ANTIMICROBIAL ACTIVITIES OF AQUEOUS EXTRACT OF RIPE *ANNONA MURICATA LINN.* (SOURSOP) FRUIT PULP ON CLINICAL ISOLATES

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Abstract

Introduction: Antimicrobial resistance poses a great challenge and so is of great public health concern. Traditional healers employ a variety of medicinal plants, including *Annona muricata Linn.*, to treat infectious diseases, especially those caused by multidrug resistant microorganisms.

Aim: To assess the antimicrobial properties of ripe *Annona muricata Linn.* fruit pulp against some clinical isolates.

Materials and Methods: Ripe *Annona muricata Linn.* fruit pulp, distilled water, and clinical isolates were used in this study. *Annona muricata* fruits was obtained from Nnewi, Anambra State, Nigeria. The in-vitro antibacterial and anti-fungal activities of aqueous extracts of ripe *Annona muricata Linn.* fruit pulp was determined using the agar disc diffusion technique. This investigation relied on use of both bacterial and fungal isolates. The organisms were collected from Nnamdi Azikiwe University Teaching Hospital Nnewi's stock culture. Cultures were transported to the laboratory by resuscitating them in peptone water, then sub culturing them into a nutrient agar medium and incubating them at 37°C for 24 hours. Data was presented using frequency counts.

Results: Aqueous extracts of ripe *Annona muricata* fruit pulp showed antibacterial and anti-fungal activity, with zones of inhibition diameter as large as 36mm for *Staphylococcus aureus* and 30mm for *Proteus spp.*, *Klebsiella spp.* (33mm), *Pseudomonas aeruginosa* (20mm), *E. coli* (24mm), *Candida albicans* (30mm) and *Aspergillus* spp. (30mm) all demonstrated considerable susceptibility to the ripe *Annona muricata Linn.* fruit pulp extracts.

Conclusion: The study concluded that the aqueous extracts of ripe *Annona muricata Linn.* fruit pulp exhibited some antimicrobial activity to both clinical bacterial and fungal isolates, which may explain why it is used locally to treat diarrhoea and other illnesses.

Keywords: *antimicrobial activity, Annona muricata, clinical isolates, aqueous extract*

Introduction

Antimicrobial resistance is increasing on a daily basis, necessitating the creation of novel antimicrobials[1]. More so, hospital acquired infections have grown to a larger extent across the globe affecting humanity in the development of ailments as well as developing high resistance to drugs[2]. Multidrug resistance is an important public health issue; importantly, the formation of multidrug resistant microorganisms is an evolutionary process based on selection for organisms with increased resistance to antibiotic dosages[3,4].

Because of the growing failure of chemotherapeutic agents and development of antibiotic resistance in pathogenic microorganisms[5], which pose a critical public health challenge across the globe[4]; the quick emergence of bacteria resistance that occurs globally tends to endanger antibiotic efficacy[6-8]. Scientists are increasingly focusing on plant medicine, which entails screening a variety of medicinal plants for antimicrobial activity in search for breakthroughs to have better medications against microbial illnesses and its resistance[9,10].

Plants have gained significance in the treatments of various ailments globally, which results from their bioactive contents present[11,12]. However, their pharmacological activities are linked to their secondary active metabolites present, such as terpenoids, flavonoids, saponins, etc, amongst others[12,13]. *Annona muricata* L. is a medicinal plant of high significance, which has gained several pharmacological importance in scientific researches because of their secondary metabolite such as saponins, terpenoids, flavonoids, glycosides Etc.[14,15]. Several pharmacological impact of the plants especially the fruits have been used to combat arthritis, diarrhea, and neuronal diseases[14].

A report showed that the leaf and fruit skin of *A. muricata* possess remarkable activity with 15 – 17 mm inhibition diameter (DIH) against the test organisms[16]. Abdulsalami et al. indicated a significant inhibition against *S. aureus and S. typhi* following aqueous and ethanolic leaf extract of *Annona muricata* [17]. However, there are limited literatures following the antimicrobial impact of aqueous extract of ripe fruit of *Annona muricata Linn.* against some clinical isolates of bacteria pathogens, which the study investigates.

Materials And MethodsPlant collection and Identification: Ripe *Annona muricata Linn.* fruits were purchased from Nkwo market, Nnewi, Anambra State and were identified and authenticated by a Taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria and was assigned a herbarium number (**NAUH4[A]**) which was deposited in the herbarium catalogue.

Materials: The materials employed in the study were ripe fruits of *Annona muricata Linn.*, electronic blender (QLINK Multifunction food processor, QMFP-128, Turinar Corp., Shang-Hai, China), Whatman filter paper *(Sigma Aldrich, WHA1001040, USA)*, Wistar rats (*Rattus norvegicus*), *rotatory evaporator (Digital TT-52, Techmel & Techmel, USA)*, thermostat oven (DHG-9023A PEC MEDICAL USA), incubator, autoclave, *refrigerator (100L NX150C, Nexus, Hong Kong)*, measuring cylinder, beaker, Petri dishes, forceps, laboratory oven, Bunsen burner, wire loop, Nutrient agar(FLUMEDIA/HI FLOWN GLOBAL RESOURCES LTD), Saboraud dextrose agar (SDA), clinical isolates of bacteria and fungi , and personal protective equipment (PPE).

Ethical Approval: Ethical approval was obtained from Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi campus, Nnewi Ethics Committee with reference number: NAU/FHST/2021/MLS66.

Extract Preparation

Ripe fruit of *Annona muricata* Linn, (soursop) was purchased from Eke Amobi market at Okofia community, Nnewi North Local Government Area, Anambra State, washed in running tap water to remove dirt and air-dried under ambient temperature. The ripe fruit of *Annona muricata Linn.* was cut open with a kitchen knife exposing the pulp and the seeds were removed. Two hundred and fifty gram (250g) of the fruit pulp was milled using an electronic blender in 400mls of lukewarm distilled water for 48hours. It was filtered using a clean handkerchief and further filtration was done using What man No.1 filter paper into a clean glass beaker.

The residue was weighed after filtering and the liquid was measured. The filtrate was concentrated using a thermostat oven (DHG-9023A PEC MEDICAL USA) at 45°C into a thick concentrate. The aqueous extract was preserved in airtight container and kept in a refrigerator at 4°C for further usage. The extraction method was done with modifications as described according to the method employed by Al-Attar and Abu Zeid[18].

Samples collection of Clinical Isolates: Bacterial isolates from clinical samples (urine, HVS) were used for this study. The organisms were obtained from the stock culture of Nnamdi Azikiwe University Teaching Hospital Nnewi. Cultures were brought to the laboratory conditions by resuscitating the organisms in peptone water and thereafter sub-cultured into nutrient agar and Sabouraud Dextrose Agar (SDA) medium and incubated at 37°C for 24 hours and 2 weeks respectively. Pure cultures of bacteria and fungi from clinical samples were made on fresh media by repeated subculture on sterilized nutrient agar using streak plating techniques. Purified colonies were stored in slants of Bijou bottle at 4°C for 24 hours, and was characterized and identified using the standard taxonomic schemes of Cowen[19].

Isolation and identification of organisms: After a proper culture of an organism, bacterial colonies were selected using their morphological characteristics (size, pigmentation, elevation, consistency)[19]. Fungi isolates was established after growth has been confirmed, where subcultures **were prepared using inocula from dif**ferent organisms in the mixed cultures to obtain a pure culture. It was done by transferring hyphal tips from the colony edge of the mixed cultures to fresh plates of SDA using flamed sterilized blades. After sub-culturing, the plates were incubated at 25°C until pure cultures were obtained. The Petri dishes of pure cultures of the test fungi were sealed tightly to prevent contamination. The resulting pure cultures were used for characterization, and subsequent identification of the fungi isolates with the aid of a compound microscope and identification guides[20].

Antimicrobial Activity and Quality Control: Agar disc diffusion method was used to determine the in-vitro antimicrobial activity of aqueous extracts of ripe *Annona muricata Linn.* Fruit pulp Nutrient Agar media for bacteria was prepared according to manufacturer's instruction (FLUMEDIA/HI FLOWN GLOBAL RESOURCES LTD).

Sabouraud Dextrose Agar(SDA) for fungal isolate was done according to the manufacturer's instructions (HIMEDIA/HiMedia Laboratories PVT. LTD India). The antibacterial activity was performed by filter paper disc diffusion technique[21].

Filter paper disc (Whatman No.1,6mm diameter) was placed in glass Petri dishes and sterilized in hot air oven. The media (10g nutrient agar in 200ml distilled water, autoclaved at 115°C for 30 minutes) cooled to 50°C. The sterile nutrient agar medium was poured into the sterile Petri dishes and allowed to solidify. Pure bacterial isolate was swabbed with a sterile wire loop. Each 6mm diameter disc was impregnated with 0.2ml of aqueous plant extracts. The disc was used after drying them in an incubator at 40°C to remove any trace of solvent. Discs were introduced onto the surface of the medium using sterile forceps. The plates were incubated at 37°C for 24 hours to obtain zones of inhibition.

The study quality control (QC) was done by using standard operational procedure for laboratory investigation and media preparation. Sample collection were carried out using aseptic technique and the samples labeled properly. Cultures and isolation were done under aseptic conditions.

Data presentation: Tables and figures were used for data presentation. Table was used for presenting zone of inhibition diameter (mm) as observed for different microbial isolates against the aqueous extract of ripe *Annona muricata Linn.* fruit pulp, whereas the antimicrobial sensitivity plates for both bacterial and fungal isolates were presented as figures**.**

Results

Table 1: Anti-microbial activities of aqueous extracts of ripe *Annona muricata Linn.* fruit pulp at concentration of 0.2ml per 6mm diameter filter paper disc.

Micro organisms	**Zone of Inhibition diameter (mm)for *Annona muricata Linn.* fruit pulp**
	Aqueous Extract
Staphylococcus aureus	36
Klebsiella spp.	15
Pseudomonas aeruginosa	20
Escherichia coli	35
Proteus spp.	38
Candida albicans	24
Aspergillus spp.	25

KEY:

Zone of inhibition Diameter (mm)	**Interpretive criteria**
< 14 - 25	Resistant
26 - 32	Intermediate
33 - 8	Sensitive (susceptible)

Source: CLSI, (2012)[21]; cited in Okeke-Nwolisa *et al.* (2018)[22]

PLATE 1: Culture plates before incubation

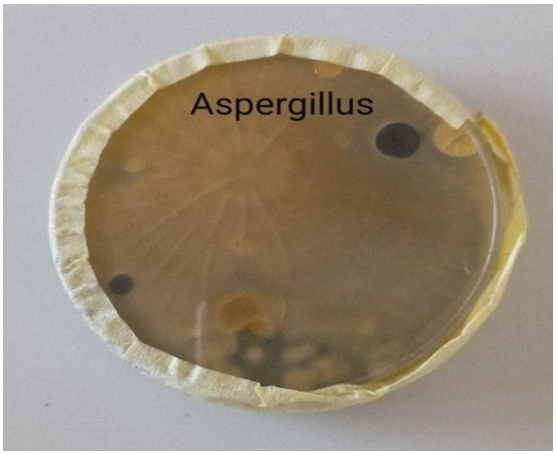

PLATE 2: *Aspergillus spp.* Sensitivity disc after incubation at 37°C for 2 weeks.

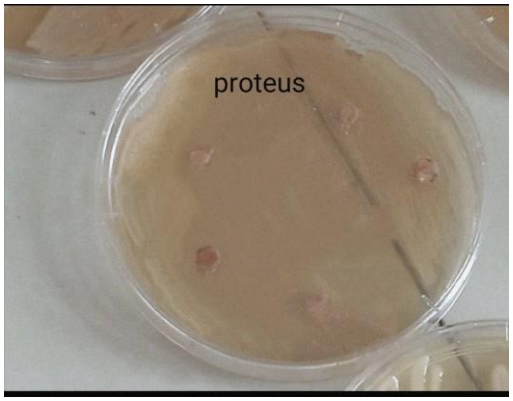

PLATE 3: *Proteus spp.* sensitivity after incubation 37°C for 24 hours.

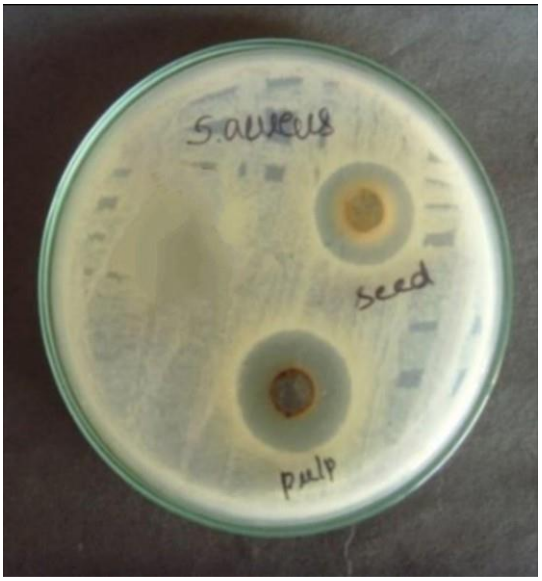

PLATE 4: *Staphylococcus aureus* sensitivity disc after incubation 37°C for 24 hours.

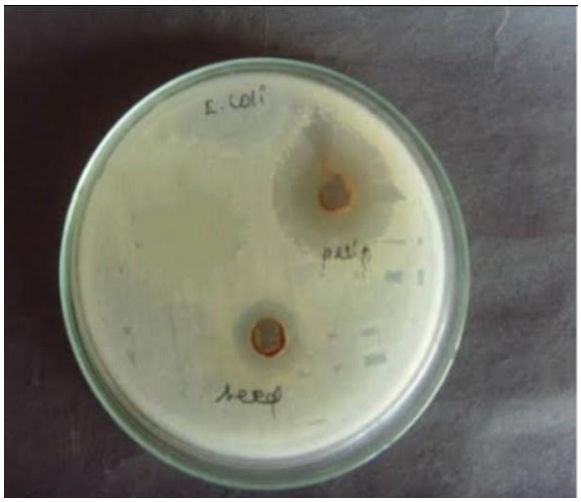

PLATE 5: *Escherichia coli* sensitivity disc after incubation 37°C for 24 hours.

Discussion

Medicinal plants are known for their significance in the management of bacterial and fungal infections because of the high antioxidant and antimicrobial activities they possess[22]. Interestingly, Annona muricata Linn. plant parts have medicinal values of great relevance in the scientific world [23,24]. This study investigated the antimicrobial activities of aqueous extract of ripe Annona muricata Linn. Fruit pulp on clinical isolates. The study findings showed a considerable antimicrobial susceptibility of the test bacterial and fungal isolates to the aqueous extract of Annona muricata Linn. fruit pulp as indicated by zone of inhibition diameter in Table 1.for S. aureus (36mm; susceptible), E. coli (35mm; susceptible) and Proteus spp. (38mm; susceptible). However, some bacterial isolates and the fungal isolates showed zone of inhibition diameter within the resistant range as shown in Table 1 for Klebsiella spp. (15mm; resistant), P. aeruginosa (20mm; resistant), Candida albicans (24mm; resistant) and Aspergillus spp. (25mm; resistant). The mechanism (s) of action following the exhibition of high antibacterial activities of A. muricata Linn.fruit pulp is attributed to the presence of terpenoids, flavonoids, and alkaloids [23,25]. Furthermore, the zone of inhibition exhibited by these bacterial isolates against the aqueous extract of A. muricata Linn. fruit pulp was between 20 to 38 mm, which is in line with the findings of Olugbuyiro et al.[26], revealing a zone of inhibition of antibacterial activity of Annona muricata leaf extracts of 20 to 42 mm. Abdel-Rahman et al.[27] reported antibacterial activity of Annona muricata leaf extract against S. aureus, P. aeruginosa, E. coli, which has agreement with the study findings revealing resistance to S. aureus, Klebsiella spp., P. aeruginosa, E. coli. Iyanda-Joe et al.[16] reported resistance to S. aureus, Klebsiella spp., P. aeruginosa following the fruit skin of Annona muricata extract with a zone of inhibition diameter of 15-17 mm, which is highly resistant and corroborates the study report. Pinto et al.[2] showed similarity to the study findings demonstrating antibacterial activity of methanolic leaf extract of A. muricata L. against S. aureus, which results from the presence of alkaloids.

Neglo et al.[28] reported that Methicillin-resistant Staphylococcus aureus showed a strong antimicrobial resistance, with zone of inhibition diameter of 3.5mm, against the peel and seed extracts of A. muricata, which does not agree with the study findings.

Also, acetogenins showed a high resistant against E. coli with zone of inhibition of 11-15.67mm, which has agreement with the study result following isolation of acetogenins from Annona muricata [29]. Vinothini and Growther,[30] demonstrated high antibacterial activity of S. aureus (13-24mm), Klebsiella pneumoniae (14mm), E. coli (14mm), following aqueous fruit extract of Annona muricata Linn, which corroborates the study findings. Lawal et al.[31]

Lawal et al.[31] showed antibacterial effect of Annona muricata leaves against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae, which corroborates the study findings.

The study findings showed that zone of inhibition diameter for C. albicans and Aspergillus spp., were 24 mm and 25mm respectively, which are within the resistant range, according to CLSI [32] as cited in Okeke-Nwolisa et al. [21]. However, antibiogram activity of the aqueous extract of ripe Annona muricata Linn. fruit pulp indicated a high resistance towards these fungal isolates. Possibly, the reason for the resistance to the aqueous extract of ripe Annona muricata Linn. fruit pulp by C. albicans could be attributed to same mechanism of escape of conventional drugs through the over expression of efflux pumps, alteration of the cell membrane and biofilm formation, which are the most dominant resistance strategies[33]. Thus, the use of medicinal plant (A. muricata) has several mechanisms, which remain unclear following its antifungal effect. The study report has similarity to the findings of Mgbeahuruike et al.[34] revealing a high resistant of 17.60 mm of C. albicans, which corroborates to the study findings of 24 mm, which is within the range. Campos et al.[35] reported a high fungal resistant of leaves of Annona muricata on Candida albicans strain, which agrees with the study findings revealing an antifungal impact with a zone of inhibition diameter of 24 mm. Abdel-Rahman et al.[27] indicated an antifungal impact of Annona muricata seed extracts on C. albicans following terpenoids activities, which is in line with the study findings. Report of Vinothini and Growther, [30] showed that C. albicans exhibited considerable resistance with zone of inhibition diameter of 18-22mm to the aqueous extract of ripe Annona muricata Linn. fruit pulp which is in line with the study findings. Lawal et al.[31] showed antifungal effect of Annona muricata leaves against Candida albicans and Aspergillus niger, which corroborates the study findings.

Conclusion

The study concluded that the aqueous extracts of ripe Annona muricata Linn. fruit pulp exhibited considerable antimicrobial activity to both clinical bacterial and fungal isolates, which may explain why it is used locally to treat diarrhoea and other illnesses.

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