Research Article

International Journal of Pharmacy and Industrial Research

Available Online at: www.ijpir.com

ISSN		
	Print	2231 – 3648
	Online	2231 – 3656

PHARMACOGNOSTICAL, PHYTOCHEMICAL, *IN-VITRO* TOXICITY AND *IN-VITRO* HEPATOPROTECTIVE INVESTIGATION OF *BRIDELIA TOMENTOSA* ROOT

*[1]Syed Dawood Noor, [1]Kathiresan Krishnasamy, [2]Ramy S Behbehani

[1]Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, India

[2]Pharmaceutical & Herbal Drug Registration and Control Admin,
Drug and Food Control Board, Ministry of Health, Kuwait.

Abstract

The present study was aimed to investigate the pharmacognostical, phytochemical, *in-vitro* toxicity and *in-vitro* hepatoprotective activity of *Bridelia tomentosa* root. Pharmacognostical and phytochemical analysis were carried out as per standard procedures. *In-vitro* toxicity study was performed using MTT assay on BRL3A cell line. *In-vitro* hepatoprotective activity was performed using MTT assay on paracetamol treated BRL3A cell line. The phytochemical screening showed the presence of different phytoconstituents. Petroleum ether extracts showed the presence of phytosterols, gums and mucilage's. Chloroform extracts showed the presence of glycosides and steroids. Methanol extract showed the presence of saponins, tannins, phenolic compounds and flavonoids. HPLC analysis showed the presence of various phytochemical. However, Gallic acid, Rutin and Quercetin were the major phytochemical. The CTC_{50} of *Bridelia tomentosa* root was greater than 1000 µg/ml. Hence, the powdered root of *Bridelia tomentosa* haven't shown any significant toxicity towards BRL3A cell line. *Bridelia tomentosa* root has offered protection for BRL3A cell line against paracetamol at the dose of 500 µg/ml. Further, increase in concentration to 1000 µg/ml has not offered protection. The study concludes that the *Bridelia tomentosa* roots is safe and offers significant hepatoprotection against paracetamol. However, this pilot study data require further validation using molecular level studies and clinical trials.

Keywords: *Bridelia tomentosa* Root, BRL3A Cell Line, Flavonoids, Hepatoprotective Activity, MTT Assay.

Introduction

The liver plays a significant role in the detoxification, digestion and metabolic functions but many factors cause liver damage leading to the development of hepatic cirrhosis and liver cancer. In spite of medical advances, hardly there are any drugs that stimulate liver utility, offer protection to the liver from damage or help regeneration of hepatic cell. Hence, there is an urgent need for safe hepatopotective agent. Plants have been used in the traditional system of medicine from time immemorial, particularly among tribal communities. The World Health Organization (WHO) has listed 20,000 medicinal plants globally and India's contribution is about 15–20%[1, 2].

Author for Correspondence:
Syed Dawood Noor,
Department of Pharmacy, Annamalai University,
Chidambaram, Tamil Nadu, India.
E-mail: syeddawood973@hotmail.com

According to the WHO estimate, about 80% of the population in the developing countries depends directly on plants for their healthcare [3, 4]. In India, about 2000 drugs used are of plant origin [5].

Bridelia tomentosa is a small tree belongs to Euphorbiaceae family distributed in the forest areas of the central and eastern parts of Bangladesh, India and Myanmar[6]. *Bridelia tomentosa* is being used traditionally to treat various ailments including treatment of colic, traumatic injury, epidemic influenza, neurasthenia and liver disorders[7]. The present study was aimed to investigate the pharmacognostical, phytochemical, *in-vitro* toxicity and *in-vitro* hepatoprotective activity of *Bridelia tomentosa root*.

Materials and methods

Materials

The chemicals used in the study were of analytical grade and were obtained from Rankem (India) and Merck (India). The roots of *Bridelia tomentosa* (Fig.1) were collected from Sashachalla forest, Tirupathi and authenticated by Dr. K. Madhava Chetty (Field Botanist, Assistant Professor, Department of Botany, Sri Venkateswara University).

Organoleptic and macroscopic evaluation of the root

Various organoleptic and macroscopic characters including colour, shape, size, taste and odour were evaluated for the root of *Bridelia tomentosa* [8-10].

Microscopic evaluation of the root

A piece of fresh *Bridelia tomentosa* root was dipped in a test tube containing sufficient water and boiled for few minutes. The softened piece was transversally sliced into fine section, which was stained using 0.1%w/v phloroglucinol and observed under microscope [11].

Powder preparation

The collected fresh roots were washed in running water and cut into small pieces to facilitate drying. The dried root pieces were pulverised into coursed powder, which was packed in a black plastic bag and stored in an air-tight container for further work [12].

Powder microscopy

The powdered root was soaked in water along with 2-3 drops of phloroglucinol (0.1% w/v) for few minutes. Soaked powder was spread neatly on a glass slide, which was stained using 1% safranine for few minutes and excess safranine was removed carefully using water. A drop of glycerine was added on to the stain and a cover slip was placed on it. Excess water was removed with the help of blotting paper and the slide was observed under the microscope. The characteristic features of cell components were observed and their photographs were taken using photomicrography [13-15].

Moisture content

About 2 grams of powdered root was transferred into a shallow weighing bottle and the contents were distributed evenly to a depth not exceeding 10 mm. The loaded bottle was heated at 105°C in hot air oven and weighed at different time intervals until a constant weight was obtained. Moisture content was calculated as follows Moisture content (%) = [(Initial weight) – (Oven dry weight) / (Oven dry weight)] x 100 [16].

Total ash value

About 2 grams of powdered root was weighed into a tared silica container and incinerated at 450°C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated as follows % Total Ash = [(Initial weight) – (Final weight) / (Final weight)] x 100 [17].

Acid insoluble ash

Ash obtained from total ash was boiled with 25 ml of 2 N HCl for few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tared silica crucible and incinerated at 450°C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated as follows % Acid insoluble ash = [(Weight of dish + contents after drying) – (Weight of empty dish) / (Weight of original sample)] x 100 [18].

Water soluble ash

Ash obtained from total ash was boiled with 25 ml of distilled water for few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tared silica crucible and incinerated at 450°C in muffle furnace until free from carbon. The crucible was cooled and weighed.

Percentage of water-soluble ash was calculated with reference to air-dried substance. The percentage of water soluble ash value was calculated using formula % Water soluble ash = [(Weight of dish + residue) – (Weight of empty dish) X 4 / (Weight of original sample)] x 100 [19].

Alcohol soluble extractive value

About 5 grams of powdered root was macerated with 100 ml of alcohol in a stoppered flask with frequent shaking during first 6 hours and allowed to stand for 18 hours and then filtered after 24 hours. About 25 ml of the filtrate was evaporated in a tared dish at 105°C and weighed and the alcohol soluble extractive value was calculated as follows % Alcohol extractive value = [(weight of the residue) / (Weight of the root)] x 100 [18].

Preparation of extracts

About 500 grams of course powdered root was weighed and subjected to successive extraction process using different solvent including petroleum ether, chloroform, ethyl acetate and methanol at 50°C temperature for 3 days. After 3 days, the solvent were filtered through muslin cloth and the filtrate was concentrated using rotary evaporator (Buchi Rotary evaporator), until a soft mass obtained and then preserved in desiccator [20, 21].

Table No. 01: Percentage (%) yield of extract using various solvents

Solvents	% yield
Petroleum ether	5.6
Chloroform	18.21
Ethyl acetate	13.26
Methanol	52.53

Phytochemical Analysis

The extracts of *Bridelia tomentosa* root was subjected to preliminary phytochemical screening for the detection of various chemical constituents [22].

Test for carbohydrates

A small quantity of various extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates [23].

Fehling's test

The filtrate was treated with each 1 ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates [23].

Test for glycosides

A small quantity of various extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate was hydrolysed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to the following tests to detect the glycosides [23].

Legal's test

To the filtrate 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides [23].

Borntrager's test

Filtrate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides [23].

Detection of fixed oils and fats

Filter paper test

Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicated the presence of fixed oils [24].

Saponification test

Few drops of 0.5 M alcoholic potassium hydroxide was added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats [24].

Detection of Proteins and Free Amino acids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests [25].

Million's test
Small quantities of various extracts were treated with Million's reagent. Red colour formed shows the presence of proteins and free amino acids [25].

Biuret test
To the prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids [25].

Ninhydrine test
The extracts were treated with Ninhydrine reagent. Purple colour produced shows the presence of proteins and free amino acids [25].

Detection of saponins
The extracts were diluted with 20 ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins [26, 27].

Detection of tannins and phenolic compounds
Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins using following test (a) Small quantities of various extracts were treated with 5% Ferric chloride solution. Appearance of violet colour shows the presence of tannins and phenolic compounds. (b) Small quantities of various extracts were treated with 1% solution of gelatin containing 10% sodium chloride. Appearance of white precipitate shows the presence of tannins and phenolic compounds. (c) Small quantities of various extracts were treated with 10% lead acetate solution. Appearance of white precipitate shows the presence of tannins and phenolic compounds.

Detection of phytosterols
Small quantities of various extracts were dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols [28].

Salkowski test
To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols [28].

Liebermann Burchard test
The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols [28].

Detection of Alkaloids
Small quantities of the various extracts were taken separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests (a) Small quantities of various filtrates were treated with Mayer's reagent. Appearance of cream precipitate shows the presence of alkaloids. (b) Small quantities of various filtrates were treated with Dragendroffs reagent. Appearance of orange brown precipitate shows the presence of alkaloids. (c) Small quantities of various filtrates were treated with Hager's reagent. Appearance of yellow precipitate shows the presence of alkaloids. (d) Small quantities of various filtrates were treated with Wagner's reagent. Appearance of reddish brown precipitate shows the presence of alkaloids [29].

Detection of Flavonoids
Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids. Similarly, to the small portion of each extract, concentrated sulphuric acid was added. Appearance of yellow orange colour indicates the presence of flavonoids. Likewise, small quantities of the extracts were dissolved in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids [30].

Detection of Gums and Mucilage's
A small quantity of various extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilage [30].

High Performance Liquid Chromatographic (HPLC) Analysis
A Shimadzu SPD10A equipped with a UV-Vis Abs.-Variable Wave detector was used. The column used for the study was Phenomenex 5 µm

C18 (2) 100 Å, LC Column 250 x 4.6 mm. HPLC grade water was adjusted the pH 3 using 10% orthophoshoric acid and this solution was sonicated for 15 min than filtered through 0.45 micron filter paper, from this 300 ml was transferred to reagent bottle and 700 ml of HPLC grade methanol was added and sonicated for 15 min. The mobile phase ratio was 70:30. The flow rate was set at 1 ml/min. The absolute calibration curve method was used for the calculation of the concentration [31, 32].

Sample solutions procedure

About 30 mg of powdered root was taken in 10 ml standard flask and make up the volume with methanol. The final concentration of the extract solution was 3000µg/ml, this solution was injected and chromatogram was recorded.

Standard solutions preparation

About 10 mg of Gallic acid was taken in 10 ml standard flask and make up the volume with methanol from this 0.1 ml taken and diluted to 10 ml . The final concentration was 100 µg/ml, this solution was injected and chromatogram was recorded. About 10 mg of Rutin was taken in 10 ml standard flask and make up the volume with methanol. The final concentration was 1000 µg/ml, this solution was injected and chromatogram was recorded. About 10 mg of Quercetin was taken in 10 ml standard flask and make up the volume with methanol. The final concentration was 1000 µg/ml, this solution was injected and chromatogram was recorded.

***In-vitro* toxicity using MTT Assay**

The monolayer cell culture (BRL3A cell line) was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 24 hour in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted at 24 h interval. After 24 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line. Growth inhibition = 100 – [(Mean OD of individual test group) / (Mean OD of control group) x 100] [33-37].

***In-vitro* Hepatoprotective activity**

The monolayer cell culture (BRL3A cell line) was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. 50 µl of DMEM with 4000 µg paracetamol/ 50 µl of different non-toxic concentrations of test drugs were added. The plates were then incubated at 37° C for 24 h in 5% CO_2 atmosphere. After 24 h, the cell supernatants were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage cell viability was determined, based on which the percentage protection offered by test and standard drugs was calculated over the ethanol control [38].

Results and discussion

Macroscopic study of root

Macroscopic examination of the root (Fig. 1) shows that it consists of 8-10 cm long, 2-4 cm thick pieces which are usually branched, shallow, tough, creamish-brown externally, bark thin, outer surface rough due to longitudinal wrinkles, with fracture hard, characteristic odour and pungent taste.

Fig. No. 01: *Bridelia tomentosa* Root

Microscopic study of root

Transverse sections study of the root show xylem vessels, medullary rays and phloem. Xylem shows a wide zone, consisting of lignified pitted vessels found in single as well as in groups of 2-3, scattered throughout xylem region. Medullary rays consist of bi-to triseriate, lignified and radially elongated parenchymatous cells, narrow in the xylem region and wider in the phloem region. Phloem consists of isodiametric, thin-walled, parenchymatous cells, a few containing rhomboidal crystals of calcium oxalate.

Powder microscopy

Root powder appears dull yellow, showing fragments of cork cells about 4-5 rows of tangentially elongated, thick-walled cells; Cortex cell consists of thin-walled polygonal sclerenchymatous cells; lignified stone cells and phloem, lignified fibres, non-lignified fibres and rhomboidal shaped calcium oxalate crystals. Powder characteristics of the root have been shown in Fig 3.

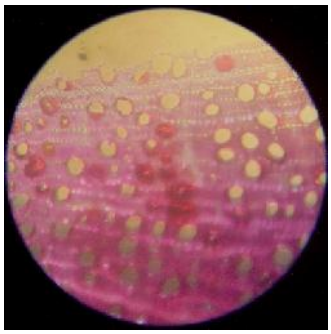

Fig. No. 02: Transverse Section of *Bridelia tomentosa* root

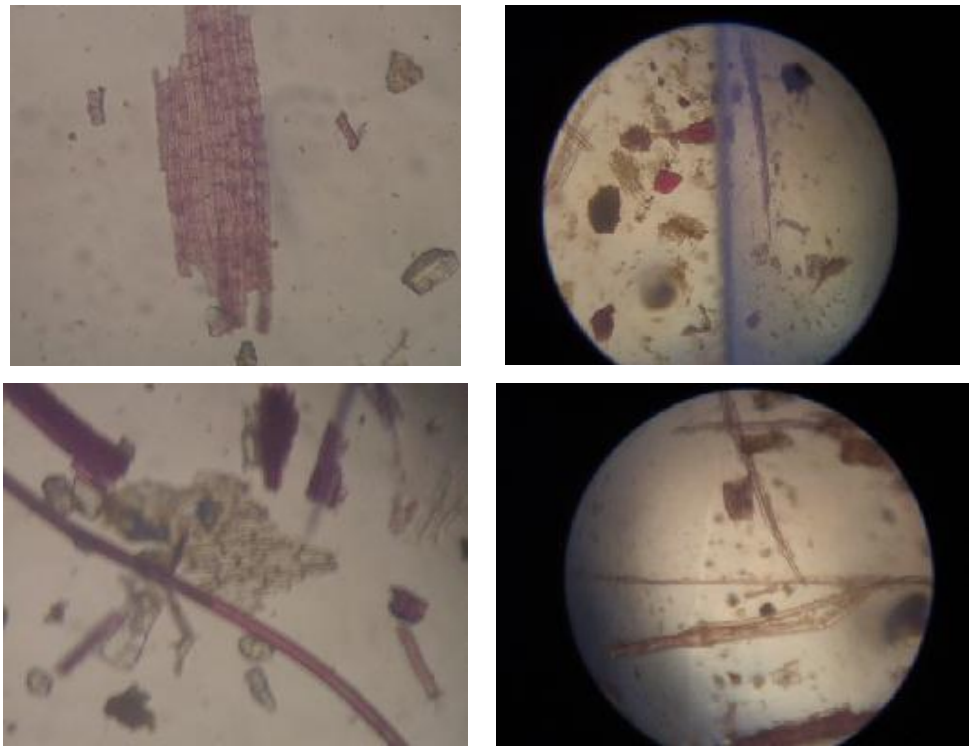

Fig. No. 03: Powder characteristics of *Bridelia tomentosa* root

Physicochemical analysis

In physiochemical analysis, various parameters like Moisture content, total ash value, acid insoluble ash, water soluble ash and extractive values were determined in triplicate and summarized in Table 2.

Table No. 02: Physicochemical analysis of *Bridelia tomentosa* root

Parameters	Values obtained on dry weight basis (% W/W)
Moisture Content	6.2 ± 0.05
Total Ash Value	5.1 ± 0.14
Acid insoluble Ash	0.81 ± 0.35
Water soluble Ash	0.42 ± 0.21
Alcohol soluble extractive value	7.3 ± 0.06

Table No. 03: Qualitative Phytochemical analysis of *Bridelia tomentosa* roots

S. No.	Tests	*Bridelia tomentosa* root extract			
		Pet Ether	Chloroform	Ethyl acetate	Methanol
1	**Carbohydrates & Glycosides**				
	Molisch's test	-	-	-	-
	Fehling's test	-	-	-	-
	Legal's test	-	+	-	-
	Borntrager's test	-	+	-	-
2	**Proteins & Free amino acids**				
	Million's test	-	-	-	-
	Biuret test	-	-	-	-
	Ninhydrine test	-	-	-	-
3	**Saponins**	-	-	-	+
4	**Tannins and Phenolic Compounds**				
	5% Ferric chloride solution	-	-	-	+
	10% sodium chloride	-	-	-	+
	10% lead acetate solution	-	-	-	+
5	**Phytosterols**				
	Salkowski test	+	+	-	-
	Liebermann Burchard test	+	+	-	-
6	**Alkaloids**				
	Mayer's reagent	-	-	-	-
	Dragendroff's reagent	-	-	-	-
	Hager's reagent	-	-	-	-
	Wagner's reagent	-	-	-	-
7	**Flavonoids**				
	Aq. NaOH	-	-	-	+
	Conc. H_2SO_4	-	-	-	+
	Shinoda's test	-	-	-	+
8	**Gums and Mucilage's**	+	-	-	-

Phytochemical Analysis

The phytochemical screening demonstrated the presence of different phytoconstituents (Table 3), which could be responsible for various pharmacological activities. Petroleum ether extracts showed the presence of phytosterols, gums and mucilage's. Chloroform extracts showed the presence of glycosides and steroids. Methanol extract showed the presence of saponins, tannins, phenolic compounds and flavonoids.

HPLC Analysis

HPLC analysis of powdered root of *Bridelia tomentosa* shows the presence of various phytochemical (Table 4 & Fig 4). However, Gallic acid, Rutin and Quercetin were the major phytochemical which were compared with respective standards (Fig. 5-7).

Table No. 04: HPLC analysis of *Bridelia tomentosa* root

Parameters	Gallic Acid	Rutin	Quercetin
Purity	99	95	98
Sample area	269973.094	44252.965	2135.122
Standard area	4655800	3191292.5	14722178
Sample weight mg	30	30	30
Standard weight mg	10	10	10
Sample dilution ml	10	10	10
Standard dilution ml	100	10	10
Phytochemicals in 30 mg of extract	0.1914 (0.7%)	0.4391 (1.75%)	0.0047 (0.013%)

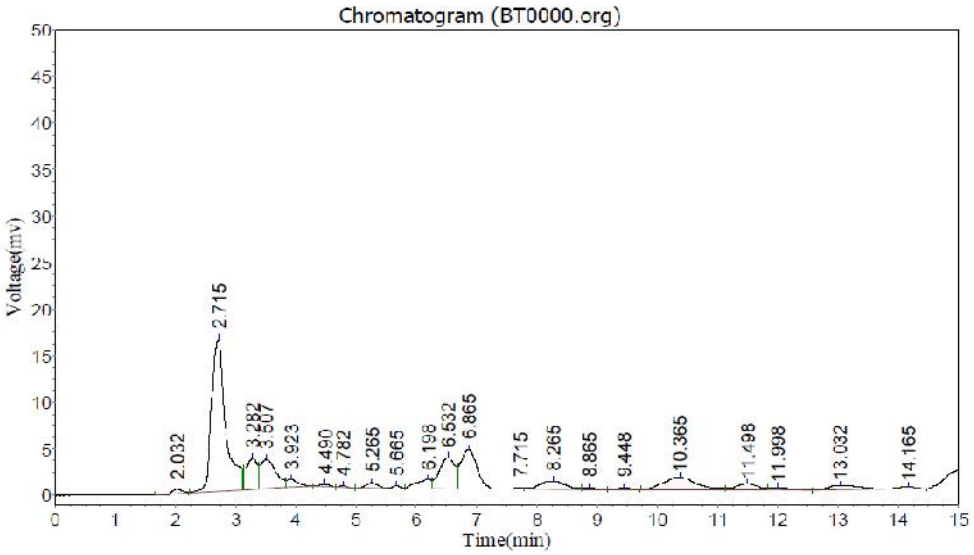

Fig. No. 04: HPLC chromatogram of powdered root of *Bridelia tomentosa*

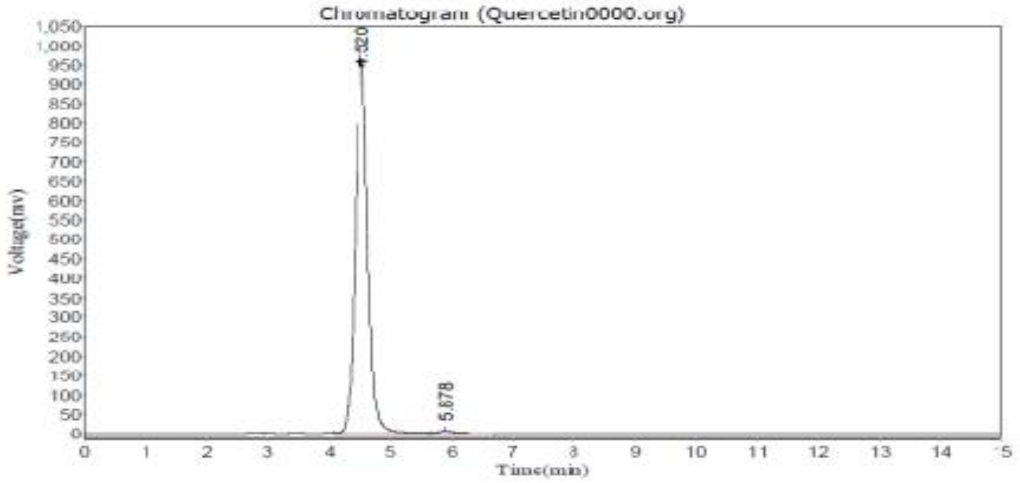

Fig. No. 05: HPLC chromatogram of quercetin

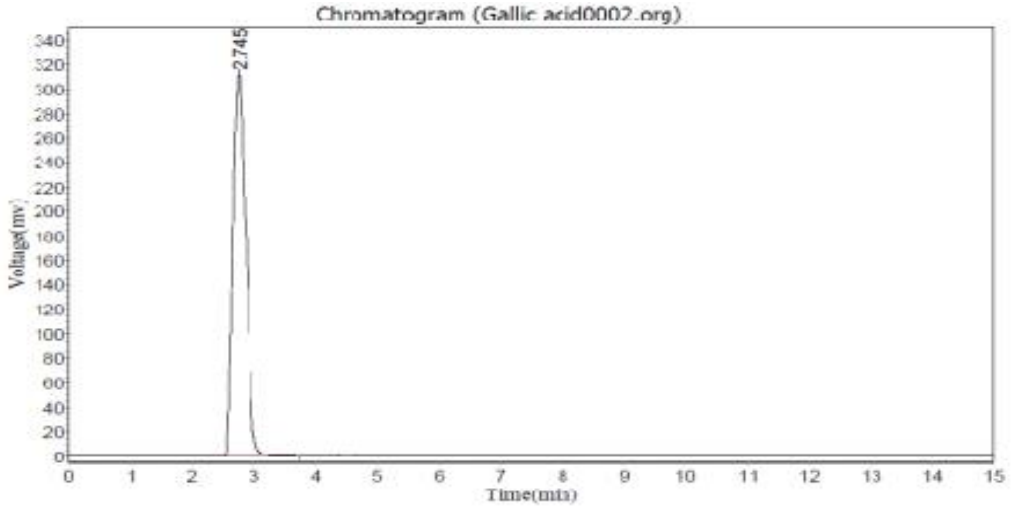

Fig. No. 06: HPLC chromatogram of gallic acid

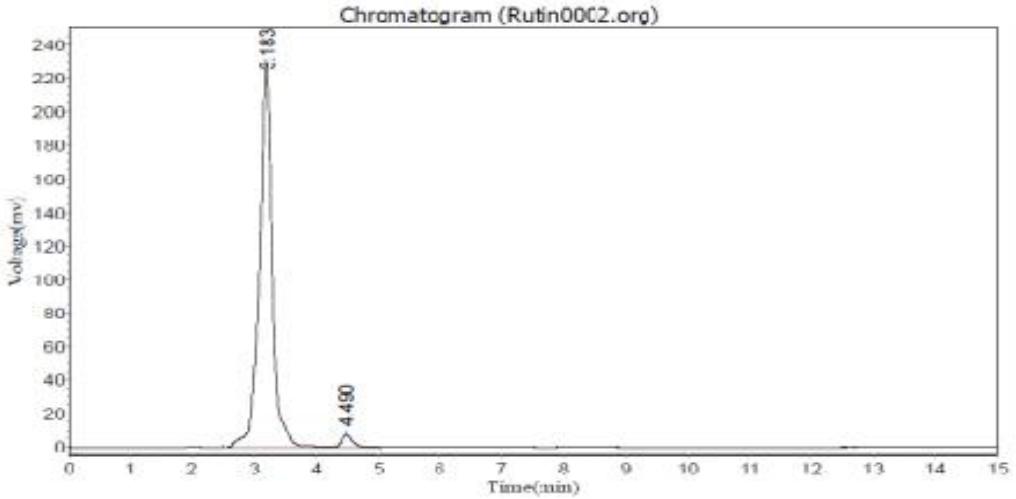

Fig. No. 07: HPLC chromatogram of Rutin

***In-vitro* toxicity using MTT assay**

The CTC_{50} of root of *Bridelia tomentosa* was greater than 1000 µg/ml. Hence, the powdered root of *Bridelia tomentosa* haven't shown any significant toxicity towards BRL3A cell line (Table 5).

Table No. 05: *In-vitro* toxicity of *Bridelia tomentosa* root in BRL3A cell line by MTT assay

S. No.	Test Concentration (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	1000	22.74±1.0	
2	500	17.71±3.1	
3	250	16.78±0.8	>1000
4	125	12.13±1.2	
5	62.5	9.67±0.4	

***In-vitro* hepatoprotective activity**

Bridelia tomentosa root has offered protection for BRL3A cell line against paracetamol at the dose of 500 µg/ml (Table 6). Further, increase in concentration to 1000 µg/ml has not offered protection. However, standard drug silymarin has offered significant protection for BRL3A cell line against paracetamol at the dose of 200 µg/ml.

Table No. 06: *In-vitro* Hepatoprotective activity using paracetamol treated BRL3A cell line

S. No.	Test Drugs	Test Concentration (µg/ml)	% Protection offered over Paracetamol control (µg/ml)
1	Bridelia tomentosa root	1000	0.00±3.64
2	Bridelia tomentosa root	500	17.26±4.02
3	Silymarin	200	48.83±7.67

Conclusion

In this present study, we have investigated the pharmacognostical, phytochemical, *in-vitro* toxicity and *in-vitro* hepatoprotective activity of *Bridelia tomentosa* root. The phytochemical screening of various extracts have shown the presence of different phytoconstituents, which are responsible for various pharmacological activities. However, methanolic extract showed the presence of saponins, tannins, phenolic compounds and flavonoids. The root haven't shown any significant toxicity towards BRL3A cell line as the CTC_{50} was greater than 1000 µg/ml. The root has offered protection for BRL3A cell line against paracetamol at the dose of 500 µg/ml. The study concludes that the *Bridelia tomentosa* roots is safe and offers significant hepatoprotection against paracetamol. However, this pilot study data require further validation using molecular level studies and clinical trials.

Reference

1. Gupta R, Chadha KL. Medicinal and aromatic plants in India: Advances in Horticulture, Medicinal and Aromatic Plants. New Delhi, Malhotra Publishing House, 1995, pp. 1-44.
2. Singh HB. Alternative source for some conventional drug plants of India: Ethnobotany and Medicinal Plants of Indian Subcontinents. Jodhpur, India. Scientific Publishers: 2000, pp. 63-78.
3. Pareek SK. Medicinal plants in India: Present status and future prospects: Prospects of Medicinal Plants. New Delhi; Indian Society for Plant Genetic Resources, NBPGR Campus, 1996, pp. 5-14.
4. Mukhopadhyay S. Conservation, protection and biodiversity of medicinal plants: Prospects of Medicinal Plants. New Delhi, Indian Society for Plant Genetic Resources, 1998, pp. 15-28.
5. Dikshit VK. Export of medicinal plants from India: need for resource management: Biodiversity - North-east India Perspectives: People's Participation in Biodiversity Conservation. Shillong; NEBRC, North-Eastern Hill University, 1999, pp. 85-88.
6. Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era. *Archives of Medical Research.*, 36(6), 2005, 697-705.
7. Srivastava J, Lambert J, Vietmeyer N. Medicinal plants: An expanding role in development. World Bank Technical Paper, 1996, 320.
8. Khatoon S, Singh N, Kumar S, Srivastava N, Rathi A, Mehrotra S. Authentification and evaluation of an important ayurvedic drug - Ashoka bark. *Indian J Tradit Knowl.*, 2009, 68, 393-400
9. Anonymous. Indian Pharmacopoeia. Edn 2, New Delhi, Government of India, 1966.
10. Evans WC. Trease and Evans, Pharmacognosy. Edn 15, Edinburgh London New York Philadelphia St Louis Sydney Toronto, W.B. Saunders, 2002, pp. 519-547.
11. Kokate CK. Practical Pharmacognosy. New Delhi, Vallabh Vrakashan, 2010, pp. 149-156.
12. Wallis TE. Practical Pharmacognosy. Edn 4, Hyderabad, PharmaMed Press, 2011.
13. Dinesh Kumar, Ajay Kumar and Om Prakash. Pharmacognostic study of Lantana camara Linn. Root. *Asian Pacific Journal of Tropical Disease*, 2012; S42-S45.
14. Krishnan KS. The Useful Plants of India. Reprinted, CSIR, New Delhi, India, Publications and Information Directorate, 1992, pp. 86-87.
15. Savithramma N, Linga Rao M, Suhrulatha D. 2011. Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research.*, 8, 2011, 579-584.
16. Kokate CK. Practical Pharmacognosy. Edn 1, New Delhi, Vallabh Vrakashan, 1986a, pp 15-30.
17. Ministry of Health and Family Welfare. Ayurvedic Pharmacopoeia of India. Edn 1, New Delhi, Government of India, Ministry of H and FW, Department of Indian System of Medicine & Homoeopathy, 1(1), 2001, pp. 14 - 143.

18. Siddiqua A, Premakumari KB, Sultana R, Vithya, Savitha. Antioxidant activity and estimation of total phenolic content of Muntingia calabura by colorimetry. *International Journal of Chem Tech Research.*, 2(1), 2010, 205-208.
19. Kyari MZ. Extraction and characterization of seed oils. *Int. Agrophysics.*, 22, 2008, 139-142.
20. Sajjad Khan M, Nitin Nema, Kharya MD, Salma Khanam. Chromatographic estimation of maturity based phytochemical profiling of *Ipomoea mauritiana. International Journal of Phytomedicine.*, 1, 2009, 22-30.
21. Harborne JB. Methods of extraction and isolation. Phytochemical Methods: London; Chapman & Hall. 1998, pp. 60-66.
22. Mohammed Sarfaraj Hussain, Sheeba Fareed, Mohammed Ali. Preliminary Phytochemical and Pharmacognostical Screening of the Ayurvedic Drug *Hygrophila auriculata* (K. Schum) Heine. *Pharmacognosy Journal.*, 23(3), 2011, 28 – 40.
23. Nishaa S, Vishnupriya M, Sasikumar JM, Gopalakrishnan VK. Phytochemical Screening and GC-MS Analysis of Ethanolic Extract of Rhizomes of *Maranta arundinacea* L. *Research Journal of Pharmaceutical Biological and Chemical Sciences.*, 4(2), 2013, 52-59.
24. Kokate CK, Prohit AP, Gokhale SB. Pharmacognosy. Edn 4, Pune, Nirali Prakashan, 1996, pp - 12.
25. Khandalwal KR. Practical Pharmacognosy. Edn 4, Pune, Nirali Prakashan, 1998, pp - 37.
26. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol Wright-Scientechnica, 1975b, pp. 36-45.
27. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Edn 9, Pune, Nirali Prakashan, 2002, pp 220-222.
28. Radhika B, Nasreen Begum, Srisailam K. Pharmacognostic and Preliminary Phytochemical Evaluation of the leaves of Bixa orellana, *Phcog J.*, 2(7), 2010, 132-136.
29. Jain S, Sharma P, Jhade D, Sharma NK, Paliwal P, Ahirwar D. Pharmacognostic screening and phytochemical evaluation of Acacia leucophloea root. *Int J Green Pharm.*, 5(2), 2011, 155-159.
30. Peach K, Tracy MV. Modern Methods of Plant Analysis. Springer-Verlag, Heidelberg, 1955.
31. Rich E, Schibli A. HPTLC for the Analysis of Medicinal Plants. Thieme Medical Publisher, Inc, ISBN 13:978-1-58890-409-6(US)
32. Madhira Geetha, Vijayalakshmi A. Pharmacognostic and Phytochemical Investigation of *Givotia rottleriformis* Griff. Ex Wight Bark. *Journal of Pharmacognosy and Phytochemistry.*, 2(3), 2013, 130-136.
33. Francis D and Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods.*, 89, 1986, 271-277.
34. Vasanth P, Raghu HC, Vijayan P, Dhanaraj SA, Rao MC, Rao VJ and Nitesh K. *In vitro* and *in vivo* hepatoprotective effect of total alkaloid fraction of *Hygrophila auriculata* leaves. *Indian Journal of Pharmacology.*, 42(2), 2010, 99-104.
35. Wei-Lun Wong, Mahmood Ameen Abdulla, Kek-Heng Chua, Umah Rani Kuppusamy, Yee-Shin Tan, and Vikineswary Sabaratnam. Hepatoprotective Effects of Panus giganteus (Berk.) Corner against Thioacetamide- (TAA-) Induced Liver Injury in Rats. *Evidence-Based Complementary and Alternative Medicine.*, 2012, 10.
36. Mohamed Saleem TS, Madhusudhana Chetty S, Ramkanth S, Rajan VST, Mahesh Kumar K, Gauthaman K. Hepatoprotective herbs—a review. *International Journal of Research in Pharmaceutical Sciences* 1(1), 2010, 1–5.
37. Ao ZH, Xu ZH, Lu ZM, Xu HY, Zhang XM, Dou WF. Niuchangchih (Antrodia camphorata) and its potential in treating liver diseases. *Journal of Ethnopharmacology* 121(2), 2009, 194–212.
38. Alshawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of Orthosiphon stamineus extract on thioacetamide-induced liver cirrhosis in rats. *Evidence-Based Complementary and Alternative Medicine.*, 2011.