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PHYTOCHEMICAL AND CYTOTOXICITY INVESTIGATIONS OF *DILLENIA INDICA* L. GROWN IN EGYPT

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ABSTRACT

Objective *Dillenia indica* L. (Dilleniaceae), is found to have good medicinal and therapeutic values and is being used by tribal and folk communities of various regions. Although its fruit is edible, it is not very much well known by people in Egypt. Its richness in phenolics and triterpenoids had led to test for its antioxidant and cytotoxic activities and isolation of its components. **Methods:** Preliminary phytochemical screening analysis of the ethanol extracts of leaves, stems and fruits, of *D. indica* L., was done. Isolation of some phenolics from fruit extracts was done using usual chromatographic techniques. Triterpenoid have been analyzed by gas chromatography-mass spectrometry. The antioxidant activity of the fruit extracts was measured using the stable free radical DPPH assay. The cytotoxic activity of the ethanol extracts of leaves, stems and fruits, was tested using two cell lines: colon carcinoma cell line (HCT-116) and liver carcinoma cell line (HEPG2) adopting SRB method. **Results:** The preliminary phytochemical screening of the ethanol extracts of the leaves, stems and fruits of *D. indica* L., revealed the presence of steroids, terpenoids, tannins, saponins, anthocyanins and flavonoids and absence of alkaloids and cardiac glycosides. Seven compounds had been isolated from the aqueous methanolic extract of the fruit of *D. indica* L. namely Quercetin-3-O-glucopyranoside (1), Rhamnetin-3-O-rhamnopyranoside (2), Apigenin-6-C-arabinopyranosyl-8-C-glucopyranoside (3), Apigenin-6, 8-di-C-glucopyranoside (4), Quercetin-3, 7-di-O-glucopyranoside (5), Myricetin (6) Myricetin-3-O-glucopyranoside (7). The fruits extract showed very strong free radical scavenging activity

with IC_{50} value of 30. 38 µg/mL. Only the ethanol extract of *D. indica* L. stems showed significant cytotoxicity activity against HCT-116 and HEPG2 carcinoma cell lines with IC_{50} = 9.8 µg and IC_{50} = 20.1 µg respectiviely, while The ethanol extract of leaves and fruits failed to exert a considerable effect on HCT-116 and HEPG2 carcinoma cell lines. Sixteen triterpenoids compounds were identified in the leaves, stems and fruits. Lupeol was the major component in leaves, stem and fruits being of 32.72, 37.49 and 16.48% respectively, followed by betulinaldehyde 20.28, 23.29 and 16.06% and betulinic acid 18.41, 22.29 and14.62% respectively. **Conclusion:** *D. indica* L. can be considered as a natural medicinal plant with a potential antioxidant and anticancer activities due to its bioactive ingredients.

INTRODUCTION

Dilleniaceae is a family of 10 genera and about 300 species of trees, shrubs, and woody vines (or rarely herbs) of the tropics and subtropics. The genus Dillenia has 60 species, of which D. *indica,* D. *pentagyna,* D. *alata,* D. *suffruticosa,* D. *papuana,* D. *excelsa,* D. *serrata,* D. *ovata,* D. *phllipinensis* etc. are found to have good medicinal value, These plants are being used by tribal and folk communities of various regions, (Dickison, 1979).

Dillenia indica Linn. (Elephant Apple or Wood apple universally); is an evergreen large shrub or small to medium- sized tree that grows all over Asia, from India, Bangladesh and Sri lanka, east to southwestern China and Vietnam, and south through Thailand to Malaysia and Indonesia. It bears green fruits which resemble large apples. Hence, it got its name, "elephant apple". It is a tropical acidic fruit, the elephant apple itself is very fleshy and is enclosed in a hard husk, is eaten both ripe and unripe, with Astringent and resinous notes. It also has a strong smell, along with numerous small seeds. (Pradhan & Badola, 2008), Traditionaly the fruit shows laxative properties and is used for relieving abdominal pain. The bark and leaves have astringent effect. The juice of *D. indica* L. leaves; bark and fruits are mixed and given orally for the treatment of cancer and diarrhea (Alam *et al*., 2010). *D. indica* L. is reported to possess antidiabetic, hypolipidemic, antioxidant, CNS depressant, hepatoprotective, anti-inflammatory, antileukemic and antimicrobial activities (Kritikar and Basu, 2003, Abdille *et al.,* 2005, Bhakuni *et al*., 1969, Yeshwante *et al*., 2009, Apu *et al.,* 2010, Sood *et a.l.*, 2005). Different prepared extracts of *D. indica* L. have been reported to contain wide range of phytoconstituent flavonoidssteroids, triterpenoids, phenolics, saponins and fixed oil (Pavanasasivam and Suktanbawa, 1975, Banerji *et al.*, 1975).

The present article approaches for phytochemical investigations and therapeutic importance of *D. indica* L.grown in Egypt which is found to have good medicinal value and therapeutic values by tribal and folk medicine in various regions. Also, fruits of *Dillenia indica* are eaten raw but not very much well known by people (Dubey *et al*., 2009; and Sharma & Pegu, 2011).

MATERIALS AND METHODS

Plant material

The leaves, stems and fruits of *D. indica* L. were collected from the Zoo Garden, Giza, Egypt (June, 2012) The collected plant materials were botanically authenticated in the Herbarium of the Botany Dept., Faculty of Science, Cairo University. Also a voucher specimen (No. dd 6/212) of the plant materials were kept in Laboratory of phytochemistry, National Organization for Drug Control and Research. Cytotoxic activity was carried out in The Regional Center for Mycology & Biotechnology Al-Azhar University. All the experiments have been done in NODCAR laboratories as well as central laboratory for chemical analysis, Horticulture Research Institute.

The fruits were washed in distilled water. They were cut into small pieces and shade dried for a week. Later the dried fruits were mechanically grounded into a coarse powder. Leaves were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under shade after complete shade drying, it was powdered. The stems were first sun dried and then ground into a coarse powder using a grinding machine.

Chemicals

All analytical grade chemicals used for the experiments were purchased from Sedico Pharmaceutical Co., 6 October City, Egypt.

General equipment

UV spectra were recorded on Shimadzu, model 2401. ESI-MS spectra on Micromass Quattro-LC triple quadruplemass spectrometer equipped with a Z-Spray electro-spray ion source, while NMR measurements were carried out using Jeol EX-500 spectroscopy; 500 MHz (1H NMR) and 125 MHz (13C NMR) . (GC-MS) analyses of triterpenoid fractions of leaves, stem and fruits, were performed on an Agilent Model 6890 GC with split/splitless injector interfaced to an Agilent 5973. Mass Selective Detector. (electron input energy 70 eV, filament current 220Ma, source temperature 160ºC, multiplier voltage 1500V, interface

temperature 300ºC). Data were acquired and process by a HP Vectra 486 Chemstation computer in full scan mode (50-650) or selected ion mode.

Preparation of samples

Plant material (250 g) of leaves, stem and fruits, of *D. indica* L., were separately extracted with 70% ethanol. The extractives were filtered through fresh cotton bed and finally with Whatman No. 1 filter paper, each filtrate was evaporated with a rotary evaporator at low temperature (40°-50°C) and reduced pressure to provide crude ethanol extracts. The residue was re-dissolved in ethanol and used for the determination phytochemical Screening and the cytotoxic activity.

Phytochemical Screening

The phytochemical screening was carried out on the aqueous extract and alcholic extract of the powdered specimens using standard procedures to identify the constituents as reported in various books and research articles. (Sofowara, 1993; Trease and Evans 1983; Harborne. 1984).

Extraction and isolation of the flavonoidal constituents

500 g of the air dried powdered *D. indica* L. fruits were exhaustively extracted by percolation in ethanol (70%) at room temperature. The ethanol extract was evaporated under reduced pressure at low temperature (40 C) to yield 50g dried extract. The residue was suspended in water (400ml), defatted with petroleum ether (40 - 60^{o}C). The suspension was partitioned successively with chloroform, ethyl acetate and n-butanol (each of 5 x 300 ml). The chloroform, ethyl acetate and n-butanol fractions were evaporated to yield 5, 8 and 10 g respectively. Ethyl acetate fraction (8 g) was subjected to a polyamide column (250 g, 120 x 5 cm) eluted using water- ethanol mixtures starting with 100% water up to pure ethanol. Fractions (250 ml) each were collected and monitored on PC developed with 15% acetic acid and n-butanol / acetic acid / water (BAW, 4:1:5,upper layer) as solvent systems. Identical fractions were pooled together to yield 5 collective fractions (I – V). Flavonoid rich fractions fractions IV and V. Fraction IV, [H_2O / EtOH (25 / 75)], contained one major spot. It was purified on a sephadex LH-20 column (40 x 1cm), to yield compound1 (10 mg). Fraction V, (EtOH 100%), contained two major spots. It was subjected to PC using BAW (4:1:5 upper layer) as solvent system followed by sephadex LH-20 column for further purification to yield compounds 2 and 3, (12 and 10 mg, respectively).The n- butanol fraction (10 g) was found to contain five major spots (R = 0.83, 0.71, 0.55, 0.41,0.32) on PC using 15% acetic acid as

solvent system. It was subjected to a polyamide column (250 g, 120 x 5 cm) using water / ethanol mixtures starting with 100% water and decreasing polarity with ethanol till 100% ethanol. Fractions were collected and monitored on PC as mentioned above. Similar fractions were pooled together to yield 5 collective fractions (I – V). Fractions rich in phenolic compounds were fractions III – V. Fractions III, [H_2O / EtOH (50/50)] and IV, [H_2 O / EtOH (25/75)], each contained one major spot. They were separately purified on a sephadex LH-20 column (40 x 1cm), to yield compounds 4 (7 mg) and 5 (10 mg), respectively. Fraction V, (EtOH 100%), contained two major spots. It was subjected to PC developed with (acetic acid 15%) as solvent system followed by sephadex LH-20 column for further purification to yield compounds 6 and 7, (20 and 8 mg, respectively).

GC-MS analysis of terpenoids fraction

3 g dried powders of each leaves, stems and fruits of *D. indica* L., were extracted with ethyl acetate during 15 min at 40°C (30 mL, three times). After filtration, the acidic compounds were extracted with aqueous KOH 5% (10 mL, three times) followed by the extraction of the basic compounds with aqueous HCl 5% (10 mL, three times). The organic fraction, which contained the neutral compounds, was washed with 30 mL of water and concentrated in a rotatory evaporator to 30 mL, being then centrifuged during 10 min at 6000 rpm, to remove the suspended particles. The solvent was evaporated to dryness, giving a residue, which was dissolved in CHCl3 for GC-MS analysis. Figure 1 summarizes the procedure of the extraction process. (Cordeiro *et al.*, 1999).

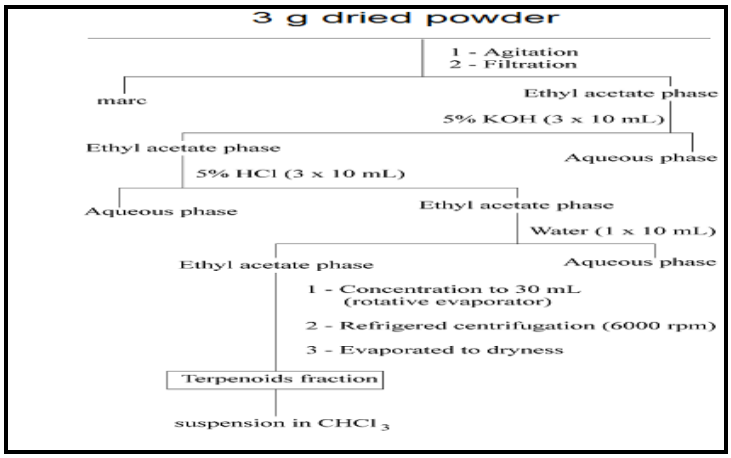

Figure 1. Extraction procedure of terpenoids of *D. indica* L.

Antioxidant activity

The antioxidant activity of the alcoholic extract of the fruits of *D. indica* L. were evaluated by using the 2, 2'diphenylpicrylhydrazyl (DPPH) assay (Cuendet *et al*., 1997; Burits and Bucar, 2000). Fifty microliters of the extracts were added to 5 ml of a 0.004% (w/v) of DPPH in methanol (100% v/v). After, a 30 min incubation period at room temperature the absorbance at 517 nm was compared to DPPH in ethanol without an extract sample (blank). The percent inhibition of free radical formation (I %) was calculated as;

I% = (A blank- A sample / A blank) × 100

Where;

A blank is the absorbance of the control reaction (containing all reagents except the extract) and A sample is the absorbance of the mixture containing the extract.

The IC_{50} (defined as the concentration of extract required to produce 50% of the maximum inhibition) was calculated from graphing inhibition percentage against extract concentration. Determinations were carried out in triplicate.

Cytotoxic assay procedures

Human tumor cell lines Authentic culture, HCT-116 and Hep-G2 cells were obtained frozen under liquid nitrogen (–180ºC) from the American Type Culture Collection. The tumor cell lines were maintained by serial subculturing in The Regional Center for Mycology & Biotechnology, Cairo, Egypt.

Culture media

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

Assay method for cytotoxic activity

The cytotoxicity against HCT116 and Hep-G2 cells was performed in The Regional Center for Mycology & Biotechnology, Cairo, Egypt, according to the SRB assay method (Skehan *et al*., 1996). Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as a reference drug. HCT116 and Hep-G2 cells were plated in multiwell plates (5x104–105 cells/well in a fresh media) for 24 h before treatment with the tested sample to allow

attachment of cells to the wall of the plate. Then, 200 μL aliquot of serial dilution of alcoholic extracts of leaves, stem and fruits, of *D. indica* L., (5.0, 12.5, 25, 50 μg/ml) were added and the plates were incubated for 24, 48 and 72 hrs at 37°C in a humidified incubator containing 5% CO_2 in air. Control cells were treated with vehicle alone. Following 24, 48 and 72 hrs treatment, cells were fixed, washed and stained with Sulforhodamine B stain (Sigma,USA). Colour intensity was measured in an EL ISA reader spectrophotometer (Tecan Group Ltd.-Sunrise, Germany).

RESULTS AND DISCUSSION

Table (1): The preliminary phytochemical screening of the ethanol extracts of the leaves, stems and fruits of *D. indica* L.

Fruits	Stems	Leaves	Compounds
+	+	+	Steriods
++	+++	++	Terpenoids
+	+	++	Tannins
-	-	+	Saponins
++	-	-	Anthocyanins
+++	+	++	Flavonoids
-	-	-	Alkaloids
-	-	-	Cardiac glycosides

(+) Present; (-) Absent; (++) More prominent; (+++) Highly prominent.

Results of phytochemical analysis

Phytochemical analysis of the plant extracts revealed the presence of steroids, terpenoids, tannins, saponins, anthocyanins and flavonoids in alcoholic extracts of leaves, stems and fruits of *D. indica* L., absence of alkaloids and cardiac glycosides (Table-1). Terpenoids present in high amount in alcoholic extract of the stems, while flavonoids present in high amount in alcoholic extract of the Fruits. According to phytochemical screening fruits were used for flavonoids isolation, determination of antioxidant activity and stems were used for terpenoids analysis by GC-MS.

Isolation of the flavonoid constituents

The flavonoids of the fruits of *D. indica* L. were isolated from the ethyl acetate and butanol fractions of alcoholic extract, seven flavonoids were identified as follow:

1-Quercetin-3-O-glucopyranoside

Rf: 0.57 (BAW), 0.08 (H2O), 0.38 (15% AcOH). UV/Vis λmax (MeOH): 256, 267sh, 298sh, 359; (+NaOMe): 272, 326, 408; ($AlCl_3$): 275, 304sh, 333sh, 429; (+AlCl3+ HCl): 269,

300sh, 358sh, 403; (+NaOAc): 271, 323, 373; (+NaOAc +H_3BO_3): 262, 301sh, 379. 1H NMR in DMSO-*d*6: δ 7.66 (1H, d, J= 8.5 Hz, H-6'), δ 7.55 (1H, d, *J* =2 Hz, H-2'), δ 6.80 (1H, d, *J*= 8.5 Hz, H- 5'), δ 6.37 (1H, d,*J* =2 Hz, H-8), δ 6.17 (1H, d, *J*= 2 Hz, H-6), δ 5.37 (1H,d, *J*= 7.5 Hz, H-1"), δ 3–4 (5H, m). ESI-MS/MS m/z $[M^{-}H]^{-}$:463,301.

2- Rhamnetin-3-O-rhamnopyranoside

Rf: 0.71 (BAW), 0.25 (H_2O), 0.54 (15% AcOH). UV/Vis ƛmax (MeOH): 257, 264 sh., 350; (+NaOMe): 267, 303sh, 398; (+$AlCl_3$): 274, 304sh, 427; (+$AlCl_3$ + HCl): 271, 301sh, 352, 399; (+NaOAc): 260, 302sh, 366; (+NaOAc +H_3BO_3): 262, 290sh, 372. 1H NMR in DMSO-*d*6: δ 7.33 (1H, d, J= 2 Hz, H-20), δ 7.27 (1H, dd, J= 2, 8.2 Hz, H-60), d 6.85(1H, d, J= 8.1 Hz, H-50), δ 6.69 (1H, d, J =2 Hz, H-8), δ 6.39 (1H, d, J =2 Hz, H-6), δ 5.28 (1H, d, J= 2.5 Hz, H-100), δ 3.86 (3H, s, OCH_3), δ 0.81 (3H, d, J= 5.5 Hz , CH_3-rhamnose). ESI-MS/MS: m/z $[M^{-}H]^{-}$:461,301.

3. Apigenin-6-C-arabinopyranosyl-8-C-glucopyranoside

Rf: 0.13 (BAW), 0.36 (H_2O), 0.48 (15% AcOH). UV/Vis ƛmax (MeOH): 273, 333, (+NaOMe): 284, 332, 399; (+$AlCl_3$): 279,305,344,387sh; (+AlCl3+HCl): 280, 304, 343, 387sh; (+NaOAc):282, 308sh, 392; (+NaOAc+H_3BO_3): 275, 320, 347sh. 1HNMR in DMSO-*d*6: d 7.9 (2H, d, J=7.6Hz, H-2', H-6'), δ 6.85(2H, d, J=7.9 Hz, H-30, H-50), δ 6.5 (1H, s, H-3), δ 4.8 (1H, d, J=8Hz, H-1"), δ 4.6 (1H, d, J=8Hz, H-1‴), δ 3.2–4 (9H, m). 13C NMR in DMSO-*d*6: d 182.6 (C-4), 164.5 (C-2), 161.6(C-5), 161.6 (C-7), 158.7 (C-4'), 155.6 (C-9), 129.4 (C-2'), 129.4(C-6'), 121.9 (C-1'), 116.4 (C-3'), 116.4 (C-5'), 108.4 (C-6), 105.3(C-8), 103.9 (C-10), 103 (C-3), 82.2 (C-5‴), 79 (C-3"), 74.6 (C-1"), 74 (C-2"), 73.7 (C-1‴), 71.5 (C-2‴), 71 (C-3‴), 70.6 (C-4‴),69.6 (C-4"), 69 (C-5"), 61.4 (C-6‴). ESI- MS/MS: m/z $[M^{-}H]^{-}$ 563, 401, 271, corresponding to a molecular weight of 564 and a molecular formula of $C_{26}H_{28}O_{14}$.

4. Apigenin-6, 8-di-C-glucopyranoside

Rf: 0.13 (BAW), 0.34 (H_2O), 0.48 (15% AcOH). UV/Vis ƛmax (MeOH): 273, 333, (+NaOMe): 283, 332, 400; (+$AlCl_3$): 280,305, 346, 388; (+$AlCl_3$ + HCl): 279, 305, 345, 387; (+NaOAc):282, 309sh, 3902; (+NaOAc +H_3BO_3): 274, 323,349sh. 1H NMR in DMSO-*d*6: δ 7.9 (2H, d, J= 8.5 Hz, H-2', H-6'), δ 6.85 (2H, d, J= 8.5 Hz, H-3', H-5'), δ 6.5 (1H, s,H-3), δ 4.8 (1H, d, J= 8.6 Hz, H-1"), δ 4.6 (1H, d, J= 8.6 Hz, H-1‴), δ 3–4 (10H, m). ESI- MS/MS: m/z $[M^{-}H]^{-}$593, 431, 271 corresponding to a molecular weight of 594 and a molecular formula of $C_{27}H_{30}O_{15}$.

5. Quercetin-3, 7-di-O-glucopyranoside

Rf: 0.18 (BAW), 0.33 (H_2O), 0.63 (15% AcOH). UV/Vis ƛmax (MeOH): 258, 267sh, 355; (+NaOMe): 270, 308sh, 405; ($AlCl_3$): 270, 309sh, 436; (+$AlCl_3$ +HCl): 266, 303sh, 357sh, 406; (+NaOAc): 261, 310sh, 387; (+NaOAc+H_3BO_3): 263, 306sh, 377. 1H NMR in DMSO-*d*6: 7.55(2H, dd, J =2.5 Hz, H-20; J = 7.5 Hz, H-60), δ 7.15 (1H, d, J= 8.5 Hz, H-50), δ 6.80 (1H, d, J= 2 Hz, H-8), δ 6.45 (1H, d, J= 2 Hz, H-6), δ 5.35 (1H, d, J= 8 Hz, H-1"), δ 4.85(1H, d, J= 8 Hz, H-1‴), δ 3–3.8 (10H, m). ESI- MS/MS: m/z $[M-H]^-$625,463,301

6. Myricetin (3, 5, 7, 3', 4', 5'-heahyroxy-flavone)

Rf: 0.43 (BAW), 0.05 (H_2O), 0.26 (15% AcOH). UV/Vis ƛmax (MeOH): 265, 300 sh, 361; (+NaOMe): 269, 328sh, 408;(+$AlCl_3$): 272, 310, 427; (+$AlCl_3$ + HCl): 271, 306, 362,411; (+NaOAc): 270, 401; (+NaOAc +H_3BO_3): 266, 391.1H NMR in DMSO-*d*6: δ 7.15 (2H, d, J =2 Hz, H-2', H-6'), δ 6.33 (1H, d, J = 2 Hz, H-8), δ 6.16 (1H, d, J= 2 Hz, H-6). ESI- MS/MS: m/z $[M-H]^-$:317,151.

7. Myricetin-3-O-glucopyranoside

Rf: 0.48 (BAW), 0.07 (H_2O), 0.27 (15% AcOH). UV/Vis ƛmax (MeOH): 264, 285 sh, 357; (+NaOMe): 272, 397; (+$AlCl_3$):272, 361sh, 411; (+$AlCl_3$ +HCl): 270, 361, 402; (+NaOAc):269, 362; (+NaOAc+ H_3BO_3): 265, 384. 1H NMR in DMSO-*d*6: δ 7.20 (2H, d, J = 2 Hz, H-2', H-6'), δ 6.35(1H, d, J = 2 Hz, H-8), δ 6.20 (1H, d, J= 2 Hz, H-6), δ 5.42(1H, d, J= 8.5 Hz, H-1"), δ 3–3.8 (5H, m). ESI- MS/MS: m/z $[M-H]^-$479,317.

DPPH radical scavenging activity

The ethanol extract of *D. indica* L. fruits showed a concentration dependent scavenging activity by quenching DPPH radicals. The fruits extract had very strong free radical scavenging activity with IC_{50} value of 4.38 mg/mL. The antioxidant activity of *D. indica* L. fruits is due to its higher phenolic content.

GC-MS analysis of Terpenoids

The results pertaining to GC-MS analysis led to the identification of number of terpenoids from the ethyl acetate extract of the leaves, stems and fruits of *D. indica* L. Sixteen compounds were identified (Table 2), the stems extract had the highest numbers of compouds followed by leaves and fruits extracts. Lupeol was the major component in the leaves, stems and fruits of *D. indica* L., being of 32.72 ,37.49 and 16.48% respectively, followed by

Betulinaldehyde 20.28, 23.29 and 16.06% respectively and betulinic acid 18.41,22.29 and 14.62% respectively.

Table 2: Compounds identified in the triterpenoids fractions of leaves, stem and fruits, of *D. indica* L., extracts.

No	Peak area%			Composition (MF)	MW	Compound Name
	fruits	stems	leaves			
1.	-	-	1.93	$C_{29}H_{47}O_2$	437	20-hydroxy-lupan-3-one
2.	16.48	37.49	32.72	$C_{30}H_{48}O$	424	Lupeol (3β-hydroxy -lup-20(29)-ene)
3.	4.11	8.23	8.75	$C_{30}H_{48}O_2$	442	Betulin (3β-28-dihydroxy-lup-20(29)-en)
4.	11.3	18.40	15.70	$C_{29}H_{48}O_2$	412	Stigmasterol(3β,22E-Stigmasta-5,22-dien-3-ol)
5.	10.4	15.70	14.2	$C_{29}H_{50}O$	414	β - Sitosterol (3*β*-Stigmast-5-en-3-ol)
6.	-	2.75	1.33	$C_{30}H_{48}O_4$	473	2α,3α- dihydroxy lup-20(29)-en-28-oic acid
7.	15.1	4.11	1.65	$C_{30}H_{48}O_2$	440	6 *β*-hydroxy lup-20(29)-en-3-one
8.	-	1.18	-	$C_{31}H_{48}O_5$	501	3β-Acetoxy-20-oxo-29-norlupan-28-oic acid
9.	9.50	1.29	5.42	$C_{30}H_{48}O_2$	442	3 α,23-dihydroxy lup-20(29)-ene
10.	16.06	23.29	20.28	$C_{30}H_{48}O_2$	441	Betulinaldehyde(3 *β*-hydroxylup-20(29)-en-28-al)
11.	-	7.56	-	$C_{30}H_{50}O_3$	459	3β,28,30-triol -Lup-20-en
12.	2.4	1.30	-	$C_{30}H_{50}O_3$	458	6*β*-28-dihydroxy lup-20(29)-en-3-one
13.	-	0.56	3.54	$C_{29}H_{48}O_3$	443	20-hydroxylupan-3,6- dione
14.	-	4.62	-	$C_{30}H_{50}O_2$	442	30-hydroxy lup-20(29)-en-3-one
15.	3.40	1.34	1.11	$C_{30}H_{50}O_3$	459	6*β*-20-dihydroxy lupan-3-one
16.	14.6	22.29	18.41	$C_{30}H_{48}O_3$	457	betulinic acid (3 *β*- hydroxy- lup-20(29)-en-28-oic acid)

Cytotoxic assay

The ethanol extracts of leaves, stem and fruits of *D. indica* were tested against human cancer cell lines: colon carcinoma cell line (HCT-116) and liver carcinoma cell line (HEPG2).Only the ethanol extract of *D. indica* L., stems showed significant cytotoxicity and dose-dependent cytotoxicity against the tested human cancer cell lines (Table 3 and 4) with IC_{50} = 9.8 and 20.1 µg /ml respectiviely, while The ethanol extracts of *D. indica* L., leaves and fruits failed to exert a considerable effect on HCT-116 and HEPG2 carcinoma cell lines. IC_{50} values that were greater than 30µg/ml considered insignificant, and vice versa.

Table 3: The cytotoxic activity of the ethanol extracts of leaves, stems and fruits, of *D. indica* L., on (HEPG2) carcinoma cell line using SRB method.

Viability % of (HEPG2) carcinoma cell line			Sample conc. (µg/ml)
Fruits	Leaves	Stem	
95.86	95.86	6.21	50
98.69	98.69	25.14	25
100	100	40.75	12.5
100	100	62.53	6.25
100	100	78.46	3.125

100	100	91.38	1.56
100.00	100.00	100.00	0

Table 4: The cytotoxic activity of the ethanol extracts of leaves, stems and fruits, of *D. indica* L., on (HCT-116) carcinoma cell line using SRB bioassay in vitro.

Viability % of (HCT-116) carcinoma cell line			Sample conc. (µg/ml)
Fruits	Leaves	Stem	
98.42	97.88	26.82	50
100	99.04	41.07	25
100	100	63.72	12.5
100	100	81.38	6.25
100	100	92.16	3.125
100	100	98.67	1.56
100.00	100.00	100.00	0

DISSCUION

From literature survey, it is revealed that different parts of *D. indica* L. contain many primary and secondary metabolites and considered as a rich source of triterpenoids, flavonoids, tannins and various other phytoconstituents (Kumar *et al.*, 2010). This is in agreement with our preliminary Phytochemical screening results which showed that alcoholic extract of *D. indica* L. fruits contain the highest amount of phenolic compounds, while the alcoholic extract of stems contain the highest amount of triterpenoids, so isolation of flavonoids and antioxidant activity have been done for the fruits extract. Seven flavonoidsn compounds Were isolated for first time from the ethanolic extract of *D. indica* L. Fruits: Quercetin-3-O-glucopyranoside (1), Rhamnetin-3-O-rhamnopyranoside (2), Apigenin-6-C-arabinopyranosyl-8-C-glucopyranoside (3), Apigenin-6,8-di-C-glucopyranoside (4), Quercetin-3, 7-di-O-glucopyranoside (5), Myricetin (6) Myricetin-3-O-glucopyranoside(7)**.** Previously, Munmee *et al*,2012 and Nazma *et al*, 2009 had reported that the edible fruits of *D. indica* L. have strong antioxidant activity which may be related to the high amount of phenolic compounds, in our study we approved that *D. indica* L .tree grown in Egypt has the same strong antioxidant(IC_{50} = 4.38 mg/mL). From GC-MS results; Lupeol was the major compoud in leaves, stems and fruits of *D. indica* L., followed by Betulinaldehyde and betulinic acid, these compouds were isolated previously from the stem extract of *D. indica* L.by Nazma *et al*, 2009 as well as Srivastava &Pande, 1981 and Parvin *et al*. 2009. The ethanol extract of the stem of *D. indica* L. contains more terpenoids than that of the leaves and fruits, which may be related to its significant cytotoxic activity against the tested carcinoma cell lines (HCT-116 and HEPG2), these results agreed with the literatures which

reported that terpenoids posses powerful anticancer activities (Kumar *et al*., 2010, Gomathi Periasamy *et al.*, 2015).

CONCLUSION

It has become clear that in Egypt some plants and herbs (have powerful antioxidant and anticancer activity) might provide effective anti cancer therapeutics. Such extracts should be more widely used in developing countries for prevention and treatment of dangerous diseases like cancer. The extracts should be considered as good sources for drug discovery. These plants may have very good medicinal potential which can be further explored for preparation of formulations.

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