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Bioactivities and Phytochemical Studies of *Acrocarpus fraxinifolius* Bark Wight Arn

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ACROCARPUS is a genus of flowering plants in the legume family Fabaceae which considered as a large and economically important family. This study aimed to carry out the biological activity screening on the total ethanol and successive extracts of *Acrocarpus fraxinofolius (A. fraxinofolius)* bark, for the first time. The biological activity studied embraced, management of diabetes in alloxan induced diabetic rats, cytotoxic activity against four human tumor cell lines and hepatoprotective activity against CCl_4-induced hepatotoxicity in rats and the activity was studied by assaying the serum marker enzymes like AST, ALT, and ALP. Concerning this, the petroleum ether extract (PEE) showed the most bioactive extract where, the anti-diabetic activity exhibited by 100mg of PEE extract was 74.38% relative to metformin. It also showed a significant anti-proliferative activity against MCF-7 (IC_{50}=2.35µg), Hela (IC_{50}=3.85µg) and HEPG-2 (IC_{50}=9.54µg) compared with Doxorubicin as reference drug. The hepatoprotective activity of the PEE was evidenced by a significant decrease in the liver function enzymes, i.e. AST, ALT and ALP by 29.18%, 28.26%, and 34.11%, respectively, using silymarin as the reference drug, compared to their concentration levels in an untreated group with liver damage induced by CCl_4. Based on the above outcomes, further phytochemical investigation including GC/MS analysis of its fractions, GLC analysis of its sterol fraction, column chromatography and TLC fractionation of PEE to separate its bioactive compounds were conducted.

Keywords: *Acrocarpus fraxinifolius*, Antidiabetic, Column chromatography, Cytotoxic, Gas chromatography–mass spectrometry, Gas-liquid chromatography, Hepatoprotective.

Introduction

The Fabaceae (old name Leguminosae) or bean and pea family, is the third largest family of angiosperms after Asteraceae (sunflowers) and Orchidaceae (orchids), and second only to Poaceae (grasses) in terms of economic and agricultural importance. Legumes constitutes many naturalized species harvested as crops for human and animal utilization as well as for fiber, fuel, oils, fertilizers, timber. Legumes are used traditionally in folk medicines [1], but also indicate significance in modern medicine. The legume family, Fabaceae, contain a genus known as *Acrocarpus* which includes two tree species. It belongs to the subfamily Caesalpinioideae.

Nowadays medicinal products from plant origin are safe in contrast to the synthetics that are considered as unsafe to environment and human. The trend of utilization of natural products has increased [2-5]. Diabetes can cause serious problems. It can damage your eyes, kidneys, and nerves, therefore, the search for naturally potent and healthy hypoglycemic agent has become an area of research. It was reported that plants from the family Fabaceae hold definite promise in the management of Diabetes mellitus [6-10]. Cancer also is the second leading cause of death, the incidence of various forms of cancer is now rapidly rising worldwide, herbal remedies have been used to cure cancer. Utilization of plant phytoconstituents in the treatment of cancer has been of recent interest [11]. Medicinal plants, the promising sources for biologically active compounds having anticancer properties, the goals of using them as sources of therapeutic agents and their role in the discovery and development

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of conventional drugs for the treatment of cancer were reviewed [12-16]. Plants of the family Fabaceae are rich in phytoconstituents; hence, they were effective therapeutic agents for numerous diseases specially as cancer chemo preventive [17,18].

Development of effective and side effects lacking hepatotoxic therapy is the aiming research direction in healthcare pharmacy. The use of natural remedies from medicinal plants for the treatment of liver diseases has a long history and plants of the family Fabaceae were best examples [18-22].

Concerning the above into account, the main goal of this work is to carry out the biological activity screening on the total ethanol and successive extracts of the bark including, management of diabetes in alloxan induced diabetic rats, cytotoxic activity against four human tumor cell lines {cervix carcinoma cell line[HELA], breast carcinoma cell line, liver carcinoma cell line[HEPG-2], colon carcinoma cell line[HCT-116]} and hepatoprotective activity against CCl_4-induced hepatotoxicity in rats and the activity was studied by assaying the serum marker enzymes like AST, ALT, and ALP. The results obtained will determine the most active extract that will further be fractionated and subjected to phytochemical study to isolate, purify and identify the bioactive compounds from this extract. To our knowledge, the current work was performed for the first time on *A. fraxinofolius* bark.

Material and Methods

Plant collection

The *A. fraxinofolius* bark was collected from Zoo garden, and authenticated by Mrs. Treasa Labib, Plant Taxonomy Consultant at the Ministry of Agriculture and former director of El-Orman Botanical Garden, Giza, Egypt.

Preparation of total ethanol and successive extracts

One Kg of the powdered plant was exhaustively extracted by ethanol, the extract was evaporated to dryness (total ethanol extract).

Five hundred gm of the powdered plant was successively extracted in a continuous extraction apparatus, with the following successive solvents with increasing polarities: petroleum ether, chloroform and ethyl acetate. After each complete extraction with one solvent, the powdered plant was dried and extracted with the next solvent. All extracts were separately evaporated to dryness.

Bioactivity studies

Experimental animals and diet

Animals, adult albino rats, of Sprauge Dawely Strain weighing 130-150 g and Albino mice weighing 25-30 g. Animals were obtained from the animal house colony of the National Research Centre, Dokki, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water. Normal diet, it consisted of vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5% and starch 54.3%. Doses of drugs, doses of the drugs were calculated according to the previous method [23] and were administered orally by a gastric tube.

Determination of antidiabetic activity

Anti-diabetic activity of total ethanol and successive extracts was carried out according to Trinder method 1969[24], using metformin as standard drug.

Alloxan –induced diabetes in rats

Induction of diabetes mellitus in rats was done using intraperitoneal injection of a single dose of alloxan (150 mg / kg body wt.). Diabetes was confirmed by determining the blood glucose levels. Rats with blood glucose levels between 200 and 400 mg/dl were considered diabetics and were employed in the study.

Investigation of anti-diabetic effect of the different extracts

Six groups of rats, each consisted of 10 rats were used in this experiment. A group consisted of untreated diabetic rats considered as a positive control. The second to fifth groups were diabetic rats treated with an oral daily dose of 100mg/kg of each extract for four weeks. The last group consisted of diabetic rats treated with reference drug, metformin in a dose of 100 mg/kg body wt. daily for four weeks. Glucose levels will be measured at 0 time, 2 and 4 weeks after administration.

Determination of blood glucose concentration

Blood glucose was determined at 0 time, 2 and 4 weeks after administration of the extracts or reference drug, blood samples were collected from the retro orbital venous plexus through the canthus of anaesthetized rats after an overnight fasting and serum was isolated by centrifugation. Glucose was determined enzymatically according to the method described by Trinder 1969 [24].

Hepatoprotective activity test

Induction of liver damage

Induction of liver damage in rats was induced according to the method of Klassen and Plaa 1969 [25] by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride in liquid paraffin.

Experimental design

Six groups of male Albino rats, each of six rats were divided as follows: First group: the control group received a daily oral dose of 1 ml saline for 7 days before and after liver damage. Four groups were treated with a daily oral dose of 100 mg/kg b. wt. of the total ethanol and successive extracts for 7 days before and after liver damage. The last group: was treated with a daily oral dose of 25 mg/kg b.wt. silymarin as a standard for 7 days before and after liver damage. Followed by overnight fast, whole blood was obtained from the retro orbital venous plexus through the eye canthus of anesthetized rats. Blood samples were collected at zero time, 7 days before and after CCl_4 injection, 72 hours after CCl_4 injection. Serum was isolated by centrifugation. Serum AST, ALT [26], and ALP [27] were measured. Results of biological activity tests were statistically analyzed by the Studen's *'t'* test.

Cytotoxicity test

Cytotoxic effect was accomplished on four human tumor cell lines (Cervix carcinoma cell line[HELA], Breast carcinoma cell line [MCF-7], Liver carcinoma cell line[HEPG-2] and Colon carcinoma cell line[HCT-116] using SulphoRhodamine-B (SRB) method [28].

The bioactivity studies revealed that the most active extract was PEE that further fractionated and subjected to the flowing phytochemical studies:

Phytochemical studies

Saponification of PEE [29].

Five grams of the residue of PEE were refluxed for 6 hrs. with 0.5 N alcoholic KOH (100 ml) in a boiling water bath. The saponified extract was concentrated to 1/3 its volume. The cooled reaction mixture was diluted with an equal volume of distilled water and exhaustively extracted with ether (till negative test for sterols). The combined ethereal extract was washed several times with water till free from alkalinity and dehydrated over anhydrous sodium sulphate. After evaporation of ether to dryness, the residue (unsaponifiable matter, USM) was kept for analysis *via* GC/MS. The aqueous alkaline solution remaining after extraction of the unsaponifiable matter was acidified with hydrochloric acid to liberate the fatty acids which were extracted several times with ether. The combined ethereal extract was washed several times with distilled water till free from acidity, and then filtered over anhydrous sodium sulphate. The filtrate was evaporated to dryness.

Preparation of fatty acids methyl ester [29].

The residue of fatty acids obtained was dissolved in 50 ml absolute methanol, mixed with 0.25 ml sulphuric acid, refluxed for about three hrs., cooled, diluted with about 100 ml distilled water and transferred to a separating funnel. The resulting fatty acid methyl esters were extracted several times with ether. The combined ethereal extract was washed several times with water until free from acidity and dehydrated over anhydrous sodium sulphate. The solvent was evaporated and the residue (fatty acids methyl ester, FAME) was kept for GC/MS analysis.

GC/MS analysis

The FAME and USM fractions were subjected to GC/MS analysis adopting the following conditions: Capillary column of fused silica (5% phenyl methyl polysiloxane), 30m length, 0.25mm I.D. and 0.25 µm thickness, DB-5, carrier gas helium at 13 psi; oven temperature 50-280^{o}C at a rate of 5^{o}C/min for USM and FAME; ion source temperature 220^{o}C; ionization voltage 70ev; accelerated voltage 2000 v; volume injected 1µl. The identification of the compounds was accomplished by comparing their retention times and mass spectral data with those of the library (Wiley Int. USA) and NIST (Nat. Inst. St. Technol., USA) and/or published data [30].

VLC chromatography

Twenty grams of the petroleum ether extract were subjected to fractionation on VLC column using silica gel (G 60 F 254) (Merck , Darmstadt, Germany) as the stationary phase and petroleum ether (60-80) as the mobile phase. Fifty fractions were collected and monitored by TLC using the solvent system: benzene-acetone (9:1) and spraying with 10% H_2SO4 and heating at 110°C. Similar fractions were pooled and subjected to preparative TLC using the same solvent system. Three bands were separately scratched and eluted by chloroform, having the following R_f values and colours: 0.5 (pink), 0.61 (brown), 0.66 (faint brown) corresponding to the sterol fraction and two triterpenoid compounds 1&2, respectively.

Conditions of GLC analysis of the sterol band

Column: capillary column HP-5, (5% Phenyl Methyl Siloxane), 30 m length, 0.32 mm id and 0.25µm film thickness, initial temp.:80°C, initial time: 1.00 min. rate: 8°C/min, final temperature: 300°C, inlet temp.: 250°C, detector: 300°C (FID), flow: 2 ml/min, carrier gas: N2 30 ml/min, H2 30 ml/min, Air 300 ml/min.

Results and Discussion

Bioassay guided fraction is a procedure whereby each successive extract of the studied plant is evaluated in a bioassay system and only active extract is further fractionated and chemically investigated for its bioactive compounds. In our work the results of the screening assay showed that the PEE possessed potent cytotoxic, antidiabetic and hepatoprotective activities as follows:

Anti-diabetic activity

The specified extract has shown a remarkable anti-diabetic activity at 100mg after four weeks of treatment as revealed by the significant decrease in blood glucose level by 41.61% with potency of 74.38% compared to untreated diabetic group using metformin as reference drug, as illustrated in Table 1. These results are consistent with the reported activity for the identified major compounds *via* GC/MS of unsaponifiable matter content including ß-sitosterol [31, 32].

TABLE 1: Effect of total ethanol and successive extracts of *A. fraxinofolius* bark (100mg/kg body wt.) on blood glucose level in Diabetic male albino rats n=6

Diabetic rats groups	Zero Mean±S.E	2W Mean±S.E	4W Mean±S.E	% Of change	Potency%
untreated (control)	249.8±7.2	258.3±7.6	266.7±8.9	-	-
treated with total ethanol	265.2±9.1	220.5±7.4*	160.3±2.9*	40.32	73.34
treated with petroleum ether	261.4±9.1	213.5±8.2*	152.6±5.4*	41.61	74.38
treated with chloroform	261.1±9.5	229.6±8.7*	192.9±7.6*	26.12	46.69
treated with ethyl acetate	258.3±9.5	209.7±8.6*	175.3±5.4*	39.61	60.11
treated with metformin	266.9±8.2	194.8±7.6*	117.6±5.8*	55.94	100

* indicates statistical significance (P < 0.05).

Hepatoprotective activity

Similarly, the bioactivity guided fractionation of *A. fraxinifolius* bark revealed that PEE, enriched with phytosterols, has a potent hepatoprotective effect as evidenced by significant decrease in liver function enzymes, *i.e.* AST, ALT and ALP by (29.18%, 28.26% and 34.11%, respectively) using silymarin as reference drug, compared to their concentration levels in untreated group with liver damage induced after 72 h of CCl_4 administration as shown in Figures 1-3.

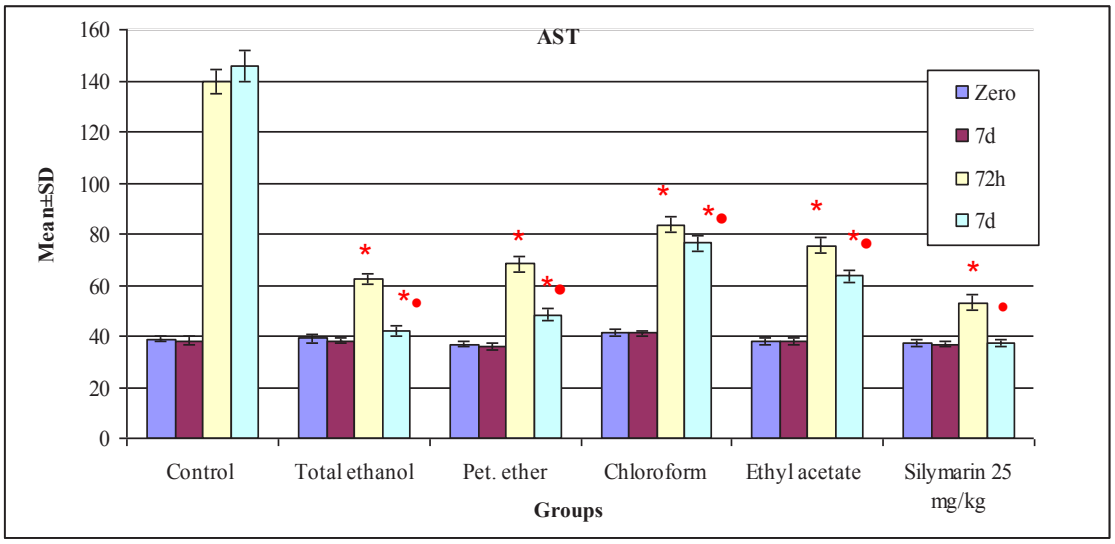

Fig. 1: Histogram of the effect of total ethanol and successive extract of A. fraxinofolius bark on serum enzyme level AST in liver damaged rats

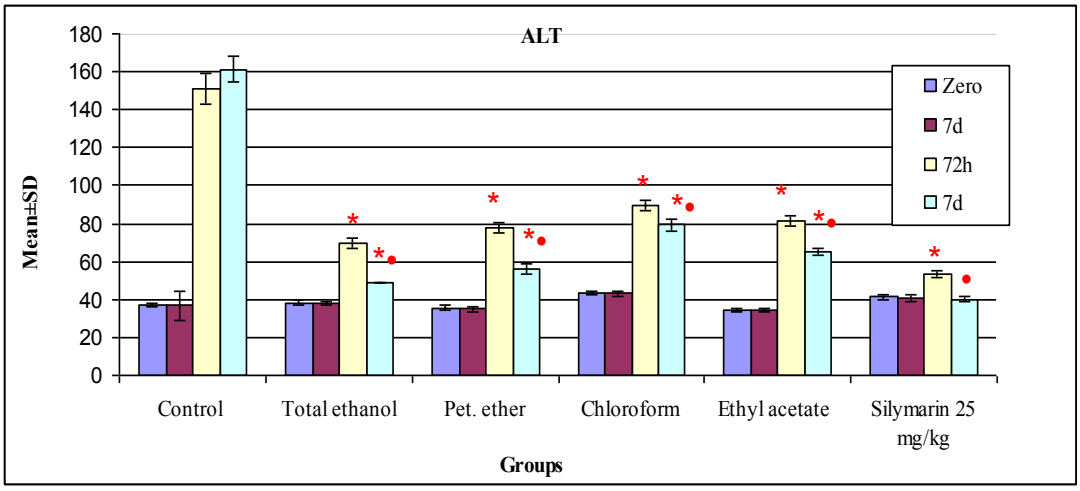

Fig. 2: Histogram of the effect of total ethanol and successive extract of A. fraxinofolius bark on serum enzyme level ALT in liver damaged rats

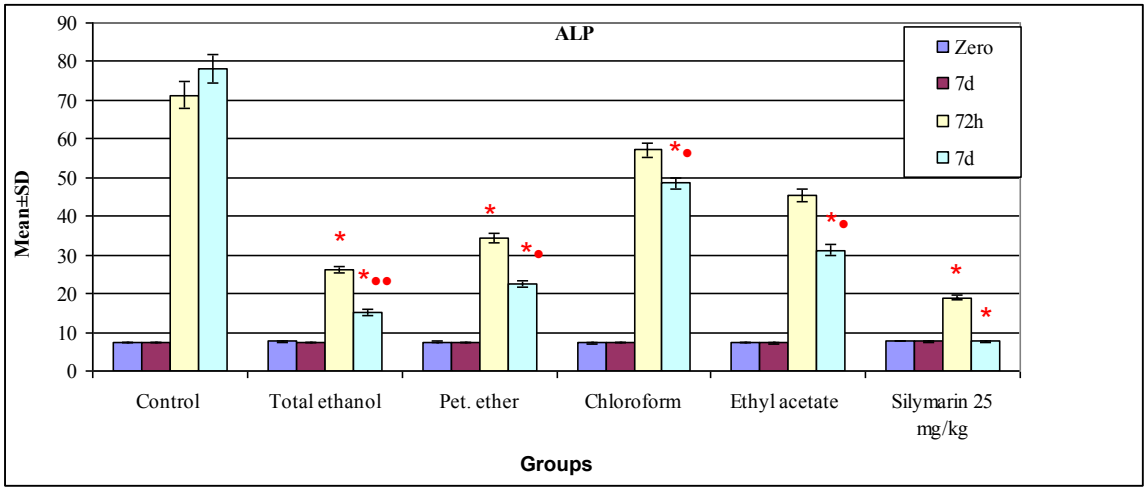

Fig. 3: Histogram of the effect of total ethanol and successive extract of A. fraxinofolius bark on serum enzyme level ALP in liver damaged rats

Cytotoxic activity

Cytotoxic activities of successive extracts of the plant showed variable activities where the PEE showed the most potential cytotoxicity against MCF-7 (IC_{50}=2.35µg), Hela (IC_{50}=3.85µg) and HEPG-2 (IC_{50}= 9.54µg) as depicted in Table 2. Since plant extracts are considered active as anti-cancer agents at $IC_{50} \leq 30$ µg/mL (Suffness and Pezzuto 1990), thus, petroleum ether extract of *A. fraxinifolius* bark can be considered as a very potent anti-proliferative agent that should be recommended for further clinical studies. These results are consistent with the previously reported studies of its main active constituents including cycloeucalenol and obtusifoliol [33-36].

Thence, the bioactivity guided fractionation for

TABLE 2: IC_{50} (µg/mL) of total ethanol and successive extracts of *A. fraxinofolius* bark on different tumor cell lines

Tumor cell line	Total ethanol	Pet. ether	Chloroform	Ethyl acetate	Dox.
HELA	108.67	3.85	0.495	20.234	2.7 ± 2.4
HEPG2	50.05	9.54	45.32	66.93	2.3 ± 1.2
MCF-7	26.56	2.35	140.98	37.72	3.7 ± 2.0
HCT116	6.97	78.03	59.81	284.41	12.7 ± 2.8

the bark successive extracts revealed that the PEE possessed significant antidiabetic activity, powerful hepatoprotective effect and potent cytotoxic activity, compared to the other successive extracts, consequently, we encouraged to further fractionate the PEE and phytochemically investigate these fractions and the results were as follows:

Investigation of petroleum ether extract

Table 3 illustrated GC/MS analysis of the unsaponifiable matter of *A. fraxinifolius* bark which revealed its enrichment with a variety of characteristic triterpenes and sterols as major compounds. Where 52 compounds were identified constituting 97.03% of the total composition. Cycloeucalenol was found to be the major compound representing 32.52% followed by obtusifoliol (26.50%), ß-Sitosterol (13.74%), 4α-metylfecosterol (5.42%), n-nonacosane (4.91%). Twelve phenyl hydrocarbons were detected representing (1.29%). Phytosterols exist in all foods of plant origin and are generally reported to have several bioactive properties [37]. They contribute to lower serum cholesterol levels and are also considered to have anti-inflammatory, anti-bacterial, anti-atherosclerotic, anti-oxidative, anti-ulcerative, anti-tumor properties in humans [38-40]. Cycloeucalenol, obtusifoliol and 4α-methylfecosterol, are important bioactive secondary metabolites due to the wide range of their biological activities. They show mainly antimicrobial, cytotoxic, antitumoral, anti-viral, anti-inflammatory, hepatoprotective and insecticidal activities [41]. Thus, PEE has shown a remarkable anti-diabetic activity as mentioned previously.

On the other hand, GC/MS analysis of the fatty acid methyl esters depicted in Table 4 revealed the identification of 33 fatty acids representing 90.71% of the total fatty acid constituents. Methyl-9,12-octadecadienoate (40.39%), methyl hexadecanoate (23.64%), dimethyl hexadecandioate (3.96%), methyl hexacosanoate (3.76%) were found to be the major compounds. The percent identified for the total unsaturated fatty acids were 41.9%. In addition, about 7% of dicarboxylic acids were identified, dicarboxylic acids have been proved to be safe in both experimental animals and humans, and their use has recently been proposed in diabetes due to their effect in improving glucose metabolism in type 2 diabetes [42]. Figure 4a and 4b showed structures of the major fatty acid methyl esters and the major identified sterols, respectively.

Compounds isolated from the petroleum ether extract

Column fractionation of the petroleum ether extract of *A. fraxinifolius* bark revealed the identification of the following:

Sterol fraction

GLC analysis of the sterol fraction revealed that it consisted of a mixture of cholesterol (7.22%), campesterol (13.30%), stigmasterol (10.00%) and β-sitosterol (69.48%). These sterols have strong protection against carbon tetrachloride hepatotoxicity, as previously reported by Wong *et al*., 2014[43].

Compound 1

IR υ max (KBr) cm^{-1}: 3402 (OH), 2920 & 2853 (CH, CH_2, CH_3), 1462 (CH_3), 1735 (C=O), 1652, 1380 (gem dimethyl), 1240, 885 (terminal CH_2), 842, 688, 617.

EIMS, *m/z* (rel. int.): 440 $[M]^+$ (35), 425$[M-Me]^+$ (47), 422 $[M-H_2O]^+$ (100), 407 (95), 379 (77), 300(58), 407 (94), 259 (30) 203 (25), 175 (30), 147 (38), 135 (48), 121 (44), 107 (58), 95 (68), 69 (85).

Based on the above data, compound 1(Fig. 5a) is identified as salacianol [21β-hydroxylup-20(29)-en-3-one] which are coincident with those reported for the same compound isolated from *Salacia beddomei* bark [44].

Compound 2

Isolated as white crystals (10 mg) (Fig. 5b). It was identified as β-amyrin as compared by authentic sample on TLC plates (both have identical R_f values in different solvent systems and same colour with sulphuric acid).

TABLE 3: GC/MS of unsaponifiable matter of *A. fraxinifolius* bark

No.	Name of compound	Rt	BP	M^+	MF	Area%
1	n-Tridecane	15.81	57	184	$C_{13}H_{28}$	0.04
2	1-Tetradecene	18.37	55	196	$C_{14}H_{28}$	0.36
3	n-Pentadecane	21.18	57	212	$C_{15}H_{23}$	0.03
4	Butylated hydroxyl toluene	21.62	205	220	$C_{11}H_{16}O_2$	0.46
5	4-Phenyl-decane	22.39	91	218	$C_{16}H_{26}$	0.07
6	1-Hexadecene	23.51	55	224	$C_{16}H_{32}$	0.81
7	n-Hexadecane	23.65	57	226	$C_{16}H_{34}$	0.04
8	6-Phenyl undecane	24.48	91	232	$C_{17}H_{28}$	0.04
9	5-Phenyl undecane	24.56	91	232	$C_{17}H_{28}$	0.09
10	4-Phenyl undecane	24.79	91	232	$C_{17}H_{28}$	0.08
11	3-Phenyl undecane	25.27	91	232	$C_{17}H_{28}$	0.06
12	1-Heptadecene	25.64	55	238	$C_{17}H_{34}$	0.04
13	n-Heptadecane	26.00	57	240	$C_{17}H_{36}$	0.05
14	2-Phenyl undecane	26.14	105	232	$C_{17}H_{28}$	0.09
15	6-Phenyl dodecane	26.73	91	246	$C_{18}H_{30}$	0.13
16	5-phenyl dodecane	26.84	91	246	$C_{18}H_{30}$	0.09
17	4-Phenyl dodecane	27.11	91	246	$C_{18}H_{30}$	0.06
18	3-Phenyl dodecane	27.58	91	246	$C_{18}H_{30}$	0.35
19	1-Octadecene	28.12	55	252	$C_{18}H_{36}$	0.89
20	n- Octadecane	28.24	57	254	$C_{18}H_{38}$	0.07
21	2-Phenyl dodecane	28.44	105	246	$C_{18}H_{30}$	0.11
22	6-Phenyl tridecane	28.90	91	260	$C_{19}H_{32}$	0.12
23	6,10,14- trimethyl-2-pentadecanone	29.28	43	268	$C_{18}H_{36}O$	0.22
24	1-Nonadecene	30.07	55	266	$C_{19}H_{38}$	0.07
25	n-Nonadecane	30.36	57	268	$C_{19}H_{40}$	0.05
26	1-Eicosene	32.29	55	280	$C_{20}H_{40}$	0.55
27	n- Eicosane	32.39	57	282	$C_{20}H_{42}$	0.08
28	Kaur-16-ene	33.41	257	272	$C_{20}H_{32}$	0.28
29	1-Heneicosene	34.10	55	294	$C_{21}H_{42}$	0.33
30	Heneicosane	34.33	57	296	$C_{21}H_{44}$	0.28
31	Phytol	34.68	71	296	$C_{20}H_{40}O$	0.36
32	1-Docosene	36.16	55	308	$C_{22}H_{44}$	0.23
33	n-Docosane	36.20	57	310	$C_{22}H_{46}$	0.25
34	1-Triecosene	37.83	55	322	$C_{23}H_{46}$	0.57
35	n-Tricosane	37.97	57	324	$C_{23}H_{48}$	0.10
36	1-Tetracosene	39.58	55	336	$C_{24}H_{48}$	0.10
37	n-Tetracosane	39.67	57	338	$C_{24}H_{50}$	0.13
38	Tricosanol	41.27	57	340	$C_{23}H_{48}O$	1.33
39	n-Hexacosane	42.89	57	366	$C_{26}H_{54}$	0.32
40	n-Heptacosane	44.46	57	380	$C_{27}H_{56}$	1.99
41	n-Octacosane	45.91	57	394	$C_{28}H_{58}$	0.33
42	Squalene	46.40	69	410	$C_{30}H_{50}$	0.17
43	n-Nonacosane	47.49	57	408	$C_{29}H_{60}$	4.91
44	n-Triacontane	48.87	57	422	$C_{30}H_{62}$	0.15
45	Lanosterol	50.16	69	426	$C_{30}H_{50}O$	0.08
46	n-Hentricontane	50.69	57	436	$C_{31}H_{64}$	0.31
47	1-Dotriacontene	53.66	55	448	$C_{32}H_{64}$	0.11
48	4α-Methylfecosterol	54.32	397	412	$C_{29}H_{48}O$	5.42
49	Stigmasta-5,22-dien-3-ol	55.03	412	412	$C_{29}H_{48}O$	1.47
50	Obtusifoliol	56.35	411	426	$C_{30}H_{50}O$	26.50
51	ß-Sitosterol	56.91	414	414	$C_{29}H_{50}O$	13.74
52	Cycloeucalenol	58.56	393	426	$C_{30}H_{50}O$	32.52
	Total identified					97.03
	Non identified					2.97

TABLE 4: GC/MS of saponifiable matter of *A. fraxinifolius* bark

No.	Compound	R_t	B.P.	M^+	M. Formula	Area%
1	Methyl octanoate	9.16	74	158	$C_9H_{18}O_2$	0.03
2	Methyl nonanoate	11.05	74	172	$C_{10}H_{20}O_2$	0.07
3	Dimethyl heptanedioate	13.26	115	188	$C_9H_{16}O_4$	0.05
4	Methyl-4-oxononanoate	13.67	98	186	$C_{10}H_{18}O_3$	0.06
5	Methyl undecanoate	14.44	74	200	$C_{12}H_{24}O_2$	0.06
6	Methyl-9-oxononanoate	14.69	74	186	$C_{10}H_{18}O_3$	0.12
7	Dimethyl octanedioate	14.88	129	202	$C_{10}H_{18}O_4$	0.33
8	Methyl dodecanoate	16.02	74	214	$C_{13}H_{26}O_2$	0.90
9	Dimethyl nonanedioate	16.45	152	216	$C_{11}H_{20}O_4$	1.40
10	Dimethyl decanedioate	17.86	199	230	$C_{12}H_{22}O_4$	0.15
11	Methyl tetradecanoate	18.89	74	242	$C_{15}H_{30}O_2$	1.67
12	Dimethyl undecanedioate	19.24	98	244	$C_{13}H_{24}O_4$	0.12
13	Methyl pentadecanoate	20.19	74	256	$C_{16}H_{32}O_2$	1.55
14	Methyl-9-hexadecenoate	21.22	55	268	$C_{17}H_{32}O_2$	0.17
15	Methyl hexadecanoate	21.76	74	270	$C_{17}H_{34}O_2$	23.64
16	Methyl heptadecanoate	22.69	74	284	$C_{198}H_{34}6O_2$	0.25
17	Methyl-9,12-octadecadienoate	24.65	67	294	$C_{19}H_{34}O_2$	40.39
18	Methyl nonadecanoate	24.96	74	312	$C_{20}H_{40}O_2$	2.53
19	Dimethyl hexadecandioate	25.40	98	314	$C_{20}H_{40}O_2$	3.96
20	Methyl-6,9,12-octadecatrienoate	25.66	292	292	$C_{19}H_{32}O_2$	0.53
21	Methyl-11-eicosenoate	25.80	55	324	$C_{21}H_{40}O_2$ $C_{21}H_{40}O_2$	0.81
22	Methyl eicosanoate	26.78	74	326	$C_{21}H_{42}O_2$	1.61
23	Dimethyl octadecanedioate	27.35	98	342	$C_{20}H_{38}O_4$	0.59
24	Methyl docosanoate	28.03	74	354	$C_{23}H_{46}O_2$	2.04
25	Methyl tricosanoate	28.91	74	368	$C_{24}H_{48}O_2$	0.26
26	Dimethyl icosanedioate	29.22	98	370	$C_{22}H_{42}O_4$	0.10
27	Methyl tetracosanoate	29.88	74	382	$C_{25}H_{50}O_2$	2.64
28	Methyl pentacosanoate	30.71	74	396	$C_{26}H_{52}O_2$	0.41
29	Dimethyl docosanedioate	30.99	98	398	$C_{24}H_{46}O_4$	0.09
30	Methyl hexacosanoate	31.63	74	410	$C_{27}H_{54}O_2$	3.76
31	Methyl heptacosanoate	32.41	74	424	$C_{28}H_{56}O_2$	0.23
32	Methyl octacosanoate	33.16	74	438	$C_{29}H_{58}O_2$	0.12
33	Methyl triacontanoate	34.99	74	466	$C_{31}H_{62}O_2$	0.07
	Total identified compounds	-	-	-		**90.71**
	Non-identified					**9.29**

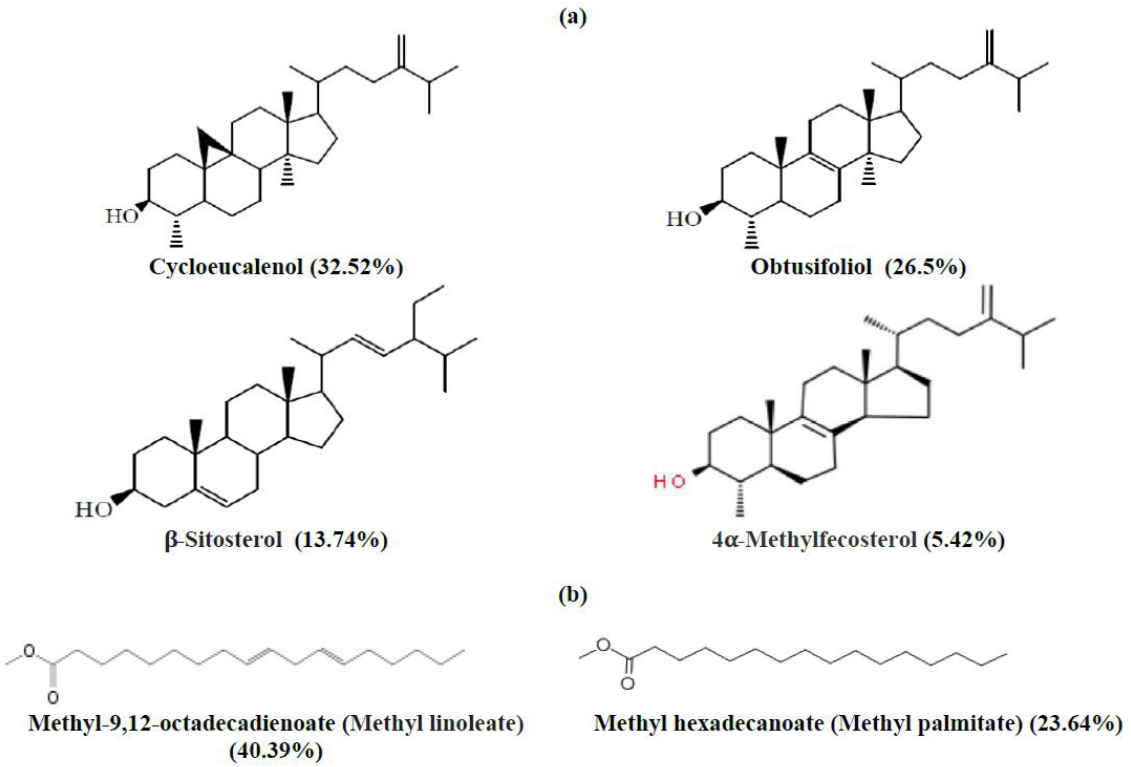

Fig. 4 : Structure of the major identified sterols and fatty acid methyl esters

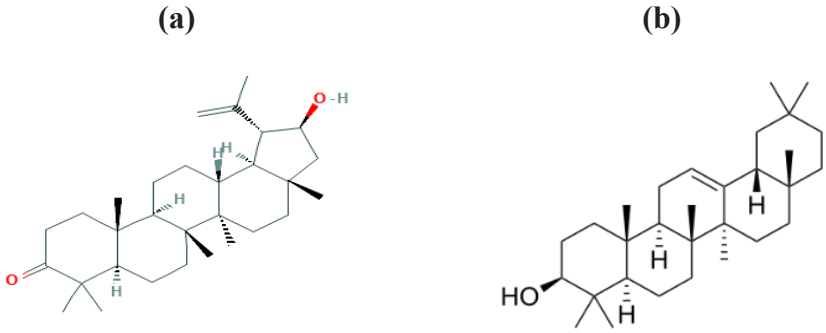

Fig. 5 : Structures of the two isolated compounds : (a) Salacianol and (b) β-Amyrin

Conclusions

In this work, results of the bioactivities screening assay revealed that the PEE possessed potent cytotoxic, antidiabetic and hepatoprotective activities and this forced attention of the authors to extensively investigate the phytoconstituents of the petroleum ether extract (PEE) of *A. fraxinofolius* bark by GC/MS analysis. The latter led to identification of 52 compounds constituting 97.03% of the total composition of the unsaponifiable matter fraction and 33 fatty acids representing 90.71% of the total fatty acid constituents. GLC analysis of the sterol fraction revealed the identification of cholesterol (7.22%), campesterol (13.30%), stigmasterol (10.00%) and β - sitosterol (69.48%). Two triterpenoidal compounds were also isolated and structurally identified as 21-β-hydroxylup-20(29)-en-3-one and β-amyrin.

The above-mentioned results indicated

the enrichment of the PEE with valuable phytoconstituents, so it could be used as hepatoprotective agent and for the treatment of diabetes mellitus and cancer. In future studies clinical trials should be done as well as cooperation with drug companies to formulate these extracts in a suitable dosage form.

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Conflict of interests

There are no conflicts of interest.

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