Original Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF ETHANOL AND AQUEOUSEXTRACT OF *Annanomuricata*LEAVES.

ABSTRACT(mention where the study was done and when)

Phytochemical screening and antimicrobial activities of the ethanol and aqueous extractof *Annanomuricata*were extracted. The phytochemical screening of both ethanol and aqueous extract were carried out using standard method and the result revealed the present of Alkaloid, Tannin, Flavonoid, volatile oil, Triterpene, and Saponin in the ethanol extract of *Annanomuricata*and Tannin, Flavonoid, Alkaloid, Volatile oil, Triterpene, Saponin in the aqueous extract of *AnnanoMuricata*. The antibacterial activity was carried out using dics(check the spelling) diffusion method and the results showed a reasonable zone of inhibition against testedorganisms, with *Escherichia coli*being the most inhibited (27mm) at concentration (1mg/ml) with ethanol extract followed by *salmonella typhi*(25mm) at the same concentration with aqueous extract. At least concentration (0.125 mg/ml), almost all the organisms showed a least zone of inhibition (6mm) with the exception of *Salmonella typhi*(9mm) with the aqueous extract and *Proteus vulgari*(9mm) with the ethanol extract of *Annanomuricata*. 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.

Introduction (references mentioned in this section should be numbered)

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 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[Nostro*et al*., 2000]. 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 (Krishnaiah*et al*., 2007).

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 (Bashir, 2012). 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[Namukobea*et al.*, 2011].The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases [Manikandan*et al*., 2011].

The present research investigated the phytochemicals and antibacterial activitiesof*AnnanoMuricata*leaves extract*A.muricata*belongs to *Annonaceae* family and also known as guayabano, soursoap and graviola[Moghadamtousi*et al.*, 2015a].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 18cm long and covered with spine like structure. The pulps are soft white and with agreeable sour flavor (Ross, 2003).

Materials and Methods

Sample collection and preparation.

Fresh leaves of guayabano (*Annanomuricata*) were collected locally from Professor'sQuarters ModibboAdamaUniversity of Technology (MAUTECH) Yola. The plant leaves were used for the purpose of their phytochemical analysis. The leaves *ofA. muricata*wereseparated 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 wereweighed and used for phytochemical analysis.

Sample extraction

Powdered*A. muricata*leaves wereweighed 100g each into a container labeled A for *A.muricata*leaves. About 400ml of ethanol was added to the sample of *A. muricata*leaves. The mixture was then left for 72 hours (for thorough extraction). The extract were then filtered first through a whatmann filter paper No. 42 (125mm) and then through cotton wool, the extract is there after concentrated using a rotary evaporator with the water bath set at 60^0C to one-tenth its original volume and then finally freeze dried. The dried residue (crude extract) was then stored at 4^0C. Aliquot portion of the crude plant extract residue were weighed and used for phytochemical screening.

Phytochemical screening

Phytochemical screening was performed using standard procedures (Sofowora, 1993 – **to be numbered**). Assessing the presence of the following compounds classes: tannins; alkaloids; saponins; flavonoids; triterpenoids; alkaloid and phenol.

Test Organisms

The bacterial usedinclude *Escherichia coli, Staphylococcus aureus, Staphylococcus epidemidis, Proteus vulgari, Salmonella typhi*and MRSA**(expand)**.

Determination of Antimicrobial Activity(the references should be numbered in this section)

The antimicrobial activities of the ethanolic and aqueous extract of *A. muricata*were determined using disc diffusion method (Mitscher*et al.*, 1972). 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 intriplicates. An inhibition zone of 8mm or greater was considered as a good antimicrobial activity (Ali *et al.*, 2001). According to Ogunwande (2001), a cleared zone bigger than 10mm was interpreted as sensitive while smaller than 9mm was interpreted as resistance Extracts that showed positive activity in the preliminary screening wereserially 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 (Habsah*et al.*, 2000).

Results and Discussion(the references mentioned in this section to be numbered)

Phytochemical screening of Ethanol and Aqueous extract *ofAnnanomuricata.*

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 *AnnanoMuricata* leaves of the plant. Saponins, tannins, flavonoids, alkaloids, volatile oils 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 present of Saponins, tannins, flavonoids, alkaloids, Alkaloid, Volatile oils, 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 (Yahaya*et al.*, 2014) 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 (Shagal*et.al., 2012* and Sara *et al.*, 2018).

The chemical constituents present in the extracts have some therapeutic values. Tannins are plant metabolites well known for their antimicrobial properties (Tschesche, 1971). Flavonoids have both antifungal and antibacterial activities. They possess anti-inflammatory activity (Iwu, 1984 and Ogundaini, 2005). Flavonoids, terpenes and alkaloid are known to have antimicrobial and bactericidal properties against several (Usman*et al*,2007).

Table 1: Phytochemical screening of Ethanol and Aqueous extract *ofAnnanomuricata*

Bioactive compounds	***Aqueous extract***	***Ethanolic extract***
Saponins	+	+
Glycosides	-	–
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Volatile oils	+	+
Phenolic compound	–	+
Triterpene	+	+

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

Table 2: The inhibition zone **(give unit of measurement here)**of ethanol extract of *Annanomuricata*against some selected bacteria (mg/ml)

Concentration (mg/ml)	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Staphylococcus epidemidis*	*Proteus vulgari*
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

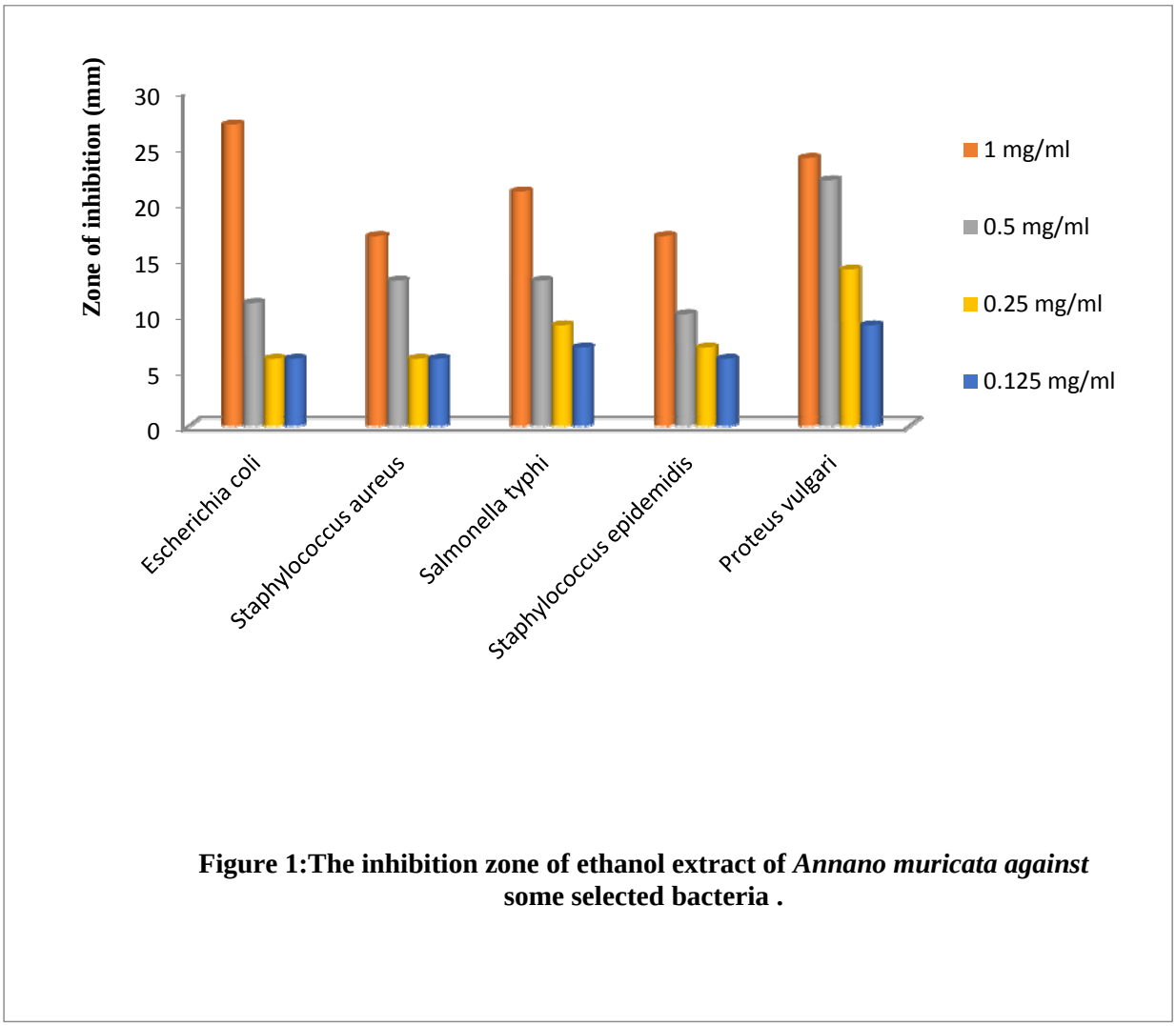

Figure 1:The inhibition zone of ethanol extract of *Annano muricata against* some selected bacteria .

Table 3: The inhibition zone**(give unit of measurement here)** of Aqueous extract of *Annanomuricata against* some selected bacteria (mg/ml)

Concentration (mg/ml)	Escherichia coli	Staphylococcus aureus	Salmonella typhi	Staphylococcus epidemidis	Proteus vulgari
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

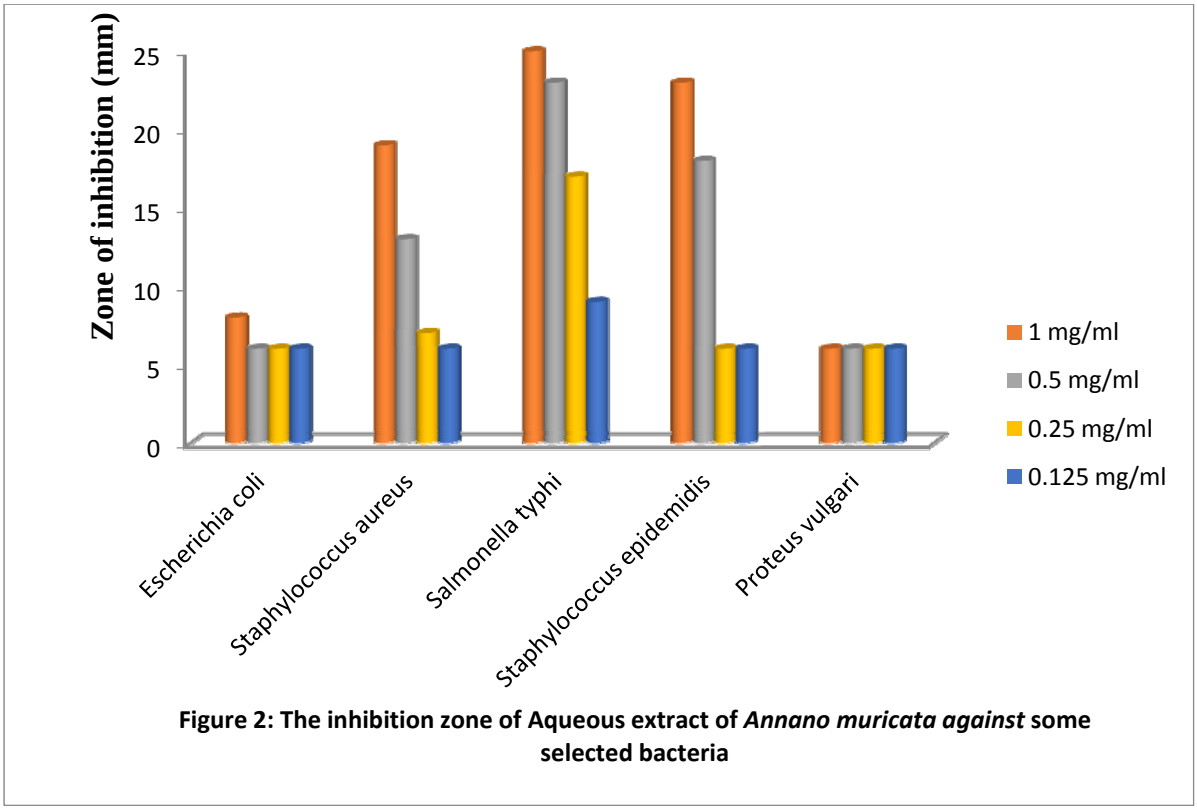

Figure 2: The inhibition zone of Aqueous extract of *Annano muricata against* some selected bacteria

The antibacterial activity of both the ethanol and aqueous extract of the leaves of *A. muricata*shows reasonable zone of inhibition against tested microorganism (Table 2&3). The ethanol extract showed the highest zone of inhibition (27mm) with*E. coli* than the Aqueous extract which gave (8mm) on the same organism at the same concentration (1mg/ml), followed by *S. typhi*(25mm) on an Aqueous extract while the ethanol extract showed (21mm) on the same organism also on the same concentration (0.5mg/ml).At (0.125mg/ml), both the ethanol and

aqueous extract showed least inhibition zone (6mm) against almost all the microorganism except *S. typhi*for aqueous extract and *P.vulgari*,*S. typhi*for ethanol extract of *Annanomuricata.*The results of this work agrees with the work of (Dahiru*etal.*, 2013) 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(to be numbered and mentioned sequentially from introduction, method and discussion sections)

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