

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

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## 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 IC<sub>50</sub> value of 31.68 µg/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 CTC<sub>50</sub> 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 CCl<sub>4</sub> 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.<sup>1</sup> 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.<sup>2</sup> 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 effectual 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.<sup>3</sup>

*Bridelia scandens* belonging to the family Euphorbiaceae, is a perennial herb, native to Southeast Asia, southern China, and the Indian Subcontinent.<sup>4</sup> It is used in Ayurveda system of medicine as Pashnubeda.<sup>5</sup> Leaf<sup>6,7</sup> and stem bark<sup>8</sup> extracts is used to treat jaundice. Leaf extract is used to cure allergy,<sup>9</sup> treat

oral problems.<sup>10</sup> Leaf and root extracts are used to treat inflammation, scabies, dermatitis.<sup>11</sup> Root is also used to cure herpes.<sup>12</sup> Bark is used to treat cough, fever and asthma, and also possess hypotensive and hypoglycaemic activity. Fruits are edible. Seeds possess hemagglutinating properties<sup>7,13,14</sup> Decoction of wood of *B. scandens* administered orally to treat malaria disease.<sup>15</sup>

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 antihepatotoxic activity by using *in vitro* parameters.

## MATERIAL AND METHODS

### Plant material and extraction

*Bridelia scandens* was collected from Biligiri Rangana hills (BR hills) of Chamaraajanagar district, Karnataka, India. Authenticated by Dr. Shiddamallayya N, at National Ayurveda Dietetics Research Institute,

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Department of AYUSH, Govt. of India, Bangalore and was deposited for future references (No: RRCBI-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.<sup>16,17,18,19,20</sup>

#### 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 1997<sup>21</sup> 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 50°C, add 2.5 ml of 10% TCA (trichloroacetic acid) and centrifuge at 3000 rpm for 10 mins. Collect 2.5ml of supernatant and mix it with 2.5 ml of water and 0.5 ml of 0.1% aqueous FeCl<sub>3</sub>. The amount of iron ferricyanide complex could be determined by measuring the formation of per's Prussian blue at 700 nm.<sup>22</sup> A higher absorbance indicates higher reducing power.

##### ORAC assay

ORAC (Oxygen Radical Absorbing Capacity) assay was performed to scavenge the peroxy 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 37°C for 30 mins Synergy™ HT multi-detection microplate reader. Add 25 µl of AAPH (153 mM, 2,2'-Azobis (2-methylpropionamide 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.<sup>23</sup>

##### Hepatoprotective activity on BRL3A cell line<sup>24,25,26</sup>

##### 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 (NCCS), Pune, India. It was cultured in DMEM (Dulbecco's modified eagles 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% CO<sub>2</sub> at 37°C 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 25cm<sup>2</sup> 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 with 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 count was accustomed to 1.0 x 10<sup>5</sup> 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 37°C for 3 days in 5% CO<sub>2</sub> 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 37° C for 3 days in 5% CO<sub>2</sub> 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<sub>50</sub>, 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

##### Determination of Hepatoprotective activity

Non-toxic or safe concentration chosen was ethanol extract for *B. scandens* to determine antihepatotoxic property. The protocol was followed as mentioned for MTT assay. To nontoxic test concentration (50 µl), add 50 µl of DMEM with nontoxic concentration (1% CCl<sub>4</sub>) of toxicant were added. Micro plate reader was used to measure the absorbance at a wavelength of 540 nm and percentage cell viability was determined. Based on which the Percentage protection offered by plant extract and standard silymarin was calculated over the DMSO control.

% Cell protection = Mean O.D of individual test group / Mean O.D of control 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 IC<sub>50</sub> 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

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.

### Hepatoprotective activity

Hepatoprotective activity of BSE was examined on BRL3A cell line. BSE exhibited CTC<sub>50</sub> 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<sub>4</sub> 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 phytoconstituents 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.<sup>27,28</sup>

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.<sup>29</sup>

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 peroxy radical. This also examines the antioxidant capacity of all the antioxidants present in the test.<sup>23,30</sup> 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.<sup>31,32</sup> Antioxidants from natural products detoxify the toxins, scavenging of free radicals, removes excess

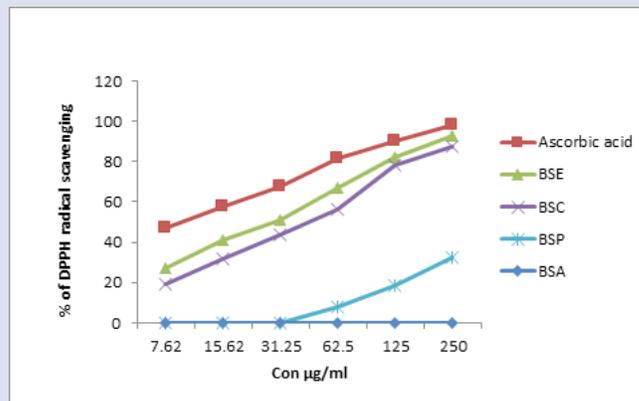
**Table 1: Phytochemical analysis of different extract of *B.scandens***

Tests	BSP	BSC	BSE	BSA
1 Alkaloids	-	-	+	-
2 Carbohydrates	-	+	+	+
3 Flavonoids	-	+	+	-
4 Phenols	-	+	+	+
5 Glycosides	-	-	+	+
6 Saponins	-	-	-	-
7 Phytosterols	+	+	+	-
8 Resins	+	-	-	-
9 Tannins	-	-	+	-
10 Terpenes	-	-	+	-
11 Proteins	-	-	-	-

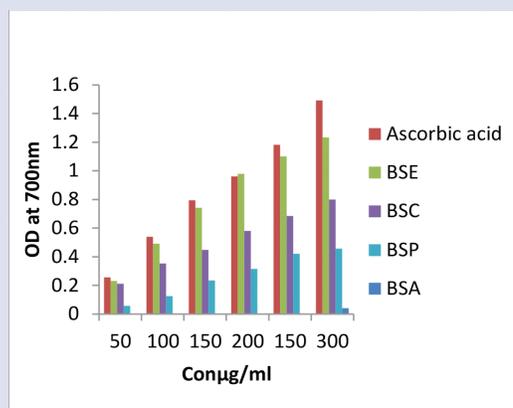
+ indicates Present, - indicates not determined

BSP (*B.scandens* petroleum ether extract), BSC (*B.scandens* chloroform extract), BSE (*B.scandens* ethanol extract), BSA- *B.scandens* aqueous extract.

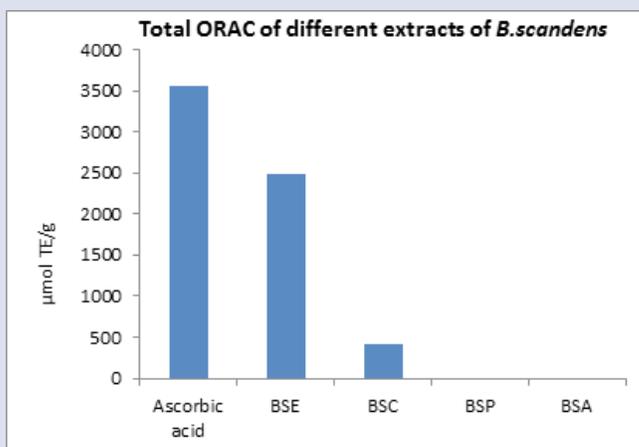
**Graphs: Antioxidant activity of *B.scandens***



**Graph 1: DPPH radical scavenging activity of *B.scandens***



**Graph 2: Reducing power assay of *B.scandens***



**Graph 3: ORAC assay of *B.scandens***

BSP (*B.scandens* petroleum ether), BSC (*B.scandens* chloroform extract), BSE (*B.scandens* ethanol extract), BSA- (*B.scandens* aqueous extract).

**Table 2: Cytotoxic property of ethanol extract of *B.scandens* on BRL3A cell line by MTT assay**

Con µg/ml	BSE
62.5	4.8±1.47
125	11.1±0.5
250	16.9±0.5
500	29.7±2.3
1000	45.3±1.1
CTC <sub>50</sub> (µg/ml)	>1000

BSE- *Bridelia scandens* ethanol extract

**Table 3: Hepatoprotective study of BSE on BRL3A cell line**

Test Drug	con µg/ml	% Viability
Normal cells	-	100
DMSO	0.25%	98±0.4
CCl <sub>4</sub>	1%	20.5±0.5
Silymarin+ CCl <sub>4</sub>	200	76.3±0.34
BSE+ CCl <sub>4</sub>	200	57±1.8 <sup>a,b</sup>
BSE+ CCl <sub>4</sub>	100	38.6±1.9 <sup>b</sup>
BSE+ CCl <sub>4</sub>	50	10.3±2.3 <sup>b</sup>

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

<sup>a</sup>Significance level : P <0.05, compared to DMSO cells

<sup>b</sup>Significance Level : P <0.05, compared to Toxicant treated cells

BSE- *B.scandens* ethanol extract

ROS and anti-lipid peroxidation.<sup>33</sup> Antioxidant activity of BSE proves that leaf of *B.scandens* can be used to treat liver disease.

The cell line testing for hepatoprotective activity is a primary level screening, used to investigate medicinal plants. *In vitro* cytotoxicity and hepatoprotective activity in BRL3A cell line (functions as rat liver cells) was carried out for BSE. MTT, tetrazolium dye is used widely to assess the cell viability and also used to determine nontoxic dose for BSE and found to be above 1000 µg/ml. CCl<sub>4</sub> induced hepatotoxicity is a widely used and accepted model to test protective role of the drug because of variation associated with CCl<sub>4</sub> – induced liver injury is similar to that of acute viral hepatitis.<sup>31,32,33</sup> CCl<sub>4</sub> is activated by cytochrome P<sub>450</sub> dependent monooxygenases to form trichloromethyl radical and trichloromethyl peroxy free radical. These free radicals lead to severe liver damage. *B.scandens* tells about its traditional usage to cure liver disease, thus BSE at 200µg/ml have tried to protect the liver cells from CCl<sub>4</sub> damage. This may be due to the antioxidant potency of the BSE and its phytoconstituents.

## CONCLUSION

Medicinal plants are always beneficial for mankind by resolving the health issues. Leaf of *B.scandens* used traditionally to cure liver disease like jaundice proved to be efficient in shielding the liver cells by its strong antioxidant potential and protecting the normal cells. Detailed *in vivo* studies on liver protection activity are in progress to support the data obtained.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATION USED

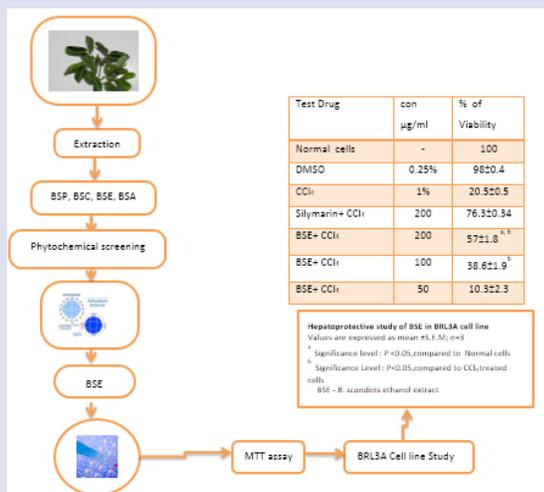
**MTT:** 3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide; **FBS:** Fetal Bovine serum; **PBS:** Phosphate Buffered Saline; **DMEM:** Dulbecco's Modified Eagle's Medium; **DMSO:** Dimethyl Sulfoxide; **PBS:** Phosphate Buffered Saline; **BRL3A:** Buffalo Rat liver; **TPVG:** Trypsin Phosphate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

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## GRAPHICAL ABSTRACT



## SUMMARY

- 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 CCl<sub>4</sub> toxicity. To expand, *In vivo* hepatoprotective studies for the ethanol extracts *B. scandens* being carried out to assess its antihepatotoxic function.

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