Original Research Article

PHYTOCHEMICAL SCRENNING AND MICROCIDIAL ACTIVITY OF THE ETHANOLIC AND AQUEOUS EXTRACT OF *Annona muricata* AGAINST SOME PATHOGENIC BACTERIA

ABSTRACT

Objective: To investigates the phytochemical composition and evaluates the microbial activity of the ethanolic and aqueous extracts of *Annano muricata* against some pathogenic bacteria.
Method: The leaves 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, 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 vulgari, 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 ethanol 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 ethanol 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 ethanol extract of *Annano muricata*.
Conclusion: The results thus support the use of the plants traditionally to treat chronic diarrhea, fever, diabetes, malaria and suggest its usage in the formulation of new antibacterial drugs.

Keywords: *Annano 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 leaves of guayabano (*Annano muricata*) were collected in June, 2018 from Professor's Quarters Modibbo Adama University of Technology (MAUTECH) Yola. The plant leaves were used for the purpose of their phytochemical analysis. The leaves of *A. muricata* were 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 leaves were weighed and used for phytochemical analysis.

Sample extraction

Powdered *A. muricata* leaves were weighed 100 g each into a container labeled A for *Annano muricata* leaves.

Extraction was carried out for 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 in a flask. Each of the soaked samples was stirred, sealed with aluminium foil and then left for 72 hours at room temperature (for thorough extraction) and the supernatant decanted. Thereafter, the extract was 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 residue (crude extract) was then stored at 4^{o}C. Aliquot portion of the crude plant extract residue 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, alkaloid and phenol.

Microrganisms

The bacterial used include *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidemidis*, *Proteus vulgari*, *Salmonella typhi* and Methicillin-resistant *Staphylococcus aureus* (MRSA).

Determination of Antimicrobial Activity

The antimicrobial activities of the ethanolic and aqueous extract 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. Extracts that showed positive activity in the preliminary screening were serially diluted in DMSO (two-fold) and loaded on the filter paper discs. These serially diluted concentrations of the extracts were assayed in triplicate to determine the minimum inhibitory concentrations (MIC) i.e. the minimum concentration per disc to inhibit growth of' the test microorganisms [13].

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***	***Ethanolic 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)	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Salmonella typhi* (mm)	*Staphylococcus epidermidis* (mm)	*Proteus vulgari* (mm)
1	27	17	21	17	24
0.5	11	13	13	10	22
0.25	6	6	9	7	14
0.125	6	6	7	6	9

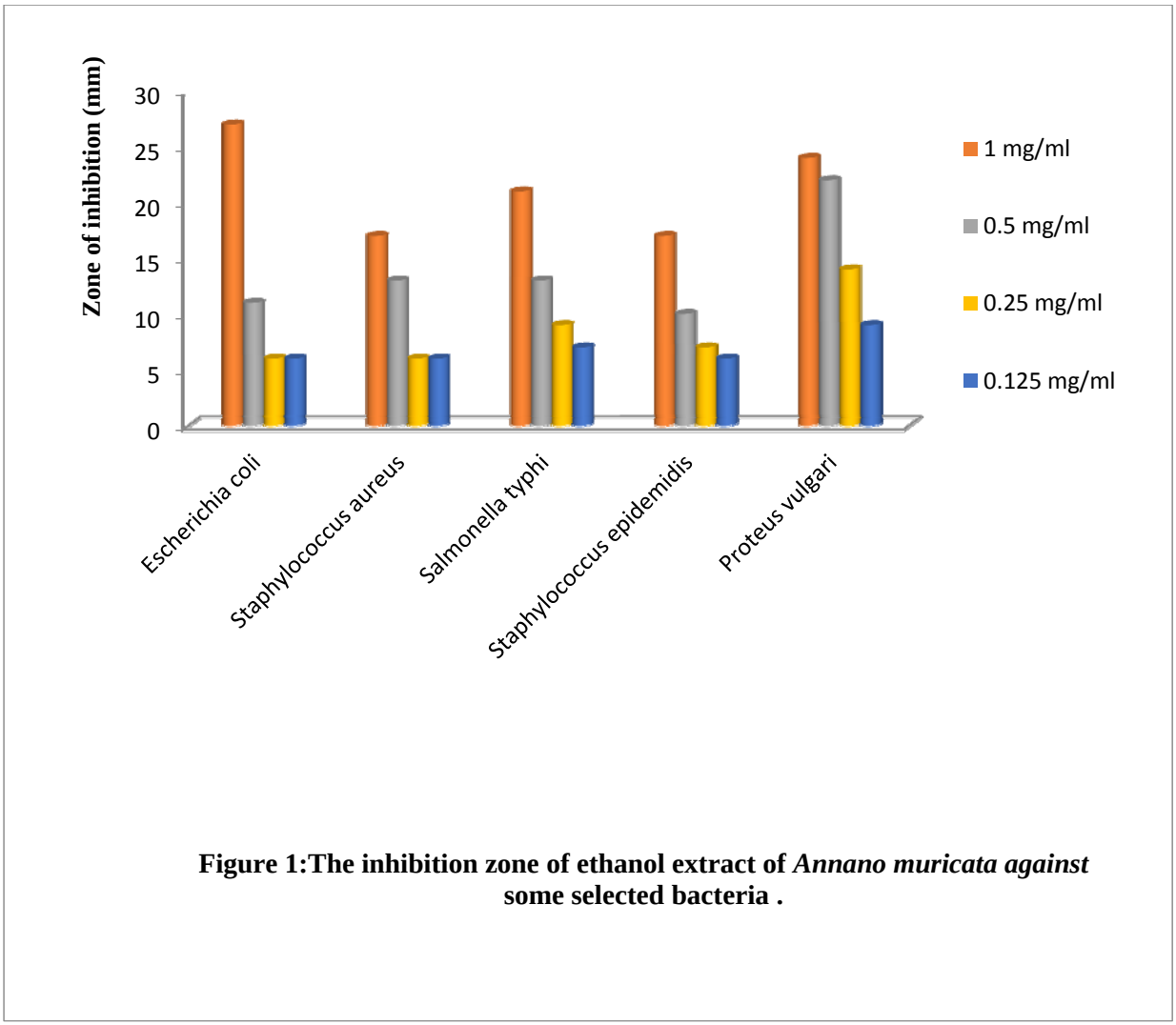

Figure 1:The inhibition zone of ethanol extract of *Annano muricata against* some selected bacteria .

Table 3: The inhibition zone of Aqueous extract of *Annano 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	19	25	23	6
0.5	6	13	23	18	6
0.25	6	7	17	6	6
0.125	6	6	9	6	6

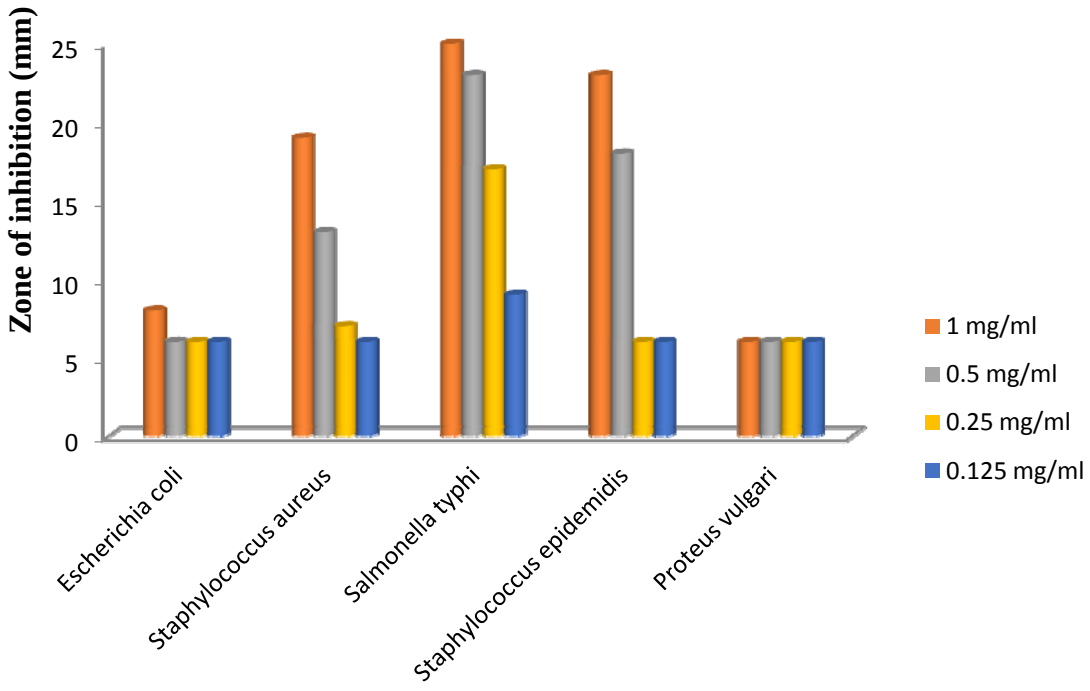

Figure 2: The inhibition zone of Aqueous extract of *Annano muricata against* some selected bacteria

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), 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). 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 *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.

Reference

[1] Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA (2000) Extraction methods and bio autography for evaluation of medicinal plant antimicrobial activity. LettApplMicrobiol 30: 379-384.

[2] Krishnaiah D, Sarbatly R, Bono A (2007) Phytochemical antioxidants for health and medicine: A move towards nature. BiotechnolMolBiol Rev 1: 97-104.

[3] Bashir, Z.A. (2012). In-Vitro Antimicrobial Activity of Membrane-Acting Antibiotics Action against Streptococci'. *Journal of Applied Pharmaceutical Sciences,* **2**(1*2):* 042 – 047.

[4] Namukobea, J., Kaseneneb, J.M., Kiremere, B.T., Byamukama, R., Kamatenesi-Mugisha, M., Krief, S., Dumontet, V., John D. and Kabasa, J.D. (2011) Traditional plants used for medicinal purpose by local communities around northern sector of Kibole National park, Uganda. *Journal of Ethnopharmacol.*,***136*:Pp**236 – 255.

[5] Manikandan, S., Ganesapanian, S., Singh,S.M and Kumar.agur, A M.K. (2011)"Emerging of multiple drug resistance human pathogens from urinary tract infections".*Curr. Res. Bacteriology***. 4**, **Pp** 9–15.

[6] Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, & Kadir HA. (2015a) *Annonamuricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *Int. J. Mol. Sci*. 16(7): 15625-15658.

[7] Ross IA. (2003) *Annona muricata*. In Medicinal Plants of the World, 133-142: Springer.Tanaya G, &Dewi RNS, (2015).*Anonna muricata Linn* Leaf Effect in Inhibiting SGPT Elevation.*Althea Medical Journal* 2(1): 86-89.

[8] Harborne. J. B (1973) Phytochemical method. A Guide to Modern Technique of Plant Analysis, 2nd Edition Chapman and Hall, New York, NY.

[9] Sofowora, E.A (1993) *Medicinal plants and traditional medicine in Africa*, spectrum books limited, Ibadan, Nigeria. Text book chapter 1 and 2.

[10] Mitscher LA, LeuRP, Balhala MS, Beal JI, White R. (1972) Antimicrobial agents from higher plants. Introduction, rational and methodology, liayadia 35:157.

[11] Ali NA A, Julich WD, Kusnick C, Lindequist U (2001). Screening of Yemeni medicinal plants foranti bacterial and cytotoxic activities. Jethnophamacol.74:173.

[12] Ogunwade IA (2001) Composition patterns of the essential oils of the leaves of Eucalyptus, Thuja, Callitris&Melaleuca species growing in Nigeria. PhD Thesis

Department of chemistry university of Nigeria.

[13] Habssah, M., Amram, M., Makeen, M. M., Lajis, N. H., Kikuzaki, H., Nakatani, N., Rahama, A. A., Ali, A. M (200) Screening of *Zingiberacaeae* extracts for Antioxidant activities. *Journal of Ethnopharmacology* 72: 403-410

[14] Yahaya G, Faten A, Fred W, Hany A. (2014) Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annonamuricata* (Graviola)., Asian Pacific Journal of Tropical Biomedicine

[15] Shagal. M. H.,Kubmarawa, .D. and Alim. H. (2012) Preliminary phytochemical investigation and antimicrobial evaluation of roots, stem-bark and leaves extracts of *Diospyrosmespiliformis*. International Research Journal of Biochemistry and Bioinformatics (ISSN-2250-9941) Vol. 2(1) pp.011-015

[16] Galo Yahaya Sara., Samaila Dauda., Andrew Emmanuel., Yusuf Yakubu Bhutto and Innocent Joseph (2018) Phytochemical Screening and Antimicrobial Activity of Leaf and Stem-bark Aqueous Extracts of *Diospyrosmespili formis*. *International Journal of Biochemistry Research & Review.* Volume 22 (3): Pp 1-8.

[17] Tschesche. R. (1971) *Advances in the chemistry of anti-biotres substances from higher plants; pharmacognosy international congress*. Heidelberg New York: Verlog, Berlin.

[18] Iwu. M. M, (1984) "Plant flavonoids in biology and medicine," in *Proceeding of 4th Annual Conference of the Nigeria Society for Pharmacology. University of Nigeria Nnsukka*.

[19] Ogundaini, A.O (2005) *From greens into medicine taking a lead from nature Inaugural lecture Series No.176.* Nigeria: O.A.U Press Ltd, Ile-Ife,

[20] Usman.H, F. I. Abdulrahman, and A. A. Ladan, (2007) "Phytochemical and antimicrobial evaluation of tribulusterrestris.L Zygophylaceae) growing in Nigeria Res," *J. of BIOSC.Medwel J.*, vol. 2, pp. 244-247.

[21] Dahiru D., Malgwi A.R., Sambo H. S (2013) Growth inhibitory effect of *sennasiamea* leaf extracts on selected microorganisms. *American journal of medicine and medical science*. 3(5):103-107.