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Anticancer activity of lactuca steriolla growing under dry desert conditionof Northern Region in Saudi Arabia

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Abstract

Medicinal herbs are also significant source of synthetic and herbal drugs. So far, pharmaceutical companies have screened more than 25,000 plants for anti-cancer drugs. So we done to discover the anticancer activity of the terpinoid compound isolated from terrestrial Saudi plants, the MeOH extract of the aerial parts of the plant lactuca steriolla family Asteraceae was in vitro investigated for cytotoxicity against HCT116, A549, HepG2 and MCF-7 cell lines, and resulted in vitro show high cytotoxic activity against breast cancer with for MeOH extract and good cytotoxicity against liver cancer of hexane extract, respectively, Organic extracts , were fractionated through classic chromatography. The steroids stigmasterol acetate, β-sitosterol and campesterol were identified in hexane extract. Triterpenes, germincol, lupeol, α-amyrin, β-amyrin, olean 18-ene, lupeol acetate were isolated from methanol extract. Steroids and 14b-perganane and 4- piprdenone were isolated from methanol extract and identified by GC-MS. while triterpenes identified byH^1 NMR and C^{13}NMR. Essential oils of the fresh leaves were obtained by hydro distillation and analyzed by GC-MS

Keywords: Asteraceae, Lactuca serriolla, triterpene, volatile oil, breast anticancer

Introduction

The Asteraceae family compasses the largest family of angiosperms with approximately 23,000 species, 1,535 genera and represents approximately 10% of all world flora .The plants of Asteraceae family are studied respecting their chemical composition and biological activity. [1]

A broad survey of available world literature showed that at least 98 wild Lactuca spp. (Asteraceae) have been described taxonomically. The distribution of the genus Lactuca worldwide includes 17 species in Europe, 51 in Asia, 43 in Africa, and 12 in the Americas (mostly the North American subcontinent). Species originating in Asia, Africa, and the Americas form ca. 83% of known Lactuca spp. richness; however, they are very poorly documented from the viewpoint of taxonomic relationships, ecogeography, and variability. The phytogeography of Lactuca spp. regarding

their distribution on different continents and in relation to the structure of the lettuce gene pool is discussed. A more detailed analysis of geographical distribution and habitats is given for some species (L. serriola, L. saligna, L. virosa, L. perennis, L. quercina, L. tatarica), which represent the primary, secondary, and tertiary gene pools of cultivated lettuce (L. sativa). Original and synanthropic distributions of Lactuca spp. and their occurrence in natural and secondary habitats are discussed, along with the representation of wild Lactuca spp. in world gene-bank collections. Global biodiversity of Lactuca spp. and their representation in germplasm collections are poorly documented. Future studies of taxonomy, phytogeography, ecology, phylogenetic relationships, and genetic diversity are needed for a more complete understanding of this genus and taxonomically related genera.[2]

Lettuce, Prickly (Lactuca serriola): The whole plant is rich in a milky sap that flows freely from any wounds. This hardens and dries when in contact with the air. The sap contains 'lactucarium', which is used in medicine for its anodyne, antispasmodic, digestive, diuretic, hypnotic, narcotic and sedative properties. Lactucarium has the effects of a feeble opium, but without its tendency to cause digestive upsets, nor is it addictive. It is taken internally in the treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough, rheumatic pain etc. Concentrations of lactucarium are low in young plants and most concentrated when the plant comes into flower It is collected commercially by cutting the heads of the plants and scraping the juice into china vessels several times a day until the plant is exhausted[3]

The genus lactuca are chosen because it have very important properties, The plant L. Virosa contains flavonoids, which have strong anti-oxidant properties, contain Coumarins, and N-methyl-β-phenethylamine. It has also been found to be a galactogogue for many women (a substance which increases breast milk), particularly when used in combination with alfalfa. Wild Lettuce commonly refers to the more bitter cousins of common, The three main species of this group are Lactuca Virosa, Lactuca Canadensis, and Lactuca Serriola. They have been used in herbal medicine throughout history mainly as a sleep aid, the wild relations are edible [3]

Many studies of the medicinal qualities of Wild Lettuce have been made. Several chemicals which constitute a mild sedative and cough suppressant can be found in the wild Lactuca species. The two main chemicals are Lactucopicrin and Lactucin.
When a stem or leaf from a Wild Lettuce plant is broken or cut, it will bleed a thick milky sap. The dried sap is often referred to as Lettuce Opium, though it contains no opiates. This sap can be extracted many ways, but the most common is by soaking plant material in alcohol. After several weeks, then plant filtered [4]. Any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups and are consistent with this concept as the combination of secondary products in aparticular plant is taxonomically distinct[5] . Arid and semi arid plants are good sources for the production of various types of secondary metabolites which make them resistant to various environmental stress e.g. scarcity of water, salinity, pathogens etc, they are also important for the primary metabolism of plants. These compounds include alkaloids,

flavonoids, steroids, phenolics, terpenes, volatile oils etc. Man has been exploiting these natural plant products for use in medicines.[5]

As part of our ongoing collaborative effort to discover the anticancer activity of the compound isolated from terrestrial plant sources , it is well known that natural products have played an important role in the discovery of useful antitumor agents especially clinically relevant anticancer drugs; such as taxol, camptothecin, vinblastine and vincristine, which were discovered from higher plants. In recent investigation we concern on bio guided assay fractionation to identify and isolate the active compound responsible on the anticancer effect of lactuca serriola, desert plant growing in Northern region in Saudi Arabia.

Materials and methods

2.1. Plant material

The whole plant of lactucas seriolla, was collected from Northern region (Arar /Rafaha road) during spring season at 2012, lactucas **seriolla** an annual or biennial weed with prickly leaves that emit a milky sap when cut. Prickly lettuce is most commonly a weed of nurseries, orchards, roadsides, and agronomic crops and is found throughout the Saudi Arabia, Northern region. The plant samples were kindly identified by Dr/ Ahmed Morsy Professor in Botany department, Desert Research Center. A voucher specimen of the plant materials were kept in the Herbarium of Desert Research Center. No drcc30/950. The plant material was air dried in shade then separated root, steam ,flower and grinded to fine powder for phytochemical and biological investigation.

2.2General experimental conditions

Thin layer chromatography (TLC) (Silica gel G-60 F_{254} Merck, Germay). Column chromatography (Silica gel G-60, 70-230 mesh). Nuclear Magnetic Resonance (NMR) spectra were measured at 600. 17 and 150.91 MHz for 1H- and ^{13}C-NMR respectively, with a JEOL ECA 600 spectrometer. Solvent systems; I- chloroform -methanol (9:1), II- ethyl acetate – methanol – acetic acid – water (65:15:10:10) and III--chloroform– methanol: ether (9:1: 0.5) were used. Visualization of chromatograms was achieved under UV (254 and 365 nm) before and after spraying with vanillin sulphoric acid[6]

2.3 **Extraction and isolation**

The dried samples**(1 kg)** dry weight were separately soxhlet extracted in petroleum ether on a water bath for 24 hrs, petroleum ether extract which subjected to GC -MS .The reaming powder of investigated plants was extracted by percolation in (70 %) methanol and filtered off. The extraction repeated four times. The combined methanol extracts were concentrated under reduced pressure in rotary evaporator till dryness at temperature not exceeding 40^{o}C. The dried extract was dissolved in water (500 ml) and extracted successively using petroleum ether, chloroform, ethyl

acetate and methanol by separating funnel. Each extract was dried over anhydrous sodium sulphate and concentrated again as before.

Isolation and identification of constituents from the organic extracts

Methanol extract (12 g) was subjected to liquid chromatography (LC) and eluted with 100% hexane, increasing polarity gradullay100% chloroform and ethyl acetate, The collected fractions were obtained according to TLC manner using system ethyl acetate: methanol: (9:1). Three fractions **(A, B, C)** were obtained containing terpenoid derivatives.

Fraction **A** (1.5g) was introduced to column silica gel column chromatography, eluted with hexane: CH_2CL_2 (80:20) increasing polarity to reach pure CH_2CL_2, 5 sup fraction A1,A2.A3,A4 and A5 collected after visualized under UV lamp, Two major bands of terpenoid nature were detected, sup fraction A1,A2 were purified by introduced to column sephadex LH-20 using eluting system methanol/water. Two pure compounds were obtained.(lupeol, lupeol acetate) The processes of fractionation of the crude extracts, as well as their fractions, were monitored by comparative thin layer chromatography (TLC) having as objectives the observation of the purity degree of products of fractionation and detection of fractions with similar chromatographic profiles, for its subsequent combination. For both, were used chromatoplates of aluminum covered with silica gel 60 with fluorescence indicator (Macherey-Nagel - UV_{254}),[7]

Also fractions **B** was subjected to liquid chromatography (LC)) and eluted with 100% hexane and ethyl acetate. Fractions (200 ml each) were collected. The collected fractions were obtained according to TLC manner using system ethyl acetate: methanol (9:1), containing triterpene compounds, which identified by ^{1}HNMR,^{13}CNMR and comparison by authentic sample ,(reference compound). The preparative chromatography (CCC) of the fractions **B** (2.4g) yielded 5 fractions one of these fractions B2 was submitted to recrystallization with hexane and drops of ethyl acetate and ethanol. From fraction B2 were obtained 30 mg of colorless crystals(germincole),other compound known as(α-amyrin, β-amyrin, oleanan and α-bisbol) were identified also by ^{1}H NMR ^{13}C NMR and authentic sample.

The hexane extract and fraction C, were analyzed through a chromatography system in gaseous phase coupled to a mass spectrometry (GC-MS). The analysis was performed using Shimadzu-QP2010 equipment, with capillary column DB-5MS Agilent (30 m x 0.25 mm, film thickness 0.25 µm), under the following conditions: Helium was used at pressure of 182.20 kPa, with flow of 1.50 mL/min; temperature in the injector was 260 ºC; the initial temperature of the column was 250 ºC staying for 12 min, increasing to 280 ºC (6 ºC/min) and being conserved by 20 min. The ionization mode used was the electronic impact at 70 eV. The 5-α-cholestane (Sigma) was used as internal standard, being calculated the relative retention (RR) of each constituent of the analyzed mixture. The constituents of the sample were identified through the analysis of their mass spectra and for comparison of their RR with RR

standards of steroids and terpene the quantification of each constituent of the mixture was accomplished through the relative area of the chromatogram peaks.

Essential oil extraction and GC-MS analysis

(Fresh leaves (500 g) were submitted separately to hydro distillation and cohobation in Clevenger apparatus for 3.5 h. After extraction, each essential oil was separate from water by decantation and was desiccated through slow percolation in simple filtration system containing Na_2SO_4 anhydrous (Synth). The essential oils were maintained under low temperature and protected from light until the moment of analysis. .

The GC-MS analyses were carried out on a Shimadzu GC-MS-QP2010 gas chromatography-mass spectrometer equipped with capillary column DB-5-MS Agilent (30 m x 0.25 mm, film thickness 0.25 µm) under the following conditions: Helium was used as the carrier gas at pressure of 81.90 kPa, with flow of 1.33 mL/min; the temperature in the injector was 250 ºC; the temperature of the oven progressed from 60 to 240 ºC to 3 ºC/min. The ionization mode used was the electronic impact at 70 eV. Later, under the same experimental conditions, each oil was co injected with a homologous series of linear hydrocarbons (C_9-C_{25})-Alltech, to accomplishment calculations of the retention index (RI) of each constituent of the samples applying Van Den Dool & Kratz Equation [8]. The compounds identification was performed by analysis and comparison of the mass spectra with database of Wiley 7 library and by comparison of RI with those of the literature [9-17]. The relative quantification of the components of each sample was obtained through the relative area of the peaks in the chromatograms.

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% antibioticantimycotic 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 [18]. Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the referencedrug. 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 alcoholic extract and isolated compound (5.0, 12.5, 25, 50 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.

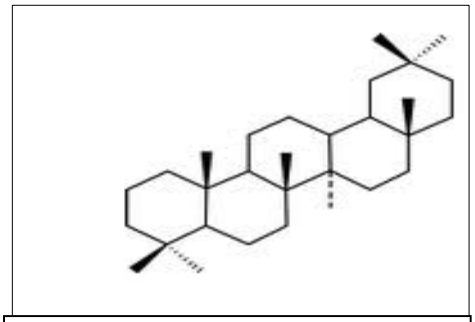
Result and discussion:

Eleven compounds were obtained from organic extracts from aerial parts (leaves plus branches plus inflorescences) of L.serriola: three common compounds from hexane extract, seven compounds from methelene chloride fraction and other two compound were identified from methanol extract. The fractionation of the hexane extract led to the isolation and the identification of three steroids, The stigmasterol acetate was the majority constituent (55.92%), following for the β sitosterol (39.64%) and campesterol (0.42%). RRs of the components of the mixture compared RRs of steroids standards(refer). Also Eicosonal, Tricosene, Icosyne , Eicosyne, α -Bisbole and alpha.-Bisabolene epoxide were identified by GC-MS in hexane extract .

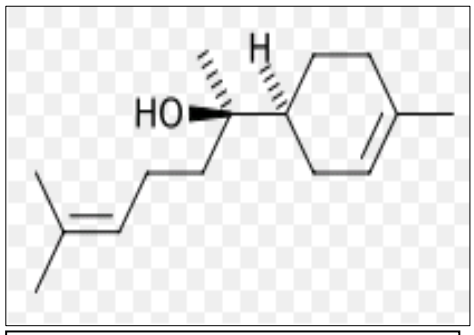
The fraction A, B, obtained from the partition of methanol extract, after successive preparative chromatography yielded seven pure triterpenes compounds **1-7**, identified through GC-MS and then isolated ,purficated and identified by H^1NMR,C^{13}NMR and comparison by litterer [19,20]. The lupeol **1**, lupeol acetate **2**, major component of fraction A1 ,that in our study presents all identified triterpenes in the methelene chloride fraction were not detected in previous studies accomplished with that vegetable species: germincol **3,** α-amyrin **4** ,β-amyrin **(**olean 12 ene) **5** Oleanane 6 and DELTA.18- oleanene (Germanicen **7)** in fraction B2 . The triterpenes found are characteristic of the the Asteraceae family which presents in all the tribes those metabolites with oleane skeletons (such as germincol ,geramcen β-amyrin),) and lupeane (β-amyrin, lupenon) [20,21].

Methanol fraction when detected on GC- MS give14 B-pregnane (**8)**and 4 **-Piperidinone (9).**4-Piperidinone is a derivative of piperidine with the molecular formula C5H9NO. Molar Mass 99.13 g/mol (4-Piperidone is used as an intermediate in the manufacture of chemicals and pharmaceutical drugs. [22]

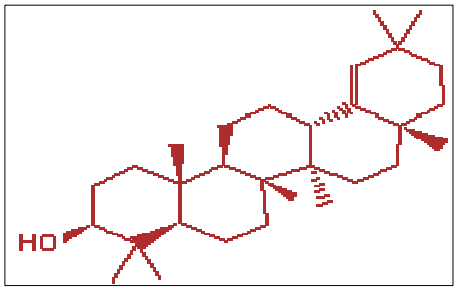
Schema of isolated compound from plant Lactuca serriola



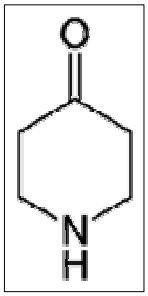
Oleanane 6



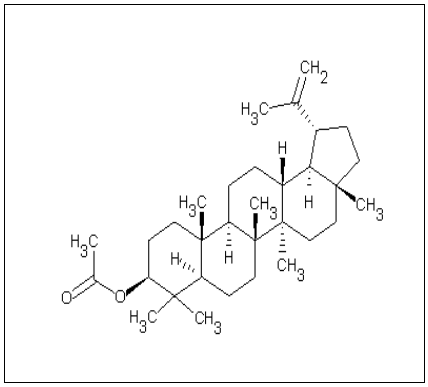
α-bisblol



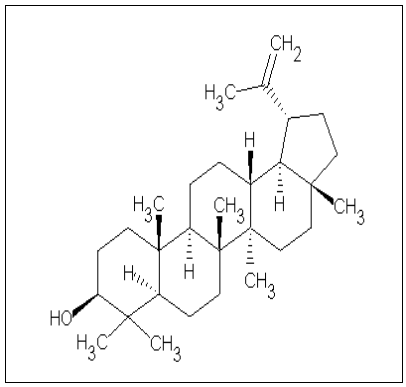
Olean 18-ene 3-0l **3**



4-piperdenon

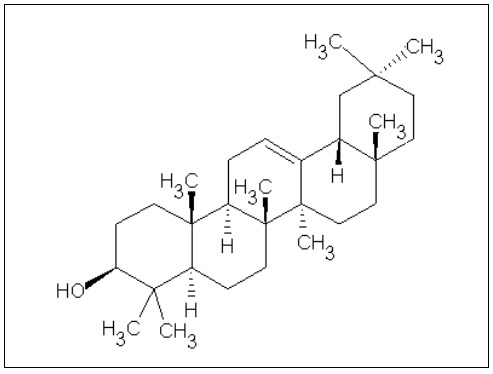


Lupeol acetate **2**

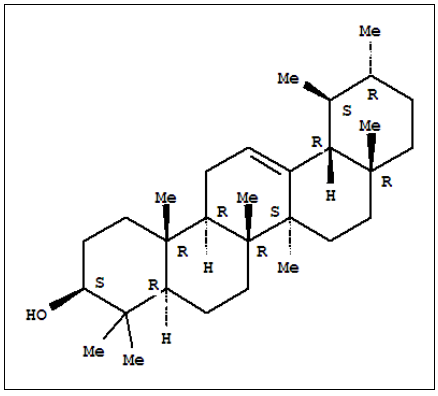


Lupeol **1**

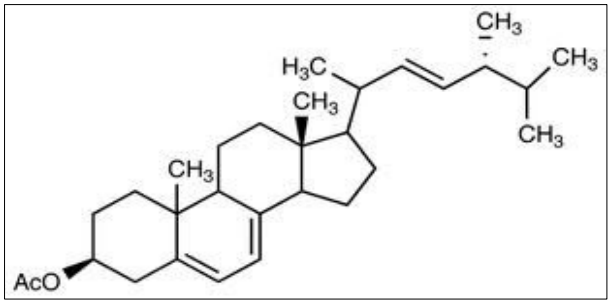
,



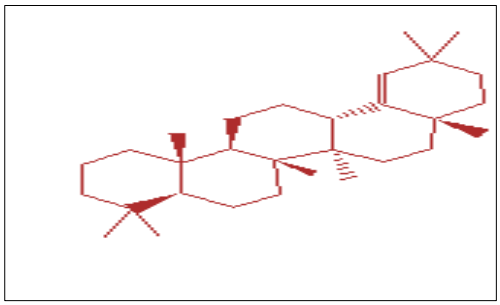
β – Amyrin **5**



α Amyrin 4



Stegmasterol acetate



Olean-18-ene 7

The essential oil

The chemical components identified in each sample of essential oil and hex extract as well as their retention indexes by GC MS are in the Table 1. Were identified 98.04% of components of the essential oil of leaves and the major constituents of essential oils:were α-pinene, limonene, germacrene D, trans-β-caryophyllene, caryophllene oxide and santolina triene. The oil has a pale yellow color and fragrant pleasant odor. The hexane extract has the same odor but yellowish green color. Most of their components could be identified unambiguously by direct comparison (mass fragmentation, retention index) with published data as well as computer library search [23]. The unidentified components mainly consisted of a mixture of oxygenated monoterpenes and sesquiterpenes, whose individual of the oil was identified. The oil also contain oxygenated monoterpene compounds , Thujol , limonene oxide, Eucalyptol , carveol, . β-Pinene. P-menthadiene (alpha.-Phellandrene) and santolina triene, Viridiflorol, Other minor constituents

were camphene, Isoterpinene, α-terpinene, p-cymene, sesqsabinenehydrate, terpin-4-ol, α-terpineol and Bornyl represent the sesquiterpenes in our sample examination of the n-hexane-extract.

Table (1) The essential oil components of Lactuca serriola

Molecular weight MW.	Molecular Formula	Chemical Compound	
154	$C_{10}H_{18}O$	Eucalyptol	1
154	$C_{10}H_{18}O$	Alpha-Terpinol	2
136	$C_{10}H_{16}$	Santolina triene	3
152	$C_{10}H_{16}O$	Camphor	4
222	$C_{15}H_{26}O$	Viridiflorol	5
222	**$C_{15}H_{26}$ O**	Sesqui sabinene hydrate	6
154	$C_{10}H_{18}O$	Gamma-Terpinol	7
152	$C_{10}H_{16}O$	limonene oxide	8
204	C15H24	Germacrene D	9
136	C10H16	LIMONENE	10
196	$C_{12}H_{20}O_2$	Bornyl	11
204	**$C_{15}H_{24}$**	B-Caryophyllene	12
220	$C_{15}H_{24}O$	caryophllene oxide	13
222	$C_{15}H_{26O}$	Globulol	14
204	C15H24	alpha.-selinene	15
152	C10H16O	CARVEOL	16
204	C15H24	α-humulene(Caryophyllene)	17
136	C10H16	B –pinene	18
136	C10H16	alpha.-Phellandrene	19

154	**$C_{10}H_{18}O$**	Thujol	20
136	C10H16	camphene,	21
156	$C_{10}H_{20}O$	Isocitronellol	22
136	C10H16	Isoterpinene	23

Assay method for cytotoxic activity:

The cytotoxicity against HepG2, A549 , HCT116, Mcf 7 and A549 were performed in the National Cancer Institute, according to the method by Skehan Adriamycin® (Doxorubicin+10 mg vials (Pharmacia, Sweden) were used as the reference drug.

The cell lines were plated in 96-multiwell plates (10^4cells/well) for 24 hrs before treatment with the tested sample to allow attachment of cells to the wall of the plate. Then, 50 µl aliquot of serial dilution of crude extract (5.0, 12.5, 25 and 50 µg/ml) was added and the plates were incubated for 48 hrs at 37°C in a humidified incubator containing 5% CO_2 in air. Cells were fixed, washed and stained with Sulforhodamine B stain (Sigma, USA). Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer (Sigma, USA). Colour intensity was measured in an ELISA reader spectrophotometer (Tecan Group Ltd.-Sunrise, Germany).

Table (2): Cytotoxic activty of methanol extract of lactuca serriola against cultured different cell lines in vitro

Human Cell line / Conc. Ppm	% of Inhibition			
	100	50	25	12.5
A549	31.6±0.2287*	16.9±0.1732*	6.4±0.2887*	0.0±
HCT116	-0±	0±	0.0±	0.0±
HepG 2	33.1±0.4619*	13.9±0.05774*	6.78±0.6351*	0.0±
MCF7	80.2±0.2309*	50.6±0.1155*	19.7±0.019	6.7±0.012

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

Table (3): Cytotoxicity of hexane (essential oil) fraction against cultured different human cell line in vitro

Human Cell line / Conc. Ppm	% of Inhibition ± SEM			
	100	50	25	12.5
A549	15.7±0.0151	16.9±0.047	6.4±0.030*	0±
HCT116	-101±0.029	-48±0.016	-16±0.009*	0±
HepG 2	20.6±0.023	5.7±0.0121	1.3±0.059*	0±
MCF7	0±	0±	0±	0±

Each value represents the inhibition growth related to Doxorubicin. P< 0.005

Activity > 75%: high, 75-50% :good, 50-25 % : normal and <25%: weak activity.

The cytotoxic activity of crude hexane and methanol extract of lactuca serriola 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 crud methanol extract at concentration100, 50 and 25 µg/ml showed high cytotoxicity against MCF7, while good cytotoxicity at the concentration at 100 µg/ml against HePG and 100 and 50 µg/ml against HepG2 and A549 showed normal activity when compared with doxorubicin. While hexane extract showed normal activity when compared with doxorubicin against A549and HePG in vitro but no activity against MCF7 and HCT116

Discussion

The methanol extracts prepared from leaves and stems of L seriola showed cytotoxic activity against A549, HePG, MCF7 and HCT116. Bioguided-fractionation of both extracts lead to the identification of cytotoxic fractions, containing lupeol acetate ,Lupenol, and β, and α amyrin, germanicol and Olean-18-en. Pentacyclic triterpenes are one group of promising secondary plant metabolites. potential of triterpenes belonging to the lupane, oleanane or ursane group, treat cancer by different modes of action. Betulinic acid is a highly promising anticancer drug after inducing apoptosis in melanoma cell lines in vitro and in vivo, experimental work focused on the apoptosis inducing mechanisms of betulinic acid and other triterpenes. Antitumour effects were subsequently confirmed in a series of cancer cell lines from other origins, for example breast, colon, lung and neuroblastoma (24). In addition, in the last decade many studies have shown further effects that justify the expectation that triterpenes are useful to treat cancer by several modes of action. Thus, triterpene are known mainly for their anti angiogenic effects as well as their differentiation inducing effects, In particular, lupane-type triterpenes, such as betulin, betulinic acid and lupeol, display anti-inflammatory activities which often accompany immune modulation. Triterpene acids as well as

triterpene monoalcohols and diols also show an antioxidative potential. The pharmacological potential of triterpenes of the lupane, oleanane or ursane type for cancer treatment seems high; although up to now no clinical trial has been published using these triterpenes in cancer therapy (in this paper we found the high activity of methanol extract against breast cancer and the methanol extract fractionation give these class of terpene (oleanan and lupane) combined together and this agree with these reference , in second next paper we study the anticancer effect in vitro of these compound . They provide a multitarget potential for coping with new cancer strategies. Whether this is an effective approach for cancer treatment has to be proven. Because various triterpenes are an increasingly promising group of plant metabolites, the utilization of different plants as their sources is of interest.

Triterpenoids have been reported to act as selective catalytic inhibitors of human DNA topoisomerases (25) which it play important roles in replication, transcription, recombination and chromosome segregation at mitosis [26], **other fraction contain** Piperidinone(C_5H_9NO) ,14β-PREGNANE which responsible for increasing of substance which increases breast milk as L. Virosa has also been found to contain Coumarins, and N-methyl-β-phenethylamine. also been found to be a galactogogue for many women (a substance which increases breast milk)(3).

Lupeol, a dietary triterpene found in certain fruits, vegetables, and medicinal plants, has potent anti-inflammatory, anticarcinogenic, antimutagenic, and antimalarial activity. It suppresses the growth of hepatocellular carcinoma cell lines SMMC7721 and HepG2 with IC_{50} values of 45 and 48.5 μM and melanoma cell lines Mel 928 and Mel 1241 with IC_{50} values of 75 and 72 μM.[1,2] At 0.76 g/kg lupeol causes a significant decrease in the blood pressure of stroke-prone hypertensive rats and reduces expression of hepatic genes involved in triglyceride.[27]

Betulin elicits anticancer effects in tumour primary cultures and cell lines in vitro whereas, it exhibited anti proliferative effect, alterd tumor cells morphology, decreased their motility and induced apoptotic cell death [28] On the other hand, Thao [29] have been reported that α-amyrin exhibited weak cytotoxicity against A549 and HL-60 cancer cell lines. This result is agree with result obtained by **Boglárka[30]** who studied, the ant proliferative activities of aqueous and organic extracts prepared from 26 Hungarian species of the tribes Cynereae and Lactuceae (Asteraceae) were tested in vitro against HeLa (cervix epithelial adenocarcinoma), A431 (skin epidermoid carcinoma) and MCF7 (breast epithelial adenocarcinoma) cells by using the MTT assay. Of the tested 200 extracts of different plant parts obtained with n-hexane, chloroform, 50% methanol and water, 16 extracts displayed noteworthy cell growth inhibitory activity (>50% inhibition at a concentration of 10 μg/mL). The IC_{50} values of these extracts were determined, and their direct cytotoxic effects were measured. High differences between the antiproliferative and cytotoxic activities

Where hexane extract show moderate activity when fractionated and analyzed show it contain mixture of volatile oil and sesquterpene as B-Caryophyllene, caryophyllene oxide which identified by GC combined with MS is the most widespread method of Sesquiterpene. Piperdenon and prgenane also can be analyzed by GC, GC–MS, TLC and OPLC. Here GC MS is the method of choice[31]

Sylvester, [32] sure our opinion in present study for the good activity of hexane extract against human lung carcinoma cell line A-549,also the major compound of essential oil of Myrica gale is beta- caryophyllene and caryophyllene oxide , Sylvestre study the anticancer activities of these extracts of Myrica gale L.(Myricaceae), were assessed against human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line, DLD-1. The 60-min fraction showed higher anticancer activity against both tumor cell lines with an IC_{50} value of 88 +/- 1 microg/ml. The 30-min fraction had an IC_{50} value of 184 +/- 4 microg/ml for A-549 and 160 +/- 3 microg/ml for DLD-1. The higher cell growth inhibition induced by the 60-min fraction, as compared to the 30-min fraction, could be due to sesquiterpene(beta- caryophyllene (9.31-10.97%) and caryophyllene oxide content was detected in oil were the major components.

References

[1] Boulos L (1999) Flora of Egypt Check-list. Al Hadara Publishing Co., Cairo, Egypt, . 419-420.
[2] Ales A. Ivana, D. Vira, F. and Dave A. (2004). Geographical distribution of lactuca, Botanical Review ,Vol. 70, No. 3, 328-356.
[3] Leila J., Ghassem H. B., Hamide S. (2011) Introduction the medicinal species of Asteraceae family in Ilkhji and Sharafaldin regions of Esat Azarbaijan in Iran, Journal of American Science; 7(5):455458]. (ISSN: 1545-1003). http://www.americanscience
[4] Bradley S., Andrew A., Katrina C. , Deborah J., Jenny L. , John C. , Oswald O ., Richard W., David K. , Steven J. (2006) Identification and comparison of natural rubber from two Lactuca speciesPhytochemistry,Vol.67, 23 2590–2596.
[5] Wink M. (1999) Introduction biochemistry, role and biotechnology of secondary metabolites. In: "Biochemistry of plant secondary metabolism" (M. Wink, ed.), Sheffield Academic Press and CRC Press, Annual Plant Reviews, Vol. 2, 1-16, 1999
[6] Barton D. R., Brooks C. W. (1951) Triterpenoid. Part I., Morolic acid a new triterpenoid sapogenin. Journal Chemical Society; 257±77
[7] WAGNER H. , BLADT S. (1996) Plant drug analysis: a thin layer chromatography atlas, 2nd ed. New York: Springer.118
[8]Van Den Dool H. , Kratz P.D. (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11: 463-471.
[9] Adams R. P. (1995) Identification of essential oil components by gas chromatography/mass spectroscopy. Illinois: Allured Publishing Corporation.
[10] Tellez M. R. , Canel C. , Rimando AM, Duke S.O. (1999) Differential accumulation of isoprenoids in glanded and glandless Artemisia annua L. Phytochemistry52, 1035-1040. [Links]
[11[Araujo E.C., Silveira E.R., Lima M.A., Neto M.A., Andrade IL, Lima M.A., Santiago G.S., Mesquita A.M. (2003) Insecticidal activity and chemical composition of volatile oils from Hyptis martiusii Benth. J Agr Food Chem 51: 3760-3762. [Links]
[12] Priestap H. A., Van Baren C.M., Di Leo Lira P., Coussio J.D., Bandoni A.L. (2003) Volatile constituents of Aristolochia argentina. Phytochemistry 63: 221-225. [Links]
[13] El-Sayed A. M., Heppelthwaite V.J., Manning L.M., Gibb A.R., Suckling D.M. (2005) Volatile constituents of fermented sugar baits and their attraction to lepidopteron species. J Agr Food Chem 53: 953-958. [Links]
[14] Morteza-Semnani K., Saeedi M., Akbarzadeh M.(2007) Essential oil composition of Teucrium scordium L. from Iran. Acta Pharm 57: 499-504.
[15] Lamien-Meda A., Schmiderer C., Lohwasser U., Börner A., Franza C., Novaka J. (2010) Variability of the essential oil composition in the sage collection of the Genebank Gatersleben: a new viridiflorol chemotype. Flavour Fragr J 25: 75-82.
[16] Chaichana J., Niwatananum W., Vejabhikul S., Somna S., Chansakaow S. (2009) Volatile constituents and biological activities of Gardenia jasminoides. J Health Res 23: 141-145.

[17] Botrel P.P., Pinto J., Figueiredo F.C., Bertolucci S. V., Ferri P.H.(2009) Teor e composição química do óleo essencial de Hyptis marrubioides Epling (Lamiaceae) em diferentes genótipos. Rev Bras Pl Med 11: 164-169. [Links]
[18] Skehan,. R Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, JWarren, . H. Bokesch, T. Kenney, S. and Boyd , R. (1990). New colorimetric cytotoxicity assay for anti-cancer drug screening. Journal of the National Cancer Institute 82: 1107-1112.
[19] Bohlmann F., Ludwig G.W., Jakupovic J., King RM, Robinson H 1983. A daucanolide and further farsene derivatives from Ageratum fastigiatum. Phytochemistry 22: 983-986.
[20]- Hostettmann-Kaldas M., Nakanishi K. 1979, Moronic acid, a simple triterpenoid keto acid with antimicrobial activity isolated from Ozoroa mucronata. Planta Medica; 37: 358±60
[21]- Gonzµlez AG, Amaro J, Fraga BM, Luis JG. 3-Oxo-6-b-hydroxyolean-18-en-28-oic acid from Orthopterygium huanay. Phytochemistry1983; 22: 1828±30.
[22] A Short Approach to Highly Functionalised Homochiral Piperidinones", S.A. Hermitage and M.G. Moloney, Tetrahedron:Asymmetry, 1994, 5, 1463-1464
[23]- Engel R, Gutmann M, Hartisch C, Kolodziej H., Nahrstedt A. (1998) Study of the composition of the volatile fraction of Hamamelis virginiana. Planta Med 64: 251–258.
[24] Laszczyk, M. N (2009) Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy, Planta Med. Dec;75(15):1549-60.
[25] Wada S. A. , Iida R. (2001) Tanaka Screening of triterpenoids isolated from Phyllanthus flexuosus for DNA topoisomerase inhibitory activity. J. Nat Prod;64:1545–7.
[26] Beretta G.L. Perego P. Zunino F. (2008) Targeting topoisomerase I: molecular mechanisms and cellular determinants of response to topoisomerase I inhibitors. Expert Opin Ther Targets;12:1243–56.
[27] Naghma K., Farrukh A., Hasan M. (2008) Cancer Chemoprevention Through Dietary Antioxidants: Progress and Promise Antioxidants , Redox Signaling. March Vol. 10, No. 3: 475-510.
[28] Rzeski, A .Stepulak, M. Szymariski, M . Juszczak, A. Grabarska, M. Sifringer, J. Kaczor, M. Kandefer (2009). Betulin elicits anticancer effects in tumor primary cultures and cell lines in vitro. Basic and Clinical Pharmacology and Toxicology, 105: 425-432.
[29] Thao, N. T. P., Hung, T. M., Lee, M. K., Kim, J. C., Min, B. S. & Bae, K. (2010). Triterpenoids from Camellia japonica and Their Cytotoxic Activity, Chemical Pharmaceutical Bulletin,58(1): 121- 124. ISSN: 0009-2363.
[30] Boglárka C., L., Zsuzsanna H., Borbála R., István Z., Imre M., Tamás R., George F., Judit H.,(2009), Ant proliferative activity of Hungarian Asteraceae species against human cancer cell lines. Part II. Phytother Res .
[31] Irmgard M. (2002) Review of the analytical techniques for sesquiterpenes and sesquiterpene lactones, ; Institute of Pharmaceutical Biology, Journal of Chromatography A, 967 115–130.
[32]- Sylvestre, M. , Legault J. , Dufour D., Pichette A. (2005) Chemical composition and anticancer activity of leaf essential oil of Myrica gale L. Phytomedicine. 2005 Apr ;12 (4):299-304 15898708.

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