PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACTS OF LEAF AND SEED OF *Annona muricata* (SOURSOP) LINN

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Abstract: *Annona muricata* belongs to the *Annonaceous* family which has been of great importance to humans since ancient times. Soursop have contributed to the world pharmacologically in providing antimicrobial, anti-arthritis, antioxidant, anti-inflammation and anticancer properties beneficial to man. The research was conducted to determine the phytochemical screening and antibacterial activity of ethanol extract of the leaf and seed of *Annona muricata* Linn (Soursop). The phytochemical screening and antibacterial activity were done according to standard methods. Terpenoid and flavonoid were present while the antibacterial activity indicated zones of inhibition at a concentration of 100 mg/ml; 28 mm (*Staphylococcus aureus*), 11 mm (*Streptococcus mutant*), 16 mm (*Pseudomonas aeroginosa*) and 25 mm (*Eschericha coli*) for the seed extract. The leaf indicated a dose dependent activity when compared to ciprofloxacin. The study has shown that the extracts of *A. muricata* contained rich bioactive chemical constituents with significant antibacterial activity.

Keywords: *Annona muricata*, antibacterial, leaf, phytochemicals, seed, ethanol extract

Introduction

Plants are potent biochemical materials which have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals (Prashant *et al*., 2011). According to World Health Organization (WHO), more than 80% of the total world's population still depends on the traditional medicines in order to satisfy their primary health care needs (WHO, 2003). In the early 14th Century, herbs were used for treating diseases based on the similarity in shape between the leaf of the plant and the part of the body suffering from the diseases e.g. plant with heart shaped leaves where used for treating heart diseases, while plant exuding milky juices were believed to increase lactation in women (Katendered *et al.,* 1995). Plant based natural constituents can be derived from any part of the plant: bark, leaves, flowers, roots, fruits and seeds. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories.

Soursop, also known as *Guanabana* is one of the exotic fruits prized for its very pleasant, sub-acid, aromatic and juicy flesh (Morton, 1987). The fruit pulp contains 80% water, 1% protein, 18% carbohydrate and fair amounts of vitamin B1 and B2 (Umme *et al.,* 1997). The pulp is also used to make fruit nectar, smoothies, fruit juice drinks, as well as candies, sorbets, and ice cream flavourings. It is consumed as a desert fruit. The seeds are flat, hard and contain all that can be used for paint or insecticide (Rice *et al.,* 1991). The crushed seeds are used as a vermifuge and anthelmintic against internal and external parasites and worms, as an astringent for diarrhoea and dysentery and to increase mother's milk after childbirth (lactagogue, which augment established lactation) (Gills, 1992). The plant possess the major activities as herb remedy of arthritis, asthma, bronchitis, skin rashes, malaria, liver ailments and leishmaniasis (Sejal and Jayvadan, 2016). This research is aimed at conducting a preliminary research on the phytochemicals and antibacterial activity of the leaves and seeds of sour sop.

Materials and Method

Sample collection

Annona muricata leaves and fruit were collected from Upper Sakponba Area of Benin City, Edo State. The plant was identified and authenticated by Dr Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin where a voucher specimen was prepared and herbarium specimen number UBH_A 356 was deposited. Theleaves and seeds were washed under running tap water and air dried and then homogenised into powder using a mechanical grinder and stored in a clean container.

Extraction

Ethanol extracts of the plant leaf and seed were respectively prepared by soaking 100 g of the dry powdered plant parts each in 1000 ml (1L) of absolute ethanol at room temperature for 48 h. The extracts were then filtered first through a Whatmann filter paper No. 42 (125 mm) and through cotton wool. The extract was thereafter concentratedusing a rotary vacuum set at 40^oC to about one-tenth the original volume of the extract. A portion of the dried residue (crude extracts) were used for phytochemical screening.

Phytochemical screening

Phytochemical screening were performed on the both extracts using the procedures by Sofowora (1993), Trease and Evans (1989), as well as Odebiyi and Sofowora (1978).

Test for glycosides

1mlof the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc. H_2SO_4. A brown ring is required for the presence of glycoside.

Test for saponins

0.5 g of plant extract was shaken with water in a test-tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was used as a standard.

Test for flavonoid

2 ml of the extract was boiled with distilled water and filtered. 5 ml of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colourless solution is indicative of a positive test.

Test for phenolic compounds

1 ml of the plant extract was added to 5 ml of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was added. A pale yellow colouration of indicative of positive test.

Test for tannins

To 2 ml of the extract, 10ml of distilled water was added and boiled for 5 minutes and then filtered into halves. To about 2 drops of the filtrate, ferric ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.

Test for Eugenols

2 ml of the extract was mixed with 5% KOH solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale yellow precipitate is indicative of a positive test

Test for steroids

2 ml of acetic anhydride was added to 0.5 g plant extract in 2 ml of dilute H_2SO_4. Acolour change from violet to blue or green is required for the presence of steroids.

Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform and 3mls of conc. H_2SO_4 was carefully added down the side of the inner wall of test tube to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

Test for alkaloids

2 mls of Picric acid was added. A yellowish precipitate test is a positive test.

Antibacterial activity of ethanol extract of soursop leaf and seed

Bacteria

The bacterial clinical isolates employed in this study and obtained from the University of Benin Teaching Hospital, Benin City were *Staphylococcus aureus, Eschericha coli, Pseudomonas aeruginosa and Streptococcus mutant*.

Media

Nutrient broth and nutrient agar, both products of Himedia Laboratories Mumbai (India) were used in this study. The composition of the medium was Beef extract -3.0 g, peptone -5.0 g, sodium chloride -8.0 g, agar-15.0 g.

Agar well diffusion assay

The antimicrobial activity of the extracts was determined by using agar well diffusion technique. Nutrient agar plates were seeded with 0.1 ml of an overnight culture of each bacterial (10^6 CFU/mL). The 24 h broth culture of each bacterium were used to seed molten nutrient agar at 45°C, allowed to set and a well was made by sterile standard cork borer (6.0 mm in diameter and 200 µl (0.2 ml) of various concentration of soursop plant extract added into each well. Then bacterial plates incubated at 37°C for 24 h after which diameter of zones of inhibition were measured. (Monica, 2006).

Determination of minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC values of the leaf and seed extract of soursop were determined using two fold micro-dilution to prepare concentrations of 100, 50, 25 and 12.5 mg/ml of each extract and a drop of the bacterial suspension that had been previously diluted to 10^6 cfu/ml were a sceptically incorporated into molten nutrient agar and allowed to set. The plates were incubated to at 37°C for 24 hours. The lowest concentration preventing visible growth for each of the test organisms was recorded as the MIC. Ciprofloxacin was used as the positive control.

Determination of minimum bactericidal concentration (MBC)

Minimum bactericidal concentration is the lowest concentration of antibacterial agent required to kill a particular bacterium. The MBC was identified by determining the lowest concentration of antibacterial agents that reduces the viability of the initial bacterial inoculum by $\geq$ 99.9%. Nutrient agar plates were divided into different sections and labelled with the different concentration on the base of the plates; these were used to plate content of each MIC aerobically at 37°C for 18-24 h, after which MBC were recorded. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

Results and Discussion

The results of the phytochemical screening of the ethanolic extract of the leaves and seeds of *Annona muricata* are shown in Table 1. The MIC and MBC zones of inhibition the soursop leaf and seed extracts are shown in Tables 2 and 3.

Phytochemical constituents

The phytochemical screening of *Annona muricata* plant parts (Table 1) showed that glycosides, alkaloids, saponins, and tannin were present in ethanolic extract of leaves and seeds. Alkaloids have been reported to have anti- inflammatory and anti-microbial properties (Kumar and Tandon, 1979). Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al.*, 1995). *Annona muricata* is used for the treatment of cardiac infections along with other ailments such as cough, and chest pain in Jamaica, Haiti, and the West Indies (Taylor, 2002). Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells (Usunobun, 2012). Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al*., 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Steroids was present only in the leaf extract and absent in the seed. Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Terpenoids and flavonoids were present in the ethanol extract of leaf but absent in the seed.Flavonoids possess antioxidant activity and may play a beneficial role in cancer prevention, and offer some protection against diabetes and atherosclerosis (Doughari, 2012).

Table1: Phytochemical constituents of ethanol extract of Soursop leaves and seeds

Phytochemicals	Leaves extract	Seeds extract
Glycosides	+	+
Steroids	+	-
Terpenoids	+	+
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Phenolics	-	-
Eugenols	+	+

+ = Present; - = Absent

Antibacterial activity of Annona muricata leaf and seed extract on isolates

The ethanolic extract of *Annona muricata* leaves was tested for the minimum inhibition concentration and minimum bactericidal concentration of the growth of the following microorganisms: *Staphylococcus aureus, Eschericha coli, Pseudomonas aeruginosa* and *Streptococcus mutant.* The antibacterial activity of the leaves extract (Table 2) indicated a significant activity with zones of inhibition; 15 mm (*S. aureus*), 12 mm (*S. mutant*), 13 mm (*P. aeroginosa*) and 14 mm (*E. coli*) at a high dose of 100 mg/ml. However, as concentration of the extract decreased, the zones of inhibition were also reduced. This indicate that the extract showed a dose dependent activity. In comparison, this result corroborates the findings of Bussmann *et al.* (2010) whose reports indicated high antibacterial activity when the plant extract was screened in aqueous and ethanol solvents while the fruit skin and leaf extract of *Annona muricata* also gave high antibacterial potency in the work of Solomon-Wisdom *et al.* (2014).

Table 2: Minimum inhibitory concentration (MBC) for Soursop leaves

Microorganism	Minimum Inhibitory Concentration (MIC) (mg/ml)					
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Ciprofloxacin 0.01 mg/ml	MBC 12.5 mg/ml
	Zone of inhibition *(mm)					
S. aures	15	12	10	9	15.6	9
S. mutant	12	11	10	9	14.6	8
P. aeroginosa	13	12	11	10	18	10
E. coli	14	13	12	11	13.4	10

MBC- Minimum bacteriacidal concentration
(-) - No activity, <10 mm- non significant activity
10 – 19 mm - Significant activity, >20 mm – high activity
(National Committee for Clinical Laboratory Standard (NCCLS, 1993)
*Average of three observations adjusted to the nearest whole number

Table 3: Minimum inhibitory concentration (MBC) for Soursop seed

Microoganism	Minimum Inhibitory Concentration (MIC) (mg/ml)					
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Ciprofloxacin 0.01 mg/ml	MBC 12.5 mg/ml
	Zone of inhibition *(mm)					
S. aureus	28	21	14	12	15	9
S. mutant	11	9	NG	NG	14	11
P. aeroginosa	16	12	9	7.5	18	6.8
E. coli	25	19	13	11	13	6.5

MBC- Minimum bacteriacidal concentration
NG- No growth
(-) - No activity, <10 mm- non significant activity
10 – 19 mm – Significant activity, >20 mm – high activity
(National committee for clinical laboratory standard (NCCLS, 1993)
*Average of three observations adjusted to the nearest whole number

In Table 3, the seed extract showed high activity against *S. aureus* (28 mm), and *E. coli* (25 mm) at the same dose of 100 mg/ml. This suggests that the seed extract will selectively inhibit infections of *S. aureus* and *E. coli* more than the leaf extract. When compared with the standard drug ciprofloxacin, the seed extract show a good activity as an antibacterial agent. This is possibly due to the fact that extract of high polarity showed antibacterial activity than the others as reported by Alade and Irobi (1993) However, alcohol extracts of *Annon amuricata* has generally be reported to have high potency against bacteria than other polar solvents like water (Viera *et al.*, 2010). The minimum bacteriacidal concentration (MBC) were observed at 12.5 mg/ml for all test organisms in Table 2 (leaf extract and in Table 3 (seed extract).

Conclusion

The phytochemical investigation conducted on *Annonamuricata* in this work had shown that it contains different essential bioactive phytochemicals. These bioactive phytochemicals can be used in treating and preventing some biological diseases such as inflammation, cancer, diabetes and arthritis. The antibacterial test clearly show that both the ethanol extract of the leaves and seeds have suitable agents inhibiting bacteria.

Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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