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Phytochemical and biological Investigation of *Carica papaya* Linn. Leaves cultivated in Egypt (Family Caricaceae)

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Abstract

Carica papaya Linn. is well known for its nutritional and therapeutic properties. Growing, successfully in Egypt environment, we analyzed content of essential minerals, aqueous ethanol extract of leaves for lipid constituents and main alkaloidal component, examination of *in-vivo* potential as antioxidant and hepatoprotective, anticancer and antimicrobial activity. Leaves and fruits are good source of micro and macro essential minerals. Squalene, 2-hexadecen-1-ol, β - sitosterol, campesterol and palmitic acid were the major identified lipid constituents. Carpaine, alkaloid was isolated and identified by different spectroscopic means. 80% Ethanol extract of leaves retained reduced glutathione which were highly compatible to those receiving vitamin E, it retained liver serum levels to which were compatible to the results of those receiving silymarin and demonstrated improvement in deleterious effect induced by CCl₄. The extract had moderate effect, while carpaine showed high activity against ovary carcinoma. It had moderate antibacterial activity against *Escherichia coli*. This plant has highly economic importance.

Keywords: *Carica papaya* leaves, Lipid compounds, Aqueous ethanol extract, Carpaine, CHO-k1 cell line (ovary carcinoma), *Escherichia coli*

Introduction

The plant kingdom possesses a wide variety of natural substances that have showed significant health properties with few or no documented side effects. These medicinal plants are rich in phytochemical compounds that can provide valuable therapeutic effects for various metabolic diseases such as diabetes, adiposity and cardiovascular complications, offering a source of dietary ingredients that affect human physiological function [1, 2, 3] and are increasingly used in the manufacture of food or consumed directly as raw ingredients, being less harmful than synthetic products. So, it is worthy to develop some edible compositions with prospects for the expansion of an alternative ways of managing many diseases in the future.

Papaya (*Carica papaya* L.), a member of the family Caricaceae, is an herbaceous plant that is usually cultivated for its edible pleasant fruit which provides good nutritional value and easy digestion. Different parts of *Carica papaya* (leaves, barks, roots, fruits and seeds) are used in folk medicine to treat a broad range of diseases [4, 5]. The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power, such as ascorbic acid, α -tocopherol, flavonoids, cyanogenic glucosides and glucosinolates [6, 7].

Upon continued study of the potential role of *Carica papaya* leaves constituents, we aim to analyze the lipid constituents, contents of minerals in comparison with ripe fruits, and isolate and purify the active principle, carpaine. *In-vivo* examination of leaves aqueous ethanol extract potential as antioxidant and hepatoprotective. In addition, evaluate its activity against various cancer cell lines as well as its potential antimicrobial activity.

Materials and methods Plant material

Fresh leaves of *Carica papaya* Linn. were collected in November-2016 from the plant cultivated in Al-Aziziyah Gardens Resort, Cairo Alexandria Desert Road, kilo 63, Giza. The plant was kindly authenticated by Dr. Kamal Zayed, professor of Ecology, Faculty of science, Cairo University. The collected leaves were dried in an air oven at 40 °C for three days, then grounded using an electric mill into coarse powder and kept in tightly-closed containers. A voucher specimen was kept at the herbarium of NRC. A sample of ripe fruits was also taken, lyophilized and stored.

Extraction and isolation of carpaine

2kg of powdered leaves were defatted by extraction with petroleum ether (b.r. 40-60 °C) in a Soxhlet apparatus. The combined petroleum ether extract was evaporated. The defatted marc was extracted with 80% aqueous ethanol by percolation in a room temperature. After complete exhaustion, the extract was filtered. The combined filtrates were dried under pressure. The residue (300 g) was divided into two equal portions. One portion was subjected for biological investigation. For the preparation of the total alkaloids, the second portion of 80% ethanol residue was dissolved and taken with 2N hydrochloric acid, kept overnight in a refrigerator, filtered, then extracted with successive portions of ether. The aqueous acidic solution was shaken with excess zinc dust, filtered, and the filtrate was rendered alkaline (pH 10) with concentrated ammonium hydroxide. The total alkaloids were extracted with chloroform, and the chloroform layer was evaporated in vacuo. A pale yellowish brown residue was obtained, dissolved in acetone and left overnight to give pure crystals which were analyzed and identified as carpaine using ESI /MS and NMR Joel-500MHz for ¹H-NMR.

Phytochemical characterization of lipid content

2.5g of petroleum ether extract was subjected to saponification according to the method reported by [8]. The unsaponifiable matter weighed 0.28g. The unsaponifiable fraction was analyzed by GC/MS whereas the fatty acids content weighed 0.8g. The free fatty acids obtained from saponification were methylated according to the method by [8]. The fatty acid methyl esters were analyzed using GLC technique Preparation of lipid constituents.

GC/MS analysis

The analysis was carried out using a trace GC ultra-Gas chromatographs (Thermo scientific corp., USA) coupled with a thermo mass spectrometer detector (ISQ single Quadrupole Mass spectrometer). The injected volume was 0.2µl where helium was used as a carrier gas at a flow rate of 1.0ml/ min and a split ratio of 1:10 using the following temperature program: 80°C for 1min, rising at 4.0°C/ min to 300°C, and held for 1min. The injector and detector were held at 240°C. Mass spectra were obtained by electron ionization at 70eV, using a spectral range of m/z 40–450.

GLC analysis

The analysis was performed using Gas chromatography-mass spectrometry (GC-MS) methodology using Hewlett Hp 6890 series, gas chromatograph fitted with an injector, and fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness), composed of a mass spectrophotometer GC-MS Finnigan mat SSQ7000 and electron impact ionization detector(EI) with an ion energy of 70eV. The injector temperature was 230 °C. The oven temperature was held at 60 °C for 1 min, then increased to 230 °C at a heating rate of 5 °C/min, then to 300 °C at 10 °C/min and held for 1 min. The carrier gas was helium at a flow rate of 1.5 ml/min. Injection volume was 1µl. The identification of compounds was achieved by comparing the retention times with those of authentic compounds and by matching their mass spectra with National Institute of Standards and Technology 05 MS (NIST) library data.

Chemical evaluation of leaves and fruits

Micro elements (Cu ++, Fe++, Mn++ and Zn++) as well as macro elements (K+, Ca++, N+and P+) of leaves were

determined adopting atomic absorption method and compared with those of fruits [9]. Biological evaluation of *Carica papaya* Linn. leaves

Antioxidant and hepatoprotective activities Animals

Thirty-six male albino rats of Sprague Dawley strain of 130-140g b.wt. were obtained from the Animal House of the Horticulture Research Institute, Giza, Egypt. The procedure in this study was subjected to the scrutiny of the NRC (FWA 00014747) Animal Ethics Committee and was approved before beginning of the experiment (certificate no. 19087). Animals were acclimatized to conventional laboratory conditions, and maintained at room temperature (20-25 °C) with free access to water and food. Using Biodiagnostic kit, the antioxidant activity was investigated using glutathione reductase assay [10], and biodiagnostic kits for assessment of serum enzymes: AST, ALT and ALT were obtained from Biodiagnostic Company.

Experimental design

The rats were randomly divided into six experimental groups. All groups were injected intra- peritoneal with carbon tetrachloride (150mg/kg b.wt.) to induce liver damage except for the negative group [11]. A positive control, liver damaged group, the negative group receiving a daily oral dose of 1 ml saline, the third and fourth groups received a daily dose of 100 mg and 200mg/kg b. wt., respectively, of 80% ethanol extract of *Carica papaya* leaves. An antioxidant reference group, receiving 7.5mg/kg vitamin E, and for hepatoprotection, the last group received a daily dose of 25 mg/kg silymarin. After one week, blood samples were collected from retro orbital venous plexus through eyes canthus of anaesthetized rats and allowed to coagulate for 30 minutes at 37°C. Serum was isolated by centrifugation. ALT and AST were determined by method described by [12], while ALP was determined according to method of [13]. The data obtained were analyzed using Student's test [14]. The liver of different groups was removed and fixed in 10% formal saline, 5µm thick paraffin sections were stained with haematoxlin and eosin and investigated histologically by light microscope [15].

Cytotoxic assay Cell culture

All materials and reagents for the cell cultures were purchased from Lonza (Verviers, Belgium). CHO-k1 cell line (ovary carcinoma), HepG-2 cell line (hepatocellular carcinoma) and HCT-116 cell line (colon carcinoma) were obtained from VACSERA Tissue Culture Unit, Giza, Egypt. The cell lines were maintained as monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 4mM L-glutamine, 100 U/mL penicillin, and 100µg/mL streptomycin sulfate. The monolayers were passaged at 70–90% confluence using a trypsin- EDTA solution. All the cells were incubated in a humidified atmosphere at 37 °C with 5% CO₂.

Cytotoxicity assay

Cytotoxicity studies were performed using a modified MTT (3-[4, 5]-2, 5-diphenyltetrazolium bromide) assay [16]. Exponentially growing tumor cells were seeded at a density of 5x10⁴ cell/well in Corning® 96-well tissue culture plates, incubated for 24 h. 100µL of increasing concentrations of the leaves extract, isolated carpaine and vinblastine Sulfate (reference) were added in culture medium (final DMSO concentration in medium = 0.5% v/v). About six vehicle

controls with media or 0.5% DMSO were run for each 96 well plate as a control. After 48 h of incubation, MTT solution in PBS (5 mg/mL) was added to each well, including the untreated control, after which the incubation was resumed for further 4 h. The formation of intracellular formazan crystals (mitochondrial reduction product of MTT) was confirmed by a phase contrast microscopic examination. Then the medium was removed, and 50 μ L of DMSO was added to each well to dissolve formed formazan crystals with shaking for 10 min (200 rpm). Dissolved crystals were quantified by reading the absorbance at 590 nm (OD) on a microplate reader (Sunrise™ microplate reader, Tecan Austria GmbH, Grödig, Austria). The cell viability was determined by comparing the average OD values of the control wells with those of the samples (quadrate to octuplet treatments), both represented as % viability [control (0.1% DMSO only) = 100%]. The 50% inhibitory concentration (IC₅₀), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using GraphPad Prism software (San Diego, CA. USA) [17, 18].

Antimicrobial assay Micro-organisms

Two bacterial strains were used in this study, *Escherichia coli*, and *Staphylococcus aureus*. The fungi strains, *Aspergillus flavus* and *Candida albicans* were also used.

Agar-well diffusion assay

Antibacterial and antifungal tests of 80%ethanolic extracts of *Carica papaya* leaves were performed by agar well diffusion method as described by [19]. Known concentrations of the extract were prepared from stock solution (100 mg/ml DMSO). To the solidified nutrient agar medium, 5mm wells were cut. An un-inoculated plate served as media control and test bacteria swab inoculated was used as an organism control. A plate swab inoculated with test bacteria with a well filled with DMSO was used as treatment control. To the test plates an aliquot of each concentration of the extract was filled after

swab inoculation of test bacteria. All the plates were allowed to incubate at 37 C for 24-48h in bacteriological incubator. After incubation the clear zone around the well was measured in mm scale defined as zone of inhibition of the test bacteria. The antibacterial activity was evaluated by measuring the zones of inhibition against the test micro-organisms. All tests were repeated three times. Minimum inhibitory concentrations (MICs) of *Carica papaya* leaves extract was determined according to the method described by [20] in sterile 96-well microplates with a final volume in each microplate well of 200 μ L. The MIC was defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth after incubation.

Statistical analysis

The design of all experiments was completely randomized and the obtained data were statistically analyzed using standard error (\pm SE) according to the method described [21].

Results

Identification of isolated carpaine

Carpaine was crystallized from 80% ethanol extract by purifying the crude alkaloidal mixture. The pure crystals obtained were identified on the basis of the mass, and 1H-NMR spectral data which were confirmed by comparison with that reported in the literature [22].

Investigation of lipoidal constituents

The results obtained from GC/MS analysis for unsaponifiable fraction of *Carica papaya* L. leaves extract indicated that it consists mainly of a mixture of a series of n-alkanes and sterols (17.83% and 60.43%, respectively). Squalene (11.07%) and 2-hexadecen-1-ol (9.33%) were the major n-alkanes constituents, whereas β -sitosterol (22.46%) and campesterol (7.25%) were the major identified sterols, as shown in Fig 1, Table 1.

Table 1: GC/MS analysis of unsaponifiable matter (USM) of *Carica papaya* leaves:

No.	Compound	Retention Time (Rt) (Min.)	RRT*	% Area	Molecular ion peak	Base peak
1	1-Nonadecene	34.52	0.51	0.61	280	70
2	2-pentadecanone 6,10,14 trimethyl	37.63	0.56	4.09	268	58
3	Unidentified	40.75	0.60	0.59	--	--
4	2-hexadecen-1-ol	44.12	0.65	9.33	296	71
5	4,8,12,16 tetramethyl heptadecan-4-olide	49.54	0.73	2.59	324	99
6	Unidentified	53.20	0.79	0.56	--	--
7	Squalene	58.20	0.86	11.07	410	69
8	1-heptatriacotanol	60.07	0.89	1.21	536	69
9	Unidentified	62.37	0.92	1.99	--	--
10	Unidentified	63.66	0.94	0.81	--	--
11	Campesterol	65.71	0.97	7.25	400	43
12	Stigmasterol	66.15	0.98	4.49	412	55
13	β -Sitosterol	67.65	1	22.46	414	43
14	Stigmasta-5,24(2)-dien-3-ol, (3, 24Z)-	67.92	1.003	5.17	412	55
15	,1 -Cyclolanost-24-en-3-ol, acetate, (3)-	69.57	1.03	7.23	468	69
16	9,19-cycloanostan-3-ol 24- methylene-,(3a',24S)	70.93	1.05	2.76	440	69
Total identified hydrocarbons					17.83%	
Total identified sterols					60.43%	
Total unidentified compounds					3.95%	

RRT*= Relative retention time of β -Sitosterol=1 with RT=67.65 USM (unsaponifiable matter).

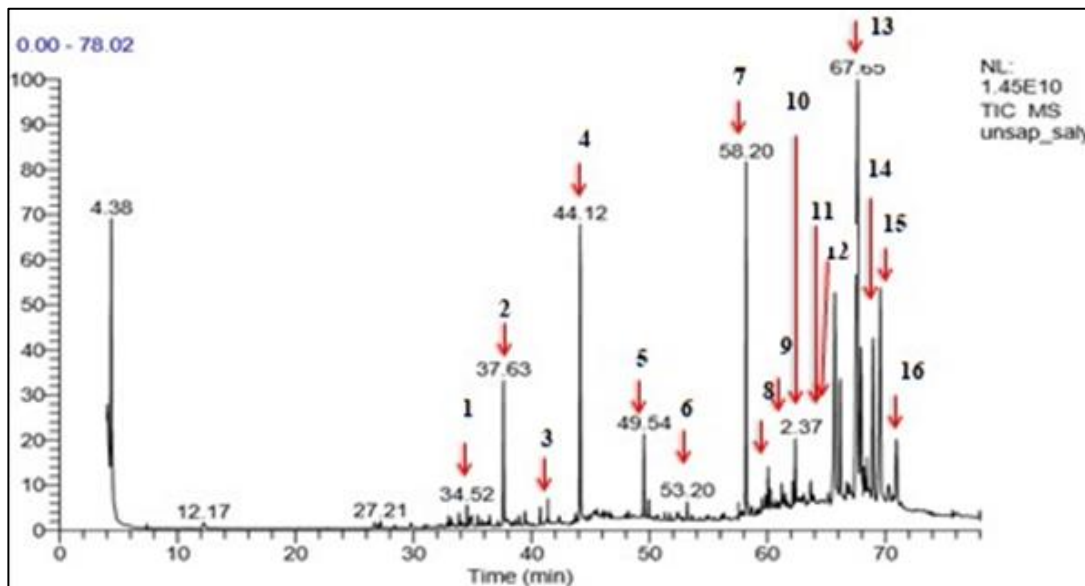


Fig 1: GC/MS analysis of unsaponifiable matter (USM) of *Carica papaya* L. leaves

GLC analysis of the fatty acids of *Carica papaya* leaves extract, as methyl esters showed palmitic acid (33.17%) as the major identified fatty acid and also revealed the presence of

α -linolenic acid (13.67%) and oleic acid (13.22%) as shown in Fig 2, Table 2. r

Table 2: GLC analysis of fatty acids methyl esters of *Carica papaya* leaves

No.	Compound	Retention time Rt	RRT*	Area%
1	Myristic acid (C 14)	16.97	0.79	2.40
2	Pentadecylic acid (C 15)	19.11	0.89	2.44
3	Palmitic acid (C 16)	21.38	1	33.17
4	Palmitoleic acid (C 16:1)	22.20	1.03	4.96
5	Stearic acid (C 18:0)	25.07	1.17	2.23
6	Oleic acid (C 18:1)	25.35	1.18	13.22
7	Linoleic acid (C 18:2)	26.16	1.22	9.87
8	α -Linoleic acid (C 18:3)	27.13	1.26	13.67
9	Arachidic acid (C 20:0)	29.04	1.35	6.53
10	Behenic acid (C22:0)	30.67	1.43	1.14
Total identified fatty acid				89.68%
Total identified saturated fatty acid				47.94%
Total identified unsaturated fatty acid				41.74%
Total unidentified compounds				10.31%

*RRT= Relative retention time (related to Palmitic acid; RRT =1).

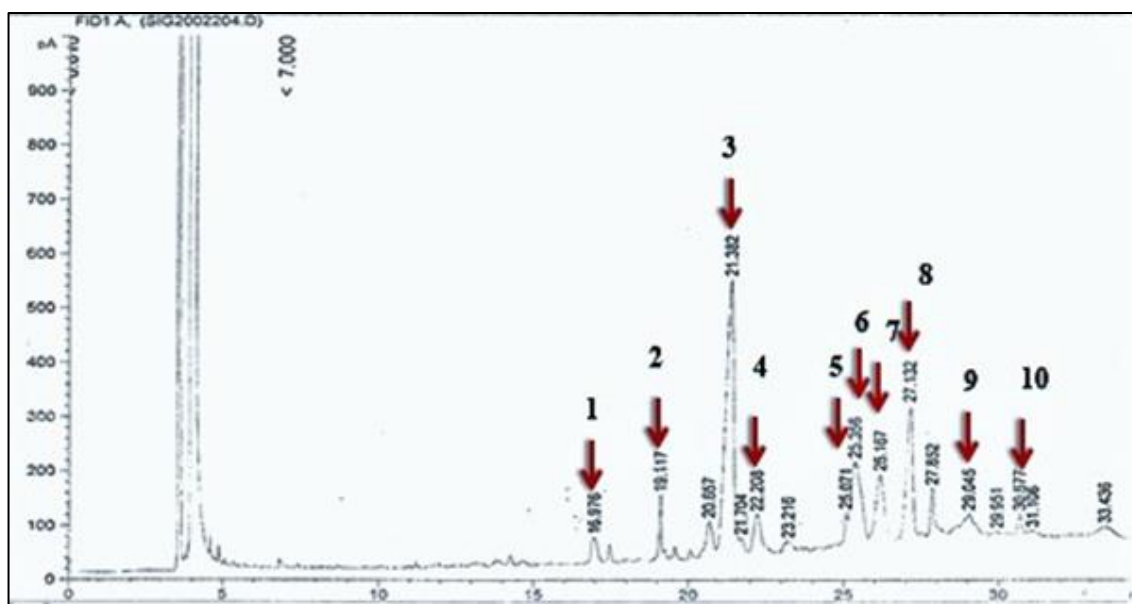


Fig 2: GLC analysis of fatty acids of *Carica papaya* L. leaves as methyl esters.

Chemical evaluation of leaves and fruits

The results revealed that the leaves and fruits of *Carica papaya* L. are good sources of micro and macro elements as shown in Table 3. Leaves and fruits contain an adequate quantity of Zinc and 4.80mg/kg) and iron (28.98 and 11.70 mg/kg) respectively. Copper is present in a reasonable amount (0.54 mg/kg) in leaves and higher amount (1.96 mg/kg) in fruits. Manganese is present in adequate amount (1.60 and

0.80mg/kg) in leaves and fruits, respectively. Calcium is present in sufficient amount (2500mg/kg) in leaves and lower amount (600 mg/kg) in fruits. Phosphorus is present in reasonable amount in leaves (4100mg/kg) but in much lower amount (70mg/kg) in fruits. Potassium content is present in a high amount (1370 and 1429mg/kg) for both leaves and fruits, respectively.

Table 3: Micro/Macro elements of *Carica papaya* L. on dry/wt basis (mg/kg).

Mineral	Leaves	Fruits
Micro elements		
Copper	0.54	1.96
Manganese	1.60	0.80
Zinc	4.60	4.80
Iron	28.98	11.70
Macro elements		
Calcium	2500	600
Potassium	1370	1429
Phosphorus	4100	70
Sodium	ND	ND

ND: not determined

Antioxidant and hepatoprotective activities

The normal rats of the negative group showed reduced glutathione (36.8 ± 0.9 mg/dl) which was dropped to (22.3 ± 0.4 mg/dl) with the liver damaged rats of the positive group. The rats receiving the daily dose of 100 mg and 200mg/kg b. wt., respectively, of 80% ethanol extract of *Carica papaya* leaves retained reduced glutathione to (35.1 ± 0.6 and 36.7 ± 1.1 mg/dl respectively) which were highly compatible to those receiving vitamin E (36.6 ± 0.8 mg/dl) as shown in Table 4.

Table 4: Antioxidant activity of 80% ethanol extract of *Carica papaya* leaves and vitamin E in albino rats (n=6) using glutathione method.

Group	Reduced glutathione (mg/dl) Mean \pm S.E	% of Change
Negative group (Vehicle 1 ml saline)	36.8 ± 0.9	-
Positive group (CCl4)	$22.3 \pm 0.4^*$	39.4
+ Ethanol extract (100 mg/kg)	35.1 ± 0.6	4.62
+ Ethanol extract (200 mg/kg)	36.7 ± 1.1	0.27
+ Vitamin E. (7.5 mg/kg)	36.6 ± 0.8	0.54

* Statistically significant different from control group at $P < 0.01$.

Regarding the hepatoprotective activity, the normal rats of the negative group showed ALT, AST and ALP serum levels of (68.3 ± 2.4 , 54.2 ± 1.7 and 62.7 ± 2.4 u/L, respectively) which were significantly increased in the positive group to (227.6 ± 5.9 , 238.2 ± 6.1 and 181.2 ± 4.3 u/L, respectively). The rats receiving the daily dose of 100mg/kg b. wt of 80% ethanol extract of *Carica papaya* leaves decreased ALT, AST

and ALP serum levels to (172.6 ± 2.8 , 143.7 ± 3.4 and 91.7 ± 1.8 u/L, respectively), and those receiving the daily dose of 200 mg/kg b. wt retained ALT, AST and ALP serum levels to (69.7 ± 2.2 , 68.4 ± 2.5 and 65.2 ± 1.3 u/L, respectively) which were compatible to the results of those receiving silymarin (25 mg/kg), where ALT, AST and ALP serum levels are (64.9 ± 2.1 , 51.2 ± 3.9 and 64.6 ± 3.1 u/L, respectively) as shown in (Table 5).

The histological examination of the liver cells (hepatocytes) of negative group rats showed normal architecture Fig 3a, while in positive group, the rats treated with CCl4 showed loss of hepatocellular architecture with massive vacuolar and fatty changes (steatosis), dilated and congestion blood vessel and thick fibrous band around the vessel, inflammation (leukocyte infiltration), and congested blood sinusoids Fig 3b. The histological changes in the liver of rats treated with the extract at dose level of 100mg/kg showed slight improvement in pathological changes in the form of no fatty changes (steatosis), no collagen fibers but vacuolar changes and leukocyte infiltration around portal vein and some hepatocyte appeared necrotic were noticed Fig 3c.

In the group of rats treated with the extract at dose level of 200mg/kg, the examination demonstrated more improvement in deleterious effect induced by CCl4, although some inflammation (leukocyte infiltration) around portal vein still present Fig 3d. Notice that, examination of liver sections in group of rats treated with silymarin showed normal hepatic architecture and the injury induced by CCl4 was regulated by the administration of silymarin Fig 3e.

Table 5: ALT, AST and ALP activities in CCl4 treated rats, given 80% ethanol extract of *Carica papaya* leaves (100 and 200 mg/kg) and silymarin in male albino rats (n=6).

Treated groups	ALT (u/L) Mean \pm S.E	AST(u/L) Mean \pm S.E	ALP (u/L) Mean \pm S.E
Negative group (Vehicle 1 ml saline)	68.3 ± 2.4	54.2 ± 1.7	62.7 ± 2.4
Positive group (CCl4)	$227.6 \pm 5.9 \bullet$	$238.2 \pm 6.1 \bullet$	$181.2 \pm 4.3 \bullet$
+ Ethanol extract (100 mg/kg)	$172.6 \pm 2.8^* \bullet$	$143.7 \pm 3.4^* \bullet$	$91.7 \pm 1.8^* \bullet$
+ Ethanol extract (200 mg/kg)	$69.7 \pm 2.2^*$	$68.4 \pm 2.5^*$	$65.2 \pm 1.3^*$
+ Silymarin (25 mg/kg)	$64.9 \pm 2.1^*$	$51.2 \pm 3.9^*$	$64.6 \pm 3.1^*$

* Statistically significant different from CCl4 control group at $P < 0.01$.

* Statistically significant different from vehicle at $P < 0.01$.

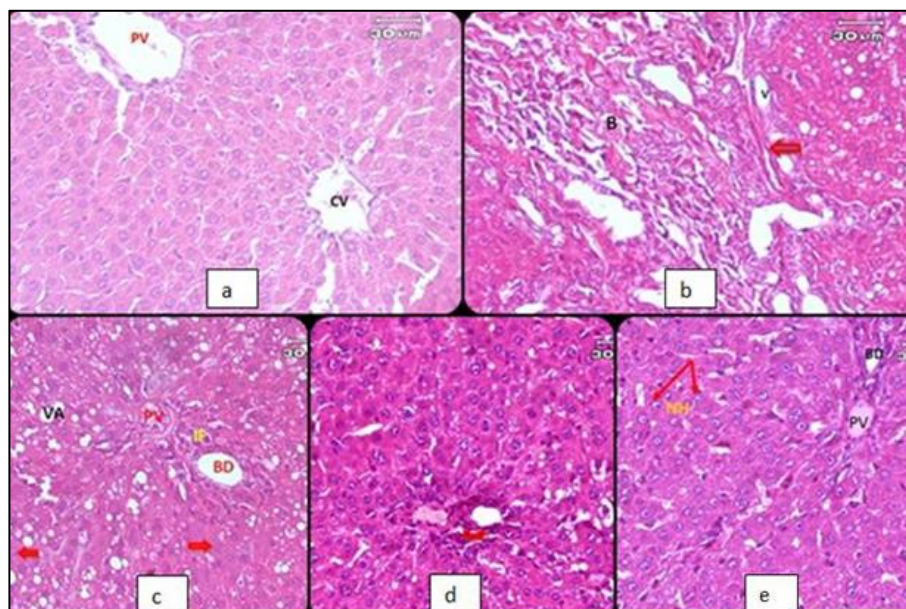


Fig 3: Photomicrographs of liver sections of the rats' groups.

a: liver section of a negative group showing the general appearance of the hepatic lobules. Notice the central vein (CV) and the peripherally situated portal area with branches of portal vein (PV), b: liver section of positive group (another filed), showing thick fibrous bands (B) formed of many fibroblast, and collagen fibers (red arrow). The bands run in septa between the hepatocytes nodules and extent around the blood vessels (v), c: liver section of rat treated with CCl₄ and extract at 100 mg/kg showing slight improvement in histological changes although vacuolar changes (VA) and leukocyte infiltration (IF) around portal vein (PV) and bile duct (BD) and some hepatocyte appeared necrotic (red arrow), d: liver section of rat treated with CCl₄ and extract at 200 mg/kg showing more improvement in pathological changes in the form of the hepatocyte more or less appeared normal but some inflammation around bile duct were detected, e: liver section of rat treated with CCl₄ and silymarin showed normal hepatic architecture (NH), normal portal vein (PV) and bile duct (BD).

Cytotoxic assay

The present study aimed to evaluate 80% ethanol extract of leaves and isolated carpaine against three types of human cancer cell to determine their effective concentration causing 50% loss of cell viability (IC₅₀ values). The results revealed that the extract had a variable activity, showing moderate effect against CHO-k1 cell line (ovary carcinoma), IC₅₀ value = $178 \pm 4.2 \mu\text{g/ml}$, when compared to vinblastine sulfate which showed IC₅₀ value = $9.65 \pm 0.74 \mu\text{g/ml}$, weak effect against HepG-2 cell line (hepatocellular carcinoma) and HCT-116 cell line (colon carcinoma) giving IC₅₀ values = 87.1 ± 3.9 and $112 \pm 1.9 \mu\text{g/ml}$, respectively, on comparison with vinblastine sulfate which showed IC₅₀ values = 2.93 ± 0.22 and 3.5 ± 0.23 , respectively, on the two cell lines. Carpaine showed a high activity against CHO-k1 cell line (ovary carcinoma), IC₅₀ value $28.4 \pm 3.2 \mu\text{g/ml}$.

Antimicrobial assay

The antibacterial screening (expressed as inhibition zone diameter, mm/mg) revealed that the 80% ethanol extract of leaves of *Carica papaya* L. had moderate antibacterial activity against the gram negative bacteria, *Escherichia coli*

(13 mm/mg) when compared to ampicillin (25 mm/mg) and weak activity against the gram positive bacteria, *Staphylococcus aureus* (14 mm/mg) compared to ampicillin (21 mm/mg). It showed weak antifungal activity against *Candida albicans*, 9 mm/mg), and no activity against *Aspergillus flavus* compared with amphotericin B (21 mm/mg) the antifungal standard used.

Discussion

Plants consumed by humans contain thousands of dietary polyphenols [23], the effect of which are of great value because of their antioxidative properties. These compounds quench reactive oxygen and nitrogen species generated in biological systems, thus break the free radical chain reaction of lipid peroxidation [24]. Compounds of these plants can be used to balance the oxidative stress associated in many diseases [25, 26]. Papaya, like other consumed fruit contains a number of functional compounds essential in promoting human health [27, 28]. The present work aimed at appraising the chemical profile of leaves of papaya (*Carica papaya* L.) cultivated in Egypt with reference to its prospective bioactive components which assess its antioxidant activity, to encourage impending applications in disease prevention.

The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power such as saponins, cardiac glycosides, flavonoids, phenolic acids, ascorbic acid, tocopherol and alkaloids [29, 30, 31, 32]. Previous studies suggest that the alkaloids in papaya leaves could contribute to health benefits [33]. In this study, carpaine alkaloid was isolated from the aqueous ethanol extract of leaves with a considerable percentage (0.06). The GC-MS analysis of the petroleum ether extract revealed that squalene (11.07%), β -sitosterol (22.46%) and campesterol (7.25%) were the major components of unsaponifiable fraction. The GLC analysis of the petroleum ether extract revealed that the major fatty acid methyl ester was palmitic acid (33.17%) which exhibits antioxidant activity [34]. Papaya also contains various bioactive minerals that are valuable for the proper physiology of human health, because of their nutritional importance [35]. Our results revealed that the leaves and fruits contain reasonable amounts of mineral micro and macro elements.

It is a challenge to find the ways of treatment for the common liver diseases, the management of which is in need to innovate pharmaceuticals having more effectiveness and less toxicity [36]. About 0% of the world's population especially developing countries have employed plant material as traditional medication for healthcare, due to the variety of chemical compounds for liver protection [37].

The antioxidant activity of *Carica papaya* leaves extracts was assayed *in-vivo* by glutathione reductase assay and the results showed that 200mg/kg b. wt. was efficient as vitamin E to hamper the oxidative effect of CCl₄. While the results of the hepatoprotective properties of the extracts showed significant reduction of ALT, AST and ALP serum levels with the administration of 200mg/kg b. wt. to challenge those observed by silymarin.

Previous studies have examined the effect of *Carica papaya* as hepatoprotective by the reduction of the transferases [38, 39]. However, our study included its protective potential on liver histology and hepatic profile, where treatment with 200mg/kg b. wt. of the extract improved liver anatomy with normalization of histopathological damage observed after CCl₄ induced hepatotoxicity. Notice that, this hepatoprotective potential might be related to the antioxidant effect played by phenolic compounds [40, 6].

Evaluation of the growth inhibitory activity of *Carica papaya* leaves extract against the tested human cancer cell lines indicates that it inhibits cell growth and stimulates anti-tumor effects. Notice that, the CHO-k1 cell line (ovary carcinoma) was the most sensitive to the treatment with the extract (IC₅₀ value = 178 ± 4.2 µg/ml), and highly sensitive to carpaine treatment (IC₅₀ value 28.4 ± 3.2 µg/ml) when compared to reference which showed (IC₅₀ value = 9.65 ± 0.74 µg/ml).

These results are in accordance with previous evidence that *C. papaya* with abundant bioactive phytochemicals has the potential to be of use in combating cancer [41]. Antimicrobials of plant origin effective in the treatment of infectious diseases and in the same time mitigating many of side effects often associated with synthetic agents have been considerably discovered [42]. Among these, different plant parts of *Carica papaya* have been investigated and the leaf extract was found to be the most potent [43, 44]. Our study dealt with the activity of the leaves extract against different pathogens and it agreed with previous studies [45] that it has potential natural antimicrobial compounds with possibility for medicinal purposes applications.

Conclusion

In conclusion, the present study showed that *Carica papaya* L. is a rich source of minerals and considerable amounts of phenolic compounds [46, 6], which appear to be responsible for its significant antioxidant potential and may contribute to the prevention and cure of diseases. Our results encourage wide cultivation and local consumption. Further investigation to determine the potential use of papaya leaves in the functional food industry could be considered.

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Competing interests

The authors declare that they have no competing interests.

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