

Original Research Article

PHYTOCHEMICAL SCREENING AND MICROCIDAL ACTIVITY OF THE ETHANOLIC AND AQUEOUS EXTRACTS OF *Annona muricata* AGAINST SOME PATHOGENIC BACTERIA

ABSTRACT

Objective: To investigate the phytochemical composition and evaluate the microbial activity of the ethanolic and aqueous extracts of *Annona muricata* against some pathogenic bacteria.

Method: The leaves of *Annona muricata* from *Annonaceae* family which is used as herbal remedies for the cure of many ailments by natives in northern part of Nigeria, were collected in June, 2018 from Professor's Quarters, Modibbo Adama University of Technology (MAUTECH) Yola, air dried, pulverised, extracted by simple overnight maceration techniques and analyzed. Aqueous extracts of the aforementioned parts of the plant were screened phytochemically for its chemical constituents and subjected to antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Salmonella typhi* and *Methicillin-resistant Staphylococcus aureus* (MRSA).

Results: The results revealed the presence of alkaloid, tannin, flavonoid, volatile oil, triterpene, and saponin in the ethanolic extract of *Annona muricata* and tannin, flavonoid, alkaloid, triterpene, saponin in the aqueous extract of *Annona muricata*. The results of the antimicrobial activity carried out using disc diffusion method showed a zone of inhibition against tested organisms, with *Escherichia coli* being the most inhibited (27 mm) at concentration (1 mg/mL) with ethanolic extract followed by *salmonella typhi* (25 mm) at the same concentration with aqueous extract. At least concentration (0.125 mg/mL), almost all the organisms showed a zone of inhibition (6 mm) with the exception of *Salmonella typhi* (9 mm) with the aqueous extract and *Proteus vulgaris* (9 mm) with the ethanolic extract of *Annona muricata*.

Conclusion: The present study conclusively demonstrate that *Annona muricata* is a better source of various phytochemicals like tannins, alkaloids, saponins, flavonoids, triterpenoids, phenol and also justify the use of the plant as bactericidal agent for the treatment of so many diseases.

Keywords: *Annona muricata*, Phytochemical, Antimicrobial

Introduction

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any or few adverse side effect especially when compared with synthetic drugs. Medicinal plants are useful for healing and curing of human disease due to the presence of phytochemical constituents

[1]. There is abundant number of medicinal plants and only small amounts of them were investigated for its biological and pharmacological activities. Phytochemicals occurred naturally in the medicinal plants such as leaves, vegetables and roots that have defense mechanism and protect from various disease. Phytochemicals are primary and secondary compounds. The primary compounds include Proteins, Chlorophyll and common sugars while the secondary compounds have terpenoids, alkaloids and phenolic compounds [2].

In some years back, there is a little enhancement in the development of antimicrobial compounds in an effort to check the harmful effects of microorganisms [3]. Bacterial disease results when the harmful bacteria enter the organism then multiply and invade the body's defense mechanism. These pathogenic bacteria enter the body through inhalation, ingestion or damaged skin tissue. The inability of the immune system to stop the bacteria from reproducing and spreading consequently results in the symptoms of bacterial disease [4]. The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases [5].

The present research investigated the phytochemicals and antibacterial activities of *Annano Muricata* leaves extract. *A. muricata* belongs to *Annonaceae* family and also known as guayabano, soursoap and graviola [6]. Graviola fruit is sweet and full of health beneficial components with high moisture content. Flowers are in yellow or greenish-yellow color, solitary and large. Fruit is 18 cm long and covered with spine like structure. The pulps are soft white and with agreeable sour flavor [7].

Materials and Methods

Sample collection and preparation.

Fresh leave of guayabano (*Annano muricata*) was collected in June, 2018 from Professor's Quarters Modibbo Adama University of Technology (MAUTECH) Yola. The plant leave was used for the purpose of their phytochemical analysis. The leave of *A. muricata* was separated from the stalk, washed and air dried at room temperature and then pulverized, crushed into fine powder and weighed. Aliquot portion of the powdered leave was weighed and used for phytochemical analysis.

Sample extraction

Powdered *A. muricata* leave was weighed 100 g each into two containers labeled A for ethanolic extract and B for aqueous extract.

Extraction were carried out for both aqueous and ethanolic extracts of the leave of *Annano muricata* by using overnight maceration techniques [8]. About 100 g each of the powdered *Annano muricata* leave material were macerated in 400 mL of ethanol and 400 mL of distille water respectively in a flask. Each of the soaked samples (A and B) were stirred, sealed with aluminium foil and then left for 72 hours at room temperature (for thorough extraction) and the supernatant decanted. Thereafter, the extracts (A and B) were filtered through a Whatman No. 42 (125 mm) filter paper concentrated using a rotary evaporator with the water bath set at 60. °C to one-tenth its original volume and then finally freeze dried. The dried portions (crude extract) of ethanolic and aqueous extract of *A. muricata* leave were then stored at 4°C. Aliquot portions of the crude plant extracts were weighed and used for phytochemical screening.

Phytochemical screening

Phytochemical screening was performed using standard procedures [9]. Assessing the presence of the following compounds classes: tannins, alkaloids, saponins, flavonoids, triterpenoids, Glycosides and phenol.

Microrganisms

The bacterial used include *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidemidis*, *Proteus vulgari*, *Salmonella typhi* and Methicillin-resistant *Staphylococcus aureus* (MRSA). All the microorganisms were obtained from the stock culture of the Federal Teaching Hospital (FTH), Gombe state. Cultures were brought to Department of Microbiology laboratory conditions by subjecting the organisms in peptone water and thereafter sub cultured into nutrient agar medium and incubated for 24 hours at 37°C.

Determination of Antimicrobial Activity

The antimicrobial activities of both ethanolic and aqueous extracts of *A. muricata* were determined using disc diffusion method [10]. Petri dish containing 10 mL of Mueller Hinton agar medium were seeded with 24 hours old culture of selected bacterial and fungal

strains. Sterile filter paper discs (9 mm in diameter) containing 1000-5000 ppm of ethanolic and aqueous extract dissolved in DMSO, was placed on the surface of the medium. DMSO and water alone served as negative controls. A standard disc containing Amoxicilline antibiotic drug (30µg/disc) was used as a positive control. Incubation was carried out for 24 hours at 37°C. The assessment of antimicrobial activity was based on the measurement of diameter of inhibition zone formed around the disc (diameter of inhibition zone minus diameter of the disc). An average zone of inhibition was calculated in triplicates and an inhibition zone of 8 mm or greater was considered sensitive [11]. According to [12], a cleared zone bigger than 10 mm was interpreted as sensitive while smaller than 9 mm was interpreted as resistance.

Results and Discussion

Phytochemical screening of Ethanol and Aqueous extract of *Annano muricata*.

From the results obtained (Table 1), the preliminary phytochemicals investigation revealed that most of the bioactive compounds tested for, were present in the ethanolic and aqueous extract of *Annano Muricata* leaves. Saponins, tannins, flavonoids, alkaloids and triterpenes were found to be present whereas phenolic compound and Glycosides were below detectable levels in the aqueous extracts of *Annano muricata*. The ethanol extract of *Annano muricata* reveal the presence of Saponins, tannins, flavonoids, alkaloids, phenolic compound and triterpenes were all present while glycosides happens to be the only compound absent in the ethanol extract of the plant. The result of phytochemicals investigation of this study was in line with that of [14] and varies from that of the other researchers. The variation may be due to the part of the plant used, age of the plant, percentage humidity, climatic condition, soil condition, geographical location, time of harvesting or method of extraction [15; 16].

The chemical constituents present in the extracts have some therapeutic values. Tannins are plant metabolites well known for their antimicrobial properties [17]. Flavonoids have both antifungal and antibacterial activities. They possess anti-inflammatory activity [18] and [19]. Flavonoids, terpenes and alkaloid are known to have antimicrobial and bactericidal properties against some pathogenic bacteria [20].

Table 1: Phytochemical screening of Ethanol and Aqueous extract of *Annano muricata*

Bioactive compounds	Aqueous extract	Ethanol extract
±Saponins	+	+
Glycosides	-	-
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Phenolic compound	-	+
Triterpene	+	+

Key: (+) = Compound is Present, (-) = Compound is absent.

Table 2: The inhibition zone of ethanol extract of *Annano muricata* against some selected bacteria (mg/mL)

Concentration (mg/mL)	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Salmonella typhi</i> (mm)	<i>Staphylococcus epidermidis</i> (mm)	<i>Proteus vulgaris</i> (mm)
1	27±0.41	17±1.10	21±1.08	17±1.78	24±1.41
0.5	11±0.82	13±0.41	13±0.82	10±1.47	22±0.82
0.25	6±0.81	6±1.41	9±0.40	7±1.08	14±0.41
0.125	6±0.71	6±1.63	7±1.08	6±0.70	9±0.41

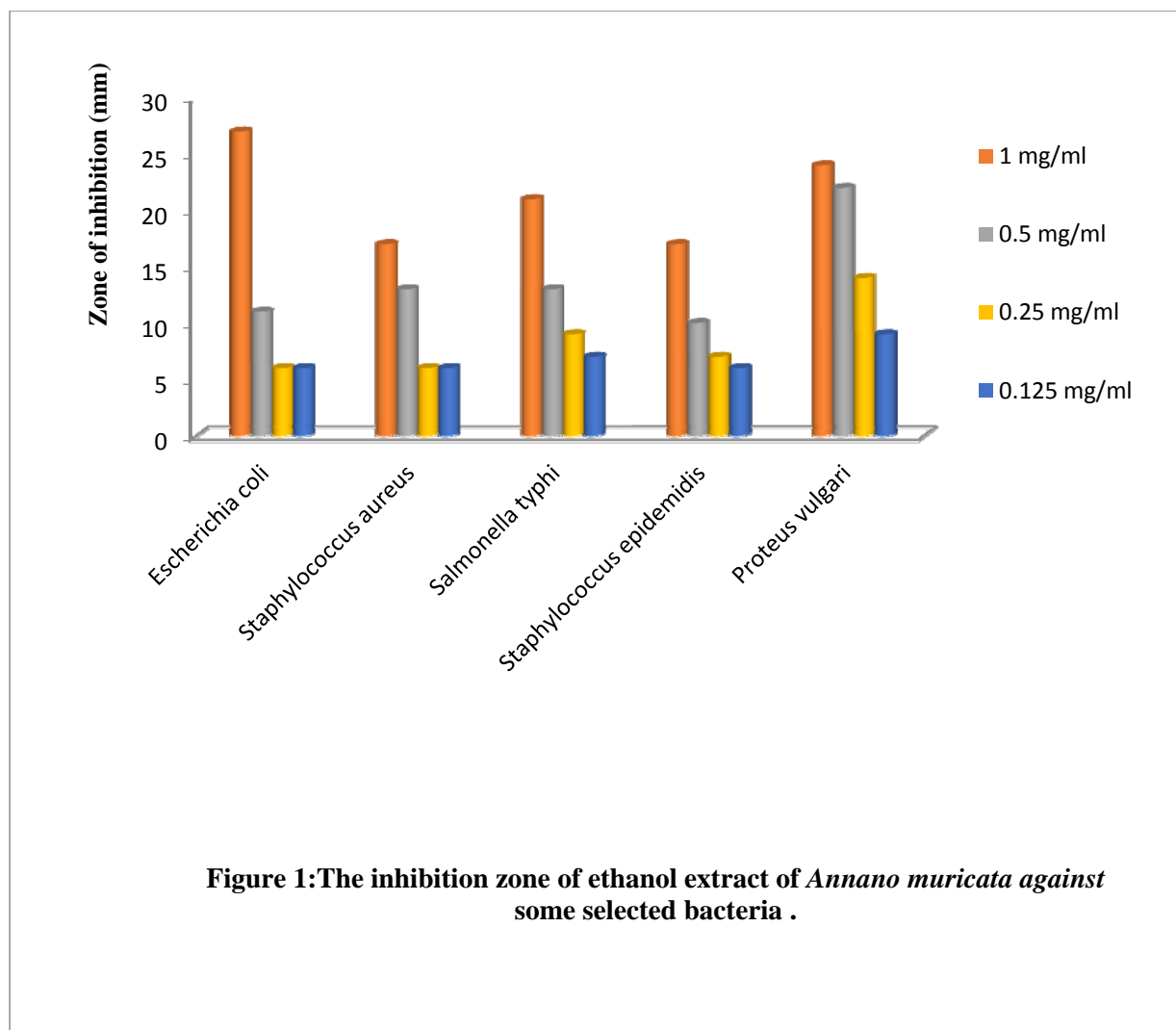
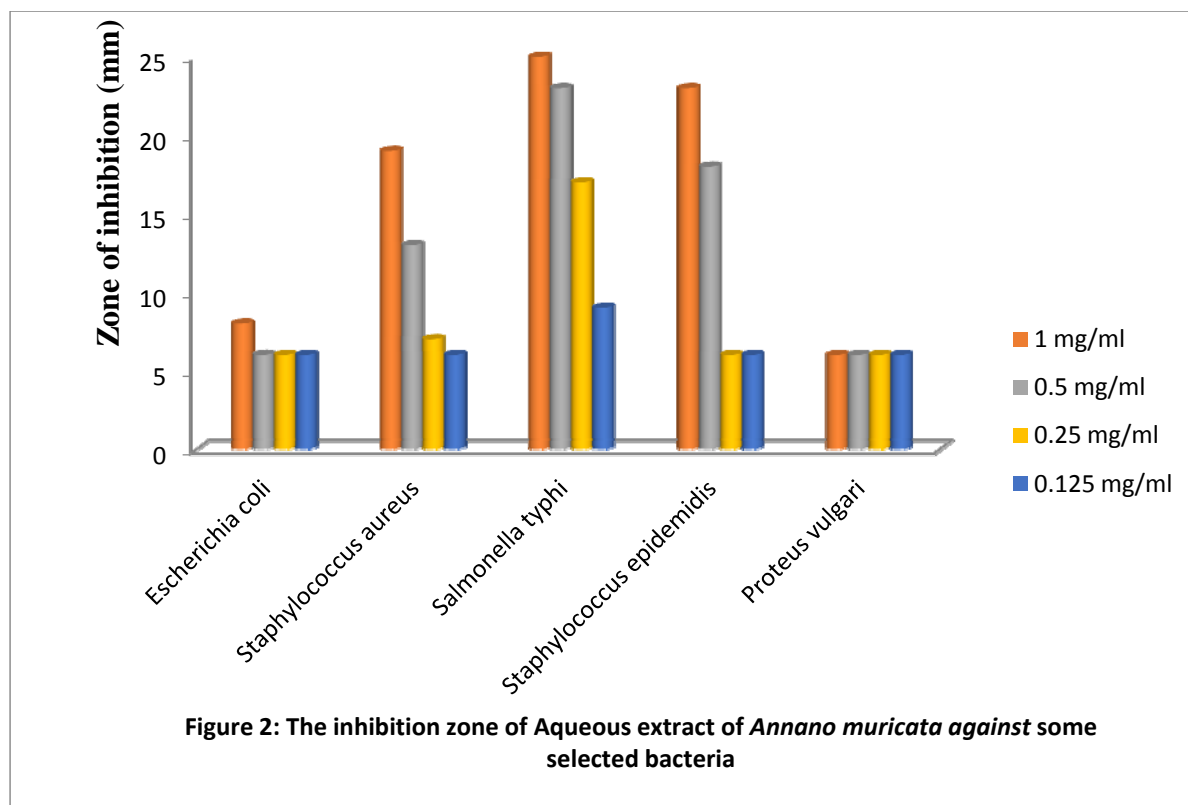


Table 3: The inhibition zone of Aqueous extract of *Annano muricata* against some selected bacteria (mg/mL)

Concentration (mg/mL)	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Salmonella typhi</i> (mm)	<i>Staphylococcus epidermidis</i> (mm)	<i>Proteus vulgaris</i> (mm)
1	8±0.41	19±1.47	25±1.22	23±1.08	6±0.82
0.5	6±0.71	13±1.08	23±0.57	18±0.70	6±0.43
0.25	6±0.42	7±0.44	17±1.77	6±0.44	6±0.41
0.125	6±0.81	6±1.08	9±0.24	6±1.63	6±0.43



The antibacterial activity of both the ethanol and aqueous extract of the leaves of *A. muricata* shows zone of inhibition against tested microorganism (Table 2 and 3). The ethanol extract showed the highest zone of inhibition (27 mm) with *E. coli* than the aqueous extract which gave (8 mm) on the same organism at the same concentration (1 mg/mL) as shown in (Figure 1 and 2), followed by *S. typhi* (25 mm) on an aqueous extract while the ethanol extract showed (21 mm) on the same organism also on the same concentration (1 mg/mL) (Figure 1 and 2). Figure 1 and 2 showed that, at (0.125 mg/mL), both the ethanol and aqueous extract showed least inhibition zone (6 mm) against almost all the microorganism except *S. typhi* for aqueous extract and *P. vulgaris*, *S. typhi* for ethanol extract of *Annano muricata*. The results of this work agrees with the work of [21] that the highest zone of inhibition produced by the ethanol extract demonstrate that ethanol was a better extracting medium for the phytochemical with antimicrobial activity.

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