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GC-MS Analysis of the Essential Oil of Leaves and Rhizomes of *Alpinia zerumbet* (Pers.) B.L. and *In Vitro* Hepatoprotection and Cytotoxicity Study.

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ABSTRACT

The investigated essential oils of rhizomes and leaves of *Alpinia zerumbet* (Pers.) B. L. Burtt & R. M. Smith, were evaluated using GC-MS analysis. The yield of the essential oil of the leaves was 0.45% V/W, while that of the rhizomes was 0.25% V/W. The major compounds found in the leaves oil were: 1,8-cineole (24.21%) and terpinen-4-ol (19.67%), while the major compounds found in the rhizomes oil were terpinen-4-ol (17.15%) and turmerone (15.62%). GLC investigation of the unsaponifiable fraction indicates the presence n-pentacosane (26.35%), n-octane (8.89%) and β-sitosterol (4.01%). GLC investigation of the fatty acids methyl esters indicate the presence of saturated fatty acids: tetradecanoic acid (10.13%) and unsaturated fatty acids: oleic acid (9.87%). The hexane fraction showed no hepatotoxicity till concentration 1000 µg/ml. The hexane exerted 90% hepatoprotection at a concentration 25 µg/ml. The aqueous extract achieved 50% cytotoxicity at 50 µg/ml on the U937 cells (human leukemic monocyte lymphoma); the methanol extract did not show any cytotoxic activity till the concentration of 100 µg/ml. The aqueous and methanolic extracts had no cytotoxic effect on PBM cells (peripheral blood mononuclear cell), till a concentration of 100 µg/ml.

Keywords: *Alpinia zerumbet* (Pers.) B.L., essential oil, hepatoprotection, cytotoxicity

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INTRODUCTION

Family Zingiberaceae is a very important family, it constitutes a vital group of rhizomatous medicinal and aromatic plants characterized by the presence of volatile oils and oleoresins [1]. It comprises around 53 genera and over 1200 species native to tropical regions [2,3]. Among them is the genus Alpinia which constitutes 250 species, making it the largest genus of the Zingiberaceae [2,3].

Alpinia zerumbet (Pers.) B. L. Burtt and R. M. Smith (syn.: *Alpinia nutans* Roscoe and *Alpinia speciosa* Schum), family Zingiberaceae, is commonly known as shell flower [2,4]. It is a perennial ginger growing widely in the subtropics and tropics. It is used in folk medicine for its anti-inflammatory, bacteriostatic and fungistatic properties [5].

Our present work aims for the evaluation of the hepatoprotective activity of the essential oil of the leaves and rhizomes of the plant growing in Egypt, it deals also with the evaluation of its antioxidant and hepatoprotective activity.

MATERIAL AND METHODS

Plant material

The rhizomes of *Alpinia zerumbet* (Pers.) B. L. Burtt & R. M. Smith, used in this study were cultivated in El-Orman garden, Cairo, Egypt and were introduced to the garden of the national research center, Cairo, Egypt, where they yielded well flourished flowering plants. The rhizomes were collected from the same place. The leaves and rhizomes of the plant under investigation were air dried, powdered and kept in tightly closed amber coloured glass containers and protected from light at low temperature as possible.

Essential oil

500g of each of fresh leaves and rhizomes of *Alpinia zerumbet* were subjected to hydrodistillation (E.P. 2005) [6]. The two oils obtained were separately dried over anhydrous sodium sulfate and kept in a dark glass container at 4˚C till analysis.

Apparatus and techniques

Unsaponifiable matter and Fatty acids methyl esters

Gas liquid chromatography Hewellett HP 6890 series. Gas liquid chromatography (GLC) analysis of the unsaponifiable matter was carried out according to the following conditions: Capillary column HP-1(methyl siloxane) 30 m length, I.d. 0.53 mm, film thickness 2.65 µm, Temperature programming:- Initial Temperature 60˚C, Initial Time 2 min., program rate 10˚C/min., Final temp. 280˚C, Final time 30 min., Injection temperature. 260˚C, Detector (FID), T=300˚C, Flow rate of carrier gas N_2: 30 ml/min., H_2: 35 ml/min., Air: 300 ml/min. Gas liquid chromatography of the fatty acid methyl ester was carried out using the following conditions: Column HP-5 (phenyl methyl siloxane) 30 m length/ 320 µm and film thickness: 0.25 µm. Flow rate of carrier gases nitrogen and hydrogen was 20 ml/ min. and that of air was 200 ml/ min. The oven temperature was 70°C, injector temperature was 250°C, Detector temperature was 280°C and using temperature programming starting with 110°C for 2 min., increased to 240°C by the rate of 4°C/ min. then isothermally for 25 min.

About 75 g. of the air dried powdered rhizomes of *Alpinia zerumbet* was exhaustively extracted with n-hexane [7]. The hexane extract was evaporated and the residue (1.76 g) was dissolved in boiling acetone (100 ml), cooled and the amorphous formed precipitate was separated out. The acetone soluble fraction was saponified (N/2 alc. KOH) and the unsaponifiable matter (0.37 g) was separated. The aqueous alkaline solution left after the separation of the unsaponifiable matters, was acidified with 5% Sulphuric acid (H_2SO_4) and the liberated fatty acids were extracted with diethyl ether till exhaustion. The ethereal extract containing the free fatty acids were combined, washed with distilled water (2 × 50 ml) till free from acidity and dehydrated over anhydrous sodium sulphate and filtered. The solvent was evaporated to dryness under reduced pressure. The weight of the residue was 0.56g.

The 0.56 g of the fatty acid fraction was methylated by dissolving in 10 ml methanol and 5 ml of boron trifluoride (BF_3). The mixture was refluxed for 5 minutes on a boiling water bath. The mixture was cooled and the methanol was evaporated under reduced pressure. The residue was diluted with 20 ml distilled water and then extracted with diethyl ether (5 × 30 ml). The combined ethereal extracts were washed with distilled water till neutral to litmus paper, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure to dryness. The weight of the residue was 0.51g representing 51% of the total lipoidal matter. The fatty acids methyl esters were subjected to GLC analysis.

Essential oil

The essential oil was subjected for Gas chromatography/ Mass spectrometry (GC/MS analysis). GC/MS analysis of the oil was carried out using Aglient 6890 Gas Chromatograph , Aglient Mass Spectrometric Detector, with a direct capillary interface and fused silica capillary column HP-5MS (30 m × 320 µm × 0.25 µm film thickness). Samples were injected under the following conditions:

Helium was used as carrier gas at approximately flow rate of 1.0 ml/min., pulsed splitless mode. The solvent delay was 3 min. and the injection size was 1.0 µl. The GC oven temperature was programmed at an initial temperature of 60°C for 3 min, then heated to 260 ° C at rate of 8°C/min. Detector and injector temperatures were set at 280°C and 250°C, respectively. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The identification of the chemical constituents of the oil was determined by their GC retention times, retention indices and interpretation of their mass spectra were confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database with those of authentic samples or published data [8]. The retention indices were calculated for all of the volatile constituents using a homologous series of C_8-C_{20} n-alkanes.

Free radical scavenging activity (DPPH)

The decrease of the absorbance at 516 nm of the DPPH solution after the addition of the sample (hexane fraction) was measured in a glass cuvette. An aliquot of 0.1 mL methanol solution of DPPH was mixed with methanolic solution of the samples, so that the relative concentration of the hexane extract versus the stable radical in the cuvette was 0.13 then the solution with tested sample was shaken vigorously. The absorbance was monitored after 20 min. after being kept in the dark against a blank of methanol without DPPH. All tests were run in duplicate and averaged. The antioxidant activity of these samples were compared with trolox where:

$$\% \text{ RSA} = 100 \text{ X} \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}$$

Results are expressed as radical scavenging activity (%RSA) as shown in table (4)

Rat hepatocytes monolayer culture

Isolation and preparation of rat hepatocytes monolayer culture

A primary culture of rat hepatocytes was prepared according to the Seglen method [9], which was modified by Kiso *et al.,*[10] using a Wistar male rat (250-300g). The rat was obtained from the animal house of the NRC (National Research Centre, Cairo). Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide For Care and Use of Laboratory Animals [11].

IC_{50} determination on rat hepatocytes monolayer culture

After 22-24h, the rat hepatocyte monolayer was washed twice with phosphate buffer saline (PBS). In order to determine IC_{50}, different concentrations were prepared for the hexane fraction (100-1000 µg mL^{-1}). After 2h of cells incubation with the extract, cell viability was determined using the MTT assay. The assay was performed according to the method of Mosmann, [12] modified by Carmicheal *et al*. [13]. Absorbance of formasan crystals produced by viable cells was read at 540 nm and 630 nm dual wavelength using the

Automatic Kinetic Microplate Reader (Labsystems Multiskan RC reader). The experiment was repeated three times, and the mean absorption of each concentration was calculated. The IC_{50} was graphically determined from the concentration that yielded an absorption coinciding with the 50% of cells that received no extract.

Evaluation of hepatoprotective activity

The primary rat hepatocyte monolayer was prepared according to the method described in (Seglen and Kiso) [9,10]. Different concentrations were prepared from the hexane fraction (12.5-100 µg/mL) using the serial dilution technique by dissolving in DMSO (1% maximum concentration). For each concentration, three replicates were carried out; in addition to positive control, that was 50 µg/mL Silymarin. The plate was incubated for 2h at 37˚C and 5% CO_2, then washed twice with PBS. A 200 µL of 25 mM paracetamol was added to each well. After 1h of cells incubation with the paracetamol, cell viability was determined using the MTT assay. The assay was performed according to the method of Mosmann [12] modified by Carmicheal *et al*. [13]. The concentration of the extract was able to protect the cells from the hepatotoxic effect of paracetamol by 100% , hence it was considered hepatoprotective.

Cytotoxicity assay on leukaemia cell lines:

Human leukaemia cell lines, namely histiocytic lymphoma cells U937 (ATCC CRL 1593) were maintained in RPMI 1640 medium (Sigma Chemical) supplemented with 1% (v/v) heat-inactivated Fetal calf serum (FCS), 100 units/ml penicillin G, 100 µg/ml streptomycin and 0.025 µg/ml amphotericin B, in an incubator at 37 ˚C and 5% CO_2.

200µl of leukaemia cells (U937), were seeded onto 96-well microplates, with a density of 0.25×10^5 cells/ml. For each well 1 µl of different concentrations (1, 3, 10, 30 &100 µg/ml) of the methanolic and aqueous extracts were added in triplicates. After 72 hours of the incubation in 5% CO_2 at 37˚C, a mixture containing 50 µl XTT [2,3-bis-(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-caboxanilide)] test solution (2 ml XTT + 40 µl electroncoupling reagent) was added to each well. After 72 hours of incubation at 37˚C in the 5% CO_2 incubator, the absorbance of the samples was measured at a wavelength of 450 nm and a reference wavelength of 650 nm using an ELISA reader (Multiskan EX Labsystem, Helsinki, Finland). The cytotoxicity of the extracts was determined by comparing the response of cell lines to the extracts with the response of cell lines to DMSO controls. Data are expressed as percentage of inhibition, calculated according to the following formula:

The 50% inhibition concentration (IC_{50}) was the concentration of the extracts that inhibited the growth of leukaemia cell lines by 50%.

Cytotoxicity assay on Normal Cells (peripheral blood mononuclear cell) PBMC

Washing buffer preparation

20 ml of sterile phosphate buffered saline (PBS) were taken from 500 ml bottle of sterile (PBS), and were put in 50 ml sterile falcon tube. The pH was adjusted to 7.2-7.4 after the addition of 2 mM tetra-ethylene glycol bis (2-aminoethylether)-N,N,N'N'-tetraacetic acid (EDTA). 0.5% Bovin serum albumin (BSA) (2.5g/500 ml) was then added to the solution. Then the solution was filtered back through 0.2 µm pore filter to the 500 ml bottle, and stored at 4°C.

Four sterile falcon tubes were prepared; each one contains 15 ml of Biocoll solution. The biocoll solution was warmed at room temperature.

Two sterile falcon tubes were prepared; each one contains 15 ml of the washing buffer. The washing buffer was warmed at room temperature.

50 ml of peripheral blood was withdrawn from the forearm vein of human volunteers brought from the Red Cross. 5ml 4.33% tri-Na-citrate was added to 50 ml fresh blood. Then the blood was mixed with the washing buffer in a ratio 1:1.

15 ml of the blood was overlayed carefully on the 15 ml of the Biocoll, then it was centrifuged at 700 g for 30 minutes at room temperature.

The plasma was sucked away carefully using 5 ml Gilson.

The white bands (PBMC) were recovered and were placed in two new falcon tubes, and were filled up to 40 ml with washing buffer. Then they were centrifuged at 200 g for five mins, this washing was repeated twice.

The PBMC were maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 100 units/ml penicillin G, 100 µg/ml streptomycin and 0.025 µg/ml amphotericin B. The assay was conducted on 2.5 $\times 10^6$ cells/ml PBMC (200µl/well) in a 96-well microplate, with 1 µl of various concentrations of samples (1, 3, 10, 30 &100 µg/ml), in triplicate. Culture plates were incubated at 37˚C for 72 hours in a 5% CO_2 incubator. At the end of incubation time, the cytotoxicity of extracts against PBMC was assayed with XTT method. Data were calculated according to the method described above.

RESULTS AND DISCUSSION

Table 1: Results of GLC analysis of the unsaponifiable matter of the rhizomes of *Alpinia zerumbet* (Pers.):

Identified components	Retention time	Relative Retention Time (RR_t)*	Relative area percentage
n-hexane (C-6)	4.457	0.21	3.00
n-heptane (C-7)	6.824	0.27	1.66
n-octane (C-8)	7.910	0.31	8.89
n-nonane (C-9)	8.604	0.34	5.21
n-decane (C-10)	9.971	0.39	3.16
n-undecane (C-11)	10.789	0.42	1.71
n-dodecane (C-12)	12.760	0.50	2.85
n-tridecane (C-13)	13.784	0.54	1.04
n-tetradecane (C-14)	14.217	0.56	2.84
n-pentadecane (C-15)	15.173	0.60	5.91
n-hexadecane (C-16)	16.432	0.65	1.37
n-heptadecane (C-17)	17.830	0.70	4.76
n-octadecane (C-18)	18.532	0.73	1.77
n-nonadecane (C-19)	20.975	0.83	2.39
n-eicosane (C-20)	22.073	0.87	1.91
n-uncosane (C-21)	22.641	0.89	1.41
n-pentacosane (C-25)	25.267	1	26.35
n-hexacosane (C-26)	25.878	1.02	0.65
n-heptacosane (C-27)	27.587	1.09	0.12
n-octacosane (C-28)	28.500	1.12	0.33
n-nonacosane (C-29)	29.210	1.15	0.16
Campasterol	33.398	1.32	0.43
Stigmasterol	34.657	1.37	1.97
β-sitosterol	36.390	1.44	4.01
α-amyrin	38.930	1.54	1.75
		Total identified hydrocarbons	77.48
		Total identified sterols	8.17
		Total unidentified compounds	14.35

* Retention time relative to (n-pentacosane). Retention time: 25.267

Unsaponifiable matter

GLC analysis of the unsaponifiable fraction proved to be a mixture of triterpenes, sterols and a series of hydrocarbons. Identification of the compounds was carried out by comparison of their retention times with the available reference compounds. The percentage of the unsaponifiable matter in the rhizomes of *Alpinia zerumbet* was 37% in relation to the lipoidal matter. GLC analysis of the unsaponifiable matter (table 1), revealed the presence of 25 identified components, representing 85.65% of the total unsaponifiable matter.

Concerning sterols, they constitute the minor percent in the unsaponifiable matter of the plant under investigation. They reach 8.17% in relation to the unsaponifiable matter. β-sitosterol was the major sterol in the unsaponifiable matter reaching 4.01%. α-amyrin was the major triterpene in the unsaponifiable matter reaching 1.75%. Hydrocarbons constitute the major percent of the unsaponifiable matter of the plant under investigation. They constitute 77.48% of the unsaponifiable matter. n-pentacosane was the major hydrocarbon (26.35%), followed by n-octane (8.89%) and n-pentadecane (5.91%).

Table 2: Results of GLC analysis of the fatty acid methyl esters of the rhizomes of *Alpinia zerumbet* (Pers.):

Identified components	Retention time	Relative Retention Time (RR_t)*	Relative Area percentage
Undeanoic acid $C_{11:0}$	10.258	0.71	0.58
Lauric acid $C_{12:0}$	11.475	0.80	8.40
Tridecanoic acid $C_{13:0}$	12.751	0.89	2.55
Tetradecanoic acid $C_{14:0}$	14.320	1	10.13
Pentadeanoic acid $C_{15:0}$	16.202	1.13	7.98
Palmitic acid $C_{16:0}$	16.802	1.17	4.35
Heptadecanoic acid $C_{17:0}$	19.105	1.33	2.59
Heptadecaeinoic acid $C_{17:1}$	19.658	1.37	2.47
Stearic acid $C_{18:0}$	20.945	1.46	4.63
Oleic acid $C_{18:1}$	21.243	1.48	9.87
Linoleic acid $C_{18:2}$	22.316	1.55	5.94
Linolenic acid $C_{18:3}$	23.276	1.62	3.01
γ-linolenic acid $C_{18:3}$	23.771	1.65	1.22
Arachidic acid $C_{20:0}$	24.574	1.71	2.87
Behenic acid $C_{22:0}$	28.478	1.98	1.07
Erucic acid $C_{22:1}$	28.585	1.99	1.15
Tricosanoic acid $C_{23:0}$	31.579	2.20	0.61
Lignoceric acid $C_{24:0}$	33.501	2.33	9.15
	Total identified fatty acids		78.57
	Total identified saturated fatty acids		56.11
	Total identified unsaturated fatty acids		22.46
	Total unidentified compounds		21.43

* Retention time relative to (tetradecanoic acid) Retention time: 14.320

Fatty acid methyl esters

GLC analysis of the fatty acid methyl esters proved to be a mixture of saturated fatty acid and unsaturated fatty acids. Identification of the compounds was carried out by comparison of their retention times with the available reference compounds. The percentage of the saponifiable matter of the plant under investigation was 56%. GLC analysis of the fatty acid methyl esters (table 2) revealed the presence of 18 components, representing 78.57% of the total fatty acids methyl esters. The percent of the saturated and the unsaturated fatty acids were 56.11% and 22.46%, respectively. The major saturated fatty acid is teradecanoic acid which constitutes 10.13%, while the major unsaturated fatty acid is oleic acid which constitutes 9.87%.

Essential oil

Quantitative analysis of the chemical composition of the investigated essential oils of the two samples are shown in Table (3). Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oil of the fresh leaves revealed the presence of 24 identified compounds, while that of the fresh rhizomes revealed the presence of 27 identified compounds. Chemical identification of the oil constituents was conducted based on their retention time, retention indices and mass spectral data, as well as by computer search of mass spectral databases.

The essential oil isolated from the fresh leaves of the plant under investigation comprises 24 identified components constituting 96.6% of the total oil composition in which 1,8-cineole is the major constituent, it constitutes 24.21% followed by Terpinen-4-ol which constitutes 19.67% of the total oil composition.

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Table 3: GC/MS analysis of the volatile oil of the leaves and rhizomes of *Alpinia zerumbet* (Pers.):

R.Rt	K.I.	Oil Constituent	Area % Leaves	Area % Rhizomes
0.81	1463.66	α- Thujene	5.79	
0.82	1470.11	α- Pinene	0.07	0.63
0.89	1506.7	Sabinene	16.28	1.78
0.96	1538.8	α- Phellandrene	0.10	0.24
0.94	1544.24	α- Terpinene	2.98	1.73
1	1554.47	1,8- Cineole	24.21	9.18
1.04	1575.77	γ- Terpinene	12.82	3.00
1.08	1590.62	Cis sabinene hydrate	0.29	
1.10	1596.09	Terpinolene	1.42	0.77
1.13	1615.18	Linalool		3.50
1.16	1627.76	Trans sabinene hydrate	2.69	
1.21	1654.5	Cis-P-2-Menthen-1-ol	0.55	
1.22	1657.35	Camphor		0.66
1.29	1691.9	Terpinen-4-ol	19.67	17.15
1.31	1702.63	Endo fenchyl acetate		2.56
1.39	1741.39	Cuminic aldehyde		2.43
1.40	1745.84	Bornyl acetate	0.12	
1.46	1771.88	Safrole		1.44
1.50	1788.01	α- Terpinyl acetate		1.83
1.54	1807.51	Daucene	0.14	
1.56	1816.11	Eugenol		4.23
1.60	1842.12	Methyl eugenol		1.53
1.62	1852.98	Trans-caryophyllene	4.53	5.10
1.63	1863.71	Aromandendrene	0.04	
1.66	1881.75	α-humulene	0.43	0.80
1.69	1894.91	Ar-curcumene		0.52
1.70	1903.39	γ- Curcumene		0.31
1.72	1913.29	Valencene		0.62
1.72	1913.29	γ- Gurjunene	0.11	
1.74	1926.34	γ- Cadinene	0.80	1.66
1.76	1935.43	Selina-3,7(11)-diene	0.33	
1.79	1951.06	Elemicin		13.72
1.80	1960.49	E-Nerolidol	0.41	
1.84	1981.6	Caryophyllene oxide	2.18	1.38
1.87	1997.35	Carotol	0.19	1.71
1.95	2042.69	Ar- Turmerone		15.62
1.96	2045.41	α- Eudesmol	0.45	

The chromatogram of the essential oil isolated from the fresh rhizomes of the plant under investigation comprises 27 identified components constituting 94.36% of the total oil composition in which Terpinen-4-ol is the major constituent, it constitutes 17.15% followed by Ar-turmerone which constitutes 15.62% of the total oil composition.

The total oxygenated compounds constituted the highest percentage of the components in the essential oil of the fresh leaves and rhizomes of the plant under investigation. They constitute 50.76% and 76.94% in the oil of leaves and rhizomes, respectively.

The total oxygenated monoterpenes constituted the higher percentage of the components in the essential oil of the fresh leaves and rhizomes of the plant under investigation. They constitute 47.53% and 66.38% in the oil of leaves and rhizomes, respectively.

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Radical scavenging activity

Table 4: The radical scavenging effect of samples on DPPH radical

Tested samples	Mean absorbance. (n)*	Antioxidant activity %
Trolox	0.01	98.8
Hexane fraction	0.489	20

***n=3**

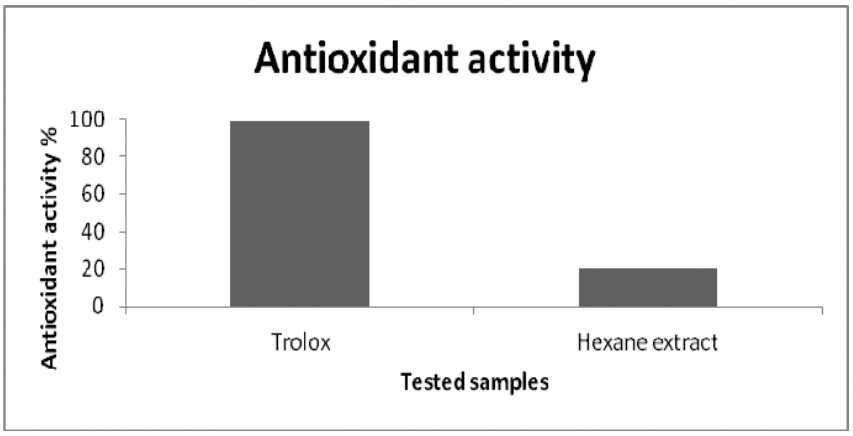

Figure 1: Total Antioxidant Activity of Hexane Extract in Comparison with Trolox

From the obtained results in table (4) and figure (1), it is clear that the hexane fraction exhibited low antioxidant activity (20%).

Hepatotoxicity

The assay was applied with a broad range of concentrations of the studied extract (from 125 to 1000 µg/mL) on the monolayer of rat hepatocytes. It revealed that the hexane fraction showed no hepatotoxicity till a concentration of 1000 µg/ml, as shown in tables (5) and figure (2).

Table 5: The hepatotoxicity of different concentrations of the hexane fraction of the rhizomes of *Alpinia zerumbet* (Pers.) B.L.

Sample conc. µg/ml	Mean absorbance percentage (n=3)
	Hexane Fr.
125	67
250	76
500	87
1000	96

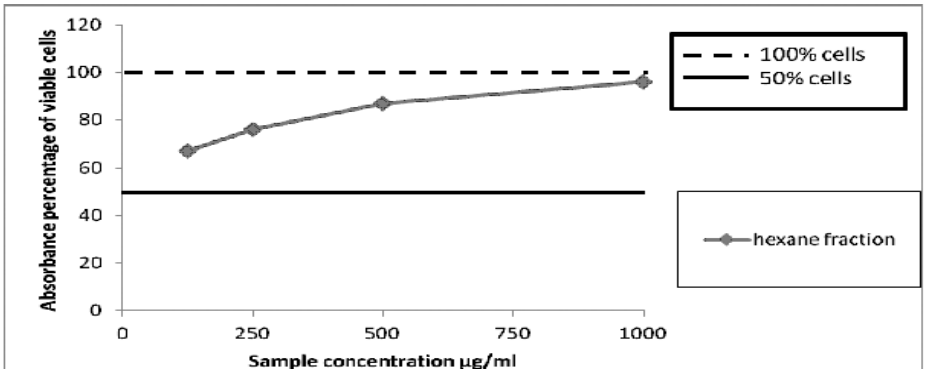

Figure 2: Viability of Monolayer of Rat Hepatocytes after 2 hrs Treatment with Different Concentrations of the Hexane Fraction Using MTT Colourimetric Assay. Each Point Represents the Mean (n=3)

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The assay was applied with a broad range of concentrations of the studied extracts (from 12.5-100 µg/ml) on monolayer of rat hepatocytes. It revealed that the hexane fraction exerted 90% hepatoprotection at a concentrations 100 µg/ml as shown in tables (6) and figure (3).

Table 6: The hepatoprotective activity of different concentrations of the hexane fraction of the rhizomes of *Alpinia zerumbet* (Pers.) B.L.

Sample conc. µg/ml	Mean absorbance percentage
	Hexane Fr.
12.5	72
25	89
50	89
100	90

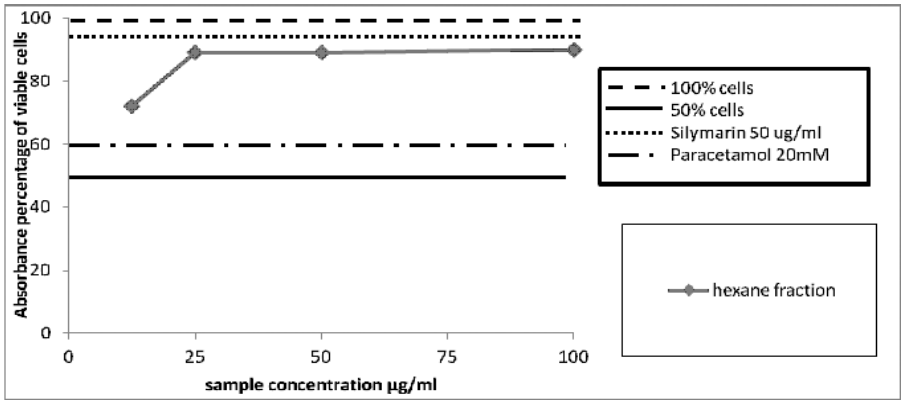

Figure 3: Viability of monolayer of rat hepatocyte after 2 hrs treatment with different concentrations of hexane fraction, followed by treatment with 20 mM paracetamol for 2hr. in comparison with 50 µg/ml silymarin as control using MTT colourimetric assay. Each point represents the mean (n=3)

Cytotoxicity Evaluation of different extracts of *Alpinia zerumbet* rhizomes on U937 and PBM Cells

The aqueous and methanol extracts of *Alpinia zerumbet* rhizomes were investigated for their cytotoxicity effect on U937 and PBM cells, where the results are as follow:

Cytotoxcity Assay on U937 Cells

The results revealed that the IC_{50} of aqueous extract of *Alpinia zerumbet* rhizomes is at 50µg/ml. The methanol extract sample of the plant did not show IC_{50} till a concentration of 100µg/ml, as shown in table (7) and figure (4).

Table 7: Cytotoxicity of Different Concentrations of *Alpinia zerumbet* (Pers.) rhizomes extracts on U937 Cells.

Sample Name	Sample concentration µg/ml	Absorbance at 450 nm			Percentage of Viable Cells
		Value 1	Value 2	Value 3	
Aqueous extract	0	723	714	750	100 ± 3.7
	1	881	806	812	115 ± 8.2
	3	668	654	592	78 ± 7.9
	10	659	749	641	76 ± 18.6
	30	680	602	656	71 ± 7.8
	100	506	378	362	7 ± 15.4
Methanolic extract	0	682	813	758	100 ± 12.5
	1	782	757	794	103 ± 3.6
	3	674	716	704	86 ± 4.1
	10	720	716	670	89 ± 5.3
	30	573	628	581	64 ± 5.6
	100	725	701	728	75 ± 2.8

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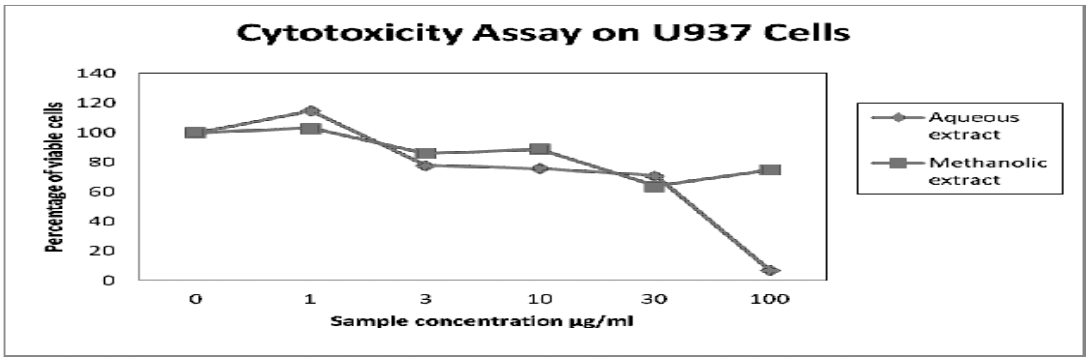

Figure 4: Viability of U937 cells after 72 hrs treatment with different concentrations of the methanolic and aqueous extracts, using XTT colourimetric assay. Each point represents the mean (n=3)

Cytotoxcity Assay on PBM Cells

The aqueous and the methanolic *Alpinia zerumbet* (Pers.) rhizomes, had no cytotoxic effect on the PBMC till a concentration of 100µg/ml, as shown in table (8) and figure (5).

Table 8: Cytototoxicity of different concentrations of *Alpinia zerumbet* (Pers.) rhizomes extracts on PBM cells

Sample Name	Sample concentration µg/ml	Absorbance at 450 nm			Percentage of Viable Cells
		Value 1	Value 2	Value 3	
Aqueous extract	0	745	749	694	100 ± 5.1
	1	692	674	750	94 ± 6.6
	3	659	630	717	87 ± 7.3
	10	746	734	673	95 ± 6.5
	30	751	675	684	87 ± 6.9
	100	554	749	784	95 ± 20.5
Methanolic extract	0	682	813	758	100 ± 12.5
	1	782	757	794	103 ± 3.6
	3	674	716	704	86 ± 4.1
	10	720	716	670	89 ± 5.3
	30	573	628	581	64 ± 5.6
	100	725	701	728	75 ± 2.

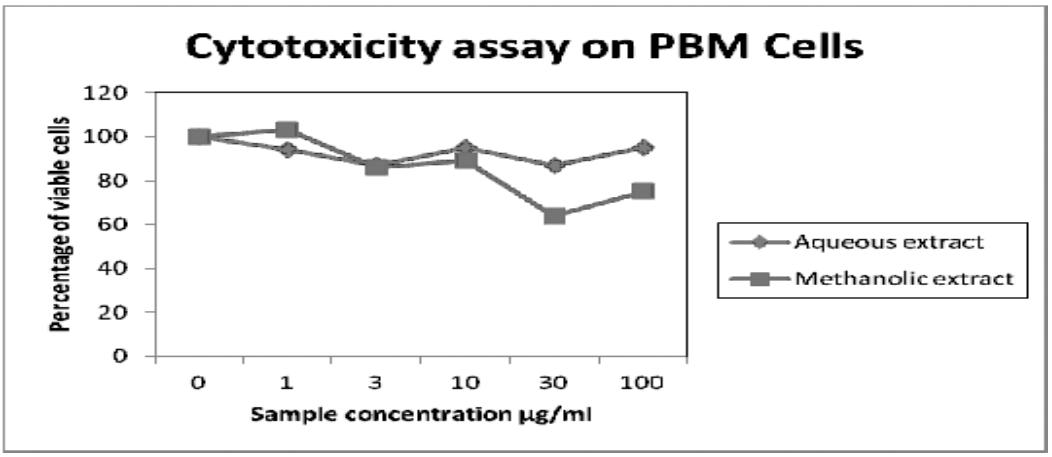

Figure 5: Viability of PBM Cells after 72 hrs treatment with different concentrations of the methanolic and aqueous extracts using XTT colourimetric assay. Each point represents the mean (n=3)

CONCLUSION

The percentage of the unsaponifiable matter in the rhizomes of *Alpinia zerumbet* was 37% in relation to the lipoidal matter, including 25 identified components, representing 85.65% of the total unsaponifiable matter. The essential oil of the fresh leaves revealed the presence of 24 identified compounds, while that of the fresh rhizomes revealed the presence of 27 identified compounds.

The hexane fraction exhibited low antioxidant activity (20%), showed no hepatotoxicity till concentration 1000 µg/ml and exerted 90% hepatoprotection at a concentration 25 µg/ml.

The aqueous extract achieved 50% cytotoxicity at 50 µg/ml on the U937 cells (human leukemic monocyte lymphoma); the methanol extract did not show any cytotoxic activity till the concentration of 100 µg/ml. Whereas, the aqueous and methanolic extracts had no cytotoxic effect on PBM cells (peripheral blood mononuclear cell), till a concentration of 100 µg/ml.

Statistical Analysis

Data were subjected to analysis of variance and treatment means were compared by an approximate Student's t-test ($P<0.05$). All treatments in experiments described consisted of three replicates.

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