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Studies on larvicidal, anthelmintic and antimicrobial efficacy of *Putranjiva roxburghii* Wall (*Putranjivaceae*)

S.J.Sudharshan[1,*], A.Chinmaya[1], N.C.Valleesha[1], T.R.Prashith Kekuda[2], Rajeshwara N.Achur[1], M.L.Sujatha[1], Namitha C.Yadav[1], S.V.Praveen Kumar[3]

[1]Dept. of Studies and Research in Biochemistry, School of Chemical Sciences, Jnanasahyadri, Shankaraghatta-577451, Karnataka, (INDIA)

[2]Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, (INDIA)

[3]Dept. of Studies and Research in Microbiology, Shivagangothri, Tholhunse, Davangere-577002, Karnataka, (INDIA)

E-mail : sscr_26@rediffmail.com

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ABSTRACT

Putranjiva roxburghii Wall (Syn. *Drypetes roxburghii* Wall) belonging to the family *Putranjivaceae* (Putranjiva family), commonly called Putranjiva, is a deciduous, evergreen tree and is used in cold, fever, rheumatism and inflammation. The study describes the larvicidal, anthelmintic and antimicrobial potential of methanolic extract of seeds of *D. roxburghii in vitro*. Preliminary phytochemical investigation revealed the presence of tannins, 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 as compared to standard drug. A dose dependent larvicidal activity was observed. Extract at concentrations 2.5 and 5mg/ml caused 100% larval mortality. The inhibitory activity may be due to the active principles present in the extract. Isolation of active constituents and *in vivo* experiments in animal models are needed to support the ethnomedicinal use of the plant.

KEYWORDS

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

INTRODUCTION

From ancient civilization various parts of different plants were used to eliminate pain, control suffering and counteract disease. Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principal natural source of medicines. The plants used as drugs are fairly innocuous and relatively free from toxic effects or were so toxic that lethal effects were well known. The nature has provided the storehouse of remedies to cure all ailments of mankind. There is no doubt that plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis[1-3]. It is estimated that there are 250000 to 500000 species of plants on earth[4]. A relatively small

percentage (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes[5]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents[6]. Mosquitoes are the most important single group of insects acting as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis and others[7]. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides[8]. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature[9]. *Putranjiva roxburghii* Wall (Syn. *Drypetes roxburghii* Wall), commonly called by name Putranjiva, belongs to the family *Putranjivaceae* (Putranjiva family) is a deciduous, evergreen tree of about 18m tall having grey bark and is used in cold, fever, rheumatism and inflammation. Bark is used to treating cold and fever[10]. The present study deals with extraction, phytochemical analysis, antimicrobial, anthelmintic and larvicidal activity of seeds of *D. roxburghii* Wall.

Figure 1 : *Putranjiva roxburghii* Wall

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 (SRNMN/Bot/DR/1452) 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[11]. The methanol extract was subjected to preliminary phytochemical analysis[12]

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[13]. 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 10% DMSO), Standard (Chloramphenicol, 10mg/ml) and Control (DMSO) 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[13]. 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 10% DMSO) and control (10% DMSO) 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.

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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*[14]. 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[15].

Larvicidal activity of methanolic extracts

The second instar Larvae of *Aedes aegypti* mosquito were collected from water stagnated area, and identified in the Dept. of Entomology, UAS, Shivamogga, Karnataka, India. The larvae were maintained under suitable temperature and humidity. Different concentrations of methanolic extract (1, 2.5 and 5mg/ml) were prepared in 10% DMSO and added to sterile labeled beakers containing about 100ml of water. Twenty larvae were placed in each of the beakers containing extracts. A control was kept containing 10% DMSO. After adding the larvae, the beakers were kept in the growth room maintained at room temperature. The larvicidal effect of extracts was determined by counting the number of dead larvae after 24 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. Each test was repeated thrice and the percentage of larval mortality was calculated[16].

RESULTS AND DISCUSSION

An extract yield of 13.5% was obtained. The methanol extract showed the presence of tannins, alkaloids, saponins, steroids and flavonoids while terpenoids were not detected in the extract (TABLE 1). TABLE 2 reveals the antibacterial activity of methanol extract. It was found that the Gram positive bacteria were found to be affected to more extent by the extract than the Gram negative bacteria. There was no difference in the susceptibility of Gram negative bacteria tested. The susceptibility of bacteria is in the order *B. cereus* > *S. aureus* > *S. typhi* and *E. coli*. Inhibition zone was not observed in case of Control (DMSO). Standard antibiotic also exhibited broad activity against Gram positive bacteria than Gram negative bacteria. 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[17]. The result of antifungal activity of methanol extract of D. roxburghii is showen in TABLE 3. Marked inhibition of A. flavus (ZOI 2.0cm) was observed which is followed by A. niger (1.9) and A. nidulans (1.6). No inhibition zone was observed in case of control. The results of anthelmintic activity of methanol extract is shown in TABLE 4. The extract at concentration 5mg/ml was found to cause paralysis (19 minutes) and death (38 minutes) of worms in a relatively shorter period of time than the standard drug 1% piperazine citrate (29 and 44 minutes for paralysis and death respectively). The methanolic extract demonstrated promising activities against the larvae of *Aedes aegypti*. The results depicted in TABLE 5 shows the dose depended activity of extract. In case of extract concentration 2.5 and 5mg/ml, 100% mortality of larvae was observed. At 1mg/ml concentration, 55% mortality was observed. In control, no mortality of larvae was observed. Earlier studies observed that phytochemicals have major role in mosquito control programme[18,19]. It is observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract having mosquito larvicidal activity[16]. It is suggested that the saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death. The deficiency of dissolved oxygen and active presence of the antioxidant saponin molecule might be the reason for larval death. However, much study is required to find out the mechanism by which saponin kills the larvae[20]. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow

fever[21]. The larvicidal property could be mainly due to phytoconstituents such as alkaloids, tannins, saponins present in the extract.

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	-	2.5	1.4
E. coli	-	2.4	1.4
S. aureus	-	2.5	1.5
B. cereus	-	2.6	1.6

Results are average of three trails

TABLE 3 : Antifungal activity of methanol extract against *Aspergillus* species

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

Results are average of three trials

TABLE 4 : Anthelmintic activity of methanol extract

Treatment	Average time in minutes	
	For Paralysis	For Death
Control (Saline)	-	-
DMSO	-	-
Extract 5mg/ml	19	38
Standard (1%)	29	44

Results are average of three trials

TABLE 5 : Larvicidal effect of different concentrations of methanol extract

Treatment	Concentration (in mg/ml)	Number of larvae dead	% larval mortality
P. roxburghii	1	11/20	55.00
	2.5	20/20	100.00
	5	20/20	100.00
Control (DMSO)	10%	-	-

Phytoconstituents present in plants are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms[22]. 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[23]. 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[24]. 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[25]. A sensitive HPTLC method for estimation of amentoflavone, a bioactive principle from *Biophytum sensitivum* (Linn.) DC. and *P. roxburghii* Wall. was developed[26]. Ethnopharmacological and phytochemical screening of *P. roxburghii* Wall. was carried[27]. Cytogenetic toxicity 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[28]. 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[29].

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CONCLUSION

The results of study revealed the potential of plant extract to inhibit bacteria, fungi, worms and larvae *in vitro*. The extract could be used to treat infections caused and transmitted by these agents. Plants offer an alternative source of control agents of these parasites or pathogens because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment. Further experiments have to be carried to isolate compounds and to reveal the potential in vivo in animal models.

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