

Original Research Article

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

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 pneumoniae* 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 3.125µl/ml. Minimum Bactericidal Concentration (MBC) required to kill the test organisms were also recorded. While CWE needs 100µl/ml to kill each of the test organisms, but HWE and ME both required concentrations of 50µl/ml to completely kill *E. coli* and *K. pneumoniae*. For *P. mirabilis*, the HWE needed a concentration of 400µl/ml, while ME needed a concentration of 100µl/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.

Keyword: *Annona senegalensis* Antibacterial, Methanolic Extracts, Bactericidal, Isolates

INTRODUCTION

Background of the Study

It is of great concern that majority of the currently available antimicrobials which are mainly synthetic are almost inefficient and could elicit terrible effects to recipients (van Vuuren, 2008 and Udegbonam *et al.*, 2014). 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 (Roca *et al.*, 2015). 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 world all over. Many potent phytochemicals or secondary metabolites with antimicrobial effects have been isolated from plant species (Salazar-Aranda, *et al.*, 2011), and these have been used in traditional medicine for treatment of various ailments including infectious disease, cancer, and neglected tropical disease (Alam *et al.*, 2019 and Francesca *et al.*, 2015). Antimicrobials from plant species are greatly important in overcoming the global problem of antimicrobial resistance spike involving the use of orthodox drugs (Willcox and Bodeker, 2004). It is also known that there are various reports and documentations on the medicinal use of plant species (Arbonnier, 2004).

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 (Arbonnier 2004). Also, the bark could also be chewed and applied on fresh wounds and a

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combination of barks and roots crushed together can be applied on snake bite wounds (Arbonnier 2004). 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. Arbonnier (2004) 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. Nanti *et al.*, (2018) 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 (Keay, 1964). 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 (Nanti *et al.*, 2018). 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*).



Photograph of *A. senegalensis*

***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 (Mare, *et al.*, 2021).

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 (Mobley and Belas, 1995). *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 (Reham and Samira, 2020). 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 (Reham and Samira, 2020). Bacteria embedded in crystalline biofilms become highly resistant to conventional antimicrobials as well as the immune system (Reham and Samira, 2020)

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 (Podschun and Ullmann, 1998). Classically, these infections occur in hospitalized or otherwise immunocompromised patients and are routinely treated with β -lactams and other antibiotics effective against *Enterobacteriaceae* (Mandell, 2005). 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 (Laport *et al.*, 2009 and Nasir *et al.*, 2015). Unfortunately, the rate at which microorganisms develop AMR outpaces the rate of discovery/development of new drugs (Schwarz *et al.*, 2017). 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).

MATERIALS AND METHODS

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° E and latitude 8.9° N in Karu, Nasarawa State, Nigeria (Ihuma, *et al.*, 2022 in press).

Methodology

The stem bark of *A. senegalensis* were collected and taken to the Microbiology laboratory, Department of Biological Sciences, Bingham University, Karu, Nasarawa State for extraction. ~~The plant species sample~~ the stem bark were air-dried at room temperature ($23 \pm 2^\circ\text{C}$) away from sunlight and milled to coarse particle ~~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 (Rahman *et al.*, 2017)

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Phytochemical Screening

Phytochemical screening was performed using methods described by ~~done for Alkaloid, Flavonoid, Tannin, Saponin and Glycoside as well as Phenol~~ (Rahman *et al.*, (2017)). ~~The extracts stem bark were screened for the following classes of phytochemicals: alkaloids, flavonoids, saponins, tannins Glycoside as well as Phenols.~~

Test microorganisms (the Isolates)

Three ~~standard~~ test microorganisms, namely *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia* ~~were obtained 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*.

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Determination of Minimum Inhibitory Concentration

Microtiter plate-based antibacterial assay were used in determination of the Minimum Inhibitory Concentration (MIC) of *A. senegalensis* stem bark extracts. Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled, and a volume of 100µL 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, 50µL. Serial dilution was performed such that each well had 50µL of the test material in serially descending concentrations. To each well, 10µL of resazurin indicator was added. Finally, 10µL of bacterial suspension was added to each well. A broad spectrum antibiotic was used as control (Ciprofloxacin). The plates were prepared in triplicates and placed in an incubator set at 37°C for 18 to 24 hours. The color change is then assessed visually. Any colour change from purple

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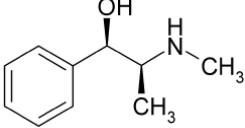
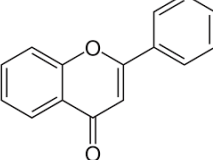
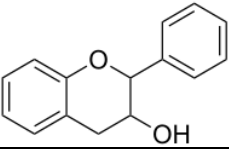
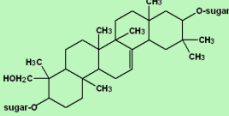
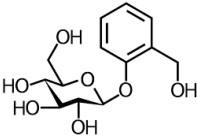
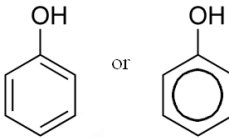
to pink or colorless was recorded as positive. To determine the Minimum Bactericidal Concentration (MBC), the dilution representing the MIC and two of the more concentrated test products were plated and enumerated to determine the viable cfu/ml.

UNDER PEER REVIEW

Results

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

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		+	+	++

(Rahman *et al.*, 2017)

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

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Table 12: MIC (µl/ml) and MBC(µl/ml) of *A. senegalensis* extracts on test organisms

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

DISCUSSION

The extract of *A. senegalensis* stem bark has significant amounts 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 [the work of Adigun et al., \(2015\)](#) ~~who also shows 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 ~~those of the present what it is in this~~ study, this ~~might be due to~~ differences ~~can be attributed to the in~~ weight of extract used or probably the protocol/method of testing for these components. Also, Nwonuma et al., (2015) reported ~~that, the presence of~~ phytochemicals ~~on a study on the leave extract of this plant species show with significantly~~ high proportions of saponin. This result affirms to studies reported ~~by in various literature~~ (Inouye, 2006; [Kannabiran, et al., 2009](#); [Guil-Guerrero, et al., 2016](#); [Khorshidiana, et al., 2018](#); ~~Guil-Guerrero, et al., 2016~~; [Abubakar, et al., 2018](#); [Pisoschi, et al., 2018](#) and ~~Kannabiran, et al., 2009~~) ~~that shows on~~ the presence of active components/phytochemicals of drug active components/phytochemicals which ~~are~~ 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 3.125µ/ml to the test micro organism. 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 Nanti et al., (2018), who 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 Ochieng et al., (2017) who 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.*

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mirabilis needs a relatively higher concentration of the extract in order to be killed. The table showed that it needed 100µ/ml 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*.

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 is the presence of the active drug components otherwise referred to as phytochemicals. 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).

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