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Preliminary Phytochemical Analysis and Antimicrobial Screening of *Hygrophilla spinosa*

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Abstract: In the present study the methanolic and aqueous extracts of *Hygrophilla spinosa* were subjected to preliminary phytochemical analysis and antimicrobial activities against certain pathogenic microorganisms. The phytochemical analysis revealed the presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Sterols, Fats & Oils, Phenols, Tannins, Flavanoids and Proteins. The antimicrobial activity was more in methanolic extract than the aqueous extract.
Key words: Antimicrobial activity, phytochemical analysis, *Hygrophilla spinosa.*

INTRODUCTION

Hygrophilla spinosa is an annual herb (60 cm), thorny and red-stemmed, is widely found in the tropical regions of India. *Hygrophilla spinosa* is popularly called Gokhulakantha (Hindi). It has bright blue colour flowers and small ,flat dark seeds (1).The leaves are whorled, 6 at node, each possesses sharp yellow coloured spines on its axil (2).

The roots, leaves and seeds of *H.spinosa* have been used in Indian system of medicine as diuretic and are employed to cure jaundice, dropsy, rheumatism and diseases of urinogenital tract .The leaves of *H.spinosa* are useful in diarrhoea and dysentery, inflammation, abdominal troubles, anaemia, anuria (3).

In recent times the traditional indigenous medicines are considered as an alternative against antibiotics. The medicinal values of *H.spinosa* is known from time immemorial (4).The objective of the present study is to investigate the effectiveness of the leaf extracts of *H.spinosa* (aqueous and methanolic extracts) against few selected pathogens using appropriate standard methods.

MATERIAL AND METHODS

Plant material and Extracts Preparation

The fresh leaves of *H.spinosa* were collected from near by areas of Thanjavur, Tamilnadu, were brought to the laboratory and shade dried to crisp. They were then subjected to pulverization to get coarse powder. The coarse powder was subjected to soxhlet extraction separately and successively with methanol and water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50ºC).Both extracts were put in separate air tight containers and stored in a refrigerator.

Qualitative phytochemical analysis

Qualitative phytochemical analysis were done by using the procedures of Ref.(5). Alkaloids, Carbohydrates, Tannins and Phenols, Flavanoids, Gums and Mucilages, Phytosterol, Proteins and Aminoacids,Fixed oils, Fats, Volatile oil and saponins were qualitatively analyzed.

Microbial cultures

The microorganisms used in the present study were procured from National Chemical Laboratory (NCC), Pune.

Screening for Antimicrobial activity

The disc diffusion method was followed for antimicrobial susceptibility tests. The plates were prepared by pour plate technique using Muller Hinton agar for bacterial strains and potato dextrose agar for fungal strains with the proper concentration of the inoculums. The filter paper discs impregnated with aqueous and methanolic extracts of *H.spinosa* were placed at suitable distance on the plate .The plates were incubated at 37ºC for 24 h and were examined after 24 h.The zone of inhibition was measured in mm and recorded.

Table 1. Preliminary phytochemical analysis of aqueous and methnolic extracts of *H.spinosa*

S.No	Test	Result
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Saponins	+
5.	Sterols	+
6.	Fats & oil	+
7.	Resins	+
8.	Phenols	+
9.	Tannins	+
10.	Flavanoids	+
11.	Proteins	+
12.	Diterpenes	-

+ = presence and - = absence of phytohemicals

Table 2. The antimicrobial activity of aqueous and methnolic extracts of *H.spinosa*

S.N.	Name of the organism	Zone of inhibition (mm)				
		Aqueous extract	Methanolic extract	Cephalosporin (50mg)	Amikacin (50mg)	Fluconazole (20mg)
Bacteria						-
1.	*Staphylo coccus aureus*	11	12	11	13	-
2.	*Escherichia coli*	13	16	18	17	-
3.	*Rhodo coccus*	13	18.5	17	13	-
4.	*Bacillus subtilis*	12	11	20	16	-
Fungi						
1.	*Aspergillus niger*	11	14	-	-	16
2.	*Trichoderma reesei*	12	11	-	-	14
3.	*Saccharomyces cerevisiae*	16	17	-	-	19

RESULTS AND DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources .These plant based traditional medicinal system continues to play an essential role in health care, with about 80 % of the world's inhabitants relying mainly on traditional medicines for their primary health care (6).

The bioactive components including thiocyanate, nitrate, chloride and sulphate, beside other

water soluble components which are naturally occurring in most plant materials, are known to be bactericidal and fungicidal in nature thus conferring the anti microbial property to plants (7).

The present work has been aimed to evaluate the antimicrobial efficacy of methanolic and aqueous extracts of the plant *H.spinosa*. The methanol extract showed higher antimicrobial activity than aqueous extract and it was evident from the zone of inhibition. The antibacterial activity of the methanolic extract *H.spinosa* is maximum for *Rhodococcus* (18.5 mm zone of inhibition).Among the fungal species, the plant extract showed maximium antifungal activity towards *S.cerevisiae*.

Various workers have already specified that gram positive bacteria are more susceptible towards plant extracts as compared to gram negative bacteria (8). Phytochemicals can be used as a natural blueprint for the development of a drug (9). Phytochemical constituents like alkaloids, flavanoids, tannins etc., are found to be effective antimicrobial substances against a wide range of micro organisms (10).

The preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, glycosides, saponins, sterols, fats and oils, resins, phenols,flavanoids and proteins (Table 1).Regarding the antimicrobial activity , it was more against bacterial strains and fungal strains. The maximum activity was observed against *Rhodococcus* (18.5 mm).Among the two extracts, methanolic extract was more potent than the aqueous extract. Further studies are required to establish the exact nature and mechanism of the active principles present in the extract.

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