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Evaluation of anthelmintic, antioxidant and anti-inflammatory potential of methanolic extract of *Artocarpus lacucha* leaves

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ABSTRACT

Artocarpus lacucha is a medicinal plant of Moraceae family and conventionally used in diverse disorder. Investigation and design of this study was carried out to evaluate its antioxidant, anthelmintic and anti-inflammatory activity with methanolic extract of *Artocarpus lacucha* leaves. Thrombolytic activity was evaluated by using *Perthima posthuma*. For antioxidant potential total phenolic content and DPPH free radical scavenging activity test were conducted. Hypnotic solution and heat induced hemolysis was evaluated for anti-inflammatory activity. The methanolic extract of *Artocarpus lacucha* showed a significant anthelmintic activity in dose dependent manner that was comparable with that of standard drugs albendazole (10 mg/ml). Five different concentrations (10, 20, 30, 40 and 50 mg/ml) were used, whereas the albendazole drug at 10 mg/ml used as standard. The total phenolic content of the methanolic extract was found 74.4±0.291 while compared with gallic acid as standard. The IC_{50} value for the DPPH test was found 26.95±0.009. It indicates plant extract showed moderate antioxidant property. In case of membrane stabilizing assay, the methanolic extract (10 mg/ml) showed maximum effect with a value of 15.06 and 20.16% inhibition of hemolysis caused by hypotonic solution and heat respectively. On the other hand standard acetyl salicylic acid (0.10 mg/ml) revealed 53.71 and 56.32% inhibition of hemolysis induced by hypotonic solution and heat correspondingly. These pharmacological activities showed that the methanolic extract has potential anthelmintic, antioxidant and anti-inflammatory activity. Further research would help to reveal all its potency for safer new drugs in medical science.

Key Words: *Artocarpus lacucha,* Anthelmintic, Antioxidant, Anti-inflammatory

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INTRODUCTION

In developing countries like Bangladesh helminthes is a most frequently occurring disease. Despite of development of some arena of health facilities in last previous years, helminthes is still a problem due to malpractice of anthelmintics which develop anthelmintic resistance in nematode. Various available synthetic drugs used for helminthiasis have possible side effects. As a result the search for medicinal plants for anthelmintic potential remains increasing worldwide.[1,2] Again, Oxidative stress generates different reactive oxygen species (ROS) which is accountable for damage of many cellular components and causes several human disorders. ROS causes destruction of DNA, lipids, proteins and release pro-inflammatory cytokines. Antioxidant plays an important role in removal of ROS and has relevance in prevention and therapeutics of diseases. Poly phenolic compounds have been testified for antioxidant activity.[3,4] For this, antioxidant activity research has become a major research recently.

In inflammatory disease, it is believed that lysosomal membrane stabilization inhibits inflammatory reaction by releasing of lysosomal constituents like bacterial enzymes and proteases.[5] Red blood cell (RBC) membrane act as lysosomal membrane and stabilization of RBC membrane resembled lysosomal membrane stabilization. This can be done by drug effects on RBC membrane. Anti-inflammatory agent is the choice of drugs that causes RBC membrane stabilization, subjected to hypnotic stress by releasing hemoglobin (Hb) from RBC.[6]

Artocarpus lacucha is a medicinal plant of the family of Moraceae. It is widely distributed in the Indian subcontinent and South East Asia region.[7] It is known as Monkey Jack. It is deciduous trees with large dense spreading crown which can grow up to 15-18 meters tall spreading head bark roughy.[8] It comprises anti-inflammatory, antiviral, anticancer, anti HIV activity.[9] The ripened and variously modified walls of a plant ovary extract have shown dose dependent antibacterial, antioxidant, anthelmintic and insecticidal activity. Its sap and bark juice is used externally to boils, pimples, cuts and

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wounds. Seeds are used as purgatives. The seed and bark of this plant has a significant role in case of liver disease.[10] Its fruits contain excellent source of antioxidant such as vitamin C and beta carotene.[11] This plant antioxidant has effective role to protects the coronary heart disease and maintain normal health.[8] World Health Organization figures that for primary health care about 80% of the world population rely on traditional medicinal plants.[12] A lot of scientist suggests that use of tropical fruits can minimize the risk of diseases like diabetes, cancer, coronary heart disease, neurodegenerative disorder. It was also established that antioxidant; anti-inflammatory, antibacterial and cytotoxic properties have been found in medicinal plants and used as useful therapeutic compounds.[13]

This present research was conducted to evaluate the anthelmintic, antioxidant and anti-inflammatory potential of methanolic extract of *Artocarpus lacucha* leaves.

MATERIALS AND METHODS

Collection and preparation of plant material

The fresh leaves of *Artocarpus lacucha* were collected from Noakhali, a coastal region of Bangladesh on 26th August 2018 and plant material was taxonomically distinguished by taxonomist and botanist of Bangladesh National Herbarium of Bangladesh whose voucher copy is kept in our laboratory for future reference.

Extraction of plant material

The selected *Artocarpus lacucha* leaves were separated from undesirable parts of plants and dried in sun light for one week and then grounded into coarse powder. The dried 500 gm of powder material was soaked in 1500 ml of distilled methanol. Then the powder material was kept in desiccator at room temperature with periodic stirring and shaking for 20 days. The extract was filtered through whatman filter paper. Then the filtrate of methanolic extract was evaporated under ceiling fan and in a water bath until dried. Thus it rendered a thick greenish black color paste.

Chemicals and reagents

DPPH (1, 1-diphenyl, 2-picrylhydrazyl) was obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine Chem. Ltd. Gallic acid was procured from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Methanol was purchased from Merck, Darmstadt, Germany. Other chemicals required for this present study were provided from pharmacology laboratory of Department of Pharmacy, Noakhali Science and Technology University. All other chemicals and reagents were of analytical grade.

Anthelmintic activity

The anthelmintic activity of metahnolic plant extract was carried out by the method of Ajaiyeoba *et al.*[14] with a slight modification. In this test, *Perthima posthuma* was used because of its similarities of physiological and anatomical features of human intestinal worms. Earthworms were collected from damp soil of Noakhali region and it was washed with normal saline to remove soil and other dust material. Normal saline water was used as control and albendazole (10 mg/ml) was used as reference standard. The earthworms were divided into different groups with equal size & each group containing five worms. 100 ml formulations containing five different concentrations of methanolic extracts of *Artocarpus lacucha* leaves (10, 20, 30, 40 and 50 mg/ml in distilled water) were prepared. The time for paralysis of worms was noted closely. The times of death of the worms were recorded after ensuring that worms neither moved when shaken vigorously or when dipped in warm water at 50°C.

Antioxidant activity

Total phenolic content

The quantity of total phenolic content in plant extract was detected by using Folin-Ciocalteau reagent. It was determined as mg of gallic acid equivalent per gram using the equation found from a standard gallic acid calibration curve.[15]

DPPH free radical scavenging activity

The free radical scavenging activity of the methanolic extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical was determined by the method elucidated by Gupta *et al.*[16] Methanolic extracts of the *Artocarpus lacucha* leaves were fixed in respect to solvent system from which serial dilutions were carried out to obtain the concentrations of 12.5, 25, 50,100 µg/ml. In this assay, 2 ml of 0.1 mM methanolic DPPH solution was added to 2 ml of extract solution at different concentrations and the contents were stirred continuously for 15 seconds. Then the solutions were kept at dark place at room temperature for 30 min for reaction to occur. After 30 min, absorbance was measured against a blank at 517 nm with UV/Visible spectrophotometer (Model 205, Germany). The percentage of DPPH radical-scavenging activity of each plant extract was calculated from:

$$\text{DPPH radical-scavenging activity (I \%)} = [(A_0 - A)/A_0] \times 100$$

Where, A_0 is the absorbance of the control solution (containing all reagents except plant extract); A

is the absorbance of the DPPH solution containing plant extract. IC50 values for the plant extract and standard were obtained by analysis of the respective percentage scavenging of DPPH radical

Membrane stabilizing activity

The membrane stabilizing activity of leaves extract was measured by inhibition of hypnotic solution hemolysis of human erythrocytes following method by Rahman *et al.*[6]

Statistical analysis

All the above assays were conducted in triplicate and repeated three times each for consistency of results and statistical function. The data were expressed as mean±SD and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett't' test using SPSS software versions 20. $p < 0.05$ was considered statistically significant.

RESULTS

Anthelmintic activity

Time required for paralysis and death time of earthworms for mathanolic extract of *Artocarpus lacucha* and reference drug are given in Table 1 and presented in as bar diagram (Concentration Vs Time) in Figure 1.

In case of extract the paralysis time at different concentrations including 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml were 75.50, 66, 53, 41 and 34.50 minutes respectively, while on the contrary death time were 88, 79, 65, 53 and 45.5 minutes respectively. But in comparison to standard drug albendazole (10 mg/ml) the paralysis time was 40 whereas death time was 55 minutes respectively.

Antioxidant Activity

Total phenolic content determination

Total phenolic content of *Artocarpus lacucha* was estimated which is presented in Table 2. Total phenolics of extracts were determined and compared with the standard solutions of gallic acid equivalents using a reference standard curve ($y=0.002x+0.107$; $R^2= 0.889$). The amount of total phenolic content for leaves of *Artocarpus lacucha* was 74.4±0.291 mg of GAE/g. This result suggests that mild antioxidant property.

DPPH radical scavenging activity

The leaves extract of *Artocarpus lacucha* showed the free radical scavenging with IC_{50} value of 26.95±0.009 µg/ml and maximum inhibition found was 84.89%. The standard ascorbic acid showed IC_{50} value of 1.93±0.027 µg/ml and maximum inhibition found was 95.86%. Figure shows the scavenging activity of extracts and standard.

Anti-inflammatory activity

The anti-inflammatory activity of methanolic extract of *Artocarpus lacucha* presented in Table 3 and 4; and Figure 3 and 4. It showed that methanolic extracts dose dependently increase the anti-inflammatory activity, whereas 10 mg/ml concentration significantly presented 15.06 and 20.16% inhibition of hemolysis by hypotonic solution and heat

Table 1 Anthelmintic activity of methanolic extract of *Artocarpus lacucha* leaves

Treatment	Concentration (mg/ml)	Time taken for paralysis in min (mean±SEM)	Time taken for Death in min (mean±SEM)
Control	-	-	-
ME	10	75.5±0.036	88±0.007
ME	20	66±0.071	79±0.077
ME	30	53±.014	65±0.014
ME	40	41±0.071	53±0.035
ME	50	34.5±0.424	45.5±0.007
Albendazole	10	40	55

ME = Methanolic extract.

Table 2 Determination of total phenolic contents of *Artocarpus lacucha* leaves.

Extract	Absorbance of the sample	Average absorbance	Total phenolic content (mg of GAE/g) of extracts
Methanolic extract	1.596	1 ±0.008	74.4±0.291
	1.587		
	1.603		

Values represent mean±SD (n=3) of duplicate analysis.

Table 3 **Effect of extract of leaves of *Artocarpus lacucha* on hypotonic solution induced hemolysis of erythrocyte membrane**

Treatment	Concentration (mg/ml)	Optical density of hypnotic solution	% inhibition of hemolysis
Control	---	3.701±0.003	
ME	2	3.438±0.002***	7.11±0.022
ME	4	3.282±0.002***	11.34±0.007
ME	6	3.250±0.003***	12.19±0.014
ME	8	3.144±0.003***	15.03±0.30
ME	10	3.122±0.002***	15.06±0.041
Acetyl salicylic acid	0.10	1.713±0.001***	53.71±0.005

Values are expressed as mean±SD; ***p < 0.001 significantly different as compared to control

Table 4 **Effect of extract of leaves of *Artocarpus lacucha* on heat induced hemolysis of erythrocyte membrane**

Treatment	Concentration (mg/ml)	Optical density of sample		% inhibition of hemolysis
		Heated Solution	Unheated Solution	
Control	---	1.093±0.010	-	-
ME	2	0.885±0.010***	0.864±0.012***	9.32±0.072
ME	4	0.777±0.047***	0.741±0.053***	10.24±0.113
ME	6	0.719±0.047***	0.657±0.055***	14.30±0.038
ME	8	0.585±0.079***	0.462±0.100***	19.48±0.074
ME	10	0.404±0.060***	0.230±0.077***	20.16±0.047
Acetyl salicylic acid	0.10	0.683±0.027***	0.155±0.071***	56.32±0.158

Values are expressed as mean±SD; ***p < 0.001 significantly different as compared to control.

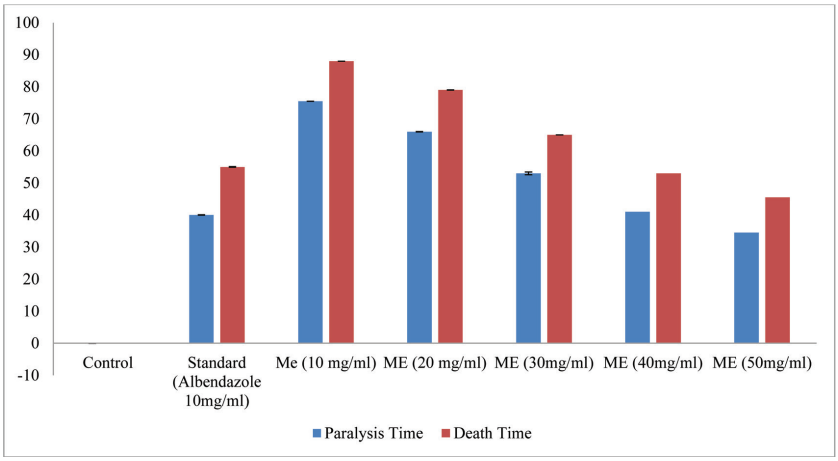

Figure 1 Paralysis time and death time of *Artocarpus lacucha* leaves

induced hemolysis respectively. Acetyl salicylic acid (ASA) 0.10 mg/ml used as reference standard in membrane stabilization which showed 53.71 and 56.32% inhibition of hemolysis, respectively induced by hypotonic solution and heat induced hemolysis.

DISCUSSION

This study was conducted to evaluate the several properties of methanolic extract of *Artocarpus lacucha* leaves including anthelmintic, antioxidant and membrane stability potential.

As this study designed to evaluate the anthelmintic activity it showed that methanolic extract produces dose dependent paralysis ranging from loss of movement which gradually leads to death. Albendazole is an anthelmintic agent causes inhibition of microtubule polymerization by binding to b-tubulin leading to immobilization ranging from paralysis to death of susceptible GI parasites and their clearance from the GI tract require several days after treatment.[17] The concentration of 50 mg/ml extracts showed a high anthelmintic activity in comparison to standard drug albendazole. The time for paralysis and death of worms decreases with the gradual increase in concentration. Previous studies recommended that alkaloid, tannin, phenol and terphnoids may be responsible for anthelmintic activity.[18,19] Further studies have to be carried out for isolation and characterization of the active components to establish an effective drug resource scientifically.

In antioxidant test total phenolic content and DPPH radical scavenging activity was determined. Free radicals are reactive oxygen species which may cause cell and tissue damage. This sort of damage is known as oxidative damage. Several bodily mechanisms are involved in the production of free radicals which may results the production of measurable end products, primarily malondialdehyde.[20] Antioxidants are useful nutrient found in most fruits and vegetables that eliminate the free radicals. DPPH radical scavenging activity determine the ability of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and reacts with an antioxidant compound which can donate hydrogen. Phenolic content can be responsible for the antioxidant activity of the crude extract of *Artocarpus lacucha*. It has been reported to have antioxidative action in biological systems and scavenging of singlet oxygen and free radicals. Previous studies reported that the higher the phenolic contents, the higher its antioxidant potential.[21,22] In our study, we found methanolic extract have significant phenolic contents. In DPPH free radical scavenging activity methanolic extract showed lower scavenging activity than standard ascorbic acid.

The crude methanolic extract of leaves of *Artocarpus lacucha* were subjected to explore anti-inflammatory effect following standard procedure and the attained results were statistically

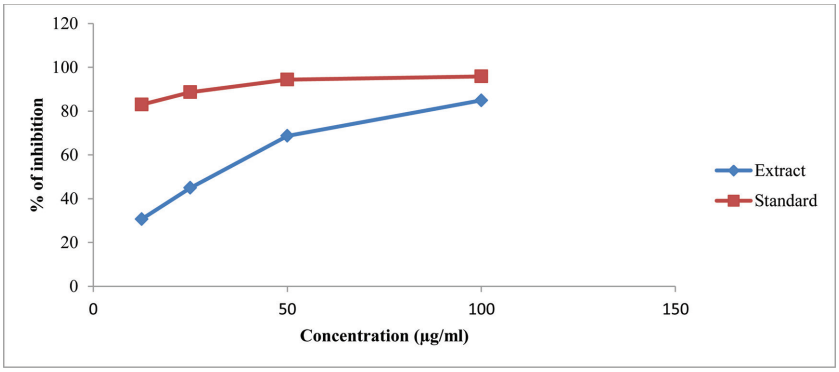

Figure 2 DPPH radical scavenging activity of crude methanolic extract of *Artocarpus lacucha* leaves and standard ascorbic acid

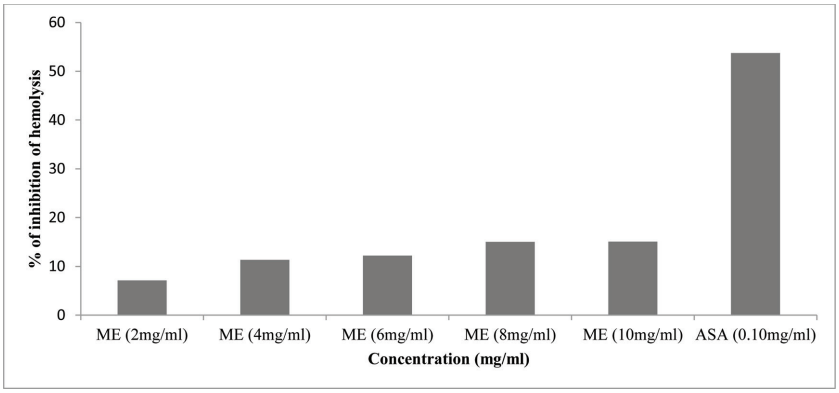

Figure 3 Effect of different conc. of *Artocarpus lacucha* on hypotonic solution induced hemolysis of erythrocyte membrane

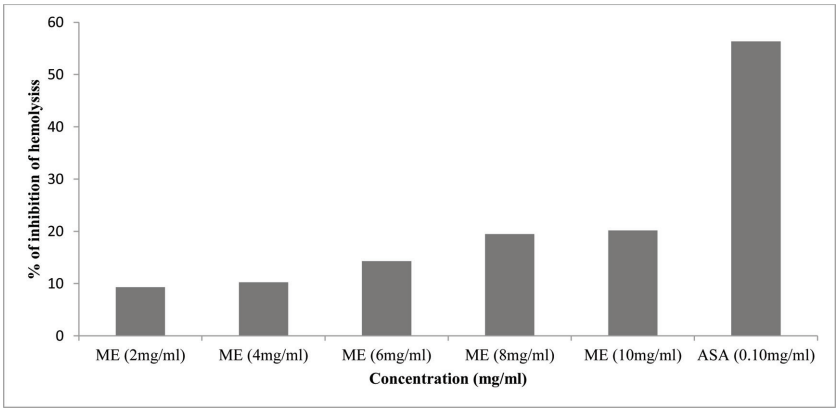

Figure 4 Effect of different conc. of *Artocarpus lacucha* on heat induced hemolysis of erythrocyte membrane

represented in Table 2 and 3; and Figures 3 and 4. It is believe that the vitality of cells depends on the integrity of their membranes.[23] When RBC is exposed to hypnotic solution deposition of fluid within the cell is related to the rupturing of cell membrane. This damage further renders free radical induced lipid peroxidation which in turn leads to cellular damage.[24,25] Therefore it can be expected that compounds with membrane stabilizing properties offer significant protection of cell membrane against injurious substances.[26] The results of the study showed that *Artocarpus lacucha* leaves extract possesses anti-inflammatory property, as it showed membrane stabilizing effect and it offers significant protection of the erythrocyte against lysis induced by hypotonic solution and heat induce condition. Methanolic extract inhibited the hypnotic solution and heat induced hemolysis of RBC at different percentages that was analogous to membrane stabilizing potential by standard acetyl salicylic acid. As standard acetyl salicylic acid showed better activity than experimental methanolic extract, but it can be a good source of anti-inflammatory agent with fewer to no side effects. Many previous experimental studies showed that plants with flavonoids show membrane stabilizing potential.[6]

CONCLUSION

The findings of the present study provide convincing evidence that methanolic extract of *Artocarpus lacucha* leaves possess remarkable anthelmintic, antioxidant and anti-inflammatory activity. However, extensive studies are required to isolate and determination of the bioactive compounds and determine the precise mechanisms is necessary for the noticed biological activities of this plant.

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DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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