# *IN VITRO* ANTIOXIDANT ACTIVITY OF *BOERRHAVIA DIFFUSA* L*

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## Abstract

*The objective of the present study was to evaluate the antioxidant activity of the methanolic extract of Boerrhavia diffusa* L *invitro. The invitro antioxidant activity was evaluated by determining the inhibitory effect of the extract on lipid peroxide formation, superoxide radical formation and nitric oxide radical formation by standard techniques. The results showed that the extract produced dose dependant inhibition of lipid peroxide formation, superoxide radical formation and nitric oxide radical formation invitro. This proved the anti oxidant potential of the plant invitro which might contribute to the different pharmacological actions of the plant and its use in traditional ayurvedic formulations.*

**Keywords***: Boerrhavia diffusa L., antioxidant, lipid peroxide formation, superoxide radical , nitric oxide radical*

The plant *Boerrhavia diffusa* L. has been recommended as an ingredient of various laxative, diuretic and emetic preparations in different dosage forms in the ancient Ayurvedic text 'Charaka Samhita' (Charaka, 1949). Chopra *et al*. (1956) and Abraham (1975) described the use of the plant in asthma, oedema, jaundice, snake poisoning and blood pressure.

Increased interest in the therapeutic potentials of medicinal plants as antioxidants has emerged recently(Jimoh *et al*., 2009), since the synthetic antioxidants were found to produce deleterious side effects.

*Boerrhavia diffusa* (known as Spreading Hogweed in English, Thazhuthama in Malayalam and Punarnava in Sanskrit) has been found ubiquitously in Kerala as a perennial weed and has been used by Ayurvedic Physicians since ages for the treatment of inflammations (Kirtikar and Basu, 1975). The potential of the plant as a natural antioxidant was investigated *in vitro* in the present study.

## Materials and Methods

### Reagents and Animals

All the chemicals used were of analytical grade. Mice of either sex weighing 20 to 25 g bred in the Small Animal Breeding Station of College of Veterinary and Animal Sciences, Mannuthy were used. All procedures were done as per the guidelines of Institutional Animal Ethics Committee. Mice were starved overnight before the experiment and liver was collected and homogenised in tris buffer (1:4) to prepare the liver homogenate for the estimation of Thio Barbituric Acid Reacting Substances (TBARS ).

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**Extract**

The whole plants were collected from the premises of Veterinary College and identified by the Medicinal Plant Division of College of Horticulture, Vellanikkara. They were dried under shade and powdered with a mechanical grinder. The dried plant material (500 g) was extracted with Soxhlet apparatus using methanol for about 48 h. The solvent was removed from the extract under reduced pressure by using rotary vacuum evaporator.

**Effect on lipid peroxidation**

Effect on lipid peroxidation was performed by the measurement of TBARS by the model described by Okhawa *et al*. (1979). The reaction mixture consisted of mice liver homogenate 100 µl, potassium chloride 100 µl and methanolic extract (0.5 mg%) in varying volumes (50 µl-1000 µl). The mixture was made upto 1 ml with tris buffer and incubated at 37°c for an hour. To 0.2 ml each of the reaction mixture, 200 µl sodium dodecyl sulphate, 1.5 ml acetic acid and 1.5 ml thiobarbituric acid were added. All the tubes were incubated in a boiling water bath for one hour. After cooling, added butanol:pyridine 5 ml to all the tubes, mixed well and centrifuged at 3000 rpm for 15 min. Control was kept with all the reagents except the extract. Optical density at 560 nm was read in a spectrophotometer and the per cent inhibition was calculated.

**Effect on Superoxide radical formation**

This was done by the NBT reduction method of McCord and Fridovich (1969). In brief, to the methanolic extract solution (2 mg/ml) in varying volumes (10 µl - 250 µl), added 0.1 ml Nitro Blue Tetrazolium (1.5 mM), Sodium Cyanide solution (0.0015 in 0.1 Media) and made upto 3 ml using phosphate buffer. Then added 0.5 ml riboflavin solution. The optical density was measured at 560 nm after exposing all the tubes to bright light for 15 min and the per cent inhibition was calculated.

**Effect on inhibition of nitric oxide radical formation**

This was performed by the method described by Green *et al*. (1982). Briefly, to various volumes of the extract (2 mg/ml concentration) sodium nitroprusside solution (10 nM in PBS) was added to make up to 4 ml and incubated at 25°C for 150 min. To 0.5 ml of the mixture from each tube, 0.5 ml of Greens reagent was added and the optical density was measured at 546 nm and the percent inhibition was calculated.

## Results and Discussion

The superoxides generated by photoreduction of riboflavin reduced the NBT salt, the reduced form of which gave a blue colour that was measured at 560 nm. The amount needed for 50 per cent inhibition (IC 50 value) was 400 µg/ml (Table 1) There was dose dependant inhibitory effect on superoxide radical formation *invitro*.

**Table 1:** Effect on inhibition of superoxide radicals formation *in vitro*

| Vol. of drug (µl) | Concentration (µg) | Per cent inhibition |
|---|---|---|
| 10 | 20 | -1.9 |
| 50 | 100 | 39.01 |
| 100 | 200 | 42.99 |
| 200 | 400 | 50.38 |
| 250 | 500 | 56.08 |
| C | No drug | — |

Similarly the ferrous ascorbate system induced TBARS in mice liver homogenate *invitro* which is a measure of lipid peroxidation. There was dose dependant inhibition of lipid peroxide formation also, the IC 50 being 200 µg/ml (Table 2). Nitric oxide radicals generated from sodium nitroprusside at physiological pH were also found to be inhibited by the extract in a dose-dependant manner with the IC50 value being 8 µg/ml(Table 3) .

**Table 2:** Effect on inhibition of lipid peroxide formation *in vitro*

| Vol. of drug (µl) | Concentration (µg) | Per cent inhibition |
|---|---|---|
| 50 | 25 | -17.65 |
| 100 | 50 | 7.4 |
| 150 | 75 | 23.53 |
| 200 | 100 | 26.47 |
| 300 | 150 | 30.88 |
| 400 | 200 | 48.53 |
| 500 | 250 | 86.76 |
| 1000 | 500 | 94.12 |
| C | No drug | — |

**Table 3:** Effect on inhibition of nitric oxide radical formation *in vitro*

| Vol. of drug (µl) | Concentration (µg) | Per cent inhibition |
|---|---|---|
| 250 | 500 | -4.95 |
| 500 | 1 mg | 3.47 |
| 1 ml | 2 mg | 24.75 |
| 2 ml | 4 mg | 45.05 |
| 4 ml | 8 mg | 51.0 |
| C | No drug | — |

The screening studies for antioxidant properties of medicinal and food plants have been increasingly performed now with the hope of finding safer remedies for disorders related to excessive oxidation of cellular substrates like cancer and certain neurohumoral diseases (Karuna *et al*., 2009). Secondary metabolites from medicinal plants act as antioxidants through a variety of processes like direct anti-radical action, chain breaking of free radical propagation, interaction with transition metals, inhibition of ROS generating enzymes like xanthine oxidase, nitric oxide synthase etc. and improving endogenous cellular antioxidant mechanisms like upregulation of the activity of SOD (Halliwell and Whiteman, 2004).

The plant *B. diffusa* has been found to contain a variety of phytochemicals like flavones (eg. 5-7-dehydroxy-3'4'-dimethoxy-6-8-dimethyl flavone), reducing sugars, triterpenes like â-sitosterol, alkaloids like punarnavine, tannins, amino acids like alanine and aspartic acid and lignins like liriodendrin etc. (Asolkar *et al*., 1992). Phenolic compounds from plants possess the ability to absorb and neutralise free radicals. Similarly, flavonoids present in most plants also exhibit *in vitro* and *in vivo* antioxidant

activity (Dorman *et al*., 2003).The antioxidant effect of the extract may therefore be attributed to its phenolic constituents.

Ferrous ions (Fe2+), in the presence of oxygen and phosphate ions ($PO_4^{2-}$), exist only transiently at physiological pH, before being auto-oxidised to Ferric ions ($Fe^{3+}$). During this process, an electron is transferred from iron to oxygen to form a superoxide radical anion and hydroperoxyl radical ($HO_{2.}$) by Fenton reaction. The concentration dependant, high reducing power of the extract leads to reduction in the transition of iron and consequent generation of superoxide and hydroperoxyl radicals. Similarly, the extract was also found to decrease the nitric oxide radical formation which is an indication of decrease in the oxidative stress that occurs as a result of oxidative peroxynitrite radicals in the system. The upregulation of inducible nitric oxide synthase enzyme has been proven in patients suffering from stress and related disorders leading to increased production of nitric oxide, which can initiate inflammatory process in the body (Harvey *et al*., 2004).

In conclusion, the results showed that the alcoholic extract of *B. diffusa* possessed antioxidant effect *in vitro*, which might be one of the mechanisms involved in the beneficial effect of the extract.

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