Asian Journal of Biology

14(2): 35-41, 2022; Article no.AJOB.84276
ISSN: 2456-7124

Antibacterial Activities of *Annona senegalensis* Pers. (Annonaceae) Extracts on *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia*

A. Z. Koggie [a], J. O. Ihuma [a*] and P. H. Nyikwagh [a]

[a] Biological Sciences Department, Bingham University, Karu, Nasarawa State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author AZK designed the methodology and supervised the study. Authors JOI and PHN managed the analysis and the literature of the study. Author JOI also prepared the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2022/v14i230212

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84276

Original Research Article

Received 22 December 2021
Accepted 25 February 2022
Published 28 February 2022

ABSTRACT

This study investigated the presence of phytochemicals in the stem bark of *Annona senegalensis.* Phytochemicals extracted were alkanoid, flavonoid, tannin, saponin, and phenol, and the three extracts were: Cold Water Extract (CWE), Hot Water Extract (HWE) and Methanol Extracts (ME). The extracts of *A*. *senegalensis* were tested on *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia* isolates in order to measure their antimicrobial and bactericidal activities. Irrespective of the method of extraction, the Minimum Inhibitory Concentration (MIC) was found to be 3.125 µl/ml. Minimum Bactericidal Concentration (MBC) required to kill the test organisms were also recorded. While CWE needs 100 µl/ml to kill each of the test organisms, HWE and ME both required concentrations of 50 µl/ml to completely kill *E. coli* and *K. pneumonia.* For *P. mirabilis,* the HWE needed a concentration of 400 µl/ml, while ME needed a concentration of 100 µl/ml to kill the organism. It was therefore concluded that the extracts of *A*. *senegalensis* had the potential to inhibit the growth (MIC) or even kill (MBC) the studied microorganisms at particular concentrations. This study is preliminary; therefore, an *in vivo* study should be undertaken to further test the potency and toxicity of the extracts with the view of standardizing the dosage and educating the traditional medicine practitioners on how to achieve healing without causing harm to patients.

**Corresponding author: E-mail: jeromeihuma@gmail.com;*

Keywords: A. senegalensis; antibacterial; methanolic extracts; bactericidal, isolates.

1. INTRODUCTION

1.1 Background of the Study

It is of great concern that majority of the currently available antimicrobials which are mainly synthetic are significantly inefficient and could elicit terrible effects to recipients [1,2]. Due to this concern, all experts proposed strategies/ solutions to tackling the Anti-Microbial Resistant (AMR) crisis.

Consequently, many initiatives and programs have been set up by many countries/ organizations with the aim of developing new, effective, and safe antimicrobials [3]. Screening of plant species for possible organic antimicrobial is therefore currently of essence.

Nearly all cultures from ancient times to the present day have used plant species extracts or plant parts in different forms and formulations as a source of medicines; consequently, different remedies were developed across the globe. Many potent phytochemicals or secondary metabolites with antimicrobial effects have been isolated from plant species [4], and these have been used in traditional medicine for treatment of various ailments such as those of infectious disease, cancer, and neglected tropical disease [5,6]. Antimicrobials from plant species are greatly important in overcoming the global problem of antimicrobial resistance spike involving the use of orthodox drugs [7]. It is also known that there are various reports and documentations on the medicinal use of plant species [8].

Although, many plant species extracts were already in use, in this study, extracts of *Annona senegalensis* Pers. (Annonaceae) was specifically tested on *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia* isolates in order to ascertain if the plant species has antibacterial properties.

The stem bark of *A. senegalensis* for instance, was reported to be useful in the treatment of guinea worms and other worms, toothache, gastroenteritis, snakebite, diarrhea and respiratory infections [8]. Also, the bark could also be chewed and applied on fresh wounds and a combination of barks and roots crushed together can be applied on snake bite wounds [8]. It was also reported that, the gum from the bark of the *A. senegalensis* can be used in closing up cuts and wounds, and the leaves have been used in the treatment of pneumonia and also as a tonic to promote general wellbeing. [8] further reported that leaves of some certain plant species including *A. senegalensis* are used in treating wounds, skin diseases and other ailments such as conjunctivitis, insect bites, haemorrhoids, fever, female sterility, convulsions, jaundice and guinea worm. [9] also reported the use of the stems, leaves, fruits and roots of *A*. *senegalensis* in the treatment of skin cancer, cough and to heal wounds.

A. senegalensis Family: Annonaceae commonly known as Wild Custard Apple has been described as a multi-stemmed deciduous shrub observed to grow 2-6 meters 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 [10]. The fruit, leaves and flowers of A*. senegalensis* have been reported to have edible implications. The leaves of A*. senegalensis* are sometimes used as vegetables; dry leaves are reported to contain 8.2% protein [9]. The extracts of *A. senegalensis* plant species obtained from various methods were used on selected test microbial isolates (*E*. *coli*, *P*. *mirabilis* and *K*. *pneumonia*).

Fig. 1. Photograph of A. senegalensis with unripe fruit

Escherichia coli is a type of bacteria that normally lives in the intestines, and can also be found in the gut of some animals (part of the normal flora of both human and animal digestive tract). Most types of *E*. *coli* are harmless and even help keep the digestive tract healthy. But some strains can

cause diarrhea when contaminated food and water are consumed. *E coli* are a large and versatile group of pathogens that can cause widespread disease and affect multiple organ systems [11].

Another test organism in this study is *P. mirabilis.* It is a common pathogen responsible for complicated urinary tract infections (UTIs) that could sometimes cause bacteremia [12]. *P. mirabilis* is a Gram-negative bacterium that is a frequent cause of catheter-associated urinary tract infections (CAUTIs). Its ability to cause such infections is mostly related to the formation of biofilms on catheter surfaces [13]. In order to form biofilms, it expresses a number of virulence factors. Such factors may include adhesion proteins, quorum sensing molecules, lipopolysaccharides, efflux pumps, and urease enzyme. A unique feature of *P. mirabilis* biofilms that build up on catheter surfaces is their crystalline nature owing to their ureolytic biomineralization. This leads to catheter encrustation and blockage and, in most cases, is accompanied by urine retention and ascending UTIs [13]. Bacteria embedded in crystalline biofilms become highly resistant to conventional antimicrobials as well as the immune system [13].

Klebsiella pneumonia species are found ubiquitously in nature, including in plants, animals, and humans. They are the causative agents of several types of infections in humans, including respiratory tract infections, urinary tract infections (UTIs), and bloodstream infections [14]. Classically, these infections occur in hospitalized or otherwise immunocompromised patients and are routinely treated with β-lactams and other antibiotics effective against *Enterobacteriaceae* [15]. However, antibiotic-resistant *K. pneumoniae* and hypervirulent *K. pneumoniae* strains have emerged separately across the world.

Thus, researchers/scientists are now looking at every ecological niche including soil, plant, animal, and marine for potentially new and safe antimicrobial agents [16]. Unfortunately, the rate at which microorganisms develop AMR outpaces the rate of discovery/development of new drugs [17]. This necessitated this study with the intent of providing empirical evidence to traditional claims of the potency of this plant materials in the treatment of illnesses as captured by literature and to also provide data on the accurate concentration needed to achieve effective treatment without causing harm to diseased individuals being treated (i.e. to avoid toxicity).

2. MATERIALS AND METHODS

2.1 Study Area

The laboratory work was carried out in the Microbiology Laboratory, Department of Biological Sciences, Bingham University, Karu, Nasarawa State. Bingham University is approximately 25km from the Federal Capital Territory (FCT), Abuja and is located at longitude 7.6^{o} E and latitude 8.9^{o} N in Karu, Nasarawa State, Nigeria [18].

3. METHODOLOGY

The stem bark of *A. senegalensis* were collected randomly from Bingham University landscape, identified by experts, and taken to the Microbiology laboratory, Department of Biological Sciences, Bingham University, Karu, Nasarawa State for extraction. The plant species samples were air dried and pounded into powder. Then, a weighed portion of the powder was extracted by cold maceration in 1000ml of methanol and shaken intermittently for 72 hours followed by sieving with a muslin cloth and filtered with a Whatman No. 1 filter paper. The filtrate was concentrated on the rotary evaporator and lyophilized to get the dry solid residue [19].

3.1 Phytochemical Screening

Phytochemical screening was done for Alkaloid, Flavonoid, Tannin, Saponin and Glycoside as well as Phenol [19].

3.2 Test Organisms (The Isolates)

Using [20] as a guide, three standard test organisms, namely *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia* sourced from National Veterinary Research Institute, Vom, Plateau State, were used to test the sensitivity of Cold Water Extract (CWE), Hot Water Extract (HWE) and Methanolic Extract (ME) of the stem bark of *A. senegalensis*.

3.3 Determination of Minimum Inhibitory Concentration (MIC)

Microtiter plate-based antibacterial assay were used in determination of the Minimum Inhibitory Concentration (MIC) of *A. senegalensis* stem

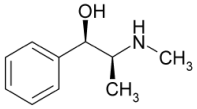
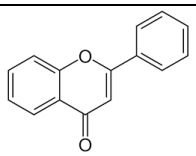
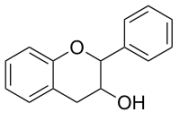
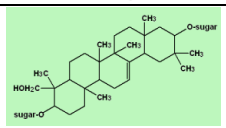
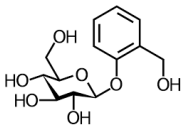
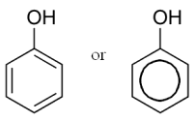
bark extracts [20]. Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled, and a volume of 100 µL of test material in 10% (v/v) DMSO and 10 mg/ml methanolic extracts were pipetted into the first row of the plate and to all other wells, 50 µL. Serial dilution was performed such that each well had 50µL of the test material in serially descending concentrations. To each well, 10 µL of resazurin indicator was added. Finally, 10 µL of bacterial suspension was added to each well [20]. A broad spectrum antibiotic was used as control (Ciprofloxacin). The plates were prepared in triplicates and placed in an incubator set at 370C for 18 to 24 hours. The color change is then assessed visually. Any colour change from purple to pink or colorless was recorded as positive. To determine the Minimum Bactericidal Concentration (MBC), the dilution representing the MIC and two of the more concentrated test products were plated and enumerated to determine the viable cfu/ml [20].

4. RESULTS

The result in Table 1 showed the phytochemical constituents obtained from the stem bark extracts of *A. senegalensis* which includes alkaloid, flavonoids, tannins, saponin and phenol.

Table 2 shows results of the minimum concentration of the extract required to prevent the growth of the test organisms or to kill the test organism. Irrespective of the extracting solvents, the Minimum Inhibitory Concentration (MIC) was found to be 3.125 µl/ml. The table also shows results of the Minimum Bactericidal Concentration (MBC) required to kill the test organisms. While cold water extract needs 100µl/ml to kill each of the test organisms, hot water and methanol extracts have varying concentrations of the extract to completely kill the test organisms.

Table1. Phytochemical Constituents of the of *A. senegalensis*

S/No	Phytochemical	Structure	HWE	CWE	ME
1	Alkaloid		+	+	+
2	Flavonoid		+	+	+
3	Tannin		+ +	+	+++
4	Saponin		+	+	+
5	Glycoside		-	-	-
6	Phenol		+	+	++

[19]

Table 1. MIC (µl/ml) and MBC(µl/ml) of *A. senegalensis* extracts on test organisms

Test organism	Extraction Methods	MIC (µl/ml)	MBC(µl/ml)
K. pneumonia	CWE	3.125	100
	HWE	3.125	50
	ME	3.125	50
E. coli	CWE	3.125	100
	HWE	3.125	50
	ME	3.125	50
P. mirabilis	CWE	3.125	100
	HWE	3.125	400
	ME	3.125	100

5. DISCUSSION

The extract of *A. senegalensis* stem bark has significant amount of phytochemicals as seen in Table 1, having more of tannins followed closely by phenol and relatively same amounts of flavonoid, saponin and alkaloid while the presence of glycosides was not seen. This agrees with [21] on the presence of phytochemicals in custard apple (i. e. *A. senegalensis*), however in his study the volume of flavonoid and tannins were relatively higher than what it is in this study, this might be due to differences in weight of extract used or probably the protocol/method of testing for these components. Also, [22] reported the presence of phytochemicals on a study on the leave extract of this plant species with significantly high proportions of saponin. This result affirms to studies reported in various literature [23,24,25] on the presence of drug active components/ phytochemicals which obviously are responsible for the treatment effect seen when crude extracts are used in the treatment of various illnesses in traditional medical practice.

The MIC of *A. senegalensis* extract in this study is the same irrespective of the extraction solvent as all three solvents used showed a 3.125 µ/ml to the test organisms. This result signifies that the least concentration of *A. senegalensis* needed to prevent the growth of these selected bacterial is 3.125µ/ml. This outcome however differs from studies by [9], that reported different values (200µ/ml) for the inhibitory concentration of solvent with methanolic extract having relatively lower values than water. It however agrees with the study by [26] that reported 3.461µ/ml.

Also, as shown in Table 2, the results of the MBC (µl/ml) of Cold Water Extract (CWE), Hot Water Extract (HWE) and Methanolic Extract (ME) of *K. pneumonia, E. coli* and *P. mirabilis*, were [100:50:50], [100:50:50] and [100:400:100] respectively. The results showed that *P. mirabilis* needs a relatively higher concentration of the extract in order to be killed. The table showed that it needed 100µ/mil of ME and 400 µ/ml of HWE extracts to kill the bacteria. Also, the result shows that *K. pneumonia* and *E. coli* need lesser concentrations of these extracts to kill the bacteria. The MBC needed were 100µ/ml for CWE of *A. senegalensis* and 50µ/ml for HWE and ME respectively. This shows that these two bacteria need relatively lower concentrations of these extracts to be killed when compared to *P. mirabilis.*

6. CONCLUSION

It is therefore concluded that the extracts of *A. senegalensis* had the potential to inhibit the growth (MIC) or even kill (MBC) the studied microorganisms at particular concentrations. This study is preliminary; therefore, an in vivo study should be undertaken to further test the potency and toxicity of the extracts with the view of standardizing the dosage and educating the traditional medicine practitioners on how to achieve healing without causing harm to patients. This study also shows that there are presence of the active drug components otherwise referred to as phytochemicals in the plant species. These substances are important in both orthodox and traditional medical practice, and could be used to treat a number of infectious diseases. However, care should be taken in order to avoid poisoning due to inappropriate administration of dosage, hence further research needs to be undertaken to study the dosage and toxicity in a living cell (in vivo).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Van Vuuren SF. Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology. 2008;119(3):462-472.
2. Udegbunam SO, Udegbunam RI, Muogbo CC, Anyanwu MU, Nwaehujor CO. Wound healing and antibacterial properties of methanolic extract ofPupalia lappacea Juss in rats. BMC Complementary and Alternative Medicine. 2014;14(1):157.
3. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, Kahlmeter G. The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections. 2015;6:22-29.
4. Salazar-Aranda R, Pérez-Lopez LA, Lopez-Arroyo J, Alanís-Garza BA, Waksman de Torres N. Antimicrobial and antioxidant activities of plants from northeast of Mexico. Evidence-Based Complementary and Alternative Medicine; 2011.
5. Alam MN, Almoyad M, Huq F. Polyphenols in colorectal cancer: Current state of knowledge including clinical trials and molecular mechanism of action. BioMed Research International. 2018;1-29. Available:https://doi.org/10.1155/2018/4154185.
6. Francesca P, Patrizio P, Annalisa P. Antimicrobial resistance: A global multifaceted phenomenon. Pathog Glob Health. 2015;109 (7):309–318.
7. Willcox ML, Bodeker G. Traditional herbal medicines for malaria. British Medical Journal. 2004;329:1156–1159.
8. Arbonnier M. Trees, shrubs and lianas of West African dry zones. Cirad, Margraf Publishers Gmbh, Mnhn, Paris, France. 2004;155. DOI: 10.3389/fcimb.2020.00414
9. Nanti GGCG, Bi SAN, Zahoui OS, Traore F. Comparative study of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiacaceae) effects on glycemia in rats. Journal of Complementary Medicine Research. 2018; 7(1):1-7.
10. Keay RWJ, Onochie CFA, Standfield DP. Nigeria Trees. 1964;1 & 2.
11. Mare AD, Ciurea CN, Man A, Tudor B, Moldovan V, Decean L, Toma F. Enteropathogenic *Escherichia coli*—A summary of the literature. Gastroenterol. Insights. 2021;12:28–40. Available:https://doi.org/10.3390/gastroent12010004
12. Mobley HL, Belas R. Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. Trends Microbiology. 1995;3: 280-284.
13. Reham W, Samira H. Proteus mirabilis Biofilm: Development and therapeutic strategies. Frontiers in Cellular and Infection Microbiology. 2020;10:1-14.
14. Podschun R. Phenotypic properties of *Klebsiella pneumoniae* and K oxytoca isolated from different sources. Zentralbl. Hyg. Umweltmed. 1990;189:527–535.
15. Mandell GL. Mandell, douglas, and bennett's principles and practice of infectious diseases. Philadelphia, PA: Elsevier; 2005.
16. Laport MS, Santos OCS, Muricy G. Marine sponges: Potential sources of new anti-microbial drugs. Current Pharmaceutical Biotechnology. 2009;10(1):86-105.
17. Schwarz S, Loeffler A, Kadlec K. Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. Advances in Veterinary Dermatology. 2017;8:95-110.
18. Ihuma JO, Agida IO, Nashima TN. Assessment of fungi species associated with a multicultural orchard and cultivated land in bingham university landscape, Karu Nasarawa State, Nigeria. Research in Ecology. 2022;4(6):7-16. Available:https://ojs.bilpublishing.com/index.php/re
19. Rahman Gul, Syed Umer Jan, Syed Faridullah, Samiullah Sherani, Nusrat Jahan. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. Scientific. World Journal. 2017;Article ID 5873648,1:1-7. Available:https://doi.org/10.1155/2017/5873648
20. Cheesbrough M. District laboratory practice in tropical countries. 2nd Edition, Cambridge University Press, Cambridge, United Kingdom; 2010.
21. Adigun MO, Afolabi OO, Daniels FV, Okonofua CC, Lateef SA. Antibiotic susceptibility profile of microbial isolates from open wounds to common antibiotics. Nigerian Journal of Microbiology. 2018; 32(1& 2):4148-4157.
22. Nwonuma CO, Irokanulo EO, Jolaiya AE, Ore A. Effect of aqueous leaf extract of

Annona senegalensis on selected testicular function indices of wistar rats. American Journal of Life Sciences. 2015; 3(3):203-212.

23. Inouye S, Uchida K, Takizawa T, Yamaguchi H. Evaluation of the effect of terpenoid quinines on *Trichophyton metagrophytes* by solution and vapor contact. Journal of Infectious Diseases-Springer. Insights. 2006;12:28–40. Available:https://doi.org/10.3390/gastroent 12010004
24. Jamkhande PG, Ghante MH, Barde SR. Antimycobacterial, antimicrobial, anti-oxidant activities and in silico PASS investigations of root fractions and extract of *Cordia dichotoma* Forst. Orient Pharm Exp Med. 2019;19:485–496. vhttps://doi.org/10.1007/s13596-019-00399-5
25. Kannabrian K, Khanna VG, Rajakumar G. Biolarvicical compound gymnemagenol isolated from leaf extract of miracle fruit plant against malaria and filariasis vectors. frontiersin.org Parasitology Research. 2009;109:1373-1386.
26. Anthony OO, Omar HS, Ibrahim H, Mataka AM, Ochieng JO, Parkia M, Oyoo OT. Assessment of anti-fungal activity of *Annona senegalensis* plant parts on *Malassezia globosa*. Natural Product Chemistry and Research. 2017;5(7):1-5. DOI: 10.4172/2329- 6836.1000291

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/84276