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STUDIES ON PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIOXIDANT POTENTIALS FROM THE ROOTS OF *BOERHAVIA ERECTA*. L.

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ABSTRACT: *Boerhavia erecta* is a weedy herb of the family Nyctaginaceae and is commonly available in almost all places. The present study is so designed for phytochemical screening of the plant as well as its antibacterial and antioxidant activities of the root extract of *B. erecta*. The whole plant was collected in and around Chennai. Phytochemical screening of acetone extract of B. erectarevealed the presence of flavonoids, tannins, phlobatannin, saponin, terpenoids, glycosides. The antibacterial activity of the crude extract was determined by the well diffusion method against four pathogenic bacteria. At higher concentratio the extract showed good zone of inhibition against *E.coli* and *P. aeruginosa*. DPPH assay, Reducing power and Hydroxyl radical scavenging assay of acetone extract was studied and compared with standard Quercetin and Ascorbic acid.

INTRODUCTION: In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries [1]. The plants supply us with large number of excellent "chemicals" which form sources for different types of drugs.

The present trend in modern medicine is towards a change from the use of cellulose coated medicinal pills to extracts of plant supplied either in pure forms or in synthetic versions for curing many human ailments.

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Thus plants have provided the blue prints for the modern medicine. Natural products perform various functions, and many of them have interesting and useful biological activities [2]. Free radicals are molecules containing one or more unpaired electrons in atomic or molecular orbitals. There is an increasing evidence that abnormal production of free radicals leads to increased oxidative stress on cellular structures and causes changes in molecular pathways that underpins the pathogenesis of several important diseases, including cardiovascular diseases, neurological diseases, cancer, and in the process of physiological ageing [3]. Antioxidants are vital substances that possess the ability to protect the body from damage caused by free radical induced oxidative stress [4].

MATERIALS AND METHODS:

Plant Material: The plant material of the root of *B. erecta* were collected from in and around Chennai. It was identified using standard books. The root

part were shade dried and crushed into fine powder with electric blender. The powdered sample was sealed in polythene bags and was stored in desiccators until further use.

Preparation of acetone extract:
Dried and powdered root part of *B. erecta* (100 g) were extracted using soxhlet with 100% acetone (1:5 W/V) for about 72 hours. The extractwas removed and concentrated to dryness in rotary vacuum evaporator below 50°C and stored until needed for the bioassays at -4 °C.

Phytochemical screening:
The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents and they were identified by the characteristic colour changes using standard procedures as described by [5, 6, 7].

Determination of Antibacterial Activity of *B.Erecta:*
In vitro antibacterial activity of the acetone root extracts of *B.erecta* was screened against a total of the four bacterial strains.

Microbial strains and inoculum preparation:
The microorganisms used in this study were human pathogens namely *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus.*

Bacterial strains stock cultures were maintained at 4°C on Muller Hinton Agar medium. Active cultures were prepared by inoculating fresh nutrient broth medium with a loopful of cells from the stock cultures at 37°C for overnight. To get desirable cell counts for bioassays, overnight grown bacterial cells were subcultured in fresh Muller Hinton Broth at 37°C.

Well diffusion method:
The well diffusion test [8, 9, 10] was performed using MHA medium. The medium was prepared and autoclaved at 15 lbs pressure (121ºC) for 15 minutes immediately cooled in a 50-55 ºC water bath after removed from the autoclave. The cooled medium was poured into sterile petriplates to a uniform depth of 4 mm; this is equivalent to approximately 25 mL in a 90 mm plate. Once the medium was solidified, then the culture was inoculated on the medium. Within 15 minutes of adjusting the density of the inoculum, a sterile cotton swab was dipped into the standardized bacterial suspension. The sterile swab was used to streak on the surface of the MHA containing plates. The plates were allowed undisturbed for 3 to 5 min to absorb the excess moisture. Sterilized 9 mm cork borer was used to make agar wells, 25µg, 50µg, 75µg of extract stock solutions were placed into each wells and 100% DMSO as a control. Positive control was made by tetracycline 30 µg which were suspended in 100% DMSO solvent. Zone of inhibition (ZI) were measured by 1mm accuracy scale prescribed method and calculated the zone of inhibition percentage also by the following formula

Percentage of inhibition = I/diameter of the petriplate in mm×100.

***In vitro* antioxidant activity:**
DPPH free radical scavenging activity:
The acetone extract of the root of *B.erecta* were subjected to *in vitro* antioxidant assay [11]. For acetone extracts reaction mixture consisted of 1 mL of 0.1 mM diphenyl-p-picrylhydrazyl radical (DPPH) in methanol and 1 mL of different concentrations (16 µg/mL, 32µg/mL, 64µg/mL, 125µg/mL, 250µg/mL, 500µg/mL and 1000µg/mL) were prepared. Acetone extracts of root were prepared and diluted to concentrations (16 µg/mL, 32 µg /mL, 64µg/mL, 125µg/mL, 250µg/mL, 500µg/mL and 1000µg/mL).1.0 mL of DPPH and 1.0 mL of methanol were used as control. The reaction mix was left in the dark at room temperature for 30 minutes. The OD was measured using spectrophotometer at 517 nm. Quercetin was used as standard. The inhibition percentage was calculated according to the formulae.

$$\text{Inhibition percentage} = A_C - A_S / A_C \times 100.$$

Where, A_C- Absorbance of Control; A_S- Absorbance of Sample. The 50% inhibitory concentration (IC_{50}) values were calculated by plotting an x, y scatter trendline with regression equation.

Reducing power assay: The reducing power of crude acetone extracts of the root of *B.erecta* were

determined by the method of (Oyaizu, 1986) [13]. Various concentrations of the plant extracts in 1.0 mL of solvent were mixed with phosphate buffer (2.5mL) and potassium ferriccyanide (2.5mL) and incubated at 50°C for 20 minutes. Trichloroacetic acid (10%; w/v) 2.5 mL were added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes whenever necessary.

The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride (0.1%; w/v) solution 0.5 mL. The Absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid with various concentrations was used as the standard reference. Increase in absorbance of the reaction mixture indicates the increase in reducing power.

Hydroxyl radical scavenging activity:

The hydroxyl radical scavenging activity of crude acetone extracts of the root of *B.erecta* was determined by Klein *et al.* (1991) [14]. Various concentration of extracts were taken in different test tubes and evaporated to dryness. 1mL of iron EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5mL of EDTA (0.018%) and 1mL of DMSO (0.85% v/v in 0.1M phosphate buffer, PH 7.4) were added to these tubes and the reaction was initiated by adding 0.5mL of 0.22% ascorbic acid. Test tubes were capped tightly and heated on water bath at 80-90°C for 15 minutes. The reaction was terminated by the addition of 1 mL of ice cold TCA (17.5%; w/v) solution.

Then 3 ml of Nash reagent (75 g of ammonium acetate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and made upto 1L with distilled water) was added to all tubes and left at room temperature for 15 minutes for colour development. Quercetin was used as standard reference. The intensity of the yellow colour formed was measured spectrophotometrically at 412 nm.

Hydroxyl radical scavenging activity (%) = A_C-$A_S/A_C \times 100$.

Where, A_C-Absorbance of Control; A_S-Absorbance of Sample

RESULTS AND DISCUSSION:

Phytochemical screening: The qualitative studies of crude acetone extract of *B. erecta* indicates the presence of flavonoids, tannins, phlobatannin, saponin, terpenoids, glycosides and absence of steroids. (**Table 1**). The crude extract of *B. erecta* was qualitatively analysed for the presence of flavonoids, tannins, phlobatannin, terpenois, saponin, glycosides, steroids of which steroids alone showed a negative result According to [15] from the ethanol extract of leaves of *B.erecta* showed the presence of flavonoid, alkaloid, glycosides, steroids, phenols, tannins and saponins. According to Rajeswari *et al.,* 2010 from the methanol extract of *B.erecta* showed the presence of phenol and flavonoids. In the present study from the acetone extract of root of *B.erecta* showed the presence of flavonoid, tannins, phlobatannin, saponin, terpenoids, glycosides.

Antibacterial activity:

Antibacterial activities of the crude extracts were tested against four pathogenic bacteria and they were compared with standard antibiotic tetracycline by measuring the zone of inhibition diameter and expressed in mm showed in (**Table 2; Fig.1**). The average zone of inhibition ranges from 15- 19mm.

Highest inhibiton was observed against the growth of *E.coli*, *P.aeruginosa* with the zone of inhibition 18mm and 19mm respectively. Antibacterial activities of the crude extracts were tested against four pathogenic bacteria and they were compared with standard antibiotic tetracycline by measuring the zone of inhibition and expressed in mm as showed in (**Table: 2; Fig.1**). The average zone of inhibition ranges from 15- 19mm. Highest inhibiton was observed against the growth of *E.coli*, *P.aeruginosa* with the zone of inhibition 18mm and 19mm respectively. According to [15] the ethanolic extract of leaves of *B.erecta* at the concentration of 150µg the maximum inhibition zone against the bacterial strains. Moderate response was seen against *B.subtilis* and *Aeromonas hydrophila* and minimal response against *P. aeruginosa*.

***In-vitro* antioxidant activity:**

DPPH Radical Scavenging Assay: The DPPH radical scavenging activity from the acetone extract

of root have strong scavenging activity. Maximum inhibition activity for root extract was observed at 1000µg/mL respectively. Antioxidant activity of root extract was compared with standard Quercetin. IC_{50} value of root extract less than Quercetin, showed more activity. (**Table 3**). The DPPH test is based on the ability of stable free radical to decolourize in the presence of antioxidant is a direct and reliable method for determining radical scavenging action. According to Parameswari *et al.*, 2013 the ethanol extracts of *B.erecta* showed IC_{50} value of 800 µg and ascorbic acid with an IC_{50} value of 600 µg. In the present study the maximum inhibition activity for root extract was observed at 1000µg/mL with IC_{50} value 7.11µg/mL with reference to standard Quercetin IC_{50} value was 8.65µg/mL.

Reducing Power Assay:

Reducing power was assayed from the acetone extract of root of *B.erecta* showed increasing activity of Fe^{3+}. Strong reducing power was observed in lower concentration of fruit extract. The concentration ranged from 64 – 1000 µg/mL. The acetone extract of root of *B.erecta* was very potent and the power of the extract increased with the quantity of sample. The concentration of extract in root showed absorbance of 0.412 at 1000µg/mL. When compared with standard ascorbic acid the root extract showed less activity. (**Table4**). Reducing power assay determine the reductive capabilities of the extract compared with ascorbic acid which has determined using potassium ferricyanide reduction method. According to [17] the ethanol extracts of *B.erecta* exhibited absorbance of 0.38 at 1000µg/mL concentration. In the present study the concentration of extract of root showed absorbance of 0.412 at 1000µg/mL when compared with reference to ascorbic acid the root extract showed less activity.

Hydroxyl radical scavenging assay:

Acetone extract ofthe root of *B.erecta* showed excellent chelating activity. The maximum chelating activity was found to be 89.03 % at 1000µg/mL concentration with IC_{50} value 7.69µg/mL with reference to standard ascorbic acid IC_{50} value was 8.63 µg/mL respectively. (**Table 5**). Hydroxyl radical scavenging assay determine the capabilities of the extract compared with ascorbic acid using Fenton method. According to [17] the ethanol extracts of *B.erecta* exhibited minimum activity of 25.42% at 200µg/mL and maximum activity of 60.72% at 1000µg/mL. In the present study the concentration of extract of root showed maximum inhibition at 1000µg/mL with IC_{50} value 7.69µg/mL with reference to standard ascorbic acid IC_{50} value was 8.63µg/mL respectively.

The antibacterial activity and antioxidant activity of *B. erecta*is substantiated by the phytochemicals present in the roots. They can be further purified and studied for therapeutic activity.

TABLE: 1: QUALITATIVE ANALYSIS OF *B.ERECTA* OF ACETONE EXTRACTS.

Phytochemicals	*P. acidus*
	Fruit
Flavonoid	+
Tannin	+
Phlobatannin	+
Saponin	+
Terpenoids	+
Glycosides	+
Steroids	-

(+) – presence; (-) - absence

TABLE 2: ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACTS OF *B. ERECTA*AGAINST HUMAN PATHOGENS

Sample	Pathogens	Concentration of acetone extracts (µg)					
		25		50		75	
		ZI	% I	ZI	% I	ZI	% I
B.erecta	*E. coli*	15.33±1.02	17.03±0.90	16.66±2.00	18.51±1.05	17.00±2.01	18.88±1.26
	P. aeruginosa	14.66±2.01	16.28±1.21	15.66±3.01	17.41±1.26	17.33±2.07	19.25±1.29
	B. cereus	15.66±1.03	17.41±0.93	14.33±1.03	15.92±0.95	16.66±1.12	16.66±1.21
	S. aureus	16.33±1.44	18.14±1.03	15.00±2.01	16.66±1.19	16.00±1.34	17.77±1.34

Values are mean ± standard deviation of triplicates

TABLE 3: DPPH ASSAY OF ACETONE EXTRACTS OF *B.ERECTA*

Samples	Concentration of acetone extracts (µg/mL)	Percentage of Inhibition (%)	IC_{50}
B.erecta	8	56.22± 0.21	7.11
	16	65.77± 0.25	
	32	70.62± 1.10	
	64	78.66± 1.46	
	125	79.87 ±0.55	
	250	81.82± 0.64	
	500	87.63± 0.25	
	1000	91.36± 0.81	
Quercetin	3	35.32±2.47	8.65
	6	43.26±3.02	
	9	52.56±3.67	
	12	65.45±4.58	
	15	68.66±4.80	
	18	79.86±5.59	
	21	93.98±6.29	

Values are mean ± standard deviation of triplicates

TABLE 4: REDUCING POWER ASSAY OF ACETONE EXTRACTS OF *B.ERECTA*

Samples	Concentration of acetone extracts (µg/mL)	Absorbance at 700 nm
B. erecta	64	0.188± 0.016
	125	0.218± 0.018
	250	0.289± 0.025
	500	0.341± 0.030
	1000	0.412± 0.041
Ascorbic acid	64	0.328±0.022
	125	0.480± 0.033
	250	0.530±0.037
	500	0.620±0.043
	1000	0.780±0.054

Values are mean ± standard deviation of triplicates

TABLE 5: HYDROXYL RADICAL SCAVENGING ACTIVITY OF ACETONE EXTRACTS OF *B.ERECTA*

Samples	Concentration of acetone extracts (µg/mL)	Percentage of Inhibition (%)	IC_{50}
B.erecta	8	51.94± 0.41	7.69
	16	59.45± 1.46	
	32	66.46± 0.55	
	64	73.92± 0.71	
	125	82.19± 0.33	
	250	85.05± 0.77	
	500	86.32± 0.41	
	1000	89.03± 1.46	
Quercetin	2	10.29±5.54	8.63
	4	13.29±5.04	
	6	35.04±2.224	
	8	48.78±1.22	
	10	59.76±5.58	
	12	75.44±4.25	
	14	96.76±4.44	

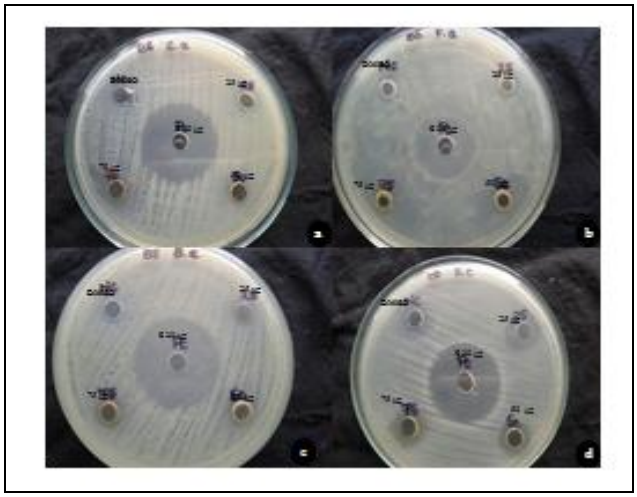

FIG.1: ANTIBACTERIAL ACTVITY OF B. ERECTA OF CRUDE ACETONE EXTRACTS
a. ***S. auresus*****, b.** ***P. Aeruginosa*****, c.** ***B. cereus*****, d.** ***E.coli***

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