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ANTIBACTERIAL PROPERTIES OF *ZINGIBER OFFICINALE*, *ASTONIA BOONEI, PICRILIMA NITIDA* AND *MOMORDICA CHARANTIA* AGAINST DIARRHOEAL-CAUSING ORGANISM.

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ABSTRACT: The antimicrobial properties of the ethanol extracts of *Zingiber officinale* (fruits), *Alstonia boonei* (stem bark), *Picrilima nitida* (seeds) and *Momodica charantia* (leaves) were investigated against diarrhoeal causing bacterium *Vibro cholerae*. All extracts were shown to possess different/varied degrees of antibacterial activity with diameter of the zones of inhibition between 11mm and 16mm. The extract of *P. nitida* was the most active of the four plant extracts tested, while *M. charantia* was the least active. The MIC values recorded ranged between 62.5mg/ml for the most active and 500mg/ml for the least active respectively. The MBC values ranged between concentrations of 250mg/ml to above 500mg/ml

KEY WORDS: *In-vitro*, Antimicrobial, *Vibro cholerae*, Diarrhoeal, Medicinal plants.

INTRODUCTION

Cholera is an acute diarrhoeal infection caused by injection of the bacterium *Vibro cholerae*. Transmission occurs through direct faecal-oral contamination or through ingestion of contaminated water and food (Steffen *et al*., 2003). Cholera is an extremely virulent disease that affects both children and adults. Its incidence varies across the developing world. Unlike other diarrhoeal diseases, it can kill healthy adults within hours (Amin *et al*., 1995). Individuals with lower immunity, such as malnourished children or people living with HIV, are at greater risk of death if infected by cholera (WHO, 2003).

Since 2005, the re-emergence of cholera has been noted in parallel with the ever-increasing size of vulnerable populations living in unsanitary conditions (Zucherman *et al*., 2007). Cholera remains a global threat to public health and one of the key indicators of social development. While the disease is no longer an issue in countries where minimum hygiene standards are met, it remains a threat in almost every developing country (Deen *et al*., 2008). The number of cholera cases reported to WHO during 2003, rose dramatically, reaching the level of the late 1990s. A total of 236 896 cases were notified from 52 countries including 6311 deaths, an overall increase of 79% compared to the number of cases reported in 2005 (WHO, 2005). It is estimated that only a small proportion of cases-less than 10% are reported due to social and economic considerations (Vijesh *et al*., 2009). The burden is grossly underestimated. The majority of patients can be treated adequately through administration of oral rehydration salts. Appropriate antibiotics can be given to severe cases to reduce the volume of rehydration fluid needed and shorten the duration of *Vibro* excretion (Bhatnager *et al*., 2007). However, routine treatment with antibiotics or mass chemoprophylaxis has no effect on the spread of cholera and can have adverse effects by increasing antimicrobial resistance (WHO, 1990). In the light of this, there is the need to seek alternative and effective therapy with no microbial resistance in the herbal medicine. Traditionally, especially in the developing countries, plants have been used as sources of treatment of diseases including diarrhoeal diseases (Hostettman *et al.,* 2002). Presently, the use of plants extracts as alternative form of medical treatment is enjoying great popularity since the late 1990s. Thus, the present study is designed to evaluate the antibacterial properties of four traditionally used medicinal plants- *Zingiber officinale* (fruits), *Alstonia boonei* (stem bark), *Picrilima nitida* (seeds) and *Momodica charantia* (leaves) against diarrhoeal causing bacteria *"Vibro cholerae".*

MATERIALS AND METHODS

Plant Materials

The samples of *Zingiber officinale* (fruits), *Alstonia boonei* (stem bark), *Picrilima nitida* (seeds) and *Momodica charantia* (leaves) plants were brought from Ekeonuwa market Owerri, Imo State, Nigeria and were identified and authenticated by Dr. Mbagwu F.N a Taxonomist of the Department of Plant Science and Biotechnology of Evan Enwerem University, Owerri with voucher specimens deposited at the University herbarium. They were air-dried and then ground to fine powder with an electric blender.

Extraction

Ground materials of the plant samples weighing 300g were Soxhlet extracted with ethanol and water in succession for 12hours. The different extracts were each concentrated by evaporation until dry under vacuum. The residues of the plants extracted were resuspended in ethanol to a concentration of 500mg/ml and stored.

Organism

The organisms used were 50 clinical strains of *Vibro cholerae* obtained from the Federal Medical Centre Owerri, Imo state. (The organisms were used for the analysis immediately after collection from the hospital within 2 hours of collection).

Evaluation of Antimicrobial Activity

The preliminary antimicrobial screenings of the plant extracts were carried out using the agar diffusion technique (Singleton, 1999).

Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates were seeded with 0.1ml of 1/100 dilution of an overnight culture of the bacterial isolate and allowed to

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stand. A standard cork borer of 6mm diameter was used to cut uniform wells on the agar surface into which 0.2ml of the test solution of each extract (500mg/ml conc.) was added. The plates were incubated at 37ºC for 24 hours after which diameter of zone of inhibition were measured. Ethanol was included separately in each plate as solvent control.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of the Plant Extracts

The MICs of the active plant samples were determined using the broth dilution method (Adeniyi *et al*., 2000). The extracts were serially diluted using Mueller Hinton broth to final concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25g/ml and 15.63mg/ml. The tubes were then inoculated with 0.1ml suspension of the test organism and incubated at 37ºC for 24 hours. The MIC was taken as the lowest concentration that inhibited the growth of the organism. From the tubes with inhibited growth, 0.1ml of the content was plated out onto the surface of agar medium and then incubated for 24 hours at 37ºC. The MBC is taken as the lowest concentration without growth of organism on the agar plate.

RESULTS AND DISCUSSION

The antimicrobial properties of the ethanol extracts of *Zingiber officinale* (fruits), *Alstonia boonei* (stem bark), *Picrilima nitida* (seeds) and *Momodica charantia* (leaves) were investigated. The results of the antibacterial screening of the plant extracts against *V. cholerae* are shown in Table 1. All extracts were shown to possess different/varied degrees of antibacterial activity with diameter of the zones of inhibition between 11mm and 16mm. These results are however relatively weak and as such the plant extracts could be said to be bacteriostatic. The antimicrobial activities of these extracts could not be connected with the presence of the solvent (ethanol) since the solvent control did not exhibit any antimicrobial activity.

The extract of *P. nitida* was the most active of the four tested plant extracts, having the highest diameter of zone of inhibition of 16mm. This was closely followed by *Z. officinale* with 15mm diameter zone of inhibition. *A. boonei* and *M. charantia* had 12mm and11mm diameter zones of inhibition respectively.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the active extracts were determined using the agar dilution method to ascertain the effectiveness of the extracts (Table 1). The MIC values recorded ranged between 62.5mg/ml for *P. nitida* and 500mg/ml for *M. charantia* respectively while MBC values ranged between concentrations of 250mg/ml to above 500mg/ml.

The results obtained from this study agreed with some previous studies while contradicting others. *M. charantia* leaves extract though the least active of the plants investigated had considerable activity as reported by Brantner & Grein (1994). *P. nitida,* the most active of the tested plants is used locally in Southern Nigeria especially as decoction for diarrhoeal conditions. The result obtained in this study buttresses this practice and also confirms earlier report by researchers on the plant as possessing antidiarrhoeal activity (Kouitcheu *et al.,* 2006). Results of *Z. officinale* however contradicts findings of Poonam *et al*. (2010) who stated that *Z. officinale* studied for its antimicrobial profile and effect on virulent features of diarrhoeal pathogens, viz colonization of epithelial cells and production of enterotoxin showed no antimicrobial activity. Although it inhibited the production of cholera toxin, it had no effect on the action of this toxin. However, the bacterial colonization of HEp-2 cells were reduced. Thus, *Z. officinale* exhibited its antidiarrhoeal activity by affecting bacterial and host cell metabolism (Poonam *et al*., 2010). This result, though in contrast to Poonam's (2010) findings, corroborates findings of Akoachere *et al*.(2002) who reported the antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens..

Many pharmacological studies have been undertaken on these plants and reports ranging from possession of anticancerous activity to antiviral activities have been recorded (Ali *et al*., 2008). However, a number of compounds such as tannins and flavonoids etc have been reported to be present in these plants (Sandberg & Bruhn, 1979). These compounds are known to have pharmacological activities including antidiarrhoeal activity. This has been attributed to inhibition of intestinal motility, antimicrobial action and antisecretory effects (Galvez *et al*., 1993; Miranda *et al.,* 1993; Tona, 1999).

This work is a basic approach to finding the antibacterial activities of *Zingiber officinale* (fruits), *Alstonia boonei* (stem bark), *Picrilima nitida* (seeds) and *Momodica charantia* (leaves). Therefore, there is the need for further works on the types of phytoconstituents and purification of individual groups of bioactive components which may reveal the exact potential of the plant to inhibit several pathogenic microbes.

Table 1: Antimicrobial Activities of the Plants crude Ethanol Extracts and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in mg/ml of the Plant Extracts.

Plants	Zone of Inhibition (mm)	MIC (mg/ml)	MBC (mg/ml)
Z.officinale	15	250	250
A.boonei	12	500	500
P.nitida	16	62.5	250
M.charantia	11	500	Above 500

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