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Cytotoxicity, Antioxidant Activities, GC-MS and HPLC Fingerprint Analyses of Different Extracts of *Desmodium tortuosum* (Sw.) DC

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ABSTRACT

The family Fabaceae is the third-largest flowering plant family, and the genus Desmodium has exhibited a wide range of biological activities and a variety of chemical constituents. In the present study, different extracts of Desmodium tortuosum were evaluated for their cytotoxic and antioxidant activities, as well as their total phenolic content (TPC). The antioxidant activities were estimated using the 1,1'-diphenyl-2-picraylhydrazyl free radical (DPPH), while the cytotoxic activity was evaluated via the brine shrimp lethality test (BSLT). The antioxidant activity results revealed that the DPPH radical scavenging activity (SC50) ranged from 1.12 to 61.22 µg/ml with respect to ascorbic acid (SC50 = 7.45 µg/ml). Among all tested fractions, 90% methanol was the most active. On the other hand, the cytotoxic activities were arranged as follows: n-BuOH (LC50 = 310), EtOAc (LC50 = 350), and 70% methanol (LC50 = 380). High-Performance Liquid Chromatography-Fingerprint analyses were used to determine the chemical composition and relative proportions of phenolic compounds. GC-MS analysis indicated the presence of fatty acids and other compounds. The major identified compounds were Benzene (1-butyloctyl) (11.88%) and Himachalene <α-> (11.08%) for the ethyl acetate extract and 10-Undecenoic acid, methyl ester (25.50%) for unsaponifiable matter.

Keywords: Antioxidant, Cytotoxicity, *Desmodium tortuosum*, GC-MS; HPLC.

INTRODUCTION

The family Fabaceae is the third-largest flowering plant family, consisting of about 482 genera and 1,200 species of evergreen trees, herbs, water plants, and shrubs [1]. A wide array of nutrients, such as proteins, amino acids, and fatty acids, are found in Fabaceae plants [2]. Among them, fatty acids fundamentally compose lipid molecules, hormones, and cell membranes, serving as an energy source for cells and playing a key role in energetic metabolic and structural activities [3]. Many species in this family are renowned in traditional medicine for their use as anti-perspirants, diuretics [4], and in the treatment of nephritis [5], diabetes, leukemia, uterine cancer [6], diarrhea, cough, cramps, and sores of the mouth [7]. Fabaceae plants are found all over the world, growing in various environments and climates [8]. Legume products contribute to the world economy through food, pharmaceuticals, medicine, chemicals, and fertilizers. Legumes are also utilized as insecticides, molluscicides, and anti-fungal agents [1].

The genus Desmodium contains about 350 species, mainly distributed in tropical and subtropical regions worldwide, with approximately 28 species in China. Most of its plants are herbs, shrubs, or sub-shrubs, but rarely trees. Besides their popularity as feeding stuffs, they are also used in traditional medicine [9]. Desmodium styracifolium (Osbeck) Merr. has been recorded in the Chinese Pharmacopeia for the treatment of urolithiasis, painful urination, and cardio-cerebrovascular diseases

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[10]. Chemical studies of D. styracifolium have shown the presence of isoflavanones, coumaronochromone [11], saponin, and alkaloids [12-13]. Other species of Desmodium, including D. gangeticum and D. adscendens, are used ethnomedicinally worldwide. Phytochemical research on both species has led to the isolation of alkaloids, phospholipids, sterols, flavones, and triterpenoid saponins. They exhibit a wide spectrum of in vitro and in vivo pharmacological activities, such as antileishmanial, immunomodulatory, smooth muscle relaxant, anti-inflammatory, anti-ulcer, antidiabetic, antiviral, antioxidant, and hepatoprotective activities [14].

During the past 30 years, the Brine Shrimp Lethality Assay has been widely used as a toxicity test for a variety of plant products. This test has been successively employed for bioassay-guided fractionation of active cytotoxic and antitumor agents, demonstrating several advantages such as rapidness, simplicity, low requirements, robustness, where the cysts are commercially and readily available, inexpensive, and with high degrees of repeatability [15].

The overproduction of reactive oxygen species (ROS), such as superoxide anion (O2), hydrogen peroxide, hydroxyl radical (OH), and peroxyl radical (ROO), may induce oxidative stress in the human body, consequently causing degenerative and pathological damages, such as aging, cancer, cardiovascular diseases, Alzheimer's disease, and inflammation. Certain environmental factors, such as stress, cigarette smoking, and some drugs, are also associated with the elevation of free radicals in the human body, so antioxidants play an important role in protecting the body from oxidative stress. Recently, antioxidant activities of medicinal plants or plant-derived chemical compounds and health foods are being investigated comprehensively [16].

The characterization of oils and fats has mainly focused on the principal components, which constitute the saponifiable fraction comprising over 95% of oils and fats. The unsaponifiable matters present in vegetable oils and fats are usually composed of sterols, fatty alcohols, tocopherols, triterpene alcohols, and hydrocarbons, each with individual biological importance [17-18]. Desmodium tortuosum plants were recently cultivated in Egypt, and there is limited information in the literature regarding their chemical content and biological activities. The present study aims to determine the chemical content and evaluate the total phenolic contents, cytotoxic and antioxidant activities of their different extracts. Conventional gas chromatography–mass spectroscopy (GC-MS) was adopted for composition analysis to identify the content of aliphatic and fatty acid compounds, while High-Performance Liquid Chromatography-Fingerprint Analyses was used to quantify different groups of phenolic components in the plant.

MATERIALS AND METHODS

Chemical, reagents and equipments

All solvents and reagents used were of analytical grade. 1,1'-diphenyl-2-picrylhydrazyl (DPPH) free radical and Folin-Ciocalteu's reagent (FCR) were purchased from Sigma-Aldrich Co. Gallic acid and ascorbic acid were purchased from Merck Chemical Co. All solvents and acids (methanol, petroleum ether, chloroform, ethyl acetate, n-butanol) were obtained from Sigma-Aldrich Co. The absorbance measurements for antioxidant activity were recorded using the UV-Vis spectrophotometer Spectronic 601 (Milton Roy, USA).

Plants materials

Desmodium tortuosum (Sw.) DC peels were collected from Elephantine Island in Aswan, Egypt. The plant was added to the Egyptian flora as a new species in Egypt in 2004. The identity of the plant was established by Dr. Fatma Abdallah Mohamed, Lecturer in the Plant Department, Faculty of Science, Aswan University. A voucher specimen was kept in the Department of Medicinal Chemistry, Theodor Bilharz Research Institute (TBRI) under the number (Dt-2012). The plant materials were air-dried in a shaded place at room temperature and then powdered using an electric mill. Finally, they were stored in a tightly closed container in a dark place until the extraction process.

Extraction

Small-scale extraction was carried out by taking samples from the dry powder of the fresh leaves of the plant (10 g). The samples were then separately extracted with different solvents (100 ml x 4): 100% methanol and methanol-water mixtures (90%, 85%, and 70%) at room temperature for one week with daily shaking. The extracts were filtered, and the extraction process was repeated four times. Each extract was filtered using Whatman filter paper No.1 and concentrated using a rotary evaporator (Buchi, Switzerland) at (50 ± 2°C), resulting in a known weight of each crude methanol extract. The crude extracts were collected and stored at room temperature in the dark for further processing.

Air-dried plant leaves (0.25 kg) were extracted by soaking in aqueous methanol (MeOH: water; 90: 10; v/v) at room temperature. After filtration, the collected filtrate was concentrated using a rotary vacuum evaporator under pressure and low temperature. The dried 90% methanol extract (31 g) underwent a defatting process to remove unwanted substances using petroleum ether (60-80°C). Subsequently, the fractionation process was performed by dissolving 28 g of the 90% defatted extract in distilled water. The completely soluble part was then partitioned using dichloromethane, ethyl acetate, and n-butanol, resulting in 2.81 g, 2.0 g, 3 g, and 10 g for dichloromethane, ethyl acetate, and n-butanol, respectively.

The n-butanol extract (7 g) was fractionated on a polyamide column chromatography [(Ø 7.0 × 120 cm)]. Elution started with water, followed by a gradual increase of methanol. Based on comp-TLC and PC with the use of UV light, individual 120 fractions (250 ml each) were collected into seven collective fractions (I-VII). Two groups of fractions (4-12 [I]) and (73-78[III]) (0.9 g & 1.3 g, respectively) were chosen for further chemical and biological analysis with other extracts.

Extraction of lipid constituents

About 50 g of the dried, powdered Desmodium tortuosum were extracted with n-hexane in a Soxhlet apparatus. The combined n-hexane extract was passed through fuller's earth to remove the colored pigments, filtered, dried over anhydrous sodium sulfate, and evaporated under vacuum at 40°C until dryness to create a pale-yellow residue (35 g).

Saponification of *n*-hexane extract

The n-hexane extract (25 g) was saponified by refluxing with 100 ml N/2 alcoholic KOH. The alcoholic solution was concentrated to about 20 ml and diluted with cold distilled water. The unsaponifiable constituents were extracted by partition with successive portions of diethyl ether (3×100 ml). The combined ether extract was washed with distilled water, dehydrated over anhydrous sodium sulfate, and evaporated under vacuum until dryness to give a yellowish-brown semi-solid residue of unsaponifiable matter (5 g), which was subjected to GC analysis.

Assessment of the total phenolic contents

The total phenolic content of the extracts was quantified using Folin-Ciocalteu's method adapted to a 96 well–plate. This method closely follows that used by previous workers, Diko et al. 2002 [19]. Thus, 20 µL of each plant extract dissolved appropriately in distilled water was mixed with 100 µL F-C reagent freshly diluted 1/10 with distilled water. After 5 min incubation at room temperature, 80 µL of 7.5% Na2CO3 solution was added. The whole was left for 30 min at room temperature in the dark with slight shaking. The absorbance was measured at 735 nm in a microplate-reader (Biochrom Asys UVM 340) against blank. Gallic acid was used as the standard. The results (average of triplicate analyses) were expressed in mg/g of extract, gallic acid equivalent (GAE).

Antioxidant activity

Free radical scavenging antioxidant activity using DPPH assay

The scavenging activity of the stable 1,1'-diphenyl-2-picrylhydrazyl free radical was determined by the method described by Marwah et al. 2007 [20]. Briefly, the reaction medium contained 2 ml of 100 µM DPPH purple solution in methanol and 2 ml of plant extract; ascorbic acid was used as

the standard. The reaction mixture was incubated in the dark for 20 min, and the absorbance was recorded at 517 nm. The assay was carried out in triplicate. The DPPH radical scavenging activity was calculated according to the equation: % DPPH radical scavenging activity = [1 - ($A_{sample}/A_{control}$) x 100], where $A_{control}$ and A_{sample} are the absorbencies of control and sample after 20 min, respectively. The SC50 (concentration of the sample required to scavenge 50% of DPPH radicals) values were determined. The decrease in the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity.

Cytotoxicity (Brine shrimp lethality test)

Brine shrimp lethality bioassay test A solution of instant ocean sea salt (Aquarium System, Ohio) was made by dissolving 2.86 gm in distilled water (75 ml). Fifty milligrams of Artemia salina Leach eggs (Artemia, Inc., California) were added to a hatching chamber. The hatching chamber was kept under an inflorescent bulb for 48 hrs for the eggs to hatch into shrimp larvae. Twenty milligrams of the tested extract was dissolved in 2 ml of methanol or the solvent in which it was soluble, and from this, 500, 400, 300, 200, 100, 50, 5 µl of each solution was transferred into vials corresponding to 1000, 800, 600, 400, 200, 100, and 10 µg/ml, respectively. Each dose was tested in triplicate. The vials and the control containing 500 µl of the solvent were allowed to evaporate to dryness in about 48 hrs at room temperature. Four and a half milliliters of instant ocean sea solution were added to each vial, and 10 larvae of Artemia salina (taken 8-72 hrs after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5 ml with sea salt solution immediately after adding the shrimp. Twenty-four hours later, the number of surviving shrimp at each dosage was counted and recorded. LC50 values were determined with 95% confidence intervals by analyzing the data. The data were analyzed, and LC50 values were calculated and carried out according to the Reed-Muench method. Potassium dichromate was used as the standard [22-23].

HPLC-DAD-ESI/MS/MS conditions

Separation and determination of phenolic compounds were performed by reverse-phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) using an Alltima C18, 5mm column (150 mm × 4.6 mm id) with a guard column Alltima C18, 5mm (Alltech). The solvent system used was a gradient of A (CH3 COOH 2.5% v/v), B (CH3 COOH 8% v/v), and C (acetonitrile). The best separation was obtained with the following gradient: at 0 min, 5% B; at 20 min, 10% B; at 50 min, 30% B; at 55 min, 50% B; at 60 min, 100% B; at 100 min, 50% B and 50% C; at 110 min, 100% C until 120 min. The solvent flow rate was 1 ml/min, and the separation was performed at 35°C. The injection volume was 10 µl for each sample solution. Phenolic compounds were assayed by external standard calibration at 280 nm and expressed in µg/100ml. All values were the mean of two injections [23]. HPLC analysis was carried out according to the reported procedures [24-26].

GC-MS analysis

Gas-chromatography-mass spectroscopy (GC-MS) analysis was performed according to the reported procedures [27-28]. On the other hand, fatty acid compositions of the three oils were investigated via GC-MS analysis according to the method described [29&17]. The identification of the chemical components and the interpretation of GC-MS spectrum were carried out according to the database of National Institute Standard and Technique (NIST08s) (WILEY8) [30].

Statistical analysis

The obtained antioxidant and total phenolic contents results were presented as mean ± S.D., and the statistical procedures were performed using SPSS 13.0 program. Computations were based on Finney, 1971 [31]. For comparisons, the Chi2 test (pairwise versus control) was performed. Data were presented as a percentage. The limit for statistical significance was set at $p \leq 0.05$ (significance level of 95%).

RESULTS AND DISCUSSION

1. Quantification of total phenolic contents (TPCs)

The results in Table (1) showed the total phenolic contents date as the following order, [4 -12] fractions (162.63 ± 2.38)

> [73 -78] fractions (115.70 ±7.15) > 85 % methanol (97.79 ± 6.31) > 90% MeOH (88.15 ± 2.38) > EtOAc (64.74 ± 6.31) > Pet. ether (61.98 ± 4.13) > 70 % methanol (60.15 ± 1.88) > *n*-BuOH (57.75 ± 3.98) (mg GAE /g dry extract).

2. Antioxidant activity

2.1. Free radical scavenging antioxidant activity using DPPH assay

The results in Table (1) revealed that the antioxidant activities (expressed as SC_{50} values in µg/ml) were arranged in the following order; 90% MeOH (1.12 ± 2.36 µg/ml) > CH_2Cl_2 (2.12 ± 4.08 µg/ml) > Pet. ether (2.53 ± 4.08 µg/ml) > *n*-BuOH (2.93 ± 4.09 µg/ml) > EtOAc & Fraction [73-78] (3.46 ± 4.08 µg/ml) > Fraction [4-12] comparison with ascorbic acid as standard with SC_{50}= 7.50 µg/ml.

Table (1): *In vitro* antioxidant activities and total phenolic contents of different extracts and fractions of *D. tortuosum*

Sample	DPPH SC_{50} [µg/ml][a]	TPC (mg GAE /g dry ext.)[b]
100% MeOH	30.90 ± 2.81	1.31 ± 0.18
90% MeOH	1.12 ± 2.36	88.15 ± 2.38
85% MeOH	61.22 ± 4.08	97.79 ± 6.31
70% MeOH	40.81 ± 4.08	60.15 ± 1.88
Pet. ether	2.53 ± 4.08	61.98 ± 4.13
CH_2Cl_2	2.12 ± 4.08	39.94 ± 2.38
EtOAc	3.46 ± 4.08	64.74 ± 6.31
n-BuOH	2.93 ± 4.09	57.75 ± 3.98
Fr. III	3.46 ± 4.08	115.70 ± 7.15
Fr. I	3.76 ± 6.22	162.53 ± 2.38
Ascorbic acid	7.50	-------

Results are presented as mean ± S.D. (n = 3).
[a]DPPH results values are presented in SC_{50} values (µg/ml).
[b]TPC results are presented as mg gallic acid equivalent/g dry extract (mg GAE/g ext.).

Cytotoxicity

1. Brine shrimp lethality bioassay test

The results present in Table (2) revealed that the cytotoxic activities of the tested samples are in the order: *n*-BuOH (LC_{50}= 310), EtOAc (LC_{50}= 350), 70 % methanol (LC_{50}= 380). Both 85 % and pure methanol extracts showed very week activity.

Table (2): Mortality percent of Brine Shrimp of different extracts of *Desmodium tortuosum* plant

Dose ext. Conc.	Mortality								
	100%MeOH	70%MeOH	85%MeOH	Pet. ether	CH_2Cl_2	EtOAc	*n*-butanol	90%MeOH	H_2O
1000	68.12	98.81	65.12	79.17	63.79	100	100	100	12.12
800	26.85	98.18	29.41	40.90	41.18	97.5	98.81	100	5.08
600	22.22	71.70	6.06	7.14	22.08	92.72	96.43	61.70	0
400	6.38	44.78	0	0	7.77	58.97	65.79	33.33	0
200	0	30.93	0	0	0	9.1	18.18	4.69	0
100	0	23.62	0	0	0	0	0	0	0
20	0	19.11	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
LC_{50}	850	380	880	820	825	350	310	500	-

HPLC-fingerprint analysis

In this study, fourteen standard phenolic compounds were used as reference compounds (Table 3 and Figure 1). The obtained results revealed variable amounts of these standard compounds present in the tested samples (Table 3 and Figures 2-4).

Table (3): Areas under peaks and concentrations of the ethyl acetate extract, fractions [III] and fractions [I] of *Desmodium tortuosum* against fourteen standard phenolic compounds

Compounds	Standard			EtOAc		Fr. III		Fr. I	
	Rt,	Area	Conc.	Area	Conc .	Area	Conc.	Area	Conc.
Gallic acid	3.116	829.87	40	251.48	12.12	184.41	10.18	1117.40	61.67
Chlorogenic acid	3.515	335.32	46.9	000	0.00	38.63	6.18	3360.69	537.32
Catechin	3.844	612.25	148.5	49.66	12.05	000	0.00	000	0.00
Caffeic acid	4.973	1068.72	30	424.88	11.93	80.72	2.51	824.82	25.67
Syringic acid	5.357	882.99	30	725.16	24.64	98.10	3.78	1244.33	47.96
Rutin	5.774	983.74	120	136.13	16.61	000	0.00	631.64	86.11
Ellagic acid	6.901	757.02	70	59.08	5.46	42.89	4.45	000	0.00
Coumaric acid	7.680	946.65	20	120.48	2.55	21.57	0.53	526.83	12.86
Vanillin	8.387	1007.61	30	253.4	7.54	76.72	2.59	526.02	17.77
Ferulic acid	9.022	857.80	20	000	0.00	000	0.00	333.67	9.08
Naringenin	9.450	836.92	20	339.42	8.11	000	0.00	5373.64	150.72
Propyl gallate	10.277	409.00	8.33	599.80	12.22	1214.67	27.79	2154.92	49.31
Quercetin	10.711	868.80	60	2297.64	158.68	1304.04	100.53	7089.20	546.52
Cinnamic acid	11.200	1280.68	10	2044.55	15.96	2439.54	21.46	541.86	4.77

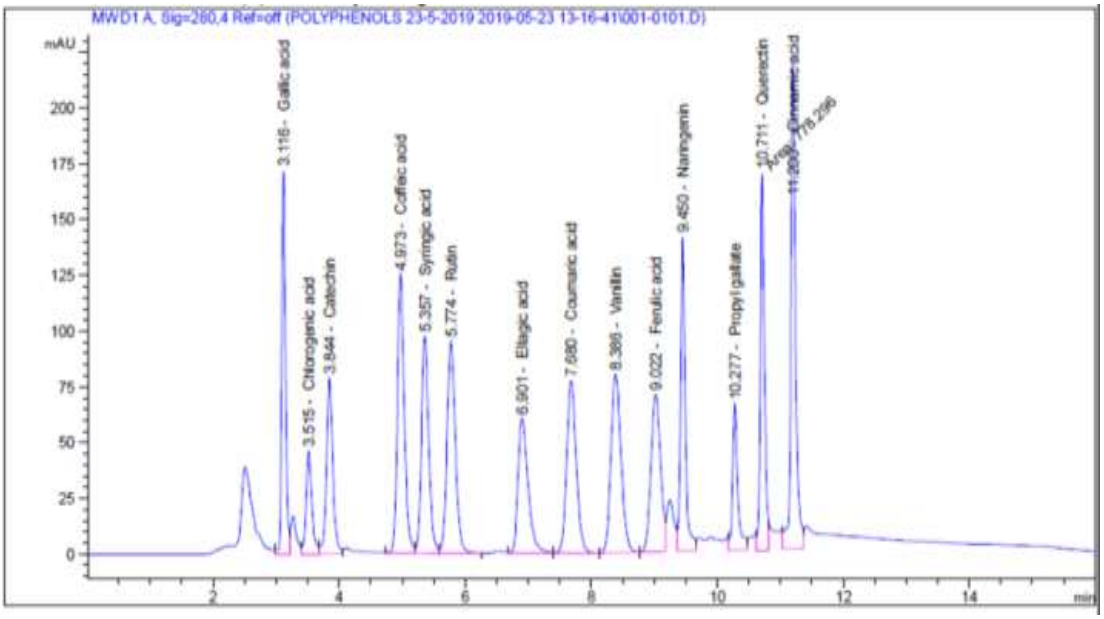


Fig. 1. High performance liquid chromatography chromatogram of fourteen standard phenolic compounds

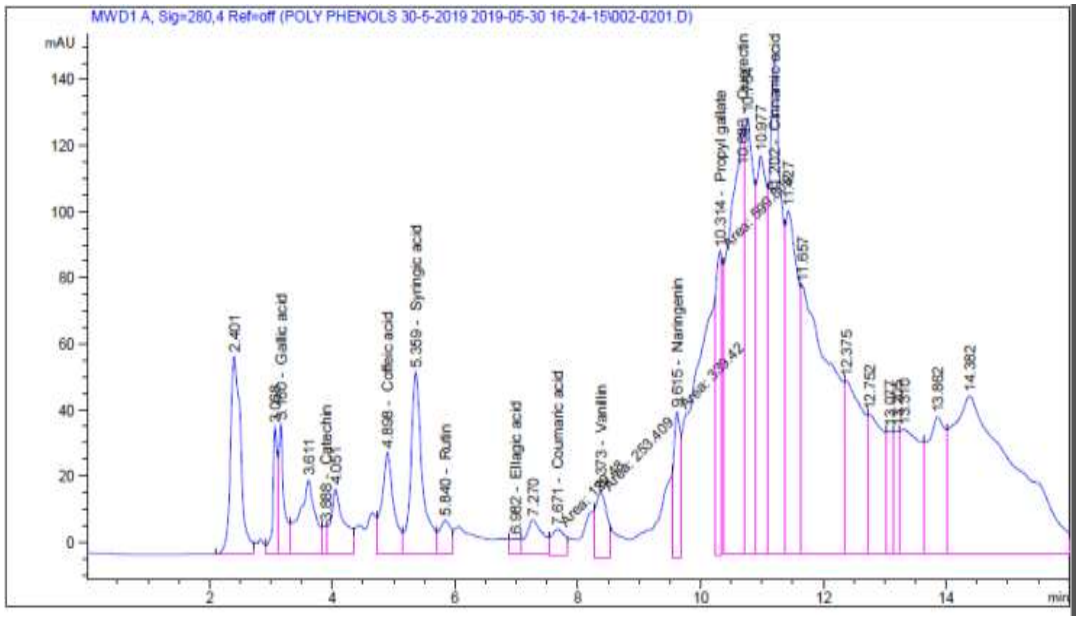


Fig. 2. High-performance liquid chromatography-fingerprint chromatogram of the ethyl acetate extract

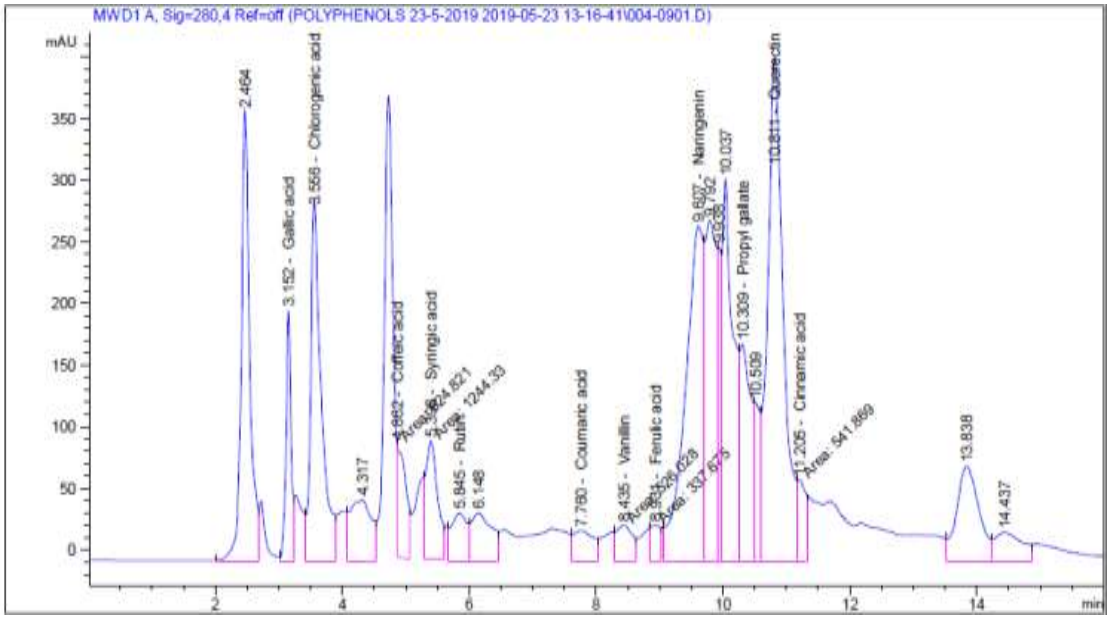


Fig. 3. High-performance liquid chromatography-fingerprint chromatogram of the *n*-butanol fractions (4-12)

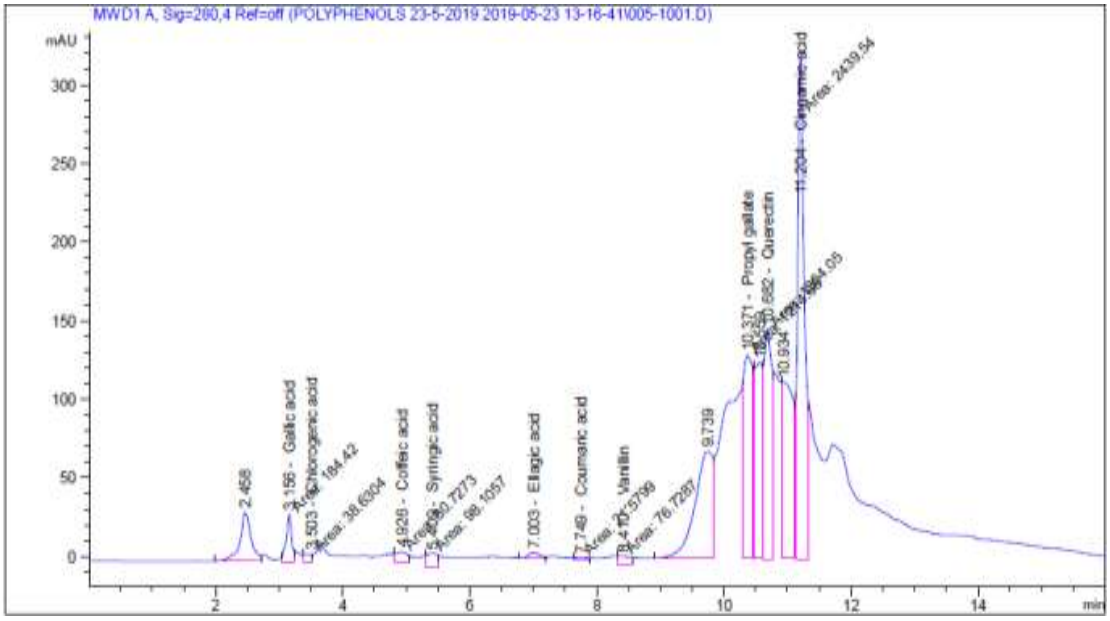


Fig. 4. High-performance liquid chromatography-fingerprint chromatogram of the *n*-butanol fractions (73-78)

5. GC-MS analysis

Chemical composition of the ethyl acetate extract of Desmodium tortuosum, as shown in (Figure 5 and Table 1S), indicates the presence of thirty-three compounds representing 89.44% of the total extract composition. The qualitative GC-MS analyses of the unsaponifiable extract resulted in the identification of thirty-two compounds (Figure 6 and Table 2S).

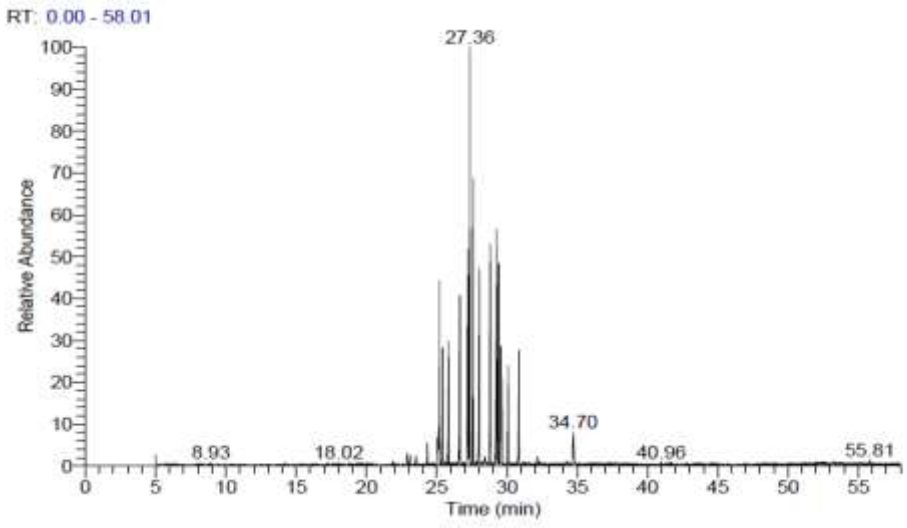
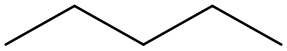
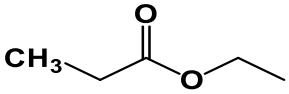
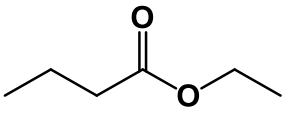
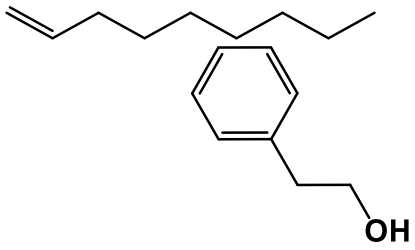
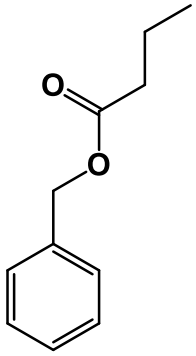
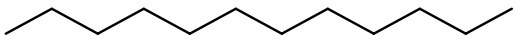
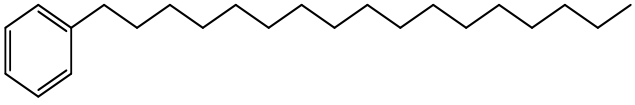
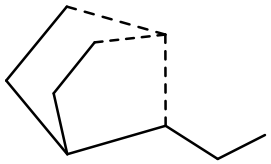
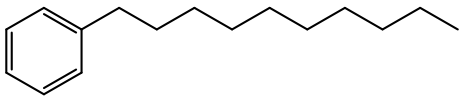
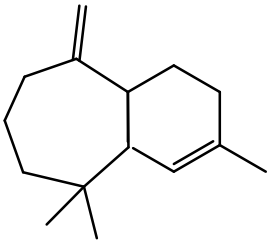
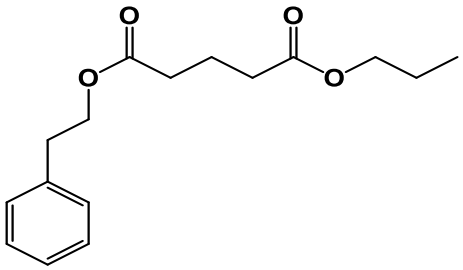
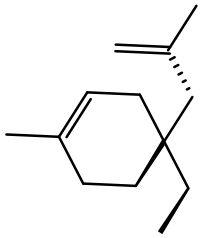
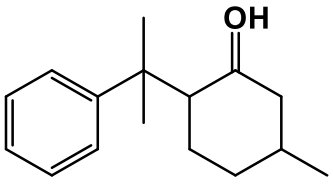
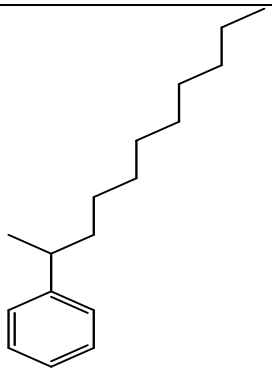
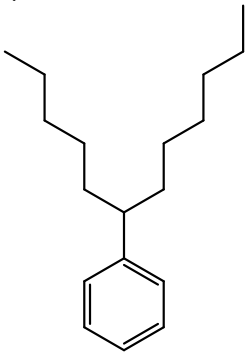
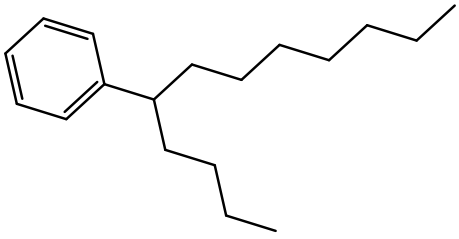
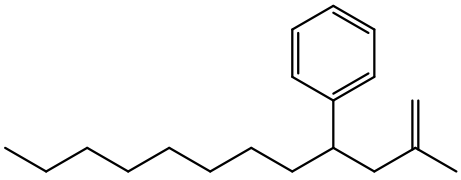
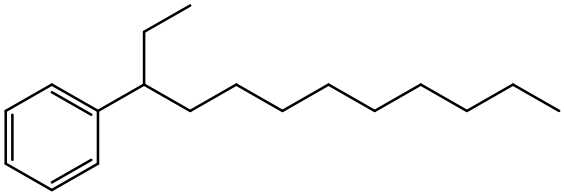


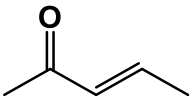
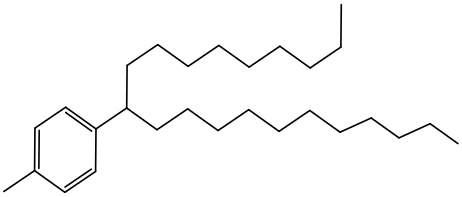
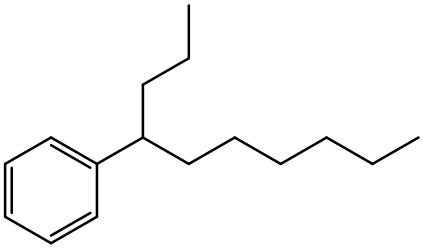
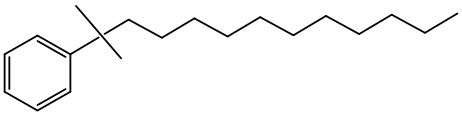
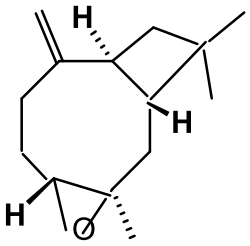
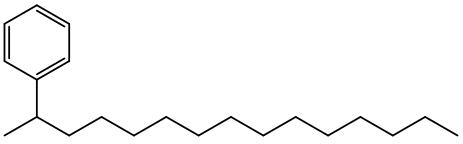
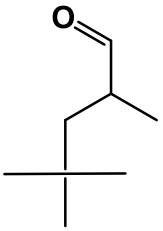
Fig. 5. GC-MS analysis of the ethyl acetate extract of *Desmodium tortuosum*.

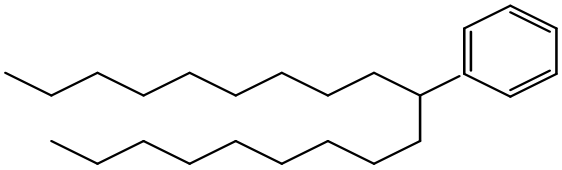
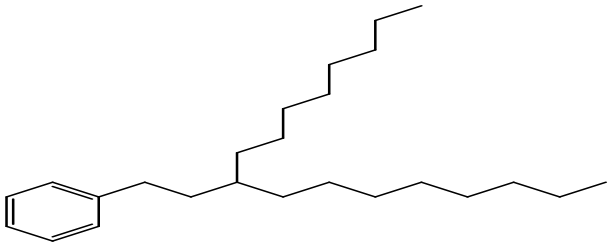
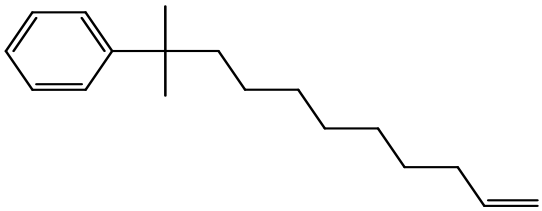
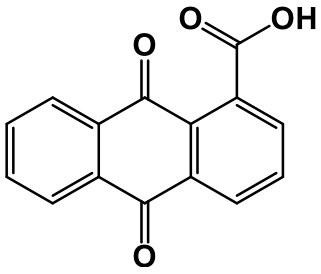
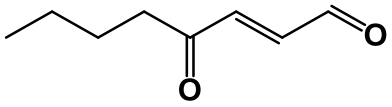
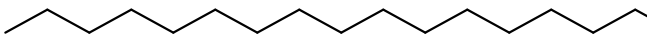
Table 1S: Chemical constituents identified in the ethyl acetate extract of *Desmodium tortuosum* using GC-MS analysis

No	M.W.	M.F.	R.T.	Area %	Name	Structure
1	86	C_6H_{14}	1.62	0.16	Hexane <N->	
2	102	$C_5H_{10}O_2$	2.1	0.07	Propionate	
3	130	$C_7H_{14}O_2$	4.38	0.08	Propyl Butrate	
4	128	$C_{8H6}O$	4.93	3.36	Heptanone<2-Methyl -4->	----
5	140	$C_{10}H_{2O}$	6.8	0.53	Decene	
6	122	$C_8H_{10}O$	10.98	7.43	Phenyl Ethyl Alcohol	
7	178	$C_{11}H_{14}O_2$	20.85	5.31	Benzyl Butyrate	
8	196	$C_{14}H_{28}$	22.95	0.21	Tetra-decene	
9	358	$C_{26}H_{46}$	23.01	0.33	Benzene,(1-propylheptadecyl)	

No	M.W.	M.F.	R.T.	Area %	Name	Structure
10	120	C_8H_8O	23.51	0.35	Norbornadiene7carbaldehyde	
11	218	$C_{16}H_{26}$	24.34	0.64	Benzene, decyl	
12	204	$C_{15}H_{24}$	25.2	11.08	Himachalene <α->	
13	320	$C_{19}H_{28}O_4$	25.58	0.13	Glutaric acid, isohexyl phenethyl ester	
14	204	$C_{15}H_{24}$	25.85	1.20	Acoradiene	
15	232	$C_{16}H_{24}O$	25.95	0.06	Cyclohexanol,5-methyl-2-(1-methyl-1-phenylethyl)	

No	M.W.	M.F.	R.T.	Area %	Name	Structure
16	232	$C_{17}H_{28}$	26.64	5.78	Benzene,(1-methyldecyl)	
17	246	$C_{18}H_{30}$	27.23	9.96	Benzene, (1-pentylheptyl)	
18	246	$C_{18}H_{30}$	27.35	11.88	Benzene, (1-butyloctyl)	
19	246	$C_{17}H_{26}O$	27.58	8.21	Undecanal, 3-phenyl	
20	246	$C_{18}H_{30}$	28.02	6.50	Benzene, (1-ethyldecyl)	

No	M.W.	M.F.	R.T.	Area %	Name	Structure
21	112	$C_7H_{12}O$	28.42	0.43	3-Hexen-2-one,5-methyl	
22	358	$C_{26}H_{46}$	28.89	0.07	Benzene,1-(1-heptyldodecyl) 4-methyl	
23	218	$C_{16}H_{26}$	29.64	2.28	Benzene, (1-propylheptyl)	
24	274	$C_{20}H_{34}$	30.08	3.05	Tridecane,2-methyl-2-phenyl	
25	220	$C_{15}H_{24}O$	30.62	3.73	Caryophyllene Oxide	
26	260	$C_{19}H_{32}$	30.84	3.42	Benzene, (1-methyldodecyl)	
27	128	$C_8H_{16}O$	31.17	0.07	2,4,4-Trimethyl-2-penten-1-ol	

No	M.W.	M.F.	R.T.	Area %	Name	Structure
28	348	$C_{23}H_{40}O_2$	31.28	0.09	Benzaldehyde dioctyl Acetal	
29	344	$C_{25}H_{44}$	31.53	0.16	Benzene, (3-octylundecyl)	
30	246	$C_{18}H_{30}$	32.01	0.09	Benzene,(1,1-dimethyldecyl)	
31	252	$C_{15}H_8O_4$	32.18	0.32	Anthraquinone-1-carboxylic acid	
32	140	$C_8H_{12}O_2$	34.71	2.30	4-Oxo-trans-2-octenal	
33	252	$C_{18}H_{36}$	38.6	0.07	Octadecene <1->	

9 Isoprene compounds
3 Monoterpene compounds
16 Sesquterpenes
2 Diterpene compounds
4 Sestraterpene compounds
15 Oxygenated compounds
18 Deoxygenated compounds
Total 89.44%

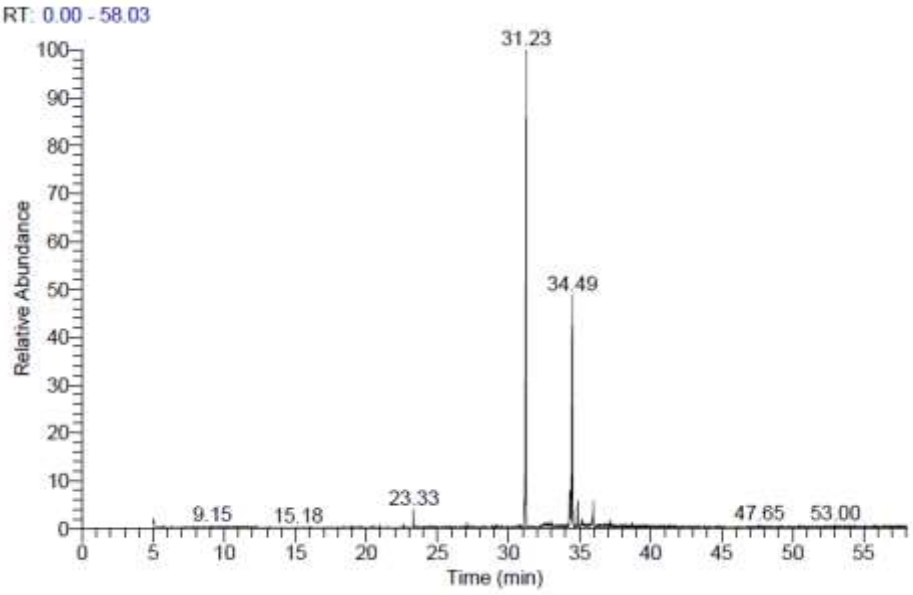
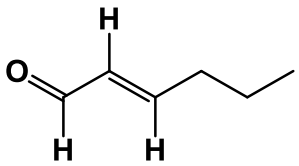
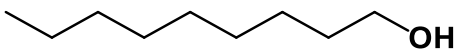
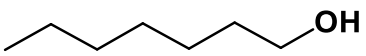
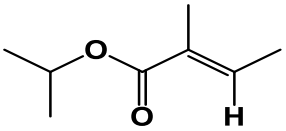
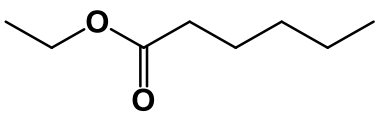
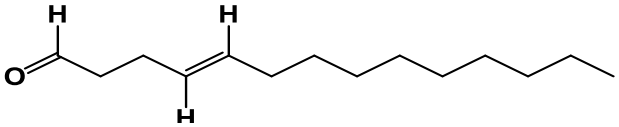
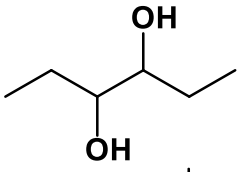
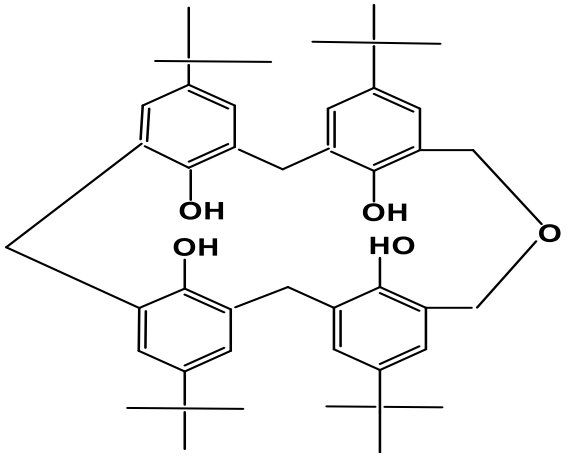
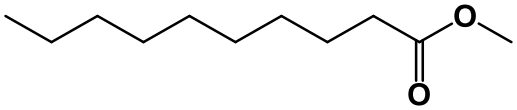
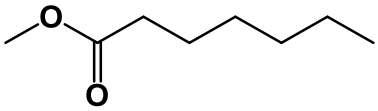
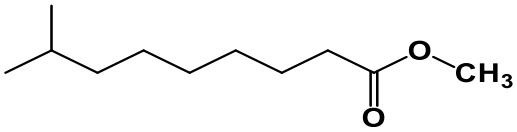
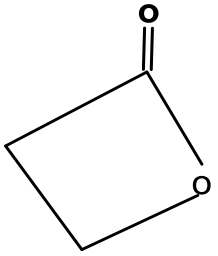
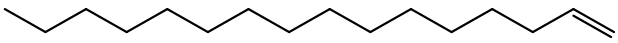
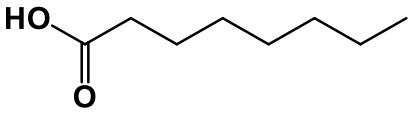
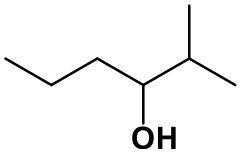
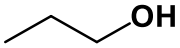
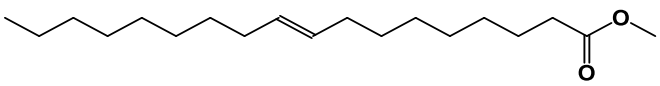
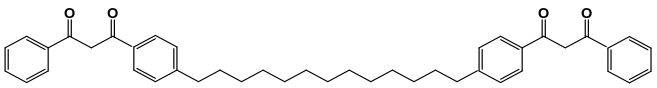
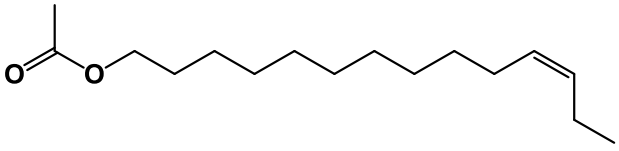
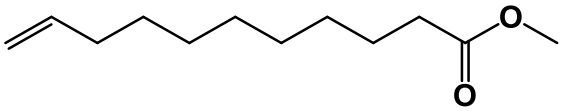


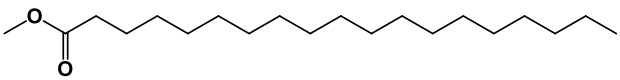
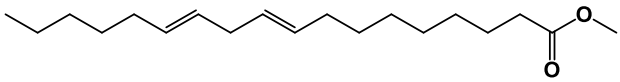
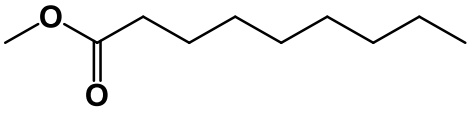
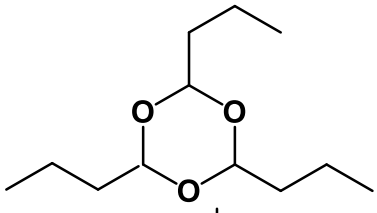
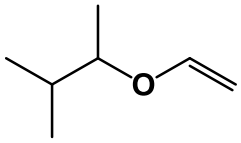
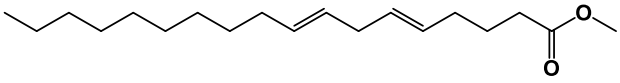
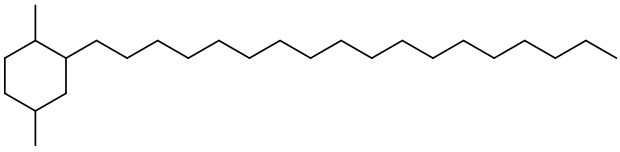
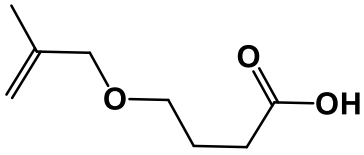
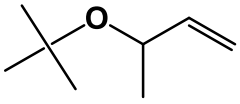
Fig. 6. GC-MS analysis of methanol extract of *Desmodium tortuosum*.

Table 2S: Chemical constituents identified in the unsaponifiable matter of *Desmodium tortuosum* using GC-MS analysis

NO	M.W.	M.F.	R.T.	Area %	Name	Structure
1	98	$C_6H_{10}O$	3.57	0.17	Hexanal<2->	
2	128	$C_9H_{20}O$	4.45	0.77	Nonane<N->	
3	116	$C_7H_{16}O$	6.1	22	Heptanol<N->	
4	142	$C_8H_{14}O_2$	6.23	0.19	Isopropyl Tiglate	
5	144	$C_8H_{16}O_2$	6.97	0.78	Ethyl Hexanoate	

NO	M.W.	M.F.	R.T.	Area %	Name	Structure
6	156	$C_{11}H_{24}$	10.6	0.44	Undecane <N->	
7	704	$C_{33}H_{36}O_{17}$	10.14	0.16	6-C-Xylosyl-8-Cgluco-Sylapigenin permethylated derivative	
8	182	$C_{12}H_{22}O$	20.94	0.26	4-Dodecen-1-aL	
9	118	$C_6H_{14}O_2$	25.58	0.17	Meso-3,4-Hexanediol	
10		$C_{45}H_{58}O_5$	26.70	0.19	7,13,19,25-Tetratertbutyl27,28,29,30 etrahydroxy 2,3-bishomo-3-O- -Xacalix [4] arene	
11	186	$C_{11}H_{22}O_2$	27.08	0.55	Decanoic acid, methyl ester	
12	144	$C_8H_{16}O_2$	28.81	0.22	Heptanoic acid, methyl ester	
13	186	$C_{11}H_{22}O_2$	29.15	0.39	Methyl 8-methylnonanoate	

NO	M.W.	M.F.	R.T.	Area %	Name	Structure
14	72	$C_3H_4O_2$	29.33	0.17	Beta-PROPIOLACTONE	
15	664	$C_{36}H_{56}O_{11}$	30.34	0.20	Tetra-acetyl derivative Of- 25-hydroxybrassinlide	
16	224	$C_{16}H_{32}$	31.13	0.22	Hexadecene <1->	
17	144	$C_8H_{16}O_2$	32.52	0.78	Octanoic acid	
18	116	$C_7H_{16}O$	32.66	0.19	3-Hexanol,2-methyl	
19	60	C_3H_8O	32.77	0.30	1-Propanol	
20	296	$C_{19}H_{36}O_2$	33.03	0.63	9-Octadecenoic acid (Z),methyl ester	
21	628	$C_{43}H_{48}O_4$	33.78	0.18	3Hydroxy1(4{13[4(3-hydroxy 3-phenyl acryloyl) phenyl] tridecyl} phenyl)3-phenylprop-2-en1one	
22	254	$C_{16}H_{30}O_2$	34.33	3.31	11-Tetradecer	
23	198	$C_{12}H_{22}O_2$	34.49	25.50	10-Undecenoic acid,methyl ester	

NO	M.W.	M.F.	R.T.	Area %	Name	Structure
24	312	$C_{20}H_{40}O_2$	34.8	2.63	Nonadecanoic acid,methyl ester	
25	294	$C_{19}H_{34}O_2$	35.17	1.13	9,12 Octadecadienoic acid (Z, Z), methyl ester	
26	172	$C_{10}H_{20}O_2$	35.25	0.25	Nonanoic acid, methyl ester	
27	216	$C_{12}H_{24}O_3$	35.32	0.20	2,4,6-Tripropyl1, 3,5-trioxane	
28	102	$C_5H_{10}O_2$	35.60	0.18	Formic acid, 2-methylpropyl ester	
29	294	$C_{19}H_{34}O_2$	35.98	3.07	5,8-Octadecadienoic acid, methyl ester	
30	364	$C_{26}H_{52}$	36.55	0.29	Cyclohexane,1,4-dimethyl 2-octadecyl	
31	158	$C_8H_{14}O_3$	38.22	0.33	4(2-Methyl-2-propenoxy) butanoic acid	
32	114	$C_7H_{14}O$	38.69	0.52	1-Propene,3 (1,1-dimethyl ethoxy)	

12 Isoprene compounds
1 Diterpene compounds
1 Sesquaterpene compounds
2 Other compounds
Total 41.28%

7 Monoterpene compounds
1 Sestreterpene compounds
1 Tetraterpene compounds
29 Oxygenated compounds

5 Sesquiterpene compounds
1 Triterpene compounds
1 Polyterpene compound
2 Deoxygenated compounds

1- Quantification of total phenolic contents (TPCs)

The results of the total phenolic content showed a remarkable variation among the tested samples, which may be due to the diversity of secondary metabolites in each individual sample. The results were in the following order: [I] fractions (162.63 µg/ml) > [III] fractions (115.70 ± 7.15 µg/ml) > 85% methanol (97.79 ± 6.31 µg/ml) > 90% MeOH (88.15 ± 2.38 µg/ml) > EtOAc (64.74 ± 6.31 µg/ml) > Pet. ether (61.98 ± 4.13 µg/ml) > 70% methanol (60.15 ± 1.88 µg/ml) > n-BuOH (57.75 ± 3.98 µg/ml) (mg GAE/g dry extract). Previous reports showed a highly positive correlation between total phenolic contents and antioxidant activities, reflecting the effective role of phenolic compounds as free radical scavengers [32-34].

2. Antioxidant activity

2.1. Free radical scavenging antioxidant activity using DPPH assay

The oxidative stress associated with generation of destructive effects and harmful health problems as cancer, immunosuppression, inflammation, ischemic heart disease, atherosclerosis, aging, diabetes, and Alzheimer's disease [35-36]. Therefore, the naturally occurring compounds exhibit a strong antioxidant potential due to their abilities to mask the reactive species. Different extracts of *Desmodium tortuosum* were evaluated for their free radical scavenging antioxidant activity using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay. The results revealed that the antioxidant activities (expressed as SC_{50} values in µg/ml) were arranged in the following order; 90% MeOH (1.12 ± 2.36 µg/ml) > CH_2Cl_2 (2.12 ± 4.08 µg/ml) > Pet. ether (2.53 ± 4.08 µg/ml) > *n*-BuOH (2.93 ± 4.09 µg/ml) > EtOAc equal to Fraction [III] (3.46 ± 4.08 µg/ml) > Fraction [I] (3.76 ± 6.22 µg/ml) in comparison with ascorbic acid as standard with SC_{50}= 7.50 µg/ml. Moreover, numerous studies have demonstrated that the high activity antioxidant maybe due to the presence of a complex pattern of the bioactive polyphenolic compounds [37-38].

3. Cytotoxicity

3.1. Brine shrimp lethality bioassay test

The BLST assay is a quick and inexpensive method used to evaluate the lethality of various extracts, fractions or compounds [39]. The results present revealed that the cytotoxic activities of the tested samples are in the order: *n*-BuOH (LC_{50}= 310), EtOAc (LC_{50}= 350), 70 % (LC_{50}= 380). Methanol extracts showed very week activity. According to the global guidelines, values above 1000 µg/ml are considered nontoxic, from 1000 to 500 µg/ml are weakly toxic, and that below 500 µg/ml are toxic [40].

4. HPLC-fingerprint analysis

HPLC-fingerprint analysis is a simple and quick technology widely used to identify the chemical components of plant extracts compared to standard materials [41-42]. Ethyl acetate and two fractions of n-butanol extracts [(I) and (III) &] of D. tortuosum leaves were subjected to chemical profiling through the HPLC-fingerprint technique to identify their chemical ingredients and to characterize the available classes of secondary metabolites [43]. In this study, fourteen standard phenolics were used as reference compounds, including gallic acid (1), chlorogenic acid (2), catechin (3), caffeic acid (4), syringic acid (5), rutin (6), ellagic acid (7), coumaric acid (8), vanillin (9), ferulic acid (10), naringenin (11), propyl gallate (12), quercetin (13), and cinnamic acid (14). The obtained results revealed the variable amounts of the above-mentioned standard compounds in the tested samples. Quercetin, syringic acid, rutin, and cinnamic acid were detected as the major ingredients. On the other hand, coumaric acid, ellagic acid, vanillin, and naringenin were detected as minor components in the ethyl acetate extract. Both chlorogenic acid and ferulic acid are absent. Moreover, quercetin, propyl gallate, cinnamic acid, and gallic acid were recognized as major constituents in fraction [III]. Caffeic acid, coumaric acid, vanillin, and ellagic acid were observed as minor ingredients in the n-butanol fraction [III], and the absence of catechin, rutin, ferulic acid, and naringenin (Table 6 and Figure 4). Also, quercetin, chlorogenic acid, naringenin, gallic acid, and rutin were detected as major constituents in the methanolic fraction [I], where cinnamic acid, ferulic acid, and coumaric acid were detected as minor components, and the absence of catechin and ellagic acid (Table 3 & Figure 4).

Numerous reports stated that there are strong positive relations between the amounts of phenolic compounds in the tested samples and their bioactivities. Due to the unique chemical skeleton and heavy hydroxylation patterns of phenolic compounds, these compounds act as potent free radical scavengers [43-49].

GC-MS analysis

The chemical composition of the ethyl acetate extract of Desmodium tortuosum indicates the presence of thirty-three compounds, representing 89.44% of the total extract composition. These compounds were identified qualitatively based on their retention times and mass spectral fragmentation patterns. They are mainly categorized into fifteen oxygenated compounds (45.4%) and eighteen non-oxygenated compounds (54.54%), classified into six groups: one saturated aliphatic compound (3%), three unsaturated aliphatic compounds (9%), seven oxygenated aliphatic compounds (21%), eighteen aromatic compounds attached to an aliphatic chain (54%), three sesquiterpene compounds (9%), and one anthraquinone compound (3%). The major identified compounds include Benzene (1-butyloctyl) (11.88%), Himachalene <α-> (11.08%), Benzene (1-pentyl heptyl) (9.96%), Undecanal, 3-phenyl (8.21%), Phenyl Ethyl Alcohol (7.43%), Benzene (1-ethyldecyl) (6.50%), Benzene (1-methyldecyl) (5.78%), and Benzyl Butyrate (5.31%).

Qualitative GC-MS analyses of the unsaponifiable matter of D. tortuosum resulted in the identification of thirty-two compounds (Table 5), representing 41.19% of the total extract composition. These compounds were identified qualitatively based on their retention times and mass spectral fragmentation patterns. They are categorized into twenty-seven oxygenated compounds (84%) and five deoxygenated compounds (15%). All compounds are classified into six groups: twenty-five oxygenated aliphatic compounds (78%), one saturated aliphatic compound (3%), one unsaturated aliphatic compound (3%), one flavonoid derivative compound (3%), one steroid derivative compound (3%), and three aromatic compounds conjugated with an aliphatic chain (9.3%). The major identified compounds include 10-Undecenoic acid, methyl ester (25.50%), 5,8-Octadecedienoic acid methyl ester (3.07%), and Nonadecanoic acid methyl ester (2.63%).

The data for both extracts indicate that D. tortuosum is rich in different types of aliphatic fatty acids. Most of the identified compounds are cited in the literature for their various biological activities. For example, 1-Octadecene has shown antibacterial, antioxidant [50], and anticancer effects [51]. Dodecanoic acid methyl ester exhibited antibacterial, antiviral, and antifungal properties [52], while 1-Hexadecene demonstrated antibacterial, antifungal [53-55], and antioxidant activity [56]. 9-Octadecenoic acid (Z) methyl ester has been reported for its antioxidant and anticancer effects [41-57]. The major identified compound Benzene (1-butyloctyl) has antimicrobial activity [58].

CONCLUSION

Different extracts of D. tortuosum were prepared and evaluated for their biological and chemical characteristics. Among all the tested extracts, the 90% methanol extract showed the highest antioxidant activity. On the other hand, the cytotoxic test showed weak toxic effects, with the most active extract being n-BuOH (LC_{50}= 310). Regarding its chemical content, HPLC-Fingerprint Analyses indicated the presence of phenolic compounds in both the ethyl acetate extract and the two butanol fractions, which are responsible for antioxidant activity. Additionally, GC-MS analysis determined the presence of many fatty acids and other aliphatic compounds with a variety of biological activities. The plant is considered a candidate for further chemical and pharmaceutical investigations.

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REFERENCES

1. Lewis G., Schrire B., Mackinder B., Lock M. Legumes of the World. Royal Botanic Gardens, Kew, Richmond, Surrey, UK. 2005.
2. Saraçoðlu H.T., Zengin G., Akin M., Aktümsek A. A comparative study on the fatty acid composition of the oils from five Bupleurum species collected from Turkey. *Turk. J. Biol.* 2012; 36: 527–532.
3. Baum S.J., Kris-etherton P.M., Willett W.C., Lchtenstein A.H., Rudel L.L., Maki K.C., whelon J., Ramsden T. Fatty acids in Cardiovascular health and disease: Acomparative update. *J. Clin. Phychiatry*. 2012; 6: 216-234.
4. Ghaffari MA, Chaudhry BA, Uzair M, Imran M and Ashfaq K. Total phenolic and flavonoid content, cytotoxic, immunomodulatory and anti-inflammatory potential of whole plant of *Astragalus criticus* (Fabaceae). Trop. J. Pharm. Res. 2021; 20: 2109-2115.
5. Ei-Hawiet AM, Toaima SM, Asaad AM, Radwan MM and EL-Sebakhy NA. Chemical constituents from *Astragalus annularis* Forssk and Atrimestris L. Fabaceae. Rev. bras. Farmacogn. 2010; 20(6): 1
6. Fofona S, Ouedraogo M, Esposito RC, Ouedraogo WP, Delporte C, Antwerpen PV, Mathieu V and Guissou TP. Systematic Review of Potential Anticancerous Activities of Erythrins senegalensis DC (Fabaceae). *Plants*. 2022; 11,19: 1-22.
7. Maroyi A. Medicinal uses of the Fabaceae Family in Zimbabwe: A Review. Plants. 2023, 12, 1255:1-26.
8. Mahbubr Rahman AHM *and* Parvin MIA Taxonomic studies on the Family Fabaceae (Weeds) at Rajshahi University Campus. *Plants*. 2015; 3(3): 20-25.
9. Tsafack B.T., Ponou B.K., Teponno R.B., Nono R.N., Jenett-Siems K., Melzig M.F., Park, HJ and Tapondjou LA, Integracide K. A New Tetracyclic Triterpenoid from Desmodium uncinatum (Jacq.) DC. (Fabaceae). *Nat. Prod. Sci*. 2017; 23(2): 113-118.
10. Cheng X., Guo C., Yang Q., Tang X., Zhang C. Isolation and identification of radical scavenging components of seeds of *Desmodium styracifolium*. *Chem. Nat. Compd*. 2017; 53 (1): 36-39.
11. Phan M.G., Phan T.S., Matsunami K., Otsuka H. Flavonoid compounds from *Desmodium styracifolium* of Vietnamese origin. Chem. Nat. Compd. 2010; 46(5): 797-798.
12. Taylor W.G., Sutherland D.H., Richards K.W. Soyasaponins and Related Glycosides of *Desmodium canadense* and *Desmodium illinoense.* Nat. Prod. J. 2009; 2: 59-67.
13. Ma X., Zheng C., Hu C., Rahmam K. and Qin L. The genus *Desmodium* (Fabaceae)-traditional uses in Chinese medicine. Phytochem and Pharmaco. J Ethnopharmacol. 2011; 138: 314-332.
14. Rastogi, S., Pandey, M.M. and Rawat, A.K.S. An 2011 Ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and Desmodium adscendens (Sw.) DC. J. Ethnopharmacol. 136: 283–296.
15. Hamidi M.R., Jovanova B. and Panovska T.K. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. Macedonian Pharm. Bull. 2014; 60 (1): 9-18.
16. Kemal M.E., Bakchiche B., Kemal M., Cheraif K., Kara Y., Bardaweel S.K., Miguel M.G., Yildiz O. and Ghareeb M.A. Six Algerian plants: Phenolic profile, antioxidant, antimicrobial activities associated with different simulated gastrointestinal digestion phases and antiproliferative properties. *J. Herb. Med*. 2023; 38: 100636.
17. Manorama R., Chinnasamy N., Rukmini C. Multigeneration studies on red palm oil, and on hydrogenated vegetable oil containing Mahua oil. Food Chem. *Toxicol*. 1993; 31: 369–375.

18. Schlag S, Huang Y, Vetter W. GC/EI-MS method for the determination of phytosterols in vegetable oils. *Anal Bioanal Chem*. 2022; 414(2):1061-1071.
19. Dicko M.H., Hilhorst R., Gruppen H., Traore A.S., van Berkel W.J.H., Voragen A.G.J. Comparison of Content in Phenolic Compounds, Polyphenol Oxidase, and Peroxidase in Grains of Fifty *Sorghum* Varieties from Burkina Faso. J Agric. *Food Chem*. 2002; 50: 3780–3788.
20. Marwah R.G., Fatope M.A., Al Mahrooqi R., Varma G.B., Al Abadi H. and Al burtamani S. Antioxidant capacity of some edible and wound healing plants in Oman. *Food Chem*. 2007; 101(2):465-470.
21. Clarke G., Ting K.N., Fry J. High correlation of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants* 2013; 2: 1-10.
22. Ipsen J., Feigi P. Bancroft's Introduction to Biostatistics. 2nd ed. Harper & Row. New York, 1970; Chapter 15.
23. Miya T.S., Holck H.G.O., Yim G.K.W., Mennear J.H., Spratto G.R. 1973 Laboratory Guidein In: Pharmacology. 4th ed. Burgess Publishing, Minneapolis; 1237.
24. Abd El-Rahman A.A.A., Abd El-Aleem I.M., Refahy L.A. and El-Shazly M.A. Total phenolic content, cytotoxic and antioxidant activities of *Quisqualis indica* (Linn.) growing in Egypt. Der Pharma Chem. 2016; 8(3):53-59.
25. Abdel-Wareth M.T.A., Ghareeb M.A. Bioprospecting certain freshwater-derived fungi for phenolic compounds with special emphasis on antimicrobial and larvicidal activity of methyl gallate and p-coumaric acid. *Egypt. J. Chem*. 2018; 61(5): 500-510.
26. Nasr S.M., Ghareeb M.A., Mohamed M.A., Elwan N.M., Abdel-Aziz A.A., Abdel-Aziz M.S. HPLC-fingerprint analyses, *in vitro* cytotoxicity, antimicrobial and antioxidant activities of the extracts of two *Cestrum* species growing in Egypt. *Pharmacognosy Res*. 2018; 10(2): 173-180.
27. Shawky B.T., Nagah M., Ghareeb M.A., El-Sherbiny G.M., Moghannem S.A.M., and Abdel-Aziz M.S. Evaluation of antioxidants, total phenolics and antimicrobial activities of ethyl acetate extracts from Fungi grown on rice straw. J. Renew. *Mater*. 2019; 7(7): 667-682.
28. Khalaf O.M., Abdel-Aziz M.S., El-Hagrassi A.M., Osman A.F., Ghareeb M.A. Biochemical aspect, antimicrobial and antioxidant activities of *Melaleuca* and *Syzygium* species (Myrtaceae) grown in Egypt. *J. Phys. Conf. Ser*. 2021; 1879(2): 022062.
29. Litchfield C. Analysis of Triglycerides. Academic Press. New York & London. 1972 p.32.
30. Adams R.P. Identification of the essential oils by ion trap mass spectrometry. Academic press INC, London 19891-310.
31. Finney D.J. Estimation of the median effective dose. In: Probit Analysis. 3rd ed. Great Britain: Cambridge University; 1971; 20-49.
32. Clarke G., Ting K.N., Fry J. High correlation of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants*. 2013; 2: 1-10.
33. Ghareeb M.A., Saad A.M., Ahmed W.S., Refahy L.A., Nasr S.M. HPLC-DAD-ESI-MS/MS characterization of bioactive secondary metabolites from *Strelitzia nicolai* leaf extracts and their antioxidant and anticancer activities *in vitro*. *Pharmacogn. Res*. 2018a; 10(4): 368-378.

34. Ghareeb M.A., Mohamed T., Saad A.M., Refahy L.A., Sobeh M. and Wink M. HPLC-DAD-ESI-MS/MS analysis of fruits from *Firmiana simplex* (L.) and evaluation of their antioxidant and antigenotoxic properties. *J. Pharm. Pharmacol*. 2018b; 70(1): 133–142.
35. El-Sayed M.M., El-Hashash M.M., El-Wakil E.A., Ghareeb M.A. Total phenolic contents and antioxidant activities of Ficus sycomorus and Azadirachta indica. *Pharmacology online*. 2009; 3: 590-602.
36. El-Sayed M.M., Mahmoud M.A., El-Nahas H.A., El-Toumy S.A., El-Wakil E.A., Ghareeb M.A. Bio-guided isolation and structure elucidation of antioxidant compounds from the leaves of *Ficus sycomorus*. *Pharmacology online*. 2010; 3: 317-332.
37. Zahin M., Farrukh A., Iqbal A. Broad spectrum antimutagenic activity of antioxidant active fraction of *Punica granatum* L. peel extracts. Mutat. Res. 2010; 703: 99-107.
38. Zaki S.A., Somia H.A., Nehal R.A., Ferial A.I. Phenolic compounds and antioxidant activities of pomegranate peels. Int. J. Food Eng. 2015; 1: 73-76.
39. Sam T.W. Toxicity testing using the Brine shrimp: Artemia salina. In: Bioactive Natural Products Detection Isolation and Structural Determination. S.M. Colegate and R.J. Molyneux (eds.), (18thed), Boca Raton, CRC Press. 1993; 441-456.
40. Refahy L.A., Farghaly T.A., Abdel-Aziz M.S. and Mohamed T. Antimicrobial, Antioxidant and Cytotoxic Potential of *Caesalpinia pulcherrima* Flower. *Glob. J. Pharmacol*. 2015; (2): 150-158.
41. Di X., Chan K.K., Leung H.W., Huie C.W. Fingerprint profiling of acid hydrolyzates of polysaccharides extracted from the fruiting bodies and spores of lingzhi by high performance thin layer chromatography. *J Chromatogr A*. 2003; 1018(1): 85-95.
42. Wang L., Tian X., Wei W., Chen G., Wu Z. Fingerprint analysis and quality consistency evaluation of flavonoid compounds for fermented *Guava* leaf by combining high-performance liquid chromatography time-of-flight electrospray ionization mass spectrometry and chemometric methods. *J Sep Sci*. 2016; 39: 3906-16.
43. Ghareeb M.A., Sobeh M., Rezq S., El-Shazly A.M., Mahmoud M.F., Wink M. HPLC-ESI-MS/MS profiling of polyphenolics of a leaf extract from *Alpinia zerumbet* (Zingiberaceae) and its anti-inflammatory, anti-nociceptive, and antipyretic activities *in vivo*. Molecules 2018c; 23: 3238.
44. Rice-Evans C.A., Miller N.J., Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med*. 1996; 20: 933-56.
45. Rice-Evans C., Miller N., Paganga G. Antioxidant properties of phenolic compounds. *Trend Plant Sci*. 1997; 2: 152-9.
46. Dilshad R. and Batool R. Antibacterial and antioxidant potential of Ziziphus jujube, Fagonia arabica, Mallotus phillipensis and Hemidesmus indicus. *Jordan Journal of Pharmaceutical Sciences*. 2022; 15(3): 413-427.
47. Ghareeb MA, Sobeh M, Aboushousha T, Esmat M, Mohammed HS, El-Wakil ES. Polyphenolic profile of *Herniaria hemistemon* aerial parts extract and assessment of its anti-cryptosporidiosis in a murine model: *In silico* supported *in vivo* study. *Pharmaceutics*. 2023; 15: 415.
48. Teggar N, Bakchiche B, Abdel-Aziz ME, Bardaweel SK, Ghareeb MA. Chemical composition and biological evaluation of Algerian propolis from six different regions. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(2): 184-197.
49. Naskar A., Dasgupta A. and Acharya K. Antioxidant and cytotoxic activity of Lentinus fasciatus. *Jordan Journal of Pharmaceutical Sciences*. 2023; 16(1): 72-81.

50. Mishra P.M., Sree A. Antibacterial Activity and GCMS Analysis of the Extract of Leaves of Finlaysonia obovata (A Mangrove Plant). *Asian J. Plant Sci*. 2007; 6: 168-172.
51. Lee Y.S., Kang M.H., Cho Y.S., Jeong C.S. Effects of constituents of Amomum xanthioides on gastritis in rats and on growth of gastric cancer cells. *Arch. Pharm. Res*. 2007; 30: 436.
52. Özçelik B., Aslan M., Orhan I., Karaoglu T. Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera*. Microbiol. Res. 2005; 160(2): 159-64.
53. Hsouna A.B., Trigie, M., Mansour R.B., Jarraya R.M., Damak M., Jaoua, S. Chemical composition, cytotoxicity effect and antimicrobial activity of Ceratonia siliqua essential oil with preservative effects against Listeria inoculated in minced beef meat. *Inter. J. Food Microbiol*. 2011; 148(2): 66-72.
54. Yogeswari S., Ramalakshmi S.N., Muthu J.M. Antimicrobial and Antioxidant Properties of a Bacterial Endophyte, Methylobacterium radiotolerans MAMP 4754, Isolated from Combretum erythrophyllum Seeds. *Global J. Pharmacol*. 2012; 6: 65.
55. Mou, Y., Meng, J., Fu, X., Wang, X., Tian, J., Wang, M., Peng, Y., Zhou. L. Antimicrobial and Antioxidant Activities and Effect of 1-Hexadecene Addition on Palmarumycin C2 and C3 Yields in Liquid Culture of Endophytic Fungus Berkleasmium sp. Dzf12. *Molecules*. 2013; 18: 15587.
56. Syeda F.A, Habib-ur-Rahman A.M. Khan, Choudahry M.I., Atta-Ur-Rahman. Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bioassays of crude extract of *Iris germanica*. *Inter. J. Genetics Mol. Biol*. 2011; 3: 95.
57. Hema, R., Kumaravel, S., Alagusundaram. GC/MS Determination of Bioactive Components of Murraya koenigii. *J. Am. Sci*. 2011; 7: 27.
58. Rastogi, S., Pandey, MM and Rawat, AKS. An Ethnomedicinal, phytochemical and pharmacological profile of Desmodium gangeticum (L.) DC. and Desmodium adscendens (Sw.) DC. *J. Ethnopharmacol*. 2011; 136(2): 283-296.

السمية الخلوية، الأنشطة المضادة للأكسدة، تحليلات GC / MS وHPLC لمستخلصات ديسموديوم تورتوسوم DC (Sw.)

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2 قسم النبات، كلية العلوم، جامعة أسوان، مصر.

ملخص

تعد عائلة Fabaceae ثالث أكبر عائلة نباتية مزهرة، وقد أظهر جنس ديسموديوم مدى واسعًا في الأنشطة البيولوجية ومجموعة متنوعة من المكونات الكيميائية. تم تقييم انشتطها السامة. فى الدراسة الحالية ل ديسموديوم تورتوسوم تم اختبار مستخلصات مختلفة منه لتقييم انشتطها السامة للخلايا وكمضادات اكسدة وتقدير المحتوى الفينولى الكلى. تم إجراء الأنشطة المضادة للأكسدة بشكل كمي باستخدام الشقوق الحرة 1.1'- ثنائي فينيل -2-بيكرايل هيدرازيل (DPPH) ولقد تم تقييم النشاط السام للخلايا عن طريق اختبار اماتة يرقات الجمبري الملحي .(BSLT) أظهرت نتائج النشاط المضاد للأكسدة أن فعالية الكسح الجذري لـ DPPH (SC50) تراوحت من 1.12 إلى 61.22 ميكروغرام / مل بالمقارنة بحمض الأسكوربيك (SC50 = 7.45 ميكروغرام / مل)، من بين جميع الأجزاء المختبرة وجد أن 90٪ الميثانول هو الأكثر نشاطًا. من ناحية أخرى ، وجد أن الأنشطة السامة لمستخلص البيوتانول على الخلايا هو الأكثر سمية بالمقارنة بالمستخلصات الأخرى. تم إجراء تحليلات كروماتوجرافيا سائلة عالية الأداء (HPLC-fingerprint) لتقدير التركيب الكيميائي ونسبة المركبات الفينولية ، وقد حدد تحليل كروماتوغرافيا الغاز – مطياف الكتلة (GC-MS) وجود الأحماض الدهنية والمركبات الأخرى. المركبات الرئيسية التي تم تحديدها هي البنزين (1-بيوتيلوكتيل) (11.88٪) وهيماشالين <11.08) <-α ٪ (لمستخلص أسيتات الإيثيل و 10-حمض أونديسينويك ، إستر الميثيل (25.50٪)، للمواد غير السائلة.

الكلمات الدالة: مضادات الأكسدة، السمية الخلوية، ديسموديوم تورتوسوم، كروماتوجرافيا الغاز، مطياف الكتلة، تحليلات كروماتوجرافيا سائلة عالية الأداء.

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