Journal of Biomedicine and Biosensors
ISSN (Online): 2583-3170
www.gpub.org

Volume 2, Issue 2: 43 – 59, 2022

Evaluation of the Antimicrobial and Phytochemical Properties of Annona Muricata L. (Soursop)

C.N. Obum-Nnadi[1], C.M Ezenwa[2], Dennis Amaechi[1,*], N.B. Ohabughiro[2], P.A. Nnagbo[2], K. S. Nwokorie[3*], C.S. Okoli[4]

[1] Department of Microbiology, Veritas University, Abuja, Nigeria
[2] Department of Microbiology, Imo state University, Owerri, Nigeria
[3] Nottingham Trent University, Nottingham, United Kingdom
[4] Department of Applied Microbiology, Nnamdi Azikiwe University, Awka, Nigeria

Received: 17.5.2022 • Accepted: 30.5.2022

Abstract: The ethanol, methanol and aqueous extracts, of dried leaves, bark root and mixture of bark, root and leaves of Annona muricata l (soursop) were screened for its phytochemical constituents and invitro antibacterial properties on the following hospital isolates: Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Salmonella typhi. Methods: The well in agar and disc diffusion techniques were used to assay for the antimicrobial properties and the result showed that the ethanol and methanol extracts of the leaf at different concentrations inhibited the growth of all the organisms. The aqueous extracts of the leaf inhibited the growth of only the E. coli. The ethanol and methanol extracts of the root inhibited the growth of S. pyogenes, and E. coli. The bark extract inhibited the growth of S. aureus, Salmonella typhi,and E. coil. The mixture of the bark, root and leaf extract inhibited the growth of S. pyogenes only. Results: The Minimum inhibitory Concentration (MIC) of the ethanol, methanol and aqueous extracts of the bark, root, leaf and the mixture of the bark, leaf and root determined by the tube dilution method falls within the range of 65.25mg/mI, 125mg/ml to 250mg/ml accordingly for the sensitive organisms. The Minimum Bactericidal Concentration (MBC) of both the ethanol and methanol extracts was found to be between 250mg/ml and 125mg/mI for some of the organisms investigated. No MBC was recorded for aqueous extracts of all the sensitive isolates. The phytochemical screening revealed the presence of saponin, anthroaquinone, anthranoid, phenol. Alkaloid, tannin, Phylobatannin, and cardiac glycosides. Conclusion: The alcoholic extracts retained the antimicrobial properties of the plant more than the aqueous extract.

Keywords: Annona muricata l. (soursop), Phytochemical analysis, S. aureus, E. coli, S. pyogenes, S.typhi.

1. Introduction

Multidrug-resistant bacterial strains are a developing global public health concern that justifies investments in the hunt for alternate infection-treatment methods (Robert, 2003; Albuquerque, *et al*.,

* Corresponding Author: amaechitoexcel@yahoo.com & n_charitez@yahoo.co.uk

2007). As a result, *Annona muricata L*. or graviola (soursop) which belongs to the family Annonaceae is one of the medicinal plants of interest that can serve as source of alternative bactericidal agent (Karen 2011). Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. *A. muricata l.* is a broad leave, flowering evergreen tree. The aroma is similar to pineapple; the flavour of the fruit is described as combination of strawberries and apple, and sour citrus flavour notes, contrasting with an underlying creamy texture reminiscent of coconut and banana (Morton, 2018).

A. muricata l. has been known to cure diabetes, high blood pressure, malaria parasite, diarrhea, cough and cancer. All these research was carried out on the fruit, seed and stem. There has not been any documentation on the root, leaf and bark as an antimicrobial. No, specified bacteria has been documented to have been attacked in vivo and in vitro by any of the root leaf and bark but *Vibrio cholera* and *S. aureus* has been reported to be sensitive to the *A. muricata L*. pod (Sao P., 2010).

Plants are used medicinally in a variety of ways, including the administration of roots, barks, stems, leaves, flowers, seeds, and extracts from whole plants. Among the plants with demonstrated antibacterial properties is Allium sativum, which is effective against Escherichia coli, Salmonella species, Staphylococcus aureus, fungal, yeast, and viral infections (lnyang *et al*., 2008; Scarlton 2011). There is yet no documentation on the antimicrobial properties of Nigerian *Annona muricata L*. (soursop) leaf, Root and bark. According to the most recent information available, Annona muricata L. has been the subject of laboratory research since the 1940s, when it has been demonstrated to:

- Effectively targeted and eliminated malignant cells in twelve distinct cancer types, including colon, Breast, prostate, lung, and pancreatic cancer (Kojima, 2004).
- Be 10,000 times more effective at destroying colon cancer cells than Adriamycin a typical Chemotherapeutic agent (Nicolas, 1997).
- Unlike chemotherapy, selectively hunts out and kills cancer cells while leaving healthy cells alone (Gonzalez-Coloma, 2002).

The activity of A. muricata against both a standard strain and a clinical isolate of Herpes simplex virus were demonstrated by an ethanol extract of A. muricata stem bark (Padma *et al.,* 1998). Jarramillo, *et al*., (2007) reported that in an experiment, acetogenins extracted from the fruit pericarp were responsible for anti-leishmanial activity demonstrated in an invitro experiment. Osorio*et al*, (2007) also demonstrated an invitro activity of the leaf extract against some Leishmania species and Trypanosomacruzi (Luna, *et al*., 2006).

Many bioactive compounds and phytochemicals have been discovered in Annona muricata L., which has been studied by scientists since the 1940s. This scientific study proved its numerous applications in natural medicine. The earliest studies were between 1941 and 1962. Several studies by different researchers demonstrated that the bark as well as the leaves had hypotensive, antispasmodic, vasodilator, and smooth muscle relaxant and cardio depressant activities in animals (Bories*et al*. 1991; Antonu*et al*, 1993; Robert *et al*., 2003). In a plant screening programme conducted by the National Cancer Institute in 1976, the leaves and stem of Annona muricata L. exhibited active cytotoxicity against cancer cells. Since then, researchers have followed up on this research. Much of the research has focused on annonaceousacetogenins, a novel class of phytochemicals. The potent antitumor, pesticidalandlor insect antifeedant properties of these annonaceousacetogenins have been reported and patented. *Annona muricata l.* produces these natural compounds in leaf, bark and twig tissues, and they have been documented to possess both highly antitumor and pesticidalproperties (Kojima, 2004).

Researchers reverified *Annona muricata l.* leaf has hypotensive properties in rats again in 1991 (Robert *et al*., 2003). Several studies over the years have demonstrated that leaf, bark, root, stem and seed extracts of *Annonamuricata l.* are antibacterial in vitro against numerous pathogens and that the bark has antifungal properties (Feng, 1962: Gbeassor, *et al.*, 1990; Robert, *et al*, 2003). *Annona muncata l.* seeds demonstrated activeanti parasitic properties in a 1991 study, and a leaf extract showed to be active against malaria in two other studies in 1990 and 1993. The leaves root, and seeds of *A. muricata l.* demonstrated insecticidal properties with the seed demonstrating strong insecticidal activity in an early 1940 study. In a new 1997 clinical study, novel alkaloids were found in its fruit with anti-depressive effect in animals (Gbeassor *et al*, 1990; Bones 1991). It produces these natural compounds in leaf, bark and twig tissues, and they were been documented to possess both highly antitumor and pesticide properties (Robert*et al*., 2003).

Mode of conduct recent research conducted in three distinct laboratories has revealed that acetogenins are excellent inhibitors of complex 1 in the mitochondrial electron transport systems of numerous organisms, including tumour cells. Numerous incredibly potentacetogenins have been uncovered through research on numerous Annona species. Active compounds from this plant and other Annona plants have been submitted to the NIH anti-AIDS screen by Purdue University and their work is continuing with a number of other active plant species in the Annona plant family. Thus far, Purdue and/or its staff have filled at least 9 US and international patents on their work around the anti-tumorous and insecticidal properties and uses of these acetogenins (Chang*et al*., 2005; Karim-Kos *et al.*, 2008; Hamizah *et al*., 2014).

Three distinct research groups have identified novel anticancer and selectively poisonous chemicals from the seeds and leaves of Annona muricata l. and published eight clinical trials describing their findings. One study found that the acetogenin in Annona muricata l. was 10,000 times more potent than adriamycin in killing colon cancer cells (a chemotherapy drug). Ongoing cancer research is being conducted on Annona muricata L., and four additional studies were published in 1998 that narrowed down the photochemicals with the strongest anticancer and antiviral activities (Kojima, 2004; Karim-Kos *et al.*, 2008; Hamizah *et al*., 2014).

2. Materials and Methods

2.1. Study Location

This research on *A. muricata L.* plant was carried out in Imo State University Owerri. 1mo State University is situated at Owerri Municipal Local Government in 1mo State. It falls within the south eastern part of the country. 1t is made of various colleges/faculties like; college of medicine and health sciences, faculty of sciences, business college, faculty of law, faculty of humanity, faculty of Engineering, faculty of Education, faculty of Social sciences, faculty of Agric and faculty of environmental sciences. Each faculty is made up of various schools and each school has different departments. It is part of the rain forest zone and the trees found there are vacado pear, mango, orange, soursop (*A. muricata l.*) grape, lemon, lime and cashew in alia

2.2. Sample Collection and Extraction

- Fresh leaves, bark, of *A. muricata l.* were collected locally from the mother plant at Imo State University Owerri.
- The samples were washed thoroughly with clean water, dried and homogenized separately with corona hand grinder.

- Each of the 30g homogenized bark, leaves and roots were mixed separately with 1000ml of 95% ethanol, methanol and water.
- Each preparation was shaken every 30 minutes for 6 hours and allowed to stand for 48 hours.
- Each preparation was filtered with No 1 what man's filter paper, they were placed separately in a beaker and put into a water bath to be evaporated to dryness at37°C for 48 hours (Inyang *et al*., 2008) to get them dried. This was not achieved until they were poured into a crucible in a Petri dish. After the 24 hours, all the extracts became evaporated to dryness.
- The same procedure was repeated for each of the leaves, bark and root with water and methanol to get the aqueous and methanol extracts of each respectively.
- 10g each of the homogenized bark leaves and root were mixed together in100ml of 95% ethanol and also allowed to undergo the same process to get the pooled extract.
- 10g each of the homogenized leaves, bark and root were also mixed together**.**
- Both the pooled alcohol mixture and the pooled aqueous mixture were shaken separately every 30 minutes for 6 hours and allowed to stay for 48 hours.
- Each of the preparations was filtered with No1wattman filter paper and evaporated to dryness in a hot air over to get the dried pooled alcohol extract and dried pooled aqueous extract respectively.

2.3. Alcohol Soxhlet Extraction Method

- The homogenized leaves were put separately into a thimble.
- 100mI of absolute alcohol was poured into a round bottom flask and connected to the soxhlet extractor.
- This set up was heated with a condenser fixed to the soxhlet extractor.
- As it heats, the extracts underwent influx and efflux reaction.
- This procedure was repeated up to 4 times until the content of the leaves has been completely extracted.
- The above procedure from step I to step 5 was repeated separately for the bark, root and the mixture of the
- Bark, root and leaves of the plant respectively.
- The methanol extracts, each of the bark, root and mixture were collected likewise.

2.4. Collection Of Bacterial Isolates

- Clinical bacterial isolates were obtained from the microbiology laboratory of General Hospital Umuguma Owerri. These organisms include; *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Salmonella typhi.*
- Each of these organisms was sub-cultured on a sterile plate of chocolate agar,nutrient agar, blood agar, and Salmonella - Shigella agar according to the type of the organism. The identity of the organisms was Confirmed using the following biochemical test: - Gram's stain, methyl red test, motility test, indole.

2.5. Biochemical Test

The following biochemical tests were carried out according to the standard procedure to confirm the isolates: Methyl Red test, Motility test, Indole test, Citrate utilization test, Coagulase test, Bile solubility test, hydrolysis test, Catalase test and Optochin sensitivity test.

2.6. Evaluation of Antimicrobial Activity:

Innoculum Size Standardization:.

Serial dilutions of the organisms were made to reduce the inoculums.

Concentration to 10^{-2} per ml Mac Farland Standard:

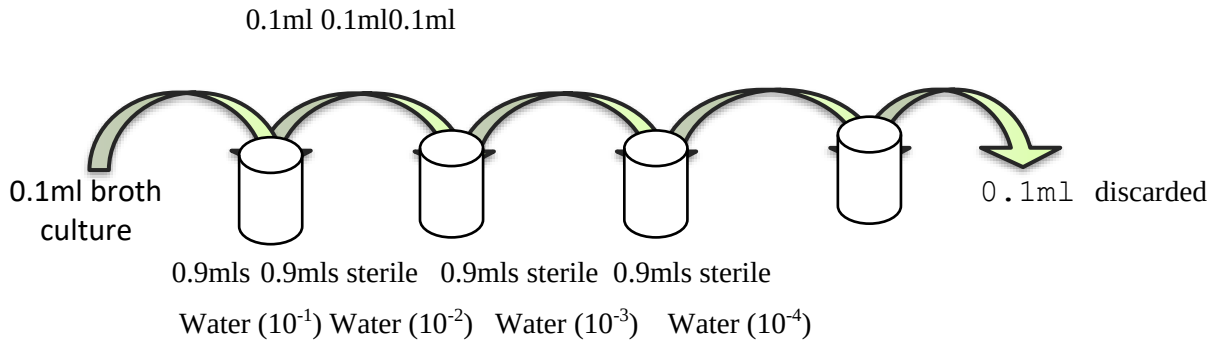
0.9ml of sterile water was added to 4 test tubes set up in a row.

0.1ml of the broth culture of one of the organism was added to the 2^{nd} tube this was first row.

It was mixed very well and 0.lml of the mixture transferred to the 2' tube this was repeated to the last tube and 0.lml discarded from the last tube.

This procedure (a — C) was repeated for the remaining five organisms.

The dilution factor of 10^{-2} was used for sensitivity testing to test the effectiveness of the extracts.



Dilution of the Extracts:.

Doubling dilutions of each of the extracts were made in 250mg/ml, 125mg/ml,62.5mg/ml, 31.25mg/ml, and 15.63mg/ml concentrations respectively. This was achieved by using the method below;

Preparation of the Discs:

Each of the raw soxhlet extract which contain 1000mg/ml was diluted 1:4 dilution to250mg/ml.No I what man's filter was perforated to get about I000pieces of paper discs of 8mmdiameter each.100 pieces each were labeled to represent a particular concentration of each of the extracts such as 250mg/ml, 125mg/ml, 31.25mg/ml, and 15.63mg/ml respectively. Each of the labeled 100peices was put into a transparent tube and was sterilized. About 100 pieces which represent a particular concentration of one of the extracts were soaked in 2ml of That extracts and left for 2 hours undisturbed. This was done so that each paper disc will contain 0.02m1(20u1) of the concentration of the extract applied on it.

Evaluation of Antibacterial Activity Using Disc:

Diffusion Method:

- A loop of the organism of test tube 2 with strength of 10^{-2} per ml of each of the bacterial isolate was Spread on the surface of sterile Mueller Hinton Agar plates.
- The discs about 8mm diameter containing different concentrations of the extract were picked with sterile Forceps and placed at different areas on the surface of each plate before incubating at 37°C for 24 hours.
- Each plate contains 4 paper disc.
- A control plate was also set according to NCTC standard for each of the isolates
- Antimicrobial activity of extract against test organisms was determined by measuring the zones of Inhibition as millimeter diameter of the respective discs and plotting the means value of triplicate Readings.

Evaluation of Antibacterial Activity Using Agar Well Diffusion Method:.

A Mueller Hinton Agar plate was prepared according to the manufacturers direction and poured in 20ml amount in a petri dish. A loopfull of the test organism from the tube containing 10^{-2}cfu/ml concentration of each of the test.Isolates were spread on the surface of the20ml Mueller Hinton Agar plate with a sterile glass spreader. These were allowed for 30 minutes pre-diffuse and a Number 4 cork borer was used to bore hole of 8mm. Diameter on each of the agar plates containing the 6isolates. A volume of 0.02ml (20ul) of each of the different extract was used to fill the agar wells made in the Mueller Hinton agar plates and NCTC Standard antibiotics for each of the isolates served

as control. The plates were allowed to stand for 1 hour to allow the drug to pre-diffuse into the agar and were Incubated at 37°C for 24 hours.

After incubation, the zones of inhibition were recorded as resistant or susceptible using the criteria for Viable antimicrobial activity for plant extracts. When D1≥ 8mm it is taken to be susceptible, while D1≤ 8mm is taken as resistance.

Minimum inhibitory concentration of the extracts was determined only on extracts exhibiting apparent Ones of Inhibition against test organism greater than D1≥ 8mm.

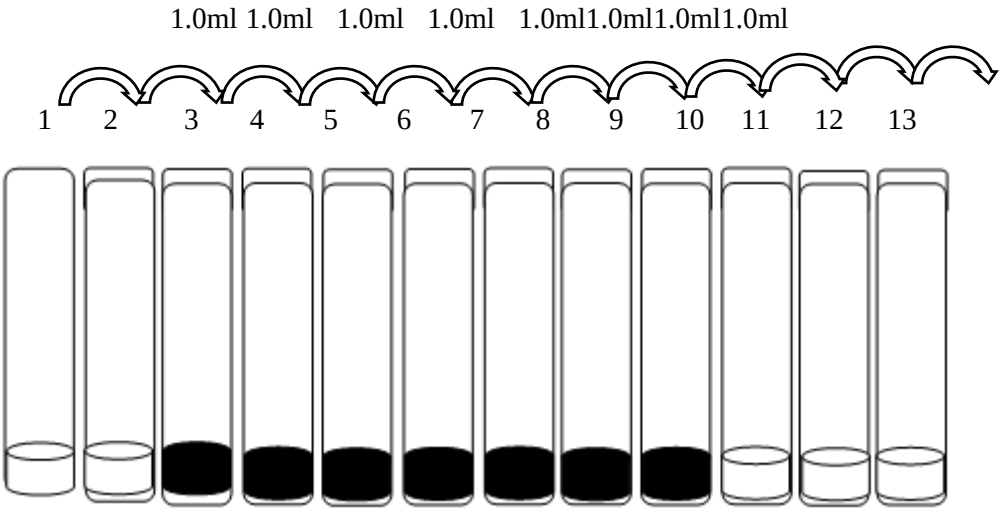
NOTE: 0.02m1 volume of the extract was used to fill the hole of 8mm diameter because it was the same Volume I used in preparing the paper disc.

Determination of Minimum Inhibitory Concentration (Mic In Mg/Ml) Using The Tube Dilution Method:

The MIC was determined only for ethanol leaf, root, bark and mixture of the bark, leaf and root extracts: methanol leaf, root, bark and mixture of the bark, leaf and root extracts; and aqueous leaf extract.

Dilution of the Extracts:

- The original concentration of each of the extracts was l000mg/ml, 1 in 4 dilution of each of the extract was made to get an extract concentration of 250mg/ml.
- Each of the extracts was sterilized by filtration using me Number 1 wattman filter paper
- 13 test tubes were set up in a row for each of the extracts.
- 1ml sterile Mueller-Hinton broth was pipette into tubes 2-10, 11 and 13.
- 2ml Mueller-Hinton broth was pipette into tube 12.
- Tube 11 was used as the inoculum control; tube 12 was the broth control and tube13 was the extract control.
- 1 ml of leaf extract with concentration of 250mg/ml was pipette into tubes 12 and 13.
- A doubling dilution was prepared from tube 2 up to tube 10 in 1mI amount to get the following concentrations 250mg/ml, 125mg/mi, 62.5mgIml, 31 .25mgImI,15.63mg/mi, 7.8mg/ml, 3.9 mg/ml. 1.g5mg/ml. 098 mg/ml and 0.49 mg/ml concentrations respectively.
- 1ml of isolate I was pipette into tubes 1 to 11.
- The same procedure was repeated for the remaining isolates.
- The tubes were incubated at 37°C.
- It was observed for turbidity or cloudiness which indicates a positive result.
- The lowest concentration showing no turbidity is the MIC of the extract.
- Procedure 4-13 were repeated for the bark extract, root extract and the mixture of the bark, leaf, and root extract each of the isolate.



Conc: 250 125 62.6 31.25 15.63 7.8 3.9 1.95 0.98 0.48 IC BC EC

In mg/ml

Key:

IC = Inoculum control

BC = Broth control

EC = Extract control

☐ = Turbidity

In this illustration above, the MIC is 125mg/ml. each value below the tubes represents the concentration in mg/ml.

Determination of The Minimum Bactericidalconcentration (MBC)

The MBC was determined by sub-culturing 0.01ml of the highest concentration of the extract which showed visible growth and all the tubes showing no visible signs of growth in the MIC tube dilution test, to fresh extract-free media like- Blood Agar, MacConkey Agar, and Chocolate Agar.

Screening For Phytochemical Properties

Test for Saponins.

- 10ml of distilled water was added to 2m1 each of the extracts in a test tube.
- The mixture was shaking vigorously (Hostettman, 1991).
- It was examined for persistent frothing even after heating which indicates the presence of saponins.

Test for Anthranoid.

The method of Trease and Evans (1989) was adopted for this test.

- To 2m1 each of the extracts, 5ml of 0.5ml potassium hydroxide was added and mixed properly.
- 6 drops of acetic acid was added.
- 2ml of Toluene was also added.
- To the upper layer formed, 2ml of 0.5m potassium hydroxide was added.
- The mixture was observed for a change in color (that color change from its original color to another color) which is an indication of a positive test while no color change is an indication of a negative test.

Test for Anthroquinone.

- To about 2m1 each of the extracts, 5m1 of 10% ammonia was added and mixed by shaking vigorously.
- It was observed for a color change which is an indication of a positive test (thecolor change is from its original color to another color). No color change is an indication of a negative test (Trease *et al,* 1983).

Test for Phenol.

The method of Harborne (1973) was employed.

- 10ml of each of the extracts was mixed with 8m1 of distilled water in a test tube.
- 6m1 of ferric chloride solution was added to the mixture.
- A color change to light brown was checked for, which is an indication of a positive test.

Test for Alkaloid.

- To about 2ml each of the extracts, 5m1 of 1% aqueous Hydrochloric acid was added and placed in a water bath for 3minutes.

- Later, 3 drops of Mayer's reagent was added (Soforowa*et al.,* 1993).
- It was observed for a white precipitate which is an indication of a positive test. No precipitation is an indication of negative test**.**

Test for Tannins.

The method of Harborne (1973) was employed.

- To 1ml each of the extracts 1% ferric chloride was added.
- It was checked for a color change which indicates positive test.

Test for Phylobatanning.

- To 2m1 each of the extracts, 1% aqueous hydrochloric acid was added and boiled.
- It was observed for the presence of white precipitate which indicates positive test (Trease, *et al,* 1989).

Test for Cardiac Glycosides.

- The Salkowski test was employed in this test.
- To 1ml each of the extracts, 2m1 of chloroform was added and then 2m1 of concentrated hydrogen tetraoxosulphate (vi) acid was added to form a lower layer.
- It was examined for a reddish brown color at the interface which indicates appositive test, while none is an indication of a negative test (Obi, 2008).

Questionaire.

A questionnaire was distributed to about 500 people. The aim was to test the People knowledge about the medicinal properties of *Anonna muricata l.*

3. Result

The ethanol extract of the leaves, bark and root of *Annona muricata l*. collected manually by soaking in ethanol was not active to all the isolates used, such as; *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella species* and. The methanol and ethanol extracts of the leaves, bark, root and the mixture of the bark, root and leaves showed some antibacterial activities against some of the test isolates. This is summarized in tables 1 to 3 below.

Table 1 showed that the ethanol extract of *A. muricata l*. leaf is effective against all the isolates used. *S. pyogenes*and *E. coli* are sensitive to the root. *S. aureus, Salmonella species and E. coli* are sensitive to the bark, but *S. pyogenes* resistant to it. The mixture of the bark, leaf and root is active against *S. aureus*and *S. pyogenes*, but *Salmonella species* and *E. coli* are resistant to it. These organisms were sensitive to the extracts singly but resistant to their mixture. In order words, the leaf is active against both the Gram-negative and Gram-positive isolates investigated. The Gram-negative isolates are sensitive to the mixture of the bark, leaves and root while the effectiveness of the bark and root varies on the isolates investigated on.

Table 2 which is the methanol extract showed almost the same result with that of the ethanol extract but with higher activity. This indicates that methanol extract more constituents of the plant than ethanol. The leaf aqueous extract in Table 3 show edlittle antibacterial activity to only *Streptococcus pyogenes* and *Escherichia coli*. All other parts of the plant aqueous extract revealed no antibacterial property to the isolates.

Table 1. Ethannol Soxhlet Extract Activity Pattern Of Annnona Muricata (Soursop) Recorded In Diameter (Mm)

Plant Test Isolates

Parts

A Muricata l	***Staphylococcus aureus***	***Strept. pyogenes***	***Salmonella typhi***	***E.coli***
Leaf	22mm	18mm	20mm	22mm
Root	Resistant	20mm	Resistant	16mm
Bark	14mm	Resistant	14mm	17mm
Mixture of Leaf, root And bark	15mm	14mm	Resistant	Resistant

Note: The size of paper disc and well is 8mm hence the resistant isolates measure 8mm in diameter. Each plate contains 4 discs.

Table 2. Methanol Soxhlet Extract Activity Pattern of *Annona Muricata L.* (Soursop) Recorded In Diameter (Mm)

Plant Test Isolates

Parts

A Muricata l	***Staphylococcus aureus***	***Strept. pyogenes***	***Salmonella typhi***	***E.coli***
Leaf	24mm	20mm	25mm	26mm
Root	Resistant	32mm	Resistant	16mm
Bark	18mm	Resistant	17mm	19mm
Mixture of Leaf, root And bark	15mm	18mm	Resistant	Resistant

Note: The size of paper disc and well is 8mm hence the resistant isolates measured 8mm in diameter. Each plate contains 4 discs.

Table 3. Activity Pattern of Aquenous Extract of a Muricata L. (Soursop) Recorded In (Mm).

Plant Test Isolates

Parts

A Muricata l	*Staphylococcus aureus*	*Strept. pyogenes*	*Salmonella typhi*	*E.coli*
Leaf	Resistant	26mm	Resistant	15mm
Root	Resistant	Resistant	Resistant	Resistant
Bark	Resistant	Resistant	Resistant	Resistant
Mixture of Leaf, root And bark	Resistant	16mm	Resistant	Resistant

Note: The size of paper disc and well is 8mm hence the resistant isolates measured 8mm in diameter. Each plate contains 4 discs.

The mixture of leaf, root and bark which has only 1/3 of the leaf was inactive to all the isolates except the strept. Phypgenes because some chemical reaction could have taken place in the cause of the mixture. The Minimum Inhibitory Concentration (MIC) of each of the sensitive extract is also summarized in the tables 4 to 6 below. The Minimum Bactericidal Concentration (MBC) of the sensitive extracts is also summarized in table 7.In table 4-6, the figures above are the Minimum Inhibitory Concentration (MIC) of each of the extract. This ranges from 250mg/ml to 62.5mg/ml. Tables 7-8shows the Minimum Bactericidal Concentration of the alcohol and methanol extracts of the leaf, bark and the root of the plant, none of the alcohol or methanol mixture of the bark, leaves and root revealed a minimum bactericidal value. The aqueous extract also did not reveal a minimum bactericidal value.

Table 4. Minimum Inhibitory Concenntration (Mic In Mg/Ml) of Methanol Extract Of Leaves, Bark, Root And The Mixture Of The Bark, Leaves And Root Against Sensitive Pathogens.

Plant Test Isolates

A Muricata l	*Staphylococcus aureus*	*Strept. pyogenes*	*Salmonella typhi*	*E.coli*
MIC (mg/ml) Leaf	62.5	62.5	62.5	62.5
MIC(mg/ml) Root	R	62.5	R	250

MIC(mg/ml) Bark	250	R	250	250
MIC(mg/ml) Mixture of Leaf, roof And bark	250	250	250	R

Note:

R = Turbidity

Table 5. Minimum Inhibitory Concentration Of Aqueous Extract Of Leaf, Bark, Root And Mixture Of All Against Sensitive Pathogens

Plant Test Isolate

A Muricata l	***Staphylococcus aureus***	***Strept. pyogenes***	***Salmonella typhi***	***E.coli***
MIC (mg/ml) Leaf	R	250	R	250
MIC(mg/ml) Root	R	R	R	R
MIC(mg/ml) Bark	R	R	R	R
MIC(mg/ml) Mixture of leaf, roof andbark	R	R	R	R

Note:

R = Turbidity

Table 6. Minimum Inhibitory Concentration Of Alcohol Extract Of Leaf, Bark, Root And Mixture Of All Against Sensitive Pathogens

Plant Test Isolate

A Muricata l	***Staphylococcus aureus***	***Strept. pyogenes***	***Salmonella typhi***	***E.coli***

MIC (mg/ml) Leaf	125	250	250	250
MIC(mg/ml) Root	250	250	125	250
MIC(mg/ml) Bark	R	R	250	250
MIC(mg/ml) Mixture of leaf, roof and bark	R	R	250	R

Note:

R = Turbidity

Table 7. Minimum Bactericidal Concentration (Mbc) Of Alcohol Extract Of Leaf, Bark And Root Of Soursop

Plant

Plant Test Isolate

A Muricata 1.	***Staphylococcus aureus***	***Strept. pyogenes***	***Salmonella typhi***	***E.coli***
MBC (mg/ml) Leaf	125	250	250	125
MBC(mg/ml) Bark	250	250	250	250
MBC(mg/ml) Root	R	R	250	250
MIC(mg/ml) Roof	R	R	125	R

Note:

R = Turbidity

Table 8. Minimum Bacterial Concentration (Mbc) Of Methanol Extracts Of Leaf Bark And Root Of A Muricata L. (Soursop) On Sensitive Selected Pathogens

Plant Test Isolate

A Muricata l	***Staphylococcus aureus***	***Strept. pyogenes***	***Salmonella typhi***	***E.coli***
MBC (mg/ml) Leaf	125	250	250	125
MBC(mg/ml) Bark	R	125	R	R
MBC(mg/ml) Root	R	R	R	R

Note:

R = Turbidity

However, the ethanol, methanol and aqueous extract of A. muricata L. leaf, bark, root and the mixture of the three contain the following phytochemicals Saponins,Anthroquinone. Anthranoid, Phenol, Alkaloid, Tannin, Phytobatannin and Cardiacglycosides as summarized in tables 9A and 9B respectively. In table 9, the phytochemical analysis of methanol and alcohol soxhlet extracts of root, bark, leaves and mixture of all reveals the presence of Sapoonin in only the bark and Cardiac glycosides in only the leaf. The bark, leaf and mixture of all contain Anthraquinone. Anthranoid was found only in leaf and the mixture of all. Phenol and Tannin was present in the alcoholic extract of the leaf and root. Alkaloid was found in all the extracts of *Annona muricata l.* Phylobatanin was found only in the alcoholic extract of the root and the mixture.

The phytochemical analysis aqueous extracts of root, bark, leaves and mixture of all displayed in table 10, reveals the presence of Anthraquinone, Anthranoid, Phenol, Alkaloid, tannin, Phylobactanin and Cardiac glycosides in the root. The aqueous extract of the bark contains Sapoonin, Anthraquinone, and Alkaloid, tannin, Phylobactanin and Cardiac glycosides. The aqueous extracts of the leaf and mixture contains Phenol, Alkaloid, tannin and Phylobactanin.

The Qualitative Phytochemical Analysis Of Methanol, Alcohol And Aqueous Extracts of Root, Bark, Leaves And Mixture Of Leaf, Bark And Root of Soursop (*A. Muricata L.*)

Table 9. Qualitative Phytochemical Analysis of Methanol And Alcohol Soxhlet Extracts of Root, Bark, Leaves And Mixture of All**.**

S/N	**Phytochemicals**	**Root**	**Bark**	**Leaf**	**Mixture**
A	**Sapoonin**	**-**	**+**	**-**	**-**
B	**Anthraquinone**	**-**	**+**	**+**	**+**
C	**Antranoid**	**-**	**-**	**+**	**+**
D	**phenol**	**+**	**-**	**+**	**-**

E	**Alkaloid**	+	+	+	+
F	**Tanin**	+	-	+	-
G	**Phylobatanin**	+	-	-	+
H	**Cardiac glycosides**	-	-	+	-

Note:

+ = Positive

- = Negative

Table 10. Qualitative Phytochemical Analysis of Aqueous Extracts of Root, Bark, Leaves And Mixtures of All

S/N	Phytochemicals	Root	Bark	Leaf	Mixture
A	**Sapoonin**	-	+	-	-
B	**Anthraquinone**	+	+	-	-
C	**Antranoid**	+	-	-	-
D	**phenol**	+	-	+	+
E	**Alkaloid**	+	+	+	+
F	**Tanin**	+	+	+	+
G	**Phylobatanin**	+	+	+	+
H	**Cardiac glycosides**	+	+	-	-

Note:

+ = Positive

- = Negative

4. Discussion

Result obtained in this study showed that the methanol extract of the *Anonna muricata L*. Leaf has an antimicrobial property against all the isolates used in varying degrees. This indicates the presence of the active ingredient in the leaves which is toxic to the isolates. The methanol extract of the root revealed its effectiveness against *Streptococcus pyogenes,* and *E. coli.* The methanol extract of the bark is active against *E. coli, Salmonella species* and *Staphylococcus aureus*. The methanol extract of the mixture of the bark, root and leaves is active against *S. aureus*and *Streptococcus pyogenes*. It agrees with the work of Viera*et al.,* (2010).

The result also revealed that the alcohol extract of the leaf is active against all the isolates. The alcohol extract of the bark is active against all. The alcohol extract of the root is effective to *E. coli, Salmonella species*. The alcohol extract of the mixture of the bark, leaf and root is effective against *Staphylococcus aureus* and *Streptococcus pyogenes*which support the work done by Mithun*et al,* (2016). The leaf aqueous extract is effective against only *E. coli*, but ineffective to all other isolates. The aqueous extract of the bark, root and its mixture is effective against all other isolates. The high

resistance pattern of the isolate to the aqueous extracts may be due the chemical reaction that occurs during the process or due to the contaminants that may be associated with the procedure of its extraction as it is difficult to ensure an aseptic method of aqueous extraction. This is subject to more research as there are not much documentations of this.

Methanol soxhlet extract gave the highest antimicrobial effect, followed by the ethanol soxhlet extract. This may be due to the fact that methanol extracts more of the active ingredients than the ethanol because it is highly volatile than ethanol. Again the soxhlet extraction method gives a better result than the aqueous method of extraction, this agrees with the work of Olugbuyiro *et al.*, (2017). That could be the reason why the isolates resisted the ethanol extracts that was collected using the local method. The actual milligram that can be taken orally to treat these organisms in vivo cannot be ascertained through this study as the MBC of both the methanol and ethanol extracts gave a higher concentration. Again no laboratory animal was used to check the in vivo sensitivity of the isolates to the extracts. This is open to further research.

This research revealed the presence of the following photochemical, Anthraquinone, Saponins, Anthranoid, Phenol, Alkaloid, tannin, Phylobactanin and Cardiac glycosides in either the bark, leaf, root and the mixture which supports the plant to be used as traditional medicine. This agrees with the work of Vinothini Lali, (2016) and Nandhini*et al.,* (2018). The findingsin this research agrees to some extent with study by Vimala *et al.*,(2012) which said that ethanolic extract of soursop leaves has secondary metabolite such as flavonoid, tannin, alkaloid, saponin and steroid, but does not agree with the fact that Saponin is present in the leaf as it was completely absent in both alcoholic and aqueous extract. In this study, Anthroquinone was absent in the aqueous extract of the leaf but present in the alcoholic extract of the leaf, this does not absolutely agree with the work of Olugbuyiro *et al.,* (2017).

5. Conclusion

The findings in this research have shown the effectiveness of the ethanol extract of *Annona muricata l.* (soursop) leaves and bark on bacteria like *Staphylococcus aureus, E. coli, Streptococcus pyogenes,* and *Salmonella species.* It also revealed the effectiveness of the root against *Salmonella species*. The methanol extract of its leaves is effective against *E. coli Staphylococcus aureus, Streptococcus pyogenes*and *Salmonella species.* The methanol extract of the bark and root was shown to be effective against *Streptococcus pygenes* used, respectively. The phytochemical present in each of the bark, root, leaves and mixture of the bark, leaves and root include saponin, anthroquinone, anthropoid phenol, tannin, phylobatannin, and cardiac glycosides

My new discovery in this study is that when the leaves, bark and root of *A. muricata l.* are mixed together, their antimicrobial property will be reduced even when each of the three are highly effective against a particular isolate. This could be as a result of a chemical reaction which may take place and change their mode of action. The presence of the phytochemicals in each of the plant parts used justifies it as a medicinal plant, but when the bark leaves and bark extracts are mixed together, it will also reduce its phytochemical property which could also be as a result of a chemical reaction.

Recommendation.

These findings can be of commercial interest to both pharmaceutical companies and the Nigerian Raw materials Research Institute, in the production of new drugs. Patients infected with any of these isolates who prefer taking herbal concoction for treatment of infection should embark more on taking *A. muricata l.* plant bark, leaves and bark. While taking this concoction as a patient or trying to work

on it as a pharmaceutical company for the production of new drug, none of the bark, leaves or root should be used together to avoid inhibition of its antimicrobial property.

References

[1] Albuquerque, W.F., Macrae, A, Sousa, O.V., Viera, G.H.F., Viera. R.HS (2007). Multiple dry resistant Staphylococcus aureus strain isolated from a fish market and from fish handlers. Braz. J. Microbial.38: 131-134.

[2] Antonu, M.D. (1993). Screening of the flora of Puerto Rico for potential anti-malarial bioactive. Int. J. Pharmacog31 (4): 255-258.

[3] Artemis. http:/Hassan.hubpageS .com/hub/mythical- God -And-Creatures. Boris, C. (1991). "Anti-parasitic activity "of Annona muricata and Annona cherom-liaseeds".Planta med. 57(5); 434436.

[4] Chang F. R. (2001). Novel cytotoxic annonaceous acerogenins from Annona muricata J. Nat. Prod. 64(7).925-3I.

[5] Feng, P.C. (1962). "Pharmacological screening of some west Indian medicinal plants". Phytottier. Res.4 (3): 115-117.

[6] Gonzalez- Coloma, A.(2002). "Selective action of acetogenin mitochondrial complex 1 inhibitors". Z Naturfosch. 57(11-12): 1028-34.

[7] Harborne (1973). Phytochemical method; a guide to modem techniques of plants analysis. chapman and Hall, New York. Pp 73-75.

[8] Hamizah S., Roslida, Fezah, O., Tan, K. L., Tor, Y. S, Tan, C.I. (2014).Chemopreventive Potential of Annona Muricata l leaves on chemically-Induced Skin Papillomagnesis in Mice. Asian Pacific JCancer Prev. 13:2533-2539.

[9] Hostettman, K. (1991). "Saponins". Cambridge University press, London. Pp 72-81.

[10] Inyang, C.U., Adegoke, A. A. (2008). Antimicrobial properties and preliminary phytochemical screening of Chromoiaenaodorata (siam or sapysa weed Leaf). Nig. J. Microbiol. 22(1): 1652-1659.

[11] Jaramillo M. C. Arango, (3. L, Gonzalez M C. Robledo, S. M., Velez, I. D. (2007). Cytotoxicity and antileishmanial activity of Annonomuriceta pericarp Fitot. 71(2): 183-186.

[12] Karen B. (2011) Alternative Nature Online Herbal.Medicinal herbs uses. http://www.aItnature.com/index.html.Accessed20th December, 2018.

[13] Karem-Kos, H.E., de Vries E., Soerjomatarami, et al (2008). Recent Trends of Cancer in Europe: a combined approachof incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer, 44: 1345-89.

[14] Kojima, N.(2004)."Systematjc synthesis of antitumor Annonaeceousacetogenins". Yakugaku zasshi. 124(100): 673-81.

[15] Luna, J. s., De carvatho, J. M., De Lima, M. R.(2006). Acetogenins in Annona muricata l. (annooaceae) leaves are potent molluscicides. Nat. Prod. Res. 20(3): 253-257.

[16] Mithun Pai,B. H.,Gururagavendra R., Ramya S., and Ashwini R. (2016).Anti-microbial Efficacy of Soursop Leaf Extract (Annona muricata) on Oral Pathogens: An In-vitro Study. J Clin Diagn Res.10(11): ZC01–ZC04.

[17] Morton, F. J, Miami, F. L. (2018). "Soursop,Annona muricata". West Lafaye, IN: Fruits of Warm Climates. New Crop Resource Online Program, center for New crops and Plant Products, Department of Horticulture and Landscape Architecture, Purdue University. Pp75-80.

[18] Nandhini, R., Pakutharivu T., Rubalakshmi G. Nirubama K.and Prabhakaran S., (2018). Phytochemical Screening and Antimicrobial Activities of Ever Green Medicinal Plant Annona Muricata -"An Edible Vaccine"Eur. J Pharm and Med Resear, 5(7), 276-281. www.ejpmr.com Accessed 23rd April, 2019.

[19] Nicolas. H. (l997)."Structure activity relationships of diverse Annonaceous acetogenins against multidrug resistant human mamryadenocacinoma (mcf-7ÍAdr) cells". J. Med. Chem. 40(13). 2102-6.

[20] Obi, M. (2008). Antimicrobial and phytochemical analysis of the roots, bark and leaf extracts of spondiasmombin. Unpublished.

[21] Olugbuiro J. A.O., Omotosho, O. E., Taiwo,O. S., Ononiwu, F. O., Banwo, A. S. , Akintokun, O. A.,Obaseke, O.S. and Ogunleye, O. M.(2017). Antmicrobial Activities and Phytochemical Properties of Annona muricata Leaf. Convenant Journal of Physical and Life Science. 5(2): 40-49.

[22] Oluwatomi O. (2010). Nigerian Tribune Web Team. African Newspapers of Nigeria plc.

[23] Osorio, E., Arango G. J., Jimenez, N. (2007). Antiprotozoal and cytotoxic activities in vitro of Colombian Anrionaceae, J. Ethanoptiarmacol111 (3): 630-6350.
[24] Padma, p., Pramod N. P., Thyagarajan S. P., Khosa, R. L. (1998). Effect of the extract of Annona muricata and Petunia nytaginiflora on He,pes simplex virus; J. Ethanopharmacol 61(1): 81-83.
[25] Robert, D., Willix, J. R. (2003). Medicine from the rain forest-Graviola. Heal. And long. News.10:3.
[26] Sao P, (2010). Revista do Instituto de Medicina Tropical. Rev. Inst. Med. 52: 3.
[27] Scarlton, (2011). http://Scarlton .hubpages.com. Accessed 13th Nov, 2018.
[28] Soforowa, A., (1993). Medicinal plants and tradition medicine in Africa. Spectrum book Ltd Ibadan, Nigeria. Pp 9-25
[29] Taylor L., (1998). Herbal secrets of the Rainforest. Prima. http://blackherbal. Com/graviola.htm.
[30] Trease, G. G., Evans, W. C. (1989). Pharmacolognosy. Baillese and Tindalle, London 11th ed. Pp 243-551.
[31] Viera GHF, Mourão JA, Ângelo ÂM, Costa RA, Vieira RHSDF (2010). Antibacterial effect (in vitro) of moringa oleifera and annona muricata against gram positive and gram negative bacteria. Rev Inst Med Trop Sao Paulo.52 (3):129–32.
[32] Vimala J. R, Rose A. L, Raja S. (2012). A study on the phytochemical analysis and corrosion inhibition on mild steel by Annona Muricata .L leaves extract in 1 N hydrochloric acid. Der Chemica Sinica., 3(3): 582-588. [Dikutip tanggal 8 Februari 2014].
[33] Vinothni, R., and Lali Growther. (2016). Antimicrobial and Phytochemical Analysis of Methanolic and Aqueous extracts of Annona muricata l (leaf and Fruit). Int. J. Curr. Microbial. App. Sci. 5(10) 617-625.