Research Article

EVALUATION OF PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *RAUVOLFIA TETRAPHYLLA*

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ABSTRACT

The present study aimed at evaluating the *in vitro* antimicrobial activity of aqueous and methanolic extracts of the medicinal plant *Rauvolfia tetraphylla* against six bacteria; three Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Leuconostoc lactis*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and against four fungi (*Aspergillus niger, Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus*). For antimicrobial test, well diffusion technique was used and the zone of inhibition of microorganisms was measured in mm. The methanolic extract of *Rauvolfia tetraphylla* presented the highest anti *Streptococcus pyogenes* activity and was effective against all bacterial strains tested except *Staphylococcus aureus* and *Escherichia coli*. Extracts of aqueous and methanol ether were ineffective in inhibiting the fungal growth or showed poor inhibition. The phytochemical analysis of both the extracts revealed the presence of steroid compound. The fluorescence analysis under visible light by treatment with different chemical reagents showed different colour changes. The presence of alkaloids, flavonoids, tannins, steroids and saponins was confirmed during preliminary phytochemical screening.

Keywords: *Rauvolfia tetraphylla*, Gram-negative, Gram-positive, well diffusion.

INTRODUCTION

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian System of Medicine in the oral and Codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7800 manufacturing units in India. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[1]. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections[2].

The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day-medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics[3, 4]. Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents[5, 6]. However, in this work is designed to evaluate the phytochemical components, antibacterial and antifungal activity of leaf extract of *Rauvolfia tetraphylla* on selected bacterial and fungal strains.

MATERIALS AND METHODS

Collection of Plant materials

Fresh Plant leaf of *Rauvolfia tetraphylla* was collected from Chengam, Thiruvannamalai District, Tamil Nadu, India; they were identified with the help of Gamble's flora.

Preparation of powder

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis[7]. The positive tests were noted as Minimum amount (+), Moderate amount (++), Rich amount (+++), High rich amount (++++) and absent (-).

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia[8]. The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activity.

Qualitative phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, proteins, amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology, Sector 39-4, Chandigarh, India.

The pathogenic fungal strains *Aspergillus niger, Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial Studies

Bacterial Media (Muller Hindon Media)

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal studies

Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer.

Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method[10]. The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

RESULTS AND DISCUSSION

The investigation on organoleptic study of leaf powder of *R. tetraphylla* indicated the characters like colour, stability and smell. The colour of the leaf powder showed green colour. The stability and smell of the leaf powder were also tested. The stability of the leaf is pasty and on analysis the leaf powder gives a characteristic odour (Table 1).

Table 1: Organoleptic study of *Rauvolfia tetraphylla* leaf powder

S. No	Parameters	Characteristic feature
1	Colour	Green
2	Stability	Pasty
3	Smell	Odor

The results of preliminary phytochemical analysis of both the extracts revealed the presence of various phytoconstituents like carbohydrates, alkaloids, steroids, tannins, phenols, saponins, fixed oils & fats, gums & mucilages and flavonoids and absence of proteins, and volatile oil (Table 2).

Table 2: Analysis of fluorescence characters of leaf powder of *Rauvolfia tetraphylla* in different chemical reagents

S. No	Chemical reagent	Appearance
1	Powder colour	Green
2	5% NaOH	Light green
3	10% NaOH	Dark green
4	Con. H_2SO_4	Brown
5	Acetic Acid	Light green
6	1N NaOH in H_2O	Light brown
7	5% KOH	Green
8	50% HNO_3	Dark brown
9	5% $FeCl_2$	Green
10	1N HCl	Green
11	Con.HNO_3	Light brown
12	1N NaOH in Ethanol	Dark green
13	50% H_2SO_4	Green
14	50% HCl	Green
15	Con. HCl	Green

The leaf powder was treated with various chemicals exhibited various colours in the visible light. When the powder was treated with aqueous 1 N NaOH and 50% H2SO4 the leaf powder exhibited varied Light brown and green colours in visible light and the results are depicted in Table 3.

Out of six bacterial cultures tested, the highest antibacterial activity was recorded on the Gram-positive culture - *Streptococcus pyogenes* by the methanol extract of *R. tetraphylla*, wherein the zone of inhabitation was 33 mm. The antibacterial effect of aqueous extract was comparatively less, but high antibacterial activity was recorded by the above bacteria with a highest zone of inhabitation value of 29 mm (Table 4).

Table 3: Results of phytochemical screening of aqueous leaf extracts of *Rauvolfia tetraphylla*

S.No.	Name of the compounds	Name of the test	Status of the substances	
			Aqueous extract	Methanol extract
1	Carbohydrates	Fehling's Benedict's	+ +	+ +
2	Alkaloids	Mayer's Hager's Wagner's Dragen Dorfff's	++ ++ ++ +++	+++ ++ + ++++
3	Steroids	Chloroform + Acetic acid + H_2SO_4	++	+
4	Tannins & Phenols	10% Lead acetate 5% Ferric chloride 1% gelatin	+++ ++ +++	+++ + +++
5	Saponins	Foam test	+	+
6	Fixed oils & Fats	Spot test	++	++
7	Gums & Mucilage	Alcoholic precipitation	++++	+
8	Proteins	Biuret test	-	-
9	Flavonoids	NaOH / HCl	+	++
10	Volatile oils	Hydro distillation method	-	-

++++ - High rich amount; +++ - Rich amount; ++ - Moderate amount; + - Minimum amount; - - Absent

Table 4: Inhibition zone of Aqueous and Methanol extracts of *Rauvolfia tetraphylla* against bacterial pathogens

S. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	*Escherichia coli*	-	-	-	-	-	-
3	*Leuconostoc lactis*	16±2.4	18±3.7	21±7.5	13±2.4	23±4.2	28±3.7
4	*Salmonella typhi*	-	-	-	19±4.9	23±4.2	28±1.4
5	*Pseudomonas aeruginosa*	18±3.7	20±2.8	23±2.8	-	-	-
6	*Streptococcus pyogenes*	15±3.7	18±2.8	29±3.7	18±1.4	21±2.8	33±3.7

Table 5: Inhibition zone of Aqueous and Methanol extracts of *Rauvolfia tetraphylla* against fungal pathogens

S. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	*Aspergillus flavus*	-	-	-	-	14±3.7	23±3.7
2	*Mucor indicus*	-	-	-	-	-	-
3	*Aspergillus niger*	-	-	-	-	12±2.8	21±1.4
4	*Rhizopus indicus*	-	-	-	09±2.4	18±3.7	23±2.8

The results of antifungal activity are given in Table 5. The tested fungal strains were most susceptible to methanol followed by aqueous extract. Methanol extract were most effective against *Aspergillus flavus* and *Rhizopus indicus*, weakly active against *Aspergillus niger* and inactive against *Mucor indicus*. The aqueous extract expressed nil activity against the tested fungus. In the recent years, there were many reports on the antimicrobial activity of plant extracts against human pathogenic bacteria[11-13].

Suresh *et al*. (2008) reported the best antimicrobial activity of ethanol extract obtained from *Rauvolfia tetraphylla*, which showed maximum activity against *E. coli* and *Enterobacter aerogenes*, and various tested fungi such as *A. niger* and *Penicillium* spp, were found to be more sensitive to crude extract when compared to others.[14]

CONCLUSION

Root bark of *R. obscura* acts as antidiarrhoeic agent by triple pronounced antibacterial, antiamoebic and antispasmodic action[15]. From the present study it is evident that *R. tetraphylla* have potential antimicrobial activity. The antibacterial activity of plant extracts was not likely to be due to any one main active constituent but to the combined action of additional other compounds[16]. Similar result was obtained from the antimicrobial activities *of Rauvolfia tertraphylla* and *Physalis minima* leaf and callus extracts[17]. Several phytoconstituents like flavanoids[18], phenolics and polyphenols[19], tannins[20], terpenoids[21], sesquiterpenes[22] etc., are effective antimicrobial substances against a wide range of microorganisms.

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