

Original Article

PROTECTIVE APTITUDE OF *BORASSUS FLABELLIFER* ROOT EXTRACTS AGAINST PARACETAMOL-INDUCED LIVER TOXICITY AND *MYCOBACTERIUM TUBERCULOSIS* (H37 RV)

VEDA PRIYA GUMMADI^{1*}, MALLIKARJUNA RAO TALLURI², KEERTHANA DIYYA¹, GANGA RAO BATTU¹

¹AU College of Pharmaceutical Science, Andhra University, Visakhapatnam, Andhra Pradesh 530003, ²Ana Cipher Clinical Research Organization, Ramanthapur, Hyderabad, Telangana 500013
Email: gummadi.veda88@gmail.com

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ABSTRACT

Objective: *Borassus flabellifer* is a dumb palm tree, different parts of it have been used as food and in traditional medicine. The root parts (rhizomes) are used for phytochemical analysis and evaluation of hepatoprotective and anti-tuberculosis activities.

Materials: Hepatoprotective activity of *Borassus flabellifer* root extracts were studied on paracetamol induced liver toxicity in rats and anti-tuberculosis activity on *Mycobacterium tuberculosis* [H37 Rv strain] quantitative determination using Microplate Alamar Blue assay (MABA) method.

Results: The phytochemical analysis of different extracts *B. flabellifer* roots showed the presence of sterols, terpenoids, glycosides, carbohydrates, proteins, flavonoids, alkaloids, phenols, tannins, saponins and oils. The methanolic extract showed more phenolic and alkaloid contents on their quantification. Ethyl Acetate, Chloroform and Methanol extracts of *B. flabellifer* showed the dose-dependent percentage protection of on paracetamol-induced liver toxicity. The methanol extract showed more activity and is comparable with standard drug Liv 52 on altered liver biomarker enzymes AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels with percentage protection 70.58%, 68.91%, 69.30% 71.18% and 70.73%. The ethyl acetate extract showed more anti-tuberculosis activity than methanol extract with MIC 6.25µg/ml but the extracts showed lower activity compared to standard drugs ciprofloxacin, Streptomycin and pyrazinamide of the MIC values are 6.25, 3.125, 3.125 µg/ml.

Conclusion: *B. flabellifer* roots possess different phytochemical constituents and those extracts possess hepatoprotective and anti-tuberculosis activities.

Keywords: *Borassus flabellifer*, Roots, Paracetamol, Liver, Tuberculosis

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INTRODUCTION

The liver is a vital organ in the human body, take part in ample metabolisms like carbohydrates, proteins, lipids metabolisms and mainly detoxification [1-2]. As of these functions, it is awful essential to maintain the liver healthy. But, different diseases (natural or drug-induced), causing the liver failure finally leads to death [3-5]. Drug-induced liver diseases (DILDs) are more common compared to natural liver diseases around the world [6-7]. The DILDs are cirrhosis, cancers, alcoholic liver diseases and non-alcoholic liver diseases are main causes for the liver failure [8-10]. Modern medical science developed many medicines for the treatment of liver diseases [11-12], and because of its regeneration capacity, the critical was liver transplantation [13]. But, all these treatments require drugs therapy. These treatments are not satisfactory and the long-term use of drugs may cause different side effects [14]. So, inadequate treatment of liver diseases and significant side effects of drugs, identification of new therapeutic agents are urgently needed. Herbal medicines provide significant formulations for treating various diseases including liver diseases with more efficient and minimize side effects [15-16]. In recent times, many researchers recognizing new drugs from different medicinal plants and those drugs are also precursors for synthesis new bioactive molecules for treating various diseases including liver diseases [17-18]. However, there was numerous medicinal flora were remained on the world without identification their medicinal uses.

Borassus flabellifer is a dumb palm tree belongs to the family resident to Indian sub-continent and Africa belongs to the family Arecaceae. Different parts of *B. flabellifer* have been used as medicine for diverse illnesses in traditional medicine [19]. Different phytochemical constituents like steroids, glycosides, vitamins etc were reported from *B. flabellifer* [20]. Different biological activities like cytotoxicity, antiarthritic activity, antibacterial activity, analgesic activity, antipyretic activity and hypoglycemic activity were reported by many researchers through their studies [21-24]. But, there were no scientific

reports on the antioxidant activity of rhizome parts of *B. flabellifer*. In this regards, the present work carried out to evaluate the antioxidant activity of *B. flabellifer* rhizome (roots) part.

MATERIALS AND METHODS

Chemicals and drugs

The chemicals and solvents used in the current study were analytical grade. Diagnostic kits were purchased from span diagnostics Ltd, Gujarat, India. Silymarin was purchased from sigma chemicals, USA. Paracetamol tablets were purchased from the local medical shop.

Preparation of extracts

The *B. flabellifer* root parts were collected at near Bheemili region, Visakhapatnam, Andhra Pradesh and authenticated by the taxonomist Rtd. Prof. M. Venkaiah, Depart of Botany, Andhra University (AUCP/DP and P/BGR/BF-82). Freshly collected materials were dried under shade and then were milled to obtain a powder. The powdered material was separately extracted with different solvents ethyl acetate, chloroform and methanol successively using a Soxhlet apparatus. Finally, collected solutions were concentrated to dryness under vacuum by using Rota-vapor to get the dry extract and were stored in desiccators for further use.

Phytochemical analysis

The phytochemical analysis was carried out to *B. flabellifer* extracts using standard test procedures for identification of their chemical constituents [25].

Total phenolic content

Total phenolic content was determined using the folin-ciocalteu reagent. Briefly, folin-ciocalteu colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue

absorption with a maximum of 765 nm. The intensity of the light absorption at that wavelength is proportional to the concentration of phenols. By using standard gallic acid calibration curve, measure the concentration of phenolic content in gallic acid total equivalents using unit's mg/gm. (GAE) [26-27].

Total alkaloid content

The plant extract (1 mg/ml) was dissolved in 2 N hydrochloric acid (HCl) and then filtered. The pH of phosphate buffer solution was adjusted to neutrality with 0.1 N sodium hydroxide (NaOH). 1 ml of this solution was transferred to a separating funnel and then 5 ml of Bromocresol green (BCG) solution along with 5 ml of phosphate buffer was added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flasks and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of mean±SEM [26, 28].

Selection of animals

Albino rats of single-sex weighing between 180-250 gm were obtained from M/s. Mahaveer Enterprises, Hyderabad, India. The animals were housed under controlled environmental conditions (temperature of 22±1 °C with an alternating 12hr light-dark cycle and relative humidity of 60±5%), one week before the start and also during the experiment. They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, and water *ad libitum* during the experiment. Food was withdrawn 12hr before the terminating experiment and water were allowed *ad libitum*. The protocols were approved by Institutional Animal Ethics Committee and the lab was approved by CPCSEA, Government of India (Regd. No. 516/01/A/CPCSEA).

Acute toxicity studies

The acute toxicity study was conducted for extracts of *B. flabellifer* extracts as per OECD guidelines 423 (OECD.2001) and regulations. Albino rats of single-sex were selected into three groups of consisting of 6 animals. They were maintained for one week before the experiment, under room temperature and allowed free access to water and diet. The animals were subjected for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 3 groups at regular intervals of time, i.e., 1, 2, 4, 8, 12 and 24 h.

During this time, the animals were under observation to note different conditions like skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain, respiratory movements and finally their mortality.

Evaluation of hepatoprotective activity using paracetamol-induced hepatotoxicity

Paracetamol-induced hepatotoxicity in rats' model was used for evaluation of hepatoprotective activity for the *B. flabellifer* extracts

[29]. Animals were divided into twelve (XII) groups (N=6). The animals of Group I served as control (vehicle) were given only saline (2 ml/kg b.w., per orally) for seven days. The animals of group II were administered with 2% w/v solution in water for 7 d. The animals of group III were administered with Liv 52 (25 mg/kg per day, p. o.) for 7 d. Group IV to XII animals was administered with Ethyl acetate, Chloroform and methanol extracts of *B. flabellifer* (125, 250 and 500 mg/kg respectively) for 7 d. Except group I, all groups were administered with paracetamol (200 mg/kg b.w., s. c.) as a 2% w/v solution in water on the 5th day. Rats of all the groups were anaesthetized by chloroform, after 48 hr paracetamol administrations. The blood was collected from the retro-orbital plexus and the collected blood samples were immediately centrifuged at 2400rpm for 15 min. When serum clearly separated out, the serum was analyzed for AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels using commercial diagnostics reagent kits and Autoanalyzer [26]. After collection of blood samples, animals were sacrificed and the liver was collected, washed with saline and were stored in 10% formalin for histopathological studies.

Histopathological studies

Histopathological studies of the liver were performed by cutting 5 mm sections of the tissues by using a microtome and fixing in 10% formalin solution and staining with haematoxylin and eosin [30].

Statistical analysis

All the data were expressed as mean±SEM. Results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 groups. ***p<0.001; **p<0.01; *p<0.05; ns= Non significance.

Anti-tuberculosis activity

The anti-tubercular activity of ethyl acetate and methanol extracts of *B. flabellifer* were assessed against *M. tuberculosis* [H37 RV strain: ATCC No-27294] using microplate Alamar Blue assay (MABA) [31]. Control wells without the tested extracts and sterility controls were assayed simultaneously. Briefly, 100µl of prepared extracts were mixed 100µl *M. tuberculosis* reference strain H37Rv and Middle Brook 7H9 broth was added to test (Drug/Control wells) in the first row wells. Then two-fold dilution was done with a micropipette to next wells and each concentration was tested as triplicates. Finally, each well was covered with paraffin and incubated at 37 °C for 5-7days. After incubation, 25µl of 0.02% w/v resazurin was added to each well and incubated again at the same conditions for color development. The growth of organisms was observed by visual using the alamar blue oxidation-reduction dye.

The dye generally indicated the cellular growth, in this non-fluorescent blue color oxidized and become fluorescent pink color. On reduction, visual pink color indicates the presence of mycobacterial grow. The confirmation of the activity, the extracts were screened to know the minimum inhibitory concentration (MIC) at 100 to 0.2µg/ml.

Table 1: Nature of phytoconstituents in different extracts of *B. flabellifer*

| Phytochemical constituents | <i>B. flabellifer</i> | | |
|----------------------------|-----------------------|----|----|
| | EAE | CE | ME |
| Phytosterols | ++ | + | + |
| Terpenoids | + | - | ++ |
| Glycosides | + | + | ++ |
| Saponins | - | - | + |
| Flavonoids | - | ++ | + |
| Tannins | + | + | + |
| Carbohydrates | - | + | ++ |
| Alkaloids | + | ++ | ++ |
| Amino acids | - | - | + |
| Oils | + | + | - |
| Phenols | + | + | ++ |

+,++= Present,-- Absent
*EAE= Ethyl acetate extract; CE= Chloroform extract; ME= Methanolic extract

Table 2: Total phenolic and alkaloid contents (mg/gm) of *B. flabellifer* extracts

| Name of the extract | TPC (mg/gm)# | TAC (mg/gm)# |
|---------------------|--------------|--------------|
| Ethyl acetate | 06.23±1.54 | 03.28±2.18 |
| Chloroform | 10.46±0.32 | 08.11±1.02 |
| Methanol | 16.08±0.49 | 13.75±1.45 |

* TPC= Total Phenolic content; TAC= Total alkaloidal content, # Values are in mean±SEM

RESULTS

Phytochemical analysis

B. flabellifer extracts exposed the presence of different phytochemical constituents on phytochemical analysis. The extracts revealed the presence of different phytochemical constituents like sterols, terpenoids, glycosides, carbohydrates, proteins, flavanoids, alkaloids, phenols, tannins, saponins and oils. The extracts gave different results for different phytochemical tests (table 1). The quantitative estimation of phenolic and alkaloid contents in three different extracts was varied, methanolic extract showed more content compared to chloroform and ethyl acetate extracts (table 2).

Acute toxicity studies

The extracts of *B. flabellifer* roots were tested for the toxicity at a dose of 2000 mg/kg and observed they are safe because there was no sign of mortality and no behavioural changes in the extracts group rats.

Hepatoprotective activity

Ethyl acetate, chloroform and methanol extracts of *B. flabellifer* were assessed at doses of 125 mg/kg b. w, 250 mg/kg b. w and 500 mg/kg b. w on Paracetamol-induced liver toxicity in rats for their hepatoprotective activity. The percentage protection produced by the standard (Liv 52) and extracts of *B. flabellifer* was calculated based on SGOT, SGPT, ALP, Total serum bilirubin and total protein levels in end of the experiment in each case (table 3).

Group I was treated with vehicle showed no significant changes, group II was treated paracetamol, there is a significant changes (Negative), The animals of group III was induced with paracetamol and was treated Liv 52 (Positive) showed the significant restoration with percentage protection 94.79%, 92.97%, 96.49%, 97.57% and 90.24% in levels of biomarker enzymes of liver (AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein) levels.

The percentage protection produced by ethyl acetate extract (BFEAE) on AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels at 125 mg/kg, 250 mg/kg and 500 mg/kg b. w were 11.66%, 10.16%, 9.62% 11.28% and 13.41%, 28.12%, 30.31%, 28.34%, 31.25% and 29.27%, 51.3%, 47.19%, 50.96% 48.61% and 53.66% respectively (fig. 1).

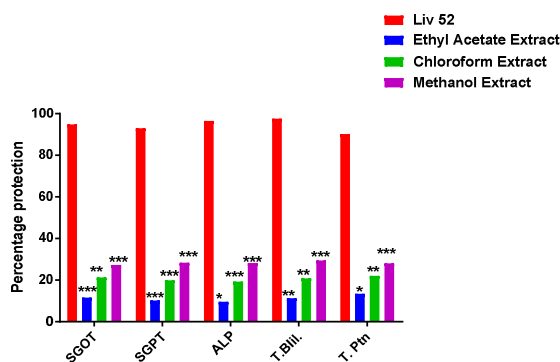


Fig. 1: Percentage protection produced by different extracts of *B. flabellifer* at a dose of 125 mg/kg, Results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 groups, ***p<0.001; **p<0.01; *p<0.05; ns= non significance (N=6), T. Bil= Total bilirubin; T. Ptn= total protein

The percentage protection produced by the chloroform extract (BFCE) on AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 21.26%, 19.84%, 19.20% 20.83% and 21.95%, 34.09%, 39.06%, 37.39% 41.67% and 42.68%, 58.85%, 58.91%, 62.27% 65.97% and 62.80% respectively (fig. 2).

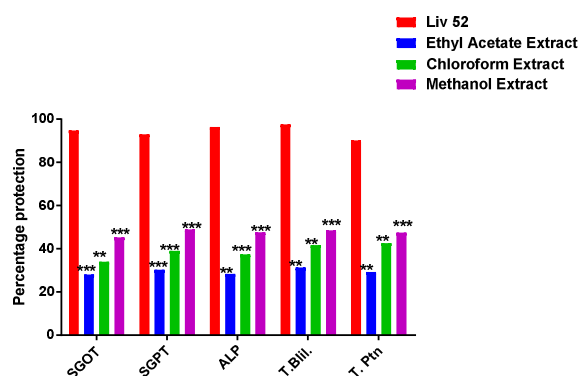


Fig. 2: Percentage protection produced by different extracts of *B. flabellifer* at a dose of 250 mg/kg, results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 groups. ***p<0.001; **p<0.01; *p<0.05; ns= non significance (N=6), T. Bil= total bilirubin; T. Ptn= total protein

The percentage protection produced by the methanol extract (BFME) on AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 27.23%, 28.28%, 28.20% 29.51% and 28.05%, 45.34%, 49.06%, 47.69% 48.61% and 47.56%, 70.58%, 68.91%, 69.30% 71.18% and 70.73% respectively (fig. 3).

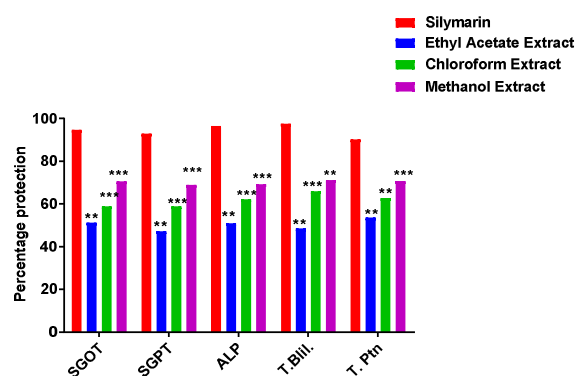


Fig. 3: Percentage protection produced by different extracts of *B. flabellifer* at a dose of 500 mg/kg, results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 groups. ***p<0.001; **p<0.01; *p<0.05; ns= non significance (N=6), T. Bil= total bilirubin; T. Ptn= total protein

Histopathological studies performed in this study showed that, control group (treated with normal saline) animals showed the normal hepatic cellular structure with normal hepatic cells, well

sinusoids. The paracetamol intoxicated liver (group II), shows evidence of the necrosis (fig. 4 (A)), haemorrhage and inflammation. These pathological changes were reduced in the retreated groups of

animals treated with *B. flabellifer* extracts (fig. 4 (D and E) at different doses. The high dose of extracts i.e. 500 mg/kg b.w. showed more restoration of the damaged liver due to the paracetamol.

Table 3: Enzymes of levels of groups I to XII animals due to the effect of different extracts of *B. flabellifer*

| Name of the drug | Name of enzymes | | | | |
|-----------------------|-----------------|-------------|--------------|------------------|-----------------|
| | AST# (U/l) | ALT# (U/l) | ALP# (U/l) | T. bil # (mg/dl) | T. ptn# (gm/dl) |
| Control | 89.17±1.11 | 52.33±1.73 | 183.50±0.85 | 0.25±0.01 | 6.93±0.06 |
| Paracetamol | 332.17±2.18 | 173±5.22 | 548.67±21.60 | 2.17±0.06 | 4.20±0.04 |
| Liv 52 25 mg//kg b.w. | 101.83±1.72 | 59.83±1.11 | 195.67±1.56 | 0.29±0.01 | 6.67±0.08 |
| BFEAE 125 mg/kg b. w | 303.83±1.45 | 148.17±1.08 | 496.50±1.67 | 1.95±0.07 | 4.57±0.06 |
| EA 250 mg/kg b. w | 263.83±2.24 | 126.67±1.74 | 431.67±1.38 | 1.57±0.03 | 5.00±0.05 |
| BFEAE 500 mg/kg b. w | 263.83±2.24 | 108.67±1.05 | 353.33±3.80 | 1.23±0.06 | 5.67±0.07 |
| BFCE 125 mg/kg b. w | 280.50±1.91 | 148.17±1.08 | 463.33±2.91 | 1.77±0.06 | 4.80±0.05 |
| BFCE 250 mg/kg b. w | 249.33±2.29 | 117.33±1.52 | 400.33±3.12 | 1.37±0.06 | 5.37±0.06 |
| BFCE 500 mg/kg b. w | 189.17±3.00 | 96.17±1.74 | 314.17±3.52 | 0.90±0.04 | 5.92±0.07 |
| BFME 125 mg/kg b. w | 266.00±1.93 | 128.83±0.83 | 432.17±1.83 | 1.60±0.05 | 4.97±0.08 |
| BFME 250 mg/kg b. w | 222.00±1.26 | 106.67±1.33 | 364.67±2.29 | 1.23±0.06 | 5.50±0.07 |
| BFME 500 mg/kg b. w | 160.67±1.33 | 85.50±1.54 | 289.83±2.79 | 0.80±0.05 | 6.13±0.04 |

#Values are in mean±SD (N=6)

Anti-tubercular activity

The ethyl acetate and methanol extracts were evaluated for antitubercular activity because of less availability of chloroform extract. The result of *in vitro* antitubercular activity of extracts revealed that

those extracts inhibited the growth of *M. tuberculosis* H37Rv at various at concentrations of 0.8-100µg/ml (table 4 and fig. 5). The ethyl acetate extracts have more active compared to methanol extracts with MIC values 6.25 and 25µg/ml and their results were comparable with standard drug ciprofloxacin.

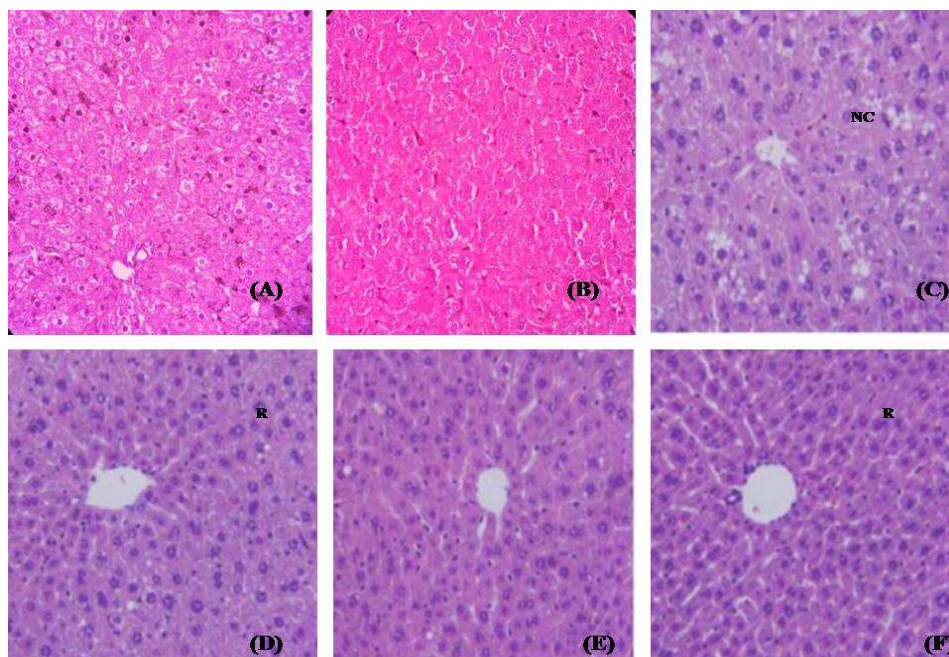


Fig. 4: Histopathological studies of liver tissue in different groups' animals in paracetamol-induced hepatotoxicity treated with different extracts of *B. flabellifer*, [A] control (Group I), [B] toxic group tissue without treatment (Group II), [C] Toxic group treated with Liv 52 (Group III), [D] toxic treated with BFEAE at 500 mg/kg b.w. (Group VI), [E] toxic treated with BFCE at 500 mg/kg b.w. (group IX), [F] Toxic treated with BFME at 500 mg/kg b.w. (group XII), * NC-necrosis; R-regeneration

Table 4: Minimum inhibitory concentrations (MIC) of anti-tubercular activity of ethyl acetate and methanolic extracts of *B. flabellifer*

| Sample name | <i>Mycobacterium tuberculosis</i> H37Rv strain MIC (µg/ml) |
|---------------|--|
| BFEAE | 12.5 |
| BFME | 25 |
| Pyrazinamide | 3.125 |
| Streptomycin | 3.125 |
| Ciprofloxacin | 6.25 |

BFEAE-*B. flabellifer* ethyl acetate extract; BFME-*B. flabellifer* methanol extract

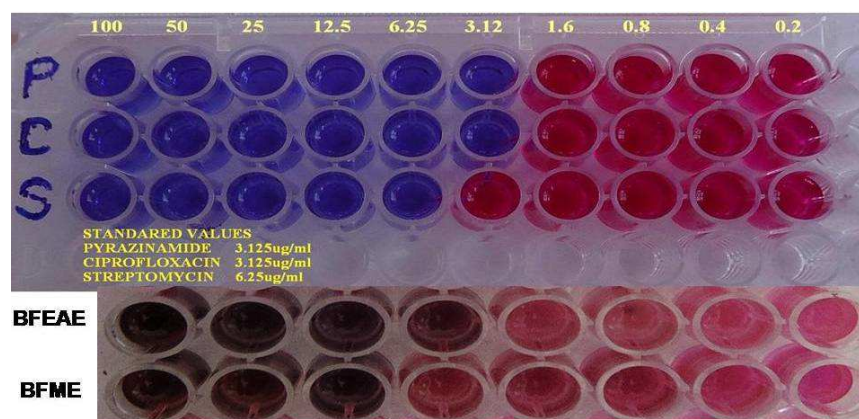


Fig. 5: the Anti-tubercular activity of ethyl acetate and methanolic extracts of *B. flabellifer* at different concentrations

DISCUSSION

In recent years, the study of ethno botanical and floristic characteristics of medicinal plants has been enhanced because of new diseases evolution, diseases causing microorganisms getting resistance to currently using drugs and cause of side effects in long-term use of modern medicines [32]. However, there was little known about the medicinal plants, which have been using traditional medicine and some of these medicinal plants were having scientific evidence [33]. In this point of view, the present work carried out on one of the medicinal plant (*B. flabellifer*) and different parts of it was used as food and in the treatment of diseases. But, there were very little works reported scientifically on root parts of *B. flabellifer*. The phytochemical analysis of different extracts *B. flabellifer* showed the presence of different phytochemical constituents like steroids, phenols, alkaloids, carbohydrates etc., in other parts' phytochemical analysis reported in fruits [34-35] and the phytochemical analysis of ethanolic extract of roots [36]. However, the results of present study mild differences were observed in the phytochemical analysis of ethanolic, ethyl acetate and chloroform extracts, this may be due to regional variation of the *B. flabellifer*. There were some earlier reports about variation in the presence of different phytochemical constituents depends plants' growing region [37-39]. The methanolic extract showed the more phenolic and alkaloid contents compared to other extracts.

The extracts were tested for their safety before going to evaluate their hepatoprotective activity on animals, they were no mortality was found and at the maximum dose i.e. at 2000 mg/kg b.w. The current research, hepatoprotective activity of *B. flabellifer* root parts was an initial report on paracetamol-induced liver intoxication in rats. Ethyl acetate, Chloroform and Methanol extracts of *B. flabellifer* were showed dose-dependent hepatoprotective activity in the preventive treatment of liver intoxication with paracetamol. The extracts showed maximum protection at higher dose i.e. 500 mg/kg b.w. They have restored the liver biomarker enzymes (SGOT, SGPT, ALP, total bilirubin and total protein) levels compared to liver toxicity groups without any treatment (group II) and their protective effect was comparable with standard drug Liv 52. The protective effect of tested extracted was supported by liver histopathological studies on different group animals (fig. 4). Normal group animals were possess normally appear liver tissue with the good architecture of size, hepatic cells, but toxic group without treatment showed the inflammation, oversized, cirrhosis, haemorrhage and necrotic liver tissue with abnormal sinusoids and portal region. The other group's animals treated with ethyl acetate, chloroform and methanol extracts prevent the liver from above pathological conditions of the liver when paracetamol induction for liver toxicity and showed almost normal liver structure as a control group.

In our previous studies, these extracts showed antibacterial activity on different bacterial strains [40]. So, studies continued on anti-tuberculosis activity using *M. tuberculosis* (H37 RV strain). The results of the present study showed good anti-tubercular activity

and the results are comparable with the standard drugs ciprofloxacin and streptomycin. Interestingly, the ethyl acetate extract showed more anti-tubercular activity compared to hepatoprotective and antibacterial activity.

There have been different new drugs introducing for treating diseases and quick attaining of resistance towards them forcing researchers to identify new drugs from different with efficient activity and cost-effective [41]. The plants possess wide diversity of chemical constituents including steroids, alkaloids, phenols, glycosides and etc. Therefore, medicinal plants are still the main source for new therapeutic agents with less side effects, low cost, easy acceptance of patients and plants are cultivable without environmental pollution [42]. The results of the present study and previous studies by us demonstrating that *B. flabellifer* roots possess different bioactive compounds with different biological activities like antioxidant, antibacterial, hepatoprotective and like us, Titas Debnath *et al.* (2013) claimed that hypoglycemic activity of *B. flabellifer* roots [43]. It was may be assumed that extracts possesses antioxidant [44-46] and hepatoprotective activities against drugs induced hepatotoxicity. Furthermore, the *B. flabellifer* roots extracts showed anti-tuberculosis activity. However, the results obtained in the present study are warranted for further studies on different medicinal plants used as food and traditional medicine.

CONCLUSION

The present research reveals that *B. flabellifer* roots possess different phytochemical constituents and those extracts possess hepatoprotective and anti-tuberculosis activities and in our previous studies, these extracts reported about their antibacterial and antioxidant activities. The further work is needed to isolate and characterize the responsible bioactive molecules from these extracts as well as from different medicinal plants for development of effective and safe medicines.

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AUTHORS CONTRIBUTION

1. Mallikarjuna Rao Talluri-correlated the results of experimental work and helped preparation of the manuscript.
2. Veda Priya and Keethana Diyya-designed the experimental work and performed lab work
3. B. Ganga Rao-guided how to plan the experimental design and monitored the lab work correlated the experimental findings with results of other workers and corrected the manuscript.

CONFLICT OF INTERESTS

The authors report no conflicts of interest

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