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Nutritional, antioxidant and medicinal potential of *Artocarpus lacucha*, an underutilized fruit from Manipur, India

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ABSTRACT

The present study dealt with the nutritional composition, antioxidant potential, and enzyme inhibitory activities of the methanolic extract of *Artocarpus lacucha* fruit. The fresh fruit contained 75.5±2.45% moisture, 0.24±0.04% crude protein, 0.63±0.08% crude fat, and 1.26±0.12% ash. It also included 2.47±0.06% crude fiber, 9.48±0.52% reducing sugar, and 13.41±0.73 mg/100g vitamin C. The total phenolic and flavonoid contents were recorded as 19.6±0.6 mg GAE/g and 8.57±0.8 mg QE/g extract, respectively. The methanolic extract exhibited antioxidant activity with IC_{50} values of 144.0±2.94 µg/ml for the ABTS assay and 300.1±20 µg/ml for the DPPH assay. The FRAP assay indicated a reducing power of 0.161±0.01 mM Fe Eq/g extract. Enzyme inhibitory activities were observed with IC_{50} values of 150±4.6 µg/ml for ACE inhibition, 1050±34.2 µg/ml for tyrosinase inhibition, and 725.4±28.2 µg/ml for xanthine oxidase inhibition, highlighting its potential for pharmaceutical applications.

Figure : 01 References : 18 Tables : 02
KEY WORDS : Angiotensin converting enzyme, Antioxidant activity; *Artocarpus lacucha*; Tyrosinase, Xanthine oxidase

Introduction

Artocarpus lacucha, commonly known as monkey jack, is an underutilised tropical fruit-bearing tree widely recognized for its nutritional and pharmaceutical significance. This species belongs to the Moraceae family and is valued for its diverse bioactive compounds. The fruit, leaves, and bark of *Artocarpus lacucha* have been traditionally used in various cultures for their health benefits. Nutritionally, the fruit is rich in vitamins, minerals, and dietary fiber. The fruit is sweet when ripe and can be eaten fresh and unripen fruits can be used to prepare curries, pickles and sauces[18]. Pharmacologically, *Artocarpus lacucha* is a powerhouse of bioactive compounds such as flavonoids, phenolics, and carotenoids, which contribute to its anti-inflammatory, antimicrobial, and antidiabetic properties. Many *Artocarpus* species are used in Southeast Asian traditional medicine for treating inflammation, malarial fever, ulcers, abscesses, and diarrhea. The leaves stimulate milk production, treat syphilis, and act as a vermifuge. Leaf ash is applied to ulcers, and warm leaves are used for wound healing[8].

Despite its rich nutritional profile and promising pharmaceutical properties, *Artocarpus lacucha* remains largely underutilized and relatively unknown outside its native regions. Often overshadowed by more commercially popular fruits, this species has yet to reach its full potential in both agricultural and medicinal sectors. The underutilization of *Artocarpus lacucha* can be attributed to a lack of awareness, limited cultivation, and minimal research investment compared to other more commonly known fruits and medicinal plants[7].

The pharmaceutical properties of *Artocarpus lacucha* leaf extracts have been extensively studied and documented by various researchers[12]. However, studies specifically focusing on the antioxidant and other

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medicinal properties of *Artocarpus lacucha* fruit extracts are relatively scarce. This gap in research highlights the need for further exploration of the fruit's bioactive compounds and their potential health benefits. Hence the present study dealt with the study on nutritional profiling and pharmaceutical potential of *Artocarpus lacucha* fruit.

Materials and Methods

Collection and identification of plant materials

Plant materials were collected from Takyel Imphal West of Manipur, India (24.797134ºN 93.905842ºE). The *Artocarpus lacucha* tree and fruit are presented in figure 1a and 1b. The leaf of the plant was brought to laboratory and herbarium was prepared. The plant material was identified and authenticated by Dr. Y. Sanatombi Devi, Department of Life Science Manipur University, (accession no. 001351dated 01.08.2023).

Nutritional analysis

The proximate content such as moisture, crude protein, fat, crude fiber, and ash content of fruit sample were determined according to AOAC methods[2]. Carbohydrate content was calculated by difference method. Vitamin C (ascorbic acid) content of the fruit was determined by spectrophotometric method (2,4-dinitrophenyl hydrazine)[15]. Estimation of reducing, non-reducing, total sugar and titratable acidity were performed[13].

Sample preparation for bioactivity assay

The edible pulp of *Artocarpus lacucha* was oven-dried at 50°C and then ground into a fine powder using a kitchen blender. 10 g of the powder was mixed with 100 ml of methanol in a 250 ml flask and shaken at 160 rpm using a Spinix orbital shaker (Tarson) at room temperature for 24 hours. The extraction process was repeated every 24 hours for three consecutive days. The resulting supernatants were combined and filtered through Whatman no. 1 filter paper. After filtration, the pooled extract was concentrated using a rotary vacuum evaporator (IKA, Germany) at 40°C. The dried extract, in solid crystal form, was then transferred to an airtight container and stored at -20°C until further use for bioassay.

Antioxidant assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay

Sample was dissolved in methanol (20 mg/ml) and used as stock for the antioxidant assay. 0.1 ml sample of different concentration was mixed with DPPH solution prepared in methanol (A_{517}=1.0±0.01) incubated for 30min at room temperature at dark. Decolourisation of purple colour was read at 517nm and calculated its percentage radical scavenging activity and IC_{50} value was determined (%RSA)[16].

$$\%RSA = \frac{Acontrol - Asample}{Acontrol} X100$$

Fig. 1(a) : *Artocarpus lacucha* tree

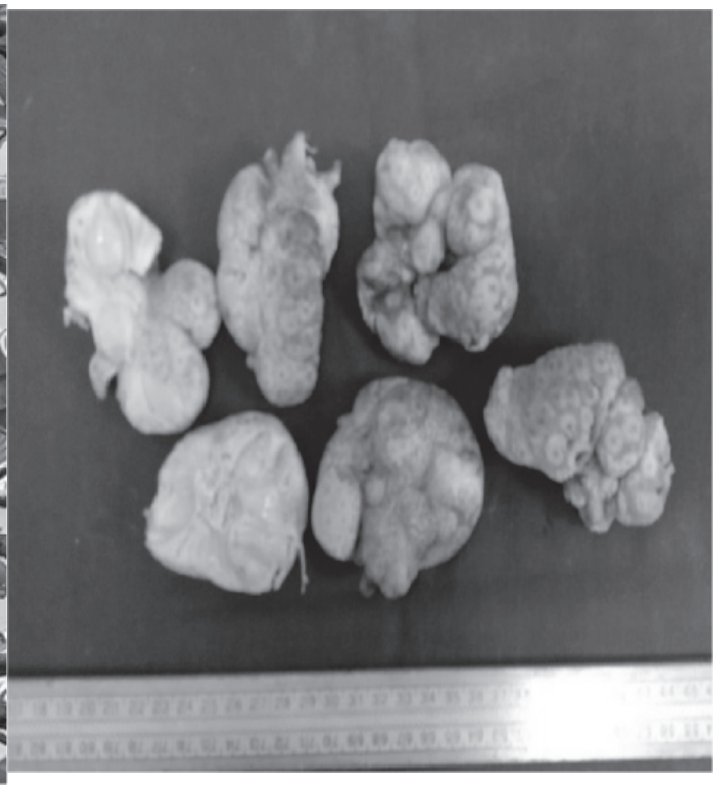

Fig. 1(b) : *Artocarpus lacucha* fruit

2,22 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

ABTS radical scavenging activity was performed[16]. The reaction mixture containing 0.1mL of extract was mixed with 1.9mL ABTS radical (A734=1.0±0.01) then incubated in the dark, and the absorbance was read at 734nm after 30 minutes. RSA and IC_{50} of the extract were calculated as described above

Ferric reducing antioxidant power (FRAP) assay

0.1mL extract was reacted with 1.9mL FRAP reagent and incubated for 5min at room temperature and the absorbance was read at 593nm. $FeSO_4$ solution (5–30µg/mL) was used as standard and ferric reducing antioxidant power was expressed as mM ferrous equivalent per gram of the extract (mM Fe Eq/g Ext.)[16].

Total Phenolic and flavonoid assay

The total phenolic and flavonoid content of the fruit extract was analyzed by Folin's reagent and aluminium chloride methods, respectively[16]. Phenolic content was expressed in milligram of gallic acid equivalent per gram of extract (mg GAE/g), whereas flavonoid content was expressed in milligram of quercetin equivalent per gram of extract (mg QE/g).

Angiotensin I converting enzyme (ACE) inhibitory assay

The ACE inhibitory activity was assessed[9]. Briefly, the 0.5 ml reaction mixture consisted of 40 µmol potassium phosphate buffer (pH 8.3), 300 µmol NaCl, 1.5 µmol hippuryl-L-histidyl-L-leucine (HHL), varying concentrations of fruit extract, and the enzyme (5 mU). The mixture was incubated at 37°C for 30 minutes, after which the reaction was terminated by placing the tubes in a boiling water bath for 10 minutes. Following this, 3.5 mL of 0.2 M phosphate buffer (pH 8.3) and 1.5 mL of triazine solution (3% in dioxane) were added, and the mixture was stirred vigorously until the turbid solution became clear. The tubes were then centrifuged at 10,000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 382 nm. Control samples (without inhibitor) and standard samples (with the inhibitor captopril) were also tested. Percent enzyme inhibition was calculated using the following formula.

$$\text{Percent Inhibition}(\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where Ac=Absorbance of the control at 382 nm, As=Absorbance of the sample at 382 nm

The concentration of the sample required for 50% inhibition of the enzyme (IC_{50}) was also determined.

TABLE-1 : Proximate constituent of *Artocarpus lacucha*

Parameter	% Composition (g/ 100g) FW
Moisture	75.5±2.45
Protein	0.24±0.04
Fat	0.63±0.08
Ash	1.26±0.12
Crude fibre	2.47 ±0.06
Reducing Sugar	9.48±0.52
Non reducing sugar	1.21±0.09
Total Sugar	10.76±0.04
Total Soluble solids	10.76±0.05
Titratable acidity	3.28±0.02
Others carbohydrate	5.86±0.87
Vit C (mg/100g)	13.41±0.73

Tyrosinase inhibitory assay

Anti-tyrosinase activity was assessed using mushroom tyrosinase and kojic acid as a positive control[9]. To evaluate the activity, 20 µL of the enzyme (dissolved in 50 mM phosphate buffer pH 6.5) was mixed with 100 µL of methanolic fruit extract (100 mM phosphate buffer, pH 6.8). The final volume of the reaction mixture was adjusted to 360 µL with assay buffer. Next, 360 µL of dihydroxyphenylalanine (16 mM in assay buffer) was added and the mixture was incubated at room temperature for 5 minutes and absorbance was read at 480 nm. The absorbance of the reaction mixture without the sample served as the control. The percent enzyme inhibition and IC_{50} value was calculated using the above formula.

Xanthine oxidase inhibitory assay

The xanthine oxidase inhibitory activity assay was conducted[7]. 0.2ml different concentration of sample prepared in phosphate buffer (0.1M pH 7.5) and 0.lml

xanthine oxidase enzyme (0.2U/ml) was mixed in a 2ml tube then preincubated for 15minutes at 37°C. The reaction was started after the addition of 0.1ml substrate (0.15M xanthine) and incubated for 30minutes at 37°C. After 30 minutes, 0.1ml HCl (0.5M) was added and read at 293nm. Allupurinol was used as standard drug. For control reaction, 0.2ml buffer and 100µl enzyme was used and for the reagent blank substrate was added only after addition of HCl. The percent enzyme inhibition and IC_{50} value was calculated using the above formula.

Results

Nutritional composition of *Artocarpus lacucha* fruit

The matured fresh fruit of *Artocarpus lacucha* contained moisture (75.5±2.45%), crude protein (0.24±0.04%), crude fat (0.63±0.08%), and ash (1.26±0.12%), crude fiber (2.47 ±0.06%), reducing sugar (9.48±0.52%), non-reducing sugar (1.21±0.09%), total sugar (10.76±0.04%), total soluble solids (10.07±0.05%) and other carbohydrates (5.86±0.87%). Titratable acidity and Vitamin C content was recorded as 3.28±0.02% and 13.41±0.73 mg/100g (Table-1).

Pharmaceutical properties of *Artocarpus lacucha* fruit extract

The total phenolic and flavonoid content of the fruit extract were recorded as 19.6±0.6 mg GAE/g and 8.57±0.8b mg GAE/g respectively. The results for antioxidant activity and enzyme inhibitory activities of the methanolic extract of *Artocarpus lacucha* are summarized in (Table-2). The IC_{50} values for ABTS and DPPH radical scavenging activities were recorded as 144.0±2.94 µg/ml and 300.1±20 µg/ml, respectively. In comparison, the standard ascorbic acid showed IC_{50} values of 3.0±0.45 µg/ml for the ABTS assay and 5.4±0.3 µg/ml for the DPPH assay. Additionally, in the FRAP assay, the methanolic fruit extract exhibited a reducing power of 0.161±0.01 mM Fe Eq/g extract. The methanolic extract of *Artocarpus lacucha* fruit exhibited IC_{50} values of 150 ± 4.6 µg/ml for ACE inhibitory activity, 1050 ± 34.2 µg/ml for tyrosinase inhibitory activity, and 725.4 ± 28.2 µg/ml for xanthine oxidase inhibitory activity.

Discussion

The *Artocarpus lacucha* fruit contained high amount of moisture (75.5 ± 2.45%). It was reported[17] that *Artocarpus lacucha* fruit contained 82% moisture. The presence of reducing sugar (9.48 ± 0.52%) and total sugar (10.76 ± 0.04%) suggests that the fruit is a good source of instant energy, which is typical of fruits that are consumed for their energy content [5]. The relatively high titratable acidity (3.28 ± 0.02%) and Vitamin C content (13.41 ± 0.73 mg/100g) contribute to its potential antioxidant and preservative properties, which are beneficial for overall health[4].

The fruit is rich in phenolic (19.6 ± 0.6 mg GAE/g extract) and flavonoid content (8.57 ± 0.8 mg QE/g extract) which reflects its antioxidant potential. Phenolic compounds are widely recognized for their ability to neutralize free radicals and reduce oxidative stress, which can mitigate the risk of chronic diseases[15]. Some workers[12] have studied the antioxidant activities in various parts (leave, barks, twig and fruits) of *Artocarpus lacucha* ethanolic extract. The total phenolic and flavonoid content in ethanolic fruit extract was reported as 0.94±0.11 g GAE/100 g extract and 1.66±0.25 g QE/ 100 g extract. The IC_{50} values for ABTS and DPPH radical scavenging activities of the present fruit extract were recorded as 144.0 ± 2.94 µg/ml and 300.1 ± 20 µg/ml, respectively, indicating moderate antioxidant activity. The standard ascorbic acid has displayed higher

TABLE-2 : Bioactivity of *Artocarpus lacucha* fruit extract

Bioactivity	Methanol fruit extract	Positive control
DPPH (µg/ml)	300.1±20	Ascorbic acid:5.4±0.3
ABTS (µg/ml)	144.0±2.94	Ascorbic acid:3.0±0.4
FRAP (mM Fe Eq/g sample)	0.161±0.01	Ascorbic acid:10.78±0.16
ACE inhibitory (µg /ml)	150±4.6	Captropril: 0.005
Tyrosinase inhibitory (µg /ml)	1050±34.2	Kojic acid: 28.5±2
Xanthine oxidase (µg/ml)	725.4±28.2	Allopurinol :77±0.08

antioxidant activity than the studied fruit extract.

The methanolic extract of *Artocarpus lacucha* fruit demonstrated inhibitory activities against ACE (angiotensin-converting enzyme), tyrosinase, and xanthine oxidase. To the best of our knowledge, this is the first report detailing the ACE and xanthine oxidase inhibitory activities of *Artocarpus lacucha* fruit extract. As for the antityrosinase activity, it was reported that a combination of *Artocarpus lakoocha* and *Glycyrrhiza glabra* extracts exhibited tyrosinase inhibitory activity and melanin pigment reduction[11].

The methanolic extract's IC_{50} value for ACE inhibitory activity (150 ± 4.6 µg/ml) is lower than those for tyrosinase (1050 ± 34.2 µg/ml) and xanthine oxidase (725.4 ± 28.2 µg/ml). This suggests that the fruit extract is particularly effective in inhibiting ACE, which is relevant for hypertension management[10]. Previous studies have highlighted the significance of ACE inhibitors in managing blood pressure and cardiovascular health, making *Artocarpus lacucha* fruit a promising candidate for further exploration in this area[1].

Xanthine oxidase plays a crucial role in purine metabolism by catalyzing the oxidation of hypoxanthine to xanthine and subsequently xanthine to uric acid[6]. The enzyme's involvement in the generation of reactive oxygen species (ROS) links it to oxidative stress and various pathological conditions, including gout and cardiovascular diseases. Inhibitors of xanthine oxidase, such as allopurinol, are commonly used in the treatment of hyperuricemia and its associated disorders[3]. The antioxidant and xanthine oxidase inhibitory properties of *Artocarpus lacucha* suggest its potential as a pharmaceutically valuable compound for managing these conditions.

Conclusion

Artocarpus lacucha fruit exhibits a unique profile with antioxidant activity and significant ACE inhibitory potential. Its ability to inhibit ACE suggests potential therapeutic benefits, particularly in cardiovascular health. The fruit's moderate levels of phenolic and flavonoid compounds, along with its enzyme inhibitory activities, underscore its potential use in dietary supplements or functional foods. Further research is warranted to explore its potential therapeutic applications and its bioactive compounds.

Declaration of conflict of interest

The authors declare that they have no conflict of interest.

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