

EVALUATION OF INVITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *HEMEDESMUS INDICUS* ROOT EXTRACT

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ABSTRACT

The present research study highlights the antibacterial and antioxidant activities of *Hemedesmus Indicus* root extract. The coarsely powder of *Hemedesmus Indicus* root was subjected to invitro antibacterial activity, the ethanolic extract of root at a concentration of 50mg/ml was tested against gram negative bacteria *Klebsiella Pneumonia* (ATCC33495), *Pseudomons Aeruginosa* (ATCC10662), *Escherichia Coli* (ATCC10536), *Bacillus Subtilis* (ATCC11774), *Staphylococcus Aereus* (ATCCBAA1026) and the zone of inhibition was compared with standard drug Gentamicin 20 ug /ml. The *Hemedesmus Indicus* root extract at a concentration of 100 μ g/ml was tested for its free radical scavenging activity adopting various methods like DPPH, Nitric oxide and reducing power assay. The extract exhibited significant antioxidant activity after comparing with standard drug gallic acid and the results were tabulated.

Keywords: *Hemedesmus Indicus*, Gentamicin, Gallic Acid, Antibacterial, Antioxidant Activity.

INTRODUCTION

Since the time immemorial our traditional system of medicine and folkloric claiming several medicinal plants as whole or their parts are being used in all types of skin diseases successfully against several bacterial and fungi. The medicinal preparations available in the market are not effective or has developed resistance resulting in reoccurrence again^{1,2}. The literature survey on this medicinal plant *Hemedesmus Indicus* not much pharmacological work has been carried out and the natives are using this plant has folkloric for treatment of various ailments. Hence the researcher made a sincere attempt to evaluate the anti bacterial and anti oxidant activities on this medicinal plant root extract.

MATERIALS AND METHODS

Collection of Plant

The root of the medicinal plant *Hemedesmus Indicus* was collected from interior parts of Maredumilli forest region of East Godavari District, Andhra Pradesh and the plant was authenticated by taxonomist Prof. Dr. S.B.Padal.

Preparation of the Extract

The root of the plant was dried under the shade coarsely powdered and was subjected to extraction process using soxhlet apparatus using ethyl alcohol for 72 hours. The solvent was evaporated and the crude extract was dried in a dessicator for few days and this extract powder was used for evaluation of anti bacterial and antioxidant activities.

Antibacterial Activity

The various bacterial strains like *Klebsiella Pneumonia*, *Pseudomons Aeruginosa*, *Escherichia Coli*, *Bacillus Subtilis*, *Staphylococcus Aereus* were procured from microbes specialty lab in Rajahmundry, East Godavari District, Andhra Pradesh.

The Antibacterial activity is determined according to the standard method described³ using Agar cup plate method. 20 ml of sterile nutrient agar medium was poured in sterile petri dishes and allowed to solidify. The petri dishes were incubated at 37^o for 24 hours to check for sterility. The medium was seeded with organisms by Pour plate method using sterile agar broth 1 ml culture. Bores were made on the medium using borer. *Hemedesmus Indicus* root extract was dissolved in water to obtain 50 mg/ml, standard drug Gentamicin at 20ug /ml was taken as standard reference. All the plates were kept in a refrigerator at 2^o to 8^o for a period of 3 hours, later they were incubated at 37^o for 24 hours. The diameter zone up inhibitions were measured and recorded and the results are tabulated in table 1. The maximum zone of inhibition is seen with *Pseudomonas Aeruginosa* 14.2mm± 0.47 as compared to Gentamicin 17mm± 0.54.

Antioxidant Activity

DPPH Radical Scavenging Assay⁴

Hemedesmus Indicus root extract was subjected to DPPH radical scavenging assay, the extract at a concentration of 100ug/ml showed maximum inhibition is 76.20% and the results was compared with standard drug gallic acid 2.5 ug/ml 79.20%. The results are tabulated in table 2.

Nitric Oxide Scavenging Activity⁵

The root extract of *Hemedesmus Indicus* was subjected to nitric oxide scavenging assay and the extract showed maximum inhibition at 100 ug/ml is 47.60% and the result is compared with standard drug gallic acid 2.5 ug/ml 53.20%. The results are tabulated in table 3.

Reducing Power Assay⁶

The concentration of *Hemedesmus Indicus* root extract 100 ug/ml and standard drug gallic acid 2.5 ug.ml were prepared using

distilled water, 1% of potassium ferricyanide, 10% trichloro acetic acid, 0.1% ferric chloride and 0.2M phosphate buffer were prepared using distilled water. Gallic acid was taken as the reference standard. Then 1 ml of each concentration of both extract and standard were taken separately and mixed with 1 ml of 0.2M phosphate buffer (p^H 6.6) and 1 ml of potassium ferricyanide. Incubate all these samples at 50^oC for 20 min. Then add 1 ml of 10% trichloro acetic acid and centrifuge at 2000 RPM for 10 min. Now separate the upper layer (2.5ml) and then add (2.5ml) distilled water, 0.5ml of freshly prepared ferric chloride. Finally measure the absorbance at 700nm. The results are tabulated in Table 4.

RESULTS AND DISCUSSION

The ethanolic root extract of *Hemedesmus Indicus* produced significant antibacterial activity against both gram positive and gram negative bacteria and the zone of inhibitions for various organisms are quiet encouraging and the maximum zone of inhibition is produced with *Pseudomonas Aeruginosa* 14.2mm as compared to standard drug Gentamicin which produced zone of inhibition 17mm. The *Hemedesmus Indicus* root extract exhibited good anti oxidant activity, the scavenging activity of the extract showed maximum inhibition in DPPH radical assay 76.20% against standard drug gallic acid 79.20%, similarly in Nitric oxide and reducing power assay methods the root extract produced significant anti oxidant activity with maximum percentage of inhibition 47.60% against 53.20% and Reducing power response of 0.662 against standard drug gallic acid 0.724 respectively.

CONCLUSION

The results of antibacterial and antioxidant activities are excellent and the plant root extract of *Hemedesmus Indicus* is said to be a very important medicinal plant in Ayurveda and can be considered as a useful folkloric medicine for the treatment of these properties. Further attempts need to be stressed to pursue extensive research work and isolate possible bioactive molecules responsible for all these biological activities.

Table 1: Antibacterial activity of Hemedesmus Indicus root extract

S.No.	Organism used	Extract of <i>Hemedesmus Indicus</i> Zone of inhibition in mm	Standard Drug Gentamicin 20 ug /ml Zone of inhibition in mm
1	<i>Klebsiella Pneumonia</i> (ATCC33495)	13.6±0.51	19±0.60
2	<i>Pseudomonas Aeruginosa</i> (ATCC10662)	14.2±0.47	17± 0.54
3	<i>Escherichia Coli</i> (ATCC10536)	12± 0.38	20± 0.38
4	<i>BacillusSubtilis</i> (ATCC11774)	11.8±0.54	19± 0.51
5	<i>Staphylococcus Aereus</i> (ATCCBAA1026)	12 ± 0.70	18 + 0.72

Values are mean ± SEM n= 3, zone of inhibition in mm, Standard drug Gentamicin

Table 2: DPPH Radical Scavenging Assay of Hemedesmus Indicus root extract

S.No.	Test	Concentration ug/ml	% of inhibition
1	<i>Hemedesmus Indicus</i> root extract	100	76.20 ± 0.48
2	Gallic Acid (Standard)	2.5	79.20 ± 0.60

Values are Mean + SEM n = 3

Table 3: Nitric Oxide Scavenging Activity of Hemedesmus Indicus root extract

S.No.	Test	Concentration ug/ml	% of inhibition
1	<i>Hemedesmus Indicus</i> root extract	100	47.60 ± 1.60
2	Gallic Acid (Standard)	2.5	53.20 ± 3.20

Values are Mean + SEM n = 3

Table 4: Results of Absorbance in Reducing power assay

Tested Material	Concentration (ug /ml)	Reducing power Absorbance
<i>Hemedesmus Indicus</i> root extract	100	0.662 ± 0.01
Gallic Acid (Standard)	2.5	0.724 ± 0.012

Values are Mean + SEM n = 3

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