CHAPTER I

Evaluation of phytochemicals and antioxidant activity in the leaf and stem bark extracts of *L. floribunda*

1.1 Introduction

Using medicinal plants for the treatment of various health problems has been an ancient practice in the world. With the current global shift to obtain drugs from plant sources, much attention is given to herbal medicines and thus medicinal plants are gaining much importance. A wide range of pharmacologically active compounds differing in terms of structures and therapeutic properties are found in the plants used in traditional medicine. Hence, a good number of plants have been screened for the presence of natural antioxidants responsible for the maintaining health, reducing the oxidative damage and other related diseases (Rhumzhum *et al.*, 2012).

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). Phytochemicals also known as phytonutrients are natural non-essential chemical compounds found in plants that give plants their color, flavor and smell. They are found in vegetables, grains, legumes, beans, fruits, herbs, nuts, roots, leaves and seeds that are largely responsible for the medicinal properties and health benefits of medicinal herbs. Since Vedic times, medicinal plants and their products are being used in India and that has resulted in the development of an important system of medicine-Ayurveda. The use of plants and other natural products in the treatment of diseases has been compiled in Charaka Samhita and Sushruta Samhita that give a detailed account of various diseases and their treatment strategies using plants as medicines (Das and Sharma, 2001).

The biochemical processes taking place in the human body produce oxygen-centered free radicals and other reactive oxygen species (ROS) as byproducts. Overproduction of free radicals result in oxidative stress and cause damage to biomolecules leading to pathogenesis of more than 50 diseases, such as atherosclerosis, cancer, diabetes, ageing, autoimmune disorders, cardiovascular
disorders and neurodegenerative diseases like Alzheimer’s and Parkinson’s in humans.

Plants are rich in free radical scavenging molecules, such as phenolic compounds, nitrogenous compounds, vitamins, terpenoids and other secondary metabolites, with good antioxidant activity. It is reported that many of these antioxidant compounds possess various biological activities such as anti-inflammatory, antimicrobial, antiatherosclerotic, cytotoxic, antidiabetic, antimutagenic, anticancerous and neuroprotective activities (Cai et al., 2003).

Antioxidants are agents that are capable of scavenging the free radical by inhibiting oxidation indirectly through iron chelation and quenching of triplet oxygen. Synthetic antioxidants such as BHT and BHA are found to be toxic and carcinogenic in animal models and hence there is a need to replace them with natural antioxidants from plant origin which cost less but more safe (Li et al., 2007). Recently, there has been a great deal of interest in phenolic substances present in edible plants, fruits, and vegetables that hold antioxidants and health promoting phytochemicals as potential therapeutic agents. The phenolics and flavonoids present in plant extracts attribute to antioxidant and hepatoprotective activity against liver injury (Aseervatham et al., 2013). Nowadays, emphasis is laid on natural antioxidants of plant origin and their health benefits as they can counteract reactive oxygen species (ROS) by acting as reducing agents, hydrogen donors and free radical quenchers (Huda-Faujan et al., 2009).

Liver is one of the largest and vital organs in human body and the chief site for metabolism, storage, excretion and detoxification of substances. Liver diseases are a serious challenge to international public health mainly caused by toxic chemicals such as antibiotics, aflatoxin, carbon-tetrachloride and xenobiotics (Yadav et al., 2011). Since, synthetic drugs used to treat liver disorders have serious side effects, there is a demand for the herbal hepaprotective medicines with minimum side effects.

Hepatotoxicity is defined as an injury to liver that is associated with impaired liver function caused by the exposure to a drug or non-infectious agent. Paracetamol is a commonly used antipyretic and analgesic agent. Continuous use of agents like paracetamol, tetracycline, anti-tubercular drugs, oral contraceptives of hormonal
origin and chemical food preservatives cause liver damage. Drug-induced liver injury is an unresolved problem and often limits drug therapy in clinical practice. Liver disorders and hepatocellular carcinoma are posing threat to mankind as they account for nearly 20,000 deaths every year. Forty different herbal formulations are available in ayurvedic system of medicine rich in phytochemicals having hepatoprotective activity such as phenols, monoterpenes, carotenoids, flavonoids and alkaloids (Sheikh et al., 2012).

Increased lipid peroxidation during the liver microsomal metabolism of alcohol results in hepatitis and cirrhosis (Harikumar et al., 2011). Hepatotoxic compounds induce lipid peroxidation and results in oxidative damage in liver cells. But the natural antioxidants present in herbs, fruits and vegetables offer resistance against oxidative damage and inhibiting lipid peroxidation. The plant extracts provide hepatoprotection through mechanisms such as antioxidative, anti-lipid peroxidative, anti-fibrotic, and liver-regenerating effects (Luper, 1998).

There are reports on various species of *Litsea* containing biologically active phytochemicals like aporphine alkaloids, butanolides, sesquiterpenes, flavonoids, terpenoids and fatty acids with strong antioxidant property as well as hepatoprotective property. In *L. floribunda*, the plant selected for the investigation, no information has been documented in the literature pertaining to the presence of phytochemicals, antioxidant and hepatoprotective potentials. Therefore, there is a need for the evaluation of phytochemicals, antioxidant activity and hepatoprotective potentials in *L. floribunda*, an endemic species of Western Ghats.

### 1.2 Materials and methods

#### 1.2.1 Collection of the Plant Material

*L. floribunda* (Bl.) Gamble was collected from the forests of Madikeri located in the Western Ghats (12°01’ to 12°27’N and 75°16’ to 75°33’E), Kodagu District, Karnataka, India (Fig 1.1) and identified on the basis of species descriptions of Flora of the Presidency of Madras (Gamble, 1957). A herbarium specimen of the species is deposited in the herbarium collection of the Department of Studies in Botany, University of Mysore, Manasagangotri, Mysuru and in the Botanical Survey of India (BSI), Western Range, Pune, India with the Accession No. 136244. Plant parts like
healthy leaves and stem bark were collected in zip lock polyethylene bags and brought to the laboratory.

1.2.2 Sample processing and extract preparation

The collected plant parts were washed with water and then rinsed with distilled water. Plant parts were dried under shade and then in a hot air oven at 40°C for 12 h and powdered. The powdered samples were stored in airtight polyethylene bags until use. Fifty grams of dried leaf and bark powder were submitted to successive extraction separately with 350 ml each of solvents in the order of polarity Hexane>chloroform>ethyl acetate >ethanol > methanol >water at room temperature in a Soxhlet apparatus for 48 h. The liquid obtained after solvent extraction was dried using a rotary flash evaporator (Superfit Model PBU-6D, India) and the residue obtained is stored as solvent extracts (Akshatha et al., 2015). The extracts were stored in labeled and pre-weighed glass vials. The aqueous extracts of leaf and stem bark were prepared according to the procedure of Hebbar et al. (2015) by boiling 500 g of the powdered materials in distilled water with continuous stirring 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. The dried powder was scraped, weighed and stored as the dry aqueous extract and used for the study of biological activities.

1.2.3 Phytochemical screening

Qualitative phytochemical screening of solvent extracts was conducted employing standard methods (Harborne, 1973 and 1998). One gram each of the leaf and bark extracts were dissolved in the respective solvents and tested for the presence of phytochemicals like tannins (Ferric chloride test), flavonoids (Ferric chloride test), alkaloids (Mayer’s, Wagner’s and Dragendorff’s tests), saponins (Foam test), cardiac glycosides (Lieberman’s, Salkowski’s, and Keller-Killani’s test), terpenoids (Salkowski’s test), anthraquinones (Borntrager’s test), phlobatannins (Precipitate test), and reducing sugars (Fehling’s test).

1.2.4 Chemicals

All the reagents were purchased from Qualigens fine chemicals, Mumbai, India. The solvents (polar and non-polar) were obtained from SD fine chemicals.
limited, Mumbai, India. The drug Silymarin (500 mg) was purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). All other chemicals used were of analytical grade.

1.2.5 Preparation of reagents for phytochemical screening

1.2.5.1 Dragendorff’s reagent:

Solution A: 0.17 g of Bismuth nitrate was dissolved in 2.0 ml of glacial acetic acid followed by 8.0 ml of distilled water.

Solution B: 4.0 g of potassium iodide was dissolved in 10.0 ml of glacial acetic acid and 20.0 ml of distilled water was added.

The solutions A and B were mixed and diluted to 100 ml using distilled water.

1.2.5.2 Ferric chloride solution (0.1 %, w/v): 0.1 g of ferric chloride was dissolved in small amount of distilled water and the volume was made up to 100 ml using distilled water.

1.2.5.3 Sodium hydroxide solution (10%, w/v): 10 g of sodium hydroxide was dissolved in distilled water and the volume made up to 100 ml using distilled water.

1.2.5.4 Sulphuric acid (2%, v/v): 2.0 ml of concentrated sulphuric acid was dissolved in 98.0 ml of distilled water

1.2.5.5 Hydrochloric acid (1%, v/v): 1.0 ml of concentrated hydrochloric acid was dissolved in 99.0 ml of distilled water.

1.2.5.6 Hydrochloric acid (10%, v/v): 10 ml of concentrated hydrochloric acid was dissolved in 90.0 ml of distilled water.

1.2.5.7 Ammonia solution (1%, v/v): 1.0 ml of ammonia solution was added to a measuring cylinder and the volume was made up to 100 ml using 99 ml of distilled water.

1.2.5.8 Ammonia solution (10%, v/v): 10 ml of ammonia solution was added to a measuring cylinder and the volume was made up to 100 ml using 90 ml of distilled water.
1.2.5.9 **Mayer's reagent**: A mixture of 1.36 g of mercuric chloride and 5.0 g of potassium iodide was dissolved in 100 ml of distilled water.

1.2.5.10 **Wagner’s reagent**: 2.0 g of iodine and 6.0 g of Potassium iodide was dissolved in 100 ml of distilled water.

1.2.5.11 **Sodium hydroxide solution** (20%, w/v): 20 g of sodium hydroxide was dissolved in distilled water and the volume was made up to 100 ml using distilled water.

1.2.5.12 **Sodium hydroxide solution** (6%, w/v): 6.0 g of sodium hydroxide was dissolved in distilled water and the volume was made up to 100 ml using distilled water.

1.2.6 Preliminary qualitative phytochemical tests

1.2.6.1 **Test for Saponins (Foam test)**: One ml of the plant extract and 2.0 ml of distilled water were taken and shaken vigorously. A stable persistent froth indicated the presence of saponins.

1.2.6.2 **Test for Tannins (Ferric chloride Test)**: One mg of the extract was mixed with 1.0 ml of water and heated on water bath. The mixture was filtered and 1.0 ml of ferric chloride (FeCl₃) was added to the filtrate. A greenish black precipitate indicated the presence of tannins.

1.2.6.3 **Test for Alkaloids**: There are three different tests for alkaloids

**Dragendorff’s Test**: 1.0 ml extract was stirred with 5.0 ml dilute Hydrochloric acid on a steam bath, filtered and 1.0 ml of Dragendorff’s reagent was added. An orange red precipitate indicated the presence of alkaloids.

**Wagner’s Test**: 0.2 g of the extract was mixed with 1% HCl and boiled. After cooling, few drops of Wagner’s reagent were added. A brownish red precipitate indicated the presence of alkaloids.

**Mayer’s Test**: 0.2 g of the extract was added to few drops of Mayer’s reagent. A yellow precipitate indicated the presence of alkaloids.
1.2.6.4 Test for Glycosides: There are three different tests for glycosides

a. Lieberman’s Test: 0.5 g extract was dissolved in 2.0 ml of acetic anhydride and after cooling sulphuric acid was added. The color change from violet to blue to green indicated the presence of glycosides.

b. Salkowski’s Test: 0.5 g of the extract was dissolved in 2.0 ml of chloroform and H₂SO₄ was added. A reddish brown color at the interphase indicated the presence of glycosides.

c. Keller-Killiani’s test: 5.0 ml of extract was treated with 2.0 ml of glacial acetic acid containing one drop of 1% ferric chloride solution. A brown ring in the interface indicates a deoxysugar characteristic of cardenolides.

1.2.6.5 Terpenoids (Salkowski’s Test) test: 0.2 ml of the extract was mixed with 2.0 ml of chloroform and one ml of conc. sulphuric acid was carefully added to form a layer. A reddish brown color in the interface indicates positive result for the presence of terpenoids.

1.2.6.6 Flavonoids (Ferric chloride test): 0.2 ml of the extract was added to Ferric chloride (10%) and the mixture was shaken. A wooly brownish precipitate indicated the presence of flavonoids.

1.2.6.7 Phlobatannins (Precipitate test): One ml of the extract was added to 2.0 ml of HCl (1%) and the mixture was boiled. Deposition of a red precipitate indicated the presence of phlobatannins.

1.2.6.8 Anthraquinones (Borntrager’s test): 0.5 g of the extract was boiled with HCl (10%) for a few minutes in a water bath and the contents were filtered. A few drops of ammonia (10%) and equal amount of chloroform was added to the filtrate and heated. The formation of rose pink color indicated the presence of anthraquinones.

1.2.6.9 Test for reducing sugars (Fehling’s test): 2.0 ml of the extract was mixed with equal volumes of Fehling A and Fehling B and boiled. The production of yellow or brownish-red precipitate of indicated the presence of reducing sugars in the given sample.
1.2.7 Antioxidant activity

1.2.7.1 Preparation of reagents for the estimation of total phenolic content

**Phosphate buffer (0.2 M, pH 7.4):** 3.12 g of sodium dihydrogen orthophosphate (NaH$_2$PO$_4$. 2H$_2$O) and 2.839 g of di-sodium hydrogen orthophosphate anhydrous (Na$_2$HPO$_4$) was weighed accurately and dissolved in 100 ml of distilled water separately and labeled as X and Y. From this stock solution 19.0 ml of X and 81.0 ml of Y was mixed to obtain the phosphate buffer.

**Gallic acid (1 mg/ml):** One mg/0.001 g of gallic acid was weighed accurately and dissolved in 1.0 ml of phosphate buffer (0.2 M).

**Folin-Ciocalteau (FC) reagent (1:2):** FC reagent of 1:2 dil was prepared by adding 2.0 ml of distilled water to one ml of FC reagent.

**Sodium carbonate (20%, w/v):** 20 g of sodium carbonate was dissolved in 100 ml of distilled water.

1.2.8 Preparation of reagents for the antioxidant assays:

1.2.8.1 DPPH assay:

**DPPH:** 4 mg of DPPH (0.004 g) was dissolved in 100 ml of methanol.

1.2.8.2 Ferrous Reducing Antioxidant Power assay (FRAP):

**Acetate Buffer of pH 3.6:** 3.1 g of sodium acetate trihydrate (CH$_3$COONa.3H$_2$O) was dissolved in 16.0 ml of acetic acid glacial and the volume was made up to one liter using distilled water and the pH 3.6 was adjusted by adding 1N HCl or 1N NaOH.

**Ferric chloride hexahydrate (FeCl$_3$.6H$_2$O, 20mM):** About 345 µl of conc. HCl was added into a measuring cylinder and the volume was made up to 100 ml using distilled water.

**TPTZ (2, 4, 6-Tripyridyl-S-triazine, 10 mM):** 0.0312 g of TPTZ was dissolved in 10 ml of 40 mM HCl.
Working solution: 25 ml of acetate buffer of pH 3.6, 2.5 ml of TPTZ (10 mM) and 2.5 ml of FeCl₃.6H₂O (20 mM) were mixed to get the fresh working solution.

1.2.8.3 Reducing power assay:

Phosphate buffer (0.2 M, pH 6.6): 2.839 g of di-sodium hydrogen orthophosphate anhydrous (Na₂HPO₄) and 3.12 g of sodium dihydrogen orthophosphate (NaH₂PO₄.2H₂O) was dissolved in 100 ml of distilled water separately and labeled as X and Y. The pH was adjusted by mixing the two stock solution using pH meter.

Potassium ferrocyanide (1%, w/v): One g of potassium ferrocyanide was dissolved in 100 ml of distilled water.

Trichloroacetic acid (10%, TCA): Ten g of TCA was dissolved in 100 ml of distilled water.

Ferric chloride (0.1%): 0.1 g of ferric chloride was dissolved in 100 ml of distilled water.

1.2.9 The antioxidant activity was evaluated by the following methods:

1.2.9a Determination of Total Phenolic Content (TPC): The total phenolic content in the L. floribunda extracts was estimated by the Folin–Ciocalteau (FC) method using Gallic acid as standard (1mg/ml) following the procedure of Volluri et al. (2011). Plant extracts were taken in different concentrations (50-250 μl) and were made up to 1000 μl using PBS (20 mM, pH-7.4). One ml of FC reagent was added to the mixture and kept for 3-4 minutes. 2.0 ml of Na₂CO₃ was added later and it was incubated in dark for 45 minutes. After the specified period of incubation, the optical density of the samples was measured at 765 nm using UV-Vis spectrophotometer (T60, TTL Technologies, Bengaluru, India). The total phenolic content was expressed in terms of Gallic acid equivalence (μg/g GAE, calculated as mean value ±SD (n=3). The values of the test samples were plotted using the standard curve. All the tests were carried out in triplicates.

1.2.10 Radical Scavenging by 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH): Radical scavenging activity of the plant extracts against stable 1,1 - diphenyl - 2- picryl
hydrazyl (DPPH-0.1mM) was determined by the method of Pannagpetch et al. (2007). Plant extracts were taken in different concentrations (20-100 μl) and were made up to 250 μl using distilled water. 2.0 ml of DPPH solution prepared in methanol was added to the extracts and mixed. A blank sample was prepared without plant extracts by taking one ml of methanol and one ml of DPPH. The test tubes were incubated for 20 minutes by keeping them in dark condition and the optical density of samples was measured at 517 nm using UV-Vis spectrophotometer. The reference standard, L-ascorbic acid of different concentration (5-25 μl) was used and the experiment was carried out in triplicates.

The percent scavenging activity was calculated as follows:

% radical scavenging = Ac - As x100/Ac

Where Ac = absorbance of the control; As = absorbance of the sample

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.

1.2.11 Ferrous Reducing Antioxidant Power Assay (FRAP assay): The antioxidant power of the extracts was estimated by following the methods of Benzie and Strain (1996) with ferrous reducing antioxidant power assay. The reagent was freshly prepared by dissolving 10 mM of Tripyridyltriazine (TPTZ) in 40 mM of hydrochloric acid, 20 mM of ferric chloride (FeCl₃) in distilled water and 300 mM of acetate buffer (pH 3.6). The working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ and 2.5 ml of FeCl₃ at 37⁰C temperature. The plant extracts of different concentration (20-100 μl) were made up to 100 μl by adding methanol. 3.0 ml of working solution was added to the test tubes and kept at 30⁰C for four minutes. Blank sample was prepared with methanol and a working solution. The absorbance of samples was measured against blank at 593 nm spectrophotometrically. The experiments were performed in triplicates. Ferrous sulphate (FeSO₄) (5-25 μl) 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.

1.2.12 Reducing Power Assay: The reducing power of the solvent extracts was measured following the procedure of Oyaizu (1986). Plant extracts (25-125 μl) of
various concentrations were made up to 500 μl by adding phosphate buffered saline (PBS) to which 2.5 ml of Potassium ferricyanide \([K_3Fe(CN)_6]\) was added and the mixture was incubated for 20 minutes at 50\(^\circ\)C. Later, 500 μl of Trichloro acetic acid (TCA-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 \(FeCl_3\) (0.01% w/v) was carefully added. The absorbance of the colored samples was measured at 700 nm against a blank using UV-Vis spectrophotometer. Blank sample was prepared using all the reagents without plant extracts and the experiments were carried out in triplicates. An increase in the optical density reading value of the reaction mixture indicated the increased reducing power of the extracts.

1.2.13 Hepatoprotective activity of \textit{L. floribunda} extracts

1.2.13.1 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±2\(^\circ\)C, relative humidity 55±2% and light and dark cycles of 12L: 12D. They were provided with standardized pellet feed and drinking water \textit{ad libitum}. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. No. 706/CPCSEA dt. 1.10.2002 (copy enclosed). All the experimental procedures were carried out in accordance with the guidelines of CPCSEA.

1.2.13.2 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 were administered with the ethanolic and aqueous extracts of \textit{L. floribunda} orally in single increasing doses 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. They were observed continuously for two hours and then occasionally for four hours and finally for overnight mortality. The dose up to 2500 mg/kg b.w. was well tolerated without producing any alteration in gross behavior signs of toxicity and mortality. The dose selected for the study was 10% of the maximum tolerated dose that is 250 mg/kg p.o. Another higher dose was selected- 500 mg/kg b.w. for administration to the rats.
1.2.13.3 Hepatoprotective study design

The present study design was 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. Blood was collected from the retro-orbital plexus. The rats were sacrificed and livers 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 samples were analyzed for marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum bilirubin, total protein levels (Prism Diagnostics Pvt., Ltd, Thane, India), and alkaline phosphatase (ALP) (Spinreact, SA, Spain).
1.2.13.4 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 48 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 40X magnification of Research microscope (Labomed Optix) and photographed.

1.2.14 Statistical analysis

The results obtained were subjected to statistical analysis using SPSS program (version 16.0). The biochemical parameters or the marker enzymes were statistically analyzed using “one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test”. The statistical data were expressed as mean “Standard Error Mean (SEM)” (n=3 for the antioxidant assays where n=5 in hepatoprotectivity studies).

1.3 Results

1.3.1 Percentage yield of extracts

The extracts stored in preweighed vials were subjected for percentage yield calculation. The percentage yield of the extracts that was obtained after evaporation was calculated using the following formula and represented in Table 1.1.

\[
\text{Percentage extraction yield} = \frac{\text{Weight of the product obtained after evaporation}}{\text{Weight of the powdered sample taken initially}}
\]

The phytochemical analysis of *L. floribunda* solvent extracts of leaf and stem bark indicated the presence of saponins, tannins, terpenoids, flavonoids, cardiac glycosides and reducing sugars (Table 1.2 and Fig. 1.2).
Table 1.1 Percentage yield (w/w) of extracts from leaf and stem bark of *L. floribunda*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>*Yield of extraction (%)</th>
<th>Leaf</th>
<th>Stem bark</th>
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<tbody>
<tr>
<td>Hexane</td>
<td>0.78 g</td>
<td>0.56 g</td>
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<tr>
<td>Chloroform</td>
<td>1.22 g</td>
<td>2.94 g</td>
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<td>Ethyl acetate</td>
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<td>Ethanol</td>
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<tr>
<td>Methanol</td>
<td>2.18 g</td>
<td>1.08 g</td>
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*100 g of powdered leaf and stem bark samples used for extraction*

Saponins were reported in the hexane, ethanol and aqueous leaf extracts and in the ethyl acetate, ethanol and methanolic stem bark extracts, tannins were reported in the ethyl acetate leaf extract and aqueous leaf and stem bark extracts, hexane and ethyl acetate leaf extracts and hexane, chloroform and methanolic stem bark extracts showed the presence of terpenoids, cardiac glycosides were reported to be present in hexane, chloroform, ethyl acetate, ethanol and methanolic leaf and ethyl acetate and methanolic stem bark extracts, flavonoids are present in the chloroform and ethyl acetate leaf extracts and hexane, chloroform, ethanol, methanol and aqueous extracts, chloroform extract of leaf and aqueous extracts of leaf and stem bark of *L. floribunda* showed the presence of reducing sugars. Alkaloids, anthraquinones and phlobatannins were not reported from the phytochemical screening of *L. floribunda* extracts. The analysis revealed the presence of terpenoids, glycosides and flavonoids as major phytoconstituents in both the extracts.
Fig. 1.1 *L. floribunda* collected from the forests of Madikeri located in the Western Ghats Kodagu District, Karnataka, India

a. Habit

b. Close up of inflorescence

c. Branch bearing fruits with persistent perianth-tube
Table 1.2  Phytochemical screening of leaf and stem bark extracts of *L. floribunda*

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Solvent extracts</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Aqueous</th>
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<td>Tannins</td>
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<tr>
<td>Alkaloids</td>
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<td>Wagner's test</td>
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<td></td>
<td>Keller-Killiani’s test</td>
<td>+ +</td>
<td>- +</td>
<td>+ +</td>
<td>+ +</td>
<td>- +</td>
<td>- -</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>- +</td>
<td>+ +</td>
<td>+ +</td>
<td>- +</td>
<td>- -</td>
<td>- +</td>
<td>- +</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>- -</td>
<td>+ -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>+ +</td>
</tr>
</tbody>
</table>

(+) = Positive result of the test, (-) = Negative result of the test; L = Leaf, B = Stem bark
Fig. 1.2 Qualitative detection of phytochemicals in *L. floribunda* extracts.

P=Positive; N=Negative
1.3.2 Total Phenolic Content

All solvent extracts of *L. floribunda* contained phenolics in various quantities (Table 1.3). In the leaves, the hexane extract depicted very low content (2.6±0.1 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.

1.3.3 Antioxidant Assays

1.3.3.1 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 IC$_{50}$ values of the leaf and stem bark solvent extracts (Table 1.3). The aqueous and the hexane extract of leaves possessed IC$_{50}$ values in the range of 25.3 ±0.4 μg/ml to 216.1±3.0 μg/ml, respectively. Similarly, in the stem bark extracts, IC$_{50}$ values of 19.9±0.1 μg/ml was observed for the ethanol extract, while, 240.1 ± 1.5 μg/ml was recorded for the hexane extract. Lower IC$_{50}$ value indicates higher activity and vice versa. The results were compared to the scavenging activity of the standard ascorbic acid (IC$_{50}$ = 14.9±0.3 μg/ml).

1.3.3.2 Ferrous Reducing Antioxidant Power Assay (FRAP Assay)

The reducing power of *L. floribunda* extracts were measured and the values are represented (Table 1.3). 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 ±1.0 & 532.2±1.5 μM Fe (II) /g were observed for the stem bark and leaf aqueous extracts, respectively.

1.3.3.3 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±0.1), and the absolute ethanol extract of stem bark (3.2±0.2). The results represented in Table 1.3 indicate that there was an increase in the reducing power of plant extracts as the extract concentration increased.
Table 1.3 Total phenolic content and antioxidant assay results of solvent extracts of leaf and stem bark of *L. floribunda*

<table>
<thead>
<tr>
<th>Extract</th>
<th>TPC (mg GAE/g)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>FRAP (µM Fe (II)/g)</th>
<th>Reducing Power (Absorbance)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf extracts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>2.6±0.1</td>
<td>216.1±3.0</td>
<td>2.5±0.4</td>
<td>0.58±0.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2.7±0.1</td>
<td>211.7±4.3</td>
<td>1.4±0.2</td>
<td>0.38±0.0</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>70.2±1.0</td>
<td>44.2±0.6</td>
<td>150.9±1.3</td>
<td>0.66±0.1</td>
</tr>
<tr>
<td>Absolute ethanol</td>
<td>114.8±0.2</td>
<td>29.0±0.2</td>
<td>179.4±1.4</td>
<td>2.16±0.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>82.7±1.0</td>
<td>48.4±0.8</td>
<td>495.7±0.6</td>
<td>1.83±0.1</td>
</tr>
<tr>
<td>Aqueous</td>
<td>98.9±0.7</td>
<td>25.3±0.4</td>
<td>532.2±1.5</td>
<td>2.90±0.1</td>
</tr>
<tr>
<td><strong>Stem bark extracts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>5.1±0.3</td>
<td>240.1±1.5</td>
<td>9.3±0.7</td>
<td>0.7±0.4</td>
</tr>
<tr>
<td>Chloroform</td>
<td>3.5±0.1</td>
<td>154.3±1.0</td>
<td>3.3±0.4</td>
<td>0.4±0.7</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>96.9±0.1</td>
<td>19.9±0.1</td>
<td>331.5±0.5</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Absolute ethanol</td>
<td>117.4±0.1</td>
<td>21.5±0.8</td>
<td>320.7±0.4</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>71.9±0.1</td>
<td>37.3±0.8</td>
<td>309.2±0.9</td>
<td>2.5±0.7</td>
</tr>
<tr>
<td>Aqueous</td>
<td>112.4±0.74</td>
<td>24.2±0.2</td>
<td>503.9±1.0</td>
<td>2.1±0.2</td>
</tr>
</tbody>
</table>

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

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

1.3.3.4 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 1.4). Treatment of rats with the 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 (G III).
Treatment of rats with the aqueous extract of leaf of *L. floribunda* (250 mg/kg and 500g/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 and 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 the 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 the standard drug Silymarin.

1.3.3.5 Histopathological studies

The liver samples of Group I (normal) rats showed normal liver with lobules and hepatocytes (Fig.1.3A). Group III constituting the toxic group showed distortion of hepatic architecture with foci of lymphocytes intervening the sinusoids which suggested the toxic effects (Fig.1.3C).

Group II (drug induced) showed normal hepatic morphology with hepatic lobules and hepatocytes (Fig.1.3B). 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 (Fig.1.3D, 1.3E and 1.3F). 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 (Fig.1.3G, 1.3H and 1.3I). 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 (Fig.1.3J and 1.3K).
Table 1.4 Effect of extracts of *L. floribunda* on serum biochemical parameters in Paracetamol-induced liver damage in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum biochemical parameters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGOT (U/L)</td>
<td>SGPT (U/L)</td>
<td>ALP (U/L)</td>
<td>Total (mg/dL)</td>
<td>Bilirubin (mg/dL)</td>
<td>Total Protein (mg/dL)</td>
</tr>
<tr>
<td>G I</td>
<td>Normal</td>
<td>85.8±1.15</td>
<td>67.8±2.8</td>
<td>99.8±2.1</td>
<td>0.67±0.03</td>
<td>6.92±0.1</td>
<td>5.91±0.2</td>
</tr>
<tr>
<td>G II</td>
<td>Silymarin (100 mg/kg)</td>
<td>115± 1.2**</td>
<td>84.6±2.2**</td>
<td>117.8±3.0**</td>
<td>0.89±0.22**</td>
<td>7.46±0.8**</td>
<td>6.88±0.4**</td>
</tr>
<tr>
<td>G III</td>
<td>Paracetamol</td>
<td>181.1± 2.4*</td>
<td>166.8±1.8*</td>
<td>255.4±2.2*</td>
<td>2.81±0.17*</td>
<td>4.46±0.4*</td>
<td>10.68±0.3*</td>
</tr>
<tr>
<td>G IV</td>
<td>LE 250</td>
<td>163.2 ±1.4</td>
<td>158.4±1.5</td>
<td>239.4±3.0</td>
<td>2.10±0.12</td>
<td>5.90±0.2</td>
<td>8.99±0.45</td>
</tr>
<tr>
<td>G V</td>
<td>LE 500</td>
<td>162.4 ±1.7</td>
<td>151.1±0.8</td>
<td>232.3±3.2</td>
<td>1.96±0.08</td>
<td>5.50±0.08</td>
<td>8.81±0.38</td>
</tr>
<tr>
<td>G VI</td>
<td>LA 250</td>
<td>152.1± 1.4</td>
<td>138.8±1.1</td>
<td>184.6±1.7</td>
<td>1.79±0.03</td>
<td>5.22±0.07</td>
<td>8.73±0.27</td>
</tr>
<tr>
<td>G VII</td>
<td>LA 500</td>
<td>154 .3±0.0</td>
<td>125.4±2.4</td>
<td>157.4±2.9</td>
<td>1.66±0.06</td>
<td>5.13±0.14</td>
<td>8.51±0.31</td>
</tr>
<tr>
<td>G VIII</td>
<td>BE 250</td>
<td>149.4 ±1.7</td>
<td>127.4±2.4</td>
<td>170.1±2.5</td>
<td>2.02±0.03</td>
<td>5.80±0.10</td>
<td>8.13±0.27</td>
</tr>
<tr>
<td>G IX</td>
<td>BE 500</td>
<td>147.6± 1.2</td>
<td>125.8±2.4</td>
<td>161.1±2.9</td>
<td>1.83±0.02</td>
<td>6.22±0.13</td>
<td>7.95±0.38</td>
</tr>
<tr>
<td>G X</td>
<td>BA 250</td>
<td>131.1± 2.0**</td>
<td>116.8±1.6**</td>
<td>148.6±2.5**</td>
<td>1.39±0.01**</td>
<td>6.04±0.07**</td>
<td>7.31±0.21**</td>
</tr>
<tr>
<td>G XI</td>
<td>BA 500</td>
<td>124.2±4.0**</td>
<td>102.8±1.8**</td>
<td>132.4±1.4**</td>
<td>1.37±0.01**</td>
<td>6.73±0.08**</td>
<td>7.04±0.34**</td>
</tr>
</tbody>
</table>

*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); SGOT=serum glutamate oxaloacetate transaminase; SGPT= serum glutamate pyruvate transaminase; ALP=Alkaline Phosphatase; U/L=Unit/liter; mg=milligram; dL=deciliter; g=grams; G=grouping of animals; *P<0.05 is considered significant when compared with normal group (GI); **P<0.05 is considered significant when compared by standard group (GII) by Duncan’s Multiple Range Test.
Fig. 1.3 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- GVI (BE250); G- GVII (BE500); H- GVIII (LA250); I- GIX (LA500); J- GX (BA250); K- GXI (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.
1.4 DISCUSSION

In the present investigation, *Litsea*, an endemic species of the Western Ghats was considered for the screening of phytochemicals, antioxidant and hepatoprotective activities.

The phytochemicals are the secondary metabolic products produced by plants for their own defense and also bearing potential benefits for humans like exhibiting antioxidant, antimicrobial, antidepressant, muscle relaxants and anti-inflammatory and many other activities (Briskin, 2000).

In the present study, the solvent extracts of *L. floribunda* were tested for various antioxidant assays such as radical scavenging by DPPH Assay, FRAP Assay and Reducing Power Assay and also for their total phenolic contents. The phenolic compounds such as flavonoids, phenolic acids and tannins contribute to antioxidant capacity of plants and possess biological activities such as anti-inflammatory and anticarcinogenic activities. The presence of flavonoids as major phytoconstituents are reported to be having the antioxidant activity in the plant extracts (Tiwari *et al.*, 2011). Further, phenolic compounds react as hydrogen donors, neutralize free radicals and thus are responsible for free radical scavenging and antioxidant activities of plants (Mitra and Uddin, 2014). 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 good free radical scavenging activity of the extracts. A linear correlation between the amount of total phenolic content and antioxidant capacity has been documented (Rabeta *et al.*, 2013). Tannins and phenolic compounds present in the bark of *L. monopetala* were responsible for antioxidant activity (Arfan *et al.*, 2008).

The DPPH assay is a sensitive assay done for the antioxidant screening of plant extracts. DPPH is a stable free radical at room temperature which accepts an electron or hydrogen radical to form a stable molecule. DPPH changes its color from violet to yellow when the radical is reduced to the corresponding hydrazine. The degree of discoloration indicates the radical scavenging potential of the sample (Lu *et al.*, 2014). The reductive capability of DPPH radicals was determined by the decrease in the absorbance at 517 nm which is induced by antioxidants. The presence of flavonoids, reducing sugars and tannins in the bark extract of *L. glutinosa* was responsible for the antioxidant activity as the extract showed DPPH and H₂O₂ radical
scavenging activity (Devi and Meera, 2010). IC\textsubscript{50} value (19.9 ± 0.1) of ethyl acetate extract of stem bark, IC\textsubscript{50} value (25.3 ± 0.4 µg/ml) of the aqueous extract of leaf, IC\textsubscript{50} value (24.2 ± 0.2 µg/ml) of the aqueous extract of stem bark, IC\textsubscript{50} value (21.5 ± 0.8 µg/ml) of absolute ethanol extract of stem bark and IC\textsubscript{50} value (29.0 ± 0.2 µg/ml) of absolute ethanol extract of leaf in the present study which is closer to scavenging activity of standard ascorbic acid with IC\textsubscript{50} value-14.9 µg/ml suggests that the extracts do have moderately good antioxidant activity and they could be important source of plant antioxidants.

The FRAP assay is considered as a novel method for assessing antioxidant power (Benzie and Strain, 1996). FRAP assay depends upon the principle of reduction of ferrous (Fe III) to ferric ion at low pH to form an intensive blue colored ferrous tripyridyltriazine (Fe II-TPTZ) complex. The reductive activity was measured at 593 nm spectroscopically. The high reducing power of aqueous leaf (532±1.5 µM Fe (II) /g) and bark (503.9 ±1.0 µM Fe (II) /g) extracts could be due to capacity of the extracts to reduce ferrous ion (Fe III) to ferric ion (Fe II) at low pH to form an intensive blue colored ferrous tripyridyltriazine (Fe II-TPTZ) complex. The higher FRAP values gives higher antioxidant capacity because FRAP value is based on reducing ferric ion where antioxidants are the reducing agents (Rabeta et al., 2013). 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 (Mitra and Uddin 2014). The principle of reducing power assay is that the substances having reduction potential convert potassium ferricyanide (Fe\textsuperscript{3+}) to potassium ferrocyanide (Fe\textsuperscript{2+}) which then reacts with ferrous chloride to form ferric ferrous complex antioxidants. The reducing power of Litsea monopetala was very high due to the presence of reductants causing the reduction of the Fe\textsuperscript{3+}/ferricyanide complex to the Fe\textsuperscript{2+} state (Arfan et al., 2008). The reducing power of L. glutinosa is high due to the presence of polyphenols causing reduction of Fe\textsuperscript{3+} and bark is more potent in reducing capacity than leaf (Choudhary et al., 2013).

Potassium ferricyanide + Ferric chloride $\rightarrow$ Potassium ferrocyanide + Ferric chloride
The complex formation leads to a colour change from yellow to different shades of green and blue which has an absorption maximum at 700 nm. The presence of radicals i.e. antioxidants causes the conversion of Fe$^{2+}$ ferricyanide complex to ferrous form in this method (Arulpriya et al., 2012). The Fe$^{2+}$ concentration can be monitored by measuring the formation of Prussian blue at 700 nm. A higher absorbance at 700 nm indicates a higher reducing power. The reducing power and radical scavenging activity of methanolic extract of *L. deccanensis* may be the reason for its pharmacological actions like cardio protective activity which in turn may be due to the presence of phenolic compounds and flavonoids present in the plant extracts (Kumar et al., 2011). The high reducing power in aqueous leaf extract (3.266±0.02) and stem bark extract (2.907 ±0.09) of *L. floribunda* may be due to its strong reducing power potential. The results indicate that the phytochemicals confirmed may be 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 the pathways and formation of toxic metabolite (Shenoy et al., 2012). Paracetamol induced hepatotoxicity is due to its toxic metabolite, N-acetyl-p-benzoquinoneamine (NAPQI) which is normally detoxified by glutathione. In paracetamol toxicity, overload of NAPQI 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 this study, the aqueous and absolute ethanol extracts of stem bark and leaves of *L. floribunda*, depicting potent IC$_{50}$ values in the DPPH assay was selected and evaluated 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 was assessed by histopathological evaluation and the levels of various biochemical parameters like liver marker enzymes in the 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 the 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 hepatoprotectivity of the extracts. So far, hepatoprotective potentials have been described and documented for one species, *L. coreana var. lanuginose*, 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 extracts tested in the present study, *L. floribunda* and its phytochemicals may offer potential therapeutic benefits as antioxidants and anti-hepatotoxic agents.