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Phytochemical analysis and antibacterial activities of aqueous and ethanolic crude extracts of soursop (*Annona muricata*) leaves against selected clinical pathogens

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Abstract

This study investigates phytochemical composition and the antimicrobial activities of aqueous and ethanol extracts of soursop (*Annona muricata*) leaves against some selected bacterial pathogens. Susceptibility effects of these extracts were determined by disc diffusion method against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Streptococcus pyogenes* and *Staphylococcus aureus.* The results showed that the efficacy of the aqueous extracts was more pronounced against the test bacterial, especially *P. aeruginosa* at minimum inhibitory concentration (MIC) of 0.2 mg/mL. The ethanol extract was more pronounced again *S. pyogenes* with diameter of zones of inhibition ranging from 15.00 – 32.00 mm at concentrations of 0.2 – 0.8 mg/mL respectively. The pathogens were susceptible to the aqueous extracts of the leave at concentrations between 0.4 – 0.8 mg/mL. Phytochemical compounds such as tannins, saponins, terpenoids, alkaloids, glycosides, and phenols were detected in *A. muricata* leaf extract. The significant antimicrobial properties of the leaf extract could be attributed to the presence of these bioactive compounds. Thus, this investigation proves to an extent that the Soursop leaf extracts when used against microorganisms, has sufficient antimicrobial property.

Keywords: *Annona muricata*; Antibacterial; Clinical pathogens; Crude extracts; Phytochemical

1. Introduction

The use of plants as medicine is a worldwide phenomenon; plants not only provide safe and cost effective remedies, they are also available and accessible at affordable prices. The use of resources already available, forms the basic core of any public health practice and what is better than plants as medicine as they are associated with fewer side effects and no known resistance to microorganisms[1]. Ethno medicine may be broadly defined as the use of plants by humans as medicines but can be more accurately called ethnobotanic medicine [2].

Traditional system of medicine which depends mainly on medicinal plants is rich in ethnomedical knowledge of the uses of medicinal plants in the treatment of infectious conditions [3]. These medicinal plants that are employed in traditional medicine, represents potential sources of cheap and effective standardized herbal medicines (phytomedicine) and leads in the discovery of novel molecules for the development of new chemotherapeutic agents. Several infectious diseases including malaria, diarrhea, dysentery, gonorrhea and fungal infections have been successfully managed in traditional medical practice employing medicinal plants [4].

The effectiveness of chemotherapeutic agents depend s on many factors, some of which include; the route of administration and location of the infection, the presence of interfering substances, the concentration of the drug in the body, the nature of the pathogen, the presence of drug allergy, and another factor that should not be overlooked is the resistance of microorganisms to the drug. A great number of antibacterial agents exist for various purposes; some of

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these are usually in the form of plants. The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, phlobatannins, flavonoids, and a host of other chemical compounds referred to as secondary metabolites that are present in them [5]. Medicinal plants have played a major role in the treatment of various diseases including bacterial and fungal infections.

Annona muricata or infamously known as soursop is one of Malaysian exotic fruits from family *Annonaceae*. The tree is a low branching and bushy but slender plant. It can reach a height of 25 to 30 ft. It is a typical tropical tree with heart shaped edible fruits with which the flesh is white and juicy. The leaves are lanceolate with glossy and dark green in color. This species are widely distributed in most of tropical countries [6]. *A. muricata L*. has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use. All parts of the soursop plant are used in natural medicine in the tropics, including the bark, leaves, roots, fruit, and fruit seeds. Different properties and uses are attributed to the different parts of the tree. Generally, the fruit and fruit juice are taken for worms and parasites, to cool fevers, to increase mother's milk after childbirth, and as an astringent for diarrhea and dysentery. The crushed seeds are used against internal and external parasites, head lice, and worms. The barks, leaves, and roots are considered sedative, antispasmodic, hypotensive, and nervine, and a tea is made for various disorders toward those effects [7].

Along with the increasing public interests on medicinal plants, recently there are lots of researches done on various potential medicinal plants, One of them is *Annona muricata* (*A. muricata*) commonly known as soursop. However, previous research on *A. muricata L*. has focused on the bark of the tree and roots for pharmaceutical purposes [8] and little attention has been paid to the leaves which is more commonly used in traditional medicine remedies. To date, there are only few research publications about phytochemical screening of *A. muricata L.* leaves and their antimicrobial activity against Gram-positive and Gram-negative bacteria [9].

Preliminary phytochemical analysis revealed the presence of secondary metabolites like tannins, steroid, cardiac glycosides, etc. were present in trace amounts in the leaves of *A. muricata* [10]. Other phytochemical analysis of the nbutanolic leaf extract of *A. muricata* revealed the presence of flavonoids, terpenoids, tannins, cardiac glycosides and reducing sugars. Whereas, the extract showed the absence of saponins, steroids, phlobatannins, oil and anthraquinones tested [11]. The phytochemical screening of the *A. muricata* different plant parts also showed the presence of flavonoids, terpenoids, reducing sugar, anthraquinone, tannins and cardiac glycosides. Phytoconstituents in the leaves of *A. muricata* contain an alkaloidal principle named 6-Hydroxyundulatine and other alkaloids [12]. The aims of this study are to determine the phytochemical constituents in the leaves of *Annona muricata*, and to examine the efficacy and potency of the crude aqueous and ethanolic extracts of *Annona muricata* leaves against some clinical pathogens.

2. Material and methods

2.1. Collection of sample

The leaves of *Annona muricata* was collected at Falegan Area of Ado-Ekiti, Nigeria in August, 2021. The leaves were identified in the Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti. The fresh samples were washed under running tap water, air-dried at room temperature of 25 ^{o}C for about 2 weeks and milled into fine powder using a Thomas Willey Milling machine and then stored in sterile container for further analysis.

2.2. Isolation and identification of organisms

Different isolates were obtained from Federal Medical Centre (FMC) Ido, Ekiti State. These isolates include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Streptococcus pyogenes*. The bacteria were identified using conventional methods and were maintained on Nutrient agar slants at 4 ^{o}C in the refrigerator until required.

2.3. Extraction of bioactive components from the plant material

Extraction method described by Ajenifuja *et al*. [13] was employed. One hundred grams (100g) of the powdered plant material (*A. muricata*) was poured into different beakers and 500 ml and 300 ml of distilled water and methanol were poured into each beaker respectively. The contents are stirred using a sterile glass rod and allowed to stand for 24 hours at room temperature (25 ^{o}C ± 1). The contents were filtered through a filter paper (Whatman No. 1) and the filtrate concentrated and evaporated using water-bath at the temperature of +95 ^{o}C. Extracts are then kept at 20^{o}C prior use.

2.4. Phytochemical screening of plant extract

2.4.1. Test for alkaloids

0.4 g of the plant extract was diluted with 8 mL of 1% HCl and the mixture was then boiled and filtered. 2 mL of filtrate was treated separately with (a) few drops of potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids.

2.4.2. Test for saponins

The ability of saponins to produce emulsion with oil was used for the screening test. 20 mg of plant extract was boiled in 20 mL of distilled water in a water bath for 5 min and then filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and was shaken vigorously for froth formation. 3 drops of olive oil was mixed with froth, shaken vigorously and was observed for emulsion development.

2.4.3. Test for terpenoids

Presence of terpenoids in the plant extract was carried out by taking 5 mL (1 mg/ml) of the extract and mixed with 2 mL of chloroform, and then followed by 3 mL of concentrated H_2SO_4. A reddish brown coloration of the interface confirmed the presence of terpenoids.

2.4.4. Cardiac Glycosides Determination

5 ml (10 mg/ml in methanol) of the plant extract was mixed with 2 mL of glacial acetic acid having one drop of $FeCl_3$ solution. To the mixture obtained 1 mL of concentrated H_2SO_4 was then added to form a layer. The presence of brown ring of the interface indicated deoxyl sugar characteristic of cardiac glycolsides (Trease and Evans 1989).

2.4.5. Test for tannins

50 mg of the plant extract was boiled in 20 mL of distilled water and filter. A few drops of 0.1% $FeCl_3$ was added in the filtrate and observe for colour change; brownish green or a blue-black coloration was taken as evidence for the presence of tannins [4].

2.4.6. Test for Phenols

A portion of the extract was treated with aqueous 5% ferric and observed for formation of deep blue or black colour.

2.5. Reactivation of organisms

Colonies were picked with a flamed inoculated loop and cultured in test tube of McConkey broth, incubated in an incubator at 37^{o}C for 18 hours. Subsequently, a loop full of the suspension was streaked on McConkey agar and incubated at 37^{o}C for 24 hours.

2.6. Determination of the Degree of Antibacterial Potency

The disk diffusion method described by Clinical and Laboratory Standard Institute [14] was employed. Various concentrations of the extracts were prepared in test tubes (2.5 mg/ml – 0.5 mg/ml). Disks obtained from Whatman No. 1 filter paper was sterilized in an oven at 160^{o}C for 30 minutes and soaked in the extracts for 24 hours. A loopful of the final dilution (10^3) of the test bacterial suspension was spread on an oven-dried nutrient agar. The disk of different concentrations of the extracts were placed at equidistance on the agar and incubated at 37 ^{o}C for 24 hours. Zones of inhibition were measured in millimeters (mm) with a meter rule. Whatman No. 1 filter paper disks were placed at the center of each agar plates as a control.

2.7. Statistical Analysis

Three replicates of each sample were taken and experiments were repeated thrice. The statistical analysis was done by ANOVA and significance of differences between replicates were measured at 5% ($P<0.05$).

3. Results

The phytochemical constituents present in soursop leaves are shown in table 1. The phytochemicals screened are found to be presents in sample at certain percentages and amount. The quantity of phenols, terpenoids and tannins and were found to be 12.536 mg/100 g, 8.78 mg/100 g and 2.59 mg/100 g respectively. Alkaloids had the highest percentage value of 2.21%. Saponins and glycosides are low in quantity with values of 0.45% and 0.0039% respectively.

Table 2 shows the antibacterial activity of aqueous extracts of *A. muricata* on *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. pyogenes* and *S. aureus*. *E. coli* had the highest diameter of zones of inhibition of 18.00 – 32.00 mm at the concentrations of 0.4 – 0.8 mg/mL respectively, no zone of inhibition at 0.2 mg/mL concentration, *P. aeruginosa* had diameter of zones of inhibition of 15.00 – 22.00 mm at the concentrations of 0.2 – 0.8 mg/mL; *S. pyogenes* had diameter of zones of inhibition of 13.00 – 20.00 mm at the concentrations of 0.4 – 0.8 mg/mL respectively, no zone of inhibition at 0.2 mg/mL concentration. *K. pneumoniae* had no zone of inhibition at 0.2 mg/mL concentration but had 15.00 – 21.00 mm at concentrations of 0.4 – 0.8 mg/mL respectively. *S. aureus* only had zones of inhibition of 15.00 mm and 21.00 mm at concentrations of 0.6 mg/mL and 0.8 mg/mL respectively.

The antibacterial activity of the ethanol extracts of *A. muricata* leaf on the test organisms is presented in table 3. *S. pyogenes* had the highest diameter of zones of inhibition of 15.00 – 32.00 mm at the concentrations of 0.2 – 0.8 mg/mL respectively. *P. aeruginosa* also had diameter of zones of inhibition of 10.00 – 32.00 mm at the concentrations of 0.2 – 0.8 mg/mL; *E. coli* had diameter of zones of inhibition of 19.00 – 27.00 mm at the concentrations of 0.2 – 0.8 mg/mL respectively. *K. pneumoniae* had zones of inhibition of 13.00 – 24.00 mm at concentrations of 0.2 – 0.8 mg/mL respectively. *S. aureus* had lowest diameter of zones of inhibition of 11.00 – 19.00 mm at the concentrations of 0.2 – 0.8 mg/mL respectively.

Table 1 Phytochemical screening of soursop (*Annona muricata*) leaves

Parameters	Qualitative	Quantitative
Alkaloid	+	2.21%
Saponins	+	0.45%
Glycosides	+	0.0039%
Tannins	+	2.59 mg/100 g
Terpenoids	+	8.78 mg/100 g
Phenols	+	12.536 mg/100 g

Table 2 Antibacterial activity of aqueous extracts of soursop (*Annona muricata*) leaf on selected human pathogens

Test organisms	Diameter of zones of inhibition (mm)				
	Concentrations (mg/mL)				
	0.2	0.4	0.6	0.8	Control
Escherichia coli	0.00 ± 0.00^{a}	18.00 ± 0.01^{c}	26.00 ± 0.00^{b}	32.00 ± 0.00^{d}	Nil
Klebsiella pneumoniae	0.00 ± 0.00^{a}	15.00 ± 0.02^{b}	17.00 ± 0.04^{c}	21.00 ± 0.02^{d}	Nil
Pseudomonas aeruginosa	15.00 ± 0.02^{b}	18.00 ± 0.01^{c}	19.00 ± 0.03^{d}	22.00 ± 0.01^{a}	Nil
Streptococcus pyogenes	0.00 ± 0.00^{a}	13.00 ± 0.03^{d}	19.00 ± 0.02^{b}	20.00 ± 0.01^{c}	Nil
Staphylococcus aureus	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	15.00 ± 0.01^{c}	21.00 ± 0.02^{d}	Nil

Values are mean ± SD of triplicate determination; Samples with different superscripts within the same column were significantly ($p\leq0.05$) different

Table 3 Antibacterial activity of ethanol extracts of soursop *(Annona muricata*) leaf on selected human pathogens

Test organisms	Diameter of zones of inhibition (mm)				
	Concentrations (mg/mL)				
	0.2	**0.4**	**0.6**	**0.8**	**Control**
Escherichia coli	19.00±0.02^{b}	19.00±0.02^{b}	19.00±0.02^{b}	27.00±0.01^{a}	Nil
Klebsiella pneumoniae	13.00±0.03^{a}	16.00±0.02^{c}	19.00±0.02^{b}	24.00±0.03^{a}	Nil
Pseudomonas aeruginosa	10.00±0.02^{b}	11.00±0.02^{c}	22.00±0.01^{b}	32.00±0.02^{a}	Nil
Streptococcus pyogenes	15.00±0.03^{b}	28.00±0.01^{d}	29.00±0.03^{c}	32.00±0.03^{a}	Nil
Staphylococcus aureus	11.00±0.02^{a}	17.00±0.01^{c}	19.00±0.02^{b}	19.00±0.02^{b}	Nil

Values are mean ± SD of triplicate determination; Samples with different superscripts within the same column were significantly ($p \leq 0.05$) different

4. Discussion

Phytochemical compounds tannins, saponins, terpenoids, alkaloids, glycosides, and phenols were detected in *A. muricata* leaf extract. The significant antimicrobial properties of the leaf extract could be attributed to the presence of these bioactive compounds. Tannins exert their antimicrobial effects through mechanisms such as membrane disruption, binding to proteins, enzyme inhibition, substrate deprivation and metal ion complexation [15]. Medicinal plants that have tannins as their main component are used in the treatment of intestinal disorders such as diarrhea and dysentery [16]. Alkaloids produce antimicrobial effects by interfering with processes such as deoxyribonucleic acid (DNA) replication and ribonucleic acid (RNA) transcription which are vital to microbial functioning [15]. Saponnins are classes of glycosides which demonstrate antifungal properties [16]. Synergistic interactions between some of these chemical groups may produce greater activity against pathogenic microorganisms.

The antimicrobial activity of aqueous and ethanol extracts of *A. muricata* (soursop) leaves against the five clinical isolates presented in Tables 2 and 3 indicated the assessment of the potency of the leaf extracts from the observation for inhibition zones that occurred on the cultured plates. Among all the extracts used in this study, the aqueous extract of *A. muricata* leaves was found to be the most active against the tested bacterial organisms. The susceptibility of the aqueous extract was more pronounced on *P. aeruginosa* at lowest concentration of 0.2 mg/mL, though its efficacy was more observed against *E. coli* showing the highest potency ranging from the diameter of inhibition of 18.00 – 32.00 mm at the concentration ranging from 0.4 – 0.8 mg/mL (Table 4.1) The results of these susceptibility tests are in accordance with those obtained by Vijayameena *et al.* [17] who had proven the susceptibility of bacteria such as *Staphylococcus aureus, Bacillus, Pseudomonas aeruginosa, Klebsiella pneumonia* to barks and leaves extracts of *A.* This justifies the traditional uses of this plant for the treatment of bacterial infections.

Plants have been used as medicines from time immemorial. The main advantages of using plants as alternative medicine include its diversity and flexibility of use, their availability and affordability in the region and mainly to reduce adverse reactions. The widespread acceptance of plants in low- and middle-income countries, its comparatively low cost and the relatively low level of technological input required, make them the ideal alternative to costly therapies. Hence, plant extracts may prove to be better and safer alternatives if they are supported by scientifically based evidence [18]. The use of Soursop extract on microorganisms has a strong traditional foundation; many countries in the world use this extract for treatment of various diseases. In countries like Peru, Brazil and Togo the extracts have been used for various treatments such as liver disorders, diarrhoea, dysentery, fevers, hypertension, sores, internal ulcers and diabetes [19].

The mode of action of Soursop extract against microorganisms is presently unknown but the common mechanism as to how they act against microbes, insects, and herbivores in their natural environment might prevail. Biologically as to what makes Soursop potent against microorganisms is the presence of acetogenins. These are bioactive compounds found in the annonacea family, these acetogenins, are known to have tumoricidal, anti-malarial, anti-helmintic, anti-viral, and anti-microbial effects, suggesting many potentially useful application. Of the annonaceous-acetogenins, bullatacin, an acetogenin is a powerful tumoricidal and antibacterial agent [18].

5. Conclusion

The present study demonstrated the in-vitro efficacy of Soursop fruits extracts which was highest against *E. coli* followed by *K. pneumoniae* and *S. pyogenes*. Hence, this study proves to an extent that the Soursop leaf extracts when used against microorganisms, has sufficient antimicrobial property. The findings of this study form a good basis for selection of the plant for further phytochemical and pharmacological investigation, suggesting antibacterial properties that can be used as antimicrobial agents in new pharmaceuticals for the therapy of infectious diseases caused by bacterial pathogens.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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