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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL STANDARDIZATION OF *RHINACANTHUS NASUTUS* (WHITE CRANE)

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Abstract
Plants are the most exclusive source of drugs for the majority of the words population and plant products constitute about 25% at prescribed medicines phytochemical tests have been performed in about 5,000 and nearly 1,100 species are exclusively exploted in 80% Ayurvedic, 46% Unani and 33% Allopathi medicine. The values of the present data indicated that ethanolic extract (5.8%) and aqueous extract (6.8%) showed higher extractive values when compared to other solvents. Ethyl acetate showed the least extraction value (0.9%). At the maximum concentration tested, (250µg) the organisms showed maximum sensitivity and the leaf extract proved to be better than the antibiotic disc Chloramphenicol (30mcg) which recorded 24mm as a zone of inhibition. The promising alkaloid, Rhinacanthin has potent medicinal applications. It exhibits anti proliferate activity, antimicrobial activity against dermatophytes and inhibits platdent aggregation. In spite of it's potential. It remains unexplored and underutilized and hence chosen for the present study.

Key Words: BSA; Antibacterial quinines; *Rhinacanthus*.

Introduction

According to WHO, traditional medicine (including herbal drugs) comprising therapeutic practices that have been in existence, and 75-80 per cent of the world's populations use herbal drugs particularly in the developing countries. Plants are the most exclusive source of drugs for the majority of the world's population and plant products constitute about 25% of prescribed medicines (Farnworth and Bingel, 1977). A large number of Indian plants has been screened for biological activity by central drug Research Institute. Cancer is an insidious disease affecting mankind in every country. Screening of plant extracts for anticancer activity started in 1961 by National cancer Institute of the U.S.A. and upto 1981 (20 years). Various compounds such as pharmaceuticals, antimicrobials, secondary metabolites such as coumarins, flavanoids, anthocyanin tannis, and tannis precursosrs, anthraquinones, napthoqinones, sterols, and triterpenes, cartonoids and nicortine have produced using tissue culture technique (Deeni et al., 1991). The promising alkaloids, Rhinacanthin has potent medicinal application. It exhibits anti proliferate activity, antimicrobial activity against dermatophytes and inhibits platelent aggregation. Anti microbial activity of some plants, their moieties and their ethno botanical study of some tribals have been reported (Acharya et al., 2004).

Materials and Methods

The drug powder as well as the extracts were subjected to phytochemical Screening, invitro cytotoxicity studies, and antibacterial activity.

Preliminary phytochemical screening

Preliminary phyto chemical screening of the drug powder as well as extracts were carried out as per the methods and tests given by Dey and Raman (1957).

1. Detection of saponins

Foam Test: The susbstance was diluted with 20 ml of distilled water and then agitated in a graduated cylinder for 10 minutes. A one centimeter layer of foam indicates the presence of saponins.

2. Detection of proteins

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a) Ninhydrin Test: The substance was treated with Ninnydrin reagent. "The purple colour was formed with the extract which shows the presence of proteins.

b) Biuret Test: To the substance, equal volume of 5% sodium hydroxide and 1% copper sulphate isolation was added. A violet colour formation indicated the presence of amino acids.

3. Detection of tannins

a) Lead acetate Test

b) Gelatin Test

4. Detection of steroids

a) Libermann – Burchardt Test

5. Detection of terpenoids

a) Salkowski Test

6. Detection of carbohydrates

a) Molisch's Test

b) Fehling;s Test

c) Benedict's Test

7. Test for flavanoids

a) Shinado's Test

At the maximum concentration tested, (250 µg) the organisms showed maximum sensitivity and the leaf extract proved to be better than the antibiotic disc Chloramphenicol (30mcg) which recorded 24mm as a zone of inhibition)

The cultivation prospects of an indigeneous plant, Rhinacanthus nasutus (Linn) Kurz which is commonly used for extracting a dye and various medicinal purposes was dealt. Maximum rooting percentage (75%) was observed in apical spoot cuttings treated with 2000 ppm of IBA followed by 2000 ppm of IAA (Nilanjana Das, 2006).

Results and Discussion

The Phytochemical screening of the drug powder as well as the extracts were carried out to test the presence of saponin, protein, tannin, steroids, terpens, sugars, flavanoid, coumarin, quinine, lignin, alkaloid, phenois, glycosides and antyraquinones. The values of the present data indicated that ethanolic extract (5.8%) and aqueous exract (6.8%) showed higher extractive values when compared to other solvents. Mallavadhani et al., (2002) reported that alcoholic extract was the best one for extracting the active principle than others.

The present findings suggest that the secondary metabolites of *Rhinacanthus nasutus* can be used as antibacterial agent in new drugs for therapy against these pathogens (Table 1, 2)

Table 1. Physico chemical constants

Sl. No.	Parameters	Values
1.	Total ash content	7.84%
2.	Acid insoluble Ash	1 72%
3.	Water soluble Ash	2.74%
4.	Foreign matter	1 .5%
5.	Moisture content	5.1%

Table 2. Successive extractive values

Sl. No.	Parameters	Values %w/w
1.	Hexane	5.1%
2.	Chloroform	2.3%
3.	Benzene	1 .3%
4.	ethyl Acetate	0.9%
Solubility		
1.	Ethanol	5.8%
2.	Water	6.8%

Table 3. Estimation of primary metabolites

Sl. No.	Estimation of	mg/g
1.	Sugars	35
2.	Protein	16.150

Table 4. Anticancer activity of *Rhinacanthus nasutus* water extract

Sl. No.	Concentration	Total number of cells	Viable cells	Dead cells	Percentage
1.	250 µg/ml	71	70	1	1.42%
2.	500 µg/ml	77	74	3	3.8%
3.	1000 µg/ml	71	65	6	8.45%
4.	2000 µg/ml	47	41	6	12.7%

Table 5. Alcohol extract

Sl. No.	Concentration	Total number of cells	Viable cells	Dead cells	Percentage
1.	250 µg/ml	62	22	40	64.5%
2.	500 µg/ml	76	13	63	83%
3.	1000 µg/ml	63	4	59	93%
4.	2000 µg/ml	103	2	101	98%

Ethyl acetate showed the least extraction value (0.9%). The ethanolic extract of *Rhinacanthus nasutus* leaves was used for invitro screening of antibacterial activity against some human pathogens. All the pathogenic organisms showed sensitivity by forming a good zone of inhibition except E. Coli which showed high resistance. Following Chemical standards were determined for the plant, *Rhinacanthus nasutus*, Purity, and strength. The present findings suggest that the secondary metabolites of *Rhincacanthus nasutus* can be used as antibacterial agent in new drugs for thereapy against these pathogens. The total Carbohydrate content and protein present in *Rhinacanthus nasutus* was calculated as 35mg/G and 1615mg/G. (Table 3,4 & 5)

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