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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL SCREENING OF METHANOL EXTRACT OF *ANNONA MURICATA* L. LEAVES

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Antibacterial activity, methanol extract, zone of inhibition, sensitivity test, antibiotics, phytochemicals.

ABSTRACT

The plant *Annona muricata* (soursop) is used in traditional medicine for the treatment of many disease conditions such as diabetes, dysentery and malaria. Hence, the need for a scientific study to justify some of the curative claims associated with its use by local healers. To achieve this, we carried out cold maceration extraction on the leaves using methanol as extracting solvent. Phytochemical analysis of the crude extract shows that flavonoids, saponins, and tannins were present, while alkaloids and anthraquinones were absent.

The extract was tested for anti-microbial activities on both bacterial and fungal isolates using ciprofloxacin and econazole as positive controls respectively. The isolates used in this study were *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. Results show that the extract was active on *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* but inactive on *Candida albicans* and *Aspergillus niger*, an indication that the plant extract possessed only antibacterial, and no antifungal, activities. The zone of growth inhibition ranged from 12 to 20 mm with variable MIC and MBC values. These results support the use of the soursop leaf concoction as herbal medication for the treatment of various bacterial diseases.

INTRODUCTION

For thousands of years now, man has depended so much on the use of different parts of plants for remediation against different ailments, within the realm of traditional or herbal medicine, including snake-bite, conjunctivitis, burns, abdominal pains, peptic ulcer, diarrhea, dysentery, chronic ulcer, measles, hepatitis, arthritis and rheumatism (Esuoso and Odetoun, 2005). Since plants produce many chemical compounds, some of which are the sources of drugs used in orthodox medicine (Sofowora, 2001), but a number of which are known poisons or have the potential of being poisonous, there is growing interest on the safety of various traditional medicine formulations (Bubayero, 1998; Sofowora, 2001). However, an increasing reliance on the use of medicinal plants in Chinese and Indian Medicine, and in many developing nations may be linked to the extraction and development of several drugs and chemotherapeutics from these plants (Sack and

Forehlich, 1982). Chemical constituents in this plant that have physiological actions in the human and animal body are called phytochemicals (Zaidi, 1998). Some of the important bioactive compounds found in medicinal plants are alkaloids, glycosides, resins, gums, mucilages etc. These are important raw materials in many orthodox drug formulations (Shinawie, 2002).

A. muricata, commonly known as soursop, is a member of the Annonaceae family comprising approximately 130 genera and 2300 species (Mishra *et al.*, 2013). *A. muricata* is native to the warmest tropical areas in South and North America, but can now be found widely distributed in the tropical and subtropical parts of the world, such as India, Malaysia and Nigeria (Adewole and Caxton-Martins, 2006). This plant has an erect growth with a large canopy height-to-diameter ratio, although it tends to be low-branching and bushy. It is 4-8m tall, slender, evergreen tree, when fully matured. The

leaves have short petioles, and are oblong-ovate to cylindrical, 14-16cm in length and 5-7cm in width (Tindall, 1977).

In South America and tropical Africa, including Nigeria, the leaves of *A. muricata* are used in ethno-medicine as anti-tumor and anti-inflammatory agent (Adewole and Ojewole, 2009). This study was, therefore, designed to study the antimicrobial potency of the leaf extract of this plant against selected microorganisms, bacteria and fungi, after extraction with methanol as extracting solvent.

MATERIALS AND METHODS

Reagents and Chemicals

All reagents and chemicals used were of analytical grade and purchased from reliable dealers.

Test Organisms

The test organisms used for this analysis were clinically isolated bacteria and fungi obtained from Department of Microbiology, Ahmadu Bello University Zaria. The bacterial isolates were *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, while the fungal isolates were *Aspergillus niger* and *Candida albicans*

Sample Collection and Identification

The leaves of soursop (*Annona muricata*) were collected from Poly Quarters, Kaduna South L.G.A, Kaduna State, Nigeria. The species was identified (with voucher number 1726) at the Herbarium in the Department of Botany, Ahmadu Bello University Zaria. After washing with water to remove dirt, the leaves were dried in open air under shade, and then weighed. They were ground with mortar and pestle in order to obtain a powdered mass which was then kept in closed pockets of plastic.

Preparation of Extract of Soursop Leaves

Exactly 80 g powder of the air dried sample was added to 10 times of the solvent (methanol). The sample was kept in dark for 6 days for cold maceration with intermittent shaking. At the expiration of this duration, the solution was filtered through a Whatman filter paper No. 1 and the filtrate was evaporated to dryness (Ayensu, 1978).

Phytochemical Screening

Phytochemical screening was conducted in order to ascertain the presence of secondary metabolites including alkaloids, flavonoids, saponins, tannins,

and anthraquinones in accordance with standard procedures reported by Sofowora (1982) and Trease and Evans (2002).

Preparation of Extract for Antimicrobial Analysis

Exactly 1g of the methanol extract was dissolved in DMSO to obtain the concentration of 100 mg/ml. This was serially diluted to give concentrations of 50mg/ml, 25 mg/ml, 12.5mg/ml and 6.25 mg/ml.

Culture Media

The culture media used in this study were Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), Potato dextrose agar (PDA) and Nutrient agar (NA). The mentioned media were used for sensitivity test, determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). All media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 15 min.

Determination of Inhibitory Activity (Sensitivity Test) of the Extract Using Agar Well Diffusion Method

The standardized inocula of both the bacterial and fungal isolates were streaked on sterilized Mueller Hinton and Potato dextrose agar plates respectively with the aid of sterile swab sticks. Four wells were punched on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentrations of the extract prepared which were 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract.

The inoculated plates with the extract (Figure 1) were allowed to stay on the bench for one hour to enable the extract to diffuse on the agar. The plates of Mueller Hinton agar were then incubated at 37°C for 24 h while the plates of potato dextrose agar were incubated at room temperature for 5 days.

At the end of the incubation periods, the plates were observed for any evidence of inhibition which appeared as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimeter and the result was recorded.



Figure 1: Culture media showing zone of inhibition for sensitivity test against bacterial isolates
Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the extract was determined in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2012) micro-broth dilution method using the Mueller Hinton broth as a diluent. The lowest concentration of the extract showing inhibition for each organism when the extract was tested during sensitivity test was serially diluted in the test tube containing Mueller Hinton broth. The organisms were inoculated into each tube containing the broth and the extract. The inoculated tubes were incubated at 37°C for 24 h.

At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion; the lowest concentration is the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extract. This was to ascertain whether antimicrobial effect of the extract was bacteriostatic or bactericidal.

A sterilized wire loop was dipped into the test tubes that did not show turbidity (clear) in the MIC test and streaked on a sterile nutrient agar plates. The plates were incubated at 37°C for 24 h. At the end of incubation period, the plates were observed for the presence or absence of growth.

RESULTS AND DISCUSSION

From the 80 g starting material of powdered soursop leaves, 7.02 g of the methanol extract was obtained, amounting to 8.78% yield. Determination of extraction yield gives insight into the ability of a

solvent to extract the bioactive constituents in plants (Basri *et al.*, 2014). Different yields are obtained due to differences in solvent polarity indices (Nur Syukriah *et al.*, 2014). Following the “like dissolves like” principle (Pathmanathan *et al.*, 2010; Gupta *et al.*, 2012), higher values are usually obtained by using polar solvents compared to non-polar ones, an indication that the polar bioactive components of plants are more than their non-polar counterparts (Joshua and Takudzwa, 2013).

The result of the phytochemical screening of the leaf of *Annona muricata* is shown in Table 1.

Table 1: Result for the Phytochemical Screening of the Methanol Extract of *A. muricata*

TESTS	Result
Saponins	+
Flavonoids	+
Tannins	+
Alkaloids	-
Anthraquinones	-

(+) = present, (-) = absent

The result shows that *Annona muricata* methanol leaf extract contained saponins, flavonoids and tannins while alkaloids and anthraquinones were absent. Similar results were obtained by Foong and Hamid (2012), Abiodun *et al.* (2011), and Vijayameena *et al.* (2013). Phytochemicals elicit both biochemical and pharmacological activities on living cells. According to Okwu and Emineke (2006), saponins possess anti-inflammatory activity and may be responsible for most biological effects related to cell division and growth in humans. Roa *et al.* (1995) posited that saponins may limit the growth and viability of cancer cells by reacting with the cholesterol rich membranes of such cells.

Flavonoids and phenolics have potential anticancer applications since they have been found to exhibit free radical-scavenging activities important in preventing or reducing oxidative cell damage (Pourmoradet *et al.*, 2006; Ugwu *et al.*, 2013).

Tannins confer astringent characteristics to herbs and are found in many local concoctions used for the treatment of diarrhea and dysentery (Bajai, 2001). This may explain why *Annona muricata* is found among the list of medicinal plants used for

the treatment of microbial infections. Tannins may also have anti-inflammatory, anti-ulcerative and anti-tumor applications. (Adegboye *et al.*, 2008; Okwu and Emineke, 2006).

The results of the antimicrobial susceptibility tests, expressed in terms of antimicrobial activity (sensitivity test), diameter of zones of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Tables 2, 3, 4 and 5 respectively.

Table 2: Sensitivity test at Varying Concentrations (mg/ml) of Methanol Extract Compared with Ciprofloxacin and Econazole

Test organisms	100	50	25	12.5	Ciprofloxacin	Econazole
<i>S. aureus</i>	S	S	S	R	S	R
<i>S. typhi</i>	S	S	R	R	S	R
<i>K. pneumoniae</i>	S	S	S	R	S	R
<i>P. aeruginosa</i>	S	S	S	R	S	R
<i>C. albicans</i>	R	R	R	R	R	S
<i>A. niger</i>	R	R	R	R	R	S

R= Resistant, S =Sensitive

Table 3: Zone of Inhibition (mm) from Sensitivity Test

Test organisms	100	50	25	12.5	Ciprofloxacin	Econazole
<i>S. aureus</i>	20	18	15	0	36	0
<i>S. typhi</i>	14	12	0	0	37	0
<i>K. pneumoniae</i>	18	14	12	0	35	0
<i>P. aeruginosa</i>	16	14	12	0	36	0
<i>C. albicans</i>	0	0	0	0	0	32
<i>A. niger</i>	0	0	0	0	0	40

Table 4: Minimum Inhibitory Concentration (MIC) (mg/ml) of Methanol Extract against Test Organisms

Test organisms	50	25	12.5	6.25
<i>S. aureus</i>	-	MIC	++	++
<i>S. typhi</i>	MIC	+	++	++
<i>K. pneumoniae</i>	-	MIC	+	++
<i>P. aeruginosa</i>	-	MIC	++	++

- = no turbidity, + =low turbidity, ++ =moderate turbidity

Table 5: Minimum Bactericidal Concentration (MBC) (mg/ml) of Methanol Extract against Test Organisms

Test organisms	100	50	25	12.5
<i>S. aureus</i>	-	MBC	+	++
<i>S. typhi</i>	MBC	+	+	++
<i>K. pneumoniae</i>	MBC	+	+	+
<i>P. aeruginosa</i>	MBC	+	+	+

- = no colony growth, + = scanty colony growth, ++ = moderate colony growth

The antimicrobial activities (sensitivity test), zone of minimal inhibitory concentration (MIC) and minimal bactericidal (MBC) concentration of the *Annona muricata* extract were studied using six microorganisms: *S. aureus*, *K. pneumoniae*, *S.typhi*, and *P.aeruginosa* as bacterial isolates, and *C. albicans* and *A.niger* as fungal isolates. None of the fungal species was sensitive to the extract at concentrations of 12.5-100 mg/ml (Table 2) while all the bacterial isolates showed susceptibility with zone of inhibition ranging between 12 and 20 mm compared to the 35-37 mm for ciprofloxacin as positive control. The large zones of inhibition (Table 3) recorded for the extract signifies that the extract was considerably active and this might be due to the presence of variety of bioactive constituents in the extract such as tannins and saponins (Abo *et al.*, 2000). Interestingly, the extract was inactive against *Candida albicans* and *Aspergillus niger* while these organism were sensitive to Econazole, meaning that the extract did not contain antifungal activity.

Annona muricata is often employed as a line of treatment for bacterial diseases like pneumonia and diarrhea. Our findings indicate that the plant may actually possess a broad spectrum anti-bacterial activity. This may serve as an important basis for exploring further the pharmacological and physiological activities of this plant (Solomon-Wisdomet *al.*, 2014).

The Minimum Inhibitory Concentration of the methanol extract was found to be 50 mg/ml for *S. typhi* and 25 mg/ml for *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Table 4). This is consistent with the findings of Haro *et al.* (2014).

The methanol extract of *Annona muricata* was bactericidal on *Staphylococcus aureus* at concentration of 50 mg/ml while for *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* the concentration of the extract that killed these organisms was found to be 100mg/ml (Table 5). Our findings support the justification for the ethno-botanical uses of *Annona muricata*.

CONCLUSIONS

Annona muricata is used in the traditional medicine for the treatment of diseases and symptoms such as diabetes, headaches, and sleeplessness. These applications may be rooted in the phytochemical constituents of the plant such as tannins and saponins. These are secondary metabolites produced by the plants which have been found to contain antimicrobial activity. Therefore, the use of

this plant as concoction for the treatment of several disease conditions may not be totally misplaced.

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