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 *Annano muricata* against some pathogenic bacteria.

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

Results: The result revealed the presence of alkaloid, tannin, flavonoid, volatile oil, triterpene, and saponin in the ethanolic extract of *Annano muricata* and tannin, flavonoid, alkaloid, triterpene, saponin in the aqueous extract of *Annano 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 vulgari* (9 mm) with the ethanolic extract of *Annano muricata*.

Conclusion: This study conclusively demonstrate that Annona muricata is a better source of various phytochemicals like: tannin, alkaloid, saponin, flavonoid, triterpenoid, phenol and also justify the use of the plant as bactericidal agent for the treatment of so many diseases.

Keywords: Phytochemical, Antimicrobial, *Annano muricata* (hereafter *A.muricata*)

Introduction

In recent times, 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 in curing of human related diseases due to the presence of phytochemical constituents [1].

There are abundant numbers of medicinal plants and only small amount of them were investigated for its biological and pharmacological activities or uses. Phytochemicals occur naturally in the medicinal plants such as leaves, vegetables and roots that have curative importance against diseases. Phytochemicals are primary and secondary compounds. The primary compounds include protein, chlorophyll and common sugar while the secondary compounds have terpenoid, alkaloid and phenolic compound [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 diseases result when the harmful bacteria enter the organism, then it multiplies 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 diseases [4].The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases [5].

This research work investigate the phytochemicals and antibacterial activity of *A. muricata* leaf extracts. *A. muricata* belong to *Annonaceae* family and is also known as guayabano, soursoap and graviola [6]. Graviola fruit is sweet and full of health beneficial components with high moisture content. The flower of the plant is yellow or greenish-yellow, solitary and large. The fruit is 18 cm long and covered with spine like structure. The pulp are soft, white and with agreeable pungent flavour [7].

Materials and Methods

Sample Collection and Preparation.

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

Sample Extraction

The grinded powder of *A.muricata* leaf used in the analysis was put into two different containers labelled A and B, each weighing 100g. Container A contain ethanolic extract and container B contain aqueous extract and extraction were carried out on both the aqueous and ethanolic extracts of the plant's leaf using overnight maceration technique [8]. 100g each of the powdered plant's leaf were macerated in 400 mL of ethanol and 400 mL of distilled water respectively in a volumetric flask. Each of the soaked samples (A and B) were stirred and sealed with aluminum foil and then left for 72 hours under 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^{o}C to one-tenth its original volume and then finally freeze dried. The dried portions of ethanolic and aqueous extracts of *A.muricata* leaf powder were then stored at 4^{o}C. Aliquot portion of the crude plant extracts were weighed and used for phytochemical screening.

Phytochemical Screening

The phytochemical screening was performed using a standard procedure according to [9]. Assessing the presence of the following classes of compounds: tannin, alkaloid, saponin, flavonoid, triterpenoid, Glycoside and phenol.

Microorganisms

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

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 disc (9 mm in diameter) containing 1000-5000 ppm of ethanolic and aqueous extract dissolved in DMSO and 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 triplicate and an inhibition zone of 8 mm or greater was considered sensitive [11]. According to [12], a cleared zone bigger than 10mm was interpreted as sensitive while smaller than 9mm was interpreted as resistance.

Results and Discussion

Phytochemical Screening of Ethanol and Aqueous Extract of *Annano Muricata.*

From the result obtained (Table 1), the preliminary phytochemicals investigation revealed that most of the bioactive compounds tested of, were present in the ethanolic and aqueous extracts of *A.Muricata* leaf. Saponin, tannin, flavonoid, alkaloid and triterpene were found to be present whereas phenolic compound and glycoside were below detectable level in the aqueous extract of *A.muricata*. The ethanol extract of *A.muricata* reveal the presence of*: Saponin, tannin, flavonoid, alkaloid, phenolic compound and triterpene* were all present while glycoside happen to be the only compound absent in the ethanol extract of the plant. The result of phytochemical investigation of this study was in line with the work of [14] and varies from that of the other researchers. The variation may be due to: the part of the plant used, the age of the plant, the percentage humidity, the climatic condition, the soil condition, the geographical location, the time of harvesting or the method of extraction [15, 16].

The chemical constituent present in the extracts have some therapeutic values. Tannin are plant metabolites well known for their antimicrobial properties [17]. Flavonoid have both antifungal and antibacterial activities. They possess anti-inflammatory activity [18] and [19]. Flavonoid, terpene 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 *A. muricata*

Bioactive compounds	***Aqueous extract***	***Ethanolic extract***
Saponin	+	+
Glycoside	-	–
Tannin	+	+
Flavonoid	+	+
Alkaloid	+	+
Phenolic compound	–	+
Triterpene	+	+

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

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

Concentration (mg/mL)	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Salmonella typhi* (mm)	*Staphylococcus epidermidis* (mm)	*Proteus vulgari* (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

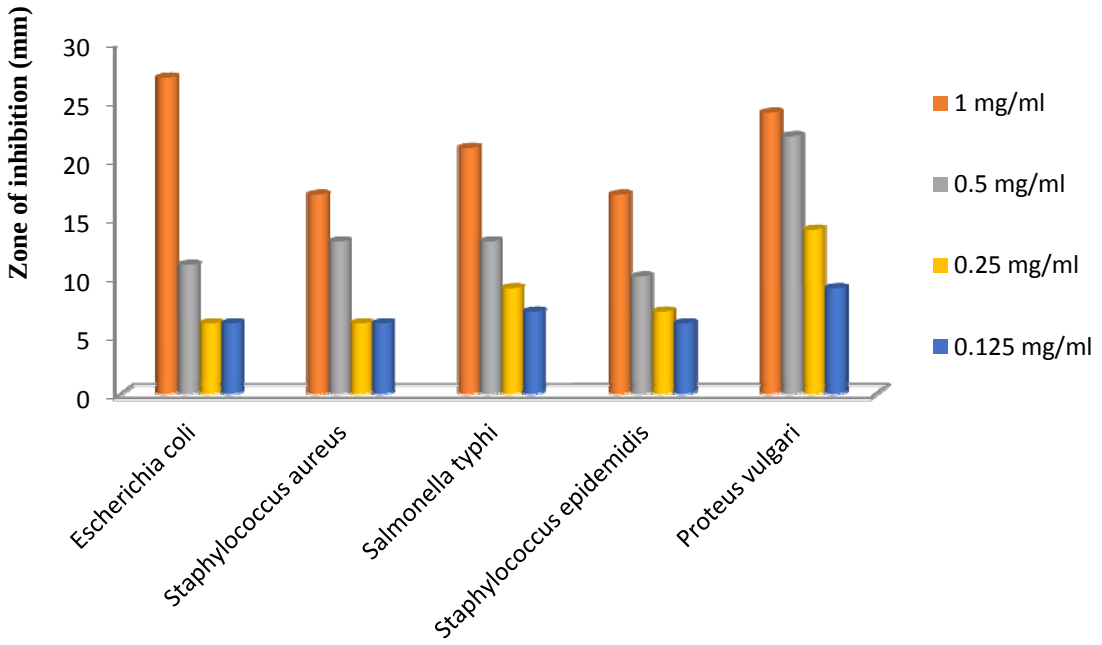

Figure 1:The inhibition zone of ethanol extract of *A. muricata against* some selected bacteria .

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

Concentration (mg/mL)	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Salmonella typhi* (mm)	*Staphylococcus epidermidis* (mm)	*Proteus vulgari* (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

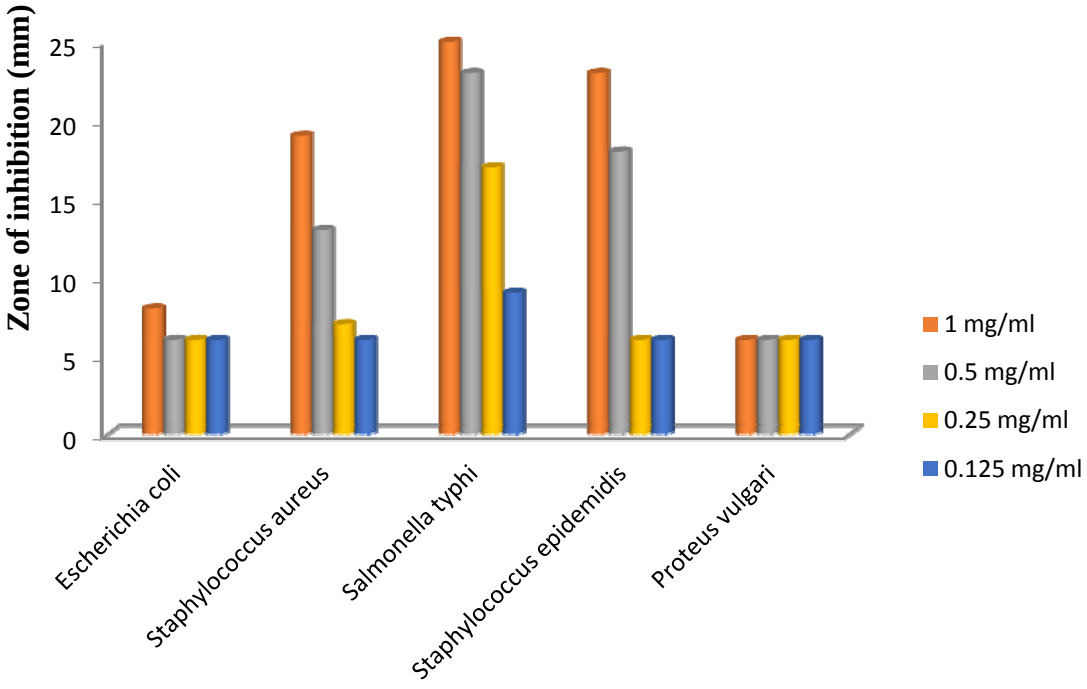

Figure 2: The inhibition zone of Aqueous extract of *A. muricata against* some selected bacteria

The antibacterial activity of both the ethanol and aqueous extract of the leaf 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. vulgari*, *S. typhi*for ethanol extract of *A. muricata.* The results of this work agree 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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