Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.6 No.3, 2014

Antitcancer effect and Seasonal variation in oil constituents of Santolina chamaecyparissus

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Abstract
The volatile components of the aerial parts of *Santolina chamaecyparissus*, an aromatic plant of North region, Saudi Arabia, were analyzed in two season by GC and GC-MS. Among the 39 compounds identified, major components were Curcumene, alpha-terpinol, p-cymene, 1,8-cineole and caryophllene oxide. Quantitative variations have been characterized following the season. Oil extract of the aerial parts of the medicinal plant Santolina, was *in vitro* investigated for cytotoxicity against A549, HCT116, HepG2 and MCF-7 cell lines, and resulted show high cytotoxic activity against HepG2 with (97.7 %), but resistance to other cell lines, The percentages of inhibition related to the reference drug (doxorubicin), as long as people are looking to alternative forms of medicine and relaxation such as aromatherapy in this review we confirm the use of one of these medicinal plant as antitumor agent.
Keywords: S. *chamaecyparissus*, volatile oil , anticancer, Caryophyllene oxide

INTRODUCTION

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as **volatile oils**, **ethereal oils** or **aetherolea**, or simply as the "oil of" the plant from which they were extracted, such as oil of olive. An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oils do not form a distinctive category for any medical, pharmacological, or culinary purpose. Various essential oils have been used medicinally at different periods in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer, and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatments and treatment of cancers in particular, are now subject to regulation in most countries.[1]
Cancer is a hyper proliferative disorder that involves transformation, deregulations of apoptosis, proliferation, invasion, angiogenesis and metastasis. Extensive research during the last 30 years has revealed much about the biology of cancer [2]. Normal body cells grow, divide and die in an orderly fashion. If DNA damage occurs, it is repaired in most of the formation, the most relevant are those activating proto-oncogenes and inactivating tumor suppressor genes. The majority of cancers in humans are induced by carcinogenic factors present in our environment including our food [3].

Aromatic plants have been used since ancient times for their medicinal properties. These properties can be partially or wholly attributed to their volatile oil fractions (essential oils) [4]. The diverse therapeutic potentials of essential oils have attracted the attention of many researchers to investigate their anticancer activity, and it has been found that the mechanism of action of essential oils is dissimilar to that of most classic cytotoxical chemotherapeutic agents.

Volatile oils, constituents are mainly monoterpenes and sesquiterpines which are hydrocarbons with the general formula (C5H8)n. [5] Oxygenated compounds derived from the hydrocarbons include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. It is estimated that there are more than 1000 monoterpene and 3000 sesquiterpene structures. Other compounds include phenylpropenes and specific compounds containing sulfur or nitrogen. Hundreds of new natural substances are being isolated and identified every year, but data concerning their biological activities are known for only some. The volatile compounds of oils are aromatic or odoriferous that occurs in plants as such or less frequently may result from the degradation of glycerides by enzyme action[6]. Medicinal uses proposed by sellers of essential oils vary from skin treatments to remedies for cancer [7].

In this present studies we study the role of volatile oil in antitumor activity of plant *santolina,* family Asteraceae , *Santolina* is a genus containing approx. 10 species of aromatic shrubs. S. *chamaecyparissus* is a hardy aromatic, dwarf fragrant, dense mound with attractive grayish-silver foliage, evergreen shrub native to the W. and Central Mediterranean area (growing wild for example in Spain, Tunisia & Morocco, and being

naturalized in parts of Britain). It is often grown in gardens for its attractive wooly silver-grey leaves born on woody stems, and for its yellow flowers [8].

Santolina chamaecyparissus is widely used in Mediterranean folk medicine. The flowers are used for their anaigesis, antiinflamatory, antiseptic, antispasmodic, bactericidal, fungicidal, digestive and vulnerary properties, and is used in phytotherapy [9].

The idea of developing a drug that selectively destroy disease cells without damaging healthy cells was proposed by Paul Ehrlich, almost a century

ago; he called his hypothetical drug the "magic bullet" [10]. Thereafter, over the past several decades, many scientists have focused their attention on the development of ideal drugs that specifically target the site of action. Although little progress has been made in this field, the advent of nanomedicine and our understanding of cellular and molecular biology have opened new avenues to transform the Ehrlich's concept into clinical reality [11]. The targeted drug delivery system is comprised of three components: a therapeutic agent, atargeting moiety, and a carrier system. The drug can be either incorporated by passive absorption or chemical conjugation into the carrier system. The choice of the carrier molecule is of high importance because it significantly affects the pharmacokinetics and pharmacodynamics of the drugs. A wide range of materials, such as natural or synthetic polymers, lipids, surfactants and dendrimers, have been employed as drug carriers [12-15] .Among these, polysaccharides have received increasing attention because of their outstanding physical and biological properties.[16]

In this present investigation we study the chemical constituent of essential oil isolated from *santolina* collected from Northern region (Arar) in Saudi Arabia and study the antitumor effect of oil extract, then compare the these chemical constituent with pervious study which investigate the role of these compound as secondary metabolite in adaptation of the plant to their environment .

2. Experimental

2.1. Plant material

Flowering aerial parts of *Santolina* were collected from wild population growing in the garden of faculty of girl , Northern Region (Arar) in Saudi Arabia in March 2012, (spring) and in last June, 2012(summer), The identity of the plants have been kindly verified by Prof. Dr. A. Kamal, Faculty of Science, Northern Border University. Voucher specimens were deposited in the Herbarium of Faculty of Science, Northern Border University, (girl department).

2.2 Sample preparation

Essential oil isolation

The fresh aerial parts of *Santolina* (500 gm plant powder) collected in winter and the same amount in summer were extracted by hydro-distillation and the solvents were removed subsequently under reduced pressure. The oil samples are kept for anticancer analysis.

3.3. Analysis

The constituents of the volatile oils obtained from the hydro- distillation were analyzed by GC-MS as reported [17]. Compounds were identified by comparison of their retention indices (RI), (C9 to C24 n-alkane mixture) and mass spectra with those reported in the literature [17-21].

Cytotoxicty assay procedures

Human tumor cell lines

Authentic cultures of HCT116 (Human colon carcinoma),A549 (non small cell lung adenocarcinoma), Hep-G2 (Human hepatocellular liver carcinoma) and MCF-7 (Human breast carcinoma) cells were obtained in frozen state under liquid nitrogen (-180ºC) from the American Type Culture Collection. The tumor cell lines were maintained by serial sub-culturing in the National Cancer Institute, Cairo, Egypt.

Culture media

HCT116, Hep-G2 and MCF-7 cells were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 1% antibiotic a ntimycotic mixture (10.000 U/ml K-penicillin, 10.000 µg/ml streptomycin sulphate and 25 µg/ml amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

Assay method for cytotoxic activity

The cytotoxicity against HCT116, Hep-G2 and MCF-7 cells were tested in the National Cancer Institute, according to the SRB (Sulforhodamine B) assay using MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) method by Skehan et al. 22]. Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the reference drug. HCT116, Hep-G2 and MCF-7 cells were plated in 96-multiwell plates (5×104–105 cells/well in a fresh media) for 24 h before treatment with the tested sample to allow attachment of cells to the wall of the plate. Then, 200 µl aliquot of serial dilution with DMSO (100%) of

extract (12.5, 25, 50, 100 µg/ml) were added and the plates were incubated for 24, 48 and 72 h at 37°C in a humidified incubator containing 5% CO2 in air.
Control cells were treated with vehicle alone. Four wells were prepared for each individual dose. Following 24, 48 and 72 h treatment, cells were fixed, washed and stained with Sulforhodamine B stain (Sigma, USA). Colour intensity was measured in an ELISA reader spectrophotometer (Tecan Group Ltd.-Sunrise, Germany).

Statistical analysis

All values were expressed as the mean of percentage of inhibition cells of the three replicates for each treatment. Data were subjected to SPSS (ver.8.0). $P<0.05$ was regarded as significant.

Result and discussion

The oil extracted from Santolina by hydrodistillation has a pale yellow color and fragrant pleasant odor. Most of their components could be identified unambiguously by G.C.-MS. through direct comparison (mass fragmentation, retention index) with published data as well as computer library search. The unidentified components mainly consisted of a mixture of oxygenated monoterpenes and sesquiterpenes, whose individual of the oil was identified. The oil consisted mainly of oxygenated monoterpene compounds rich in Curcumene, Thujol, limonene oxide, Bornyl, Eucalyptol, alpha.-Bisabolene epoxide, Carveol,. caryophllene oxide and Camphor as in Table (**1**).
Other minor constituents were camphene, β-pinene, α-terpinene, *p*-cymene, santolina triene, *cis*-sabinene hydrate, limonene, *trans*-sabinene hydrate, α-terpineol and farnesol , represent in our sample , CIS- Piperitone oxid and vanillin also identified by comparison the (**RI** and Mass fragment) with literature [21]. The results obtained differ from all those reported elsewhere in the literature for this species . For the first time thujol, carveol and Curcumene were found in the plant.
The chemical composition of the essential oil of *S. chamaecyparissus ssp.* Were analysis in two season winter and summer and there are a little differentiation in a concentration but some compound disappear in summer eg Limonene, santolina trien, β-pinene, curcummin and carveol, while some compound appear eg. Oxazolidin. Some compound as caryophllene oxide found in higher concentration than winter.
Yet other studies such as that of Villar *et al*. [23] analyzing the essential oil of of *S. chamaecyparissus* ssp. *squarrosa* plants from Valencia show a different distribution of components – here with camphor at 25%, alloaromadendrene 19%, p-cymene, 1,8-cineole 10%, alpha-muurolene 7% with thujone at 0.2%. However, the examination of essential oils from several *S. chamaecyparissus* subspecies, both wild (Spanish insular & peninsular) and cultivated (Spanish & British), reveals a slightly more complex story. Pérez-Alonso and Velasco-Negueruela [24], analyzed samples from cultivated plants characteristically showed a preponderance of artemisia ketone (27.8-35.6%) & Tcadinol (4.8 to 23.6%).

Antitumor activity of oil extract

The cytotoxic activity of crude oil extract of S*antolina chamaecyparissus* was *in vitro* assessed against A549, HCT116, HepG2 and MCF7. The percentages of inhibition related to the reference drug (doxorubicin) are given in Table (2). The oil extract at concentration 100 and 50 µg/ml showed high cytotoxicity (97% at 100 ppm) against HepG2, and low activity (11.8) at the concentration 100 µg/ml against A549 while the extract didn't show any inhibition against MCF7and HCT116 at any concentrations, related to the reference drug (doxorubicin).
The results show the oil extract of S*antolina chamaecyparissus* have high cytotoxity against **Human hepatocellular carcinoma cell line,** and activity increase with increasing concentration. this may be related to the chemical constituents of oil which contain high ratio of bisbolol and Caryophllene oxide, there are many review exhibited these compounds have anticancer activity**.**
Increasing attention has been paid to sesquiterpenes for their potentially useful biological activities, especially the anti-tumor activity [25-27]. α-Bisabolol , is sesquiterpene alcohol with very low toxicity, has been widely used in fragrances and cosmetic preparations for hundreds of years [28]. Recent years, this oily compound has been studied for its effect of increasing bacterial resistance to antibiotics and antimicrobials, [29] antimutagenic activity,[30] inhibitory effect on the genotoxic damage [31]and depigmenting effect .[32]
Research groups in Italy also have studied the apoptosis-inducing and anti-tumor activities of a-bisabolol, and found that a-bisabolol was able to selectively induce apoptosis in several malignant tumor cells through the mitochondrial pathway, probably by targeting lipid rafts on cell membranes [33-34].However, the molecular mechanism underlying a-bisabolol cytotoxicity in tumor cells has not been well elucidated.

Table (1) The essential oil components of *Santolina chamaecyparissus* oil Extract.

Concentration% Spring	Concentration% Summer	Chemical Compound
1.49	1.37	Eucalyptol(1,8-Cineole)
0.80	1.49	Alpha-Terpinol
0.39	0.43	β- terpinol
1.82	0.70	Camphor
1.82	0.70	Camphanone
1.27	1.94	limonene oxide
1.20	---	Limonene
0.77	1.82	Bornyl
1.4	---	B-Caryophyllene
1.54	3.62	Caryophllene oxide
0.89	--	Carveol
0.35	----	β-pinene
0.39	1.49	CIS sabinene hydrate
0.39	1.49	Trans sabinene hydrate
0.89	0.87	alpha.-Bisabolene epoxide
0.35	----	Santolina triene
1.27	1.4	Thujol
1.27	2.93	Limonene diepoxide
1.0	1.2	Zingiberone
0.22	0.22	Farnesol
------	0.93	Oxazolidin
1.9	1.15	Alpha bisabolol,
2.3	---	Curcumene
----	1.37	P-Cymen-8-ol

Wei Chen et al , [35] study, the effect of a-bisabolol on the viability of PC-3,Hela, ECA-109, and HepG2 cells was examined by MTT assay. Cells were treated with a-bisabolol at different concentrations (0–20 mM) for 24 h. the data indicated that a-bisabolol exhibited a cytotoxic effect in a dose-dependent manner, As 70% of HepG2 cells was killed after 24 h of treatment with 10 mM a bisabolol, only 30% of Hela and ECA-109 cells were killed, whereas PC-3 cell line appeared to be much more resistant to a-bisabolol. The results showed that a-bisabolol seemed to have a stronger death effect towards human liver carcinoma cell line HepG2. This all reference confirm our study.

Table (2): Cytotoxic activity of oil extract of S*antolina chamaecyparissus* against cultured different cell lines *in vitro*

Human Cell line / **Conc. ppm**	**% of Inhibition**			
	100	**50**	**25**	**12.5**
A549	11.8	0	0	0
HCT116	-13	0	0	0
HepG 2	97.7	70.1	38.9	18.5
MCF-7	-20.5	0	0	0

Each value represents the inhibition growth related to Doxorubicin.
Activity > 75%: high, 75-50% :good, 50-25 % : normal and <25%: weak activity.
P<0.005

In vitro research by Gill et al, [35] looked at the effect of virgin olive oil phenols on colorectal carcinogenesis, Using specific cell lines, they investigated processes involved in cancer initiation, promotion and metastasis - the three main stages in cancer development and concluded olive oil phenols exert beneficial effects in all three stages. The oil extract was shown to reduce DNA damage (initiation), increase barrier function (promotion) and reduce cell invasion of surrounding tissue(metastasis).

An excellent survey on the uses of fragrances and essential oils as medicaments was published by[36] It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels.

At certain levels of dosage, the volatile oils saturate the membranes and show effects similar to those of local anesthetics. They can interact with the cell membranes by means of their physiochemical properties and molecular shapes, and can influence their enzymes, carriers ion channels and receptors. The authors describe various studies concerning the physiological effects on humans. These include brain stimulation, anxiety-relieving sedation and antidepressant activities, as well as increasing the cerebral blood flow. The studies also describe the effects of odors' on cognition, memory, and mood. The fragrance compounds are absorbed by inhalation and are able to cross the blood-brain barrier and interact with receptors in the central nervous system. Bioassays used for the description and explanation of volatile oil action, are usually carried out on mice, rats and toads e.g. the influence of peppermint oil on intestinal transport [37], the effect of volatile oils on the skin penetration [38], so the presence of these volatile oil in *santolina* with high value and fragrances odor make the plant used as anti -inflammatory agent and inhaling of burns. Beside their effect against liver cancer.

CONCLUSION

The results clearly show that ecological factors influence the selection of medicinal plants in this region. Climate and substrate are the most important ecological factors that influence the distribution and abundance of plants, which are the biological factors that affect medicinal plant selection. So the present study suggest that the plant *santolina* could be a potential source as natural anti-tumor that could have great importance to inhibition of liver cancer.

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