

Research Article

Received on: 08-05-2015
Accepted on: 17-05-2015
Published on: 15-06-2015

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Pharmacognostic and Antimicrobial Screening of Leaf Extracts of *Annona muricata*

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ABSTRACT

Background: Medicinal plants have continued to attract attention in the global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs obsolete in the treatment of infections.

Aim: To determine the pharmacognostic and antimicrobial effect of the leaf of *Annona muricata*.

Materials and methods: The pharmacognostic evaluation of the leaf of *A. muricata* was carried out using microscopic, macroscopic, chemomicroscopic, and phytochemical methods; while the antimicrobial activity was determined using agar cup diffusion technique.

Results: The Chemomicroscopic evaluation revealed the presence of lignin, starch, and cellulose. Phytochemical analysis revealed the presence of alkaloids, tannins, saponins and carbohydrates. The extract showed varied inhibitory effects against the test-microorganisms *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans* at concentrations 10-100 µg/ml.

Conclusion: The results of this study demonstrated the antimicrobial potential of *Annona muricata* and the basis for its inclusion in herbal preparations used to treat infectious diseases.

Key-words: Pharmacognostic, antimicrobial activity, *Annona muricata*

Cite this article as:

Felix N. Osuala, Sylvester C. Ohadoma, Pharmacognostic and Antimicrobial Screening of Leaf Extracts of *Annona muricata*, Asian Journal of Pharmaceutical Technology & Innovation, 03 (12); 2015. www.asianpharmtech.com

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INTRODUCTION

The emergence of strains of potentially pathogenic microorganisms that are resistant to most routinely used antibiotics has put pressure on the continuous search for plants that have antimicrobial properties. *Annona muricata* Linn (Annonaceae) is a low branching, bushy tree and native to Caribbean and Central America but now widely cultivated in tropical climate throughout the world.^[1] It is also known as soursop due to the slightly acidic taste of the riped fruit; chop-chop in colloquial English, akpummiri in Igbo, tuwobiri in Hausa, ekooyinbo in Yoruba all in Nigeria; guanabana in Spain, sauersack in Germany, anonemuriquee in French, graviola in Portugal, and mstafeli in Swahili.^[2] It is reputed to be a versatile medicinal remedy against many diseases.^[1] The leaves are said to possess antispasmodic action and are used in Jamaica and Brazil for the management of bronchial spasm.^[3] Antidiabetic action and are used in Peru.^[4] Other reported actions of *A. muricata* included anticancer and anti-herpes ^[7,6] and antihyperlipidemia^[8] In this study, *Annona muricata* was screened pharmacognostically to determine the secondary metabolites present and investigate the possible antimicrobial activities.

MATERIALS AND METHODS

Collection and preparation of plant materials:

The fresh leaves of *Annona muricata* were collected from Elele, Rivers State, Nigeria, and authenticated at the Department of Pharmacognosy, Madonna University, Elele, where herbarium specimen was deposited. Three hundred grams of the ground leaves previously dried in the oven at 50% were extracted with successive quantities of chloroform and methanol in soxhlet extractor. The extracts were concentrated under reduced pressure in a rotary evaporator and the yields were recorded (Table 1).

Macroscopic and microscopic evaluations:

The microscopic character of the leaf –size, shape, surface, venation, petiole, apex, margin, base, texture, odour, colour; and microscopic characters-stomata (type and distribution), epidermal cells (nature), epidermal trichomes (type and distribution), cell inclusions (calcium oxalate crystals) were carried out using the standard method.^[10] The epidermal membranous layers of *A. muricata* leaf was carefully peeled off and mounted on a clean slide with dilute glycerine after clearing with chloral hydrate. The transverse section of the leaf fragment was covered with a clean cover slide and observed under the microscope (x 400 magnification).

Phytochemical screening:

Phytochemical analysis of the methanol and chloroform extracts were carried out using the standard procedure ^[9]

Chemomicroscopic screening:

The chemomicroscopic analysis of the two extract were done using the standard method [10]. For lignin test, then powdered sample was placed in a few drop of phloroglucinol and concentrated HCl, and observed under the microscope for a pink colour. For starch test, the powdered sample was placed in N/50 iodine solution and observed for a blue-black coloration. For calcium oxalate test, the powdered sample was palced in concentrated HCl and observed under the microscope for disappearance of oxalate crystals which indicate a positive test, for cellulose test, the powdered sample was placed in iodine and 80% H₂SO₄ and observed under the microscope and observed for a blue-black colour, which indicates a positive test.

Antimicrobial screening:

Agar cup diffusion method was employed to determine the activity of the crude extracts on the following: Gram-negative and Gram-positive bacteria and fungi: *Staphylococcus aureus* NCTC 6571; *Bacillus subtilis*

(Lab.stock); *Escherichia coli* NCTC 9001; *Pseudomonas aeruginosa* NCTC 6750; *Aspergillus niger* and *Candida albicans* (Lab. Stock). A 0.1 ml of a 10⁻² dilution of an overnight broth culture of each organism (containing an inoculum size 10⁶-10⁷ cells/ml) seeded into molten but cooled 20 ml nutrient agar (pH 7.4 oxide) was used for bacteria and Sabouraud dextrose agar (pH 5.4 Oxide) was used to prepare fungal plates by surface spread method [11] The extracts prepared 20 and 100 µg/ml were dropped into the 7 mm diameter wells bored in the agar. Ampicillin (Beecham, England) 25 µg/ml and 1% w/v tioconazole (Pfizer, NY) 0.5 mg/ml were used as positive control, and 50% methanol or chloroform as negative control. The plates were incubated at 37°C for 24 h for bacteria and at 25°C for 48-72 h for fungi. The diameter of the zone inhibition was measured to determine antimicrobial activity (Table 2).

RESULTS

The percentage yields of extracts were higher with methanol (14.94%) than with chloroform (4.64%) (Table 1). The microscopy showed a size of 6.25 ± 20 cm long, 2.5 ± 6.25 cm wide, oblong shape, smooth surface, pinnate venation, 1.25 ± 5 cm petiole, acuminate apex, entire margin, acute base, glossy texture when fresh, dark-green colour at the upper surface, light green colour beneath, brown colour when dry, and aromatic odour. The microscopy showed epidermal cellwall that is straight and beaded; epidermal trichomes present on both upper and lower epidermis. Chemomicroscopy revealed the presence of lignin, starch, and cellulose, and phytochemical analysis showed the presence of alkaloids, tannins, saponins, and carbohydrate. With respect to the antimicrobial activity, *Annona muricata* extracts showed a slight antimicrobial activity at 20 mg/ml but very significant broad-spectrum activity (active on all the tested organisms) at 100 mg/ml.

DISCUSSION

The percentage yield of methanol and chloroform extracts was in the preponderence of the former. This may mean that the majority of the constituents are in highly polar form.[12] The microscopic and microscopic characteristics

are in agreement with annonaceae^[2,1,13] The chemomicroscopy showed the presence of lignin, starch and cellulose but revealed the absence of calcium oxalate. This report is consistent with the finding in pharmacological screening of plant decoctions commonly used in cuba folk medicine.^[5] The phytochemical screening showed that *A. muricata* were rich in secondary metabolites such as alkaloids, tannins and saponins which are very important constituents when searching for pharmacologically active compounds. Further, the antimicrobial activities recorded against the test organisms showed that *Annona muricata* leaf extract had broad spectrum of activity. The high content of saponin and tannin could be the basis of its antimicrobial action.^[13] which is in accordance with the claim that plants rich in saponins and tannins have antimicrobial property.^[14].

Table 1: Percentage yield of *Annona muricata* Linn

Plant	part used	Solvent	% yield
<i>Annona Muricata</i> Linn	leaf	Chloroform	4.64
		methanol	14.94

Table 2: Phytochemical constituents of *Annona muricata* Linn

Plant	Part used	solvent	saponins	cardenolids	Tannins	Alkaloids	steroids	anthraquinones
<i>Annona Muricata</i> Linn	Leaf	chloroform	++	-	++	++	++	-
		methanol	+++	++	+++	+++	++	+

Table 3: Antimicrobial screening of *A. muricata* extracts

Sample	Extract	conc (mg/ml)	S. aureus	B. subtilis	E. coli	P. aeruginosa	Asp. niger	C. albicans
<i>A. muricata</i>	chloroform	20	10.0	10.0	9.0	-	11.0	11.5
		100	11.0	11.0	11.0	12.0	15.0	14.0
	Methanol	20	11.0	11.0	11.0	-	11.0	11.0
		100	12.0	12.0	12.5	14.0	16.0	16.0
Ampicillin		25µg/ml	20.5	19.0	18.0	15.0	NT	19.0
Tioconazole		0.5mg/ml	NT	NT	NT	NT	NT	20.0

- = not active; NT = not tested; cup diameter = 7mm; comparative analysis of antimicrobial activity of three cephalosporins on clinical isolates.

CONCLUSION

The antimicrobial activities of *A. muricata* in this study justified the traditional use of this plant in the treatment of infectious disease and encouraged further studies with the view to isolating and characterizing the specific active components of the plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

Source of support: Nil

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