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Biochemical profiling of leaf, stem bark and root of *Enantia chlorantha* native to Akwa Ibom, Nigeria: A comparative analysis

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ABSTRACT

The present investigation was carried out to explore the bio-activities of *Enantia chlorantha Oliv* (Annonaceae) commonly known as African yellow wood. The root, stem and leaf of *E. chlorantha* were extracted with ethanol in soxhlet extractor. Their phytochemical analysis revealed presence of alkaloids, flavonoids, saponins, phenols, steroids, cardiac glycosides, terpenoids, anthraquinones, proteins, aldehydes/ketones and carboxylic acids in all the extracts except leaves extract which contain tannins in addition. The extracts were screened in vitro for their antibacterial activities against Gram-positive bacteria (*Bacillus thuringensis strain EB-151, Bacillus tequilensis strain ADIP3, Bacillus cereus strain SB2, Bacillus pumilus strain m414, Sphingobacterium mizutaii strain AUMC b-161, Bacillus subtilis strain AIMST 2ME1, Bacillus cereus strain CF7 and Lysinbacillus sphaericus III*) and Gram-negative bacteria (*Providencia rettgeri strain RCB 200, Proteus vulgaris strain MWG 20141026, Alcaligenes faecalis strain L48, Pseudomonas aeruginosa strain PG1*, *Pseudomonas aeruginosa strain 335K55* and *Escherichia coli strain sanji.*); out of which eight of strains were susceptible to the extracts and six were resistant to all the extracts. This study has opened a frontier for the possible application of the extracts in the control of potential pathogens.

Capsule Summary: Leaf, bark and root extracts of *Enantia chlorantha* (African yellow wood) contain bio-actives capable of inhibiting the growth of some bacterial isolates investigated and *E. chlorantha* showed promising bio-activity.

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INTRODUCTION

The incidence of deadly infections caused by pathogenic microorganisms has been on the increase worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in developing countries (Al-Bari et al., 2006). The increasing pervasiveness of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of resistible bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy et al.,

2005; Rojas et al., 2006). For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana et al., 2007). Medicinal plants are a pool of natural compounds with beneficial effects. Certain compounds identified in the extracts of leaves, root barks, stem barks and seeds such as alkaloids, saponins, phenolic compounds and others are endowed with extremely important biological activities (Bisignano et al., 1999).

Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993). It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2001; Sharif and Banik, 2006; Doughari et al., 2007). *Enantia chlorantha* (African yellow wood): a therapeutic plant used traditionally in southwestern Nigeria is a member of Annonaceae's family and has various uses (Adesokan *et al*., 2008). It is a decorative tree which may grow as tall as 30m high with crowded foliage and spreading crown (Apkulu et al., 1994). "The stem bark is used in the treatment of leprosy spots, as haemostatic agent and uterus stimulant" (Obadoni and Ochuko, 2001). The possibility of using the plant in situations like rickettsia fever, typhoid fever, infective hepatitis or jaundice was also reported by Gill (1992) and Edeoga et al. (2005). Studies have revealed that the stem of *E. chlorantha* has broad spectrum antimicrobial activity, antimalarial and antipyretic properties (Adesokan et al., 2008). In Cameroon, stem extract is prepared to care for jaundice and urinary tract infections (Adjanohoun et al., 1996; Ndah et al., 2013). The newest and most effective antibiotics are not easy to come-by by many Africans living villages. These antibiotics are also related with some serious side-effects. A healing plant, such as African yellow wood, is always on hand and inexpensive. The present investigation was carried out to explore the bio-activities of *Enantia chlorantha Oliv* (Annonaceae) commonly known as African yellow wood

MATERIAL AND METHODS

Plant materials

Roots, barks and leaves of the plant used for the study were collected from Ikot Abia Enin in Mkpat Enin Local Government Area of Akwa Ibom State, Nigeria.

Preparation of extracts

Freshly collected leaves, barks and roots of *Enantia chlorantha* were air-dried and ground to a semi-powder. About 30 g of each of the semi-powdered sample was extracted with 250 mL of ethanol for 12 h in a soxhlet extractor equipped with a reflux condenser. The ethanol was removed from the extract using rotary evaporator to give a gel-like solid, which was dissolved in ethanol/water mixture (4:1) and filtered using vacuum pump. The filtrate of each of the sample from the plant was used for preliminary phytochemical screening and antibacterial experiment.

Phytochemical screening of the extracts

The methods employed for phytochemical screening were those previously described by Gupta (2012).

Microbial strains

Ten bacterial strains were used in the study, among these were four Gram negative, namely: *Pseudomonas aeruginosa strain PG1*, *Providencia rettgeri strain RCB 200, Proteus vulgaris strain MWG 20141026, Alcaligenes faecalis strain L48, Pseudomonas aeruginosa strain 335K55,* and *Escherichia coli strain sanji* and eight Gram-positive, namely: *Bacillus tequilensis strain ADIP3, Bacillus cereus strain SB2, Bacillus pumilus strain m414, Sphingobacterium mizutaii strain AUMC b-161, Bacillus subtilis strain AIMST 2ME1, Bacillus cereus strain CF7, Lysinbacillus sphaericus III,* and *Bacillus thuringensis strain EB-151*. Bacteria were maintained on nutrient agar slants at 4°C.

Evaluation of antibacterial activity

Agar diffusion method was employed for the antibacterial activities of the compounds. From overnight broth cultures of the various *bacterial* isolates, a 1× 10^8 cell/mL McFarland standard was prepared and 0.1mL aseptically transferred to sterile Petri dishes before adding 20 mL molten Brian heart infusion agar cooled to 50°C. The content was thoroughly mixed and then allowed to solidify. Five holes (5.0 mm) were made in each plate using a cork borer and 0.2 ml of the various extracts concentrations (100mg/mL, 150mg/mL, 200 mg/mL and 250 mg/mL) of all the compounds transferred into each hole aseptically using a pipette. Plates were allowed to stand for pre-diffusion for 1h before incubation at 29±2°C for 24 h. Ethanol served as control. Zones of inhibition were measured and the average calculated.

RESULTS AND DISCUSSION

Bio-actives agents

All plants produced bewildering variety of phytochemicals like primary metabolites (carbohydrates, fats, proteins) and secondary metabolites (Alkaloids, flavonoids, steroids, saponins, polyphenols, etc.) for their normal metabolic activities (Asif, 2012). These secondary metabolites showed various biological activities and act in plant defense mechanisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions.

Table 1: Phytochemical investigations of crude ethanolic extract of *Enantia chlorantha*

Phytochemicals	Leaf extract	Bark extract	Root extract
Alkaloids			
(a) Dragendorff's test	+++	+++	+++
(b) Mayer's test	+++	+++	+++
(c) Wagner's test	+++	+++	+++
Flavonoids			
Alkali test	+++	+++	+++
Protein			
Biuret test	+++	+++	+++
Gelatin	+++	+++	+++
Tannins			
(a) Hydrolysable tannins	++	-	-
(b) Condensed tannins	-	-	-
Steroids			
(a) Liebermann test	++	++	+++
(b) Salkowski test	++	+	-
(c) Chloroform test	++	+	+++
Terpenoids			
(a) Liebermann test	++	+	-
(b) Salkowski test	-	-	-
(c) Chloroform test	-	-	++
Cardiac glycosides			
(a) Liebermann test	-	++	+++
(b) Keller Killianic test	-	+	-
Anthraquinones			
(a) Bontrager's test	++	+++	++
(b) Bontrager's modification	+	++	++
Saponin	+++	++	+++
Phenol			
(a) Ferric Chloride test	-	-	-
(b) Acetic acid test	-	-	-
(c) Iodine solution test	++	+++	++
Aldehydes/Ketones	+	+	++
Carboxylic acids	++	±	±

+++ = high; ++ = moderate; + = low; ± = negligible; - = absent.

The secondary metabolites have therapeutic actions, which produced drugs (Lai, 2004). It is therefore necessary to identify the phytochemical components of local medicinal plants usually employed by herbalists in the treatment of diseases (Banso and Adeyemo, 2007). The present study revealed the presence of medicinally active phytoconstituents in different parts of the plants viz., root, stem and leaf. Analysis of plant extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids, cardiac glycosides, terpenoids, anthraquinones, proteins, aldehydes/ketones and carboxylic acids in different parts of the plant. The results of the phytochemical analysis of *Enantia chlorantha* extracts are as summarized in (Table 1). Similar findings were also reported and well documented by Akpan et al., (2017). The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. For example, saponins are a special class of glycosides which have soapy characteristics and have been reported to be active antifungal agents. Antimicrobial properties of a number of tannins, flavonoids, alkaloids have been reported. Not only have antimicrobial properties been ascribed to these plant phytochemicals, but other biological activities including modulation of the immune system of these compounds in plants (Jagtap et al., 2014). Tannins were found in only leaves extract, while cardiac glycosides were found in both barks and roots extracts, which revealed the different parts have different bioactive components that might be used as bioactive agents (Akpan et al., 2017).

In vitro antibacterial activity

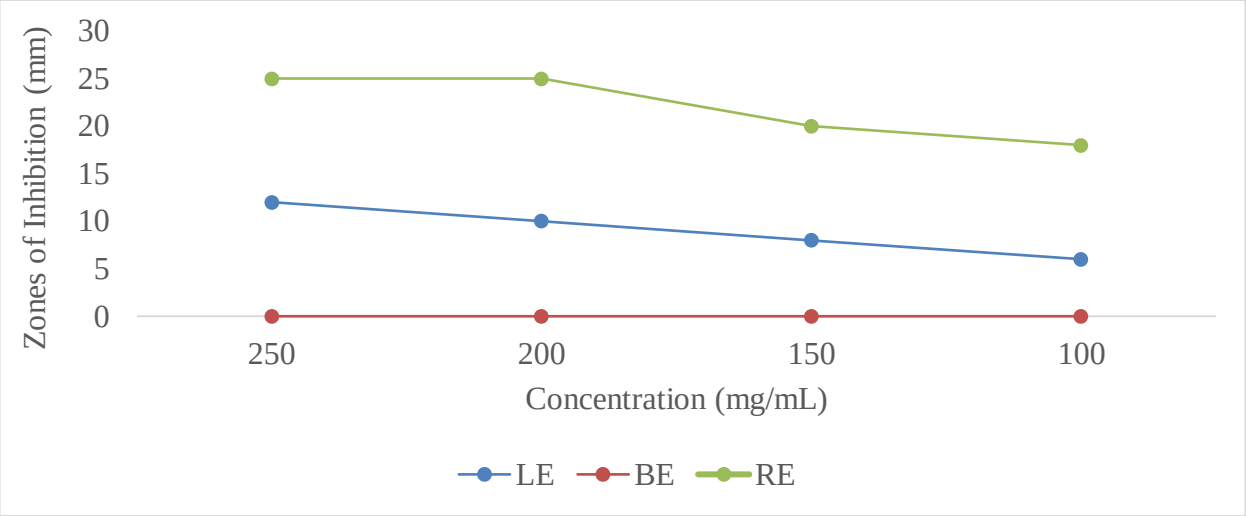

Fig. 1: Activity of plant extracts on *Proteus vulgaris strain MWG 2014102* (LE = ethanol extract of leaves of *E. chlorantha*; BE = ethanol extract of barks of *E. chlorantha* RE = ethanol extract of roots of *E. chlorantha*)

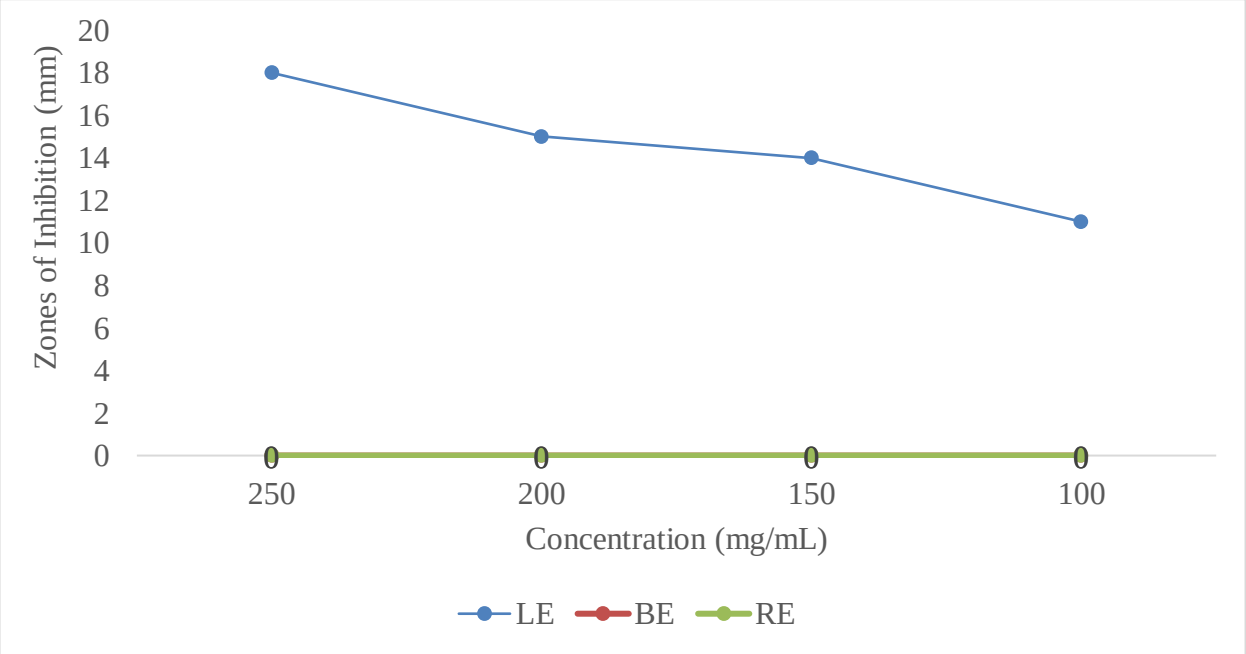

Fig. 2: Activity of plant extracts on *Alcaligenes faecalis strain L48*

All the extracts were screened in vitro for their antibacterial activities against *Pseudomonas aeruginosa strain PG1*, *Bacillus tequilensis strain ADIP3, Bacillus cereus strain SB2, Bacillus pumilus strain m414, Sphingobacterium mizutaii strain AUMC b-161, Bacillus subtilis strain AIMST 2ME1, Bacillus cereus strain CF7, Lysinbacillus sphaericus III, Providencia rettgeri strain RCB 200, Proteus vulgaris strain MWG 20141026, Alcaligenes faecalis strain L48, Pseudomonas aeruginosa strain 335K55, Bacillus thuringensis strain EB-151* and *Escherichia coli strain sanji.* The antibacterial screening was carried out by well in agar diffusion method at different concentrations of each extract. The zone of inhibition was expressed in mm while the concentrations of the extracts were expressed in mg/mL. Zones of inhibition of each pathogen was observed at the concentrations; 100mg/mL, 150mg/mL, 200mg/mL and 250mg/mL of the extracts. The results are displayed in Figures 1 to 8.

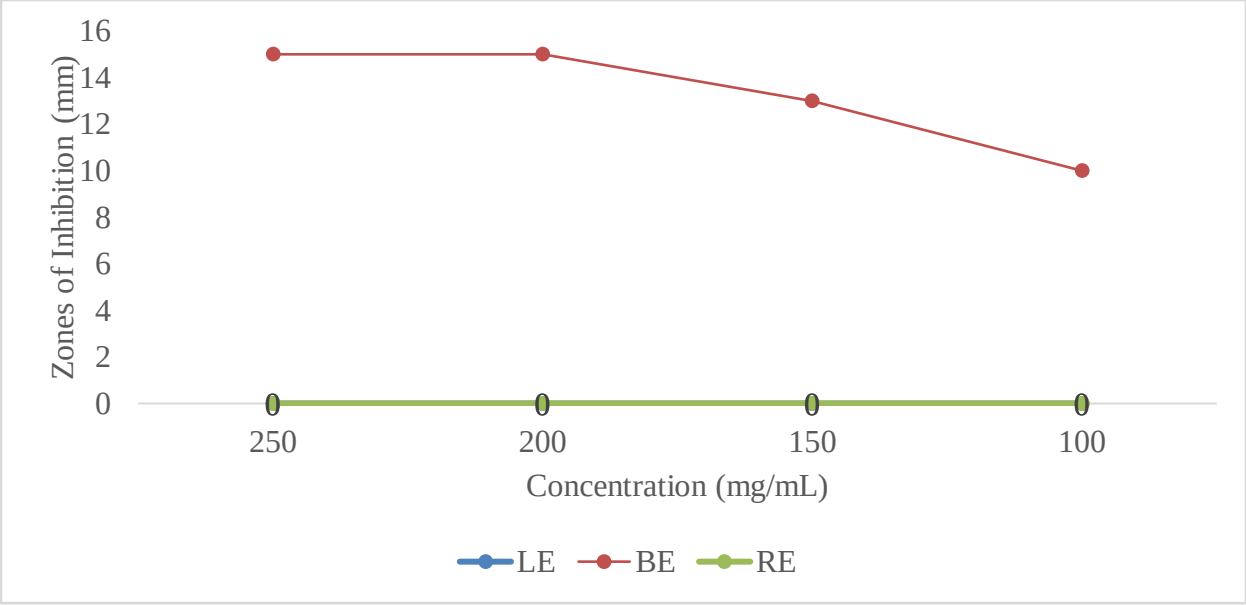

Fig. 3: Activity of plant extracts on *Pseudomonas aeruginosa strain 335K55*

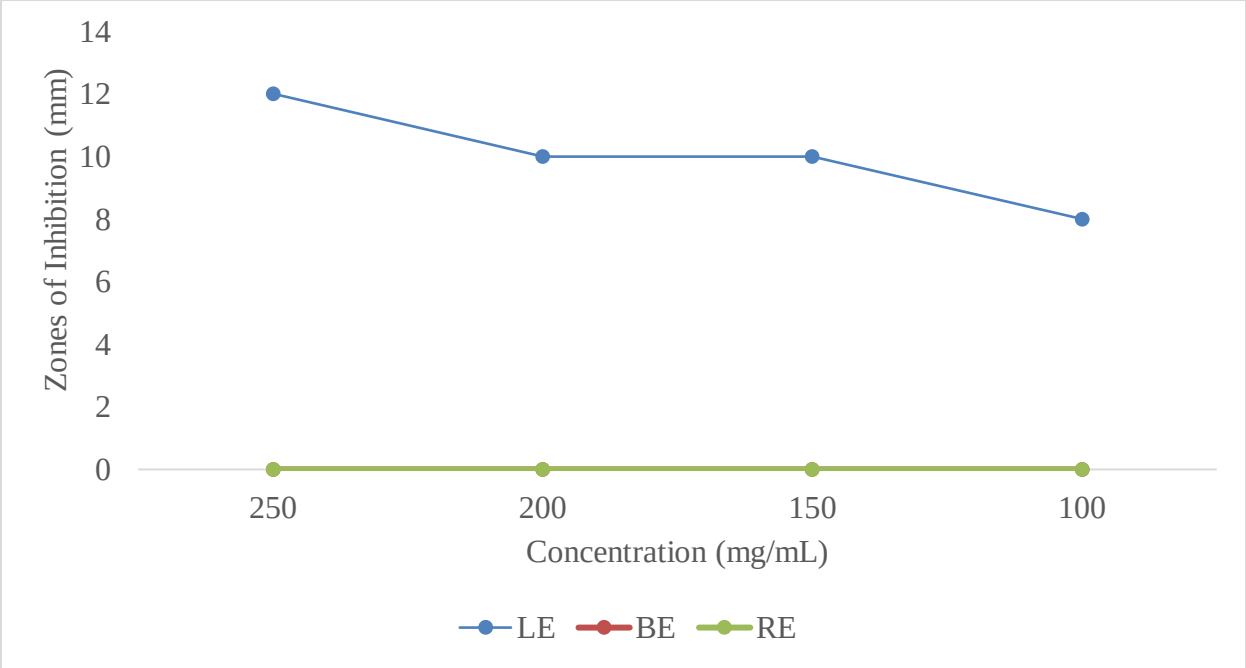

Fig. 4: Activity of plant extracts on *Bacillus thuringensis strain EB-151*

It was found that at all the concentrations, none of the extracts displayed activity against *Lysinbacillus sphaericus III, Providencia rettgeri strain RCB 200, Bacillus tequilensis strain ADIP3, Bacillus pumilus strain m414, Bacillus subtilis strain AIMST 2ME1,* and *Bacillus cereus strain CF7* microbial strains. Each of the extract showed extensive antibacterial effect against some of the tested Gram-negative bacteria (*Proteus vulgaris strain MWG 20141026, Alcaligenes faecalis strain L48, Pseudomonas aeruginosa strain 335K55, Pseudomonas aeruginosa strain PG1*, and *Escherichia coli strain sanji*) and some Gram-positive bacteria (*Bacillus thuringensis strain EB-151, Bacillus cereus strain SB2* and *Sphingobacterium mizutaii strain AUMC b-161*).

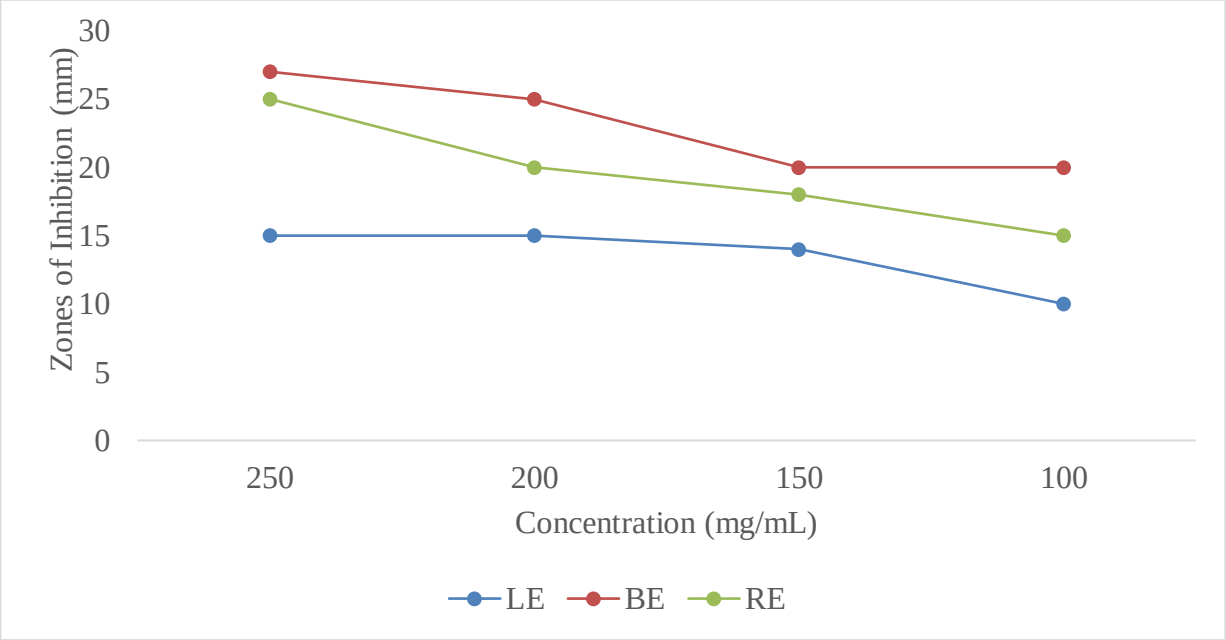

Fig. 5: Activity of plant extracts on *Escherichia coli strain sanji*

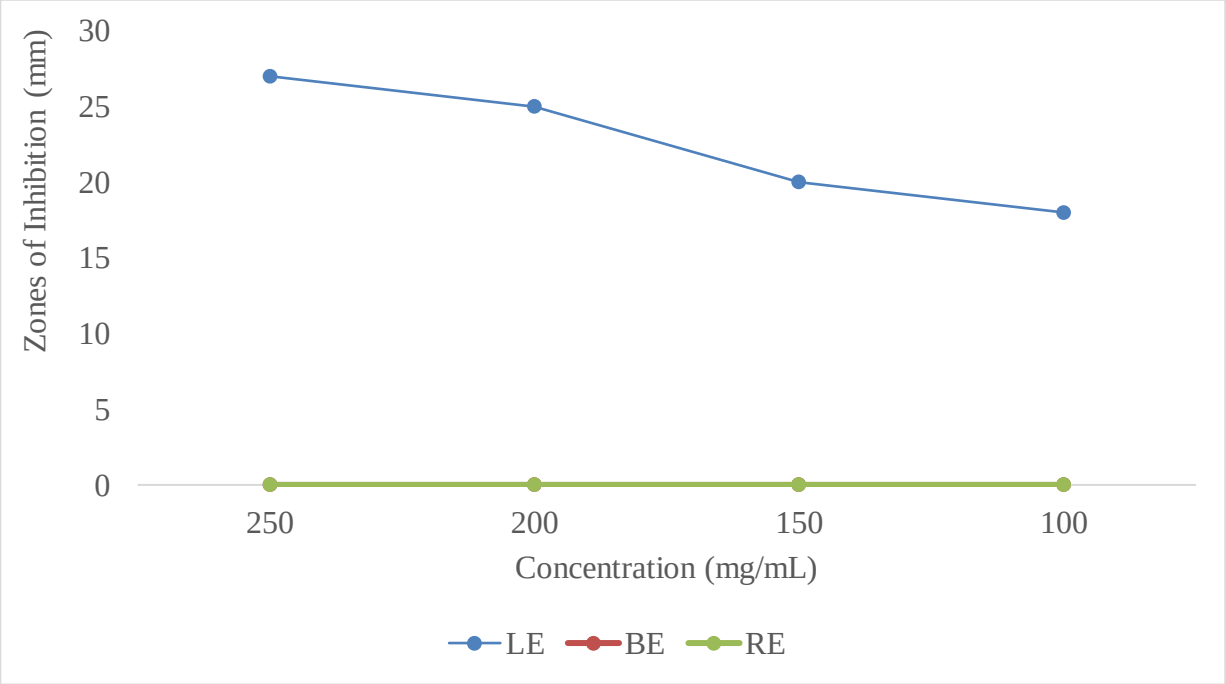

Fig. 6: Activity of plant extracts on *Pseudomonas aeruginosa strain PG1*

Bark extracts of *E. chlorantha* (BE) showed activity to two tested Gram-negative bacteria (*Pseudomonas aeruginosa strain 335K55, Escherichia coli strain sanji,*) and two Gram-positive bacteria (*Bacillus cereus strain SB2,* and *Sphingobacterium mizutaii strain AUMC b-161*) (Figures 3, 5, 7 and 8). Leave extracts were found active against four Gram-negative bacteria (*Proteus vulgaris strain MWG 20141026, Escherichia coli strain sanji, Alcaligenes faecalis strain L48,* and *Pseudomonas aeruginosa strain PG1*) and three Gram-positive bacteria (*Bacillus cereus strain SB2, Bacillus thuringensis strain EB-151,* and *Sphingobacterium mizutaii strain AUMC b-161*) (Figures 1-8).

On the other hand, two Gram-negative bacteria (*Proteus vulgaris strain MWG 20141026* and *Escherichia coli strain sanji*) and two Gram-positive bacteria (*Bacillus cereus strain SB2* and *Sphingobacterium mizutaii strain AUMC b-161*) were susceptible to the root extracts of *E. chlorantha* (Figures 1, 5, 7 and 8). The extensive antibacterial activity of the three extracts against Gram-positive and Gram-negative bacteria is in accordance with the findings reported by Rahman et al. (2009) on "antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam – against some human pathogenic bacteria". Figure 1 reveals that RE was more active than LE in their activity against *Proteus vulgaris strain MWG 20141026* at all concentration. Among all the pathogens tested, *Bacillus cereus strain SB2,*

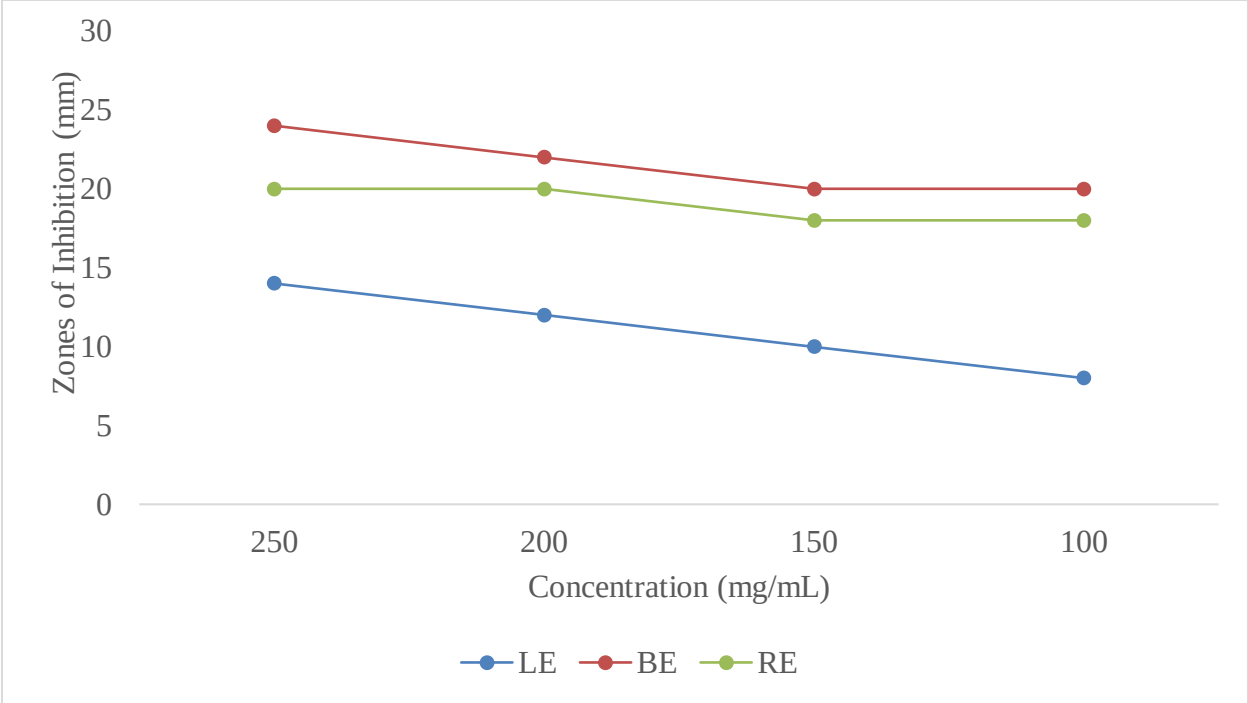

Fig. 7: Activity of plant extracts on *Bacillus cereus strain SB2*

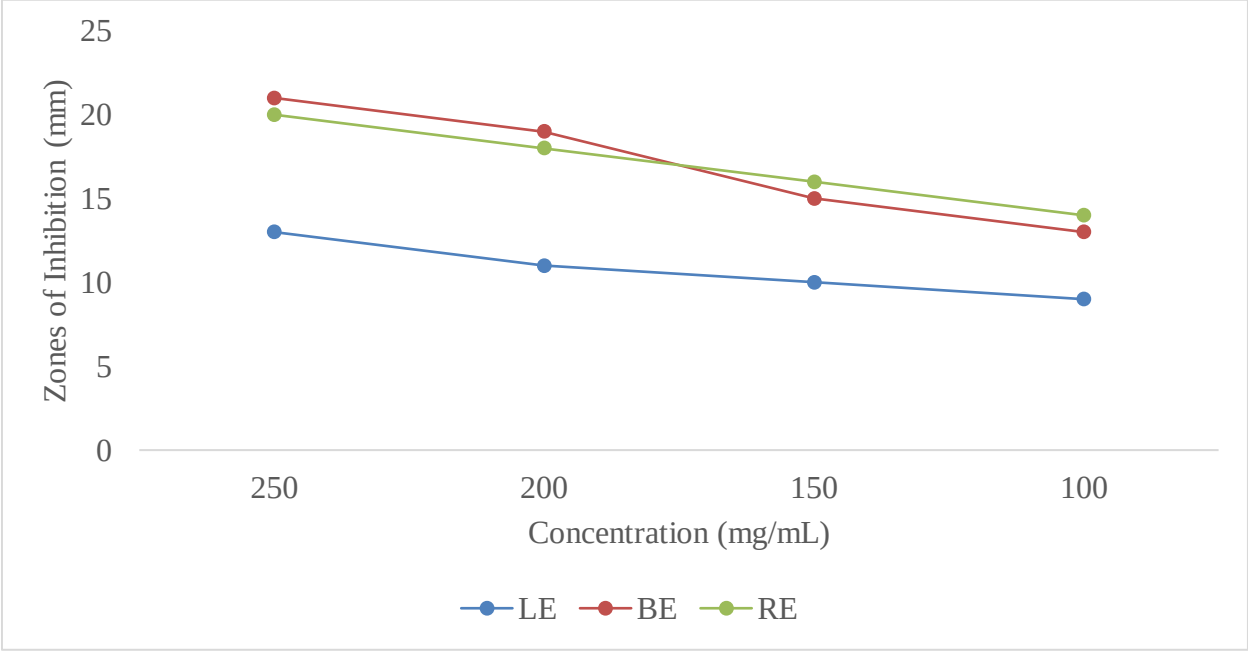

Fig. 8: Activity of plant extracts on *Sphingobacterium mizutaii strain AUMC b161*

Escherichia coli strain sanji, and *Sphingobacterium mizutaii strain AUMC b161* were susceptible to all the three extracts (Figures 5, 7 and 8). *Alcaligenes faecalis strain L48, Bacillus thuringensis strain EB-151* and *Pseudomonas aeruginosa strain PG1* were resistant to all the extracts except LE (Figures 2, 4 and 6) while *Pseudomonas aeruginosa strain 335K55* was only susceptible to BE (Figure 3). In all the tested pathogens, the activity of the extracts varied with variation in their concentrations. The activity of the plant against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the plant (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007; Rahman et al., 2009). The broad-spectrum antibacterial principles in LE, BE and RE are likely to be tannins, flavonoids, steroids, saponins, and alkaloids in them (Jagtap et al., 2014; Zablotowicz et al., 1996). Secondary metabolites like flavonoids are synthesized by plants in response to microbial infection (Jamine et al., 2007). Alkaloids are the largest group of phytochemicals causing toxicity against cells of foreign organisms (Nobori et al., 1994). Alkaloids have been reported to be responsible for the antibacterial activity in some plants (Doughari, 2006; Osbourn, 2003). Earlier studies have shown that the antimicrobial properties of alkaloids may be associated with inhibition of nucleic acid, protein and membrane phospholipid synthesis (Shelton, 1991) or their ability to intercalate with DNA (Phillipson and O'Neill, 1987). A wider activity observed in LE may be due to the presence of tannins which is absent in BE and RE. Also, higher activity exhibited by BE and RE against *Escherichia coli strain sanji*, *Bacillus cereus strain SB2*, and *Sphingobacterium mizutaii strain AUMC b161* (Figures 5, 7 and 8) may be as a result of cardiac glycosides found in them which is not present in LE. The antibacterial activity of flavonoids, can be explained by the mechanism of toxicity towards the microorganisms which is made by specific interactions, such as the establishment of hydrogen bonds with the cell walls proteins or enzymes, the chelation of metal ions, inhibition of bacterial metabolism, sequestration of substances necessary for the growth of bacteria (Bessam and Mehdadi, 2014). Polyphenols such as tannins and flavonoids such as epigallocatechin, catechin, myricetin, quercetin, (Shan et al., 2007) and luteolin (Askun et al., 2009) are important antibacterial substances. According to a research reported by Wang et al. (2010) the antibacterial activity of pomegranate is due to the presence of tannins such as ellagitannins and flavonoids. Tannins have been reported to prevent the microbial growth by precipitating microbial proteins (Prasad et al., 2008). The growth of many molds, yeasts, bacteria and viruses are inhibited by tannins (Hegde et al., 2012). Saponin possess antimicrobial property due to its ability to cause leakage of certain enzymes and proteins from the cell (Zablotowicz et al., 1996). Flavonoids are also reported to be hydroxylated phenolic substances synthesized by the plants in response to microbial infection and has the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999; Pandey and Gupta, 2014). According to Bessam and Mehdadi (2014), flavonoids act at several levels. It appears that the B ring is important in the intercalation with nucleic acids, and thus inhibits DNA and RNA synthesis. They can also inhibit the DNA gyrase E. coli. Again, hydroxylation of the B ring by flavonoids appears to be essential for the activity (Cushnie and Lamb, 2005). Tannins are known to form irreversible complexes with proline-rich proteins resulting in the inhibition of cell protein synthesis, bind proteins and adhesins, inhibit enzymes and complex with cell walls (Ahmad et al., 2006; Shimada, 2006; Pandey and Gupta, 2014). Steroids have been documented to have antibacterial properties. The steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Raquel, 2007; Pandey and Gupta, 2014).

CONCLUSIONS

The present investigation was focused on the antibacterial activity and phytochemical analysis in the root, stem and leaf extracts of *E. chlorantha* in ethanol against fourteen bacteria. The results were promising and revealed that the highest activity was recorded in: bark extracts in *Escherichia coli strain sanji*, *Bacillus cereus strain SB2*, *Sphingobacterium mizutaii strain AUMC b161*; leaf extracts in *Pseudomonas aeruginosa strain PG1* and root extracts in *Proteus vulgaris strain MWG 2014102*, whereas leaf extracts and bark extracts exhibited pronounced activity against *Alcaligenes faecalis strain L48*, *Bacillus thuringensis strain EB-151* and *Pseudomonas aeruginosa strain 335K55* respectively. The obtained results may provide a support to use of the plant in traditional medicine. Based on this, further chemical and pharmacological investigations to isolate and identify minor chemical constituents in *E. chlorantha* and to screen other potential bioactivities is recommended.

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