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Chemical and Biological Activities of Deverra triradiata Hochst. ex. Boiss. Aerial parts from St. Catherine, Southern Sinai, Egypt

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Abstract

The present study describe the phenolic and flavonoid profiles of the ethyl acetate extract of *D. triradiata* which demonstrated by high-performance liquid chromatography (HPLC) to prove the presence of 16 compounds, nine major compounds namely, querectin (46.37 mg/g), vanillin (11.94), daidzein (10.02), ellagic acid (4.76), propyl gallate (3.89), naringenin (3.27), gallic Acid (3.18 mg/g), rutin (2.7) and chlorogenic Acid (2.58).

The chemical constituents of unsaponifiable portion of petroleum ether 60-80° extract which analyzed by gas-liquid chromatography (GLC) proved the presence of 14 hydrocarbons and three sterol; The major compounds are: n-eicosane (16.94%), n-docosane (8.22 %), n-heptadecane (7.13 %), squalene (6.89%), n-tricosane (1.63 %), n-hendecane (1.63 %), cholesterol (1.12 %), campesterol (3.81 %) and stigmasterol (5.27 %).

From saponifiable portion five unsaturated fatty acids were indicated as, α-Linolenic acid (ALA), (17.95 %), arachidonic acid (11.37), linolenic acid (LA) (6.12), oleic acid (3.36) and palmitoleic acid (3.01) and also four saturated fatty acid named , palmitic acid (31.61), myristic acid (7.17), Lauric acid (1.67) and stearic acid (1.47) were indicated .

Total phenolic (tannic acid equivalent), total tannins (tannic acid equivalent) and total flavonoids (quercetin equivalent) were (67.8±0.39, 31.4 ± 0.63 and 4.7 ± 0.077 mg/g d.w.), respectively.

The in *vitro* antioxidant activity using the stable free radical DPPH (2,2-diphenyl-1-pycrylhydrazyl) method of the petroleum ether and ethyl acetate extracts were carried out . The percentage of maximal inhibition for extracts in DPPH are 18.42 ± 0.39 and 43.58±0.52 %, respectively compared with percentage of gallic acid 91.29 ±0.72µg/ml, which proved that ethyl acetate extract possesses a distinct radical scavenging effect, which may be attributed to its high polyphenolic content.

The in *vitro* cytotoxic activity by using SRB assay, of petroleum ether showed promising activity against (MCF7), (PC3) and (HCT 116) with IC_{50} 18.8, 9.3 and 9.5 ug/ml, respectively, compared with normal cell. Baby Hamster kidney (BHK) with IC_{50} 37, while, the ethyl acetate extract showed weak activity against (MCF7), (PC3) and (HCT) with IC_{50} 42, 42 and 37 ug/ml, comparable to (BHK) normal cell with IC_{50} 59 µg/ml.

All identified phenolic compounds are therapeutically active compounds with anticancer potency and antioxidant capacities which were reported for the first time.

Keywords: *Deverra triradiata* (Apiaceae), HPLC, GLC, Total phenolic, Total tannins,Total flavonoids, Antioxidant, cytotoxicity

Introduction

The Apiaceae (previously known as the Umbel Family: Umbelliferae) is one of the largest plant families in the world. This family comprises approximately 450 genera and 3700 species worldwide **[1]** . The members of this family are well known as vegetables, culinary and medicinal plants . The Apiaceae family contains more than 20 species

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and they are used in folk medicine which possesses a range of compounds that have many biological activities. Some of the main properties are ability to induce apoptosis, antibacterial, hepatoprotective, anti-asthmatic inhibitory and antitumor activities **[2].** The genus Deverra DC.Species: *Deverra triradiata* Hochst. ex Boiss. with two species Family: Apiaceae Lindl., these species are widespread in South Sinai, Egypt , *Deverra* is used by the Egyptians to prepare carminative drinks and used to alleviate stomach pain, to combat intestinal parasites and used as anti-asthmatic. The present study aims to demonstrate the *Deverra triradiata* as sources of antioxidants with potential medicinal properties,[**3].**

Experimental

Plants material

Fresh wild plants of *Deverra triradiata* Hochst. ex. Boiss were collected from Wadi El Marwa, St.Catherine, South Sinai, Egypt at wet stage on July 2017.The taxonomic identification of the plant material was confirmed using well identified herbarium specimens and authenticated by Dr. Omran Ghaly,Desert Research Center. A voucher herbarium specimen was deposited in the herbarium of Desert Research Center (CAIH) with Code Number are (CA1H-1015-R).

Preparation of plant extracts for chromatographic analysis

Successive extract of (200 gm) dried plant powder by using Soxhlet apparatus with (250 ml) petroleum ether 60-80^{o} then (250 ml) for ethyl acetate, finally ethanol (250 ml). The extraction processes carry on till the solvent in siphon tube of Soxhlet apparatus become colorless, the extracts were then filtered using filter papers and concentrated under vacuum on a rotary evaporator. Yielded extracts were 6.78, 4.40 and 2.32 gm, respectively. The dried plant crude extracts kept in refrigerator for analysis.

Determination of hydrocarbons, sterols and fatty acid in petroleum ether fraction by Gas Liquid Chromatography (GLC):

The petroleum ether fraction was saponified by refluxing with 1mol KOH in 95% ethanol for one hr. The solution was cooled, water add and then extracted by diethyl ether. The ether extract was washed several times with water, dried over anhydrous Na_2SO_4. The non-saponifiable mater (hydrocarbon and sterol) was obtained by removal of ether solvent in a rotary evaporator.

The washing water was added to the aqueous layer, which was acidified with 6 N Hcl and extracted by diethyl ether. The saponifiable mater (free fatty acids) was obtained after washing the extract with water, drying it over anhydrous Na_2SO_4 and removing the solvent using rotary evaporator.

I- ***Gas Liquid Chromatography (GLC) of unsaponifiable hydrocarbon and sterol:***

The relative percentage of each unsaponifiable compounds determined by using triangulation method according to **Nelson *et al.*,[4].** By using Gas-Liquid Chromatography (GLC) GCV Pye-Unicam

II. ***Gas Liquid Chromatography (GLC) of saponifiable fatty acids:***

The extracted fatty acids of plant and the standards were converted to the corresponding methyl esters using ether solution of diazomethane. The methyl ester of the fatty acids was analyzed with GC apparatus. The fraction of fatty acids methyl ester was conducted using (GLC) column.

Peak identification was performed by comparing the relative retention time of each compound with those of standard materials. The relative proportions of each individual compound were estimated as the ratio of the partial area to the total area.

Determination of phenolic and flavonoid compound from ethyl acetate fraction of Deverra triradiata by high-performance liquid chromatography (HPLC)

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse Plus C_{18} column (4.6 x 250 mm). The mobile phase consisted of water (A) and 0.02% trifloroacetic acid and acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in liner gradient as follows: 0 min (80% A); 0–5 min (80% A); 5-8 min (40% A); 8-12 min (50% A). The multi-wavelength detector was monitored at λ_{max} 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35°C.

Total active constituents from aqueous alcoholic extract:

I- Estimation of total phenolic and total tannins contents:

Total phenolic and total tannins were estimated according to Giagourta *et al*., (2012) **[5],** using Folin-Ciocalteau reagent (1N) (Commercially available folin-ciocalteau reagent (2N) (Loba, India) and Polyvinyl Polypyrrolidone (PVPP) (Sigma, UK).

The concentrations of total phenolic and total tannins were calculated from the standard curve using the following equation:

$$Y = 0.0042X + 0.0296, R^2 = 0.9825$$

$$\text{Concentration of phenolic (g\%)} = \frac{\text{tannic acid equivalent (µg/ml)} \times \text{volume of total extract(ml)}}{\text{weight of sample(g)} \times 10^6 \times \text{volume used (ml)}} \times 100$$

Percentage of tannins (g %) = total phenolic (g %) – non tannin phenolic (g %).

Estimation of total flavonoids contents:

Total flavonoids were estimated by the aluminum chloride assay colorimetric according to (Malla, et al. 2013)**.[6]** .The flavonoid concentration (g %) was calculated from the standard curve using the following equation:

$$y = 0.0066x, r^2 = 0.9866$$

$$\text{Concentration of flavonoids (g\%)} = \frac{\text{quercetin equivalent (µg/ml)} \text{X volume of total extract(ml)}}{\text{sample weight(g)} \times 10^6 \times \text{volume used (ml)}} \times 100$$

Antioxidant activity

Chemical reagents for antioxidant evaluation :

The antioxidant activity of the *Deverra triradiata* extract was measured on the basis of the scavenging activity of the stable DPPH (2,2-diphenyl-1-pycrylhydrazyl) free radical according to the method described by (Katalinicet al., 2006**),[7]**

Preparation of plant extract for antioxidant evaluation:

About 10 g of the grounded plant were soaked in 1 Liter of 80% ethyl alcohol and put in a shaker device at 100 rounds per minute for 72 hours at room temperature and stored in refrigerator for 4 days. The extracts were then filtered using filter papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4º C and the antioxidant test was done directly with in five minutes.

Percentage of inhibition of DPPH activity:

Radical scavenging activity of plant extract against the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich Chemie, Germany) was determined spectrophotome trically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) using UV spectral data at λmax: 517nm Estimation was done to the method of (Katalinicet al.,2006) **[7].** with slightly modified method of Brand Williams, Cuvelier, and Berset, C**. [8].** Where the extract solution was prepared by dissolving 0.025 g of the dry extract in 10 ml of methanol. The solution of DPPH in methanol (6×10−5 M) was freshly prepared, before UV measurements. Three ml of this solution were mixed with 9 different concentrations of the samples. The resulting solutions were kept in the dark for 30 min at room temperature and then the decrease in absorbance was measured. Absorbance of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

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

Where: AB is the absorbance of blank sample and AA is the absorbance of the tested samples

Materials for cytotoxic activity

Human tumor carcinoma cell lines used are three cell lines , Breast carcinoma cell line, MCF7 (ATCC-22), Prostate cancer cell line, PC-3 (ATCC-1435) and Colon carcinoma cell line, HCT- 116 (ATCC-247) where the normal cell lines ,Baby hamster kidney cells line ,BHK-21 (ATCC-10). The cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Extracts were prepared by dissolving 1:1 Stock solution and stored at -20°C in dimethylsulfoxide (DMSO) at 100 mM. Different concentrations of the extract were used (5-100 ug/ml).

Methods of Cytotoxic Assay

Potential cytotoxicity of petroleum ether and ethyl acetate extracts of *D. triradiata* was tested by using sulforodamine B (SRB assay) **[9].** to measure the potential cytotoxicity using the method cells were seeded in 96-multiwell plate (104 cell/well) for 24 h before treatment to allow attachment of the cells . Different concentrations of the extract under test were added to the cell monolayer in triplicate wells. The monolayer cells were incubated with the extract for 48 h at 37˚C and in atmosphere of 5% CO2 , cells were then fixed, washed and stained with Sulforodamine B stain, excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader, the relation between surviving fraction and extract concentration is plotted to get the survival curve of

each tumor cell line after application of different concentrations of the extract.

Results:

I. Identification of unsaponifiable constituents (hydrocarbons and sterols) of D. triradiata by GLC:

The unsaponifialbe composition of D. triradiata by using GLC analysis, which contains 14 hydrocarbons and three sterols and the highest concentration of hydrocarbons n-eicosane (16.94%), n-docosane (8.22 %) and n-heptadecane (7.13 %), squalene (6.89%) while the lowest percentage concentration of hydrocarbons was n-tricosane (1.63 %) and n-hendecane (1.63 %). Where as percentages of sterol were cholesterol "phytosterols" (1.12 %), campesterol (3.81 %) and stigmasterol (5.27 %). (Table 1 and Figure 1)

Table (1): GLC of Hydrocarbons and Sterols in aerial parts of *D. triradiata* .

No. of C atoms	Compound name	*D. triradiata*	
		RT	Area (%)
(C11)	n-Hendecane	9.58	1.63
(C12)	n-Dodecane	10.18	1.80
(C13)	n-Tridecane	12.07	3.74
(C14)	n-Tetradecane	12.77	5.06
(C15)	n-Pentadecane	13.76	2.82
(C16)	n-Hexadecane	14.37	4.89
(C17)	n-Heptadecane	14.97	7.13
(C18)	n-Octadecane	15.48	5.46
(C19)	n-Nonadecane	16.62	4.94
(C20)	n-Eicosane	17.88	16.94
(C21)	n- Heneicosane	19.62	3.97
(C22)	n-Docosane	20.15	8.22
(C24)	n-Tricosane	9.58	1.63
C:30	Squalene	25.11	6.89
Sterols			
C:27	Cholesterol "phytosterols"	28.87	1.12
C:28	Campesterol	29.58	3.81
C:29	Stigmasterol	30.62	5.27

Where: RT = Retention time.

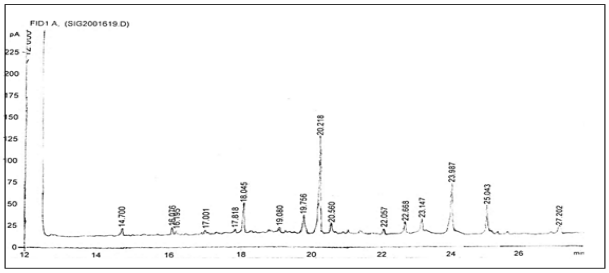
II. Identification of saponifiable (Fatty acid) constituents:

The fatty acid composition of D. triradiata by using GLC analysis contains nine of fatty acid with different ranges of concentrations divided into four saturated fatty acid and five unsaturated fatty acid the highest concentration of fatty acid palmitic acid (31.61 %), α-linolenic acid (ALA) (17.95 %) and arachidonic acid (11.37 %) and the lowest concentration was stearic acid (1.47%). As in Figure 2 and Table 2.

Table (2): GLC analysis of Fatty acids in aerial parts of D. triradiata .

No. of C atom	Common name	*D. triradiata*	
		RT	Area (%)
C12:0	Lauric acid	16.08	1.67
C14:0	Myristic acid	18.05	7.17
C16:0	Palmitic acid	20.22	31.61
C18:0	Stearic acid	22.06	1.47
C16:1	Palmitoleic acid	20.56	3.01
C18:1	Oleic acid	22.67	3.36
C18:2	Linoleic acid (LA)	23.15	6.12
C18:3	α-Linolenic acid (ALA)	23.99	17.95
C20:4	Arachidonic acid	25.04	11.37

Figure 2. GLC chromatogram of Fatty acids in aerial parts of D. Triradiata



Identification of phenolic and flavonoid compound from ethyl acetate fraction of Deverra triradiata by HPLC analysis:

The HPLC data showed 14 phenolic compounds , the highest concentration are querectin (46.37 mg/gm), vanillin (11.94 mg/gm) and daidzein (10.02 mg/gm), ellagic acid (4.76 mg/g), propyl Gallate (3.89 mg/g), naringenin (3.27 mg/g), gallic Acid (3.18 mg/g), rutin (2.7 mg/g 4) and chlorogenic Acid (2.58 mg/g) while, coumaric acid (0.36 mg/gm) was the lowest concentration. And only one alkaloids caffeine (0.61 mg/g) , (Figure 3 and Table 3).

Figure 3. HPLC chromatogram of flavonoids and phenolic acids in Ethyl Acetate extract of aerial parts of *D. triradiata*.

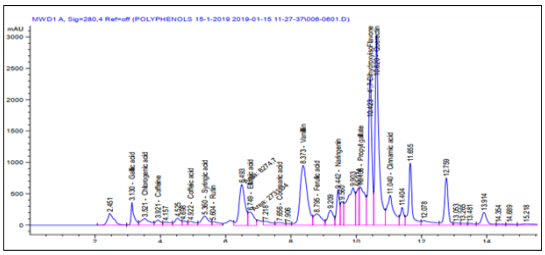


Table (3): HPLC chromatogram of flavonoids and phenolic acids in Ethyl acetate extract of aerial parts of *D. triradiata*.

No	Phytochemical Compounds	*D. triradiata*	
		RT (min.)	Conc. (mg/g)
1	Gallic Acid	3.13	3.18
2	Chlorogenic Acid	3.52	2.58
3	Catechin	3.82	0.00
4	Caffeine	3.921	0.61
5	Coffeic Acid	4.92	0.68
6	Syringic Acid	5.36	1.91
7	Rutin	5.60	2.74
8	Ellagic Acid	6.75	4.76
9	Coumaric Acid	7.66	0.36
10	Vanillin	8.37	**11.94**
11	Ferulic Acid	8.80	1.51
12	Naringenin	9.44	3.27
13	Propyl Gallate	10.14	3.89
14	4`.7-Dihydroxyisoflavone	10.42	**10.02**
15	Querectin	10.62	**46.37**
16	Cinnamic Acid	11.04	1.34

Total active materials from aqueous-alcoholic extract:

The total phenolic acids (as mg/g d.w. tannic acid equivalent), total tannins (as mg/g d.w. tannic acid equivalent) and total flavonoids (as mg/g d.w. quercetin equivalent) of D. triradiata were (67.8 ± 0.39), (31.4 ± 0.63) and (4.7 ± 0.077), respectively. **(Table 4)**

Table (4): Total active materials of *D. triradiata*

1	Total flavonoids (quercetin equivalent)	4.7 ± 0.077
2	Total phenolic (tannic acid equivalent)	67.8 ± 0.39
3	Total tannins (tannic acid equivalent)	31.4 ± 0.63

Antioxidant activity by DPPH-radical scavenging assay:

The free radical scavenging activity of the petroleum and ethyl acetate extracts of D. triradiata has been tested by DPPH radical method using gallic acid as standard. The concentration ranged from 1-100 µg/ml. The zero inhibition was considered for the solution which contained only DPPH without any plant extract. The results are showed in (Table 5) . The percentage of maximal inhibition for extracts of D. triradiata in DPPH radical scavenging assay. (Mean ±S.E) at 1000 ug/ml of petroleum ether and ethyl acetate extracts are (18.42 ± 0.39 and 43.58±0.52%, respectively) , compared with percentage of standard gallic acid of 91.29 ± 0.72 µg/ml, which proved that ethyl acetate extract possesses a distinct radical scavenging effect, which may be attributed to its high polyphenolic content.

Table (5): Antioxidant activity by DPPH-radical scavenging ,IC_{50} of extracts from *D. triradiata* on carcinoma cell line.

IC_{50} (ug/ml)				
Extracts	BHK	MCF7	PC3	HCT
Petroleum ether	37	18.8	9.3	9.5
Ethyl acetate	59	42	42	37

Cytotoxic activity:

Potential cytotoxicity of petroleum ether and ethyl acetate extracts of D. triradiata with different concentrations were tested against MCF7 (Breast carcinoma cell line), PC3 (prostate cancer cell line), HCT (Colon carcinoma cell line) in comparison with normal BHK (Baby hamster kidney cells line). (Table 6 and Figure 4,5)

The petroleum ether extract showed potent cytotoxic activity for breast (MCF7) carcinoma cell line with IC50 18.8 ug/ml with effective about 49.2% compared to normal (BHK) cell line, strong potent cytotoxic activity for prostate (PC3) carcinoma cell line with IC50 9.3 ug/ml with effective about 74.9%, strong potent cytotoxic activity for colon (HCT) carcinoma cell line with IC50 9.5 ug/ml with effective about 74.3% of normal BHK cell line .While, the ethyl acetate extract showed weak activity against both of breast (MCF7) and prostate (PC3) carcinoma which the colon (HCT) carcinoma cell lines with IC50 37 ug/ml, 42 ug/ml and 42 ug/ml respectively with weak effectively about 28.8 , 37 and 37 % as compared with IC50 of BHK normal cell line .

According to the American Cancer Institute (NCI) announced that the cytotoxic activity criteria for the crude extract is an IC50 < 20 ug/ml. So the petroleum ether extract had a significant cytotoxic activity for all carcinoma cell line, while ethyl acetate extract had a weak cytotoxic activity for all carcinoma cell line. [10].

Table (6): The percentage of maximal inhibition for extracts of *D. triradiata* in DPPH radical scavenging assay.

Tested extracts	% scavenging DPPH
Gallic acid	91.29 ± 0.72
Ethyl acetate	43.58 ± 0.52
Petroleum ether	18.42 ± 0.39

(Mean ±S.E).

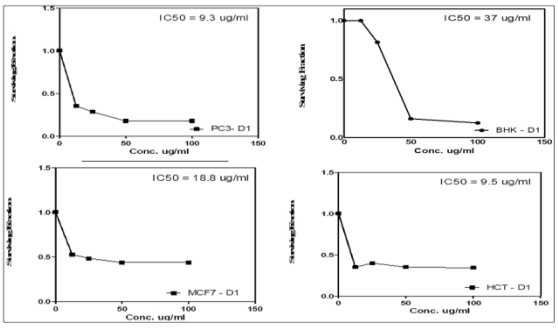
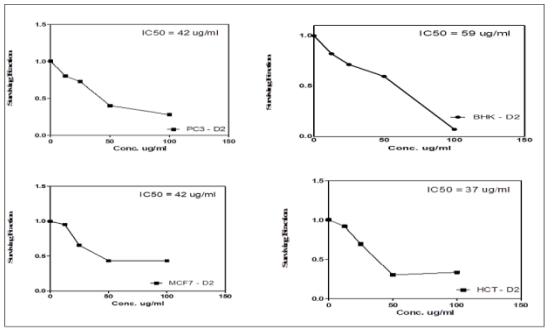


Fig. (4):Percentage of survival fraction against concentration (ug/ml) of petroleum ether extract of *D*



survival fraction against concentration (ug/ml) of ethyl acetate extract of *D. triradiata* on carcinoma cell line.

Dissection :

The sterol fraction comprises of β-sitosterol, cholestrol and stigmasterol. Stigmasterol had been investigated for its pharmacological prospects such as antihypercholestrolemic, antitumor antiosteoarthritic , hypoglycaemic, cytotoxicity, antioxidant, antimutagenic, anti-inflammatory and CNS effects.[11]. Eicosane have antitumor activity against the human gastric SGC-7901 cell line and the triterpene squalene was consider antioxidant, antitumor and Immunostimulant,[12] . Campesterol, a naturally occurring plant sterol, is known to reduce cholesterol and have anticarcinogenic effects,[13] .The unsaturated fatty acids (UFAs), including the ω-3 agent ALA, have been observed to exhibit cytotoxicity or growth inhibition against a variety of malignant cell lines.[14]. The saturated fatty acids palmitic acid and myristic acid have specific roles in acylation of membrane proteins, which are essential in the anchoring of those proteins to the plasma membrane, while the ω-6 polyunsaturated fatty acid arachidonic acid and Linoleic acid are the principal precursor for production of eicosanoids, such as thromboxanes, prostaglandins, and leukotrienes that have many regulatory functions.[15]. The phenolic acids have been stated to have several useful properties, such as estrogenactivity, anti-inflammatory activity, antimicrobial activity, antioxidant activity, enzyme inhibition, vascular activity, anti-allergic activity and cytotoxic anti-tumor activity. [16, 17, 18] . Quercetine is a polyphenolic flavonoid and a bioactive compound with potential chemoprotective properties such as anti-inflammatory, antioxidant, and anticancer and antibacterial,[19, 20] . Quercetin offers many health-promoting advantages, including improvement of eye diseases, allergic disorders, cardiovascular health, arthritis, reducing risk for cancers [21, 22] . Naringenin was ascribed to many biological functions, including antitumor, antioxidant, antiviral, anti-inflammatory, antibacterial, antiadipogenic and cardioprotective impact.[23,24]. Vanillin having antioxidant and anticancer potential,[25,26].Daidzein (4`.7-Dihydroxy isoflavone) is an isoflavone of substantial nutritional value, and is derived primarily from soy plants. This pharmacological activity is attributed to diverse metabolites including trihydroxy isoflavone. [27]

Conclusion:

The chemical study showed that the D. triradiata plant extract contain a mixture of phytochemicals, flavonoids, phenolic compounds and alkaloids. The quantitative total flavonoids and total phenol screening indicated that the ethyl acetate extract has the highest contents of flavonoids and phenols and the DPPH assay showed that the plant has potent antioxidant activity which can be an excellent choice for biological and chemical analysis, and can be further subjected for the isolation of the therapeutically active compounds with anticancer potency.

Conflict of interest

The authors declared that this study was performed in absence of any conflict of interest

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