African Journal of Biotechnology Vol. 6 (22), pp. 2502-2505, 19 November, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark

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Accepted 2 November, 2007

The antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark at varying concentrations of 50, 100 and 150 mg/ml was investigated by measuring the zones of inhibition produced after incubation on nutrient agar. *Staphylococcus aureus* and *Bacillus substilis, Escherichia coli*, *Salmonella typhymurium* and *Pseudomonas aeruginosa* were used as test organisms. The results revealed that the zones of inhibition on the bacterial isolates increased ($P < 0.05$) as the concentration of the plant extract increased. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration produced by the extract on the gram positive bacteria were significantly lower ($P < 0.05$) than the gram negative bacteria. The results revealed that the aqueous extract of *Enantia chlorantha* stem bark possessed antibacterial activities on the clinical isolates with more antibacterial effect on the gram-positive than the gram-negative bacteria. The identified alkaloids might be responsible for the antibacterial activities.

Key words: Antibacterial potentials, aqueous extracts, alkaloids, *Enantia chlorantha*.

INTRODUCTION

Over the years, there have been several reports on the resistance to antibiotics by these strains of organism (Ozumba, 2003). This has therefore necessitated the use of medicinal plants as antibiotics. One of such plant is *Enantia chlorantha*. *E. chlorantha* Oliv [Annonaceae] is an ornamental tree of up to 30 m high, with dense foliage and spreading crown. The stem is fluted, the bark fissured geometrically and the outer bark is thin and dark brown; the inner bark is light brown above and pale cream beneath. This plant is commonly found in the forest and coastal areas of West Africa, and the Democratic Republic of Congo (Vivien and Faure, 1985; Iwu, 1993). Wafo et al. (1999) reported the antiviral activity of aqueous extract of the dried stem bark of the plant, while the potentials of the plant extract in relieving pyrogen-induced fever in albino rats was reported by Agbaje and Onabanjo (1988). In Cameroon, stem bark extract is used to treat jaundice and urinary tract infections (Adjanohoun et al., 1996).

The more current and most effective antibiotics are very expensive and out of reach of many Africans, majority of whom reside in the rural areas. These antibiotics are also associated with some serious side-effects. A medicinal plant, such as *E. chlorantha*, is readily available and affordable. This study is therefore aimed at providing scientific evidence to the acclaimed antibacterial potentials of *E. chlorantha* stem bark.

MATERIALS AND METHODS

Plant

The plant was obtained from Ore, Ondo State, Nigeria. It was identified and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria where voucher specimen was deposited at the herbarium.

Test organisms

Staphylococcus aureus, *Bacillus substilis*, *Escherichia coli*, *Salmonella typhymurium*, and *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria. The cultures maintained on nutrient agar were stored at 4°C.

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Table 1. Qualitative and quantitative phytochemical analysis of *Enantia chlorantha*.

Phytochemical	Qualitative	Quantitative	Percentage
Phenolics	++	1.12±0.00	18.77
Flavonoids	+	0.40±0.02	6.71
Alkaloids	++	2.76±0.02	46.26
Glycosides	±	0.086±0.01	1.44
Saponins	++	1.60±0.02	26.99
Tannins	nd	0.00	0.00
Phlebotanins	nd	0.00	0.00
Steroids	nd	0.00	0.00

++ = Strongly positive, + = positive, ± = weakly positive, and nd = not detected.

Preparation of aqueous extract of *Enantia chlorantha* stem bark

Stem barks of *E. chlorantia* were sun-dried for 7 days until a constant weight was obtained. This was ground into powder using an electric blender (Blender/Miller III, model MS-223, Taiwan, China) and later stored in air-tight plastic container until required (Adesokan and Akanji, 2003). 25 g of the powder was extracted in 100 ml of distilled water for 48 h at room temperature with constant shaking using magnetic stirrer. The resulting mixture was then filtered through muslin cloth and the filtrate obtained was concentrated on a water bath to give yellowish slurry. The resulting residue was reconstituted in distilled water to give the desired doses of 50, 100 and 150 mg/ml.

Qualitative and Quantitative phytochemical analysis

Simple chemical tests were carried out to detect the phytochemicals present in the plant according to the methods of Odebiyi and Sofowora (1978). The quantification of each component was also carried out following the procedure described by El-Olemy et al. (1994).

Bacterial inoculation and incubation with extract

Nutrient agar and nutrient broth (oxoid) were prepared according to the manufacturers' recommendations. The agar-well diffusion method was used for the inoculation of the bacteria. Plates containing 30 ml of sterile nutrient broth each were inoculated with standardized innocula (1.5 x 10^8 cells/ml) (Olafimihan and Fawole, 2003) using sterile Pasteur pipette. Wells of 5 mm diameter were made at the centre of each plate and 0.15 ml of the various concentrations of the plant extract was dispensed into each wells. The extract was allowed to diffuse into the medium for 1hr at room temperature. This was then incubated at for 24 h at 37°C after which the zones of growth inhibition were measured and recorded in millimeter. The control was set up in a similar manner except that the extract was replaced with sterile distilled water.

Determination of minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) by the aqueous extract of the plant stem bark at the dose levels of 12.5, 25.0, 50.0, 100.0 and 150.0 mg/ml was carried out by the method as described by National Committee for Clinical Laboratory Standard (1990). Briefly, 0.1 ml of varying concentrations of the plant extract was introduced into the test tubes containing 9 ml of nutrient broth and standard bacterial cells. The test tubes were incubated at 37°C for 24 h. Controls were set up with the test organisms using distilled water instead of the plant extract. The minimum inhibitory concentration was taken as the tube with the least concentration of the extract with no visible growth after incubation.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration of the plant extract on the clinical bacterial isolates was done according to the method highlighted in National Committee for Clinical Laboratory Standard (1990). Briefly, 1 ml was pipetted from the mixture obtained in the determination of MIC stage was streaked out on the nutrient broth for 24 h. The least concentration of the extract with no visible growth was taken as the minimum bactericidal concentration.

Statistical analysis

Data are mean of three replicates ± SEM and were subjected to Duncan's Multiple Range test (Montgomery, 1976).

RESULTS AND DISCUSSION

Table 1 shows the identified phytochemicals while Table 2 shows diameter of zones of inhibition of bacterial growth at varying concentrations of the aqueous extract of *E. chlorathia* stem bark. For *S. aureus,* the zone of inhibition increased significantly ($p < 0.05$) with increasing concentrations of the extract. The same pattern was observed for each of the other isolates. However, the various concentration of the plant extract produced wider zones of inhibition for *S. aureus* and *B. substilis* when compared to the zones of inhibition of the gram-negative isolates. This implied that the gram-positive bacteria were more susceptible to the extract than the gram-negative bacteria, possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative species (Nikaido, 1999). In addition, since the zones of inhibition is equal to or greater than the standard, it shows that the test organisms are sensitive to the plant extract.

S. aureus was the most susceptible bacterium, an ob-

Table 2. Inhibitory zones of bacteria growth on culture media by varying concentrations of aqueous extract of *Enantia chlorantha*.

Aqueous extract	*S. aureus* (mm)	*B. subtillis* (mm)	*E. coli* (mm)	*S. typhimurium* (mm)	*P. aeruginosa* (mm)
50 mg/ml	7.00 ±0.29b	6.00 ±0.29b	5.20 ±0.12a	5.10 ±0.12a	5.10 ±0.15a
100 mg/ml	10.00 ±0.12c	10.00 ±0.29c	7.00 ±0.17b	5.10 ±0.15a	5.00 ±0.12a
150 mg/ml	14.83 ±0.20c	12.10 ±0.15c	10.10 ±0.20c	9.00 ±0.17b	6.10 ±0.15b

Values are means of three determinations ± S.E.M. Values in each vertical column carrying different letters are significantly different from one another ($p < 0.05$).

Table 3. Minimum inhibitory concentrations (MIC) for the aqueous extract of *E. chlorantha* (mg/ml).

Extract	*S. aureus*	*B. subtillis*	*E. coli*	*S. typhimurium*	*P. aeruginosa*
Aqueous	25.00 ±0.50a	48.00 ±2.50a	100.00 ±0.50b	100.00 ±0.50b	100.50 ±5.00b

Values are means of three determinations ± S.E.M. Values in each vertical column carrying different letters are significantly different from one another ($p < 0.05$).

Table 4. Minimum bactericidal concentration (MBC) of the aqueous extract of *E. chlorantha* on the bacteria (mg/ml).

Extract	*S. aureus*	*B. subtillis*	*E. coli*	*S. typhimurium*	*P. aeruginosa*
Aqueous	90.00 ±0.50a	100.00 ±5.00a	130.00 ±0.50b	150.00 ±5.00b	150.00 ±0.50b

Values are means of three determinations ±S.E.M. Values in the row carrying different letters a and b are significantly different ($p < 0.05$).

servation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by active principles of the extract of *E. chlorantha*. In contrast, *P. aeruginosa* showed the least susceptibility to the extract. This may be due to the fact that *P. aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (MDRs), which is in agreement with previous observation (Nikaido, 1999).

The anti-bacterial activity observed in this study was concentration-dependent. Tables 3 and 4 showed the values for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the isolates, respectively. The MIC and MBC for the gram-positive bacteria were significantly lower ($p < 0.05$) than those of the gram-negative bacteria. The MIC values obtained for the entire test organisms were very high, ranging from 25 to 100 mg/ml, when compared to the values of 0.01-10 µg/ml usually recorded for typical antibiotics. This difference may be due to the fact that the extract used was in the impure form and would definitely contain substances which do not have antibacterial activities (George et al., 2002). The basic quantitative measures of the *in vitro* activity of antibiotics and plant extract with antibacterial potentials are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (that is colonies on a plate or turbidity in broth culture) under standard conditions while the MBC is the lowest concentration of the antibiotic that kills 99.9% of the original inoculum in a given time. The lower values of MIC in this study are indications that the organisms are not resistant to the plant extract as antibiotic plant material.

Previously, Lewis (2001) extracted palmatine, coloumbamine and jatrorrhizine, which are alkaloids, as major phytochemicals with amphipatic cations from *E. chlorantha.* These amphipatic cations are said to be preferred substrates by most multidrug resistance pumps (Lewis, 2001). Earlier workers (Jennings and Ridder, 1983) also discovered that these phytochemicals intercalate DNA of microorganism, as the mechanism of their antibacterial activity.

Conclusion

In conclusion, extract of aqueous of *E. cholantha* have broad-spectrum antibacterial activities with gram-positive bacteria investigated showing more susceptibility to the aqueous plant extract of *E. chlorantha* stem bark at all the doses when compared with the gram-negative bacteria. The broad-spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as antibacterial agent in folklore

medicine of Nigeria and may thus be useful in the treatment of bacterial infections.

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