976; Mass Spectral Library, 2002), and some reference compounds, and those described by (Adams, 2001; Mimica-Dukić *et al.*, 2003; Vagionas *et al.*, 2007).

3.3 Phenolic compouds study

3.3.1 Extraction of phenolics

For preparation of crude extracts, plant extracts were prepared using different polarities solvents (n-hexane, chloroform and methanol 80%, respectively) according to a standard protocol.

Prepared plant material (leaves, stems and flowers, separately) (20 g) was transferred to dark-coloured flasks and macerated for 24h, three times (48 hours $\times$ 3) with hexane (200 ml) at room temperature (25 °C) protected from sunlight, and mixed several times daily with a sterile glass rod. After 24 hours, infusions were filtered through Whatman N°.1 filter paper and residue was extracted successively with equal volume of chloroform and methanol After 48 houres, the process was repeated three times, supernatants were evaporated to dryness under vacuum at 40 ºC using rotary evaporator (Brinkmann rotavapor, Model # R,). The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 ºC after determination of dry weight.

3.3.2 Total phenolic content yield calculation

The yield of each extraction is calculated by the ratio between the mass of the TPC extracted and the mass of the treated plant material. The yield expressed as a percentage is calculated by the following formula:

$$\frac{MHE}{MVE} \times 100 \tag{3.2}$$

Y: Yield.

MHE: Mass of TPC extracted in (g).

MVE: Mass of dried plant material.

3.3.3 Derivatization

Derivatization was carried out Briefly, $ChCl_3$ and MeOH extracts (1.0 mg) was added to 20 μl of pyridine and 50 μl of Bis (trimethylsilyl) trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS) 99:1.

The obtained solution was incubated in the oven at a temperature of 60 °C for 60 min to prepare the trimethylsilyl (TMSi) derivatives (Li and Barz, 2005).

3.3.3.1 Statistical analysis

All tests were performed in triplicate and results were expressed on the basis of dry matter weight. Data were expressed as mean ± SD. The means were compared by using the three-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests. The differences

between individual means were deemed to be significant at $p < 0.05$. All analyses were performed by using the SPSS v. 9.1.3 program

3.3.4 Analysis of phenolic extracts using gas chromatography – mass spectrometry (GC-MS)

The GC-MS system consisted of an Agilent 7890 A gas chromatography system with split injection (250 °C; 1:10), coupled to an Agilent MS model 5975 C MSD with triple axis detector (Agilent Technologies, USA). 1 μL was injected into the GC-MS injection port, 30 m HP5-MS column [(5% phenyl)-methylpolysiloxane, 0.25 mm i.d., df = 0.25 μm] (Johnson-Ajinwo and Li, 2014). The gas chromatography began with an oven temperature of 60 °C for 2 min, which increased to 300 °C at the rate of 10 °C/min, and was then held at 300 °C for 4 min under a constant helium pressure (10 psi). The mass spectrometry utilized an electron ionization (EI) source at 70 eV with an ion source temperature of 230 °C. Raw GC-MS data were processed using Turbo Mass software (Agilent Technologies, USA) to obtain data table with compound name, retention time, peak height and peak area percentage. The compounds were identified by matching their EI-MS spectra with those in the NIST/EPA/NIH (National Institute of Standards and Technology) mass spectral library 2011 Mass Spectral Library using MSD ChemStation (Agilent Technologies, USA).

3.3.5 Evaluation of cytotoxicity activity of phenolic extracts

Cytotoxicity of chloform and methanolic extracts have been evaluated against human ovarian cancer lines (OVCAR-4; FOUV-1; COV-362) and normal epithelial celles (HOE) using SRB assay as described by (OBrien *et al.*, 1964).

Resazurin is a non-fluorescent dye reduced to fluorescent resorufin by living cells. The fluorescent is proportional to the quantity of cells. Consequently, this assay allows to determine inhibition of cell growth and cytotoxicity.

3.3.5.1 Cell growth conditions

Human ovarian cell lines were grown in Roswell Park Memorial Institute (RPMI 1640; Lonza) medium supplemented with 10% foetal bovine serum (FBS; Lonza), 50 μg/ml penicillin/streptomycin (Lonza) and 2 mM glutamine (Lonza).

3.3.5.2 Trypsinization of adherent cells

All cells were routinely sub-cultured when they were more than 80% confluent as determined using an Olympus CKX41 light microscope.
To detach adherent cells from the culture flask for routine passage or experimentation, cells were washed with 1 ml phosphate buffered saline (PBS; Lonza) and subsequently exposed to 1 ml 0.01% trypsin (Lonza) in PBS.
To encourage detachment, cells were incubated at 37°C and gently agitated.
Following detachment, the trypsin was neutralized by the addition of 1 ml RPMI containing 10% FBS and cells were transferred into a sterile 15 ml polypropylene tube (Sarstedt).
Cells were centrifuged at 150 g for 3 minutes at room temperature in a Thermo Scientific Heraeus Megafuge 8 centrifuge. The supernatant was carefully aspirated and the cell pellet was re-suspended in a fresh cell culture medium. For routine passage, cells were transferred to T25 or T75 sterile tissue culture flasks (Sarstedt). For experimentation, at least 100 cells were counted using a Neubauer haemocytometer to determine cell number and an appropriate number of cells were transferred to tissue culture plates (96-well, Sarstedt) as described for each experimental procedure.

3.3.5.3 Cryopreservation of cells

Cells of a low passage number were collected by trypsinization (section 2) when they had reached approximately 50% confluence in a T75 tissue culture flask, to ensure that the cells were growing in the logarithmic phase.
The cell pellet was re-suspended in 1.2 ml growth medium containing 10% FBS and 8% dimethyl sulfoxide (DMSO, Sigma-Aldrich), and 0.2 ml aliquots were transferred into 2 ml cryovials (Triple Red). Cryovials were incubated overnight in a "Mr Frosty" freezing container (Nalgene) containing isopropanol (Sigma-Aldrich), at -80°C in a Nuaire -86°C Ultra low freezer, and the following day transferred into liquid nitrogen until required.

3.3.5.4 Reviving ryopreserved Cells

Frozen cells in a cryovial obtained from the liquid nitrogen were rapidly thawed in a Grant JB Series water bath (Grant Instruments) at 37°C and then added to 5 ml pre-warmed growth medium in a 15 ml polypropylene tube. Cells were then centrifuged at 150 g for 3 minutes at room temperature

and the pellet was re-suspended in 8 ml growth medium.
The resulting cell suspension was transferred into a T25 tissue culture flask and incubated for 24 hours at 37°C. Subsequently, the growth medium was replaced to remove residual DMSO and dead cells, and adherent cells were grown to an appropriate density for experimentation or sub-culture.

3.3.5.5 Cell growth assays

Following trypsinization and quantification, cells (5000 cells/well, except for HOE, where 2000 cells/well were used) were seeded in 96-well plates in 80 μl of growth medium. After incubation for 24 hours, 20 μl of 18 different concentrations of the drug or drug extract at 5 times the required concentration and a drug solvent control was added to the cells.After incubation for a further 72 hours, the growth medium was removed and the cells in each well were fixed in 100 μl 10% trichloroacetic acid (TCA, Sigma-Aldrich) on ice for 30 minutes.
The TCA was then removed by washing the plates three times in water and the cells were left to air dry, before staining in 0.4% sulforhodamine B (SRB, Sigma-Aldrich) in 1% acetic acid (Sigma-Aldrich) for 30 minutes.
After removing excess SRB by washing the wells three times in 1% acetic acid and drying, the dye was solubilized in 100 μL 10 mM Tris (pH 10, Sigma-Aldrich) and the absorbance at 570 nm (A570) was determined using a BioTek Synergy 2 multi-mode microplate reader.

3.3.5.6 Statistical analysis to determine IC_{50} value

Data obtained from cell growth assays was analyzed using the GraphPad Prism software (GraphPad Software, Inc.).
Non-linear regression was used to fit a four-parameter (Hill-equation) sigmoidal dose-response curve, and subsequently, the concentration at which 50% of cell growth was inhibited (IC50) was determined.
Both the mean average (mean) and standard deviation (SD) were calculated using IC_{50} values from repeat experiments. Statistically analysed using the Student's t-test by MS-Excel software, Differences were considered significant at $P < 0.05$.

Chapter 4

Results and Discussion

4. Results and Discussion

4.1 Essential oil results

4.1.1 Extraction and determination of essential oils

Plants EOs preparations of *C. sphaerocephala*, *A. maritimus*, *B. sylvestris*, *Ar. campestris* (Asteraceae family) also *L. stoechas* (Lamiaceae family) were carried out via hydrodistillation using a clevenger type apparatus and identified by GC–MS. The relative amount (g/100g powdered material) of EOs (w/w % of dry plant materials) extracted from different plants have been calculated. The content of the EOs depends on several factors, the most important being genetic characteristics, stage of development, environmental and agronomic factors, and drying and storage conditions (Calín-Sánchez *et al.*, 2013).

In the studied species, the percentage yields of the essential oil given here refer to the weight of dried plant material and varies in the different parts of the plant.

4.1.1.1 Yield (w/w %) of Asteraceae species

Table 4.1 shows the variance and the sum of squares analysis of EOs .

TABLE 4.1: Sum of squares analysis.

Source	DDL	Sum of squares	Average of squares
Organs	2	0.17	0.09
Species	3	0.30	0.09
Organs*Species	6	1.02	0.17

Results of the analysis of variance indicated that the interaction effect between organs and species was highly significant ($p < 0.0001$) for all EO yields considered.

Table 4.2 summarises the results obtained from the interaction between studied species organs.

The results show that leaves and flowers of all species recorded significantly the best yields in EO with no changes between theme ($p = 0.05$) with values of (0.20 ± 0.20 and 0.22 ± 0.25, respectively), compared by stems which they recorded significantly the lowest yield in EO ($p < 0.0001$) with value of (0.07 ± 0.06). The analysis of variance indicated that organ effect was highly significant ($p < 0.0001$) for all EO yields considered.

TABLE 4.2: Essential oil yields (%) from different organs of studied species.

Modality	**Mean ± SD**
Flowers	0.22 ± 0.25^{a}
Leaves	0.20 ± 0.20^{a}
Stems	0.07 ± 0.06^{b}

Data are given as mean ± SD (n = 3).
Means having different superscripts (a-b) within the same column are significantly different at (p <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5% (Duncan test).
SD: standart deviation.

The important observed variations could be related to morphological differentiation occurred during phenological cycle. According to the "source-sink transportation" hypothesis, Kramer and Kozlowski (1979) revealed that metabolites and photosynthesis are diverted to secondary metabolism pathways in flowers and fruits after anthesis. On the other hand, enhancement of essential oil accumulation in leaves and flowers could have an ecological and biological significance. It is noteworthy that floralscent play various important roles in the interaction between plants and their surrounding as pollinators, phytophageous, etc (Cunningham *et al.*, 2004; Dudareva *et al.*, 2004).

Table 4.3 summarises the results from the quantitative determination of EO.

TABLE 4.3: Essential oil yields (%) of different studied species.

Modality	**Mean ± SD**
C. sphaerocephala	0.23 ± 0.31^{a}
B. sylvestris	0.22 ± 0.23^{a}
Ar . campestris	0.18 ± 0.05^{b}
A. maritimus	0.02 ± 0.02^{c}

Data are given as mean ± SD (n = 3).
Means having different superscripts (a-c) within the same column are significantly different at (p <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5% (Duncan test).
SD: standart deviation.

Results show that *C. sphaerocephala* and *B. sylvestris* recorded significantly the best EOs yields with no changes between theme (p >0.05) with values of (0.23 ± 0.31 and 0.22 ± 0.23, respectively) compared by *Ar. campestris* (0.18 ± 0.05), while the lowest yield has been registered significantly for *A. maritimus* (0.02 ± 0.02) ($p < 0.0001$).

Table 4.4 summarises the yields of different parts of plants.

TABLE 4.4: Essential oil yields (%) from different organ/species.

Modality	Mean ± SD
Flowers * *C. sphaerocephala*	0.64 ± 0.01^{a}
Leaves * *B. sylvestris*	0.53 ± 0.03^{b}
Leaves * *Ar . campestris*	0.23 ± 0.07^{c}
Flowers * *Ar . campestris*	0.17 ± 0.02^{d}
Stems * *Ar . campestris*	0.15 ± 0.05^{d}
Flowers * *B. sylvestris*	0.07 ± 0.02^{e}
Stems * *B. sylvestris*	0.06 ± 0.02^{ef}
Stems * *C. sphaerocephala*	0.04 ± 0.03^{ef}
Leaves * *A. maritimus*	0.03 ± 0.03^{ef}
Stems * *A. maritimus*	0.01 ± 0.01^{f}
Flowers * *A. maritimus*	0.01 ± 0.01^{f}
Leaves * *C. sphaerocephala*	0.01 ± 0.003^{f}

Data are given as mean ± SD (n = 3).
Means having different superscripts (a-e) within the same column are significantly different at (p <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5% (Duncan test).
SD: standart deviation.

Our results show that leaves record significantly the best yields for the following species, *B. sylvestris*, *Ar. campestris* and *A. maritimus*, while flowers recorded significantly the best yield for *C. sphaerocephala*.
C. sphaerocephala flowers (0.64 ± 0.01) recorded significantly the best yield in EO compared by the other organs of different studied species ($p < 0.0001$), while *B. sylvestris* leaves recorded the second best yield (0.53 ± 0.03), following by *Ar. campestris* leaves (0.23 ± 0.07), then flowers and stems of this later whose shows no changes in term of EO yields (p = 0.05) with values of (0.2 ± 0.10 and 0.15 ± 0.05, respectively).
The lowest yield was significantly registered for, *B. sylvestris* stems and flowers (0.06 ± 0.02 and 0.07 ± 0.02, respectively), then, *C. sphaerocephala* stems and leaves (0.04 ± 0.03 and 0.01 ± 0.01, respectively) and finally *A. maritimus* leaves, flowers and stems (0.03 ± 0.02, 0.01 ± 0.01 and 0.01 ± 0.005, respectively) with no significant changes in the EO (p >0.05).
Results show that EO yields varied significantly among the studied organs and species ($p < 0.0001$). These variations could be attributed to some differences such as location, climate, environment, harvest period, berry maturity and variety type (Dudareva *et al.*, 2004).

4.1.1.1.1 ***Centaurea sphaerocephala* L.**

The hydrodistillation of *C. sphaerocephala* L. leaves, stems and flowers yielded yellow, dark yellow and light yellow EOs, respectively, without a particular smell. The yields of EOs, based on dry weight vary between leaves, stems and flowers.

Figure 4.1 shows the essential oil yields of *C. sphaerocephala* L. leaves, stems and flowers.

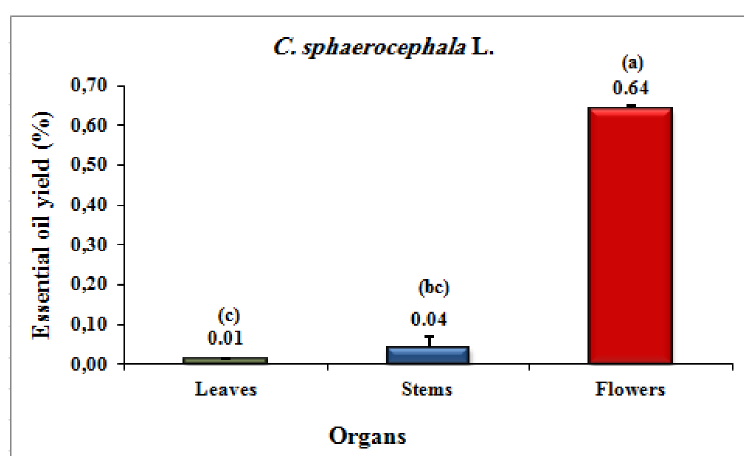


FIGURE 4.1: Essential oil yield (%) of *C. sphaerocephala* L.

Data are given as mean ± SD (n = 3).

EO yields with different subscripts (a–c) were significantly different at $p < 0.05$ (Duncan test).

Figure 4.1 shows that *C. sphaerocephala* flowers was characterized significantly by a high level of EO with an appreciable percentage (0.64 ± 0.01) compared by stems and leaves (0.04 ± 0.03 and 0.01 ± 0.00, respectively).

The analysis of variance indicated that organ effect was highly significant ($p < 0.0001$) between leaves/flowers and stems/flowers , while effect between leaves/stems is not significant ($p = 0.185$). According to our results inflorescences produced more essential oil than stems and leaves (>63 and 60% more, respectively). The same finding is reported for other medicinal plants such *Chaerophyllum macropodum* (Ebrahimabadi *et al.*, 2010), *Eucalyptus oleosa* (Marzoug *et al.*, 2011) and *Artemisia absinthium* (Riahi *et al.*, 2013).

The yield recorded for Algerian *C. sphaerocephala* L. can be considered high compared to some other plants that are used industrially as a source of EOs. A lower level was obtained for the same

species in Italy (0.12%) (Senatore *et al.*, 2006).

The yield recorded for Algerian *C. sphaerocephala* L. flowerheads can be considered high compared to some other *Centaurea* species flowerheads, a lower level was obtained in Italain species for *C. cineraria* L. subsp. umbrosa (Lacaita) Pign and *C. napifolia* L. (0.02 and 0.08%, respectively) (Senatore and Bruno, 2003) as well as *C. pseudoscabiosa* subsp. pseudoscabiosa Boiss. et Babse. and *C. hadimensis* Wagenitz. in Turkey (0.05 and 0.06%, respectively) (Flamini *et al.*, 2002; Ertugrul *et al.*, 2003).

Higher levels were obtained for *C. calcitrapa* L. in Italy (0.20%) (Asadipour *et al.*, 2005), *C. eryngioides* Lan., *C. iberica* Trev. var. hermonis Boiss. in Lebanon (0.18 and 012%) (Yayli *et al.*, 2005) and *C. moschata* L. in Egypt (0.12%) (Saleh *et al.*, 1981).

Concerning the genus *Centaurea* a low yield of 0.01% was recorded for the species *C. aphanina*. Sm. subsp. Mixta (DC.) Runemark. and *C. spruneri* Boiss. et Heldr. aerial part (Lazari *et al.*, 1999; Binder *et al.*, 1990b) as well as the aerial parts of *C. pelia* DC., *C. zuccariniana* DC. and *C. thessala*. Hausskn. subsp. drakiensis.(Freyn et Sint.) Georg (0.05% for all the three of them) (Lazari *et al.*, 2000).

Whereas this yield reach 0.07% for *C. kotschyi* (Boiss. et Heidr.) Hayek var. kotschyi (Senatore and Bruno, 2003) growing in Turkey and 0.09% for *C. mucronifera* DC. and *C. chrysantha* Wagenitz, capitula (Ertugrul *et al.*, 2003).

Moderate yields were obtained from the aerial part for *C. sessilis* Willd. *C. armena* Boiss. and *C. aladagensis* Wagenitzfrom Turkey (0.25, 0.18 and 0.1%, respectively), as well as *C. aucheri* (DC.) wagenitz and *C. depressa* N.B. from Iran (0.16% for the both) and higher levels were obtained for *C. calcitrapa* in USA (18, 6.1 and 5.5 µg/g) (Esmaeili *et al.*, 2005).

4.1.1.1.2 *Bellis sylvestris* L.

The hydrodistillation of *B. sylvestris* L. leaves, stems and flowers yielded yellow, aromatic and a liquid EOs.

Figure 4.2 shows the essential oil yields of *B. sylvestris* L. leaves, stems and flowers.

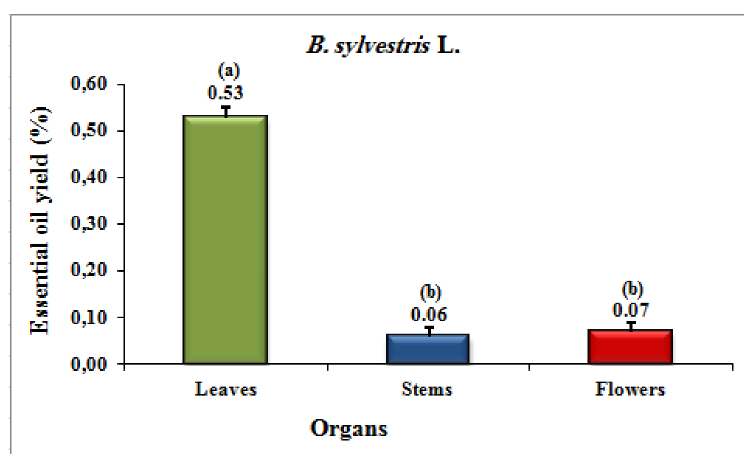


FIGURE 4.2: Essential oil yield (%) of *B. sylvestris* L.

Data are given as mean ± SD (n = 3).

EO yields with different subscripts (a–b) were significantly different at ($p < 0.05$ (Duncan test).

Figure 4.2 shows that *B. sylvestris* leaves record significantly the best EO yield (0.53 ± 0.02) than flowers and stems (0.07 ± 0.02 and 0.06 ± 0.02, respectively).

The analysis of variance indicated that organ effect was highly significant ($p < 0.0001$) between leaves/flowers and stems/flowers , while effect between leaves/stems is not significant ($P = 0.748$).

According to our results leaves produced more essential oil than flowers and stems. Similar results were reported earlier on others species, *Rutta chalepensis* leaves contained more EO than stems and flowers (Tounsi *et al.*, 2011), also on *Ocimum basilicum* L., (Chalchat and Özcan, 2008) and on *Myrtus communis* var. italica (Fadil *et al.*, 2018), as well as on *Rosmarinus officinalis* L. (Yosr *et al.*, 2013) reporting that leaves have the highest essential oil yield.

Also, Tuberoso *et al.* (2006), reported that *Myrtus communis* L. leaves showed always the highest yields, from 10- to 30- or 40-fold higher than the berries.

4.1.1.1.3 *Asteriscus maritimus* (L.) Less.

The hydrodistillation of *A. maritimus* (L.) Less. leaves yielded yellowish-green with an aromatic pleasant odour. While the oil obtained from stems is pale yellow with an aromatic fragrant-pleasant odour. EO obtained from flowers is light yellow with strong odour.

The analysis of variance indicated that organ effect was not significant (p >0.0001) between all organs.

Figure 4.3 shows the essential oil yields of *A. maritimus* (L.) Less. leaves, stems and flowers.

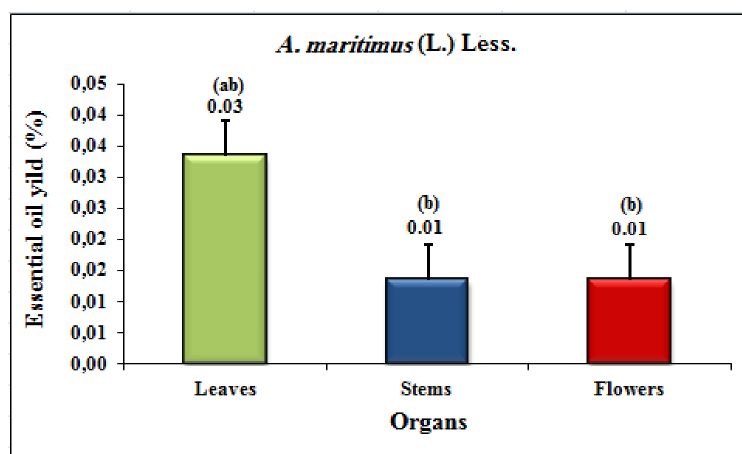


FIGURE 4.3: Essential oil yield (%) of *A. maritimus* (L.) Less.

Data are given as mean ± SD (n = 3).

EO yields with different subscripts (a–c) were significantly different at $p < 0.05$ (Duncan test).

Figure 4.3 shows that, In all organs, the average in leaves, stems and flowers was not significantly (0.03 ± 0.02, 0.01 ± 0.02 and 0.01 ± 0.02). Thus, EOs are synthesized either in translucent glands, or insecretory canals that may be localized in leaves, petals, sepals and pistil (Ciccarelli *et al.*, 2001).

Our results were in agreement with those of Chalchat and Özcan (2008), which reported that flowers, leaves and stems of *Ocimum basilicum* L. yielded (0.5%, 1.0% and 0.05% v/w, respectively), also Tounsi *et al.* (2011) reported that leaves have the highest essential oil yield.

Arnold *et al.* (1991) reported that a maximum EO yields were found with 1.3% in leaves of *Thlaspi cyprium* ssp. cyprium. Higher levels were obtained for *Senecio polyanthemoides* leaves copared by stems and flowers (0.23, 0.17 and 0.10 %) (Oladipupo and Adebola, 2009).

Fresh leaves and flowers part of *Bidens pilosa* Linn. var. Radiata were yielded 0.08% and 0.06%,

w/w (Deba *et al.*, 2008).

Mentha arvensis L.f. piperascens Malinvaud ex Holmes, leaves, stems and flowers, gave oil yields of 1.56%, 0.06% and 1.50%, respectively (Rajeswaran *et al.*, 2008). In general *A. maritimus* recorded a low amount of EO, our results are in agreement with earlier studies carried out on other species of the genus *Asteriscus* in the Mediterranean region (Alilou *et al.*, 2014).

4.1.1.1.4 *Artemisia campestris* L.

The hydrodistillation of *A. campestris* L. leaves, stems and flowers yielded light blue colored essential oil with a strong odor. The yields of the EOs, based on dry weight vary between these organs.

Figure 4.4 shows the essential oil yields of *A. campestris* L. leaves, stems and flowers.

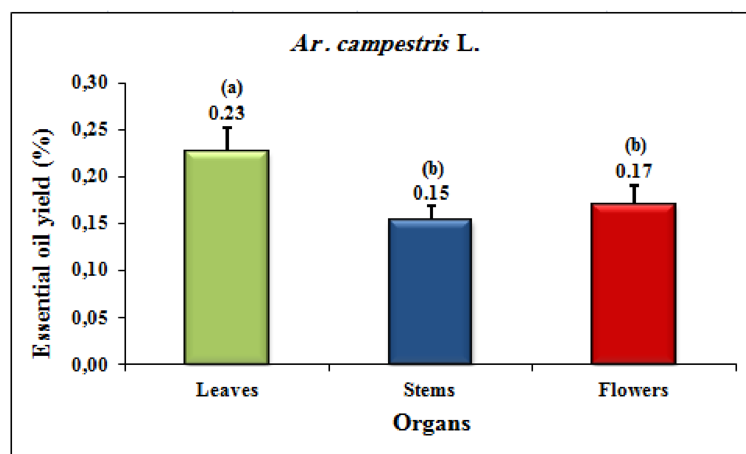


FIGURE 4.4: Essential oil yield (%) of *A. campestris* L.

Data are given as mean ± SD (n = 3).

EO yields with different subscripts (a–c) were significantly different at $p < 0.05$ (Duncan test).

Figure 4.4 shows that the highest yield was recorded significantly for leaves (0.23 ± 0.06) followed by flowers and stems (0.17 ± 0.02 and 0.15 ± 0.05, respectively).

The analysis of variance indicated that organ effect was hight significant between leaves and stems as well as leaves and flowers (p = 0.05), while this effect was not significant between stems and flowers (p = 0.098).

According to our results leaves produced more essential oil than flowers and stems, similar results were reported earlier on others species. On *Rutta chalepensis* leaves contained more EO than stems

and flowers (Tounsi *et al.*, 2011). *Ar. campestris* L. flowers from Iran contained more EO (0.49%) than leaves and stems (0.18 and 0.05%, respectively) (Kazemi *et al.*, 2009).

Concerning the genus *Artemisia* in Algeria, *Ar. absinthium* L. yielded (1.5%) (Orhan *et al.*, 2010), and the same Tunisian species gave a highest yield for flowers (2.98%), whereas leaves exhibited a lower yield (1.87%) (Riahi *et al.*, 2013), whil Moroccan species gave (0.57%) (Derwich *et al.*, 2009).

Essential oils' yields of *Ar. absinthium* L. obtained from different geographical areas of Europe vary between 0.1% and 1.1% (Orav *et al.*, 2006).

Concerning the genus *Artemisia* a low yield of 0.17% was recorded for the species *Ar. scoparia* (Singh *et al.*, 2009) whereas this yield reach 0.5% for *Ar. mesatlantica* (Benchekroun *et al.*, 2012). Higher levels were obtained for *Ar. herba alba* species in Tunisia (0.68–1.93%) (Mohsen and Ali, 2009) and *Ar. arborescens* L. in Lebanon (1.7%) (El Beyrouthy *et al.*, 2011).

The yield recorded for Algerian *Ar. campestris* can be considered low compared to some other plants that are used industrially as a source of essential oils.

4.1.1.2 Yield (w/w %) of Lamiaceae species

4.1.1.2.1 *Lavandula stoechas* L.

Table 4.5 shows the variance and the sum of squares analysis of EOs .

TABLE 4.5: Variance analysis.

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Organs	2	0.024	0.012	70.641	< 0.0001
Species	0	0.000			
Organs * Species	0	0.000			

The analysis of variance indicated that the interaction effect between organs and species was highly significant ($p < 0.0001$) for all essential oil yields considered.

Table 4.6 summarises the yield of different parts of *L. stoechas* L.

TABLE 4.6: Essential oil yields (%) from different organ.

Modality	Mean ± SD
Leaves	0.14 ± 0.01 [a]
Flowers	0.07 ± 0.02 [b]
Stems	0.01 ± 0.003 [c]

Data are given as mean ± SD (n = 3).
Means having different superscripts (a-c) within the same column are significantly different at 5% (Duncan test).
Values followed by the same small letter did not share significant differences at 5% (Duncan test).
SD: standart deviation.

Figure 4.5 shows the essential oil yields of *L. stoechas* L. leaves, stems and flowers.

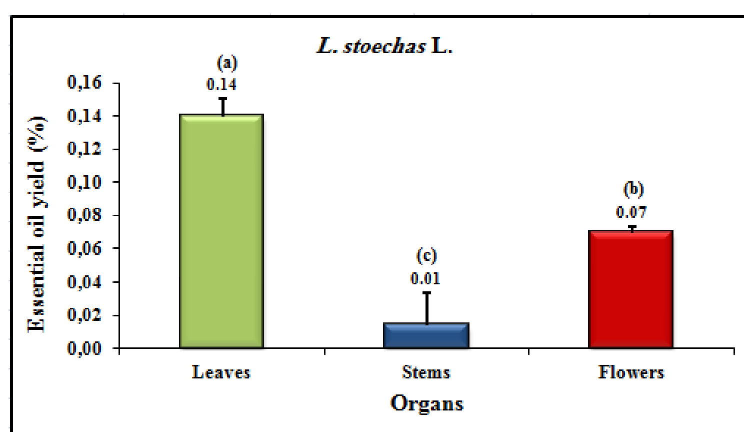


FIGURE 4.5: Essential oil yield (%) of *L. stoechas* L.

Data are given as mean ± SD (n = 3).

EO yields with different subscripts (a–c) were significantly different at $p < 0.05$ (Duncan test).

Figure 4.5 and **table 4.6** show that *L. stoechas* leaves recorded significantly the best yield in EO compared with flowers ($p = 0.001$) and stems ($p < 0.0001$) with value of (0.14 ± 0.01), followed by flowers (0.07 ± 0.02), while stems recorded the lowest yield (0.01 ± 0.003) ($p < 0.002$).
Results show that EO yields varied significantly among the studied organs and species ($p < 0.0001$).
The analysis of variance indicated that organ effect was highly significant ($p < 0.0001$) for all EO yields considered.
The important observed variations could be related to morphological differentiation occurred during phenological cycle. According to the "source-sink transportation" hypothesis, Kramer and Kozlowski (1979) revealed that metabolites and phytosynthesis are diverted to secondary metabolism pathways in flowers and fruits after anthesis. Stems releases small quantities of volatile organic

compounds, which could be induced by mechanical damage or by herbivore- or pathogen infection (Loughrin *et al.*, 1994; Paré and Tumlinson, 1997; Arimura *et al.*, 2004).

Volatile emission in flowers and accumulation in leaves increase during the early stages of organ development (when leaves are young and not fully expanded or when flowers are ready for pollination) and then either remaining relatively constant or decreasing over the lifespan of the organ (Gershenzon *et al.*, 2000; Dudareva *et al.*, 2004). This correlates to our study which revealed that the EO was concentrated in flowers of *L. stoechas*.

The analysis of variance indicated that organ effect was highly significant ($p < 0.05$) between leaves/ stems, while effect between leaves/flowers and flowers/stems is not significant. According to our results leaves produced more essential oil than flowers and stems. In contrast, *L. stoechas* flowers contained more essential oils than leaves (>60% more), and also on *L. latifolia* (Muñoz-Bertomeu *et al.*, 2007) and *L. dentata* L. species (Touati *et al.*, 2011).

4.1.2 Determination of essential oil extracts using GC-MS

Biological activities of essential oils varied significantly according to the chemical composition and chemotypes (Yashphe *et al.*, 1987).

4.1.2.1 Asteraceae species

4.1.2.1.1 *Centaurea sphaerocephala* L.

Table 4.7 shows the chemical composition of EOs (components and their percentage) hydrodistilled from leaves, stems and flowers.

Altogether 76 components were identifiend, 30, 61 and 32 compounds in leaves, stems and flowerheads , respectively, representing (93.6, 99 and 92.3% of the total oil in leaves, flowerheads and stems respectively).

TABLE 4.7: Chemical composition (%) of *Centaurea sphaerocephala* L. essential oil.

Pic N○	Rt	Compounds	Leaves %	Stems %	Flowers %
1	10,129	3,5-Di-tert-butyltoluene	0.4	0.2	-
2	11,675	4-Carene	0.5	0.1	-
3	13,515	α-Curcumene	-	0.2	-

Chemical composition (%) of *Centaurea sphaerocephala* L. essential oil. Continued...

Pic N○	**Rt**	**Compounds**	**Leaves %**	**Stems %**	**Flowers %**
4	13,933	2,4-Di-tert-butylphenol	-	0.3	-
5	14,043	Dodecane	-	0.2	-
6	14,466	Elemol	-	0.3	-
7	14,706	Lauric acid	0.8	-	0.6
8	14,858	1-Hexadecene	-	0.3	-
9	14,9	Spathulenol	-	1.6	-
10	14,921	Isolongifolene, 9,10-dehydro-	0.5	0.2	-
11	15,099	1-Bromo-4-methylbicyclo(2.2.2)octane	-	0.2	-
12	15,229	β-Selinene	-	0.1	-
13	15,322	Caryophyllene oxide	-	0.2	-
14	15,459	n-Octadecyl Trichlorosilane	-	0.1	-
15	15,871	(+)-γ-Gurjunene	0.8	0.2	-
16	16,093	n-Heptadecan	-	-	0.4
17	16,115	α-Bisabolol	-	2.7	-
18	16,127	Cis-α-Bisabolene	0.9	0.4	-
19	16,486	4-Methyldodecane	0.8	0.1	-
20	17,037	Myristic acid	-	0.9	1.2
21	17,231	n-Pentadecane	0.2	0.2	-
22	17,248	’Tridecane, 1-iodo-	0.9	0.1	-
23	17,702	Cis-pinane	-	-	0.5
24	17,813	6,10,14-Trimethylpentadecan-2-one	**10.2**	2.4	**6.4**
25	17,835	3,8-Dimethyldecane	0.3	0.2	-
26	17,971	1-Tridecene	0.2	0.4	-
27	18,169	Diisobutylphthalat	-	1.5	-
28	18,18	Dibutyl phthalate	-	-	1.6
29	18,201	’Di-sec-butyl phthalate	-	-	0.8
30	18,331	n-Nonadecane	-	-	0.6
31	18,625	2-Bromododecane	-	0.4	-

Chemical composition (%) of *Centaurea sphaerocephala* L. essential oil. Continued...

Pic N○	Rt	Compounds	Leaves %	Stems %	Flowers %
32	18,643	Palmitic acid methyl ester	-	0.7	1.2
33	19,399	Palmitic acid ('n-Hexadecanoic acid)	**18.5**	**21.3**	**14.7**
34	19,773	Propan-2-yl tetradecyl sulfite	-	0.1	-
35	19,897	Palmitic acid, trimethylsilyl ester	-	12	2
36	20,02	Pentacosane	0.5	0.1	-
37	20,142	2-Methyloctacosane	0.5	0.1	-
38	20,658	Phytol	0.9	3.1	2.6
39	20,778	3-Methyltridecane	0.2	0.3	-
40	20,651	2-Methyltetracosane	0.2	0.3	-
41	21,072	Linoleic Acid	**10.8**	**16.2**	**6.8**
42	21,112	Arachidonic acid	-	-	0.7
43	21,151	Germacrene D	-	-	**5.7**
44	21,153	1,5-Cyclododecadiene, (Z,Z)-	-	-	4.3
45	21,544	1,19-Eicosadiene	0.5	0.1	-
46	21,575	Phytol, acetate	1.8	1.7	1
47	22,003	Tributyl citrate	-	0.6	-
48	22,039	9-Octyl-heptadecane	-	0.2	-
49	22,242	Heptadecane	-	0.2	-
50	22,259	7-Hexyltridecane	-	1.4	-
51	23,117	Tetracosane	1.5	-	1.7
52	23,956	Eicosane, 10-methyl-	-	-	1.5
53	23,957	Octadecane	1.9	0.2	2.7
54	24,19	1-Docosene	0.2	0.5	-
55	24,396	N-Docosane	0.5	0.4	1
56	24,492	Bis(2-ethylhexyl) phthalate	0.1	-	0.5
57	24,771	Eicosane, 9-octyl-	1.2	-	2
58	24,78	Tricosane	4.6	0.8	4.6
59	25,041	2-Methyl-6-propyl-dodecane	-	-	1.6
60	25,51	2-(Octadecyloxy)ethan-1-ol	0.5	0.7	2.6

Chemical composition (%) of *Centaurea sphaerocephala* L. essential oil. Continued...

Pic N○	**Rt**	**Compounds**	**Leaves %**	**Stems %**	**Flowers %**
61	25,56	Heneicosane	0.5	0.8	**5.8**
62	25,596	Heptacosane	**6.8**	**5.7**	3.1
63	25,815	7-Hexyldocosane	1.2	0.6	-
64	25,964	Eicosane	3.3	0.3	3.8
65	26,033	β-Sitosterol trimethylsilyl ether	2	-	1.7
66	26,052	Hexacosane	-	1.7	4.2
67	26,317	Tetracosane	-	0.4	-
68	26,346	Octacosane	5.6	**4.7**	4.4
69	27,176	Nonacosane	**6.6**	**5.1**	-
70	27,831	2-Methylhexadecane	0.5	0.5	-
71	27,823	3-Methylheptadecane	0.9	0.2	-
72	28,038	Triacontane	0.1	0.3	-
73	28,064	Tetratetracontane	1.8	1.7	-
74	27,435	Heneicosane, 11-decyl-	1.9	1.5	-
75	28,712	Heptadecane, 2-methyl-	0.5	0.1	-
76	29,093	Hentriacontane	1	0.9	-
		Chemical classes			
		Monoterpene hydrocarbons	0.5	0.3	0.5
		Sesquiterpene hydrocarbons	2.2	0.9	5.7
		Oxygenated sesquiterpenes	-	4.8	-
		Oxygenated compounds	43.6	49.4	40.7
		Hydrocarbons	44.4	30.7	41.7
		Others	2.9	12.9	3.7
		Total identified	**93.6%**	**99%**	**92.3%**

Rt : Retention time (min)

(-) not detected

The oxygenated compounds (40.7–49.4%) and hydrocarbons (30.7–44.4%) fraction was dominant

in the three of the EOs analysed, although with some differences concerning the main components. The contribution of monoterpene hydrocarbons and sesquiterpene hydrocarbons in the EOs were (0.3-0.5% and 0.9-5.7%, respectively), while the oxygenated sesquiterpenes were presented only in stems with 4.8%.

Among hydrocarbons, some of theme showed high concentrations, heneicosane (5.8% in flowers), tricosane (4.6% in both leaves and stems) and octacosane (5.6, 4.7 and 4.4% in leaves, stems and flowers, respectively), whereas the major components of this fraction in stems were heptacosane (5.7%), nonacosane (5.1%) and octacosane (4.7%).

The contribution of hydrocarbones in the EOs of *C. sphaerocephala* flowerheadss, leaves and stems were 44.2; 41.7 and 30.0%, respectively.

The EO from leaves, flowerheads and stems were also contained substantial amounts of fatty acid, 27.2 and 51.1%, respectively.

Heptacosane and tricosane have been the main hydrocarbons (4.9 and 8.0% respectively) of the flowerhesd oil of *C. sphaerocephala* (Senatore *et al.*, 2003).

Sesquiterpene fraction was constituted by several components, in stems tricyclic sesquiterpenes predominated, with spathulenol (1.6%) being the only components that attained relative percentages higher than 1.5%. whereas the only sesquiterpenes present in the flowerheads was germacrene (5.7%).

In leaves, stems and flowers, palmitic acid (n-Hexadecanoic acid) and linoleic Acid were the major constituents of the EOs with ratio of (21.3, 14.7 and 18.5%; 10.8, 16.2 and 6.8%, respectively), followed by, 6,10,14-trimethyl- (10.2 and 6.4%, in leaves and stems respectively), germacrene D (5.7% only in stems), and nonacosane (6.6 and 5.1%, in leaves and flowers, respectively).

Hexadecanoic acid has been identified as the major compound (25.6 %, 50.6 % and 40.0 %, respectively) of the EOs of the aerial parts of *C. wagenitzii* Hub.-Mor., *C. tossiensis* Freyn et Sint. and *C. luschaniana* Heimerl from Turkey (Köse *et al.*, 2016). Also Karamenderes *et al.* (2008) found that the major component of the EO from *C. calolepis* were hexanoic acid and carvacrol.

Among the compounds identified in the oil of *C. sphaerocephala*, alkanes and alkenes (C15–C29), palmitic acid and some fatty acid methyl esters were the dominating constituents, and they accounted for 84.1% of the oil. These compounds are typical constituents of epicuticular waxes (Engel *et al.*, 1993; Reverchon and Senatore, 1994). The presence of 1-pentadecene in stems' oil (0.2%), identified in this oil is probably due to the palmitic acid (NEY and BOLAND, 1987).

The content of palmitic acid was also remarkable in the oil of leaves, stems and flowers.

The absence of important flavour components in the three EOs accounts for the poor flavour quality of these EOs.

Several investigations (Buttery *et al.*, 1986; Binder *et al.*, 1990a; Lazari *et al.*, 2000; Flamini *et al.*, 2002; Baser *et al.*, 2002; Senatore and Bruno, 2003; Ertugrul *et al.*, 2003; Dural *et al.*, 2003; Yayli *et al.*, 2005; Asadipour *et al.*, 2005; Flamini *et al.*, 2006) on the EOs of various *Centaurea* genus showed that germacrene D (21.7–61.0%) and hexadecanoic acid (6.5–30.7%) characterized most species, whereas germacrene present only in the flowerheads (5.7%) in our study and hexadecanoic acid is the major components in the flowerheads and stems with (14.7 and 6.8% respectively).

A previous paper (Binder *et al.*, 1990b) reported benzene (4.26 μg/g), caryophyllene (3.09 μg/g), germacrene D (1.80 μg/g) and 2-pentanone (1.39 μg/g) as compounds present in the volatiles obtained from acetone extracts of flower tissues in > 1 μg/g concentration.

In our study caryophyllene oxide was presented only in stems EO (0.2%), also and phytol was presented in leaves, flowers and stems (0.9, 3.1 and 2.6%) whereas this compounds were the main component in *C. sphaerocephala* aerial part oil with (27%) (Dob *et al.*, 2009) and *A. segetalis* Ten (4.2%) growing in Montenegro (Radulović *et al.*, 2009).

While, pentadecanone, 6,10,14-trimethyl was detected as a third major compound with 6.4 %, many reports on EO of Algerian *Centaurea* species have been detected this compound compound with a high percentages. For exemple; aerial parts of *C.pullata* (14.9%) (Dob *et al.*, 2009) and *A. segetalis* Ten (4.2%) growing in Montenegro (Radulović *et al.*, 2009).

In the best of our knowledge, there is no literature reports that has been done on *C. sphaerocephala* L. leaves, stems and flowers.

4.1.2.1.2 *Bellis sylvestris* L.

Table 4.8 shows the chemical composition of EOs from *B. sylvestris* stems, GC–MS analysis resulted in the identification of 52 compounds (representing 84.3% of the oil).

TABLE 4.8: Chemical composition (%) of *Bellis sylvestris* L. essential oil.

Pic N°	Rt	Compounds	Stems %
1	27.032	α-Curcumene	0.5
2	27.098	Isocaryophillene	0.4

Chemical composition (%) of *Bellis sylvestris* essential oil. Continued...

Pic N°	Rt	Compounds	Stems %
3	27.355	Valencene	2.1
4	27.517	Cycloheptasiloxane, tetradecamethyl-	0.5
5	27.986	α-Panasinsenes	0.4
6	28.543	cis-α-Bisabolene	0.2
7	29.46	(-)-Spathulenol	0.5
8	29.861	Ethyl iso-allocholate	0.3
9	31.566	Hexadecamethyl-cyclooctasioxane	0.4
10	31.639	17-Pentatriacontene	0.2
11	31.888	α-Bisabolol	1
12	32.497	Tetradecanal	0.2
13	33.311	Azuleno(2,1-b)thiophen-3(2H)-one	**27.2**
14	33.752	Himachalene	0.3
15	34.023	Benzene, 1-methyl-3,5-bis[(trimethylsily l)oxy]-	0.6
16	34.852	2-Naphthaleneac etaldehyde, 1,4-dihydro-α,α-dimethyl-1,4-dioxo-	0.2
17	34.867	Phenoxathiin	**16.3**
18	35.27	2-Pentadecanone, 6,10,14-trimethyl-	0.6
19	35.769	Diisobutylphthalat	1.1
20	36.172	Octahydroanthracene	0.5
21	36.356	Eicosane	0.3
22	36.803	Benzofuran, 4, 7-dimethyl-	0.3
23	37.544	n-Hexadecanoic acid	1.3
24	37.61	Dibutyl phthalate	1.8
25	38.292	Eicosane, 10-methyl-	0.3
26	38.696	Oxirane, heptadecyl-	0.5
27	39.847	n-Heptadecanol-1	0.2
28	40.163	Heneicosane	0.3
29	40.405	Phytol	0.4
30	40.566	Hexadecanal	0.3
31	41.278	Pentacosane	**5.7**

Chemical composition (%) of *Bellis sylvestris* essential oil. Continued...

Pic N°	**Rt**	**Compounds**	**Stems %**
32	41.718	11α-Hydroxyprogesterone	0.3
33	41.923	docosane	0.4
34	42.371	Octadecanal	**4.1**
35	43.075	Acetyl tributyl citrate	0.4
36	43.412	Eicosanol	0.5
37	44.168	Estradiol	0.3
38	44.388	2,3,6-Trimethylnaphthoquinone	0.6
39	44.549	4-β-Phorbol	1.3
40	45.011	α-Naphthoquinone	**4.8**
41	45.738	Oxirane, tetradecyl-	0.6
42	46.831	Hexacosane	1
43	47.351	Nonacosane	0.2
44	47.608	Diisooctyl phthalate	0.3
45	47.924	Cyclodecasiloxane, eicosamethyl-	0.3
46	49.405	Eicosane, 10-heptyl-10-octyl-	0.2
47	49.611	Oleic acid, 3-(octadecyloxy) propyl ester	0.8
48	49.794	Hexatriacontane	1.4
49	50.278	Octadecane, 1, 1-[1,3-propanediylbis (oxy)]bis-	0.4
50	51.107	Lanosta-7,9(11),20-triene-3beta,18-diol, diacetate	0.2
51	51.202	Heptacosane	0.8
52	51.694	Tetrapentacontane, 1,54-dibromo-	0.5
		Chemical classes	
		Monoterpene hydrocarbons	0.9
		Sesquiterpene hydrocarbons	4
		Hydrocarbons	14.6
		Oxygenated compounds	42.1
		Others	19.6
		Total identified	**84.3%**

Chemical composition (%) of *Bellis sylvestris* essential oil. Continued...

Pic N°	**Rt**	**Compounds**	**Stems %**

Rt : Retention time (min)

The contribution of oxygenated compounds and hydrocarbons in the EO of *B. sylvestris* stems were (42.1 and 14.6%, respectively). The oil also contained substantial amounts of sesquiterpene hydrocarbons (4%), and small amount of monoterpene hydrocarbons (0.9%).

Furthermore, this analysis revealed that the major constituents of stems were azuleno(2,1-b) thiophen-3(2H)-one (27.2%), phenoxathiin (16.3%), pentacosane (5.7%), α-naphthoquinone (4.8%) and octadecanal (4.1%).

To the best of our knowledge, there is no any report on the chemical composition of *B. sylvestris* stems EOs in the literature.

Scognamiglio *et al.* (2016) isolated 28 secondary metabolites from leaves of *B. sylvestris* belonging to different classes, two megastimane derivatives and a glycoside of 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone, were reported for the first time.

However, there are few reports on the chemical composition of the EOs from the other plants belonging to the genus *Bellis*; in leaves and flowers of *B. perennis*, the common daisy, Avato *et al.* (1997) found that polyacetylenes were one of the major chemical classes (18–21%) mainly consisting of two new C10 acetylenic compounds as methyl deca-4,6-diynoate (2,8-tetrahydromatricaria ester) and deca-4,6-diynoic acid.

Gudej and Nazaruk (2001) also isolated that three flavonol glycosides, isorhamnetin 3-*O*-β-d-galactopyranoside, isorhamnetin 3-*O*-β-d-(6-acetyl)-galactopyranoside and kaempferol 3-*O*-β-d-glucopyranoside from flowers of *B. perennis*.

Holme and Sörensen (1954) Isolated a trans-lachnopyllum este from *B. perennis* L. roots.

4.1.2.1.3 ***Asteriscus maritimus* (L.) Less.**

The EO obtained from *A. maritimus* stems using hydrodistillation having yellow colour with an aromatic fragrant odour. The identified components, their retention time and their proportions are summarized in **table 4.9**.

TABLE 4.9: Chemical composition (%) of *Asteriscus maritimus* (L.) Less. essential oil.

Pic N°	Rt	Compounds	Stems%
1	18.479	Decanal	0.4
2	23.291	Terpinyl acetate	2.5
3	24.113	2H-2,4α-Ethanonaphthalene, 1,3,4,5,6, 7-hexahydro-2, 5,5-trimethyl-	0.5
4	24.245	Cadinene	1.9
5	25.001	(-)-Tricyclo[6,2,1,0(4,11)]undec-5-ene,1,5,9,9-tetramethyl-(isocaryophyll ene-I1)	0.3
6	25.353	α-Guaiene	0.4
7	25.411	1,4-Dimethoxy-2-tert-butylbenzene	1
8	27.517	Cycloheptasiloxane, tetradecamethyl-	0.5
9	27.766	α-Caryophyllene alcohol	0.8
10	27.869	Diepi-α-cedrene epoxide	1
11	28.25	Epiglobulol	2.3
12	28.954	Methyl 4-tert-butyl-thiobenzoate	0.6
13	29.416	Isolongifolene, 7,8-dehydro-8a-hydroxy-	**4.5**
14	29.629	Globulol	2.1
15	29.871	Tetradecane, 2, 6,10-trimethyl-	0.5
16	29.945	Anisole, o-octyl-	0.4
17	30.275	Calarene epoxide	0.4
18	30.766	Humulane-1,6-dien-3-ol	**5.1**
19	31.155	α-Guaiene	0.5
20	31.566	Hexadecamethyl-cyclooctasioxane	0.9
21	31.903	1H-Inden-1-ol, 2,4,5,6,7,7a-hexahydro-4,4, 7a-trimethyl-	1.4
22	32.021	γ-Eudesmol	**17.4**

Chemical composition (%) of *Asteriscus maritimus* (L.) Less. essential oil. Continued...

Pic N°	Rt	Compounds	Stems%
23	32.16	Salsoline	0.5
24	32.497	Tetradecanal	0.4
25	32.607	9α-Acetoxy-3,5, 8-trimethyltricyclo [6,3,1,0(1, 5)]dodec-2-ene	0.3
26	33.275	Octanal, 2-(phenylmethyle ne)-	0.3
27	33.942	9H-Fluorene, 9-methyl-	0.6
28	34.023	Benzene, 1-methyl-3,5-bis[(trimethylsily l)oxy]-	1.1
29	34.478	Phosphorin, 2,4, 6-tris(1,1-dimethylethyl)-	0.4
30	34.852	2-Naphthaleneac etaldehyde, 1,4-dihydro-α,α-dimethyl-1,4-dioxo-	**5.9**
31	35.255	Hexahydrofarnesyl acetone	1
32	35.769	Diisobutylphthalat	1.2
33	35.96	1-Hexadecanol	0.4
34	36.172	Octahydroanthracene	1.1
35	36.356	Eicosane	0.3
36	36.715	1-Hexen, 2-(p-anisyl)-5-methyl-	0.4
37	36.803	Benzofuran, 4, 7-dimethyl-	0.7
38	37.61	Dibutyl phthalate	0.7
39	38.476	2-(1-Methyl-1,2, 3,4-tetrahydro-isoquinolin-1-yl)-propan-2-ol	0.5
40	38.945	Propanoicacid,2-methyl-,2-[3-[(acetyloxy)methyl]oxiranyl]-5-methylphenyl ester	0.8
41	40.163	Heneicosane	0.5
42	40.405	Phytol	0.4
43	40.544	3(2H)-Benzofuranone, 4,7-dimethyl-	1.3
44	41.285	Triacontane	1.2
45	42.371	Octadecanal	0.5
46	43.632	Tetracosane	0.5
47	44.087	Hexadecanal	0.4

Chemical composition (%) of *Asteriscus maritimus* (L.) Less. essential oil. Continued...

Pic N°	Rt	Compounds	Stems%
48	44.557	Spheroidenone	0.6
49	45.254	Tetratetracontane	0.6
50	45.738	Oxirane, tetradecyl-	1.8
51	46.831	Hexacosane	1.3
52	48.342	Octadecane	1.1
53	48.848	Hentriacontane	0.5
54	49.398	Flurandrenolide	1.4
55	49.794	Hexatriacontane	0.5
56	49.802	Triacontane	2.2
57	50.278	Octadecane, 1, 1-[1,3-propanediylbis (oxy)]bis-	0.6
58	50.696	Octadecane,3-ethyl-5-(2-ethyl)	0.4
59	50.887	Cyclotrisiloxane, 2,4,6-trimethyl-2,4,6-triphenyl-	1.6
60	51.115	Thymol blue	0.6
61	51.217	Octacosane	1.6
62	51.694	Tetrapentacontane, 1,54-dibromo-	0.7
		Chemical classes	
		Sesquiterpene hydrocarbons	3.6
		Oxygenated sesquiterpenes	16.2
		Hydrocarbons	12.3
		Oxygenated compounds	36.3
		Others	13.1
		Total identified	**82.3%**

Rt : Retention time (min)

Totally, 62 components were identified representing (82.3%) of the oil. Although, oxygenated compounds were dominant in the EO (36.3%), the distribution of oxygenated sesquiterpenes and hydrocarbons were (16.2 and 12.3 %), the oil also contained a small amount of sesquiterpene hydrocarbons (3.6%).

The major compounds of EO of *A. maritimus* were γ-eudesmol (17.4 %), 2-naphthaleneac etaldehyde, 1,4-dihydro-α, α-dimethyl-1,4-dioxo- (5.9%), humulane-1,6-dien-3-ol (5.1%) and isolongifolene, 7,8-dehydro-8α-hydroxy- (4.5%).

EOs of *A. maritimus* stems from other countries have been reported to possess same chemical compounds with different percentage; Medimagh *et al.* (2012) showed that stems' oil of Tunisian *A. maritimus* was very rich on hexdecanoic acid (49.18%), phytol (15.57%), 1-heneicosene (6.53%) and γ-eudesmol (4.45 %) However, literature of *A. maritimus* EOs reported that, myrtenyl acetate (44.2%), terphenyl (17.5%), (Z)-β-farnesene (12.9%), myrtenol (5%) and terpinen-4-ol (4.5%) were represented as the major components in *A. maritimus* aerial part oil extracted in Italy (Fraternale *et al.*, 2001). α- pinene (27.5%) and p-cymene (10.0%) as major components in *A. maritimus* stems and leaves in Tunisia (ALI *et al.*).

Additionally, in comparison with other Algerian species and subspecies, Chaib *et al.* (2017) identified that the major components of *A. vulgaris* aerial parts were cis-chrysanthenyl acetate (31.1%), myrtenyl acetate (15.1%), kessane (11.5%).

Previous studies on *Asteriscus* species and subspecies have revealed the abundance, 6-oxocyclonerolidol (30.72%), t-cadinol (14.50%), α-pinene (4.22%), α-bisabolone oxide (3.56%), humulene epoxyde II (3.5%), and bisabolone (3.5%) in *A. graveolens* subsp. odorus aerial parts from Morocco (Alilou *et al.*, 2014); 6-oxo- cyclonerolidol (74.9 and 50.4%) and 6-hydroxycyclonerolidol (11.8 and12.2%) were the major components in leaf and stems respectively , while cis-8-acetoxychrysanthenyl acetate (48.5%), cis-chrysanthenyl acetate (13.4%), t-Cadinol (11.4%), 6-oxocyclonerolidol (7.8%), a-oxobisabolene (5.7%) in *A. graveolens* (FORSSK.) LESS. flowers from Morocco; (Cristofari *et al.*, 2012); 6-oxocyclonerolidol (66.7%), 6-hydroxycyclonerolidol (8.8%), and intermedeol (4.3%) in *A. graveolens* aerial parts from Marroco (Znini *et al.*, 2011); 6-oxocyclonerolidol 19 (74.9%) and 6-hydroxycyclonerolidol 23 (11.8%) in Marrocan *A. graveolens* leaves respectively (Znini *et al.*, 2012) 1,5-dihydroxy-6,7-dimethylocta-3,5-diene (8.62%), carveol (6.07%), α-pinene (3.83%), cedrenol (3.10%), a-phellandrene (2.12%), and α-himachalene (1.89%) in *Nauplius graveolens* flowering parts from Sinai (Fahmy, 2003).

Some major constituents of previous studies on the EO of *Asteriscus* plants such as 1,8- cineole, β-phellandrene, m-cymene, exo-2-hydroxycineole, trans-chrysanthenyl acetate, menthyl acetate, 2,6-dimethyl-1,6-heptadien-4-yl acetate, 6-oxocyclonerolidol, d-cadinene and phytol were not identified in our *A. maritimus* stems EO.

For instance, 1,5- dihydroxy-6,7-dimethylocta-3,5-diene, a-phellandrene, a-himachalene, or d-cadinol, which were reported as major components of stems' oil of *A. graveolens* in the literature, were not detected in this study. Since nerolidol derivatives have been described as chemical constituents of *Asteriscus* species (Jakupovic *et al.*, 1987), these compounds can be considered as chemical markers of this genus.

These variations in the main constituents reported in the literature can be explained by the influence of environmental and methodological factors (Bakkali *et al.*, 2008) also the soil composition, plant organ, vegetative cycle phase and climate (Miguel *et al.*, 2005).

In the best of our knowledge, there is no literature reports that has been done on *A. maritimus* stems.

4.1.2.1.4 *Artemisia campestris* L.

The EO obtained from stems using hydrodistillation having yellow colour with an aromatic fragrant odour.

Table 4.10 shows the chemical composition of the EO of *Ar. campestris* L. stems.

TABLE 4.10: Chemical composition (%) of *Artemisia campestris* L. essential oil.

Pic N°	Rt	Compounds	Stems %
1	23.291	Terpinyl acetate	0.4
2	24.399	Pent-1-yn-3-ene, 4-methyl-3-phenyl-	0.5
3	27.032	α-Curcumene	0.3
4	27.098	Isocaryophillene	0.3
5	27.517	Cycloheptasiloxane, tetradecamethyl-	0.3
6	29.035	$\pm$-Trans-Nerolidol	0.6
7	29.46	(-)-Spathulenol	0.4
8	29.614	Caryophyllene oxide	0.4
9	29.861	Ethyl iso-allocholate	0.4
10	30.795	γ-Muurolene	**4.6**
11	31.155	α-Guaiene	0.5
12	31.566	Hexadecamethyl-cyclooctasioxane	0.5
13	31.639	17-Pentatriacontene	2.3
14	31.888	α-Bisabolol	0.7

Chemical composition (‰) of *Artemisia campestris* L. essential oi . Continued...

Pic N°	Rt	Compounds	Stems ‰
15	33.121	Phorbol	0.4
16	34.023	Benzene, 1-methyl-3,5-bis[(trimethylsily l)oxy]-	0.7
17	34.852	2-Naphthaleneac etaldehyde, 1,4-dihydro-α,α-dimethyl-1,4-dioxo-	**22.1**
18	35.769	Diisobutylphthalat	0.4
19	36.187	6,8-Difluoro-2,2, 4,4,6,7,7,8,9,9-decamethyl-[1, 3,5,2,4,6,7,8, 9]trioxahexasil onane	0.5
20	38.864	Falcarinol	**12**
21	40.838	Propanoic acid, 2-(3-acetoxy-4, 4,14-trimethylandros t-8-en-17-yl)-	0.3
22	41.278	Pentacosane	**4.3**
23	44.117	Lanosta-7, 9(11),20-triene-3β,18-diol, diacetate	0.4
24	41.718	11α-Hydroxyprogesterone	0.6
25	41.923	docosane	0.5
26	41.931	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.5
27	42.371	Octadecanal	1.2
28	42.694	5,8,11-Eicosatriynoic acid, methyl ester	0.9
29	43.42	1-Eicosanol	0.4
30	43.625	Heptadecane, 9-hexyl-	0.4
31	44.557	Spheroidenone	0.5
32	45.048	9-Desoxy-9x-chloroingol 3,7, 8,12-tetraacetate	0.3
33	45.254	Tetratetracontane	2.2
34	45.576	16α,17α-Epoxypregnenolone	0.3
35	45.738	Oxirane, tetradecyl-	1
36	46.082	Lanosta-7, 9(11),20(22)-triene-3 β,18-diol, diacetate	0.3
37	46.339	Lanosta-7, 9(11)-diene-3 β, 18,20-triol, 3,18-diacetate, (20R)-	0.4
38	46.398	Ingenol triacetate	0.4
39	46.831	Hexacosane	0.6
40	47.484	Pentatriacontane	2.6

Chemical composition (%) of *Artemisia campestris* L. essential oi . Continued...

Pic N°	Rt	Compounds	Stems %
41	47.924	Cyclodecasiloxane, eicosamethyl-	0.4
42	48.342	Octadecane	0.8
43	48.679	15,17,19,21-Hexatriacontatetrayne	0.7
44	48.848	Hentriacontane	0.5
45	48.995	Androst-4-en-6-one, 3,17-diacetoxy-	0.5
46	49.097	9,19-Cyclolanost-24-ene-3,26-diol, diacetate	0.5
47	49.259	Gedunin	0.3
48	49.398	Flurandrenolide	0.4
49	49.611	Oleic acid, 3-(octadecyloxy) propyl ester	0.8
50	49.794	Hexatriacontane	1.2
51	49.941	1,3-Dichloro-1,3-bis(norbornadien-2-yl)-1,3-bis(3-trimethylsilylpropyl) disiloxane	0.3
52	50.029	Octasiloxane, 1, 1,3,3,5,5,7,7,9, 9,11,11,13,13, 15,15-hexadecamethyl-	0.8
53	50.278	Octadecane, 1, 1'-[1,3-propanediylbis (oxy)]bis-	0.7
54	50.330	1,8,15,22-Tetraaza-2,7,16, 21-cyclooctacosa netetrone	0.3
55	50.440	Cholestane, 3,5-dichloro-6-nitro-, (3β,5α,6β)-	0.3
56	50.645	Hexa-t-butylselenatrisiletane	0.5
57	50.667	Cholic acid	0.3
58	50.836	Tetratriacontane	3.7
59	51.115	Thymol blue	0.6
60	51.202	Heptacosane	1.8
61	51.694	Tetrapentacontane, 1,54-dibromo-	2.3
		Chemical classes	
		Monoterpene hydrocarbons	0.8
		Sesquiterpene hydrocarbons	6.1
		Oxygenated sesquiterpens	24.7
		Oxygenated diterpenes	0.8

Chemical composition (%) of *Artemisia campestris* L. essential oi . Continued...

Pic N°	Rt	Compounds	Stems %
		Hydrocarbons	19.9
		Oxygenated compounds	38.1
		Others	15.4
		Total identified	**84.9%**

Rt : Retention time (min)

A total of 61 constituents comprising (84.9%) were characterized from the EO.

The oxygenated compounds (38.1%) and hydrocarbons (19.9%) fraction was dominant in the EO analysed, although with some differences concerning the main components.

The contribution of oxygenated sesquiterpens and sesquiterpene hydrocarbons in the EO of *Ar. campestris* stems were (24.7 and 19.9%, respectively). The oil also contained a small amount of monoterpene hydrocarbons and oxygenated diterpenes (0.8%, Both).

2-naphthaleneac etaldehyde, 1,4-dihydro-α,α-dimethyl-1,4-dioxo- (22.1%), Falcarinol (12%), γ-muurolene (4.6%) and pentacosane (4.3%).

β-pinene (35%) and 1, 8-cineole (14.4%), p-cymene (11.2%) and myrcene (10.9) were the two major constituents of leaves' oil of *Ar. campestris* from Tunisia (Dhifi *et al.*, 2017).

In fact, the study on the stems' oil is very few, a yield of (0.05%) and α-pinene and spathulenol (29.2% both) were reported as the major components of stem oil from *Ar. campestris* growing in Iran (Kazemi *et al.*, 2009).

In comparison with other Algerian studies on *Ar. campestris*, Bakchiche *et al.* (2014) mentioned that EO yield was of 0.33% for aerial parts, in their EO, the major constituents in were α-pinene (75.8%) and sabinene (16%).

Moreover, hydrodistillation of fresh aerial parts of *Ar. campestris* originating from Algeria resulted in 1.0 % as yield of a yellowish EO, and the authors identified 48 compounds (Ghorab *et al.*, 2013), their EO was characterized by the main presence of β-myrcene (16.2 %), α-pinene (14.18 %), trans-β-ocimene (12.61%), *P*-cymene (8.15%) and camphor (5.85%).

EOs of Tunisian and Italian *Ar. campestris* were mainly composed by β-pinene (24.0-49.8%) (Bellomaria *et al.*, 2001; Akrout *et al.*, 2003). The same authors found that the species growing in

Tunisia and Algeria have been reported to contain important amounts of α-pinene (5.9-12.5%) and (18%) of the whole oil, respectively.
Belhattab *et al.* (2011) collected leaves of *Ar. campestris* growing in Southern Algeria, EO with a yield of 0.66%. They reported as main components α-terpenyl acetate and α-pinene (19% and 18% respectively) followed by camphor (9%), camphene (8%), limonene and borneol (5% both).
Caryophyllene oxide (8.5-38.8%) has also been reported from the EO of *Ar. campestris*, growing in Lithuania (Judzentiene *et al.*, 2010).
Additionally, in comparison with other Algerian species and subspecies, (Boukhalkhal *et al.*, 2018) found the total percentages for identified components were varying from 64.34 to 89.24%, and from 72.15 to 86.94% for both subspecies glutinosa and eu-campestris, respectively.

4.1.2.2 Lamiaceae species

4.1.2.2.1 *Lavandula stoechas* L.

The EO obtained from leaves, stems and flowers using hydrodistillation having yellow colour with an aromatic fragrant odour.
GC–MS analysis of *L. stoechas* leaves, stems and flowers resulted in the identification of 51, 27 and 21 compounds (representing 97.1, 84.1 and 88.9% of the total oil).
Table 4.11 shows the chemical composition of *L. stoechas* L.' EOs.

TABLE 4.11: Chemical composition (%) of the *Lavandula stoechas* essential oil.

Pic N∘	Rt	Compounds	Leaves%	Stems %	Flowers %
1	8.136	Tricyclene	0.8	1.9	-
2	8.547	α-Pinene	1.5	2.1	-
3	9.083	Camphene	**6.8**	**7.8**	**10.2**
4	9.237	Bicyclo[3.1.0]hex-2-ene.4-methylene-1-(1-methylethyl)-	0.6	-	-
5	11.723	*p*-Cymene	0.8	-	-
6	11.958	Eucalyptol	1.9	3.2	3.3
7	13.851	Camphenilone	0.3	-	0.2

Chemical composition (%) of the *Lavandula stoechas* essential oil . Continued...

Pic N○	Rt	Compounds	Leaves%	Stems %	Flowers %
8	14.093	Fenchone	**4.7**	-	0.3
9	14.504	Linalool	0.3	0.9	0.2
10	14.614	Linalyl anthranilate	0.6	0.2	-
11	15.457	(R)-2.2.3-Trimethylcyclopent-3-en-1-acetaldehyd	0.6	0.1	-
12	16.323	(-)-Camphor	**27.1**	**25.2**	**30.2**
13	16.807	Pinocarvone	0.3	-	-
14	16.954	Borneol	1.3	-	-
15	17.401	Terpinen-4-ol	0.6	-	-
16	17.709	$p.\alpha.\alpha$-Trimethylbenzyl alcohol	0.4	-	-
17	18.157	(-)-Myrtenol	1.2	-	-
18	18.597	4.6.6-T4.6.6-Trimethylbicyclo[3.1.1] hept-3-en-2-one	0.6	0.9	0.9
19	18.978	(Z)-carveol.2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol.cis-mentha-1.8-dien-6-ol	0.4	0.3	-
20	19.844	Carvone	0.3	0.3	-
21	21.347	L-Bornyl acetate	4.9	**10.2**	**8.9**
22	21.538	Carvacrol	0.6	-	-
23	22.616	Myrtenyl acetate	5	-	-
24	23.812	(+)-Cyclosativene	0.6	-	-
25	26.556	L-Carveol	0.3	0.3	-

Chemical composition (%) of the *Lavandula stoechas* essential oil . Continued...

Pic N○	Rt	Compounds	Leaves%	Stems %	Flowers %
26	26.981	4α.8α-Dimethyl-5.6.7.8-tetrahydronaphthalen-2-ol	0.3	0.6	0.2
27	27.179	β-Selinene	0.5	0.9	-
28	27.282	Naphthalene.1.2.3.4-tetrahydro-5.6.7.8-tetramethyl-	0.3	1.1	0.5
29	27.392	Germacrene D-4-ol	1	0.2	0.9
30	27.715	(-)-trans-Pinocarvyl acetate	0.4	-	0.4
31	27.927	(-)-cubenol	1.1	-	-
32	28.111	(+)-Delta-cadinene	1	-	-
33	28.602	α-Calacorene	0.4	1.2	-
34	28.742	(+)-α-Longipinene	0.7	2.2	-
35	28.910	α-Pinene10-(2-oxopropyl)-	0.5	-	-
36	29.504	(-)-Spathulenol	0.3	-	-
37	29.556	α-Cedren-9-β-ol	0.3	0.2	-
38	29.636	Caryophyllene oxide	1.7	-	-
39	29.923	Ledol	**8.6**	**7.2**	**10.2**
40	30.187	Globulol	**6**	**15.9**	**18.2**
41	30.289	Isoaromadendrene epoxide	0.4	0.4	-
42	30.355	2,5-Di-tert-butylquinone	0.3	0.2	-
43	30.685	Cubenol	1.5	0.1	0.5
44	30.891	Luciferin aldehyde	0.4	0.2	0.5
45	31.008	T-muurolol	0.8	-	0.7

Chemical composition (%) of the *Lavandula stoechas* essential oil . Continued...

Pic N○	Rt	Compounds	Leaves%	Stems %	Flowers %
46	31.214	β-Eudesmol	0.3	-	0.3
47	31.734	Cadalene	0.4	-	1.2
48	31.830	Longiverbenone	0.7	-	-
49	32.505	Cedren-13-ol. 8-	0.6	-	0.2
50	36.994	Linalyl phenylacetate	0.3	-	-
51	37.969	Fumaric-acid dimyrtenyl ester	0.4	0.1	0.2
		Chemical classes			
		Monoterpene hydrocarbons	10.5	11.8	10.2
		Sesquiterpene hydrocarbons	3.6	4.3	1.2
		Oxygenated monoterpenes	46.1	41	43.9
		Oxygenated diterpenes	0.4	0.1	0.2
		Oxygenated Sesquiterpenes	26.3	25	31
		Oxygenated compounds	4.5	0.8	1.9
		Hydrocarbons	0.3	1.1	0.5
		Total identified	**91.7%**	**84.1%**	**88.9%**

Rt : Retention time (min)

(-) not detected

The most abundant compound class was constituted of leaves, stems and flowers EOs containing Oxygenated monoterpenes (46.1, 41 and 43.9%, respectively) with camphor, myrtenyl acetate and L-bornyl acetate as the most abundant representative, following by monoterpenes hydrocarbons (10.5, 11.8 and 10.2%, respectively), with camphene, and α-pinene as the most abundant compounds.

Oxygenated sesquiterpenes composed the third most abundant compound class (26.3, 25 and 31%, respectively), with fenchone and ledol as the most abundant representative.

The EOs were, however, poor in sesquiterpene hydrocarbons (3.6, 4.3 and 1.2%) and oxygenated diterpenes (0.4, 0.1 and 0.2%).

Furthermore, this analysis revealed that the major constituents of the EOs extracted from level over 4%, were (-)-camphor (27.1, 25.2 and 30.2%), ledol (8.6, 7.2 and 10.2%), camphene (6.8, 7.8 and 10.2%), globulol (6, 15.9 and 18.2%) and L-bornyl acetate (4.9, 10.2 and 8.9%) in leaves, stems and flowers, respectively, while myrtenyl acetate (5%) present only in leaves EO, and fenchone was found in leaves and flowers (4.7 and 0.3%).

Also, other compounds as eucalyptol (syn:1,8-cineole) (1.9, 3.2 and 3.3%), cubenol (1.5%), (-)-cubenol (1.1%), and (+)-delta-cadinene (1%) have been found in a proportion over 1%.

Whereas caryophyllene oxide (1.7%) was found only in leaves EO, and germacrene, are present in small amount (1, 0.2 and 0.9%, in leaves, stems and flowers, respectively).

According to the existing literature, the oil of *L. stoechas* consists of linalyl acetate, 1,8-cineole, fenchone and camphene (Guenther, 1960; Gildemeister-Hoffmann, 1961).

In a more recent study of *L. stoechas* its EO seems to consist almost exclusively of fenchone and camphor and the most reports chemotype is fenchone/camphor chemotype record in Algeria (Dob *et al.*, 2006; Benabdelkader *et al.*, 2011), Morocco (Zrira and Benjilali, 2003), Tunisia (Bouzouita *et al.*, 2005 ; Messaoud *et al.*, 2012), Italy (Angioni *et al.*, 2006), Greece (Tzakou *et al.*, 2009), Hatay, Turkey (Dadalioglu and Evrendilek, 2004), Cyprus (Valentini *et al.*, 1993), Turkey (Giray *et al.*, 2008; Kaya *et al.*, 2012), Greece (Kokkalou, 1988), Portugal (Matos *et al.*, 2009), Greece (Hassiotis, 2010), Italy (Zuzarte *et al.*, 2013), Spain (Carrasco *et al.*, 2015) and Corsica, France (Ristorcelli *et al.*, 1998).

In a few studies conducted in Spain, Turkey and Crete, some populations with a fenchone/1,8-cineol chemotype were distinguished, that were growing in the same phytogeographic area as others with a fenchone/camphor chemotype from Spain (Garcia Vallejo, 1992), Crete, Greece (Skoula *et al.*, 1996), Algeria (Chekoual *et al.*, 2018), Greece (Hassiotis and Orfanoudakis, 2018) and Turkey (Kirmizibekmez *et al.*, 2009; Inan *et al.*, 2013). In Turkey, a 1,8-cineol/camphor chemotype was described (Arabaci and Bayram, 2006).

Camphor is found as the major component in our sample with 27.1% from leaves, from this point, our result is in agreement with some studies of this species, which its oil consist mostly of camphor with different chemotype camphor/fenchone, from Algeria (Belarbi *et al.*, 2018), Turkey (Topal *et al.*, 2008, Akgün *et al.*, 2001; Gursoy *et al.*, 2009) and Italy (Marongiu *et al.*, 2010). Also camphor/bornyl acetate from Turkey (Mokhtarzadeh *et al.*, 2018).

Some other studies reported eucalyptol/δ-cadinene from Iran (Ebadollahi *et al.*, 2010), linalyl acetate/linalool from Tunisia (Msaada *et al.*, 2012), pulegone/menthol (Gören *et al.*, 2002), α-thujone/camphor from Turkey (Sertkaya *et al.*, 2010) and 1.8-cineole/finalool from Cyprus (Piccaglia *et al.*, 1993).

According to the existing literature, there are only five studies about leaves LsEO, two of them were reported that camphor is the major component; they investigated leaves EO of *L. stochas* growing wild in Turkey (Topal *et al.*, 2008) and Italy (Marongiu *et al.*, 2010).

L. stoechas leaves EOs studied differed greatly from other published *L. stoechas* EOs with respect to less abundant compounds.

p-cymene was revealed as a fairly abundant constituent (6.5% of the total oil content) in the EO of another Algerian population (Dob *et al.*, 2006), in contrast to the EOs of the present study, where it was, at best, detected at (0.8% of the total oil content) **(table 4.11)**. Inversely, the same study on an Algerian population (Dob *et al.*, 2006), did not detect any myrtenyl acetate, which was an important constituent of the present EOs (5%) and a characteristic component of other *L. stoechas* EOs (Garcia Vallejo, 1992) also present in other *L. stoechas* EOs (Dadalioglu and Evrendilek, 2004; Angioni *et al.*, 2006; Giray *et al.*, 2008; Kirmizibekmez *et al.*, 2009; Ristorcelli *et al.*, 1998).

Examination of our results revealed that limonene, fenchol, cis-sabinol, viridiflorol, cis-pinene-3-ol, and thymol, previously found in *L. stoechas* (Kokkalou, 1988; Garcia-Vallejo *et al.*, 1990; Valentini *et al.*, 1993; Skoula *et al.*, 1996; Akgün *et al.*, 2001; Kim and Lee, 2002), could not be identified.

The composition of the EOs reported here differed from any other by the presence of higher quantities of camphene (6.8%) and globulol (6%), and the absence of linalyl acetate, b-phellandrene, longifolene, and germacrene D, excepted the Eos extracted from flowering aerial part of some Algerian wild population of *L. stoechas* where these last components were absent too (Benabdelkader *et al.*, 2011).

The variability in oil composition was present even in several *Lavandula* species, and these variations may be sufficient to allow the distinction of different chemotypes, or the result of an adaptative process to particular ecologic conditions.

4.2 Total phenolic content results

4.2.1 Extraction and determination of total phenolic contents

Plants extracts preparations of *C. sphaerocephala*, *A. maritimus*, *Ar. campestris* and *B. sylvestris* (Asteraceae) also *L. stoechas* (Lamiaceae) were carried out using various polar (methanol), intermediate (chloroform) and non polar (n-Hexane) solvents according to a standard protocol. The most commonly used solvent is methanol (Dave, 2009). According to the method, dried plant material (20 g) was ground in warring blender, transferred to dark-coloured flasks and mixed with 200 ml of solvents with different polarities (Hexane, Chloroform and Methanol) respectively. Prepared plant material was transferred to dark-coloured flasks and macerated for 24h and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 ^{o}C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 °C.
The relative yields (g/20g powdered material) of the total phenolic content extracted from different plants of Asteraceae and Lamiaceae family were calculated.

4.2.2 Yield (w/w %) of total phenolic content

It is well known that polyphenols are widely distributed in plants, they are sometimes present in high concentrations (Harborne, 1993), especially in medicinal plants and many edible plants (Hagerman *et al.*, 1998).

4.2.2.1 Yield (w/w %) of Asteraceae species

Table 4.12 shows the variance and the sum of squares analysis of TPC yields.

TABLE 4.12: Sum of sqaures analysis.

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Organs	2	11.089	5.544	9.979	0.000
Solvent	1	66.490	66.490	119.669	< 0.0001
Species	3	3.506	1.169	2.103	0.111
Organs * Solvent	2	18.506	9.253	16.654	0.0001
Organs * Species	6	47.697	7.950	14.308	< 0.0001
Solvent * Species	3	19.300	6.433	11.579	< 0.0001

The analysis of variance indicated that the interaction effect between organs and species, organs and solvent as well as solvent and species were highly significant for all phenolic content yields considered ($p < 0.05$).

Table 4.13 summarises the results obtained from the interaction between the studied species organs.

TABLE 4.13: Essential oil yields (%) from different organs of studied species.

Modality	**Mean ± SD**
Leaves	2.68 ± 2.06^{a}
Stems	1.92 ± 1.77^{b}
Flowers	1.79 ± 0.82^{b}

Data are given as mean ± SD (n = 3).
Means having different superscripts (a-b) within the same column are significantly different at (p <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5% (Duncan test).
SD: standart deviation.

Table 4.13 shows that leaves (of all species) recorded significantly the best yields in TPC (2.68 ± 2.06), followed by stems and flowers, which they recorded significantly the lowest yield with no changes in term of TPC, with values of (1.90 ± 1.60 and 1.79 ± 0.82, respectively) ($p < 0.05$).
The analysis of variance indicated that organ effect was high between leaves and flowers (p = 0.0001) as well as between leaves and stems (p = 0.001), while there is no significant changes between flowers and stems (p = 0.564).

Table 4.14 summarises the results obtained from the solvent used effect.

TABLE 4.14: TPC yieds (%) depending on the different solvent used.

Modality	**Mean ± SD**
MeOH	3.09 ± 1.84^{a}
$ChCl_3$	1.17 ± 0.56^{b}

Data are given as mean ± SD (n = 3).
Means having different superscripts (a-b) within the same column are significantly different at (p <0.05) (Duncan test).
SD: standart deviation.

Table 4.14 shows that MeEs recorded significantly the best yields in TPC (3.09 ± 1.84) compared by ChEs (1.17 ± 0.56).
Previous results revealed that methanol and ethanol were better solvents than the others in extracting phenolic compounds from the extracts due to their polarity and good solubility for phenolic components from plant materials (Siddhuraju and Becker, 2003; Zhou and Yu, 2006).

Usually, polar solvents (e.g. methanol, ethanol and water) are used to get the highest extraction yields of phenolic compounds and methanol is the best one, however if glycoside derivatives are present, the aqueous fraction is enriched (Tsao and Deng, 2004).

Table 4.15 summarises the results from the quantitative determination of TPC.

TABLE 4.15: TPC yields (%) of different studied species

Modality	**Mean ± SD**
A. maritimus	2.51 ± 3.37^a
B. sylvestris	2.05 ± 1.92^a
C. sphaerocephala	1.99 ± 1.24^a
Ar. campestris	1.98 ± 0.70^a

Values followed by the same small letter did not share significant differences at 5 % (Duncan test).
SD: standart deviation.

Table 4.15 shows that all species (*A. maritimus, B. sylvestris, C. sphaerocephala* and *Ar. campestris*) recorded approximately the same yields of TPC ($P > 0.05$) (2.51 ± 3.37, 2.05 ± 1.92, 1.99 ± 1.24 and 1.98 ± 0.70, respectively).

Table 4.16 summarises the interaction effect between organs and solvents.

TABLE 4.16: TPC yields (%) of different organs depending on the solvent used.

Modality	**Mean ± SD**
Leaves * MeOH	3.83 ± 1.86^a
Stems * MeOH	2.82 ± 2.02^b
Flowers * MeOH	2.67 ± 0.87^c
Flowers * $ChCl_3$	1.43 ± 0.65^d
Leaves * $ChCl_3$	1.02 ± 0.27^d
Stems * $ChCl_3$	0.97 ± 0.59^d

Means having different superscripts (a-d) within the same column are significantly different at ($p < 0.05$) (Duncan test).
Values followed by the same small letter did not share significant differences at 5 % (Duncan test).
SD: standart deviation.

Table 4.16 shows that leaves MeEs recorded significantly the highest yields (3.83 ± 1.86), followed by Stems' MeEs(2.82 ± 1.80), then flowers (2.67 ± 1.39), while ChEs of flowers, leaves and stems recorded the lowest yields (1.43 ± 0.58, 1.02 ± 0.31 and 0.97 ± 0.53 respectively) and there is no difference between these organs in terme of TPC ($p > 0.05$).

Table 4.17 summarises the yield of different parts of plants.

TABLE 4.17: TPC yields (%) from different organ/species.

Modality	Mean ± SD
Leaves * *B. sylvestris*	3.54 ± 2.58^{a}
Stems * *A. maritimus*	3.32 ± 2.80^{a}
Leaves * *A. maritimus*	3.2 ± 2.69^{a}
Flowers * *Ar. campestris*	2.69 ± 0.68^{ab}
Leaves * *C. sphaerocephala*	2.66 ± 1.49^{ab}
Flowers * *B. sylvestris*	2.12 ± 0.52^{bc}
Stems * *C. sphaerocephala*	1.96 ± 1.28^{bcd}
Stems * *Ar. campestris*	1.92 ± 0.09^{bcd}
Flowers * *C. sphaerocephala*	1.36 ± 0.60^{cde}
Leaves * *Ar. campestris*	1.33 ± 1.28^{cde}
Flowers * *A. maritimus*	1.02 ± 1.14^{de}
Stems * *B. sylvestris*	0.48 ± 0.20^{e}

Means having different superscripts (a-e) within the same column are significantly different at $p < 0.05$ (Duncan test).
Values followed by the same small letter did not share significant differences at 5 % (Duncan test).
SD: standart deviation.

Table 4.17 shows that *B. sylvestris* leaves, *A. maritimus* stems and leaves recorded significantly the best yields in TPC (3.54 ± 2.58, 3.32 ± 2.80, 3.2 ± 2.69, respectively) with no changes in TPC (p >0.05), followed by, *Ar. campestris* flowers and *C. sphaerocephala* leaves (2.69 ± 0.68 and 2.66 ± 1.49 respectively), then *B. sylvestris* flowers (2.12 ± 0.52), followed by stems of *C. sphaerocephala* and *Ar. campestris* (1.96 ± 1.28 and 1.9 2 ± 0.09, respectively).

C. sphaerocephala flowers and *Ar. campestris* leaves recorded a low yields (1.36 ± 0.60 and 1.33 ± 1.28, respectively) with no difference (p>0.05), followed by *A. maritimus* flowers (1.02 ± 1.14), while *B. sylvestris* stems recorded the lowest yield in TPC (0.48 ± 0.20).

Our results show that TPC yields varied significantly among the studied organs and species ($p < 0.05$). These variations could be attributed to some differences such as location, climate, environment, harvest period, berry maturity and variety type and could be related to the extreme conditions of growth, arid ecosystem and aggression conditions (solar exposure and big altitude until 2800 m) (Zengin *et al.*, 2016).

Table 4.18 summarises the interaction effect between species and solvents.

TABLE 4.18: TPC yields (%) of different studied species depending the solvent used.

Modality	Mean ± SD
MeOH * *A. maritimus*	4.17 ± 2.39^a
MeOH * *B. sylvestris*	3.02 ± 2.31^b
MeOH * *C. sphaerocephala*	3.00 ± 0.95^b
MeOH * *Ar. campestris*	2.18 ± 0.80^c
$ChCl_3$ * *Ar. campestris*	1.78 ± 0.56^c
$ChCl_3$ * *B. sylvestris*	1.07 ± 0.61^d
$ChCl_3$ * *C. sphaerocephala*	0.98 ± 0.29^d
$ChCl_3$ * *A. maritimus*	0.85 ± 0.20^d

Means having different superscripts (a-d) within the same column are significantly different at (p <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5 \% (Duncan test).
SD: standart deviation.

Results show that *A. maritimus* MeE recorded significantly the highest yields (p<0.05) with value of (4.17 ± 2.39), followed by MeEs of *B. sylvestris* and *C. sphaerocephala* (3.02 ± 2.31 and 3.00 ± 0.95, respectively) with no significant difference (p>0.05).

No significant changes in terms of TPC (p = 0.30) were indicated between *Ar. campestris* MeE and ChE with values of (2.18 ± 0.80 and 1.78 ± 0.56, respectively) (p = 0.265).

It could be noticed also from the results that no significant changes in terms of TPC (p >0.05) were indicated between ChEs of *B. sylvestris*, *C. sphaerocephala* (1.07 ± 0.61, 0.98 ± 0.29 and 0.85 ± 0.20, respectively) and ChE of *A. maritimus* (p <0.05) which they recorded significantly the lowest yields.

It could be noticed from the results that the highest extract yield was obtained by methanol extraction of all plant materials, compared by chloroform.

In conclusion chloroform efficiency as a solvent was lower than the efficiencies of all other solvents (Tsao and Deng, 2004).

4.2.2.1.1 *Centaurea sphaerocephala* L.

Figure 4.6 shows the TPC MeE and ChE yields of *C. sphaerocephala* L. leaves, stems and flowers.

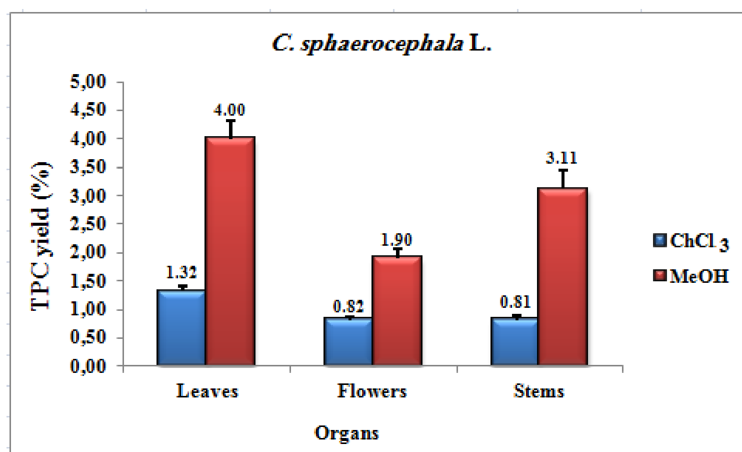


FIGURE 4.6: Total phenolic content yield (%) of *C. sphaerocephala* L.

Figure 4.6 and **table 4.17** show that there is no significant changes in terms of TPC (p >0.05) were indicated between leaves, stems, as well as between stems and flowers of *C. sphaerocephala*, but there is a high significant change between leaves and flowers(p = 0.185), organs recorded yields values of (2.66 ± 1.49, 1.96 ± 1.28 and 1.36 ± 0.60, in leaves, flowers and stems, respectively).

It could be noticed from **figure 4.6** and **table 4.18** that methanol was significantly (p <0.0001) better solvent than chloroform in extracting phenolic compounds from the extracts (3.00 ± 0.95 and 0.98 ± 0.29, respectively) due to their polarity and good solubility for phenolic components from plant materials.

Usually, polar solvents (e.g. methanol, ethanol and water) are used to get the highest extraction yields of phenolic compounds and methanol is the best one, however if glycoside derivatives are present, the aqueous fraction is enriched (Tsao and Deng, 2004).

Our results are similar with those found by Erol-Dayi *et al.* (2011), which found that TPC of aqueous and methanolic extracts were similar in *C. calcitrapa*, also the highest TPC was observed in the aqueous extract of *C. ptosimopappa*. In *C. spicata* and *C. ptosimopappa*, aqueous extracts contained approximately two fold phenolics than methanolic extracts.

Literature reports that there is no study that has been done on TPC of *C. sphaerocephala*.

4.2.2.1.2 *Bellis sylvestris* L.

Figure 4.7 shows the TPC yields of *B. sylvestris* L. (leaves, stems and flowers).

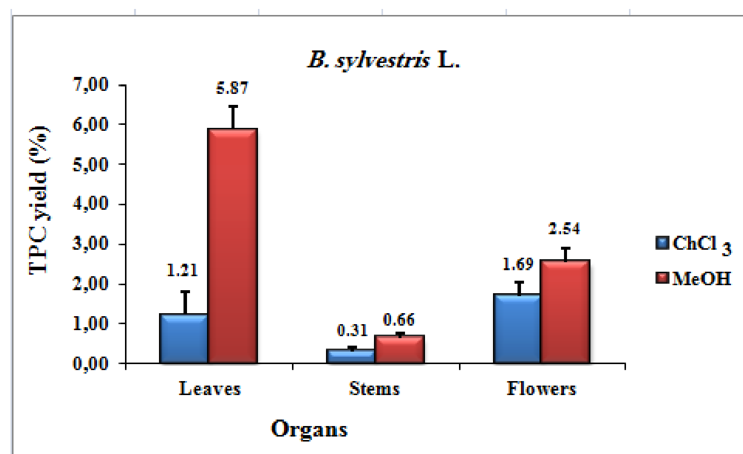


FIGURE 4.7: Total phenolic content yield (%) of *B. sylvestris* L.

Figures 4.7 showed that *B. sylvestris* leaves record significantly the best EO yield (p <0.05) with value of (3.54 ± 2.58), followed by flowers, (2.12 ± 0.52), while stems recorded significantly the lowest yield (p <0.05) with value of (0.48 ± 0.20).

The results obtained from the solvent used show that MeE recorded significantly (p <0.0001) the best yields in TPC (3.02 ± 2.31) compared by ChE (1.07 ± 0.61).

Moreover, the difference in total phenolic content could be due to the chemical composition of extract but also to the solvent polarity.

Literature reports that there is no study that has been done on TPC of *B. sylvestris* stems.

Concerning the genus *Bellis*, Ninomiya *et al.* (2016) evaluated the TPC of Albanian *B. perennis* flowers and found that the highest extract yield was obtained by methanol extraction (25.8 % from the dried material), followed by ethyl acetate (6.7 %).

4.2.2.1.3 *Asteriscus maritimus* (L.) Less.

Figure 4.8 shows TPC yield of *A. maritimus* (L.) Less. (leaves, stems and flowers).

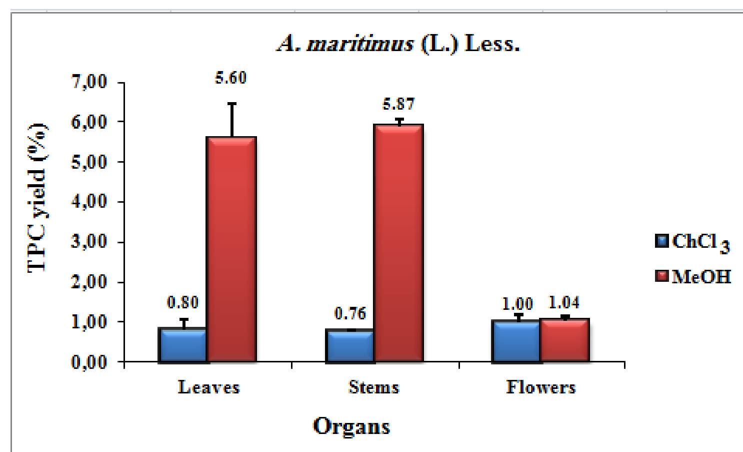


FIGURE 4.8: Total phenolic content yield (%) of *A. maritimus* (L.) Less.

Figures 4.8 shows that no significant changes in terms of TPC (p >0.05) were indicated between leaves and stems and recorded the best yield compared with flowers with values of (3.32 ± 2.80, 3.2 ± 2.69 and 1.02 ± 1.14, respectively).

The results obtained from the solvents used show that MeE recorded significantly (p<0.0001) the best yields in TPC (4.17 ± 2.39) compared by ChE (0.85 ± 0.20).

Literature reports that there is no study that has been done on TPC of Algerian *A. maritimus* organs. Ramdane *et al.* (2017) reported that the highest phenolic content in the Algerian *A. graveolens* was obtained with the ethyl acetate fraction, followed by n-Butanol fraction, then Hydro MeOH extract, while the ethanol fraction gave the lowest yields. Similar results were previously dilate by Haddouchi *et al.* (2014).

The high amount of phenolic compounds from *A. maritimus* could be related to the extreme conditions of growth, arid ecosystem and aggression conditions (Chan *et al.*, 2015; Zengin *et al.*, 2016). Moreover, it is proven that ethyl acetate is the most suitablesolvent for extraction of bioactive compounds from this plant. Infact, most of the phenolic compounds were soluble in non-polaror weakly polar solvents as ethyl acetate (Akrout *et al.*, 2012; Khlifi *et al.*, 2013).

The difference in total phenolic content could be due to the chemical composition of extract but also to the solvent polarity.

4.2.2.1.4 *Artemisia campestris* L.

Figure 4.9 shows the TPC yield of *Ar. campestris* L. (leaves, stems and flowers).

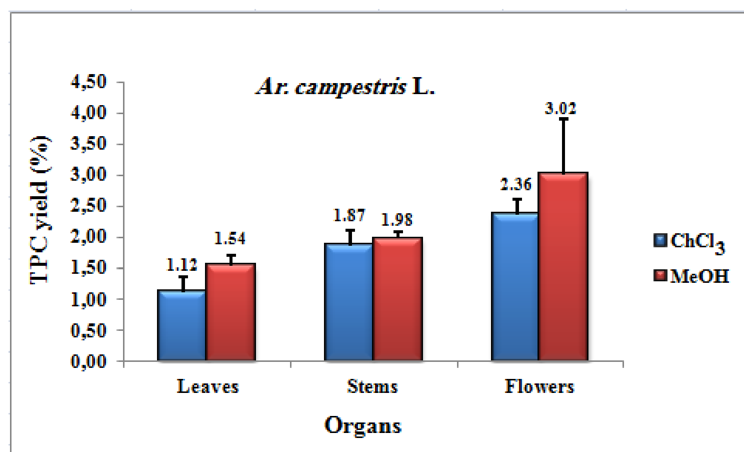


FIGURE 4.9: Total phenolic content yield (%) of *Ar. campestris* L.

Figures 4.9 shows that there is no significant changes in terms of TPC (p >0.05) were indicated between flowers and stems as well as between stems and leaves, while flowers recorded a highly significant changes (p >0.05) compared with leaves, *A. campestris* organs recorded TPC yields values of (2.69 ± 0.68, 1.92 ± 0.09 and 1.33 ± 1.28, in flowers, stems and leaves, respectively).

Table 4.18 showed that there is no significant changes in terms of TPC (p >0.05) were indicated between methanol and chloroform, even though methanol was provided large amount compared with chloroform (2.18 ± 0.80 and 1.78 ± 0.56, respectively).

Literature reports that there is no study that has been done on TPC of *Ar. campestris* organs. Concerning the genus *Artemisia*, Younsi *et al.* (2016) found that the TPC yield of MeE of Tunisian *A. herba-alba* aerial part was 27.65 ± 0.08 mg GAE/g DW.

The TPC observed in our study, were lower than that reported for samples from the southern part of Tunisia (Akrout *et al.*, 2011; Khlifi *et al.*, 2013) or for other *Artemisia* species such as *Ar. princeps, Ar. arborescens, Ar. annua, Ar. ludoviciana, Ar. oleandica*, and *Ar. stelleriana* Carvalho and *Ar. vulgaris* (Melguizo-Melguizo *et al.*, 2014).

The contents and the composition of total phenolic of *Artemisia* species varied according to plant origin, the solvent of extraction and the analytical method used (Seddik *et al.*, 2010).

4.2.2.2 Yield (w/w %) of Lamiaceae species

4.2.2.2.1 *L. stoechas* L.

Table 4.19 shows the variance and the sum of squares analysis of TPC .

TABLE 4.19: Sum of squares analysis.

Source	DDL	Sum of squares	Average of squares	F	Pr > F
Organs	2	13.689	6.845	88.754	< 0.0001
Solvent	1	18.261	18.261	236.762	< 0.0001
Species	0	0.000			0.488
Organs * Solvent	2	9.418	4.709	61.055	< 0.0001
Organs * Species	0	0.000			< 0.0001
Solvent * Species	0	0.000			0.010

The analysis of variance indicated that the effect between organs and solvent, organs and species as well as solvent and species were highly significant ($p < 0.05$) for all phenolic content yields considered.

Table 4.20 shows the yield of different parts of the plant.

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TABLE 4.20: TPC yields (%) from different organ of *L. stoechas* L.

Modality	Mean ± SD
Flowers	3.47 ± 2.09^{a}
Leaves	2.50 ± 0.26^{b}
Stems	1.34 ± 1.12^{c}

Means having different superscripts (a-c) within the same column are significantly different at (p <0.05) (Duncan test).

Values followed by the same small letter did not share significant differences at 5 % (Duncan test).

SD: standart deviation.

The analysis of variance indicated that organ effect was highly significant ($p = 0.039$) for all TPC yields considered.

Table 4.20 shows that the highest yield in TPC of different part of *L. stoechas* was required significantly from flowers (3.47 ± 2.09), followed by leaves (2.50 ± 0.26), while stems required the

lowest yield (1.34 ± 1.12) ($p < 0.0001$).

According to our results flowers produced more TPC than leaves and stems, similar results were reported earlier on other species, *Rutta chalepensis* leaves contained more EO than stems and flowers (Tounsi *et al.*, 2011), also on *Ocimum basilicum* L., (Chalchat and Özcan, 2008) and on *Myrtus communis* var. italica (Tounsi *et al.*, 2011), as well as on *Rosmarinus officinalis* L. (Yosr *et al.*, 2013) reporting that leaves have the highest essential oil yield.

On the other hand, enhancement of phenolic compounds accumulation in leaves and flowers could have an ecological and biological significance. It is noteworthy that floralscent play various important roles in the interaction between plants and their surrounding as pollinators, phytophagTPCus, etc. (Vainstein *et al.*, 2001; Cunningham *et al.*, 2004).

Table 4.21 summarises the interaction effect between species and solvents.

TABLE 4.21: TPC yields (%) depending the solvent used.

Modality	Mean ± SD
MeOH	3.45 ± 1.47^{a}
$ChCl_3$	1.43 ± 0.91^{b}

Means having different superscripts (a-c) within the same column are significantly different at (*p* <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5% (Duncan test).
SD: standart deviation.

Table 4.21 shows that MeE of *L. stoechas* recorded significantly the highest yields (3.45 ± 1.47), followed by ChE (1.43 ± 0.91).

It could be noticed from the results that the highest extract yield was obtained by methanol extraction compared by chloroform. So, it could be concluded that the higher polar solvents were more efficient in extracting phenolic compounds than the less polar solvents. These results are almost similar to those reported by Hernández-Hernández *et al.* (2009).

Previous results revealed that methanol and ethanol were better solvents than other solvents in extracting phenolic compounds from the extracts due to their polarity and good solubility for phenolic components from plant materials (Siddhuraju and Becker, 2003; Zhou and Yu, 2006).

Table 4.22 summarises the interaction effect between organs and solvents.

TABLE 4.22: TPC yields (%) of different organs depending on the solvent used.

Modality	Mean ± SD
Flowers * MeOH	5.37 ± 0.15 [a]
Leaves * MeOH	2.63 ± 0.21 [b]
Leaves * ChlCl$_3$	2.37 ± 0.28 [b]
Stems * MeOH	2.33 ± 0.45 [c]
Flowers * ChlCl$_3$	1.57 ± 1.13 [d]
Stems * ChlCl$_3$	0.34 ± 0.32 [e]

Means having different superscripts (a_e) within the same column are significantly different at (p <0.05) (Duncan test).
Values followed by the same small letter did not share significant differences at 5 % (Duncan test).
SD: standart deviation.

Figure 4.10 shows the TPC yield (%) of *L. stochas* L.

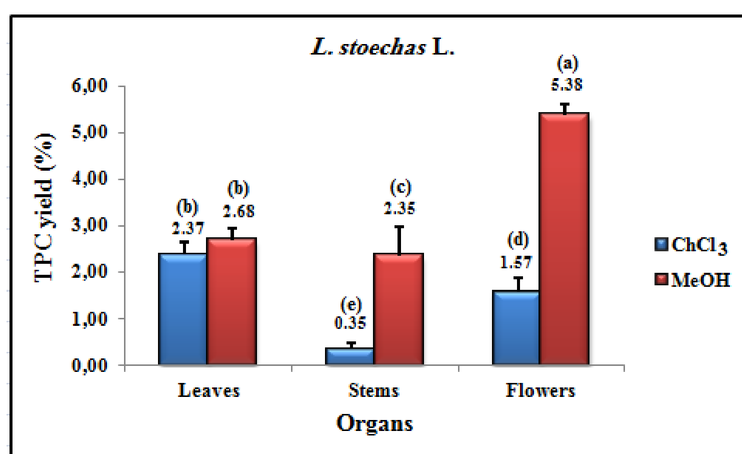

FIGURE 4.10: Total phenolic content yield (%) of *L. stochas* L.

Table 4.20 and **figure 4.10** show that flowers MeE recorded significantly the highest yields (5.37 ± 0.15) (p < 0.0001), followed by leaves MeE and stems ChE (2.63 ± 0.62 and 2.33 ± 0.28), wich no significant changes in terms of TPC were indicated between theme (p = 0.102), then MeE of stems and ChEs of flowers and stems, with yields values of (2.33 ± 0.45, 1.57 ± 0.30 and 0.35 ± 0.13, respectively), also there is no significant difference between stems MeE and leaves ChE (1.50 ± 0.98) (p = 0.349). Stems ChE recorded the lowest yield (0.62 ± 0.32).

In fact, the study on the TPC leaves, stems and flowers is very few, previous studies reported that the contents in TPC is higher than those observed in the ethanol or water extracts of *L. stoechas* from Turkey (Gülçin *et al.*, 2004b), in *L. officinalis* water extract from Iran (Bouayed *et al.*, 2007)

and in methanol extract of *L. angustifolia* from Lithuania (Miliauskas *et al.*, 2004), also, in *L. multifida* and *L. stoechas* extracts, as well as hexane and aqueous fraction in *L. intermedia* from Spain (Torras-Claveria *et al.*, 2007).

Alizadeh and Aghaee (2016) found that the total phenols from two different populations of Iranian *L. stricta* varied from 61.05 to 64.45 mg GAE/g dry weight.

Messaoud *et al.* (2012) reported that MeE of *L. coronopifolia* aerial part from Tunisia had a high contents of the total phenolic and flavonoid contents, followed by *L. stoechas* extracts.

However, total phenol contents were lower than those estimated in acetone extracts from Taiwanese *Lavandula* species and varieties (Lee *et al.*, 2011).

On the other hand, there are unavailable data concerning *L. stoechas* leaves, stems and flowers separately polyphenols and for the first time it was studied regarding its total polyphenols content using different solvent.

4.2.3 Determination of total penolic extracts using GC-MS

studied species were extracted with n-hexane. After evaporation of n-hexane in vacuo, the aqueous phase was successively partitioned with $ChCl_3$ and MeOH.

4.2.3.1 Asteraceae species

4.2.3.1.1 *Centaurea sphaerocephala* L.

The identified compounds, their retention time, and percentage compositions are given in **table 4.23** where the components are listed in order of their elution on apolar column (HP5-MS).

Table 4.23: Chemical composition (%) of *C. sphaerocaphala* L. chloroform extracts.

Peak N°	Rt	Compounds	Leaves %	Stems %	Flowers %
1	19,874	Palmitic acid, trimethylsilyl ester	-	2.2	3.4
2	21,993	Acetyl tributyl citrate	-	-	1.3
3	23,114	Octadecane	-	0.5	-
4	23,169	Diisooctyladipat	-	-	2.9
5	23,356	Acrylonitrile,2-chloro-3,3-bis-(4-nitrophenoxy)-	-	8.4	-
6	23,382	2-Hydroxycyclohexane-1-carboxylic acid, di-TMS	**23.9**	-	-
7	23,951	Pentacosane	-	1.8	2
8	23,955	Eicosane	-	2.4	-
9	24,053	Cyclohexanepropanoic acid, α,α -dichloro-β- [(trimethylsilyl)oxy]-, ethyl ester	1.1	-	-
10	24,284	Octanoic acid, trimethylsilyl ester	**8**	-	-
11	24,286	2-Methylglutaconic acid, bis(trimethylsilyl) ester	**31.1**	**9.3**	-
12	24,288	Arachidic acid	-	-	**6.6**
13	24,291	α-Linolenic acid, trimethylsilyl ester	**12.1**	**11.9**	-
14	24,294	Thymidine, 3’-*O*-TBDMS.	-	-	**13.7**
15	24,448	Bis(Trimethylsilyl)(2R,3R)-2-hydroxy-3-[(trimethylsilyl)oxy]butanedioate	-	7.8	-
16	24,443	(-)-Isolongifolol, trimethylsilyl ether	-	2.9	**9.2**
17	24,447	3Hydroxy-2,3-didehydrosebacic acid,tris(trimethylsilyl) derivative	3.1	-	-
18	24,455	Arachidonic acid, TMS derivative	**5.8**	**10.5**	**22.1**
19	24,761	Octacosane	-	6	2.1
20	24,762	Camphoric acid, bis(tert-butyldimethylsilyl) ester	0.8	-	-

Chemical composition (%) of *C. sphaerocaphala* L. chloroform extracts. Continued...

Peak N°	Rt	Compounds	Leaves %	Stems %	Flowers %
21	24,836	19-Norandrosterone-diOTMS	-	-	2.9
22	24,837	Octadecane, 1-chloro-	-	2	-
23	24,909	Batilol	-	2.7	-
24	24,911	(-)-Isolongifolol, trimethylsilyl ether	0.7	-	-
25	25,034	2,4-Imidazolidinedione,5-(3-methoxyphenyl)-3-methyl-5-phenyl-1-(trimethylsilyl)-	-	5.3	-
26	25,268	Hexadecane, 2-methyl-	-	3.6	-
27	25,539	cis-5,8,11-Eicosatrienoic acid, trimethylsilyl ester	1.8	-	-
28	25,542	Heptacosane	-	-	**10**
29	25,543	Pentadecane	-	**7.7**	-
30	25,542	Imidazo[4,5-e][1,4]diazepin-8(1H)-one,4,5,6,7-tetrahydro-4,7-dimethyl-5-thioxo-	2.6		-
31	25,936	Ethyltrimethylsilyl methylmalonate	-	4.7	-
32	25,976	β-Sitosterol trimethylsilyl ether	-	5.9	-
33	26,013	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	-	-	1.8
34	26,298	Tetracosane	-	-	2.2
35	27,113	Tricosane	1	-	-
36	27,118	Nonacosane	-	2.9	**10.1**
37	27,508	Silane, (hexacosyloxy) trimethyl-	0.8	-	-
38	28,016	Hexacosane	-	-	2.2
39	29,055	Heptacosane, 1-chloro-	-	1.1	-
40	28,939	2-Isopropenyl-4α,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1.8	-	-
41	29,054	Hentriacontane	-	-	7.3

Chemical composition (%) of *C. sphaerocaphala* L. chloroform extracts. Continued...

Peak N°	Rt	Compounds	Leaves %	Stems %	Flowers %
42	29,193	7,7-Dimethyl-(5Z,8Z)-eicosadienoic acid,trimethylsilyl ester	1.8	-	-
		Total identified	**96.4%**	**99.6%**	**99.8%**

Rt : Retention time (min)

(-) not detected

The detailed results of GC/MS analysis indicated that the $ChCl_3$ extract contained 15, 20 and 16 compounds with 96.4, 99.6 and 99.8 % recovery, in leaves, stems and flowers respectively.

The main constituents of the ChE from leaves were identified as fatty acids and found to be 2-methylglutaconic acid, bis(trimethylsilyl) ester (31.1%), 2-hydroxycyclohexane-1-carboxylic acid, di-TMS (23.9%) and α-linolenic acid, trimethylsilyl ester (12.1%), this latter was the major constituent of stems extract (11.9%). Other compounds present in appreciable amounts were octanoic acid, trimethylsilyl ester (8.0%), and arachidonic acid, TMS derivative (5.8%), this latter was the major constituent of flowers extract (22.1%) followed by thymidine, 3'-*O*-TBDMS (13.7%), nonacosane (10.1%) and heptacosane (10%). Arachidonic acid, trimethylsilyl ester was also the second major constituent of stems extract (10.5%) followed by 2-Methylglutaconic acid, bis(trimethylsilyl) ester (9.3%), bis(trimethylsilyl) (2R,3R)-2-hydroxy-3-[(trimethylsilyl)oxy]butanedioate (7.8%) and pentadecane (7.7%). There is no study on the chemical composition of ChE of the different organes of *C. sphaerocephala*, while there are few studies have been reported the chemical composition of the aerial part of this species, previous paper (Geppert *et al.*, 1983) reported cincin (I) and unknown lacton , as lactones present in the lactone fraction obtained from ChE of aerial part of *C. sphaerocephala* harvested from Portugal (Coimbra), while we didn't find any lactone in our extract this is do maybe to the variability of the organ used and the geographic region (Miguel *et al.*, 2005). While, our *C. sphaerocephala* stems ChE is deficient in different metabolites, several phenolic compounds have been isolated and characterized from this species and other species belonging to the genus *Centaurea*,in particular arctiin and arctiin and arctigenin ATG that have been identified as the major compounds in *C. sphaerocephala* ssp. Polyacantha (Bastos *et al.*, 1990).

However, there are few reports on the chemical composition of the total phenolic content from

the *C. sphaerocephala*, found that the main constituent of the aerial parts was (-)-arctiin followed by its congeners (-)-matairesiriol and (-)-arctigenin, this study led also to isolated of two very closely related isomeric sesquilignans $C_{30} H_{32} 0_9$ (lappaol A and isolappaol A) as well as 2-(2-Thienylethylnyl)-5-(1,2- di/iydroxyethyl)-thiophene (Liu *et al.*, 2010).

Previous phytochemical studies of the species *sphaerocephala* (ssp. sphaerocephala and ssp. polyacantha) led to the isolation of sesquiterpene lactones and lignans, sesquilignans and dithienylacetylene (Bruno *et al.*, 1994; Bastos *et al.*, 1990) respectively. These variations in the chemical composition reported in the literature can be explained by the influence of environmental and methodological factors (Bakkali *et al.*, 2008) also the soil composition, plant organ, vegetative cycle phase and climate (Miguel *et al.*, 2005).

A previous paper (Geppert *et al.*, 1983) reported salonitenolide (I) and cincin (II) (germacranolide group) as lactones present in the lactone fraction obtained from ChE of the aerial part of *C.calcitrapa* (Heister ex fabr.) Hayek harvested from Rumania (Cluj) and Bulgaria (Burgas) respectively.

4.2.3.1.2 ***Bellis sylvestris* L.**

The detected compounds of ChE from *B. sylvestris* resulting from the GC-MS analysis after derivatization were summarized in **table 4.24**.

TABLE 4.24: Chemical composition (%) of *B. sylvestris* L. chloroform extract.

Peak N°	Rt	Compounds	Stems %
1	19,871	Palmiticc acid, trimethylsilyl ester	**5.5**
2	21,624	1,19-Eicosadiene	3.7
3	21,985	Tributyl acetylcitrate	0.2
4	22,724	Eicosoxy-trimethylsilane	**7.5**
5	23,173	Hexanedioic acid, bis(2-ethylhexyl) ester	0.5
6	23,488	Arachidic acid, trimethylsilyl ester	**12.8**
7	23,953	Octadecane	2.2
8	24,372	1-Docosanol, trimethylsilyl ether	4.8
9	24,484	Bis (2-ethylhexyl) phthalate	0.2
10	24,764	Heptacosane	6.1
11	25,085	Docosanoic acid, trimethylsilyl ester	**11.6**

Chemical composition (%) of *B. sylvestris* L. chloroform extract. Continued...

Peak N°	**Rt**	**Compounds**	**Stems %**
12	25,551	Heneicosane	**10.5**
13	25,981	3,5-Dicaffeoylquinic acids	3.2
14	25,914	Tetracosan-1-ol trimethylsilyl ether	3.3
15	26,019	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	0.3
16	26,303	Octacosane	2.9
17	27,119	Nonacosane	4.3
18	27,517	Silane, (hexacosyloxy)trimethyl-	3.7
19	27,958	Luteolin-7-*O*-b-D-glucuronide	1.3
20	28,025	Triacontane	2.5
21	29,061	Hentriacontane	**5.8**
22	29,428	C(14α)-Homo-27-nor-14β-gammaceran-3α-ol	4.2
		Total identified	**97%**

Rt : Retention time (min)

The major compounds were found to be saturated fatty acids (arachidic acid, trimethylsilyl ester (12.8%), docosanoic acid, trimethylsilyl ester (11.6%), palmiticc acid, trimethylsilyl ester (5.5%), as well as alkanes (Heneicosane (10.5%), Eicosoxy-trimethylsilane (7.5%), Heptacosane (6.1%)), this is maybe due to the fact that the device recognizes more volatile than non-volatile compounds with a large molecular weight.

The results indicated that the ChE of *B. sylvestris* stems contained 22 Compounds representing (97%), one identified as phenolic acids (3,5-dicaffeoylquinic acid (13)), another one as C-glycosyl flavonoids (luteolin-7-*O*-b-D-glucuronide (19)), similar compounds were isolated and identified in the MeE of *B. sylvestris* leaves from Italy (Scognamiglio *et al.*, 2016) and *B. perennis* (Karakas and Turker, 2013; Karakas *et al.*, 2015). It has been also previously shown that phenolic constituents of *B. perennis* include flavonoids (Gudej and Nazaruk, 2001), anthocyanins (Toki *et al.*, 1991) and phenolic acids (caffeic, ferulic, sinapic, *p*-coumaric, and salicylic acids) (Grabias *et al.*, 1995).

There is no study on the chemical composition of the ChE of *B. sylvestris* stems, while there are few studies have been reported the chemical composition of the leaf MeE of this species.

While we didn't find any saponins in our extract, Schöpke *et al.* (1994), were isolated two saponins from the MeE of *B. sylvestris* aerial part from Italy, one of these was a new triterpenoid saponins named besysaponin, the second was previously isolated from *B. perennis* named bellissaponin BS1. Also Scognamiglio *et al.* (2012) were isolated new six oleanane saponine for the first time from ME of *B. sylvestris* leaves collected from Italy; this is do maybe to the variability of the solvent polarity, as well as the organ used and geographic region.

While, our *B. sylvestris* stems ChE is deficient in different metabolites, several saponins have been isolated and characterized from other species belonging to the genus *Bellis*. In particular, phenolic acids and flavonoid glycosides (Gudej and Nazaruk, 2001) have been previously isolated from *B. perennis* flowers and roots, and triterpenoid saponins (Li *et al.*, 2005; Yoshikawa *et al.*, 2008; Morikawa *et al.*, 2008, Morikawa *et al.*, 2010; Morikawa *et al.*, 2011).

As well as, three triterpene saponins (i.e. besysaponins C12, OD2, UB1) and other related compounds isolated from *B. perennis* have been previously reported (Schöpke *et al.*, 1994), from *B. annua* (Schöpke *et al.*, 1996a) and *B. sylvestris* (Schöpke *et al.*, 1996b).

Gallic acid monohydrate, caffeic acid, rutin hydrate, luteolin-7-*O*-β-D glucoside, kaempferol, myricetin, quercetin, coumarin and apigenin were the main phenolic compounds in the MeE of *B. perennis* aerial part (Karakas *et al.*, 2015).

4.2.3.1.3 Asteriscus maritimus (L.) Less.

The identified compounds, their retention time, and percentage compositions of *A. maritimus* stem are given in **table 4.25** where the components are listed in order of their elution on apolar column (HP5-MS).

TABLE 4.25: Chemical composition (%) of *A. maritimus* (L.) Less. chloroform extract.

Peak N°	Rt	Compounds	Stems %
1	10,59	Glycerol, tris (trimethylsilyl) ether	0.1
2	13,946	Butane, 1,2,3,4-tetrakis[(trimethylsilyl)oxy]-	0.5
3	13,947	Meso-Erythritol, tetrakis(trimethylsilyl) ether	0.4
4	16,36	Xylitol, 1,2,3,4,5-pentakis-*O*-(trimethylsilyl)-	0.6
5	16,638	L-(-)-Arabitol, pentakis(trimethylsilyl) ether	0.1
6	23,952	Octadecane	5

Chemical composition (%) of of *A. maritimus* (L.) Less. chloroform extract. Continued...

Peak N°	Rt	Compounds	Stems %
7	23,98	β-sitosterol	**10.2**
8	24,154	Docosane	0.6
9	24,31	Asterisulphoxide	5.1
10	24,329	Undecane, 2,5-dimethyl-	0.1
11	24,765	Heptacosane	0.6
12	24,769	Octacosane	0.2
13	24,897	Chlorogenic acid	1.9
14	25,037	Hexacosane, 9-octyl-	1.3
15	25,44	Asterisulphone	1.2
16	25,555	Tricosane	0.5
17	25,568	Patuletin-7-*O*-[6-*O*-caffeoyl-2-*O*-[(S)-3-hydroxyisobutanoyl]glucopyranoside]	**5.2**
18	25,573	α-D-Glucopyranoside,1,3,4,6-tetrakis-*O*-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-*O*-(trimethylsilyl)-	0.7
19	25,614	Patuletin-7-?-β-[-6-?-[(S)-3-hydroxyisobutanoyl]glucopyranoside]	0.2
20	25,789	Oxo-6,7,9,10-tetrahydrohumulen-1,12-olide	0.5
21	25,804	Tricosane, 2-methyl-	1.4
22	25,987	Luteolin	**10.2**
23	26,14	Patuletin-7-*O*-[6-*O*-caffeoylglucopyranoside]	**20.4**
24	26,246	β-Amyrin trimethylsilyl ether	4.1
25	26,441	Asteriscunolides	0.3
26	26,554	8-oxo-α-humula-6Z,9Z-dien-12-oic acid	0.2
27	27,121	Nonacosane	1.1
28	27,223	10-isobutyryloxy-8,9-epoxythymyle	2.3
29	27,58	Patuletin-7-O-β-D- glucopyranoside	0.6
30	28,018	Heneicosane	3.4
31	28,029	Quercetin-3-O-β-rutinoside	0.8
32	28,03	Tetracosane	2.7
33	28,596	4-hydroxy-2-methoxybenzaldehyde	1.2

Chemical composition (%) of of *A. maritimus* (L.) Less. chloroform extract. Continued...

Peak N°	Rt	Compounds	Stems %
34	29,06	Hentriacontane	0.9
35	29,068	Hexacosane	**9.5**
36	29,089	Coniferaldehyde	1.6
37	29,176	Heptadecane, 9-octyl-	0.1
38	30,023	methylcaffeate	0.3
39	31,256	8-hydroxy-9,14-diisobutyryloxythymol	1.2
40	32,012	8,9-dihydroxy-10-isobutyryloxythymol	0.9
41	32,125	Nerolidol	0.1
42	23,089	Docosane	0.5
		Total identified	**97.1%**

Rt : Retention time (min)

The detailed results of GC-MS analysis of *A. maritimus* indicated that stemes ChE contained 37 compounds with 97.1% recovery, this study revealed that this extract is rich in flavonoids and polyphenols, where the main constituents were found to be: patuletin-7-*O*-(6-*O*-caffeoylglucopyranoside) (10.4%), luteolin (10.2%), β-sitosterol (10.2%), patuletin-7-*O*-(6-*O*-caffeoyl-2-*O*-[(S)-3-hydroxyisobutanoyl] glucopyranoside) (5.2%) and asterisulphoxide (5.1%) . This latter and asterisulphone (1.2%) are sulphurated diacetylenes isolated for the first time by (Medimagh-Saidana *et al.*, 2014) from Tunisian *A. maritimus* (L.) Less.

In addition we found another known compounds like 8-hydroxy-9,14-diisobutyryloxythymol (0.9%) (González *et al.*, 1986), coniferaldehyde (1.6%) (Kim *et al.*, 2003), 4-hydroxy-2-methoxybenzaldehyde (1.2%) (Lerrick *et al.*, 2008) and methylcaffeate (0.3%) (Xiang *et al.*, 2011).

Stems extract of *A. maritimus* afforded, as other species of this genus, the humulen derivatives with low amount: asteriscunolides (0.3%) and 8-oxo-α-humula-6Z,9Z-dien-12-oic acid (0.2%), Rauter *et al.* (2001) were isolated this two compounds for the first time from *A. vogelii* aeriel part, collected in the northern coast of Santiago Island, in Cabo Verde. As well as an nerolidol derivative (0.1%).

From the genus *Asteriscus*, two of the three species have been studied chemically and the typical constituents are humulene derivatives such as asteriscunolide A-D (l-4) (San Feliciano *et al.*, 1984;

El Dahmy *et al.*, 1985).

A previous paper (Jakupovic *et al.*, 1986) reported humulene derivatives such as asteriscunolide A-D are typical constituents of *A. sericeus* aerial part harvested from Spain. Furthermore two nerolidol derivatives (6-oxo-cyclonerolidol and 6u-Hydroxycycfonerolidol) were present.

4.2.3.1.4 ***Artemisia campestris* L.**

The detected compounds of ChE extracted from *Ar. campestris* stems resulting from the GC-MS analysis after derivatization were summarized in **table 4.26**.

TABLE 4.26: Chemical composition (%) of *A. campestris* L. chloroform extract.

Peak N°	Rt	Compounds	Stems %
1	18,456	Chlorogenic acid	**12.9**
2	20,128	Quercetin-rha-glu	**6.0**
3	21,125	Luteolin	**10.8**
4	23,952	Octadecane	0.8
5	23,761	7-*O*-methyl 8-hydroxyapigenin	3.3
6	24,329	Undecane, 2,5-dimethyl-	4.9
7	24,765	Heptacosane	1.3
8	24,943	3,7,-*O*-dimethyl kaempferol	**9.1**
9	25,037	Hexacosane, 9-octyl-	**6.0**
10	25,555	Tricosane	1.1
11	25,572	α-D-Glucopyranoside,1,3,4,6-tetrakis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-*O*-(trimethylsilyl)-	2.0
12	25,804	4-methoxy-cinnamic acid	3.9
13	26,24	1,4-Dicaffeoylquinic acid	1.4
14	26,246	β-Amyrin trimethylsilyl ether	0.6
15	26,311	Octacosane	3.3
16	27,118	Heptadecane, 9-octyl-	1.6
17	27,128	Nonacosane	4.9
18	27,456	3,4,5-Tricaffeoylquinic acid	3.6
19	28,018	Heneicosane	3.7

Chemical composition (%) of *A. campestris* L. chloroform extract. Continued...

Peak N°	**Rt**	**Compounds**	**Stems %**
20	28,03	Tetracosane	1.4
21	29,06	Hentriacontane	4.8
22	29,143	Petunidin-3-*O*-acetyl glucoside	2.7
23	29,168	Hexacosane	4.5
24	29,089	Docosane	0.5
25	29,123	3,4-Dicaffeoylquinic acid	3.2
		Total identified	**88.3%**

Rt : Retention time (min)

Of the 25 detected compounds, one was identified as C-glycosyl flavonoid (quercetin-rha-glu (2) (6.1%)), five compounds as phenolic acids (chlorogenic acid (1) (12.9%), 4-methoxy-cinnamic acid (12) (3.9%), 1,4-dicaffeoylquinic acid (13) (1.4%), 3,4,5-tricaffeoylquinic acid (18) (3.6%), 3,4-dicaffeoylquinic acid (25) (3.2%), similar phenolic compounds were identified in *Ar. herba alba* (ASSO.) from Tunisia (Younsi *et al.*, 2016) except compound (12) which found in*Ar. Campestris* L. (Riedel *et al.*, 2010).

These findings are in accordance with other studies conducted on phenolic rich extracts of *Ar. campestris* L. from Algeria and on methanolic, water and ethyl acetate extracts of the aerial part of Tunisian *A. campestris* L. which found that these preparations contained chlorogenic and caffeic acids as well as isochlorogenic acids A, B and C (Djeridane *et al.*, 2007; Sebai *et al.*, 2014; Megdiche-Ksouri *et al.*, 2015).

Similar phenolic compounds were identified in limited species of *Artemisia* such as *Ar. annua*, (Han *et al.*, 2008), *Ar. rupestris* (Gu *et al.*, 2012) and rarely reported in *Ar. herba-alba*.

Different quinic acid derivatives, reported for the genus *Artemisia* (Han *et al.*, 2008; Carvalho *et al.*, 2011; Dahmani-Hamzaoui *et al.*, 2012; Melguizo-Melguizo *et al.*, 2014) have been also observed in *Ar. campestris* .

Thus, the use of GC-MS after derivatization will contribute the increase of the reliability and the accuracy of the chemical methods used to separate polyphenols in the species. The identification of caffeoylquinic acids and c-glycosyl flavonoids suggests that the species could play an important role

as inhibitor against carcinogens, mutagens, and diabetes (Patel and Mishra, 2011). The potential effects of these compounds as analgesic and inhibitor of human immunodeficiency virus (HIV) infection had been also reported (dos Santos *et al.*, 2005; Nakajima *et al.*, 2007).

On the other hand, the flavonos subclass seems to be represented mainly by two compounds (luteolin (3) (10.8%), 7-*O*-methyl 8-hydroxyapigenin (5) (3.3%)), also one compound was identified as flavonols (3,7,-*O*-dimethyl kaempferol (10) (9.1%)) and only one anthocyanin (petunidin-3-*O*-acetyl glucoside (22) (2.7%), this latter was the only anthocyanin contained in MeE of the aerial part of *Ar. campestris* L. from Tunisia (Megdiche-Ksouri *et al.*, 2015) where it identified for the first time. These findings are in accordance with other studies conducted on phenolic rich extracts of *Ar. campestris* L. from various countries (El-Ghazouly and Omar, 1984; Djeridane *et al.*, 2006; Karabegović *et al.*, 2011; Akkari *et al.*, 2014; Sebai *et al.*, 2014; Megdiche-Ksouri *et al.*, 2015).

4.2.3.2 Lamiaceae species

4.2.3.2.1 *Lavandula stoechas* L.

The detected compounds, their retention time, and percentage of ChEs extracted from *L. stoechas* leaves, stems and flowers, resulting from the GC-MS analysis after derivatization were summarized in **table 4.27**. Components are listed in order of their elution on apolar column (HP5-MS).

TABLE 4.27: Chemical composition (%) of *L. stochas* L. chloroform extract.

Peak N°	Rt	Compounds	Leaves%	Stems%	Flowers%
1	10,125	Chlorogenic acid	**17.5**	2.3	-
2	11,287	Apigenin-6,8-di-C-glu	**15**	-	2.3
3	15,287	Quercetin-rha-glu	**9.8**	2.3	-
4	17,616	D-Psicofuranose,pentakis(trimethylsilyl) ether (isomer 2)	2.9	3.6	5.6
5	17,702	Benzo[1,2-b:4,3-b]dithiophene,1-methyl-	3	-	-
6	17,796	Sorbopyranose,1,2,3,4,5-pentakis-*O*-(trimethylsilyl)-, L-	2.9	5.2	1.3
7	18,584	Glucopyranose,pentakis-*O*-trimethylsilyl-	3.5	-	-

Chemical composition (%) of *L. stochas* L. chloroform extract. Continued...

Peak N°	Rt	Compounds	Leaves%	Stems%	Flowers%
8	18,866	*O,O,O*'-Tris-trimethylsilylmalonate	5.5	4.2	**10.3**
9	19,295	Inositol,1,2,3,4,5,6-hexakis-*O*-(trimethylsilyl)-, D-chiro-	5.6	**9.3**	4.5
10	19,473	-β-D-(+)-Mannopyranose,pentakis (trimethylsilyl) ether	4.5	**18.2**	**10.3**
11	20,649	Myo-Inositol,1,2,3,4,5,6-hexakis-*O*-(trimethylsilyl)-	1.5	5.6	4.5
12	24,376	Docosane	2.9	-	-
13	25,586	[4-Bromo-2-(hydrazono-phenyl-methyl)-phenyl]- carbamic acid, ethyl ester	1.3	5.3	-
14	25,895	1,4-Dicaffeoylquinic acid	4.1		**12.3**
15	25,594	Sucrose, octakis(trimethylsilyl) ether	6.4	**10.3**	-
16	26,248	3,4,5-Tricaffeoylquinic acid	**8.1**	-	**14.6**
17	30,258	3,4-Dicaffeoylquinic acid	**17.2**	**25.6**	**29.8**
		Total identified	**99.7%**	**91.9%**	**95.5%**

Rt : Retention time (min)

Of the 17, 12 and 10 detected compounds in leaves, stems and flowers, respectively, five compounds as phenolic acids were detected, one was identified as c-glycosyl flavonoid (quercetin-rha-glu (3)) only in leaves and stems (9.8 and 2.3%, respectively), one present in leaves, stems and flowers is 3,4-dicaffeoylquinic acid (17) (17.2, 25.6 and 29.8%), one present only in leaves and stems is (chlorogenic acid (1) (17.5%), two of theme are only present in leaves and flowers are: 1,4-dicaffeoylquinic acid (14) (4.1 and 12.3% respectively), 3,4,5-tricaffeoylquinic acid (16) (8.1 and 14.6, respectively%), similar phenolic compounds were identified in *A. herba alba* from Tunisia (Younsi *et al.*, 2016).

In spite of the large research number about the chemical profile of *L. stoechas* L., most of these studies were about the EO composition of the plant (Carrasco *et al.*, 2015).

Contrary to our finding, rosmarinic acid has a large distribution among the Lamiaceae family species (Ceylan *et al.*, 2015; Algieri *et al.*, 2016), while we didn't found this compound in ChE. Chlorogenic acid was identified to be among the major phenolics of *L. stoechas* ChE, Farah *et al.* (2008), revealed that this compound and its chemical derivatives were clinically shown to be highly bioavailable.

Furthermore, in a recent study, a significantly high level of chlorogenic acid bioavailability was also observed after *in vitro* digestion of *Hypericum perfoliatum* (Celep *et al.*, 2017).

Flavone glycosides; particularly, apigenin -6,8-di-C-glu was also identified to be among the major phenolics of *L. stoechas* ChE, the same finding is reported for other *L. stoechas* (Upson *et al.*, 2000; Gabrieli and Kokkalou, 2003).

4.2.4 *In vitro* anti-ovarian activity

Generally, in the search for new anticancer agents, the most common study methods are the screening tests against a panel of cancer cell lines. In this study, we used SRB method which is based on the ability of metabolically active cells to convert the purple SRB dye to a spectrophotometrically quantifiable sulforhodamine B product .

The chloroform and methanol extract of 5 species of the genus, *Centaurea*, *Bellis*, *Asteriscus*, *Artemisia* and *Lavandula* (Altogether 18 extracts) were tested for their effects on the proliferation of OVCAR-4, FUOV-1 and COV-362 ovarian cancer cell lines using a cell growth assay. Carboplatin (CBPT) was used as positive control and Human epithelial cell line (HOE) as negative control.

Cytotoxicity was expressed as the concentration of extract inhibiting cell growth by 50% (IC_{50}). The IC_{50} value of the extracts was evaluated to a serial dilution started from 40 μg /ml.

Among the solvent with different polarity, mainly ChEs were found to be active compared with the CBPT. In some cases, MeEs were also effective.

Figure 4.11 A typical plate in resazurin microtitre-plate assay showing the color change due to cytotoxic effect of TPC.

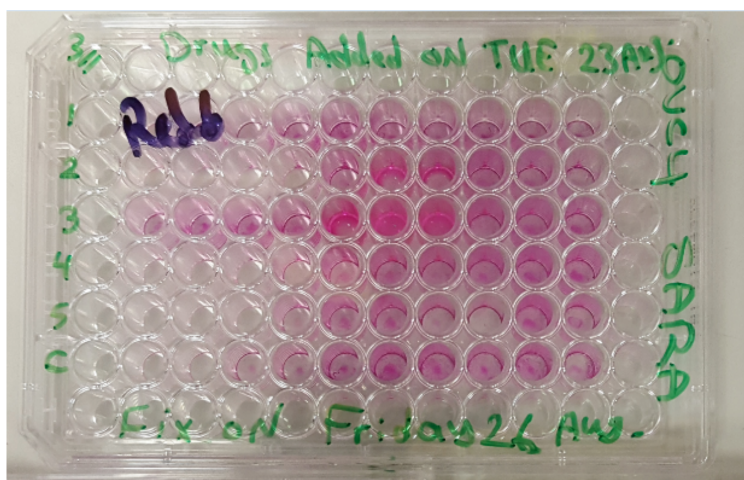

FIGURE 4.11: A typical plate in resazurin microtitre-plate assay

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Extract is usually regarded as interesting for *in vitro* cytotoxic activity if IC_{50} < 100 μg/ml (Sahranavard *et al.*, 2012), therefore no extract results were regarded as non-cytotoxic to each of the cell lines tested. Also, the National Cancer Institute Guidelines specify that extracts with IC_{50} values <20 μg/ml can be regarded as active (Suffness and Pezzuto, 1990). When the present results are considered from this aspect, it may be speculated that many of the tested species are promising sources of new natural products.

The American National Cancer Institute (NCI) claims that compounds having an IC_{50} value that is equal to or less than 4 μg/mL could be potential anti-cancer drugs on cancerous cell lines (Alejandre-García *et al.*, 2015).

4.2.4.1 Asteraceae species

4.2.4.1.1 *Centaurea sphaerocephala* L.

Cytotoxicity of chloroform and methanol extracts of *C. sphaerocephala* L. (leaves, stems and flowers) has been evaluated for their growth inhibitory activity against human ovarian cancer cell lines (OVCAR-4; FOUV-1; COV-362) and normal epithelial cells (HOE) using SRB assay.
Consequently, this assay allows to determine inhibition of cell growth and cytotoxicity.

The extracts showed somewhat greater potencies for the OVCAR-4, FUOV-1 and COV-362 cancer cells compared to normal cells (selectivity index (SI)), (**Table 4.28**).

TABLE 4.28: IC_{50} values (μg/ml) of different cells incubated with different concentrations of *C. sphaerocephala* L. chloroform and methanol extracts.

Drug and Species	**Plant part**	**Solvent**	**Selectivity index (SI)**	**Cytotoxic activity against**			
				OVCAR-4	**FOUV-1**	**COV-365**	**HOE**
CBPT				8.2±1.3	8.4±0.8	6.9 ± 3.2	10.1±1.6
	Leaves	Chloform		11.1±7.2	8.3±4	**22.9±6.9 * #**	8.9 ±2.8
			SI	<1	1.1	<1	
		Methanol		10.2 ± 6.5	11.6±4.5	17±5.5 *	11.7±2.3
			SI	1.1	1	<1	
	stems	Chloform		16.5 ± 8	**38.8±5.3 *#**	16.1±8.6	15.6±2.4
sphaerocephala			SI	<1	<1	<1	
L.		Methanol		15.6±9.6 #	6.6±1.4 #	**60.9±20.5 *#**	56.9±1.1
			SI	3.6	8.6	1.2	
	Flowers	Chloform		6.3±1.6 #	11.1±3.9	**63±19.5 *#**	19.4±4.9
			SI	3.1	1.7	<1	
		Methanol		18.6±6.6 *	8.7±4.6	39.8±10.7 *	21.9±5.4
			SI	1.2	2.5	<1	

* *P* <0.05 compared with CBPT.

\# *P* <0.05 compared with HOE.

Data is represented as mean ± SD (N = 3).

CBPT: Positive control.

Cytotoxicity against OVCAR-4

Table 4.28 shows that, among the different extracts and solvent with different polarity, flowers MeE was significantly found to be active compared with CBPT (*P* <0.05) with IC $_{50}$ value of (18.6 ± 6.6 µg/ml), also flowers ChE and stems MeE were be significantly active compared with HOE (SI = 3.1 and 3.6, respectively) with IC_{50} values of (6.3 ± 1.6 and 15.6 ± 9.6 µg/ml, respectively). In general, ChEs of leaves and stems extracts showed less cytotoxicity compared with flowers. This implies that compounds that are naturally present in flowers are converted into more toxic compounds and raises safety issues about the extracts.

Figure 4.12 shows the mean dose-response curves of *C. sphaerocephala* L.' chloroform extracts of leaves, stems and flowers on OVCAR-4 ovarian cancer cell line.

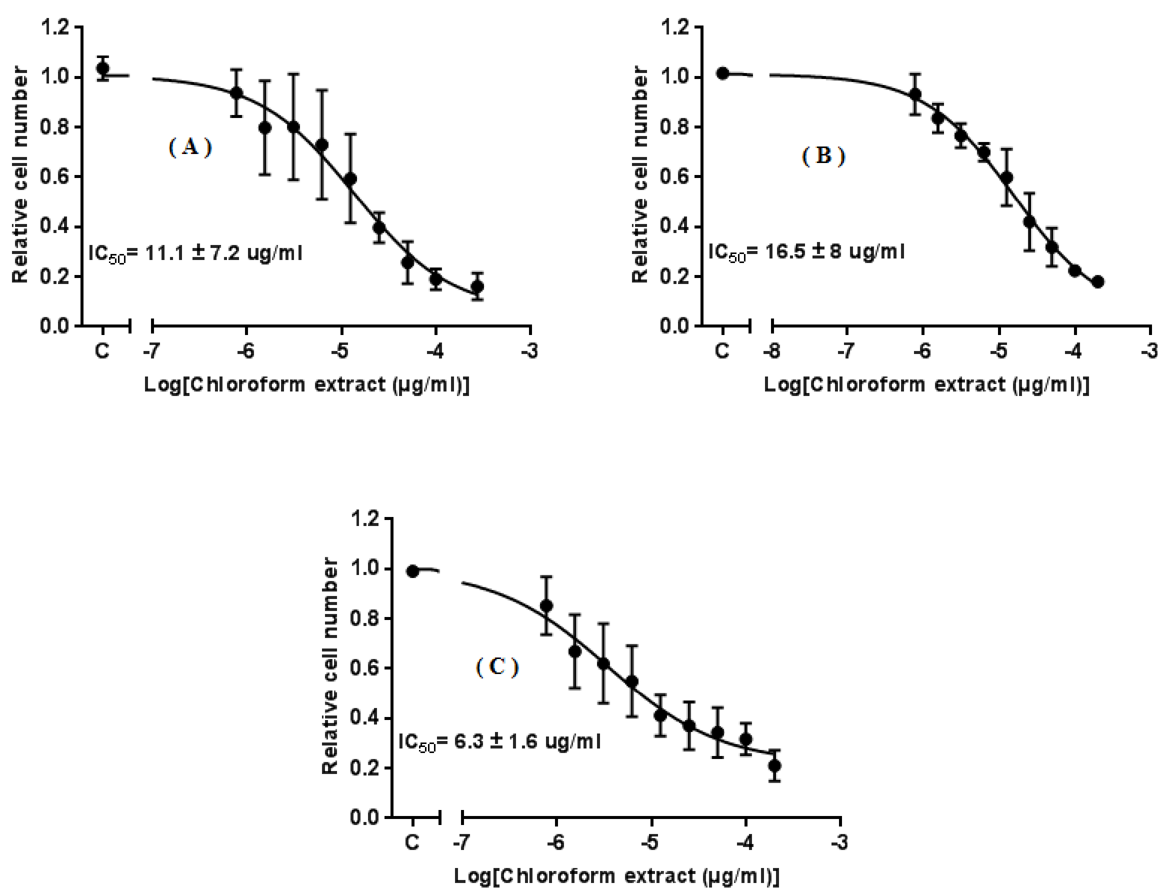

FIGURE 4.12: Mean dose-response curves of *C. sphaerocephala* L.' chloroform extracts of leaves (A) stems (B) and flowers (C) on the OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean $\pm$ SD (n = 3).

Figure 4.13 shows the mean dose-response curves of *C. sphaerocephala* L.' methanol extracts of leaves, stems and flowers on OVCAR-4 ovarian cancer cell line.

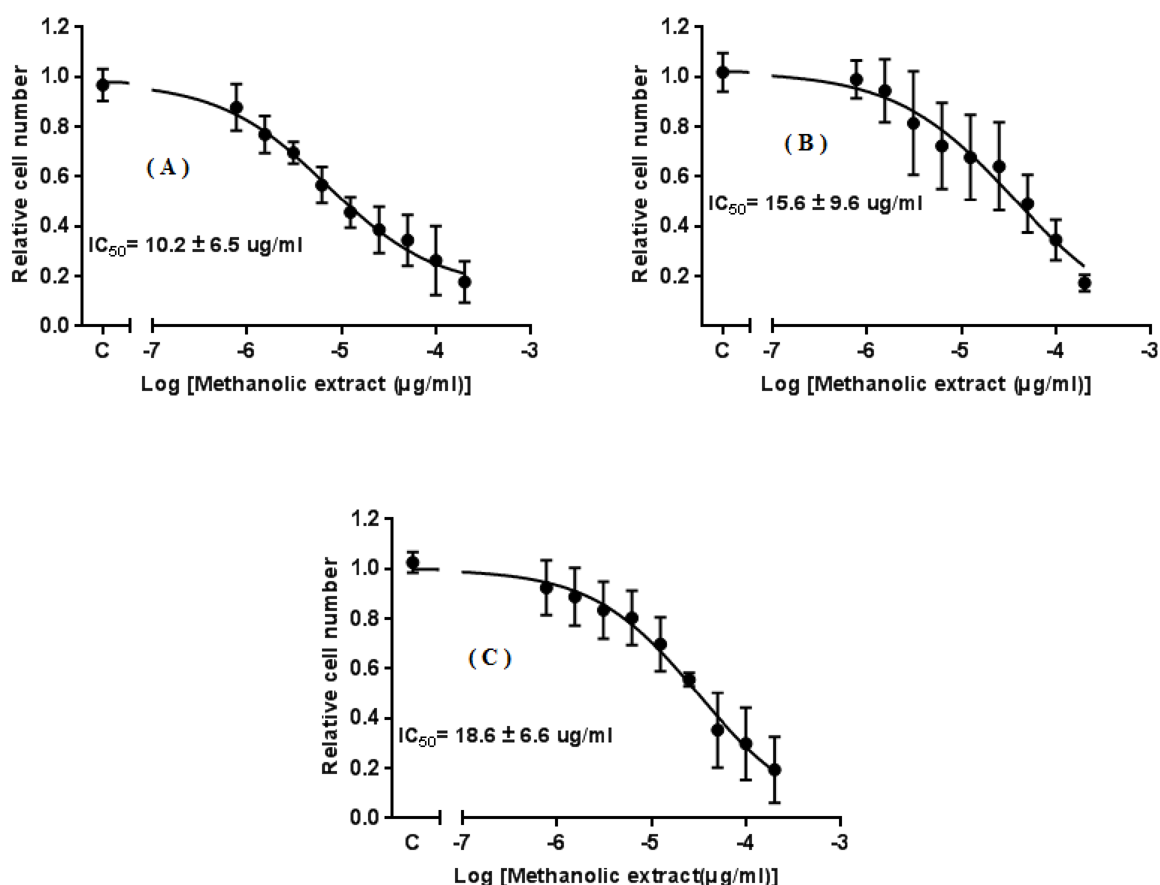

FIGURE 4.13: Mean dose-response curves of *C. sphaerocephala* L.' MeEs of leaves (A) stems (B) and flowers (C) on the OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Cytotoxicity against FOUV-1

Table 4.28 shows that, among the different extracts and solvent with different polarity, stems' ChE was significantly found to be active compared with CBPT (P = 0.0002) and with HOE (P = 0.003) (SI<1) with IC_{50} value of (38.8 ± 5.3 µg/ml), also stems' MeEs was be significantly active compared with HOE (P = 0.006) (SI = 8.6) with IC_{50} value of (6.6 ± 1.4 µg/ml). While the other extracts demonstrated quite cytotoxic activity.

Figure 4.14 shows the mean dose-response curves of *C. sphaerocephala* L.' chloroform extracts of leaves, stems and flowers on FOUV-1 ovarian cancer cell line.

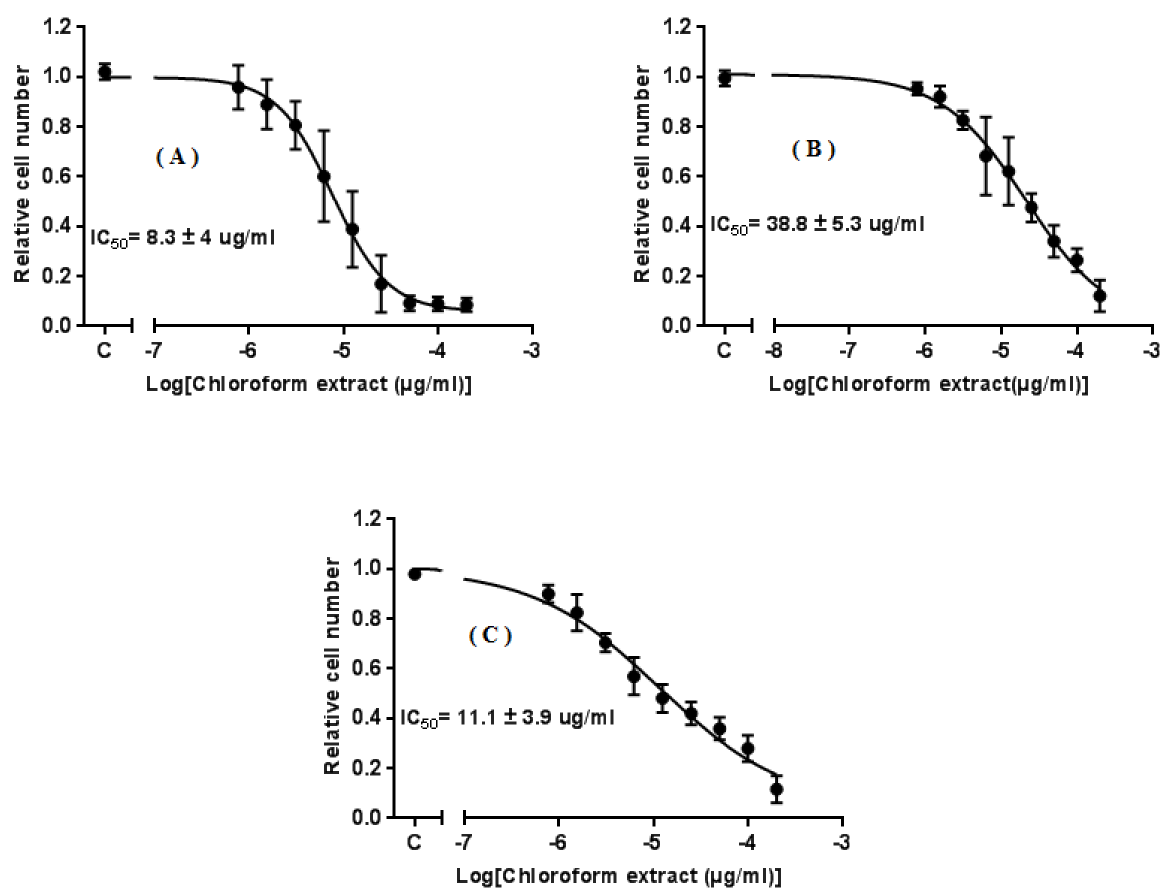

FIGURE 4.14: Mean dose-response curves of *C. sphaerocephala* L.' ChEs of leaves (A) stems (B) and flowers (C) on the FOUV-1 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Figure 4.15 shows the mean dose-response curves of *C. sphaerocephala* L.' methanol extracts of leaves, stems and flowers on FOUV-1 ovarian cancer cell line.

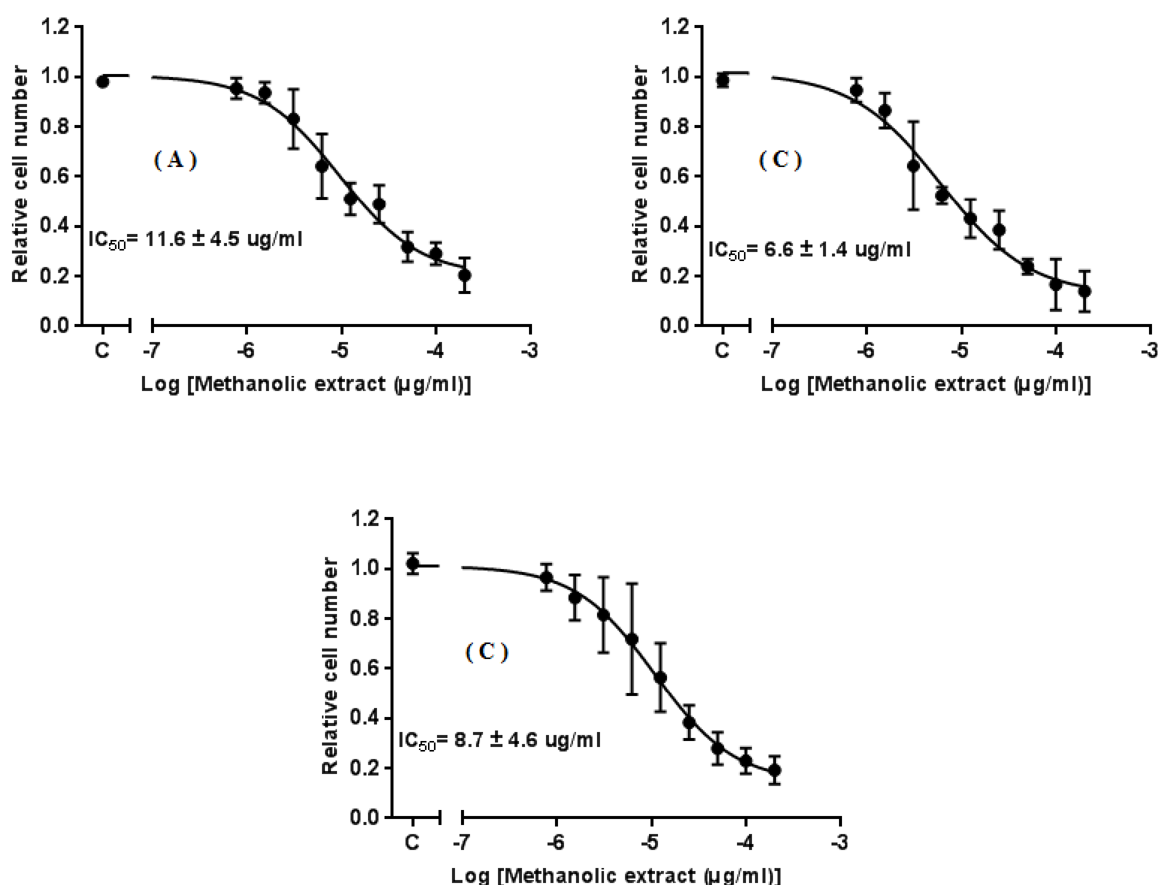

FIGURE 4.15: Mean dose-response curves of *C. sphaerocephala* MeEs of leaves (A) stems (B) and flowers (C) on the FOUV-1 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Cytotoxicity against COV-362

Table 4.28 shows that, among the different extracts and solvent with different polarity, all extracts were significantly found to be active compared with CBPT (P <0.05) except the ChEs of stems with IC_{50} value of (16.1 ± 5.5 µg/ml), while ChE of leaves and flowers (SI <1, for both) and Stems' MeEs(SI = 1.2) were be significantly active compared with HOE with IC_{50} value of (22.9 ± 6.9, 60.9 ± 20.5 and 63 ± 19.5 µg/ml, respectively).

In general, Stems' MeEs showed more cytotoxicity compared with the other organs (**table 4.28** and **figure 4.16**).

These results also show that *C. sphaerocephala* has cytotoxic activity against the three different studied cancer cell lines.

Results demonstrated in **table 4.28** clearly show that ChEs of leaves and flowers also MeE of stems showed somewhat greater potencies for the COV-362 cancer cells compared to normal cells (selectivity index (SI)) as well as CBPT, and this later was the most cytotoxic across the board.

Figure 4.16 shows the mean dose-response curves of *C. sphaerocephala* L.' chloroform extracts of leaves, stems and flowers on COV-362 ovarian cancer cell line.

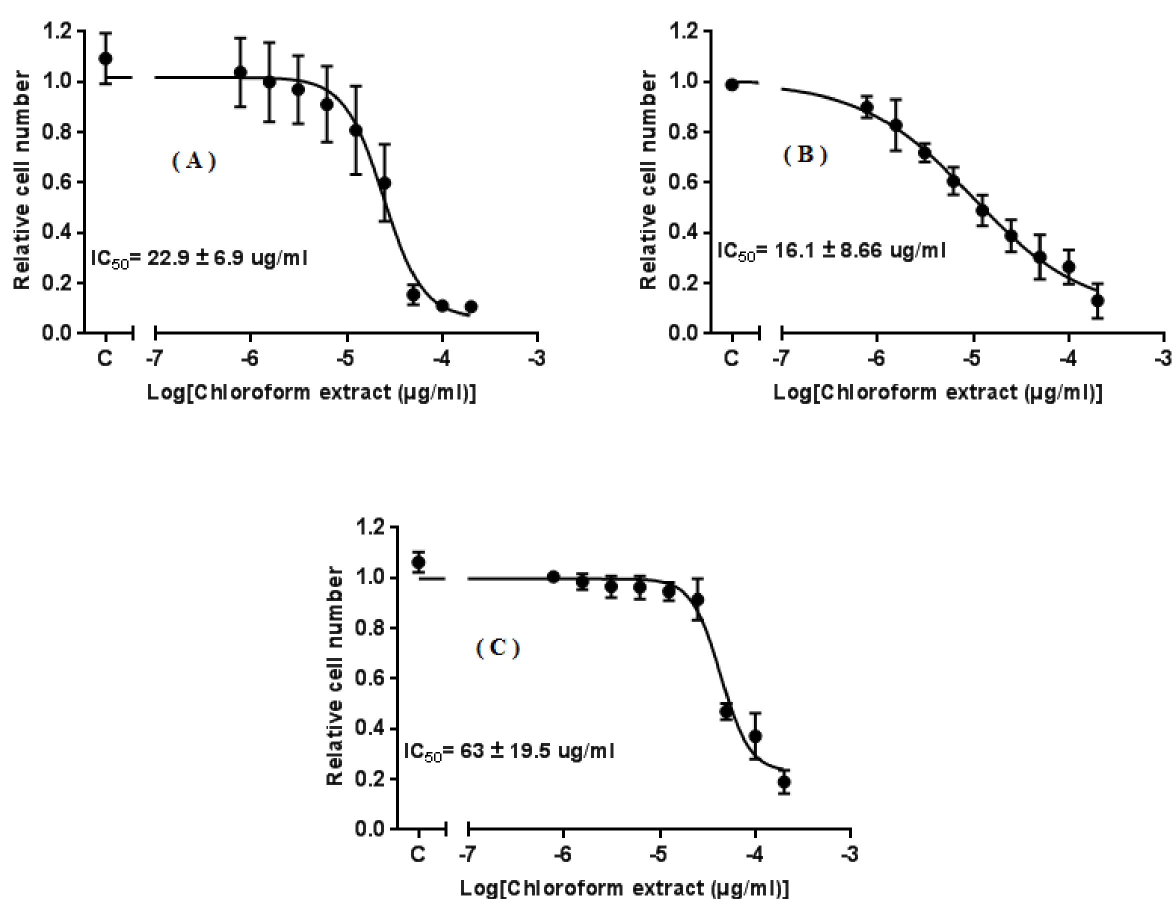

FIGURE 4.16: Mean dose-response curves of *C. sphaerocephala* L.' ChEs of leaves (A) stems (B) and flowers (C) on the COV-362 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Figure 4.17 shows the mean dose-response curves of *C. sphaerocephala* L.' methanol extracts of leaves, stems and flowers on COV-362 ovarian cancer cell line.

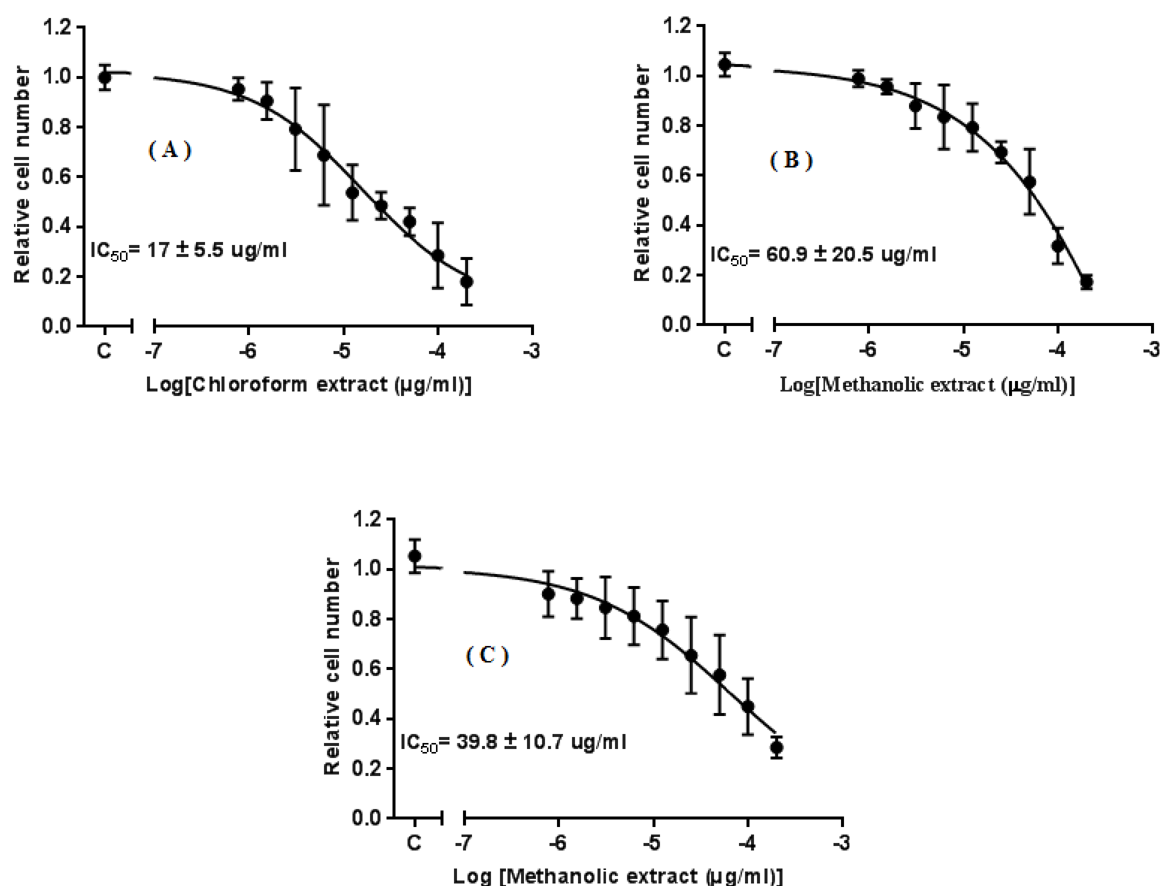

FIGURE 4.17: Mean dose-response curves of *C. sphaerocephala* L.' ChEs of leaves (A) stems (B) and flowers (C) on the COV-362 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

To the best of our knowledge, this is the first report on the assessment of cytotoxicity of *C. sphaerocephala* L.' leaves, flowers and stems extracts against OVCAR-4; FOUV-1 and COV-362 cell lines.

Most ChEs and MeEs of *C. sphaerocephala* exhibited a cytotoxic activity against the three ovarian cell lines, and the OVCAR-4 cells were slightly more sensitive than the other two cell lines, as demonstrated by the IC_{50} values.

Similarly, the crude extracts and isolated compounds of some *Centaurea* sp. found to exhibit of cytotoxic activity (Medjroubi *et al.*, 2005; Shoeb *et al.*, 2007b; El-Najjar *et al.*, 2008; Seghiri *et al.*, 2009) against several cancer cell lines.

As far as our literature survey could ascertain, little is known about the antiovarian cancer activity of *Centaurea* species, Erol-Dayi *et al.* (2011) tested the methanolic and aqueous extracts of *C. calcitrapa* for their cytotoxic activity on HeLa and Vero cell lines by the MTT assay.

The results indicate that aqueous extracts exhibit very low cytotoxic activity. It was found that MeE caused more inhibition than other MeEs on both cell lines.

However, there are few reports on the anti-colon cancer activity of *Centaurea* species present that chlorogenic acid isolated from the aerial part MeE of *C. gigantea* exhibited considerable anti-colon cancer activity (IC_{50}=79.0 lM) (Shoeb *et al.*, 2007a).

While none of the extracts (n-Hexane, chloroform and methanol) demonstrated any significant cytotoxicity against the CaCo-2 colon cancer cell line (IC_{50} = >1000 μg/ mL), the isolated lignans (matairesinoside and arctiin) isolated from *C. urvillei* subs. Armata and *C. mucronifera* showed considerable cytotoxicity against CaCo-2 colon cell line, while none of the MeEs of these species demonstrated any significant cytotoxicity against this cell line (IC_{50} = 220 and 288 μM) (Shoeb *et al.*, 2007b).

Our results showed that the highest activity was demonstrated by MeEs of the plants parts, in contrast, Forgo *et al.* (2012) in an *vitro* screening of *C. jacea* on the proliferation of cultured human tumour cell lines (HeLa, MCF7 and A431) detected earlier an extremely high cytotoxicity of ChE. However, further studies should be carried out to enlighten the mechanism of antiproliferative activity of both MCF-7 and PANC-1 cells. These findings are consistent with literature data about the importance of *Centaurea* species as cytotoxic agents against ovarian cell lines.

These results also show that *C. sphaerocephala* has cytotoxic activities against three different cancer cell lines; this is maybe to the presence of sesquiterpenes which are regarded as one of the major classes of natural compounds with a large spectrum of biological activities including cytotoxic activity (Bork *et al.*, 1997).

Many polyphenols and flavonoids have been shown to inhibit proliferation and angiogenesis of tumor cells *in vitro* (Senatore *et al.*, 2003) and inhibit carcinogenesis and tumorigenesis in animal experiments (Senatore and Dasanu, 2016; Senatore *et al.*, 2016).

However, ChE and MeE of *Centaurea* species were not analysed in terms of their antiproliferative activities.

Some of our extracts are not active but it has previously been observed that it is not surprising to have active compounds from inactive fractions or extracts as the amounts of active compounds

present in the amounts of fractions or extracts tested can be very small to show any activity (Shoeb *et al.*, 2006).

4.2.4.1.2 *Bellis sylvestris* L.

Cytotoxicity of chloform and methanol extracts of *B. sylvestris* L. has been evaluated for their growth inhibitory activity against human ovarian cancer cell line (OVCAR-4 and FOUV-1) and normal epithelial cells (HOE) using SRB assay. Consequently, this assay allows to determine inhibition of cell growth and cytotoxicity.

The results of the cytotoxic activity were listed in **table 4.29**.

In the preliminary screening, extract prepared with chloroform (A) and methanol (B) from *B. sylvestris* stems were investigated for their cytotoxic effect.

TABLE 4.29: IC_{50} values (μg/ml) of different cells incubated with different concentrations of *B. sylvestris* L. chloroform and methanol extracts.

Drug and Species	Plant part	Solvent	Selectivity index (SI)	Cytotoxic activity against		
				OVCAR-4	FOUV-1	HOE
CBPT				8.2±1.3	8.4±0.8	10.1±1.6
B. sylvestris L.	**Stems**	Chloform		58.4±19.8*	14.5±8#	86.5±12.6
			SI	1.5	6	
		Methanol		16.4±10.5	**14.8±2.6*#**	67.3±7.1
			SI	4.1	4.5	

[*] $P < 0.05$ compared with CBPT.

[#] $P < 0.05$ compared with HOE.

Data is represented as mean ± SD (N = 3).

CBPT: Positive control.

Cytotoxicity against OVCAR-4

Table 4.29 shows that, among the different extracts and solvent with different polarity, stems ChE of *B. sylvestris* was significantly found to be active on OVCAR-4 cells compared with CBPT (*P* = 0.004) with IC_{50} value of (58.4 ± 19.8 μg/ml), while MhE show slight effects (*P* = 0.24) with IC_{50} values (16.4 ± 10.5 μg/ml).

Morever, **table 4.29** shows that both ChE and MeE were be significantly not active compared with HOE (SI = 1.5 and 4.1, respectively) (*P* = 0.06 and 0.29, respectively).

Figure 4.18 shows the mean dose-response curves of *B. sylvestris* L.' stems, chloroform (A) and methanol extracts on the OVCAR-4 ovarian cancer cell line.

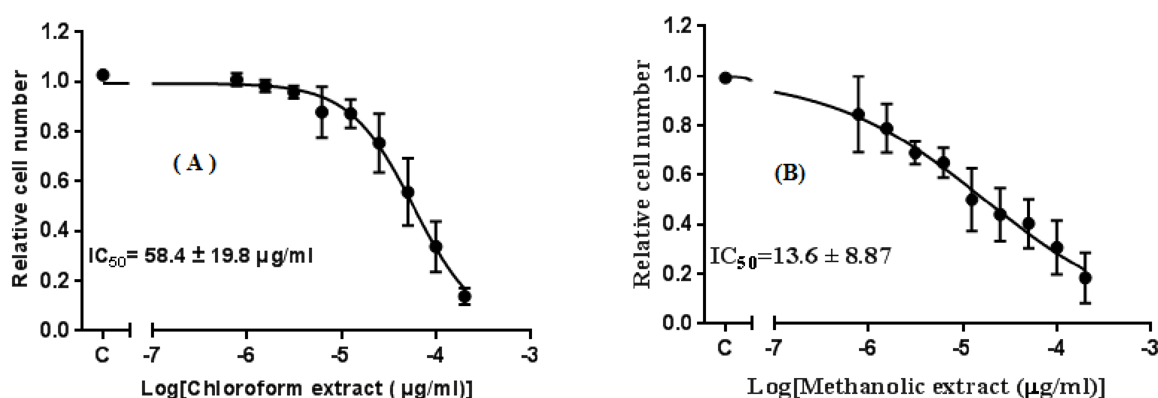

FIGURE 4.18: Mean dose-response curves of *B. sylvestris* L.' stems, chloroform (A) and methanol extracts on the OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean $\pm$ SD (n = 3).

Cytotoxicity against FOUV-1

Table 4.29 shows that MeE was significantly found to be active compared with CBPT (*P* = 0.02) with IC_{50} values of (14.8 ± 2.6 μg/ml), while ChE was not active (*P* = 0.25) with IC_{50} values of (14.5 ± 8 μg/ml). Moreover, both (ChE and MeE) were found to be significantly active compared with HOE (SI = 6 and 4.5, respectively) with (*P* = 0.0002 and 0.01, respectively).

In general, MeEs studied showed more cytotoxic activity compared with ChEs **Table 4.29**.

This implies that compounds that are naturally present are converted into more toxic compounds and raises safety issues about the extracts.

Stems' MeE is active against both FOUV-1 also HOE, these effects are selective and non-toxic for normal cells, it may be useful in the development of novel drugs to combat ovarian cancer.

Figure 4.19 show the mean dose-response curves of *B. sylvestris* L.' stems, chloroform (A) and methanol extracts on the FOUV-1 ovarian cancer cell line.

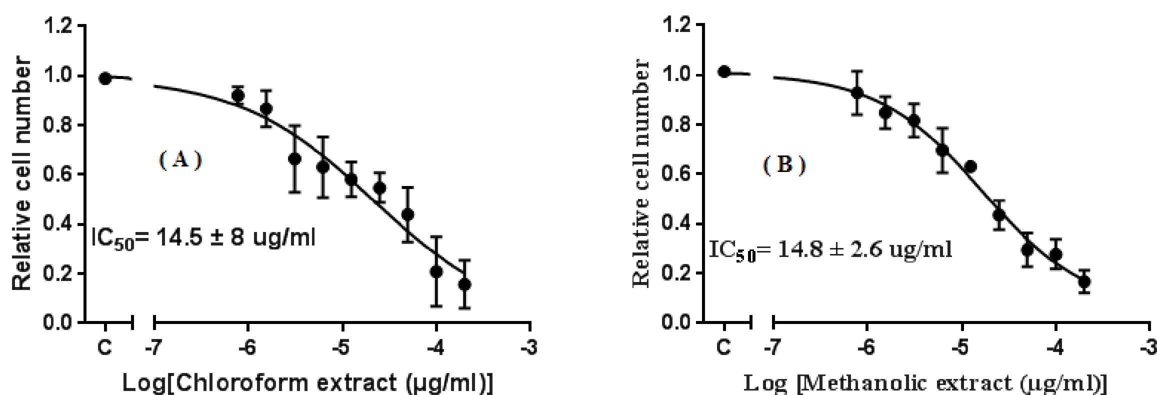

FIGURE 4.19: Mean dose-response curves of *B. sylvestris* L.' stems, Chloroform (A) and Methanol (B) extracts on the FOUV-1 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

In general, MeE of stems was the most cytotoxic across the board against FOUV-1, ChE also resulted a cytotoxicity in the OVCAR-4 cell line.

For comparison, the IC_{50} in cell growth assays using normal human ovarian epithelial (HOE) cells we found that all extract are active only against FOUV-1.

Stems' MeEs and flowers are active against both OVCAR-4 and HOE, these effects are selective and non-toxic for normal cells, they may be useful in the development of novel drugs to combat ovarian cancer.

Literature reports that there is no study that has been done on the cytotoxic activity of of *B. sylvestris* phenolic compounds, while other biological activities of this species have been studied, Scognamiglio *et al.* (2012) have been evaluated the phytotoxic activity of the methanol extract of *B. sylvestris* leaves against *Aegilops geniculata* Roth., and revealed that all the compounds, at the highest concentrations, showed strong phytotoxicity against the leaf development.

Scognamiglio *et al.* (2016), tested the compounds isolated from *B. sylvestris* for their antimicrobial activity against some microorganisms associated with urinary tract infections (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus aureus* and *Candida albicans*) and revealed that the

bacterial strains showed variable degrees of susceptibility to the compounds. Selected compounds were evaluated for their anti-biofilm properties against *Pseudomonas aeruginosa* and *Candida albicans*. Caffeic and rosmarinic acids were the once showing a higher reduction rate of biofilm formation.

As far as our literature survey could ascertain, little is known about the biological avtivities of the species *B. sylvestris*, Ninomiya *et al.* (2016) revealed that perennisaponins A–M (8–20) isolated from *B. perennis* flowers methanol extract, exhibited anti-proliferative activities against human digestive tract carcinoma HSC-2, HSC-4, and MKN-45 cells. Among them, perennisaponin (IC_{50} = 11.2, 14.3, and 6.9 lM, respectively) showed relatively strong activities.

Karakaş *et al.* (2011) demondtrated that *B. perennis* may produce biphasic effects on both anxiety-like behaviour and learning performance of the rats.

Prabhakar *et al.* (2014) tested the anticancer activity of the methanol extract of chalcone derivatives against the A2780 ovarian cancer cell line and it was observed that the tested compounds were effective.

4.2.4.1.3 ***Asteriscus maritimus* (L.) Less.**

Cytotoxicity of chloform and methanol extracts of *A. maritimus* (L.) Less. has been evaluated for their growth inhibitory activity against human ovarian cancer cell line (OVCAR-4 and FOUV-1) and normal epithelial cells (HOE) using SRB assay. Consequently, this assay allows to determine inhibition of cell growth and cytotoxicity.

The results of cytotoxic activity were listed in **table 4.30**.

In the preliminary screening, extract prepared with chloroform (A) and methanol (B) from *A. maritimus* stems were investigated for their cytotoxic effect.

Table 4.30: IC_{50} values (μg/ml) of different cells incubated with different concentrations of *A. maritimus* (L.) Less. chloroform and methanol extracts.

Drug and Species	Plant part	Solvent	Selectivity index (SI)	Cytotoxic activity against		
				OVCAR-4	**FOUV-1**	**HOE**
CBPT				8.2±1.3	8.4±0.8	10.1±1.6
A. maritimus (L.) Less.	**Stems**	Chloform		35.6±8.4	9.5 ±4.2#	35.5±12.6
			SI	<1	4.5	
		Methanol		56.9±13.9	40.9±11.8*	40.3±7.1
			SI	<1	<1	

[*] *P* <0.05 compared with CBPT.

[#] *P* <0.05 compared with HOE.

Data is represented as mean ± SD (N = 3).

CBPT: Positive control.

Cytotoxicity against OVCAR-4

Table 4.30 shows that both ChE and MeE found to be significantly not active compared with CBPT (P = 0.102 and 3.336, respectively) with IC_{50} values of (35.6 ± 8.4 and 56.9 ± 13.9 μg/ml). Moreover, they were both (ChE and MeE) found to be significantly not active compared with HOE (SI = <1, for both) (P = 0.76 and 0.976, respectively).

Figure 4.20 shows the mean dose-response curves of *A. maritimus* stems chloroform extract (A) and methanol (B) on the OVCAR-4 ovarian cancer cell line.

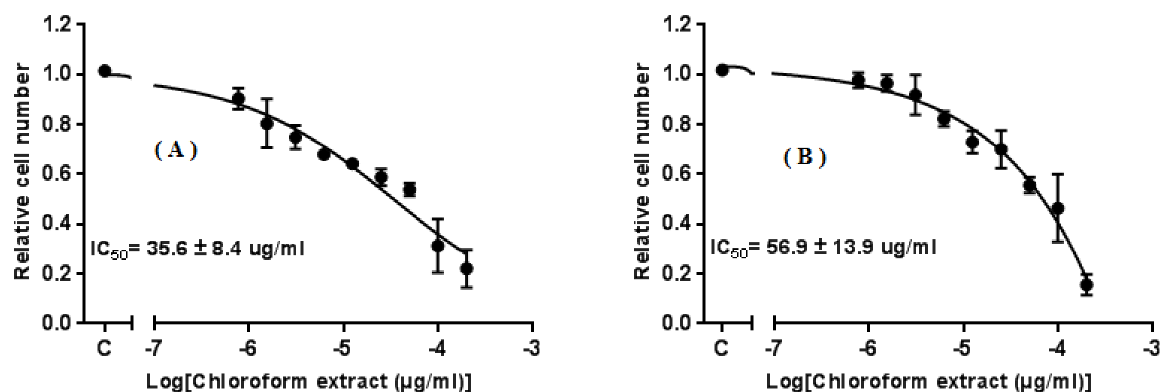

FIGURE 4.20: Mean dose-response curves of *A. maritimus* stems chloroform (A) and methanol (B) extract on the OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Cytotoxicity against FOUV-1

Table 4.30 shows that MeE was found to be significantly active compared with CBPT (*P* = 0.008) with IC_{50} values of (40.9 ± 11.8 μg/ml), while ChE was found to be significantly not active (*P* = 0.63) (9.5 ± 4.2) Moreover, ChE was found to be significantly active compared with HOE (SI = 3.7) (*P* = 0.037), while MeE was found to be active (SI <1) (*P* = 0.247).

Figure 4.21 show the mean dose-response curves of *A. maritimus* stems chloroform extract (A) and methanol (B) on the FOUV-1 ovarian cancer cell line.

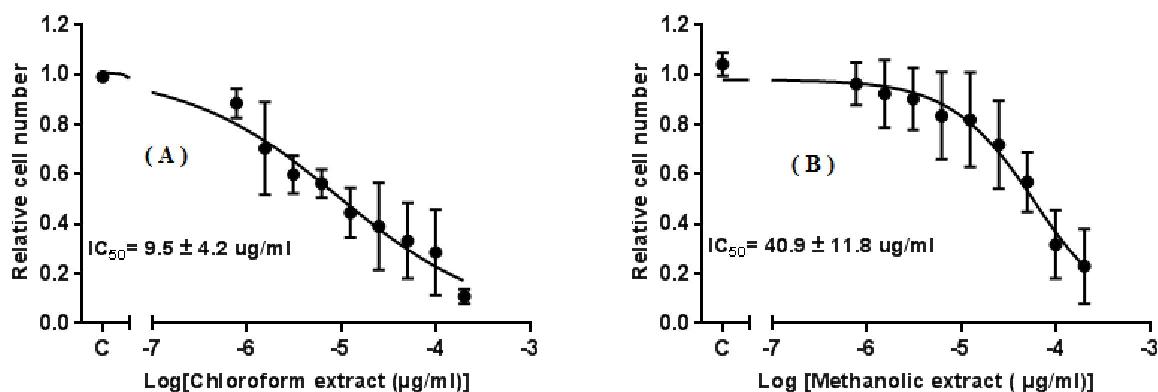

FIGURE 4.21: Mean dose-response curves of *A. maritimus* stems chloroform (A) and methanol (B) extract on the FOUV-1 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

The results of the cytotoxicity assay exhibit an interesting trend across all the cell lines. Almost MeE were found to be active only on OVCAR-4 cell line. While ChE exhibited significant activity on FOUV-1 cell line, and did not exhibit an activity on OVCAR-4 cell line comparable to CBPT. The same trend was found in the other higher dilutions also.

While there are many studies demonstrate that the antitumor action of *A. maritimis* extracts, it is difficult to find literature about its possible anticancer against ovarian cancer cell lines.

ChE of *A. maritimus* contained hight concentration of chlorogenic acid, quercetin-rha-glu, luteolin, 3,4-dicaffeoylquinic acid and 1,4-dicaffeoylquinic acid, which have been described by their strong anti-Alzheimer's disease (Orhan *et al.*, 2007; Choi and Choi, 2014), antioxydant and antiacetyl-cholinesterase activity (Younsi *et al.*, 2016). So, these compounds might be responsible also for all or part of the cytotoxic activity.

4.2.4.1.4 *Artemisia campestris* L.

Cytotoxicity of chloform and methanol extracts of *Ar. campestris* L. has been evaluated for their growth inhibitory activity against human ovarian cancer cell line (OVCAR-4 and FOUV-1) and normal epithelial cells (HOE) using SRB assay. Consequently, this assay allows to determine

inhibition of cell growth and cytotoxicity.

MeEs of *Ar. campestris* L.' stems, showed somewhat greater potencies for the OVCAR-4 and FOUV-1 cancer cells **(table 4.31)**.

TABLE 4.31: IC_{50} values (μg/ml) of different cells incubated with different concentrations of *Ar. campestris* L. chloroform and methanol extracts.

Drug and Species	Plant part	Solvent	Selectivity index (SI)	Cytotoxic activity against		
				OVCAR-4	FOUV-1	HOE
CBPT				8.2±1.3	8.4±0.8	10.1±1.6
***Artemisia* campestris L.**	**Stems**	Chloform		58.2±8.9 #	43.5±11.7 #	86.5±12.6
			SI	1.5	2	
		Methanol		**36.5±7.2 * #**	**19.7±1.7*[1] #[2]**	67.3±7.1
			SI	1.8	3.4	

* P <0.05 compared with CBPT.

\# P <0.05 compared with HOE.

Data is represented as mean ± SD (N = 3).

CBPT: Positive control.

Cytotoxicity against OVCAR-4

Table 4.31 shows that MeE was found to be significantly active compared with CBPT (P = 0.005) with IC_{50} values of (36.5 ± 19.2 μg/ml), while ChE was found to be significantly not active (P = 0.23) (58.2 ± 8.9).

Moreover, both ChE and MeE were found to be significantly active compared with HOE (SI = 1.5 and 1.8, respectively) (P = 0.033 and 0.021).

Cytotoxicity against FOUV-1

Table 4.31 shows that MeE was found to be significantly active compared with CBPT (P = 0.05) with IC_{50} values of (19.7 ± 1.7 μg/ml), while ChE was found to be significantly not active (P = 0.43) (43.5 ± 11.7).

Moreover, both ChE and MeE were found to be significantly active compared with HOE (SI = 2 and 3.4, respectively) (P = 0.0001 and 0.001).

Figure 4.22 shows the mean dose-response curves of *Ar. campestris* stems chloroform (A) and methanol (B) extracts on the OVCAR-4 ovarian cancer cell line.

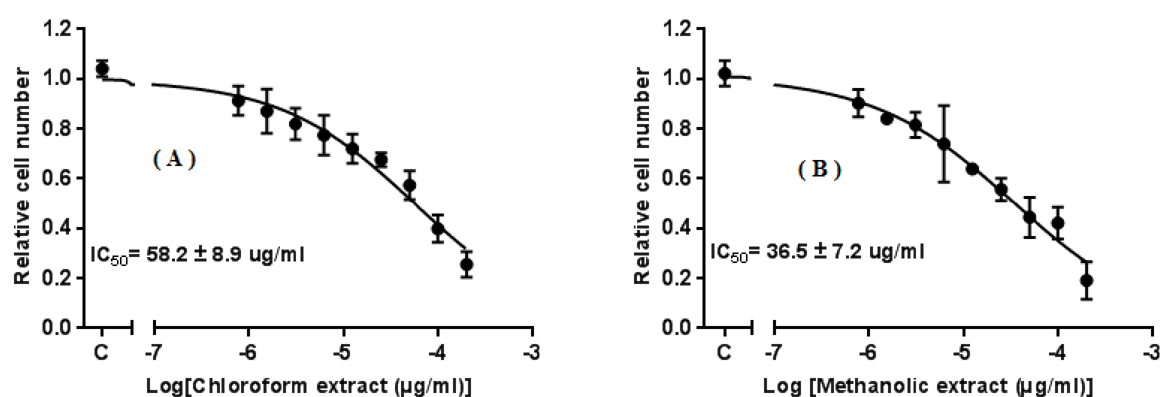

FIGURE 4.22: Mean dose-response curves of *Ar. campestris* L.' stems chloroform (A) and methanol (B) extracts on OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Table 4.31 shows that MeE was found to be significantly active compared with CBPT (P = 0.005) with IC_{50} values of (36.5 ± 19.2 μg/ml), while ChE was found to be significantly not active (P = 0.23) (58.2 ± 8.9).

Figure 4.23 shows the mean dose-response curves of *Ar. campestris* L.' stems chloroform (A) and methanol (B) extracts on FOUV-1 ovarian cancer cell line.

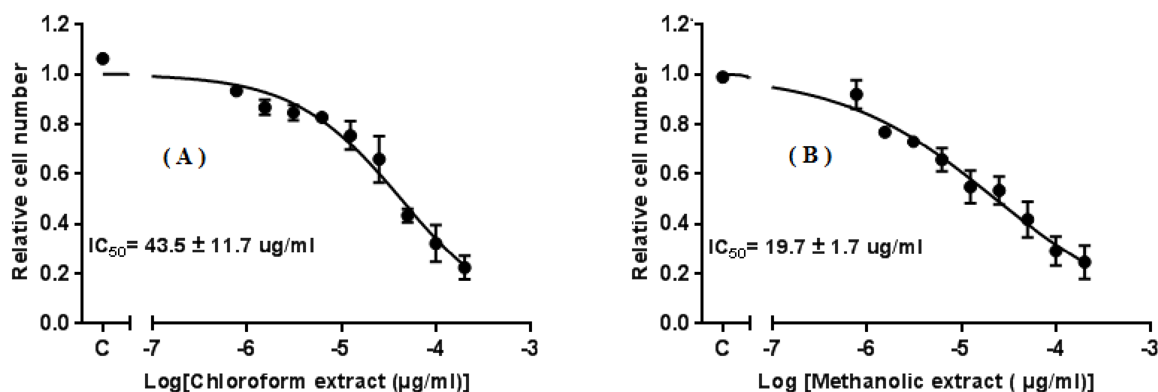

Figure 4.23: Mean dose-response curves of *Ar. campestris* L.' stems chloroform (A) and methanol (B) extracts on FOUV-1 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean $\pm$ SD (n = 3).

Among the different extracts and solvent with different polarity, MeE of *Ar. campestris* was significantly found to be active compared with CBPT (P<0.05) against the two studied celle lines, while ChE showed slight effects on compared with CBPT (P>0.05), these effects are selective and non-toxic for normal cells, it may be useful in the development of novel drugs to combat ovarian cancer.

While there are many studies demonstrate that the antitumor action of *A. campestris* extracts, it is difficult to find literature about its possible anticancer against ovarian cancer cell lines.

ChE of *A. campestris* contained hight concentration of chlorogenic acid, quercetin-rha-glu, luteolin, 3,4-dicaffeoylquinic acid and 1,4-dicaffeoylquinic acid, which have been described by their strong anti-Alzheimer's disease (Orhan *et al.*, 2007; Choi and Choi, 2014), antioxydant and Antiacetylcholinesterase Activity (Younsi *et al.*, 2016). So, these compounds might be responsible also for all or part of the cytotoxic activity.

Akrout *et al.* (2010) have been evaluated the antitumor activity of some extracts of different polarity (essential-oils, hexane, ethanol–water extract and infusion extract) obtained from two Tunisian herbal plants, *A. campestris* L. and *Thymelaea hirsuta* against HT-29 (human adenocarcinoma cell line), and revealed that all extracts tested except the hexane extract, showed growth inhibitory activity.

In a recent study (Orhan *et al.*, 2010), ethanol extract of *A. herba-alba* had exhibited antiacetylcholinesterase activity against AChE and butyrylcholinesterase enzymes, while acetone extract had been unable to inhibit either enzyme.

4.2.4.2 Lamiaceae species

4.2.4.2.1 *Lavandula stoechas* L.

Cytotoxicity of chloform and methanol extracts of *L. stoechas* L. has been evaluated for their growth inhibitory activity against human ovarian cancer cell line (OVCAR-4) and normal epithelial cells (HOE) using SRB assay.
Consequently, this assay allows to determine inhibition of cell growth and cytotoxicity.

Cytotoxicity against OVCAR-4

Table 4.32 shows that, among the different extracts and solvent with different polarity, stems ChE and MeE were significantly found to be active compared with CBPT (P = 0.02, for both) with IC $_{50}$ values of (34.2 ± 8.4 and 34.7 ± 8 µg/ml), also flowers MeE was significantly active (P = 0.02) with IC $_{50}$ values of (17.8 ± 0.8 µg/ml), while ChE of this organ also the two extracts of leaves were be found to be not active compared with CBPT (P = 0.187, 0.858 and 0.547, respectively) with IC $_{50}$ values of (20.6 ± 13.1, 8.8 ± 4.9 and 7.7 ± 0.8 µg/ml, respectively). Compared with HOE both leaves and flowers, ChE and MeE were found to be active (P <0.05) with (SI = 1.4, 4.1, 1.7 and 3.7, respectively).
In addition, stems MeE was found to be active while ChE was not (SI = 1.2 and <1, respectively).
In general, *L. stoechas* flowers extracts were found to be moderately active, while stems extracts were proved to have marked efficacy, while leaves extracts showed less cytotoxicity **(table 4.32)**. This implies that compounds are naturally present in stemss are converted into more toxic compounds and raises safety issues about the extracts.
In general, stems was the most cytotoxic across the board against OVCAR-4, flowers also resulted a cytotoxicity in the OVCAR-4 cell line. In contrast leaves showed low-level of cytotoxicity.
For comparison, the IC_{50} in cell growth assays using normal human ovarian epithelial (HOE) cells we found that all extract are active ecxept ChE of stems.
Stems' MeEsand flowers are active against both OVCAR-4 and HOE, these effects are selective and

non-toxic for normal cells, they may be useful in the development of novel drugs to combat ovarian cancer.

TABLE 4.32: IC_{50} values (μg/ml) of different cells incubated with different concentrations of *L. stoechas* L. chloroform and methanol extracts.

Drug and Species	Plant parts	Solvent	Selectivity index (SI)	Cytotoxic activity against OVCAR-4	HOE
CBPT				8.2±1.3	10.1±1.6
L. stoechas L.	**Leaves**	Chloform		8.8±4.9#	10±4.7
			SI	1.1	
		Methanol		7.7±0.8#	31.9±6.7
			SI	4.1	
	Stems	Chloform		34.2±8.4*	16.2±0.6
			SI	<1	
		Methanol		**34.7±8*#**	39.3±1.8
			SI	1.1	
	Flowers	Chloform		20.6±13.1#	20.1±3.1
			SI	1.7	
		Methanol		**17.8±0.8*#**	65.1±10
			SI	3.7	

[*] *P* <0.05 compared with CBPT.

[#] *P* <0.05 compared with HOE.

Data is represented as mean ± SD (N = 3).

CBPT: Positive control.

Figure 4.24 shows the mean dose-response curves of *L. stoechas* L.' ChEs of leaves (A) flowers (B) and stems (C) on the OVCAR-4 ovarian cancer cell line.

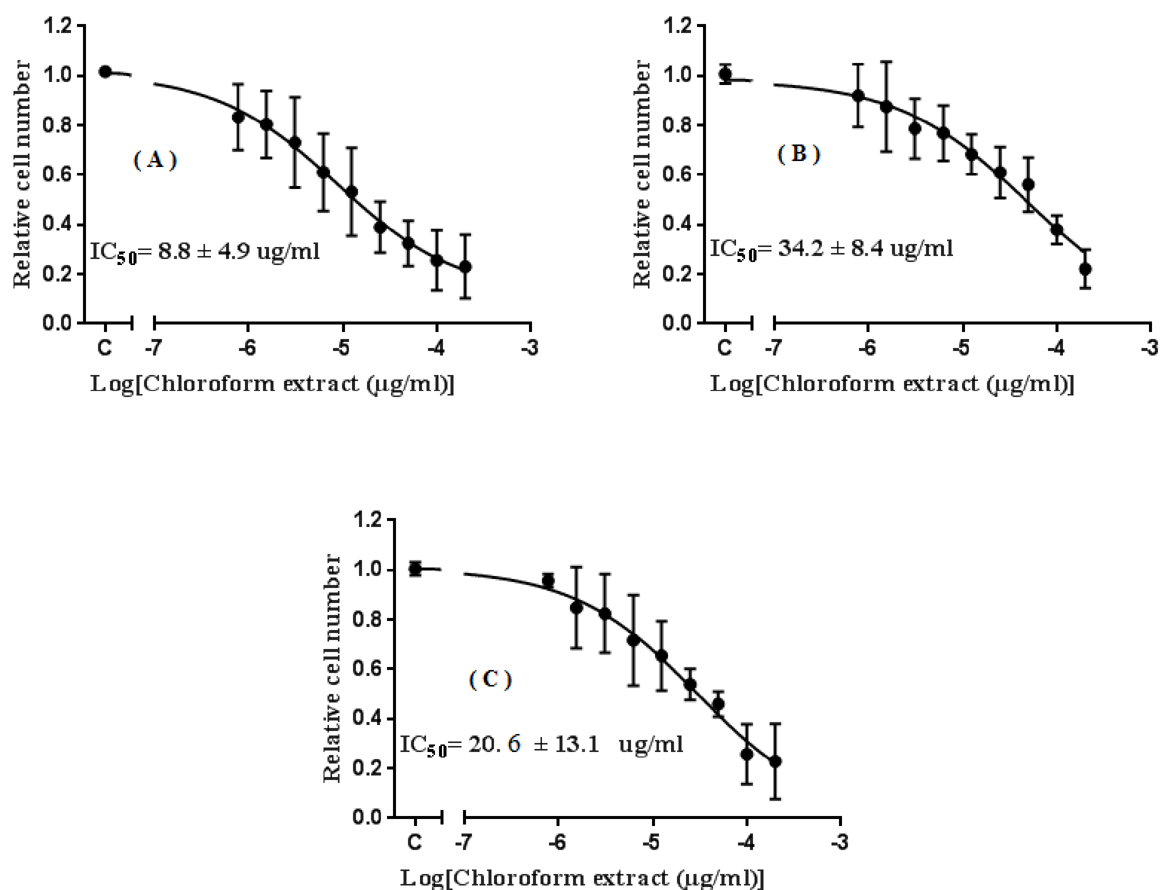

FIGURE 4.24: Mean dose-response curves of *L. stoechas* L.' ChEs of leaves (A) flowers (B) and stems (C) on OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean $\pm$ SD (n = 3).

Figure 4.25 shows the mean dose-response curves of *L. stoechas* L.' MeEs of leaves (A) flowers (B) and stems (C) on the OVCAR-4 ovarian cancer cell line.

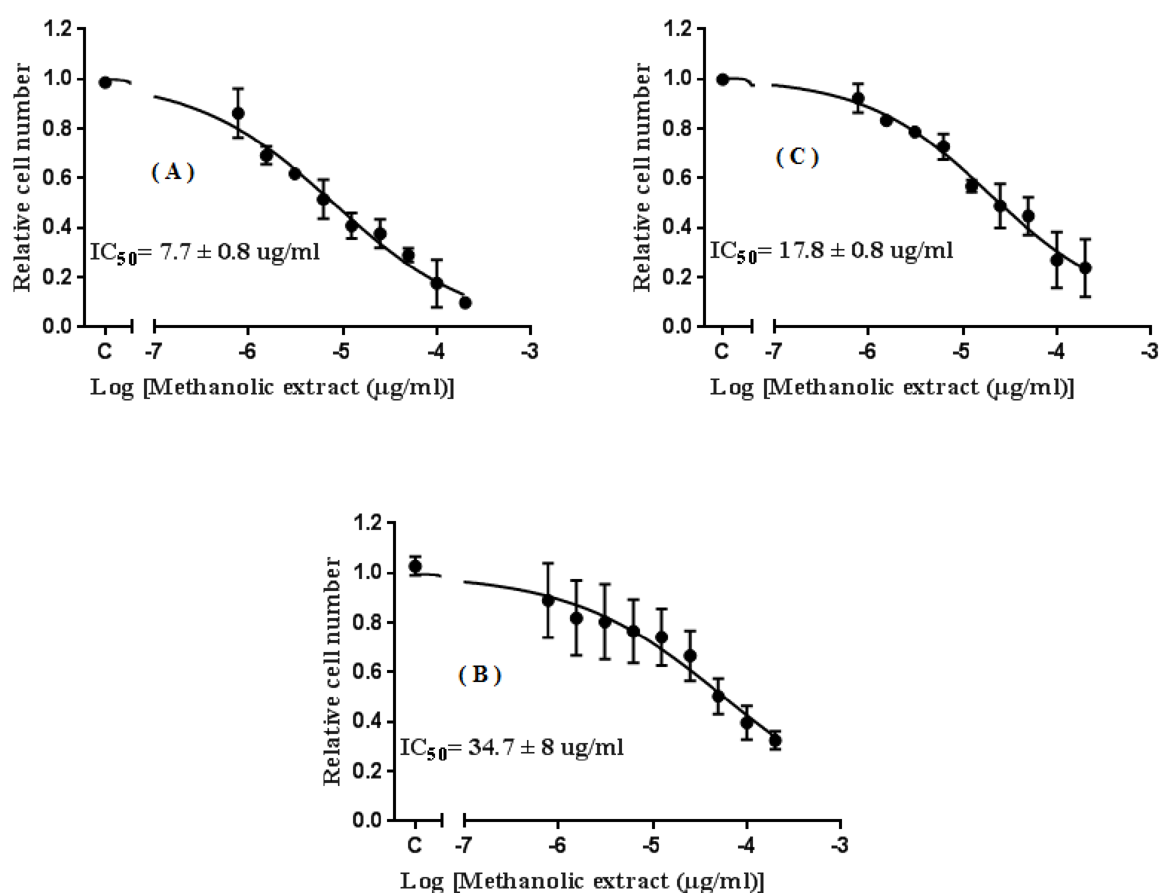

FIGURE 4.25: Mean dose-response curves of *L. stoechas* L.' MeEs of leaves (A) flowers (B) and stems (C) on the OVCAR-4 ovarian cancer cell line. The mean values of their IC_{50} are expressed as mean ± SD (n = 3).

Results of a recent study pointed out the *in vitro* immunomodulatory and anti-inflammatory properties of the hydroalcoholic extract of the aerial parts of *L. stoechas*, the authors attributed these effects to the polyphenolic content (Algieri *et al.*, 2016). Costa *et al.* (2014) and Celep *et al.* (2018) revealed a slight trend of gastric effect on rosmarinic acid in *L. viridis* extracts. However, the results of another study pointed out substantial decrease in rosmarinic acid content of sage and savory after digestion (Gião *et al.*, 2012). So, these compound might be responsible for all or part of the cytotoxic activity of this species. Falé *et al.* (2013) studing on Caco-2 cells revealed that the increase in the concentration of luteolin and apigenin induced a similar increase in the bioavailability of rosmarinic acid. These results can be interpreted as the interactions with coexistent components in a plant matrix might directly affected the bioavailability of compounds.

Conclusion and Perspectives

Conclusion and perspectives

In spite of the large research number about the chemical profile of medicinal plants, most of these studies were about the essential oil (EO) and total phenolic composition (TPC) of the aerial parts of these plants. However our study was conduct to occur the yields and the chemical profile of different organs (leaves, stems and flowers) separately, of some Asteraceae and Lamiaceae species. Additionally, phenolic compounds are well known for their positive effects on human health as well as their role in decreasing the incidence of serious chronic diseases such as cancer, diabetes, Alzheimer's, etc. Therefore, the purpose of the current study was to assess the potential anticancer of the total phenolic compounds present in the different parts of each single species (chloroform and methanol extracts) as well as clarification the chemical profile of the essential oil extracted from each species organ.

The yield and the chemical composition of EOs and TPC extracts of five Algerian species belonging Asteraceae and Lamiaceae families have been conducted.

The EO yield results of Asteraceae species showed that leaves and flowers of all studied species recorded significantly (p <0.05) the best yields in terms of EO with values of (0.22 ± 0.26 and 0.20 ± 0.22, respectively), compared by stems which recorded significantly the lowest yield (0.07 ± 0.06), whereas, *C. sphaerocephala* and *B. sylvestris* recorded significantly (p <0.0001) the best yields of EO (0.23 ± 0.31 and 0.22 ± 0.23, respectively) compared by *Ar. campestris* (0.18 ± 0.05), while the lowest yield has been registered for *A. maritimus* (0.02 ± 0.02).

Lamiaceae EO results showed that leaves of *L. stoechas* recorded significantly ($p < 0.05$) the best yields in EO (0.14 ± 0.01), compared by flowers and tems which they recorded significantly the lowest yield (0.07 ± 0.02 and 0.01±0.003, respectively) with no significant changes in terms of EO (p = 0.12).

In the best of our knowledge there is no study on the chemical composition of EO of the different organs (leaves, stems and flowers) of all species under investigation. To the best of our knowledge, this is the first report on the chemical composition *C. sphaerocephala* leaves, stems and flowers. Also there is no literature reports that has been done on of *B. sylvestris* stems EO.

Concerning *A. maritimus* and *Ar. campestris* stems there is no any report in the literature on the Algerien species.

According to the existing literature, there are only five studies about only leaves LsEO.

On the basis of the number of compounds, GC-MS analysis results showed the following order : *C. sphaerocephala* (76) >*A. maritimus* (62) >*Ar. campestris* (61) >*B. sylvestris* (52) >*L. stoechas* (51). To the best of our knowledge, this is the first report on TPC yield of *C. sphaerocephala* and *B. sylvestris* stems, also there is no literature reports that has been done on TPC yield of Algerian *A. maritimus* separate organs. On the other hand, there are unavailable data concerning *A. campestris* and *L. stoechas* leaves, stems and flowers separately polyphenols and for the first time it was studied regarding its total polyphenols content using different solvent.

In spite of the large research number about the chemical profile of *L. stoechas* L., most of these studies were about the EO composition of the plant.

In the other hand, TPC of Asteraceae species results showed that leaves of all studied species recorded significantly the best yields in TPC (2.43 ± 1.94), followed by leaves and stems (1.90 ± 1.60 and 1.79 ± 0.82, respectivelt).

Results of *L. stochas* TPC yield showed that flowers recorded significantly ($p < 0.05$) the best yields in TPC (3.47 ± 2.09), followed by leaves (2.50 ± 0.26) while stems required the lowest yield (1.34 ± 1.12) ($p < 0.0001$). The results obtained from the interaction between solvent used and species studied as well as organs showed that MeOH extracts recorded significantly the best yields in TPC compared by $Chcl_3$ extracts (3.09 ± 1.84 and 1.17 ± 0.56, respectively in Asteraceae species) and (3.45 ± 1.47 and 1.43 ± 0.91, respectively in Lamiaceae species).

In the best of our knowledge there is no study on the chemical composition of ChE of the different organes (leaves, stems and flowers) of all species under investigated.

On the basis of the number of compounds, GC-MS analysis after derivatization showed the following order of the number of compounds : *C. sphaerocephala* (42) and *A. maritimus* (42) >*Ar. campestris* (25) >*B. sylvestris* (22) >*L. stoechas* (17).

Generally, in the search for new anticancer agents, the most common study methods are the screening tests against a panel of cancer cell lines. In this study, cytotoxicity of chloroform and methanol extracts has been evaluated for their growth inhibitory activity against human ovarian cancer cell lines (OVCAR-4; FOUV-1; COV-362) and normal epithelial cells (HOE) using SRB method which is based on the ability of metabolically active cells to convert the pale yellow SRB dye to a spectrophotometrically quantifiable purple formazan product.

To the best of our knowledge, this is the first report on the assessment of phenolic compounds cytotoxic activity of all studied species, against ovarian cancer cell lines. Also literature reports

that there is no study that been done on the biological activities of separate organs species.

Compared to normal cells and CBPT, results demonstrated that ChEs of leaves and flowers also MeE of stems of *C. sphaerocephala* L. showed somewhat greater potencies for the COV-362 cancer cells. Moreover, ChE of stems has been found to be significantly active against FOUV-1 cancer cells ($P<0.05$) .

Stems MeE of *B. sylvestris* has been significantly found to be active compared with CBPT and HOE ($P<0.05$) against FOUV-1, while ChE was found to be active against OVCAR-4 compared with CBPT and against FOUV-1 compared with HOE.

No extract of *A. maritimus* have been found to be active against OVCAR-4 and FOUV-1 compared with both CBPT and HOE, while MeE and ChE have been found significantly to be active compared with CBPT and HOE ($P<0.05$) against FOUV-1, respectively.

However, *Ar. campestris* MeE of stems were found to be active against both OVCAR-4 and FOUV-1 compared with CBPT and HOE, while ChE have been found significantly to be active compared with only HOE ($P<0.05$) against OVCAR-4 and FOUV-1.

textitL. stochas MeEs of stems and flowers *L.stoechas* have been found significantly to be active compared with CBPT and HOE. ($P<0.05$) against OVCAR-4.

The results of cytotoxic activity of the phenolic compounds extracts indicated that the extracts contain active compounds. Especially, methanolic extracts. Apparently, there is a strong positive relation between polyphenols content and cytotoxic activity, which suggests that the active compounds on extracts are phenolic compounds.

In conclusion, to our knowledge this is the first detailed report on the secondary metabolites of the separate organs of studied species. Evaluation of cytotoxic activity of TPC in the present study establishes the potential of this extracts as an cytotoxic agent. However, the effect of TPC on the cytotoxic activity targets was found to be associated with the chemical profile of every extract.

These effects are selective and non-toxic for normal cells, they may be useful in the development of novel drugs to combat drug-resistant ovarian cell line.

Some of our extracts are not active but it has previously been observed that it is not surprising to have active compounds from inactive fractions or extracts as the amounts of active compounds present in the amounts of fractions or extracts tested can be very small to show any activity (Shoeb *et al.*, 2006).

These effects are selective and non-toxic for normal cells, it may be useful in the development of

novel drugs to combat ovarian cancer.

The higher level of cytotoxic activity of MeEs and ChEs extracts of *C. sphaerocephala* and MeEs of *B. sylvestris*, *Ar. campestris* and *L. stoechas* suggested that lipophilicity might have an impact on the cytotoxic activity of the species studied (except *L. stoechas*), where the less polar (high lipophilic) compounds showed the strongest cytotoxic effect. However, it is known that lipophilicity is one of the major factors that influence the transport, absorption, and distribution of chemicals in biological systems.

These results are preliminary and it would be interesting to test the activity of high purified fractions and isolate the responsible molecules which underlie the various detected activities in different extracts by more efficient methods. In any case it is important to highlight that the tests were performed in vitro. It is thus mandatory to confirm these findings by in vivo studies so as to obtain useful information for eventual therapeutic or dietary interventions.

Bibliography

Abdullah, Marwan Ibrahim, Abed, Mohammed Najim, and Richardson, Alan. Inhibition of the mevalonate pathway augments the activity of pitavastatin against ovarian cancer cells. *Scientific reports*, 7(1):8090, 2017.

Abed, Mohammed Najim, Abdullah, Marwan Ibrahim, and Richardson, Alan. Antagonism of bcl-x l is necessary for synergy between carboplatin and bh3 mimetics in ovarian cancer cells. *Journal of ovarian research*, 9(1):25, 2016.

Aburjai, Talal and Natsheh, Feda M. Plants used in cosmetics. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 17(9):987–1000, 2003.

Adams, RP. Quadrupole mass spectra of compounds listed in order of their retention time on db-5. identification of essential oils components by gas chromatography/quadrupole mass spectroscopy. *Quadruple mass spectroscopy*, 2001.

Ahmed, Ahmed A, Ishak, MS, Micheal, HN, El-Ansari, MA, and El-Sissi, HI. Flavonoids of asteriscus graveolens. *Journal of Natural Products*, 54(4):1092–1093, 1991.

Akgün, Nalan A, Akgün, Mesut, Dinçer, Salih, and Akgerman, Aydin. Supercritical fluid extraction of lavandula stoechas l. ssp. cariensis (boiss.) rozeira. *Journal of Essential Oil Research*, 13(3): 143–148, 2001.

Akkal, S, Benayache, F, Medjroubi, K, Tillequin, F, and Seguin, E. Flavonoids from centaurea furfuracea (asteraceae). *Biochem. Syst. Ecol*, 31(6):641–643, 2003.

Akkari, Hafidh, Rtibi, Kais, B'chir, Fatma, Rekik, Mourad, Darghouth, Mohamed Aziz, and Gharbi, Mohamed. In vitro evidence that the pastoral artemisia campestris species exerts an anthelmintic effect on haemonchus contortus from sheep. *Veterinary Research Communications*, 38(3):249–255, 2014.

Akrout, Ahmed, El Jani, Hajer, Amouri, Sondes, and Neffati, Mohamed. Screening of antiradical and antibacterial activities of essential oils of artemisia campestris l., artemisia herba alba asso, & thymus capitatus hoff. et link. growing wild in the southern of tunisia. *Recent Research in Science and Technology*, 2(1), 2010.

Akrout, Ahmed, Gonzalez, Lidia Alarcon, El Jani, Hajer, and Madrid, Pablo Campra. Antioxidant and antitumor activities of artemisia campestris and thymelaea hirsuta from southern tunisia. *Food and Chemical Toxicology*, 49(2):342–347, 2011.

Akrout, Ahmed, Mighri, Hedi, Krid, Mabrouka, Thabet, Fatma, Turki, Hamida, El-Jani, H, and Neffati, M. Chemical composition and antioxidant activity of aqueous extracts of some wild medicinal plants in southern tunisia. *International Journal of Life Science and Medical Science*, 2(1):1–4, 2012.

Akrout, Ahmed A, Chemli, Rachid, Simmonds, Monique, Kite, Geoffrey, Hammami, Mohamed, and Chreif, Imed. Seasonal variation of the essential oil of artemisia campestris l. *Journal of Essential Oil Research*, 15(5):333–336, 2003.

Akssira, Mohamed, Mellouki, Fouad, Salhi, Ali, Alilou, Hakim, Saouf, Abderrahmane, El Hanbali, Fadwa, Arteaga, Jesus F, and Barrero, Alejandro F. Naupliolide, a sesquiterpene lactone with a novel tetracyclic skeleton from nauplius graveolens subsp. odorus. *Tetrahedron letters*, 47(38): 6719–6721, 2006.

Al-Naggar, Redhwan A, Osman, Muhamed T, Bobryshev, Yuri V, and Kadir, Samiah Yasmin Abdul. Ovarian cancer: knowledge of risk factors and symptoms among working malaysian women. *Middle-East Journal of Scientific Research*, 14(4):549–553, 2013.

Al-Snafi, Ali Esmail. *Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants*. Rigi Publication, 2015.

Al-Zubairi, Adel S, Abdul, Ahmad Bustamam, Abdelwahab, Siddig Ibrahim, Peng, Chew Yuan, Mohan, Syam, and Elhassan, Manal Mohamed. Eleucine indica possesses antioxidant, antibacterial and cytotoxic properties. *Evidence-Based Complementary and Alternative Medicine*, 2011, 2011.

Alejandre-García, Ivonne, Álvarez, Laura, Cardoso-Taketa, Alexandre, González-Maya, Leticia, Antúnez, Mayra, Salas-Vidal, Enrique, Díaz, J Fernando, Marquina-Bahena, Silvia, and Villarreal, María Luisa. Cytotoxic activity and chemical composition of the root extract from the mexican species linum scabrellum: Mechanism of action of the active compound 6-methoxypodophyllotoxin. *Evidence-Based Complementary and Alternative Medicine*, 2015, 2015.

Algieri, Francesca, Rodriguez-Nogales, Alba, Vezza, Teresa, Garrido-Mesa, Jose, Garrido-Mesa, Natividad, Utrilla, M Pilar, González-Tejero, M Reyes, Casares-Porcel, Manuel, Molero-Mesa, Joaquin, del Mar Contreras, Maria, *et al.* Anti-inflammatory activity of hydroalcoholic extracts of lavandula dentata l. and lavandula stoechas l. *Journal of ethnopharmacology*, 190:142–158, 2016.

ALI, Nejib BEN, BENDAOUD, Houcine, and ROMDHANE, Mehrez. Study of asteriscus maritimus (l.) essential oil encapsulation with almond gum-gelatin by complex coacervation method.

Alilou, Hakim, Asdadi, Ali, Hassani, LM Idrissi, González-Mas, C, Blázquez, M Amparo, and Akssira, M. Antifungal and antioxidant activity of asteriscus graveolens subsp. odorus essential oil. *J Nat Sci Res*, 4(10):1–10, 2014.

Alizadeh, Ardalan and Aghaee, Zahra. Essential oil constituents, phenolic content and antioxidant activity of lavandula stricta delile growing wild in southern iran. *Natural product research*, 30 (19):2253–2257, 2016.

Ambrosio, Allison J, Suzin, Daphne, Palmer, Edwin L, and Penson, Richard T. Vintafolide (ec145) for the treatment of folate-receptor-α positive platinum-resistant ovarian cancer. *Expert review of clinical pharmacology*, 7(4):443–450, 2014.

Anderberg, AA and Eldenäs, P. Tribe inuleae. *K. Bremer, Asteraceae, Cladistics and Classification*, pages 273–291, 1994.

Angioni, Alberto, Barra, Andrea, Coroneo, Valentina, Dessi, Sandro, and Cabras, Paolo. Chemical composition, seasonal variability, and antifungal activity of lavandula stoechas l. ssp. stoechas essential oils from stem/leaves and flowers. *Journal of agricultural and food chemistry*, 54(12): 4364–4370, 2006.

Aniya, Yoko, Shimabukuro, Minako, Shimoji, Manami, Kohatsu, Mayumi, Gyamfi, Maxwell Afari, Miyagi, Chika, Kunii, Daisuke, Takayama, Fusako, and Egashira, Toru. Antioxidant and hepatoprotective actions of the medicinal herb artemisia campestris from the okinawa islands. *Biological and Pharmaceutical Bulletin*, 23(3):309–312, 2000.

Arabaci, Olcay and Bayram, Emine. Evaluation of chemical composition, essential oil and morphological traits in wild populations of lavandula stoechas l. in the mediterranean environment. *Asian Journal of Chemistry*, 18(1):371, 2006.

Arfan, M, Khan, GA, and Ahmad, N. Steroids and terpenoids of the genus ajuga. *JOURNAL OF THE CHEMICAL SOCIETY OF PAKISTAN*, 18(2):170–174, 1996.

Arimura, Gen-ichiro, Huber, Dezene PW, and Bohlmann, Jörg. Forest tent caterpillars (malacosoma disstria) induce local and systemic diurnal emissions of terpenoid volatiles in hybrid poplar (populus trichocarpa× deltoides): cdna cloning, functional characterization, and patterns of gene expression of (-)-germacrene d synthase, ptdtps1. *The Plant Journal*, 37(4):603–616, 2004.

Arnold, N, Bellomaria, B, Valentini, G, and Rafaiani, SM. Comparative study on essential oil of some teucrium species from cyprus. *Journal of ethnopharmacology*, 35(2):105–113, 1991.

Asadipour, Ali, Mehrabani, Mitra, and Najafi, Moslem Lari. Volatile oil composition of centaurea aucheri (dc.) wagenitz. *DARU Journal of Pharmaceutical Sciences*, 13(4):160–164, 2005.

Aung, Theingi, Halsey, Jim, Kromhout, Daan, Gerstein, Hertzel C, Marchioli, Roberto, Tavazzi, Luigi, Geleijnse, Johanna M, Rauch, Bernhard, Ness, Andrew, Galan, Pilar, *et al.* Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77 917 individuals. *JAMA cardiology*, 3(3):225–234, 2018.

Avato, Pinarosa, Vitali, Cesare, Mongelli, Piernatale, and Tava, Aldo. Antimicrobial activity of polyacetylenes from bellis perennis and their synthetic derivatives. *Planta medica*, 63(6): 503–507, 1997.

Baba Aissa, F. Les plantes médicinales en algérie. *coédition Bouchene et ad. Diwan, Alger*, page 29, 1991.

Bahekar, Satish, Nagpure, Shailesh, *et al.* A review on medicinal plants used in scorpion bite treatment in india. *Mintage Journal of Pharmaceutical and Medical Sciences*, pages 1–6, 2016.

Bakchiche, Boulanouar, Gherib, Abdelaziz, Maatallah, Mohamed, and Miguel, MG. Chemical composition of essential oils of artemisia campestris and juniperus phoenicea from algeria. *International Journal of Innovation and Applied Studies*, 9(4):1434, 2014.

Bakkali, Fadil, Averbeck, Simone, Averbeck, Dietrich, and Idaomar, Mouhamed. Biological effects of essential oils–a review. *Food and chemical toxicology*, 46(2):446–475, 2008.

Baldovini, N, Muselli, A, Ristorcelli, D, Tomi, F, and Casanova, J. Variabilite chimique de lavandula stoechas l. ssp. stoechas de corse. *RIVISTA ITALIANA EPPOS*, 9:773–780, 1998.

Bankhead, CR, Collins, C, Stokes-Lampard, H, Rose, P, Wilson, S, Clements, A, Mant, D, Kehoe, ST, and Austoker, J. Identifying symptoms of ovarian cancer: a qualitative and quantitative study. *BJOG: An International Journal of Obstetrics & Gynaecology*, 115(8):1008–1014, 2008.

Barrero, AF, Oltra, JE, Rodriguez, I, Barragan, A, Gravalos, DG, and Ruiz, P. Lactones from species of centaurea. cytotoxic and antimicrobial activities. *FITOTERAPIA-MILANO-*, 66:227–227, 1995.

Başer, K Hüsnü Can, Özek, Gülmira, Özek, Temel, and Duran, Ahmet. Composition of the essential oil of centaurea huber-morathii wagenitz isolated from seeds by microdistillation. *Flavour and fragrance journal*, 21(3):568–570, 2006.

Baser, KC, Özek, T, Demirci, F, and Boydag, I. The essential oil of anthemis cretica l. subsp. leucanthemoides (boiss.) grierson. *Acta Pharmaceutica Turcica*, 44:189–194, 2002.

Bastos, Margarida MSM, Kijjoa, Anake, Cardoso, José M, Gutiérrez, Alicia B, and Herz, Werner. Lignans and other constituents of centaurea sphaerocephala ssp. polyacantha. *Planta medica*, 56 (04):403–405, 1990.

Baytop, T. Turkiye'de bitkiler ile tedavi (gecmiste ve bugun). *Nobel Tip Kitabevleri, Istanbul*, 1999.

Belarbi, Nadia, Dergal, Fayçal, Chikhi, Ilyes, Merah, Salah, Lerari, Djahida, and Bachari, Khaldoun. Study of anti-corrosion activity of algerian l. stoechas oil on c38 carbon steel in 1 m hcl medium. *International Journal of Industrial Chemistry*, 9:115–125, 2018.

Belhattab, R, Boudjouref, M, Barroso, JG, Pedro, LP, and Figueirido, AC. Essential oil composition from artemisia campestris grown in algeria. *Advances in Environmental Biology*, pages 429–433, 2011.

Bellomaria, B, Valentini, G, and Biondi, E. Chemotaxonomy of artemisia variabilis ten. and a. campestris l. ssp. glutinosa (ten.) briq. et cavill.(asteraceae) from italy. *Journal of Essential Oil Research*, 13(2):90–94, 2001.

Benabdelkader, Tarek, Zitouni, Abdelghani, Guitton, Yann, Jullien, Frédéric, Maitre, Dany, Casabianca, Hervé, Legendre, Laurent, and Kameli, Abdelkrim. Essential oils from wild populations of algerian lavandula stoechas l.: composition, chemical variability, and in vitro biological properties. *Chemistry & biodiversity*, 8(5):937–953, 2011.

Benchekroun, Hassan, Gaudet, Gérard, and Lohoues, Hervé. Some effects of asymmetries in a common pool natural resource oligopoly. 2012.

Benchelah, A-C, Bouziane, H, and Maka, M. Fleurs du sahara, arbres et arbustes, voyage au coeur de leurs usages avec les touaregs du tassili. *Phytothérapie*, 2(6):191–197, 2004.

Benli, Mehlika, Kaya, Ilhan, and Yigit, Nazife. Screening antimicrobial activity of various extracts of artemisia dracunculus l. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease*, 25(6):681–686, 2007.

Binder, Ronald G, Turner, Charles E, and Flath, Robert A. Comparison of yellow starthistle volatiles from different plant parts. *Journal of agricultural and food chemistry*, 38(3):764–767, 1990a.

Binder, Ronald G, Turner, Charles E, and Flath, Robert A. Volatile components of purple starthistle. *Journal of agricultural and food chemistry*, 38(4):1053–1055, 1990b.

Bnouham, Mohamed, Mekhfi, Hassane, Legssyer, AbdelKhaleq, and Ziyyat, Abderrahim. Ethnopharmacology forum medicinal plants used in the treatment of diabetes in morocco. *Int J Diabetes & Metabolism*, 10:33–50, 2002.

Bogdan, Christian and Ding, Aihao. Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor α and interleukin-1 in macrophages. *Journal of leukocyte biology*, 52(1):119–121, 1992.

Bork, Peter M, Schmitz, M Lienhard, Kuhnt, Michaela, Escher, Claudia, and Heinrich, Michael. Sesquiterpene lactone containing mexican indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor nf-κb. *FEBS letters*, 402(1):85–90, 1997.

Bouasla, Asma and Bouasla, Ihcène. Ethnobotanical survey of medicinal plants in northeastern of algeria. *Phytomedicine*, 2017.

Bouayed, Jaouad, Piri, Khosro, Rammal, Hassan, Dicko, Amadou, Desor, Frédéric, Younos, Chafique, and Soulimani, Rachid. Comparative evaluation of the antioxidant potential of some iranian medicinal plants. *Food Chemistry*, 104(1):364–368, 2007.

Boudjelal, Amel, Henchiri, Cherifa, Sari, Madani, Sarri, Djamel, Hendel, Noui, Benkhaled, Abderrahim, and Ruberto, Giuseppe. Herbalists and wild medicinal plants in m'sila (north algeria): An ethnopharmacology survey. *Journal of ethnopharmacology*, 148(2):395–402, 2013.

Boukhalkhal, Sarah, Gourine, Nadhir, Pinto, Diana CGA, Silva, Artur MS, and Yousfi, Mohamed. Variability of the chemical composition and the antioxidant activity of the essential oils of two subspecies of artemisia campestris l. growing in algeria. *Journal of Food Measurement and Characterization*, 12(3):1829–1842, 2018.

Boulanouar, Bakchiche, Abdelaziz, Gherib, Aazza, Smail, Gago, Custódia, and Miguel, M Graca. Antioxidant activities of eight algerian plant extracts and two essential oils. *Industrial Crops and Products*, 46:85–96, 2013.

Bouzouita, N, Kachouri, F, Hamdi, M, Chaabouni, MM, Aissa, R Ben, Zgoulli, S, Thonart, Philippe, Carlier, A, Marlier, Michel, and Lognay, GC. Volatile constituents and antimicrobial activity of lavandula stoechas l. oil from tunisia. *Journal of Essential Oil Research*, 17(5):584–586, 2005.

Broxterman, Henk J, Gotink, Kristy J, and Verheul, Henk MW. Understanding the causes of multidrug resistance in cancer: a comparison of doxorubicin and sunitinib. *Drug resistance updates*, 12(4-5):114–126, 2009.

Bruno, Maurizio and Herz, Werner. Sesquiterpene lactones and flavones from centaurea cineraria subsp. umbrosa. *Phytochemistry*, 1988.

Bruno, Maurizio, Fazio, Caterina, Passananti, Salvatore, Paternostro, Maria Pia, Díaz, Jesús G, and Herz, Werner. Sesquiterpene lactones from centaurea sphaerocephala ssp. sphaerocephala. *Phytochemistry*, 35(5):1371–1372, 1994.

Bruno, Maurizio, Vasallo, Nadia, Fazio, Caterina, Gedris, Thomas E, and Herz, Werner. Sesquiterpene lactones of two centaurea species from sicily. *Biochemical systematics and ecology*, 1998.

Butow, Phyllis N, Price, Melanie A, Bell, Melanie L, Webb, Penelope M, Friedlander, Michael, Group, Australian Ovarian Cancer Study, Investigators, Life Study, *et al.* Caring for women with ovarian cancer in the last year of life: A longitudinal study of caregiver quality of life, distress and unmet needs. *Gynecologic Oncology*, 132(3):690–697, 2014.

Buttery, Ron G, Maddox, Donald M, Light, Douglas M, and Ling, Louisa C. Volatile components of yellow starthistle. *Journal of agricultural and food chemistry*, 34(5):786–788, 1986.

Byers, Tim, Levin, Bernard, Rothenberger, David, Dodd, Gerald D, Smith, Robert A, Detection, American Cancer Society, and on Colorectal Cancer), Treatment Advisory Group. American

cancer society guidelines for screening and surveillance for early detection of colorectal polyps and cancer: update 1997. *CA: a cancer journal for clinicians*, 47(3):154–160, 1997.

Cakilcioglu, U, Sengun, MT, and Turkoglu, I. An ethnobotanical survey of medicinal plants of yazkonak and yurtba districts of elaz province, turkey. *J. Med. Plant. Res*, 4(7):567572, 2010.

Calín-Sánchez, Ángel, Figiel, Adam, Lech, Krzysztof, Szumny, Antoni, and Carbonell-Barrachina, Ángel A. Effects of drying methods on the composition of thyme (thymus vulgaris l.) essential oil. *Drying Technology*, 31(2):224–235, 2013.

Calvo, Joel, Quintanar, Alejandro, and Aedo, Carlos. Typification of four species names of bellis (compositae). *Nordic journal of botany*, 30(6):668–670, 2012.

Carrasco, Alejandro, Ortiz-Ruiz, Vanessa, Martinez-Gutierrez, Ramiro, Tomas, Virginia, and Tudela, Jose. Lavandula stoechas essential oil from spain: Aromatic profile determined by gas chromatography–mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. *Industrial crops and products*, 73:16–27, 2015.

Carvalho, Isabel S, Cavaco, Teresa, and Brodelius, Maria. Phenolic composition and antioxidant capacity of six artemisia species. *Industrial crops and products*, 33(2):382–388, 2011.

Cavanagh, HMA and Wilkinson, JM. Biological activities of lavender essential oil. *Phytotherapy research*, 16(4):301–308, 2002.

Celep, Engin, İnan, Yiğit, Akyüz, Selin, and Yesilada, Erdem. The bioaccessible phenolic profile and antioxidant potential of hypericum perfoliatum l. after simulated human digestion. *Industrial Crops and Products*, 109:717–723, 2017.

Celep, Engin, Akyüz, Selin, İnan, Yiğit, and Yesilada, Erdem. Assessment of potential bioavailability of major phenolic compounds in lavandula stoechas l. ssp. stoechas. *Industrial Crops and Products*, 118:111–117, 2018.

Ceylan, Y, Usta, K, Usta, A, Maltas, E, and Yildiz, S. Evaluation of antioxidant activity, phytochemicals and esr analysis of lavandula stoechas. *Acta Phys Pol A*, 128:483–487, 2015.

Chaib, Faiza, Allali, Hocine, Bennaceur, Malika, and Flamini, Guido. Chemical composition and antimicrobial activity of essential oils from the aerial parts of asteriscus graveolens (forssk.) less.

and pulicaria incisa (lam.) dc.: Two asteraceae herbs growing wild in the hoggar. *Chemistry & biodiversity*, 14(8):e1700092, 2017.

Chalchat, Jean-Claude and Özcan, Mehmet Musa. Comparative essential oil composition of flowers, leavesand stems of basil (ocimum basilicum l.) used as herb. *Food Chemistry*, 110(2):501–503, 2008.

Chalchat, Jean-Claude, Cabassu, Patrick, Petrovic, SD, Maksimovic, ZA, and Gorunovic, MS. Composition of essential oil of artemisia campestris l. from serbia. *Journal of Essential Oil Research*, 15(4):251–253, 2003.

Chambers, Ann F, Groom, Alan C, and MacDonald, Ian C. Metastasis: dissemination and growth of cancer cells in metastatic sites. *Nature Reviews Cancer*, 2(8):563, 2002.

Chan, Pei Teng, Matanjun, Patricia, Yasir, Suhaimi Md, and Tan, Tek Song. Antioxidant activities and polyphenolics of various solvent extracts of red seaweed, gracilaria changii. *Journal of applied phycology*, 27(6):2377–2386, 2015.

Chekoual, Lilia, Aissat, Abdelkader, Ait-Kaci Aourahoun, Karima, and Benabdelkader, Tarek. The effect of ultrasound pre-treatment on the yield, chemical composition and antioxidant activity of essential oil from wild lavandula stoechas l. *Journal of Essential Oil Bearing Plants*, 21(1): 253–263, 2018.

Choi, Beomjoon and Choi, Beom-Jin. The effects of perceived service recovery justice on customer affection, loyalty, and word-of-mouth. *European Journal of Marketing*, 48(1/2):108–131, 2014.

Christophers, Brett. The state and financialization of public land in the united kingdom. *Antipode*, 49(1):62–85, 2017.

Ciccarelli, Daniela, Andreucci, Andrea Cesare, and Pagni, Anna Maria. Translucent glands and secretory canals in hypericum perforatum l.(hypericaceae): morphological, anatomical and histochemical studies during the course of ontogenesis. *Annals of botany*, 88(4):637–644, 2001.

Clancy, Suzanne. Dna damage & repair: mechanisms for maintaining dna integrity. *Nature Education*, 1(1):103, 2008.

Costa, Patrícia, Grosso, Clara, Gonçalves, Sandra, Andrade, Paula B, Valentão, Patrícia, Bernardo-Gil, M Gabriela, and Romano, Anabela. Supercritical fluid extraction and hydrodistillation for the recovery of bioactive compounds from lavandula viridis l'hér. *Food Chemistry*, 135(1): 112–121, 2012.

Costa, Patrícia, Grevenstuk, Tomás, da Costa, Ana M Rosa, Gonçalves, Sandra, and Romano, Anabela. Antioxidant and anti-cholinesterase activities of lavandula viridis l'hér extracts after in vitro gastrointestinal digestion. *Industrial Crops and Products*, 55:83–89, 2014.

Cragg, Gordon M and Newman, David J. Plants as a source of anti-cancer agents. *Journal of ethnopharmacology*, 100(1-2):72–79, 2005.

Cragg, Gordon M, Newman, David J, and Snader, Kenneth M. Natural products in drug discovery and development. *Journal of natural products*, 60(1):52–60, 1997.

Cragg, Gordon M, Grothaus, Paul G, and Newman, David J. Impact of natural products on developing new anti-cancer agents. *Chemical reviews*, 109(7):3012–3043, 2009.

Cristofari, Gregory, Znini, Mohamed, Majidi, Lhou, Mazouz, Hamid, Tomi, Pierre, Costa, Jean, and Paolini, Julien. Chemical diversity of essential oils from asteriscus graveolens (forssk.) less.: Identification of cis-8-acetoxychrysanthenyl acetate as a new natural component. *Chemistry & biodiversity*, 9(4):727–738, 2012.

Cruet-Hennequart, Séverine, Glynn, Macdara T, Murillo, Laura S, Coyne, Seamus, and Carty, Michael P. Enhanced dna-pk-mediated rpa2 hyperphosphorylation in dna polymerase η-deficient human cells treated with cisplatin and oxaliplatin. *DNA repair*, 7(4):582–596, 2008.

Csupor, Dezső, Blazsó, Gábor, Balogh, Ágnes, and Hohmann, Judit. The traditional hungarian medicinal plant centaurea sadleriana janka accelerates wound healing in rats. *Journal of ethnopharmacology*, 127(1):193–195, 2010.

Cunningham, John Paul, Moore, Chris J, Zalucki, Myron P, and West, Stuart A. Learning, odour preference and flower foraging in moths. *Journal of Experimental Biology*, 207(1):87–94, 2004.

Curry, Susan J, Byers, Tim, Hewitt, Maria, *et al.* Potential of screening to reduce the burden of cancer. 2003.

Dadalioglu, Itir and Evrendilek, Gulsun Akdemir. Chemical compositions and antibacterial effects of essential oils of turkish oregano (origanum minutiflorum), bay laurel (laurus nobilis), spanish lavender (lavandula stoechas l.), and fennel (foeniculum vulgare) on common foodborne pathogens. *Journal of agricultural and food chemistry*, 52(26):8255–8260, 2004.

Dahmani-Hamzaoui, Nacéra, Salido, Sofía, Linares-Palomino, Pablo J, Baaliouamer, Aoumeur, and Altarejos, Joaquín. On-line radical scavenging detection and characterization of antioxidants from artemisia herba-alba. *Helvetica Chimica Acta*, 95(4):564–576, 2012.

Danh, Luu Thai, Triet, Ngo Duy Anh, Zhao, Jian, Mammucari, Raffaella, Foster, Neil, *et al.* Comparison of chemical composition, antioxidant and antimicrobial activity of lavender (lavandula angustifolia l.) essential oils extracted by supercritical co 2, hexane and hydrodistillation. *Food and bioprocess technology*, 6(12):3481–3489, 2013.

Dave, R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, 3(13):981–996, 2009.

de Leon, M Ponz. Oncogenes and tumor suppressor genes. In *Familial and Hereditary Tumors*, pages 35–47. Springer, 1994.

De Natale, Antonino and Pollio, Antonino. A forgotten collection: the libyan ethnobotanical exhibits (1912-14) by a. trotter at the museum o. comes at the university federico ii in naples, italy. *Journal of ethnobiology and ethnomedicine*, 8(1):4, 2012.

Deba, Farah, Xuan, Tran Dang, Yasuda, Masaaki, and Tawata, Shinkichi. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from bidens pilosa linn. var. radiata. *Food control*, 19(4):346–352, 2008.

Derwich, Elhoussine, Benziane, Zineb, and Boukir, Abdellatif. Chemical compositions and insectisidal activity of essential oils of three plants artemisia sp: Artemisia herba-alba, artemisia absinthium and artemisia pontica (morocco). *Electronic Journal of Environmental, Agricultural & Food Chemistry*, 8(12), 2009.

Dhifi, Wissal, Jilani, Imtinen Ben Haj, Jazi, Sabrine, El Beyrouthy, Marc, Mnif, Wissem, *et al.* Essential oil chemical characterization and allelopathic potential of artemisia campestris l growing in tunisia. *Journal of Microbiology, Biotechnology & Food Sciences*, 7(3), 2017.

Di Foggia, Giacomo. *Tracce di cinefilia su Facebook*. PhD thesis, alma, 2014.

Dinç, Muhittin and Doğu, Süleyman. Anatomical characteristics of turkish steno-endemic origanum leptocladum boiss.(lamiaceae). *Modern Phytomorphology*, 3:25–28, 2013.

Djeddi, Samah, Argyropoulou, Catherine, and Skaltsa, Helen. Secondary metabolites from centaurea grisebachii ssp. grisebachii. *Biochemical Systematics and Ecology*, 36(5-6):336–339, 2008.

Djeridane, A, Yousfi, M, Nadjemi, B, Boutassouna, D, Stocker, P, and Vidal, N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food chemistry*, 97 (4):654–660, 2006.

Djeridane, A, Yousfi, M, Nadjemi, B, Vidal, N, Lesgards, JF, and Stocker, P. Screening of some algerian medicinal plants for the phenolic compounds and their antioxidant activity. *European Food Research and Technology*, 224(6):801–809, 2007.

Djidel, Saliha *et al.* Radical scavenging, reducing power, lipid peroxidation inhibition and chelating properties of extracts from artemisia campestris l. aerial parts. *Annual Research & Review in Biology*, 4(10):1691, 2014.

Dob, T, Dahmane, D, Berramdane, T, and Chelghoum, C. Chemical composition of the essential oil of artemisia campestris. l. from algeria. *Pharmaceutical biology*, 43(6):512–514, 2005.

Dob, T, Dahmane, D, Agli, M, and Chelghoum, C. Essential oil composition of lavandula stoechas. from algeria. *Pharmaceutical biology*, 44(1):60–64, 2006.

Dob, Tahar, Dahmane, Dahmane, Gauriat-Desrdy, Bénédicte, and Daligault, Virgine. Essential oil composition of centaurea pullata l. *Journal of Essential Oil Research*, 21(5):417–422, 2009.

Dobignard, A and Chatelain, C. Index synonymique et bibliographique de la flore d'afrique du nord. conservatoire et jardin botaniques de la ville de genève (ch), 2010.

Doll, Richard and Peto, Richard. The causes of cancer: quantitative estimates of avoidable risks of cancer in the united states today. *JNCI: Journal of the National Cancer Institute*, 66(6): 1192–1308, 1981.

dos Santos, Michel David, Gobbo-Neto, Leonardo, Albarella, Laura, de Souza, Glória Emília Petto, and Lopes, Norberto Peporine. Analgesic activity of di-caffeoylquinic acids from roots of lychnophora ericoides (arnica da serra). *Journal of Ethnopharmacology*, 96(3):545–549, 2005.

Dudareva, Natalia, Pichersky, Eran, and Gershenzon, Jonathan. Biochemistry of plant volatiles. *Plant physiology*, 135(4):1893–1902, 2004.

Dural, Huseyin, Bagci, Yavuz, Ertugrul, Kuddisi, Demirelma, Hakki, Flamini, Guido, Cioni, Pier Luigi, and Morelli, Ivano. Essential oil composition of two endemic centaurea species from turkey, centaurea mucronifera and centaurea chrysantha, collected in the same habitat. *Biochemical Systematics and Ecology*, 31(12):1417–1425, 2003.

Ebadollahi, Asgar, Safaralizadeh, Mohammad H, and Pourmirza, Ali A. Fumigant toxicity of essential oils of eucalyptus globulus labill and lavandula stoechas l., grown in iran, against the two coleopteran insect pests; lasioderma serricorne f. and rhyzopertha dominca f. *Egyptian Journal of Biological Pest Control*, 20(1), 2010.

Ebrahimabadi, Abdolrasoul H, Djafari-Bidgoli, Zahra, Mazoochi, Asma, Kashi, Fereshteh Jookar, and Batooli, Hossein. Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of chaerophyllum macropodum boiss. *Food Control*, 21(8):1173–1178, 2010.

El Beyrouthy, MAAN, Labaki, M, Cazier, F, Najm, S, and AbouKaïs, A. Chemical composition of the essential oil of the artemisia arborescens l. growing wild in lebanon. *LSJ*, 12(1):71–78, 2011.

El Dahmy, S, Jakupovic, J, Bohlmann, F, and Sarg, TM. New humulene derivatives from asteriscus graveolens. *Tetrahedron*, 41(2):309–316, 1985.

El-Ghazouly, MG and Omar, AA. Flavonoid constituents of artemisia campestris. *Fitoterapia (Italy)*, 1984.

El-Mokasabi, FM. Floristic composition and traditional uses of plant species at wadi alkuf, al-jabal al-akhder, libya. *American-Eurasian J Agric Environ Sci*, 14(8):685–697, 2014.

El-Najjar, Nahed, Dakdouki, Saadah, Darwiche, Nadine, El-Sabban, Marwan, Saliba, Najat A, and Gali-Muhtasib, Hala. Anti-colon cancer effects of salograviolide a isolated from centaurea ainetensis. *Oncology reports*, 19(4):897–904, 2008.

Engel, Ralf, Gülz, Paul-Gerhard, Herrmann, Thorsten, and Nahrstedt, Adolf. Glandular trichomes and the yolatiles obtained by steam distillation of quercus robur leaves. *Zeitschrift für Naturforschung C*, 48(9-10):736–744, 1993.

Erol-Dayi, Ö, Pekmez, M, Bona, M, Aras-Perk, A, and Arda, N. Total phenolic contents, antioxidant activities cytotoxicity of three centaurea species: C. calcitrapa subsp. calcitrapa, c. ptosimopappa c. spicata. *Free Radicals and Antioxidants*, 1(2):31–36, 2011.

Ertugrul, Kuddisi, Dura, Huseyin, Tugay, Osman, Flamini, Guido, Cioni, Pier Luigi, and Morelli, Ivano. Essential oils from flowers of centaurea kotschyi var. kotschyi and c. kotschyi var. decumbens from turkey. *Flavour and fragrance journal*, 18(2):95–97, 2003.

Esmaeili, Akbar, Rustaiyan, Abdolhossein, Nadimi, Marjan, Masoudi, Shiva, Tadayon, Fariba, Sedaghat, Sajjad, Ebrahimpur, Nayyereh, and Hajyzadeh, Elmira. Volatile constituents of centaurea depressa mb and carduus pycnocephalus l. two compositae herbs growing wild in iran. *Journal of essential oil research*, 17(5):539–541, 2005.

Fadil, Mouhcine, Fikri-Benbrahim, Kawtar, Rachiq, Saad, Ihssane, Bouchaib, Lebrazi, Sara, Chraibi, Marwa, Haloui, Taoufik, and Farah, Abdellah. Combined treatment of thymus vulgaris l., rosmarinus officinalis l. and myrtus communis l. essential oils against salmonella typhimurium: Optimization of antibacterial activity by mixture design methodology. *European Journal of Pharmaceutics and Biopharmaceutics*, 126:211–220, 2018.

Fahmy, HS. Chemical composition and antimicrobial activity of the volatile oil of nauplius graveolens (forssk.) less. *J Environ Sci (Mansoura, Egypt)*, 26:307, 2003.

Falé, Pedro L, Ascensão, Lia, and Serralheiro, Maria LM. Effect of luteolin and apigenin on rosmarinic acid bioavailability in caco-2 cell monolayers. *Food & function*, 4(3):426–431, 2013.

Farah, Adriana, Monteiro, Mariana, Donangelo, Carmen M, and Lafay, Sophie. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *The Journal of nutrition*, 138(12): 2309–2315, 2008.

Farnsworth, Norman R, Akerele, Olayiwola, Bingel, Audrey S, Soejarto, Djaja D, and Guo, Zhengang. Medicinal plants in therapy. *Bulletin of the world health organization*, 63(6):965, 1985.

Fathalla, MF. Incessant ovulation and ovarian cancer–a hypothesis re-visited. *Facts, views & vision in ObGyn*, 5(4):292, 2013.

Ferchichi, Loubna, Merza, Joumaa, Landreau, Anne, Le Ray, Anne Marie, Legseir, Belgacem, Seraphin, Denis, and Richomme, Pascal. Occurrence of isocoumarinic and phenolic derivatives in artemisia campestris l. subsp. campestris. *Biochemical systematics and ecology*, 2006.

Fismer, Kate Louise and Pilkington, Karen. Lavender and sleep: A systematic review of the evidence. *European Journal of Integrative Medicine*, 4(4):e436–e447, 2012.

Flamini, Guido, Ertugrul, K, Cioni, PL, Morelli, Ivano, Dural, H, and Bagci, Y. Volatile constituents of two endemic centaurea species from turkey: C. pseudoscabiosa subsp. pseudoscabiosa and c. hadimensis. *Biochemical Systematics and Ecology*, 30(10):953–959, 2002.

Flamini, Guido, Tebano, M, Cioni, PL, Bagci, Y, Dural, H, Ertugrul, K, Uysal, T, and Savran, A. A multivariate statistical approach to centaurea classification using essential oil composition data of some species from turkey. *Plant Systematics and Evolution*, 261(1-4):217–228, 2006.

Forgo, Peter, Zupkó, István, Molnár, Judit, Vasas, Andrea, Dombi, György, and Hohmann, Judit. Bioactivity-guided isolation of antiproliferative compounds from centaurea jacea l. *Fitoterapia*, 83(5):921–925, 2012.

Formisano, Carmen, Rigano, Daniela, Senatore, Felice, Celik, Sezgin, Bruno, Maurizio, and Rosselli, Sergio. Volatile constituents of aerial parts of three endemic centaurea species from turkey: Centaurea amanicola hub.-mor., centaurea consanguinea dc. and centaurea ptosimopappa hayek and their antibacterial activities. *Natural product research*, 22(10):833–839, 2008.

Foster, Talia, Brown, T Michelle, Chang, Jane, Menssen, Hans D, Blieden, Marissa B, and Herzog, Thomas J. A review of the current evidence for maintenance therapy in ovarian cancer. *Gynecologic oncology*, 115(2):290–301, 2009.

Fraga, Pere, Castro, Mercedes, and Rossello, Josep A. A new annual species of bellium (asteraceae) from the balearic islands. *Botanical journal of the Linnean Society*, 154(1):65–77, 2007.

Fraternale, D, Giamperi, L, Ricci, D, and Manunta, A. Composition and insecticidal activity of the essential oil of asteriscus maritimus l. less. *RIVISTA ITALIANA EPPOS*, 32:33–39, 2001.

Gabrieli, C and Kokkalou, E. A new acetylated glucoside of luteolin and two flavone glucosides from lavandula stoechas ssp. stoechas. *Die Pharmazie*, 58(6):426–427, 2003.

Garbacki, Nancy, Gloaguen, Vincent, Damas, Jacques, Bodart, Patricia, Tits, Monique, and Angenot, Luc. Anti-inflammatory and immunological effects of centaurea cyanus flower-heads. *Journal of Ethnopharmacology*, 68(1-3):235–241, 1999.

Garcia-Vallejo, MC, Garcia-Vallejo, I, Velasco-Negueruela, A, *et al.* Essential oils of genus lavandula l. in spain. In *Proceedings of the 11th International Congress of essential oils, fragrances and flavours. New Delhi, India, 12-16 November, 1989 Vol. 4 Chemistry-analysis and structure.*, pages 15–26. Aspect Publishing, 1990.

Garcia Vallejo, MI. Essential oils of iberian lavandulas: chemotaxonomy trials. 1992.

Gast, M. Armoise. *Encyclopédie berbère*, (6):905–908, 1989.

Gayther, Simon A and Pharoah, Paul DP. The inherited genetics of ovarian and endometrial cancer. *Current opinion in genetics & development*, 20(3):231–238, 2010.

Geppert, Barbara, Drożdż, Bohdan, Kiełczewski, Michał, and Holub, Mirosław. Sesquiterpene lactones. xxiii. isolation of sesquiterpene lactones from centaurea l. species. *Acta Societatis Botanicorum Poloniae*, 52(1):23–34, 1983.

Gershenzon, Jonathan, McConkey, Marie E, and Croteau, Rodney B. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology*, 122(1):205–214, 2000.

Ghorab, H, Laggoune, S, Kabouche, A, Semra, Z, and Kabouche, Z. Essential oil composition and antibacterial activity of artemisia campestris l. from khenchela (algeria). *Der Pharmacia Lettre*, 5(2):189–192, 2013.

Gião, Maria S, Gomes, Susana, Madureira, Ana R, Faria, Ana, Pestana, Diogo, Calhau, Conceição, Pintado, Manuela E, Azevedo, Isabel, and Malcata, F Xavier. Effect of in vitro digestion upon the antioxidant capacity of aqueous extracts of agrimonia eupatoria, rubus idaeus, salvia sp. and satureja montana. *Food Chemistry*, 131(3):761–767, 2012.

Gilani, AH, Aziz, N, Khan, MA, Shaheen, F, Jabeen, Q, Siddiqui, BS, and Herzig, JW. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of lavandula stoechas l. *Journal of Ethnopharmacology*, 71(1-2):161–167, 2000.

Gildemeister-Hoffmann, P. Die ätherischen öle, 1961.

Giray, E Sultan, Kırıcı, Saliha, Kaya, D Alpaslan, Türk, Murat, Sönmez, Özgür, and Inan, Memet. Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of lavandula stoechas. *Talanta*, 74(4):930–935, 2008.

Gonçalves, Sandra and Romano, Anabela. In vitro culture of lavenders (lavandula spp.) and the production of secondary metabolites. *Biotechnology Advances*, 31(2):166–174, 2013.

Gonzalez, AG, Darias, V, Alonso, G, Boada, JN, and Feria, M. Cytostatic activity of sesquiterpene lactones from compositae of the canary islands. *Planta medica*, 33(04):356–359, 1978.

González, Antonio G, Barrera, Jaime Bermejo, Rosas, F Estevez, Hernández, Angel C Yanes, Espineira, J, and Joseph-Nathan, P. Thymol derivatives from schizogyne glaberrima. *Phytochemistry*, 25(12):2889–2891, 1986.

Gören, Ahmet C, Topçu, Gülaçtı, Bilsel, Gökhan, Bilsel, Mine, Aydoğmusç, Zeynep, and Pezzuto, John M. The chemical constituents and biological activity of essential oil of lavandula stoechas ssp. stoechas. *Zeitschrift für Naturforschung C*, 57(9-10):797–800, 2002.

Grabias, B, Dombrowicz, E, Kalemba, D, and Swiatek, L. Phenolic acids in flores bellidis and herba tropaeoli. *Herba Polonica*, 41(3):111–114, 1995.

Greenwood, Peter Humphry. The haplochromine fishes of the east african lakes; collected papers on their taxonomy, biology and evolution (with an introduction and species index). 1981.

Greuter, Werner. Save asteriscus, sink nauplius (compositae). *Fl. Medit*, 7:41–48, 1997.

Gu, Dongyu, Yang, Yi, Abdulla, Rahima, and Aisa, Haji Akber. Characterization and identification of chemical compositions in the extract of artemisia rupestris l. by liquid chromatography coupled to quadrupole time-of-flight tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 26(1):83–100, 2012.

Guarino, Carmine, De Simone, Luciana, and Santoro, Simona. Ethnobotanical study of the sannio area, campania, southern italy. 2008.

Gubbels, Jennifer AA, Claussen, Nick, Kapur, Arvinder K, Connor, Joseph P, and Patankar, Manish S. The detection, treatment, and biology of epithelial ovarian cancer. *Journal of ovarian research*, 3(1):8, 2010.

Gudej, J and Nazaruk, J. Flavonol glycosides from the flowers of bellis perennis. *Fitoterapia*, 72 (7):839–840, 2001.

Guenther, E. Determination of physical and chemical properties. the essential oils (vol iii). toronto, new york and london, 1960.

Gülçin, İ, Sat, IG, Beydemir, Sß, and Küfrevioglu, ÖI. Evaluation of the in vitro antioxidant properties of broccoli extracts (brassica oleracea l.). *Italian journal of food science*, 16(1), 2004a.

Gülçin, Ìlhami, Şat, İ Güngör, Beydemir, Şükrü, Elmastaş, Mahfuz, and Küfrevioğlu, Ö İrfan. Comparison of antioxidant activity of clove (eugenia caryophylata thunb) buds and lavender (lavandula stoechas l.). *Food Chemistry*, 87(3):393–400, 2004b.

Gupta, Gaorav P and Massagué, Joan. Cancer metastasis: building a framework. *Cell*, 127(4): 679–695, 2006.

Gurbüz, İlhan and Yesilada, Erdem. Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from centaurea solstitialis l. ssp. solstitialis by using various in vivo and biochemical techniques. *Journal of ethnopharmacology*, 112(2):284–291, 2007.

Gursoy, Ulvi Kahraman, Gursoy, Mervi, Gursoy, Orhan Vedat, Cakmakci, Lutfu, Könönen, Eija, and Uitto, Veli-Jukka. Anti-biofilm properties of satureja hortensis l. essential oil against periodontal pathogens. *Anaerobe*, 15(4):164–167, 2009.

Güven, Kιymet, Celik, Sezgin, and Uysal, Ismet. Antimicrobial activity of centaurea. species. *Pharmaceutical Biology*, 43(1):67–71, 2005.

Haddouchi, Farah, Chaouche, Tarik Mohammed, Ksouri, Riadh, Medini, Faten, Sekkal, Fatima Zohra, and Benmansour, Abdelhafid. Antioxidant activity profiling by spectrophotometric methods of aqueous methanolic extracts of helichrysum stoechas subsp. rupestre and phagnalon saxatile subsp. saxatile. *Chinese journal of natural medicines*, 12(6):415–422, 2014.

Hagerman, Ann E, Riedl, Ken M, Jones, G Alexander, Sovik, Kara N, Ritchard, Nicole T, Hartzfeld, Paul W, and Riechel, Thomas L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of agricultural and food chemistry*, 46(5):1887–1892, 1998.

Hamilton, William, Peters, Tim J, Round, Alison, and Sharp, Deborah. What are the clinical features of lung cancer before the diagnosis is made? a population based case-control study. *Thorax*, 60(12):1059–1065, 2005.

Hamilton, William, Peters, Tim J, Bankhead, Clare, and Sharp, Deborah. Risk of ovarian cancer in women with symptoms in primary care: population based case-control study. *Bmj*, 339:b2998, 2009.

Hammer, Katherine A, Carson, Christine F, and Riley, Thomas V. Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, 86(6):985–990, 1999.

Hammiche, Victoria and Maiza, Khadra. Traditional medicine in central sahara: pharmacopoeia of tassili n'ajjer. *Journal of ethnopharmacology*, 105(3):358–367, 2006.

Han, Jian, Ye, Min, Qiao, Xue, Xu, Man, Wang, Bao-rong, and Guo, De-An. Characterization of phenolic compounds in the chinese herbal drug artemisia annua by liquid chromatography coupled to electrospray ionization mass spectrometry. *Journal of pharmaceutical and biomedical analysis*, 47(3):516–525, 2008.

Hanamanthagouda, Manohar Shirugumbi, Kakkalameli, Siddappa Bhimashya, Naik, Poornananda Madhava, Nagella, Praveen, Seetharamareddy, Harisha Reddy, and Murthy, Hosakatte Niranjana. Essential oils of lavandula bipinnata and their antimicrobial activities. *Food Chemistry*, 118(3):836–839, 2010.

Hänsel, R, Keller, K, Rimpler, H, and Schneider, G. Drogen ad: Betula, 1992.

Harborne, JB. Plant toxins and their effects on animals. *Introduction to Ecological Biochemistry. Academic Press, London*, pages 71–103, 1993.

HassaniM, El, Douiri, EM, Bammi, J, Zidane, L, Badoc, A, and Douira, A. Plantes médicinales de la moyenne moulouya (nord-est du maroc). *Ethnopharmacologia*, 50:39, 2013.

Hassiotis, Christos N. Chemical compounds and essential oil release through decomposition process from lavandula stoechas in mediterranean region. *Biochemical Systematics and Ecology*, 38(4): 493–501, 2010.

Hassiotis, Christos N and Orfanoudakis, Michail. The impact of lavandula stoechas l. degradation on arbuscular mycorrhizal fungi, in a mediterranean ecosystem. *Applied soil ecology*, 126: 182–188, 2018.

Hernández-Hernández, E, Ponce-Alquicira, E, Jaramillo-Flores, Maria Eugenia, and Legarreta, I Guerrero. Antioxidant effect rosemary (rosmarinus officinalis l.) and oregano (origanum vulgare l.) extracts on tbars and colour of model raw pork batters. *Meat Science*, 81(2):410–417, 2009.

Hiller, K, Schöpke, Th, Wray, V, and Schulten, H-R. Zur struktur der hauptsaponine von bellis perennis l. *Pharmazie*, 43(12):850–852, 1988.

Hmamouchi, Mohammed. *Les plantes médicinales et aromatiques marocaines*. Editions, 1999.

Holme, DAGNY and Sörensen, NILS ANDREAS. Studies related to naturally occurring acetylene compounds. xv. the isolation of trans-lachnophyllum ester from bellis perennis l. *Acta chem. scand.*, 8:280, 1954.

Hose, S. Der wermut-artemisia absinthium l. arzneipflanze fur kranke und» kultige. *Zeitschrift fur Phytotherapie*, 23(4):187–194, 2002.

Inan, Memet, Kaya, Durmus Alpaslan, and Albu, Madalina Georgiana. The effect of lavender essential oils on collagen hydrolysate. *Rev Chim*, 64:1037–42, 2013.

Islem, Saadaoui, Hayet, Ilahi, Christopher, Robin Bryant, and Hichem, Rejeb. Contribution to the study of the flora in the central-west of tunisia: landscape dynamics and evaluation of plant biodiversity of mountain bouchebka. *International Journal of Innovation and Applied Studies*, 6(2):257, 2014.

Jain, Harsh V and Meyer-Hermann, Michael. The molecular basis of synergism between carboplatin and abt-737 therapy targeting ovarian carcinomas. *Cancer research*, pages canres–3174, 2010.

Jakupovic, J, Jia, Y, Pathak, VP, Bohlmann, F, and King, RM. Bisabolone derivatives and sesquiterpene lactones from centaurea species. *Planta medica*, 52(05):399–401, 1986.

Jakupovic, J, Lehmann, L, Bohlmann, F, and Hogdson, A Arroyo. Nerolidol derivatives from asteriscus sericeus. *Phytochemistry*, 26(10):2854–2855, 1987.

Jamila, Fakchich and Mostafa, Elachouri. Ethnobotanical survey of medicinal plants used by people in oriental morocco to manage various ailments. *Journal of ethnopharmacology*, 154(1):76–87, 2014.

Jaspars, M and Lawton, LA. Cyanobacteria-a novel source of pharmaceuticals. *Current opinion in drug discovery & development*, 1(1):77, 1998.

Jelovac, Danijela and Armstrong, Deborah K. Recent progress in the diagnosis and treatment of ovarian cancer. *CA: a cancer journal for clinicians*, 61(3):183–203, 2011.

Jensen, Mette Munk, Erichsen, Kamille Dumong, Björkling, Fredrik, Madsen, Jacob, Jensen, Peter Buhl, Sehested, Maxwell, Højgaard, Liselotte, and Kjær, Andreas. Imaging of treatment response to the combination of carboplatin and paclitaxel in human ovarian cancer xenograft tumors in mice using fdg and flt pet. *PloS one*, 8(12):e85126, 2013.

Johnson-Ajinwo, Okiemute Rosa and Li, Wen-Wu. Stable isotope dilution gas chromatography–mass spectrometry for quantification of thymoquinone in black cumin seed oil. *Journal of agricultural and food chemistry*, 62(24):5466–5471, 2014.

Judzentiene, Asta, Budiene, Jurga, Butkiene, Rita, Kupcinskiene, Eugenija, Laffont-Schwob, Isabelle, and Masotti, Veronique. Caryophyllene oxide-rich essential oils of lithuanian artemisia campestris ssp. campestris and their toxicity. *Natural product communications*, 5(12):1981–1984, 2010.

Kadereit, Joachim W and Jeffrey, Charles. *Flowering plants. eudicots: asterales*, volume 8. Springer Science & Business Media, 2007.

Kaijser, J, Bourne, Tom, Valentin, Lil, Sayasneh, A, Van Holsbeke, Caroline, Vergote, Ignace, Testa, ANTONIA CARLA, Franchi, D, Van Calster, Ben, and Timmerman, Dirk. Improving strategies for diagnosing ovarian cancer: a summary of the international ovarian tumor analysis (iota) studies. *Ultrasound in obstetrics & gynecology*, 41(1):9–20, 2013.

Kaplan, Warren. Background paper 6.5 cancer and cancer therapeutics. *World Health Organization (ed) Priority medicines for Europe and the world: update*, pages 6–5, 2013.

Karabegović, Ivana, Nikolova, Milena, Veličković, Dragan, Stojičević, Saša, Veljković, Vlada, and Lazić, Miodrag. Comparison of antioxidant and antimicrobial activities of methanolic extracts of the artemisia sp. recovered by different extraction techniques. *Chinese Journal of Chemical Engineering*, 19(3):504–511, 2011.

Karakaş, F Pehlivan, Karakaş, A, Coşkun, H, Uccedil, A, *et al.* Effects of common daisy (bellis perennis l.) aqueous extracts on anxiety-like behaviour and spatial memory performance in wistar albino rats. *African Journal of Pharmacy and Pharmacology*, 5(11):1378–1388, 2011.

Karakas, Fatma Pehlivan and Turker, Arzu Ucar. An efficient in vitro regeneration system for bellis perennis l. and comparison of phenolic contents of field-grown and in vitro-grown leaves by lc-ms/ms. *Industrial crops and products*, 48:162–170, 2013.

Karakaş, Fatma Pehlivan, Karakaş, Alper, Boran, Çetin, Türker, Arzu Uçar, Yalçin, Funda Nuray, and Bilensoy, Erem. The evaluation of topical administration of bellis perennis fraction on circular excision wound healing in wistar albino rats. *Pharmaceutical biology*, 50(8):1031–1037, 2012.

Karakas, Fatma Pehlivan, Yidirim, Arzu Birinci, Bayram, Recep, Yavuz, Muhsine Z, Gepdiremen, Akcahan, and Turker, Arzu Ucar. Antiproliferative activity of some medicinal plants on human breast and hepatocellular carcinoma cell lines and their phenolic contents. *Tropical Journal of Pharmaceutical Research*, 14(10):1787–1795, 2015.

Karamenderes, Canan, Khan, Shabana, Tekwani, Babu L, Jacob, Melissa R, and Khan, Ikhlas A. Antiprotozoal and antimicrobial activities of centaurea. species growing in turkey. *Pharmaceutical biology*, 44(7):534–539, 2006.

Karamenderes, Canan, Bedir, Erdal, Pawar, Rahul, Baykan, Sura, and Khan, Ikhlas A. Elemanolide sesquiterpenes and eudesmane sesquiterpene glycosides from centaurea hierapolitana. *Phytochemistry*, 68(5):609–615, 2007.

Karamenderes, Canan, Demirci, Betul, and Baser, K Hüsnü Can. Composition of essential oils of ten centaurea l. taxa from turkey. *Journal of essential oil research*, 20(4):342–349, 2008.

Kaya, DURMUS ALPASLAN, Inan, MEMET, Giray, Elife Sultan, and Kirici, Saliha. Diurnal ontogenetic and morphogenetic variability of lavandula stoechas l. ssp stoechas in east mediterranean region. *Rev Chim*, 63:749–53, 2012.

Kazemi, Masoud, Tabatabaei-Anaraki, Maryam, Rustaiyan, Abdolhossein, Motevalizadeh, Alireza, and Masoudi, Shiva. Chemical composition of the essential oils obtained from the flower, leaf and stem of artemisia campestris l. from iran. *Journal of Essential Oil Research*, 21(3):197–199, 2009.

Kery, A, Tawaij, HAA, and Al-Kazraji, N. Methylated flavones from centaurea phyllocephala l. *Herba Hungarica*, 24:183, 1985.

Khaled-Khodja, Nabyla, Boulekbache-Makhlouf, Lila, and Madani, Khodir. Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some lamiaceae. *Industrial crops and products*, 61:41–48, 2014.

Khlifi, Daycem, Sghaier, Rabiaa Manel, Amouri, Sameh, Laouini, Dhafer, Hamdi, Mokhtar, and Bouajila, Jalloul. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of artemisia herba-alba, ruta chalpensis l. and peganum harmala l. *Food and chemical toxicology*, 55:202–208, 2013.

Kim, Hoon, Ralph, John, Lu, Fachuang, Ralph, Sally A, Boudet, Alain-M, MacKay, John J, Sederoff, Ronald R, Ito, Takashi, Kawai, Shingo, Ohashi, Hideo, *et al.* Nmr analysis of lignins in cad-deficient plants. part 1. incorporation of hydroxycinnamaldehydes and hydroxybenzaldehydes into lignins. *Organic & biomolecular chemistry*, 1(2):268–281, 2003.

Kim, Nam-Sun and Lee, Dong-Sun. Comparison of different extraction methods for the analysis of fragrances from lavandula species by gas chromatography–mass spectrometry. *Journal of Chromatography a*, 982(1):31–47, 2002.

Kinghorn, A Douglas, Pan, Li, Fletcher, Joshua N, and Chai, Heebyung. The relevance of higher plants in lead compound discovery programs. *Journal of natural products*, 74(6):1539–1555, 2011.

Kirmizibekmez, Hasan, Demirci, Betuel, Yeşilada, E, Başer, KH, and Demirci, F. Chemical composition and antimicrobial activity of the essential oils of lavandula stoechas l. ssp. stoechas growing wild in turkey. *Natural product communications*, 4(7):1001–1006, 2009.

Koca, Ufuk, Süntar, Ipek Peşin, Keles, Hikmet, Yesilada, Erdem, and Akkol, Esra Küpeli. In vivo anti-inflammatory and wound healing activities of centaurea iberica trev. ex spreng. *Journal of ethnopharmacology*, 126(3):551–556, 2009.

Kokkalou, Eugenios. constituents of the essential oil from lavandula stoechas growing wild in greece. *Planta medica*, 1988.

Korkmaz, H and Gürdal, A. Effect of artemisia santonicum l. on blood glucose in normal and alloxan-induced diabetic rabbits. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 16(7):675–676, 2002.

Köse, Y Bülent, İşcan, G, Demirci, B, Başer, KHC, and Celik, S. Antimicrobial activity of the essential oil of centaurea aladagensis. *Fitoterapia*, 78(3):253–254, 2007.

Köse, Yavuz Bülent, İşcan, Gökalp, Göger, Fatih, Akalın, Gülşen, Demirci, Betül, and Başer, Kemal Hüsnü Can. Chemical composition and biological activity of centaurea baseri: new species from turkey. *Chemistry & biodiversity*, 13(10):1369–1379, 2016.

Koshy, M, Vijayananthan, A, and Vadiveloo, V. Malignant ovarian mixed germ cell tumour: a rare combination. *Biomedical imaging and intervention journal*, 1(2), 2005.

Koukoulitsa, Ekaterini, Skaltsa, Helen, Karioti, Anastasia, Demetzos, Costas, and Dimas, Kostas. Bioactive sesquiterpene lactones from centaurea species and their cytotoxic/cytostatic activity against human cell lines in vitro. *Planta medica*, 68(07):649–652, 2002.

Koulivand, PH, Ghadiri, M Khaleghi, and Gorji, A. Lavender and the nervous system, evid. *Based Complement. Altern. Med*, 681304, 2013.

Kramer, PJ and Kozlowski, TT. Physiology of woody plants academic press. *New York*, 811, 1979.

Kujawska, Monika and Hilgert, Norma I. Phytotherapy of polish migrants in misiones, argentina: Legacy and acquired plant species. *Journal of Ethnopharmacology*, 153(3):810–830, 2014.

Kumarasamy, Y, Middleton, M, Reid, RG, Nahar, L, and Sarker, SD. Biological activity of serotonin conjugates from the seeds of centaurea nigra. *Fitoterapia*, 74(6):609–612, 2003.

Kumari, Aruna and Sharma, RA. A review on millingtonia hortensis linn. *Int. J. Pharm. Sci. Rev. Res*, 19(2):85–92, 2013.

Kurman, RJ. Origin and molecular pathogenesis of ovarian high-grade serous carcinoma. *Annals of Oncology*, 24(suppl_10):x16–x21, 2013.

Kurman, Robert J and Shih, Ie-Ming. The origin and pathogenesis of epithelial ovarian cancer-a proposed unifying theory. *The American journal of surgical pathology*, 34(3):433, 2010.

Lazari, Diamanto M, Skaltsa, Helen D, and Constantinidis, Theophanis. Volatile constituents of centaurea raphanina sm. subsp. mixta (dc.) runemark and c. spruneri boiss. & heldr.(asteraceae), growing wild in greece. *Flavour and fragrance journal*, 14(6):415–418, 1999.

Lazari, Diamanto M, Skaltsa, Helen D, and Constantinidis, Theophanis. Volatile constituents of centaurea pelia dc., c. thessala hausskn. subsp. drakiensis (freyn & sint.) georg. and c. zuccariniana dc. from greece. *Flavour and fragrance journal*, 15(1):7–11, 2000.

Ledermann, Jonathan, Harter, Philipp, Gourley, Charlie, Friedlander, Michael, Vergote, Ignace, Rustin, Gordon, Scott, Clare, Meier, Werner, Shapira-Frommer, Ronnie, Safra, Tamar, *et al.* Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *New England Journal of Medicine*, 366(15):1382–1392, 2012.

Lee, Chia-Jung, Chen, Lih-Geeng, Chang, Ting-Lin, Ke, Wei-Ming, Lo, Ying-Fei, and Wang, Ching-Chiung. The correlation between skin-care effects and phytochemical contents in lamiaceae plants. *Food chemistry*, 124(3):833–841, 2011.

Leporatti, Maria Lucia and Ghedira, Kamel. Comparative analysis of medicinal plants used in traditional medicine in italy and tunisia. *Journal of Ethnobiology and Ethnomedicine*, 5(1):31, 2009.

Lerrick, Reinner Ishaq, Purwono, Bambang, and Matsjeh, Sabirin. The synthesis of 4-hydroxy-3-methoxybenzonitrile from vanillin: a precursor of synthesis of 3 -methoxydaidzein. In *Proceeding of the International Seminar on Chemistry*, pages 30–31, 2008.

Li, Wei, Asada, Yoshihisa, Koike, Kazuo, Nikaido, Tamotsu, Furuya, Tsutomu, and Yoshikawa, Takafumi. Bellisosides a–f, six novel acylated triterpenoid saponins from bellis perennis (compositae). *Tetrahedron*, 61(11):2921–2929, 2005.

Li, Wen-Wu and Barz, Wolfgang. Biotechnological production of two new 8, 4-oxynorneolignans by elicitation of echinacea purpurea cell cultures. *Tetrahedron letters*, 46(17):2973–2977, 2005.

Li, Wen-Wu and Barz, Wolfgang. Structure and accumulation of phenolics in elicited echinacea purpurea cell cultures. *Planta medica*, 72(3):248, 2006.

Li, Wen-Wu, Johnson-Ajinwo, Okiemute Rosa, Uche, Fidelia Ijeoma, *et al.* Advances of plant-derived natural products in ovarian cancer therapy. *International Journal of Cancer Research and Prevention*, 9(1):81–105, 2016.

Lis-Balchin, M Lavander. The genus lavandula. *Medicinal and Aromatic Plants–Industrial Profiles. London*, page 268, 2002.

Liu, He, Zhang, Yupu, Sun, Yantao, Wang, Xue, Zhai, Yujuan, Sun, Ye, Sun, Shuo, Yu, Aimin, Zhang, Hanqi, and Wang, Yinghua. Determination of the major constituents in fruit of arctium lappa l. by matrix solid-phase dispersion extraction coupled with hplc separation and fluorescence detection. *Journal of Chromatography B*, 878(28):2707–2711, 2010.

Loughrin, John H, Manukian, ARA, Heath, Robert R, Turlings, TC, and Tumlinson, James H. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proceedings of the National Academy of Sciences*, 91(25):11836–11840, 1994.

Lukanova, Annekatrin and Kaaks, Rudolf. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. *Cancer Epidemiology and Prevention Biomarkers*, 14(1):98–107, 2005.

MacEwan, David J. Tnf ligands and receptors–a matter of life and death. *British journal of pharmacology*, 135(4):855–875, 2002.

Mahmoudi, Y. La thérapeutique par les plantes communes en algérie. *Palais du livre, Blida*, 1990.

Mans, Dennis RA, Da Rocha, Adriana B, and Schwartsmann, Gilberto. Anti-cancer drug discovery and development in brazil: targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. *The oncologist*, 5(3):185–198, 2000.

Marongiu, Bruno, Piras, Alessandra, Porcedda, Silvia, Falconieri, Danilo, Maxia, Andrea, Frau, Maria Assunta, Molicotti, Paola, and Zanetti, Stefania. Composition and biological activity of supercritical co2 extract of some lamiaceae growing wild in sardinia (italy). *Journal of Essential Oil Bearing Plants*, 13(5):625–632, 2010.

Martín, J, Torrell, M, Korobkov, AA, and Vallès, J. Palynological features as a systematic marker in artemisia l. and related genera (asteraceae, anthemideae)-ii: Implications for subtribe artemisiinae delimitation. *Plant Biology*, 5(1):85–93, 2003.

Marzoug, Hajer Naceur Ben, Romdhane, Mehrez, Lebrihi, Ahmed, Mathieu, Florence, Couderc, François, Abderraba, Manef, Khouja, Mohamed Larbi, and Bouajila, Jalloul. Eucalyptus oleosa essential oils: chemical composition and antimicrobial and antioxidant activities of the oils from different plant parts (stems, leaves, flowers and fruits). *Molecules*, 16(2):1695–1709, 2011.

Mason, Edna. Note on the presence of mycorrhiza in the roots of salt marsh plants. *New Phytologist*, 27(3):193–195, 1928.

Massada, Y. Analysis of essential oil by gas chromatography and spectrometry. *John Willey & Sons, New York, NY*, page 334, 1976.

Masso, JL and Adzet, T. Hypoglycaemic activity of centaurea aspera l (author's transl). *Revista espanola de fisiologia*, 32(4):313–316, 1976.

Matondo, Abel, Jo, Yong Hwa, Shahid, Muhammad, Choi, Tae Gyu, Nguyen, Minh Nam, Nguyen, Ngoc Ngo Yen, Akter, Salima, Kang, Insug, Ha, Joohun, Maeng, Chi Hoon, *et al.* The prognostic 97 chemoresponse gene signature in ovarian cancer. *Scientific reports*, 7(1):9689, 2017.

Matos, Fabíola, Miguel, Maria G, Duarte, João, Venâncio, Florência, Moiteiro, Cristina, Correia, Ana ID, Figueiredo, A Cristina, Barroso, José G, and Pedro, Luís G. Antioxidant capacity of the essential oils from lavandula luisieri, l. stoechas subsp. lusitanica, l. stoechas subsp. lusitanica x l. luisieri and l. viridis grown in algarve (portugal). *Journal of Essential Oil Research*, 21(4): 327–336, 2009.

McGrogan, Barbara T, Gilmartin, Breege, Carney, Desmond N, and McCann, Amanda. Taxanes, microtubules and chemoresistant breast cancer. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1785(2):96–132, 2008.

McWhinney, Sarah R, Goldberg, Richard M, and McLeod, Howard L. Platinum neurotoxicity pharmacogenetics. *Molecular cancer therapeutics*, 8(1):10–16, 2009.

Médail, F and Quézel, P. Conséquences écologiques possibles des changements climatiques sur la flore et la végétation du bassin méditerranéen. *Bocconea*, 16(1):000–000, 2003.

Medimagh, Sined, Daami-Remadi, Mejda, Jabnoun-Khiareddine, Hayfa, Jannet, Hichem Ben, *et al.* Chemical composition, antimicrobial and anti-acetylcholinesterase activities of essential

oils from the tunisian asteriscus maritimus (l.) less. *Mediterranean Journal of Chemistry*, 2(2): 459–470, 2012.

Medimagh, Sined, Daami-Remadi, Mejda, Jabnoun-Khiareddine, Hayfa, Naffati, Mohamed, Ben Jannet, Hichem, and Hamza, M'hamed Ali. Chemical composition and in vitro evaluation of antimicrobial and anti-acetylcholinesterase activities of the root oil from asteriscus maritimus (l.) less growing in tunisia. *Journal of Essential Oil Bearing Plants*, 16(4):443–450, 2013.

Medimagh-Saidana, S, Daami-Remadi, M, Abreu, P, Harzallah-Skhiri, F, Ben Jannet, H, and Hamza, MA. Asterisulphoxide and asterisulphone: two new antibacterial and antifungal metabolites from the tunisian asteriscus maritimus (l.) less. *Natural product research*, 28(18):1418–1426, 2014.

Medjroubi, Kamel, Benayache, Fadila, and Bermejo, Jaime. Sesquiterpene lactones from centaurea musimomum. antiplasmodial and cytotoxic activities. *Fitoterapia*, 76(7-8):744–746, 2005.

Megdiche-Ksouri, Wided, Trabelsi, Najla, Mkadmini, Khaoula, Bourgou, Soumaya, Noumi, Amira, Snoussi, Mejdi, Barbria, Rahma, Tebourbi, Olfa, and Ksouri, Riadh. Artemisia campestris phenolic compounds have antioxidant and antimicrobial activity. *Industrial Crops and Products*, 63:104–113, 2015.

Melguizo-Melguizo, D, Diaz-de Cerio, E, Quirantes-Piné, R, Švarc-Gajić, J, and Segura-Carretero, A. The potential of artemisia vulgaris leaves as a source of antioxidant phenolic compounds. *Journal of functional foods*, 10:192–200, 2014.

Messaoud, C, Chograni, H, and Boussaid, M. Chemical composition and antioxidant activities of essential oils and methanol extracts of three wild lavandula l. species. *Natural product research*, 26(21):1976–1984, 2012.

Miara, MD, Boutabia, L, Telaïlia, S, and Vela, E. Apparition de senecio angulatus (asteraceae) en algérie. 2018.

Miguel, M Graça, Duarte, João, Figueiredo, A Cristina, Barroso, José G, and Pedro, Luis G. Thymus carnosus boiss.: effect of harvesting period, collection site and type of plant material on essential oil composition. *Journal of Essential Oil Research*, 17(4):422–426, 2005.

Miladi, Hanene, Slama, Rihab Ben, Mili, Donia, Zouari, Sami, Bakhrouf, Amina, and Ammar, Emna. Essential oil of thymus vulgaris l. and rosmarinus officinalis l.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens. *Natural Science*, 5(6):729–739, 2013.

Miliauskas, G, Venskutonis, PR, and Van Beek, TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, 85(2):231–237, 2004.

Mimica-Dukić, Neda, Božin, Biljana, Soković, Marina, Mihajlović, Biserka, and Matavulj, Milan. Antimicrobial and antioxidant activities of three mentha species essential oils. *Planta medica*, 69(05):413–419, 2003.

Minami, Motoyasu, Suzuki, Minori, Hosokawa, Keizo, Kondo, Seizo, Oka, Kenji, and Shibata, Toshiro. Preliminary survey of taxonomical problems, pharmacognostical characteristics, and chloroplast dna polymorphisms of the folk medicinal herb artemisia campestris from the ryukyu islands, japan. *Journal of natural medicines*, 64(2):239–244, 2010.

Minisini, AM, Tosti, A, Sobrero, AF, Mansutti, M, Piraccini, BM, Sacco, C, and Puglisi, F. Taxane-induced nail changes: incidence, clinical presentation and outcome. *Annals of Oncology*, 14(2): 333–337, 2003.

Mohsen, Haouari and Ali, Ferchichi. Essential oil composition of artemisia herba-alba from southern tunisia. *Molecules*, 14(4):1585–1594, 2009.

Mokhtarzadeh, Sam, Demirci, Betül, Ağalar, Hale Gamze, Khawar, Khalid Mahmood, and Kırımer, Nese. In vitro propagation and volatile compound characterization of lavandula stoechas l. subsp. stoechas-an economically important source of essential oil. 2018.

Moon, Therese, Cavanagh, Heather MA, and Wilkinson, Jenny M. Antifungal activity of australian grown lavandula spp. essential oils against aspergillus nidulans, trichophyton mentagrophytes, leptosphaeria maculans and sclerotinia sclerotiorum. *Journal of Essential Oil Research*, 19(2): 171–175, 2007.

Morat, Philippe, Jaffré, Tanguy, Tronchet, Frédéric, Munzinger, Jérôme, Pillon, Yohan, Veillon, Jean-Marie, Chalopin, Monique, Birnbaum, Philippe, Rigault, Frédéric, Dagostini, Gilles, *et al.* Le référentiel taxonomique florical et les caractéristiques de la flore vasculaire indigène de la nouvelle-calédonie. *Adansonia*, 34(2):179–222, 2011.

Morikawa, Toshio, Li, Xuezheng, Nishida, Eriko, Ito, Yuki, Matsuda, Hisashi, Nakamura, Seikou, Muraoka, Osamu, and Yoshikawa, Masayuki. Perennisosides i- vii, acylated triterpene saponins with antihyperlipidemic activities from the flowers of bellis perennis. *Journal of natural products*, 71(5):828–835, 2008.

Morikawa, Toshio, Li, Xuezheng, Nishida, Eriko, Nakamura, Seikou, Ninomiya, Kiyofumi, Matsuda, Hisashi, Oda, Yoshimi, Muraoka, Osamu, and Yoshikawa, Masayuki. Medicinal flowers. part 29. acylated oleanane-type triterpene bisdesmosides: Perennisaponins g, h, i, j, k, l, and m with pancreatic lipase inhibitory activity from the flowers of bellis perennis. *Helvetica Chimica Acta*, 93(3):573–586, 2010.

Morikawa, Toshio, Li, Xuezheng, Nishida, Eriko, Nakamura, Seikou, Ninomiya, Kiyofumi, Matsuda, Hisashi, Hamao, Makoto, Muraoka, Osamu, Hayakawa, Takao, and Yoshikawa, Masayuki. Medicinal flowers. xxxii. *Chemical and Pharmaceutical Bulletin*, 59(7):889–895, 2011.

Morley, Karen L, Ferguson, Peter J, and Koropatnick, James. Tangeretin and nobiletin induce g1 cell cycle arrest but not apoptosis in human breast and colon cancer cells. *Cancer letters*, 251 (1):168–178, 2007.

Mouhid, Lamia, Corzo-Martínez, Marta, Torres, Carlos, Vázquez, Luis, Reglero, Guillermo, Fornari, Tiziana, and Ramírez de Molina, Ana. Improving in vivo efficacy of bioactive molecules: An overview of potentially antitumor phytochemicals and currently available lipid-based delivery systems. *Journal of oncology*, 2017, 2017.

Moura, Ana Paula Gomes, Beltrão, Daiene Martins, Pita, João Carlos Lima Rodrigues, Xavier, Aline Lira, Brito, Monalisa Taveira, Sousa, Tatyanna Kelvia Gomes de, Batista, Leônia Maria, Carvalho, João Ernesto de, Ruiz, Ana Lúcia Tasca Gois, Della Torre, Adriana, *et al.* Essential oil from fruit of xylopia langsdorffiana: antitumour activity and toxicity. *Pharmaceutical biology*, 54(12):3093–3102, 2016.

Msaada, Kamel, Salem, Nidhal, Tammar, Sonia, Hammami, Majdi, Jamal Saharkhiz, Mohamed, Debiche, Nizar, Limam, Ferid, and Marzouk, Brahim. Essential oil composition of lavandula dentata, l. stoechas and l. multifida cultivated in tunisia. *Journal of Essential Oil Bearing Plants*, 15(6):1030–1039, 2012.

Muñoz-Bertomeu, Jesús, Arrillaga, Isabel, and Segura, Juan. Essential oil variation within and among natural populations of lavandula latifolia and its relation to their ecological areas. *Biochemical systematics and Ecology*, 35(8):479–488, 2007.

Nakajima, Yoshimi, Shimazawa, Masamitsu, Mishima, Satoshi, and Hara, Hideaki. Water extract of propolis and its main constituents, caffeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions. *Life sciences*, 80(4):370–377, 2007.

Nam, Eun Ji, Yun, Mi Jin, Oh, Young Taik, Kim, Jae Wook, Kim, Jae Hoon, Kim, Sunghoon, Jung, Yong Wook, Kim, Sang Wun, and Kim, Young Tae. Diagnosis and staging of primary ovarian cancer: correlation between pet/ct, doppler us, and ct or mri. *Gynecologic oncology*, 116(3): 389–394, 2010.

Negrete, Rosa E, Backhouse, Nadine, Cajigal, Ivan, Delporte, Carla, Cassels, Bruce K, Breitmaier, Eberhard, and Eckhardt, Gert. Two new antiinflammatory elemanolides from centaurea chilensis. *Journal of ethnopharmacology*, 40(3):149–153, 1993.

Newman, David J and Cragg, Gordon M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of natural products*, 75(3):311–335, 2012.

NEY, Peter and BOLAND, Wilhelm. Biosynthesis of 1-alkenes in higher plants: A model study with the composite carthamus tinctorius l. *European journal of biochemistry*, 162(1):203–211, 1987.

Ninomiya, Kiyofumi, Motai, Chiaki, Nishida, Eriko, Kitagawa, Niichiro, Yoshihara, Kazuya, Hayakawa, Takao, Muraoka, Osamu, Li, Xuezheng, Nakamura, Seikou, Yoshikawa, Masayuki, *et al.* Acylated oleanane-type triterpene saponins from the flowers of bellis perennis show antiproliferative activities against human digestive tract carcinoma cell lines. *Journal of natural medicines*, 70(3):435–451, 2016.

Noumi, Zouheir, Dhaou, Saad Ouled, Derbel, SALMA, Chaieb, Mohamed, *et al.* The status of asteraceae in the arid and saharan flora of north african region: case of tunisia. *Pak J Bot*, 42(3): 1417–22, 2010.

Oberprieler, Christoph, Himmelreich, S, Källersjö, M, Vallès, J, Watson, LE, and Vogt, Robert. Tribe anthemideae cass. 2009.

OBrien, TrP, Feder, N, and McCully, Mi E. Polychromatic staining of plant cell walls by toluidine blue o. *Protoplasma*, 59(2):368–373, 1964.

of North America Editorial Committee, Flora. *Flora of North America: Volume 23: Magnoliophyta: Commelinidae (in Part): Cyperaceae*, volume 23. Oxford University Press on Demand, 1993.

Oladipupo, Lawal and Adebola, Oyedeji. Chemical composition of the essential oils of the flowers, leaves and stems of two senecio polyanthemoides sch. bip. samples from south africa. *Molecules*, 14(6):2077–2086, 2009.

Orav, Anne, Raal, Ain, Arak, Elmar, Muurisepp, M, and Kailas, Tiiu. Composition of the essential oil of artemisia absinthium l. of different geographical origin. In *Proceedings-estonian Academy of Sciences Chemistry*, volume 55, page 155. TRUEKITUD OU, 2006.

Organization, World Health. *National cancer control programmes: policies and managerial guidelines*. World Health Organization, 2002.

Organization, World Health, on Biological Standardization, WHO Expert Committee, *et al. WHO Expert Committee on Biological Standardization: sixty-eighth report*. World Health Organization, 2018.

Orhan, I Erdogan, Belhattab, R, Şenol, FS, Gülpinar, AR, Hoşbaş, S, and Kartal, M. Profiling of cholinesterase inhibitory and antioxidant activities of artemisia absinthium, a. herba-alba, a. fragrans, marrubium vulgare, m. astranicum, origanum vulgare subsp. glandulossum and essential oil analysis of two artemisia species. *Industrial crops and products*, 32(3):566–571, 2010.

Orhan, Ilkay, Kartal, Murat, Tosun, Fatma, and Şener, Bilge. Screening of various phenolic acids and flavonoid derivatives for their anticholinesterase potential. *Zeitschrift für Naturforschung C*, 62(11-12):829–832, 2007.

Ouyahya, A. Clé de détermination des espèces marocaines du genre artemisia l. *Al Biruniya Rev. Mar. Pharm.*, 6:31–91, 1990.

Özdemir, Bahittin Bozdağ Canan and Sepet, Hakan. Karyotype analysis on the species of bellis l.(asteraceae) in turkey. *Caryologia*, 64(3):251–255, 2011.

Ozols, Robert F. Recurrent ovarian cancer: evidence-based treatment. *Journal of Clinical Oncology*, 20(5):1161–1163, 2002.

Palá-Paúl, J, Usano-Alemany, J, and Soria, AC. Essential oil composition of asteriscus maritimus (l.) less. from spain**. *J Plant Biol Soil Health*, 1(2):4, 2014.

Paleri, V, Mehanna, H, and Wight, RG. Tnm classification of malignant tumours 7th edition: what's new for head and neck? *Clinical Otolaryngology*, 35(4):270–272, 2010.

Panagouleas, Constantinos, Skaltsa, Helen, Lazari, Diamanto, Skaltsounis, Alexios-Leandros, and Sokovic, Marina. Antifungal activity of secondary metabolites of centaurea raphanina ssp. mixta, growing wild in greece. *Pharmaceutical biology*, 41(4):266–270, 2003.

Parada, Montse, Carrió, Esperança, Bonet, Maria Àngels, and Vallès, Joan. Ethnobotany of the alt emporda region (catalonia, iberian peninsula): plants used in human traditional medicine. *Journal of Ethnopharmacology*, 124(3):609–618, 2009.

Paré, Paul W and Tumlinson, James H. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant physiology*, 114(4):1161–1167, 1997.

Parmar, MKea, Ledermann, JA, Colombo, N, Delaloye, JF, Kristensen, GB, Wheeler, S, Swart, AM, Qian, W, Torri, V, Floriani, I, *et al.* Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the icon4/ago-ovar-2.2 trial. *Lancet (London, England)*, 361(9375):2099–2106, 2003.

Parvez, Aslam, Choudhary, M Iqbal, Akhter, Farzana, Noorwala, Mushtaq, Mohammad, Faryal V, Hasan, Naim M, Zamir, Talat, and Ahmad, Viqar Uddin. Perovskone: a triterpene with a novel carbon skeleton from perovskia abrotanoides. *The Journal of Organic Chemistry*, 57(16): 4339–4340, 1992.

Patel, MB and Mishra, SH. Hypoglycemic activity of c-glycosyl flavonoid from enicostemma hyssopifolium. *Pharmaceutical biology*, 49(4):383–391, 2011.

Pearce, Celeste Leigh, Chung, Karine, Pike, Malcolm C, and Wu, Anna H. Increased ovarian cancer risk associated with menopausal estrogen therapy is reduced by adding a progestin. *Cancer*, 115 (3):531–539, 2009.

Pezzuto, John M. Plant-derived anticancer agents. *Biochemical pharmacology*, 53(2):121–133, 1997.

Piccaglia, Roberta, Marotti, M, Giovanelli, E, Deans, SG, and Eaglesham, Elizabeth. Antibacterial and antioxidant properties of mediterranean aromatic plants. *Industrial crops and Products*, 2 (1):47–50, 1993.

Piccart, Martine J, Bertelsen, Kamma, James, Keith, Cassidy, Jim, Mangioni, Constantino, Simonsen, Ernst, Stuart, Gavin, Kaye, Stan, Vergote, Ignace, Blom, Rene, *et al.* Randomized intergroup trial of cisplatin–paclitaxel versus cisplatin–cyclophosphamide in women with advanced epithelial ovarian cancer: three-year results. *Journal of the National Cancer Institute*, 92(9):699–708, 2000.

Pieroni, Andrea, Janiak, V, Dürr, CM, Lüdeke, S, Trachsel, E, and Heinrich, Michael. In vitro antioxidant activity of non-cultivated vegetables of ethnic albanians in southern italy. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 16(5):467–473, 2002.

Pignata, S, Cannella, L, Leopardo, D, Bruni, GS, Facchini, G, and Pisano, C. Follow-up with ca125 after primary therapy of advanced ovarian cancer: in favor of continuing to prescribe ca125 during follow-up. *Annals of oncology*, 22(Suppl_8):viii40–viii44, 2011.

Pinato, Odra, Musetti, Caterina, Farrell, Nicholas P, and Sissi, Claudia. Platinum-based drugs and proteins: Reactivity and relevance to dna adduct formation. *Journal of inorganic biochemistry*, 122:27–37, 2013.

Popović, Zorica, Smiljanić, Miroslava, Matić, Rada, Kostić, Miroslav, Nikić, Predrag, and Bojović, Srdjan. Phytotherapeutical plants from the deliblato sands (serbia): traditional pharmacopoeia and implications for conservation. 2012.

Prabhakar, Visakh, Balasubramanian, Ranganathan, Sathe, Priyanka, Murali Krishna, C, and Juvekar, Aarti. In vitro anticancer activity of monosubstituted chalcone derivatives. *Int. J. Tumor Ther*, 3(1):1–9, 2014.

Quézel, Pierre and Santa, Sébastien. Nouvelle flore de l'algérie et des régions désertiques méridionales. Technical report, 1963.

Quezel, Pierre, Santa, Sébastien, Schotter, O, *et al.* Nouvelle flore de l'algerie et des regions desertiques meridionales-v. 1-2. 1962.

Quirk, Jeffrey T and Natarajan, Nachimuthu. Ovarian cancer incidence in the united states, 1992–1999. *Gynecologic oncology*, 97(2):519–523, 2005.

Radulović, Niko S, Blagojević, Polina D, Zlatković, Bojan K, and Palić, Radosav M. Chemotaxonomically important volatiles of the genus anthemis l.–a detailed gc and gc/ms analyses of anthemis segetalis ten. from montenegro. *Journal of the Chinese Chemical Society*, 56(3): 642–652, 2009.

Rafie, Shahram, Namjoyan, Forough, Golfakhrabadi, Fereshteh, Yousefbeyk, Fatemeh, and Hassanzadeh, Alireza. Effect of lavender essential oil as a prophylactic therapy for migraine: a randomized controlled clinical trial. *Journal of Herbal Medicine*, 6(1):18–23, 2016.

Rajeswaran, Anand, Trojan, Andreas, Burnand, Bernard, and Giannelli, Massimo. Efficacy and side effects of cisplatin-and carboplatin-based doublet chemotherapeutic regimens versus non-platinum-based doublet chemotherapeutic regimens as first line treatment of metastatic non-small cell lung carcinoma: a systematic review of randomized controlled trials. *Lung cancer*, 59(1): 1–11, 2008.

Ramdane, Farah, Essid, Rym, Mkadmini, Khaoula, Hammami, Majdi, Fares, Nadia, Mahammed, Mahfoud Hadj, El Ouassis, Dahmane, Tabbene, Olfa, Limam, Ferid, and Hadj, Mohamed Didi Ould. Phytochemical composition and biological activities of asteriscus graveolens (forssk) extracts. *Process Biochemistry*, 56:186–192, 2017.

Ramirez, Ingrid, Chon, Hye Sook, and Apte, Sachin M. The role of surgery in the management of epithelial ovarian cancer. *Cancer Control*, 18(1):22–30, 2011.

Rauter, Amélia P, Branco, Isabel, Bermejo, Jaime, González, Antonio G, Garcia-Grávalos, Maria D, and San Feliciano, Arturo. Bioactive humulene derivatives from asteriscus vogelii. *Phytochemistry*, 56(2):167–171, 2001.

Rebbas, K and Bounar, R. Études floristique et ethnobotanique des plantes médicinales de la région de m'sila (algérie). *Phytothérapie*, 12(5):284–291, 2014.

Reverchon, Ernesto and Senatore, Felice. Supercritical carbon dioxide extraction of chamomile essential oil and its analysis by gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, 42(1):154–158, 1994.

Riahi, Leila, Chograni, Hnia, Elferchichi, Miryam, Zaouali, Yosr, Zoghlami, Nejia, and Mliki, Ahmed. Variations in tunisian wormwood essential oil profiles and phenolic contents between leaves and flowers and their effects on antioxidant activities. *Industrial crops and products*, 46: 290–296, 2013.

Riedel, Heidi, Cai, Zhenzhen, Kütük, Onur, and Smetanska, Iryna. Obtaining phenolic acids from cell cultures of various artemisia species. *African Journal of Biotechnology*, 9(51):8805–8809, 2010.

Risch, Harvey A. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *Journal of the National Cancer Institute*, 90(23):1774–1786, 1998.

Risinger, April L, Riffle, Stephen M, Lopus, Manu, Jordan, Mary A, Wilson, Leslie, and Mooberry, Susan L. The taccalonolides and paclitaxel cause distinct effects on microtubule dynamics and aster formation. *Molecular cancer*, 13(1):41, 2014.

Ristorcelli, Dominique, Tomi, Félix, and Casanova, Joseph. 13c-nmr as a tool for identification and enantiomeric differentiation of major terpenes exemplified by the essential oil of lavandula stoechas l. ssp. stoechas. *Flavour and fragrance journal*, 13(3):154–158, 1998.

Roh, Sang Young, Hong, Sook Hee, Ko, Yoon Ho, Kim, Tae Hee, Lee, Myung Ah, Shim, Byoung Yong, Byun, Jae Ho, Woo, In Sook, Kang, Jin Hyoung, Hong, Young Seon, *et al.* Clinical characteristics of primary peritoneal carcinoma. *Cancer research and treatment: official journal of Korean Cancer Association*, 39(2):65, 2007.

Rooth, Carolyn. Ovarian cancer: risk factors, treatment and management. *British journal of nursing*, 22(Sup17):S23–S30, 2013.

Rosen, Daniel G, Yang, Gong, Liu, Guangzhi, Mercado-Uribe, Imelda, Chang, Bin, Xiao, Xue Sherry, Zheng, Jingfang, Xue, Feng-Xia, and Liu, Jinsong. Ovarian cancer: pathology, biology, and disease models. *Frontiers in bioscience: a journal and virtual library*, 14:2089, 2009.

Rotblatt, Michael and Ziment, Irwin. *Evidence-based Herbal Medicine. 1*. Philadelphia, PA: Hanley & Belfus, 2002.

Rusak, Gordana, Krajačić, Mladen, and Pleše, Nada. Inhibition of tomato bushy stunt virus infection using a quercetagetin flavonoid isolated from centaurea rupestris l. *Antiviral research*, 36(2): 125–129, 1997.

Saadi, A, Brada, M, Kouidri, M, Dekkiche, H, and Attar, F. Chemical composition and content of essential oil of lavandula multifida from algeria. *Chemistry of Natural Compounds*, 52(1): 162–164, 2016.

Safarzadeh, Elham, Shotorbani, Siamak Sandoghchian, and Baradaran, Behzad. Herbal medicine as inducers of apoptosis in cancer treatment. *Advanced pharmaceutical bulletin*, 4(Suppl 1):421, 2014.

Sahranavard, SHAMIM, Naghibi, FARZANEH, and Ghffari, S. Cytotoxic activity of extracts and pure compounds of bryonia aspera. *Int J Pharm Pharmaceut Sci*, 4(3):541–543, 2012.

Saleh, MM, Awad, N, and EL KHOLY, SA. Gas–chromatographic analysis of the volatile oil of centaurea moschata l. 1981.

San Feliciano, A, Barrero, AF, Medarde, M, del Corral, JM Miguel, Aizpiri, A Aramburu, and Sánchez-Ferrando, F. Asteriscunolides a, b, c and d, the first humulanolides; two pairs of conformationally stable stereoisomers. *Tetrahedron*, 40(5):873–878, 1984.

San Feliciano, A, Medarde, M, del Corral, JM Miguel, Aramburu, A, Gordaliza, M, and Barrero, AF. Aquatolide. a new type of humulane-related sesquiterpene lactone. *Tetrahedron Letters*, 30 (21):2851–2854, 1989.

Sarafraz, M and Ahmadi, K. Paraclinical evaluation of side-effects of taxanes on auditory system. *Acta Otorhinolaryngologica Italica*, 28(5):239, 2008.

Sarg, TM, El-Dahmy, SI, Ateya, AM, and Abdel-Fattah, HA. Two new bisabolone hydroperoxides and biological activity of asteriscus graveolens. *Fitoterapia*, 65:241–244, 1994.

Sarker, Satyajit D, Savchenko, Tamara, Whiting, Pensri, Šik, Vladimir, and Dinan, Laurence N. Moschamine, cis-moschamine, moschamindole and moschamindolol: four novel indole alkaloids from centaurea moschata. *Natural Product Letters*, 9(3):189–199, 1997.

Sassi, Ahlem Ben, Harzallah-Skhiri, Fethia, and Aouni, Mahjoub. Investigation of some medicinal plants from tunisia for antimicrobial activities. *Pharmaceutical biology*, 45(5):421–428, 2007.

Scherrer, Ayalla M, Motti, Riccardo, and Weckerle, Caroline S. Traditional plant use in the areas of monte vesole and ascea, cilento national park (campania, southern italy). *Journal of Ethnopharmacology*, 97(1):129–143, 2005.

Schöpke, Th, Hiller, K, Wray, V, and Nimtz, M. Application of ms-ms for the rapid, comparative analysis of saponin mixtures as exemplified by the deacylated and partially deacylated triterpenoid saponins of bellis annua. *Planta medica*, 62(04):336–340, 1996a.

Schöpke, Thomas, Hiller, Karl, Wray, Victor, Köppel, K-D, Yamasaki, Kazuo, and Kasai, Ryoji. Triterpenoid saponins from bellis sylvestris, i. structures of the major deacylsaponins. *Journal of natural products*, 57(9):1279–1282, 1994.

Schöpke, Thomas, Al-Tawaha, Chaled, Wray, Victor, Nimtz, Manfred, Meyer, Anke, and Hiller, Karl. Triterpenoid saponins from the aerial parts of aster bellidiastrum. *Phytochemistry*, 40(5): 1489–1492, 1995.

Schöpke, Thomas, Hiller, Karl, Wray, Victor, Nimtz, Manfred, Yamasaki, Kazuo, and Kasai, Ryoji. Triterpenoid saponins from bellis sylvestris. 2. structures of partially deacylated saponins. *Journal of natural products*, 59(4):355–359, 1996b.

Schorge, John O, Modesitt, Susan C, Coleman, Robert L, Cohn, David E, Kauff, Noah D, Duska, Linda R, and Herzog, Thomas J. Sgo white paper on ovarian cancer: etiology, screening and surveillance. *Gynecologic oncology*, 119(1):7–17, 2010.

Schulte, Paul A and Perera, Frederica P. *Molecular epidemiology: principles and practices*. Academic Press, 1998.

Scognamiglio, Monica, D'Abrosca, Brigida, Fiumano, Vittorio, Chambery, Angela, Severino, Valeria, Tsafantakis, Nikolaos, Pacifico, Severina, Esposito, Assunta, and Fiorentino, Antonio. Oleanane saponins from bellis sylvestris cyr. and evaluation of their phytotoxicity on aegilops geniculata roth. *Phytochemistry*, 84:125–134, 2012.

Scognamiglio, Monica, Buommino, Elisabetta, Coretti, Lorena, Graziani, Vittoria, Russo, Rosita, Caputo, Pina, Donnarumma, Giovanna, Fiorentino, Antonio, *et al.* Phytochemical investigation and antimicrobial assessment of bellis sylvestris leaves. *Phytochemistry Letters*, 17:6–13, 2016.

Scully, Robert E. Classification of human ovarian tumors. *Environmental Health Perspectives*, 73: 15, 1987.

Sebai, Hichem, Jabri, Mohamed-Amine, Souli, Abdelaziz, Hosni, Karim, Selmi, Slimen, Tounsi, Haifa, Tebourbi, Olfa, Boubaker, Samir, El-Benna, Jamel, and Sakly, Mohsen. Protective effect of artemisia campestris extract against aspirin-induced gastric lesions and oxidative stress in rat. *Rsc Advances*, 4(91):49831–49841, 2014.

Seddik, Khennouf, Nadjet, Iratni, Daoud, Baghiani Abderrahmane Harzallah, and Lekhmici, Arrar. Antioxidant and antibacterial activities of extracts from artemisia herba alba asso. leaves and some phenolic compounds. *Journal of Medicinal Plants Research*, 4(13):1273–1280, 2010.

Seghiri, Ramdane, Boumaza, Ouahiba, Mekkiou, Ratiba, Benayache, Samir, Mosset, Paul, Quintana, Jose, Estevez, Francisco, Leon, Francisco, Bermejo, Jaime, and Benayache, Fadila. A flavonoid with cytotoxic activity and other constituents from centaurea africana. *Phytochemistry Letters*, 2(3):114–118, 2009.

Senatore, Felice and Bruno, Maurizio. Composition of the essential oil of pallenis spinosa (l.) cass.(asteraceae). *Flavour and fragrance journal*, 18(3):195–197, 2003.

Senatore, Felice, Rigano, Daniela, De Fusco, Raffaele, and Bruno, Maurizio. Volatile components of centaurea cineraria l. subsp. umbrosa (lacaita) pign. and centaurea napifolia l.(asteraceae), two species growing wild in sicily. *Flavour and fragrance journal*, 18(3):248–251, 2003.

Senatore, Felice, Landolfi, Sergio, Celik, Sezgin, and Bruno, Maurizio. Volatile components of centaurea calcitrapa l. and centaurea sphaerocephala l. ssp. sphaerocephala, two asteraceae growing wild in sicily. *Flavour and fragrance journal*, 21(2):282–285, 2006.

Senatore, Frank J and Dasanu, Constantin A. Synchronous gastric and ampullary adenocarcinomas in a hairy cell leukemia patient treated with pentostatin eight years prior. *Journal of Oncology Pharmacy Practice*, 22(3):543–547, 2016.

Senatore, Frank J, Murali, Shruti, and Dasanu, Constantin A. Accelerated premalignant polyposis and second colon cancers: incriminating immunosuppression, radiotherapy, and systemic chemotherapy agents. *Journal of gastrointestinal cancer*, 47(2):152–156, 2016.

Senhaji, B, Ben Hmamou, D, Salghi, R, Zarrouk, A, Chebli, B, Zarrok, H, Warad, I, Hammouti, B, and Al-Deyab, SS. Asteriscus imbricatus extracts: Antifungal activity and anticorrosion inhibition. *Int J Electrochem Sci*, 8:6033–6046, 2013.

Sertkaya, Erdal, Kaya, Kamuran, and Soylu, Soner. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (tetranychus cinnabarinus boisd.)(acarina: Tetranychidae). *Industrial Crops and Products*, 31(1):107–112, 2010.

Sharafzadeh, Shahram and Zare, Mahdi. Effect of drought stress on qualitative and quantitative characteristics of some medicinal plants from lamiaceae family: a review. *Advances in Environmental Biology*, 5(8):2058–2062, 2011.

Shemluck, Melvin. Medicinal and other uses of the compositae by indians in the united states and canada. *Journal of ethnopharmacology*, 5(3):303–358, 1982.

Shoeb, Mohammad, Celik, Sezgin, Jaspars, Marcel, Kumarasamy, Yashodharan, MacManus, Stephen M, Nahar, Lutfun, Thoo-Lin, Paul K, and Sarker, Satyajit D. Isolation, structure elucidation and bioactivity of schischkiniin, a unique indole alkaloid from the seeds of centaurea schischkinii. *Tetrahedron*, 61(38):9001–9006, 2005.

Shoeb, Mohammad, MacManus, Stephen M, Kumarasamy, Yashodharan, Jaspars, Marcel, Nahar, Lutfun, Thoo-Lin, Paul Kong, Nazemiyeh, Hossein, and Sarker, Satyajit D. Americanin, a bioactive dibenzylbutyrolactone lignan, from the seeds of centaurea americana. *Phytochemistry*, 67(21):2370–2375, 2006.

Shoeb, Mohammad, Jaspars, Marcel, MacManus, Stephen M, Celik, Sezgin, Nahar, Lutfun, Kong-Thoo-Lin, Paul, and Sarker, Satyajit D. Anti-colon cancer potential of phenolic compounds from the aerial parts of centaurea gigantea (asteraceae). *Journal of natural medicines*, 61(2):164, 2007a.

Shoeb, Mohammad, MacManus, Stephen M, Jaspars, Marcel, Kong-Thoo-Lin, Paul, Nahar, Lutfun, Celik, Sezgin, and Sarker, Satyajit D. Bioactivity of two turkish endemic centaurea species, and their major constituents. *Revista Brasileira de Farmacognosia*, 17(2):155–159, 2007b.

Siddhuraju, Perumal and Becker, Klaus. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (moringa oleifera lam.) leaves. *Journal of agricultural and food chemistry*, 51(8):2144–2155, 2003.

Silva, JS, Moura, MD, Oliveira, RAG, Diniz, MFF, and Barbosa-Filho, JM. Natural product inhibitors of ovarian neoplasia. *Phytomedicine*, 10(2-3):221–232, 2003.

Singh, Harminder Pal, Mittal, Sunil, Kaur, Shalinder, Batish, Daizy R, and Kohli, Ravinder K. Chemical composition and antioxidant activity of essential oil from residues of artemisia scoparia. *Food chemistry*, 114(2):642–645, 2009.

Skliar, MI, Toribio, MS, and Oriani, DS. Antimicrobial activity of centaurea diffusa. *Fitoterapia*, 76(7-8):737–739, 2005.

Skoula, Melpomeni, Abidi, Chedly, and Kokkalou, Eugene. Essential oil variation of lavandula stoechas l. ssp. stoechas growing wild in crete (greece). *Biochemical Systematics and Ecology*, 24(3):255–260, 1996.

Snader, M and McCloud, Thomas G. Ethnobotany and drug discovery: the experience of the us national cancer institute. *Ethnobot. Search New Drugs*, 185:178, 1994.

Sobiczewski, Piotr, Dańska-Bidzińska, Anna, Rzepka, Jakub, Kupryjańczyk, Jolanta, Gujski, Mariusz, Bidziński, Mariusz, and Michalski, Wojciech. Evaluation of selected ultrasonographic parameters and marker levels in the preoperative differentiation of borderline ovarian tumors and ovarian cancers. *Archives of gynecology and obstetrics*, 286(6):1513–1519, 2012.

Sonmezdag, Ahmet Salih, Kelebek, Hasim, and Selli, Serkan. Identification of aroma compounds of lamiaceae species in turkey using the purge and trap technique. *Foods*, 6(2):10, 2017.

Sprowl, Jason A, Reed, Kerry, Armstrong, Stephen R, Lanner, Carita, Guo, Baoqing, Kalatskaya, Irina, Stein, Lincoln, Hembruff, Stacey L, Tam, Adam, and Parissenti, Amadeo M. Alterations in tumor necrosis factor signaling pathways are associated with cytotoxicity and resistance to taxanes: a study in isogenic resistant tumor cells. *Breast Cancer Research*, 14(1):R2, 2012.

Stanev, S, Zagorcheva, T, and Atanassov, I. Lavender cultivation in bulgaria 21st century developments, breeding challenges and opportunities. *Bulgarian Journal of Agricultural Science*, 22(4): 584–590, 2016.

Stavropoulou, Maria I, Angelis, Apostolis, Aligiannis, Nektarios, Kalpoutzakis, Eleftherios, Mitakou, Sofia, Duke, Stephen O, and Fokialakis, Nikolas. Phytotoxic triterpene saponins from bellis longifolia, an endemic plant of crete. *Phytochemistry*, 144:71–77, 2017.

Steinhoff, B. Escop monographs-a scientific basis for herbal medicinal products in europe under specific aspects of the regulatory situation. In *II WOCMAP Congress Medicinal and Aromatic Plants, Part 4: Industrial Processing, Standards & Regulations, Quality, 503*, pages 71–74, 1997.

Stevens, KL and Merrill, GB. Sesquiterpene lactones and allelochemicals from centaurea species. In *ACS Symposium series-American Chemical Society (USA)*, 1985.

Steyn, Krisela, Damasceno, Albertino, *et al.* Lifestyle and related risk factors for chronic diseases. *Disease and mortality in sub-Saharan Africa*, 2:247–65, 2006.

SUCHY, M and HEROUT, V. Identity of the bitter principle from centaurea stoebe (l.) sch. et thell. with cnicin. *Coll. Czech. Chem. Commun*, 27:1510–1512, 1962.

Sudhakar, Akulapalli. History of cancer, ancient and modern treatment methods. *Journal of cancer science & therapy*, 1(2):1, 2009.

Suffness, M and Pezzuto, JM. Methods in plant biochemistry: assays for bioactivity. *Academic Press, London*, pages 71–133, 1990.

Sunil, C, Saxena, A, Choudhury, RK, and Pant, LM. Neutron yield and dose equivalent from heavy ion interactions on thick target. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, 534(3):518–530, 2004.

Sweeney, Jillian C and Soutar, Geoffrey N. Consumer perceived value: The development of a multiple item scale. *Journal of retailing*, 77(2):203–220, 2001.

Tan, Ren Xhiang, Zheng, WF, and Tang, HQ. Biologically active substances from the genus artemisia. *Planta medica*, 64(04):295–302, 1998.

Taylor, Douglas D and Gercel-Taylor, Cicek. Microrna signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecologic oncology*, 110(1):13–21, 2008.

Tempany, Clare MC, Zou, Kelly H, Silverman, Stuart G, Brown, Douglas L, Kurtz, Alfred B, and McNeil, Barbara J. Staging of advanced ovarian cancer: comparison of imaging modalities—report from the radiological diagnostic oncology group. *Radiology*, 215(3):761–767, 2000.

Tlili, Nizar, Elfalleh, Walid, Hannachi, Hedia, Yahia, Yassine, Khaldi, Abdelhamid, Ferchichi, Ali, and Nasri, Nizar. Screening of natural antioxidants from selected medicinal plants. *International journal of food properties*, 16(5):1117–1126, 2013.

Toki, Kenjiro, Saito, Norio, and Honda, Toshio. Three cyanidin 3-glucuronylglucosides from red flowers ofbellis perennis. *Phytochemistry*, 30(11):3769–3771, 1991.

Topal, Ummihan, Sasaki, Mitsuru, Goto, Motonobu, and Otles, Semih. Chemical compositions and antioxidant properties of essential oils from nine species of turkish plants obtained by supercritical carbon dioxide extraction and steam distillation. *International Journal of Food Sciences and Nutrition*, 59(7-8):619–634, 2008.

Topcu, G, Ayral, MN, Aydin, A, Gören, AC, Chai, HB, and Pezzuto, JM. Triterpenoids of the roots of lavandula stoechas ssp. stoechas. *Die Pharmazie*, 56(11):892–895, 2001.

Torras-Claveria, Laura, Jauregui, Olga, Bastida, Jaume, Codina, Carles, and Viladomat, Francesc. Antioxidant activity and phenolic composition of lavandin (lavandula x intermedia emeric ex loiseleur) waste. *Journal of agricultural and food chemistry*, 55(21):8436–8443, 2007.

Tortorella, Lucia, Vizzielli, Giuseppe, Fusco, Domenico, Cho, William C, Bernabei, Roberto, Scambia, Giovanni, and Colloca, Giuseppe. Ovarian cancer management in the oldest old: improving outcomes and tailoring treatments. *Aging and disease*, 8(5):677, 2017.

Touati, Bechir, Chograni, Hnia, Hassen, Imed, Boussaïd, Mohamed, Toumi, Lamjed, and Brahim, Nadia Ben. Chemical composition of the leaf and flower essential oils of tunisian lavandula dentata l.(lamiaceae). *Chemistry & Biodiversity*, 8(8):1560–1569, 2011.

Tounsi, Moufida Saidani, Wannes, Wissem Aidi, Ouerghemmi, Ines, Msaada, Kamel, Smaoui, Abderrazak, and Marzouk, Brahim. Variation in essential oil and fatty acid composition in different organs of cultivated and growing wild ruta chalepensis l. *Industrial crops and products*, 33(3):617–623, 2011.

Traboulsi, Abdallah F, Taoubi, K, El-Haj, Samih, Bessiere, J M, and Rammal, Salma. Insecticidal properties of essential plant oils against the mosquito culex pipiens molestus (diptera: Culicidae). *Pest Management Science*, 58(5):491–495, 2002.

Trimbos, Baptist, Timmers, Petra, Pecorelli, Sergio, Coens, Corneel, Ven, Koen, Van der Burg, Maria, and Casado, Antonio. Surgical staging and treatment of early ovarian cancer: long-term analysis from a randomized trial. *Journal of the National Cancer Institute*, 102(13):982–987, 2010.

Tsao, Rong and Deng, Zeyuan. Separation procedures for naturally occurring antioxidant phytochemicals. *Journal of chromatography B*, 812(1-2):85–99, 2004.

Tuberoso, Carlo IG, Barra, Andrea, Angioni, Alberto, Sarritzu, Erika, and Pirisi, Filippo M. Chemical composition of volatiles in sardinian myrtle (myrtus communis l.) alcoholic extracts and essential oils. *Journal of agricultural and food chemistry*, 54(4):1420–1426, 2006.

Tulp, Martin and Bohlin, Lars. Rediscovery of known natural compounds: nuisance or goldmine? *Bioorganic & medicinal chemistry*, 13(17):5274–5282, 2005.

Tzakou, Olga, Bazos, Ioannis, and Yannitsaros, Artemios. Essential oil composition and enantiomeric distribution of fenchone and camphor of lavandula cariensis and l. stoechas subsp. stoechas grown in greece. *Natural product communications*, 4(8):1103–1106, 2009.

Uche, Fidelia Ijeoma, Abed, Mohammed N, Abdullah, Marwan I, Drijfhout, Falko P, McCullagh, James, Claridge, Timothy WD, Richardson, Alan, and Li, Wen-Wu. Isochondodendrine and 2-norcocsuline: additional alkaloids from triclisia subcordata induce cytotoxicity and apoptosis in ovarian cancer cell lines. *RSC Advances*, 7(70):44154–44161, 2017.

Ugur, Aysel, Duru, Mehmet Emin, Ceylan, Ozgur, Sarac, Nurdan, Varol, Omer, and Kivrak, Ibrahim. Chemical composition, antimicrobial and antioxidant activities of centaurea ensiformis hub.-mor.(asteraceae), a species endemic to mugla (turkey). *Natural Product Research*, 23(2): 149–167, 2009.

Ulubelen, A, Gören, N, and Olcay, Y. Longipinene derivatives from lavandula stoechas subsp. stoechas. *Phytochemistry*, 27(12):3966–3967, 1988.

Upson, T and Andrews, S. The genus lavandula. portland, 2004.

Upson, Tim M, Grayer, Renée J, Greenham, Jennifer R, Williams, Christine A, Al-Ghamdi, Farag, and Chen, Fen-Hui. Leaf flavonoids as systematic characters in the genera lavandula and sabaudia. *Biochemical Systematics and Ecology*, 28(10):991–1007, 2000.

Vagionas, K, Graikou, K, Ngassapa, O, Runyoro, D, and Chinou, I. Composition and antimicrobial activity of the essential oils of three satureja species growing in tanzania. *Food Chemistry*, 103 (2):319–324, 2007.

Vainstein, Alexander, Lewinsohn, Efraim, Pichersky, Eran, and Weiss, David. Floral fragrance. new inroads into an old commodity. *Plant physiology*, 127(4):1383–1389, 2001.

Valdés, Benito, Talavera, Salvador, and Fernández-Galiano, Emilio. *Flora vascular de Andalucía occidental*, volume 2. Ketres, 1987.

Valentini, G, Arnold, N, and Bellomaria, B. [comparative chemical study between four wild populations of lavandula stoechas l. stoechas from cyprus [fenchone, camphor]].[french]. *Plantes Medicinales et Phytotherapie*, 1993.

Van den Dool, H. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr. A*, 11:463–471, 1963.

Van der Schueren, E, Kesteloot, Katrien, and Cleemput, Irina. Federation of european cancer societies. full report. economic evaluation in cancer care: questions and answers on how to alleviate conflicts between rising needs and expectations and tightening budgets. *European Journal of Cancer*, 36(1):13–36, 2000.

Vanhaelen-Fastré, Renée and Vanhaelen, Maurice. High-performance liquid and thin-layer chromatography of coumarin anti-coagulants and their degradation products. *Journal of Chromatography A*, 129:397–402, 1976.

Wall, Monroe E, Wani, M Cook, Cook, CE, Palmer, Keith H, McPhail, AT, , and Sim, GA. Plant antitumor agents. i. the isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminata1, 2. *Journal of the American Chemical Society*, 88 (16):3888–3890, 1966.

Wani, Mansukhlal C, Taylor, Harold Lawrence, Wall, Monroe E, Coggon, Philip, and McPhail, Andrew T. Plant antitumor agents. vi. isolation and structure of taxol, a novel antileukemic

and antitumor agent from taxus brevifolia. *Journal of the American Chemical Society*, 93(9): 2325–2327, 1971.

Wiklund, Annette. The genus nauplius (asteraceae-inuleae). *Nordic journal of botany*, 7(1):1–23, 1987.

Wright, CW. Medicinal and aromatic plantsüindustrial profiles, artemisia, 2002.

Wu, Shasha, Sun, Youping, Niu, Genhua, Pantoja, Graciela Lizette Grimaldo, and Rocha, Arturo Castro. Responses of six lamiaceae landscape species to saline water irrigation. *Journal of Environmental Horticulture*, 34(1):30–35, 2016.

Xiang, Meixian, Su, Hanwen, Hu, Jinyue, and Yan, Yunjun. Isolation, identification and determination of methyl caffeate, ethyl caffeate and other phenolic compounds from polygonum amplexicaule var. sinense. *Journal of Medicinal Plants Research*, 5(9):1685–1691, 2011.

Yashphe, J, Feuerstein, I, Barel, S, and Segal, R. The antibacterial and antispasmodic activity of artemisia herba alba asso. ii. examination of essential oils from various chemotypes. *International journal of crude drug research*, 25(2):89–96, 1987.

Yayli, Nurettin, Yaşar, Ahmet, Güleç, Canan, Usta, Asu, Kolaylı, Sevgi, Coşkunçelebi, Kamil, and Karaoğlu, Şengül. Composition and antimicrobial activity of essential oils from centaurea sessilis and centaurea armena. *Phytochemistry*, 66(14):1741–1745, 2005.

Yesilada, Erdem, Gurbuz, Ilhan, and Shibata, Hirofumi. Screening of turkish anti-ulcerogenic folk remedies for anti-helicobacter pylori activity. *Journal of Ethnopharmacology*, 66(3):289–293, 1999.

Yesilada, Erdem, Gürbüz, Ilhan, Bedir, Erdal, Tatli, Irem, and Khan, Ikhlas A. Isolation of anti-ulcerogenic sesquiterpene lactones from centaurea solstitialis l. ssp. solstitialis through bioassay-guided fractionation procedures in rats. *Journal of ethnopharmacology*, 95(2-3):213–219, 2004.

Yoshikawa, Masayuki, Li, Xuezheng, Nishida, Eriko, Nakamura, Seikou, Matsuda, Hisashi, Muraoka, Osamu, and Morikawa, Toshio. Medicinal flowers. xxi. structures of perennisaponins a, b, c, d, e, and f, acylated oleanane-type triterpene oligoglycosides, from the flowers of bellis perennis. *Chemical and Pharmaceutical Bulletin*, 56(4):559–568, 2008.

Yosr, Zaouali, Hnia, Chograni, Rim, Trimech, and Mohamed, Boussaid. Changes in essential oil composition and phenolic fraction in rosmarinus officinalis l. var. typicus batt. organs during growth and incidence on the antioxidant activity. *Industrial Crops and Products*, 43:412–419, 2013.

You, Jueng Soo and Jones, Peter A. Cancer genetics and epigenetics: two sides of the same coin? *Cancer cell*, 22(1):9–20, 2012.

Younsi, Faten, Trimech, Rym, Boulila, Abennacer, Ezzine, Olfa, Dhahri, Samir, Boussaid, Mohamed, and Messaoud, Chokri. Essential oil and phenolic compounds of artemisia herba-alba (asso.): Composition, antioxidant, antiacetylcholinesterase, and antibacterial activities. *International journal of food properties*, 19(7):1425–1438, 2016.

Youssef, SA, Ibraheim, ZZ, and Attia, AA. Flavonoids from asteriscus pygmaeus (dc.) coss & dr grown in egypt. *BULLETIN OF PHARMACEUTICAL SCIENCES-ASSIUT UNIVERSITY*, 18: 33–33, 1995.

Yun, Kyeong Won, Maun, Anwar, and Kim, Jong Hee. Effects of the aqueous extract fromartemisia campestris ssp. caudata on mycorrhizal fungi colonization and growth of sand dune grasses. *Journal of Plant Biology*, 50(3):358–361, 2007.

Zeller, Constanze and Brown, Robert. Therapeutic modulation of epigenetic drivers of drug resistance in ovarian cancer. *Therapeutic advances in medical oncology*, 2(5):319–329, 2010.

Zengin, Gokhan, Cakmak, Yavuz Selim, Guler, Gokalp Ozmen, and Aktumsek, Abdurrahman. In vitro antioxidant capacities and fatty acid compositions of three centaurea species collected from central anatolia region of turkey. *Food and Chemical Toxicology*, 48(10):2638–2641, 2010.

Zengin, Gokhan, Nithiyanantham, Srinivasan, Locatelli, Marcello, Ceylan, Ramazan, Uysal, Sengul, Aktumsek, Abdurrahman, Selvi, Palanisamy Kalai, and Maskovic, Pavle. Screening of in vitro antioxidant and enzyme inhibitory activities of different extracts from two uninvestigated wild plants: Centranthus longiflorus subsp. longiflorus and cerinthe minor subsp. auriculata. *European Journal of Integrative Medicine*, 8(3):286–292, 2016.

Zhang, LL, Liu, JX, Fang, XQ, and Nie, GQ. Effects of surface piezoelectricity and nonlocal scale on wave propagation in piezoelectric nanoplates. *European Journal of Mechanics-A/Solids*, 46: 22–29, 2014.

Zhang, Shu, Balch, Curt, Chan, Michael W, Lai, Hung-Cheng, Matei, Daniela, Schilder, Jeanne M, Yan, Pearlly S, Huang, Tim HM, and Nephew, Kenneth P. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer research*, 68(11):4311–4320, 2008.

Zhou, Kequan and Yu, Liangli. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in colorado. *LWT-Food Science and Technology*, 39(10):1155–1162, 2006.

Znati, Mansour, Ben Jannet, Hichem, Cazaux, Sylvie, Souchard, Jean Pierre, Harzallah Skhiri, Féthia, and Bouajila, Jalloul. Antioxidant, 5-lipoxygenase inhibitory and cytotoxic activities of compounds isolated from the ferula lutea flowers. *Molecules*, 19(10):16959–16975, 2014.

Znini, M, Cristofari, G, Majidi, L, Ansari, A, Bouyanzer, A, Paolini, J, Costa, J, and Hammouti, B. Green approach to corrosion inhibition of mild steel by essential oil leaves of asteriscus graveolens (forssk.) in sulphuric acid medium. *Int J Electrochem Sci*, 7:3959–3981, 2012.

Znini, MOHAMED, Cristofari, Gregory, Majidi, Lhou, Mazouz, HAMID, Tomi, Pierre, Paolini, JULIEN, and Costa, Jean. Antifungal activity of essential oil from asteriscus graveolens against postharvest phytopathogenic fungi in apples. *Natural product communications*, 6(11):1763–1768, 2011.

Zrira, S and Benjilali, B. The constituents of the oils of lavandula stoechas l. ssp. atlantica br.-bl. and l. stoechas ssp. stoechas from morocco. *Journal of Essential Oil Research*, 15(2):68–69, 2003.

Zuzarte, M, Gonçalves, MJ, Cavaleiro, C, Cruz, MT, Benzarti, A, Marongiu, B, Maxia, Andrea, Piras, Alessandra, and Salgueiro, L. Antifungal and anti-inflammatory potential of lavandula stoechas and thymus herba-barona essential oils. *Industrial Crops and Products*, 44:97–103, 2013.

Appendix

4.2.5 Chemical and standard compounds using in the essential oil study

Dimethylsulfoxide, 3-[4,5-dimethylthiazol-2,5- diphenyltetrazolium bromide] (DMSO), Isopropan-2-ol, Diethyl ether (EtO_2) homologous series of C_9-C_{24} n-alkanes and various reference chemicals (α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, limonene, p-cymene, β-ocimene, δ-terpinene, 1,8- cineol, γ-terpinene, linalool, menthone, borneol, menthol, terpinene-4-ol, α-terpineol, estragole, dihydrocarveol, dihydrocarvone, pulegone, carvone, pipretone, thymol, fenchone, fenchyl alcohol, fenchyl acetate, anethole, piperitenone oxide, *p*-anisaldehyde α-copaene, β-bourbonene, β-elemene, β-caryophyllene, β-cubebene, α-bergamotene, α-caryophyllene, γ-muurolene, germacrene D, γ-cadinene and caryophyllene oxide etc.) used to identify the constituents were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Glacial acetic acid, Trichloroacetic acid (TCA), analydrous sodium sulphate and methanol were purchased from Fisher Scientific, U.K. Trypsin-EDTA solution, Tris base, N,*O*-bis(trimethylsily) tri-fluoroacetamide (BSTFA) with 1% chlorotrimethylsilane (TMCS), Phosphate buffered saline (PBS) and dimethylsulfoxide (DMSO) were purchased from Sigma–Aldrich. RPMI 1640 medium media, DMEM media, fetal bovine serum (FBS), penicillin-streptomycin solution, and glutamine were purchased from Lonza, Switzerland. Sulforho-damine B (SRB) sodium salt, carboplatin (CPBT), and chloroform from TCI (Tokyo Chemical Industry). Pyridine and Hexane from ACROS organics.

4.2.5.1 Chemical and reagents using in total phenolic compounds study

All chemicals used were analytical grade. Methoxyamine hydrochloride (MeOX) and N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alkane standard of C_8-C_{20} and C_{21}-C_{40} were also purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents like chloroform, ethanol, and methanol (MeOH) were obtained from Merck (Germany).

Abstract

Although, there have been large improvements in cancer treatment over the last two decades, the ineffectiveness of most chemotherapeutic drugs is still a major cause of cancer death worldwide. Medicinal plants offer naturally occurring agents that may play an important role in the treatment of cancer by offering unique active drugs or their templates for clinical uses. Cytotoxicity screening of plant extracts from wild species may lead to the discovery of novel agents for cancer therapy. Five Algerian species belonging Asteraceae family *Centaurea sphaerocephala* L., *Bellis sylvestris* L., *Asteriscus maritimus* (L.) Less. and *Artemisia campestris* L.) and one belonging Lamiaceae family (*Lavandula stoechas* L.) have been used. Therefore, the purpose of the current study was to assess the potential anticancer of the total phenolic compounds (TPC) present in the different parts of each single species (chloroform (ChE) and methanol (MeE) extracts), as well as the yield calculation and the chemical profile of essential oil (EO) and TPC extracted from each organ. The EO yield results of Asteraceae species showed that leaves and flowers recorded significantly the best yields (0.22 ± 0.25 and 0.20 ± 0.20, respectively), compared by stems (0.07 ± 0.06), whereas, *C. sphaerocephala* and *B. sylvestris* recorded significantly the best yields (0.23 ± 0.31 and 0.22 ± 0.23, respectively) compared by *Ar. campestris* (0.18 ±0.05), while the lowest yield has been registered for *A. maritimus* (0.02 ±0.02). Also, leaves of *L. stoechas* recorded significantly the best yields (0.14 ±0.01), compared by flowers and stems (0.07 ± 0.002 and 0.01± 0.003, respectively). GC-MS analysis of the chemical constituents of the isolated oils mainly revealed the presence of monoterpene hydrocarbons, sesquiterpenes hydrocarbons, oxygenated monoterpenes, and oxygenated sesquiterpenes. In the other hand, TPC results of Asteraceae species showed that leaves of all studied species recorded significantly the best yields in TPC (2.68 ± 2.06), followed by flowers and stems (1.92 ± 1.77 and 1.79 ± 0.82, respectively). Also *L. stochas* flowers recorded significantly the best yields in TPC (3.47 ± 2.09), followed by leaves (2.50 ± 0.26) while stems required the lowest yield (1.34 ± 1.12). In addition, MeEs recorded significantly the best yields compared by ChEs (3.09 ±1.84 and 1.17 ±0.56, respectively in Asteraceae species) and (3.45 ±1.47 and 1.43 ±0.91, respectively in Lamiaceae species). Further, GC-MS analysis after derivatization of the ChE of each plant organ was performed and the cytotoxicity of ChEs and MeEs were evaluated by SRB essay on ovarian cancer (OC) cell lines (OVCAR-4, FOUV-1 and COV-362), human normal epithelial cells (HOE) and carboplatin (CBPT) were used as negative and positive controls, respectively. Compared to HOE and CBPT, results demonstrated the following conclusion, leaves and flowers's ChEs also stems's MeE of *C. sphaerocephala* L. showed somewhat greater potencies for the COV-362 cancer cells. Moreover, stems's ChE has been found to be significantly active against FOUV-1. Stems's MeE of *B. sylvestris* has been significantly found to be active against FOUV-1. Contrary, no extract of *A. maritimus* have been found to be active against OVCAR-4 and FOUV-1. However, *Ar. campestris*'s MeE of stems were found to be active against both OVCAR-4 and FOUV-1. Stems and flowers's MeEs of *L. stoechas* have been found significantly to be active against OVCAR-4.

Keywords: Essential oil, total phenolic compounds, Asteraceae, Lamiaceae, cytotoxic activity.

Résumé

Bien que le traitement du cancer ait considérablement progressé au cours des deux dernières décennies, l'inefficacité de la plupart des médicaments chimiothérapeutiques reste une cause majeure de décès par cancer dans le monde. Cependant, Les plantes médicinales constituent une source naturelle des agents susceptibles de jouer un rôle important dans le traitement du cancer en produisant des principes actifs pour des utilisations cliniques. Le screening d'extraits par cytotoxicité de certaines espèces de plantes sauvages, pourrait permettre de découvrir de nouveaux agents anticancéreux. Cinq espèces algériennes appartenant à deux familles, Asteraceae (*Centaurea sphaerocephala* L., *Bellis sylvestris* L., *Asteriscus maritimus* (L.) Less. et *Artemisia campestris* L.) aussi, Lamiaceae (*Lavandula stoechas* L.) ont été utilisés. Le présent travail s'est concentré sur l'extraction, le rendement et l'identification des huiles essentielles (EO) et du contenu phénolique (TPC) (extraits chloroformique (ChE) et méthanolique (MeE)) de chaque organe des plantes utilisées. Ainsi que l'évaluation du potentiel anticancéreux des composées phénoliques à été réalisé.

Les résultats du rendement en EO des Asteraceae ont montré que les feuilles et les fleurs de toutes les espèces étudiées enregistraient significativement les meilleurs rendements (0.22 ± 0.25 et 0.20 ± 0.20, respectivement), par apport aux tiges (0.07 ± 0.06), alors que *C. sphaerocephala* et *B. sylvestris* ont enregistré significativement les meilleurs rendements (0.23 ± 0.31 et 0.22 ± 0.23, respectivement) par rapport à *Ar. campestris* (0.18 ± 0.05), alors que le rendement le plus faible a été enregistré pour *A. maritimus* (0.02 ± 0.02).

Les feuilles de *L. stoechas* a enregistré significativement les meilleurs rendements (0.14 ± 0.01) en comparaison avec les fleurs et les tiges (0.07 ± 0.002 et 0.01 ± 0.003, respectivement). L'analyse par GC-MS des constituants chimiques des huiles isolées a révélé principalement la présence d'hydrocarbures monoterpéniques et sesquiterpéniques, de monoterpènes et de sesquiterpènes oxygénés.

D'autre part, les résultats de TPC des Asteraceae ont montré que les feuilles enregistraient significativement les meilleurs rendements (2.68 ± 2.06), suivies par les fleurs et les tiges (1.92 ± 1.77 et 1.79 ± 0.82, respectivement). Aussi *L. stochas* fleurs a enregistré significativement les meilleurs rendements (3.47 ± 2.09), suivis par les feuilles (2.50 ± 0.26), et les tiges (1.34 ± 1.12).

En outre, MeEs ont enregistré significativement les meilleurs rendements comparés par ChEs (3.09 ± 1.84 et 1.17 ± 0.56, respectivement chez les Asteraceae) et (3.45 ± 1.47 et 1.43 ± 0.91, respectivement chez les Lamiaceae). Aussi, une analyse GC-MS après dérivatisation des ChEs de chaque organe de la plante a été réalisée et la cytotoxicité des ChEs et MeEs a été évalué par la méthode (SRB) sur quelques lignées responsable du cancer épithélial de l'ovaire (OC) sont (OVCAR-4, FOUV-1 et COV-362), où les cellules épithéliales normales (HOE) et le carboplatine (CBPT) ont été utilisés respectivement comme contrôles négatifs et positifs.

En comparaison avec HOE et CBPT, les résultats ont démontré que ChEs des feuilles et des fleurs aussi MeE des tiges de *C. sphaerocephala* L. ont montré une puissance supérieure pour les cellules cancéreuses COV-362, de plus, ChE des tiges était significativement active contre FOUV-1. De l'autre coté, MeE des tiges de *B. sylvestris* a été significativement actif contre le FOUV-1. Par contre, aucun extrait de *A. maritimus* n'a été trouvé actif contre OVCAR-4 et FOUV-1. Cependant, une cerrtaine activité contre OVCAR-4 et FOUV-1 a été demontré par MeE des tiges d'*Ar. campestris* et finalement MeEs de tiges et de fleurs de *L. stoechas* étaient trouvé significativement actifs contre OVCAR-4.

Mots clés: Huile essentielle, Composés phénoliques, Asteraceae, Lamiaceae, cytotoxicité.