COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOL ROOT EXTRACTS OF *Azadirachta indica* ROOTS EXTRACT ON SOME BACTERIAL PATHOGENS

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Abstract: *Azadirachta indica* commonly known as neem is a plant of the family Meliaceae used as traditional medicine for the treatment of many infections. The research was carried out to determine the antimicrobial effects of aqueous and ethanolic extracts of the neem plant root. Clinical samples of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used as test organisms. Crude extracts were prepared by dissolving 50 g of the dried powder of the plant root in a sterile beaker of 250 ml of the solvent and kept for 72 h at room temperature with periodic shaking and daily filtration using Watt-man No.1 filter paper and evaporation under reduced pressure to dryness using rotary evaporator. Agar well diffusion method was done for the antimicrobial sensitivity test of the extracts against the pathogens under study. Extract concentrations of 200, 150, 100 and 50 mg/ml, respectively were added into 4 wells in the inoculated petri dishes, allowed to stand for 1 h in the refrigerator and then incubated at 37ºC for 24 h. Ethanol extracts showed relatively stronger antimicrobial activity against aqueous extracts across the studied organisms. *E. coli* was found to have greater sensitivity to aqueous preparation at 200 mg/ml with mean diameter of zone of inhibition of 14.0±0.6 while *P. aeruginosa* was found to have greater sensitivity ethanolic extract at 200 mg/ml with mean diameter of 21.0±0.8. *A. indica* root extracts is a resource of herbal medicine and can be used against Gram negative bacteria such as *E. coli* and Gram positive organisms such as *S. aureus*.

Keywords: Antimicrobial, *Azadirachta indica*, extract, infections, sensitivity

Introduction

Medicinal plants have a long history and their use is widespread in both developed and developing countries, about 80% of the world's population relies mainly on traditional therapies which involve the use of plants and animal (WHO, 1993). During the last few decades, the incidence of microbial infections has increased dramatically. Continuous development of antimicrobial drugs in treating infections has led to emergence of resistance among various microorganisms including bacteria which results to persistence and spread of infections (WHO, 2014). Studies shows very high rates of resistant in bacteria such as *Escherichia coli* against antibiotics such as cephalosporin and fluoroquinolones, *Klebsiellapneuomoniae* against cephalosporin, *Enterococci* resist vancomycin and *Staphylococcus aureus* against methicillin (Nikaido, 2009; WHO, 2014). It is known that more than 400,000 species of tropical flowering plants have medicinal properties (Marinelli, 2005; IUCN Species Survival Commission, 2007; Odugbemi, 2009). Some plant decoctions are of great value in the treatment of diarrhea or gastrointestinal disorder, urinary tract infection, skin infection, infertility, wound and cutaneous abscesses (Langmead and Rampton, 2001; Ergene *et al.*, 2006).

The plant under study is believed to have originated in Asia (Assam and Burma of south Asia, Pakistan, Siri Lanka, Thailand, Malaysia and Indonesia). The tree also grows in tropical and subtropical areas around the world (Verker and Wright, 1993). It has successfully been established in Australia, Haiti, West Africa, the Dominican Republic, Ecuador, Puerto Rico, the Virgin Islands and in the continental United State in Florida, Oklahoma and Arizona (Jacobson, 1990). It is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activity (Biswas *et al.*, 2002).

The plant has been in use since ancient times to treat a number of human ailment (Parrotta and Chaturvedi, 1994; Biswas *et al.*, 2002) and also as household pesticide (Chattopadhyay *et al*., 1993). Numerous biological and pharmacological activities have been reported (Alzohairy, 2016). Extracts from the bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthosis and respiratory disorders (Ketkar and Ketkar, 1995).

Azadirachta indica shows therapeutics role in health management due to rich source of various types of ingredients. The most important active constituent is azatdirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain bioactive ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. Quercetin and ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin (Alzohairy, 2016).

Recent investigations have confirmed its role as anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and antitumour activities (Alzohairy, 2016). The tree is still regarded as 'Village dispensary' and known over 2000 yearsas one of the most versatile medicinal plantshaving a wide spectrum of activity (Lakshmanan and Subramanian, 1996). The enteric bacteria such as *E. coli* and *P. aeruginosa* are among the most important group of bacteria that are medically important that may occasionally be associated with diseases in humans and animals, also *S. aureus* have been found to be implicated in opportunistic infections in man and other animals (Todar, 2008; Gupte, 2010; O'Keeffe *et al.*, 2015; O'Gara, 2017). Many researchers have contributed with regard to antibacterial implication of *A. indica* including Bhowmik (2010); Santhosh and Navaratnam (2013); Pankaj (2014); Tiwari *et al*. (2014); Francine *et al*. (2015), Maina *et al*. (2015), Ahmad *et al.* (2019); Karthika *et al*. (2019); Trivedi *et al*. (2019), among others, hence the need to conduct a research of this kind in order find out the most potent extraction procedure to ascertain high sensitivity extract against disease causing microbes for the benefit of mankind.

Materials and Methods

Sample collection

Portions of healthy roots were cut off from the neem tree at Federal University Wukari using clean knife and immediately transferred into a clean polythene bag as employed by Cheesbrough (2006). The collected plant material was taken to a plant scientist at the Botany Department Federal University Wukari for confirmation and then to the Microbiology laboratory where it was processed.

The stock organisms were obtained from the National Veterinary Research Institute Vom (NVRI) Jos, Plateau State. They were confirmed using cultural and biochemical tests as approved by Ochei and Kolhatkar (2008).

Preparation of ethanol and aqueous extract

Using a weighing balance, 50 g of the dried powder of the plant root extract was measured and transferred into a sterile beaker and then 250 ml of 80% ethanol was added and kept for 72 h at room temperature with periodic shaking and daily filtration using Watt-man No. 1 filter paper (Orhue *et al*., 2014) and evaporation under reduced pressure to dryness using rotary evaporator to produce a crude extract as done by Sukhdev *et al.* (2008). The procedure was repeated three times. The same procedure was followed in the preparation using distilled water to obtain the aqueous extract.

Preparation of inoculum

A loop full of the colony of the stock organisms were inoculated in three different properly labeled sterile test tubes containing 1 ml Mueller Hinton broth (MHB) and incubated for 24 h at 37ºC. The resulting turbidity was compared to 0.5 McFarland Turbidity standards (MTS) as approved by Ochei and Kolhatkar (2008). The bacterial suspension was kept at 4ºC prior to antibacterial assay.

Preparation of the growth medium

Thirty-eight grams (38 g) of Muller Hinton Agar was added to 1000 ml distilled water and autoclaved at 121°C for 15 min at 15 lbs, allowed to cool and then poured in sterile Petri plates up to a uniform thickness of approximately 4 mm and allowed to solidify (Cheesbrough, 2006).

Antibacterial assay of neem root extract

The agar well diffusion method was done on Muller Hinton Agar (MHA) for the antimicrobial assay of the extracts against the pathogens under study. A sterile cotton swab was soaked into the bacterial suspension, rotated and then compressed against wall of the test tube to expel any excess fluid. The swab was then streaked on the surface of MHA plate three times over the entire plate surface to ensure a uniform growth. A sterile cork borer was then used to make 4 wells (6 mm diameter) on the medium. Under aseptic conditions each of the wells was filled up with four prepared concentrations of the extracts 200, 150, 100 and 50 mg/ml, respectively. The plates were allowed to stand for 1 h in the refrigerator for diffusion of the extract to take place and then incubated at 37ºC for 24 h. The set up was repeated tree times for each test organism. The diameters of zones of inhibition were measured (in mm) and the mean values were calculated as done by Cheesbrough (2006) and Aneja and Joshi (2009).

About 50 mg of amoxil powder was added to sterile water and made up to 100 ml antibiotic solution, after thoroughly shaking for 5 min in a volumetric flask, 1 ml solution was taken and mixed with 3 ml distilled water. This 4 ml solution was the desired antibiotic solution that was used as control (Modak *et al*., 2015).

Results and Discussion

Antimicrobial effects and the minimum inhibitory concentrations of ethanol and aqueous extracts of *Azajirachta indica* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was observed in this research.

The result obtained showed different zones of inhibition on aqueous extract at the concentration of 200 mg/ml on the tested organisms as illustrated in Table 1, the extract showed effectiveness against *E. coli* (15.0±0.5 mm), *P. aeruginosa* (10.0±0.8 mm) and *S. aureus* (14.0±0.6 mm), respectively as shown in Table 1. The highest sensitivity was observed in *E. coli* across the tested concentrations.

Table 1: Antimicrobial effects of the aqueous *A. indica* root extract on the organisms under study

Organism	Mean Diameter of zone of inhibition on bacteria (mm)				
	Amoxil	**50 mg/ml**	**100 mg/ml**	**150 mg/ml**	**200 mg/ml**
E. coli	29.0	0.0±0.0	0.0±0.0	13.0±0.5	13.0±0.5
P. aeruginosa	27.0	0.0±0.0	0.0±0.0	11.0±0.7	10.0±0.8
S. aureus	26.0	0.0±0.0	0.0±0.0	0.0±0.0	14.0±0.6

Table 2: Antimicrobial effects of the ethanolic extract of *A. ndica* root on the organisms under study

Organism	Mean Diameter of zone of inhibition on bacteria (mm)				
	Amoxil	**50 mg/ml**	**100 mg/ml**	**150 mg/ml**	**200 mg/ml**
E. coli	28.0	0.0±0.0	13.5±1.0	15.5±1.0	17.5±0.6
P. aeruginosa	26.0	0.0±0.0	17.0±0.5	19.0±1.4	21.0±0.8
S. aureus	27.0	0.0±0.0	10.0±0.4	11.0±0.9	13.0±0.7

Table 2 shows antimicrobial effects of the Ethanol extract of *A. ndica* root on the organisms under study, higher zones of inhibition was found with extract concentration of 200 mg/ml of 21.0±0.8 for *P. aeruginosa*, 17.5±0.6 for *E. coli*, and 13.0±0.7 for *S. aureus*, the highest effect was found with *P. aeruginosa* across the concentrations.

Plants are a source of large number of drugs which are known to possess the antibiotic properties in the traditional system. These plants have some kind of secondary metabolites that are responsible for their antibacterial properties and thus treat large number of diseases (Sonal and Pankaj, 2014; Yash *et al.*, 2014). *Azajirachta indica* has been in use since ancient times to treat a number of human ailment (Parrotta and Chaturvedi, 1994; Biswas *et al.*, 2002) more importantly the enteric gastrointestinal tract diseases and has been used to treat various ailments from prehistory to the contemporary (Todar, 2008; Santhosh and Navaratnam, 2013). The antimicrobial potential of *A. indica* root extract may be due to its compositions; indeed, the phyto-constituents alkaloids, glycosides, flavanoids and saponins which are components of importance and accounts to the plant's antimicrobial properties (Hafiza, 2000).

The result obtained in this research showed sensitivities (mean zones of inhibitions)to both aqueous and ethanol root extracts at various concentrations which are higher at 200 mg/ml on the tested organisms as illustrated in Tables 1 and 2; the aqueous extract (at 200 mg/ml) showed mean zones of inhibitions against *E. coli* (15.0±0.5 mm), *P. aeruginosa* (10.0±0.8 mm) *and S. aureus* (14.0±0.6 mm), respectively as shown in Table 1 and 21.0±0.8 for *P. aeruginosa*, 17.5±0.6 for *E. coli*, and 13.0±0.7 for *S. aureus* (Table 2) with regard to ethanol extract, no effect was found in concentrations less than 150 mg/ml for aqueous extract and 100 mg/ml for ethanolic extracts, respectively.

With regard to *E. coli*, both aqueous and ethanol extracts showed activities (0.0±0.0, 13.0±0.5 and 13.0±0.5 mm), however ethanol extract has higher activity (13.5±1.0,

15.5±1.0 and 17.5±0.6), all at concentrations of 100, 150 and 200 mg/ml, respectively. This contradicts the work of Yash *et al.* (2014) who observed the maximum antibacterial activity in aqueous extract of neem seed against *E. coli* and no activity with root extract. Moreover, ethanol root extract showed diameter of inhibition of 17 mm as compared to this study with mean diameter of 17.5±0.6 mm. The differences in the findings may be due to parts of the plant used in the research as different plant parts may vary in active ingredients. Gnanakalai and Gopal in 2016 found no effect on *E. coli* for both aqueous and ethanol extracts of leaves and bark have which is attributed to the differences in parts of the plant analyzed.

P. aeruginosa was found to have sensitivity to both the aqueous (with mean values of 0.0±0.0, 11.0±0.7 and 10.0±0.8 mm) and ethanolic (with mean values of 17.0±0.5, 19.0±1.4 and 19.0±1.4 mm) extracts in this study, moreover, the highest sensitivity values were obtained compared to *E. coli* and *S. aureus* as in Tables 1 and 2. The activity of the ethanolic extract is in line with the finding of Bharathi *et al*. (2018) who found zones of inhibitions of 25 and 30 mm in extract of methanol and 17 and 22 mm, respectively in ethyl alcohol all at concentrations of 100 and 200 mg/ml. The little discrepancy with our research may be due to difference in chemical properties between the solvents used. Both aqueous and ethanol extracts of leaves and bark have no effect on *P. aeruginosa* as shown by Orhue *et al.* (2014) which is attributed to the differences in the plant parts studied.

The activity of the extract against *S. aureus* was also ascertained, ethanol extract was found to be 27 more effective as the activity was observed within the range of concentration of 100, 150 and 200 mg/ml (10.0±0.4, 11.0±0.9 and 13.0±0.7, respectively) against the aqueous extract which was found to have activity only at concentration of 200 mg/ml in aqueous preparation (14.0±0.6) and this is in concordance with the finding of Francine (2015) who found that ethanol extract from both dry and fresh extracts of neem were more effective against *S. aureus* compared to water extracts. He however found out that ethanol twig extracts at 50% w/v (µg/ml) showed statistically significant ($P \leq 0.05$) growth inhibition of *S. aureus* at 48 h incubation but sensitive at the same concentration with aqueous extracts, which is contrary to the finding of this study and may be due to difference in extract formulation.

Conclusion

Azadirachta indica root extracts demonstrated bactericidal potential against both gram positive and gram-negative bacteria which indicate that the plant can be employed as a source of antibacterial. Information derived from this study can change depending on Neem parts used, the solvent used; moreover, the root extracts can be a good resource for herbal drugs that can be used against Gram negative bacteria such as *E. coli* and Gram organisms such as *S. aureus.*

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Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

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