

PHYTOCHEMICAL AND BIOLOGICAL ACTIVITIES OF TWO ASTERACEAE PLANTS *SENECIO VULGARIS* AND *PLUCHEA DIOSCORIDIS* L.

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Abstract

Identifying ornamental plants as new natural anticancer sources is always of great effective for the ornamental and horticultural industries. The investigator for *Senecio vulgaris* were identified by GC-MS to give a total of 14 sesquiterpenoids, represents 98.53% of the total oil, while *Pluchea dioscoridis* L. shows the standardization of 30 compounds essential oil represents 91.28% of the total oil. *Senecio vulgaris* essential oil showed a high brine shrimp lethality with LC₅₀ value of 8 µg/ml followed by *Pluchea dioscoridis* L with LC₅₀ value of 24 µg/ml. Following cytotoxic activity was screened by MTT viability test against HePG2 cell line. Inhibitory activity against Hepatocellular carcinoma cells of *S. vulgaris* and *P. dioscoridis* L essential oil extract under the experimental conditions exhibited a promising IC₅₀ of 5.63 and 11.50 µg/ml, respectively compared to Doxorubicin as a control. The results demonstrated the potential of the two plants essential oils for cancer treatments; therefore, in the future *in vivo* experiments are required for further understanding of the effect of these plants on cancer cells to be useful in medicine.

Keywords: *Senecio vulgaris*, *Pluchea dioscoridis* L, cytotoxic activity, volatile oil

Introduction

Medicinal plants are an important category of the plants that includes some chemical constituents in their bodies and are benefit for therapy purposes. These are also different in their habits like herbs, shrubs, trees, climber and creepers etc. (Patel, 2014). Natural products from plants afforded an opportunity for a new medicinal an approach for new drugs.

Cancer is a group of diseases that fall under the name of a cancer (Adebajo *et al.*, 2012). Liver cancer is one of the most common types of cancers known today, as it represents 22,000 men and 9,000 women in the United States (Emre and McKenna 2004; Ahmed *et al.*, 2009). Not only that, but also among the challenges the knowledge of phytochemicals that are primarily responsible for any biological activity within the plant and to accurately identify this activity (Breslin and Andrew, 2017) (Puhan *et al.*, 2017).

Senecio vulgaris family: Asteraceae is one of the most important plants that fall under the family of Asteraceae (Dan Tenaglia, 2007). The groundsel of *Senecio vulgaris* was tested and explored for its impact on human health as it was discovered to act as a diaphoretic, an antiscorbutic, a purgative, a diuretic and an anthelmintic, and not only that it worked on the expelling of gravel of the kidneys and reins (Jin *et al.*, 2018) (Lin *et al.*, 2018). *Pluchea dioscoridis* family: Asteraceae is a plant species that has many forms and characteristics that distinguish from the rest of the species (Buapool *et al.*, 2013). *Pluchea* is one of the most important types of plants that are used in many treatments, urging that it has a high ability to kill cancer cells and actually the process of apoptosis (Gridling *et al.*, 2009). The current article revealed study of the chemical constituents and biological activities of *Senecio vulgaris* and *Pluchea dioscoridis* L including cytotoxicity test using brine shrimps larva and HepG2 cell lines (hepatocellular carcinoma cell lines).

Methods

Experimental

Collection and identification of plant materials

The plant materials of *S. vulgaris* and *P. dioscoridis* L were collected during flowering period from desert Egypt - Alexandria road at Egypt, in March. Identification of the plants was confirmed by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. A voucher specimen no. Sv 1 and Pd 1, respectively has been deposited at Medical Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

General Experimental Procedures

Chemicals and Reagents Used

The used solvents and reagents used in this study were all analytically graded, as; hydrochloric acid, ammonium hydroxide, citric acid, salicylic acid, mercuric chloride, diethyl ether, sodium sulfate, sulfuric acid, chloroform, acetic anhydrides, ammonium solution, magnesium powder, sodium hydroxide, ferric chloride, α -naphthol, and ethanol. Other solvents/reagents used for brine shrimp assay were saline (Instant Oceanic, Marine land Labs, USA) and brine shrimp's eggs (Artemia Inc., California). Also for MTT assay other chemicals were used as Dimethyl sulfoxide (DMSO), trypan blue dye (obtained from Sigma St. Louis, Mo., USA), Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA (obtained from Lonza Belgium). Cell lines that used were from the American Type Culture Collection (ATCC, Rockville, MD) as it was Hepatocellular carcinoma cell line HepG-2. All solvents, materials and acids were fetched from Merck Chemical Company and Sigma-Aldrich Company.

Equipments Used

Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of your samples were performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 μ m film thickness). The column oven temperature was initially held at 50 C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C

/min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Extract preparation

The powdered fine of each plants sample (500g) was extracted with 85% methanol; the solution was covered and shaken every 30 min for about six (6) hours and allowed to stand for about 48 hours in room temperature. Then, solution was shaken and filtered using Whatman filter paper (No.1). Then, the solvent was removed by evaporation using a rotary evaporator apparatus under reduced pressure at temperature below 55°C to keep stabilization of the methanol constituents.

Essential oil Extraction

Significantly, the essential oils extraction methods from plants affect the essential oil composition and chemical constituents. Also it should be select the most convenient and appropriate method to concentrate the biologically active targeted compound into an essential oil. Also, it should be concentrate all the active components from the extract if the activity is not based on a single compound but based on a mixture. Generally, according to that the most essential oils constituents are small, lipophilic and volatile; the consideration key is the need of separation of these compounds from the materials of aqueous plant. There are several methods which have been developed but the most recent reports which describing the used methods to made an essential oils extraction, It is only will be review.

The essential oil of the fresh plants (2 Kg each) was obtained by hydrodistillation using a Clevenger-type apparatus for 5 h. After drying with anhydrous sodium sulphate and filtration then

extracted with diethyl ether, the oil obtained was stored at refrigerator until use.

Brine shrimp lethality test

The extracts, fractions and pure isolated compounds were routinely evaluated in a test for lethality to brine shrimp larvae 3, with minor modifications. Toxicities of compounds were tested at 50, 100, 300, 800 and 1000 ppm in 10 mL sea-water solutions with 1% DMSO (v/v). Ten, one-day nauplii were used in each test and survivors counted after 24 h. Three replications were used for each concentration. A parallel series of tests with the standard potassium dichromate solution ($DL_{50} = 20-40$ ppm) and the blank control were always conducted. The lethal concentration for 50% mortality after 24 h of exposure, the chronic LC_{50} and 95% confidence intervals were determined using the probit method 10, as the measure of toxicity of the extract or fractions. LC_{50} values greater than 1000 ppm for plant extracts were considered inactive (Hamed et al., 2015b; Hamed et al., 2016).

Antitumor Assays by MTT Method

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were subcultured two to three times a week. For antitumor assays, the tumor cell lines were suspended in medium at concentration 5×10^4 cell/well in Corning 96-well tissue culture plates, and then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve twelve concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO₂ for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the

pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as $[(OD_t/OD_c)] \times 100\%$ where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) (Mosmann, 1983; Hassanein et al., 2010).

Statistical analysis

The results were expressed as the average standard deviation of the same parallels using the SPSS 13.0 program. Microsoft Excel 2003 had been used for measuring the anticancer activity results.

Results and Discussion

Recently we notice a much attention is being paid to extracts and biologically active plant species used in folklore medicine.

Phytochemical screening

The results of the phytochemical screening (Trease, 1989) carried out on the methanolic extract of *Senecio vulgaris* whole plant is shown in table 1 below. As an analytical responses, the precipitate formation and the color intensity has been used to these tests. The result shows the presence of alkaloids, steroids, glycosides, tannins, flavanoids and terpenoids, saponins, alkaloids and tannins. Using the phytochemical qualifications, plant methanol extract was evaluated for the usual plant secondary metabolites. It was concluded that *Pluchea dioscoridis* L plant contains Saponins, Triterpenes, Alkaloids, Carbohydrates, Tannins, Flavonoids and Steroids, by the results which have been obtained which shown in Table 2.

Essential oil GC-MS mass analysis

Essential oils are recognized and well accepted in chemical industry, more recently in academia, even in everyday life (Teranishi et al., 1993). Generally, essential oils are produced by specific species of plants and known as aromatic substances. Since ancient times, there are most of these oils which are flavoring agents and used as raw material fragrances. It was once thought called essential because, it was represented that the original plant essence (Foderaro, 1991). Also since ancient times, essential oils have been used as medicines. Because of many traditional folk medicines which are based mainly on materials of plant, from this standpoint in many areas the most widely used natural products are essential oils.

From many plant parts, the essential oils can be extracted which including twigs, fruit peels, leaves, seeds, fruits, stems, flowers, and roots. Particularly for physiological effects of essential oils on the plants themselves, the roles of their have not been well studied. Also for many years, essential oils have been known that have important roles the plant as repellents and on the other hand, as insect attractants. The materials of essential oils have some major classes which include diterpenoids, Mono- and sesqui-. Also essential oils have been reported to have therapeutical activity or pharmacological activity (Sticher, 1977). The understanding of the relationship between biological activity and essential oils, is very important because of the use of essential oils is becoming more popular and in everyday life as important for many practical applications. Furthermore, considerable quantities of essential oils are consumed from a food sources variety and the exposition to volatiles from plants, are being in forested and gardens areas.

The investigators were identified by GC-MS to give a total of 14 sesquiterpenoids, represents 98.53% of the total oil including; Butylated Hydroxytoluene (2) (63.87% of oil), 4,4'-Ethylenebis(2,6-di-tert-butylphenol) (14) (8.29%), Phthalic acid, di(2-propylpentyl) ester (13) (3.98%), Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (10) (3.27%), Glycerol 1-palmitate (7) (3.11%), 1,3-Propanediol, 2-methyl-2-(1-methylpropyl)-, Dicarbamate (5) (2.43%),

Heptacosane (9) (1.94%) and 4-Nitrophthalamide (4) (1.85%) as the major constituents (Table 3,4).

Butylated Hydroxytoluene (BHT) do as doing other closely related phenol antioxidants, it has low acute toxicity. The LD₅₀ of 2, 6-di-tert-butylphenol as an example, it is greater than 9 g/kg (Helmut et al., 2002). BHT has shown in early stage research, as an anti-viral activity (Chetverikova and Inozemtseva, 1996).

The Table 5 shows the standardization of 30 compounds essential oil through GC-MS represents 91.28% of the total oil. The major compounds observed in the essential oil chemical composition used in this work were sesquiterpens: 2H-Cyclopropa[a]-naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1aà,7à,7aà,7bà)- (25) 55.42%, 1,6-Octadien-3-ol, 3,7-dimethyl-, acetate (2) 7.45%, Benzene,1-methyl-4-(1-methylethyl)- (6) 4.14%, Bicyclo[3.1.0]hexane (5) 3.12%, Guaia-1(10),11-diene (16) 2.19%, (1R,3aR,4aR,8aR)-1,4,4,6-Tetramethyl-1,2,3,3a,4,4a,7,8 octahydrocyclopenta[1,4]cyclobuta[1,2]benzene (15) 1.71%, 1,4,7,-Cycloundecatri-ene,1,5,9,9-tetramethyl-, Z,Z,Z- (14) 1.68% table 5,6.

In the toxicity, antimicrobial, anti-parasitic, anticancer, the rate of interest has been raised by the wide use of limonene in soft drinks, many other flavoring products and cosmetics. Limonene is a compound which composed of two isoprene units, and it is rendered to be a potential antioxidant compound because the presence of two double bonds. According to a study published in *Bioorganic and Medicinal Chemistry* and made by Keinan et al., In 2005, (Keinan et al., 2005) this study is working on validating this property and it was found that the pulmonary membrane is easily being saturated by limonene and this will be benefit because It will protect the lung cells from other oxidant agents and either endogenous or exogenous ozone. Because of Limonene's chemo-preventive properties which work against many types of cancer, it has been well researched by many scientists. The Administration of either a pure form of limonene or as an orange peel oil constituent (>90% d-limonene), It will be inhibit the development of the skin cancer, lung cancer, odent mammary and fore-stomach cancers which is chemically induced (Haag et al., 1992).

The trans-Verbenol would be obtained from the biotransformation, which is produced at lower concentrations in an *in vitro* effect against the tumor cells (IC₅₀ value ¼ 77.8 mg/mL) (Paduch et al., 2016).

The paclitaxel passage through the membrane is being facilitated by beta-caryophyllene and thus also strengthens its anticancer activity (Legault and Pichette, 2007). The cytotoxic activity of the β-caryophyllene oxide against SNU-1, HeLa, AGS, HepG2, and SNU-16 cells has been evidenced, respectively with 3.95, 12.6, 13.55, 16.79, and 27.39 μM of IC₅₀ values. Also the results have been showed that the cytotoxicity of β-caryophyllene oxide in both time-dependent manner and dose-dependent manner has been evidenced (Jun et al., 2011).

The phytol has been tested in all of the cell lines by testing the induced cytotoxic response which is concentration-dependent, which respectively has been demonstrated that to be most effective against the prostate adenocarcinoma PC-3 cells and the breast adenocarcinoma MCF-7 (IC₅₀ 8.79 ± 0.41 μM and 77.85 ± 1.93 μM). The other five tumors (A-549, HeLa, Hs294T, HT-29, and MDA-MB-231) have been towards in the IC₅₀ values, which is ranged between 15.51 to 69.67 μM. However, the mild toxicity against (MRC-5) the foetal lung fibroblast cells has been detected at the used concentrations (IC₅₀ 124.84 ± 1.59 μM) (Pejin et al., 2014).

Brine shrimp bioassay

The Brine Shrimp (*Artemia* sp.) Lethality Assay (BSLA) (Pisutthanan et al., 2004) is a general bioassay which appears that it is capable of detecting a bioactivity broad spectrum present in plant crude extracts. BSLA is used a guide for the detection of antitumor, as an indicator for general toxicity and pesticidal compounds (Meyer et al., 1982). The ease of performing and the low cost of the assay and the inexpensive brine shrimp eggs commercial availability makes BSLA a very useful bench top method (McLaughlin et al., 1991). For the isolation from plant extracts of bioactive compounds, BSLA assay has been noted as a useful tool (Sam, 1993). As a preliminary study of antitumor agents and cytotoxic agents, the successfully test has been the *In vivo* lethality test (Ramachandran et al., 2010). Thus, this present work

findings would give an information baseline on the most plant species promising that could be used for the development as a basis of new tools of great therapeutic importance.

Oil extract of *S. vulgaris* exhibited a high brine shrimp lethality with LC₅₀ value of 8 µg/ml. This is followed by methanol extract with LC₅₀ values of 13 µg/ml (figure 1,2). The degree of lethality was directly proportional to the concentration of the extract. Based on the results obtained, the brine shrimp lethality of *S. vulgaris* extracts were found to be concentration-dependent. The observed lethality of the plant extracts to brine shrimps indicated the presence of promising cytotoxic activity and probably antitumor constituents of *S. vulgaris* plant. According to Meyer et al., crude plant extract is toxic (active) if it has an LC₅₀ value of less than 1000 µg/mL while non-toxic (inactive) if it is greater than 1000 µg/mL (Meyer et al., 1982).

It was appeared that; From the Brine shrimp assay which was found that in the control sets almost all the shrimp survived throughout the observed period (24 h) while the LC₅₀ value was seems to be 22 µg/ml and 24 µg/ml for Methanol and oil extracts, respectively (figure 3,4).

Anticancer activity

Cancer is a group of genetic disease which comprises specific hallmarks. They include evading growth suppressors, sustaining proliferative signaling, resisting cell death, activating invasion and metastasis, apart from evading immune destruction and reprogramming of energy metabolism, enabling replicative immortality, and inducing angiogenesis (Hanahan and Weinberg, 2011).

Primary liver cancers (PLC) have two major forms include intrahepatic cholangiocarcinoma (ICC) and Hepatocellular carcinoma (HCC), respectively accounting for approximately 5% and 90% (Kumar et al., 2011; Sithithaworn and Haswell-Elkins, 2003). The most common cancer widespread in the world is the incidence of each. In Cambodia, Thailand and Laos, where viral hepatitis is endemic, the high annual mortality rates caused by HCC (Newell et al., 2008).

In traditional medicine, *Senecio* with different species have been used as diaphoretic, diuretic, anthelmintic, for the scurvy treatment as poultice, in sickness of stomach treatment it said to be useful

(Sahu et al., 2011), as calmative and antipyretic, to treat against lung and cholera diseases (Qureshi et al., 2007). the *S. scandens*, *S. wallichii* and *S. graciliflorus*, have been used in Rasuwa district of Nepal, to treat increase appetite, fever, headache, for indigestion and to care chest pain. Also the *S. stabianus*'s hexane fraction was reported to inhibit the cancer cell lines viability (Tundis et al., 2009).

A large number of studies in these recent years have been documented their chemical constituents and the essential oils efficacy, including against cancer (Rasoanaivo et al., 2003; Zapata et al., 2014) they have been used as a source of new bioactive natural products. Therefore, the chemical constituents and essential oils are natural products used against various types of tumors with high pharmacological potentials.

Inhibitory activity against Hepatocellular carcinoma cells of *S. vulgaris* essential oil extract was detected using MTT assay under these experimental conditions with a promising IC₅₀ of 5.63 ± 0.4 µg/ml compared to Doxorubicin as a control which showed anticancer activity at (4 µg/ml) (Hassanein et al., 2010) (Fig 5). *P. dioscoridis* L plant essential oil showed growth inhibition in Liver cancer cell lines at 11.50 µg/ml. These results are shown in (Fig. 6). Data obtained from MTT reduction assay.

Some of certain medicinal plants extracts, various secondary metabolites in plant was showing a response of positive apoptotic in a human cancer cell lines variety (Taraphdar et al., 2001).

In conclusion

According to the existing literature in conjunction with the observed results, *S. vulgaris* and *P. dioscoridis* L are anticipated that, especially its oil extracts and methanol, for mitigating human diseases, this might be used for mitigation. The tentative identification or identification of compounds have been allowed for investigate the GC/MS analyses. For better understanding of the impact, this will further extensively trigger research of *Senecio vulgaris* and *P. dioscoridis* L plants on health and thus may be utilized for raising antitumor drug of interesting pharmaceuticals of plant origin.

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Table 1: Phytochemical screening of *Senecio vulgaris* plant

Test Name:	<i>Senecio vulgaris</i>
Test for volatile oil	Positive
Saponins	Positive
Carbohydrates	Positive
Triterpenes	Positive
Steroids	Positive
Alkaloids and/or nitrogenous bases	Positive
Tannins	Positive
Flavonoids	Positive

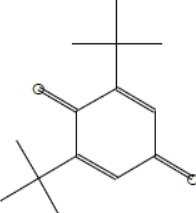
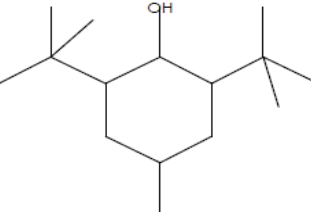
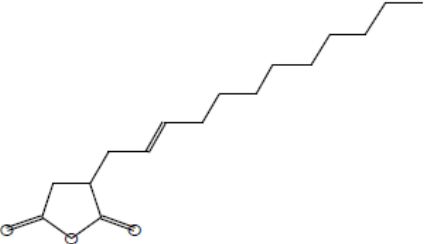
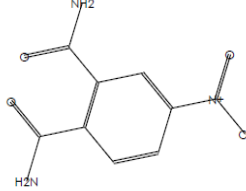
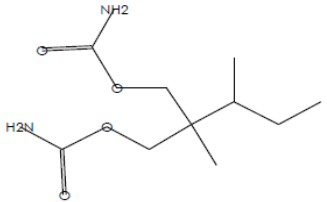
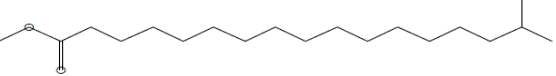
Table 2: Phytochemical screening of *P. dioscoridis* L

Test Name:	<i>P. dioscoridis</i> L
Test for volatile oil	Positive
Saponins	Positive
Carbohydrates	Positive
Triterpenes	Positive
Steroids	Positive
Alkaloids and/or nitrogenous bases	Positive
Tannins	Positive
Flavonoids	Positive

Table 3: Results of GC/MS analyses of *Senecio vulgaris* plant.

Peak No.	R _t	Area %	M.F.	Identified Compounds
1	21.08	1.83	C ₁₄ H ₂₀ O ₂	2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-
2	22.48	63.87	C ₁₅ H ₂₄ O	Butylated Hydroxytoluene
3	23.22	1.53	C ₁₆ H ₂₆ O ₃	2-Dodecen-1-yl(-)succinic anhydride
4	28.99	1.85	C ₈ H ₇ N ₃ O ₄	4-Nitrophthalamide
5	31.19	2.43	C ₁₀ H ₂₀ N ₂ O ₄	1,3-Propanediol, 2-methyl-2-(1-methylpropyl)-, Dicarbamate
6	37.34	1.74	C ₁₉ H ₃₈ O ₂	Heptadecanoic acid, 16-methyl, methyl ester
7	41.59	3.11	C ₁₉ H ₃₈ O ₄	Glycerol 1-palmitate
8	42.75	1.57	C ₂₂ H ₄₆	Docosane
9	44.68	1.94	C ₂₇ H ₅₆	Heptacosane
10	45.59	3.27	C ₂₁ H ₄₂ O ₄	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
11	46.55	1.67	C ₃₆ H ₇₄	Hexatriacontane
12	48.34	1.45	C ₃₂ H ₆₆	Docosane, 11-decyl-
13	48.68	3.98	C ₂₄ H ₃₈ O ₄	Phthalic acid, di(2-propylpentyl) ester
14	52.91	8.29	C ₃₀ H ₄₆ O ₂	4,4'-Ethylenebis(2,6-di-tert-butylphenol)

Table 4: shows the compound separated from GC/MS analyses for *Senecio vulgaris* plant.

Structure	Number
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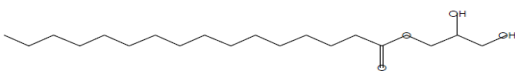


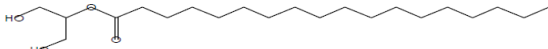

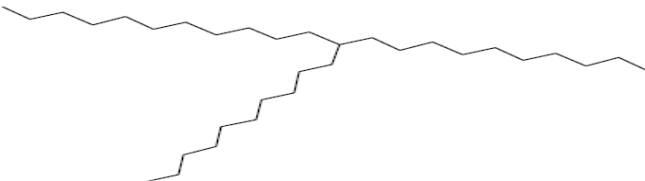
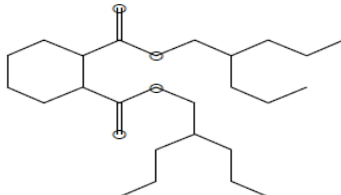
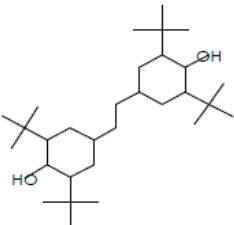
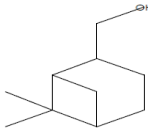
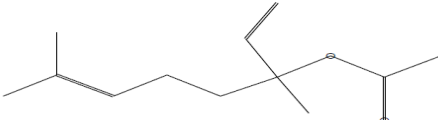
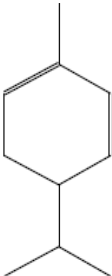
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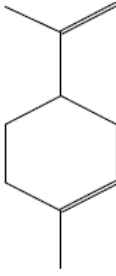
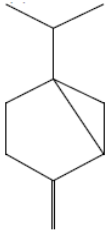
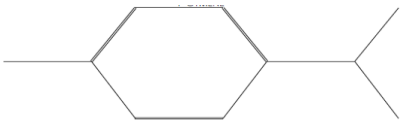
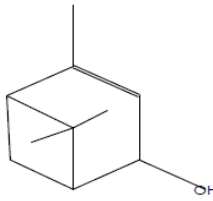
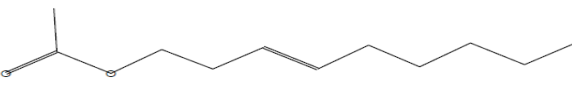
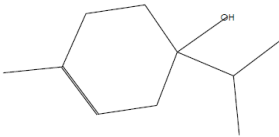
Table 5: GC/MS analyses of *Pluchea dioscoridis* L plant.

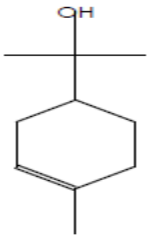
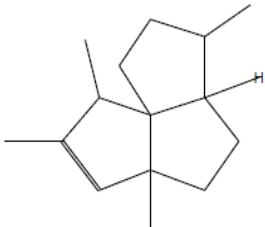
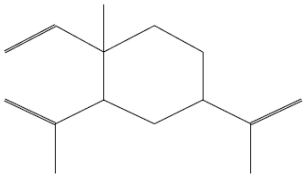
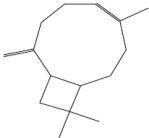
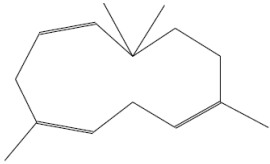
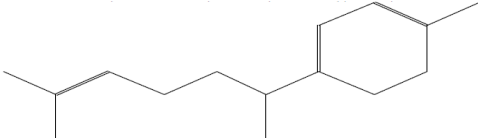
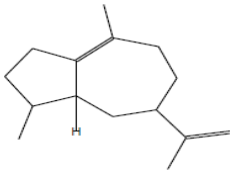
Peak No.	R _t	Area %	M.F.	Identified Compounds
1	5.17	0.66	C ₁₀ H ₁₈ O	(6,6-Dimethylbicyclo-[3.1.1]hept-2-yl)methanol
2	7.28	7.45	C ₁₂ H ₂₀ O ₂	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate
3	7.67	0.41	C ₁₀ H ₁₆	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-
4	8.22	0.37	C ₁₀ H ₁₆	D-Limonene
5	8.59	3.12	C ₁₀ H ₁₆	Bicyclo[3.1.0]hexane
6	9.08	4.14	C ₁₀ H ₁₄	Benzene,1-methyl-4-(1-methylethyl)-
7	12.97	0.27	C ₁₀ H ₁₆ O	trans-Verbenol
8	13.49	0.57	C ₁₁ H ₂₀ O ₂	Non-3-enyl acetate
9	13.73	1.05	C ₁₀ H ₁₈ O	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
10	14.42	0.74	C ₁₀ H ₁₈ O	(-)-(1S,2R,4R)-Beta-fenchol
11	17.12	0.66	C ₁₅ H ₂₄	Silphiperfol-5-ene
12	17.79	1.01	C ₁₅ H ₂₄	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1s-(1à,2á,4á)]-
13	18.83	0.73	C ₁₅ H ₂₄	Caryophyllene
14	20.04	1.68	C ₁₅ H ₂₄	1,4,7,-Cycloundecatri-ene,1,5,9,9-tetramethyl-, Z,Z,Z-
15	20.39	1.71	C ₁₅ H ₂₄	(1R,3aR,4aR,8aR)-1,4,4,6-Tetramethyl-1,2,3,3a,4,4a,7,8-octahydrocyclopenta[1,4]cyclobuta[1,2]benzene
16	21.10	2.19	C ₁₅ H ₂₄	Guaia-1(10),11-diene
17	21.19	0.68	C ₁₅ H ₂₂	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-
18	22.03	1.42	C ₁₅ H ₂₄	Guaia-3,9-diene
19	24.53	0.79	C ₁₅ H ₂₆ O ₂	Butanoic acid, 2-methyl-,3,7-dimethyl-2,6-octadienyl ester, (E)-
20	24.81	0.97	C ₁₅ H ₂₆ O ₂	(2E)-3,7-Dimethyl-2,6-octadienyl-3-methylbutanoate #
21	25.05	0.74	C ₁₅ H ₂₆ O	Tricyclo[7.2.0.0(2,6)]undecan-5-ol, 2,6,10,10-tetramethyl-(isomer 3)
22	26.34	0.38	C ₁₅ H ₂₄ O	Caryophyllene oxide
23	28.78	1.22	C ₁₅ H ₂₄ O	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1aà,4aà,7á,7aá,7bà)]-
24	30.41	0.94	C ₁₅ H ₂₄ O ₂	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-

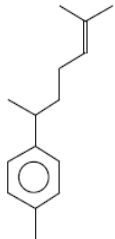
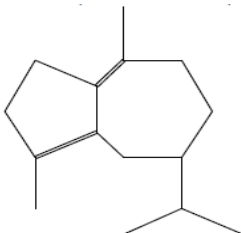
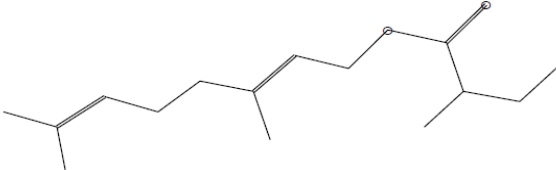
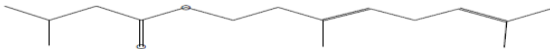
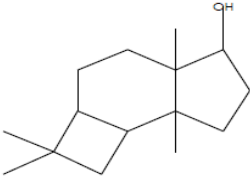
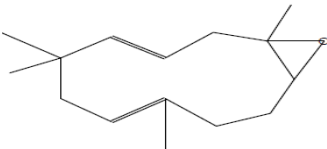
25	34.84	55.42	C ₁₅ H ₂₂ O	2H-Cyclopropa[a]-naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro- 1,1,7,7a-tetramethyl-, (1aà,7à,7aà,7bà)-
26	36.35	0.76	C ₁₃ H ₁₄ O ₃	Ethanone, 1-(7-hydroxy-2,2-dimethyl-2h-1- benzopyran-6-yl)-
27	36.87	0.59	C ₁₃ H ₁₂ O ₃	Ethanone, 1-[6-hydroxy-2-(1-methylethenyl)- 7-benzofuranyl]-
28	37.13	0.74	C ₂₀ H ₄₀ O	Phytol
29	41.19	0.43	C ₁₃ H ₁₂ O ₂	1-(6-Methoxy-1- naphthyl)ethanone
30	45.68	0.45	C ₂₁ H ₄₂ O ₄	Octadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester

Table 6: shows the compound separated from GC/MS analyses for *Pluchea dioscoridis* L plant.

Structure	Number
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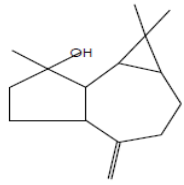
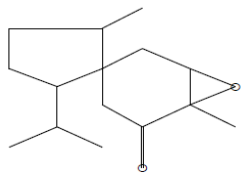
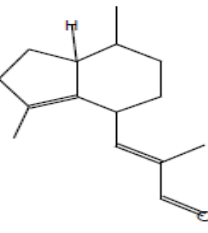
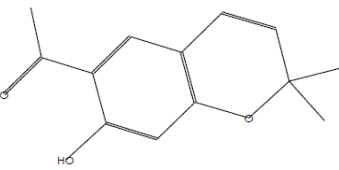
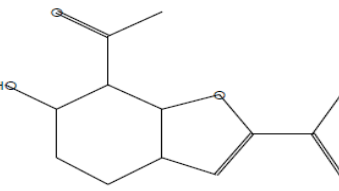
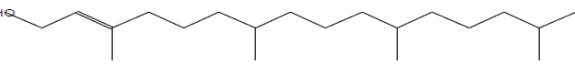
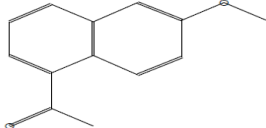
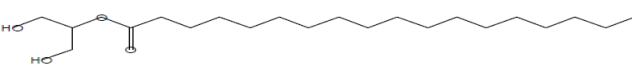
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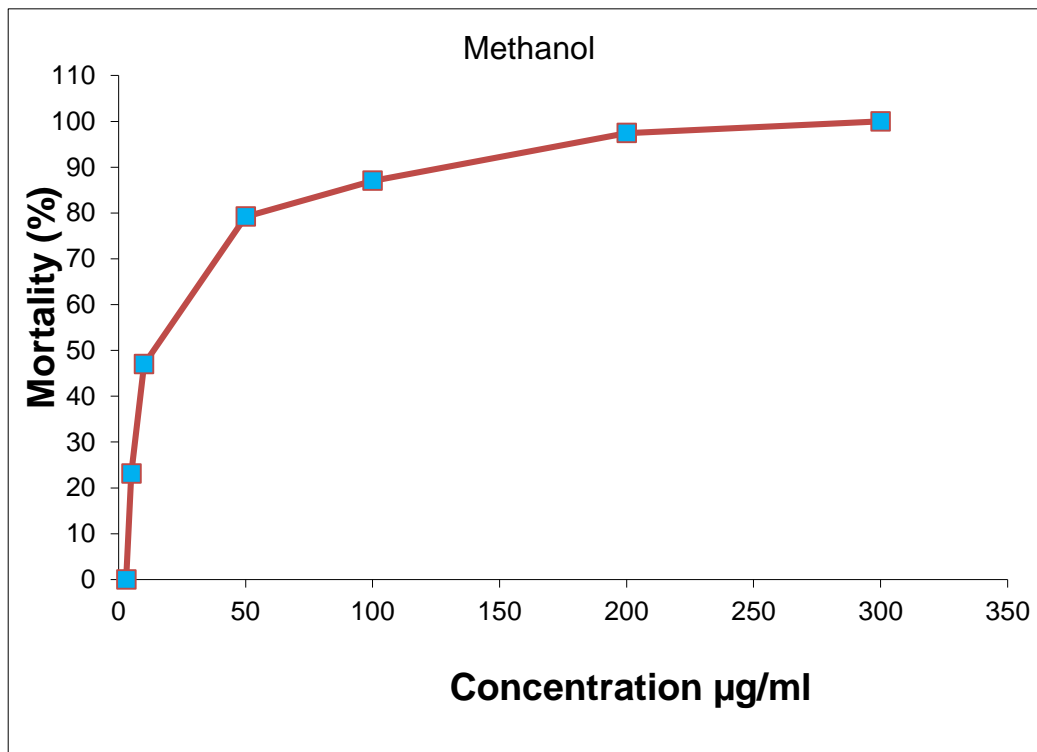
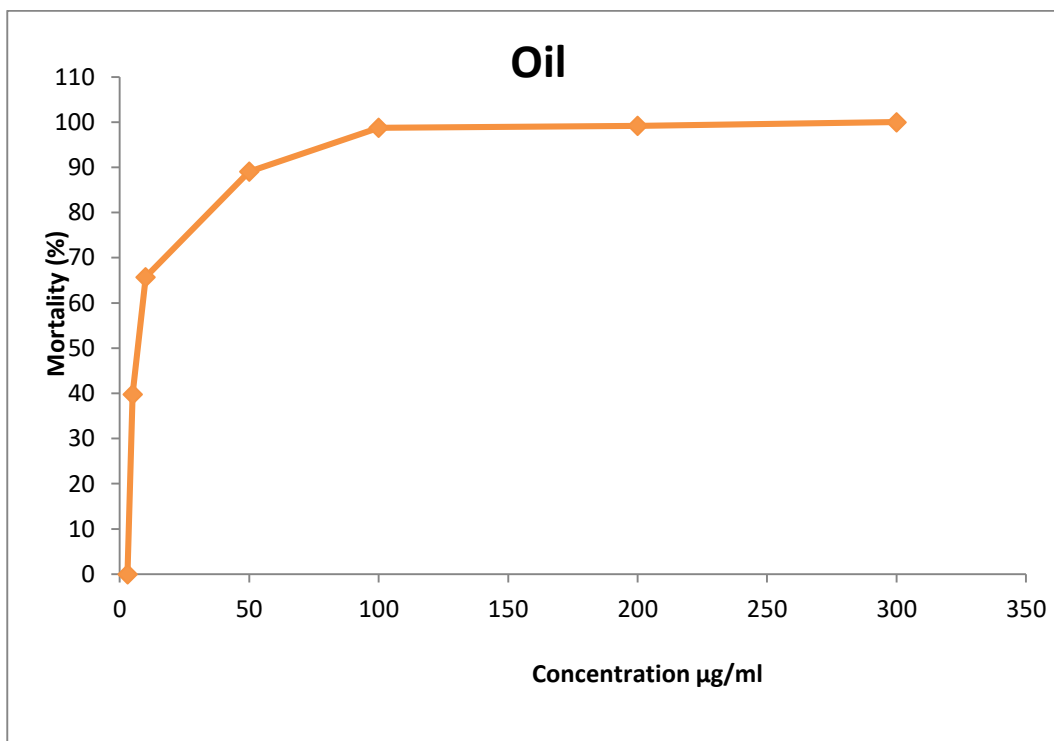
Figure 1: Brine shrimp lethality evaluation of *Senecio vulgaris* methanol extract.**Figure 2:** Brine shrimp lethality bioassay of *Senecio vulgaris* essential oil

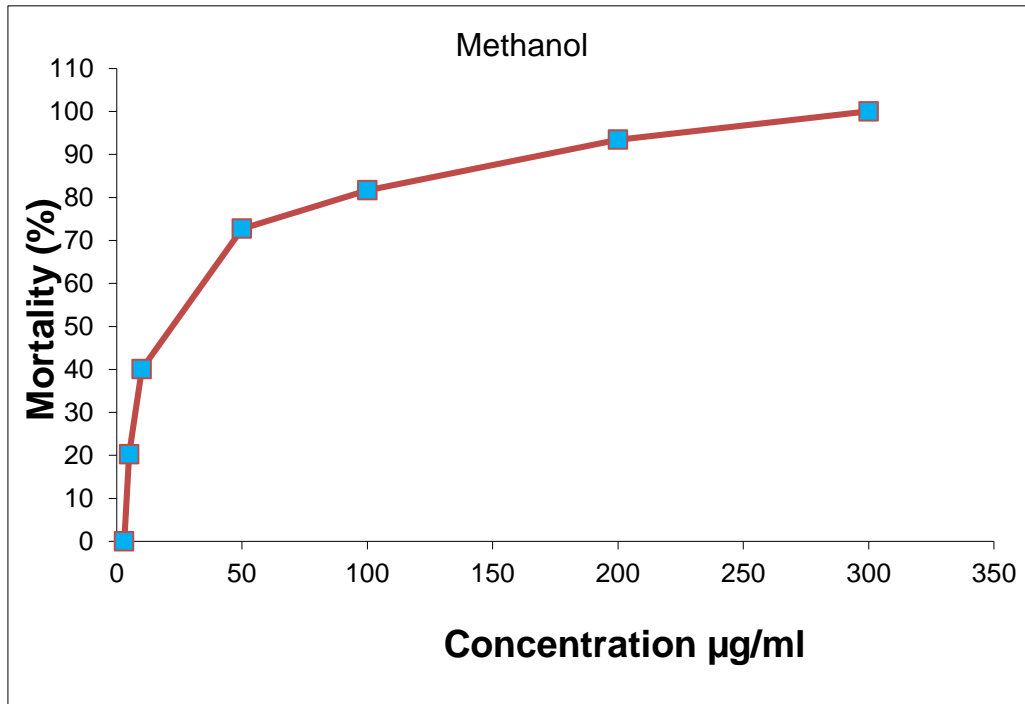
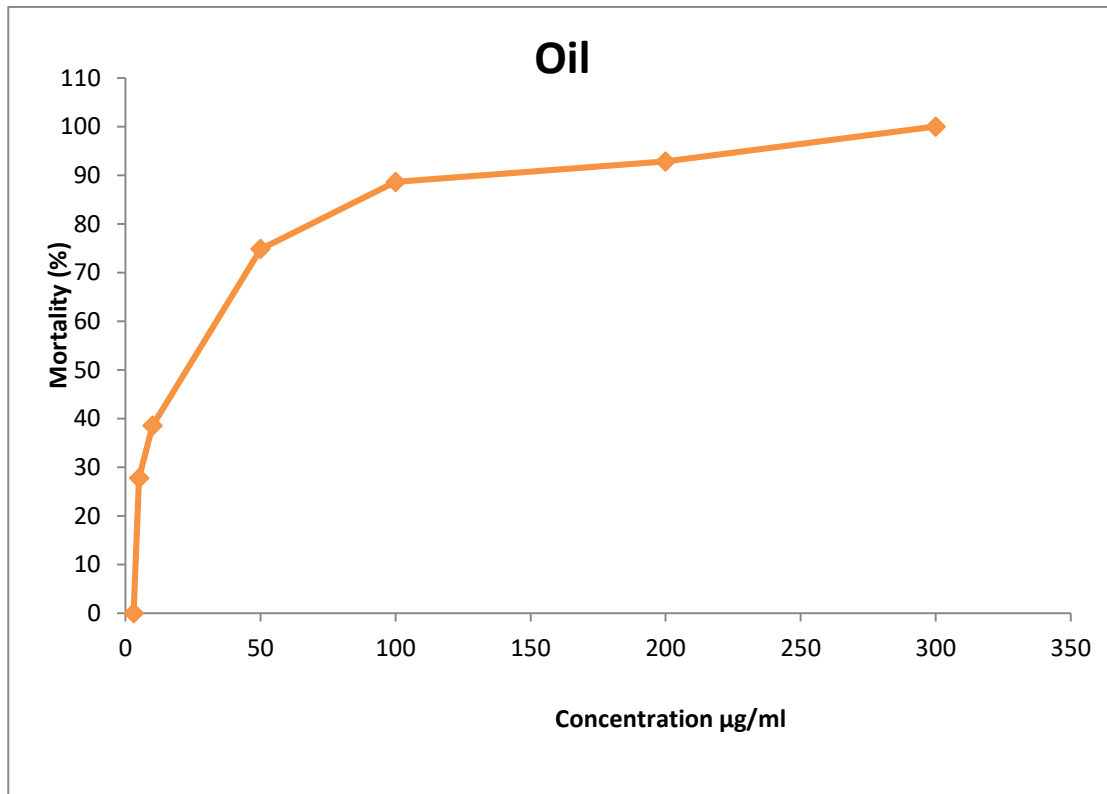
Fig. 3: Brine shrimp assay activity of methanol extract of *P. dioscoridis* L.**Fig. 4:** Brine shrimp activity assay of *P. dioscoridis* L essential oil extract.

Figure 5: The cytotoxic activity of *S. vulgaris* plant oil against HepG2 liver cancer cells.

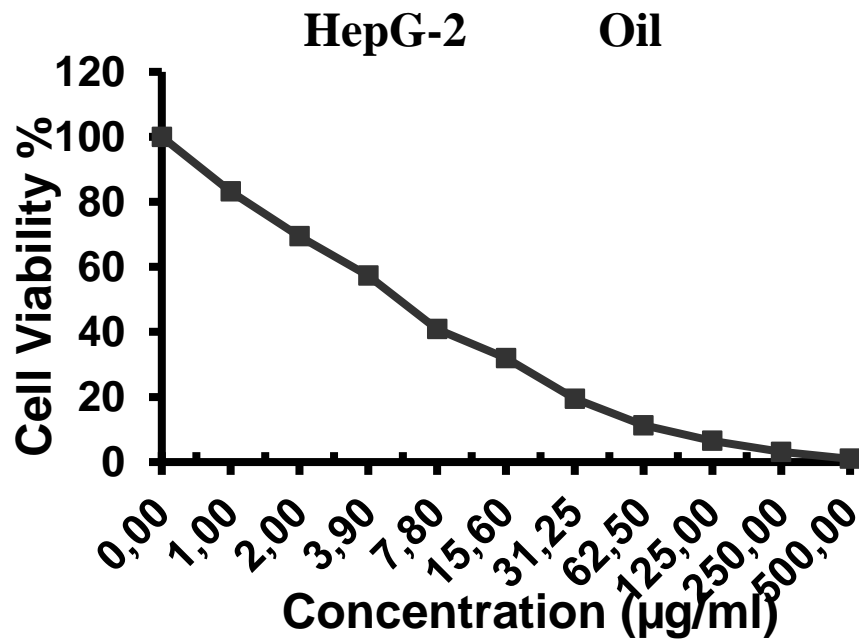


Fig. 6: The cytotoxic activity of *P. dioscoridis* L against Liver cancer cells.

