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Full Length Research Article

COMPARATIVE ANTIBACTERIAL EFFICACIES OF THE EXTRACTS OF *ENANTIA CHLORANTHA* (AWOPA) ON SELECTED CLINICAL ISOLATES

Ajijolakewu Abiodun Kamoldeen[1*], Ajide-Bamigboye Nimat Toyosi[1], Omotunde Munirat Yetunde[1], Ayoola Saheed Abiodun[1,2]

[1]Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Nigeria, PMB 1515, Ilorin
[2]Bioresources Development Centre, National Biotechnology Development Agency, Ogbomosho, Oyo State, Nigeria

*Corresponding Author Email Address: ajijolakewu.ak@unilorin.edu.ng Phone: +2348066980264

ABSTRACT
Researchers and health scientists are challenged by the phenomenon of antimicrobial resistance which has necessitated the need for the discovery of novel antimicrobial agents. This study evaluated the antimicrobial efficacy of the bark of *Enantia chlorantha* (Awopa) based on extractions using selected polar and non-polar solvents (Aqueous, Dichloromethane, Petroleum ether, and N-hexane). Antimicrobial activities were investigated against six clinical isolates (*Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*) via the agar well diffusion assay. Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) were also determined by the macro broth dilution method. The phytochemical components of the extracts were analyzed qualitatively and quantitatively. The dichloromethane extract of *E. chlorantha* expressed the highest antimicrobial activity against all the test organisms with *K. pneumonia* being the most susceptible with (21.50 mm zone of inhibition) at a concentration of 50mg/mL; while *C. albicans* was the least susceptible organism with 3.00 mm zone of inhibition at concentration of 500mg/mL. On the other hand, extract from the petroleum ether expressed no antimicrobial activities, based on the absence of the zones of inhibition around the cultures of the respective organisms. Mainly the Dichloromethane and aqueous extracts of *E. chlorantha* expressed varying organism-dependent MIC on the test isolates. While the lowest MIC of dichloromethane extract was expressed on *S. aureus* at 6.25mg/mL; the highest MIC (50mg/mL) was exerted on *C. albicans*. Phytochemical screening of the extracts revealed the presence of glycosides, oxalates, steroids, alkaloids, tannins, flavonoids, phenol, triterpenes, saponin and phytate; while terpenoid was not found in all the plant's extracts. Findings in this study suggest that the bioactive ingredients of *E. chlorantha* barks could be best extracted by polar solvents which are evident in the superior antimicrobial activities of dichloromethane and aqueous extracts. The non-polar solvents such as n-hexane and petroleum ether are poor extractant, thus necessitating the need to carefully select suitable solvent for the extraction of any plant's bioactive compounds.

Keywords: *Enantia chlorantha* (Awopa); Phytochemical screening; Polar and non-polar solvents; bioactive compounds; Antibacterial efficacies.

1.0 INTRODUCTION

Bacterial infectious diseases constitute a major public health problem all over the world and more importantly in Africa where living conditions are precarious (Etame *et al*., 2018) The incidence of infectious diseases has been greatly reduced due to the discovery of antibiotics which have been a great relief for humanity (Bevilacquia, 2011). However, the selection pressure by bacteria which is caused by, but not limited to, inappropriate prescription and indiscriminate use of antibiotics by the population is a major menace in the management of bacterial diseases for several decades (WHO, 2011). This has led to the emergence of resistant bacteria leading to higher frequency of therapeutic failures, high mortality and a high costs of treatment. It has been identified in earlier reports (Ajijolakewu *et al*., 2021; Cerceo *et al*., 2016) projected that identifying new sources of natural products with antimicrobial substances that are effective, available and low in toxicity could serve as alternative remedy against bacterial infections.

Traditional medicine based on plants is one of the oldest and diversified therapeutic systems in African countries (Mahomoodally, 2013). Plants used as medicine possess bioactive components which play vital roles in the treatment of various human diseases (Hasler *et al*., 2000). These bioactive components provide unlimited opportunity for new drugs discovery because of the readily available medicinal diversity (Cos *et al*., 2006). The antibacterial activity of plants have been demonstrated through many approaches which include crude extract preparation using variety of solvents (Iwalewa *et al*., 2009) purification of active compounds from the extracts (Voukeng *et al*., 2017; Seukep *et al*., 2016; Djeussi *et al*., 2015; Tankeo *et al*., 2015) successive extraction with solvents with increasing polarity (Njateng *et al*., 2016) and distillation. The most common approach is the solvent extraction however recent studies have established high activity with fractionation of crude extracts (Etame *et al*., 2018).

Enantia chlorantha (*Annickia chlorantha*) belongs to the *Annonaceae* family, this plant is commonly known as African yellow wood and is also called Awopa or Osu pupa (in the Yoruba language, Nigeria) (Akinwale *et al*., 2022). It is a dense forest tree found in the Southern parts of Nigeria, Gabon Angola and DRC. It is also found in the East and Southern part of Cameroon. The stem barks, leaves and roots are used in Africa to treat jaundice, urinary infection and leprosy spots (Etame *et al*., 2018). The bark of *E. chlorantha* has several medicinal properties and has been used by traditional medical practitioners in Nigeria for the treatment of skin, gastric and duodenal ulcers, and as an antimalarial (Siminialayi and Agbaje, 2005). However, there is a dearth of information on which type or form of solvent best extracts the bioactive components of the plant. There is also a need to investigate its spectrum of activities on more strains of microorganisms mostly

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implicated in common bacterial diseases. Hence, this study compares the efficacies of selected polar and non-polar solvents in order to determine the best solvent for the extraction of the bioactive principle in the bark of *E. chlorantha* and to assess the efficacies of the extracts against selected clinical isolates.

2.0 MATERIALS AND METHODS

2.1. Materials

2.1.1. *Plant material*: The barks of *E. chlorantha* were collected from the Botanical Garden, University of Ilorin, Ilorin, Kwara State. The plant samples were authenticated at the herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria.

2.1.2 *Clinical Isolates*: The test isolates_ *Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pneumoniae*- were collected from the Culture Collection Center, Microbiology Unit of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. Standard biochemical tests were carried out to confirm the identity of the test isolates in accordance with the procedure described in the Bergey's manual of systematic bacteriology (Galushko and Kuever, 2020). These microorganisms were maintained at 4°C on Mueller Hinton Agar (MHA) (Liofilchem, Italy).

2.2 Methods

2.2.1. *Extract preparation:* The barks of *E. chlorantha* were dried and pulverized into fine powder and was used for subsequent extractions. A 150 g of the powder was placed in different glass jars and 600 mL of each extractant were respectively added to the glass jars. The jars containing the substrate and solvents were placed in a shaker and subjected to 100 rpm for 48 hours. The obtained extracts were sieved through muslin cloth and then filtered using Whatman filter paper. Thereafter, the resulting solution was concentrated under vacuum at 40°C in a rotary evaporator. Concentrates were air-dried until constant weight as earlier described (Ajijolakewu and Awarun, 2015).
Reconstitution of Extracts: The respective extracts were reconstituted by dissolving 1g or 2g of the extract into 2-4 mL of dimethylsulfoxide (DMSO) to prepare two stocks of 1000 mg/mL and 500 mg/mL extract solution. Stocks were diluted until ten-folds concentrations were achieved.

2.2.2. Antimicrobial activity
a) *Preparation and Standardization of Inocula*: An 18-hours old culture of actively growing isolates was inoculated into a tube containing 3 mL Mueller Hinton Broth (Liofilchem, Italy). After 24 h, each suspension was standardized at 600 nm to 1.5 ×10^6 CFU/mL.
b*) Antimicrobial Susceptibility Testing (AST)*: Agar well diffusion assay was employed for the preliminary screening of the plant extracts against the test organisms. Plates containing MHA were inoculated with 1.5 × 10^6 CFU/mL of the inoculum of each test organisms. Thereafter, 100 µl of respective extracts was filled in a 7 mm wells made on the plate using sterile cork borer. The plates were incubated in an upright position at 37°C for 24hours. For positive control, standard antibiotics (Streptomycin) was used. The presence of zone of inhibition (ZI) indicates the antimicrobial potential of the plant extracts (Ajijolakewu & Awarun, 2015).
c*) Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):* The MIC was determined by the macro broth dilution method as described in the MO7 reference guidelines of Clinical and Laboratory Standard Institute (Jean *et al*., 2015). Test tubes containing 3 mL of MHB were inoculated with 10 µl of the extracts at varying concentrations as described in section 2.2.2.c and incubated at 37°C for 24hours. The lowest concentration with little or no detectable growth was regarded as MIC. The MBC was determined by obtaining a loopful using sterile inoculating wire loop from each test tube that showed little or no detectable changes in turbidity and was plated on fresh MHA. The plates were incubated at 37°C for 24 hours. Concentrations that showed no growth in the subcultures were regarded as bactericidal or fungicidal; while those that showed growth after 24hours are regarded as bacteriostatic or fungistatic.

2.2.3 Phytochemical Screening of Extracts.
The various extracts were screened for the presence of alkaloids, triterpenes, sterols, flavonoids, tannins, glycosides and other phytochemicals according to the common phytochemical methods according to the method described by Harbone (1973).

3.0 RESULTS AND DISCUSSION

This study evaluated and compares the polar and non-polar solvent's extracts of the stem bark of *E. chlorantha* in order to determine the best solvent for the extraction of its bioactive principles against selected clinically important isolates. The presence and the extent of bioactive components in the extract which confers the antibacterial activities were also assessed.

3.1 Antimicrobial Activity

Tables 1-4 below show the respective responses of the various clinical isolates to the antimicrobial activities of the respective extracts. The aqueous extract of the plant was efficacious on only three isolates- the *K. pneumomiae* (16mm) which was susceptible at all tested concentrations, *S. aureus* and *S. pneumoniae.* Meanwhile, the three others- *C. albicans, E.coli* and *P. aeruginosa* showed no susceptibility at all tested concentrations. On the other hand, dichloromethane extract exerted its antimicrobial activity against all the isolates with *K. pneumoniae* being the most susceptible (21.50 mm) and *E. coli* being the least susceptible. N-hexane extract showed minimal antimicrobial activity against the test organisms with the zone of inhibition ranging from 4-8mm. However, extracts of the petroleum ether exerted no antimicrobial activity against any of the six test organisms at all concentration. The standard antibiotics only exerted antimicrobial activities at higher concentrations (100 to 1000 mg/mL) against the test isolate; while lower at lower concentrations, no growth inhibition of the test organisms was recorded.

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Table 1: Antimicrobial Activity of aqueous extract of *E. chlorantha*

Concentrations	Zones of Inhibition (mm)					
(mg/mL)	*C. albicans*	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. pneumoniae*	*P. aeruginosa*
2000	__	__	16.0	8.2	__	__
1000	__	__	8.5	5.8	__	__
500	__	__	8.0	4.2	__	__
250	__	__	8.0	3.8	__	__
125	__	__	12.0	5.8	__	__
100	__	__	5.8	__	__	__
50	__	__	8.5	__	4.2	__
12.5	__	__	5.0	__	4.2	__
6.25	__	__	10.0	__	4.5	__
3	__	__	6.5	__	6.5	__
Control	__	__	__	__	__	__

Table 2: Antimicrobial Activity of Dichloromethane extract of E. chlorantha

Concentration	Zones of Inhibition (mm)					
(mg/mL)	*C. albicans*	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. pneumomiae*	*P. aeruginosa*
2000	5.2	__	__	6.0	4.0	__
1000	4.5	__	5.0	6.0	__	4.0
500	__	__	9.0	7.0	5.0	3.0
250	4.5	16	6.0	6.0	__	7.0
125	6.0	4.0	3.5	4.0	10	7.0
100	4.5	__	5.0	11.0	7.0	4.0
50	4.5	__	22.0	13.0	__	5.0
25	7.0	3.0	10.0	__	__	4.0.
12.5	7.0	__	16.0	8.0	11.0	4.0
6.25	__	__	6.0	7.0	8.0	3.0
Control	__	__	__	__	__	__

Table 3: Antimicrobial Activity of n-hexane extract of *E. chlorantha*

Concentration (mg/mL)	Zones of Inhibition (mm)					
	C. albicans	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. pneumoniae*	*P. aeruginosa*
1000	__	__	__	__	__	__
500	4.5	7.2	6.2	5.0	6.2	5.8
250	5.5	3.2	6.0	7.4	5.0	5.2
125	5.5	6.8	7.2	7.2	3.0	6.2
100	6.8	4.2	5.0	5.2	5.4	4.2
50	__	__	__	__	__	__
25	6.2	6.8	5.0	4.8	4.8	4.0
12.5	5.8	6.0	3.8	6.2	6.2	4.2
6.25	__	__	__	__	__	__
3	__	__	__	__	__	__
Control	__	__	__	__	__	__

Table 4: Antimicrobial Activity of Petroleum ether extract of *E. chlorantha*

Concentrations (mg/mL)	Zones of Inhibition (mm)					
	C. albicans	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. pneumoniae*	*P. aeruginosa*
1000	__	__	__	__	__	__
500	__	__	__	__	__	__
250	__	__	__	__	__	__
125	__	__	__	__	__	__
100	__	__	__	__	__	__
50	__	__	__	__	__	__
25	__	__	__	__	__	__
12.5	__	__	__	__	__	__
6.25	__	__	__	__	__	__
3	__	__	__	__	__	__
Control	__	__	__	__	__	__

3.2. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The various extracts exhibited organism-dependent inhibitory effects but no bactericidal efficacies were observed. Dichloromethane extract exerted MIC in all the test organisms at concentrations ranging from 6.25 to 50mg/mL. The MIC of both the aqueous and n-hexane extracts was minimum at 12.5/25 mg/mL against *S. pneumoniae* but maximum at 500 mg/mL against *S. aureus*.

Table 5: Minimum inhibitory concentration and Minimum Bactericidal Concentration of aqueous extract on test organisms

Organisms	Minimum Inhibitory Concentrations of all extracts (mg/mL)			Minimum Bactericidal Concentrations of all extracts		
	Aqueous	Dichloromethane	N-hexane	Aqueous	Dichloromethane	N-hexane
C. albicans	__	50	125	__	__	__
E. coli	__	25	100	__	__	__
K. pneumoniae	100	25	250	__	__	__
S. aureus	500	6.25	250	__	__	__
S. pneumoniae	12.5	12.5	25	__	__	__

3.3 Qualitative and Quantitative Phytochemical Analysis

The aqueous extract of *E. chlorantha* contains a high amount of glycosides (143.60 mg / 100 g of the extract) and oxalates (69.40 mg /100 g of the extract. Steroids, alkaloids, tannins, and saponin were present in moderate amounts (2.75 – 20. 67 mg / 100 g of the extract) whilst flavonoids, phytates, phenols, and triterpenes were present in low amounts (0.58 - 8.04 mg /100 g of the extract), the least available phytochemical was phenol (0.58 mg / 100 g) while Terpenoid was not detected. On the other hand, the dichloromethane extract showed relatively higher amount of glycosides (150 mg) and oxalates (108 mg) per 100 g of extract. Steroids, phytates, alkaloids, tannins, and flavonoids were present in moderate amounts whilst phenol, triterpenes and saponin were present in low amounts. The least available phytochemical was saponin, 0.3mg/100g.

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Table 6: Comparative Quantitative Analysis of Extracts of *E. chlorantha*

Phytochemical	Values (mg/100g)	
	Aqueous extract	Dichloromethane
Alkaloids	11.44	12.00
Saponin	2.75	0.36
Tannin	10.16	10.12
Phenol	0.58	0.95
Flavonoid	2.84	6.22
Terpenoid	0.00	0.00
Steroids	20.67	26.00
Glycosides	143.60	150.00
Phytates	0.95	13.00
Triterpenes	8.04	0.48
Oxalates	69.40	108.00

The antibacterial properties of medicinal plants have been demonstrated through many approaches and the plant diversity promises new drug discovery. In this study, the antibacterial efficacies of the bark of *E. chlorantha* were assessed using the extracts of various polar and non-polar solvents with a view to determine the best extractants for its bioactive antimicrobial agents.

As shown in section 3.1, all extracts of *E. chlorantha* exerted organism-dependent inhibitory effect. Both the aqueous and dichloromethane extracts showed significantly higher antimicrobial activities against all the clinical isolates (Table 1 & 2), n-hexane showed minimal antimicrobial activity (Table 3) while the extract of petroleum ether has insignificant effect on the test organisms (Table 4). This shows that the antimicrobial activity of the bark of *E. chlorantha* is largely dependent on the type of solvent used for the extraction of its active principle. Sarbadhikary and George (2022) opined that the therapeutic potential of chemically complex plant extracts depends on and varies with the interactions among compounds and their proportions within the extract.

Comparatively, the dichloromethane extract showed significantly higher antimicrobial activities compared to other extracts. All the test organisms were susceptible to the extract, albeit at different concentrations and MICs (Table 5). Umaru and Umaru (2018) projected the antibacterial activities of various concentration of dichloromethane crude extract of the plant *Leptadenia hastata* that was tested against some pathogenic bacteria such as; *K. pneumoniae*, *E. coli*, *S. aureus* and *Salmonella typhi*. All the concentrations used were active against all test organisms, the highest activity was recorded against *E. coli* and *S. aureus* at the concentrations of 500 ppm and 1000 ppm (1.03±0.06 mm, 1.23±0.06 mm and 1.23±0.06 mm, 1.33±0.06 mm) respectively. The aqueous extract has appreciable antimicrobial activities against the test organisms at varying concentrations. *K. pneumoniae* showed the highest susceptibility and an MIC of 100 mg/mL while *E. coli* and *P. aeruginosa* were the least susceptible. In the same order, Adesokan *et al*. (2005) had earlier reported that the aqueous bark extract of this plant showed antibacterial activity against *S. aureus, Bacilus subtilis*, *P. aeruginosa*, and *S. typhi* with MIC values ranging between 25 and 105 mg/mL.

On the other hand, the n-hexane extract showed little antimicrobial activity against the test organisms (Table 3); while the petroleum ether extract showed no antimicrobial activities against all the test organisms (Table 4). These two extracts are from non-polar solvents which has been projected according to Etame *et al*. (2018) as bad extractant. Generally, *K. pneumonia* was the most susceptible organism to the extracts of *E. chlorantha* (Tables 1 to 4). This has also been observed in an earlier report by Etame *et al*. (2018) where the methanolic extract of E. chlorantha has the highest antibacterial activity against *Salmonella enterica* serotype paratyphi are most sensitive whereas isolates from *E. coli* (EC 96), *E. aerogenes* (ENT 118) and *S. aureus* (MRSA 12) were found to be less sensitive. The variability observed could be attributed to the solvent used, the antibacterial test method, the soil, and the time of collection of plant parts. Hence, extracts from *E. chlorantha* could be used as a potential drug candidate for the treatment of the disease caused by the organisms.

The higher antibacterial activities of both the aqueous and dichloromethane extracts on one hand and the relatively higher activities of the dichloromethane extract with broad spectrum activities as compared with aqueous extract with limited activity could be due respectively to the presence or relatively higher composition of the bioactive principles in each of the extracts. Ajijolakewu *et al.* (2021) had opined that the higher the propensity and the extractability of important bioactive compound of plant origin, the higher the antibacterial efficacy of the plant. This condition is however dependent on which type of extractant employed. Generally, polar solvents have been shown as a good extractant as opined in various earlier researches (Ajijolakewu and Awarun, 2015; Etame *et al*., 2018 & Abike *et al*., 2020) and supported by the findings in this study. Hence, it could be concluded that the active ingredients of *E. chlorantha* barks could be best extracted by polar solvents as evident in the antimicrobial activities of aqueous and dichloromethane extracts. The non-polar solvents such as n-hexane and petroleum ether are poor extractant, thus necessitating the need to carefully select suitable solvent for plant extraction for bioactive compounds together with experimental methods.

The phytochemical analysis of the bark of *E. chlorantha* for the solvents used showed varying differences in the amount of the phytochemical present. Oxalates and glycosides occurred in greater proportions in all extracts compared to all other phytochemicals. The extracts contain steroid and tannins in the ranges of 13-25mg/100g and 10-11mg/100g respectively which is contrary to the research conducted by Adesokan *et al*. (2007) in which steroids and tannins were not detected. Considerable changes were also noticed in the quantity of phenol, phytate, alkaloid and flavonoid which may also be responsible for the antimicrobial activities observed against the test organisms.

Conclusion

E. chlorantha generally exhibited bacteriostatic effect against all isolates with activities depending on type of solvent used for extractions and the concentrations used. The dichloromethane extract has the highest antimicrobial activities on the test organisms followed by the aqueous extract. *K. pneumonia* showed the highest susceptibility to the two extracts gotten from polar solvents. The two non-polar solvents; n-hexane extract showed minimal antimicrobial activity and petroleum ether has no antimicrobial activity against all the test organism. Considering the variability observed in the antimicrobial activities and the phytochemical results of the extracts, it appears that the efficacy of *E. chlorantha* depends largely on the type of solvent used.

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