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Antimicrobial Assessment of *Annona muricata* Fruits and Its Chemical Compositions

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Authors' contributions

This work was carried out in collaboration between all authors. Author RIU designed the study, performed the chemical composition, wrote the protocol, and wrote the first draft of the manuscript. Author JNA determined the fungi associated with deterioration. Author KUU determined the antibacterial analysis and author ICI managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the antibacterial activity of *Annona muricata* fruits against some human pathogens, the fungi associated with deterioration of *Annona muricata* fruits and the chemical compositions of the fruits.
Study Design: The study was designed to test the inhibitory ability of the plant extract on human pathogens, to identify the fungi associated with deterioration of *Annona muricata* fruits and determine its chemical compositions by GC-MS.
Place and Duration of Study: Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri and Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria, between June to November 2016.
Methodology: The antibacterial activity of *Annona muricata* fruits was performed by filter paper disc diffusion technique. Pieces of the sour sop sections were plated on potato dextrose agar

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(PDA) plates each and incubated at 27ºC and the plates examined daily for the development of fungal growth. The chemicals from *Annona muricata* fruits were extracted with ethanol and subjected to GC/MS analysis and the identification of compounds was done by comparing spectrum of the unknown component with the spectrum of the known components stored in the NIST library.

Results: The results of the bacterial analysis showed that the ethanol extract inhibited all the tested organisms *S. aureus, P. mirabilis, K. pneumoniae*, *Salmonella* and *E. coli.* which justified the use of the plant in the treatment of diarrhea and other infections by Herbalists. The fungal pathogens found to be associated with the rotting of *A. muricata* fruit included *Aspergillus niger* (Tiegh)*, Aspergillus flavus* (Link)*, Rhizopus stolonifer* (Ehrenb.)*, Fusarium oxysporium* (Schlech), and *Botryodiplodia theobromae* (Pat.). The most pathogenic fungi was *B. theobromae* which caused rot of 11.48 mm^2, followed by *R. stolonifer* that caused rot of 4.03 mm^2. The least pathogenic fungus was *A. niger* causing rot area of 2.51 mm^2. The GC-MS analysis revealed that ethanolic extract of *Annona muricata* fruits contains seven compounds with 5-Hydroxymethylfurfurole constituting the bulk of the oil (29.95%), followed by 3,5-Dihydroxy-6-methyl-2,3-dihydro-4-H-pyran-4-one (28.57%). The oil contains an ester, Butanoic acid,2-methyl-3-oxo-ethyl ester (5.35%) which may be responsible for the sweet aroma of the fruit. Other compounds present were Furfural (6.25%), 4- Hydroxy-2,5-dimethyl-3-hexanone (13.84%), 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (3.13%) and Oleic acid (12.95%). These compounds possessing a wide spectrum of pharmacological activities justified the use of this plant for the treatment of ailments traditionally.

Keywords: Antibiotics; antifungal; pathogens; Annona muricata.

1. INTRODUCTION

Plants have been useful to man not only for food, shelter, and clothing but also for their use for ornamentation and health care. Due to the increasing failure of chemotherapeutic agents and antibiotics resistance exhibited by pathogenic organisms, Researchers are increasingly turning their attention to traditional medicine, screening several medicinal plants for their potential antimicrobial activities for new leads to develop better drug against microbial infections.

Annona muricata Linn also known as Soursop belongs to the family of Annonaceae. It is a widespread small tree and has its native in Central America. The fruit of *Annona muricata* Linn. is of economic value and hence cultivated and used widely as an edible food. The fruit is very delicate dark green covered with soft spines. It is relatively large and very thin shell. The flesh is white, creamy, juicy and slightly acidic, measuring 2-3 cm long, it can weigh 2.5 kg.

The flesh of the fruit consists of an edible, white pulp, some fiber, and a core of indigestible, black seeds. The pulp is also used to make fruit nectar, smoothies, fruit juice drinks, as well as candies, sorbets, and ice cream flavorings. The fruits of *Annona muricata* have been found and used as an antiparasitic, antipyretic (reduces fever) and astringent in diarrhea. They are used to reduce joint pains, treat heart conditions and reduce coughing or flu symptoms in herbal medicine [1]. The fruit contains significant amounts of vitamin C, vitamin B_1 and vitamin B_2. The soursop fruit consists of 67.5% edible pulp, 20% peel, 8.5% seeds, and 4% core by weight [2;3]. The white edible pulp contains 80–81% water, 1% protein, 18% carbohydrate, 3.43% titratable acidity, 24.5% non-reducing sugar, and vitamins B1, B2, and C. One hundred grams of raw soursop fruit yields 66 calories, 3.3 g dietary fiber, 14 mg calcium, 278 mg potassium, 20.6 mg vitamin C, 27 mg phosphorus, and 16.8 g carbohydrate [4]. It is a good source of vitamin B (0.07 mg/100 g pulp) and vitamin C (20 mg/ 100 g pulp).

Soursop gives a flavor of custard when it is ripen condition and hence has a pleasant, distinctive aroma and fibrous pulp that can be consumed because of its very juicy, creamy and sweet character. The juice is processed into various other products such as juice blends, nectars, syrups, jams, jellies and ice-creams. This unique flavor of soursop increases its processed products to possess much potential in the international market [5]. According to [5], the soursop flavor possess a maximum of 114 volatile compounds that is found to be responsible for the whole aroma profile, 44 esters, 25 terpenes, 10 alcohols, 9 aldehydes

and ketones, 7 aromatic compounds, 5hydrocarbons, 3 acids, 3 lactones and 8 other miscellaneous compounds.

All parts of *A. muricata* are used in natural medicine, including bark, leaves, roots and fruits. The stems, leaves and roots are considered sedative, hypotensive (blood pressure lowering), antispasmodic and anti-diabetic [5].

The leaves are used as a tea against catarrh (inflammation of mucous membranes). The ground seeds are used by Andean tribes against intestinal parasites [5]. Studies showed that *A. muricata* is beneficial in treating nearly twelve different types of cancer, including most common cancer like colon, breast, lung, pancreatic cancer etc. It was reported that the immune system of the cancer patients who consumed soursop during chemotherapy cycles were not affected or weakened much, as compared to other cancer patients [6].

The soursop is the most powerful anti-cancer plant on the planet, used for over 40 years in the U.S., Europe and Asia. Studies from 1998 to 2000 by [6] have shown that acetogenins found in soursop can selectively inhibit the growth of cancer cells and also inhibit the growth of tumor cells resistant to adriamycin (chemotherapeutic drug). Studies were made of soursop compared to the effect with adriamycin (known chemoteherapeutic). It was found that Soursop is 10000 times more potent, and kills cancer cells without harming healthy cells as occurs with chemotherapy, which also causes nausea, weight loss and hair, protects and elevates the immune system.

It has been observed that the fruits of *A. muricata* are attacked by various fungi which cause deterioration. Micro-organisms especially fungi have been reported to cause extensive deterioration of fruits and vegetables [7;8]. Some of these micro-organisms cause rotting, discoloration or fermentation of the fruits which affect their preservation. Production of this useful fruit, *A. muricata* is fraught with some pathological problems and these disease causing microbes include fungi, bacteria, virus and some nematodes.

In spite of the numerous uses of *A. muricata* in treatment of various ailments, research work has not been fully documented. Hence we described herein the antimicrobial assessment and chemical compositions of *Annona muricata* fruits.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The fresh ripped fruits were harvested, and the pulp soaked in ethanol for 48 hours and filtered. The filtrate was concentrated with rotary evaporator and was used for GC- MS analysis and antibacterial assessment.

2.2 Antibacterial Evaluation of the Fruits of *A. muricata*

2.2.1 Preparation of extracts

The test solution of each extract was prepared by dissolving 0.1g of the plant extracts separately in 1.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml.

2.2.2 Micro-organisms

The bacteria organisms used were *Staphylococus aureus, Klebsiella pneumoniae,* Salmonella*, Proteus mirabilis and Escherichia coli.* All the organisms were obtained from the stock culture of the Federal Medical Center, Umuahia. Cultures were brought to laboratory conditions by resuscitating the organisms in peptone water and thereafter subcultured into nutrient agar medium and incubated at 37ºC for 24 hours.

2.2.3 Antibacterial assay

The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (whatman No1, 6mm diameter) were placed in glass petri dishes and sterilized in hot air oven [9]. The media (10 g nutrient agar in 200 ml distilled water, auto-claved at 115ºC for 30 minutes) was cooled to 50ºC. The sterile nutrient agar media were poured into the sterile petri dishes and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2 ml of plant extracts and standard- Ciprofloxacin. Discs with DMSO (100 mg/ml) served as a control.

The discs were used after drying them in an incubator at 40ºC to remove any trace of solvent. Discs were introduced onto the surface of the medium. The plates were incubated at 37ºC for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each

extract to minimize error and the average of these values were tabulated in Table 1.

Table 1. Inhibition Zone Diameter (IZD) (mm) of *A. muricata* fruits on the human pathogens

Pathogens	*A. muricata* fruits
Proteus mirabilis	11 ± 0.25
Klebsiella pneumoniae	7 ± 0. 30
Staphylococus aureus	12 ± 1.50
Salmonella	13 ± 0.55
Escherichia coli	12 ± 0.20

Values are mean of triplicate determination ± standard error

2.3 Isolation and Identification of Fungal Pathogens

Following the procedures of [10], small sizes of approximately 2x2 mm were cut from soursop fruit infected with rot at interphase between the healthy and rotten portions of the tubers. The soursop pieces were first surface sterilized by dipping completely in a concentration of 10% sodium hypochlorite solution for 2 minutes. The sterilized sections to be inoculated were then removed and rinsed in sterile distilled water. The sour sop pieces were then placed on sterile paper towels to dry for about 20minutes. Three pieces of the sour sop sections were plated on PDA plates each and incubated at 27ºC. The plates were examined daily for the development of fungal growth. The colony, spores, mycelium and conidia characteristics observed were matched with those available in manual of [11,12].

2.3.1 Pathogenicity test

Healthy sour sop fruits were washed under running tap water to remove dirt. Following the procedures of [11,10], the soursop were dipped into 10% concentrated sodium hypochlorite for 2 minutes and rinsed twice in sterile distilled water, then dried with sterile paper towel .

Circular holes were drilled at the proximal and distal ends of the soursop using a sterile 10mm diameter cork borer. Spores of each isolate were dispersed into the circular holes for each tuber. Control was set up by inoculating another soursop with 1 ml sterile distilled water and all the set up were in 3 replications.

The part of the fruit bored out were carefully replaced after inoculation and tightly sealed with paraffin to prevent contamination. The soursop were then put separately into a moist white nylon bags. The nylon bags were tied with rubber band and punctured so as to allow air for conducive environment for the growth of the pathogenic fungi and were placed on laboratory benches at room temperature for 15 days.

On establishment of disease conditions, the inocula were taken again from the infected yam tubers and cultured. The resulting mixed culture were sub-cultured and the resulting pure cultures were characterized and identified as the previously isolated organisms. This was taken as evidence that they incite the disease and were thus identified as pathogenic isolates [13].

2.4 Gas Chromatography- Mass Spectrum Analysis (GC-MS)

Gas chromatography analysis was performed using GC-MS SHIMADZU QP 2010, JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25 nm x 50 m) and the conditions were as follows: Temperature programming from 80-200^{o}C held at 80ºC for 1 minute, rate 5ºC / min and at 200ºC for 20 mins. FID temperature 300ºC, injection temperature 250ºC, carrier gas nitrogen at a flow of 1ml /min, split ratio 1:75. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all analytical grade and were procured from Merck, Germany.

The interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Subsequently, the details about their molecular formula, molecular weight, structure were also obtained [14,15].

2.5 Statistical Analysis

All values are expressed as mean ± S.D. Statistical analysis were performed by Student's *t*-test. The values of p lower than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Ethanolic extracts of the fruit of *Annona muricuta* exhibited antibacterial activities on the pathogens tested as shown in Table 2. The extracts inhibited all the tested organisms *P. mirabilis, K. pneumonia*, *S. aureus*, *Salmonella* and *E. coli*. The organisms *P. mirabilis* and *E. coli* are the common cause of urinary tract infection and traveler's diarrhea [16]. *Staphylococus aureus* and *Pseudomonas aeruginosa* are the cause of severe eye infections such as blepharo conjuctivities, corneal ulcers,abscesses, styes, dacryocystitis, orbital cellutitis and blebs. The present result also shows that eating of the fruit of *Annona muricuta* may be useful in treatment of eye and nose problems. Natural antibiotics are preferred recently since the use of synthetic antibiotics have been reported to have side effects like hypersensitivity reactions, gastric disturbances, nephrotoxicity, etc.

Table 2. Measurement of extent of yam tuber rot caused by different fungal pathogens

Isolates	Mean length	Mean diameter	Area of rot(mm^2)
F. oxysporium	1.02	1.20	3.85
R. stolonifer	1.50	0.85	4.03
A. flavus	1.20	0.80	3.02
B. theobromae	1.80	2.03	11.48
A. niger	0.80	1.00	2.51

The fungal pathogens isolated from rotted *A. muricata* fruit included *Aspergillus niger* (Tiegh)*, Aspergillus flavus* (Link)*, Rhizopus stolonifer* (Ehrenb.)*, Fusarium oxysporium* (Schlech), and *Botryodiplodia theobromae* (Pat.). The pathogenicity test showed that all the isolated fungi were pathogenic to soursop. These fungal pathogens which were originally isolated from soursop caused the same symptom (soft rot). *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Fusarium oxysporium and Botryodiploidia theobromae* were associated with soft rot. The soft rots observed were characterized by softing and wetting of tissues which later became brown.

Measurement of extent of rot caused by the fungal pathogens showed that the most pathogenic fungi was *B. theobromae* which caused rot of 11.48 mm^2, followed by *R. stolonifer* that caused rot of 4.03 mm^2. The least pathogenic fungus was *A. niger* causing rot area of 2.51 mm^2.

Fig. 1. Fruits of *Annona muricata*

These fungi species isolated and identified in this study corroborated those isolated and reported by [17,18]. Similarly *Fusarium spp, Rhizopus spp, B. theobromae and Colletotrichumglo esporides* were reported to be major cause of soft rot [17] *A. flavus*is reported to produce aflatoxins, the most toxic and potent carcinogenic natural compound [19]. *A. niger* is reported to cause rot in many plants such as grape, cereal, onions, peanut, cotton, kernels, datesetc and a common contaminant of food which produces ochratoxin, fumonisin and aflatoxin [20].

The GC-MS analysis of ethanol extract of *Annona muricata* fruits depicted seven compounds with 5-Hydroxymethylfurfurole constituting the bulk of the oil (29.95%), followed by 3,5-Dihydroxy-6-methyl-2,3-dihydro-4-H-pyran-4-one (28.57%). The oil contains an ester, Butanoic acid,2-methyl-3-oxo-ethyl ester (5.35%) Other compounds present were Furfural (6.25%), 4- Hydroxy-2,5-dimethyl-3-hexanone (13.84%), 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (3.13%) and Oleic acid (12.95%). Oleic acid is a mono-unsaturated fatty acid found in animal and vegetable oils. It synergistically enhances cancer drug effectiveness [15]. For example oleic acid improved the effectiveness of herceptin, a breast cancer drug. It lowers heart attack risk and artherosclerosis, and aids in cancer prevention. High concentrations of oleic acid can lower blood levels of cholesterol and lower the risk of heart problems [21,22]. The quantity of oleic acid in the fruit of *A. muricata* is high, 12.95%. The presence of this compound may be one of the reasons *A. muricata* fruit is effective in the treatment of cancer. Another compound present

Table 3. Chemical constituents of ethanol extract of *A. muricata* fruits

Peak	Retention time	Height	Name of compound
1	3.950	6.25%	Furfural
2	6.033	13.84%	4- Hydroxy-2,5-dimethyl-3-hexanone
3	7.208	5.35%	Butanoic acid,2-methyl-3-oxo-ethyl ester
4	7.492	3.13%	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
5	8.850	28.57%	3,5-Dihydroxy-6-methyl-2,3-dihydro-4-H-pyran-4-one
6	10.358	29.95%	5-Hydroxymethylfurfurole
7	23.008	12.95%	Oleic Acid

in the fruit is 3,5-Dihydroxy-6-methyl-2,3-dihydro-4-H-pyran-4-one which has been reported to possess antimicrobial, anti-inflammatory and antiproliferative activities [15]. The compound, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4-H-pyran-4-one has also been reported to possess anticancer activity [23] and its percentage in the fruit is equally high. The ester, Butanoic acid, 2-methyl-3-oxo-ethyl ester present in the fruit may be responsible for the sweet aroma of the fruit.

4. CONCLUSION

The results of the analysis justify the use of the fruit of *Annona muricata* as bactericidal agent for the treatment of diseases, by the herbalists and as antifungal agent in treatment of mycotic infections and in the control of fungal plant disease. Ethanol extract of A. muricata fruits revealed some pharmacologically relevant compounds. A higher concentration of a potent anticancer agent, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one (DDMP) in the extract studied is an interesting finding. Though seven different compounds have been identified, it may have many more unidentified compounds in the same extract which can be isolated by adopting different extraction procedures or by using different solvents. fungal infection leads to a reduction in carbohydrate and protein contents of the soursop pulp which might have a remarkable effect on the value of the fruit, especially in the food industry.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Uchegbu RI, Bako SS. Phytochemical evaluation by GC-MS analysis of the leaves of *Annona muricata* Linn. Alvana Journal of Science, Vocational Education and Technology. 2016;2(1):92-99.
2. Bora PS, Holschuh HJ, da Silva Vasconcelos MA. Characterization of polyphenol oxidase of soursop (*Annona muricata* L.) fruit and a comparative study of its inhibition in enzyme extract and in pulp. Ciencia y Tecnologia Alimentaria (Ourense, Spain). 2004;4:267–273.
3. Onimawo IA. Proximate composition and selected chemical properties of the seed, pulp and oil of soursop (*Anona muricata*). Plant Foods Hum. Nutr. 2002;57(2):165–171.
4. Badrie N, Schauss AG. Soursop (*Annona muricata* L.): Composition, nutritional value, medicinal uses, and toxicology. In: Ronald Ross Watson and Victor R. Preedy, editors, Bioactive Foods in Promoting Health. Oxford: Academic Press. 2009;621-643.
5. Gajalakshmi S, Vijayalakshmi S, Devi Rajeswari V. Phytochemical and pharmacological properties of *Annona muricata*: A review. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(2):1-6.
6. McLaughlin and Chih H.W; 2000. Available: www.wisegeek.com
7. Amadioha AC, Uchendu PN. Post harvest control of tomato fruit rot, caused by *Fusarium solani* with extracts of *Azadirachta indica*. Discov. Innov. 2003;15(1/2):83-86.
8. Okwulehie IC, Alfred NK. Fungi associated with deterioration of sour-sop (*Anona muricata* Linn.) fruits in Abia State, Nigeria. African Journal of Microbiology Research. 2010;4(3):143-146.
9. Singh S, Bhawani Rajesh BS, Sahoo K, Subudhi E, Nayak S. Chemical

composition of turmeric oil (*Curcuma longa* L.) and its antimicrobial activity against eye infecting pathogens. Journal of Essential Oil Research. 2013;23:11-18.

10. Okigbo, R.N. and Omodamiro, O.D. Antimicrobial effect of leaf extracts of pigeon pea (*Cajanus cajaan* (L.) Millsp) on some human pathogens. Journal of herbs, spices and medicinal plants. 2006;12:117-127.
11. Fawole MO, Oso BA. Laboratory mannual of microbiology 3rd ed. Spectrum books ltd Ibadan. 1998;127–129.
12. Barnett HL, Hunter S. Illustrated genera of imperfect fungi 6th ed. Burgess publishing company. Minneapolus. 1998;250–260.
13. Psuneo W. Pictorial atlas of soil and seed fungi, morphologies of cultured fungi and key to species 2nd ed. CRC press Florida; 2002.
14. Uchegbu RI, Echeme JO, Njoku PC, Igara CE, Ngozi-Olehi LC. Identification of phytochemicals present in the seeds of *Mucuna pruriens* (utilis) by GC/MS analysis. Asian Academic Research Journal of Multidiciplinary. 2015;2(2):65–75.
15. Uchegbu RI, Akalazu JN, Ibe CO, Ahuchaogu AA, Amadikwa CU. Chemical composition of the stem extract of *Costus afer* (Bush Cane) and its antimicrobial activity. British Journal of Pharmaceutical Research. 2016;10(5):1-9.
16. Uchegbu RI, Mbadiugha CN, Ibe CO, Achinihu IO, Sokwaibe CE. Antioxidant, anti-inflammatory and antibacterial activities of the seeds of *Mucuna flagellipes.* American Journal of Chemistry and Applications. 2015;2(5):114-117.
17. Nweke CN, Ibiam OFA. Preharvest and post harvest fungi associated with soft rot of fruits of *Annona muricata* and their effects on the nutrient contents of the pulp. American Journal of Food and Nutrition. 2012;2(4):78-85.
18. Morton J. Fruits of warm climates 2nd ed. University of Florida. 2000;401.
19. Pasqualotto AC, Denning DW. An aspergilloma caused by *A. flavus*. Medical Mycology. 2008;46:275-278.
20. Ajay KG, Sushi S, Shubhi A, Rekha B. Diversity, pathogenicity and toxicology of *A. niger.* An important spoilage fungi, Research Journal of Microbiology. 2011:6(3):270–280.
21. David TW. Oleic acid – The anti-breast cancer component in olive oil. AU J.T. 2005;9(2):75-78.
22. Mendez JA, Vellon L, Colomer R, Lupu R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erbB-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin) in breast cancer WE cells with Her-2/neu oncogene amplification. European Society for Medical Oncology; 2005.
Available:http://www.annonc.oupjournals.org/cgi/content/ abstract/mdi090v1
23. Rajasekaran M, Archana R, Raviprasadh R. GC/MS analysis and identification of phytochemicals present in the leaves of *Beloperone plumbaginifolia* (Jacq.) Nees. International Journal of Bio-Engineering, Science and Technology. 2012;03(Issue 02):134-138.

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