ID: 195

Comparative analysis of *Annona muricata* Linn (soursop) leaf and fruit extracts against selected pathogens

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Abstract

Multidrug resistance to antibiotics has necessitated the need for natural products which are alternative method of antimicrobial stewardship. The use of plants, which provide safe and cost effective remedies can be properly harnessed and regulated provided there are scientific-based evidences. This study compared the bioactivity of soursop leaf and fruit extracts against selected pathogens. Extract yield as well as analysis of inherent phytochemicals of soursop fruit and leaf were estimated using standard methods. Agar well diffusion method was used for antimicrobial screening and minimum inhibitory concentration (MIC) against test isolates. 24g and 33g extract was obtained from 60g dried soursop leaves and fruits, resulting in approximately 40% and 55% yield, respectively, using methanol as extraction solvent. Qualitatively, saponins, tannins, flavonoids, terpenoids, alkaloids and phenols were present in both samples however, cardiac glycosides, anthraquinones and steroid was absent in the fruit. Quantities of the compounds ranged from 43 to 940 mg/100g (leaves) and 18 to 560 mg/100g (fruit), respectively. The fruit extract inhibited the growth of *Staphylococcus aureus* (15±0.15 mm), *Pseudomonas aeruginosa* (20±0.12 mm), *Bacillus alvei* (30±0.47 mm) and *Escherichia coli* (12±0.52 mm) while the leaf extract inhibited only *S. aureus* (14±0.91 mm) and *B. alvei* (15±0.76 mm). 200 mg/mL was the minimum inhibitory concentration of the extracts against *S. aureus* and *B. alvei* and *E. coli.* Conclusively, the findings of this study indicated that soursop leaf and fruit have varied phytochemicals. The fruit extract however showed higher antimicrobial inhibitory effects when compared to the leaf extract.

Key Words: *Plant extract, Antimicrobial, Solvent extraction, Pathogens, Soursop.*

Introduction

Annona muricata is known generally as Soursop (Atawodi, 2011). It is from the family of custard apple tree (Annonaceae), a species of a genus known for its edible fruits called Annona. The tree, with low branches is about 5–10 m tall and 15–83 cm in diameter (Orwa et al., 2009). The creamy flesh of the fruit which has a slightly acidic taste when ripe consist of 80% water, 1% protein, 18% carbohydrates and a good amount of vitamins B, B2 and C, as well as potassium and dietary fiber (Anibasa, 2014). The leaves of soursop have a dark green colour while the young leaves have a yellowish green colour, which measures up to 8-16×3-7 cm and are elliptical with short pointed tip, glossy surface, flat edges with a stalk length of about 3-7 mm (Ameta et al., 2018). Its use in medicine has come to fore as Researchers claims that it has inhibitory potential against common pathogens. In the last decades, soursop has been widely studied due to its therapeutic potential and ever since, it has attracted attention due to its bioactivity (Coria-Tellez et al., 2018). Aside having nutrients and minerals that are beneficial to the body, soursop have been proven to possess compounds that exhibited anticancerous, antitumor and antimicrobial effects (Anibasa, 2014; Ameta et al., 2018). Findings revealed that *Annona muricata* had shown antibacterial activities against species of pathogenic bacteria indicating that the antibacterial properties of this plant can be used in the prevention of diseases caused by pathogenic bacteria (Okeke-Nwolisa et al., 2023). There are natural compounds present in the stem, bark, leaves, roots, seeds and fruits of soursop, with the leaf being the most studied part of the plant, followed by the roots (Silva et al., 2022). This study was aimed at comparing the efficacy of soursop fruit and leaf extracts against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus alvei* and *Escherichia coli,* pathogens that have been widely reported to be antimicrobial resistant.

Materials and Methods

Samples and culture collection

The leaves and fruits of *Annona muricata* was collected from a household garden in Oyo State, Nigeria and identification/authentification was done at the herbarium of the Botany Department of University of Ibadan. Test isolates (*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus alvei* and *Escherichia coli*) used for the antimicrobial analysis were obtained from the Department of Medical Microbiology, University College Hospital (UCH), Nigeria.

Sample extraction

Samples were air-dried for two weeks then blended with a laboratory blender (Rico, India) and stored in a clean container at room temperature.

Extraction was done according to the modified standard procedures of Olugbuyiro et al., (2017). 60 g of each sample was added to 700 mL methanol and shaken intermittently for 48 hours. Extract was strained and filtered over anhydrous sodium sulphate and then evaporated in vacuo at 45°C. The concentrated fractions were dried in desiccator and kept for further analysis.

Estimation of extract yield

The total yield of the extracts were calculated using the formula: $\%Yield = \frac{W1}{W2} \times 100$

where W1 = weight of the extract and W2 = weight of the dry blended samples (Adam et al., 2019).

Phytochemical Screening of plant extracts

Qualitative and quantitative phytochemical screening was carried out on *A. muricata* extract to determine the presence and estimation of bioactive compounds according to the methods of Harborne, (1998) and Dohou et al. (2003).

Antimicrobial activity

The antimicrobial potential of the extracts against four test organisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus alvei*), using the well diffusion assay was done according to the method of Yusuf et al. (2021), while the minimum inhibitory concentration of the extracts was also analyzed (Usman et al., 2013).

Results and Discussion

24g and 33g extract was obtained from 60g dried soursop leaves and fruits, resulting in approximately 33% and 55% yield, respectively, using methanol as extraction solvent as indicated in table 1. The fruit was more fibrous and this could have accounted for a higher extract yield. Steroid and Cardiac glycoside and anthraquinone were not detected in the fruit extract while only steroid was negative in the leaf. Compounds like alkaloid, phenol, tannin, flavonoid, saponin and terpenoid were all detected in both the leaf and fruit of soursop (Table 2). Similar findings were established by Olugbuyiro et al. (2017) and Hasmila et al. (2019). These compounds have been reported to have biomedical relevance (Ana-Paola et al., 2020). Qantitatively, phyto-compounds were higher in the leaf than the fruit, except for terpenoid and tannin. Alkaloids, however, was the most abundant in both samples with 940 and 560 mg/100 mL, as indicated in figure 1.

Plate 1. *Annona muricata* (soursop) leaves and fruits (Source: Britannica, 2016)

Table 1. Percentage yield of *A. muricata* leaf and fruit extract using methanol as extraction solvent

Sample	Dry weight (g)	Weight of extract (g)	% yield	Characteristics features
Fruit	60	33	55	Creamy jelly-like extract
Leaf	60	24	33	Dark green jelly-like extract

Turkish Journal of Agriculture - Food Science and Technology
Türk Tarım - Gıda Bilim ve Teknoloji Dergisi
International Peer-Reviewed Journal | ISSN: 2148-127X
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Table 2. Qualitative analysis of phytochemical *compounds in A. muricata* leaf and fruit extracts

Phytochemical compounds	Soursop Fruit Extract	Soursop Leaf Extract
Alkaloid	+ve	++ve
Phenol	+ve	++ve
Cardiac glycoside	-ve	++ve
Tannin	+ve	+ve
Flavonoid	+ve	+ve
Saponin	+ve	+ve
Anthraquinone	-ve	++ve
Terpenoid	+ve	+ve
Steroid	-ve	-ve

Key: +ve = present; ++ve = abundantly present; -ve = absent

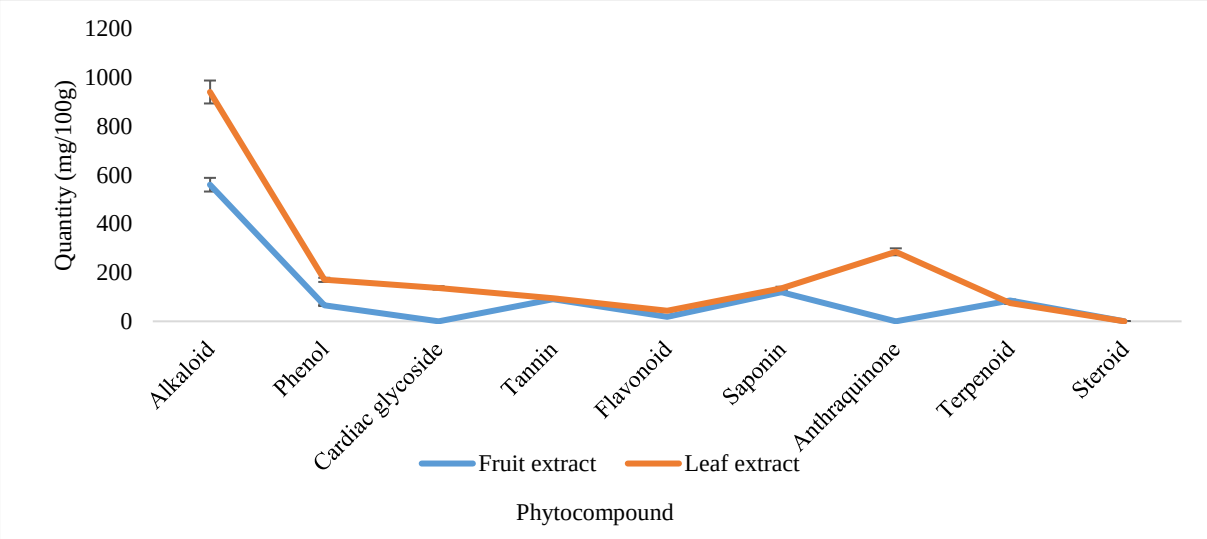

Figure 1. Quantitative analysis of phytochemical *compounds in A. muricata* leaf and fruit extracts

Antimicrobial inhibition zones ranged from 12 mm to 30 mm for the fruit extract and 14 mm to 15 mm for the leaf extract indicating that the fruit showed higher antimicrobial activity against wider range of test isolates (table 3). Both extracts had highest inhibition zones against *Bacillus alvei* however, the fruit extract showed the least zone (12 mm) against *E. coli* while the leaf extract did not inhibit the growth of *E. coli.* Iyanda-Joel et al. (2019) reported 17 ± 0.58 and 16 ± 0.58 as inhibition zones of soursop fruit ethanol extract against *S. aureus and P aeruginosa*, respectively, considering that *S. aureus* has been reported as one the most susceptible bacteria to plant extracts (Melendez and Capriles, 2006). Susceptibility of some clinical isolates (fungi and bacteria) to fruit pulp extract was also reported by Okeke-Nwolisa et al. (2023) where the growth of *E. coli., S. aureus, Candida albicans, Aspergillus niger, Klebsiella* sp. *and Proteus* sp.were inhibited with zones ranging between 20 to 36 mm. However, the zones reported in the current study were lower than the range in the studies conducted by Olugbuyiro et al. (2018) and Okeke-Nwolisa et al. (2023), using extracts from the fruit. Positive activity of the leaf extract against *S. aureus* was also demonstrated by Pinto et al. (2017), attributing reason for such to the presence of alkaloids. *E. coli* and *P. aeruginosa* were however resistant to the leaf extract in this study.

Upon reduction in the extract concentration ustilized, it was observed that the zones of inhibitions significantly reduced (table 4), even though, concentrations below 200 mg/ mL did not inhibit the isolate's growth. This is an indication that a more increased concentration could further lead to higher inhibition zones.

Table 3. Antimicrobial sensitivity of test isolates to the leaf and fruit extracts of *A. muricata*

Test isolates	Extract (250 mg/mL) / Diameter zone of inhibition (mm)	
	Fruit	Leaf
Staphylococcus aureus	15 ± 0.15	14 ± 0.91
Pseudomonas aeruginosa	20 ± 0.12	-
Bacillus alvei	30 ± 0.47	15 ± 0.76
Escherichia coli	12 ± 0.52	-

Values reported are means ± standard deviation of three replicates; Key: - = Resistance

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International Peer-Reviewed Journal | ISSN: 2148-127X
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Table 4. Minimum Inhibitory Concentration (MIC) of methanol extracts of soursop leaf and fruit against selected pathogens

Test isolates	Extract Concentration (mg/mL) / Extract / Diameter zone of inhibition (mm)					
	100		150		200	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
Staphylococcus aureus	-	-	-	-	12 ± 1.15	14 ± 0.75
Pseudomonas aeruginosa	-	-	-	-	-	05 ± 0.12
Bacillus alvei	-	-	-	-	10 ± 0.23	14 ± 0.69
Escherichia coli	-	-	-	-	-	03 ± 1.00

Key: - resistant

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