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ORIGINAL RESEARCH

Phytochemical Screening and Determination of Antioxidant and Antimicrobial Activity in Crude Extract of *Annona muricata*

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Abstract

Annona muricata (Linn) is a member of the Annonaceae family, which includes more than 2000 species and 119 genera. Although the plant is native to the warmest tropical regions of North and South America, it is currently widely distributed throughout the world's tropical and subtropical regions, including Nigeria, Malaysia, and India. A. muricata is an herbaceous plant whose leaves, bark, roots, fruits, and seeds are all utilized as natural cures for a range of ailments. The fruits and seeds have analgesic and antidiarrheal properties and are used to treat worm infestations and parasite diseases. The leaves, bark, and roots are utilized for their anti-effects that are sedative, antimalarial, antispasmodic, anti-inflammatory, and anticonvulsant. Secondary metabolites present in the plant exhibit strong anticancer effects and selective toxicity against a variety of malignant cells, without endangering healthy cells. The objective of the study was to identify the antioxidants and phytochemicals found in the leaves, seeds, and bark of A. muricata. The antibacterial property of the plant was examined using well diffusion method against different strains of microorganisms.

Keywords

Annona muricata, Phytochemical studies, Antioxidant property, Antibacterial activity

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1. Introduction:

The usage of medicinal herbs dates back thousands of years, almost as old as humankind itself. The variety of species employed, and their healing potential is great. Any plant that has compounds that can be used therapeutically or can act as a precursor for the creation of effective medications in one or more of its organs is considered a medicinal plant. These herbs also can enhance flavor, preserve food, and stop the spread of infectious diseases. The biological traits of plant species that are used all over the world are typically caused by the secondary metabolites that the plants generate. Different plant parts, such as seeds, roots, stems, flowers, leaves, rhizomes, barks, and bulbs, contain the active principles of a plant that exhibits therapeutic action. some of the most common medicinal plants include Holy Basil, Cinchona, Belladona, Opium Poppy Sarpagandha etc.

Annona muricata is an evergreen tree that stands erect and reaches a height of 5 to 7 meters. Its broad, smooth leaves are a dark green color *(Moghadamtousi et al., 2015)*. All the parts of the plant are widely used in traditional medication for treating human diseases and ailments, to mention a few, such as arthritic pain, headaches, diabetes, parasitic infections, and even cancer *(Mishra et al., 2013; De Sousa et al., 2010)*. The medicinal properties of the plant mainly reside in their bioactive components *(Agu & Okolie 2017; Baskar et al., 2007)*. The effective phytochemicals and secondary metabolites have been isolated that include mainly Annonaceous acetogeneins; essential oils, phenolic compounds, and megastigmanes found in parts of the *Annona muricata* plant *(Agu, et al., 2017; Coria-Téllez et al., 2016)*.

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Phytotherapeutic properties of *A. muricata* have been extensively illustrated in several reviews and articles such as antidiabetic, hypolipidemic, and renal protective *(Mohammed et al., 2021; Karou et al., 2011)*. Fruit and leaf infusions have been used to treat fever *(Magaña et al., 2010)*; respiratory-related problems *(Kossouoh et al., 2007; Vandebroek et al., 2010)*; gastrointestinal problems *(Samuel et al 2010; Atawodi 2011)*. In recent years it has been known to inhibit enzyme processes found on the membranes of tumor cells and is widely used in cancer treatments *(Monlgatti et al., 2013)*.

Annona muricata extracts from the leaves, stems, roots, and seeds exhibit antibacterial action against a variety of diseases *(Sundarrao et al., 1993)*. Alkaloids, flavonoids, sugars, glycosides, saponins, tannins, terpenoids, and proteins are among the phytochemicals found in *A. muricata (Edeoga et al., 2005)*. According to *Padma et al. (2001)*, *A. muricata* is used to treat inflammatory illnesses including the flu and cough. The current investigation identified the phytochemical components, antioxidant qualities, antimicrobial activity, protein, and starch contents found in different *Annona muricata* sections.

2. Materials and Methods:

2.1. Specimen collection and analysis:

The plant specimen (*Annona muricata*) *(Figure 1)* was collected locally. The stem of the plant was separated and carefully cleaned with tap water. The plant was allowed to air dry in the shade for four weeks, removing the leaves, bark, and seeds. The milling machine was used to evenly grind the plant samples into powder and the decoction was used for further studies

Fig 1: *Annona muricata plant and fruit*

2.2. Preparation of various solvent extracts:

The dry powder was soaked in distilled water for 12 hours to create an aqueous extract of the sample. The extract was used for additional research after being filtered with Whatman filter paper. A paper cone containing 5 g of powdered *A. muricata* leaves and bark was put inside the Soxhlet apparatus and extracted using methanol, ethanol, and water in a solvent-solvent partition process *(Figure 2)*. 100 ml of methanol was taken in the round bottom flask attached to this setup. The heating mantle was then used to support the entire system. The temperature was adjusted to between 65 and 80 °C.

Methanol evaporates, rises to the condenser, and then condenses back into liquid. It then descends into the cone containing the plant sample and extracts certain components, which fall into the flask with a circular bottom *(Satyanarayana, 2006)*. The aqueous extract and ethanol underwent the same procedure twice.

Fig 2: *Soxhlet extraction of leaf and bark samples of Annona muricata by different solvents*

2.3. Phytochemical screening :

Qualitative assays for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids, proteins, and anthraquinone were conducted on the leaf and bark solvent extracts by the protocol outlined by *Harborne et al. (1973)*. The following tests were carried out: protein content was estimated by the Biuret and Ninhydrin test by color change; alkaloids by Mayer's test by forming precipitate of Mayer's reagent, flavonoids by Shinoda test by formation of colored complex, carbohydrates estimation by Benedict's test and the Molisch's test. The Keller-Killani test for cardiac glycosides, the Froth test for saponins, the Lead acetate test for tannins, the Salkowski test for terpenoids, and the Ammonia test for anthraquinone. These tests are collectively used in identifying and quantifying various secondary metabolites in plant extracts. The phytochemical, pharmacological, and antidiabetic properties have widely gained attention from this plant species *(Zubaidi et al., 2023)*.

2.4. Quantitative Investigation :

Using the Anthrone method, the amount of starch was quantitatively assessed *(Hedge et al., 1962)*. Lowry's method *(Lowry et al., 1951)* was used to quantitatively analyze the estimation of protein. Based on the steady 1, 1-diphenyl 2 picryl hydroxyl (DPPH) free radical activity's radical scavenging ability, the antioxidant activity of the plant extracts was evaluated. The DPPH radical scavenging activity of solvent extracts of leaf, bark, and seeds of *Annona muricata* was determined *(Yen and Wu 1999)*. Ascorbic acid (2 mg/ml) was used as standard, and the sample was measured in triplicates. The percentage inhibition of absorbance was calculated with the concentration of antioxidants against ascorbic acid as standard reference data.

2.5. Antibacterial Activity Assay:

The well diffusion method was used to assess the antibacterial activity of *A. muricata* solvent extracts. *Pseudomonas aeruginosa, Klebsiella oxytoca,* gram-positive *Bacillus subtills*, and gram -ve *Escherichia coli* were the test bacteria that were obtained from IMTECH, Chandigarh. The bacteria were swabbed on Muller Hinton agar medium plates and the test microbial culture was spread plated. The wells were created and filled with phytochemical extracts of 100 µl and allowed to diffuse. After 24 hours of incubation at 37 °C, the zones of inhibition were assessed *(Neglo et al., 2021)*.

3. Results & Discussion:

3.1. Phytochemical analysis:

In the table, summary of the outcomes of the phytochemical screening of many leaf and bark solvent extracts. According to Table 1, the Annona muricata plant exhibits the existence of phytochemical elements such as alkaloids, flavonoids, proteins, carbohydrates, glycosides, tannins, terpenoids, and anthraquinones. In 2017, Agu et al. 2017 and Agu & Okolie 2017 measured and reported on these phytochemicals.

Table 1: *Annona muricata aqueous, methanol, and ethanol extracts were screened qualitatively for phytochemicals.*

Sl. No.	Leaf/Bark extract	Ethanol extract	Methanol extract	Water extract
1	Test for alkaloids • Mayer's test	+ +	+ +	- -
2	Test for Flavonoids • Shinoda's test	+ +	+ +	+ +
3	Test for carbohydrates • Benedict's test • Molisch's test	+ + +	+ + +	+ + +
4	Test for glycosides • Borntrager's test • Keller-Killani test	- - +	- - +	- - +
5	Test for proteins • Ninhydrin test • Biuret test	+ + +	+ + +	+ + +
6	Test for saponins • Form test	+ +	+ +	+ +
7	Test for tannins • Lead acetate test	+ +	+ +	+ +
8	Test for terpenoids • Salkowski test	- -	- -	+ +
9	Test for anthroquinones • Ammonia test	- -	- -	+ +

3.2. Quantitative evaluation of Annona muricata aqueous extract:

When compared to the leaf and bark, *Annona muricata* aqueous extract shows that the seed has a larger protein content (Lowry's method) and starch content (Anthrone method).

Table 2: *Annona muricata's aqueous extract's protein and starch contents*

Aqueous extract of *Annona muricata*	Protein content (µg/ml)	Starch content (µg/ml)
Leaf	120	15
Seed	137	43
Bark	81	13

3.3. Free radical scavenging ability of Annona muricata:

Ascorbic acid Equivalent Antioxidant Capacity (AEAC), a DPPH radical scavenging activity, was used to assess the antioxidant activities of various solvent extracts of leaves, bark, and seeds. A drop in absorbance is measured via radical scavenging. According to the ascorbic acid standard graph, the methanolic leaf extract and the aqueous seed extract show good potential for antioxidants at 1.09 ± 0.03. The highest absorbance of DPPH, a stable free radical, in ethanol is measured at 517 nm. The indicator of a drop in absorbance is called radical scavenging *(Shimada et al., 1992)*.

3.4. Antimicrobial Activity Assay:

Gram-positive and gram-negative bacteria were tested for the antibacterial ability of *A. muricata* ethanolic leaf and bark extract. The plates with *Bacillus subtilis* did not exhibit an evident zone of inhibition (measured in mm). Whereas the leaf and bark extracts were effective against gram negative bacteria, *Escherichia coll, Pseudomonas aeruginosa,* and *Klebsiella oxytoca (figure 3)*. The leaf extract was effective against *Pseudomonas aeruginosa* and bark extracts against *Escherichia coli* and *Klebsiella oxytoca (Table 3)*. The extracts of A. muricata have been evaluated for modulatory effect and employed against biofilm-forming MRSA *(Neglo et al., 2021)*

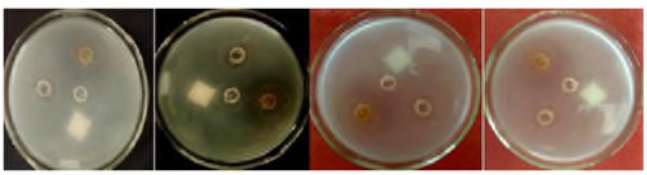

Fig 3: *Antibacterial activity of ethanolic extract of Annona muricata against (A) Pseudomonas aeruginosa; (B) Bacillus subtilis; (C) Klebsiella oxytoca and (D) E. coli*

Table 3: *Antibacterial activity of ethanolic extract of Annona muricata*

Sl. No.	Microorganism	Zone of inhibition (mm)	
		Leaf extract	Bark extract
1	*Pseudomonas aeruginosa*	6	0
2	*Bacillus subtilis*	1	3
3	*Klebsiella oxytoca*	3	6
4	*E. coli*	5	6

4. Conclusion:

A. muricata plant parts are widely used in traditional medicine for treatment protocol of various ailments. Our findings suggest that *Annona muricata's* bark and leaf extracts, whether in aqueous, methanol, or ethanolic form, contain a variety of phytochemicals with antibacterial and antioxidant properties. *A. muricata* may therefore be a potential source of phytocompounds with several avenues available for the development of formulations for treatment. It is therefore need of the hour to identify ancient plants, validate their importance, and promote their protection and cultivation.

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