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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF WHOLE PLANT OF BOERHAAVIA *DIFFUSA LINN.*

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ABSTRACT

Objective: The aim of this research was to analyze the antioxidant, anti-microbial activity and of the phytochemicals extracted from *Boerhaavia diffusa Linn.* Whole plant.

Methods: The anti-oxidant activity of the Methanol extract of *Boerhaavia diffusa* was done by using DPPH method. The antimicrobial activity was performed by using cup plate agar diffusion method.

Results: Methanol extract of *Boerhaavia diffusa* whole plant was shown to have declined in antioxidant activity with the increase in concentration. There was a considerable amount of zone of inhibition observed in antimicrobial activity when the assay was performed against *E. coli*. However, no such zone of inhibition was observed against *Bacillus subtilis*. In present study was under taken evaluate anti oxidant activity of concentrations dependent DPPH radical by methanolic extract of *B. diffusa* whole plant and ascorbic acid is for *in vitro* studies. The result revealed that zone of inhibition of Anti bacterial activity of methanol crude extract of *B. diffusa* whole plant against tested bacterial species (gram +ve and gram –ve). Methanol extract obtained by this study.

Conclusion: The present study validates the effective use of *B. diffusa* against the microbial growth and also has anti-oxidant activity.

INTRODUCTION:

Boerhaavia diffusa Linn. (Family: Nyctaginaceae) is an herbaceous plant, cultivated in fields [1, 2] spreading vine widely distributed in the tropical and subtropical regions in the world[3] .*Boerhavia* genus is a collection of 40 tropical and subtropical species. It is found as a weed during rainy seasons in Indian, Northern and Southern American Hermann *Boerhaave*, a famous Dutch physician of the 18th century, while the species got the name from its typical diffuse branching.B.diffusa is up to 1m long or more, having spreading branches. The stem is prostrate, woody, cylindrical often purplish, hairy, and its nodes.

The leaves are simple, thick, fleshy and hairy, arranged in un equal pairs, green and glabrous above and usually white underneath. The shapes of leaves are ovate, round oblong. The *plants* are a rich source of vitamins, minerals, proteins and carbohydrates. In Punjab region, the drug is useful for the eye disease and in Bombay used for dropsical swellings. The leaf juice is used in jaundice and the root whole plant is generally used in infusion in internal inflammation, laxative and also in urinary diseases. In the present study was under taken to investigate anti bacterial and oxidant activities of methanolic extract of the B.diffusa whole plant.

MATERIALS AND METHODS

Plant material

The whole plant of *Boerhaavia diffusa* was collected around Andhra university, Visakhapatnam district, India. During the month of feburary-march, 2016 and authenticated at department of botany, Prof. Padal. Andhra University, Visakhapatnam. The freshly collected whole plant (leaves, stem, roots) was washed with water and dried under shade at room temperature for 1 week. The dried *Boerhaavia diffusa* plant material was cut into small pieces and powdered in a blender. The powder material was stored in sterile air tight container for further use.

Soxhlet extraction:

The air dried powdered material of (520gms) was subjected to hot continuous extraction with soxhelt apparatus by using methanol. After complete extraction the solvent was removed using a buchi type solvent evaporator. Then the extract was obtained which is concentrated and dried completely, weighed and stored in dessicator. The extract was transferred into sterile container until for further use.

Determination of anti bacterial activity

Test Organisms:

The microorganisms used for the experiments were procured from MTCC, IMTECH, Chandigarh. Gram-positive organisms: Staphylococcus aureus, Bacillus subtilis. Gram-negative organisms: Escherichia coli, Pseudomonas aeruginosa.

Standardization of micro-organisms:

One loop-full of micro-organisms were inoculated into 100 ml of sterile medium and incubated for 24 h at 37°c for bacterial culture and for 48 h at 27°c for fungal culture. After 24 h/48 h of incubation, 1 ml of broth containing the micro-organisms was added to 9 ml of peptone water.10 fold serial dilutions were made in the range of 10^{-1} to 10^{-10}.100 µl of the dilutions ranging from 10^{-5} to 10^{-8} were spread over the sterile nutrient agar (SDA) plates and kept at 37 and 27°c for 24 / 48 hours respectively. The number of colony forming units (CFU) was counted and number of micro-organisms per 1 ml of stock culture was calculated.

Preparation of test and standard solutions:

The stock solution of test compounds was prepared by dissolving the dried extracts at a concentration of 5 and 10mg/ml in dimethylsulphoxide (DMSO) respectively. The stock solution of reference standards (Rifampicin) was prepared at a concentration of 0.6 mg/ml in sterile water. Antimicrobial activity was screened by adding 0.05 ml stock solution to each cup by micropipette.

Antibacterial assay

Determination of zone of inhibition by cup plate method

(*Indian* Pharmacopoeia.1996):

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

Table: 1. Extraction of plant material

Plant material	Solvent used	No of cycles	Yield of extract
Dried whole plant of B.diffusa	Methanol (1200ml)	4 cycles	52 g

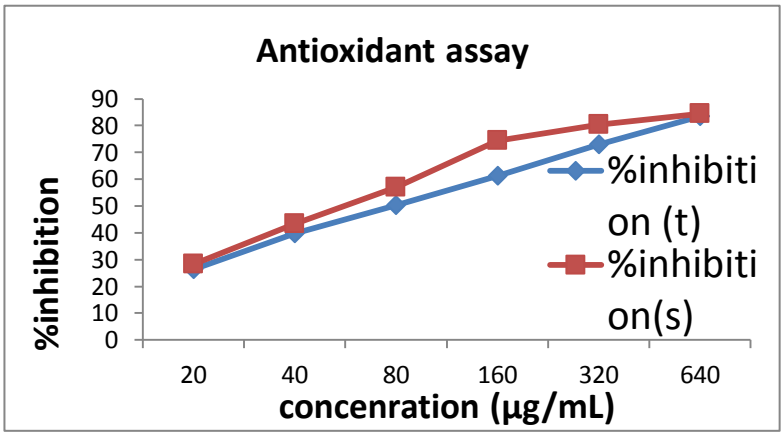
Table: 2 Anti Bacterial activity of *B.diffisa* methanol extract whole plant

s.no	Conc (µg/ml)	Zone of inhibition(mm)	
		Gram positive	Gram negative
1	100	14.5	10
2	200	16	12
3	400	20	15
4	Standard	18	16
5	Control	No zone	No zone

Table 3: Anti oxidative activity of *Boerhaavia diffusa* using DPPH Method

S.no	Conc	% Inhibition of B.diffusa (test)	%inhibition of absorbic acid (standard)
1	10 µg/ml	25.52	25.45
2	20 µg/ml	28.81	43.19
3	40 µg/ml	45.76	56.87
4	60 µg/ml	42.37	74.46
5	80 µg/ml	66.10	80.27
6	100 µg/ml	72.25	84.41

Fig. 1: *In vitro* concentration dependent %inhibition of DPPH radical by methanolic extract of *Boerhaavia diffusa* and ascorbic acid



Anti oxidant activty

Determination of 1, 1- Diphenyl-2-Picrylhydrazyl (Dpph) Radical Scavenging Activity [8]

Principle: In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl–picrylhydrazine.

Reagents: 1, 1- diphenyl-2-picrylhydrazyl (DPPH, 0.004%) solution: 4 mg of DPPH was dissolved in 100 ml of methanol and kept it overnight in dark place for the generation of DPPH radical.

Procedure:

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*., 2003. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room

temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. Concentration Dependent % Inhibition Of DPPH Radical By metanolic Extract Of *B.diffusa* whole plant and Ascorbic Acid In Invitro Studies, A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculations.

$$[(A_0-A_1)/A_0] \times 100.$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the plant extract/ ascorbic acid.

RESULTS AND DISCUSSION

The anti bacterial activity of whole plant methanolic extract of *B. diffusa* for few bacterial strains presented in table- 2. The results clearly depicted that *B. diffusa* whole extract of anti microbial activity. The zone of inhibition was observed gram positive and gram negative strains that are E.coli and S.aureus. the methanolic extract of maximum inhibition of *B. diffusa* for E. coli(10mm) and S.aureus (20 mm). Anti oxidant activity of whole plant of the *B. diffusa* concentraction dependent Dpph radial scavenging activity presented in table -3 . The methanol extract of *B. diffusa* showed 72.25% inhibition at 100µg /ml compared with 84.41% inhibition by ascorbic acid. The extract showed a dose dependent increase in activity.

CONCLUSION

In the present study aim to evaluate the results clearly depicted that *B. diffusa* whole plant of methanol extract has anti bacterial activity and invitro antioxidant and free radial scavenging activity studied on whole plant extract of *B. diffusa* revealed the presence of antioxidant activity.

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