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In-vitro antioxidant and antibacterial activities of different fractions of *Heliotropium indicum L.*

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ABSTRACT

In this study we investigated the *In-vitro* Antioxidant (DPPH radical) and Antibacterial activity of different fractions (hydro-alcoholic, methanolic, ethyl acetate and hexane) of *Heliotropium indicum* was carried out against nine selected pathogenic bacteria. In preliminary phytochemical investigation of the plant extracts, quantified the total phenolic content by taking Gallic acid as a standard, *In-vitro* antibacterial activity was evaluated for extracts by using cup plate method and In-vitro antioxidant activity by using DPPH free radical. In preliminary phytochemical investigation the plant extracts possesses Steroids, terpenoids, Saponins, Flavonoids, Carbohydrates, Glycosides, Amino acids and oils .Among the selected extracts ethyl acetate fraction of *H.indicum* showed high phenolic content and same fraction was showed more DPPH radical scavenging activity . For antibacterial activity, Ethyl acetate fraction showed good antibacterial activity against all the tested bacteria except *Priteus mirabilis, Salmonella typhimurium.* The antibacterial activity was may be due to phenolic compounds present in the extracts. In the present study we found that the different fractions of *Heliotropium indicum* showed good Antioxidant and Antibacterial activity.

Key words: *Heliotropium indicum,* DPPH radical, Total Phenolic content, *In-vitro* Antibacterial activity, *In-vitro* Antioxidant Activity.

INTRODUCTION

The history of drugs is intimately linked with plants from the earliest times and even today, plant products have extensive use in ethno medicine and traditional systems of medicine. Interest in medicinal plants as therapeutic alternates has increased enormously over the last three and half decades. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties, which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [1-3]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) [4-5]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. *Heliotropium indicum* has a pantropical distribution, but is probably native of Asia and tropical america. *H. indicum* has been used widely for centuries on warts and to treat inflammations and tumours. Throughout tropical Africa it is used as an analgesic (rheumatism), diuretic and for numerous skin problems (e.g. yaws, urticaria, scabies, ulcers, eczema, impetigo). There is ample variation in plant parts used, and in methods of preparation and administration.

In this paper, we reported the results of antioxidant and antibacterial activities of different fractions *Heliotropium indicum* in order to orient future investigations towards the finding of new, potent and safe antioxidant and antibacterial drug candidates.

MATERIALS AND METHODS

Chemicals

1, 1- diphenyl-2-picrylhydrazyl and Rifampicin were purchased from Sigma Chemical Company, St. Louis, USA), Muller Hinton agar media was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

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Test Organisms

Nine bacterial species were used. The bacterial species were purchased from National collection of industrial micro organisms (NCIM), Pune. The Bacterial species were maintained in the nutrient broth medium on placing shaker in separate culture tubes for each species separately. Out of nine, two are Gram positive Organisms *(Bacillus megaterium, Staphylococcus epidermidis)* Gram Negative *(Pseudomonas aeruginosa, Priteus mirabilis, Klebsiella pneumonia, Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli, Enterobacter cloacae)*

Culture Media

For Anti bacterial activity Muller-Hinton Agar media (Solid and Broth) was used. For maintaining the bacterial species Nutrient both was used.

Preparation of Extracts

The plant material used in present study was collected from Visakhapatnam, Andhra Pradesh and authenticated by the taxonomist Prof.M.Venkaiah , Depart of Botany, Andhra University. Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. To the coarse powder (1kg) in round bottomed flask 1 litre of alcohol (70% v/v) was added and macerated for 24 hours at room temperature. The macerated powder was packed in a soxhlet apparatus and subjected to continuous extraction with 3 litre of alcohol (70% v/v). The liquid extract was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained. The mass obtained was weighed in each case. The extract was thoroughly air dried to remove all traces of the solvent. Hexane, ethyl acetate and methanolic fractions were prepared from hydro-alcoholic crude extract by successive fractionations by using hexane, ethyl acetate and methanol as solvents (analytical grade).

Phytochemical Analysis

Phytochemical studies were carried out for hydro alcoholic crude, hexane,

ethyl acetate and methanol fractions of *Heliotropium indicum* to detect the presence of steroids, terpinoides, tannins, flavonoides, saponins, cardiac glycosides, amino acids etc following the described procedures [6-8].

Quantification of Total Phenols

Total phenolic content was determined using the Folin-Ciocalteau reagent [9]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

DPPH Free Radical Scavenging Activity

The crude extract and different fractions (hexane, ethyl acetate and methanol) of *H. indicum* were screened for DPPH radical Scavenging activity. DPPH radical scavenging activity was measured according to the method of Braca *et al.*, 2003[10]. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37°c for 30 min. and absorbance of the test mixture was read at 517nm. The All experiments were performed thrice and the results were averaged.

The percentage of inhibition of DPPH radical was calculated

$$\text{Inhibitory ratio} = (A_0 - A_1)/A_0 \times 100$$

A_0 is the absorbance of control;

A_1 is the absorbance with addition of plant extract/ ascorbic acid.

The optical density obtained with each concentration of test sample plotted taking concentration on X-axis and percentage inhibition on Y-axis, the graph was extrapolated to find the 50% inhibition concentration of test sample.

Antibacterial Activity

The antibacterial activity of hydro-alcoholic extract was determined by cup plate method. Different concentrations of the extracts were prepared by reconstituting with DMSO. The prepared Muller Hinton Agar medium was heated at 45^{0}C to get liquid state. The Muller-Hinton Agar medium was cooled at room temperature. Then, the 20 ml of Muller-Hinton Agar medium is taken in the eight test tubes, to those tubes subjected to testing bacterial inoculums (20µl). After adding the inoculums the tubes were mixed well for equal distribution of the Bacterial species in the medium wells were prepared by using metal steel borer. Different concentrations of plant extracts were placed in the wells of solidified Petri dishes.

Then the plates were incubated in incubator for 24hrs at 36^{0}C. After incubation , the zones of inhibitions were measured in mm.

RESULTS AND DISCUSSION

Phytochemical Analysis

Qualitative chemical tests indicated that the hydro-alcoholic crude extract *H.indicum* showed positive test for Steroids, Triterpenoids, Saponins, Flavonoids, Carbohydrates, Glycosides, Amino acids and oils. The methanolic fraction of *H.indicum* showed positive test Triterpenoids, Saponins, Carbohydrates, Glycosides, Amino acids and oils. The ethyl acetate fraction of *H.indicum* showed positive test Steroids, Saponins, Flavonoids, Carbohydrates, Glycosides, Amino acids and oils. The hexane fraction of *H.indicum* showed positive test Saponins, Carbohydrates and Amino acids.

Quantification of Total Phenols

The phenolic content in hydro-alcoholic crude extract, methanolic, ethyl acetate and hexane fractions of *H.indicum* was found to be 5.49, 2.96, 9.76 and 2.32 mg/g respectively. Among the selected extracts ethyl acetate fraction of *H.indicum* showed high phenolic content (Table 1).

Table 1.Phenolic content present in different fractions of *Heliotropium indicum* (mg/g)

Name of the extract	GAE(Gallic acid equivalent) mg/gm
Hydro-alc.extract	5.49
Methanolic fraction	2.96
Ethyl acetate fraction	9.76
Hexane fraction	2.32

DPPH Radical Scavenging Activity

In this study the hydro-alcoholic, methanolic, ethyl acetate and hexane fractions of *H.indicum* was found to posses concentration dependent inhibition of DPPH radical scavenging activity. The mean IC_{50} values for DPPH radical by hydro-alcoholic, methanolic, ethyl acetate and hexane fractions of *H.indicum* ,was found to be 200µg , 306µg , 75.4µg and 307µg respectively. The IC_{50} value of ascorbic acid was found to be 22 µg (Table.2).

Table 2.*In-vitro* 50% inhibition concentration (IC_{50}) of different fractions of *Heliotropium indicum* on DPPH free radical scavenging activity

Name of the extract	IC_{50} value (µg)
Hydro-alc.extract	200.0
Methanolic fraction	306.0
Ethyl acetate fraction	75.4
Hexane fraction	307.0
Ascorbic acid	22.0

The extracts hydro-alcoholic crude extract, methanolic, ethyl acetate and hexane fractions of *H.indicum* showed concentration dependent percentage inhibition of DPPH radical and better percentage inhibition was produced at a concentration of 640 µg (Fig.1). The ethyl acetate fraction showed better activity than hydro-alcoholic crude extract, methanolic and hexane fractions of *H.indicum.*

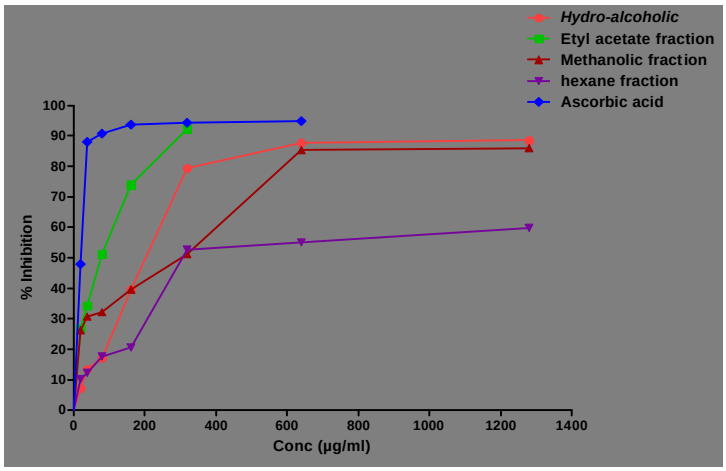

Fig 1: Concentration dependent percent inhibition of DPPH radical by different fractions of *Heliotropium indicum* and Ascorbic acid in *in-vitro* studies

The order of DPPH radical scavenging activity is as follows:

Ascorbic acid (22µg) > Ethyl acetate fraction (75.4µg) > hydro-alcoholic extract (200µg) > Methanol fraction (306µg) >Hexane fraction (307µg).

Antibacterial Activity

Hydro-alcoholic crude extract and their hexane, ethyl acetate and methanol fractions of *Heliotropium indicum* whole plant were showed significant

zone of inhibition against "Gram -positive" bacteria, *Bacillus megaterium, Staphylococcus epidermidis* and "Gram-negative" bacteria *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli* (Table3-6).

Table 3. Antibacterial activity of Hydro-alcoholic (70%v/v ethanol) extract of *Heliotropium indicum*

Organisms	Gram +Ve/-Ve	Rifampicin	Zone of inhibition(in mm) of hydro alcoholic (70%v/v ethanol) extract (50µL)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.0mg
Bacillus megaterium	+ve	31	-	09	14	18	23
Staphylococcus epidermidis	+ve	35	-	08	13	17	21
Pseudomonas aeruginosa	-ve	41	07	10	16	19	23
Priteus mirabilis	-ve	39	10	13	19	24	32
Klebsiella pneumoniae	-ve	30	-	09	15	21	24
Salmonella typhimurium	-ve	41	08	14	21	27	34
Enterobacter aerogenes	-ve	32	-	09	15	20	24
Escherichia coli	-ve	28	07	11	17	22	27
Enterobacter cloacae	-ve	36	-	09	15	20	24

Table 4.Antibacterial activity of hexane fraction of *Heliotropium indicum*

Organisms	Gram +Ve/-Ve	Rifampicin	Zone of inhibition (in mm) of hexane fraction (50µL)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.0mg
Bacillus megaterium	+ve	32	07	09	12	15	19
Staphylococcus epidermidis	+ve	35	08	12	15	18	21
Pseudomonas aeruginosa	-ve	41	07	08	10	13	16
Priteus mirabilis	-ve	38	07	12	15	17	20
Klebsiella pneumoniae	-ve	31	08	15	18	22	26
Salmonella typhimurium	-ve	40	09	18	23	25	29
Enterobacter aerogenes	-ve	33	-	10	14	17	21
Escherichia coli	-ve	27	07	09	11	15	18
Enterobacter cloacae	-ve	35	07	11	14	16	19

Table 5. Antibacterial Activity of ethyl acetate fraction of *Heliotropium indicum*

Organisms	Gram +Ve/-Ve	Rifampicin	Zone of inhibition (in mm) ofEthyl acetate fraction (50µl)				
			0.5mg	1.0mg	1.5mg	2.0mg	2.0mg
Bacillus megaterium	+ve	31	08	11	17	22	28
Staphylococcus epidermidis	+ve	35	10	13	19	24	29
Pseudomonas aeruginosa	-ve	41	09	13	19	23	28
Priteus mirabilis	-ve	37	07	12	18	23	29
Klebsiella pneumoniae	-ve	30	07	13	20	25	30
Salmonella typhimurium	-ve	41	10	13	19	23	30
Enterobacter aerogenes	-ve	32	08	12	18	23	28
Escherichia coli	-ve	28	10	13	19	24	28
Enterobacter cloacae	-ve	36	07	11	17	22	28

Among the four (one crude, hexane, ethyl acetate and methanol) fractions tested at five different doses, the ethyl acetate fraction and hydro-alcoholic crude extract 2.5 mg/50µl dose were more potent in their antibacterial activity. The order of antibacterial activity against selected Gram positive and Gram negative bacteria as follows:

Table 6 .Antibacterial Activity of methanolic fraction of *Heliotropium indicum*

Organisms +Ve/-Ve	Gram	Rifampicin	Zone of inhibition (in mm) of (50µl) methanolic fraction				
			0.5mg	1.0mg	1.5mg	2.0mg	2.0mg
Bacillus megaterium	+ve	31	-	09	13	15	21
Staphylococcus epidermidis	+ve	34	-	08	11	15	20
Pseudomonas aeruginosa	-ve	41	-	09	13	15	21
Priteus mirabilis	-ve	37	-	07	13	17	22
Klebsiella pneumoniae	-ve	31	-	09	12	19	23
Salmonella typhimurium	-ve	41	-	08	12	15	20
Enterobacter aerogenes	-ve	32	-	09	13	16	20
Escherichia coli	-ve	30	07	11	14	19	26
Enterobacter cloacae	-ve	36	-	07	12	17	21

Ethyl acetate fraction > hydro-alcoholic extract > methanolic fraction > hexane fraction.

CONCLUSION

The data clearly indicated that the hydro-alcoholic crude extract and their hexane, ethyl acetate and methanol fractions of *Heliotropium indicum* showed good antioxidant and antibacterial activity. Among the all the ethyl acetate fraction showed better activity.

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