In vitro Antioxidant and Hepatoprotective Activity of Bridelia scandens (Roxb.) Willd.

Preetham Jinadatta¹*, Kiran Sundera Raja Rao¹, Sharath Rajshekarappa², Sujan Ganapathy Pasura Subbaiah³, Mruthunjaya Kenganora⁴

ABSTRACT
Natural products are emerging out as potent and alternative therapies for many diseases. Today herbs have become the part of mankind, because of its manifold ways in targeting diseased cells with minimal effects on normal cells and tissues. The present research investigated the in vitro antioxidant activity and hepatoprotective of B. scandens leaf. Preliminary phytochemical analysis exhibited the presence of most of the constituent in ethanol extract (BSE). Antioxidant capacity of various extracts of B. scandens was examined. DPPH assay revealed that ethanol extract has a good antioxidant with IC₅₀ value of 31.68μg/ml, whereas standard ascorbic acid with 8.78 μg/ml. BSE revealed dose dependent response with increase in concentration for reducing power assay. ORAC assay directly measured the scavenging capacity and BSE (2485 trolox eq/gm) was found to be potent than other extracts. In vitro hepatoprotective activity was performed for BSE using MTT assay in BRL 3A cell line, which revealed nontoxic dose with CTC₅₀ value more than 1000 μg/ml. At the dose 200 μg/ml, BSE and standard silymarin offered cell protection of 57% and 76 % respectively. Present study concludes that B. scandens leaf extract possess antioxidant potential and protect the liver cells against CCl₄ damage. However in vivo studies are being carried out to validate the traditional usage of Bridelia scandens.

Key words: Bridelia scandens, BRL3A, ORAC, MTT, Hepatoprotective.

INTRODUCTION
Medicinal plants are still a potent source of therapeutic agent. These plants and their extracts are being used as natural sources to treat various illnesses.¹ People of India have remarkable passion for medicinal plants and its use in healing health related issues. India being populated by a substantial number of tribal groups, with enormous knowledge of using wild plants for remedying human complaints.² Today liver disease is one of the serious complaints in the world and to maintain a healthy liver is a decisive aspect for the present population. Despite of remarkable improvements in modern treatment for liver disorders, there is no effective remedy for restoration of liver functions or regeneration of hepatic cells. So, the herbal remedies are in huge demand for developed and developing country for health issues. Nature has bestowed us with herbs, which extends its use for Protection for liver from being injured or assist in revival of hepatic cells with less or no side effects.³

Bridelia scandens belonging to the family Euphorbiaceae, is a perennial herb, native to Southeast Asia, southern China, and the Indian Subcontinent.⁴ It is used in Ayurveda system of medicine as Pashnubeda.⁵ Leaf⁶ and stem bark⁷ extracts is used to treat jaundice. Leaf extract is used to cure allergy,⁸ treat oral problems,⁹ Leaf and root extracts are used to treat inflammation, scabies, dermatitis.¹⁰ Root is also used to cure herpes.¹¹ Bark is used to treat cough, fever and asthma, and also possess hypotensive and hypoglycaemic activity. Fruits are edible. Seeds possess hemagglutinating properties.¹²,¹³,¹⁴ Decoction of wood of B. scandens administered orally to treat malaria disease.¹⁵

Herbal based therapies for the liver diseases are used from long time ago and are popular world over. Still, there is no potent and effective drug available to stimulate hepatic cells and help in revival to perform vital functions. Based on the tribal usage of leaf of Bridelia scandens and medical practitioners to treat jaundice and other ailments, present study has been carried out to investigate antioxidant and antihapatotoxic activity by using in vitro parameters.

MATERIAL AND METHODS
Plant material and extraction
Bridelia scandens was collected from Biligiri Rangana hills (BR hills) of Chamarajanagar district, Karnataka, India. Authenticated by Dr. Shiddamallayya N, at National Ayurveda Dietetics Research Institute, Bangalore – 560078, Karnataka, INDIA.

Received: 18-09-2017; Review completed: 27-10-2017; Accepted Date: 20-11-2017

¹Department of Biotechnology, Dayananda Sagar College of Engineering, Kumaraswamy Layout, Bangalore-560078, Karnataka, INDIA.
²Department of Biotechnology, M.S. Ramaiah Institute of Technology, MSBIT Post Bangalore 560054, Karnataka, INDIA.
³Research and Development Centre, Indusviva International Private Limited, No.7450, Near Navayuga Toll Gate Office, NH-4, Nelamangala, Bangalore – 562123, INDIA.
⁴Department of Pharmacognosy, JSS College of Pharmacy, JSS University, Mysuru-570015 Karnataka, INDIA.

Correspondence
Preetham Jinadatta
Research Scholar, Department of Biotechnology Dayananda Sagar College of Engineering, Kumaraswamy Layout, Bangalore-560078, Karnataka, INDIA.
Phone no: +91-9448117536
E-mail: preethamjharsha@gmail.com

Pharmacognosy Journal, Vol 9, Issue 6 (Suppl), Nov-Dec, 2017
Department of AYUSH, Govt. of India, Bangalore and was deposited for future references (No: RRCRI-MUS-10121). Leaf material was shade dried, powdered and extracted successively with petroleum ether, chloroform and ethanol using soxhlet apparatus and water extract by refluxing the marc in waterbath. All the extracts of B. scandens were filtered and solvent was evaporated by using rotary evaporator.

**Phytochemical analysis**

Different extracts of *B. scandens*, BSP (*B. scandens* petroleum ether), BSC (*B. scandens* chloroform extract), BSE (*B. scandens* ethanol extract), BSA- (*B. scandens* aqueous extract) was obtained after extraction. All the extracts were considered for preliminary qualitative phytochemical analysis. Test for carbohydrates, alkaloids, phenols, flavonoids, tannins, terpenes, phytosterols, glycosides, resins were carried out using the standard methods as described.16,17,18,19,20

**In vitro Antioxidant activity**

**DPPH radical scavenging assay**

DPPH radical scavenging assay is a discoloration assay, upon reaction with hydrogen donor changes purple to yellow colour. It is evaluated by the addition of the antioxidant to a DPPH and was performed as described by cuendet et al 199721 taking ascorbic acid as standard. 4.0ml (0.2mg) of DPPH solution in methanol was added to 1 ml of various concentrations (7.62 -250µg) of plant extracts. After 30 mins of incubation period at room temperature the absorbance was read at 517 nm.

**Reducing power assay**

The capacity of the plant extracts to reduce iron III was measured by using different concentrations (50-300 µg/ml) and standard ascorbic acid. Add 2.5 ml of 0.2M phosphate buffer pH 6.6 and 2.5 ml of 1% aqueous potassium hexacyanoferrate solution. Incubate for 30mins at 50ºC, add 2.5 ml of 10% TCA (trichloroacetic acid) and centrifuge at 3000 rpm for 10 mins. Collect 2.5 ml of supernatant and mix it with 2.5 ml of water and 0.5 ml of 0.1% aqueous FeCl₃. The amount of iron ferricyanide complex could be determined by measuring the formation of perl’s Prussian blue at 700 nm.22 A higher absorbance indicates higher reducing power.

**ORAC assay**

ORAC (Oxygen Radical Absorbing Capacity) assay was performed to scavenge the peroxyl radical as well as to estimate the total antioxidant capacity of the test drug. Phosphate buffer (pH 7.4) was used to prepare the plant extracts and using sodium fluorescein as a probe in Synergy™ HT fluorescence multiplate reader. Plant extracts solution and/or 25 µl of Trolox of 1mM were allotted in 96-well micro titre plate. 150 µl of the fluorescein (4 µM) solution added to each well, mixed thoroughly, incubated at 37ºC for 30 mins Synergy™ HT multi-detection microplate reader. Add 25 µl of AAPH (153 mM, 2,2’-Azobis (2-methylpropionamidine dihydrochloride) using dispenser and mixed well. Fluorescence of standard and plants extracts were monitored continuously every minute for 1 hour using the reader plate, controlled by using KC4™ software version 3.4.23

**Hepatoprotective activity on BRL3A cell line**

**Cell lines and Culture medium**

In the present study BRL3A (Buffalo Rat liver cell line) cell line was used to assess the hepatoprotective function of potent extract (BSE). BRL3A was obtained from National Centre for Cell Sciences (NCCLS), Pune, India. It was cultured in DMEM (Dulbecco’s modified egdes medium), supplemented with 10% inactivated fetal Bovine serum(FBS),100IU/ml of penicillin,100 µg/ml of streptomycin and 5 µg/ml amphotericin in an humidified atmosphere of 5% CO₂ at 37ºC until confluent. Later the cells were dissociated with TPVG solution containing 0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS. Stock cultures were grown in 25cm² culture flasks and study was carried out in 96 microtitre plates.

**Preparation of Test Solutions**

Stock solution of 10 mg/ml concentration of plant extract was prepared by dissolving sample in DMSO and then volume was made upto DMEM, supplemented with 2% inactivated FBS. Stock was serially diluted to get lower concentrations.

**Evaluation of cell viability by MTT Assay**

MTT assay was carried out for ethanol extract of *B. scandens* to assess its nontoxic doses. A monolayer cell culture was trypsinised and counted was accustomed to 1.0 x 10⁶ cells/ml using DMEM containing 10% FBS. Approximately 10,000 cells (0.1 ml diluted suspension) were added to each 96 well microtitre plate. After 24hrs, supernatant was flicked off to form a partial monolayer of cells and was washed with medium. 100µl of different test concentrations (62.5-1000 µg/ml) was added to each well and then incubated at 37ºC for 3 days in 5% CO₂ atmosphere. Microscopic examination and observations were noted in 24hrs time interval. After 72hrs, test samples were disposed and MTT in PBS (50 µl) was added to each well. Again incubated at 37º C for 3 days in 5% CO₂ atmosphere, supernatant was removed, 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. Microplate reader at a wavelength of 540 nm was used to read the absorbance and percentage of growth inhibition was calculated using the formula. CTC_initial concentration of test drug needed to inhibit cell growth by 50% is generated from the dose-response curves for test samples.

% Growth Inhibition = 100 – Mean OD of control group / Mean OD of individual test group × 100

**RESULTS**

Leaf of *B. scandens* subjected to successive extraction resulted with four extracts, BSP (*B. scandens* petroleum ether), BSC (*B. scandens* chloroform extract), BSE (*B. scandens* ethanol extract), BSA- *B. scandens* aqueous extract. Preliminary phytoconstituents present in different extracts of leaf of *B. scandens* were revealed in Table 1. It showed that most of constituents like, carbohydrates, phenols, flavonoids, tannins, phytosterol and terpenes in ethanol extract (BSE).

**Antioxidant assays**

Antioxidant activity of different extracts of *B. scandens* was measured by DPPH scavenging assay, reducing power and ORAC assay. In DPPH assay,BSE showed potent scavenging activity with IC₅₀ value of 31.68 ± 0.40 µg/ml, whereas standard ascorbic acid with 8.78 µg/ml. Comparitively, less activity was found in BSC, negligible in BSP and nil scavenging activity in BSA (Graph 1). Reducing power of different extracts of *B. scandens* was measured (Graph 2). BSE showed high reducing power with increasing in concentration. BSC extract showed moderate activity and BSP with very less activity followed by BSA. ORAC assay was performed for all the four extracts of *B. scandens* and it showed a good oxygen radical absorbing activity.
Jinadatta et al. *B.scandens*-Antioxidant and Hepatoprotective Activity

**Hepatoprotective activity**

Hepatoprotective activity of BSE was examined on BRL3A cell line. BSE exhibited CTC$_{50}$ of more than 1000 µg/ml (Table 2). Nontoxic dose of BSE tested for its hepatoprotective activity on BRL 3A cell line intoxicated with CCl$_4$ showed that 57% shielding effect (200 µg/ml), whereas standard silymarin at 200 µg/ml exhibited 76.3% (Table 3).

**DISCUSSION**

In the present investigation phytoconsituents of various extracts of *B.scandens* was examined. In *vitro* antioxidant potential, cytotoxicity and hepatoprotective activity of *B.scandens* was evaluated. The chemicals and drugs used in the routine life damage liver cells by inducing lipid peroxidation and altering the cellular redox state piloting to oxidative stress/damage in the system. Reactive oxygen species (ROS) produced due to this stress initiate and regulate the progression of liver diseases. The inequity between the ROS production and antioxidant defense mechanisms leads to oxidative stress. This impacts the transcription of various biochemical mediators, which can modulate other affairs in the system like cholestasis, apoptosis, fibrosis, and regeneration. This leads to different types of liver diseases.$^{27,28}$

These medicinal plants are always beneficial for mankind by resolving the health issues and various studies has been conducted on traditional medicine to develop new drug for antioxidant and hepatoprotective activity.$^{29}$

Ethanol extract of *B.scandens* has phytoconstituents like phenols flavonoids, tannins, alkaloids. In the present findings, *in vitro* antioxidant activity of different extracts revealed that, compare to all the extracts used, ethanol extract is having a good scavenging and reducing capacity. ORAC assay analyse scavenging of peroxyl radical. This also examines the antioxidant capacity of all the antioxidants present in the test.$^{23,30}$ ORAC results revealed that ethanol extract of *B.scandens* as potent antioxidant than other extracts, indicating the direct measure of antioxidant ability of the extract. As the value of ORAC increases, antioxidant competence also increases and vice versa.$^{31,32}$ Antioxidants from natural products detoxify the toxins, scavenging of free radicals, removes excess capacity for BSE (2485 trolox eq/gm) and ascorbic acid (3562 trolox eq/gm) followed BSC (Graph.3). BSP and BSA didn’t show any activity.

Since ethanol extract (BSE) proved to be a good antioxidant, it was further considered to test its hepatoprotective ability on BRL3A cell line.

**Table 1: Phytochemical analysis of different extract of B.scandens**

<table>
<thead>
<tr>
<th>Tests</th>
<th>BSP</th>
<th>BSC</th>
<th>BSE</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2 Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3 Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4 Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6 Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7 Phytosterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8 Resins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9 Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10 Terpenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11 Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates Present, - indicates not determined

BSP (B.*scandens* petroleum ether extract), BSC (B.*scandens* chloroform extract), BSE (B.*scandens* ethanol extract), BSA- B.*scandens* aqueous extract.
Table 2: Cytotoxic property of ethanol extract of B.scsndens on BRL3A cell line by MTT assay

<table>
<thead>
<tr>
<th>Test Drug</th>
<th>con µg/ml</th>
<th>% Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cells</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.25%</td>
<td>98±0.4</td>
</tr>
<tr>
<td>CCl₄</td>
<td>1%</td>
<td>20.5±0.5</td>
</tr>
<tr>
<td>Silimarin + CCl₄</td>
<td>200</td>
<td>76.3±0.34</td>
</tr>
<tr>
<td>BSE+ CCl₄</td>
<td>200</td>
<td>57±1.8</td>
</tr>
<tr>
<td>BSE+ CCl₄</td>
<td>100</td>
<td>38.6±1.9</td>
</tr>
<tr>
<td>BSE+ CCl₄</td>
<td>50</td>
<td>10.3±2.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M; n=3

*Significance level : P <0.05, compared to DMSO cells

Conflict of Interest

The authors declare no conflict of interest.

ABBREVIATION USED

MTT: 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide; FBS: Fetal Bovine serum; PBS: Phosphate Buffered Saline; DMEM: Dulbecco’s Modified Eagle’s Medium; DMSO: Dimethyl Sulfoxide; PBS: Phosphate Buffered Saline; BRL3A: Buffalo Rat liver; TPVG: Trypsin Phosphatase Versene Glucose; 0.2% trypsin, 0.02% EDTA, 0.05% glucose; DPHP: 2,2-diphenyl-1-picrylhydrazyl.

REFERENCES


The present investigation on leaf of *B. scandens* shows its potential as antioxidant and hepatoprotective. Phenols and flavonoids of the plant, as a secondary metabolite serve as a good antioxidant and hepatoprotective agent. Ethanol extract with its phytoconstituents may be responsible for the present activity. It proves to be less toxic on BRL3A cell line, which was revealed by its protective effect against CCl₄ toxicity. To expand, *in vivo* hepatoprotective studies for the ethanol extracts *B. scandens* being carried out to assess its antihepatotoxic function.