December 2009 Volume 3 Issue 4

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 3(4), 2009 [193-196]

Antimicrobial and Anthelmintic efficacy of methanol extract of *Putranjiva roxburghii* Wall seed

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Received: 4th July, 2009 ; Accepted: 14h July, 2009

ABSTRACT

Putranjiva roxburghii Wall., commonly called Putranjiva, is a deciduous, evergreen tree and is used in cold, fever, rheumatism and inflammation. The study carried by us describes the antibacterial and anthelmintic potential of methanolic extract of fruits of *P. roxburghii in vitro*. The dried and powdered Seed material of *P. roxburghii* was subjected to soxhlet extraction using methanol solvent. The phytochemical analysis was carried. The antibacterial activity was checked using Agar well diffusion method and Anthelmintic assay was conducted in earthworm model. Preliminary phytochemical investigation reveled the presence of saponin, steroid, alkaloids and flavonoids in the extract. A marked antibacterial activity against Gram positive and Gram negative bacteria, known to cause food poisoning, was observed. Gram positive bacteria were more inhibited than Gram negative bacteria. Among fungi tested, *A. flavus* was found to be more susceptible followed by *A. niger* and *A. nidulans*. The extract was found to cause paralysis and death of worms in a relatively short period of time. The results reveals that methanol extract possess active principles that possess antimicrobial and anthelmintic activity. Purification of active constituents and *in vivo* experiments in animal models are needed to support the ethnomedicinal use of the plant.

KEYWORDS

Putranjiva roxburghii Wall;
Antibacterial activity;
Anthelmintic activity;
Agar well diffusion;
Soxhlet extraction.

INTRODUCTION

The antimicrobial activities of the plants may be attributed to the phytoconstituents present in them such as flavonoids, phenolics and polyphenols, tannins, alkaloids, quinones, triterpenoids, sesquiterpenoids etc. These phytochemicals have shown to possess antimicrobial activities against wide range of microorganisms[1]. *Putranjiva roxburghii* Wall., commonly called Putranjiva, is a deciduous, evergreen tree of about 18m tall having grey bark. Leaves elliptic-oblong to ovate-lanceolate, unequal sided at the base, dark green and shining inappearance. Flowers small; male in dense, rounded clusters, yellowish in colour;female flowers

FULL PAPER

solitary or 2-3 together, green. Drupes ellipsoid or globose and white-tomentose, stone pointed, rugose, very hard and normally having a single seed. It is used in cold, fever and rheumatism[2,3] and seeds in inflammation[4]. The plant is cultivated in Hong Kong Zoological and Botanical Gardens. India, Sri Lanka, Myanmar, Cambodia, Laos, Indonesia, New Guinea[3]. In the present study, we have explored the *in vitro* potential of methanolic extract of fruit material of *P. roxburghii* against bacteria causing food poisoning, opportunistic fungi and worms.

MATERIALS AND METHODS

Collection and extraction of plant material

The Seeds of *P. roxburghii* Wall were obtained from local shops of Udupi city and authenticated to identity by Dept. of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga. Voucher specimen was deposited in the department for future reference. The dried Seeds were powdered mechanically. About 150g of powdered material was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator, dried in the dessicator. The yield was recorded and the extract was kept in refrigerator until use[1]. The methanol extract was subjected to preliminary phytochemical analysis[5]

Antibacterial activity

The pure cultures of Gram positive bacteria namely *Staphylococcus aureus* and *Bacillus cereus* and Gram negative bacteria namely *Escherichia coli* and *Salmonella typhi*, obtained from Dept. of Microbiology, were screened for their sensitivity towards the methanol extracts by Agar well diffusion method[6]. 24 hours old standardized Muller-Hinton broth cultures of test bacteria were swabbed uniformly on sterile Muller-Hinton agar plates. Then wells of 6mm diameter were bored in the inoculated plates and the extract (50mg/ml of DMF), Standard (Chloramphenicol, 10mg/ml) and Control (DMF) were added into the wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition around the well was recorded. The experiment was carried in triplicates to get average reading.

Antifungal activity

The antifungal activity of methanol extracts was tested against three species of the genus *Aspergillus* namely *A. niger, A. nidulans* and *A. flavus*. The test fungi were screened for their sensitivity by Agar well diffusion method[6]. The spore suspension of test fungi were swabbed uniformly on solidified sterile Sabouraud's dextrose agar plates using sterile cotton swab. Then wells of 6mm diameter were bored in the inoculated plates and the extract (50mg/ml of DMF), Standard (Chloramphenicol, 10mg/ml) and DMF (control) were added into respectively labeled wells. The plates were incubated at room temperature for 72 hours in upright position. After incubation, the diameter of zone of inhibition was recorded.

Anthelmintic activity

The anthelmintic assay was performed on adult Indian earthworm *Pheretima pasthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. The standard drug (Piperazine citrate, 1%) and test (5mg/ml) were prepared in normal saline (0.85%) and were poured into respective labeled Petri plates (50 ml in each plate) and 6 worms of equal size (or nearly equal) were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur *when the worms were not able to move even in normal saline*. Death was concluded *when the worms lost motility followed with fading away of their body colors*[7]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased[8].

RESULTS AND DISCUSSION

The presence of various phytoconstituents in methanol extract of *P. roxburghii* is shown in TABLE 1. The phytoconstituents namely alkaloids, flavonoids, saponins and steroids were found to be present in the extract. Triterpenoids and tannins were not detected in the extract. TABLE 2 reveals the antibacterial activity of

TABLE 1 : Phytochemical groups detected in methanol extract

Group	Methanol extract
Tannin	+
Alkaloid	+
Saponins	+
Steroids	+
Terpenoids	ND
Flavonoids	+

'+' – Detected; 'ND' – Not detected

TABLE 2 : Antibacterial activity of methanol extract against food poisoning bacteria

Test bacteria	Zone of inhibition in cm		
	Control	Standard	Methanol extract
S. typhi	1.1	2.5	1.4
E. coli	1.1	2.6	1.4
S. aureus	1.0	2.4	1.5
B. cereus	1.0	2.4	1.6

Control- DMF; Standard- Chloramphenical- 10mg/ml; Extract- 50mg/ml

methanol extract. It is clear from the table that the extract possess antibacterial activity as revealed by the zone of inhibition produced around the well. Gram positive bacteria, *B. cereus* and *S. aureus*, were found to be more inhibited than Gram negative bacteria namely *S. typhi* and *E. coli*. Among bacteria tested, more inhibition was observe in *B. cereus* followed by *S. aureus*, *S. typhi* and *E. coli*. The higher resistance of Gram-negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell[9]. Among fungi tested, *A. flavus* was found to be more susceptible followed by *A. niger* and *A. nidulans* (TABLE 3). The methanol extract caused paralysis and death of worms in a relatively short period of time. Time taken for paralysis was found to be 9 minutes while death occurred in 38 minutes. DMF was also checked for its effect. It was found that the time for paralysis and death was 19 minutes and 57 minutes respectively (TABLE 4).

TABLE 3 : Antifungal activity of methanol and ethanol extracts of selected plants against *Aspergillus* species

Treatment	Zone of inhibition in cm		
	A. niger	*A. nidulans*	*A. flavus*
Methanol extract	1.9	1.6	2.0
Control (DMF)	0.9	1.0	1.0

Results are average of three trials

TABLE 4 : Anthelmintic activity of methanol extract

Treatment	Average time in minutes	
	For Paralysis	For Death
Control	-	-
DMF	19	57
Extract 5mg/ml	9	38

Results are average of three trials

Phytoconstituents present in plants are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms[10]. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins), however, other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils, etc. have also been reported[11]. It was found that the methanol extracts *P. roxburghii*. Wall was found to be one of the more effective against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* was found to be susceptible to 68% of the tested plant extracts, whereas *Pseudomonas aeruginosa* showed resistance to most of the plant extracts[12]. A sensitive HPTLC method for estimation of amentoflavone, a bioactive principle from *Biophytum sensitivum* (Linn.) DC. and *P. roxburghii* Wall. was developed[13]. Ethnopharmacological and phytochemical screening of *P. roxburghii* Wall. was carried[14]. The oil of *P. roxburghii* showed potential as a preservative for peanut seeds against spoilage by fungi and insects during storage. Volatile constituents extracted in the form of essential oils from 32 plant species were evaluated against the dominant fungi, *Aspergillus flavus* and *Aspergillus niger*, as well as *Trogoderma granarium*. The oil of *P. roxburghii* exhibited the greatest toxicity. The oil was found to be fungicidal and thermostable at its minimum inhibitory concentration of 400 ppm. The oil protected the peanut seeds completely for 6 months at 0.25 and 0.38 mL in containers of 250 mL capacity holding 200 g seeds. It did not exhibit any adverse effect on seed germination, seedling growth and general health and morphology of plants[15]. Cytogenetic toxic-

FULL PAPER

ity of leaf extract of *P. roxburghii* was tested by oral administration of leaf extract in young weaning Swiss albino mice. The results showed that the extract significantly induced mitosis-disruptive chromosomal changes in bone marrow cells. It is proposed that the extract might have interfered with the spindle and other proteins causing polyploidy, aneuploidy, c-mitosis, etc[16]. A highly stable and potent trypsin inhibitor of approximately 34 kDa was purified and characterized from *P. roxburghii* seeds by acid precipitation, cation-exchange and anion-exchange chromatography[17].

CONCLUSION

The results of study reveal the potential of plant to inhibit bacteria, fungi and worms *in vitro*. The extract could be used to treat infections caused by bacteria, fungi and helminths. Further experiments in animal models could possibly reveal the potential of plant to inhibit disease causing microorganisms.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Head of the Dept. of Microbiology, Principal, S.R.N.M.N College of Applied Sciences and N.E.S, Shivamogga for providing all facilities and moral support to conduct the work.

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