Antioxidant and immunomodulatory assay of *Boerhavia diffusa* Linn

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**ABSTRACT**

Botanical and herbal preparations for medicinal usage contain various types of bioactive compounds; the focus of the study is on the analytical methodologies, which includes the extraction, isolation, and characterisation of alkaloid fractions from *Boerhavia diffusa* Linn. The analysis of bioactive compound present in the *Boerhavia diffusa* Linn involving the procedure includes column chromatography, TLC, Immunoassay by PMN & Antioxidant assay by DPPH & FRAP method. Dietary supplementation with antioxidant properties may greatly help in the healthy metabolism of our body. A natural alkaloid which is isolated from a herbal plant *B. diffusa* Linn has antioxidant & Immunomodulatory effect & also *B. diffusa* Linn has anti-inflammatory, anti-arthritis, anti-bacterial, anti-ageing & diuretic activities as well.

**Keywords:**

*Boerhavia diffusa,*

Extraction,

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**INTRODUCTION**

Natural products from medicinal plants, either as pure compounds or as standardised extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. It has been recorded in history that medicinal herbs have been used as a form of therapy. The exploration of the chemical constituents from plants, pharmacological and phytochemical screening would provide the basis for developing the new lead molecule in strategic favour of natural product drug delivery. The aim and subject of many researchers is the discovery and development of isolating new efficient, active and less toxic molecules for systemic activity. The biologically active agents from natural sources have always been of great interest in working on various diseases.

**Herbal Plant**

*Boerhavia diffusa* Linn is a species of flowering plant in the four-‘o’ dock family which is commonly known as Punarnava (Meaning that which Rejuvenates or renew the body) *B. diffusa* Linn is widely dispersed, occurring throughout India. This wide range is explained by its small fruit, which is very sticky and grows a few inches off the ground. *Boerhavia diffusa* Linn is taken in herbal medicine for pain relief and other uses.

**Kingdom:** Plantae

**Order:** Caryophyllales

**Family:** Nyctaginaceae

**Genus:** Boerhavia

**Species:** *B. diffusa*

**MATERIALS AND METHODS**

**Chemicals:** The chemicals were purchased from Sigma, USA.

**Collection of *Boerhavia diffusa* Linn:** Fresh leaves of *Boerhavia diffusa* Linn was collected from the local village. The collected leaves were allowed to shadow dry for 25-30 days. The dried leaves were blended using an electrical blender, and the fine leaf powder was prepared.

**Extraction:** A sample of 25g of powdered plant material was suspended in 400ml of methanol in a
glass beaker and sonicated for 3 hours in an ultrasonic bath at a constant temperature of 25°C. The extract was separated by simple filtration.

**Thin Layer Chromatography in Crude sample**

TLC plate was taken, leave 2.5 cm from one end of the TLC plate & spot the sample on to the plate. Pour the appropriate developing solvent (Methanol: Ammonium hydroxide (3:2)) into the glass jar. Dip the TLC plate in the running solvent just below the sample load. Allow the solvent to run due to capillary action till it reaches nearly the end of the plate. Remove the plate from the jar and let it dry. Visualize the fluorescing spots of the sample under UV Illuminator in a cabinet. Spray Dragendorff’s reagent or Wagner reagent for visualization of the compound.

**Column Chromatography for the separation of alkaloids from Boerhavia diffusa Linn**

a) **Adsorbing the crude filtrate to silica gel**

1.0 gm of crude extract of *B. diffusa Linn* was dissolved in 10 ml of dichloromethane. 5 gm of silica gel was added to the crude extract. Gently the mixture was dried at room temperature until the silica gel becomes free-flowing.

b) **Packing the column for chromatography**

A cylindrical glass column (3mm * 4mm) was taken and plugged at the nozzle using cotton to block the free flow of slurry. The column was mounted on the stand. 100gm of fresh silica gel was taken in a 250 ml beaker. 100 ml of petroleum ether was added into the beaker and stirred well using a glass rod to make the slurry. The slurry was poured into the column without any air bubble. A conical flask was kept below the mounted column to drain out the excess solvent.

c) **Loading of crude extract on the column**

The adsorbed crude material was poured into the solvent layer above the silica gel in the packed column. A bunch of kinds of cotton was kept above the compound slurry.

d) **Elution with Chloroform: Methanol**

The column was filled with Chloroform, and the elution was continued, and each eluent was collected in separate test tubes. The column was continued until the separation of the extract of interest is done. The polarity was further increased using Chloroform: Methanol as 80: 20 ratios, for better separation of individual constituents and individual fractions, was analyzed using TLC to identify the compound of interest. Once the separation of different polarity of solvent exhibit identical constituent the addition of solvent was gradually reduced and the identical fractions were mixed and concentrated on a dark place at room temperature.

**Determination of Antioxidant property by DPPH**

Prepared 0.1 M DPPH in methanol and allowed it to completely soluble and wrapped the vial using foil as it is light sensitive. Transferred 1 ml of 0.1 M DPPH alone in a test tube and 1 ml of sample extract in a separate tube. A fresh tube was taken and added 1 ml of *B.diffusa Linn* extract and 0.1 M DPPH, and it was mixed well. Incubated the mixture in the dark for 30 minutes at room temperature and the absorbance was measured at 517 nm using 0.1 M DPPH as a standard and methanol as a blank.

The radical scavenging activity was calculated using the following formula:

\[
%\text{ inhibition} = \left(\frac{\text{Ab} - \text{Aa}}{\text{Ab}}\right) \times 100
\]

Where, Ab is the absorption of the blank sample; Aa is the absorption of the extract

**Modified Ferric Ion Reducing Antioxidant Power Assay (FRAP)**

To 1 ml of the extract 0.9% ethanol, 5 ml of distilled water, 1.5 ml of 1 M HCl, 1.5 ml 1% potassium Ferricyanide, 0.5 ml of 1% SDS & 0.2% Ferric chloride. Boil the mixture at 50°C for 20 minutes and cool it & read the absorbance at 750 nm.

Scavenging % = \{(1 – (Test absorbance/Blank absorbance)) x 100

**Immunomodulatory Assay**

a) **PMN preparation on the slide**

Capillary blood (0.2 ml) was obtained by finger prick method and was placed on a clean glass slide and spread to 1.5 x 1.5 cm. Blood was allowed to clot at room temperature for 25 minutes. The clot was removed using sterile normal saline. The Polymorphonuclear leukocytes (PMN) were found adhered to the glass surface while the rest of the blood components are washed away.

b) **Candida albicans preparation**

An overnight culture of *C.albicans* was centrifuged at 2000 rpm for 15 minutes. The cell pellet was washed four times with sterile PBS solution. The final cell button was suspended in sterile PBS.

c) **Protocol**

0.1 ml of *B.diffusa Linn* extract was flooded over the PMN layer on the slides, after which the slides were incubated at 37°C for 15 minutes. A control slide was also maintained without extract. 100 μl of C.albicans was added on PMN layer. The slide was fur-
ther incubated at 37°C for 60 minutes, after incubation, the film was washed twice with sterile normal saline. The film was fixed with methanol for 5 minutes, and diluted Giemsa stain was flooded over the film and was left undisturbed for 25 minutes. The excess stain was removed using PBS and air dried. The slide was observed under oil immersion (x 100) objective.

RESULTS AND DISCUSSION

In the present study, we report on Isolation of Alkaloids from the plant *Boerhavia diffusa* Linn using column chromatography and also the antioxidant & immunomodulatory effect of the plant *Boerhavia diffusa*.

Column chromatography

![Column chromatography](image1)

The brown colour band indicates the presence of alkaloids in the crude extract of *Boerhavia diffusa*.

**TLC after separating alkaloids**

![TLC result of alkaloid after the isolation from Boerhavia diffusa](image2)

Determination of Antioxidant property by DDPH

The molecule of 1, 1-diphenyl-2-picrylhydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, Characterization by an absorption band in ethanol solution centred at about 520nm. When a solution of DPPH is mixed with that of substances that can donate a hydrogen atom, and then this gives rise to the reduced form with the loss of this violet colour.

![Molecule of 1, 1-diphenyl-2-picrylhydrazyl](image3)

![The colour changes from Violet to Yellow indicates the positive result of Antioxidant effect](image4)

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Modified Ferric Ion Reducing – Antioxidant Power Assay (FRAP)

The antioxidant present in the sample reduces the oxidant probe and the respective product interacted with some colouring agents to form a coloured complex. In this method, the antioxidants reduce the Fe3+ to Fe2+. This ion then conjugated with ferricyanide ion to form a Prussian blue coloured product, which was spectrophotometrically measured at 700nm. The presence of SDS (Sodium Dodecyl Sulphate) prevents the formation of turbidity in the solution.

\[
\text{Fe}^3+ + \text{Antioxidant} \rightarrow \text{Fe}^2+ + \text{Oxidized antioxidant}
\]

**CONCLUSION**

The Plant *Boerhavia diffusa Linn* has got tremendous clinical significance; It has proved that it has antioxidant & immunomodulatory effects. It also has cardioprotective, hepatoprotective activity, also it has diuretic activities. The alkaloid has been separated from the herbal plant *Boerhavia diffusa Linn* by the technique Column Chromatography. The Antioxidant activity of the extract has been proved by DPPH & FRAP method; Immunomodulatory effect has also been proved by PMN method. The fine compound called punarnavine a specific alkaloid is to be isolated later from the group of alkaloids. Hence it has more effective significance when compared to the crude plant extract.

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