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**Abstract:** Natural products, particularly those from plants, have been used as vital tool to help mankind in sustaining health care delivery systems. Thus, this research aimed to determine the efficacy of *Annonasenegalensis* against some bacteria associated with wound infection. Leaf preparation of *A. senegalensis* is used as ethnomedicine for the treatment of wound and organisms associated with wound. Leaf extract of *A. senegalensis* was obtained by macerating the powdered leaf of 57.89g into 440ml of methanol which yielded 8.5g (14.68%). The phytochemical constituents of the methanol leaf extract of *A. senegalensis* were determined using standard qualitative procedure. The antibacterial activity was performed using agar well diffusion method. The phytochemical screening showed that the methanol leaf extract of *A. senegalensis* contained saponins, flavonoids, carbohydrates, balsam, tanins, phenols and cardiac glycosides however, terpenes and steroids, resins and alkaloids were absent. The various concentrations of 400mg/ml, 200mg/ml, and 100mg/ml exhibited significant mean zone of inhibition ranging from 1.45±2.17 to 2.15±3.22 against clinical strain of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound when interpreted with interpretative chart of zone size of Kirby-Bauer technique. Minimum Inhibitory Concentration (MIC) was 9.37mg/ml for both *S. aureus* and *P. aeruginosa*. Minimum Bactericidal Concentration (MBC) was 150mg/ml for *S. aureus* and 300mg/ml for *P. aeruginosa*. Therefore, the extract from *A. senegalensis* exhibited potent antibacterial activity and can probably be used for wound treatment.

**Keywords:** *Annonasenegalensis*, Studies, Antibacterial Activity, Wound Causing Bacteria, Phytochemistry

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I. **Introduction**

Medicinal plants have been used in traditional medicine for treatment of various ailments including infectious disease and many potent phytochemical or secondary metabolites possessing antimicrobial effects have been isolated from plants (Salazar-Aranda, et al., 2011). *Annonasenegalensis* Pers. (Annonaceae) commonly known as Wild Custard Apple has been described as a multi-stemmed deciduous shrub observe to grow 2-6 metres in height; though it can grow to become like a tree. It is a multi-purpose tree which grows in the wild and provides food, medicine and other materials for local people. The fruit, leaves and flowers of *Annona senegalensis* have been reported to have edible applications. The leaves of *A. senegalensis* are sometimes used as vegetables, dry leaves are reported to contain 8.2% protein, (The Useful Plants Database, 2019).

Various reports on the medicinal use of various parts of the plant have been documented. The barks are reported to be used in the treatment of guinea and other worms, toothache, gastroenteritis, snakebite, diarrhoea and respiratory infections. The bark is also chewed and applied on fresh wounds and a combination of barks and roots crushed together can be applied on snake bite wounds. The gum from the bark of the tree is used in closing up cuts and wounds (The Useful Plants Database, 2019).

The leaves have been used in the treatment of pneumonia and also as a tonic to promote general well-being (The Useful Plants Database, 2019). Arbonnier (2002) has reported further claims on the use of its leaves in treating wounds and skin diseases and ailments such as conjunctivitis, insect bites, haemorrhoids, fever, female sterility, convulsions, jaundice and guinea worm.

Nantiet al. (2018) have reported the use of the stems, leaves, fruits and roots of *Annona senegalensis* in the treatment of skin cancer, cough and to heal wounds.

Phytochemical analysis of methanolic extracts carried out on various parts of the plant have shown the presence of common metabolites alkaloids, glycosides, phlobatansins, saponins, and tanins.

Other uses of the leaves include its use as fodder by livestock, as stuffings for mattresses and pillows and in the promotion of milk production (Arbonnier, 2004).
Nwonuma et al. (2015) have reported the effect of the aqueous leaf extract of *A. senegalensis* on the selected testicular function of Wistar Rats. Their report showed that the leaf extract was capable of causing alterations in some testicular and biochemical functions which were tested.

Other reports have revealed that the extracts of the plant parts of *Annona senegalensis* could be utilized for the growth inhibition of the *Malessezialglobossa* fungus (Ochieng et al., 2017). Would healing has been described as a dynamic process which is complex, with the wound environment changing with the changing health status of the individual (Adigun et al., 2018). The aim of this research was to evaluate the effects of methanolic leaf extracts of *Annona senegalensis* on wound causing bacteria.

### II. Methodology

**Plant Material**

*Annona senegalensis* leaves were collected from the Federal College of Forestry in Jos, Plateau State Nigeria. The Plant was identified and authenticated at the Herbarium of the College.

**Test Organisms**

Clinical strain of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound were obtained from Veterinary Laboratory Vom, Plateau State, Nigeria and preserved in Microbiology unit of Plateau State Polytechnic, B/Ladi.

**Preparation and Extraction of Plant Material**

The plant leaves were grounded into a fine powder using a clean mortar and pistil and then extracted (58g of the powdered leaves in 440ml of the solvent) and maceration for three days in methanol at room temperature. Extract was filtered through a Whatman No. 1 filter paper and filtrate collected. This (Filtrate) was concentrated using water bath at 97°C.

**Phytochemical screening**

The phytochemical screening of the methanol leaf extract of *Annona senegalensis* carried out using standard qualitative procedures (Trease and Evans, 1987; Sofowora, 1986).

**Test for Alkaloids**

Few drops of the Dragendorf reagent were added to 2.0ml of extract and observed for orange colouration to indicate that alkaloid is present or otherwise.

**Test for Flavonoids**

Two ml of extract was added to 5% lead acetate solution and observed for either cream light yellow coloration confirming the presence of flavonoid.

**Test for Tanins**

To 2ml of the extract, a few drops of 10% FeCl₃ solution were added. The mixture was observed for deep bluish or greenish coloration which indicated the presence of tannins.

**Test for Saponins**

One ml of the extract was added to 4ml of distilled water and shaken vigorously, formation of froth indicated the presence of saponins.

**Terpenes and Steroids**

To 1ml of the extract was added 2ml of conc. H₂SO₄ along the side of the test tube, no formation of reddish brown ring at the interphase indicated the absence of terpens and steroids.

**Test for Balsam**

Three drops of Alcoholic Ferric chloride were added to 2ml of the extract, dark green colouration formed showed the presence of balsam.

**Test for Carbohydrates**

Five drops of the extract added to 2ml of Benedict’s reagent and placed on a hot plate for 5minutes then observed for the formation of brick red precipitate as indication for the presence of carbohydrates.

**Test for Phenol**

Two ml of the extract added to 2ml of FeCl₃ solution, deep bluish green colouration showed presence of phenol.
Studies on the Antibacterial Potency of Annona Senegalensis Pers. (Annonaceae) Extract on Bacteria

Test for Resins
To 2ml of the extract, 2 ml of acetic anhydride was added plus few drops of conc. H$_2$SO$_4$, no violet colour showed the absence of resins.

Test Cardiac glycosides
Two 2ml of the extract was dissolved in 2ml of chloroform. Conc. Sulphuric acid was added carefully. A reddish brown colour at the interphase indicated the presence of cardiac glycosides.

Antibacterial activity
Four gram of methanol extract of Annona senegalensis was dissolved in 10ml dimethylsulphoxide (DMSO) to obtain 400mg/ml as stock concentration. Subsequently, the concentration was diluted to obtain 200, 100, 50 and 25mg/ml for the sensitivity test.

Agar diffusion method as described by Perez et al., 1990 was employed for the assay. The test organisms, the clinical isolate from wound was prepared using 0.5McFarland standard. Petri-plates containing 20ml of the nutrient agar were seeded with 0.2ml of microbial strains and incubated at 37°C for 24hrs. Standard antimicrobial agent used as positive control was gentamycin. After 24hrs the Inhibition Zone Diameter were recorded and mean calculated.

Determination Minimum Inhibitory Concentration (MIC) Using the tube method (Atlas, 1995)
Two-fold serial dilution was made using nutrient broth. Five ml of a solution of the test compound (plant extract 3g/ 10ml) was added aseptically to 5ml of double strength medium and mixed by shaking. Using a fresh pipette 5ml of mixture was taken and dispensed into test tube 2 which contained 5ml single strength medium. This was mixed by shaking and from it 5ml was taken into test tube 3 aseptically, mixed and transferred to test tube 4. This procedure was repeated up to test tube 8 and 5ml from the same test tube 8 was discarded after shaking. The 9th test tube contained no test compound being the control. Finally, to each test tube (1–8), was added 0.1 ml inoculum of the test organism aseptically. The lowest concentration that inhibited the growth of bacterium was considered as MIC.

Minimum Bactericidal Concentration (MBC)
A sterile wire loop was dipped into the wells of minimum inhibitory concentration that showed no turbidity (no bacterial growth) and streaked on nutrient agar and incubated overnight. The MBC was obtained as the lowest concentration preventing the growth of bacteria (Mourouge et al., 2013).

III. Results
The phytochemical screening revealed that methanol leaf extract of Annonasenegalensis contained saponins, flavonoids, carbohydrates, balsam, tanins, phenols and cardiac glycosides, however, terpens and steroids, resins and alkaloids were absent. The mean diameter at 1.45 mm±2.17 to 2.15 mm±3.22 exhibited significant antibacterial activity as compare with interpretation chart of zone size of Kirby-Bauer technique. The result of the Inhibition Zone Diameter of extract also revealed activity against gram-positive (Staphylococcus aureus) and gram-negative (Pseudomonas aeruginosa) as shown in Table 2 below. The result of MICs of the extract revealed activity against gram-positive and gram-negative organisms. The extract exhibited an MIC of 9.37mg/ml against clinical isolate of S.aureus and P. aeruginosa. The MBC result for Staphylococcus aureus was 150mg/ml and Pseudomonasaeruginosa 300mg/ml showing bactericidal activity against tested organisms.

Table1. Phytochemical constituents of Methanol leaf extract of Annona senegalensis

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>+/-</th>
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</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Tanins</td>
<td>+</td>
</tr>
<tr>
<td>Balsams</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes and Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>+ (present), - (absent)</td>
<td></td>
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</tbody>
</table>

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The antimicrobial effect of methanol extract of *Annona senegalensis* exhibited appreciable antibacterial effects. Antibacterial effect of *Annona senegalensis* against organisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* correlated with the ethnomedicinal use of the plant in wound healing, since *Pseudomonas aeruginosa* and *Staphylococcus aureus* had been implicated in the contamination of wounds and boils (Okoh et al., 2007). The antibacterial activity against *Pseudomonas aeruginosa* is of interest because *Pseudomonas aeruginosa* has been reported to be resistance to many antibacterial agent and identified as an opportunistic pathogen which causes complications in immune compromised patients (Adeshina et al., 2010). The antibacterial activity of the root and leave extract of *Annonasenegalensis* has also been documented in a study by Apak and Olila (2006).

The better activity of *A. senegalensis* extract against *S.aureus* than on *P.aeruginosa* is in line with previous screening for antibacterial agents from leaves and seeds of plants which showed that gram-positive bacteria were less susceptible to the leaf extracts than gram-negative bacteria (Ajaiyeoba, 2002). This could due to the fact that the outer membrane surrounding the cell wall of gran-negative bacteria may restrict diffusion of hydrophilic compound through its lipopolysaccharide covering (Burt, 2004, Davidson et al., 2005). Nevertheless, the antimicrobial action of *A.senegalensis* against both gram-positive and gram-negative bacteria demonstrates that this plant might have broad spectrum therapeutic effect.

The low value of MIC (9.37mg/ml) and MBC indicates that the plant *Annona senegalensis* has effective antibacterial potency especially against *S.aureus* and *P.aeruginosaisolated from wound. This antibacterial action of *A.senegalensis* due probably to the presence of phytochemicals like saponins, flavonoids, carbohydrates, balsam, tanins, phenols and cardiac glycosides. Medicinal plants with preponderance of variety of secondary metabolites such as tannins, trepenoids, essential oil, alkaloids and flavonoids have been found in vitro to possess antimicrobial properties. It has been discovered that saponins and other flavonoid compounds even at low concentrations inhibited the growth of microorganisms and also acted as bactericidal agents at higher concentrations by coagulating protoplasm of the organisms. Saponins are sources of sapogenins which can be converted in the laboratory to animal sterols of therapeutic importance (Sofowora, 1993).

Medicinal plants have long been used in traditional medicine for treatment of various ailments including infectious diseases and many potent phytochemicals or secondary metabolites possessing antimicrobial effects have been isolated from plants (Cowan, 1999, Salazar-Aranda et al., 2011). Study has indicated that a diterpenoid, kaur-16-en-19-oic acid or kaurenolic acid, has been identified as the phytochemical constituent responsible for the antibacterial effects of root bark of Nigerian *Annonasenegalensis* Pers (Okhale et al., 2016).

Also, the stimulation of fibroblast by wound healing plant which result in differentiation of myofibroblast in wound are essential event during wound contraction, expression and prolonged fibroblast expression (scar) in wound (Bochaton-Piallat et al., 2016).

### IV. Discussion

<table>
<thead>
<tr>
<th>Organism</th>
<th>400</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>Gen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>2.15 ±3.22</td>
<td>1.50 ±2.25</td>
<td>1.30 ±1.95</td>
<td>1.25 ±0.88</td>
<td>0.00</td>
<td>3.20 ±2.26</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1.82 ±2.74</td>
<td>1.75 ±2.62</td>
<td>1.45 ±2.17</td>
<td>1.23 ±1.88</td>
<td>1.05 ±1.57</td>
<td>3.58 ±5.36</td>
</tr>
</tbody>
</table>

Table 2: Inhibition Zone Diameter (mm±) of the extract at 400mg/ml-25mg/ml

<table>
<thead>
<tr>
<th>Organism</th>
<th>300</th>
<th>150</th>
<th>75</th>
<th>37.5</th>
<th>18.75</th>
<th>9.37</th>
<th>4.68</th>
<th>2.34</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>9.37</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9.37</td>
</tr>
</tbody>
</table>

Table 3: Minimum Inhibitory Concentration (MIC) of extract

<table>
<thead>
<tr>
<th>Organism</th>
<th>300</th>
<th>150</th>
<th>75</th>
<th>37.5</th>
<th>18.75</th>
<th>9.37</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>150</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 4: Minimum Bactericidal Concentration (MBC)

<table>
<thead>
<tr>
<th>Organism</th>
<th>300</th>
<th>150</th>
<th>75</th>
<th>37.5</th>
<th>18.75</th>
<th>9.37</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Values of concentration are in mg/ml, keys:+: Present of growth, -: No or absent of growth.

V. Conclusion

The results from this study tend to support the ethnomedicinal claim of leaf of *A. senegalensis* in the treatment of bacterial infections and in wound healing.

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