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Phytochemical Screening and Evaluation of Antioxidant Potential of Ethanolic Extract of *Artocarpus lacucha* Leaves *In-vitro*

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ABSTRACT

Antioxidants, particularly the polyphenolics, defend cells from the oxidative injuries caused by free radicals originated during the cellular biochemical processes. Plants are the natural stock of phytochemicals having antioxidant capacity. This investigation explored the presence of different class of phytochemicals as well as the antioxidant potentials of the crude ethanolic leaf extract (EAL) of *Artocarpus lacucha* (Fam: Moraceae). All the tests were carried out using established procedures. Antioxidant capacity of the EAL was determined based on the total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and DPPH free radical inhibiting activity estimation. Phytochemical tests detected the occurrence of polyphenols, flavonoids, alkaloids and tannins in the EAL. The EAL contained 426.11±35.02 mg GAE/g of phenolics, 415.87±12.81 mg QE/g of flavonoids, and 123.48±11.24 mg GAE/g of tannins. In DPPH scavenging test, the EAL showed IC_{50} value 29.65 µg/mL compared to standard ascorbic acid with IC_{50} value 9.12 µg/mL. The presence of polyphenolic, flavonoid, and tannin compounds in *A. lacucha*, may be the likely contributors of the observed antioxidant properties. Further comprehensive study is required to ascertain the specific compound or group of compounds responsible for these effects.

BACKGROUND

As of now, plants remain the most abundant source of pharmaceutical intermediates, nutraceuticals, and nutritional supplements, modern and traditional medicines. The presence of some bioactive phytochemicals could be the cause. Therefore, the use and popularity of plant-based medicines is widely and steadily rising day by day. Above 80% of people of the developing nations, rely on herbal medications to meet their basic medical needs, according to World Health Organization (Canter *et. al.,* 2005).

Oxidative stress (OS) indicates the disproportion between the synthesis of reactive oxygen species (ROS) and the cellular ability to detoxify them and to restore the damage caused by ROS. When these disruptions take place, ROS like peroxides and free radicals accumulates, which can be detrimental to the cell as a whole. Certain ROS, like superoxide, have the ability to transform into more potent radical species that can seriously harm cells. By oxidizing proteins, oxidizing DNA, or lipid peroxidation, excessive OS levels induce necrosis. This damage results in ATP depletion, which halts controlled apoptotic cell death and leaves the cell in a state of simple disintegration. (Evans *et. al.,* 2004; Lee *et. al.,* 1999; Sies, 1997 & Valko *et. al.,* 2004).

When free radicals interact with biological macromolecules in healthy human cells, such as proteins, lipids, and DNA, they cause damage to those components and become stable. The pathophysiology of diabetes, cancer, atherosclerosis, cardiovascular disease, aging, and inflammatory illnesses has all been linked to the harm caused by OS (Braca *et. al.,* 2002 & Maxwell, 1995). Weakened cellular antioxidant defense systems may cause the damage to spread more widely. An antioxidant defense mechanism exists in all biological systems, which guards them against oxidative damage and fixes enzymes to eliminate harmed molecules.

Antioxidants inactivate or neutralize the radicals by electron donation and thus prevent the radical induced cellular injuries. Additionally, antioxidants convert the injurious radicals into waste products that are eventually eliminated. Antioxidants, present in dietary components such as fruits and vegetables and supplements may thus potentially prevent or lower the risk of ailments like heart diseases, stroke, altitude sickness and cancer by averting OS (Baillie *et. al.,* 2009 & Willett, 2002). A variety of bioactive phytochemicals, including polyphenols,

carotenoids, vitamins especially E and C, have been linked to these positive effects (Steinmetz *et. al.,* 1996).

Numerous plant products, including grains, fruits, vegetables, oils, spices, and herbs (both edible and inedible) contain phenolic chemicals. (Kahkonen *et. al.,* 1999 & Miliauskasa *et. al.,* 2004). However, scientific evidences related to the antioxidant capabilities of endemic plants, which are exclusively present in certain places and are only known to the local community are not adequate. Thus, assessing the antioxidant activities of these plants is still a fascinating option while looking for novel, promising natural antioxidant sources for dietary supplements and/or functional meals (Miliauskas *et. al.,* 2004 & Balasundram *et. al.,* 2006).

Artocarpus lacucha, a tropical evergreen tree of about 15-18 meters' height, is often referred to as monkey jack or monkey fruit (Family: Moraceae) (*Artocarpus lacucha*, 2014). It is extensively dispersed throughout Southeast Asia and the Indian subcontinent (Oudhia *et. al.,* 2008). The leaves are leathery, elliptic, pointed, and alternating, ranging in length from 10 to 25 cm. When combined with goat milk and other herbs, fruits have historically been used to treat skin conditions, arthritic swelling, diarrhea, and clean wounds. Indian healer's uses bark topically to remove poisons from the body. The stems are used to eradicate tapeworms. Applying powdered bark on wounds helps extract foreign matter. It is said that the bark and seed can effectively treat liver and stomach ailments.

A number of previous studies reported different pharmacological activities of *A. lacucha*. *A. lacucha* heart wood aqueous extract exhibited neuroprotective and antiglycation activities (Hasriadi *et. al.,* 2017 & Pandey *et. al.,* 2021). Fruit extract increases liver function and showed hepatoprotective effect in experimental mice (Saleem *et. al.,* 2018). Islam *et. al.,* reported significant anti-nociceptive activity of A. lacucha bark extract (Islam *et. al.,* 2019). *In-vivo* experiments using animal models found anti-inflammatory, analgesic, antidiarrheal, cytotoxic, anti-hyperlipidemic, proliferative, and wound-healing properties in leaf extract of *A. lacucha* (Nesa *et. al.,* 2015; Nazliniwaty *et. al.,* 2022 & Shafaqa *et. al.,* 2022).

As far as we are aware, no research has been done on the antioxidant potential of *A. lacucha* leaves. The current study assessed the antioxidant

properties of the ethanolic extract of *A. lacucha* leaves (EAL) *in-vitro* and conducted a phytochemical screening test to find different kinds of antioxidant components as well as other secondary metabolites.

MATERIALS AND METHODS

Collection of Fresh Leaf

Fresh *A. lucucha* leaves were collected in February 2023 from Narayanganj. Leaves were segregated from unwanted elements and plant fragments and shade dried for 15 days. Leaves were shade dried to prevent any deterioration of the phytochemicals and active ingredients might cause by direct sun. Then, the leaves were ground into coarse powder using an appropriate grinder. The powdered plant materials were kept in dry, cold, and dark environment inside an airtight container.

Preparation of Leaf Extract

The crude extract of the leaf powder was prepared using 95% ethanol following a previously reported method (Tiwari *et. al.*, 2011). Five hundred (500) milliliters of ethanol were added to a reagent bottle containing about 415 g of coarse leaf powder. After sealing, the bottle was stored for 15 days with occasional shaking for better extraction. The mixture found after passing through a cotton plug, was filtered using Whatman filter. To obtain a semisolid mass, the filtrate was concentrated under vacuum below 50°C using a rotary evaporator. Upon drying, the concentrated extract, known as the crude ethanolic extract of leaves (EAL), produced a paste with a deep green color. About 6.74% of crude extract was produced from 415 g of dried leaf powder.

Chemicals

Aluminium chloride, methanol, gallic acid (GA), and quercetin (QE) were supplied by Qualikems Fine Chem Pvt. Ltd. in Nandesari, Sodium carbonate and folin-Ciocalteu reagent (FCR) were supplied by Merck Life Science Private Ltd. in Mumbai. Ascorbic acid (AA), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), sodium hydroxide, sodium nitrite, and distilled water were procured from Merk, India via local vendors.

Phytochemical Screening

Phytochemical screening of the EAL was performed using the method of Trease *et. al.,* 1989.

Total Phenolic Content Assay (TPC)

The phenolic content of the EAL was tested using modified Folin-Ciocalteu method (Wolfe *et. al.,* 2003). In test tubes, 0.5 mL EAL solution at graded concentrations (conc.), 5 mL FCR reagent (10 times dilution water) and 4 mL 7% Na_2CO_3 solution were added; followed by 15 seconds vortexing and keeping at 40°C for 30 minutes to complete the reactions. Absorbance of each reaction mixture was taken at 765 nanometers was measured using a UV-visible spectrophotometer with a blank in place. The same process was used to prepare the blank with the exception of adding the extract. The total phenolic of the EAL based on GA equivalents, GAE/g of extract was estimated using the formula below:

$C = (c \times v)/m;$
where,
C = total phenolic compounds, mg/g extract, in GAE.
c = conc. of GA obtained from calibration curve (mg/mL).
v = volume of extract solution in mL.
m = weight of extract in g.

Total Flavonoid Content Assay (TFC)

Total flavonoids in the EAL were ascertained by using aluminum chloride in the colorimetric method (Shraim *et. al.,* 2021). In this test, 1 mL of EAL solution at different concentrations was mixed with 4 mL water and 3 mL 5% w/v sodium nitrite solution in a test tube. After 5 minutes reaction time, 0.3 mL of 10% $AlCl_3$ solution, 2 mL 1M NaOH solution, and 2.4 mL water were added to the test tubes. To ensure color development, the mixtures were left at room temperature (RT) for 15 minutes. Using a UV-visible spectrophotometer, the absorbance at 510 nm counted against blank. All reagents, except the EAL or standard contained in the blank (Miliauskas *et. al.,* 2004). The total flavonoid compound in the EAL in Quercetin equivalents (QE/g of extract) was derived by the given formula:

C = (c × v)/m; where,
C = total flavonoid compounds, mg/g EAL, in QE.
c = conc. of QE measured from the calibration curve.
v = volume of extract in mL.
m = weight of EAL in g.

Total Tannin Content Assay (TTC)

Folin-Ciocalteu method with a few modifications was adopted to count total tannin in the EAL (Tambe *et. al.*, 2014). In a test tube, 0.5 mL FCR (10 times diluted), 7.5 mL water, and 0.1 mL EAL were mixed. Then 0.9 mL distilled water and 1 mL 35% sodium carbonate were added; followed by vortexing for 15 seconds and incubating at RT for 30 minutes. Finally, absorbance of all mixtures was recorded at 725 nm with a blank in place. The process for making the blank was same, except the addition of extract or standard (Amorim *et. al.*, 2008). The total tannins of the EAL in GAE/g of EAL was counted with the given equation:

C = (c × v)/w; which indicates,
C = total tannins, mg/g of EAL.
c = conc. of GA based on calibration curve.
v = volume of EAL in mL.
w = weight of EAL in g.

DPPH Free Radical Scavenging Assay

Free radical scavenging capacity of the EAL was found using the established DPPH method (Uddin *et. al.*, 2009). To 1 mL solution of EAL test tubes, 2 mL DPPH solution were combined. Then 15 seconds mixing was followed by 30 minutes' reaction time in dark at RT. Absorbance counted spectrophotometrically at 517 nm in relation to control. The same procedures were followed to prepare the control sample, except adding the EAL or standard AA. The formula below was utilized to get DPPH radical scavenging ability of the EAL and standard:

$$\% \text{ inhibition of DPPH} = \frac{A_b - A_s}{A_b} \times 100$$

Where, A_b= absorbance of the control, and A_s= absorbance of the EAL solution

The EAL and AA concentrations were plotted against the percentage of DPPH radical inhibition to get half-maximal inhibitory concentration, IC_{50}, of the EAL and AA which reduced DPPH radical to 50%.

STATISTICAL ANALYSIS

Each experiment was performed three times in exactly the same way. The data was calculated in mean ± standard deviation (SD). Statistical and graphical analysis of experimental data was executed using Microsoft Excel 2007 (Roselle, IL, USA).

RESULTS AND DISCUSSION

Phytochemical Screening

The purpose of the phytochemical screening tests was to find out the types of phytochemicals and secondary metabolites present in the EAL. As shown in Table-1, the EAL contained alkaloids, glycosides and a relatively higher concentration of phenolics, flavonoids, and tannins.

Table-1: Secondary metabolites found in the EAL

Phytochemical Group	Test performed	Results
Carbohydrates	Fehling's test	-
	Molisch's test	-
	Benedict's test	-
Alkaloids	Mayer's test	+
	Hager's test	+
	Wagner's test	+
	Dragendroff's test	+
Glycosides	Legal test	+
	Keller-Kiliani test	+
	Conc. H_2SO_4 test	+
	$FeCl_3$ solution test	++

Phenolics	Diluted HNO_3 test	++
	Lead acetate test	++
Flavonoids	General test	++
	Lead acetate test	++
	$FeCl_3$ test	++
Tannins	$FeCl_3$ test	++
	Lead acetate test	++

++ = Indicates significantly present, + = Indicates present, - = Indicates absent

Total Phenolic Content Assay (TPC)

Polyphenols, the crucial plant derived biomolecules show antioxidant activity because of their free radicals free radical scavenging or deactivating capability (Soobrattee *et. al.*, 2005). The total amount of phenolic found in the EAL was 426.11±35.02 mg GAE/g of EAL (Table-2). This result suggests that the EAL's antioxidant activity could partly be accredited to the occurrence of polyphenolic components in it.

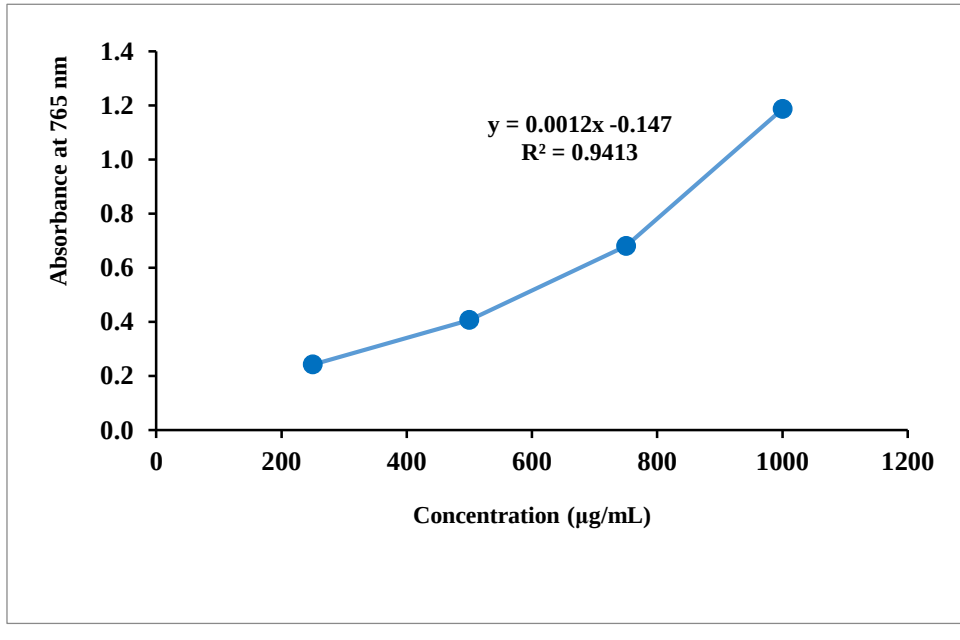


Figure-1: GA calibration curve for the determination of TPC.

Table-2: Determination of TPC in the EAL.

Mass of dry extract/mL	Absorbance	GAE concentration	GAE concentration	TPC as GAE	Mean	SD	Mean ± SD
		c	c	C			
		(µg/mL)	(mg/mL)	(mg/g)			
mg					(mg/g)		
0.001	0.402	457.500	0.458	457.50	426.11	35.02	426.11 ± 35.02
0.001	0.319	388.333	0.388	388.33			
0.001	0.372	432.500	0.433	432.50			

Each experiment was conducted thrice. Values above displayed as mean ± SD.

Total Flavonoid Content Assay (TFC)

Flavonoids are other important groups of antioxidant compounds are able to scavenge and neutralize the free radicals (Panche *et. al.*, 2016). The total flavonoid count of the EAL was 415.87±12.81 mg QE/g of dry extract (R^2 = 0.9906), as shown in Table-3. This result advocates that the presence of flavonoid molecules might be another contributor for the antioxidant activity of the EAL witness during this study.

Figure-2: Quercetin calibration curve for the determination of the TFC.

Table-3: Determination of TFC in the EAL.

Mass of dry extract/mL	Absorbance	QE concentration	QE concentration	TFC as QE	Mean	SD	Mean ± SD
		c	c	C			
		(µg/mL)	(mg/mL)	(mg/g)			
mg					(mg/g)		
0.001	0.741	411.769	0.412	411.77	415.87	12.81	415.87 ± 12.81
0.001	0.765	430.231	0.430	430.23			
0.001	0.733	405.615	0.406	405.62			

Each experiment was conducted thrice. Values above displayed as mean ± SD.

Total Tannin Content Assay (TTC)

The amount and degree of polymerization of hydroxyl groups in a molecule dictates its ability to scavenge free radicals. Because plant tannins can be oxidized more easily, the higher the number of hydroxyl groups they have, the greater will be their antioxidant property (Ariga *et. al.*, 1988 & Olejar *et. al.*, 2016). The total tannin levels of the EAL were 123.48±11.24 mg GAE/g of EAL, as indicated in Table-4. These findings demonstrate the likely contribution of tannin constituents in the antioxidant action of the EAL.

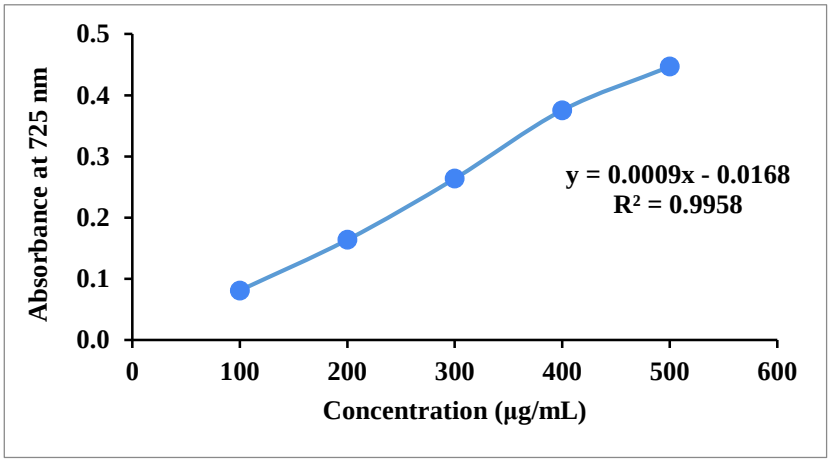


Figure-3: Gallic acid calibration curve for the TTC calculation.

Table-4: Determination of TTC in the EAL.

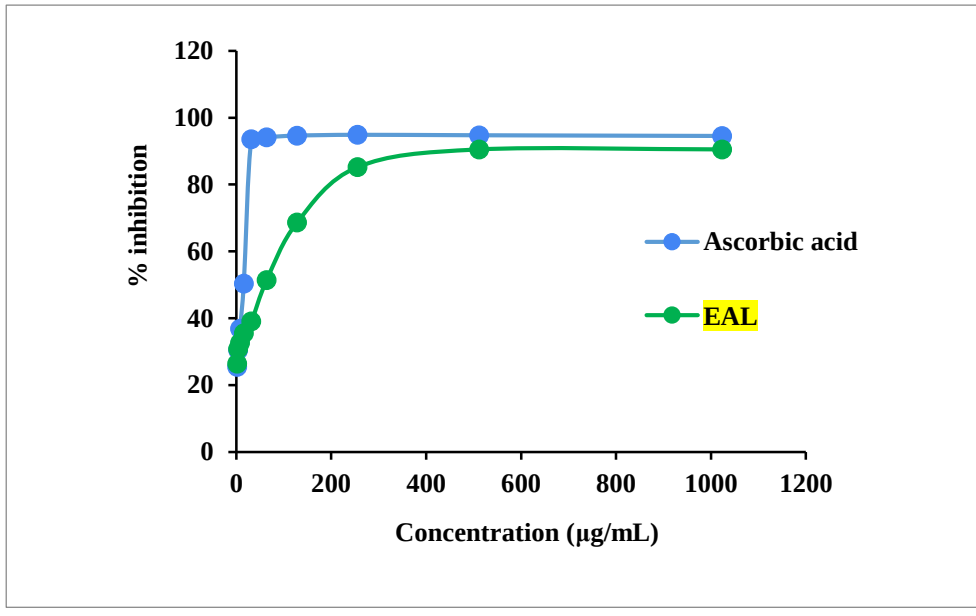
Mass of dry extract/mL	Absorbance	GAE concentration	GAE concentration	TTC as GAE	Mean	SD	Mean ± SD
		c	c	C			
		(µg/mL)	(mg/mL)				
mg				(mg/g)	(mg/g)		
0.001	0.106	136.444	0.136	136.44	123.48	11.24	123.48 ± 11.24
0.001	0.089	117.556	0.118	117.56			
0.001	0.088	116.444	0.116	116.44			

Experiments were carried out in triplicate. Values are displayed as mean ± SD.

DPPH Radical Scavenging Assay

Radical scavenging capacity is crucial to defend the injurious role of free radical induced OS, which triggers the development of many diseases with

cancer. DPPH radical scavenging is a highly recognized and extensively used method to predict ways by which antioxidants obstruct free radical generation.



Flavonoids or polyphenolics demonstrated notable capacity to scavenge or inhibit free radicals (Saeed *et. al.*, 2012). DPPH method assessed the antioxidant potential of the EAL in relation to its capacity to scavenge DPPH free radicals, with ascorbic acid serving as the reference standard. The result showed (Table-5) that the EAL has significant free radical inhibiting ability with IC_{50} value 29.65 µg/mL, relating to standard ascorbic acid with IC_{50} value 9.12 µg/mL. This outcome suggests that the EAL constituents can function as major antioxidants by donating protons to scavenge free radicals.

Figure-4: DPPH radical scavenging assay of the EAL.

Table-5: DPPH radical scavenging assay of the EAL

Group	**IC_{50} (µg/mL)**
Ascorbic acid	9.12
EAL	29.65

CONCLUSION

This investigation revealed that the EAL have significant antioxidant properties. This outcome might be ascribed to the occurrence of some secondary metabolites or bioactive substances like polyphenolics, flavonoids and tannins. Further extensive investigation is necessary to identify bioactive substances with antioxidant properties might be useful for finding antioxidant-based alternatives and preventive therapies for oxidant causing human diseases.

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