Antibacterial Effect of *Acacia Nilotica* and *Acacia Senegalensis* Fruit Extracts on *Escherichia Coli* and *Salmonella Typhi*

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Abstract

Acacia nilotica and *Acacia senegalensis* are plants native to the Sudan and Sahelian region, widely spread in northern parts of Nigeria. It has been mentioned in plants used in folk medicine to be effective against a variety of diseases affecting man. The acetone extracts of the *Acacia nilotica* and *Acacia senegalensis* fruits was tested against two human bacterial pathogens (*Escherichia coli* and *Salmonella Typhi*) using well diffusion method, MIC and MBC tests. The results revealed that the extract exhibited a broader spectrum of antibacterial activity and revealed potent bactericidal effect at low concentration of 25mg/ml and bacteriostatic at lower concentrations (as low as 12.5 mg/ml). In general, all tested bacteria were susceptible, however supporting the traditional medical application and suggest it as a source for new antibacterial drugs.

Keywords: *Acacia Spp, Escherichia coli, Salmonella Typhi*, MIC, MBC tests, Bacteriostatic

INTRODUTION

A disease is a particular abnormal condition that negatively affects the structure or function of all or part of an organism, and that is not due to any immediate external injury. Diseases are often known to be medical conditions that are associated with specific symptoms and signs. A disease may be caused by external factors such as pathogens or by internal dysfunctions. Life and diseases are related, where there is life, diseases will also be present. Man and Animals depends on plants for food, fibers, and shelter, but also plants have been used to control and ease diseases, therefore the use of plants as medicines is an ancient and reliable practice (Sunita, 2016). The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world (Kathe, 2005). They observed that about 74% of 119 plant-derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care (Mishra, 2010).

Acacia nilotica is a single stemmed plant, grows to 15-18m in height and 2-3m in diameter. Pods are 7-15cm long, green and tomentose when immature or greenish black when matured. . The pod is indehiscent, deeply constricted between the seed giving a necklace appearance. Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa (Iman, 2007). Leaves are fine and densely hairy bipinnate, pinnate 3-10 pairs, 1.3-3.8cm long, leaflets 10-20 pairs and 2-5mm long that are narrow with parallel margin, and are rounded at the apex with a central mid-rib closely crowded (Beniwal, 1992). Flowers are globular heads, 1.2-1.5cm in diameter of a bright golden yellow colour, develop either in axillary or whorly pattern on peduncles 2-3cm long located at the end of the branches (Bargal, 2009).

According to Orwal *et al., (*2009) *Acacia senegalensis* is a deciduous shrub, growing to 15 m tall and usually branched from the ground. Branches fork repeatedly and in mature trees commonly form a rounded, flat topped crown. The trunk may vary in diameter up to about 30 cm. The bark is greyish-white, although in old trees growing in the open it may be dark, scaly and thin.

Escherichia coli is a member of family *Entrobacteriaceae,* facultative, gram-negative, non-spore producing *bacilli* bacteria with most strains being motile and generally possessing both sex pili and adhere fimbriae (Haris *et al.,* 2015). *E. coli* is one of the normal colonic flora and most common cause of opportunistic infections. *Salmonella typhi* is a member of the family *Entrobacteriaceae*. It is Gram-negative bacilli, motile and non-Lactose fermenter. The genus can be divided into two species (*S. enteric and S. bongori*), based on their phenotypic profile (Tankeshwar, 2015).

Salmonella typhi causes acute gastroenteritis and when *Salmonella* infection become invasive, they can affect the bloodstream (bacterimia), bone (osteomyelitis), joint (septic arthritis), brain or nervous system (meningitis) (Tankeshwar, 2015). Typhoidal Salmonella refers to the specific Salmonella serotypes which causes typhoid fever or paratyphoid fever, including typhi, paratyphi A, paratyphi and paratyphi C. Non-typhoidal *Salmonella* refers to all other Salmonella serotypes (Tankeshwar, 2015).

MATERIALS AND METHOD

Collection and Identification of Plant material

The fruit samples of *Acacia nilotica* and *A. senegalensis* was collected around Dutse, Jigawa State Nigeria on 1st June, 2018 through the protocols described by Emad (2007). The collected samples were taken to the herbarium section of Bayero University Kano, Nigeria in a polythene bag for identification. A voucher specimen number BUKHAN186 was given to the specimens.

Extraction of the plant Materials

The collected fruit sample of *A. nilotica* and *A. senegalensis* was taken to the Laboratory, rinsed with distilled water to remove foreign materials and shade dried at room temperature for seven days (Emad, 2016). The dried fruit samples were pulverized in to powder and 50g from each sample was extracted in 250ml of acetone using the protocol of Emad (2016). The extract was filtered using Watman No 1 filter paper, rotary evaporator was used under reduced pressure to concentrate the extract to dryness at 60-80^{o}C (Haris *et al.,* 2017). The extracts was kept in a sterile bottle in a refrigerator at 4^{o}C until used (Emad, 2016).

Test Organisms

The clinical isolates of *Escherichia coli* and *Salmonella typhi* were obtained from Rasheed Shekoni Teaching Hospital of Federal University Dutse. The isolates were identified using the schemes of Cheesbrough (2006) and then sub-cultured into MacConkey agar, Eosine Methylene blue and Salmonella-Shigella agar for further confirmation (Cheesbrough, 2006).

Sub-culturing and isolation of the Test Organisms

The clinical isolates of *Escherichia coli* and *Salmonella typhi* was streaked on MacConkey agar, Eosine methylene blue and Salmonella-Shigella agar and incubated at 37^{o}C for 24hrs (Abdallah *et al.,* 2016). The pale colonies in Salmonella-Shigella agar shows the presence of both *Shigella* and *Salmonella*, while colonies of *E. coli* in MacConkey agar appears pinkish in colour, and green

metallic sheen on EMB. However, during inoculation, the plates of the media have been dried because of easier growth and identification of the colonies. The wire loop has also flamed and sterilized. The plates were placed invertedly overnight, to prevent falling of condensed water vapour on plate surface (Cheesbrough, 2006). Further identification and confirmation of the test organisms was carried out through biochemical tests and gram staining.

MacFarland Standard

MacFarland's Standard was prepared by mixing 99.5ml of 1% H_2SO_4 0.5ml of 1.175% w/v barium chloride ($BaCl_2.H_2O$). The mixture was dispensed in tubes at a volume of 3-4ml (Ocie and Kolhatkar, 2007).

Preparation and Standardization of Inoculums

The isolated test organisms were sub-cultured on to a sterile nutrient agar plates and incubated at 37^{o}C for 18-24hrs; the sub-cultured isolated were inoculated into a sterile test-tubes containing 5ml of normal saline and compared with 0.5 MacFarland's standard to get a bacterial density equivalent to approximately $1.0x10^8$ cfu/ml, and directly employed in an antibacterial testing (Cheesbrough, 2004; Emad, 2016 and Haris *et al.,* 2017).

Preparation of Concentrations

The concentrations of the crude fruit extracts from *A. nilotica* and *A. senegalensis* through double fold dilution method adopted by Haris *et al.,* (2017). Dimethyl sulfoxide (DMSO) was used as a solvent in the preparation of the different concentrations of the extracts. DMSO was also used as negative control and ciprofloxacine as positive control Four concentrations were made from each extract as follows: 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml.

Antibacterial Susceptibility testing

The antibacterial susceptibility testing was carried out using well diffusion method as described by Jahangirian *et al.,* (20013), Abdallah *et al.,* (2016) and Haris *et al.,* (2017). The plates were incubated at 37^{o}C for 24hrs, after which diameter of the growth inhibition zone was measured in millimeter using standard transparent meter rule.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum inhibitory concentration (MIC) of the acetone extract of *Acacia nilotica* and *Acacia senegalensis* fruits against the tested bacteria was evaluated as described by Ochei and Kolhatkar (2007), Emad (2016) and Haris *et al.* (2017).

To about 1ml of the crude extract at a concentration of 100 mg/ml was added to a tube containing 1 ml Mueller Hinton broth. Then, 1 ml from this tube (First tube) was transferred to the next one in two folds serial dilutions till reached the 4^{th} tube, to get the concentrations 25, 12.5, 6.25, 3.12mg/ml respectively. To additional tube 1ml of DMSO was loaded instead of the plant extract and served as negative control. The least concentration of the plant extract that did not show any visible growth was considered as MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum bactericidal concentration (MBC) of the acetone extract of *Acacia nilotica* and *Acacia senegalensis* fruits against the tested bacteria were carried out according to Haris *et al.,* 2017).

From the MIC tubes that shows no turbidity, which was considered as the positive MIC concentration, a loop full of the clear culture from were streaked onto MHA palates and incubated at 37^{o}C for 24 hrs. Absence of growth after incubation, determine positive result (Haris *et al.,* 2017). This was repeated for all of the extracts at different concentration on both *E. coli* and *S. typhi* to determine the MBC.

RESULTS AND DISCUSSION

Extracts characteristics

The of the A. nilotica extract appears to be dark brown,.while that of A. senegalensis is coffee colour. A. nilotica extract textured jelly, while dried form was observed in A. senegalensis extract.

Biochemical Test

E. coli was confirmed positive catalase and indole, and negative on oxidase and gram stain test. S. typhi was negative indole, oxidase and gram stain. Only catalase test was positive in S. typhi.

The fruits from *A.nilotica* and *A. senegalensis* was extracted using acetone as a solvent. Tables 1 and 2 shows the antibacterial sensitivity of fruit extracts of *A.nilotica* and *A. senegalensis* showing zones of inhibition at different concentrations for both *Escherichia coli* and *Salmonella typhi*. The result of this study revealed that the acetone extracts of *A.nilotica* and *A. senegalensis* had antibacterial activity when the extracts were tested on *E. coli and* S.typhi.

Table 1shows the highest zone of inhibition of 24.33±0.33 mm at 100mg/ml and a lowest inhibition zone of 10±0.00 at 12.5mg/ml of *A. nilotica* fruit extract on *E. coli.* This agrees with the findings of Emad (2016), Banso (2009), Mashram (2009) and Khan (2009). The extract on *S.typhi* shows the highest inhibition zone of 21.67±0.89 at 100mg/ml and the lowest inhibition zone of 1.67±0.67 at 12.5mg/ml of the extract, and it is in line with Sunita (2016) that examined comparative antimicrobial studies of Acacia species and *A. nilotica* exhibited highest activity against three bacteria (*Escherichia coli, Staphylococcus aureus and Salmonella typhi*). The extract of *A. senegalensis* on *E.coli* reveals a highest inhibition zone of 19.67±0.33 at 100mg/ml and moderate zone at 50mg/ml and 25mg/ml with the lowest zone of inhibition of 4.5±0.5 at 12.5mg/ml and this is in harmony with the findings of Renuca *et al.*,(2012). The extract on *S. typhi* shows a zone of 18.5±0.5 at 100mg and lowest zone of 6.5±0.5 at 12.5mg/ml.

Table 1: Antibacterial Susceptibility of test organisms to Acetone extract of *A. nilotica*

Isolates	Mean Zone of Inhibition at different concentration of extract (mm)				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	post control
Escherichia coli	24.33±0.33	13.00±0.56	12.67±0.67	10.00±0.00	19.33±0.33
Salmonella typhi	21.67±0.89	14.00±1.00	12.33±0.67	1.67±0.67	20.42±0.02

The antibacterial efficacy of these plant extracts was also evaluated by MIC and MBC assays, as shown in Table 3 and 4 and figures 1 and 2. The results revealed that the fruit extract of *A. nilotica* and *A. senegalensis* higher represented effective bacteriostatic activity against all tested bacteria, at a concentration of 25 mg/ml and on *E.coli* at 12.5mg/ml of *A. nilotica* extract. The MIC value of *A.nilotica* extract on *E. coli* (12.5mg/ml) in this research were lower than that reported by Emad

Table 2: Antibacterial Susceptibility of test organisms to Acetone extract of *A. senegalensis*

Isolates	Mean Zone of Inhibition at different concentration of extract (mm)				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	post contrl
Escherichia coli	19.67±0.89	10.67±0.67	8.5±0.5	4.5±0.5	19.33±0.33
Salmonella typhi	18.5±0.5	12.5±0.5	9.5±0.67	6.5±0.5	20.42±0.02

(2016), with the MIC value at 100mg/ml for methanolic extract. This phenomenon may be due to variability to site of isolation of tested organism, the solvent used in extraction and strains of the test organism.

Table 3: Minimum Inhibitory Concentration (MIC) of Acetone extract of *A.nilotica*

Isolates	MIC (mg/ml)			
	25	12.5	6.25	3.125
Escherichia coli	+	++	-	-
Salmonella typhi	+	-	-	-

Key: + = no turbidity observed; - = Turbidity observed; ++ = MIC value.

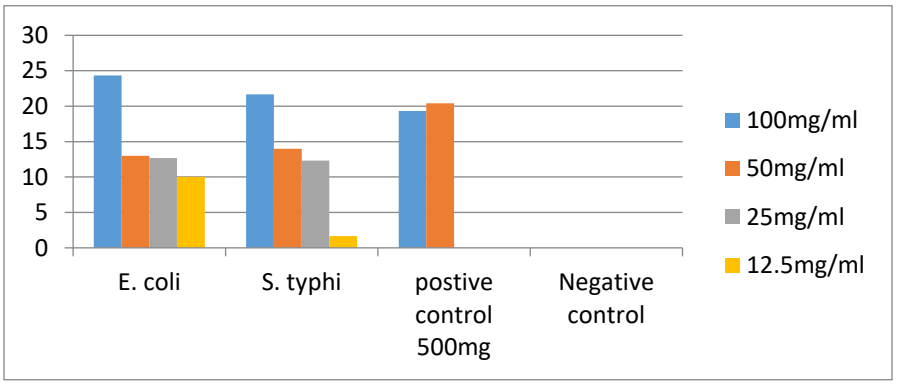

Figure 1: Antibacterial Susceptibility of test organisms to Acetone extract of *A. nilotica*

Table 4: Minimum Inhibitory Concentration (MIC) of Acetone extract of *A. senegalensis*

Isolates	MIC (mg/ml)			
	25	12.5	6.25	3.125
Escherichia coli	++	-	-	-
Salmonella typhi	++	-	-	-

Key: + = no turbidity observed; - = Turbidity observed; ++ = MIC value.

Table 5: Minimum Bactericidal Concentration (MBC) of Acetone extract of *A. nilotica*

Isolates	MBC (mg/ml)			
	25	12.5	6.25	3.125
Escherichia coli	++	-	-	-
Salmonella typhi	++	-	-	-

Key: + = no growth observed; - = Growth observed; ++ = MBC value.

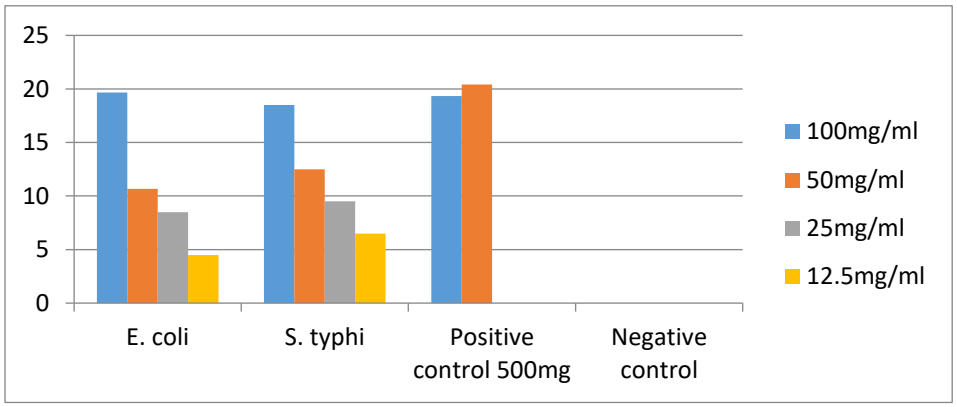

Figure 2: Antibacterial Susceptibility of test organisms to Acetone extract of *A. senegal*

Table 6: Minimum Inhibitory Concentration (MBC) of Acetone extract of *A. senegal*

Isolates	MBC (mg/ml)			
	25	12.5	6.25	3.12
Escherichia coli	++	-	-	-
Salmonella typhi	++	-	-	-

Key: + = no turbidity observed; - = Turbidity observed; ++ = MIC value.

The minimum bactericidal effect of the two plant extracts showed activity of 25mg/ml on all the test organisms. The MBC value of *A. senegalensis* were all equal to the MIC value on the test organisms. That of *A. nilotica* on *S. typhi* was equal to the MIC value and higher than the MIC value in *E. coli*. Actually, the closer in value of the MBC to MIC the more bactericidal, if the MIC value of the plant extract is also the MBC value or higher, it means good sign of bactericidal effect. This is in agreement to some extend with Emad (2016) who stated that the MIC and MBC of pods of *A. nilotica* methanolic extract were 100mg/ml for *E. coli* (figure 1 and 2, Table 5 and 6).

Accordingly, the antibacterial activity of some plant species is related to the richness of phenolic compounds such as flavonoids and tannins (Hideyeku *et al.*, 2002; Meng *et al.*, 2001). The acetone extract of *A. nilotica* and *A. senegalensis* fruits have potential bactericidal effect against all tested bacteria at a concentration of 25mg/ml. Finally, the fruits of these plants are definitely considered promising source of effective antibacterial drug. The interesting outcomes of this investigation, providing encourages to the antibiotics-researchers to give more attention to the natural plant products particularly those mentioned or applied in traditional or folk medicine.

CONCLUSION

The extract of *Acacia nilotica* and *A. senegalensis* fruits in this study showed antibacterial activity on *Salmonella typhi* and *Escherichia coli* , capable of suppressing the growth of the test organisms. This establishes the potential of the providing the scientific basis for their traditional application in folk medicine against bacterial diseases caused by the tested organisms. This study demonstrated that the extracts of fruits from these plants is as effective as modern medicine. Biological and pharmacological screening of this medicinal plants using modern tools such as

High performance thin layer chromatography and mass spectrometry to identify the exact active compound of chemotherapeutic potential responsible for the antibacterial effect of the plants, and in vivo evaluation as well as toxicological analysis to ascertain the efficacy and suitability of using the extracts may lead to the development of some new interesting bactericidal drugs.

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