IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 3 Ser. III (May – June 2019), PP 07-11
www.Iosrjournals.Org

Studies on the Antibacterial Potency of *Annona Senegalensis* Pers. (Annonaceae) Extract on Bacteria Associated with Wound Infection.

[1*]Dawang,DN, [1]Ayanlere, JR and [2]Dawang,SN
[1.] *Department of Science, Plateau State Polytechnic, B/Ladi, Nigeria*
[2.] *Department of Plant Science and Biotechnology,University of Jos,Nigeria*
Corresponding Author: Dawang,DN

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, terpens 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 2.15mm±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*

Date of Submission: 16-06-2019 Date of acceptance: 02-07-2019

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.(Annonceae) 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.

Nanti*et 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, phlobatanins, saponins, and tannins.

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 *Malesseziaglobossa* 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 theHerbarium of the College.

Test Organisms

Clinical strain of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound were obtained from Veterinary LaboratoryVom, 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 97^0C.

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% $FeCl_3$ 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.

Terpens and Steroids

To 1ml of the extract was added 2ml of conc.H_2SO_4 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 $FeCl_3$ solution, deep bluish green colouration showed presence of phenol

Test for Resins

To 2ml of the extract, 2 ml of acetic anhydride was added plus few drops of conc. H_2 $S0_4$, 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 37^0C 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 9^{th} 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 at1.45mm±2.17to 2.15mm±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. Pytochemical constituents of Methanol leaf extract of *Annona senegalensis*

Chemical constituents	+/-
Saponins	+
Alkaloids	-
Cardiac glycosides	+
Tanins	+
Balsams	+
Resins	-
Carbohydrates	+
Terpens and Steriods	-
Flavonoids	+
Phenols	+

+ (present), - (absent)

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

Organism	400	200	100	50	25	Gen
S. aureus	2.15 ±3.22	1.50±2.25	1.30±1.95	1.25 ±0.88	0.00	3.20 ±2.26
P. aeruginosa	1.82±2.74	1.75±2.62	1.45±2.17	1.23±1.88	1.05±1.57	3.58±5.36

Values in mm, are mean± are Standard Deviation, Gen.; Gentamycin

Table 3: Minimum Inhibitory Concentration (MIC) of extract

Organism	300	150	75	37.5	18.75	9.37	4.68	2.34	MIC
S. aureus	-	-	-	-	-	-	+	+	9.37
P. aeruginosa	-	-	-	-	-	-	+	+	9.37

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

Table 4: Minimum Bactericidal Concentration (MBC)

Organism	300	150	75	37.5	18.75	9.37	MBC
S. aureus	-	-	+	+	+	+	150
P. aeruginosa	-	+	+	+	+	+	300

Values of concentration are in mg/ml, +: Present of growth, -: No growth

IV. Discussion

The antimicrobial effect of methanol extract of *Annonasenegalensis* exhibited appreciable antibacterial effects. Antibacterial effect of *Annonasenegalensis* 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 (Okoli*et. al.*, 2007). The antibacterial activity against *Pseudomonas aeruginosa* is of interest because *Pseudomonas aeruginosa* had 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 *Annonasenegslensis* 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 havebroad spectrum therapeutic effect.

The low value of MIC (9.37mg/ml) and MBC indicates that the plant *Annona senegalensis* haseffective antibacterial potency especially against *S.aureus*and *P.aerouginisa*isolated from wound. Thisantibacterial action of *A.senegalensis*is 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 microorganismsand 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 kaurenoic 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).

V. Conclusion

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

References

[1]. Adeshina, G.O. Tashir, T.S andOnaolapo, J.A. (2010); Microbiological Evaluation of Packaged Pineapple Juice market in Kaduna metropolis.'*Nigeria Journal of Pharmaceutical Research* vol. 8. No. 1 pp 281-287

[2]. Adigun,M.O.,Afolabi,O.O.,Agbaje-Daniels,F.V.,Okonofua,C.C and Lateef,S. A.(2018). Antibiotic Susceptibility Profile of Microbial Isolates from Open Wounds to Common Antibiotics.*Nigerian Journal of Microbiology.* 32(1 &2):4148-4157.

[3]. Ajaiyeoba, E. Falade, M. Izabe, K. Akumka, D andGamaniel, K. (2005).Effect of *Annonasenegalensis*Rootbark Extracts on *Najanigricolisnigricolis* Venom in Rats. *Journal Ethnopharmacol*. 96:507-513

[4]. Ajaiyeoba, E.O (2002). Phytochemical and antibacterial properties of Parkiabiglobosa and extracts.*African Journal of Biomedical Research* 5, 125–129.

[5]. Apak, L. and Olila, D. (2006).'The in vitro Antibacterial Activity of *Annona senegalensis.Securudacccalongipediculate* and *Steganotteaniaaraliacea*-Uganda medicinal plant'.Africa Health Science vol. 68. No. 1-3 pp289-294

[6]. *Arbonnier, M.(2004). Trees,Shrubs and Lianas of West African Dry Zones. Cirad, Margraf Publishers Gmbh,Mnhn,Paris,France.Pp.155.*

[7]. Atlas,R.M.(1995).*Microorganisms in Our World*.Mosby Publishers Inc.Baltimore.765p.

[8]. Bochaton-Piallat, M.L. Gabbiani, G. and Hinz, B. (2016).The Myofibroblast in Wound Healing Fibrosis. Answered and unanswered question flooo Res doi:10.12688/100 research. 8190.1

[9]. Burt, S(2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology* 94, 223–253

[10]. Cowan, M.M(1999)."Plant products as antimicrobial agents," Clinical Microbiology Reviews, vol. 12, no. 4, pp. 564–582, 1999.

[11]. Davidson, J.M, Yu, F. Opalenik, S.R. (2013). The mouse excisional wound splinting model, including applications for stem cell transplantation. Nat Protoc;8: 302-309. Doi:10.1089/wound.2012.0424

[12]. Davidson, J.N., Sofos, A.L and Branen, A. (Eds.) (2005).Antimicrobials in Food. CRC Press/Taylor and Francis Group, pp. 1–9.

[13]. Mourouge, A.S., Nazlina,I. and Wan,A.Y. (2013).Bio-guided study on *Melastomamalabathricum* Linn leaves and elucidation of its biological activities.*American Journal of Applied Sciences* 10(**8**):767-778.

[14]. Nanti,G.C.G.G.,Bi,N.A.S.,Zahoui,I,S and Traore,F. (2018).Comparative Study of *Annonasenegalensis*(Annonaceae) and *Hallealedermanii* (Rubiaceae)effects on glycemia in rats.*Journal of Intercultural Ethnopharmacology*. 7(1): 1-7.

[15]. Nwonuma,C .O.,Irokanubo, E. O.,Tolaiya, A.E and Ore, A(.2015) The Effect of Aqueous Leaf Extract of *Annonasenegalensis*on selected Testicular Function Indices of Wistar Rats. *American Journal of LifeSciences*, 3(3):203-212.

[16]. Ochieng,O.A.,Omar,H.S.,Ibrahim,H.,Mataka, A .M.,Ochieng,J.O.,Parkia,M and Oyoo,O.T.(2017).Assessment of Anti-Fungal Activity of *Annonasenegalensis* Plant Parts on *Malasseziaglobosa. Nat, Prod.Chem.Res. 5(7): DOI: 104172/2329-68361000291*.

[17]. Okhale, S.E. Ediofom, A. Fatokun, O.T, Esevo, K.B and Kunle.O.F. (2016).*Annonasenegalensis*Persoon (Annonaceae): A review of its ethnomedicinal uses, Biological Activities and phytocompounds. *Journal of Pharmacognosy and Phytochemistry*, 5(2): 211-219. www.phtojournal.com

[18]. Okoli, C.O. Akah, P.A and Okoli A.S. (2007). 'Potentials of Leaves of Aspilia Africana (compositae) in Wound care; An experimental evaluation' BMC complementary and alternative medicine vol. 7, article 24

[19]. Okoye, T. C. Akah, P.A. Ezike, A.C. and Nwoke, J.C. (2011). 'Studies on the effects of *Annonasenegalensis*root bark on acute and chronic inflammation in rats'. *Journal of Pharmacy Research*, vol. 4, no. 5, pp 1443-1444.

[20]. Okoye,C.T ,Akah,A.P,Okoli,O.P, Ezike,C.A, Omeje,O.E and Odoh,S.T(2012).Antimicrobial Effects of a Lipophilic Fraction and Kaurenoic Acid Isolated from the Root Bark Extracts of Annona senegalensis Evidence-Based Complementary and Alternative Medicine
Volume 2012.

[21]. Perez, C. Paul, M. and Bazerque, P. (1990).'An antibiotic assay by the Agar Well Diffusion method.ActaBiologiac at medicine experimentalist vol. 15 pp 113-115.

[22]. Salazar-Aranda, R., Pérez-Lopez, L.A., Lopez-Arroyo, J., Alanís-Garza, B.A and Waksman de Torres, N., (2011).Antimicrobial and antioxidant activities of plants from northeast of Mexico.Evid.Based Complement.Alternat. Med., 1–6

[23]. Sofowora, E. A. (1986). State of Medicinal Plant Research in Nigeria by University Press Ile-Ife, Nigeria, pp 243 – 245.

[24]. The Useful Tropical Plants Database –tropical.the ferns.info/viewtropical.php?id=Annona+senegalensis (Retrieved 02/06/2019).

[25]. Trease, G. E. and Evans, W. C. (1989). A Textbook of Pharmacognosy, 12 th Edition. BailliereTindall, London, pp 45 - 50.

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Dawang DN " Studies on the Antibacterial Potency of Annona Senegalensis Pers. (Annonaceae) Extract on Bacteria Associated with Wound Infection." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.3 (2019): 07-11.