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Full Length Research Paper

Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts

Nayeemulla Shariff[1], M. S. Sudarshana[1], S. Umesha[2]* and P. Hariprasad[2]

[1]Department of Botany, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India. [2]Department of Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India.

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The *in vitro* antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts were studied against selected pathogenic fungi and bacteria, following broth dilution assay. Leaves and calli were extracted using absolute alcohol, benzene, chloroform, methanol and petroleum ether. Among the five solvents used, leaf and callus extracted in chloroform of both the plants were found to be more effective against pathogenic bacteria and fungi, where the minimum inhibitory concentration (MIC) ranged between 0.25 to 6 mg/ml. Absolute alcohol extracts showed MIC of 0.25 to 4 mg/ml for bacteria, whereas for fungi it ranged from 0.25 to 100 mg/ml. Extracts of benzene and petroleum ether were ineffective in inhibiting the bacterial and fungal growth or showed poor inhibition. Methanol extract showed MIC of 0.25 to 100 mg/ml against bacterial pathogens and 0.5 to 100 mg/ml against fungal pathogens. The antimicrobial activities of these two indigenous medicinal plants were discussed in the present paper.

Key words: *Rauvolfia tetraphylla*, *Physalis minima*, leaf, callus, extract, antimicrobial assay.

INTRODUCTION

Use of plants as a source of medicine has been inherited and is an important component of the health care system. There are about 45,000 plant species in India with concentrated hotspot in the region of Eastern Himalayas, Western Ghats and Andaman and Nicobar Islands. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world (Ahmedulla and Nayar, 1999). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini et al., 2004). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic bacteria and fungi.

A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. The so-called secondary metabolites (Evans et al., 1986), which are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Purohit and Mathur, 1999). The accumulation of phytochemicals in the plant cell cultures had been studied for more than thirty years and the generated knowledge had helped in realization of using cell cultures for production of desired phytochemicals (Castello et al., 2002).

The antimicrobial activity of Solanaceae and Apocynaceae members were well documented in the literature. These include *Cestrum diurnum* (Bhatta-charjee et al., 2005), *Capsicum annum* (Cichewicz and Thorpe, 1996), *Withania* spp. (Ramzi et al., 2005), *Picralima nitida* (Nkere and Lroegbu, 2005), *Nerium oleander* (Hussain and Gorsi, 2004), *Alstonia macrophylla*, *Alstonia cholaris*, *Voacanga foetida*, *Wrightia* spp. (Hadi and Bremner, 2001), *Rauvolfia*

*Corresponding authors E-mail: su@appbot.uni -mysore.ac.in, pmumesh@yahoo.com. Tel: + 91 821 2419884 (O); 2413774 (R).

serptentina (Siddique et al., 2004). The leaf extract of the herbaceous plant, *Rauvolfia tetraphylla* (Apocynaceae), is used for treatment of cholera, eye disease and fever. It is also used as antihypertensive, as well as in intestinal disorders, diarrhea and dysentery (Anon, 1969).

Physalis minima belong to the family Solanaceae, a small herbaceous annual plant grown as weed in crop fields. It is used as tonic, diuretic, laxative, applied in inflammations, enlargement of the spleen, ascites, and as a helpful remedy in ulceration of the bladder. The leaves are crushed and applied over snakebite site (Karthikeyani and Janardhanan, 2003). Fruits of this plant are used to cure spleen disorders (Anon, 1969).

The principle aim of the present work was to study the antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts in different solvents like, absolute alcohol, benzene, chloroform, methanol and petroleum ether against both human and plant pathogenic bacteria including *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas solanacearum*, *Xanthomonas axonopodis* pv. *malvacearum, Xanthomonas vesicatoria* and fungi like *Aspergillus ochreaceous*, *Aspergillus flavipes*, *Fusarium verticilloides* and *Penicillium* sp.

EXPERIMENTAL

Plant material

The fresh matured leaves of the *R. tetraphylla* and *P. minima* were collected randomly during the month of January-February, from the outskirts of Mysore, Karnataka, India. The plant species were identified by referring the standard morphological characteristic features (keys) according to the Flora of Madras Presidency.

Callus culture

Leaves and stem segments from axenic plants were cut aseptically and placed on Murashige and Skoog's medium (Murashige and Skoog, 1962) containing 30 g/l sucrose and supplemented with 3.0 mg/l BAP (Benzyl Amino purine) + 1.0 mg/ml NAA (-Naphthalene Acetic Acid) in case of *R. tetraphylla*. Whereas for *P. minima* 1.0 mg/ml BAP + 0.5 mg/ml NAA was used as standard medium and cultured at 22 ± 2°C with 16 h light and 8 h darkness. After 3 weeks, calli were subcultured on MS Basal medium and harvested after 4 weeks.

Preparation of extracts

Fresh leaf material were washed thoroughly under running tap water, shade dried and used for extraction. 4 week-old-calli, derived from the leaf were collected and dried in an oven at 50±1°C for 60-72 h. Both dried leaf and calli were homogenized to a fine powder and stored in airtight bottles.

25 g of leaf/calli powder were extracted with 150 ml of solvent (absolute alcohol, benzene, chloroform, methanol and petroleum ether) for 24 h by using Soxhlet apparatus. The extract was dried in a flash evaporator for 30 min and the left over powder was considered 100%. Different concentrations; 0.25, 0.5, 1, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 80 and 100 mg/ml were prepared by redissolving the extracted powder in the same solvent which was used in the extraction

Test microorganisms

Selected pathogenic bacteria; *Bacillus subtilis, Escherichia coli, Pseudomonas solanacearum, Xanthomonas axonopodis* pv. *malvacearum, Xanthomonas vesicatoria* and fungi like *Aspergillus ochreaceous, Aspergillus flavipes, Fusarium verticilloides* and *Penicillium* sp. were obtained from culture collection of the Department of Applied Botany and Biotechnology, University of Mysore, Mysore, India.

All the test bacterial species were maintained on nutrient agar media. 36 h old bacterial culture were inoculated into nutrient broth and incubated at 35±2°C on a rotary shaker (Janked and Kunkel, IKA Labortechnik, Germany) at 100 rpm. After 36 h incubation, the bacterial suspension was centrifuged at 10,000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1 x 10^8 cfu/ml using UV-visible spectrophotometer (Hitachi U-2000, Japan) by reading the OD of the solution to 0.45 (A_{610} nm) and used for further studies.

Fungal colonies were harvested from 9 - 10 day old cultures, which were maintained on Potato dextrose agar. The spores were suspended in sterile distilled water and the spore suspensions were adjusted to 1 x 10^8 spores/ml.

Antimicrobial assay

Antimicrobial assay was performed in 96 well, sterile, flat bottom microtiter plates, based on broth microdilution assay, which is an automated colorimetric method, uses the absorbance (optical density) of cultures in a microtiter plate (Suffredini et al., 2004; Ali and Reddy, 2000). Each well of microtiterplates was filled with 200 µl of nutrient broth/potato dextrose broth, 1 µl of test organism and 15 µl different concentrations leaf/callus extracts. For bacteria and fungi the microtiterplates were incubated at 35±2°C for 24 h. After the incubation period the plates were read at 465 nm using ELISA reader (ELX 800 MS, Biotek Instruments, Inc. USA). Minimum inhibitory concentration (MIC), which was determined as the lowest concentration of plant extracts inhibiting the growth of the organism, was determined based on the readings.

RESULTS AND CONCLUSION

The ethnobotanical screening tests of leaf and calli extracts of *R. tetraphylla* and *P. minima* in different solvents against both human and plant pathogenic bacteria and fungi using microdilution technique are depicted in Tables 1 and 2. The extracts are found to be more effective against bacteria rather than fungi. Both benzene and petroleum ether extracts were found to be ineffective or showed poor inhibition of bacterial and fungal growth.

The leaf extract of *R. tetraphylla* in Absolute alcohol showed MIC of 2.0 mg/ml against all tested bacteria except *X. vesicatoria* (4.0 mg/ml). Chloroform leaf extract showed MIC of 4.0 mg/ml against *B. subtilis* , *E. coli* and *X. vesicatoria*, whereas MIC of 1.0 and 2.0 mg/ml were found against *P. solanacearum* and *X. axonopodis* pv. *malvacearum*, respectively (Table 1).

Table 1. Minimum inhibitory concentration (MIC) of *R. tetraphylla* and *P. minima* for antibacterial activity.

Source	Solvents	MIC (mg/ml)				
		B. subtilis	***E. coli***	***P. solanacearum***	***X. axonopodis***	***X. vesicatoria***
R. tetraphylla						
Leaf extract	Absolute alcohol	2.00	2.00	2.00	2.00	4.00
	Benzene	-	-	*	-	-
	Chloroform	4.00	4.00	1.00	2.00	4.00
	Methanol	-	-	*	-	-
	Petroleum ether	-	-	-	-	*
Callus extract	Absolute alcohol	2.00	1.00	1.00	1.00	2.00
	Benzene	*	20.00	80.00	-	-
	Chloroform	2.00	4.00	6.00	4.00	4.00
	Methanol	1.00	2.00	1.00	1.00	2.00
	Petroleum ether	-	-	*	*	*
P. minima						
Leaf extract	Absolute alcohol	2.00	4.00	2.00	2.00	4.00
	Benzene	-	-	*	-	-
	Chloroform	4.00	4.00	2.00	4.00	4.00
	Methanol	10.00	30.00	2.00	4.00	4.00
	Petroleum ether	-	-	*	-	*
Callus extract	Absolute alcohol	4.00	2.00	4.00	4.00	4.00
	Benzene	-	-	-	-	-
	Chloroform	*	-	4.00	4.00	-
	Methanol	*	10.00	2.00	*	10.00
	Petroleum ether	*	*	*	*	6.00
Chloramphenicol		4.00	10.00	6.00	4.00	4.00

Values are the average of at least three determinations. -: Not active; *: shows poor inhibition of bacterial growth.

The MIC of 1.0 mg/ml was found against *E. coli*, *P. solanacearum* and *X. axonopodis* pv. *malvacearum* when absolute alcohol extract of *R. tetraphylla* callus was used. The same concentration was found to be effective against *B. subtilis*, *P. solanacearum* and *X. axonopodis* pv. *malvacearum* when methanol extract of *R. tetraphylla* callus was used (Table 1).

The MIC of 2.0 and 4.0 mg/ml was found against all the tested bacteria when absolute alcohol and chloroform extracts of *P. minima* leaf were used. Whereas absolute alcohol extract of *P. minima* callus showed MIC of 4.0 mg/ml against all the test bacteria except for *E. coli* (2.0 mg/ml) (Table 1).

R. tetraphylla leaf extract showed MIC of 2.0 and 4.0 mg/ml against all the tested fungi when extracted in absolute alcohol and chloroform, where the MIC ranged between 1.0 to 6.0 mg/ml when the *R. tetraphylla* callus extracted with absolute alcohol, chloroform and methanol were used (Table 2). *P. minima* leaf extracted with chloroform also showed MIC of 2.0 and 4.0 mg/ml against all the tested fungi. The callus extract of *P. minima* with Chloroform showed MIC of 4 mg/ml against *A. ochreaceous*, *F. verticilloides* and *Penicillium* sp. (Table 2).

Petroleum ether and benzene extracts of *R. tetraphylla* and *P. minima* (leaf and callus) were weakly active to inactive against all the tested bacteria and fungi (Tables 1 and 2).

The extracts of higher plants can be very good source of antibiotics (Fridous et al., 1990) against various fungal and bacterial pathogens. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Higher plants have also made important contributions in the areas such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblastine and vincristine, which were both obtained from the Madagascan periwinkle *(Catharanthus roseus* syn. *Vinca rosea*).

Aqueous extracts of fruits and leaves of *Capsicum fruitescens*, *Capsicum annum* (Solanaceae) and *Nerium oleandar* (Apocyanceae) were found to inhibit the germination of *Alternaria solani* spores and decrease the mycelial dry weight of *Alternaria solani* and *Saprolegnia*

Table 2. The Minimum inhibitory concentration (MIC) of *R. tetraphylla* and *P. minima* for antifungal activity.

Source	Solvents	MIC (mg/ml)			
		A. ochreaceous	***A. flavipes***	***F. verticilloides***	***Penicillium* sp.**
R. tetraphylla					
	Absolute alcohol	2.00	4.00	2.00	4.00
	Benzene	-	-	-	10.00
Leaf extract	Chloroform	4.00	4.00	2.00	2.00
	Methanol	*	*	*	2.00
	Petroleum ether	-	-	-	-
	Absolute alcohol	1.00	4.00	4.00	2.00
	Benzene	-	*	-	*
Callus extract	Chloroform	2.00	2.00	4.00	2.00
	Methanol	2.00	4.00	4.00	6.00
	Petroleum ether	-	-	-	-
P. minima					
	Absolute alcohol	4.00	30.00	-	4.00
	Benzene	-	-	-	*
Leaf extract	Chloroform	2.00	2.00	4.00	4.00
	Methanol	6.00	10.00	2.00	20.00
	Petroleum ether	-	-	*	-
	Absolute alcohol	-	10.00	20.00	4.00
	Benzene	-	*	-	*
Callus extract	Chloroform	4.00	4.00	4.00	6.00
	Methanol	4.00	-	-	*
	Petroleum ether	-	-	-	*
Fluconazole		4.00	6.00	4.00	2.00

Values are the average of at least three determinations.
-: Not active; *: shows poor inhibition of fungal growth.

parasitica (Khallil and Pak, 2001). Singh and Sudarshana (2002) tested the aqueous and ethenolic extract of *Baliospermum axillare* leaf and callus against bacteria like *X. campestris*, *P. syringae* and the fungi *F. solani* and *F. oxysporum*. The ethanolic, methanolic and chloroformic extracts of *Nerium oleander* (Apocynaceae) leaf and root showed considerable antimicrobial activity against *Bacillus pumillus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Hussain and Gorsi, 2004). Aqueous extracts of Apocynaceae and Solanaceae members from native of Amazon rain forest and Atlantic forest were also tested for their antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis* following broth microdilution method, and they showed some degree of inhibition of bacterial growth at concentrations of 100 g/ml (Suffredini et al., 2004).

In conclusion chloroformic and absolute alcoholic leaf and callus extracts of *R. tetraphylla* and *P. minima* inhibited bacterial and fungal growth. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

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