ANTIBACTERIAL ACTIVITY OF ANNONA MURICATA
(SOURSOP) LEAF EXTRACT) ON Escherichia coli and/
Pseudomonas aeruginosa

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ABSTRACT
Annona muricata (Soursop) was evaluated for its antibacterial activity against Escherichia coli and Pseudomonas aeruginosa using standard bacteriological method. The zone of inhibition observed were 17mm, 15mm, 12.5mm at a concentration of 200mg/ml, 100mg/ml, and 50mg/ml respectively for E. coli and 17mm, 15mm, 13.5mm for Pseudomonas aeruginosa for the ethanol extract of the plant leaves and slightly lower inhibition for the aqueous extract. This study reveals that Annona muricata possesses significant antibacterial activity against the tested organisms with a varying potency at different concentration. Hence can serve as an adjunct antibacterial agents. Further research should be carried out on the plant to determine the concentration needed to archive similar result in-vivo.

Keywords: Annona muricata, extract, antibacterial, E. coli,

INTRODUCTION
Generally, infections are known to be a matter of interest globally due to its resulting in the increase in rate of morbidity and death in individuals. Infectious diseases are disorders usually caused by organisms — such as bacteria, fungi, viruses or parasites. Certain microorganisms are found to be resident in and on our bodies which normally harmless and sometimes helpful but under certain conditions, some of these organisms may cause diseases or infections. These infections are known to be caused by organisms such as Escherichia coli and Pseudomonas spp which have various clinical manifestations in man and animals. Escherichia coli, a gram negative bacteria and specie of the enterobacteriaceae is well known to be the common cause of urinary tract infections and traveler’s diarrhea (Delong and Pace, 2001). Pseudomonas aeruginosa on the other hand is one of the most important bacterial agents associated with nosocomial infections (Anton and David, 2011), a common bacterial agents in patients that causes invasive infections in skin burns leading to sepsis or eye infections (Alhazmi, 2015). Infections caused by Escherichia coli and Pseudomonas
Aeruginosa have been successfully treated with a wide range of antimicrobial agents. Thus, antibiotics administration has been widely used to shorten the course of illness and duration of excretion of enterotoxigenic Escherichia coli (ETEC) in adults in endemic areas and in traveler’s diarrhea. The antibiotics administered are usually dependent on the susceptibility patterns. The antibiotics used for the treatment of these infections include fluoroquinolones, quinolones, aminoglycosides, and ciprofloxacin etc. The control and treatment of diseases caused by these organisms relies almost entirely on the use of conventional drugs, but due to escalating cost, circulation of fake and adulterated drugs, non-availability of conventional drugs and development of multiple resistance strains of bacteria, there is an urgent need to discover new antibacterials with diverse chemical structures and new mechanisms of action against infectious diseases. Thus, plant based antibacterial represent a wide untapped source of medicines offering enormous therapeutic potential (Tagboto and Townson, 2001). Some problems associated with antibiotics used for treatments include resistance to antibiotics which leads eventually to failure of the treatment (Radji et al., 2015). It is also a well-known fact that the use of conventional antibiotics have been associated with adverse side effects ranging from mild rashes, nausea, dizziness to hypersensitivity as seen in penicillin, neutropenia, gastrointestinal disorder to severe effects such as auditory damage, kidney damage and problems of the central nervous system. However, in most developing countries there is a rise in the resistance of microbial agents to antimicrobials due to lack of adequate health facilities and inability to purchase second-line antibiotics. However, plants have been useful to man not only for food, shelter and clothing but also for their use for ornamental and health care. Due to the increasing failure of chemotherapeutic agents and antibiotics resistance exhibited by pathogenic organisms, researchers are gradually and rapidly turning their attention towards traditional treatment or herbal medicine, screening several medicinal plants for their potential antimicrobial activities to develop better drug against microbial infections (Hann and Koch, 1996). Multi-drug resistant infections have become a threat to immune-compromised patients, resulting in severe infections with poor outcomes. The increase in infections caused by gram-negative and gram-positive resistant bacteria is concerning for the success of empiric treatment. Many different ways of approach in search for plants and plant products of pharmaceutical and chemical interest become important as a result of emergent bacteria resistant strains to conventional antibacterial agents. The essence of this study therefore is to assess the efficacy of the Annona muricata leaf extract against Escherichia coli and Pseudomonas species to ascertain its usefulness effectively in the treatment and prevention of diseases.

MATERIALS AND METHODS

EQUIPMENTS/MATERIALS

Incubator, refrigerator, weighing balance, Applicator stick, Wire loop, cotton wool, Aluminum foil, Petri dish, Latex gloves, measuring cylinder, Hot air oven, bursen burner autoclave conical flask, Pipette, Test tube rack, Needle and syringe, Hydrogen peroxide, Crystal violet, Safranin, Iodine, Kovac’s reagent

PLANT COLLECTION AND IDENTIFICATION

Fresh plant leaves of Annona muricata were collected from Opete town in Udu Local Government Area of Delta State, Nigeria. The leaves were taken to the Department of
Botany, Delta state university, Abraka for identification. They were then air dried at room temperature for 65 days and blended using a domestic blender. The powdered leaves were properly stored in a labeled polythene bag and kept in a cupboard prior to usage.

**PREPARATION OF EXTRACT**

**Aqueous extraction**

Weighed amount of powdered leaves (230.2g) of Annona muricata were soaked in 2000ml of distilled water for 24 hours and sieved with muslin cloth to remove fiber particles and then filtered with a filter paper into conical flasks. The filtrate was concentrated and dried using a water bath set at 400c.

**Ethanol extraction**

A net powdered leaves of 460g of Annona muricata were extracted with 80 % ethanol dissolved in 275ml of distilled water. The ethanolic extract was properly covered and kept for a week. It was then sieved using muslin cloth to remove fiber particles and filtered with a filter paper into conical flasks. The filtrate was concentrated and dried using a water bath set at 400c. The solvent free extracts were then weighed and stored in 50ml beakers at 40C.

**TEST ORGANISMS**

The bacteria used were Escherichia coli and Pseudomonas species. The organisms were obtained from the stock culture of Microbiology laboratory Delta state university, Abraka. Cultures were brought to the laboratory conditions by resuscitating the organisms in peptone water and thereafter subculture into nutrient agar and incubated at 370c for 24 hours and confirmed by several biochemical tests (Cheesebrough, 2006).

**CONFIRMATION OF TEST ORGANISMS**

All biochemical and cultural test were carried out following standard procedure as described by Cheesebrough, (2006).

**ANTIBACTERIAL ASSAY**

The method of Nostro et al., (2000) was used. A quantity (20 ml) of sterile Mueller-Hilton agar was poured into sterile Petri dishes to solidify. Using a sterile syringe, 0.1 ml of the standardized inoculum (test organisms) were inoculated into four petri dishes in duplicate, representing the various concentrations and for each of the extracts. The bacterial suspension was spread with the aid of a sterile swab stick. Using a cork borer (8mm in diameter), four wells were made in each agar plate. All plates were appropriately labeled according to the corresponding concentrations and extract used. Using a sterile syringe, 0.1ml of the diluted extracts was placed into the wells and the plates left on the work bench for 2 hours for the extracts to diffuse into the agar before incubation at 370C for 24 hrs. Ciprofloxacin antibiotic sensitivity disc (10 µg) was used as a control against test organisms. After incubation, the plates were observed for evidence of inhibition (appearance of clear zones that are completely
devoid of growth around wells). The diameters of the zones were measured using a calibrated rule in millimeters.

**DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION.**

The minimal inhibitory concentration (MIC) is the lowest concentration that prevents visible microbial growth after an overnight incubation. The MIC was determined by serial dilution as recommended by the national committee for clinical standard. Minimum inhibitory concentrations (MIC) of the extracts were determined by agar diffusion method as described. Two fold dilutions of the extracts were prepared to give final concentration in the ranges of 200, 100, 50, 25, 12.5 and 6.25 mg/ml. 4ml of each dilution was incorporated in 16ml of the appropriate melted agar medium which were poured into petri-dishes and allowed to set. A loopful of the diluted culture of each test organism was inoculated by streaking on the surface of the petri dish and then incubated for 24hrs. After incubation, the plates were examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective extracts (Nwinyi et al., 2008).

**RESULTS**

After the extraction procedures, the final weight of extracts was 12.76 g for the ethanol and 7.48g for the aqueous extract. Bacterial inhibition by extracts was evaluated visually by measuring the inhibition zone diameters around disks (disk diameter included) recorded in millimeters.

The results of the various concentration ethanol and aqueous extracts of the soursop leaf on the test microorganisms have been tabulated as follows:

<table>
<thead>
<tr>
<th>Table 4.1: Zone of inhibition of ethanol extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test organisms</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<td></td>
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</tbody>
</table>
Table 4.2: Zone of inhibition of aqueous extract (mm)

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Aqueous extract concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 100 50 25 12.5 6.25</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>14 12.5 11 9 8 7.5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>17 15 14 12 11 8</td>
</tr>
</tbody>
</table>

Table 4.3: Minimum Inhibitory Concentration of Ethanol Extract

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Ethanol extract concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 100 50 25 12.5 6.25 3.125</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>- - - - -(MIC) +</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>- - - - -(MIC)</td>
</tr>
</tbody>
</table>

Table 4.4: Minimum Inhibitory Concentration of Aqueous Extract

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Aqueous extract concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 100 5 2 12 6.25 3.125</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>- -(MIC) + + + + +</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>- -(MIC) + + + +</td>
</tr>
</tbody>
</table>

Table 4.1 and 4.2 shows the zone of inhibition size in millimeter (mm) of the two extracts on the test organisms *Escherichia coli* and *Pseudomonas aeruginosa*. It was observed that all concentrations of extracts were effective on the two test organisms and the antibacterial activities of all the extracts increased with an increase in its concentration (6.25mg/ml, 12.25mg/ml, 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) with zones of inhibition ranging from 7-18mm.
The result however demonstrated that ethanolic extract of Annona muricata was more effective on Pseudomonas aeruginosa than Escherichia coli with subsequent result with the aqueous extract but high sensitive rate on both test organisms of the initial extract when compared with the aqueous.

The minimum inhibitory concentration (MIC) of ethanol and aqueous leaf extract of Annona muricata against Escherichia coli and Pseudomonas aeruginosa were observed to be 6.25 and 100mg/ml as demonstrated in table 3 and 4 respectively.

**Figure 1.0: Curves for the plot of the zones of inhibition Escherichia coli and Pseudomonas aeruginosa against concentration of Annona muricata ethanolic leaf extract**
Concentration of aqueous leaf extract (mg/ml)

Figure 2.0: Curves for the plot of the zones of inhibition of Escherichia coli and Pseudomonas aeruginosa against concentration of Annona muricata aqueous leaf extract

DISCUSSION

In this study, the ethanol and aqueous leaf extract of Annona muricata has demonstrated some degree of efficacy against the test organisms, Escherichia coli and Pseudomonas aureginosa which confirmed bioactivity of the extract as earlier reported by Vijayaneena et al., (2013), Solomon-Wisdom et al., (2014) and Radji et al., (2015) respectively. It was observed that 81% of the ethanol extracts inhibited Pseudomonas aeruginosa while 36% inhibited Escherichiacoli with minimum inhibitory concentration range between 0.008 - 256 mgmL-1. The difference in efficiency of the ethanol and aqueous extract is actually as a result of the difference in polarity of solvents. During the extraction process, polarity influences solubility of the main active substance, leading to difference in their chemical composition and consequently, in their biological activity (Kossouoh et al., 2007). The yield of extraction and concentration of the extract solution can also intervene in the results.

Pai et al., (2016) proved that the antibacterial effects of the soursop leaf extract on these organisms are actually due to the active compounds contained in it. Soursop leaf extract has phytochemicals components such as alkaloids, flavonoids, tannins, steroids, and saponins that act as antibacterials. They emphasized that the ability of alkaloid compounds as antibacterials is actually influenced by the basic groups when in contact with the bacteria cell wall, it then react with the amino acid compounds that
make up the bacterial wall. This reaction thus leads to changes in the structure of amino acids, and bacterial DNA becomes damaged promoting bacteria lysis (Pujiyanto et al., 2018). The same process also occurs with the flavonoids. The biological activity in such processes is done by damaging the cell walls of bacteria consisting of lipids and amino acids leading to lysis (Cushnie and Andrew, 2005).

The mechanism of action of steroids as antibacterial actually is by destroying bacterial cell membranes as observed by Jannah et al., (2017). Dewi et al., (2015) in their work proved that the antibacterial action of saponins causes increase in the permeability of cell membranes and thus inhibit bacterial growth, increase the efficiency of protein synthesis and renders cells unstable which leads to cell lysis.

CONCLUSION

This study has shown that the leaf extract of Annona muricata has shown efficacy against the selected organism, Escherichia coli and Pseudomonas aeruginosa. However, the ethanolic extract show more potency than the aqueous extract.

CONFLICT OF INTEREST

All authors declares no conflict of interest

REFERENCES


