Composition of lipoidal matter and evaluation of hepatoprotective, cytotoxic, and antioxidant activities of *Khaya grandifoliola* C.DC. growing in Egypt

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Background and objectives
Liver disease is one of the most common health problems. Schistosoma and hepatic viruses are widespread in Egypt, causing severe liver damage and posing a threat to life, which has motivated researchers to discover and evaluate new hepatoprotective agents, particularly from natural sources. The hepatoprotective effect of the leaf extracts of *Khaya grandifoliola* C.DC. were investigated, after determination of acute toxicity. The anticancer activity and antioxidant potential of the plant were evaluated, as well as the phytochemical composition of petroleum ether and chloroform extracts.

Materials and methods
Different extracts of *K. grandifoliola* C.DC. leaves were prepared and tested for hepatoprotective effect against carbon tetrachloride-induced liver damage in rats using silymarin as the reference drug. Anticancer activity was tested on HEPG2 (liver carcinoma cell line), HCT116 (colon carcinoma cell line), HELA (cervix carcinoma cell line), HEP2 (larynx carcinoma cell line), and MCF7 (breast carcinoma cell line). Its potency was compared with the reference drug Doxorubicin. The antioxidant potential was evaluated using 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay and ascorbic acid as the reference drug. After saponification of the petroleum ether extract, unsaponifiable matter and fatty acid methyl esters were analyzed by GC/MS. The chloroform extract was subjected to vacuum liquid chromatography.

Results and conclusion
The ethanolic extract of the leaves showed no toxicity up to 5 g/kg. It exhibited potent cytotoxic activity against HCT116 (colon carcinoma cell line) and MCF7 (breast carcinoma cell line), compared with doxorubicin as the standard. Also the ethanolic extract has significant free radical scavenger (100% inhibition) activity, compared with ascorbic acid. The extracts showed significant hepatoprotective and curative activity. GC/MS analysis of both unsaponifiable matter and fatty acids from petroleum ether extract allowed identification of 94.29% of the total unsaponifiable matter, (hentriacontane represented the major component, 11.98%) and 80.72% of the fatty acid methyl ester content (hexadecanoic acid methyl ester represented the major component, 31.68%). Vacuum liquid chromatography of chloroform extract led to isolation of two sterol glucosides (β-sitosterol-3-*O*-β-d-glucopyranoside and β-stigmasterol-3-*O*-β-d-glucopyranoside).

Keywords:
anticancer, antioxidant, hepatoprotective, *Khaya grandifoliola* C.DC., lipids, sterols

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Introduction

Plants belonging to the family Meliaceae are distributed in tropical, subtropical, and occasionally warm temperate regions. The family comprises about 50 genera with 650 species, rich in limonoids [1]. The genus *Khaya* comprises seven species native to tropical Africa and Madagascar. Compounds other than limonoids were isolated from *Khaya grandifoliola* C.DC. (African Mahogany), which include catechin [2] and steroid hormone from bark [3]. Antimalarial [4], schistosomicidal [5], hypoglycemic and hypocholesterolemic [6] activities were reported for *K. grandifoliola* C.DC. stem bark. A review of the literature showed that only a few studies have reported on *K. grandifoliola* C.DC. grown in Egypt. Schistosoma and hepatic viruses are widespread in Egypt, causing severe liver damage and threatening life.

In a previous study, we reported the isolation of rutin and scopoletin-arabinofuranoside from ethanol extract of *K. grandifoliola* C.DC. leaves and the isolation of quercetin-3-*O*-glucosides, quercetin-3-*O*-rhamnoside, and quercetin from ethyl acetate extract; we also evaluated the desmutagenic and antimutagenic activities of the plant [7]. This study aims to investigate the hepatoprotective, anticancer, and antioxidant activities, as well as identify the lipoidal matter composition of *K. grandifoliola* C.DC. leaves.

Experimental

An NMR Jeol ECA spectrometer (JEOL Corporation, Tokyo, Japan), 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, using $CDCl_3$ as solvent, was used. All chemical shifts (δ) are given in ppm units with reference to TMS as the internal standard, and the coupling constants (*J*) are given in Hz. Gas chromatograph coupled with mass spectrometer, (GC/MS): Agilent 6890 gas chromatograph coupled with an Agilent mass spectrometric detector, 70 eV (California, USA). ESR, Brucker, Elexsys, E 500, (Frankfurt, Germany).

Materials and methods

Plant material

The fresh leaves of *K. grandifoliola* C.DC. Meliaceae grown in Egypt were collected from Giza Zoo (Giza, Egypt). The plant was identified by the plant taxonomist Dr Mohamed El-Gebaly. Herbarium sheets are kept in the Herbarium, Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Preparation of ethanolic extract

The air-dried powdered leaves of *K. grandifoliola* C.DC. (500 g) were extracted with 80% aqueous ethanol until exhaustion. The solvent was evaporated to dryness under reduced pressure at 40°C to yield 129 g (representing 25.8% of the air-dried leaves).

Preparation of successive extractives

The powder of the air-dried leaves (600 g) was successively extracted with petroleum ether, chloroform, ethyl acetate, and 95% ethanol in a Soxhlet apparatus. The solvents were evaporated to dryness under reduced pressure at 40°C, yielding 25.2, 24.3, 0.3, and 60 g, respectively (representing 4.2, 4.05, 0.05, and 10% of air-dried leaves, respectively).

Investigation of lipoidal matter

Saponification of petroleum ether extract

The petroleum ether extract (1 g) was subjected to saponification according to the method reported by Tsuda *et al.* [8]. The unsaponifiable matter weighed 0.307 g, whereas the fatty acid content weighed 0.098 g.

Preparation of fatty acid methyl esters

The free fatty acids obtained from saponification were methylated according to the method reported by Finar [9].

GC/MS analysis

Study of both the unsaponifiable and saponifiable fractions was carried out to identify their contents using GC/MS analysis. The constituents were identified by comparison of their spectral fragmentation patterns with those of the available database libraries, Wiley (Wiley International, USA) and NIST (Nat. Inst. St. Technol., USA), and/or published data [10,11]. Quantitative determination was carried out on the basis of peak area integration. GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters of *K. grandifoliola* C.DC. leaves was carried out using conditions described in Table 1.

Vacuum liquid chromatography

Successive chloroform extract was extracted with methanol. The methanol-soluble fraction was applied on vacuum liquid chromatography silica gel column using hexane/chloroform/ethyl acetate/methanol as eluent (with gradient elution 10%). Fractions eluted with 10% ethyl acetate/methanol were collected and purified on silica gel column using chloroform/methanol as eluent (with gradient elution 0.5, 2, 4, 8, 10, 20, 60, 80, and 100%) to give two compounds, I and II.

Determination of LD_{50}

The lethal dose of the total ethanol extract of *K. grandifoliola* C.DC. leaves was determined according to the method described by Miller and Tainter [12]. Male albino mice (25–30 g) were divided into groups of six animals each. Preliminary experiments were carried out to determine the minimum dose that kills all animals (LD_{100}) and the maximum dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in-between these two doses. The extract at each dose was injected into a group of six animals by subcutaneous

Table 1 Conditions of GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters

Conditions	Unsaponifiable matter	Fatty acid methyl esters
Column	Capillary column of fused silica, 30 m length, 0.32 mm ID and 0.25 μm thickness	
Stationary phase	TR-5MS (5% phenyl polysilphenylene siloxane)	
Carrier gas	Helium at 1 ml/min, 13 psi	
Temperature programming	70°C isothermal for 5 min70–290°C at a rate of 4°C/min290°C isothermal for 10 min	140°C isothermal for 5 min140–200°C at a rate of 5°C/min200°C isothermal for 3 min
Detector temperature	280°C	220°C
Ion source temperature	270°C	200°C
Ionization voltage	70 eV	

GC/MS, gas chromatograph coupled with a mass spectrometer.

injection. The mice were then observed for 24 h and symptoms of toxicity and mortality rates in each group were recorded, and median lethal dose (LD_{50}) was calculated.

Evaluation of hepatoprotective activity

Induction of liver damage

Liver damage in adult male albino rats (Sprague–Dawley strain of 130–150 g body weight) was induced by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride in liquid paraffin, according to the method reported by Klassen and Plaa [13].

Experimental design

Seventy male albino rats were randomly divided into seven groups of 10 rats each (130–150 g).

Animal groups:

(1) Group I: This was the control group, which received a daily oral dose of 1 ml saline for 1 week before and after liver damage (negative control).
(2) Groups II, III, IV, V, and VI: These groups comprised rats with liver damage pretreated with a daily oral dose of 100 mg/kg body weight of total ethanol, petroleum ether, chloroform, ethyl acetate, and ethanol extracts, respectively, for 1 week. Administration of the extracts was continued after liver damage for another week.
(3) Group VII: This group comprised rats with liver damage pretreated with a daily oral dose of 25 mg/kg body weight silymarin as a standard. Administration of the drug was continued after liver damage for another week. Whole blood was obtained from the retro-orbital venous plexus through the eye canthus of the anesthetized rat. Blood samples were collected at time point 0 and 1 week before carbon tetrachloride (CCl_4) injection and 72 h and 1 week after CCl_4 injection. Serum was isolated by centrifugation. Aspartate Aminotransferase [AST], Alanine Aminotransferase [ALT] [14], and Alkaline Phosphatase [ALP] [15] were measured. The data shown in Tables 7–9 were statistically analyzed using Student's '*t*'-test [16]. Results with *P* value less than 0.01 were considered statistically significant.

Protective activity was calculated as follows:

$$\frac{\text{Level of enzyme at 72h (after liver damage)} - \text{level at 0 time} \times 100}{\text{Level at 0 time}}.$$

Curative activity was calculated as follows:

$$\frac{\text{Level of enzyme at 72h} - \text{level at 7 days (after liver damage)} \times 100}{\text{Level at 72h}}.$$

Cytotoxicity test

Cytotoxicity of 80% ethanol extract of the plant was tested [17] as follows:

(1) The total ethanol extract of the plant was tested for cytotoxic activity against the following human tumor cell lines in the National Cancer Institute (Cairo).
   (a) HEPG2 (liver carcinoma cell line).
   (b) HCT116 (colon carcinoma cell line).
   (c) HELA (cervix carcinoma cell line).
   (d) HEP2 (larynx carcinoma cell line).
   (e) MCF7 (breast carcinoma cell line).

(2) The successive extracts of *K. grandifoliola* C.DC. leaves were tested for cytotoxic activity against HCT116 (colon carcinoma cell line) for which the total ethanol extract showed higher activity.
   (a) Cells were plated in a 96-well plate (10^4 cells/well) for 24 h before treatment with the total ethanol extract of the plant to allow attachment of the cells to the wall of the plate.
   (b) Different concentrations of the extract under test (0, 1, 2.5, 5, and 10 μg/ml) were added to the cell monolayer, with triplicate wells being prepared for each individual dose
   (c) Monolayer cells were incubated with the extract of the plant for 48 h at 37°C, in 5% CO_2.
   (d) After 48 h, cells were fixed, washed, and stained with sulforhodamine B stain (Sigma, Germany).
   (e) Excess stain was washed away with acetic acid and the attached stain was recovered with Tris-EDTA buffer (Sigma).
   (f) Color intensity was measured in an ELISA reader. The relation between surviving fraction and the plant extract concentration was plotted to get the survival curve of each tumor cell line after treatment. Potency was compared with the reference drug Doxorubicin.

Evaluation of the antioxidant activity of the total ethanol extract

The antioxidant activity of the total ethanol extract of *K. grandifoliola* C.DC. leaves was assessed by measuring its ability to scavenge the free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) using electron spin resonance (ESR) spectroscopy [18].

A solution of DPPH (5×10^{-4} mol/l) in methanol was prepared. 0.5 mg/mL of the extract in methanol and 0.5 mg/mL of Ascorbic acid in methanol were prepared.

Volumes of 1 ml DPPH solution and 1 ml MeOH were mixed and measured (the reference solution). Then, 1 ml DPPH solution and 1 ml MeOH solution

of the extract or ascorbic acid were mixed for 2 min and measured.

The ESR signal of DPPH in MeOH appeared, characterizing the free radical. The decrease in this signal after mixing with the solution of each extract is taken as an indication of the antioxidant activity, measured as double integration area (DIA):

$$\%\text{Inhibition} = \frac{[\text{DIA (DPPH)} - \text{DIA (DPPH + compound)}] \times 100}{\text{DIA (DPPH)}}.$$

Determination of the antioxidant activity of successive extracts by UV absorbance

The free radical scavenging activity of successive extracts was measured with DPPH according to the method of Shimada *et al.* [19]. The extracts were screened at 100 μg/ml, whereas the most potent active extracts (gave >90%) were assayed at 25–75 μg/ml. A volume of 1 ml of 0.1 mmol/l solution of DPPH in methanol was added to 3 ml of extract solution at different concentrations (25–75 μg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Absorbance was measured at 517 nm in a microplate reader. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

$$\text{DPPH scavenging effect (\%)} = 100 - \left[\left(\frac{A_0 - A_1}{A_0}\right) \times 100\right],$$

where A_0 was the absorbance of the control reaction, and A_1 was the absorbance in the presence of the sample [20].

Results and discussion

GC/MS of lipoidal matter

Thirty-three compounds were identified (Table 2) by GC/MS of unsaponifiable matter, representing 94.29% of its total content. The identified components comprise 55.75% unoxygenated compounds, 14.9% oxygenated compounds, 9.99% steroidal/triterpenoidal compounds, and 13.6% miscellaneous compounds. Hentriacontane (11.98%) is the major compound in the unsaponifiable fraction.

Fatty acid methyl esters

Twelve fatty acid methyl esters were identified, representing 80.72% of the total saponifiable matter (Table 3). From Table 3, it can be concluded that hexadecanoic acid, 9,12,-octadecadienoic acid, and 9,12,15-octadecatrienoic acid represent the major components (31.68, 21.17, and 17.54%, respectively). Saturated fatty acids represent 39.6% of the total fatty acid content, whereas monounsaturated, diunsaturated, and triunsaturated fatty acids represent 2.41, 21.17, and 17.54% of the total fatty acid content, respectively.

Table 2 GC/MS analysis of unsaponifiable matter from *Khaya grandifoliola* leaves

Compound	RR_t[a]	%Peak area	Molecular weight
n-Decane	0.268	2.8	142
5-Isopropyl-3,3,-dimethyl-2-methylene-2,3-dihydrofuran	0.301	6.01	152
7a-methyl-3-methylene, hexahydrobenzofurane-2-one	0.375	1.29	166
Tetradecane	0.381	1.08	198
Neopentylidene cyclohexane	0.465	1.44	152
2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	0.474	3.03	182
2-Octadecene	0.582	1.82	112
Formamide, *N,N*-diphenyl	0.583	3.32	197
2-Pentadecanone,6,10,14-trimethyl	0.612	8.76	268
Heptadecene	0.644	1.83	238
Heptadecanol	0.665	2.16	256
Nonadecane	0.710	4.5	268
Neophytadiene	0.728	1.68	278
Eicosane	0.732	3.89	282
Heineicosane	0.735	3.12	296
Docosane	0.737	1.34	310
Tricosane	0.740	1.10	324
Tetracosane	0.743	2.21	338
4,8,12,16-Tetramethylheptadecan-4-olide	0.806	4.04	324
Pentacosane	0.819	1.92	352
Heptacosane	0.898	2.64	380
Octacosane	0.925	1.29	394
Squalene	0.94	1.87	410
Triacontene	0.969	1.68	420
Triacontane	0.977	2.93	422
Hentriacontane	1	11.98	436
Ethylcholestra,5,23-diol	1.01	4.42	432
Dotriacontane	1.024	1.29	450
Tritriacontane	1.04	2.74	464
Stigmast-3,5-diene	1.100	1.54	396
Stigmast,3,5-diene-7-one	1.102	1.82	410
Stigmast,4-ene-3-one	1.11	1.2	412
β-Sitosterol	1.15	1.01	414

GC/MS, gas chromatograph coupled with a mass spectrometer; [a]RR_t: Retention time relative to hentriacontane (R_t = 53.32 min).

Compound I

^{1}H-NMR: δ (500 MHz, $CDCl_3$) 0.96 (3H, d, J = 6 Hz, Me-21), 0.89 (3H, t, Me-29), 0.85 (3H, d, J = 7 Hz, Me-26), 0.87 (3H, d, J = 7 Hz, Me-27), 0.72 (3H, S, Me-18), 1.02 (3H, s, Me-19), 3.18 (1H, m, H-3), 4.27 (1H, d, J = 8, H-1′), 5.489 (1H, br.s., H-6) ppm.

^{13}C-NMR: (125 MHz, $CDCl_3$), 140.4 (C-5), 121.2 (C-6), 76.7 (C-3), 56.2 (C-14), 55.4 (C-17), 49.6 (C-9), 45.1 (C-24), 41.8 (C-13), 39.7 (C-12), 38.8 (C-4), 36.8 (C-1), 36.2 (C-10), 35.5 (C-21), 33.3 (C-22), 31.4 (C-7), 31.4 (C-8), 29.3 (C-25), 29.2 (C-2), 28.7 (C-16), 26.0 (C-23), 25.4 (C-15), 22.64 (C-28), 20.6 (C-11), 19.1 (C-19), 19.0 (C-27), 18.9 (C-26), 11.7 (C-18), 11.7 (C-29), 100.8 (C-1′), 76.9 (C-3′), 76.8 (C-5′), 73.5 (C2′), 70.1 (C-4′), 61.1 (C-6′). Compound

II gave same spectral data signals in NMR analysis as compound I, except that another olifinc signal appeared at 136 ppm in ^{13}C, and 4.93 ppm in ^{1}H-NMR referred to additional double bond C22=C23 of stigmasterol.

Compounds I and II were obtained as white powder, and displayed red spots after being sprayed with 20% H_2SO_4. R_f's were 0.2 and 0.3, respectively, on TLC silica gel plate using chloroform/methanol 8 : 2 as the developing system.

Comparison with previously published data [21,22] confirmed the identity of compounds I and II as β-sitosterol-3-*O*-β-D-glucopyranoside and stigmasterol-3-*O*-β-D-glucopyranoside, respectively. This is the first isolation of both compounds from *K. grandifoliola* C. DC.

fx1
(I) β-Sitosterol-3-*O*-β-D-glucopyranoside.

fx2
(II) β-Stigmasterol-3-*O*-β-D-glucopyranoside.

Toxicity and hepatoprotective activity

The total ethanol extract of *K. grandifoliola* C.DC. leaves was found to be safe up to 5 g/kg body weight. The hepatoprotective activity of the tested extracts is presented in Tables 4–6.

Table 3 GC/MS analysis of fatty acids methyl esters from *Khaya grandifoliola* leaves

Compound	RR_t[a]	%Peak area	Molecular weight
Tetradecanoic acid methyl ester (methyl myristate)	0.73	1.63	242
Undecanedioic acid dimethyl ester	0.78	0.16	244
Pentadecanoic acid methyl ester	0.87	0.66	254
14-Methyl pentadecanoic acid methyl ester (14-methyl pentadecylate)	0.966	0.22	270
9-Hexadecenoic acid methyl ester (methyl palmitoleate)	0.986	1.35	268
Hexadecanoic acid methyl ester (methyl palmitate)	1	31.68	270
14-Methyl hexadecanoic acid methyl ester (14-methyl methyl palmitate)	1.10	0.48	284
8-Heptadecenoic acid methyl ester	1.15	0.40	282
Heptadecanoic acid methyl ester (methyl margarate)	1.73	0.90	284
9,12-15-Octadecadienoic acid, methyl ester (methyl linolenate)	1.28	17.54	292
9,12-Octadecatrienoic acid, methyl ester (methyl linoleiate)	1.30	21.17	294
Octadecanoic acid, methyl ester (methyl stearate)	1.356	4.63	298

GC/MS, gas chromatograph coupled with a mass spectrometer; [a]RR_t: Retention time relative to hexadecanoic acid methyl ester (R_t = 10.30 min).

Table 4 Effect of total ethanol and successive extracts of *Khaya grandifoliola* C.DC. leaves (100 mg/kg body weight) and silymarin drug on serum enzyme level [AST (μg/l)]

Groups	Time					
	0	7 days before CCl_4	72 h after CCl_4	% of increase	7 days after CCl_4	% of decrease
Control 1 ml saline	28.7 ± 0.4	28.2 ± 0.5	139.6 ± 5.9[b]	386.4	158.7 ± 6.2[a]	13.6
Total alcohol extract	29.8 ± 0.7	29.1 ± 0.8	88.6 ± 3.6[b]	197.3	64.7 ± 2.4[a,b]	26.9
Petroleum ether extract	32.4 ± 0.9	31.2 ± 1.1	96.4 ± 0.8[b]	179.5	71.8 ± 1.9[a,b]	25.5
Chloroform extract	33.1 ± 1.1	31.8 ± 1.1	71.6 ± 3.7[b]	116.3	56.3 ± 2.7	21.3
Ethyl acetate extract	32.8 ± 1.2	30.1 ± 0.9	62.3 ± 3.1[b]	89.9	41.9 ± 2.4[a,b]	32.7
Ethanol extract	31.2 ± 1.1	30.5 ± 0.7	81.3 ± 3.9[b]	160.5	61.2 ± 2.7[a,b]	24.7
Silymarin 25 mg/kg	31.6 ± 1.1	29.8 ± 0.6	51.3 ± 2.6[b]	62.3	30.9 ± 0.8[b]	39.7

CCl_4, carbon tetrachloride; [a]Significantly different from 72 h after CCl_4 at $P < 0.01$; [b]Significantly different from time point 0 at $P < 0.01$.

Table 5 Effect of total ethanol and successive extracts of *Khaya grandifoliola* C.DC. leaves (100 mg/kg body weight) and silymarin drug on serum enzyme level [ALT (μg/l)]

Groups	Time					
	0	7 days before CCl_4	72 h after CCl_4	% of increase	7 days after CCl_4	% of decrease
Control 1 ml saline	31.2 ± 0.9	30.9 ± 0.8	142.3 ± 6.2[a]	356.1	156.8 ± 6.8[a,b]	10.2
Total alcohol extract	32.4 ± 1.1	30.8 ± 0.9	59.2 ± 2.6[a]	82.7	55.9 ± 1.7[a,b]	5.5
Petroleum ether extract	28.7 ± 0.6	28.1 ± 0.7	82.3 ± 2.8[a]	186.7	61.7 ± 1.4[a,b]	25.0
Chloroform extract	39.8 ± 0.6	29.5 ± 0.7	63.2 ± 2.6[a]	58.7	46.1 ± 2.2[a,b]	27.0
Ethyl acetate extract	33.4 ± 1.1	32.6 ± 1.2	58.2 ± 2.8[a]	74.2	39.4 ± 1.7[a,b]	32.3
Ethanol extract	44.6 ± 0.8	33.9 ± 0.7	73.5 ± 3.1[a]	64.8	56.8 ± 2.3[a,b]	22.7
Silymarin 25 mg/kg	27.9 ± 0.4	27.1 ± 0.5	46.3 ± 2.1[a]	65.9	29.7 ± 0.8[b]	35.8

CCl_4, carbon tetrachloride; [a]Significantly different from time point 0 at $P < 0.01$; [b]Significantly different from 72 h after CCl_4 at $P < 0.01$.

All extracts showed protective effect when compared with the control group. The ethyl acetate extract exhibited the highest liver protective activity, as detected from the lower percentage of increase of AST level at 72 h after liver damage from the level at time point 0 (89.9%), followed by chloroform extract (116.3%), as compared with the control group (386 %) and the silymarin group (62.3%).

The ethyl acetate extract exhibited the highest curative activity to the damaged liver, as detected from the higher percentage of decrease in AST level at 7 days after liver damage from the level at 72 h (32.7%), followed by total ethanol extract (26.9%), petroleum ether extract (25.5%), and ethanol extract (24.7%), as compared with the control group (13%) and the silymarin group (39.7%).

The chloroform extract exhibited the highest protective activity to the liver, as detected from the lower percentage of increase in ALT level at 72 h after liver damage from the level at time point 0 (58.7%), followed by ethanol extract (64.8%) and ethyl acetate (74.2), as compared with the control group (356 %) and the silymarin group (65.9%).

The ethyl acetate extract exhibited the highest curative activity to the damaged liver, as detected from the higher percentage of decrease in ALT level at 7 days after liver damage from the level at 72 h (32.3%), followed by chloroform extract (27.0%), petroleum ether extract (25.0%), and ethanol extract (22.7 %), as compared with the control group (10 %) and the silymarin group (35.8%).

The ethyl acetate extract exhibited the highest protective activity to the liver, as detected from the lower percentage of increase in ALP level at 72 h after liver damage from the level at time point 0 (98.7%), followed by chloroform extract (404.1%), as compared with the control group (992 %) and the silymarin group (149.3%). The ethylacetate extract exhibited the highest curative activity to the damaged liver as detected from the higher percentage of decrease in ALP level at 7 days from the level at 72 h (48.4%), followed by chloroform extract (39.9%), petroleum ether extract (34.5%), ethanol extract (29.4%), and total ethanol (27.7%), as compared with the control group (18.8%) and the silymarin group (62.0).

In-vitro cytotoxic activity

The results of cytotoxic activity of total ethanol extract and successive extracts from *K. grandifoliola* C. DC. leaves are summarized in Table 7.

The results of Table 7 show that the total ethanol extract of the plant exhibited potent cytotoxic activity against HCT116 (colon carcinoma cell line) and MCF7 (breast carcinoma cell line), compared with doxorubicin, followed by HEPG2 (liver cell carcinoma) and HEP2 (larynx). The successive extracts were effective against HCT116 (colon carcinoma cell line).

Table 6 Effect of total ethanol and successive extracts of *Khaya grandifoliola* (C.DC.) leaves (100 mg/kg body weight) and silymarin drug on serum enzyme level [ALP (μg/l)]

Groups	Time					
	0	7 days before CCl_4	72 h after CCl_4	% of increase	7 days after CCl_4	% of decrease
Control 1 ml saline	7.1 ± 0.1	7.2 ± 0.1	72.6 ± 3.1[b]	922.5	85.8 ± 2.9[a,b]	18.18
Total alcohol extract	7.2 ± 0.1	7.1 ± 0.1	37.1 ± 1.4[a,b]	415.2	26.8 ± 0.7[a,b]	27.7
Petroleum ether extract	7.5 ± 0.1	7.4 ± 0.1	48.9 ± 1.8[b]	552.0	36.1 ± 1.5[a,b]	34.5
Chloroform extract	7.2 ± 0.1	7.3 ± 0.1	36.3 ± 2.4[b]	404.1	21.8 ± 0.9[a,b]	39.9
Ethyl acetate extract	7.4 ± 0.1	7.1 ± 0.1	28.7 ± 1.6[b]	98.7	14.8 ± 0.4[a,b]	48.4
Ethanol extract	7.4 ± 0.1	7.2 ± 0.1	41.4 ± 0.9[b]	459.4	29.2 ± 0.4[a,b]	29.4
Silymarin 25 mg/kg	7.3 ± 0.1	7.1 ± 0.1	18.2 ± 0.4[b]	149.3	6.9 ± 0.1[a]	62.0

CCl_4, carbon tetrachloride; [a]Significantly different from 72 h after CCl_4 at $P < 0.01$; [b]Significantly different from time point 0 at $P < 0.01$.

Table 7 IC_{50} (μg/ml) results of total ethanol extract and successive extracts of *Khaya grandifoliola* C.DC. leaves compared with doxorubicin on different tumor cell lines

Tumor cell line	IC_{50}					
	Total ethanol extract	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Doxorubicin
HEPG2	3.36	—	—	—	—	0.6
HELA	7.58	—	—	—	—	0.85
MCF7	2.68	—	—	—	—	0.7
HCT116	2.68	2.36	2.24	2.28	2.86	0.7
HEP2	3.36	—	—	—	—	0.4

IC_{50}, dose of the drug that reduces survival to 50 %.

The chloroform extract showed high cytotoxic activity against HCT116 (colon carcinoma cell line), followed by ethyl acetate, petroleum ether, and ethanol extract.

Determination of antioxidant activity

The antioxidant activity of the total ethanol extract and successive extracts and rutin, which was previously isolated [7] from *K. grandifoliola* C.DC. leaves, was determined by ESR and UV absorbance, respectively.

The results from recording the DIAs of DPPH by ESR were calculated after the addition of the ethanolic extract and are illustrated in Table 8. The results from absorbance of DPPH by UV were calculated after the addition of the tested extracts and are illustrated in Table 9.

It could be concluded that the total ethanol extract of the plant has potent free radical scavenging activity (100% inhibition) compared with ascorbic acid (100%).

It could be concluded that rutin free radical scavenger activity showing 100% inhibition at 50 and 100 μg/ml concentration, compared with Ascorbic acid, followed by ethyl acetate and ethanol extracts, which may be attributed to flavonoids and other phenolics in the extracts.

The cytotoxic activity of the petroleum ether and chloroform extracts could be attributed to its hydrocarbon and sterol contents. β-Sitosterol inhibits HT-29 human colon cancer cell growth [23]. An in-vivo micronucleus test showed that β-sitosterol inhibited the mutagenicity of tetracycline by 65.3% at a dose of 0.5 mg/kg mouse. At the same dose, it did not exhibit chromosome-breaking activity [24]. Derivatives of cholesterol are known to exhibit potent cytotoxic effects against different cell types [25]. The cytotoxic effect of oil containing fatty acids and sterols was reported [26].

Table 8 Antioxidant activity of the total ethanol extracts of *Khaya grandifoliola* C.DC. leaves by ESR

Extract	Concentration (mg/ml)	Double integration area (DIA)	Inhibition%
DPPH	0.197	757 178	—
Total ethanol extract	0.5	0	100
Ascorbic acid	0.5	0	100

DPPH, 1,1-diphenyl-2-picrylhydrazyl.

Table 9 Antioxidant activity of successive extracts and rutin of *Khaya grandifoliola* C.DC. leaves by UV absorbance

Concentration (μg/ml)	%Inhibition of free radicals			
	Rutin	Ethanol	Ethyl acetate	Ascorbic acid
100	100	89.6	92.5	100
50	100	75.4	81.9	100
25	90	38.8	47.7	100
12.5	61	24.8	31.2	97
3	39	—	—	45
0.75	19.3	—	—	15

UV, ultraviolet.

The hepatoprotective effect of the chloroform and ethyl acetate extract may be attributed to their chemical constituents. It has been previously demonstrated [27] that a mixture of β-sitosterol and α-amyrin exhibits protective activities against liver damage induced by CCl_4. Hashem *et al.* [7] reported the isolation of rutin and scopoletin-arabinofuranoside from the ethanol extract of *K. grandifoliola* C.DC. leaves and the isolation of quercetin-3-*O*-glucosides, quercetin-3-*O* rhamnoside, and quercetin from ethyl acetate extract. Antioxidants such as quercetin are thought to play an important role in protecting cells from oxidative stress induced by reactive oxygen species [28]. Quercetin has a hepatoprotective effect upon chronic administration in rats with CCl_4-induced fibrosis [29]. Rutin prevents the CCl_4-induced increase in serum enzymes, confirming its hepatoprotectivity [30]. Pretreatment with rutin improves the effects induced by radiation, including serum liver enzyme (ALP, AST, and ALT) activities [31]. Thus, the hepatoprotective activity of the plant may be attributed to rutin, quercetin, and β-sitosterol, which are the major compounds in the extracts.

The significant cytotoxic activity of the polar fraction could possibly be attributed to its oxidative DNA damage, a known risk factor of cancer. Quercetin has been shown to interact with some receptors, particularly an aryl hydrocarbon receptor, which is involved in the development of cancers induced by certain chemicals. Quercetin modulate several signal transduction pathways, which are associated with the processes of inflammation and carcinogenesis. Rodent studies have demonstrated that dietary administration of this flavonol prevents chemically induced carcinogenesis. Dietary quercetin is, therefore, a promising agent for cancer prevention [32].

The in-vitro effect of quercetin against human carcinoma of the larynx (HEP-2) and sarcoma-180 (S-180) cell lines was studied by Elangovan *et al.* [33]. Priming HEP-2 cells with quercetin increased cisplatin-induced apoptosis by 16.3% through the mitochondrial pathway, which improved the efficacy of chemotherapy for head and neck cancer [34].

Conclusion

The *K. grandifoliola* C.CD. leaf extract is safe, showing no toxicity profile. The total and successive extracts exhibit significant protective and curative hepatic effects

as compared with control; however, they were variable, which suggests a synergistic effect of phytoconstituents. The extracts of leaves showed potent cytotoxic and antioxidant activities. *K. grandifoliola* C.DC. leaves contain chemical compounds with hepatoprotective, cytotoxic, and antioxidant activity.

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Conflicts of interest

There are no conflicts of interest.

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