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Elemental Composition, Anticariogenic, Pancreatic Lipase Inhibitory and Cytotoxic Activity of *Artocarpus Lakoocha* Roxb Pericarp

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Abstract

Artocarpus lakoocha Roxb is belongs to the family Moraceae and is called Monkey jack and Lakoocha. In the present study, we investigated elemental composition of fruit pericarp and anticariogenic, pancreatic lipase inhibitory and cytotoxic activity of methanol extract of pericarp. The elemental analysis was determined using atomic absorption spectrophotometer. Anticariogenic activity was determined against 12 isolates of mutans streptococci by Agar well diffusion method. Pancreatic lipase activity of different concentrations of pericarp extract was tested against chicken pancreatic lipase. Cytotoxic activity was tested by Brine shrimp lethality bioassay. Among the principal elements, potassium was present in high concentration followed by magnesium, phosphorus and calcium. Among trace elements, high concentration of iron was detected followed by zinc, manganese and copper. The extract caused inhibition of cariogenic bacteria and the inhibition caused by the extract was lesser when compared to standard antibiotic. The extract caused inhibition of pancreatic lipase in a dose dependent manner and highest inhibition (82.49%) was observed at concentration 1000mg/ml. The lethal nature of extract towards brine shrimp was directly proportional to the concentration of the extract. The LC_{50} was found to be 452.49µg/ml. Preliminary phytochemical analysis showed the presence of tannins and alkaloids. The fruit may be consumed as a source of important elements. The bioactivities of the extract could be attributed to the presence of secondary metabolites. Further study is required to isolate and characterize the active constituents and to determine their bioactivities.

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Key words:

Artocarpus lakoocha Roxb., Elements, Pancreatic lipase, mutans streptococci, Agar well diffusion, Brine shrimp lethality

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INTRODUCTION

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Artocarpus lacucha Buch.-Ham. (Syn- *Artocarpus lakoocha* Roxb.) belongs to the family Moraceae and is called Monkey jack and Lakoocha. It is native to humid sub-Himalayan regions of India, South China and South-East Asia. It is cultivated in Uttar Pradesh, Bengal, Khasi Hills and Western Ghats. The bark chewed like betel nut and is used to treat skin ailments. 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, 2]. 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 wood chips prepared by boiling and then evaporating water. This preparation has been used as a traditional anthelmintic drug for treatment of tapeworm infection in Thailand [3, 4]. 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 [5]. In a previous study, the pericarp extract showed dose dependent antibacterial, antioxidant, anthelmintic and insecticidal [6]. When literatures were searched on elemental composition, anticariogenic, pancreatic lipase inhibitory and cytotoxic activity of pericarp, it was found that these studies remain unexplored. Hence, in the present investigation, the elemental analysis of powdered pericarp, anticariogenic, pancreatic lipase inhibitory and cytotoxic activity of pericarp extract of *A. lakoocha* fruit was investigated.

MATERIALS AND METHODS

Collection and Identification of Fruit

The fruits were collected during April 2010 in college campus and identified by Prof. Rudrappa D, Lecturer, Dept. of Botany and a voucher specimen (Voucher no. PK/Al-801) was kept in the department of Microbiology for future reference. The ripened fruits were washed thoroughly, pulp was separated, shade dried and powdered using blender.

Elemental Analysis of Powdered Pericarp

For elemental analysis, 1gm of powdered pericarp was digested using a mixture of concentrated nitric acid and perchloric acid (10ml). The powder was left in acid mixture for 24 hours and was digested on the hot plate until complete digestion. After digestion, 10ml of 10% nitric acid was added and left for two hours for residue to settle down. The supernatant liquid was filtered though Whatman No. 1. The filtrate was subjected for estimation of elements using Atomic absorption spectrophotometer [7].

Extraction of Powdered Pericarp

The powdered pericarp was extracted by soxhlet apparatus using methanol. The extract was filtered using Whatman filter paper No. 1 and concentrated at 40°C under reduced pressure. The condensed methanol extract was stored at 4°C until use [7].

Phytochemical Analysis of Pericarp Extract

The extract was subjected to standard qualitative tests for the detection of alkaloids (Dragendorff's reagent and Mayer's reagent), tannins (ferric chloride test), saponins (frothing test and hemolysis test), glycosides (Salkawski test and Keller-Kiliani test), sterols (Burchard test), flavonoids (Shinoda test) and terpenoids (Salkowski test) [8, 9].

Anticariogenic Activity of Pericarp Extract

The anticariogenic activity of pericarp extract was tested by Agar-well-diffusion method against 12

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isolates of mutans streptococci recovered from dental caries patients. Briefly, 24 hours old Brain heart infusion broth (HiMedia, Mumbai) cultures of isolates were swabbed uniformly on solidified sterile Brain heart infusion agar plates using sterile cotton swab. Wells of 6mm diameter were punched in the inoculated plates with the help of sterile cork borer and the extract (20mg/ml of 10% DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were added separately into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured with a ruler. The experiment was carried in triplicate to get average reading [10].

Pancreatic Lipase Activity of Pericarp Extract

The inhibitory activity of methanol extract against lipase was tested against lipase extracted from the pancreas of chicken. Lipase inhibitory activity of different concentrations of methanol extract was tested by mixing 100µl of each concentration of methanol extract, 8ml of oil emulsion and 1ml of chicken pancreatic lipase followed by incubation of 60 minutes. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH using phenolphthalein as an indicator [11]. Percentage inhibition of lipase activity was calculated using the formula: Lipase inhibition = A - B / A X 100, where A is lipase activity, B is activity of lipase when incubated with methanol extract.

Cytotoxic Activity of Pericarp Extract

The brine shrimp lethality test was conducted according to the method of Kekuda *et al.* (2010) [12]. Brine shrimp *Artemia nauplii* eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500ml of distilled water. After 36-48 hours, the phototropic shrimps were collected by pipette for bioassay. The different concentrations of pericarp extract (10-1000µg/ml) were tested in vials containing 5ml of brine and 25 shrimp in each of three replicates. The vials were incubated at 25°C and surviving shrimps were counted after 24 hours. The LC_{50} value was determined by regression analysis.

RESULTS

Qualitative Phytochemical Screening

Preliminary qualitative phytochemical analysis of methanol extract of pericarp revealed the presence of tannins and alkaloids.

Mineral Composition of Pericarp

The mineral composition of the powdered pericarp material was determined using atomic absorption spectrophotometer and the result is shown in Table 1. Among the principal elements, potassium was present in high concentration (13500 ppm) followed by magnesium (1545 ppm), phosphorus (1500 ppm) and calcium (950 ppm). Among trace elements, iron was found to be in high amount (108.1 ppm) followed by zinc (12.4 ppm), manganese (5.4 ppm) and copper (2.7 ppm).

Anticariogenic Activity of Pericarp Extract

The anticariogenic activity extract was tested against 12 isolates of mutans streptococci and the result of inhibitory activity of extract 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 the absence of zone as negative. It was found that the extract caused inhibition of isolates. The diameter of inhibition zone formed was in the range of 1.1 to 1.5 cm. Inhibition caused by antibiotic was higher than

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that of extract. DMSO did not cause any inhibition of cariogenic isolates.

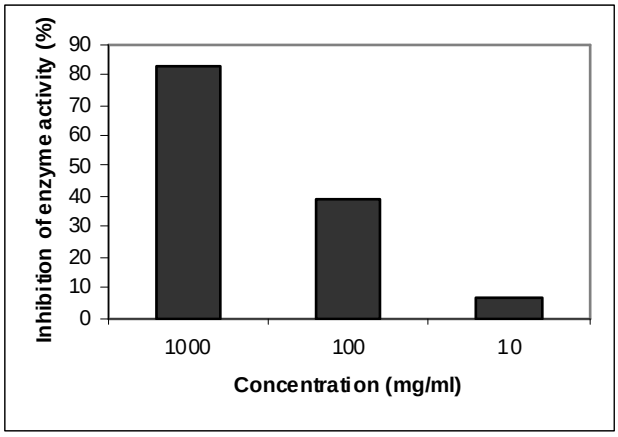
Table 1: Anticariogenic activity of pericarp extract.

Isolates	Zone of inhibition in mm		
	Pericarp extract	DMSO	Standard
1	1.1	--	2.4
2	1.1	-	2.2
3	1.5	-	3
4	1.2	-	2.5
5	1.1	-	2.4
6	1.2	-	2.5
7	1.1	-	2.3
8	1.2	-	2.7
9	1.2	-	2.6
10	1.2	-	2.6
11	1.2	-	2.4
12	1.3	-	2.6

Pancreatic Lipase Inhibitory Activity of Pericarp Extract

Inhibitory activity of different concentrations of pericarp extract on chicken pancreatic lipase was determined using olive oil as the substrate. It was found that the activity of lipase was drastically affected when incubated with the extract. The inhibitory activity was found to be concentration dependent. Highest mortality (82.49%) was observed at extract concentration 1000mg/ml (Figure 1).

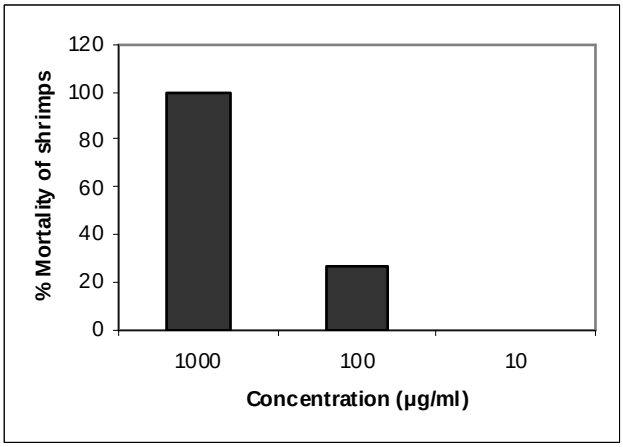
Figure 1: Pancreatic lipase inhibitory activity of pericarp extract.



Cytotoxic Activity of Pericarp Extract

Brine shrimp lethality bioassay was conducted to determine the cytotoxic nature of extract and the result is presented in Figure 2. The lethality of extract was directly proportional to the concentration of the extract. Highest mortality (100%) was observed at 1000µg/ml concentration whereas mortality was not observed at 10µg/ml extract concentration. The LC_{50} was found to be 452.49 µg/ml.

Figure 2: Brine shrimp lethality of pericarp extract.



DISCUSSION

Although minerals yield no energy, they have important roles to play in many activities in the body. Minerals are broadly classified into principal elements and trace elements. Minerals namely Ca, K, Mg, P etc forms the principal elements while Fe, Cu, Mn, Zn etc forms the trace elements. K maintains intracellular osmotic pressure and is required for the regulation of acid base balance and water balance in cells. It is required for the transmission of nerve impulse and is necessary for protein synthesis by ribosomes. P is a constituent of high energy phosphate compounds and nucleotide coenzymes. Ca is important for muscle contraction, blood coagulation, nerve transmission, membrane integrity and permeability, activation of certain enzymes, development of bones and teeth. Mg is required for the formation of bones and teeth and is necessary for proper neuromuscular function. It serves as a

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cofactor for several enzymes requiring ATP. Fe is an important component of hemoglobin and myoglobin. It is present in cytochromes and certain non-heme proteins that are involved in electron transport chain and oxidative phosphorylation. Iron is associated with effective immunocompetence of the body. Cu is an essential component of several enzymes and is necessary for the synthesis of hemoglobin, melanin and phospholipids. Cu is important for the development of bone and nervous system. Mn is required for the formation of bone, synthesis of hemoglobin, cholesterol and normal functioning of nervous system. It serves as cofactor for several enzymes and it inhibits lipid peroxidation. Zn is important in storage and secretion of insulin from pancreas. It is an essential component of several enzymes and is required for wound healing [13, 14]. In this study, the mineral composition of the powdered pericarp was determined using atomic absorption spectrophotometer. Among the principal elements, potassium was present in high concentration followed by magnesium, phosphorus and calcium. Among trace elements, iron was found to be in high concentration followed by zinc, manganese and copper.

An important strategy in the treatment of obesity includes the development of nutrient digestion and absorption inhibitors, in an attempt to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanisms. Orlistat, one of the two clinically approved drugs for obesity treatment, has been shown to act by inhibiting pancreatic lipase. Although it is one of the best drugs, it has certain unpleasant gastrointestinal side effects such as oily stools, oily spotting, and flatulence, among others. This necessitated identification of new pancreatic lipase inhibitors that lack some of these unpleasant side effects. Still now, the potential of natural products for the treatment of obesity is yet largely unexplored and might come out as an excellent alternative strategy for the development of safe and effective antiobesity drugs [15-19]. Pancreatic lipase or triacylglycerol acyl hydrolase, the principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides. Pancreatic lipase is responsible for the hydrolysis of 50–70% of the total dietary fats. It removes fatty acids from the α and α′ positions of dietary triglycerides, yielding β-monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic products. Pancreatic lipase inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as antiobesity agents. Many polyphenolics such as flavones, flavonols, tannins, and chalcones are active against Pancreatic lipase [18, 20, 21]. Studies on lipase inhibitory activity of extracts have been carried on chicken pancreatic lipase. Ethyl acetate extract of *Terminalia bellerica* Roxb seeds has shown concentration dependent inhibition of lipase [22]. The methanol extract of *Everniastrum cirrhatum* (Fr.) Hale was found to inhibit activity of chicken pancreatic lipase in a concentration dependent manner [11]. In this study, the extract caused dose dependent inhibition of lipase activity. The presence of tannins in the extract might be responsible for the enzyme inhibitory activity.

The brine shrimp lethality bioassay is a rapid, inexpensive and simple for testing efficacy of plant extracts which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. The assay is based on the ability of the extract to cause mortality of laboratory cultured *Artemia nauplii* brine shrimp. It is considered as useful tool for preliminary assessment of toxicity. This is a rapid method as the result is taken after 24 hours. It is so simple that no aseptic technique is required. It utilizes a large number of organisms for validation and a relatively small amount of sample. It does not require animal serum as needed for other methods of

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cytotoxicity testing [23-25]. Several studies have been carried on brine shrimp lethality of extracts from natural sources. Raghavendra *et al.,* (2010) [26] showed cytotoxic activity of methanol extract of *Putranjiva roxburghii* Wall (Euphorbiaceae) seeds. The extract was found to be toxic with LC_{50} of 427.74µg/ml. The cytotoxicity of methanol extract of leaves of *Abrus pulchellus* Wall (Fabaceae) using brine shrimp lethality bioassay revealed dose dependent activity with LC_{50} of 281.70µg/ml [12]. Kekuda *et al.,* (2012) [27] found cytotoxic nature of extract of a macrolichen Everniastrum cirrhatum with LC50 value of 474.06 µg/ml. In this study, the extract was found to cause mortality of brine shrimps in a dose dependent manner. The lethal nature of the extract could be attributed to the presence of secondary metabolites present in the extract.

CONCLUSION

The fruit could be consumed as an important source of various elements required for normal physiology of the body. A marked anticariogenic, pancreatic lipase inhibitory and cytotoxic activity was observed in the study and these bioactivities could be related to the presence of secondary metabolites. Further study is needed to isolate and characterize the active constituents from the extract and to determine their bio-efficacy.

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