International Journal of Frontline Research in
Science and Technology
Journal homepage: https://frontlinejournals.com/ijfrst/
ISSN: 2945-4859 (Online)

(RESEARCH ARTICLE)

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Antibacterial activity of *Annona muricata* leaves' extracts

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International Journal of Frontline Research in Science and Technology, 2023, 02(01), 024–028

Publication history: Received on 20 December 2022; revised on 31 January 2023; accepted on 02 February 2023

Article DOI: https://doi.org/10.56355/ijfrst.2023.2.1.0053

Abstract

The phenomenon of multidrug-resistant pathogens has increasingly become a cause for serious concern with regard to both nosocomial and community-acquired infections. Plants have been useful to man not only for food, shelter, and clothing but also for their use for ornamentation and health care. *Annona muricata* belongs to the family of Annonaceae and commonly known as Soursop. It is used in traditional medicine in many countries to cure various ailments and diseases. This study investigated *in vitro* antibacterial activity of *Annona muricata* Linn. leaves extracts against *Escherichia coli, Staphyococcus aureus, Acenobacter baumanni, Moraxella catarrhalis* and *Enteroccocus faecium*. Antibacterial assay was performed by Agar well diffusion method in Mueller Hinton Agar plates. N-hexane, methanol and water were used as solvents for extraction while Chloramphenicol (30 µg) served as control. The result of the present study revealed that n-hexane *A. muricata* extract exhibited highest inhibitory action against *Enterococcus faecium* (20.67 mm) and *Acinetobacter baumarii* (21.34 mm) when compared with all the treatments including chloramphenicol (16.65 mm; 15.35 mm respectively) . The result also showed that methanol, aqueous and n-hexane extracts had highest zone of inhibitions against *Escherichia coli* (25.76 mm), *Staphylococcus aureus* (18.65 mm) and *Moraxella catarrhalis* (18.65 mm) respectively. It was discovered in this study that *Annona muricata* leaves' extracts possessed activity against the tested bacteria responsible for the most common bacterial diseases with n-hexane extract showing significant inhibitory action against both Gram-positive and Gram-negative bacteria. The activity of *Annona muricata* leaves depended greatly on the solvent of extraction. The result recommends *Annona muricata* leaves as potential new effective drug for bacterial infections including multi-drug resistant bacteria and further studies should be carried out to determine its mechanism of actions.

Keywords: Pathogens; Chloramphenicol; *Annona muricata; In vitro*; Extract

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 (Uchegbu and Bako, 2016). Due to the increasing failure of chemotherapeutic agents and antibiotics resistance exhibited by pathogenic organisms, researchers are increasingly turning their attention to traditional medicine, thereby screening several medicinal plants for their potential antimicrobial activities for new leads to develop better drugs against microbial infections. *Annona muricata* Linn. (Figure 1) belongs to the family of Annonaceae and widely known as Soursop due to the sour and sweet taste of its fruit (Gavamukulya *et al*., 2017). It is a flowering evergreen tree native to Mexico, Cuba, Central America and parts of India. Different parts of *A. muricata* are widely used in traditional medicine in many countries to cure various ailments and diseases. The natives of Malaysia apply the leaf juice of a mixture of *A. muricata, A. squamosa* and *Hibiscus rosasinensis* on the head to protect against fainting, and they also use the *A. muricata* leaves to treat cutaneous (external) and internal parasites (Ong and Norzalina, 1999). The decoction of the leaves is applied topically for its anti-rheumatic and neuralgic effects, and to reduce abscesses (Mishra *et al.,* 2013). Decoction of *A. muricata* leaves is also used to reduce colds, flu, and asthma (Haiat and Bucay, 2009). The leaves, seeds, unripe fruits and roots of *A. muricata* are traditionally used as biopesticides, bioinsecticides and topical

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insect repellents in Latin America. The fruit is 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 (Uchegbu and Bako, 2016). Besides the traditional medicinal uses, *A. muricata* is also used in other fields, for instance, the fruits are widely used in the food industries in the making of syrups, beverages, candy, ice creams and shakes (Wu *et al*., 1995; Jaramillo-Flores and Hernandez-Sanchez, 2000). This study investigated *in vitro* antibacterial activity of *Annona muricata* Linn. leaves' extracts against *Escherichia coli, Staphyococcus aureus, Acenobacter baumanni, Moraxella catarrhalis* and *Enteroccocus faecium*.

Figure 1 *Anonna muricata* Linn.

2 Material and methods

2.1 Experimental plant

Annona muricata leaves were collected from the botanical garden of Adekunle Ajasin University, Akungba Akoko and authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria with voucher referencing number FHI 110177. The collected leaves were first washed under running tap water and air-dried in shade at room temperature for a month. Using a home grinder, the leaves were then ground to fine powder. The weight of the ground powder was taken and the extracts from leaves were prepared by using, methanol, n-hexane and water as solvents. The filtrate for each extraction was combined, and evaporated to dryness at room temperature.

2.2 Test microorganism

Microorganisms were obtained from the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, and Ondo State, Nigeria. Three strains of Gram-negative bacteria (*Escherichia coli*, *Acinetobacter baumarii* and*Moraxella catarrhalis*) and two strains of Gram-positive bacteria (*Staphylococcus aureus* and *Enteroccocus faecium*) were used for the assay. They were isolated on sterile nutrient agar slants and kept at -4^{o}C prior to the analysis.

2.3 Agar-well Diffusion method

Antibacterial assay of *Annona muricata* leaves' extracts was performed by Agar well diffusion method in Mueller Hinton Agar (MHA) plates. The test organisms (*Enterococcus faecium, Escherichia coli, Staphylococcus aureus, Acinetobacter baumarii* and *Moraxella catarrhalis*) were inoculated in Nutrient agar and incubated overnight at 37 °C to adjust the turbidity to 0.5 McFarland standards giving a final *inoculum* of 1.5 × 10^{8} CFU/ml. Plant extracts of 50 mg/ml concentration were prepared in DimethylSulfoxide (DMSO) and four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Three wells were filled with 50 mg/μl of methanol, n-hexane and aqueous extracts (Figure 2) respectively while the fourth well was filled with control (Chloramphenicol, 30 µg). It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 °C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antibacterial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured to the nearest millimeters. The results were obtained in triplicates (Akanji *et al*., 2018; John and Veda, 2007).

2.4 Statistical analysis

Results were expressed as Mean ± Standard Error. Data were analyzed with Statistical Package for Social Sciences (SPSS), software version 19. $P \leq 0.05$ was considered significant.

Figure 2 Agar-well diffusion plate of *Anonna muricata* extracts (50 mg/µl) against *Escherichia coli* relative to control (Chloramphenicol, 30 µg)

3 Results and discussion

Enterococcus spp. is Gram-positive, facultative and anaerobic cocci found in the intestinal flora and, less frequently, in the vagina or mouth. *Enterococcus faecalis* and *Enterococcus faecium* are the most common species found in humans (Beata *et al.,* 2021). It normally lives harmlessly in intestines. However, if it spreads to other parts of the body it can cause a more serious infection. The bacteria can get into blood, urine, or wound during surgery. From there, it can spread to different sites causing more serious infections, including sepsis, endocarditis, and meningitis. Research suggests it may have a role in the development of colon tumorigenesis as well (Cheng *et al*., 2020). *Staphylococcus aureus* is Gram-positive bacterium found on the skin and mucous membranes, and one of the most common bacterial infections in humans. They are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections (Taylor and Unakal, 2022). *Escherichia coli* is a Gram-negative bacterium that lives in intestines and also found in the gut of some animals. *Escherichia coli* is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia. *Acinetobacter baumannii* is a Gram-negative bacterium, an opportunistic pathogen, with high incidence among immune-compromised individuals, particularly those who have experienced a prolonged (> 90 d) hospital stay (Montefour, *et al*., 2008). *Acinetobacter baumannii* can cause serious infections such as ventilator-associated pneumonia, wound infections, meningitis and bacteraemias in critically ill hospital patients (Moon, *et al*., 2012). *Moraxella catarrhalis,* a Gram-negative, aerobic, oxidase-positive diplococcus is responsible for cases of acute otitis media (in children older than 3 months old), chronic and serious otitis media (fever, acute ear pain, irritability, and can escalate to sepsis and CNS infection), acute and chronic sinusitis (occasional fever, nasal or postnasal discharge, cough, fetid breath, sinus pain, and headache), upper and lower respiratory tract infections and sometimes systemic infections, meningitis, bacteraemia, endocarditis, keratitis and suppurative arthritis(Murray, *et al*., 2007).

The phenomenon of multidrug-resistant pathogens has increasingly become a cause for serious concern with regard to both nosocomial and community-acquired infections (Peleg, *et al*., 2008). Indeed, the World Health Organization (WHO) has identified antimicrobial resistance as one of the three most important problems facing human health (Bassetti, *et al*., 2011). The most common and serious multidrug-resistant pathogens have been encompassed within the acronym "ESKAPE," standing for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp*. (Rice, 2008). Plants as medicines are being used from time immemorial (Barnhill *et al*., 2012). 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 (Barnhill *et al*., 2012). Hence, plant extracts may prove to be better and safer alternatives if they are supported by scientifically based evidence (Moghadamtousi *et al.*, 2015).

In the present study, the efficacy of the leaves of *Anonnamuricata* methanol, n-hexane and aqueous extracts were determined by measuring the diameter of zone of inhibition (Table 1). All the *A. muricata* leaves extracts were sensitive against the tested pathogenic organisms. The result of the present study revealed that n-hexane *A. muricata* extract exhibited highest inhibitory action against *Enterococcus faecium* (20.67 mm)and *Acinetobacter baumarii* (21.34 mm) when compared with all the treatments including chloramphenicol (16.65 mm; 15.35 mm respectively) . The result also

showed that methanol, aqueous and n-hexane extracts had highest zone of inhibitions against *Escherichia coli* (25.76 mm), *Staphylococcus aureus* (18.65 mm) and *Moraxella catarrhalis* (18.65 mm)respectively. There was no significant difference among n-hexane extract (17.33±0.88^{b}), aqueous extract (18.65±0.89^{b}) and chloramphenicol (18.65±0.89^{b}) when tested against *Staphylococcus aureus.* Methanol extract (25.76 mm) and chloramphenicol (25.76 mm) revealed the same zone of inhibition against *Escherichia coli*. The result showed the significant ability of n-hexane extract to inhibit the growth of both Gram-positive and Gram-negative bacteria. The activity of *Annona muricata* leaves depended greatly on the solvent of extraction. The sensitivity of this plant could be traced down to its strong ethnomedicinal foundation where many countries such as Peru, Brazil and Togo use *Annona muricata* for treatment of skin infections, diarrhoea, dysentery, sores, fever, cold and internal ulcers (Moghadamtousi *et al.*, 2015).

Table 1 Diameter of zones of inhibition (mm) of *Annona muricata* leaves extracts (50 mg/μl) against bacteria

Treatments				
Bacterial	**N-hexane extract**	**Methanol extract**	**Aqueous extract**	**Chloramphenicol (control; 30 µg)**
Enterococcus faecium	20.67±0.33d	14.33±0.88b	10.66±0.33a	16.65±0.84c
Escherichia coli	22.34±0.81b	25.76±1.20c	16.66±0.85a	25.76±1.20c
Staphylococcus aureus	17.33±0.88b	12.67±0.78a	18.65±0.89b	18.65±0.89b
Moraxella catarrhalis	30.33±1.89d	25.67±0.89c	21.33±0.67b	13.67±0.57a
Acinetobacter baumarii	21.34±0.78c	14.67±0.66ab	12.76±0.33a	15.35±0.88b

Mean values with the same alphabets in rows are not significantly different from each other at P>0.05 using Duncan's New Multiple Range Test (DNMRT)

4 Conclusion

It was discovered in this study that *Annona muricata* leaves' extracts possessed activity against the tested bacteria responsible for the most common bacterial diseases with n-hexane extract showing significant inhibitory action against both Gram-positive and Gram-negative bacteria. The result recommends *Annona muricata* leaves as potential new effective drug for bacterial infections including multi-drug resistant bacteria and further studies should be carried out to determine its mechanism of actions.

Compliance with ethical standards

Acknowledgments

Appreciation goes to Mr. Akele of the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko for his technical assistance.

Disclosure of conflict of interest

No conflict of interest.

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