ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Essential oil, Antioxidant, Antimicrobial and Anticancer Activities of Egyptian *Pluchea dioscoridis Extract*

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ABSTRACT

Pluchea dioscorid is a common shrub along the banks of the Nile and islands, and also a pioneer of the moist rocky habitats. *P. dioscoridis* is one of the plants used for several remedies. The present study was undertaken to analyze and identify active volatile compounds of *Egyptian P.dioscoridis* essential oil. Antioxidant activity and polyphenols as well as flavonoids determined in *P.dioscoridis* extracts and tested against pathogenic organisms and HEPG2 liver cancer cell line. A total of 50 volatile components, including 10 monoterpenes, 11 light oxygenated compounds, 13 sesquiterpenes, 16 heavy oxygenated compounds were identified in essential oil. Amongst the volatiles, sesquiterpenes and heavy oxygenated compound were the largest groups. Farnesol *cis-trans*, farnesol ,nuciferol, *trans*-cadinol , eudesmol and methyl eicosane were major volatiles . Results showed that *P.dioscoridis* extract had a strong antioxidant effect *in vitro*. Antimicrobial activity of *P.dioscoridis* against nine highly pathogenic microorganisms of animal origin was tested. The antimicrobial activities of extract were compared with reference antibiotics using agar well diffusion method (AWD) as well as minimum inhibitory concentrations test (MIC). It is suggested that the ethanol extract of *P.dioscoridis* used as natural antimicrobial drug against Gram positive bacteria and Gram negative bacteria as well as mycotic infection with *C. albicans*. The results revealed that *P.dioscoridis* ethanol extract had strong effect against HepG2 liver cancer cell line.

Keywords: GC-MS**,** Natural antioxidant, phenolic contents, flavonoids, Minimum inhibitory concentration**,** human liver cell line

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ISSN: 0975-8585

INTRODUCTION

Pluchea dioscoridis is an important wild evergreen shrub, attaining a height of one to three meters, richly branched with hairy and glandular surfaces and belongs to family Asteraceae (Compositae). The plant is widely distributed in the Middle Eastern and surrounding African countries. In Egypt, it occurs mainly in the Nile region, oases of the western desert, Mediterranean coastal strip, Eastern Desert and Sinai Peninsula [1]. It inhabits the banks of water courses, moist habitats, abandoned fields, depression along road sand railways and demolished houses, solid and liquid refuses [2]. Some active chemical constituents have been reported in different parts of *P. dioscoridis* plant. These include volatile oils, flavonoids, triterpenoids, sesquiterpenoids, pluchecin and thiophene derivatives[3]. Some studies have indicated that several types of extracts of *P. dioscoridis* leaves exerted had a promising antimicrobial activates against some microorganisms [3, 4]. According to our knowledge, no studies reported to date have assessed the allelopathic potential of *Pluchea dioscoridis. P.dioscoridis* represents one of the traditional medicines all over the world. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to the host cells are considered for developing new antimicrobial drugs. Recently, evaluated the plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. . Many of the chemo-preventive agents are phyto-chemicals, namely non-nutritive plant chemicals with protective or disease preventive properties. Among the interesting biological properties exhibited by plant polyphenols, in recent years, there has been increasing interest regarding cancer prevention [5]. Plant polyphenols are promising candidate agents for liver cancer chemoprevention [6]. Although *P. dioscoridis* widely cultivated in Egypt, no enough studies reported its chemical and biochemical effects. The aims of the present study are: 1. isolation of essential oil and identify the bioactive volatile compounds, 2. determination of some phyto-chemicals (polyphenols and flavonoids) as well as determination of antioxidant activity *in vitro*, 3. identify the possible antimicrobial and anticancer effects of the extract of *P. dioscoridis*.

MATERIALS AND METHODS

Raw Material and Chemicals

Plant

Egyptian P. dioscoridis leaves obtained and identified from the department of medicinal and aromatic plants, ministry of agriculture, Egypt.

Chemicals

Diethyl ether, ethanol, sodium sulphate anhydrous, linoleic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, catechin, β-carotene, sodium nitrite, aluminium chloride, Tween-80 (polyoxyethylenesorbitanmonopalmitate),2,2\-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, potassium persulfate, deionized water, homologous series of *n*-alkanes (C_7-C_{21}), *tert*-butyl hydroquinone (TBHQ) were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA).

Isolation of Essential Oil

The essential oil (EO) of *Egyptian P. dioscoridis* leaves was extracted by hydro-distillation method using Clevenger's apparatus for 3 hours [7]. The yield of volatile oils was weighted and calculated in g/100 g dry plant.

Identification of Essential Oil

Gas chromatography (CG) analysis

About 2 µl of pure volatile oil was used. GC analysis was performed by using Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 mx 0.32 mm id,) was used. The oven temperature was maintained initially at 50°C for 5 min, then programmed from 50 to

250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C8-C22, Aldrich Co.) as references [8].

Gas chromatographic-mass spectrometric analysis (GC/MS)

The analysis was carried out by using a coupled gas chromatography Hewlett-Packard model (5890) / mass spectrometry Hewlett-Packard MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The GC condition was carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and published data [8]**.** The quantitative determination was carried out based on peak area integration.

Preparation of Hexane and Ethanol Extracts

Dry leaves of *P. dioscoridis* were subjected to hexane extraction as well as two different concentrations of ethanol (95% ethanol and 70% ethanol) extraction [9]. These three extracts subjected to determination of antioxidant activity *in vitro* by two different methods.

Determination of antioxidant activity

β-Carotene-linoleate scavenging assay

The antioxidant activity of hexane as well as two different ethanol extracts (70% and 95% ethanol) were evaluated using β-carotene-linoleate model system. 0.1 mg of β-carotene in 0.2 ml of chloroform, 10 mg of linoleic acid and 100 mg of Tween-20 were mixed. The solvent was removed at 40 ^{0}C under vacuum and the resulting mixture was diluted with 10 ml of water and was mixed well. To this mixture, 20 ml of oxygenated water was added. Four milliliter aliquots mixtures were pipetted into different test tubes containing 200 µl of each extract (40, 80, 160, 320 and 400 µg/ml) and TBHQ (40, 80, 160, 320 and 400 µg/ml) in ethanol. TBHQ as a standard antioxidant was used for comparative purposes. A control containing 200 µl of ethanol and 4 ml of the above emulsion was prepared. The tubes were placed at 50 ^{0}C in a water bath and the absorbance at 470 nm was taken at zero time (t= 0). The absorbance was continued to be measured until the colour of β-carotene disappeared in the control tubes (t =60 min) at an interval of 15 min[10]. A mixture prepared as mentioned above without β-carotene served as blank. All determinations were carried out in triplicate. The antioxidant activity (A.A) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula,

$$\% \text{ Inhibition} = [(AB-AA)/AB] \times 100$$

Where: AB: absorption of blank sample (t=0 min).
AA: absorption of sample solution (t=60 min).

Radical scavenging activity using DPPH assay

Antioxidant activity was also determined by DPPH assay using spectrophotometer at 517 nm. [11].Hexane extract as well as two ethanol extracts (70% and 95% ethanol) of different concentrations (40, 80, 160, 320 and 400 µg/ml) and TBHQ (40, 80, 160, 320 and 400 µg/ml) were taken in different test tubes. Four milliliter of 0.1 mM methanol solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared as the same without any extract. The changes in the absorbance of the prepared samples were measured at 517 nm. Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition} = [(AB-AA)/AB] \times 100$$

Where: AB: absorption of blank sample (t=0 min),
AA: absorption of sample solution (t=30 min).

The best extract which revealed the highest antioxidant activity subjected to total phenolic content and total flavonoids determination and tested its activity as antimicrobial and anticancer.

Determination of phenolic compounds (TPC)

The phenolic content was determined in ethanol extracts (95% and 70% ethanol) according to the Folin-Ciocalteu procedure [12]. Briefly, the extract (50 µl) was transferred into a test tube and oxidized with the addition of 250 µl of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na2CO3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as mg of gallic acid equivalent (GAE) per ml of sample.

Determination of total flavonoid content (TFC)

The total flavonoid content was determined in ethanol extracts (95% and 70% ethanol)[12]. Briefly, 250 µl of 5% NaNO2 was mixed with 50 µl of extract. After 6 min, 2.5 ml of a 10% AlCl3 solution was added. After 7 min, 1.25 ml of 1 M NaOH was added, and the mixture was centrifuged at 5000 *g* for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as mg of catechin equivalent (CE) per ml of sample.

Antimicrobial Assay

Preparation of microbial suspensions

Antimicrobial activities were carried out against nine highly pathogenic strains accused of being the main cause of food intoxication of animal byproducts; four Gram positive bacteria (*S. aureus, E.fecalis, Bacillus cereus* and *L.monocytogenes*), four Gram negative bacteria (*E.coli O157, Salmonella, Pseudomonas aeruginosa and Klebsiella pneumoniae*) and a mycotic strain (*C. albicans*) isolated from animal origin. Agar disk diffusion (qualitative method) and minimum inhibitory concentration (MIC) (quantitative method) were used in this study. Where a suspensions of bacterial and mycotic strains were freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 ml of Muller Hinton Broth for bacterial strains and Sabaroud Dextrose broth for mycotic strain. The inoculated tubes were incubated at 37°C and 28 °C for 24 hr, respectively. Serial dilutions were carried out for each strain, dilution matching with 0.5 Mc-Farland scale standard was selected for screening of antimicrobial activities. Ciprofloxacin 100µg/ml and fluconazole 100µg/ml were used as reference drugs.

Agar well diffusion method

The antimicrobial activity of *Pluchea dioscoridis* ethanol extract (70% ethanol) against bacterial and mycotic isolates was evaluated by using agar-well diffusion method [13]. Hundred ml of cell culture suspension matching with 0.5 McFarland of target isolate was spread onto the plates. For the investigation of the antibacterial and antimycotic activity, 100µl of extract (70% ethanol), ciprofloxacin (100µg/ml) and fluconazole as control positive and DMSO as control negative were added into wells of agar plates directly. Plates were left for 1 hr at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were re-incubated at 37°C and 28°C for 24 hrs for bacterial and mycotic strains, respectively. After incubation, plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for each of the samples. For an accurate analysis, tests were run in triplicate for each strain to avoid any error.

Minimum Inhibitory concentration method (MIC)

Micro-titer dilution plate quantitative method, i.e. the minimum inhibitory concentration (MIC) was used for evaluation of the antimicrobial activity of *P. dioscoridis* against inhibited organisms. Determination of MIC of extract against tested strains was achieved using 96-well sterile micro plates. Initial concentration 100 %, then two fold serial dilutions of the extract, reference drugs (ciprofloxacin and fluconazole) and DMSO as control negative, Then wells were inoculated with 100µl of tested strains (0.5 Mc-Farland, about 1×10^{8}cfu/ml) and incubated at 37°C-28°C for 24 hrs for bacterial and fungal strains, respectively. After incubation, plates were examined visually for bacterial or fungal growth precipitation. The experiment was repeated three times. The lowest concentration that showed complete hindrance of growth was taken as MIC [14].

Anti-cancer Activity

Cell culture and reagent

Human liver cancer cell line, Hep G2 cell line, were procured from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in humidified atmosphere containing 5% CO2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium. All the other reagents were procured from Sigma Aldrich (USA).

Treatment

Sterile filtered extract (70% ethanol) of *P. dioscoridis* was stored at -20°C as aliquots for further use. The cells were seeded in 6-well plate at a density 3 X 10^5 cells/well and incubated for one day at 37°C, 5% CO_2 and 95% humidity. The following day, the cells were exposed to varying concentrations (10, 20, 30,40, ...to 100 µg/ml) of extract of *P. discoridis* for 24hr at 37°C, 5% CO_2 and 95% humidity.

MTT Assay

The cells were taken for MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay [15] with minor modification. MTT assay serves as indication of viable cells with intact mitochondria. The formazancrystals formed by the dehydrogenase enzymes give colorimetric reading. The cells washed with PBS (phosphate buffer saline) were incubated with MTT (100µg/ml) in dark at 37°C for 5 hr. DMSO (dimethyl sulfoxide) was used to dissolve the formazan crystals and O. D. was read at 540nm. The absorbance of the untreated cells was considered as 100% viable cells. The percent viable cell was plotted on Y axis and concentration on X axis.

Statistical analysis

The results are reported as Mean ± Standard deviation (S.D.) for at least three times experiments. Statistical differences were analyzed by one way ANOVA test.

RESULTS AND DISCUTION

Total yield and chemical composition of essential oil of *P. dioscoridis*

The total yield of essential oil from Egyptian *Pluchea dioscoridis* leaves (relative to the amount of dried plant used) was 3.94 ± 0.48% (w/w), the value (g/100g) is mean ± standard deviation (n=3). The results revealed that, Egyptian *P. dioscoridis* is excellent source of essential oil. The identified volatile components of *P.dioscoridi* lists in table 1.A total of 50 volatile components in table 1, including 10Monoterpenes, 11 Light oxygenated compounds, 13 Sesquiterpenes, 16 Heavy oxygenated compounds were identified. Amongst the volatiles, sesquiterpenes and heavy oxygenated compound were the largest groups. Farnesol *cis-trans* (40%), nuciferol (15%), γ-eudesmol (7.83%), farnesol (6.29%), methyl eicosane (5.73%), *trans*-cadinol (2.9%) and Phytol (2.16%) were major volatiles. Grace (2002)[16] identified 36 components in the volatile oil of *Pluchea dioscoridis*, where farnesol was the major component (16.5%) accompanied by a high percentage of sesquiterpene alcohols. Oxygenated sesquiterpenes (26.4%) and sesquiterpene hydrocarbons (39.4%) represented the main constituents in the oil. El-Hamouly and Ibraheim (2003)[4] reported that the leaves of *Pluchea dioscoridis* containing 3-5% volatile oil, where 112 compounds were detected consisting mainly of sesquiterpene hydrocarbons (mainly β-maaliene and α-elemene), oxygenated sesquiterpenes (mainly α-cadinol, muurolol and caryophyllene oxide isomer). The plant also containing triterpenoid as hexacosanol, octacosanol, tetracosanol, cholesterol and campesterol.

Antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of *P.dioscoridis*

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food

manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects [17]. Accumulated evidence suggests that free radicals can be scavenged through chemoprevention utilizing natural antioxidant compounds present in medicinal plants [18]. The results showed that *P. dioscoridis* hexane extract posses the lowest value as antioxidant through both DPPH and β-carotene linolate assays as shown in table 2, while ethanol extract (both ethanolic extract, 70 % and 95 % ethanol) showed strong antioxidant activity, but 70% ethanol extract was stronger as anti-oxidant than 95 % ethanol extract as shown in tables 3 and 4 . These results may be due to the higher value of total phenolic and total flavonoid content in 70% ethanol extract than 95% ethanol extract, as shown in table 5.

Hexane is a non-polar solvent so it is unable to extract polar phenolic compounds, while ethanol is a good polar solvent so it is able to extract the phenolic compounds easly, ethanol was better solvent for extracting phenolic compounds due to their polarity. Phenolic acids and their esters have been reported as active antioxidants as well as flavonoids are antioxidants that battle and neutralize a wide variety of free radicals including nitric oxide, the hydroxyl radical and singlet oxygen [18-20]. Flavonoids exert their antioxidant effects by neutralizing all types of oxidizing radicals. Flavonoid can also act as powerful chain breaking antioxidant due to the electron-donating capacity of their phenolic groups.TPC and TFC work as potent scavenger against the artificial radical $DPPH^{\cdot}$ and physiological radicals including $ROO^{\cdot}$, $OH^{\cdot}$, and $O^{\cdot}$[18]. They could be used as an important indicator of the antimicrobial and antioxidant capacities. In addition, the TPC may be used as a preliminary screen for essential oils when intended as natural sources of antioxidants in functional foods [21].The results showed that essential oil of *P.dioscoridis* contains farnesol *cis-trans*, farnesol, nuciferol, *trans*-cadinol ,eudesmol methyl eicosane and phytol as major volatile compounds. Qamar and Sultana (2009)[22] reported that farnesol against oxidative stress was massive consequent injuries caused by cigarette smoke toxicants. Heavy oxygenated compounds especially Phytol, demonstrated a strong antioxidant effect *in vitro* in its capacity to remove hydroxyl radicals and nitric oxide as well as to prevent the formation of thio-barbituric acid reactive substances [23]. Sesquiterpens, the large volatile group in our results, was shown to be a good antioxidant as it blocks the peroxidation of arachidonic acid and inhibit auto-oxidative and microsomal lipid per-oxidation[18].

Where 70% ethanol extract achieved the highest antioxidants as well as high total flavonoids and total phenolic content so it tested as an antimicrobial activity and anti liver cancer cell line.

Antimicrobial activity of *P.discoridis* using Agar Well Diffusion (AWD) and Minimum Inhibitory Concentration (MIC)

Many Egyptian aromatic plants have strong antimicrobial activity [24]. The effect of ethanol extract of leaves of *P. dioscoridis*, used in the folk medicine, against nine bacterial strains was evaluated by the agar-well diffusion method as shown in table 6. Minimum inhibitory concentration (MIC) of ethanol extract of leave of *P. dioscoridis* is presented in table 7. The zone of inhibition above 7 mm in diameter was taken as positive result. Minimum inhibitory concentration test showing the highest dilution of *P.dioscoridis* ethanol extract causing inhibition of growth of different tested microbial strains compared with reference drugs was ranging from 3.13-25 mg /100 ml. Antimicrobial activity of *P.dioscoridis* ethanol extract against nine highly pathogenic microorganisms of animal origin was tested. The antimicrobial activities of extract were compared with reference antibiotics using agar well diffusion method (AWD) as well as minimum inhibitory concentrations (MIC), among the 8 bacterial species with an inhibition zone was 24mm (MIC 3.125µg/ml) against *Pseudomonas aeruginosa*, 22mm (MIC 3.125µg/ml) against *S. aureus, Bacillus cereus* and *L. monocytogenes* followed by 20mm (MIC 6.25µg/ml) against *E.fecalis* and *Klebsiella pneumoniae*. Lower inhibitory activity was shown against *E.coli* O157 and S. *Typhimurium* with an inhibition zone 16mm (MIC 25µg/ml) and finally *C. albicans*; mycotic strain with an inhibition zone 15mm (MIC 25µg/ml). Ethanol extract of leave of *p. disocoridis* was effective as an antibacterial all the nine bacterial species tested. Our results are identical with Obeidat *et al.,* (2012)[25] which demonstrated that aqueous extracts of *A. dioscoridis* leaves exhibited the highest potency against all pathogenic bacteria tested. A potent antimicrobial activity of farnesol was first observed with the follicular bacterium *Propioni bacterium acnes*[26] and more recently with a *halophilica rchaeon, Halofera xvolcanii* [27]. A step of mevalonate biosynthesis was proposed to be one of the FOH-sensitive sites in *H .volcanii.* Rajeswari *et al*., (2012)[28] reported that phytol is strong antimicrobial. The present study agree with the finding of Cicerale *et al.,* (2012)[29], who illustrated that the antimicrobial of *dioscoridis* is related to the presence of phenolic compounds. Also, it has been previously assessed against selected Gram-negative and Gram-positive bacteria, as well as unicellular and filamentous fungi [30]. The antimicrobial

activity of the ethanol extracts of *dioscoridis* was assessed by Zalabani *et al.,*(2013)[31]using agar dilution method and MICs and results revealed that leaf and root extracts exhibited the highest growth inhibitory activity against *Mycobacterium phlei*, *Bacillus subtilis*, *Listeriainnocua*, moderately inhibitory activity was shown against *S.aureus* and *C. albicans*.

Anti-proliferative activity of *P.dioscoridis in vitro*

Liver cancer is the main leading cause of cancer deaths worldwide. Thus, the present preliminary studies were done on human liver cancer cell line Hep G2 cell (Hepatocellular carcinoma) human line for the anticancer property of extract of *P.dioscoridis*. Extract of *P.dioscoridis* has been reported for its anti-proliferative activity against liver cancer Hep G2 cell line. The cell viability was tested by MTT ((3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay. The percentage cell viability was calculated by multiplying the ratio of O. D. of treated cells and O. D. of untreated cells by 100. The IC_{50} of the extract was found to be 66.95µg/ml on Hep G 2 (Figure 1). The results showed that Egyptian *P. dioscoridis* posses high content of phenols and flavonoids and have strong antioxidant activity. A number of *in vitro* and *in vivo* studies have shown that plant polyphenols could be used as chemopreventive agents against different cancer types including liver cancer [6]. polyphenols, particularly flavonoids, inhibit the initiation, promotion and progression of tumors, possibly by a different mechanism[18].

The results showed that farnesolcis *cis-trans*, farnesol, nuciferol, *trans*-cadinol, eudesmol and methyl eicosane were major volatile compounds of *P.dioscoridis*. Farnesol (FOH) can thus be endogenously generated, possibly by enzymic dephosphorylation of the corresponding pyrophosphate esters in mammalian cells [32]. Unexpectedly, exogenous FOH was found to inhibit the proliferation of human acute leukaemia CEM-C1 cells and human promyelocytic HL- 60 cells, respectively, thereby inducing apoptotic cell death as reflected in the predicted DNA fragmentation pattern [33]. The mammalian cells were mostly viable (84.0 %) in the medium with 25 pM FOH but drastically lost viability to 0.6% of the original level when exposed to 30 pM FOH It remains to be solved how such a modest change in FOH concentration could evoke such a strong response [34]. *In vitro* Farnesol inhibit cell proliferation and induce apoptosis in different types of malignant cells, noticing that tumor cells were more sensitive to the growth inhibition induced by this compound, than normal cells. Cells treated with farnesol showed to have a GO/G1 cell cycle arrest, reduction in CDK2 activity and an increased generation of the cyclin-dependent kinase inhibitor p27Kip1 with cyclin E/CDK2 complexes. The inhibitory effect of this terpene is suggested to be dependent on these CDK inhibitors (p21Cip1 and p27Kip1), because a down-regulation of them showed to provide a protection from the proliferation-inhibitory effect. Farnesol also proved its anti-tumor effects *in vivo*. Liver of farnesol treated rats had a number of phase I and phase II enzymes increased, which metabolize drugs and carcinogens, so farnesol might interfere in the metabolism, toxicity or carcinogenesis of drugs. The fact that farnesol has inhibitory effects on HMG-CoA reductase can be related to its anti-cancer effect. Tumor cells need an increased cholesterol biosynthesis, thus by inhibiting it, farnesol might provide its growth suppressing activity [35].

β-Caryophyllene oxide which represents (1.31%) of total volatiles, possesses several pharmalogical effects. It has been reported about its antibacterial, antifungal, immuno-modulatory, anti-inflammatory, anti-oxidative and even anti-proliferative and anti-cancer effects. Park *et al*.,(2011)[36]investigated the effect of this sesquiterpene on human prostate and breast cancer cells. The results demonstrated that β-caryophyllene oxide can inhibit the cell proliferation and down-regulation of different gene products that are related to processes of cell survival, proliferation, metastasis, and angiogenesis in human prostate and breast cancer cells.

Phytol which represents (2.16%) of total volatiles, demonstrated a strong antioxidant effect, anti-inflammatory and anti cancer [28]. Eudesmol, which represents (7.83%) of total volatile compounds, is naturally occurring sesquiterpenoid alcohol that present cytotoxic effect to cancer cells. Herein, all eudesmol isomers displayed cytotoxicity to different tumour cell lines. Bomfim *et al*.,(2013)[37] studied the mechanisms of cytotoxic action of eudesmol isomers (a-, b- and c-eudesmol) in human hepatocellular carcinoma HepG2 cells. After 24-hr incubation, HepG2 cells treated with eudesmol isomers presented typical hallmarks of apoptosis, as observed by morphological analysis in cells stained with haematoxylin–eosin and acridine orange/ethidium bromide. None of eudesmol isomers caused membrane disruption at any concentration tested. Moreover, eudesmol isomers induced loss of mitochondrial membrane potential and an increase in caspase-3 activation in HepG2 cells, suggesting the induction of caspase-mediated apoptotic cell death.

Eudesmol isomers herein are able to reduce cell proliferation and to induce tumor cell death by caspase-mediated apoptosis pathways [37].

Table 1: The chemical compositions of Egyptian *P.discoridis* essential oil

Compound	Area %	KI	Identifications Method
Monoterpenes			
α-Thujene	0.16	935	KI&Ms
α-Pinene	0.05	938	KI&Ms&ST
Verbenene	0.1	959	KI&Ms&ST
Sabinene	0.04	967	KI&Ms&ST
β-Pinene	0.35	980	KI&Ms&ST
Myrcene	0.14	988	KI&Ms&ST
δ-Carene	0.55	1009	KI&Ms&ST
α-Terpinene	0.14	1055	KI&Ms
Cymenene	0.51	1081	KI&Ms
allo-Ocimene	0.49	1101	KI&Ms
total	2.53		
Light oxygenated compounds			
O-Guaiacol	0.75	1087	KI&Ms
Ethylacetate	0.98	877	KI&Ms
5-Methyl-heptanone	0.29	942	KI&Ms
Octanone	0.19	986	KI&Ms
Linalool	0.23	1098	KI&Ms&ST
Hexyl-isobutrate	0.21	1125	KI&Ms
β-Terpineol	0.15	1132	KI&Ms&ST
Terpin-4-ol	0.06	1152	KI&Ms&ST
Methyl-Salicylate	0.28	1163	KI&Ms
Chrysanthenylacetate	1	1259	KI&Ms
Carvylacetate	0.09	1348	KI&Ms
total	4.23		
Sesquterpenes			
Isoledene	0.06	1362	KI&Ms
α-Copaene	0.15	1370	KI&Ms
Z-Caryophyllene	0.06	1399	KI&Ms
Farnesene	0.05	1407	KI&Ms
α -Gurjunene	0.08	1412	KI&Ms
β-Humulene	2.1	1468	KI&Ms
Germacrene	0.35	1474	KI&Ms
allo-Aromadenderene	0.06	1478	KI&Ms
γ-Muurolene	0.08	1501	KI&Ms
Germacrene B	0.3	1549	KI&Ms
γ-Cadinene	0.18	1557	KI&Ms
eicosene	2.43	1904	KI&Ms
Methyl eicosane	5.73	2011	KI&Ms
total	11.63		
Heavy oxygenated compound			
trans-Ionone	0.19	1512	KI&Ms
Elemol	0.08	1564	KI&Ms
Spathulenol	0.25	1578	KI&Ms
Caryophylene oxide	1.31	1596	KI&Ms
Guaiol	0.73	1612	KI&Ms
γ-Eudesmol	7.83	1623	KI&Ms
Cedrol-epi	0.5	1625	KI&Ms
α-Cadinolepi	0.44	1638	KI&Ms
α-Eudesmol	1.34	1650	KI&Ms
Cedranol-5-neo	1.94	1677	KI&Ms
Muurolol	0.66	1682	KI&Ms
trans-Cadinol	2.94	1736	KI&Ms
Farnesol	6.29	1772	KI&Ms
nuciferol	15	1777	KI&Ms
Farnesolcis-trans	40	1791	KI&Ms
phytol	2.16	1887	KI&Ms
Total	81.66		

KI: kovate index; **Ms**: Mass spectrometric; **ST**: standard compound

ISSN: 0975-8585

Table 2: Antioxidant activity (A.A) of *P. dioscoridis* hexane extract by two different methods (DPPH assay and β- carotene/ Linoleic acid assay)

Concentration of sample	% Inhibition by DPPH assay		% Inhibition by β carotene/ Linoleic acid assay	
	hexane extract	TBHQ*	hexane extract	TBHQ*
40 µg /ml	11.41 ± 0.5	65.31 ± 1.9	10.00 ± 0.6	66.21± 2.1
80 µg /ml	15.33 ± 0.9	79.59± 2.2	12.24 ± 0.8	78.4± 2.2
160 µg /ml	20.31 ± 0.9	87.35± 2.1	21.11 ± 0.9	86.27±2.9
320 µg /ml	27.80 ± 1.0	95.13± 2.5	26.73 ± 1.2	93.45±2.3
400 µg /ml	36.17± 1.1	99.0 ± 2.1	29.78± 1.0	99.41± 2.1

***** TBHQ: Tert –butyl hydroquinone, standard synthetic antioxidant.
Each value represents the mean ± S.D (Standard deviation) and mean of three replicates.

Table 3: Antioxidant activity (A.A) of *P. dioscoridis* ethanol extracts by DPPH free radical scavenging method

Concentrations of sample	% Inhibition		
	95% Ethanol extract	70% ethanol extract	Standard synthetic antioxidant (TBHQ)*
40 µg /ml	21.11 ± 1.1	29.45 ± 1.3	65.31 ± 1.9
80 µg /ml	43.21 ± 1.3	51.11 ± 1.3	79.59± 2.2
160 µg /ml	77.91 ± 1.6	78.95 ± 1.6	87.35± 2.1
320 µg /ml	82.31 ± 2.2	84.31 ± 2.1	95.13± 2.5
400 µg /ml	82.45 ± 2.1	88.60 ± 2.1	99.0 ± 2.1

*TBHQ: Tert –butyl hydroquinone, standard synthetic antioxidant.
Each value represents the mean ± S.D (Standard deviation) and mean of three replicates.

Table 4: Antioxidant activity (A.A) of *P. dioscoridis* ethanolic extracts by β- carotene/ Linoleic acid assay

Concentrations of sample	% Inhibition		
	95% ethanol extract	70% ethanol extract	Standard synthetic antioxidant (TBHQ)*
40 µg /ml	30.41 ± 1.5	33.15 ± 1.1	66.21± 2.1
80 µg /ml	49.15 ± 1.2	53.51 ± 1.2	78.4± 2.2
160 µg /ml	65.31 ± 1.9	71.95 ± 1.9	86.27±2.9
320 µg /ml	76.82 ± 2.0	80.31 ± 2.4	93.45±2.3
400 µg /ml	81.07± 2.3	86.92 ± 2.5	99.41± 2.1

*TBHQ: Tert –butyl hydroquinone, standard synthetic antioxidant.
Each value represents the mean ± S.D (Standard deviations) and mean of three replicates.

Table 5: Total phenolic content and total flavonoid content in ethanol extracts of *P. dioscoridis*

Ethanol extracts	Total Phenolic Content	Total Flavonoid Content
	mg/g extract	mg/g extract
95% ethanol	160 ± 3.31	201 ± 7.94
70% ethanol	192 ± 4.10	235 ± 10.16

Each value represents the mean ± S.D (Standard deviation) and mean of three replicates.

Table 6: Agar well diffusion test showing zone of inhibition of *P. dioscoridis* ethanol extract against different tested microbial strains compared with reference drugs.

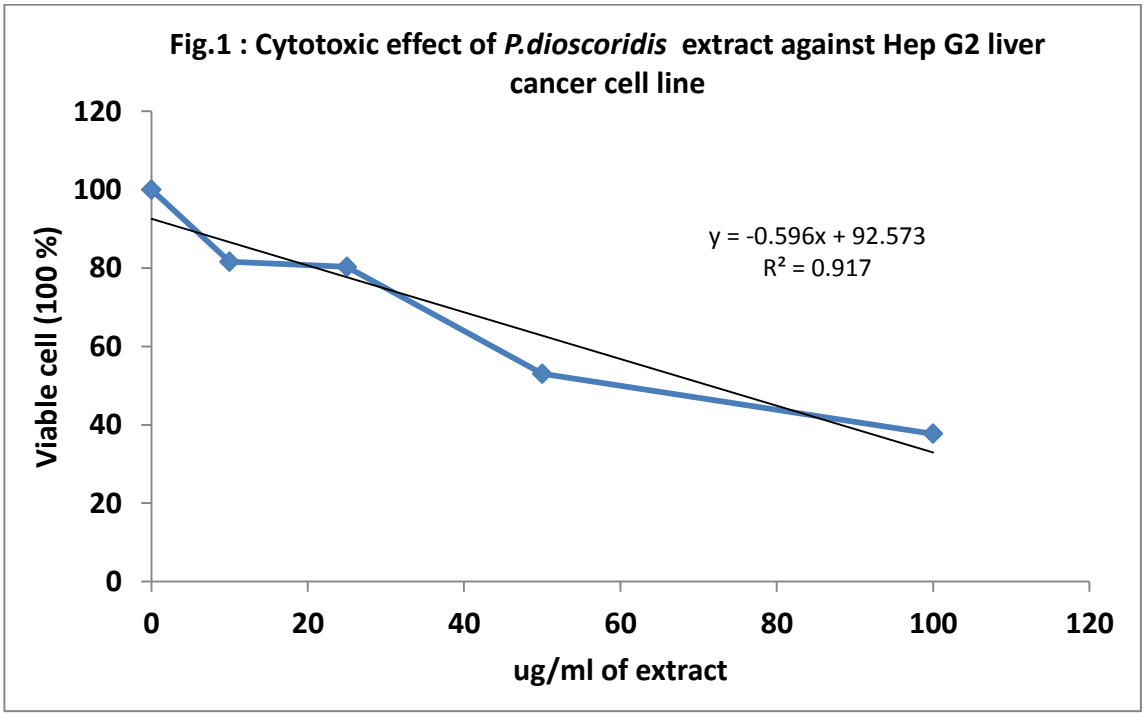
Strains	InhibitionZone [mm diameter]		
	*P.dioscoridis*ethanolic extract	Ciprofloxacin	Fluconazole
S. aureus	22±0.6	38±0.7	-
E.fecalis	20±0.3	43±0.7	-
B. cereus	22±0.4	41±0.5	-
L. monocytogens	22±0.3	48±0.7	-
***E. coli* O157**	16±0.4	40±0.6	-
S. typhimurium	16±0.2	48±0.6	-
P. aeruginosa	24±0.7	45±0.4	-
K. pneumoniae	20±0.5	42±0.6	-
C. albicans	15±0.3	-	41±0.6

Each value represents the mean ± S.D (Standard deviation) and mean of three replicates.

ISSN: 0975-8585

Table 7: Minimum Inhibitory Concentration test showing the highest dilution of *P. dioscoridis* ethanol extract causing inhibition of growth of different tested microbial strains compared with reference drugs.

Strains	Minimum inhibitory concentration (MIC) mg/100ml		
	***P.dioscoridis*ethanolic extract**	**Ciprofloxacin**	**Fluconazole**
S. aureus	3.125	0.19	-
E. fecalis	6.25	0.097	-
B. cereus	3.125	0.19	-
L. monocytogens	3.125	0.097	-
***E. coli* O157**	25	0.19	-
S. typhimurium	25	0.097	-
P. aeruginosa	3.125	0.097	-
K. pneumoniae	6.25	0.19	-
C. albicans	25	-	0.19



CONCLUSION

Egyptian *P. dioscoridis* is excellent source of essential oil which possess many bioactive volatile constituents. It has strong antioxidant and anti-liver cancer activity .It also has antimicrobial properties against Gram positive bacteria and Gram negative bacteria as well as mycotic infection with *C. albicans*. Further investigations are needed to be applied as additives, in food and pharmaceutical industries.

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