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Full Length Research Paper

Cytotoxicity and antibacterial activity of the leaf methanolic extract of *Verbena hastata*

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The phytochemical screening of the methanolic and dichloromethane extracts of *Vebena hastata* showed the presence of alkaloids, flavonoids and glycosides but these were absent in the n-hexane extract. The methanolic extract exhibited antimicrobial activity against *Salmonella typii, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae* (minimal inhibitory concentration (MIC) 0.3 ± 0.1 mg/ml) was inactive against *Proteus mirabilis, Bacillus subtilis, Proteus vulgaris* and *Escherichia coli.* It was also inactive against *Candida albicans* which implies that it cannot be used for the treatment of fungal infections. It was potent against brine shrimps with LD_{50} value of 55.7 ppm which indicates that the extract has the ability to exert a wide range of pharmacological effects. These results support the use of the leaves in the treatment of ailments such as bronchial infections.

Key words: *Verbena hastate*, antimicrobial, cytotoxicity, brine shrimps lethality.

INTRODUCTION

For centuries plants have been used for both nutritional and medicinal purposes. In Nigeria, orthodox medicine is not cheap and a large population of the people depends on traditional medicine for their healthcare needs. Over the years, these herbal drugs have been shown to be effective (Awe and Omojasola, 2003). Many plants and their parts are used for the treatment of various diseases in different parts of the world, and are being screened for antimicrobial activities and the results obtained from these scientific studies have aided in the rationalization of tradomedical use of these plants (Abo et al., 1999; Elegami et al., 2001; Islam et al., 2001; Kariba and Houghton, 2001; Khatune et al., 2001; Kishore et al., 2001 and Olafimihan, 2002). These results also show that the same plants of the same species have difference in use and activity across countries of the world. *Verbena hastata* belongs to the verbanaceae. It is found mainly in forest areas in West Africa and is a perennial herb with 4-angled, erect or mostly prostate stems and simple, opposite, serrate leaves. Traditionally the herb is referred to as a multi-purpose plant. Traditional health practitioners use the hot water extract for the treatment of all bronchial infections. Other uses include: tonic, emetic, nervine, sudorific and febrifuge (Kafaru, 1994). In South western Nigeria and especially in Ogbomoso the leaves of the plant are used for bronchial infections and for flushing the stomach. Therefore, the aim of this work was to investigate the phytochemical, cytotoxicity, antimicrobial activity and secondary metabolites of the plant leave of *V. hastata* that thrives in Ogbomoso, Nigeria.

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Abbreviation: MIC, Minimal inhibitory concentration.

MATERIALS AND METHODS

Plant collection and identification

The plants were collected from a traditional herbalist farm in Lagos, Nigeria and brought to the Department of Pure and Applied Biology, Ladoke Akintola University of Technology for identification by Prof. Osundina.

Preparation of the extract

The leaves were collected and air dried in the laboratory for two weeks and ground into fine powder using a sterilized mechanical

Table 1. Phytochemical screening of leaf extracts of *V. Hastata.*

Extract	Alkaloids	flavonoids	Steroids	saponins	tannins	Glycosides
Methanol	+	+	-	-	-	+
n-hexane	-	-	-	-	-	-
DCM	+	+	-	-	-	+

Key: - = absent; + = present, DCM = Dichloromethane.

grinder. 250 g each of the fine powder was placed in different flasks and covered with redistilled methanol, dichloromethane and n-hexane. These were allowed to stand for 48 h; after which they were filtered and concentrated using a rotary evaporator.

Phytochemical screening

The method used is described by Harborne (1973). The extract was screened for the presence of steroids, flavonoids, tannins, alkaloids and glycosides.

Test organisms

Clinical isolates of *Salmonella typii, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas mirabilis, Bacillus subtilis, Proteus vulgaris, Escherichia coli* and *P. aeruginosa* were obtained from Baptist Medical Center, Ogbomoso.

Preparation of the medium

Nutrient agar medium was prepared by dissolving 2.8 g of nutrient agar in 100 ml of distilled water. The solution was sterilized in an autoclave at 121°C for 15 min. It was cooled and poured into sterile Petri dishes to solidify. The agar depth of the medium was measured (4 cm).

Preparation of test samples

1.0 mg of the extract was dissolved in 1.0 ml of redistilled methanol. The activity of streptomycin was also determined and used as the positive control; 2.5 mg of it was dissolved in 1 ml of distilled water.

Antibacterial activity

Disc diffusion method of Kirby-Bauer (1966) was employed. This involved the use of filter paper disc as carrier for the antibacterial agent. Sterilized discs cut from Whatman no. 1 filter paper were impregnated with solutions of the antibacterial agent at different concentrations. The solvent was evaporated and the disc dried properly. The nutrient agar medium was inoculated with the test organism and the impregnated disc placed on the surface of the nutrient agar. The antibacterial agent upon contact with the agar diffused into all directions. The ability of the test organism to grow or not in the presence of the test sample was then determined within 24 h by measuring the zones of inhibition. The plates were incubated upside down at 37°C. Redistilled methanol was used as control. All test carried out were done in triplicates and the antibacterial activity was expressed as a mean of inhibition diameters (mm) produced by the leaf extract. The effect of the antibiotic, streptomycin (2.5 mg/ml) on the test organisms was determined using the same procedure as that of the antibacterial susceptibility test for the extract. The zones of inhibition were measured and recorded.

Minimum inhibitory concentration (MIC)

Disc diffusion method was used to determine the MIC of the plant leaf extract against sensitive microorganisms. Serial dilutions of the plant extract were prepared (20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mg/ml). Each of the innocula was poured into Petri-dishes and the agar was later poured and allowed to set. A sterile 3 mm cork borer was used to make wells in which the prepared serial dilutions of the extract were introduced. The plates were incubated at 37°C for 24 h. Clear zones of no microbial growth were used to determine the sensitivity of the microorganisms to the test solutions. The least concentration of the plant extract that had inhibitory effect was taken as the MIC of the extract against such microorganism.

Brine shrimp lethality test of crude extract

Sea water was put in a tank which was partitioned into dark and light areas. Brine shrimp eggs were added to the dark area and covered. The set was left in a well lit place for 48 h. The hatched eggs which swarm to the lit place were used for the bioassay. 20 mg of the extract was dissolved in 2 ml of sea water. From this solution 500, 50 and 5 µl were transferred into vials and made up to 5 ml. The corresponding concentrations were 1000, 100 and 10 µg/ml, respectively. Ten brine shrimps were transferred into each of these vials using Pasteur pipette. Replicates of each of the dose levels were prepared using sea water as control. Number of survivors, deaths and nauplii with sluggish movement were recorded 24 h later. Data were processed using probit analysis to estimate LC_{50} values at 95% confidence interval for statistical comparison of potency. LC_{50} less than 100 were considered as potent (Gupta et al., 1996).

RESULTS

The results of the phytochemical screening have indicated the presence of alkaloids, flavonoids, saponins and glycosides in the methanolic and dichloromethane extracts but absent in the n–hexane extract (Table 1). The antibacterial activity of the methanolic leaf extract was observed to be active against *S. typii, P. aeruginosa, S. aureus* and *K. pneumonia* with MIC of 0.3 ± 0.1. The antibacterial activity compares well with that of streptomycin (Table 2) where as the cytotoxicity using brine shrimp lethality assay has shown that the methanolic extract is potent with LC_{50} value of 55.7 ± 4.02 ppm (Table 3).

Table 2. Antibacterial activity and minimum inhibitory concentration of methanolic leaf extract of *V. hastata.*

Microorganism	Zones of inhibition (mm)		MIC (mg/ml)
Bacteria	**Extract (1 mg/ml)**	**Streptomycin (1 mg/ml)**	**Extract**
Salmonella typii	17 ± 0.2	26 ± 0.2	0.3 ± 0.1
Staphylococcus aureus	28 ± 0.3	21 ± 0.3	0.3 ± 0.1
Klebsiella pneumoniae	20 ± 0.2	10 ± 0.2	0.3 ± 0.1
Pseudomonas aeruginosa	32 ± 0.2	21 ± 0.4	0.3 ± 0.1
Escherichia coli	0	0	0
Proteus mirabilis	0	0	0
Fungus			
Candida albcans	0	0	0

Values are mean ± SD, n = 3, MIC = Minimum inhibitory concentration.

Table 3. Brine shrimp lethality test of methanolic leaf extract of *V. hastata.*

Conc. (µg/ml)	No. of subject	No. of living	No. of death	LC_{50} (ppm)
1000	10	1.0	9.0	
100	10	5.0	5.0	55.7 ± 4.02
10	10	7.0	3.0	

Values are mean ± SD of 3 replicates.

DISCUSSION

Phytochemical screening

Table 1 shows the phytochemical profile of the plant leaf extracts. The presence of alkaloids, flavonoids, steroids and glycosides were identified in both methanolic and dichloromethane extracts but absent in the n-hexane extract. Alkaloids comprise of a large group of nitrogenous compounds that are widely used as cancer chemotherapeutic agents (Chabner and Horwit, 1990; Noble, 1990). Alkaloids also interfere with cell division, hence the presence of alkaloids in *V. hastata* could account for the antimicrobial and brine shrimps lethality recorded in this study. Flavonoids are a group of phenolics that are found in varying amounts in foods and medicinal plants which have been shown to exert anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 2006), anti-microbial and, antihepatotoxic activities (Robert et al., 2001). They alter enzyme activities affecting cell division, proliferation, platelet aggregation and immune response. Moreover, many studies have suggested that flavonoids exhibit chemoprevention and important anticancer activities (Pei et al., 2007). Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary atherosclerotic disease (Knekt et al., 1996). The presence of flavonoids in this part of the plant may give credence to their therapeutic effects especially in the treatment of hypertension. The presence of these metabolites probably explains the various uses of this plant in traditional medicine. The glycosides are cardioactive compounds which belong to the class of triterpenoids. They act directly on the smooth muscle of the vascular system and exert a number of effects on the neural tissue thereby influencing directly the mechanical and electrical activities of the heart. Their inherent activity resides in the aglycone portion of the sugar attachment (Brian et al., 1985).

Antimicrobial activities

Table 2 shows the antibacterial activity of the methanolic leaf extract. The methanolic extract was observed to be active against *S. typii, P. aeruginosa, S. aureus* and *K. pneumoniae.* This activity suggests that the plant leaf extract possess remarkable therapeutic potential in the treatment of gastrointestinal and bronchial infection in man (Rogger et al., 1990). However the plant extract was inactive against *P. mirabilis, B. subtilis, P. vulgaris* and *E. coli.* It was also inactive against *C. albicans* which imply that it cannot be used for the treatment of fungal infections. The high potency of the leaf extract against these bacteria shows its scientific basis for its uses in traditional medicine in the treatment of different types of cough, diarrhea and dysentery. These antibacterial activities are likely due to the presence of the secondary metabolites present in the extract.

Brine shrimp lethality test

Brine shrimp lethality is a general bioassay which is

indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions. It has earlier been reported that LD_{50} values for general cytotoxicities are about one-tenth LD_{50} values in the brine shrimp test (MacLaughin et al., 1991). The leaf extract of *V. hastata* was observed to be potent against brine shrimps with LD_{50} of 55.7 ppm which is less than 100 ppm. The brine shrimps lethality test was observed to be concentration-dependent. The result indicates the ability of the extract to exert a wide range of pharmacological effects (Gupta et al., 1996). The presence of alkaloids and flavonoids and glycoside may be responsible for the observed brine shrimps lethality activity of the extract. The brime shrimp letharlity further supports the antibacterial activities of the leaf extract of *V. hastata* on some pathogenic organisms observed in this study.

Conclusion

This work has revealed further potential of this plant in the area of pharmacology as an antimicrobial agent. As a result of the high LD_{50} value in brine shrimp lethality and high antimicrobial activity, the extract is considered a safe antimicrobial agent.

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