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Research Article

ANTIMICROBIAL ACTIVITY OF LEAF AND PERICARP EXTRACTS OF *POLYALTHIA LONGIFOLIA* (ANNONACEAE)

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ABSTRACT
The present study was conducted with an aim of determining antimicrobial activity of leaf and pericarp (ripe and unripe) extracts of *Polyalthia longifolia* (Annonaceae). The leaf, ripe pericarp and unripe pericarp were shade dried and extracted using methanol in soxhlet assembly. Antibacterial activity of leaf and pericarp extracts was determined against *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* by Agar well diffusion assay. Antifungal activityof leaf and pericarp extracts was tested against *Sclerotium rolfsii* by Poisoned food technique. The extracts were effective against *S. aureus* to higher extent when compared to Gram negative bacteria. The extracts caused marked inhibition of mycelial growth of *S. rolfsii*. Unripe pericarp extract exhibited marked inhibition of bacteria and fungus when compared to other extracts. Preliminary phytochemical analysis of extracts revealed the presence of flavonoids, tannins, steroids and glycosides in leaf and pericarp extracts. The observed inhibitory potential could be ascribed to the presence of secondary metabolites in the extracts. The leaf and pericarp of *P. longifolia* can be exploited for the development of potent antimicrobial agents.
Keywords: *Polyalthia longifolia*, Agar well diffusion, Poisoned food technique, *Sclerotium rolfsii*

INTRODUCTION

The genus *Polyalthia* (Greek: poly- many and *althea*- to cure) belongs to the family Annonaceae. The species of the genus *Polyalthia* are medicinally importance due to the presence of clerodanediterpenoids and alkaloids in various parts. *Polyalthia longifolia* is a native of Sri Lanka. It is an ornamental lofty evergreen tree grown ingardens throughout the warmer parts of India. In English, it is knownas Mast tree, Fake Asoka tree, False Devadaru and Cemetryand in Ayurveda it is known as Devadaari. Almost all parts of the plant are used in the Indian traditional system of medicine including Ayurveda. In particular, the bark of *P. longifolia* has significant medicinal properties. The plant has febrifuge action and causes cardiac depression. Various phytoconstituents such asclerodanediterpenes, polyalthialdoic acid, kolavenic acid, liriodenine, noroliveroline, oliveroline-beta-N-oxide, azafluorenealkaloids etc., are present in the plant[1-3]. The plant has been used traditionally in various parts of India. As febrifuge, stem bark is used by tribals of Visakhapatnam, Andhra Pradesh[4]. Local communities of Uthiramerur of Tamil Nadu, India consume fresh stem bark juice to treat indigestion[5]. Adeevasee communities of Danta, Gujarat consume dried stem bark with butter for curing gonorrhea[6]. Tribal people of Khargone, Madhya Pradesh use stem bark to cure malignant tumor[7]. In Manchale area of Shimoga district, Karnataka, bark is used to prevent abortion in pregnant women[8]. The leaves are used to treat fever, gonorrhea, uterus ailments, mouth ulcer, heart problems and others in Vellore, Tamil Nadu, India[9]. Stem bark is used for diabetes and hypertension by tribals of Bankura district, West Bengal, India[10]. Various parts of *P. longifolia* were shown to possess antimicrobial activity. Extracts and isolated compounds from the plant have shown promising activity against bacteria and fungi. A lactone from stem extract caused inhibition of Gram positive and Gram negative bacteria[11]. Two diterpenoids, isolated from the hexane extract of the seeds demonstrated significant antibacterial and antifungal activities[12]. Solvent extracts of leaves exhibited inhibitory activity against human pathogenic yeasts *viz*., *Candia albicans* (isolated from HIV patients) and *Cryptococcus neoformans* and molds such as *Trichosporan beigelli* and *Aspergillus candidus*[13]. Aqueous extract of leaves was found to exhibit significant antifungal activity against various species of *Aspergillus*[14]. Clerodanediterpenes isolated from leaves were found to exhibit antimicrobial activity[15]. Methanol extracts of leaves and green berries were found to possess promising antibacterial activity[16]. Aqueous and methanol extract of mature leaves showed inhibition of Gram positive and Gram negative bacteria[2]. The methanol, acetone and 1, 4-dioxan fractions of leaves were shown to exhibit marked inhibitory activity against clinical isolates of Gram positive bacterial and fungal strains[17]. Flavonoids isolated from extract of bark were found to exhibit promising inhibitory activity against bacteria and fungi[18]. The treatment of sorghum seeds by ripe pericarp extract promoted germination of seeds and prevented fungal infection of seeds[19]. Aqueous and petroleum ether extracts of leaves were shown to exhibit inhibition of various mold species isolated from sorghum grains[20]. Alcoholic extract of leaves was shown to possess marked inhibitory effect against *Fusarium solani* isolated from rotted rhizomes of ginger when compared to aqueous extract[21]. Aqueous leaf extract had significant growth inhibitory effect against aflatoxin producing *Aspergillus parasiticus*[22]. An aqueous extract from leaves has shown inhibition of seed borne fungi isolated from green gram[23]. In a previous study of us, the leaf, ripe pericarp and unripe pericarp extracts of *P. longifolia* were shown to exhibit marked inhibitory activity against the mycelial growth of *Colletotrichum capsici* (from anthracnose of chilli) and drug resistant Gram positive and Gram negative bacteria from urinary tract infections[24]. In the present study, we determined antimicrobial activity of leaf, ripe and unripe pericarp extract of *P. longifolia*.

MATERIALS AND METHODS

Collection and extraction of plant material

The leaves, ripe and unripe fruits of *P. longifolia* were collected at college campus. Pericarp was separated from ripe and unripe fruits. The leaves and separated pericarps were washed well using clean water, dried under shade and powdered in a blender. For extraction, a known quantity (25 g) of each of the powdered plant material was added into separate 250 ml conical flasks containing 100 ml of methanol (Hi Media, Mumbai, India) and mixed well. The containers were left for two days with occasional stirring. Later, the contents were filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated in vacuum under reduced pressure[24].

Phytochemical analysis of leaf and pericarp extracts

The leaf and pericarp extracts were screened for the presence of phytochemicals namely alkaloids, flavonoids, tannins, saponins, glycosides and steroids by standard phytochemical tests[25-26].

Antibacterial activity of leaf and pericarp extracts

Agar well diffusion assay was carried out to investigate antibacterial activity of leaf and pericarp extracts against a Gram positive bacterium *Staphylococcus aureus* NCIM-2079 and two Gram negative bacteria *Salmonella typhi* MTCC-734 and *Klebsiella pneumoniae* NCIM-2957. The test bacteria were inoculated into test tubes containing sterile Nutrient broth (Hi Media, Mumbai, India) and incubated at 37^{o}C for 24 hours. The broth cultures were aseptically swabbed on sterile Nutrient agar (Hi Media, Mumbai, India) plates using sterile cotton swabs. Using a sterile cork borer, wells of 6 mm diameter were punched in the inoculated plates. 100 µl of leaf and pericarp extracts (20 mg/ml of 25 % DMSO [Dimethyl sulfoxide; Hi Media, Mumbai, India]), Chloramphenicol (reference antibiotic, 1 mg/ml of sterile water) and DMSO (25 %, in sterile water) were transferred into respectively labeled wells. The plates were incubated at 37^{o}C for 24 hours in upright position. The zones of inhibition formed around the wells were measured using a ruler[27].

Antifungal activity of leaf and pericarp extracts

The inhibitory effect of leaf and pericarp extracts of *P. longifolia* was tested against a phytopathogenic fungus *Sclerotium rolfsii* by Poisoned food technique. Potato dextrose agar medium was prepared, poisoned with leaf and pericarp extracts (1 mg/ml), sterilized by autoclaving, cooled to 45^{o}C and dispensed into sterile petri dishes. The control and poisoned plates were inoculated at the centre with the test fungus aseptically. The plates were incubated at 28^{o}C for 5 days. Later, the colony diameters were measured in mutual perpendicular directions using a ruler. Antifungal efficacy of extracts was recorded in termsof inhibition of mycelial growth (%) of test fungus and was calculated using the formula:

Inhibition of mycelia growth (%) = (C – T / C) x 100,
Where C is average diameter of fungal colony in controlplates and T is the average diameter of fungal colony inpoisoned plates[28]

RESULTS

Phytoconstituents detected in leaf and pericarp extracts

The result of preliminary phytochemical analysis of leaf and pericarp extracts of *P. longifolia* is shown in Table 1. The ripe and unripe pericarp showed the presence of all except alkaloids. Leaf extract was found to contain all phytochemicals except saponins.

Table 1: Phytoconstituents in leaf and pericarp extracts

Phytoconstituent	Leaf	Ripe pericarp	Unripe pericarp
Alkaloids	+	-	-
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	-	+	+
Steroids	+	+	+
Glycosides	+	+	+

'+' Detected; '-' Not detected

Antibacterial activity of leaf and pericarp extracts

Result of inhibitory effect of leaf and pericarp extractsagainst test bacteria is shown in Table 2 and Figure 1. Among testbacteria, susceptibility was recorded higher in case of *S. aureus* followed by *K. pneumoniae* and *S. tyhpi*. Among extracts, high inhibitory activity was observed in case of unripe pericarp followed by ripe pericarp and leaf extracts. Inhibition of test bacteria by reference antibiotic was higher when compared to leaf and pericarp extracts. Reference antibiotic caused higher inhibition of *S. aureus*. There was no inhibition of test bacteria in case of DMSO.

Table 2: Inhibitory effect of leaf and pericarp extracts of *P. longifolia* against bacteria

Treatment	Zone of inhibition (cm)		
	S. aureus	*S. typhi*	*K. pneumoniae*
Leaf	2.1 ± 0.1	1.5 ± 0.0	1.9 ± 0.1
Ripe pericarp	2.4 ± 0.2	1.7 ± 0.1	2.0 ± 0.1
Unripe pericarp	2.5 ± 0.1	2.0 ± 0.1	2.4 ± 0.1
Chloramphenicol	2.9 ± 0.1	2.6 ± 0.2	2.7 ± 0.1
DMSO	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Antifungal activity of leaf and pericarp extracts

The result of antifungal effect of leaf and pericarp extracts of *P. longifolia* against *S. rolfsii* is presented in Table 3 and Figure 1 and 2. Extracts were able to inhibit the growth of test fungus to a varied extent. Unripe pericarp extract was more effective in inhibiting the growth of fungus followed by ripe pericarp and leaf extract. Among extracts, unripe and ripe extracts caused >90 % inhibition of mycelial growth of *S. rolfsii* while leaf extract caused nearly 70 % growth inhibition.

Table 3: Colony diameter of *S. rolfsii*on control and poisoned plates

Treatment	Colony diameter (cm)
Control	2.6 ± 0.2
Leaf	0.8 ± 0.0
Ripe pericarp	0.2 ± 0.0
Unripe pericarp	0.1 ± 0.0

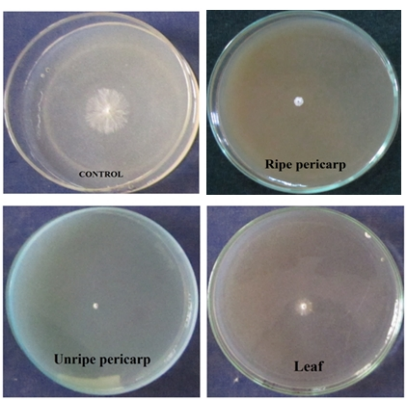

Figure 1: Growth of *S. rolfsii* in control and poisoned plates

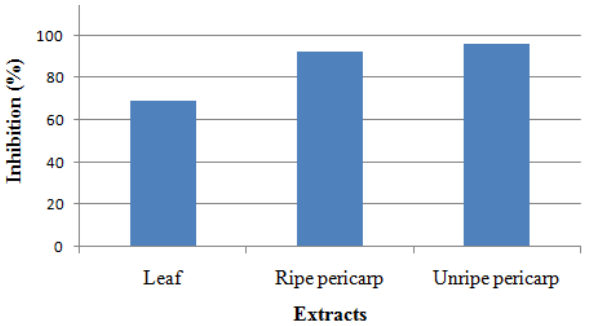

Figure 2: Inhibition of *S. rolfsii* (%) by leaf and pericarp extracts

DISCUSSION

One of the significant events in the field of chemotherapy is the discovery of antibiotics from microorganisms. These antibiotics saved countless lives and have revolutionized the field of medicine. However, the overuse and misuse of these wonder drugs resulted in appearance of microbial strains developing resistance. The wide spread use of antibiotics appears to be the major selective force for development of antibiotic resistance. Bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa, Mycobacterium tuberculosis*, *Enterococcus faecalis* and coliforms are among the most important drug resistant bacteria which have developed resistance against a range of antibiotics. These antibiotic resistant pathogens create significant problems in successful treatment of infections caused by them. This situation is alarming and hence, search for alternatives is of utmost importance. Plants are among the best alternates for the treatment of infections caused by drug resistant microorganisms. Crude extracts and purified components of plants have shown to be effective even against antibiotic resistant bacteria[27,29-33]. In the present study, we evaluated antibacterial activity of leaf and pericarp extracts against two Gram positive and three Gram negative multiple drug resistant bacteria isolated from urinary tract infections. The leaf and pericarp extracts strongly inhibited Gram positive bacteria when compared to Gram negative bacteria. Unripe pericarp caused high inhibition of test bacteria followed by ripe pericarp and leaf extract. Similar results were observed in earlier studies where extracts or compounds from *P. longifolia* caused higher inhibition of Gram positive bacteria. In an earlier study, leaf and pericarps extract exhibited stronger inhibition of Gram positive uropathogenic bacteria *viz*., *S. aureus* and *Enterococcus faecalis* when compared to Gram negative uropathogenic bacteria. Unripe pericarp displayed stronger inhibition of uropathogens followed by ripe and leaf extracts[24]. A clerodanediterpenoid isolated from leaves inhibited *S. aureus* to higher extent than Gram negative bacteria[15]. Methanol extract of mature leaves was found to inhibit Gram positive bacteria to higher extent than Gram negative bacteria as indicated by lower MIC values[2]. In a study of Uzama*et al*.[34], solvent extracts of leaves exhibited stronger inhibition of Gram positive bacteria than Gram negative bacteria as revealed by wider inhibition zones. The low antibacterial activity of leaf and pericarp extracts against the Gram negative bacteria could be ascribed to the presence of an outer membrane that possess hydrophilic polysaccharides chains and forms an additional barrier for extract[35,36]. Plants have been exploited for several purposes since time immemorial. However, plants are very prone for infection by a variety of pathogens such as bacteria, viruses, fungi and parasites. Economic losses due to pre- and post-harvestdiseases range from 5 to 50 % or even higher in developing countries. Among various phytopathogens, fungi are the main infectious agents which cause alterations in plants during developmental stages as well as post-harvest. The fungalinfection results in various quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life. Some fungi are often implicated in causing allergic or toxic disorders. A number of strategies including the use of synthetic fungicides have been employed to prevent or control phytopathogenic fungi. Among these, chemical control using synthetic fungicides is extensively used. Although this method is found effective, but their continued or repeated application lead to adverse effects such as disrupted biological control by natural enemies, widespread development of resistance in fungal pathogens to various types of fungicides, toxicity to non target organisms and environmental problems. This situation stimulated research on development of alternatives for crop protection methods which have little or no adverse effects. It has been shown that natural products mainly plants and their metabolites are shown to exhibit inhibitory effect against a range of phytopathogenic fungi[37-39]. *Sclerotium rolfsii* Sacc.is a soil borne omnivorous (saprophytic or parasitic) phytopathogenic fungus commonly referred to as rolf fungus. It affects a large number of agricultural (sweet potato, pumpkin, corn, peanut, wheat, tomato) and horticultural crops of approximately 500 species in tropical, subtropical and warm temperate countries. The wide host range, fastidious growth and ability to produce persistent sclerotia contribute to their involvement in losses of economic crops (fruits and vegetables) and difficulty in their control[40,41]. In the present study, we determined antifungal effect of leaf and pericarp extracts of *P. longifolia* against *S. rolfsii*. The leaf and pericarp extracts displayed marked inhibitory activity against *S. rolfsii* in terms of mycelial growth inhibition. Pericarp extracts exhibited stronger inhibition of fungus when compared to leaf extract. Unripe pericarp extract suppressed the growth of test fungus to high extent followed by ripe pericarp and leaf extract. Similar result was observed in our previous study in which the leaf and pericarp extracts showed inhibition of *C. capsici* isolated from anthracnose of chilli. The unripe pericarp extract inhibited *C. capsici* to higher extent when compared to leaf and ripe pericarp extracts which displayed similar inhibition of the fungus[24]. It has been shown that leaf extract exhibited marked growth inhibition of *S. rolfsii* causing collar rot of lentil[42]. The aqueous extract of leaves exhibited mycelial growth inhibition of several ground nut fungal pathogens including *S. rolfsii*[43]. In another study, leaf extract was found to suppress the growth of *S. rolfsii* causing stem rot of ground nut[44]. It has been experimentally shown that *P. longifolia* possess marked inhibitory effect against a variety of other fungi. Extracts of leaves were found to exhibit inhibitory activity against several species of *Aspergillus*[14], various fungi *viz*., species of *Drechslera*, *Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* isolated from sorghum grains[20], *Fusarium solani* isolated from rhizome rot of ginger[21], aflatoxin producing *Aspergillus parasiticus*[22] and seed borne fungi isolated from green gram[23]. It has been shown that the treatment of sorghum seeds by ripe pericarp extract prevented fungal infection of seeds[19].

CONCLUSION

A marked inhibitory potential of leaf and pericarp extracts of *P. longifolia* against Gram positive and Gram negative bacteria and *S. rolfsii* was observed in this study. The antimicrobial potential of extracts could be ascribed to the presence of secondary metabolites detected in the extracts. The leaf and pericarp extracts can be the potential candidates for development of agents active against human pathogenic bacteria and phytopathogenic fungi. Further studies on isolation and characterization of bioactive metabolites from extracts and their bioactivity determinations are to be carried out.

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