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Antibacterial Effect of some Plant Extracts on Selected Enterobacteriaceae

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ABSTRACT

*Vernonia amygdalina (*Bitterleaf), *Eucalyptus citriodora (*Eucalypt) and *Phyllanthus amarus* (Schum) were investigated for their antibacterial properties against pure cultures of clinical isolates of *Escherichia coli, Klebsiella sp., Salmonella sp.* and *Shigella sp.* The isolates were obtained from dept of Medical Microbiology and Parasitology of the University of Ilorin Teaching Hospital. Water and Ethanol were used in the crude extractions of the active constituents of the plants. Broth dilution and Agar diffusion methods were used in determining the antibacterial effects of the different plant extracts on the test organisms. The minimum inhibitory concentration (MIC) of the water extracts on the test organisms was 50mg/ml while that of the ethanolic extract ranged between 6.25 - 50mg/ml. Similarly the diameters of zones of inhibition of the plant extracts at concentration of 100mg/ml ranged between 3.0-14.0mm and 3.0-18.0mm for the water and ethanolic extracts respectively on the test organisms.

Water extracts of *vernonia amygdalina (*Bitterleaf) and *Schum* (*Phyllanthus amarus)* were not effective on majority of the test organisms. Kl*ebsiella sp.* was not inhibited by the water extracts at the test concentrations. The Ethanolic extracts of *Eucalyptus citriodora (*Eucalypt) were most effective on all the test organisms. The least and the most susceptible organisms to the extracts were *Shigella sp.* and *E. coli* respectively. The results of this study suggest the possibility of using the ethanolic extracts of these plants in treating diseases caused by the test organisms.

Key Words: Plant extracts, Antibacterial effect, Inhibition, Enterobacteriaceae.

INTRODUCTION

Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. However, nowadays these antibiotics and chemotherapeutic agents are obtained by various synthetic processes (Reiner, 1984). Most countries in West Africa especially Nigeria are richly blessed with forests containing arrays of

different herbs, shrubs and trees.

The leaves, stems, bark, roots etc of these plants are being used by the local populace and people with thin income for incurring different types of ailments because of the inadequate medical facilities across the nook and cranny of these countries.

Some of the plants which are being used medicinally in Nigeria include: Neem (*Azadirachtha indica)*, Cottonleaf (*Gossypium spp)* etc. Ebana *et al.* (1993) have shown that the ethanolic and aqueous extracts of both the roots and leaves of *Strophantus hipidis* (Arrow poison) and *Secamone afzeli* have antibacterial activity against *Neisseria gonorrheae, klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Proteus mirabilis.* Similarly, Akujobi *et al.* (2006) have worked on the antibacterial activities and phytochemical screening of *Vernonia* amygdalina and *Citrus aurantifolia*.

Enterobacteriaceae is one of the most widely studied family of bacteria. The members of this family are gram negative, rod shaped, non-sporulating and facultative anaerobes. Some members of this family are able to ferment lactose with the production of acid and gas. These are called coliforms. Examples of coliforms are *E.coli, Klebsiella, Enterobacter, Citrobacter* etc. However, others do not ferment lactose and includes *Shigella sp.* and *Salmonella sp.* Most of the members of this family enterobacteriaceae cause infection in their host.

Infection by *Salmonella sp.* is contacted by ingesting large numbers of viable bacteria in faecally contaminated food or water. The symptoms include diarrhoea and abdominal cramps with nausea and vomiting. Good sanitation and avoiding sewage contamination of water by domestic animals and fowl help to reduce the incident of Salmonella infections.

Similarly, the symptoms of bacillary dysentery caused by *Shigella dysenteriae* are initial fever and abdominal cramps, then diarrhoea with profuse bloody stools. High temperature and vomiting may also occur. The control of shigellosis requires disruption of the anal-oral route of transmission by means of good sanitary practice.

The natural habitat of *E. coli* is the alimentary tract of man and warm blooded animals; it is one of the most abundant of the intestinal flora. Various strains of *E. coli* have been implicated in the outbreaks of diarrhoeal illness and their routes of transmission have been traced to sewage contaminated drinking water. These strains of *E. coli* include: Enteropathogenic strains of *E. coli* (EPEC); Enteroinvasive *E. coli* (EIEC) and Enterotoxigenic *E. coli* (ETEC). They cause diarrhoea in children and/ or adults (Sterritt and Lester, 1988).

The local populaces rely heavily on most of these medicinal plants for treating various diseases caused by some of these agents. The plants for this study Vernonia amygdalina (Bitterleaf), *Eucalyptus* citriodora (Eucalypt tree) and *Phyllanthus amarus* (Schum) have also been used for treatments locally (Akujobi *et al.*, 2006).

Vernonia amygdalina (Bitterleaf) belongs to the family of compositae (Keay, 1989). It is particularly abundant in grassland throughout the tropics and warmer regions. It is well known as a source of chewing stick for their bitter taste. It is a popular leafy vegetable especially among the Ibos of Eastern Nigeria.

Eucalyptus is a lemon scented gum tree. Industrially, Eucalyptus oil is obtained by a process of distillation from the fresh leaves of this plant and have been used in treating colds, coughs, catarrh, cuts, scratches etc (B.P. 1993).

Schum (*Phyllanthus amarus)* is an herbaceous plant and have equally been used to cure various ailments.

Therefore, this study was primarily undertaken to confirm the acclaimed antibacterial properties of *Vernonia amygdalina*, *Eucalyptus citriodora* and *Phyllanthus amarus* based on their ethnomedical uses in Nigeria.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Eucalyptus citriodora (Eucalypt) and *Phyllanthus amarus* (Schum) were collected at the permanent site of University of Ilorin, Ilorin, Nigeria while *Vernonia amygdalina* (Bitterleaf) was purchased at Ipata Market, Ilorin, Nigeria. In all cases, fresh plant materials were used for this study and the extractions were done within 1-2 hours of collection of the plant materials. The collected plants were identified with the assistance of the Herbarium section of the department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Test Organisms

The test organisms for this study were members of the family Enterobacteriaceae, namely: *Escherichia coli*, Kl*ebsiella sp.*, *Salmonella sp.* and *Shigella sp.* The pure clinical isolates were obtained from the department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria. All the clinical isolates were checked for purity and maintained on Nutrient agar at 4^0c in the refrigerator until required for use.

Sample Preparation and Extraction

Each of the plant material was grinded and 10g of it was added to 100ml of distilled water or 70%w/v ethanol in order to obtain water or Ethanolic extract (100mg/ml). This crude extraction was done at room temperature for 24 hours. Muslin cloth was then used to filter the plant residues and the filtrate thus obtained was further purified by filtration through Whatman No 1 filter paper (Atata *et al*., 2003). This stock solution of extract was sterilized by filtration through Millipore membrane filter of 0.45µm pore-size (Ronald, 1995). The sterile extract obtained was stored in sterile capped bottles and refrigerated at 4^0c until when required for use.

Sterility Proofing of the Extracts

The extract was tested for sterility after Millipore filtration by introducing 2ml of this supposed sterile extract

into 10ml of sterile nutrient broth. Incubation was done at 37^0c for 24hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period (Ronald, 1995).

Standardization of the Bacterial Cell Suspension

Five colonies of each test organism were picked into sterile testtube containing sterile nutrient broth and incubated at 37^0c for 24 hours. The turbidity produced by this organism was adjusted and used to match the turbidity (opacity) standard prepared as described by Monica (1984).

Determination of Minimum Inhibitory Concentration (MIC) of the extracts on the test organisms

The initial concentration of the plant extract (100mg/ml) was diluted using double fold serial dilution by transferring 5ml of the sterile plant extract (stock solution) into 5ml of sterile Nutrient broth to obtain 50mg/ml concentration. The above process was repeated several times to obtain other dilutions: 25mg/ml, 12.5mg/ml, 6.25mg/ml and finally 3.125mg/ml (Ibekwe *et al.*, 2001). Having obtained the different concentrations of the extracts, each concentration was inoculated with 0.1ml of the standardized bacterial cell suspension and incubation was done at 37^0c for 24 hours. The growth of the inoculum in the broth is indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organism was taken as the Minimum Inhibitory Concentration (MIC). Negative controls were set up as follows: Nutrient broth only; Nutrient broth and sterile plant extract; and finally positive control containing Nutrient broth, and a test organism.

Determination of Zones of Inhibition

Fifteen millimetre (15ml) of sterile Nutrient agar was poured into each sterile petridish of equal size and allowed to solidify. The surface of this sterile Nutrient agar plate was streaked with the pure culture of the standardized bacterial cell suspension. A corkborer, (8mmin diameter) was sterilized by flaming and used to create ditch at the center of the plate. The hole so created was then filled with the plant extract. The plates were allowed to stand for one hour for pre-diffusion of the extracts (Esimone *et al.*, 1998) and incubation was done at 37^0c for 24hours. At the end of the incubation period, the diameter of zone of inhibition was measured in millimetre (Hugo and Russel, 1996).

Table 1. Determination of Minimum Inhibitory Concentration (MIC) of the water extracts on the test organisms.

Test organisms	**Concentrations (mg/ml)**		
	Bitterleaf	**Eucalypt**	**Schum**
E. coli	-	50.0	-
Kl*ebsiella sp.*	-	-	-

Salmonella sp.	-	-	50.0
Shigella sp.	-	-	-

-, No inhibition at the concentrations used.

Table 2. Determination of Minimum Inhibitory Concentration (MIC) of the Ethanolic Extracts on the Test Organisms.

Test organisms	**Concentrations (mg/ml)**		
	Bitterleaf	**Eucalypt**	**Schum**
E. coli	25.0	50.0	50.0
Kl*ebsiella sp.*	12.5	50.0	50.0
Salmonella sp.	6.25	25.0	12.5
Shigella sp.	50.0	50.0	50.0

Table 3. Diameters of Zones of Inhibition of the Water extracts (100mg/ml) on the test organisms.

Test organisms	**Diameters of Zones of Inhibition (mm)**		
	Bitterleaf	**Eucalypt**	**Schum**
E. coli	NI	14.0	N.I
Kl*ebsiella sp.*	NI	NI	N.I
Salmonella sp.	3.0	7.0	N.I
Shigella sp.	NI	3.0	N.I

NI: No inhibition at the concentration used.

Table 4. Diameters of zones of inhibition of the ethanolic extracts (100mg/ml) on the test organisms.

Test organisms	**Diameters of Zones of Inhibition (mm)**		
	Bitterleaf	**Eucalypt**	**Schum**
E. coli	3.0	18.0	13.0
Kl*ebsiella sp.*	4.0	17.0	13.0
Salmonella sp.	3.0	9.0	6.0
Shigella sp.	NI	5.0	3.0

NI: No inhibition at the concentration used.

RESULTS

The results of the Minimum Inhibitory Concentration (MIC) showed that majority of the test organisms were not inhibited by the water extracts at the test concentration used. However, the water extracts of Eucalypt and Schum had MIC of 50.0mg/ml on *E. coli* and *Salmonella sp.* respectively (Table 1).

In contrast, all the ethanolic extracts exerted inhibitory effect on the test organisms to different extent (Table 2) *Salmonella sp.* was most susceptible to the ethanolic extract of Bitterleaf, this was followed by *Klebsiella sp.* (MIC 12.5mg/ml) and *E. coli* (MIC 25.0mg/ml). *Shigella sp.* was the least susceptible (MIC 50.0 mg/ml). The most susceptible test organism to the ethanolic extracts of Eucalypt and Schum was *Salmonella sp.* whereas the other test organisms were inhibited to the same extent (MIC 50.0mg/ml).

The results obtained in the Agar diffusion plates followed the same trend with what was obtained in the Minimum Inhibitory tests. The water extracts of Schum was not inhibitory on all the test organisms. Similarly, the water extract of Bitterleaf only inhibit *Salmonella sp.* where it created a diameter of zone of inhibition of 3.0mm. Eucalypt inhibited all the test organisms to different extents except *Klebsiella sp.* where no inhibition was observed (Table 3) at the concentration used.

The ethanolic extracts of the plants inhibit the test organisms to different degrees except Bitterleaf which failed to show inhibitory effect on *Shigella sp*. at the concentration used. In all cases, the ethanolic extracts of Eucalypt had the most inhibitory effect on the test organisms and this was followed by Schum (Table 4).

DISCUSSION

The results of the inhibitory effects of the water extracts showed that it is less effective on the test organisms than the ethanolic extracts (Tables 1-4).

In the broth dilution tubes for the MIC, the water extract of Bitterleaf was not inhibitory on all the test organisms. Eucalypt and Schum followed similar trend being inhibitory on only 25% of the test organisms. This implies that the crude water extracts of these plants could not be suitable (at 6.25 - 50.0mg/ml) in tackling diseases caused by these test organisms as it is sometimes used by the local populace. If water must be used for extraction, Eucalypt will be most suitable (Tables 1 and 3).

Investigators in the past had also clearly shown that ethanolic extracts were more effective than water extract (Ibekwe *et al.*, 2001; and Dutta 1993). They have attributed this observation to the high volatility of ethanol which tends to extract more active compound from the sample than water. Hence, these studies followed similar trends.

Kl*ebsiella sp.* was not inhibited in the water extracts (Tables 1 and 3). However, it was inhibited by the

ethanolic extracts. This may be due to the fact that this organism produces capsule which would not be readily dissolved in water.

In all cases where possible, the ethanolic extracts of these plants should be used at a concentration up to 100mg/ml (Table 4) so as to give a better treatment margin than the maximum 50.0mg/ml obtained in the MIC tests (Table 2).

From the Agar diffusion plates, the ethanolic extracts of Eucalypt had the greatest antibacterial effects and this was followed by Schum. Bitterleaf had the least antibacterial effect on all the test organisms. It however, failed to inhibit *Shigella sp.* at the concentration used. Therefore, the order of ease of susceptibility of the test organisms to the different Ethanolic extracts were: *E. coli; Klebsiella sp., Salmonella sp.* and finally *Shigella sp.*

CONCLUSION

This investigation revealed that the water extract of Eucalypt exert appreciable antibacterial effect on all the test organisms except *Klebisiella sp.* and that the water extract of Bitterleaf is the least effective on all the test organisms. Furthermore, this study also revealed that the ethanolic extract of Eucalypt had the highest antibacterial effect on all the test organisms, followed by Schum and finally Bitterleaf. Eucalypt is therefore recommended for usage by the local populace because of its significant antibacterial effect as revealed by this study.

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