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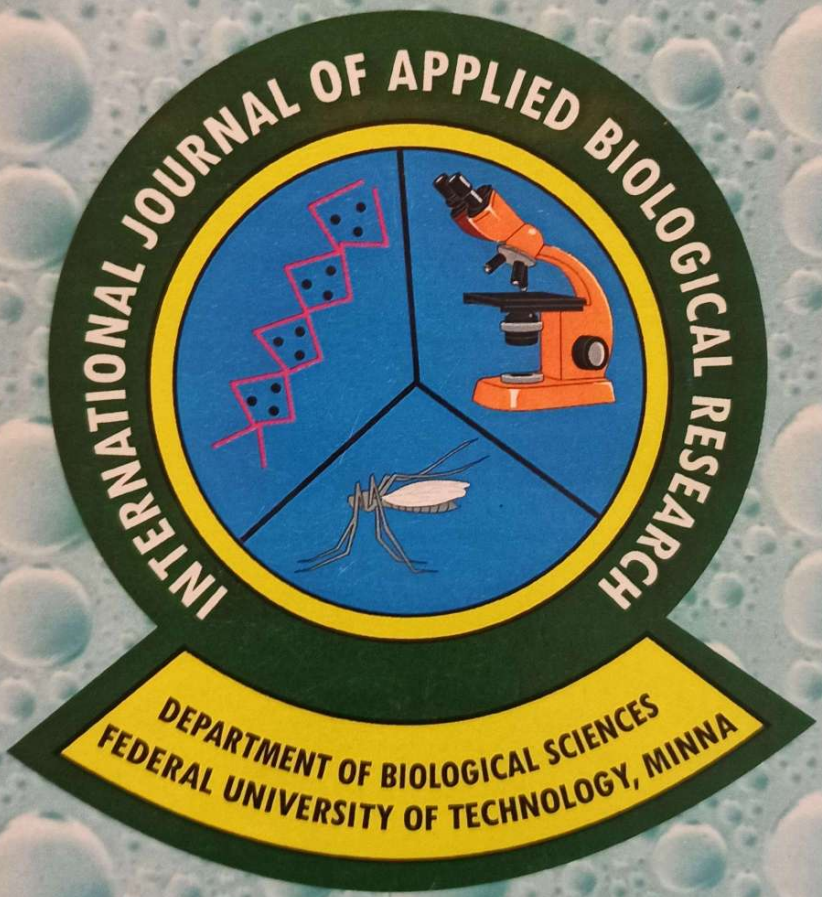

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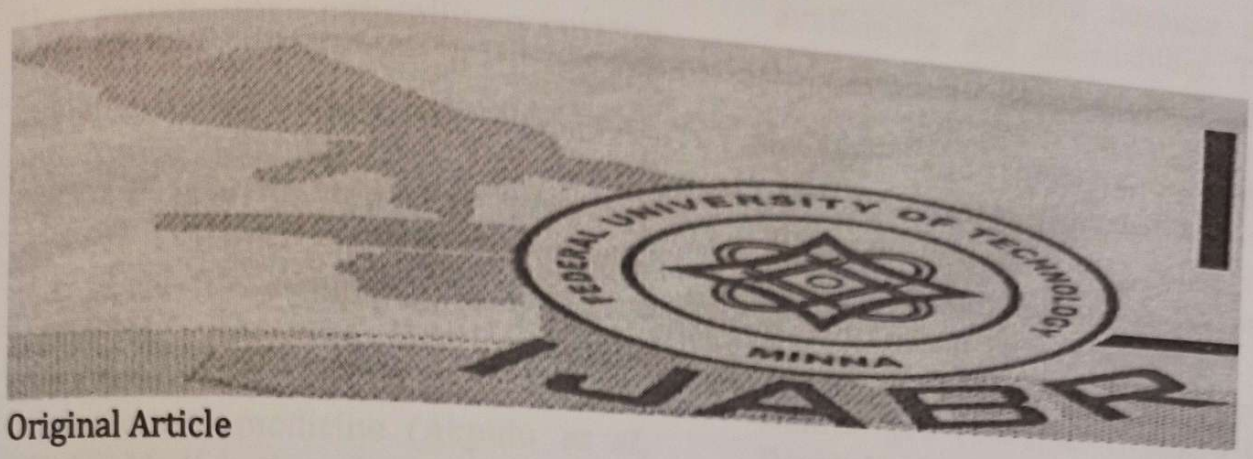

Original Article

ANTIBACTERIAL EVALUATION OF THE METHANOLIC EXTRACT OF THE LEAF AND STEM BARK OF *ENANTIA CHLORANTHA*

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ABSTRACT

Studies on the antibacterial activity of methanolic extracts of the stem bark and leaf of *Enantia chlorantha,* were carried out. The antibacterial activity of the methanolic extract was studied using agar well diffusion and tube dilution methods. The bacteria used were *Salmonella typhi, Pseudomonas aeruginosa,* and *E. coli.* The results revealed that the methanolic extract of the stem bark of *E. chlorantha* showed wider zones of inhibition on the bacterial isolates than the leaf extract. The zone of inhibition decreases with the decrease in concentration in both methanolic extract of the leaf and stem bark. The Minimum Inhibitory Concentration (MIC) of the methanolic extract of both stem bark and leaf ranged from 12.5mg/ml to 25mg/ml, while Minimum Bactericidal Concentration (MBC) of the methanolic extract of both leaf and stem bark were at 50mg/ml respectively. The result suggests that the extract possess some active components that may be used for the development of therapeutic agents for the treatment of ailments associated with the test organisms.

Key words: E*nantia chlorantha,* Antibacteria, Stembark,

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INTRODUCTION

The search for naturally occurring antimicrobial and antifungal substances in plants chemotherapy is gaining more importance (Okigbo and Ogbonnaya, 2006). 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 (Akpulu *et al.,* 1994). Medicinal and aromatic plants which are a gift of nature have been used against various diseases and infections in the world since medieval time (Khali *et al.,* 2007). The substance that can either inhibit the growth of pathogens or kill them and no or least toxicity to host cells are considered candidate for developing new antimicrobial drugs. The roles of plants in the development of new drugs is in two folds; (i) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (ii) a phytomedicine to be used for the treatment of diseases (Igbinosa *et al.,* 2009).

Medicinal plants play a key role in human and animal health care. Higher plants have been shown to be a potential source for the new antimicrobial agent. The screening of plant extract has been of great interest to scientists in the discovery of new drugs effective for the treatment of several diseases (Alim *et al.,* 2009).

Phytomedicinal preparations are becoming increasingly patronized for the treatment of ailments because orthodox medicine is not readily available in some places besides the fact that the first line drugs which have become ineffective due to resistance development. Herbal preparations are becoming more widely used by people all over the world because of their availability and affordability. The fact that most of them can be used safely without any side effect make it more popular and appealing (Udobi and Onaolapo, 2009).

Enantia chlorantha Oliv, belong to the Family of Annonaceae and commonly called African Yellow Wood. It is locally known as Awogba, Osopupa, Awopa or Dokita igbo (Yoruba), Erumeru (Igbo), Osomolu (Ikale), Kakerim (Boki) and Eremba-Vbogo (Bini) (Gbadamosi and Oni, 2004). It is an Ornamental tree of up to 30m high, with dense foliage and spreading crown. The stem is fluted while the outer bark which is thin and dark brown is fissured geometrically; while the inner bark is brown above and pale Cream beneath. (Iwu, 1993). The plant is widely distributed along the forest and coastal areas of West Africa. It is also commonly found in the forest regions of Nigeria, and the Democratic Republic of Congo (Adesokan *et al.,* 2007). In traditional medicine, the plant is used in the treatment of coated tongue, typhoid fever, malaria, jaundice, ulcer, rickettsia and infective hepatitis (Olowokudejo *et al.,* 2008; Atukpawu and Ozoh 2014).

MATERIALS AND METHODS

Collection and identification of plant

Fresh leaf and stem bark of *Enantia chlorantha* were obtained in a forest at Kajola-ibooro Village, Ado-odo Ota local government Area of Ogun state. The plant was identified and authenticated in the Department of Biological sciences, Federal University of Technology Minna Niger state, Nigeria where voucher specimen was deposited at the herbarium.

Preparation of plant extract

The leaf and stem bark of *Enantia chlorantha* obtained were thoroughly washed with tap water to remove debris. The samples were air dried for three weeks. Exposure to sunlight was avoided to prevent possible loss of active compounds. The air-dried leaf was milled into fine powder using an electric blender (Blender /Miller III, model MS-223, Taiwan, China). The stems bark was pulverized with mortar and pestle into fine powder.

Plant extraction

About 200g of the powdered plant samples (leaf and stem bark) were measured using an electric weighing balance and macerated into 1000ml of 70% methanol respectively in a sterile conical flask for 48 hours at ambient temperature with vigorous shaking at interval. The extracts were filtered first using a sterile muslin cloth and then a Whatman No. 1 filter paper. The extracts produced were concentrated to dryness on water bath. The dry extracts were weighed and tested for purity by plating them on nutrient agar for 24 hours at 37ºC and then used to prepare stock solutions for the susceptibility test.

Test organisms

The Bacteria used for this study were *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella tyhpi.*. These organisms were obtained from the laboratory of the Department of Microbiology, Federal University of Technology, Minna. The organisms were maintained on nutrient agar (NA) slant and then stored in the refrigerator at 4^{0}C until required.

Standardization of inoculum

Pure stock culture of test organisms were picked and inoculated into 5ml sterile nutrient broth and incubated for 18 hours. Aliquots of 0.1ml from overnight culture of the organism was dispensed into 5ml of sterile nutrient broth and incubated for 3 hours. Turbidity produced was adjusted to match 0.5 McFarland's standard (Coyle, 2005).

Screening for antimicrobial activity

The agar-well diffusion method was adopted for the susceptibility studies. A loopful each of 18 hours old standardized culture, adjusted to 0.5 McFarland was streaked on a sterile plate of Nutrient Agar (NA). A sterile cork borer of 7mm diameter was used to make five wells on each plate. 0.1ml of the extract of *E. chlorantha* leaves and stem bark were dispensed into each well appropriately labeled as A (200mg/ml), B (100mg/ml), C (50mg/ml), D (0.1ml water) and E (100mg/ml Ampiclox) respectively. Water served as negative control while the Ampiclox was used as positive control. Precaution was taken to avoid spillage of the extract on the surface of the media. The inoculated plates were left for one hour to allow the extracts diffuse into the agar. Each analysis was done in triplicates. The bacterial culture plates were then incubated at 37^{0}C for twenty-four hours (Coyle, 2005).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts against the test organisms was determined using the broth dilution method as described by Coyle (2005). Aliquots of 1ml of stock

extract at the concentration of 100mg/ml was added to 1ml of fresh nutrient broth and serially diluted to obtain extract concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.56mg/ml and 0.7mg/ml in seven different test tubes. Using a syringe, 1ml of standardized test organisms adjusted to 0.5 Mcfarland turbidity standard (10^8cfu/ml) was inoculated into each test tube. Controls were equally set up by using only the nutrient broth alone and test organisms without extract. The tubes were then incubated at 37^{0}C for 24hours. The tube with least concentration of extract with no detectable growth after incubation when checked visually for turbidity was taken and recorded as the MIC.

Determination of the Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the plant extract on the clinical bacterial isolates was done according to the method described by National Committee for Clinical Laboratory Standard (1990). Aliquots of 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 for 24hours to determine the minimum bactericidal concentration of the extract that will kill the organisms. The lowest concentration of the extract indicating a bactericidal effect after incubation was regarded as the Minimum Bactericidal Concentration (MBC).

RESULTS AND DISCUSSION

The methanolic extracts of the leaf and stem bark of *E. chlorantha* showed antibacterial activity against all the isolates at 200mg/ml concentrations. The methanolic stem bark extract gave wider zones of inhibition than the leaf extract (Tables 1 and 2). The zone of inhibition increased significantly ($p < 0.05$) with increasing concentrations of the extract on the isolates. The diameter of each zone of inhibition was used in this study to estimate an organism's sensitivity to a particular extract. It is not worthy to mention that all the organisms were more sensitive to the methanolic stem bark extracts of *E. chlorantha* than that of the leaves. At 50mg/ml concentration of the extracts of *E. chlorantha* leaves, *S. typhi* and *E. coli* showed resistance. The methanolic extract of *Enantia chlorantha* stem bark showed broad spectrum activity as all the isolates were sensitive to it, with zones of inhibition comparable to the antibiotics used as standard.

The Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts differed (Tables 3 and 4). The MIC values obtained for the entire test organisms ranged from 12.5mg/ml to 25mg/ml while the MBC for the organisms was at 50mg/ml. The MIC values obtained for the entire test organisms were very high when compared to the values of 0.01-10µg/ml usually recorded for typical antibiotics, these differences 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 MBC varied from the MIC, indicating that a different concentration is needed to inhibit the growth of the bacteria and an entirely different concentration to kill them (Atukpawu and Ozoh, 2014). 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 (Adesokan *et al.*, 2008).

The result of the antibacterial activity of *Enantia chlorantha* stem bark was consistent with that obtained by Razaq *et al* (2003) , Adesokan *et al.* (2008) and (Atukpawu and Ozoh 2014) .

CONCLUSION

The methanolic extracts of the stem bark and leaf of *Enantia chlorantha* showed a broad spectrum antibacterial activity against the test pathogenic bacteria. The result of this study lend credence to the use of this plant in traditional folklore and this suggests that traditional medicine could be used as guide in the continuous search for new antimicrobial agents. It also forms the basis for further investigation on the purification and structural determination of the most promising constituents for *in vivo* evaluation of toxicity of this plant in animal and human studies.

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Table 1: Zones of inhibition (in mm) of the different concentrations of the methanolic extracts of *E. chlorantha* stem bark on the test bacteria.

Methanolic extract	*S.typhi*	*P.aeruginosa*	*E.coli*
200mg/ml	24.33 ± 0.67^{d}	26.00 ± 2.08^{c}	24.33 ± 2.96^{b}
100mg/ml	16.00 ± 1.00^{c}	21.00 ± 0.58^{b}	17.67 ± 1.45^{a}
50mg/ml	11.67 ± 0.33^{b}	15.33 ± 1.45^{a}	15.33 ± 1.45^{a}
Ampliclox	0.00 ± 0.00^{a}	35.67 ± 1.33^{d}	32.00 ± 1.00^{c}
Water	-	-	-

(-) No zones of inhibition, Values are means triplicates ± S.E.M.
Values followed by the same superscript, in the same column, are not significantly different ($P>0.05$)

Table 2: Zones of inhibition (in mm) of the different concentrations of the methanolic extracts of *E. chlorantha* leaf on the test bacteria.

Methanolic extract	*S. typhi*	*P. aeruginosa*	*E. coli*
200mg/ml	22.67 ± 1.45^{c}	23.33 ± 0.88^{c}	16.00 ± 2.08^{b}
100mg/ml	14.67 ± 0.88^{b}	19.00 ± 0.58^{b}	13.33 ± 1.86^{b}
50mg/ml	0.00 ± 0.00^{a}	13.67 ± 1.20^{a}	0.00 ± 0.00^{a}
Ampliclox	0.00 ± 0.00^{a}	35.00 ± 1.15^{d}	22.67 ± 2.67^{c}
Water	-	-	-

(-) No zone of inhibition,Values are means of triplicates ± S.E.M.
Values followed by the same superscript, in the same column, are not significantly different ($P>0.05$)

TABLE 3.: Minimum Inhibitory Concentration (MIC) of methanolic extracts of the leaf and stem bark of *E. chlorantha*

Test organisms	MIC of extracts (mg/ml)	
	Leaf	Stem bark
S. typhi	25	12.5
P. aeruginosa	25	12.5
E. coli	25	12.5

TABLE 4 : Minimum bactericidal Concentration (MIC) of methanolic extracts of the leaf and stem bark of *E. chlorantha*

Test organisms	MBC of extracts (mg/ml)	
	Leaf	Stem bark
S. typhi	50	50
P. aeruginosa	50	50
E. coli	50	50