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SCREENING OF SELECTED BIOLOGICAL ACTIVITIES OF *ARTOCARPUS LAKOOCHA* ROXB (MORACEAE) FRUIT PERICARP

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ABSTRACT: *Artocarpus lakoocha* Roxb (Moraceae) is cultivated in Uttar Pradesh, Bengal, Khasi Hills and Western Ghats. Objectives of the present study were to determine antibacterial, antioxidant, anthelmintic and insecticidal efficacy of methanol extract of *A. lakoocha* fruit pericarp. Antibacterial activity was tested against by Agar well diffusion method. Antioxidant activity in terms of free radical scavenging ability was determined by DPPH free radical scavenging assay. Anthelmintic efficacy was determined using adult Indian earthworm. Insecticidal activity was tested against second and third instar larvae of *Aedes aegypti*. The extract has shown dose dependent antibacterial, antioxidant, anthelmintic and insecticidal activity. Among bacteria, *S. aureus* has shown more susceptibility than *K. pneumoniae* and *P. aeruginosa*. The extract exhibited marked antioxidant activity by scavenging DPPH free radical. The IC₅₀ value for extract was found to be 49.42µg/ml. The extract exhibited marked anthelmintic activity by causing paralysis and death of worms and the effect was found to be dose dependent. The extract concentration 100mg/ml has shown marked anthelmintic effect than standard drug. In insecticidal study, the 2nd instar larvae were shown to be more susceptible than 3rd instar larvae. Phytochemical analysis revealed the presence of tannins and alkaloids. The presence of these phytoconstituents might be responsible for the biological activities of extract tested. The extract could be used to treat free radical damage, bacterial and helminthic infections and to control insect vectors. Further studies on isolation of constituents and their bio-efficacies *in vitro* and *in vivo* are under investigation.

KEYWORDS: *Artocarpus lakoocha* Roxb, Agar well diffusion, *Pheretima posthuma*, DPPH, IC₅₀, *Aedes aegypti*

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INTRODUCTION

A *rtocarpus lakoocha* Roxb (Syn: *A. lacucha* Buch.-Ham.) is a member of the family Moraceae and is cultivated in Uttar Pradesh, Bengal, Khasi Hills and Western Ghats. It is called Monkey Jack in English and in Ayurveda it is called Lakuch, Kshudra Panas, Granthiphala and Pitanaasha. Bark when applied externally, draws out purulent matter; heals boils, cracked skin and pimples. Seeds are purgative, haemagglutinating.

Stem is vermifuge. The stem bark contains oxyresveratrol, used for tapeworm. A lectin, artocarpin, isolated from seeds, precipitates several galactomannans. It agglutinates rat lymphocytes and mouse ascites cells [1]. The lakoocha fruits are generally eaten fresh. The edible fruit pulp is believed to acts as a tonic for the liver. The raw fruits and male flowers spikes (acidic and astringent) are utilized in pickles and chutney. The brown powder called Puag-Haad in Thailand is a product of the aqueous extraction of *A. lakoocha* prepared by boiling the wood chips and then evaporating water away. This preparation has been used as a traditional anthelmintic drug for

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treatment of tapeworm infection in Thailand [2,3]. The hardwood sold as lakuch is comparable to famous teak wood, is used for constructions, furniture, boat making and cabinet work. Tree bark containing 8.5% tannin is chewed like betel nuts and is also used to treat skin ailments. It yields a durable fiber good for cordage. The wood and roots yield a lavish color dye [4]. Two isolectins, ALA-I and ALA-II, isolated from seed extracts of *A. lakoocha* possessed several similar properties such as blood type agglutination, pH optimum, pH and temperature stability, as well as binding specificity towards asialomucins [5]. Two new stilbene derivatives, lakoochins A and B, were isolated from the roots of *A. lakoocha*. Both exhibited antimycobacterial activity and showed cytotoxic activity against some cell lines [6]. Oxyresveratrol, isolated from heartwood of *A. lakoocha* has shown moderate anti-herpes simplex virus activity and anti-HIV activity against a wild-type human immunodeficiency virus type 1 [7]. Critical review of literature revealed scanty information on antibacterial, antioxidant, anthelmintic and insecticidal activity of fruit pericarp of *A. lakoocha*. Thus, the present investigation has been carried to investigate antibacterial, antioxidant, anthelmintic and insecticidal activity of fruit pericarp of *A. lakoocha*.

EXPERIMENTAL

Collection and identification of plant material

The fruits of *A. lakoocha* were collected during April 2010 from outskirts of Shivamogga, Karnataka, India. The fruit was identified by specialist and a voucher specimen (SRNMN/PK/Al-801) was deposited in the department for future reference.

Drugs and Chemicals used

Methanol (HiMedia, Mumbai), Dimethyl sulfoxide (S.D Fine Chemicals, Mumbai), Rifampicin (Ranbaxy Laboratories, New Dehli), Piperazine citrate (GlaxoSmithKline Pharmaceutical Limited, Bangalore), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH, Sigma Chem. Co., USA).

Extraction and Phytochemical analysis

The collected ripe fruits were washed thoroughly, pericarp was separated, shade dried and powdered using blender. For extraction, the powdered material was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extract was filtered, concentrated using rotary flash

evaporator and dried in the desiccator. The extract was subjected to preliminary phytochemical screening to detect secondary metabolites [8,9].

Preparation of extract for antibacterial, anthelmintic and insecticidal activity

The concentrated methanol extract was dissolved in 10% Dimethyl sulfoxide (DMSO) to get concentrations 10, 25, 50 and 100mg/ml.

Screening for Antibacterial activity of methanol extract

The antibacterial efficacy of methanol extract of pericarp was tested against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by Agar well diffusion method [10]. Briefly, 24 hours old broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 6mm with the help of sterile cork borer. The Standard drug (Rifampicin, 1mg/ml of sterile distilled water) and Control (10%DMSO) and different concentrations of extract were added to respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. Experiment was carried thrice and average reading was noted.

Screening for free radical scavenging ability of methanol extract

The radical scavenging ability of methanol extract and the Ascorbic acid (standard) was tested on the basis of the radical scavenging effect on the DPPH free radical [11]. Different concentrations of extract and standard namely 25, 50, 100, 200 and 400µg/ml were prepared in methanol. In clean and labeled test tubes, 2 ml of DPPH solution (0.002% in methanol) was mixed with 2 ml of different concentrations of extract and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517nm using UV-Vis Spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity of the extract was calculated using the formula

$$\text{Scavenging activity (\%)} = \frac{A - B}{A} \times 100$$

where A is absorbance of DPPH and B is absorbance of DPPH and extract/standard combination. The IC₅₀ value was calculated which denotes the concentration of extract required to scavenge 50% of DPPH free radicals.

Screening for Anthelmintic activity of methanol extract

In this study, adult Indian earthworm *Pheretima posthuma* was used to assess anthelmintic potential of methanol extract of pericarp due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. The worms were identified in the department of Zoology, Sahyadri Science College (Autonomous), Shivamogga. Standard drug (Piperazine Citrate, 1%) and different concentrations of methanol extract (10, 25, 50 and 100mg/ml of 10% DMSO) were poured into respective labeled petriplates containing saline. A saline control was kept. Six worms of nearly equal size 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 their motility followed with fading away of their body colors. 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 [12,13].

Screening for Insecticidal activity of methanol extract

The insecticidal efficacy of methanol extract of pericarp was determined against second and third instar larvae of *Aedes aegypti*. The larvae were col-

lected from stagnant water and identified in University of Agricultural Sciences, Shivamogga by an Entomologist. Briefly, different concentrations of methanol extract (10, 25, 50 and 100mg/ml of 10% DMSO) were added to labeled beakers containing twenty larvae. A beaker containing only water (i.e., without extract) serves as control. The larvicidal effect of the extract was determined by counting the number of dead larvae after 24 hours and the observation was continued for up to 72 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. The test was repeated thrice and the percentage of larval mortality for each concentration of extract was calculated [14].

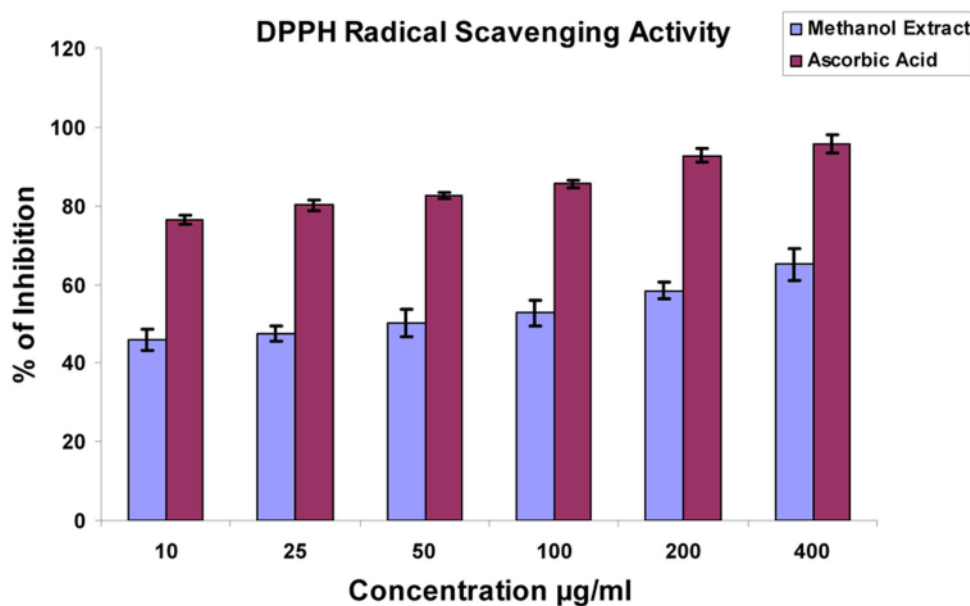
RESULTS

The preliminary phytochemical analysis of methanol extract of *A. lakoocha* fruit pericarp revealed the presence of tannins and alkaloids.

The result of antibacterial activity of methanol extract of fruit pericarp is shown in Table 1. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative. In this study, the extract has shown inhibition of test bacteria in a concentration dependent manner. Among bacteria, *S. aureus* was found to be more

Table 1: Antibacterial activity of methanol extract

Treatment	Concentration	Inhibition Zone in cm		
		<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
Control	10%	0.0	0.0	0.0
Standard Methanol extract	1 mg/ml	3.8	3.8	3.9
	10 mg/ml	1.1	0.9	0.8
	25 mg/ml	1.5	1.3	1.3
	50 mg/ml	1.9	1.7	1.6
	100 mg/ml	2.2	1.9	1.8

Figure 1: DPPH free radical scavenging activity of methanol extract and Ascorbic acid**Table 2:** Anthelmintic activity of methanol extract

Extract/ Drug	Concentration	Paralysis time (in min)	Death time (in min)
Saline	0.85%	-	-
	10 mg/ml	154	188
	25 mg/ml	113	146
Methanol extract	50 mg/ml	81	113
	100 mg/ml	74	95
	Standard	1%	85

susceptible to extract followed by *K. pneumoniae* and *P. aeruginosa*. Standard antibiotic caused more inhibition of test bacteria than methanol extract. No inhibition of test bacteria was observed in case of control i.e., 10% DMSO. It appears that overall the bacteria were found to be sensitive to extract. The reasons for this could be that the components from the plant active against microorganisms are most often obtained through solvent extraction.

Antioxidant activity of different concentrations of methanol extract of fruit pericarp and ascorbic acid in terms of free radical scavenging ability was evaluated using DPPH free radical assay (Figure 1). The extract exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH and the activity was found to be dose dependent. The scavenging activity of ascorbic acid was greater than that of methanol extract. The IC₅₀

Table 3: Insecticidal activity of methanol extract of fruit pericarp

Concentration	Mortality Percentage (%)					
	II Instar Larvae			III Instar Larvae		
	24 h	48 h	72 h	24 h	48 h	72 h
10 mg/ml	0	0	0	0	0	20
25 mg/ml	0	40	60	0	20	40
50 mg/ml	20	35	70	10	20	45
100 mg/ml	50	60	85	25	35	50

of the extract and Ascorbic acid was found to 49.42 and 06.09 µg/ml respectively.

The result of anthelmintic activity of methanol extract of fruit pericarp is depicted in Table 2. The extract exhibited marked anthelmintic effect by causing paralysis followed by death of worms and the effect was found to be dose dependent. The anthelmintic effect of extract concentration 50mg/ml was comparable with that of standard drug (1% Piperazine citrate). Anthelmintic effect by extract concentration 100mg/ml was higher when compared to that of standard drug as the time taken for causing paralysis and death of worms was shorter.

The insecticidal efficacy of different concentrations of methanol extract was evaluated against 2nd and 3rd instar larvae of *A. aegypti*. The mortality of the larvae was found to be concentration dependent. Among larvae, 2nd instar larvae were shown to be more susceptible than 3rd instar larvae. The highest mortality (85%) of 2nd instar larvae was recorded at concentration 100mg/ml on 3rd day. In case of 3rd instar larvae, highest mortality (50%) was observed on 3rd day with concentration 100mg/ml. Extract concentration 10mg/ml did not caused death of both larval stages even after 72 hours (Table 3).

DISCUSSION

Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance [15]. Interest in plants

with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics [16]. Antimicrobial activities of tannins [17], flavonoids [18], saponins [19], terpenoids [20] and alkaloids [21] have been documented. In this study, the preliminary phytochemical analysis of the methanol extract of fruit pericarp showed the presence of tannins and alkaloids. The antibacterial activity of extract in this study could be chiefly due to the presence of these phytoconstituents and is suggestive of the possible use of the plant in treatment of bacterial infections as most strains have already developed resistance to most of the currently used antibiotics.

Living tissues derive energy from aerobic metabolism and are under constant threat of damage by reactive oxygen derivatives. Such free radicals are usually short-lived species but they possess a single unpaired electron, rendering them highly reactive against biologically important macromolecules including DNA, proteins and membrane lipids. To counteract this threat to their integrity, cells have evolved a variety of defense systems based on both water soluble and lipid-soluble antioxidant species, and on antioxidant enzymes. A high proportion of the antioxidant systems of the human body are dependent on dietary constituents. Consequently, the search for natural antioxidants, especially of plant origin, has notably increased in recent years [22-24]. DPPH radical scavenging is a widely used method to evaluate the free radical scavenging ability of various materials [25]. DPPH is a stable nitrogen-centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of

hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and, therefore, radical scavengers [26]. In this study, it was found that the radical-scavenging activity of methanol extract increased with increasing concentration.

During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health [27]. The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of natural compounds for their proclaimed anthelmintic activity. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India [13]. Indigenous system of medicine reports a number of natural sources for their anthelmintic efficacy. However, their scientific evaluation as compared to commercial anthelmintics is limited. Many plants have proven to possess anthelmintic activity *in vitro* and *in vivo*. Tannins were found to possess anthelmintic activities. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death [28-29]. Preliminary phytochemical analysis revealed the presence of tannins in the methanol extract which could be responsible for the anthelmintic effect of extract. The result of the present study is suggestive that the extract could be used in the control of round worm infections such as Ascariasis, hookworm infections etc as the worms used in the study are in anatomical and physiological resemblance with the intestinal round worms.

Herbal products with proven potential as insecticide or repellent can play important role in the interruption of the transmission of mosquito borne diseases at the individual as well as community level. Some herbal products have been used as natural insecticides even before the discovery of synthetic organic insecticides [30]. Mosquitoes are the most important

single group of insects acting as vector for many tropical and subtropical diseases [31]. 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 [32]. Killing larvae of mosquitoes is a successful way of minimizing mosquito densities in breeding grounds before they reach adult stage. It largely depends on the use of synthetic chemical insecticides. But their repeated use has caused environmental problems and widespread development of resistance. Plants offer an alternative source of insect-control agents 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. It is observed that the carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins are having mosquito larvicidal activity [14]. In this study, the larvicidal effect of fruit pericarp extract was found to be dose dependent and the effect may be due to the presence of phytochemicals present in it. The extract could be used in the prevention of arboviral infections such as dengue, chickungunya etc which are transmitted by *A. aegypti*.

CONCLUSION

Plant extracts have been used in the control/treatment of free radical damage, bacterial, helminthic and mosquito borne diseases as the chemical agents have caused some ill effects and also the pathogens have developed resistance against them. The results of the present study suggest that the fruit extract selected in this study could be used in treatment of free radical damage, bacterial and helminthic infections and control of arboviral infections transmitted by the mosquito *Aedes aegypti*. The biological activities of methanolic extract in this study could be chiefly due to the presence of phytoconstituents such as tannins and alkaloids. Further studies on isolation of constituents and determining their *in vitro* and biological efficacies are under investigation.

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