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Cytotoxicity and Suppressive Effect of Leaves of *Mimusops laurifolia* on Carbon Tetrachloride-induced Liver Injury in Rats and its Bioactive Constituents

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Abstract: Since the genus *Mimusops* is one of the important genera in the Indian traditional medicine, and is represented in Egypt with species; *Mimusops laurifolia* (Forssk.) Friis., thus the plant is selected for our investigation to reveal its biological activities and phytochemically analyze its bioactive fractions. The ethanolic extract of its leaves (LEE) and its different fractions: *n*-hexane (HF), chloroform (CF), ethyl acetate (EAF) and n-butanol (BF) were evaluated for *in vivo* hepatoprotective activity against CCl_4 induced hepatic cell damage in rats and for *in vitro* cytotoxicity against human liver cancer cell line (HEPG2); consequently the bioactive constituents were defined whereby EAF evidenced statistically significant hepatoprotection. Moreover, HF and α-amyrin (major compound isolated from HF) showed promising cytotoxicity against HEPG2. Structures of isolated compounds were established on the basis of physicochemical properties and spectral analysis. The bioactive fractions were examined for the isolation of 14 compounds for the first time from *Mimusops laurifolia* (Forssk.) Friis. From the lipophilic fractions: Lupeol acetate, α-amyrin, chondrillasterol, oleanolic acid, chondrillasterol-3-O-β-D-galactoside, mearnsitrin, myricetin and quercetin were isolated. While, from EAF: mearnsitrin, myricitrin, myricetin-3-O-β-D-galactoside, quercetin-3-O-β-D-glucoside, rutin and myricetin-3-O-β-D-glucuronide were isolated. Leaves of *Mimusops laurifolia* (Forssk.) Friis can be considered as a natural medicinal plant with a potential anticancer and hepatoprotection due to its bioactive ingredients in both HF and EAF, respectively.

Key words: Anticancer, hepatoprotective, *Mimusops laurifolia*, triterpenes, flavonoids

INTRODUCTION

Liver disease in Egypt is wide-spread causing serious health problems, as well as indirect economic problems taking in consideration the loss of the country's productivity influenced by the age group affected and the high rate of mortality (Lehman, 2008). The considerable reputation of genus *Mimusops* in the Indian traditional medicine as febrifuges, astringents, purgatives and stimulants (Sahu *et al*., 1995; Shah *et al*., 2003), as well as its previously isolated constituents as betulinic acid and ursolic acid that were reported to have potent hepatoprotective and antitumour effects, initiated our interest to carry our investigation on one of its species: *Mimusops laurifolia* (Forssk.) Friis; the only species cultivated in Egypt. By reviewing the current literature, nothing could be found dealing with the chemical characters and biological activities of *M. laurifolia* except for the investigation of the saponin content of its leaves and seeds and their ulceroprotective and anti-inflammatory activities (Eskander, 2005).

M. laurifolia is a large evergreen tree (15-20 m high) with small white fragrant flowers and small, rounded or ovoid, green, sweet edible, 1-6 hard seeded fruits. It is present in Gulf of Aden and countries around the Red Sea especially Eritrea, Ethiopia and Somalia and has been cultivated in Egypt since Pharos times as it is frequently found in their tombs (Friis, 1980). The great finding of *M. laurifolia* from ancient Egypt was in the tomb of Tut-ankh-Amen consisting of several very large funeral bouquets of leafy twigs (Friis, 1980). It was also discovered in a garland at the Roman tomb in Abu Rawash (Hammouda and Fahd, 1988).

The aim of this study was to investigate the possibility of introducing *M. laurifolia* as a new medicinal plant to be used against two of the main causes

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of liver damages in Egypt; liver toxicity by chlorinated agents and liver cancer. As well as, isolating and identifying the phytoconstituents that could be responsible for these activities in their bioactive fractions.

MATERIALS AND METHODS

Plant material: Leaves of *M. laurifolia* were collected during May-July 2008 from the Egyptian museum and River Garden, El-Gazira, Cairo, Egypt. Plant material was kindly identified by Dr. Mohamed El- Gebaly, Senior Taxonomist. A voucher specimen (No. M-3) is kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

DNA fingerprinting: DNA isolation was performed using the CTAB method of Doyle and Doyle (1990). Fresh leaves (0.5 g) were powdered in liquid nitrogen, suspended in 1 mL preheated (65°C) CTAB buffer, incubated at 65°C for 1 h with occasional shaking and then centrifuged for 10 min at 1000 rpm. The supernatant was transferred to a new tube by wide pore, 0.5 mL of chloroform and isoamyl alcohol mixture (24:1) was added forming an emulsion, then centrifuged for 15 min at 14000 rpm and the aqueous layer was transferred to a new sterilized tube (avoid protein surface). The ice cold isopropanol was added to precipitate the nucleic acid (RNA, DNA) then incubate at -20°C overnight and centrifugation was happened at 14000 rpm for 20 min. The supernatant was discarded and the pellet was washed carefully twice with cold 70% ethanol, dried at room temperature and resuspended in 100 μL of sterile deionized distilled water. DNA concentration was determined by electrophoresis of 5 μL of DNA with 2 μL of loading buffer and run at 100 volt for approximately 30 min.

RAPD was performed as described by Williams *et al.* (1990) with minor modifications. Briefly, PCR amplification was performed in 25 μL reaction mix Table 1 containing 20.40 mg genomic DNA, 0.5 unit Taq polymerase (Sigma), 0.2 mM each of dATP, dCTP, dGTP, dTTP, 5 Pico mole random primer and appropriate amplification buffer. The reaction was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles using Biometera Uno thermal cycler, as follows: One cycle at 95°C for 3 min and then 44 cycles at 92°C for 2 min, 37°C for 1 min and 72°C for 2 min (for denaturation, annealing and extension, respectively). Reaction was finally incubated at 72°C for 10 min and further incubated on 4°C. Ten primers were used for RAPD analysis based on their ability to amplify Amaransis genome and producing reproducible amplification patterns.

Table 1: RAPD analysis of *M. laurifolia* with ten primers

Band No.	Molecular weight (bp)	A1	A2	A3	A4	B1	B2	B4	G1	G2	G3
1	960	-	-	-	-	-	-	+	-	-	-
2	890	-	-	-	-	-	-	-	-	-	+
3	860	-	-	-	-	-	-	+	-	-	-
4	840	-	-	-	-	-	-	-	-	-	+
5	810	-	-	+	-	-	-	-	-	-	-
6	790	-	-	-	-	-	-	-	-	-	+
7	760	-	-	-	-	-	-	+	-	+	-
8	740	+	-	-	-	-	-	-	-	-	-
9	710	-	-	+	-	-	-	-	+	-	+
10	690	-	+	-	-	-	-	-	-	-	-
11	660	+	-	-	-	-	+	-	-	+	-
12	640	-	-	-	+	-	-	+	-	-	+
13	610	-	-	+	-	+	-	-	-	-	-
14	560	-	+	-	-	-	-	+	-	+	-
15	540	+	-	-	-	-	-	-	-	-	+
16	510	-	-	+	-	+	-	+	-	-	-
17	490	+	+	-	+	-	-	-	-	-	-
18	460	-	-	+	-	-	-	-	+		+
19	440	-	+	-	-	-	+	+	-	+	-
20	390	+	-	+		+	-	-	+		+
21	340	-	-	-	-	-	-	+	-	-	-
22	310	-	+	+	-	-	-	-	-	+	
23	290	-	-	-	+	-	-	-	-	-	-
24	260	-	+	-	-	+	-	+	+	-	+
25	240	+	-	-	-	-	-	-	-	-	-
26	210	-	+	+	-	-	-	-	-	-	+
27	190	-	-	-	+	-	-	-	-	-	-
28	160		+	-	-	-	+	-	-	-	-
29	110	-	-	+	-	-	-	-	-	-	-
30	90	-	-	-	+	-	-	-	-	-	-
31	60	-	+	-	-	-	-	-	-	-	-
32	10	-	+	-	-	-	-	-	-	-	-
Total		6	10	9	5	4	3	9	4	5	10

+: Presence of band, -: Absence of bands

The amplification products were analyzed by electrophoresis in 2 % agarose in TAE buffer stained with 0.2 μg mL^{-1} ethidium bromide and photographed under UV light using a Polaroid camera. The buffer was added to the agarose then heated in a microwave tell melting, cooling to 60°C then the ethidium bromide was added. Sample was prepared by using 10 μL PCR-product and 2 μL loading buffer. One marker was used, 100 bp DNA ladder (Axygen). Results are recorded in Table 1.

Drugs and chemicals: Silymarin (Sedico Pharmaceutical Co., 6 October City, Egypt); carbon tetrachloride (Analar, El-Gomhoreya Co., Cairo, Egypt). Biochemical kits for assessment of serum enzymes (Bio-Merieux Co.). Adult male albino rats of Sprauge Dawley Strain weighing (100±30 g) for liver experiment and albino mice (20±5 g) for LD_{50}. All animals were kept on standard laboratory diet and under hygienic conditions.

Reference samples of flavonoids: mearnsitrin, myricetin, quercetin, mearnsitrin, myricitrin, myricetin-3-O-βD-galactoside, quercetin-3-O-β-D-glucoside and rutin; triterpenes: Lupeol acetate, α-amyrin and oleanolic acid (E. Merck, Darmstadt, Germany). Silica gel H for vacuum liquid chromatography (VLC) (E-Merck, Darmstadt, Germany); silica gel 60 for column chromatography (Fluka, 70-230 mesh ASTM, Germany); Sephadex LH 20 (Pharmacia). Silica gel GF_{254} precoated plates (TLC) (Fluka, Germany) using solvent systems: n-hexane-ethyl acetate (95:5 v/v, S1) and (75:25 v/v, S2) (for triterpenes and sterols), chloroform-methanol (95:5 v/v, S3) and (90:10 v/v, S4) (for sterol glycosides and flavonoid aglycones), ethyl acetate-methanol-water (100:16.5:13.5 v/v, S5) and n-butanol-acetic acid-water (4:1:5 v/v, S6) (for flavonoid glycosides). The chromatograms were visualized under UV light (366 nm) before and after exposure to ammonia vapor and spraying with $AlCl_3$ for flavonoids detection and after spraying with p-anisaldehyde/sulphuric then heated at 110°C for sterols and triterpenes detection (Stahl, 1969). Melting points (uncorrected) were determined on a D. Electrothermal 9100 (UK). Mass spectra were measured using Shimadzu QP-2010 Plus, 70 eV. ^1H (300 MHz) and ^{13}C (75 MHz) NMR were measured on Varian Mercury-VX-300 NMR instrument. NMR spectra were recorded in $CDCl_3$, DMSO and CD_3OD and chemical shifts were given in δ (ppm) relative to TMS as internal standard.

Determination of LD_{50}: The LD_{50} of the LEE was calculated according to Lorke (1983).

***In vivo* hepatoprotective activity:** Eighty animals were used for the experiment and divided into 8 groups (10 animals, each). LEE prepared from leaves of *Mimusops laurifolia* and its different fractions; HF, CF, EAF, BF were orally administered (100 mg kg^{-1} b. wt. daily) for 15 days before induction of liver damage by intraperitoneal injection of 5 mL kg^{-1} of 25% CCl_4 in liquid paraffin according to Klaassen and Plaa (1969), silymarin (25 mg kg^{-1} b.wt.) was used as a reference drug. The extracts as well as the reference drug were continued to be administered to the rats for another 15 days after liver damage. The levels of aspartate aminotransferase (AST) alanine aminotransferase (ALT) (Thewfweld, 1974) and alkaline phosphatase (ALP) (Kind and King, 1954) enzymes were measured in the blood of each group at zero time, after receiving tested drug for 15 days and 72 h after induction of liver damage and after 15 days of treatment with the tested samples. Results are shown in Table 2.

***In vitro* screening for cytotoxic activity:** LEE prepared from *M. laurifolia* and its different fractions; HF, CF, EAF, BF, as well as the isolated compound 2 (the major isolated compound from HF) were tested for their cytotoxicity at different concentrations in DMSO (0-10 μg mL^{-1}), against human liver cancer cell line (HEPG2) according to the method of Skehan *et al.* (1990). The IC_{50} values were calculated and the results are shown in Table 3 (at the National Cancer Institute, Cairo, Egypt).

Extraction and fractionation: The air-dried powdered leaves of *M. laurifolia* (2 kg) were extracted by cold percolation with 70 % ethanol till exhaustion. The ethanolic extract was evaporated under reduced pressure to give 339 g dry residue of reddish-brown LEE. An aliquot of LEE (320 g) was suspended in 400 mL distilled water and partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol saturated with water and evaporated under reduced pressure to yield 20.22, 33.74, 26.84 and 53.82 g dry residues of HF, CF, EAF and BF, respectively.

Table 2: *In vitro* cytotoxicity of *M. laurifolia* leaves on human liver cancer cell line (HEPG2)

	IC_{50} (μg $well^{-1}$)
Test sample	HEPG2
LEE	17.3
HF	14.4
CF	125.0
EAF	17.0
BF	133.1
α-amyrin	12.0
Doxorubicin	0.67

Table 3: Effect of leaves ethanolic extract of Mimusops laurifolia (Forssk.) Friis and its fractions on the serum AST, ALT and ALP levels on adult albino rats (n = 10)

Group	Zero	15 days	72 h	15 days	% reduction at 72 h
AST (μL^{-1})					
Control	29.2±1.1	28.6±0.4	138±5.1	150±5.9*	
LEE	31.3±1.1	31.1±1.2	64.7±2.9	48.2±1.8*	53.25
HF	28.9±0.6	28.7±0.4	77.2±0.6	68.2±2.2*	44.22
CF	29.2±0.7	29.6±0.8	83.4±3.7	83.4±2.3*	39.74
EAF	29.8±0.3	28.3±0.6	54.6±1.3	41.7±1.6*	60.55
BF	28.7±0.5	28.9±0.4	97.8±4.3	97.8±3.7*	29.34
Silymarin	32.4±1.1	29.8±0.9	48.7±1.3	27.3±0.6	64.81
ALT (μL^{-1})					
Control	31.6±1.1	30.2±0.6	143 ±6.8	148±6.2*	
LEE	30.7±0.9	29.8±0.7	61.4±3.1	38.1±1.3*	57.21
HF	33.6±1.3	33.5±1.2	84.2±2.9	79.8±3.1*	41.32
CF	27.3±0.4	27.5±0.4	81.5±2.4	73.6±0.1*	43.21
EAF	29.6±0.7	28.2±0.2	50.9±2.3	35.7±1.1*	64.53
BF	29.7±0.6	30.2±0.9	89.8±2.6	76.4±0.1*	37.42
Silymarin	27.8±0.5	26.5±0.4	53.6±1.8	29.2±0.8	62.65
ALP (KAU)					
Control	6.9±0.1	7.1±0.1	57.2±1.8	62.3±2.1*	
LEE	7.2±0.1	6.9±0.1	21.6±0.3	19.2±0.4*	62.24
HF	7.8±0.1	7.7±0.1	33.9±1.4	30.5±1.2*	40.73
CF	7.6±0.1	7.5±0.1	41.7±1.8	39.8±1.3*	27.10
EAF	7.4±0.1	7.2±0.1	24.7±0.6	18.2±0.4*	56.82
BF	7.5±0.1	7.4±0.1	52.2±2.4	49.3±2.1*	8.74
Silymarin	7.3±0.1	6.9±0.1	15.1±0.6	6.8±0.1	73.60

*Statistically significant from zero time at $p<0.01$, Statistically significant from 72 h after CCl_4 at $p<0.01$

Isolation of the phytoconstituents from the biologically active fractions: An aliquot of HF (20 g) was chromatographed on a VLC column (170 g silica gel H, 7× 20 cm) and eluted with gradients of n-hexane, n-hexane /chloroform, chloroform and chloroform/ethyl acetate. Fractions, 200 mL each, were collected, monitored by TLC, and similar fractions were pooled to give eight collective fractions. Collective fraction 2 (2 g, 5% chloroform in n-hexane) was purified over silica gel and eluted with n-hexane to obtain compound 1 (420 mg); collective fraction 3 (4.5 g, 10-25% chloroform in n-hexane) was crystallized from acetone to give compounds 2 (3 g); collective fraction 4 (700 mg, 30-40% chloroform in n-hexane) was chromatographed over silica gel and eluted with gradients of n-hexane/ethyl acetate mixtures to obtain compound 3 (30 mg) (at 92% ethyl acetate in n-hexane); collective fractions 6 (1.5 g, 15-45% ethyl acetate in chloroform) and 7(400 mg, 75-100% ethyl acetate in chloroform) were crystallized from acetone to give pure compounds 4 (420 mg) and 5 (13 mg), respectively. EAF (15 g) was chromatographed on a VLC column (150 g silica gel H, 7×20 cm) eluted with gradients of chloroform, chloroform/ ethyl acetate, ethyl acetate and ethyl acetate/methanol. Fractions, 200 mL each, were collected and monitored by TLC, similar fractions were pooled to give seven collective fractions. Each of the collective fractions 2 (200 mg, 25% ethyl acetate in chloroform), 3 (500 mg, 30-75% ethyl acetate in chloroform), 4 (900 mg, 80-85% ethyl acetate in chloroform) and 6 (2.4 g, 25-30% methanol in ethyl acetate) was purified over sephadex LH-20 using methanol and methanol/water mixtures to obtain compounds: 9 (40 mg, collective fraction 4), 13 and 14 (9 and 71 mg, respectively, collective fraction 6); collective fraction 5 (4.5 g, 90% ethyl acetate in chloroform, 100% ethyl acetate and 0-20% methanol in ethyl acetate) was purified over silica gel to obtain compounds 10 (92 mg), 11 (69 mg), 12 (5 mg). Compounds 6, 7 and 8 were identified in CF using CO-TLC using different solvent systems: S3 (R_f: 0.35, 0.27, 0.22, respectively) as well as Co-PLC S6 upper layer (R_f: 76, 57, 64, respectively).

Acid hydrolysis of the glycosides: Complete acid hydrolysis was carried out (Harborne *et al*., 1975) for compounds 9-14 by treating the isolated glycosides (5 mg, each) with 1.5 N HCl in aqueous methanol (1:1) for 2 h at 100°C. Each hydrolysate was then, separately, extracted with successive portions of chloroform. The chloroform extract was concentrated under reduced pressure and subjected to Co-TLC or Co-PC investigation alongside with authentic aglycones using solvent systems: S3 (R_f: 0.35, 0.27, 0.22, respectively) as well as Co-PLC S6 upper layer (R_f: 76, 57, 64, respectively). The mother liquor of each compound was neutralized with sodium bicarbonate, evaporated under reduced pressure and dissolved in isopropanol and subjected to Co-PC using authentic sugars as reference markers using S6.

Lipids in the bioactive HF: Saponification of the HF (1 g), preparation of the unsaponifiable matter (USM) (0.49 g) and the fatty acid methyl esters (0.39 g) were

Table 4: Conditions for GLC analysis of the fatty acid methyl esters and unsaponifiable matter

	Conditions	
GLC	Unsaponifiable matters	Fatty acid methyl esters
Column type	Thermo TR-5MS (5% phenyl polysil phenylene siloxane)	Thermo TR-FAME
Column dimensions	30 m×0.25 mm i.d.×0.25 μm	30 m×0.25 mm i.d. ×0.25 μm
Carrier gas	Nitrogen	Nitrogen
Flow rate	30 mL min^{-1}.	30 mL min^{-1}.
Sample size	0.5 μL of 10% chloroform solution (w/v)	0.2 μL of 10% chloroform solution (w/v)
oven temperature	160°C	100°C
Temperature programming	70°C increased to 270°C by the rate of 10°C min^{-1}, then isothermally 25 min	70°C increased to 190°C by the rate of 8°C min^{-1}, then isothermally 25 min
Injector port temperature	270°C	200°C
Detector temperature	280°C	220°C

Table 5: Results of GLC analysis of the unsaponifiable matter of the leaves of *M. laurifolia*

Peak No.	RR_t (min)	Identified compounds	Leaves (%)
1	0.07	n-tetradecane	-
2	0.08	n-hexadecane	-
3	0.16	n-heptadecane	-
4	0.59	n-octadecane	18.272
5	0.60	n-nonadecane	-
6	0.61	n-eicosane	0.314
7	0.64	n-docosane	0.381
8	0.68	n-tetracosane	0.315
9	0.81	n-hexaconsane	0.315
10	0.87	n-octacosane	0.813
11	0.90	n-nonacosane	0.298
12	0.96	Stigmasterol	-
13	0.97	Chondrillasterol	7.016
14	1.00	α-amyrin	65.675
15	1.02	n-dotriacontane	2.415
Total identified hydrocarbons (%)		23.123	
Total identified triterpenes (%)			65.675
Total identified sterols (%)		7.016	

Table 6: Results GLC analysis of fatty acid methyl esters of the leaves of *M. laurifolia*

Peak No.	RRt (min)	Identified compounds	Leaves (%)
1	0.50	Methyl caprate	4.705
2	0.69	Methyl laurate	2.042
3	0.82	Methyl myristate	1.707
4	1.00	Methyl palmitate	70.662
5	1.16	Methyl palmitoleate	1.052
6	1.32	Methyl magarate	0.380
7	1.47	Methyl stearate	0.661
8	1.56	Methyl oleate	2.384
9	1.81	Methyl linoleic	13.149
10	1.97	α-Methyl linolenate	1.720
11	2.35	γ-Methyl linolenate	0.799
12	3.98	Methyl eicosapentaenoate	-
13	4.73	Methyl docosahexaenoate	-
Total identified SAFA (%)			75.452
Total identified USAFA (%)			19.104
Total identified FA (%)			94.556

SAFA: Saturated fatty acid, USAFA: Unsaturated fatty acid

carried out, according to Vogel (1961). GLC conditions are recorded in Table 4. Results are recorded in Table 5 and 6.

RESULTS AND DISCUSSION

DNA fingerprinting: The DNA of *M. laurifolia* (Forssk.) Friis was amplified using ten random decamer primers to reveal RAPD fragments (Table 1). Each of the ten primers successfully directed the amplification of a genome-specific fingerprint of DNA fragment; all amplifications were found to be prolific.

A total of 65 different fragments have been recorded and found to be produced mainly by 4 of the ten primers (A2, A3, B4 and G3), while primer (B2) produced only 3 bands. Thus, RAPD analysis can be performed using primers A2, A3, B4 and G3 for the selective discrimination of *M. laurifolia* (Forssk.) Friis.

Acute toxicity: Study of LEE of *M. laurifolia* revealed no mortality up to a dose level of 7.2 g kg^{-1} body weight.

***In vivo* Hepatoprotective activity:** LEE, HF, CF, EAF, BF (100 mg kg^{-1} b.wt. daily, each) showed no significant change in enzymes levels after 15 days of administration (Table 2). Comparing the results of the test groups at 72 h after induction of liver damage (by 25% CCl_4) with that of the control it was observed that the LEE and EAF significantly reduced the increase in the levels of AST enzyme (53.25 and 60.55% change, respectively), ALT enzyme (57.21 and 64.53 % change, respectively) and ALP enzyme (62.24 and 56.82% change, respectively). These protective effects were comparable to that of silymarin which reduced the rise in the levels of AST, ALT and ALP by 64.81, 62.65 and 73.60%, respectively. Furthermore, administration of the extracts for another 15 days after induction of liver damage led to a significant decrease in the enzyme levels regarding their respective normal values which indicated stabilization of the hepatocyte cell membrane as well as repairing of hepatic tissue damage caused by CCl_4 (Singab *et al*., 2005). These significant results were recorded in the order of silymarin >EAF > LEE is highly attributed to mixture of flavonoids glycosides in the EAF.

***In vitro* anticancer:** Results of the cytotoxic activities of LEE, HF, CF, EAF, BF prepared from leaves of *M. laurifolia* against the human liver cancer cell line (HEPG2) compared to the reference drug Doxorubicin® (Table 3) revealed that the test samples with significantly positive results were recorded in the order of HF> EAF>

LEE, displayed by IC_{50} values 14.4, 17.0, 17.3 μg mL^{-1}, respectively. The *in vitro* anticancer effect of the HF on liver carcinoma was highly attributed to α-amyrin (the main constituent of the lipophilic part) which is apparently noticed from its significant IC_{50} (12 μg mL^{-1}) when examined alone on the same cell line.

Phytochemical investigation of the bioactive fractions: According to the biologically guided fractionation, the HF and EAF were selected for phytochemical investigation of bioactive constituents. Results showed that: Five compounds (1-5) were isolated from the lipophilic fraction as follows: lupeol acetate, α-amyrin, chondrillasterol, oleanolic acid, chondrillasterol-3-O-β-D-galactoside from HF and three flavonoid aglycones (6-8) were identified in CF namely; myricetin-4'-O-methyl ester (mearnsitrin), myricetin and quercetin. The non significant biological results of the investigated aglycones- containing fraction is mainly attributed to the less solubility of the aglycones in water. Six flavonoid glycosides (9-14)were isolated from hydrophilic EAF namely; myricetin-4'-O-methyl ether-3-O-α-L-rhamnoside (mearnsitrin), myricetin-3-O-α-L-rhamnoside (myricitrin), myricetin-3-O-β-D-galactoside, quercetin-3-O-β-D-glucoside, quercetin-3-O-β-D-rutinoside (rutin) and myricetin-3-O-β-D-glucuronoide (14). The structures of the isolated compounds were identified by comparing their m. p., chromatographic behavior (TLC and PC) and spectroscopic data (UV, MS, ^{1}H and ^{13}C-NMR) with available authentic and literature data (Mabry *et al*., 1970; Iida *et al*., 1980; Agrawal, 1989; Good and Akisha, 1997; Jahan *et al*., 1995; Nair *et al*., 1999; Seidel *et al*., 2000; Braca *et al*., 2001; Migas *et al*., 2005) with the aglycones obtained after acid hydrolysis of compounds 9-14 and identification of their sugars. Compounds 8, 11 and 14 were isolated for the first time from the family Sapotaceae. Compounds 1, 4 and 13 were isolated for the first time from the genus *Mimusops*. Our report presents the first investigation of these compounds in *M. laurifolia*.

CONCLUSION

Our study presented a profile of *M. laurifolia* (Forssk.) Friis and introduced it as an expected natural medicinal plant. We focused on the medicinal value and low toxicity of its leaves rich in bioactive constituents as shown in the investigation of the bioactive n-hexane (anticancer) and ethyl acetate (hepatoprotective) fractions. Further studies are necessary to discuss the possibility of its application in a suitable pharmaceutical form for treating liver cancer and liver toxicity induced by chlorinated agents, of common occurrence in Egypt.

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