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Effect of Stem Bark Extracts of *Enantia chloranta* on Some Clinical isolates

Razaq F. ATATA*, Alhassan SANI and Stella M. AJEWOLE

Department of biological sciences, University of Ilorin, PMB, 1515 Ilorin, Nigeria.

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Abstract

Studies on antimicrobial activity of water, methanol and ethanol extracts of the stem bark of *Enantia chlorantha,* was carried out by the agar ditch diffusion and tube dilution methods. The clinical isolates used include; *Staphylococcus aureus, Streptococcus pyogenes, Shigella sonnei, Proteus mirabilis, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Aspergillus niger,* and *Candida albicans.* The isolates were obtained from the Department of Medical microbiology and Parasitology of the University of Ilorin Teaching Hospital. Crude extracts of all solvents used inhibited the growth of all the isolates tested, except *Pseudomonas aeruginosa* . The Minimum Inhibitory Concentration (MIC) ranged between 25mg/ml to 150mg/ml depending on isolate and extracting solvent. Ethanolic extracts showed greater antimicrobial activity than the other two solvents. The killing rate of the Minimum Bactericidal Concentration (MBC) of the ethanolic extract on *S. aureus* and *P.aeruginosa* was $2^1/_2$hr, and 3hr respectively. Temperature stability study showed that the extracts are stable and active over a temperature range of 30 to121^{o}C.

Key words: *Enantia chlorantha*, antimicrobial property, plant extract

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***E-mail**: rfatata@yahoo.com **Tel**: 08035036850

INTRODUCTION

It is estimated that there are between 200 000 and 700 000 species of tropical flowering plants that have medicinal properties, this has made traditional medicine relatively cheaper than modern medicine.[1] Over the years there have been alarming reports of multiple drug resistant in medically important strains of bacteria and fungi.[2-7] The persistent increase in antibiotic resistant strains of organisms has lead to development of more potent synthetic antibiotics such as the 3rd and 4th generations of Cephalosporins by Pharmaceutical companies. These new antibiotics are scarce, costly and not affordable particularly in the developing countries and therefore make compliance difficult. There is therefore need for continuous search for new effective and affordable antimicrobial drugs. Local medicinal plants provide a source of new possible antimicrobial drugs.[8] Efforts of Scientists in establishing plants with promising antimicrobial property is yielding fruitful results as a number of plants with high antimicrobial property have been elucidated.[9-13] Among plants believed traditionally to have therapeutic effect but which receives little or less scientific research is *Enantia chlorantha*. Traditionally the bark extract is applied to ulcers and leprous spots for quick healing, decoction is used for washing wounds, bark sap is taken as decoction against diarrhea. This work therefore aims at investigating its suggested antimicrobial activity.

MATERIALS AND METHOD

Collection and preparation of plant material

Stem bark of the plant material (*E. chlorantha*) used was purchased from herb sellers at Baboko market in Ilorin Kwara State Nigeria. The plant was identified at the the Forestry Research Institute of Nigeria (FRIN) Ibadan. The plant was sun-dried for two weeks until constant weight was obtained. It was ground into powder and stored in sterile glass bottle at 30°C (room temperature)

Preparation of extracts

Three different solvents viz; distilled water, methanol and ethanol were used for extraction. Fifty gramme of the ground plant material was weighed and suspended in each of the three solvents, the mixtures were then left on shaker at 190 rev/min. for 24hrs at room temperature.[14] The extracts from each solvent was decanted, passed through muslin cloth, and then filtered with Whatman No 1 filter paper. The extracts were tested for purity by plating them on nutrient agar and incubated for 24hrs at 37°C and 25°C. The extract from each solvent was then stored in sterile brown bottle kept in refrigerator at 7°C.

Concentration of the extracts and preparation of stock solutions

The three extracts (water, methanol,and ethanol) were evaporated to semi-solid form on water bath at 100°C and 80°C respectively. The semi-solid yellow extracts obtained were further evaporated in oven at 110°C until constant weight was achieved for each extract. Each solid extract was reconstituted in their respective solvents to obtain a stock solution of 200mg/ml. The stock solutions obtained were then filtered using Millipore membrane filter (0.45um pore size).The sterile extracts obtained were stored in sterile capped bottles.

Test organisms and source.

The organisms used comprise of two Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogens*), six Gram-negative bacteria *(Escherichia coli*, *Salmonella typhi,*

Shigella sonnei, Proteus mirabilis. Pseudomonas aeruginosa, Klebsiella pneumoniae) and two fungi (*Aspergillus niger* and *Candida albicans*). The test organisms were collected from the Department of Medical microbiology and Parasitology of the University of Ilorin Teaching Hospital.

Standardization of inoculum.

Five colonies of each organism used were picked into Nutrient Broth (NB) and incubated at 37^{o}C for 18-24hrs for bacteria while *C. albicans*,and,the *A. niger* were incubated at 25^{o}C for 5-7days. Turbidity produced was adjusted to match 0.5 Mc Farland standard (10^{8}cfu/ml) which was further adjusted to 10^{5}cfu/ml and 10^{3}cfu/ml.[15]

Test for antimicrobial activity of the extracts.

Sterile Nutrient Agar (NA) and Potato Dextrose Agar (PDA) plates were prepared. An 18-24hr old standardized culture of bacterial and the *C. albicans* was separately used to flood the NA and PDA surfaces of each plate respectively[16], and excess was drained off. The seeding was done in such away that NA plates contained 10^{5}cfu/ml of bacterial isolates while PDA agar contained 10^{3}cfu/ml of fungal spores. A sterile cork borer of 5mm diameter was used to make six ditches on each plate. A 0.1ml of the extract from each solvent (equivalent to 20mg of the extract) was dropped into each appropriately labeled ditch. And into the remaining three ditches distilled water, methanol, and ethanol were used as positive controls. The inoculated plates were left on the table for 1hr to allow the extracts to diffuse into the agar.[15] The NA plates were incubated aerobically at 37^{o}C and PDA at 25^{o}C for 18-24hrs.The PDA plates containing *A.niger* was incubated for 7days at 25^{o}C. Zones of inhibition produced after incubation was measured in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC) of the extracts on the isolates.

Broth dilution method[15] was used. Varying concentrations of the extracts (200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml) were prepared. A 0.1ml of each concentration was added to each 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells and fungal spores. The tubes were incubated aerobically at 37^{o}C and 25^{o}C for 24hrs and 7days for bacterial and Fungal isolates respectively. Positive controls were equally set up by using solvents and test organisms without extracts. The tube with least concentration of extract without growth after incubation was taken and recorded as the MIC.

Determination of Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC).

A 1ml sample from the tubes used in MIC determination which did not show any visible growth after the period of incubation were streaked out on NA and PDA agar to determine the minimum concentration of the extract require to kill the organisms. These concentrations were indicated by the failure of the test organisms to grow on subsequent transfer to NA and PDA plates. The lowest concentration of the extract indicating a bactericidal effect after 24hrs Of aerobic incubation was regarded as the Minimum Bactericidal Concentration (MBC) while the lowest concentration that prevent fungal growth after 7days of aerobic incubation was recorded as the Minimum Fungicidal Concentration (MFC).

Determination of death rate of the isolates in the extract

Death rate of the most susceptible and the least susceptible bacterial used (*Staph. aureus* and *P. aeruginosa*) were carried out using Kelsey and Maurer method.[17] This was

carried out by mixing 0.5ml of 10^5cfu/ml of test isolates with 4.5ml of MBC of the ethanolic extract, 0.1ml of the mixture was taken and plated out on sterile nutrient agar at time intervals of 30mins for 300 mins. The plates were incubated at 37°C for 24hrs. The numbers of colonies developed on each plate at the time intervals were counted.

Effect of temperature on stability of extracts

The extracts were heated in a water bath at 30°c, 60°c, 80°c, 100°c, for 30 minutes and in an autoclave at 121°c for 15 minutes. After cooling, the extracts were tested for antimicrobial activity.

RESULTS

The results showed that the crude extracts have antimicrobial activity against all the isolates tested at 200mg/ml. Ethanolic extract showed greater antimicrobial activity than methanolic and aqueous extracts, as indicated by zones of inhibition (Table 1) *C. albican*s was the most susceptible isolate tested, while *P. aeruginosa* was the least susceptible isolate (Table1). Minimum Inhibitory Concentration (MIC) for each isolate (Table 2) showed that *Staphy. aureus*, *Stre. pyogenes*, *E. coli*, *K. pneumonia* and *A. niger* had MIC of 25mg/ml of ethanolic extract while *Pr. mirabilis*, *P. aeruginosa*, had MIC of 150mg/ml for all three extracts. MIC of ethanolic and methanolic extracts on *S. typhi* was 50mg/ml. The Minimum Bactericidal Concentration (MBC) of all isolates ranged between 200mg/ml-50mg/ml (Table 3). The effect of temperature on the stability of the extracts showed that the extracts were stable at temperature range of 30°c-121°c (Table 4). Time course study of ethanolic extract on *Staph. aureus*, showed that the extract was able to kill all the cells after 3/2hr of exposure (fig.1). The killing effect of the extract on *P.aeruginosa*, was gradual in the first 2hours of exposure but became drastic 30 mins later and the surviving cells were reduced to zero after 2.5 hours of exposure (fig.1).

DISCUSSION

The results of this work showed that the bark stem extracts of *Enantia chlorantha* inhibited the growth of all the bacteria and fungi tested (Table 1). This suggests that the plant extract is broad spectrum in activity and that its mode of action may not be due to inhibition of cell wall synthesis. Similar finding has been reported.[1,10].

Generally higher antimicrobial activity of the extracts was observed on *Staph. aureus*, *Stre. faecalis*, *E. coli, K. pneumoniae* and *A.niger*, this is similar to the earlier results obtained.[18] when extracts from *Aramomum melegueta* fruit was used. Whereas moderate antimicrobial activity of the extracts were observed on; *S. typhi* and *C. albican*. Ethanolic extracts showed the strongest activity followed by Methanolic extracts and aqueous extracts an indication that ethanol is a better extractant than the two other solvents used in this study. However aqueous extracts showed better antimicrobial effect (with MFC of 100mg/ml) on *A. niger* and *Candida albican* than Methanolic and Ethanolic extracts (Table3).

The strong activity of the extracts on *Staph. aureus, Sh. sonnei* and *E. coli* suggest that it may be used for the treatment of wound infection and diarrhea caused by these organisms. This work also revealed the potential use of extracts of this plant for use in the control of medically important organisms such as; *S. typhi* (causative agent of typhoid fever), *P. aeruginosa, C. albican* and *Aspergillus niger*. Similar results have

Table 1: Activity of crude extracts from the stem bark 0f Enantia chlorantha on clinical isolates**.**

Test organisms.	Diameter of zones of inhibition (mm).					
				Control		
	Aq. extract	Meth.extract.	Eth.extract.	Aq.	Meth	Eth.
S.aureus	12	25	28	0	0	0
Strept. Pyogen	17	18	25	0	0	0
Shigella sonnei	12	14	18	0	0	0
E.coli	12	16	25	0	0	0
Proteus mirabilis	12	12	13	0	0	0
Ps.aeruginosa	7	7	10	0	0	0
Kleb.pneumonia	12	12	14	0	0	0
Salmonella typhi	11	14	18	0	0	0
Aspergillus niger	14	13	25	0	0	0
Candida albicans	17	18	28	0	0	0

Aq.= Aqueous Meth = Methanol Eth = Ethanol .

Table 2: Minimum Inhibitory Concentration (MIC) of the crude Extracts of *Enantia chlorantha* on clinical isolates.

Test organisms	Minimum Inhibitory Concentration MIC(mg/ml) in		
	Aqueous	Methanol	Ethanol.
Staphylococcus aureus	25.0	25.0	12.5
Streptococcus pyogen	100.0	25.0	25.0
Shigella sonnei	100.0	50.0	50.0
Escherichia coli	100.0	50.0	25.0
Proteus mirabilis	150.0	150.0	150.0
Pseudomonas aeruginosa	150.0	50.0	25.0
Klebsiella pneumoniae	150.0	50.0	50.0
Salmonella typhi	150.0	50.0	50.0
Aspergillus niger	50.0	50.0	25.0
Candida albican	100.0	50.0	50.0

Table 3: Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC) of crude extracts of *Enantia Chlorantha* on clinical isolates.

Test organisms	MBC and MFC of the extracts (mg/ml) in		
	Aqueous	Methanol	Ethanol.
S. aureus	50.0	50.0	50.0
Streptococcus faecalis	100.0	50.0	100.0
Shigella sonnei	150.0	100.0	100.0
Escherichia coli	150.0	150.0	150.0
Proteus mirabilis	200.0	150.0	150.0
Pseudomonas aeruginosa	200.0	200.0	150.0
Klebsiella pneumoniae	200.0	200.0	150.0
Salmonella typhi	200.0	150.0	150.0
Aspergillus niger	100.0	150.0	150.0
Candida albican	100.0	150.0	200.0

Table 4: Effect of temperature on the stability and activity of crude extracts of *Enantia chlorantha.*

Test organisms	Zones of inhibition (mm) at different temperature (^{o}C)							
	30	50	60	70	80	90	100	121
S. aureus	25	25	25	25	25	25	25	25
Strept.pyogen	30	30	30	30	30	30	30	30
Shigella sonnei	15	15	15	15	15	15	15	15
E. coli	30	30	30	30	30	30	30	30
Proteus mirabilis	15	15	15	15	15	15	15	15
Ps. aeruginosa	9	9	9	9	9	9	9	9
K.pneumoniae	15	15	15	15	15	15	15	15
Salmonella typhi	15	15	15	15	15	15	15	15
Aspergillus niger	13	13	13	13	13	13	13	13
Candida albicans	20	20	20	20	20	20	20	20

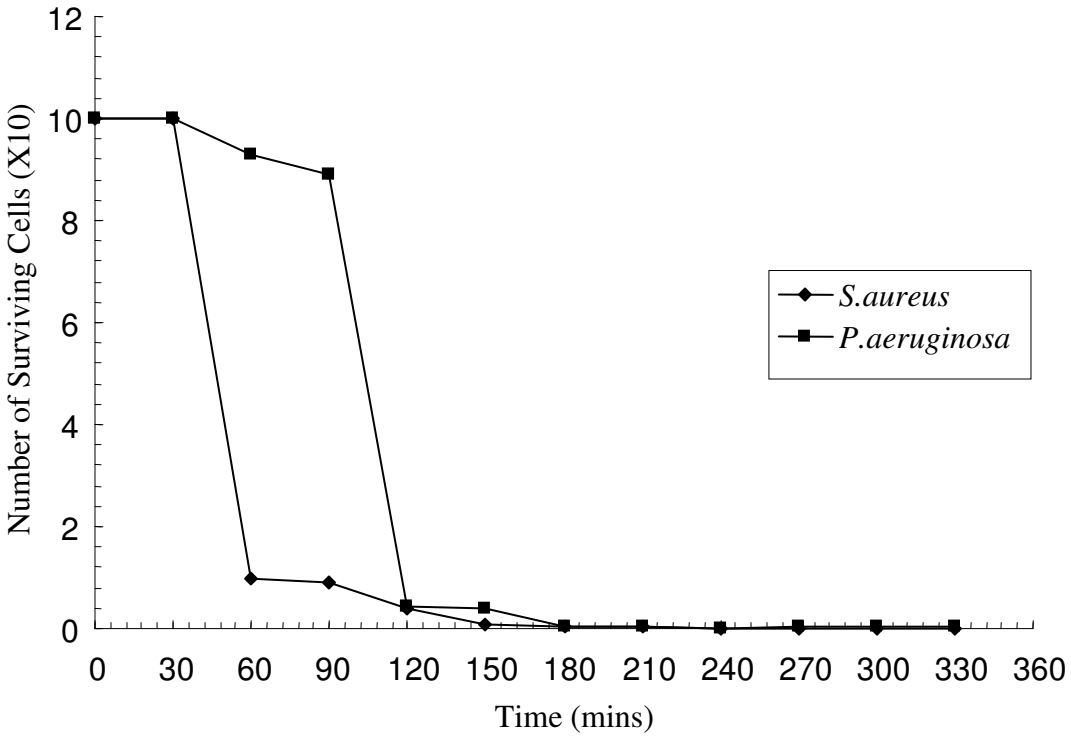

Figure 1: Death rate *of S.aureus* and *P.aeruginosa* in MBC, of *Enantia chlorantha*

been obtained with extracts from leaves of *Kalanchoe pinnate* when tested against *S.aureus, E.coli, B.subtilis, Pr.vulgaris, P.aeruginosa* and *C. albicans*[19]. The killing rate of extracts on *Staphy. aureus*, showed that the extracts has strong killing effect on the organism, it reduces the cells from 10^5cfu/mlt to less than 10^2cfu/ml within 90 minutes of exposure (fig.1). Killing rate of the extracts on *P. aeruginosa* however took longer time to reduce to less 10^2cfu/ml; it was observed that after 5hr of exposure to the extract there was sudden increase in numbers of surviving cells (fig1). It could be concluded from this study that the activity of the extracts from bark stem of *Enantia chlorantha* stem showed activity against the tested isolates and probably justify its local use. Further work on identification and purification of the extracts to find out the active principle responsible for the antimicrobial property of the plant extracts is required.

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