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Research Article

Phytochemical and Biological Studies of Total Ethanol and Petroleum Ether Extracts of *Terminalia Bentzoe* (L.) Leaves

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ABSTRACT

Terminalia is a genus of *Combretaceous* plants extensively distributed in tropical and subtropical areas. Numerous species of this genus have been utilized for medicinal purposes because of their valuable phytoconstituents. This study aimed to compare between the phytoconstituents of the hydroalcoholic extract (HAE) of *Terminalia bentzone* (*T. bentzoe*) leaves as polar extract and its petroleum ether extract (PEE) as a nonpolar extract. Concerning this, total phenolics, flavonoids, proteins and carbohydrate content of the HAE were estimated. HPLC analysis of HAE revealed identification of nine phenolic acids, two flavonoids and one anthocyanin, they were gallic, ellagic, pyrogallol, catechin, chlorogenic acid, synergic acid, pyrocatechol, cinnamic acid, paraqumaric acid, caffeic acid, quercetin and rutin arranged according to the order of elution. Colum chromatographic separation of HAE resulted in the isolation of six compounds for the first time named: gallic acid[1], ellagic[2] methyl gallate[3] catechin[4], rutin[5] and quercetin[6]. On the other hand, the PEE was fractionated to saponifiable and unsaponifiable fractions and both fractions were analysed by GC/MS. The GC/MS analysis of the saponifiable fraction revealed the identification of thirty-seven compounds as fatty acid methyl esters representing 83.51% of the total fraction, methyl hexadecanoate (39.98%) constitutes the major fatty acid methyl ester followed by methyl octadecanoate (6.62%). The analysis of the unsaponifiable fraction disclosed the presence of forty-nine compounds representing 72.32% of the total compounds, where butylated hydroxyl toluene (10.03%) constitutes the major compound. Both HAE and PEE were evaluated for their *in vitro* antioxidant potential by DPPH and β-carotene methods. The HAE superiors PEE in its antioxidant potentiality. Correspondingly, the HAE was subdued for further investigation embracing its *in vivo* antioxidant and antihyperlipidemic activities.

Keywords*: Terminalia bentzoe*; hydroalcoholic extract; petroleum ether extract; HPLC; CC; GC/MS.

INTRODUCTION

The genus *Terminalia* (Family *Combretaceae*), comprises two hundred and fifty species, is widespread in Egypt and other tropical and subtropical countries. Many valuable phytoconstituents isolated from this genus and used extensively in the development of drugs against various diseases, including triterpenoids for cardiovascular properties[1-4], tannins and flavonoids for its anticancer and antioxidant properties[5], hydrolyzable tannins for antiviral activity[6] and other biologically active ingredients used primarily for treating mild or chronic ailments[7-9]. Research work was done on various species of *terminalia* as antihyperlipidemic and antioxidant agent[10-15] which are the main topics of this work. *T. bentzoe* (L.) is a flowering evergreen tree belongs to this family. Its vernacular names were bois binjouin, benjoin, badamier , bois benzoin, bois charron. Its bark resin is used traditionally to treat skin infections and as sudorific. The bark decoction or infusion was used to treat diarrhea, dysentery and to stop bleeding. Leaf and bark decoctions are taken to treat colds, cough and bronchitis. Its wood has been used in constructions and for carpentry. Also, its leaves extract has shown to posses antiplasmodial, anti-inflammatory and cytotoxic activities[16]. To the authors' knowledge, there was a little work has been reported on the leaves of *T. bentzoe* (L.)[17-19]. Therefore, this work aims to phytochemical screening of the HAE and PEE of the leaves both qualitatively and quantitatively, in addition to investigating their *in vitro* antioxidant activities. The present research was also undertaken to investigate the *in vivo* antioxidant and antihyperlipidemic activities of the HAE leaves extract.

MATERIAL AND METHODS

Plant material

Green leaves of *T. bentzoe* (L.) were collected from the Giza Zoo Garden, Cairo, Egypt, in March 2013 and identified by Mrs. Tereez Labib, Consultant of Plant

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Taxonomy at the Ministry of Agriculture and director of the Orman Botanical Garden, Giza, Egypt. The leaves of plant were rinsed with running tap-water for about 20-30 min. till all the foreign material and soil particles detached from the leaves surface and afterward shade-dried in air at room temperature. The air-dried leaves were finely powdered utilizing an electric grinder and separately subjected for ethanolic and petroleum ether extraction.

Preparation of plant extracts

The powdered air-dried leaves of *T. bentzoe (L.*) (500g) were individually extracted to exhaustion in a soxhlet apparatus using ethanol (70%) and petroleum ether. The extracts were singly filtered through Whatman filter paper No.1 and then concentrated in a rotary evaporator at a temperature range of 40^0-50^0C. Definite yields of the HAE (22.4%) and PEE (4.6%) were obtained.

Phytochemical analysis of leaf extract

In this study, preliminary phytochemical screening (colour reactions) was operated on the HAE and PEE leave extracts according to standard methods[20-22].

Total phenolic assay

The total phenolic content (TPC) of the HAE, expressed as mg of gallic acid equivalents (GAE)/g dry weight of the extract, was estimated using the Folin–Ciocalteu reagent[23] using gallic acid as a reference standard. The estimation was carried out in triplicate and averaged.

Total flavonoid assay

The total flavonoid content (TFC) of the HAE, expressed as quercetin equivalents/100g of the dry extract, was determined according to a spectrometric method[24].

Total protein content

The protein content of the HAE was estimated as total nitrogen by the procedure adopted by Pearson using micro-Kjeldhal method[25]. The crude protein was subsequently calculated by multiplying the nitrogen content by 100/16 or 6.25. The factor 6.25 is derived from the generalization that most proteins contain 16% nitrogen.

Total carbohydrates

The total carbohydrate content was determined as glucose by the phenol-sulphuric acid method. A known weight of each dried extract (30 mg) was mixed with 10 ml H_2SO_4 (1M) and refluxed on a boiling water-bath for 2 h. The volume was adjusted to 100 ml in a volumetric flask with distilled water. To one ml of the sample solution, 1ml of 5% phenol solution was added, followed by 5 ml conc. H_2SO_4. The mixture was allowed to stand for 10 min. in a water-bath at 25ºC to 30ºC. The absorbance of the characteristic yellow-orange colour was measured at 490nm against a blank[26]. The amount of carbohydrates in the sample was determined by reference to a standard curve of glucose.

% Sugar = y/m x100

Where y and m are the mean reading of the sample weight and the slope (0.003), respectively.

High performance liquid chromatography (HPLC)

The polyphenols were analysed in HPLC according to the method described by Çam[27] with some modifications as follows: The HAE of the dried plant was analysed on the Agilent 1200 (USA) HPLC system with a diode array detector. Hichrom C18 (4.6 mmx250 mm; 5 µm particle size) column, 40ºC column temperature and 20 µl injection volume were used in the analysis. The solvent system was a gradient of water-acetic acid (98:2) (A) and methanol (B). The gradient employed was: starting with 95% A, from 95% A to 50% for 10 min, from 50% A to 30% for 5 min at a flow rate of 1.0 ml/min. The phenolic compounds were monitored at 272 nm for gallic acid, 275 nm for (-)-epicatechin, 279 nm for chlorogenic acid, syringic acid, psoralen and 356 nm for rutin. The spectra of these polyphenols were recorded between 190-400 nm. Gallic acid, (-)-epicatechin, chlorogenic acid, syringic acid, psoralen rutin were determined quantitatively by using the external standard method. The HPLC method was validated. Due as *et al.*[28] reported that the limit of detection (LOD) and the limit of quantification (LOQ) are defined as 3:1 and 10:1 peak to noise ratio. LOD and LOQ were calculated for gallic acid, chlorogenic acid, syringic acid, rutin, (-)-epicatechin and psoralen.

Investigation of lipoidal matter

The petroleum ether extract (1g) was saponified according to the method described elsewhere[29], by refluxing with 50 ml alcoholic KOH (10%) for 6 h. After distillation of ethanol and dilution with water, the unsaponifiable fraction was extracted with ether. The residue left after the evaporation of ether (USM) was weighed and kept for GC/MS analysis. The aqueous mother liquor was acidified with 10% HCl and the liberated fatty acids were extracted with ether. The residue left after evaporation of ether (FA) was weighed and subjected to methylation.

Methylation of fatty acids

Fatty acid fraction of the PEE was subjected to methylation[30], by refluxing with 50 ml absolute methanol and 3ml of H_2SO_4 for 2 h, then cooled, diluted with about 100 ml of distilled water and transferred to a separating funnel. The fatty acid methyl esters (FAMEs) were extracted several times with ether. The combined ethereal extracts, in each case, were washed several times with water until free of acidity, dehydrated over anhydrous sodium sulphate and the ether was evaporated to dryness. The fatty acid methyl esters (FAME) were kept for GC/MS analysis.

GC/MS analysis of fatty acid methyl esters and unsaponifiable matter

The FAME and USM fractions were subjected to GC/MS analysis adopting the following conditions: Capillary column of fused silica (5% phenyl methyl polysiloxane), 30m length, 0.25mm I.D. and 0.25 µm thickness, DB-5, carrier gas helium at 13 psi; oven temperature 50-280^0C at a rate of 5^0C /min for USM and FAME; ion source temperature 220^0C; ionization voltage 70 ev ; accelerated voltage 2000 v; volume injected 1µl. The identification of the compounds was accomplished by comparing their retention times and mass spectral data with those of the library (Wiley Int. USA) and NIST (Nat. Inst. St. Technol., USA) and/or published data[31].

Column chromatography of HAE

Table 1: Phytochemical screening of ethanol and petroleum ether extracts of *T. bentzoe* (L.) leaves

Phytochemical constituents	Ethanol extract	Petroleum ether extract
Carbohydrates	++	-
Fats/Oils	+	++
Saponins	-	-
Terpenoids	+	+
Steroids	-	+
Flavenoids	++	-
Phenolics/Tannins	+++	-
Cardiac glycosids	-	-
Proteins/Amino acids	++	-
Alkaloids	-	-
Anthraquinones	-	-

+ = Present ; - = Absent

Table 2: Results of HPLC analysis of ethanol extract of *T. bentzoe* (L.) leaves

Phenolic compound	Concentration
Gallic acid	36.28*
Ellagic acid	12.24
Pyrogallol	2.76
Catechin	5.18
Chlorogenic acid	4.73
Synergenic acid	5.51
Pyrocatechol	9.68
Querectin	5.52
Cinnamic acid	3.04
P-Qumaric	1.33
Rutin	2.01
Caffeic acid	1.49

*Values are expressed as relative area percentage

Forty grams of HAE were applied on diaion HP20 CC (120×5cm) and subjected to reversed phase chromatography starting elution with 100% water till 100% methanol. Five main fractions (A-E) were obtained by mixing similar fractions. Fraction B eluted with 25% methanol water was subjected to reversed phase chromatography on a polyamide S6 column starting elution with water till 100% methanol. Fractions of 500 ml each were collected, inspected on Whatman PC 1MM, using butanol:acetic acid:water, 4:1:5 (BAW), 15% and/or 6% acetic acid as solvent systems, six major fractions were obtained by mixing similar fractions (I-VI). Fraction I was chromatographed on PC eluted with 6% acetic acid/H_2O led to the isolation of compounds 1&2. Fractions II was chromatographed on PC eluted with 6% acetic acid/H_2O leading to the isolation of 3. Fraction III was subjected to PC fractionation using 15% AcOH to obtain compound 4. Fraction IV was subjected on a Sephadex LH-20 column using methanol/water 30% to obtain compound 5. Fraction V was subjected on a Sephadex LH-20 column using methanol/water 50% to obtain compound 6. The final purification of all compounds was performed on Sephadex LH-20 (Pharmazia Sweden).

In vitro antioxidant activity with DPPH method

The antioxidant activity of the extract was measured as described by Gebhard[32] using the 1,1–diphenyl–2–picrylhydrazyl (DPPH^o) free radical scavenging capacity. 1 ml of each plant extract was mixed with 3 ml of methanol solution of 0.004 % DPPH and the absorbance was read at 517 nm 30 min later. The radical scavenging activity of the samples (antioxidant activity) was expressed as percent inhibition of DPPH^o radical as following:

% Inhibition = $[(A_{control} - A_{treatment})/A_{control})] \times 100$

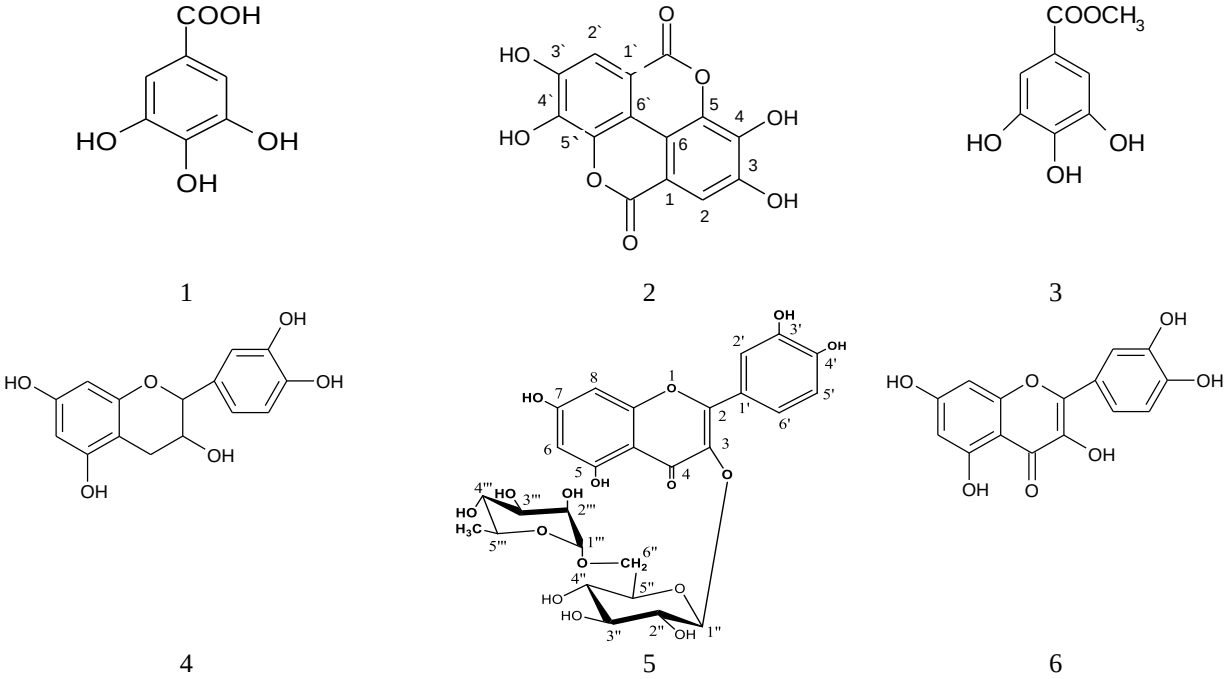

Figure 1: Chemical structure of compounds 1-6 isolated from *Terminalia bentzoe* (L.) leaves.

Table 3: GC/MS Analysis of the fatty acid methyl esters of *Terminalia bentzoe*

No.	Compound	R_t	B.P.	M^+	Rel. Area (%)	Mol. Formula
1	Methyl decanoate	19.17	186	74	0.10	$C_{11}H_{22}O_2$
2	Methyl,4-oxo-nonanoate	20.54	186	98	0.06	$C_{10}H_{16}O_3$
3	Dimethyl octandioate	22.65	202	129	0.15	$C_{10}H_{18}O_4$
4	Methyl dodecanoate	24.60	214	74	0.76	$C_{13}H_{26}O_2$
5	Dimethyl nonanedioate	25.28	216	152	1.14	$C_{11}H_{20}O_4$
6	Methyl tridecanoate	27.08	228	74	0.18	$C_{14}H_{28}O_2$
7	Dimethyl decanedioate	27.71	230	74	0.14	$C_{12}H_{22}O_4$
8	Methyl tetradecanoate	29.49	242	74	4.40	$C_{15}H_{30}O_2$
9	Methyl undecanedioate	30.04	244	98	0.19	$C_{13}H_{24}O_4$
10	Methyl-4-pentadecenoate	31.33	254	74	1.02	$C_{16}H_{30}O_2$
11	Methyl pentadecanoate	31.69	256	74	0.74	$C_{16}H_{32}O_2$
12	Methyl-7-hexadecenoate	33.36	268	55	0.08	$C_{17}H_{32}O_2$
13	Methyl hexadecanoate	34.12	270	74	39.98	$C_{17}H_{34}O_2$
14	Methyl heptadecanoate	35.86	284	74	0.42	$C_{18}H_{36}O_2$
15	Methyl-2-hydroxyhexadecanoate	36.27	286	227	0.42	$C_{17}H_{34}O_3$
16	Methyl-9,12-octadecadienoate	37.20	294	81	0.76	$C_{19}H_{34}O_2$
17	Methyl-9-Octadecadecenoate	37.32	296	55	0.85	$C_{19}H_{36}O_2$
18	Methyl octadecanoate	37.87	298	74	6.62	$C_{19}H_{38}O_2$
19	Methyl nonadecanoate	39.65	312	74	0.39	$C_{20}H_{40}O_2$
20	Methyl-10-hydroxy octadecanoate	40.13	314	255	0.56	$C_{19}H_{38}O_3$
21	Methyl-6,9,12-octadecatrienoate	40.75	292	79	1.14	$C_{19}H_{32}O_2$
22	Methyl eicosanoate	41.52	326	74	5.32	$C_{21}H_{42}O_2$
23	Methyl henicosanoate	43.17	340	74	0.72	$C_{22}H_{44}O_2$
24	Methyl-2-hydroxy eicosanoate	43.63	342	283	0.46	$C_{21}H_{42}O_3$
25	Methyl docosanoate	44.85	354	74	1.74	$C_{23}H_{46}O_2$
26	Methyl tricosanoate	46.41	368	74	1.06	$C_{24}H_{48}O_2$
27	Methyl-2-hydroxy docosanoate	46.90	370	311	0.89	$C_{23}H_{46}O_3$
28	Methyl tetracosanoate	47.95	382	74	2.06	$C_{25}H_{50}O_2$
29	Methyl-2-hydroxy tricosanoate	48.45	384	325	1.37	$C_{24}H_{48}O_3$
30	Methyl pentacosanoate	49.41	396	74	0.69	$C_{26}H_{52}O_2$
31	Methyl-2-hydroxy tetracosanoate	50.03	398	339	3.25	$C_{25}H_{50}O_3$
32	Methyl hexacosanoate	51.06	410	74	1.29	$C_{27}H_{54}O_2$
33	Methyl 2-hydroxy pentacosanoate	51.73	412	353	1.07	$C_{26}H_{52}O_3$
34	Methyl heptacosanoate	52.94	424	74	0.22	$C_{28}H_{56}O_2$
35	Methyl-2-hydroxyhexacosanoate	53.77	426	367	0.60	$C_{27}H_{54}O_3$
36	Methyl octacosanoate	55.27	438	74	1.56	$C_{29}H_{58}O_2$
37	Methyl nonacosanoate	58.03	452	74	0.12	$C_{30}H_{60}O_2$
	Total identified area				83.51	

Where: $A_{control}$, is the absorbance of the control; $A_{treatment}$, is the absorbance of the treatments.

Standard antioxidants (BHA and TBHQ, 200 ppm) were used for comparison as positive control. IC50 value was calculated using the dose inhibition curve**.**

In vitro antioxidant activity with the β-carotene bleaching method

The antioxidant activity of the extracts was evaluated using β-carotene-linoleic acid[33]. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade); 25 uL of linoleic acid and 200 mL of tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water was added with vigorous shaking for 30 min; 2500 uL of this reaction mixture was dispersed to test tubes, and 150, 250 and 350 uL portions of extracts were added. The emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHA, TBHQ and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Tests were carried out in triplicate. Inhibition of coloration of β-carotene in percentage (I%) was calculated as:

$I\% = [(A_{blank} - A_{sample})]/ A_{blank}] \times 100$

Where A_{blank} is the absorbance of the control reaction (containing of the reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Acute toxicity study

In this experiment, the animals received one oral dose of 250, 500 and 1000mg of *T. bentazoe* HAE/kg body weight. These animals were then observed for 15 days. The dead animals were counted and the mortality rate was calculated. Safety dose was monitored according to the recorded LD_{50}.

Table 4: Results GC/MS Analysis of the Unsaponifiable Matter of *Terminalia bentzoe*

No.	Compound	R_t	B.P.	M^+	Rel. Area (%)	Mol. Formula
1	2-Ethyl-1-hexanol	9.65	57	130	1.57	$C_8H_{18}O$
2	Undecane	17.80	57	1560	0.25	$C_{11}H_{24}$
3	Dodecane	19.16	57	170	0.05	$C_{12}H_{26}$
4	Tetradecene	21.04	55	196	0.11	$C_{14}H_{28}$
5	B-Ionone	23.16	177	192	0.39	$C_{13}H_{20}O$
6	Butylated hydroxyanisole	23.33	165	180	0.36	$C_{15}H_{24}O$
7	n-Pentadecane	23.93	57	212	0.11	$C_{15}H_{32}$
8	Butylated hydroxyl toluene	24.43	220	205	10.03	$C_{11}H_{16}O_2$
9	4-phenyl-decane	25.14	91	218	0.12	$C_{16}H_{26}$
10	3-phenyl-decane	25.59	91	218	0.11	$C_{16}H_{26}$
11	1-Hexadecene	26.30	83	224	2.67	$C_{14}H_{32}$
12	n-Hexadecane	26.44	57	226	0.44	$C_{14}H_{34}$
13	5-phenyl undecane	27.35	91	232	1.15	$C_{17}H_{28}$
14	4-phenyl undecane	27.59	91	232	0.71	$C_{17}H_{28}$
15	3-phenyl undecane	28.07	91	232	0.70	$C_{17}H_{28}$
15	Heptadecene	28.65	83	338	0.04	$C_{17}H_{34}$
16	Heptadecane	28.82	57	240	0.28	$C_{17}H_{36}$
17	2-phenyl undecane	28.95	105	232	1.01	$C_{17}H_{28}$
18	6-phenyl dodecane	29.57	91	246	0.83	$C_{18}H_{30}$
19	5-phenyl dodecane	29.68	91	246	1.15	$C_{18}H_{30}$
20	4-phenyl dodecane	29.95	91	246	1.21	$C_{18}H_{30}$
21	3-phenyl dodecane	30.43	91	246	1.26	$C_{18}H_{30}$
22	1- octadecene	31.01	97	252	5.22	$C_{18}H_{36}$
23	2-phenyl dodecane	31.32	105	246	1.84	$C_{18}H_{30}$
24	6-phenyl tridecane	31.78	91	260	2.33	$C_{19}H_{32}$
25	5-phenyl tridecane	31.93	91	260	1.45	$C_{19}H_{32}$
26	6,10,14-trimethyl-2-pentadecanone	32.18	58	268	5.03	$C_{18}H_{36}$ O
27	3-phenyl tridecane	32.70	91	260	2.14	$C_{19}H_{32}$
28	Nonadecane	33.24	57	268	0.44	$C_{19}H_{40}$
29	2-phenyl-tridecane	33.52	105	260	2.02	$C_{19}H_{32}$
30	Isophytol	34.27	71	296	0.08	$C_{20}H_{40}$ O
31	1-Ecosene	35.23	97	280	4.01	$C_{20}H_{40}$
32	1-Heneicosene	36.96	83	294	0.23	$C_{21}H_{42}$
33	n-Heneicosane	37.23	57	296	0.35	$C_{21}H_{44}$
34	Phytol	37.57	71	296	1.59	$C_{20}H_{40}$ O
35	1-docosene	39.06	97	308	4.35	$C_{22}H_{44}$
36	9-Triecosene	40.74	83	322	0.33	$C_{23}H_{46}$
37	n-Tricosane	40.91	57	324	0.40	$C_{23}H_{48}$
38	1-Tetracosene	42.59	97	336	1.51	$C_{24}H_{48}$
39	β-sitosterol	43.35	386	414	0.09	$C_{29}H_{50}$ O
40	n-Pentacosane	44.92	57	352	1.52	$C_{25}H_{52}$
41	β-amyrin	45.56	218	426	1.35	$C_{30}H_{50}$ O
42	1-Hexacosene	45.85	97	364	4.20	$C_{26}H_{52}$
43	n-Hexacosane	46.86	57	366	0.41	$C_{26}H_{54}$
44	n-Heptacosane	47.46	57	380	1.57	$C_{27}H_{56}$
45	n-Octacosane	48.93	57	394	2.41	$C_{28}H_{58}$
46	Squalene	49.42	69	410	0.25	$C_{30}H_{50}$
47	n-Nonacosane	50.50	57	408	1.71	$C_{29}H_{60}$
48	n-Triacontane	52.25	57	422	0.93	$C_{30}H_{62}$
49	n-Hentricontane	54.40	57	436	0.21	$C_{31}H_{64}$
	Total identified area				72.32%	

Antihypercholesterolimic activity study

Experimental design

Twenty four male albino rats were selected for this study and divided to four groups (six rats each) as follows: Group 1**,** normal healthy control rats, Group 2, hypercholesterolemic group received cholesterol, Groups 3 received cholesterol and treated with the plant extract at the same time and for the same duration, Group 4, received cholesterol and treated with the lipanthyl drug (50mg finofibrate/kg body weight) at the same time and for the same duration and served as a reference treated

Table 5: Effect of treatment with *T. bentazo*e on obesity index, body and relative liver weights of hypercholesterolemic rats

Groups	Body weight (BW)	Liver weight (LW)	Relative liver weight	Obesity index (OI)
Normal control	139.82±25.53^{d}	6.14±0.21^{a}	3.90±0.47^{a}	5.47±0.62^{bc}
Hypercholesterolemic	214.68±14.03^{ab}	5.45±0.53^{a}	2.48±0.15^{e}	7.18±1.06^{a}
Hypercholesterolemic + *T. bentazoe*	174.84±44.72^{bcd} (28.49)	5.71±1.33^{a} (8.95)	3.22±0.18^{bc} (12.05)	5.93±0.77^{b} (22.85)
Hypercholesterolemic + Drug	171.54±14.48b^{cd} (30.85)	5.73±0.41^{a} (4.56)	3.31±0.14^{b} (21.28)	4.91±0.45^{c} (41.49)

- Data are means ± SD of six rats in each group.
- BW and LW are expressed in grams.
- Relative liver weight = (LW/BW) x 100.
- OI = Body weight (kg)/ body height (m)^2
- Unshared letters between groups are the significant values at p<0.0019 for BW, p<0.00001 for relative liver weight, p< 0.0009 for OI.
- % of improvement = $\frac{\text{Hypercholesterolemic mean - treated mean}}{\text{Control mean}} \times 100$

Table 6: Effect of *T. bentazo*e HAE extract and lipanthyl drug on lipid profile and liver function levels in hypercholesterolemic rats

Parameters	Control	Hypercholesterolemic	Hypercholesterolemic + *T. bentazo*e	Hypercholesterolemic + Drug
TC	117.00±13.51^{d}	177.00±5.70^{a}	139.00±20.73^{bc} (32.47)	121.00±14.17^{cd} (47.86)
HDL-C	20.63±3.52^{a}	13.71±1.66^{c}	15.45±1.66^{bc} (8.43)	17.72±1.86^{ab} (19.43)
LDL-C	69.93±13.24^{b}	112.29±5.60^{a}	89.45±24.02^{b} (32.66)	75.80±15.41^{b} (52.18)
TG	132.17±16.72^{c}	255.02±36.49^{a}	177.37±13.17^{b} (58.75)	137.39±16.72^{c} (88.99)
AST	132.17±16.72^{c}	255.02±36.49^{a}	177.37±13.17^{b} (58.75)	137.39±16.72^{c} (88.99)
ALT	40.19±4.12^{d}	56.32±7.92^{a}	47.99±7.46^{bc} (20.72)	46.22±4.27^{cd} (25.13)

- Data are means ± SD of six rats in each group.
- Lipid profile is expressed as mg/dL and liver function indices as U/L.
- Unshared letters between groups are the significant values at p<0.00001for cholesterol, p<0.0007 for HDL-C, p<0.0179 for LDL-C, p<0.00001 for triglycerides, p<0.0001 for AST and p< 0.0003 for ALT.

Values between brackets are % of improvement = $\frac{\text{Hypercholesterolemic mean - treated mean}}{\text{Control mean}} \times 100$

group. At the end of the experiment, the liver and body weight of the rats in each group were estimated.

Diet

Control groups were fed with a standard diet (El-Kahira Co. for Oil and Soap), while hypercholesterolemic groups were fed with standard diet containing 150g lard/kg diet[34]. The modified diet was taken along with the oral administration of cholesterol to get a condition of high fat and cholesterol level[35] and to ensure triglycerides elevation[36].

Administration regimen

Administration regimens according to Adaramoye *et al*.[37] were five times/one week for nine consecutive weeks. Doses of *T. bentazoe* were selected according to the toxicity study. The selected dose was 500mg/kg body weight. Cholesterol was orally given at a dose 30 mg/animal. Lipanthyl drug (Mina Pharm., Egypt) was orally given at a dose 50mg/kg body weight[38]. The dose of lipanthyl drug was calibrated to exactly contain 50 mg of fenofibrate/kg body weight.

Sample preparations

Blood collected from each animal by puncturing the sublingual vein in a clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was stored at -80^{o}C for further determinations of lipid profile and liver function enzymes. Liver tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:9 w/v). The homogenate was centrifuged at 4^{o}C for 10 min at 3000 rpm and the supernatant was stored at -80^{o}C for further estimation of hepatic oxidative stress markers; glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD).

Biochemical determinations

Table 7: Effect of *T. bentazo*e HAE extract and lipanthyl drug on oxidative stress markers of Hypercholesterolemic rats.

Parameters	Control	Hypercholesterolemic	Hypercholesterolemic + *T. bentazo*e	Hypercholesterolemic + Drug
MDA	0.92 ± 0.03^{cd}	2.02 ± 0.15^{a}	0.97 ± 0.08^{c} 114.13	1.02 ± 0.14^{c} 108.69
GSH	51.40 ± 3.04^{a}	22.20 ± 1.92^{e}	32.20 ± 1.92^{d} (19.45)	44.60 ± 4.33^{d} (43.57)
SOD	54.60 ± 2.96^{f}	119.20 ± 4.96^{a}	90.40 ± 1.82^{c} (52.74)	86.20 ± 3.19^{cd} (60.43)

- Data are means ± SD of six rats in each group.
- Values are expressed as µmol/mg protein for MDA and µg/mg protein for GSH and SOD.
- Unshared letters between groups are the significant values at $p<0.00001$.
- Values between brackets are % of improvement =

$$\frac{\text{Hypercholesterolemic mean - treated mean x 100}}{\text{Control mean}}$$

Serum cholesterol was determined by the method of Meiattini *et al.*[39], serum high density lipoprotein cholesterol (HDL-C) was determined according to the method of Bustein *et al.*[40], serum low density lipoprotein cholesterol (LDL-C) was determined by the method of Assmann *et al.*[41], serum triglyceride (TG) was measured by the method of Fossati and Prencipe[42] using a diagnostic kit ((Spectrum, Egypt) and total protein in liver tissue was estimated by the method recorded elsewhere[43]. Aspartate and alanine amintransferases (AST& ALT) were estimated as previously described[44]**.** Malondialdehyde (MDA) was assayed according to the method of Buege and Aust[45]. Glutathione (GSH) was assayed according to a reported method[46]. Total superoxide dismutase (SOD) was assayed according to Nishikimi *et al.*[47].

Histopathological study

Liver slices were fixed in 10% paraformaldehyde and embedded in paraffin wax blocks. Sections of 4µm thick were stained with hematoxylin & eosin (H&E), then examined under a light microscope for determination of pathological changes[48].

Statistical analysis and calculations

All data were expressed as mean ± SD of six rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program. A significant value between groups was at $p<0.05$, % of change = control mean - treated mean/control mean x 100 ; % improvement = treated mean - cholesterolemic mean/control mean x 100; mortality rate = number of dead animals/total animal number x 100

RESULTS AND DISCUSSION

Phytochemical screening

The HAE and PEE yields were found to be 22.4% and 4.6%, respectively. The present study clearly confirms the presence of various phytoconstituents like terpenoids, sterols, tannins, flavonoids and phenolics in both HAE and PEE, as depicted in Table 1. These secondary plant metabolites may be responsible for their numerous medicinal effects. The total phenolic and flavonoid contents for HAE were 765.1mg/g Gallic acid equivalent and 137.87mg/g carotene equivalent, respectively, meanwhile the total protein and carbohydrate contents were 5.20 and 8.04%w/w. By and large, the total flavonoid and phenolic contents are relatively high in HAE, thence this may conceivably accountable for the more noticed radical scavenging activity of HAE than PEE, due to these compounds can promptly give atom to the radical and it has been accounted for that the amount of phenolic compounds in an extract is specifically proportional to its free radical scavenging capacity[49]. HPLC analysis of HAE permits identification of the isolated compounds with respect to their peak area. It disclosed the presence of nine phenolic acids, two flavonoids and one anthocyanin. They were gallic, (36.28%), ellagic (12.24%), pyrogallol (2.76%) catechin (5.18%) chlorogenic acid(4.73%) synergic acid (5.51%) pyrocatechol (9.68%) cinnamic acid (3.04%) paraqumaric acid (1.33 %) caffeic acid (1.49 %) querectin (5.52%) and rutin (2.01%) arranged according to the order of elution (Table 2). Chromatographic examination of the HAE of *T. betazoe* L. leaves resulted in the isolation and identification of six compounds for the first time from *T. betazoe* L. leaves, they were identified as gallic acid[1], ellagic[2] methyl galate[3] catechin[4], rutin[5] and quercetin[6] (Fig. 1). Structure elucidation of all isolated compounds was realized through R_f-values, colour reactions, chemical investigations (complete and mild acid hydrolysis) and spectral investigations (UV, NMR and MS)[50-52].

Gallic acid[1]: White amorphous powder, R_f values**:** 0**.**65 (BAW) and 0.52 (15% HOAc) on PC, UV at λ max (MeOH): 270. ^{1}H-NMR(500 MHz, DMSO-d6, ppm): 6.98 (s, H-2, H-6).

Ellagic acid[2]: Off-white amorphous powder, R_f values**:** 0**.**30 (BAW) and 0. 40 (15% HOAc) on PC, UV at λ max (MeOH): 255, 364.^{1}HNMR (500 MHz, acetone, ppm)

Methyl gallate[3]: Off-white amorphous powder, R_f values: 0.76 (BAW) and 0.60 (15% HOAc) on PC, UV at λ max (MeOH): 220, 275 nm. ^{1}HNMR (500 MHz, acetone, ppm) 7.01(s, H-2 and H-6) and 3.71 (s, methyl ester proton). ^{13}C-NMR: 120.33(C-1), 109.00 (C-2), 145.43 (C-3), 138.47 (C-4), 145.43 (C-5), 109.00 (C-6), 167.28 (C-7), 51.46 (COO-CH_3).

Catechin[4]: Pale pink amorphous powder, R_f values 0.76 (BAW) and 0.62 UV spectra (MeOH): λmax 280 nm and sh 330 nm. ^{1}H-NMR (500 MHz, acetone-d6): at δ ppm 4.47 (H-2, d, J=7.8 Hz), 4.08 (H-3, ddd,), 2.45 (H-4a, dd,

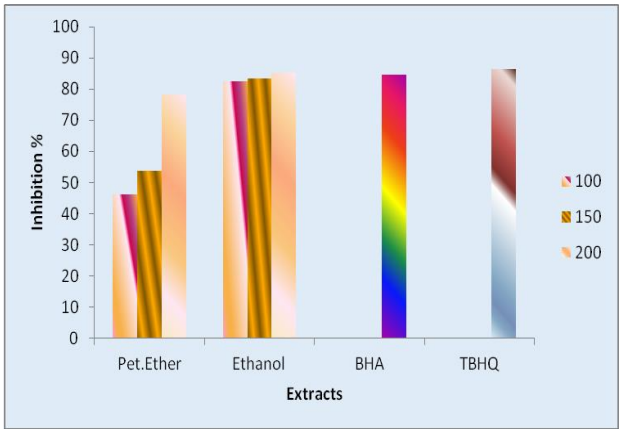

Figure 2: DPPH• radical scavenging activity of petroleum ether and ethanol extracts.

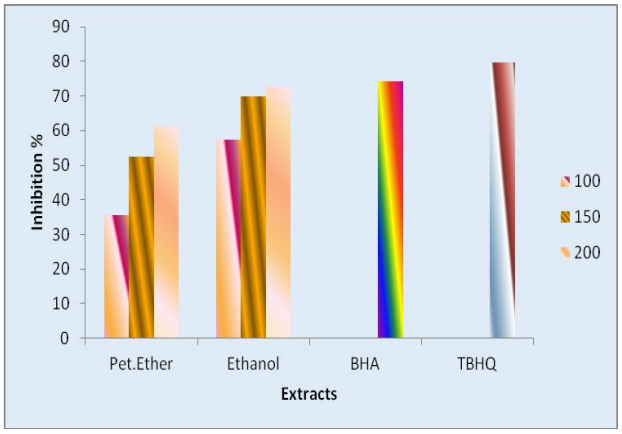

Figure 3: Antioxidant activity of petroleum ether and ethanol extracts with the β-carotene bleaching method.

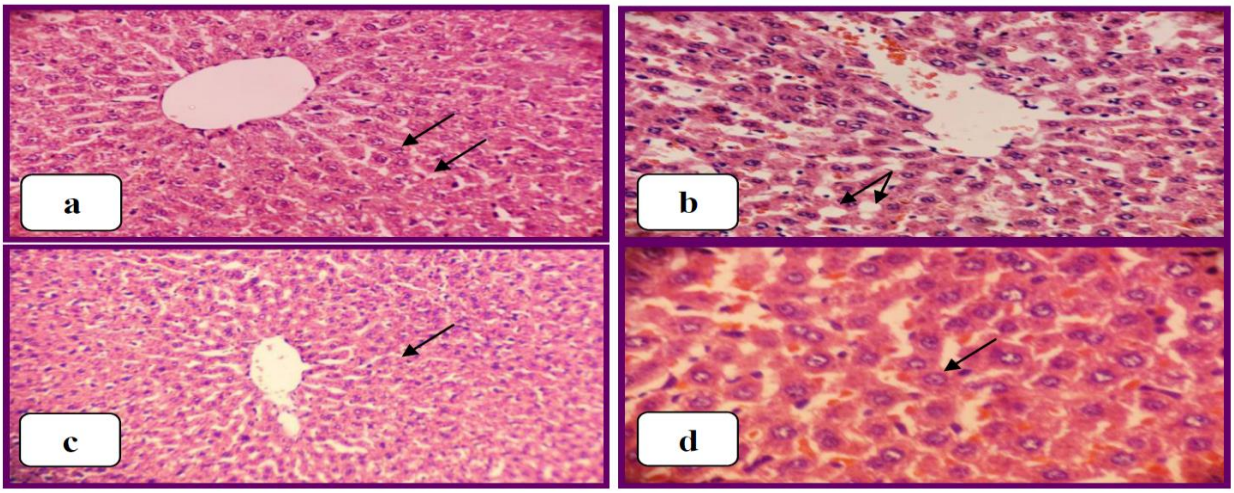

Figure 4: Haematoxylin and eosin (H&E) stain liver sections (200x) of control rats: (a) (arrows indicate normal hepatocytes), hypercholesterolemic rats, (b) (arrows indicate fat vacuoles deposition), hypercholesterolemic rats treated with plant extract, (c) (arrows indicate normal cells appearance) and hypercholesterolemic rats treated with lipanthyl drug, (d) (arrows indicate well structured hepatocytes with normal appearance).

J= 8.50 Hz, J=16.10 Hz), 2.80 (H-4e, dd, J= 5.50 Hz, J= 16.10 Hz], 5.78 (H-6, d, *J*= 2.3 Hz), 5.95 (H-8, d, *J*=2.3 Hz), 6.81 (H-2′, d, J=1.95 Hz), 6.74 (H-5′, d, J=8.00 Hz), 6.66(H-6′, dd, J=1.94 Hz, J=8.4 Hz). ^{13}C-NMR (125 MHz acetone): 27.7 (C-4), 66.3 (C- 3), 80.9 (C-2), 93.9 (C-6), 95.1 (C-8), 114.5 (C-2), 115.1 (C-5), 18.4 (C-6) and other aromatic carbons showed peaks at δ of 99.1, 130.6, 144.6, 144.8, 155.3, 156.1 and 156.4.

Rutin[5]: Yellow amorphous powder, R_f values(BAW) 0.380 and (15% HOAc) 0.66 ^{1}H-NMR: UV at λ max nm
(MeOH): 258,300,358; NaOMe: 272,327sh,410; $AlCl_3$: 275,305sh,420; $AlCl_3$/HCl: 269,301sh,358,400; NaOAc: 272,324sh,379; NaOAc/H_3B: 262, 378. ^{1}H-NMR (500 MHz, DMSO-d6): δ ppm 7.52 (2H, m H-2`/6`), 6.84 (1H, d, J = 9 Hz, H-5`), 6.36 (1H, d,J=2.5Hz, H-8), 6.20 (1H,J=2.5Hz, H-6), 5.36 (1H, d, J=7.5 Hz, H-1``), 4.38 (1H, s, H-1```), 3.90-3.20 (m, remaining sugar protons), 0.99 (3H, d, J=6 Hz, H-6```) ^{13}C NMR(125 MHz, DMSO-d6): δ ppm 177.84 (C-4), 164.70 (C-7), 161.66 (C-5), 157.15 (C-2), 156.96 (C-9), 148.90 (C-4`), 145.25 (C-3`), 133.76 (C-3), 122.12 (C-6`), 121.66 (C-1`), 116.73 (C-2`),115.72 (C-5`),104.41 (C-10), 101.66 (C-1``) 101.20(C-1```, 99.24 (C-6), 94.16 (C-8),74.58(C-3``),72.33(C-5``),72.2(C-4'''),71.05(C-2``),70.8(C-2'''),70.87(C-3''')70. 49(C-4'') 68.74(C-6``)18.20(C-6''').

Quercetin[6]: Yellow amorphous powder, R_f values **:** 0.03 (15% HOAc), 0.70 BAW. UV at λ max (MeOH): 258, 268sh, 295sh, 368. Negative ESI-MS: m/z : 301.20 . ^{1}H-NMR: 7.69 (d, *J*= 2.1, H-2'), 7.55 (dd, *J* =2.1 Hz, and 8.4 Hz, H-6'), 6.90 (d, *J*= 8.4 Hz, H-5'), 6.42 (d, *J*= 1.8 Hz, H-8), 6.20 (*d*, *J*= 1.8 Hz, H-6).

As is evident in Table 3, which illustrate the results of GC/MS analysis of the saponifiable fraction of *T. bentzoe* thirty seven compounds, representing 83.51% of the total peak area, in which methyl hexadecanoate represents the major fatty acid methyl ester (39.98%) beside 8.62% of hydroxy fatty acid methyl ester. On the other hand, the unsaponifiable matter contains the largest number of the identified compounds (49 compounds), representing 72.32% of the total peak area as depicted in Table 4, in which unsaturated hydrocarbon constitute 22.63% of the total identified compounds among these, 1-octadecene

5.22% represents the major compound, and phenyl hydrocarbon constitute 18.03% of the total identified compounds, 6-phenyl tridecane (2.33%) is the major one.

Acute toxicity test and antioxidant activity

No dead animals were seen along the experimental period and this affirms the extract safety. So, we chose the median dose of 500mg/kg b.wt. for additional biological investigations. This study declares that both HAE and PEE have potential antioxidant activities as described in Figures 2 and 3 in a dose dependent manure[53-55]. It is evident (Fig. 2) that the HAE has more potent antioxidant activity than the PEE as the former contains the highest amounts of flavonoids and polyphenols. These compounds have a ubiquitous class of alternative antioxidants and display a constructive role in avoiding free radical damage and owe its antioxidant property[56].

Antihyperlipidemic activity

Hyperlipidemia, the disorder of lipid metabolism is distinguished by exalted serum total cholesterol, low density and very low density levels of lipoprotein cholesterol. Hyperlipidemia-related lipid disorders are considered to bring about atherosclerotic cardiovascular disease[57]. Presently, available hypolipidemic drugs have been associated with a variety of side effects[53]. So the current interest has fortified the search for new lipid-lowering agents with littlest side effects from natural sources. Herbal therapy for hyperlipidemia has insignifican side effects and economic impacts in decreasing the lipid levels[58]. Previous studies[59,60] have shown the hypolipidemic and anti-dyslipidemic activity of flavonoid-rich extract, disclosing a potential role of flavonoids in the treatment of hyperlipidemia. Likewise, treatment with *T. Chebula* and its combination with gaumutra significantly diminishes the cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index and a noteworthy increment in HDL-C in serum and different tissue homogenate like aorta, liver and Heart. Anti-hyperlipidemic activity of this combination may be because the presence of anthraquinones, tannins, chebulic acid, chebulinic acids, gallic acid and ellagic acid[61]. No direct evidence for the antihyperlipidemic activity of *T. bentazoe* leaf extract has been published up to now. In this work, we demonstrated for the first time that the *T. bentazoe* leaf extract possessed potent antihyperlipidemic activities. Body and liver weights as well as the obesity index in diverse groups were depicted in Table 5. In case of body weight treatment with the extract and lipanthyl drug brought about amelioration by 28.49, and 30.85%, respectively, showing a diminishing in cholesterol and fat deposition in the liver. In the meantime, increase of liver weights was observed by 8.95, and 4.56%, respectively, and the relative liver weight was improved by 12.05 and 21.28%, respectively. The obesity index recorded amelioration after treatment with extract and the selected drug reached to 22.85and 41.49%, respectively. High fat diet induced hypercholesterolemia in rats significantly increased TC, TG and LDL-C as compared to normal rat, while HDL-C was significantly decreased[62]. Our results presented in this work showed that the administration of the HAE of *T. bentzoe* at a dose of 500mg/kg body weight leads to ameliorations in these parameters by 32.47, 8.43, 32.66 and 58.75% for TC, HDL-C, LDL-C and TG, respectively (Table 6). To correlate between the hypolipidemic and antioxidant effect of *T. bentazoe* with its active ingredients, we noticed the presence of flavonoids, polphenolic, and threnodies. Previous reports[63,64] demonstrated that flavonoid-rich fraction decreased the levels of lipids in the plasma and tissues.

Hepatoprotective and antioxidant effects

In case of liver function enzymes, AST and ALT recorded a significant increase in hyperlipidemic rats as compared with the control group. This was in accordance with the results of Awad *et al.*[62] who attributed this increase in enzyme activities to the increase of cell membrane fluidity that leads to enzyme leakage into the circulations. Treatment with plant extracts showed improvement by 58.75 and 20.72%, respectively (Table 6). This was in agreement with those reported by Sarkar *et al.*[65] who found the same results after treatment of iron intoxicated rats with *T. chebula* (*Retz.*) fruit extract. The oxidative stress markers imparted significant increment in MDA and SOD as compared with the control group, while it recorded a considerable decrement in GSH level. Treatment with plant extract indicated improvement in MDA, SOD and GSH by 114.13, 52.74 19.45%, respectively (Table 7). Some authors (Shukla et al., 2004) ascribed the enhancement of the antioxidant levels after treatment to the constituents existing in the extract which may diminish the levels of lipid peroxidation products by scavenging free radicals like hydroxyl, superoxide anion, and peroxy-free radicals or by diminishing the accessibility of lipid substrate. The *in-vitro* results of the antioxidant effect of the plant extract affirmed its role as free radical scavenging effects.

Histopathological evaluation

Light microscopic investigation of the liver sections of normal rats indicated the hepatic lobules brought about by radially arranged cords of normal liver cells that radiated from the central vein to the periphery of the lobule (Fig. 4a). Hypercholesterolemic rat livers demonstrated extensive vacuoles of lipid accumulation and loss of normal hepatic architecture and dark inflammatory infiltrates (Fig. 4b). This was in concurrence with the outcomes reported by Awad *et al.* [62] and Hamed[66] who observed the same structure of the liver after high fat diet. This was ascribed to the existence of fatty liver as a result of hypercholesterolemia and accumulation of large vacuoles of fat. Treatment with *T. bentzoe* extract showed minimal fat deposition in hepatocytes and less degenerative changes in the liver (Fig. 4c). Treatment with lipanthyl drug showed more or less normal hepatocytes appearance with very rare fat vacuoles deposition (Fig. 4d).

CONCLUSION

In conclusion, the results suggest the effectiveness of both the ethanolic and petroleum ether extracts of *T. bentzoe* leaves as in *vitro* antioxidant agents, where, the ethanolic extract showed the most potent effect due to its enrichment with flavonoids, tannins, protein and

carbohydrates. In addition, ethanol extract recorded hypolipidemic, hepatoprotective and antioxidant effect in hypercholesterolemic rats. However, studies are required in human subjects to prove its clinical efficacy as a hypolipidemic agent.

REFERENCES

1. Dwivedi, S. & Chopra, D. Revisiting *Terminalia arjuna* – An Ancient Cardiovascular Drug. *J Tradit Complement Med.*, 2014; 4(4): 224–231.
2. Laskar, M. A., and Manabendra D. C. "Screening of Some Triterpenoids Isolated from Terminalia arjuna as Inhibitors of Angiotensin Converting Enzyme to Prevent Cardiovascular Diseases." *Research & Reviews: A Journal of Drug Design & Discovery* 2014; 1(3): 12-19.
3. Pingali U, Fatima N, Nizampatnam M. Evaluation of Terminalia arjuna on cardiovascular parameters and platelet aggregation in patients with Type II diabetes mellitus. *Age* 2013;1(2):7-12.
4. Prem Verma, Muneesh, Seema Rani, Garima BhutaniPrem, V., Muneesh, S.R. & Garima B. Experimental Evaluation of *Terminalia Arjuna* (Aqueous Extract) On Cardiovascular System In Comparison To Digoxin. *IOSR-JDMS*, 2013; 7(2): 48-51.
5. Vijayalakshmi, A., Kumar, P.R., Priyadharshini S.S. & Meenaxshi, C. In Vitro Antioxidant and Anticancer Activity of Flavonoid Fraction from the Aerial Parts of *Cissus quadrangularis* (Linn.) Against Human Breast Carcinoma Cell Lines. *J.Chem.*, 2013; Article ID 150675: 1-9.
6. Lin, L.T., Chen, T.Y., Chung, C.Y., Noyce, R.S., Grindley, T.B., McCormick, C., Lin, T.C., Wang, G.H., Lin, C.C. & Richardson, C.D. Hydrolyzable tannins (chebulagic acid and punicalagin) target viral glycoprotein-glycosaminoglycan interactions to inhibit herpes simplex virus 1 entry and cell-to-cell spread. *J Virol*, 2011; 85(9): 4386-98.
7. Nema, R., Jain, P., Khare, S., Pradhan, A., Gupta, A. & Singh, D. Antibacterial and antifungal activity of *Terminalia Arjuna* leaves extract with special reference to flavanoids. *BRJMCS*, 2012; 1(5): 63-65.
8. Gangadhar, M., Bhavana, P., Datta, S. & Ganesh, M. Effect of Epigallocatedhin gallate isolated from *Terminalia belerica* fruit rind on glucoamylase activity invitro. *JAPS*, 2011; 1(6): 115-117.
9. Arif-Ullah, K. & Anwarl, H.G. Anti-Secretory & Analgesic Activities of *Terminalia belerica*. *AJB*, 2010; 09: 2717-2719.
10. Reddy, M.M., Devavaram, J.D., Dhas, J., Adeghate, E. & Emerald, B. S. Anti-hyperlipidemic effect of methanol bark extract of Wistar rats *Terminalia chebula* in male albino Wistar rats. *Pharm Biol*, 2015; 53(8): 1133-1140.
11. Sharma, S., Asija, R., Kumawat, R.S., Chaudhary, P. & Sharma, P.K. A study of anti-hyperlipidemic activity of marketed formulations of *Terminalia arjuna* powder using experimental animal model. *JBPR*, 2015; 4(1): 107-111.
12. Selvan, P.S., Velavan, S., Sagunthala, P. & Subramanian, S. Anti-hyperlipidemic activity of the bark extract of *Terminalia arjuna* in caffeine induced mice. *Biochem*, 2015; 5(8): 310-313.
13. Sinha S. & Ghosh, A.K. Hypolipidemic effect of ethanolic extract of aegle marmelos and *Terminalia arjuna* in hyperlipidemic rat model. *IJATES*, 2015; 03(2): 616-621.
14. Arya, A., Nyamathulla, S., Noordin, M. I. & Mohd, M. A. Antioxidant and hypoglycemic activities of leaf Extracts of three popular *Terminalia* species, *E-J Chem*., 2012; 9(2): 883-892.
15. Subramaniam, S., Ramachandran, S., Uthrapathi, S., Gnamanickam, V.R. & Dubey, G.P. Anti-hyperlipidemic and antioxidant potential of different fractions of *Terminalia arjuna* Roxb. bark against PX-407 induced hyperlipidemia. *Indian J Exp Biol.*, 2011; 49(4): 282-8.
16. Jonville, M.C., Kodja, H., Humeau, L., Fournel, J., De Mol, P., Cao, M., Angenot, L. & Frederich, M. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. *J. Ethnopharmacol.*, 2008; 120: 382-386.
17. El-Rafie, H.M. & Hamed, M.A. Antioxidant and anti-inflammatory activities of silver nanoparticles biosynthesized from aqueous leaves extracts of four *Terminalia* species. Advances in Natural Sciences: *Nanosci. &Nanotech.*, 2014; 5: 1-11.
18. Jonville, M.C, Kodja, H., Strasberg, D., Pichette, A., Ollivier, E., Frédérich, M., Angenot, L. & Legault, J. Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. *J Ethnopharmacol.*, 2011; 136 (3): 525-531.
19. Gurib-Fakim, A. & Demarne, F. Essential oil of *Terminalia bentzoe* (L.) L. f. subsp. rodriguesensis Wickens, *Nat.Agricult. Lib.*, 1994; (NAL, 6 (5): 533-534.
20. Vinod, D.R. Pharmacognosy and Phytochemistry 1 (Nasik: Carrier Publication), 2002; p132.
21. Harbone, J.B. Phytochemical Methods: A guide to modern techniques of plant analysis 3rd edn (London: Chapman and Hall Ltd), 1998; p 117.
22. Fransworth , N.R. Biological and phytochemical screening of plants. *J. Pharm. Sci*., 1966; 55 (3): 225-276.
23. Lim, Y.Y. & Quah, E.P.L. Antioxidant properties of different cultivars of *Portulaca oleracea*. *J Food Chem.*, 2007; 103: 734-740.
24. Chatha, S.A.S., Arshad, M.U., Arshad, W., Hussain, A.I. & Saif, M.J. Extraction regimes on antioxidant potential of rice (*Oryza ativa*) bran extracts. *J Chem Soc Pak*, 2012; 34: 1290-1294.
25. Pearson, D., "The Chemical Analysis of Food "Churchill LTD., London, 1970. 6thed.; p 9.
26. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. Calorimetric method for determination of sugars and related substances. *Anal. Chem.*, 1956; 28(3): 350- 356.

27. Çam, M., 2009. Extraction of antioxidant compound of pomegranate peels and seeds using pressurized liquid extractor with water. Ege University, The Institute of Science and Technology Food Technology Department Ph.D. Thesis, Izmir, Turkey, 271 p. (in Turkish).
28. Due as, M., Pérez-Alonso, J.J., Santos-Buelga, C. & Escribano-Bail, N.T. Anthocyanin composition in fig (*Ficus carica* L.). *J Food Compos Anal*, 2008; 21: 107-115.
29. Tsuda, K., Kishida, Y. & Hayatsu, R. Steroid studies XVII on the absolute configuration of C-24-ethyl of stigmasterol. *J. Am. Chem. Soc*, 1960; 82 (13): 3396-3399.
30. Johonson, A. R. & Davenport, J. B. "Biochemistry and Methodology of lipids" A. Division of John Wiely and sons Inc. New York, 1971; p 35 A.
31. Adams, R.P. "Identification of Essential Oil by Ion Trap Mass Spectroscopy" Academic press, INC, New York, 1995.
32. Gebhard, R. Anticholestatic activity of flavonoids from artichoke (*Cynara scolymus* L.) and their metabolites. *Med Sci Mon.*, 2001; 7: 316-320**.**
33. Kabouche, A., Kabouche, Z., Fiztufirk, M.O., Kolak, M.U. & Topcfu, G.,. Antioxidant abieetane diterpenoids from *Salvia barrelieri*. *Food Chem.*, 2007;102: 1281-1287.
34. Auger, C., Caporiccio, B., Landrault, N., Teissedre, P.L., Laurent, C. & Cros, G. Besançon, P., Rouanet, J.M. Red wine phenolic compounds reduce plasma lipids and apolipoprotein B and prevent early aortic atherosclerosis in hypercholesterolaemic golden Syrian hamsters (*Mesocricetus auratus*). *J. Nutr.*, 2002; 132(6): 1207-1213.
35. Kim, J.K. Kraemer, G.P. & Yarish, C. Physiological activity of *Porphyra* in relation to eulittoral zonation. *J Exp* Marine *Biol.*, 2008; 365: 75–85.
36. Gershkovich, P. & Hoffman, A. Effect of a high-fat meal on absorption and disposition of lipophilic compounds: The importance of degree of association with triglyceride-rich lipoproteins. *Eur. J. Pharmaceut. Sci.*, 2007; 32(1): 24–32.
37. Adaramoye, O.A., Akintayo, O., Achem, J. & Fafunso, M.A. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vasc Health Risk Manag. 2008;4(1):235-41.
38. Pietta, P.G. Flavonoids as antioxidants. *J Nat Prod.*, 2000; 63(7): 1035-1042.
39. Meiattini, F., Prencipe, L., Bardelli, F., Giannini, G. & Tarli, P. The 4- hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. *Clin. Chem.*, 1978; 24(12): 2161–2165.
40. Bustein, M., Schoirick, H. R. & Morfin, R. Rapid method for the isolation of lipoproteins from human serum by perception with polyanions. Scand. *J. Clin. Lab. Invest.*, 1980; 40: 583–595.
41. Assmann, G., Jabs, H.U., Kohnert, U. & Nolte, W., Schriewer, H. LDL-cholesterol determination in blood serum following precipitation oh LDL with polyvinylsulphate. *Clin. Chem. Acta*, 1984; 140(1): 77-83.
42. Fossati, P. & Prencipe, L. Serum triglycerides determination colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.*, 1982; 28(: 2077–2080.
43. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Bioch.*, 1976; 72: 248–254.
44. Gella, F.J., Olivella, T., Cruz, P.M., Arenas, J., Moreno, R., Durban, R, & Gomez, J.A. A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. *Clin Chem Acta*, 1985; 153: 241-247.
45. Buege, J.A. & Aust, S.D. Microsomal Lipid Peroxidation. In: Flesicher, S., Packer, L. (Eds.), Methods in Enzymology. Vol. 52. Academic Press, New-York, 1978, pp. 302–310.
46. Moron, M.S., Depierre, J.W. & Mannervik, B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta.*, 1979; 582: 67–78.
47. Nishikimi, M., Rao, N.A. & Yagi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Commun*, 1972; 46(2): 849-854.
48. Hirsch, C., Zouain, C.S., Alves, J.B., & Goes, A.M. Induction of protective immunity and modulation of granulaomatous hypersensitivity in mice using PIII, an anionic fraction of *Schistosoma mansoni* adult worm. *Parasitology*, 1997; 115: 21–28.
49. Kalia, K., Sharma, K., Singh, H.P. & Singh, B. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of Potetilla atrosanguinea Lodd. and quantification of its phenolic constituents by RP-HPLC. *J Agric Food Chem*, 2008; 56: 10129-34.
50. Agrawal, P.K. Carbone-13 NMR of flavonoids. Elsevier Science Publishing Co. Inc New York, 1989.
51. Markham, K.R. Techniques of Flavonoid Identification. Academic Press, London, 1982.
52. Mabry, T.J., Markham, K.R. & Thomas, M.B. The Systematic Identification of Flavonoids. Berlin: Springer, 1970.
53. Saravanan, R. Prasad, R.N. & Pugalandi, K.V. Effect of Piper beetle leaf extract on alcoholic toxicity in the rat brain. *J Med Food*., 2003; 6: 261–5.
54. Hazra, B**.,** Sarkar, R., Biswas, S. & Mandal, N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*. *BMC Comp. Alt. Med.*, 2010; 10: 20 (doi: 10.1186/1472-6882-10-20).
55. Sultana, B., Anwar, F. & Przybylski, R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica,Terminalia arjuna, Acacia nilotica*

and Eugenia jambolana Lam. Trees. Food Chem., 2007; 104: 1106–1114.
56. Shetgiri, P.P., Darji, K.K. & D'Mello, P.M. Evaluation of Antioxidant and Antihyperlipidemic Activity of extracts Rich in Polyphenols. *Int. J. Phytomedicine*, 2010; 2: 267-276.
57. Dhar, U., Rawal, R.S., Samant, S.S., Airi, S. & Upreti, J. People's participation in imalayan biodiversity conservation: a practical approach. *Current Sci*, 1999; 76: 36–40.
58. Berliner, J.A. and Heinecke, J.W. The role of oxidized lipoproteins in atherogenesis. *Free Radic Biol Med.*, 1996; 20: 707–27.
59. Narender, T., Khaliq, T., Puri, A. and Chander, R. The Antidyslipidemic activity of Furano-flavonoids isolated from *Indigofera tinctoria*. *Bioorg Med Chem Lett.*, 2006; 16(13): 3411-3414.
60. Sharma, B., Balomajumder, C. & Roy, P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food Chem Toxicol.*, 2008; 46(7): 2376- 2383.
61. Israni, D.A., Patel, K.V., Gandhi, T.R. Anti-hyperlipidemic activity of aqueous extract of *terminalia chebula* & *gaumutra* in high cholesterol diet fed rats. *IJPSR.*, 2010; 1(1): 48-59.
62. Awad, N.E., Seida, A.A., Hamed, M.A., Hosny, A.M. & Elbatanony, M.M. Phytochemical and *in vitro* screening of some *Ficus* and *Morus* spp. for hypolipidemic and antioxidant activities and *in vivo* assessment of *Ficus mysorensis* (Roth). *Nat. Prod. Res.*, 2012; 26: 1101-1111.
63. Nagarathna, P.K.M., Honi yalu & sudheer, M. Anti Hyperlipidemic Activity of Flavonoid of *Cassia tora*. *IJTPR* ., 2013; 5(4): 129-136.
64. Kaviarasan, K. Mohankumar, R. Narasimhan, S. Viswanathan, P. & Pugalendi, V. Antihyperlipidemic efficacy of flavonoid-rich fraction from *spermacoce hispida* seed on high-fat-diet fed rats: a dose-dependent study. *J. Food Biochem.*, 2010; 35(6): 1627–1636.
65. Sarkar, R., Hazra, B. & Mandal, N. Reducing power and iron chelating property of *Terminalia chebula* (Retz.) alleviates iron induced liver toxicity in mice. *BMC Comp. Alt. Med.*, 2012; 12: 144 (doi:10.1186/1472-6882-12-144).
66. Hamed, M.A. Beneficial effect of *Ficus religiosa* Linn. On high-fat-diet-induced hypercholesterolemia in rats. *Food Chem.*, 2011; 129(1): 162-170.