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Original Research

Elemental Analysis and Bioactivities of Ripe and Unripe Pericarp of *Polyalthia longifolia* (Annonaceae)

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Abstract

Polyalthia longifolia (Annonaceae) is an ornamental street tree having several medicinal values. The plant is used in traditional systems of medicine. The present study was conducted with an aim of estimating the content of minerals and determining bioactivities *viz*., antibacterial, cytotoxic and larvicidal activity of ripe and unripe pericarp of *P. longifolia*. The content of major and minor elements in powdered ripe and unripe pericarp was estimated by ICP-OES after microwave digestion using nitric acid. The pericarp powders were extracted using methanol and the extracts were subjected to preliminary phytochemical analysis. Antibacterial activity of pericarp extracts was determined against two Gram positive and three Gram negative bacteria by agar well diffusion assay. Cytotoxic potential of pericarp extracts was determined against two cell lines *viz*., HT-29 and MDA-MB-231 by MTT assay. Insecticidal activity, in terms of larvicidal activity of pericarp extracts was tested against II instar larvae of *Aedes aegypti*. The content of all elements except copper was highest in ripe pericarp. The content of potassium and iron was highest among major and minor elements respectively. Pericarp extracts caused dose dependent inhibition of test bacteria. Extract of ripe pericarp caused higher inhibition of test bacteria than extract of unripe pericarp. Both extracts showed concentration dependent cytotoxic effect. The cytotoxic effect of both the extract was pronounced against HT-29 than MDA-MB-231. The extracts exhibited dose dependent larvicidal effect. Among extracts, potent larvicidal activity was observed in case of ripe extract. Preliminary phytochemical analysis revealed the presence of flavonoids, tannins, saponins, steroids and glycosides in both the extracts. In conclusion, the ripe and unripe pericarp extracts of *P. longifolia* were found to contain various minerals in an appreciable quantity. The observed dose dependent bioactivities *viz*., antibacterial, cytotoxic and larvicidal activities might be ascribed to the presence of phytoconstituents. There is a great potential for the development of therapeutic agents from ripe and unripe pericarp. Further studies on isolation of active principles from pericarp extracts and their bioactivity determinations are under progress.

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INTRODUCTION

Nutrients are broadly classified into macro and micronutrients. Nutrients such as carbohydrates, proteins, fats etc. are consumed in greater quantity and are called macronutrients. Minerals and vitamins are needed in smaller quantities and hence, are called micronutrients. Mineral elements represent an average of 4% of total body mass. There is sufficient evidence that minerals, both independently or in proper balance with other minerals, have multiple functions in the body that are key for overall health of an individual. About 20 minerals are found to be essential for normal physiology of the body and are grouped into major (macro) elements and minor (trace) elements based on their daily requirements. Macroelements are required in larger amounts whereas trace elements are needed in smaller quantities. These elements play an important role in many biological reactions like enzyme reactions, transportation of gases, muscle contraction, transmission of nerve impulses and utilization of nutrients from foods. Plants are consumed directly or indirectly by humans and animals. Hence, determination of mineral contents of plants and plant products is of much interest (Lukaski, 2004; Zamberlin *et al*, 2012; Vinayaka *et al*., 2013).

Before the development of chemotherapeutic agents notably antibiotics, infectious diseases caused by bacteria, fungi, viruses and protozoa have devastated mankind. The morbidity and mortality due to these infectious diseases have been reduced after the discovery of antibiotics and their subsequent use. The use of these wonder drugs saved countless lives. However, the traditional antibacterial therapy which uses antibiotics (natural or synthetic analogues) is facing a lot of problems and the development of resistance is the most important among them. *Staphylococcus aureus* is among those pathogens which became resistant to commonly used

antibiotics posing a global threat. Vancomycin resistant enterococci, multidrug resistant tuberculosis, antibiotic resistant *E. coli*, *P. aeruginosa* etc., are among other antibiotic resistant bacteria against which most of the antibiotics are not effective. Above all, these drug resistant bacteria have the potential to inherit the resistance gene susceptible bacteria. The need for new antimicrobials in particular from natural origin such as botanicals is increasing day by day (Ojala *et al*., 2000; Hemaiswarya *et al*., 2008; Davies and Davies, 2010; Wright, 2010).

Cancer remained a major cause of death and the number of individuals affected with cancer is increasing. Cancer therapy with chemotherapeutic agents suffers from great difficulties and the most frequently are the drug resistance, toxicity and low specificity. Plant derived compounds have played important role in the development of anticancer agents. The anticancer activity of these compounds are related to the regulation of cancer related gene expression, induction of apoptosis, cell cycle arrest and /or DNA fragmentation and inhibition of different cellular enzymes (Patil *et al*., 2012; de Mesquita *et al*., 2009; Kumar *et al*., 2011). A large number of phytochemicals have shown to possess anticancer property and thus are an important source of newer cancer therapy agents. The use of complementary and alternative medicine such as herbal extracts is becoming increasingly popular in the treatment of cancer (Manchana *et al*., 2011; Patil *et al*., 2012).

Mosquitoes transmit more diseases than any other arthropod group. The mosquito-borne diseases affect millions of people throughout the world. These diseases are prevalent in several countries and India being a country with high incidence of diseases. Mosquitoes act as vectors for several life threatening diseases like malaria, yellow fever, dengue fever, chikungunya ferver, filariasis, West Nile virus infection and others (Ghosh *et al*., 2012). Hence, control of mosquitoes is crucial for prevention of diseases and to improve environment quality and public health. The use of synthetic insecticides namely organochlorines and organophosphates is the major strategy employed in mosquito control. However, it is not so beneficial due to several factors such as human, technical, operational, ecological, and economic. Moreover, the use of synthetic insecticides has been limited due to high cost, residual effect, detrimental effect on human and other non-target populations, and higher rate of biological magnification and emergence of resistance in mosquitoes against insecticides. This situation is alarming and triggered interest to look for alternate, eco-friendly, cost-effective, target specific agents to control these mosquito vectors. Exploration of natural compounds is one of the most effective alternative approaches and the significance of insecticides of botanical origin has been extensively investigated (Promsiri *et al*., 2006; Vinayaka *et al*., 2009; Ghosh *et al*., 2012).

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 clerodane diterpenoids and alkaloids in various parts. *Polyalthia longifolia* is an ornamental lofty evergreen street (avenue) tree and is considered native of Sri Lanka. It is cultivated all over Indian subcontinent. 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 (Ghosh *et al*., 2008; Katkar *et al*., 2010). The plant has been used traditionally in various parts of India. As febrifuge, stem bark is used by tribals of Visakhapatnam, Andhra Pradesh (Bapuji and Ratnam, 2009). Local communities of Uthiramerur of Tamil Nadu consume fresh stem bark juice to treat indigestion (Sugumaran *et al*., 2010). Adeevasee communities of Danta, Gujarat consume dried stem bark with butter for curing gonorrhea (Patel, 2010). Tribal people of Khargone, Madhya Pradesh use stem bark to cure malignant tumor (Mahajan *et al*., 2010). In Manchale area of Shimoga district, Karnatka, bark is used to prevent abortion in pregnant women (Poornima *et al*., 2012). The leaves are used to treat fever, gonorrhea, uterus ailments, mouth ulcer, heart problems and others in Vellore, Tamil Nadu (Sundaresan and Senthilkumar, 2013). Stem bark is used for diabetes and hypertension by tribals of Bankura district, West Bengal (Sinhababu and Banerjee, 2013).

Several bioactivities such as antimicrobial (Faizi *et al*., 2008; Dileep *et al*., 2013), antioxidant (Manjula *et al*., 2010), antitumor (Manjula *et al*., 2010), antiulcer (Malairajan *et al*., 2008), antileishmanial (Pal *et al*., 2011), hypotensive (Saleem *et al*., 2005), anti-hyperglycemic (Ghosh *et al*., 2010), anti-inflammatory (Tanna *et al*., 2009), hepatoprotective (Tanna *et al*., 2009), anticataract-ogenesis activity (Sivashanmugam and Chatterjee, 2012) others have been attributed to various parts of the plant. The treatment of sorghum seeds by ripe pericarp of fruit promoted germination of seeds and prevented fungal infection of seeds (Kekuda *et al*., 2010). In a previous study, Dileep *et al*. (2012) showed dose dependent DPPH free radical scavenging and ferric reducing activity of different solvent extracts of ripe pericarp. Ethanol extract exhibited potent activity. In another study, Dileep *et al*. (2013) observed inhibitory activity of leaf and pericarp extracts against rhizome rot causing pathogens. Recently, Kekuda *et al*. (2014) showed potent inhibitory activity of leaf and pericarp extracts against *Colletotrichum capsici* and drug resistant uropathogens. In the present study, we estimated the content of major and minor elements in the ripe and unripe pericarp powder by ICP-OES and determined antibacterial, cytotoxic and insecticidal activity of unripe and ripe pericarp extract of *P. longifolia*.

MATERIALS AND METHODS

Collection of Fruits

The ripe (purple to black) and unripe (green) fruits of *P. longifolia* (Figure 1) were collected during May 2012 from the campus of S.R.N.M.N College of Applied Sciences and authenticated by Prof. Rudrappa D, Department of Botany, S.R.N.M.N College of Applied Sciences. The fruits were washed well using clean water to remove adhering matter. The pericarp was separated from ripe and unripe fruits, shade dried and powdered using blender.

Figure 1: The ripe and unripe fruits of *P. longifolia*

Elemental Analysis by Using ICP-OES

The powdered ripe and unripe pericarp was subjected to elemental analysis by Inductively Coupled Plasma with Optical Emission Spectroscope (ICP-OES). Exactly 1.0g of ripe and unripe pericarp powder was added to 10ml of ultrapure metal free nitric acid in separate containers and digested in microwave digester (CEM). After complete digestion, the content was diluted to 25ml with distilled water. Then, the digested samples were aspirated into ICP-OES (Agilent Technologies 700series, US) in order to estimate major elements *viz*., Calcium (Ca), Potassium (K), Sodium (Na) and Magnesium (Mg) and minor elements *viz*., Manganese (Mn), Iron (Fe), Zinc (Zn), Nickel (Ni), Chromium (Cr), Lithium (Li) and Copper (Cu). The calibration standards were prepared by diluting stock multi-elemental standard solution in nitric acid (Vinayaka *et al*., 2013). The instrument configuration and experimental conditions employed in the study are represented in Table 1.

Table 1: ICP-OES Operation conditions

Parameter	Value
Power (kW)	1.2
Plasma flow (L/min)	15.0
Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75
Sample flow rate (L/min)	1.5
Replicate read time (s)	3.00
Instrument stabilization delay (s)	15.0
Sample uptake delay (s)	10.0
Pump rate (rpm)	15.0
Rinse time (s)	10.0
Spray chamber	Cyclonic type
Elements, wavelengths (nm)	Ca (422.673), Cu (327.395), Na (589.592),Cr (267.716), Fe (238.204), K (766.491), Mg(279.553), Mn (257.610), Ni (231.604), Zn (213.857), Li (670.783)

Extraction of Ripe and Unripe Pericarp Powder

For extraction, a known quantity (25g) of powdered ripe and unripe pericarp material was transferred into separate containers containing 100ml of methanol (HiMedia, Mumbai) and stirred well. The containers were left for overnight. The contents were filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper. The filtrates were condensed under reduced pressure and stored in airtight containers (Vinayaka *et al*., 2009).

Preliminary Phytochemical Analysis

The pericarp extracts were screened for the presence of phytochemicals namely alkaloids, flavonoids, tannins, saponins, glycosides, steroids and terpenoids by standard phytochemical tests (George *et al*., 2010; Kekuda *et al*., 2012).

Antibacterial Activity

The antibacterial activity of pericarp extracts was determined by Agar well diffusion assay against two Gram positive bacteria *viz*., *Staphylococcus aureus* and *Bacillus cereus* and three Gram negative bacteria *viz*., *Escherichia coli*, *Shigella flexneri* and *Vibrio cholerae*. In brief, the test bacteria were inoculated into sterile Nutrient broth (HiMedia, Mumbai) tubes and incubated overnight at 37^{o}C. The broth cultures were swabbed aseptically on sterile Nutrient agar (HiMedia, Mumbai) plates using sterile cotton swabs. Wells of 6mm diameter were punched in the inoculated plates using sterile cork borer. 100µl of ripe and unripe pericarp extracts (10 and 20mg/ml of 25% dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), standard antibiotic (Chloramphenicol, 1mg/ml) and DMSO (25%, in sterile water) were added into labeled wells. The plates were incubated at 37^{0}C for 24 hours in upright position. Using a ruler, the zones of inhibition formed around each well was measured (Kekuda *et al*., 2012).

Cytotoxic Activity

The cytotoxic potential of pericarp extracts was tested against two cell lines *viz*., HT-29 (human colon carcinoma) and MDA-MB-231 (Human breast cancer). The cell lines were maintained in DMEM, supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/ml of penicillin and 100µg/mL streptomycin. The cells were maintained in a humidified atmosphere under 5% CO_2 at 37^{o}C.

Preparation of Extracts

The extracts were dissolved in DMSO at 20 mg/ml as stock solution which was then diluted with DMEM to desired concentrations (10 to 200µg/ml). The final concentration of DMSO in each sample did not exceed 0.1% v/v in both control and test.

Cytotoxicity Assay

Cell viability was assessed by MTT [3′-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazoliumbromide] assay, which is based on the reduction of MTT into formazan dye by active mitochondria (Lee *et al*., 2009). In this assay, the cells were placed in 96-well plates at a density of 5×10^{4} cells/well in culture medium that contained 10% FBS and then incubated at 37^{o}C under 5% CO_2. After 24 hours, the cells were washed and placed in culture medium with different concentrations of extracts and reference standard (5-Fluorouracil) for 48 hours. Then, 20µl of MTT solution (5mg MTT/ml in phosphate buffered saline) was added to each well of the microtitre plate and then incubated for 4 hours at 37^{o}C. After washing, the formazan dye precipitates, which are proportional to the number of live cells, were dissolved in 100µl of DMSO. The absorbance at 570nm was then read using a microtitre plate reader. The effects of each concentration were analyzed in triplicate. The rate of cell growth inhibition (CGI) was calculated using the following formula:

$$CGI\ (\%) = (A - B / A) \times 100\%$$

where A is the absorbance of control and B is the absorbance of test. IC_{50} (Inhibitory Concentration) was calculated for both extracts and reference standard using Origin 6.0 software.

Insecticidal Activity

The insecticidal effect, in terms of larvicidal effect of different concentrations of ripe and unripe pericarp extract (0.1, 0.5 and 1.0mg/ml) was tested against II instar larvae of *Aedes aegypti* mosquito. Twenty larvae were placed in beakers containing different concentrations of pericarp extracts. A control was kept in which extract was not added. The larvicidal effect of extracts was studied by counting the number of dead larvae after 24 hours. Dead larvae were identified when the larvae failed to move even

after probing with a needle in siphon or cervical region (Vinayaka *et al*., 2009). LC_{50} (Lethal Concentration) was calculated for both the extracts by Origin 6.0 software.

RESULTS

Mineral Elements in Ripe and Unripe Pericarp

The content of major and minor elements present in ripe and unripe pericarp of *P. longifolia* is shown in Table 2. The content of all elements except copper was higher in ripe pericarp. Among major elements, the content of potassium was highest followed by calcium, magnesium and sodium. Among minor elements, the content of iron was highest followed by zinc, manganese and others.

Table 2: Elemental composition of ripe and unripe pericarp

Element	Ripe (ppm)	Unripe (ppm)
Calcium	3987.42	3628.27
Magnesium	750.28	653.91
Sodium	82.62	67.53
Potassium	19504.80	17417.90
Iron	60.69	40.54
Manganese	6.23	5.49
Zinc	12.58	10.08
Copper	5.83	6.92
Lithium	0.27	0.25
Nickel	0.43	0.35
Chromium	0.78	0.62

Phytochemicals in Ripe and Unripe Pericarp Extracts

Preliminary phytochemical analysis of methanol extract of ripe and unripe pericarp showed the presence of flavonoids, saponins, steroids, glycosides and tannins in both the extracts. Terpenoids and alkaloids were not detected in the extracts.

Antibacterial Activity of Ripe and Unripe Pericarp Extracts

Table 3 shows antibacterial activity of ripe and unripe pericarp extracts. Agar well diffusion method was employed to screen antibacterial activity of extracts against Gram positive and Gram negative pathogenic bacteria. The extracts inhibited test bacteria in a dose dependent manner and the inhibitory efficacy was higher in case of ripe extract than unripe extract as evidenced by the wider inhibition zones. However, the inhibition produced by extracts was lesser than that of standard antibiotic. Among bacteria tested, sensitivity was highest in case of *S. aureus*. There was no inhibition observed in case of DMSO.

Cytotoxicity of Ripe and Unripe Pericarp Extracts

Table 4 presents the cytotoxic effect of different concentrations of ripe and unripe pericarp extracts. Both the extracts showed cytotoxic effect and the effect was dose dependent. The ripe extract caused inhibition of HT-29 and MDA-MB-231 cells with IC_{50} value of 125.90 and 132.65µg/ml respectively. Unripe extract was more toxic to HT29 (IC_{50} 116.50µg/ml) than MDA-MB-231 (IC_{50} 139.50µg/ml). Overall, HT29 cells were more sensitive to both the extracts than MDA-MB-231 cells. However, the growth inhibition caused by the extracts was lesser when compared to 5-Fluorouracil (IC_{50} value of 15.94 and 58.18µg/ml for HT29 and MDA-MB-231 respectively). The DMSO being used as extract solubilizing agent at the concentration of 0.1% showed no effect on cell proliferation.

Table 3: Antibacterial activity of ripe and unripe pericarp extracts

Test bacteria	Zone of inhibition in cm (Mean±Standard Deviation)					
	Ripe extract		Unripe extract		Standard	DMSO
	20mg/ml	10mg/ml	20mg/ml	10mg/ml		
E. coli	2.3±0.0	1.7±0.1	2.2±0.1	1.6±0.1	2.8±0.0	0.0±0.0
S. flexineri	2.8±0.0	1.8±0.1	2.0±0.1	1.6±0.1	3.6±0.1	0.0±0.0
V. cholerae	2.4±0.1	1.9±0.0	2.3±0.0	1.7±0.1	3.5±0.2	0.0±0.0
B. cereus	2.4±0.1	1.8±0.0	2.1±0.2	1.6±0.1	3.9±0.2	0.0±0.0
S. aureus	3.2±0.2	3.0±0.2	3.1±0.2	2.8±0.2	4.5±0.2	0.0±0.0

Table 4: Cytotoxic activity of ripe and unripe pericarp extracts

Treatment	Concentration (µg/ml)	% growth inhibition of cell lines			
		HT29		MDA231	
		% growth inhibition	IC_{50} (µg/ml)	% growth inhibition	IC_{50} (µg/ml)
Ripe pericarp extract	200	63.29	**125.90**	65.70	**132.65**
	100	50.95		47.65	
	50	40.75		32.58	
	25	22.89		14.19	
	10	10.98		04.31	
Unripe pericarp extract	200	68.17	**116.50**	61.34	**139.50**
	100	52.64		49.97	
	50	39.97		27.57	
	25	22.73		17.70	
	10	11.90		04.84	
5-Fluorouracil	200	90.95	**15.94**	81.06	**58.18**
	100	82.79		70.62	
	50	70.89		47.83	
	25	57.14		33.33	
	10	45.39		23.54	

Insecticidal Activity of Ripe and Unripe Pericarp Extracts

The insecticidal activity, in terms of larvicidal effect, of ripe and unripe extract was evaluated against III instar larvae of *A. aegypti* and the result is shown in Figure 2. The extracts exhibited dose dependent larvicidal effect. Among extracts, potent larvicidal activity was observed in case of ripe extract when compared to unripe extract. The LC_{50} for ripe and unripe extract was found to be 0.514 and 0.669mg/ml respectively.

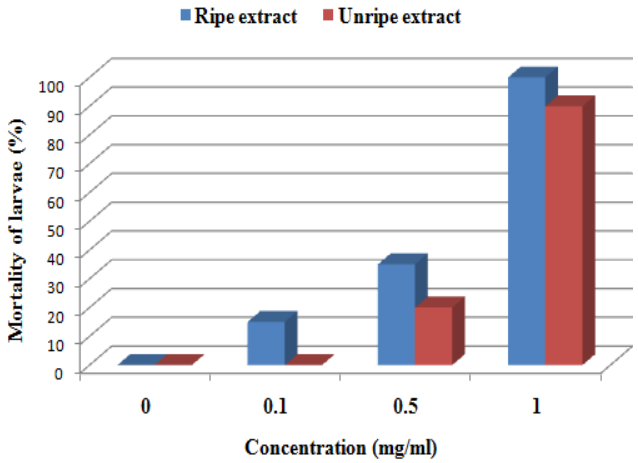

Figure 2: Mortality of larvae at different concentrations of pericarp extracts

DISCUSSION

Mineral Content of Ripe and Unripe Pericarp

Estimation of elements in plant samples requires sample digestion with single or various mixtures of concentrated acids and the sample digestion is done using different digestion equipment such as open beakers heated on hot plates, block digesters and digestion units placed in microwave ovens. The analytical techniques used for estimation of minerals are mainly based on atomic spectrometry with mono-elemental detection, such as flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry. ICP-OES has the advantage due to the multi-elemental determination. Due to this, ICP-OES has become one of the most used techniques for elemental determination and many studies being conducted to validate this method for metals analysis in a large variety of sample types including plant samples (Marin *et al*., 2011; Del Vitto *et al*., 2009; Vinayaka *et al*., 2013). In this study, we have estimated the elemental content of ripe and unripe pericarp of *P. longifolia* by using ICP-OES technique after digestion with nitric acid in microwave digester. The present study revealed that all the major elements were found at high quantity in ripe pericarp than unripe pericarp. Potassium content was highest followed by calcium, magnesium and sodium. All minor elements except copper were found to be present in high quantity in ripe pericarp when compared to unripe pericarp. Among minor elements, iron was detected at high quantity followed by zinc and others. The content of lithium was least.

Antibacterial Activity of Ripe and Unripe Pericarp Extracts

It has been shown that crude extracts and purified components from *P. longifolia* possess antimicrobial activity. A lactone from stem extract caused inhibition of Gram positive and Gram negative bacteria (Faizi *et al*., 2003). Two diterpenoids, isolated from the hexane extract of the seeds demonstrated significant antibacterial and antifungal activities (Murthy *et al*., 2005). Methanol extracts of leaves and green berries were found to possess promising antibacterial activity (Faizi *et al*., 2008). The methanol, acetone and 1,4-dioxan fractions of leaves were shown to exhibit marked inhibitory activity against clinical isolates of Gram positive bacteria and fungal strains (Chanda and Nair, 2010). Flavonoids isolated from extract of bark were found to exhibit promising inhibitory activity against bacteria and fungi (Bose *et al*., 2010). In the present study, we determined antibacterial activity of ripe and unripe pericarp extract of *P. longifolia* against a panel of Gram positive and Gram negative bacteria. The extracts were shown to exhibit dose dependent inhibitory activity against test bacteria. Ripe pericarp extract displayed higher inhibitory activity than unripe pericarp extract. However, the study of Kekuda *et al*. (2014) revealed marked inhibitory activity of unripe pericarp extract than ripe pericarp extract. It is well known that phytoconstituents namely flavonoids (Kanwal *et al*., 2009), steroids (Taleb-Contini *et al*., 2003), saponins (Biswas and Roymon, 2012), tannins (Akiyama *et al*., 2001), glycosides (Nazemiyeh *et al*., 2008) possess antibacterial activity. In our study, preliminary phytochemical analysis of ripe and unripe pericarp extracts revealed the presence of flavonoids, steroids, saponins, tannins and glycosides and presence of these phytoconstituents might be responsible for the observed inhibitory activity of extracts against test bacteria.

Cytotoxic Effect of Ripe and Unripe Pericarp Extracts

It has been shown experimentally that various parts of *P. longifolia* possess cytotoxic effect. Two clerodanediterpenes isolated from seeds of *P. longifolia* were found to exhibit significant cytotoxicity in brine shrimp lethality bioassay (Islam *et al*., 2001).The leaf extract of *P. longifolia* and its chloroform fraction exhibited significant inhibitory activity against cell proliferation of various human cancer cell lines (Verma *et al*., 2008). Ethanol extract of stem bark of *P. longifolia* is shown to exhibit antitumor activity against Ehrlich's ascites carcinoma, Dalton's ascites lymphoma cells, HeLa and MCF-7 cells (Manjula *et al*., 2010). In the present study, the survival of HT-29 and MDA-MB-231 cells in the presence of extracts and 5-fluorouracil (positive control) was assessed by MTT method. MTT assay is a simple and reliable technique for measuring cell viability and can be used for screening of cytotoxic agents. Succinate dehydrogenase, a mitochondrial enzyme, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells (Lee *et al*., 2004). In this study, the methanol extract of ripe and unripe pericarp of *P. longifolia* revealed a dose dependent cytotoxicity against two cell lines HT29 and MDA-MB-231. Antiproliferative activity of crude extracts (Cheng *et al*., 2005; Conforti *et al*., 2008; Boivin *et al*., 2009) and well as phytoconstituents such as alkaloids (Sarikaya *et al*., 2012), tannins (Sakaqami *et al*., 2000), triterpenoids (Lage *et al*., 2010), steroids (Samadi *et al*., 2010), glycosides (Tian *et al*., 2007), saponins (Liu *et al*., 2000) and flavonoids (Yanez *et al*., 2004) of plants have been documented. The cytotoxic effect of ripe and unripe pericarp extracts being observed in this study might be attributed to the presence of phytoconstituents *viz*., steroids, glycosides, saponins, flavonoids and tannins.

Insecticidal Activity of Ripe and Unripe Pericarp Extracts

Insecticides from botanical origin, mainly plant based formulations, comprise of a mixture of phytochemicals and hence there is little chance for pests to develop resistance to such botanicals. *Aedes aegypti* is a vector transmitting arboviral diseases such as dengue fever, urban yellow fever and chikungunya. The life cycle of this mosquito has four stages namely egg, four larval instars, pupa and adult. *A. aegypti* is fundamentally aquatic, however, it reaches the terrestrial environment as an adult (Promsiri *et al*., 2006; Farnesi *et al*., 2012). In the present study, we investigated insecticidal activity, in terms of larvicidal effect of ripe and unripe pericarp extracts against II instar larvae of *A. aegypti*. The extracts displayed marked dose dependent larvicidal effect. In a previous study, Murty *et al*. (1997) showed larvicidal effect of leaf extract of *P. longifolia* against *Culex quinquefasciatus*. It is experimentally shown that various phytoconstituents such as saponins, phytosterols, phenols, flavonoids and tannins possess insecticidal activity. Prenylated xanthones, tetracyclic phenols, flavonoids and saponins are reported to have activity against *A. aegypti* (Marston *et al*., 1993; Khanna and Kannabiran, 2007; Chaieb, 2010; Gautam *et al*., 2013). The larvicidal activity of pericarp extracts could be ascribed to the presence of phytoconstituents present in them.

CONCLUSIONS

The ripe and unripe pericarps of *P. longifolia* were shown to contain various mineral elements in an appreciable quantity. The ripe and unripe extracts displayed marked concentration dependent antibacterial, cytotoxic and larvicidal activity. The observed bioactivities of pericarp extract could be ascribed to the presence of phytoconstituents. There is a great potential for the development of therapeutic agents from ripe and unripe pericarp. Further studies on isolation of active principles from pericarp extracts and their bioactivity determinations are under progress.

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