

Phytochemicals, Antioxidative and *in vivo* Hepatoprotective Potentials of *Litsea floribunda* (BL.) Gamble (Lauraceae) - An Endemic Tree Species of the Southern Western Ghats, India

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Abstract

The leaf and stem bark samples of *Litsea floribunda* (Bl.) Gamble a medicinal tree species, endemic to the Western Ghats, a biodiversity 'hot spot' of India were subjected to soxhlet extraction, phytochemical analysis and antioxidant activity by employing *in vitro* screening methods. The extracts exhibiting high antioxidant activity were assessed for the hepatoprotective activity against paracetamol-induced liver damage in rats. Phytochemical analysis of the stem bark and leaf extracts indicated the presence of flavonoids, terpenoids, cardiac glycosides, tannins, saponins and reducing sugars in the hexane, chloroform, ethyl acetate, absolute ethanol, methanol and aqueous extracts, respectively. The *in vitro* antioxidant activities revealed that the absolute ethanol and the aqueous extracts of stem bark as well as leaves exhibited high activity. The aqueous (98.9±0.7 mg/g GAE), and absolute ethanol extracts (114.8±0.2 mg/g GAE) of leaf as well as the stem bark aqueous (112.4±0.7 mg/g GAE) and absolute ethanol extracts (117.4±0.1 mg/g GAE) extracts contained higher amount of total phenolics compared to other solvent extracts. On the basis of the IC₅₀ values (21.5±0.8 µg/ml to 29.0±0.2 µg/ml), the leaf/stem bark absolute ethanol and the aqueous extracts were considered for hepatoprotective studies in the *in vivo* rat model. The absolute ethanol and aqueous leaf and stem bark extracts were administered orally to 11 groups of animals in two doses (250 and 500 mg/kg b.w) with hepatotoxicity induced by paracetamol (2 mg/kg b.w.). Silymarin (100 mg/kg b.w.), the standard drug was used as a positive control. The stem bark aqueous and absolute ethanol extracts were effective in protecting the liver against the toxicity induced by paracetamol in rats. This was evident from the significant reduction in serum enzymes such as Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetate Transaminase (SGOT), alkaline phosphatase (ALP), serum bilirubin, serum total proteins and liver weight against the treated group of animals. Histopathological studies conducted by sectioning of liver samples also indicated the hepatoprotective nature of the stem bark absolute ethanol and aqueous extracts with moderately dilated hepatic vein and degenerated peripheral hepatocyte infiltration suggestive of reduced toxicity. The results obtained strongly indicate that the absolute ethanolic and aqueous extracts of *L. floribunda* stem bark has good antioxidant activity

1. Introduction

The medicinal value of the plant lies in the bioactive phytochemical constituents, which work with nutrients and fibers to form an integrated part of defense system against stress conditions and diseases (Koche et al., 2010). Living cells generate free radicals and other reactive oxygen species as a result of physiological and biochemical processes. Oxidative damage to lipids, proteins and DNA by free radicals leads to chronic diseases. Plant constituents form an important source of antioxidants that are capable of scavenging free radicals and prevent them from cell and tissue

damage and terminate the free radical chain reactions. The liver is the main site for intense metabolism and excretion. Liver diseases are a serious challenge to international public health (Yadav et al., 2011). Toxic chemicals, xenobiotics, alcohol consumption, malnutrition and medications cause liver damage due to exposure to high quantities of free radicals leading to oxidative stress (Alaqsoumi et al., 2014). Unfortunately, synthetic drugs used in the treatment of liver damage are inadequate and can have serious side effects. Therefore, there is a growing demand for the traditional herbal medicines that have hepatoprotective activity. In Ayurveda, 40 herbal formulations are used for hepatoprotection and

contain phenols, coumarins, essential oils, monoterpenes, carotenoids, flavonoids, alkaloids and glycosides (Sheik et al., 2012).

In recent years, there is a worldwide increase in the use of natural antioxidants present in herbs, fruits and vegetables as against the synthetic antioxidants. Antioxidants offer resistance against oxidative stress by scavenging free radicals and inhibiting lipid peroxidation. The property of hepatoprotection of plant extracts is through one of the mechanisms such as antioxidative, anti-lipid peroxidative, anti-fibrotic, immunomodulatory and liver-regenerating effects (Luper, 1998). The phenolics and flavonoids present in plant extracts attribute to antioxidant and hepatoprotective activity against liver injury (Aseervatham, 2013). Lauraceae, referred to as the laurel family, includes about 55 genera and 4000 species world-wide, mostly from warm or tropical regions, especially Southeast Asia and South America. The members are aromatic trees or shrubs forming laurel forests in the tropics. *Litsea* is a member of Lauraceae comprising ~200 species, in the tropical and subtropical Asia, Australia, North America and subtropical South America. 45 species are documented in India of which, 18 are endemic (Bhuniya et al., 2010a). In the traditional medicine, the leaf and bark of *Litsea* are used (Bhuniya et al., 2010b). In the Chinese medicinal system, it is used in the treatment of diarrhea, stomachache, dyspepsia, gastroenteritis, diabetes, edema, arthritis, pain etc. (Kong et al., 2015).

Litsea floribunda (Bl.) Gamble is an arborescent, endemic and predominant species of the shola vegetation in the Western Ghats, a biodiversity 'hotspot' of southern India. It is also documented from the coffee plantations and sacred groves (Bhagwat et al., 2005). The leaves of *L. floribunda* are used as one of the ingredients in the preparation of herbal shampoo, in southern India (Girish et al., 2014). In the health traditions, the local inhabitants use *L. floribunda* to treat certain gastrointestinal and respiratory disorders (personal observation). Till now, no data are available on the phytochemical profile, antioxidant and hepatoprotective potentials of this species. *Litsea* species also contain structurally diverse and biologically active phytochemicals with broad-spectral biological activities (Agarwal et al., 2011). Therefore, considering this aspect, we investigated on this less explored species of *Litsea*.

2. Materials and Methods

2.1. Collection of the Plant Material

Litsea floribunda (Bl.) Gamble was collected in the month of May 2012 during the early rainy season from the forests of the Western Ghats (012°17' to 012°27'N and 075°26' to 075°33'E), Kodagu District, Karnataka, India and identified based on taxonomical parameters. A herbarium specimen of the species is deposited in the

herbarium collection of the Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, India. Plant parts like healthy leaves and stem bark were collected in zip lock polyethylene bags and brought to the laboratory.

2.2. Sample Processing and Extract Preparation

The collected plant parts were washed with water to remove dust and then rinsed with distilled water. Later, they were dried under shade and then in a hot air oven at 40°C overnight until brittle and powdered. The powdered samples were stored in airtight polyethylene bags until use. Fifty grams of dried leaf and bark powder were extracted with solvents in the order of polarity (Hexane > chloroform > ethyl acetate > ethanol > methanol in a Soxhlet apparatus. The liquid obtained after solvent extraction was subjected to drying using a rotary flash evaporator (Superfit Model PBU-6D, India). The residue obtained after flash evaporation of solvents was designated as the dry extracts (Akshatha et al., 2015). The extracts were stored in pre-weighed glass vials and labeled. The aqueous extracts of leaf and stem bark were prepared according to the procedure of Hebbar et al. (2015) by stirring 500 g of the materials in distilled water and boiling for an hour. The extract was filtered using a double layer cheese cloth and the filtrate was evaporated to dryness in a temperature controlled water bath for 72 h (Fisher Scientific, Mumbai, India). The dried powder was scraped, quantified and designated as the dry aqueous extract and used throughout the studies.

2.3. Phytochemical Screening

Qualitative phytochemical screening of solvent extracts was conducted employing standard methods (Harborne, 1973). One gram each of the leaf and bark extracts were dissolved in one ml of respective solvents and tested for the presence of phytochemicals like tannins (Braymer's/Ferric chloride test), flavonoids (Ferric chloride and ammonia test), alkaloids (Mayer's, Wagner's and Dragendrof's tests), saponins (Foam test), cardiac glycosides (Keller-Killani test), terpenoids (Salkowski's test), anthraquinones (Borntrager's test), phlobatannins (Precipitate test), and reducing sugars (Benedict's test).

2.4. Determination of Total Phenolic Content

The total phenolic content in the extracts was estimated by the Folin-Ciocalteu (FC) method employing Gallic acid as standard (mg/mL) (Volluri et al., 2011). The plant extracts were taken in different concentrations (50-250 µg) and were made up to 1000 µL using phosphate buffered saline (PBS, 20 mM, pH-7.4). One ml of FC reagent (1:1 dilution) was added to the test tubes and kept for 3-4 minutes. Later 2.0 mL of Na₂CO₃ (10%, w/v) was added and the mixture was allowed to stand for 45 minutes under dark conditions for incubation. After the specified period of incubation, the absorbance of the samples was measured at 765 nm using UV-Vis spectrophotometer (T60, TTL Technologies,

Bangalore, India). The concentration of total phenolics was expressed in terms of Gallic acid equivalence ($\mu\text{g/g}$ GAE, calculated as mean value $\pm\text{SD}$ ($n=3$). The values of the test samples were plotted using the standard curve. All the tests were carried out in triplicates.

2.5. Determination of Antioxidant Activity

The antioxidant activity was evaluated by the following methods:

2.5.1. Radical Scavenging by 1, 1-diphenyl-2-Picryl Hydrazyl (DPPH)

The radical scavenging activity of plant extracts was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Pannangpetch et al., 2007). Plant extracts (20-100 μg) were made up to 250 μL using distilled water. Two mL of DPPH solution (0.1 mM) in methanol was added to the extracts and mixed. Blank sample was prepared with one ml of methanol and one ml of DPPH. All the test tubes were kept for incubation in the dark for 20 minutes and the absorbance was measured at 517 nm spectrophotometrically. L-ascorbic acid (5-25 μg) was used as the reference standard and the experiment was carried out in triplicates. The percent scavenging activity was calculated as follows:

$$\% \text{ radical scavenging} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

The concentration of the sample required to scavenge 50% DPPH free radical (IC₅₀) was calculated from the plotting of inhibition (%) against the concentration of extracts.

2.5.2. Ferrous Reducing Antioxidant Power Assay (FRAP assay)

The antioxidant power of the extracts was determined by Ferrous reducing antioxidant power assay (Benzie and Strain, 1996). The FRAP reagent was freshly prepared by adding 10 mM of Tripyridyltriazine (TPTZ) dissolved in 40 mM of HCl, 20 mM of FeCl₃ in H₂O and 300 mM of acetate buffer (pH 3.6). The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ and 2.5 mL of FeCl₃ · 6H₂O, with temperature rise to 37°C. The plant extracts (20-100 μg) were made up to 100 μL by adding methanol to which 3.0 mL of working solution was added and kept at 30°C for 4 minutes. Blank samples were prepared with methanol and a working solution. The absorbance of samples was taken against blank at 593 nm spectrophotometrically. The experiments were performed in triplicates. FeSO₄ (5-25 μg) was used as standard for calibration curve and the results were compared with that of standard and expressed in μM Fe (II) /g dry weight.

2.5.3. Reducing Power Assay

Reducing power of the solvent extracts was measured using standard procedure (Oyaizu, 1986). Various concentrations of plant extracts (25-125 μg) were made up to 500 μL by adding PBS. 2.5 mL of

Potassium ferricyanide [K₃Fe (CN)₆] was added and the mixture was incubated for 20 minutes at 50°C. Later, 500 μL of Trichloroacetic acid (10%, w/v) was added and the mixture was centrifuged at 2500 rpm for 10 minutes. 2.5 mL of supernatant was taken to which equal amount of distilled water and 300 μL of FeCl₃ (0.01% w/v) was carefully added. The absorbance of the colored samples was recorded at 700 nm against a blank using spectrophotometer. Blank was prepared using all the reagents without the addition of plant extracts. The experiments were carried out in triplicates. An increase in the absorbance value of the reaction mixture indicates the increased reducing power of the extracts. The average data with standard deviation were recorded for each test sample and represented.

2.6. Hepatoprotective Activity

2.6.1. Animals

Adult albino rats of either sex weighing 140-180 g were selected, housed in the animal house of Sarada Vilas College of Pharmacy, Mysore. The animals were maintained at a temperature of 23 \pm 2°C, relative humidity 55 \pm 2% and light and dark cycles of 12 L: 12D. They were provided with standardized pellet feed and drinking water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. No. 706/CPCSEA dt. 1.10.2002. All the experimental procedures were carried out in accordance with the guidelines of CPCSEA.

2.6.2. Chemicals

The drug Silymarin (500 mg) was purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). All other chemicals used were of analytical grade.

2.6.3. Acute Toxicity Studies

Healthy albino rats of either sex were chosen and divided into 4 groups ($n=5$ in each group). They were fasted overnight and administered with ethanolic and aqueous extracts of *L. floribunda* orally in a single increasing dose of 1000 mg/kg b.w., 1500 mg/kg b.w., 2000 mg/kg b.w. and 2500 mg/kg b.w. of the rats, respectively. The rats were observed continuously for 2, 4 hours and finally for overnight mortality.

2.6.4. Hepatoprotective Study Design

The present study design is as follows:

Group I: Normal was given only vehicle (Guar gum) for 10 days.

Group II: Drug control was administered with standard drug Silymarin p.o. (100 mg/kg b.w.) for 10 days plus paracetamol on the 11th day

Group III: Toxic control was given paracetamol (2 mg/kg b.w.) single dose p.o. on the 11th day.

Group IV & V were administered leaf absolute ethanol extract p.o., 250 & 500 mg/kg b.w., respectively, for 10 days and paracetamol (2 mg/kg b.w., single dose) on the 11th day.

Group VI & VII were administered stem bark absolute ethanol extract *p.o.* 250 & 500 mg/kg b.w., respectively, for 10 days and paracetamol (2 mg/kg b.w., single dose) on the 11th day.

Group VIII & IX were administered leaf aqueous extract *p.o.*, 250 & 500 mg/kg b.w., respectively, for 10 days and paracetamol (2 mg/kg b.w., single dose) on the 11th day.

Group X & XI were administered stem bark aqueous extract *p.o.*, 250 & 500 mg/kg b.w.) for 10 days and paracetamol (2 mg/kg b.w., single dose) on the 11th day. After 11 days of treatment, rats of all the groups were anesthetized by diethyl ether 48 hours post-administration. The blood was collected from the retro-orbital plexus. Rats were sacrificed and the liver was carefully dissected, cleaned for extraneous tissue and a portion of it was fixed in Cornoy's fluid (Absolute alcohol: chloroform, 3:1) for histopathological studies. The blood samples thus collected were immediately centrifuged at 2200 rpm for 15 min. The separated serum was analyzed for marker enzymes such as SGOT, SGPT, serum bilirubin, total protein levels (Prism Diagnostics Pvt., Ltd, Thane, India), and ALP (Spinreact, SA, Spain).

2.6.5. Histopathological Studies

Hepatoprotective activity was confirmed through histopathological studies on the liver of rats of all groups. The animals were sacrificed under light anesthesia after 24 hours of last dosage. The livers were dissected out, washed with normal saline and weight determined. Liver tissue was fixed in Cornoy's fluid (Absolute alcohol: chloroform, 3:1) for histopathological studies (Yadav and Dixit, 2003). Sections of all groups were observed under 10X and 40 X of Research microscopes (LabomedOptix, 40 X magnifications) and photographed.

2.7. Statistical Analysis

The results obtained were subjected to statistical analysis using SPSS program (version16.0). The results obtained were compared with the control group in antioxidant assays. The biochemical parameters or the marker enzymes were statistically analyzed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test ($P < 0.05$) considered as statistically significant. The data were expressed as mean SEM (n=3 for the antioxidant assays whereas n=5 in hepatoprotectivity study).

3. Results

3.1. Phytochemical Screening

The phytochemical analysis of *L. floribunda* carried out for the various solvent extracts of leaf and stem bark indicated that both the extracts contained saponins, tannins, terpenoids, flavonoids, glycosides and reducing sugars in common (Table 1).

Table 1. Phytochemical screening of leaf and stem bark extracts of *L. floribunda*

Solvent extracts	P											
	Hexane		Chloroform		Ethylacetate		Absoluteethanol		Methanol		Aqueous	
	L	B	L	B	L	B	L	B	L	B	L	B
Saponins	+	-	-	-	-	+	+	+	-	+	+	-
Tannins	-	-	-	-	+	-	-	-	-	-	-	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	-	+	+	-	-	-	-	+	-	-
Cardiac glycosides	+	-	+	-	+	+	+	-	+	+	-	-
Flavonoids	-	+	+	+	+	-	-	+	-	+	-	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Reducing sugars	-	-	+	-	-	-	-	-	-	-	-	+

(+) = Positive result of the test, (-) = negative result of the test

3.2. Total Phenolic Content

All solvent extracts of *L. floribunda* contained phenolics in various quantities (Table 2). In the leaves, the hexane extract depicted very low content (2.6 ± 0.05 mg/g GAE), while the absolute ethanol extract contained high values (114.8 ± 0.2 mg /g GAE) and even the stem bark extracts contained phenolics in the same range i.e., 3.5 ± 0.1 mg /g GAE to 117.4 ± 0.1 mg /g GAE.

3.3. Antioxidant Assays

3.3.1. Radical Scavenging by 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Assay

The extracts of *L. floribunda* had the potential to scavenge the DPPH radical, as evident from the IC₅₀ values of the leaf and stem bark solvent extracts (Table 2). The aqueous and the hexane extract of leaves possessed IC₅₀ values in the range of 29 ± 0.1 µg/mL to 216 ± 3.0 µg/mL, respectively. Similarly, in the bark extracts, IC₅₀ values of 22 ± 0.08 µg/mL was observed for the ethanol extract, while, 240 ± 1.5 µg/mL was recorded for the hexane extract. Lower IC₅₀ value indicates higher activity and visa-versa. The results were compared to the scavenging activity of the standard ascorbic acid (IC₅₀ = 15 µg/mL).

3.3.2. Ferrous Reducing Antioxidant Power Assay (FRAP Assay)

The reducing power of *L. floribunda* extracts were measured and the values are represented (Table 2). The chloroform extracts of both leaf and stem bark exhibited lower values for FRAP assay (1.4 ± 0.2 & 3.3 ± 0.4 µM Fe (II) /g), while high values of 503.9 ± 1.05 & 532.2 ± 1.5 µM Fe (II) /g

were observed for the stem bark chloroform and aqueous extracts, respectively.

Table 2. Total phenolic content and antioxidant assay values of solvent extracts of leaf and stem bark of *L. floribunda*.

0	TPC (mg GAE/ g)	IC ₅₀ (µg/ml)	FRAP (µM Fe (II)/g)	Reducing power (Absorbance)*
Leaf extracts				
Chloroform	2.6 ± 0.05	241.7±4.3	1.4±0.2	0.38±0.1
Ethyl acetate	70.2±1.0	44.2±0.6	150.9±1.3	0.6±0.09
Absolute ethanol	114.8±0.2	29.0±0.2	179.4±1.41	2.1±0.02
Methanol	82.7±1.0	48.5±0.8	495.7±0.6	1.8±0.06
Aqueous	98.9±0.7	25.3±0.4	532.2±1.5	2.9±0.09
Stem Bark extracts				
Chloroform	3.5±0.1	154.3±1.0	3.3±0.4	0.4±0.07
Ethyl acetate	96.9±0.1	19.9±0.1	331.5 ±0.5	1.1±0.04
Absolute ethanol	117.4±0.1	21.5±0.8	320.7±0.4	3.2±0.02
Methanol	71.9±0.1	37.3±0.8	309.2±0.9	2.5±0.07
Aqueous	112.4±0.74	24.2±0.2	503.9±1.0	2.1±0.02

All values are expressed as mean ± standard error mean (SEM) (n=3)

*Absorbance represented as two-fold dilutions of the extracts.

3.3.3. Reducing Power Assay

The extracts showed dose-dependent reducing power activity. The reducing power results showed high readings in the ethanol and aqueous extracts of leaf and stem bark. In the present study, high reducing power was noted in the aqueous extract of leaf (2.9 ±0.09), and the absolute ethanol extract of stem bark (3.2±0.02). The results represented in Table 2 indicate that there was an increase in the reducing power of plant extracts as the extract concentration increased.

3.4. Hepatoprotective Studies

Acute toxicity studies conducted to the animal groups were observed continuously for two hours and then occasionally for 4 hours and finally for overnight mortality. The dose up to 2500 mg/kg b.w., was well tolerated without producing any alteration in gross behavioral signs of toxicity and mortality. Based on the observations, the dose selected for the study was fixed at 10% of the maximum tolerated dose that is 250 mg/kg p.o. A higher dose was selected at 500 mg/kg b.w., for administration to the rats.

In the present study, there was a significant increase in the serum levels of SGOT, SGPT, ALP, bilirubin and total proteins in rats treated with paracetamol (G III) as compared to control indicating paracetamol-induced hepatotoxicity (Table 3). Treatment of rats with standard drug Silymarin (G II), and paracetamol on the last day showed decreased levels of marker enzymes (P < 0.05) in comparison to paracetamol-treated rats (GIII).

Treatment of rats with the aqueous extract of leaf of *L. floribunda* (250 mg/kg and 500 mg/kg b.w.,) did not alter the enzyme levels as compared to paracetamol-treated rats. Rats treated with the absolute ethanol extract of leaf (250 mg/kg and 500 mg/kg b.w.) and stem bark (250 mg/kg) showed slight reduction in the serum enzyme levels. The groups treated with the absolute ethanol extract of stem bark (VII;500 mg/kg) and the aqueous stem bark extract (both G X & XI; 250 mg/kg b.w. and 500 mg/kg b.w.) showed significant decrease in the enzyme levels almost close to rats treated with standard drug Silymarin (P< 0.05). The liver weight of the toxic group showed an increase (10.68±0.3g) from that of normal controls (5.91±0.2g). Treatment of rats with the absolute ethanol extract of leaf (250 mg/kg and 500 mg/kg b.w.) did not show much decrease in the mean liver weight, but rats treated with the aqueous extracts of stem bark (250 mg/kg and 500 mg/kg b.w.) showed significant decrease in the mean liver weight on par with the rats treated with standard drug Silymarin.

Table 3. Effect of extracts of *L. floribunda* on serum biochemical parameters in paracetamol-induced liver damage in rats

Treatment	Biochemical parameters					
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (mg/dL)	Liver weight(g)
GI- Normal	85.8±1.15	67.8±2.8	99.8±2.1	0.67±0.03	6.42±0.1	5.91±0.2
GII-Silymarin (100 mg/kg)	115± 1.2**	84.6±2.2**	117.8±3.0**	0.89±0.22**	7.46±0.8**	6.88±0.4**
GIII-Paracetamol	181.1± 2.4*	166.8±1.8*	255.4±2.2*	2.81±0.17*	4.46±0.4*	10.68±0.3*
G IV-LE 250	163.2 ±1.4	158.4±1.5	239.4±3.0	2.10±0.12	5.90±0.2	8.99±0.45
G V-LE 500	162.4 ±1.7	151.1±0.8	232.3±3.2	1.96±0.08	5.50±0.08	8.81±0.38
G VIII-LA 250	152.1± 1.4	138.8±1.1	184.6±1.7	1.79±0.03	5.22±0.07	8.73±0.27
G IX- LA 500	154.3±1.6	125.4±2.4	157.4±2.9	1.66±0.06	5.13±0.14	8.51±0.31
G VI- BE 250	149.4 ±1.7	127.4±2.4	170.1±2.5	2.02±0.03	5.80±0.10	8.13±0.27
G VII-BE 500	147.6± 1.2	125.8±2.4	161.1±2.9	1.83±0.02	6.22±0.13**	6.95±0.38**
G X- BA 250	131.1± 2.0**	116.8±1.6**	121.6±2.5**	1.39±0.01	6.04±0.07**	6.31±0.21**
G XI- BA 500	124.2±4.0**	102.8±1.8**	127.4±1.4**	1.37±0.01	6.73±0.08**	6.04±0.34**

LE= leaf ethanol extract, LA= leaf aqueous extract, BE= stem bark ethanol extract, BA= stem bark aqueous extract; Values represent the mean ± SEM; (n=5). 250 & 500 represent dosage of extracts (mg/kg); U/L=Unit/liter; mg=milligram; dL=deciliter;

g=grams; G=grouping of animals; *P<0.05 is considered significant when compared with GI; **P<0.05 is considered significant when compared by GII by Duncan's Multiple Range Test.

3.5. Histopathological Studies

The liver samples of Group I (normal) rats showed normal liver with lobules and hepatocytes (Figure 1A). Group III constituting the toxic group showed distortion of hepatic architecture with foci of lymphocytes intervening the sinusoids which suggested the toxic effects (Figure 1C).

Group II (drug induced) showed normal hepatic morphology with hepatic lobules and hepatocytes (Figure 1B). Group IV, V and VI showed predominantly normal morphology with occasional

areas showing lymphatic infiltration and mild distortion of lobular architecture suggesting reduced hepatic toxicity (Figures 1F, J & K). Group VII, VIII and IX rats showed considerable decrease in toxicity effects with moderately dilated hepatic vein and degenerated peripheral hepatocytes infiltration suggesting reduced toxicity (Figures 1G, H & I). Group X, XI showed severe distortion of lobules with totally misplaced portal vein and central vein suggesting toxic changes and less effect of the extracts (Figures 1D & E).

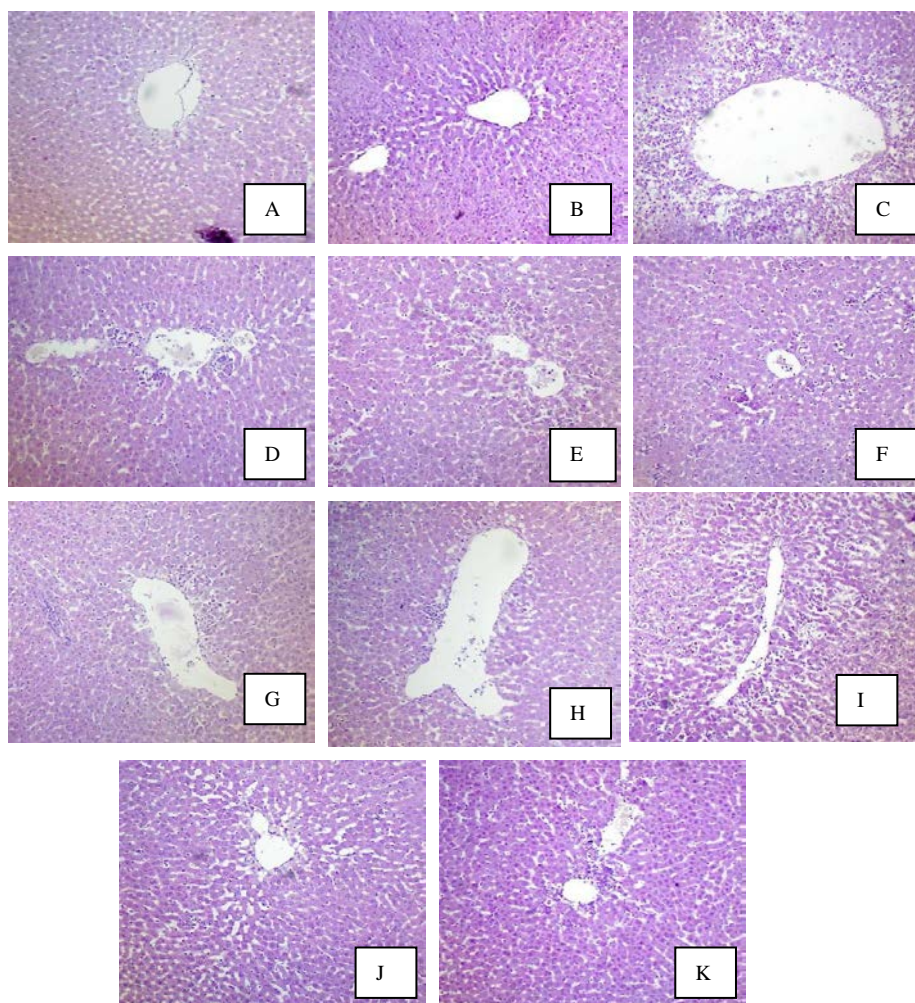


Figure 1. Histopathology of the sections of livers in the experimental groups of animals

A- GI (Normal); B- GII (Standard); C- GIII (Toxic); - D- GIV- LE250; E- GV- LE500; F- VI-BE250; G- GVII- BE500; H- GVIII LA250; I- GIX LA500; J- X BA250; K- XI BA500 BA: Stem Bark aqueous extract; BE: Stem Bark ethanol extract; LA: leaf aqueous extract; LE: leaf ethanol extract; 250 and 500= dosage of extracts (mg/kg b.w.) administered to rats.

4. Discussion

In the present investigation, an endemic *Litsea* species of the Western Ghats was considered for the screening of phytochemicals, antioxidative and hepatoprotective potentials. The Western Ghats are a long coastal hill chain extending from 8° N to 21° N latitude along the west coast of Peninsular India, and is a very narrow hill range stretching between 73°E and 77°E longitude. It covers an area of 180,000 square kilometers and comprises the major portion of the Western Ghats and Sri Lanka Hotspot, one of 34 global biodiversity hotspots for conservation and one of the two in the Indian subcontinent. This area is extraordinarily rich in biodiversity (Bawa *et al.*, 2007).

The phytochemicals are the secondary metabolic products produced by plants for their own defense and also bearing potential benefits for humans with antioxidant, antimicrobial, antidepressant, muscle relaxants and anti-inflammatory and many other activities (Briskin, 2000). The presence of flavonoids as major phytoconstituents may be responsible for the antioxidant activity of the extracts. In recent years, due to ethnomedicinal applications, *Litsea* species are being investigated for their pharmacological benefits (Ruth, 2004; Kong *et al.*, 2015). Phenolic compounds such as flavonoids, phenolic acids and tannins contribute to the antioxidant capacity of plants and possess biological activities such as anti-inflammatory and anti-carcinogenic activities (Arfan and Kader, 2006; Jia *et al.*, 2013). Correlation between the amount of total phenolic content and antioxidant capacity has been established (Li *et al.*, 2006). The presence of high phenolic content in the ethanol and aqueous extracts of both leaf and stem bark of *L. floribunda*, may be responsible for the free radical scavenging activity of the extracts.

The presence of flavonoids, reducing sugars and tannins in the bark extract of *L. glutinosa* was responsible for antioxidant activity as the extract showed notable DPPH and H₂O₂ radical scavenging activity (Ruth, 2004). The IC₅₀ values of the aqueous leaf (29.04 ± 0.12 μg/mL) and ethanolic bark (21.59 ± 0.082 μg/mL) extracts in the present study are closer to the scavenging activity of standard ascorbic acid with IC₅₀ value of 14.97 μg/mL. The IC₅₀ values obtained for the DPPH assay indeed suggests that the extracts have good antioxidant activity and they could be an important source of plant antioxidants and are comparable to the results obtained in *L. glutinosa*, a species of greater pharmacological interest (Devi and Meera, 2010). On the other hand, the leaf aqueous extract of this species exhibited a lower scavenging activity with IC₅₀ value of 30.24 μg/mL.

The Ferrous reducing antioxidant power assay (FRAP assay) is considered as a novel method for assessing antioxidant power (Rabeta and Faraniza, 2013). Higher FRAP values give higher antioxidant capacity as it is based on reducing ferrous ion when

antioxidants are the reducing agents. High reducing power of aqueous leaf (532 ± 1.5 μM Fe (II) /g) and stem bark (503.95 ± 1.05 μM Fe (II) /g) extracts could be due to the capacity of the extracts to reduce ferrous ion (Fe III) to ferric ion (Fe II) at low pH to form an intense blue colored ferrous tripyridyltriazine (Fe II-TPTZ) complex. The results thus suggest that it has powerful antioxidant activity.

The reducing capacity of extracts is another significant indicator of antioxidant activity. The reducing properties are normally associated with the presence of reductones which are responsible to exert antioxidant activities by breaking the free radical chain by donating a hydrogen atom (Lu *et al.*, 2014). The reducing power of *Litsea* extracts is high due to the presence of polyphenols causing reduction of ferric ion (Devi and Meera, 2010). High reducing power (3.2 ± 0.02) of the aqueous leaf and stem bark absolute ethanol extracts (2.9 ± 0.09) in *L. floribunda* indicate that the tannins and flavonoids detected in these extracts are responsible for the antioxidant activity.

Hepatotoxicity is an acute adverse effect of drugs in the liver. Several models are available to study and interpret hepatotoxicity levels of which, paracetamol is a widely employed analgesic and antipyretic agent. More consumption of paracetamol results in saturation of these pathways and formation of toxic metabolite (Shenoy *et al.*, 2012). The paracetamol induced hepatotoxicity is due to its toxic metabolite, N-acetyl-p-benzoquinoneamine (NAPQ1) which is normally detoxified by glutathione. In paracetamol toxicity, overload of NAPQ1 causes oxidative stress and binds covalently to liver proteins and other macro molecules resulting in hepatic necrosis and hepatic damage due to cease in detoxification (Pandey *et al.*, 2012).

In the present study, the aqueous and absolute ethanol extracts of stem bark and leaves of *L. floribunda*, depicting potent IC₅₀ values in the DPPH assay was selected for their hepatoprotective potentials. Two dosages of the extracts were administered (250 mg/kg and 500 mg/kg b.w.) on the basis of the results of the acute toxicity studies. The extent of hepatic damage is assessed by histopathological evaluation and the levels of various biochemical parameters like liver marker enzymes in serum. In hepatotoxicity, loss of integrity of hepatocyte membrane leads to cell damage. Therefore, liver marker enzymes present in the cytosol leak out, enter into serum and show an increase in the levels of marker enzymes i.e. SGOT, SGPT, ALP, and bilirubin and decreased level of total protein and albumin in serum (Chaudhari *et al.*, 2009). The animal groups treated with the absolute ethanol (VII: 500 mg/kg) and the aqueous (X: 250 mg/kg and XII: 500 mg/kg) stem bark extracts showed significant decrease in the enzyme levels almost close to rats treated with standard drug Silymarin ($P < 0.05$).

Hepatic damage induced by paracetamol resulted in elevated levels of SGOT, SGPT, ALP and bilirubin reflects the liver damage and indicates a loss of functional integrity of cell membranes in the liver which is further reflected in the histopathological studies. Synthesis of protein is one of the most important liver functions. Liver damage causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. Restoring the normal levels of protein is an important parameter for liver recovery (Navarro and Senior, 2006). Treatment with paracetamol resulted in a decrease in liver protein levels. The significant reduction in the level of marker enzymes and total protein in the serum due to the administration of stem bark aqueous and absolute ethanol extracts of *L. floribunda* treated groups provides key evidence for the hepatoprotectivity of the extracts. So far, hepatoprotective potentials have been described and documented for one species, *L. coreana* var. *lanuginosa*, commonly known as hawk tea, tested in the carbon tetrachloride-induced hepatotoxicity model (Zhao, 2013). Therefore, owing to the observed antioxidant and hepatoprotective potentialities of the various extracts tested in the present study, *L. floribunda* and its phytochemicals may offer potential therapeutic benefits.

5. Conclusion

Litsea floribunda is an important endemic species of the Western Ghats of southern India. An attempt has been made in the present study to investigate the phytochemicals, antioxidative and hepatoprotective potentials of the solvent extracts. The obtained results justify the fact that the absolute ethanol and aqueous extracts of both leaf and stem bark with high phenolic content exhibited higher antioxidant activity than other solvent extracts. These extracts contain flavonoids as well as other phenolic compounds that may be responsible for their antioxidant activities. The aqueous and absolute ethanol stem bark extracts in both lower and higher doses have shown potent antioxidant activity which is confirmed through histopathological studies. Further, studies directed towards the fractionation of extracts and identification of compounds responsible for the antioxidant and hepatoprotectivity are deemed necessary future work.

Conflict of interest

The authors declare no conflict of interest in the publication of the manuscript.

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References

- Agarwal N, Chowdhary AS, Sharma MC and Dobhal MB. 2011. Chemical constituents of plants from the genus *Litsea*. *Chem Biodivers*, **8**: 223-243.
- Akshatha JV, Prakash, HS and Nalini MS. 2015. Antioxidative and α -amylase inhibitory potentials of medicinal plants from the Western Ghats of southern India. *Pharm Lett*, **7**: 10-17.
- Alqasoumi SI. 2014. Valuation of hepatoprotective and nephroprotective activities of *Scrophularia hypericifolia* growing in Saudi Arabia. *Saudi Pharm J*, **22**: 258-263.
- Arfan M and Kader A. 2006. Antioxidant properties-tannins from *Litsea monopetala*. *Polish J Food and Nutr Sci*, **34**: 66-69.
- Aseervatham GSB, Sivasudha T, Sasikumar JM, Christabel PH, Jeyadevi R, and Ananth DA. 2013. Antioxidant and hepatoprotective potential of *Pouteria campechiana* on acetaminophen-induced hepatic toxicity in rats. *J Physiol Biochem*, DOI:10.1007/s13105-013-0274-3.
- Bawa KS, Das A, Krishnaswamy J, Karanth KU, Kumar, NS and Rao, M. 2007. Ecosystem profile: Western Ghats and Sri Lanka biodiversity hotspot, Western Ghats region. *Critical Ecosystem Partnership Fund Report*. pp. 1-3.
- Benzie IF and Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analyt Biochem*, **239**: 70-76.
- Bhagwat SA, Kushalappa CG, Williams PH and Brown ND. 2005. The role of informal protected areas in maintaining biodiversity in the Western Ghats of India. *Ecology & Soc*, **10**: 8. Online URL: <http://www.ecologyandsociety.org/vol10/iss1/art8/>.
- Bhuniya T, Singh P, and Mukherjee SK. 2010a. An account of the species of *Litsea* LAM. (Lauraceae) Endemic to India. *Bangl J Plant Taxon*, **17**: 183-191.
- Bhuniya T, Singh P, and Mukherjee SK. 2010b. *Litsea cubeba*-medicinal values-brief summary. *J Trop Med Plants*, **11**: 124-130.
- Briskin DP. 2000. Medicinal plants and phytomedicines, linking plant biochemistry and physiology to human health. *Plant Physiol*, **124**: 507-514.
- Chaudhari NB, Chittan KP, and Patil VR. 2009. Hepatoprotective activity of *Cassia Fistula* seeds against paracetamol-induced hepatic injury in rats. *Arch Pharm Sci Res*, **1**: 218-221.
- Devi P, and Meera R, 2010. Study of antioxidant, anti-inflammatory and wound healing activity of extracts of *Litsea glutinosa*. *J Pharma Sci & Res*, **2**: 155-163.
- Koche D, Shirsat R, Imran S, and Bhandange DG. 2010. Phytochemical screening of eight traditionally used ethno medicinal plants from Akola district (MS) India. *Int J Pharm Bio Sci*, **1**: 253 – 256.
- Girish KE, Pradeep KG, Sasikala K and Shivadasan KK. 2014. Plants used in traditional herbal shampoos (thaali) of Kerala, India: a documentation. *Asia Pacific J Res*, **10**: 56-63.

- Harborne JB. 1973. **Phytochemical Methods**. Chapman and Hall Ltd., London., pp. 49-188.
- Hebbar DR, Savitha G and Nalini MS. 2015. Aqueous leaf extracts of *Schefflera venulosa* and *S. wallichiana* protects the liver against Carbon tetrachloride (CCl₄) - induced hepatic damage in albino rats. *Am J Pharm Tech Res*, **5**: 328-340.
- Jia X, Dong L, Yang Y, Yuan S, Zhang Z and Yuan M. 2013. Preliminary structural characterization and antioxidant activities of polysaccharides extracted from *Litsea coreana* (Hawk tea). *Carbohydr Polym*, **95**: 195-199.
- Kong DG, Zhao Y, Li GH, Chen BJ, Wang XN, Zhou HL, Lou HX, Ren DM and Shen T. 2015. The genus *Litsea* in traditional Chinese medicine: an ethnomedical, phytochemical and pharmacological review. *J. Ethnopharmacol.*, **164**: 256-264. doi: 1016/j.jep.2015.02.020.
- Li HB, Cheng KW, Wong CC, Fan KW, Chen F and Jiang Y. 2006. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem*, **102**: 771-776.
- Lu Y, Shipton FN, Khoo TJ, and Wiart C. 2014. Antioxidant activity determination of *Citronella* and crude extracts of *Cymbopogon citratus* by three different methods. *J. Pharm. Pharmacol*, **5**: 395-400.
- Luper S. 1998. A review of plants used in the treatment of liver diseases: Part 1. *Altern Med Rev*, **3**: 410-421.
- Navarro VJ, and Senior JR. 2006. Drug-related hepatotoxicity. *N Engl J Med*, **35**: 731-739.
- Oyaizu M. 1986. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucosamine. *J Nutr*, **44**: 307-315.
- Pandey N, Barve D, Prajapati N, and Dubey BK. 2012. Evaluation of hepatoprotective activity of ethanolic extract of *Arisaema leschenaultia* Blume tuber in paracetamol-induced hepatotoxicity in Swiss albino mice. *Int J Res Pharma Biomed Sci*, **3**: 312-318.
- Pannangpetch P, Laupattarakasem P, Kukongviriyapan V, Kukongviriyapan U, Kongyingyoes B and Aromdee C. 2007. Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans*. *Sangklanakorin J Sci Technol*, **29**: 1-9.
- Rabeta MS, and Faraniza RN. 2013. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atroviridis* and *Cynomera cauliflora*. *Int Food Res J*, **20**: 1691-1696.
- Ruth B. 2004. Medicinal properties of *Litsea glutinosa* Lour. and *Litsea monopetala*. *e-Planet*, **2**: 94-95.
- Sheikh RA, Babu DJM, Venkatrao N, Irene PR, More S, and Turaskar A. 2012. Hepatoprotective activity of alcoholic and aqueous extracts of bark of *Bassia latifolia* Roxb. against paracetamol induced hepatotoxicity in rats. *Pharm Lett*, **4**: 1272-1284.
- Shenoy S, Kumar H, Thashma, Nayak V, Prabhu K, Pai P, Warriar I, Somayaji, Madhav V, Bairy KL and Kishore A. 2012. Hepatoprotective activity of *Plectranthus ambolonicus* against paracetamol-induced hepatoprotectivity in rats. *Int J Pharmacol Clin Sci*, **1**: 52-58.
- Volluri SS, Bammidi SR, Chippada SC and Vangalapati M. 2011. *In vitro* antioxidant activity and estimation of total phenolic content in methanolic extract of *Bacopa monniera*. *Rasayan J Chem*, **4**: 381-386.
- Yadav MS, Kumar A, Singh A, Sharma US and Sutar N. 2011. Phytochemical investigation and hepatoprotective activity of *Cissampelos pareira* against CCl₄-induced hepatotoxicity. *Asian J Pharm Health Sci*, **1**: 106-110.
- Yadav NP and Dixit VK. 2003. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. *J Ethnopharmacol*, **86**: 197-202.
- Zhao X. 2013. Hawk tea (*Litsea coreana* Levl. var. *lanuginosa*) attenuates CCl₄-induced hepatic damage in Sprague-Dawley rats. *Exp Ther Med*, **5**: 555-560.

