Global Journal of Pharmacology 10 (1): 13-18, 2016
ISSN 1992-0075
© IDOSI Publications, 2016
DOI: 10.5829/idosi.gjp.2016.10.01.1125

Antibacterial Activity of the Leaf and Stem Bark of *Irvingia gabonensis* (Bush Mango) Against *Escherichia coli* and *Staphylococcus aureus*

[1]O. Nworie, [2]J.O. Orji, [2]U.O. Ekuma, [2]M.V. Agah, [3]C.S. Okoli and [4]M.C. Nweke

[1]Department of Microbiology, Federal University Ndufu-alike Ikwo, Ebonyi State, Nigeria
[2]Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria
[3]Department of Applied Microbiology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[4]Immunization Unit, Ministry of Health Abakaliki, Ebonyi State, Nigeria

Abstract: Bacterial resistance to antibacterial drugs in the treatment of some bacterial infections has become a menace, therefore causing untold health challenges to patients. The antibacterial activity of hot and cold water and ethanolic extract of the leaf and stem bark of *Irvingia gabonensis* against *Escherichia coli* and *Staphylococcus aureus* was evaluated using agar-well diffusion and agar dilution methods. All the organisms were susceptible to all the extracts with the diameter of zones of inhibition ranging between 8mm-23mm for ethanolic extract, 8mm-14mm for hot water extract and 8mm-20mm for cold water extracts. The Minimum Inhibitory Concentration (MIC) ranged between 6.25mg/ml-50mg/ml. The Minimum Bactericidal Concentration (MBC) ranged between 12.5mg/ml-50mg/ml. Sapinin, flavonoids, tannins, cardiac glycosides, anthraquinones, phylobatanins and alkaloids were the phytochemical constituents detected from the *irvingia gabonensis* stem bark and leaf extract. This study suggests that leaf and bark extract of *Irvingia gabonensis* has positive antibacterial properties on the test organisms. Further exploration of these plant materials will possibly unveil its potential use for the treatment of disease caused by the test organisms.

Key words: *Irvingia gabonensis* · Bacterial resistance · Health challenges

INTRODUCTION

For a long period of time in history, plant has been valuable and indispensible sources of natural product for the health of human being and they have a great potential for producing new drugs [1,2]. Even today people who live near to the forest use plant product to cure chronic diseases. According to the World Health Organization, plants are a source of compounds that have ability to combat disease, antimicrobial, antiviral and antifungal activities [1, 3].

Day by day new dreaded diseases are arising. The rise of antibiotic resistant microorganism is one of the severe problems in health care system of the world and infectious disease are the second most serious causes of death worldwide [1, 4]. Therefore, new drugs have to be found in order to combat such diseases and it is essential to find new compounds that have antimicrobial properties. Concerning the above facts, it is worthwhile to screen plant species which have the above properties to synthesize new drugs [1].

Interestingly, African mango (*Irvingiagabonensis*) leaf and root extracts have documented inhibitory activity against several bacterial and fungi [5, 6]. For instance, leaf extract of *Irvirgiagabonensis* used as a febrifuge. In Cameroon, preparation mainly from the bark are used to treat hernia and yellow fever and as an antidote for poisoning. Kernels of *Irviginiagabonensis* are used to treat diabetes. Preparations from the bark are rubbed on to the body to relieve pains and are applied to sores and wound and against toothache. They are also taken to treat diarrhea [7]. Potential mechanism of action include membrane disruption by terpenoidsand inactivation of microbial adhesion, enzymes and cell envelope transport proteins by ellagic acid-like compounds [5].

*Escherichia coli*is a gram-negative facultatively anaerobic rod-shaped bacterium commonly found in the lower intestine of warm-blooded organism (endotherms). [8]. Most *Escherichia* strain are harmless, but some serotypes can cause serious food poisoning in human and are occasionally responsible for product recalls due to food contamination [9]. The harmless strains are part of

Corresponding Author: O. Nworie, Department of Microbiology, Federal University Ndufu-alike Ikwo, Ebonyi State, Nigeria.

the normal flora of the gut and can benefit their host by producing vitamin k_2 and preventing colonization of the intestine with pathogenicbacteria [10-12]. On the other hands *Staphylococcusaureus* is a gram-positive coccal bacterium that is member of the Firmcutesand is frequently found in the respiratory tract and on the skin. It is often positive for catalase and nitrate reduction. Although *Staphylococcusaureus*is not always pathogenic, it is a common cause of skin infections such as abscesses respiratory infections such as sinusitis and food poisoning [13].

Therefore, this study aims to investigate the antibacterial activity of *Irvingiagabonensis* (Bush mango) leaf and stem bark on *Escherichia coli* and *Staphylococcus aureus,* as well as the minimum inhibitory concentration (MIC) of the extracts were determined.

MATERIALS AND METHODS

Collections of Plant Materials and Processing: TheLeaf and stem bark of plant materials *Irvingiagabonensis (*Bush mango tree*)* were collected from Umuagara Izhiamgbovillage of OhaukwuLocal Government Area of Ebonyi state, Nigeria. The plant material were examined and authenticated by Dr. C.V Nnamaniof Applied Biology Department, EbonyiState University Abakaliki, Nigeria. The plant samples were transferred to Microbiology Laboratory Unit for processing. At arrival the leaves and bark were washed with distilled water to reduce the bacterial load and also were size reduced with sterile knife in order to facilitate drying. The plant parts were dried at room temperature in order to prevent loss of active constituents which may be thermo-labile and drying was continued until constant weights obtained.After drying, the leaves and bark were separately grounded using sterile mortar and pestle and electric grinder into fine powder and was stored for further use.

Preparation of Extracts: Here, 100g of powdered *Irvingiagabonensis* leaves and stems bark were extracted using ethanol, hot water and cold water according to [14].

Qualitative Determination of Phytochemical Components of Leaf and Bark Extract of *Irvingiagabonensis:* The following phytochemicals were qualitatively determined inleaf and bark extracts *Irvingiagabonensis*namely: tannin [15], alkaloids [16], saponin [17], phlobatannins [18]. Anthraquinones [19], flavonoid and cardiac glycoside [16].

Purification and Re-identification of the Bacterial Isolates: The bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*)were sub-cultured on nutrient agar plate and incubated at 37°C for 24 hours. There were re-identified using gram staining and conventional biochemical tests such as catalase test and coagulase tests [20, 21].

Gram Staining: Gram staining is of a great importance in the recognition and identification of gram positive bacteria and gram negative bacteria.A smear of isolate was made on a clean glass slide and allowed to air dry then the slide was passed through a Bunsen flame for 3 times to heat fix. The slide was flooded with crystal violent and it was allowed to stay for 30-60seconds. Thereafter it was washed off with distilled water, then theslide was flooded withlugol's iodine and was allowed to stay for 30-60 seconds, it was washed off with distilled water. Thereafter the slide was flooded with acetone (alcohol) and was washed away immediately with water then slide was flooded with safranin and was allowed to stay for 1-2 minutes and it was allowed to air dry for 10minutes. The dried slide was placed under microscope and was viewed with X100 (oil immersion) objective

Biochemical Test: The following biochemical tests were carried out Catalase Test and Coagulase Test[20], Methyl Red Test [21] and Indole Test.

Preparation of 0.5 McFarland Standards: Firstly, 1% chemically pure sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid on99ml of distilled water in conical flask. Also 1% of barium chloride solution was prepared in another test-tube by adding 0.5g of dehydrated Barium chloride to 50ml of distilled water. Then slowly, with constant agitation, 0.6ml of Barium chloride solution was added to 99.4ml sulphuric acid [20].

Standardization of Inoculums: Suspension of inocula was prepared in test-tube from the stock culture, which were maintained on nutrient agar slant 40°C. the density of organism inoculated on to the media for susceptibility test was determined bycomparism with the turbidity of 0.5 McFarland and standards.

According to the manufacturer's specification in 1000ml flask and sterilized by autoclaving at 121°Cfor 15minutes.

Antimicrobial Susceptibility Testing: The test organism were checked to know whether they are susceptible to the

herbal extract by carrying out antimicrobial screening using the extracts and by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC).

Antimicrobial Screening of the Extract: 20ml of sterile nutrient agar was poured into the sterile Petri- dish and allowed to gel. The surface was flooded with 2ml of 18 hours broth culture standardized according to National Committee forClinic Laboratory Standard (NCCLS, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately $1.0x10^6$cfu/ml. the surface was allowed to dry and sterile No.4 Cork borer was used to bore six holes of about 2.5cm equal size on the surface 0.1ml of the extract at different concentrations of 6.25%w/v, 12.5%w/v, 25%w/v, 50%w/v and 100%w/v was dropped into each hole and the plate was kept for about 1hour at room temperature and incubated at 37°C for 18hours. The diameter of zones of inhibition was measured after incubation to the nearestmillimeter(mm). The experiment was repeated three times and the mean diameter was taken. Amoxicillin 25mg/l was used as control [14]

Minimum Inhibitory Concentration (MIC)and Minimum Bactericidal Concentration (MBC) Determination

The Minimum Inhibitory Concentration: This was determined by Agar dilution method Ten milliliter (10ml) volume of double strength melted Mueller-Hinton agar at 45° was diluted with equal volume of the test extract in graded concentration (50%w/v, 25%w/v, 12.5%w.v, 6.25% w/v and 3.13%w/v). These were poured aseptically into sterile Petri-dishes and dried at 37° for 1hour with the lid slight raised. Standardized test bacteria (10^6cfu/ml)were aseptically inoculated on the surface of the media/agar for each concentration of the test plant extract [14]. These were incubated at 37°C for 18 hours.

The M.I.C value was taken as the least concentrations of the extract showing no detectable growth.

The Minimum Bactericidal Concentrations: This was determined by transferring incubated organism from the concentration that showed no visible growth from the M.I.C determination into a sterile nutrient agar. These were incubated at 37°C for 72 hours. The least concentration of the extract that showed no bacterial growth on the surface of the medium was taken as the M.B.C.

RESULT

Phytochemical Analysis: The phytochemical analysis of the plant extracts revealed the presence of saponins, alkaloids, cardiac glycosides, anthraquinones, tannins, flavonoid and phlobatan.

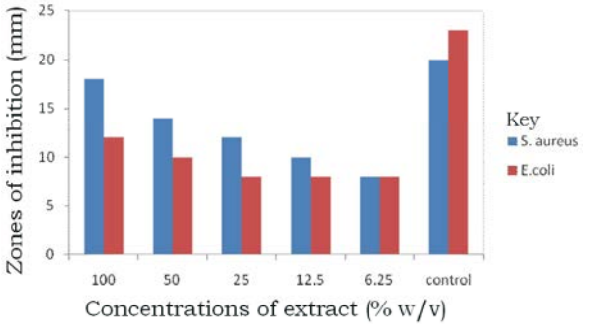

Fig. 1: Inhibitory zone diameter (mm) of ethanol bark extract of *Irvingiagabonensis* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations.

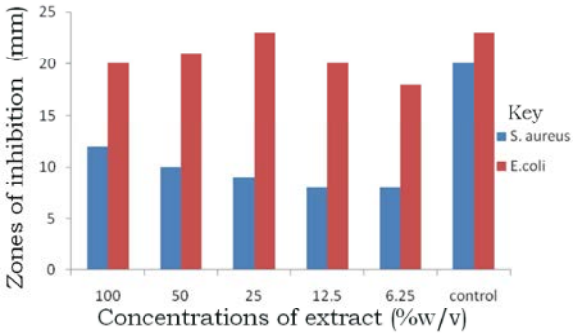

Fig. 2: Inhibitory zone diameter (mm) of ethanol leaf extract of *Irvingiagabonensis* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations.

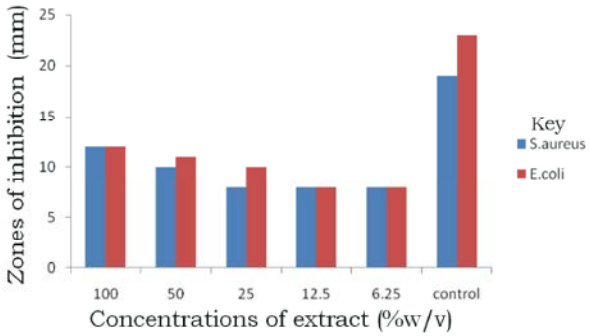

Fig. 3: Inhibitory zone diameter (mm) of hot waterbark extract of *Irvingiagabonensis* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations.

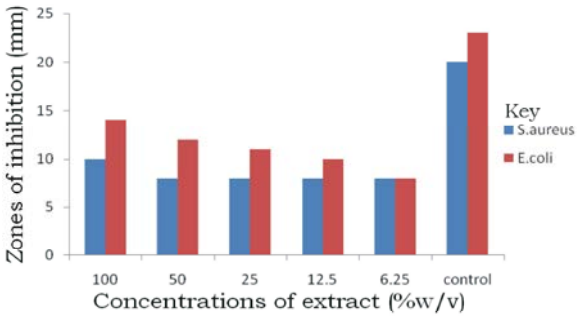

Fig. 4: Inhibitory zone diameter (mm) of hot water leaf extract of *Irvingiagabonensis* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations.

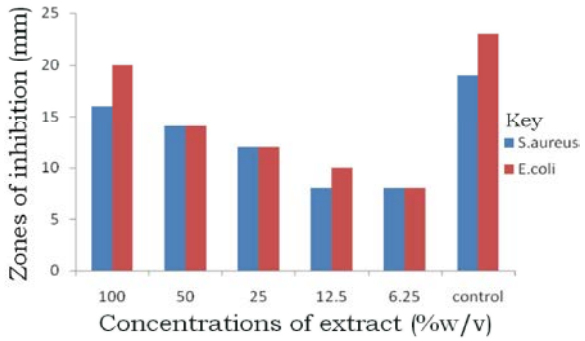

Fig. 4.5: Inhibitory zone diameter (mm) of cold water bark extract of *Irvingiagabonensis* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations.

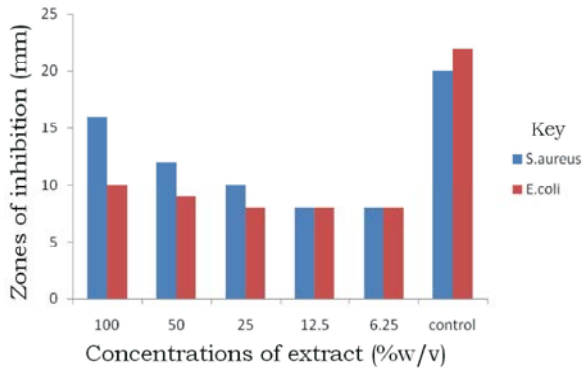

Fig. 5: Inhibitory zone diameter (mm) of cold water leaf extract of *Irvingiagabonensis* against *Escherichia coli* and *Staphylococcus aureus* at different concentrations.

DISCUSSION

All the extract showed antibacterial activity against all the tested bacterial species. This report agrees with those of [22]. [23]reported that ethanolic and water extracts of *Irvingiagabonensis*had effect against *Staphylococcusaureus* and *Escherichia coli.*

The cold water stems bark and Ethanolic leaf showed more antibacterial activities on *E.coli* than the other tested extracts as revealed by this study. This result agrees with that of [24], who reported that the stem bark and leaf extract of *Irvingiagabonensis*was very active against all the tested organisms. *Escherichia coli* appeared to be the most susceptibile to all the extracts,this result confirms those of [24]. [25]reported the antibacterial activity of *Irvingiagabonensis*against *E.coli.*

The phytochemical screening of the plant parts extract revealed the presence of saponin, flavonoids, tannins, cardiac glycoside, anthraquinones, phtobayanins and alkaloids, this report agrees with that of [26]. It has been reported that saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormone, diuretic, steroids, vitamin D and cardiac glycoside [27]. [28] has also demonstrated in his study that several compounds like saponins and flavonoids could be responsible for plant diureticThe conventional antibiotic Amoxilillin, consistently showed superior antibacterial activity then the extract similar to the results presented by other workers [29].This may be attributed to the fact that herbal medicinal product are prepared from plant and animal origin, most of the time subjected to contamination and deterioration while antibiotic are usually prepared from synthetic materials by means of reproducible manufacturing techniques procedure [30].

CONCLUSION

Irvingiagabonensis(bush mango) contains chemical constituents which posses antibacterial activity against *E.coli,* the causative agent of diarrhea, fatal dehydration, urinary tract infection and bladder infection and *Staphylococcus aureus*causative agent of skin lesion such as boils, pneumonia and gastroenteritis. The eye infection, diarrhea, healing of wound and inflammation of testicles. The chance to find antimicrobial drugs were apparent on both the leave and bark extract, therefore, the plant could be a source of new antibiotic.

REFERENCES

1. Nasamento, G.G.F., J. Locatelli, P.C. Freitas and G.L. Silva, 2000. “Antibacterial Activity of Plant Extracts and Photochemical on Antibiotic Resistant Bacteria” Brazillian Journal of Microbiology, 31: 247-256.

2. Littleton, J., T. Rogers and D. Falcone, 2005. "Novel Approaches to Plant Drug Discovery Based on High Throughout Pharmacological Screening and Genetic Manipulation". Life Science, 78: 467-475 (Pub Med).
3. Gazim, Z.C., C.M. Rezende, S.R. Fraga, T.I. Svidzinski and D.A. Cortez, 2008. "Antibacterial Activity of the Essential Oil from *Calendula officinalis* L. (Asteracea) Growing in Brazil". Brazillian Journal of Microbiology, 39: 61-63.
4. Mojab, F., M. Pouraeed, H. Mehrgan and S. Pakdaman, 2008 Antibacterial Activity of *Thymus daenensis* Methanol and Extract. Pakistan Journal of Pharmaceutical Science, 21: 210-213.
5. Kuete, V., G.F. Wabo and B. Ngamerni, 2007. Antimicrobial Activity of the Methanolic Extract, Fractions and Compounds from the Sterm Bark of *Irvingiagabonensis* (Ixonanthaceae). Journal of Ethnophamacology, 114(1): 54-60.
6. Fadare, D.A. and E.O. Ajaiyeoba, 2008. "Phytochemical and Antimicrobial Activities of the Wild Mango-*Irvingiagabonesis* Extracts and Fraction". Africa Journal of Medical Science, 37(2): 119-124.
7. Harris, D.J., 1996. A Revision of the *Irvingiaceae* in Africa Bulletin Dujardin Betarique Natural De-Belgigue, 65: 143-196.
8. Singleton, P., 1999. "Bacteria in Biology, Biotechnology and Medicine (5th edition). Wiley, pp: 444-454.
9. Vogt, R.L. and L. Dippoid, 2005. "*Escherichia coli* 0517:H7 Outbreak Associated with Consumption of Ground Beef, June-July 2002" Public Health Reports (Washington, D.C: 1974), 120(2): 178-178.
10. Bentley, R. and R. Maganathan, 1982. "Biosynthesis of Vitamin K (Menaquinone) in Bacteria" Microbiological Reviews, 46(3): 280.
11. Hudault, S., J. Guignot and A.L. Servin, 2001. *Escherichia coli* Strains Colonizing the Gastrointestinal Tract Protect Germfree Mice against *Salmonella typhimurium* Infection" Gut, 49(1): 55.
12. Reid, G., J. Howard and B.S. Gan, 2001. "Can Bacterial Interference Prevent Infection?". Trends in Microbiology, 9(9): 424-428.
13. Klutmans, J., A. Van-Belkum, H. Verbrugh and V. Van Belkum, 1997. "Nasal" Carriage of *Staphylococcus aureus*: Epidemiology, Underlying Mechanisms and Associated Risks" Cinical Microbiology Review, 10(3): 505-520.
14. Adeshina, G., O. Onujagbe and J. Onaolapo, 2009. "Comparative Antibacterial studies on The Root Stem Bark and Leaf Extract of *Parkia clappertoniana*". The International Journal of Alternative medicine, 8(2): 649.
15. Van-Burden, T.P. and W.C. Robinson, 1981. Formation of Complexes Between Protein and Tannin Acid. Journal of Agricultural Food Chemistry, 1: 77.
16. Harborne, J.B., 1973. Phytochemical Method. Chapman and Hall Ltd., London, pp: 49-188.
17. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy 11thedn., BailliereTiridel Can. Macmillian Publishers, London, pp: 45-50.
18. Oleyede, O.I., 2005. Chemical Profile of Unripe Pulp of *Carica papaya.* Pakistan Journal of Nutrition, 4(6): 379-381.
19. Sofowora, A., 1993. Medical Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan Nigeria, pp: 191-289.
20. Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries Cambridge University Press, United State of America Part2. 38-39, 64-67, 137-138.
21. Amadi, E.S., T.E. Ayogu and I.R. Iroha, 2009. Microbiology Laboratory Manchel in Department of Applied Microbiology Ebonyi State University Abakaliki, Nigeria UGUBU's Printing and Publishing Co. Abakaliki, Ebonyi State (1steds), pp: 52-54.
22. Raiji, Y., I.A. Ogunwande, J.M. Adesola and A.F. Belar, nwa, 2001. Antidiarrhegenic and anti-ulcer properties of *Irvingiagabonensis*in rat. journal of pharmaceutical biology, 39(5): 340-345.
23. Abdulrahman, F., L.S. Inyang, J. Abbah, L. Binda, S. Amos and K. Gamaniel, 2004. Effect of Aqueous Leaf Extract of *Irvingiagabonensis*on Gastrointestinal Tract in Rodent. India Journal of Experimental Biology, 42(8): 787-791.
24. George, I.N. and Y. Zhao, 2007. " Pharmacological Activity of 2,3,8- Tri-o-methyl Ellagic Acid Isolation from the Stem Bark of *Irvingiagabonensis*" African Journal of Biotechnology, 6(16): 1910-1912.
25. Okolo, C.O., P.B. Johnson, E.M. Abdurahmman, I. Abdu-Aguye and I.M. hussaini, 1995. "Analgesic Effect of *Irvingia gabonensis* Stem Bark Extract." Journal of Ethnopharmacology, 45(2): 125-129.
26. Oluwafemi, A.O., O.A. Basiru, E.O. Babatunji and B.O. Adebola, 2014. Prophylatic Effects of Ethanolic Extracts of *Irvingiagabonensis* Stem Bark Against Cadmium-Induced Toxicity in Albino Rat, pp: 65-72.

27. Adedapo, A.A., O.M. Mogbojuri and B.O. Emikpe, 2009. Safety Evaluation of the Aqueous Extract of the Leaves of *Moringaoleifera*. Journal of Medical Plants Res., 3(8): 586-592.
28. Maghrani, M., N. Zeggwagh, J. Michel and M. Eddouks, 2005. *In-vitro* Antioxidant Activities Phytochemical Analysis of Seed Extracts of *Lepidiumsativum*- a Medical Herb. Journal of Ethnophamacology, 100: 193-197.
29. Kubmarawa, D., G. Ajoku and D.A. Okrie, 2002. "Antimicrobial Spectrum of hexane of commiphorakerstingii (Burderaceae)". Technology development, 8: 29-32.
30. De, N. and E. Ifeoma, 2002. "Antimicrobial effects of component of the bark extract of neem (Azadirachtaindica A. Juss.). Technology Development, 8: 2.