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Original Article

EXTRACTS OF *EUPHOBIA HIRTA LINN* AND *PHYSALIS ANGULATA L.* AND THEIR AMALGAMATION DEMONSTRATE POTENCY AGAINST *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objective: The goal was to investigate the antibacterial activity of the extracts of *Euphobia hirta* and *Pysalis angulata* from Ghana on clinical bacteria isolate found to associate with wound and skin infections.

Methods: The aqueous and crude ethanolic extracts and extract-PEG ointment formulation as well as the combination of the extracts of *P. angulata* and *E. hirta* were tested against *Psedomonas aeruginosa* and *Staphylococcus. aureus,* using the agar diffusion bioassay. Antibacterial activities were indicated by the diameters of zones of inhibition of bacterial growth for both the test extracts as well the extract-PEG ointment formulations.

Results: Ethanolic crude extract of *E. hirta* inhibited *P. aeruginosa* at inhibition zones ranging from 24±0.08 mm at 50 µg/ml to 21.00±0.40 mm at 250 µg/ml, whilst the aqueous crude extracts showed potency against *P. aeruginosa* at varying inhibition zones ranging from 20±0.60 mm at 250 µg/ml to 4.00±0.20 mm at 50 µg/ml. Ethanolic *E. hirta* crude extracts exhibited potency against *S. aureus* and was dose dependent, with a decrease in inhibition as concentration increased, recording a minimum zone of inhibition at 13.00±0.30 mm at 250 µg/ml and maximum zone of inhibition of 16.00±0.20 mm at 50 µg/ml. Only one concentration of 100 µg/ml at 20±0.60 mm zone for the aqueous extract of *E. hirta* was potent against *S. aureus*. Ethanolic crude extract of *P. angulata* inhibited *P. aeruginosa* at zones ranging from 23±0.30 mm at 100 µg/ml to 13.00±0.20 mm at 50 µg/ml, whereas the aqueous crude extracts showed potency against *P. aeruginosa* at dose dependent concentrations ranging from 10±0.30 mm at 50 µg/ml to 13.00±0.30 mm at 250 µg/ml. Here, inhibition zone increased as concentration was increased. Ethanolic *P. angulata* crude extracts exhibited potency against *S. aureus*, recording a minimum zone of inhibition at 12.00±0.40 mm at 50 µg/ml and maximum zone of inhibition of 21.00±0.30 mm at 250 µg/ml. At lower concentration of 50 µg/ml, the combination of ethanolic *E. hirta and P. angulata* inhibited *S. aureus* at a zone of inhibition of 2 mm for the combination of the crude aqueous extracts; *P. aeruginosa* was inhibited at the zone of 1.0 mm with extract concentration of 50 µg/ml. A one-way analysis of variance (ANOVA) of the above values compared with the activity of Ciprofloxacin (positive control) indicated significant inhibitory activity by the unformulated *P angulata* and *E hirta* ethanolic crude extracts.

Conclusion: The study indicates that crude extracts of *E. hirta* and *P. angulate are* possible sources of natural antibacterial agents against both skin and wound infections caused by *S. aureus* and *P. aeruginosa*.

Keywords: Inhibition, Amalgamation, Gram-negative, PEG-ointment

INTRODUCTION

Communicable diseases are key sources of indisposition and mortality in the developing world and accounts for about 50 % of all demises. Bacterial resistance to antibiotics upsurges mortality, possibility of hospitalization and the dimension of stay in the hospital [1]. The spread of resistance, which is transportable amongst members of the enterobacteriaceae, has been ascribed to the conscription of drug resistance markers by a diversity of agents encoded on plasmids, transposon and integrons [2]. Isolation of bacteria less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is now a global problem [3]. In the developing world, the condition is equally worst because of poor sanitation and illiteracy of decent hygienic practices thus divulging an enormous number of people to infectious agents. Some of the bacteria implicated in causing enteric infections include but not limited to *E. coli, salmonella spp. Proteus spp., shigella spp., pseudomonas spp.* and the *staphylococci* [4].

These bacteria which are habitually existent as commensal have numerous virulent factors and colonize in a biofilm fashion, causing a variety of intestinal and extraintestinal diseases [5]. The success story of modern medicine lies in the incessant quest for novel drugs to counter the challenges posed by resistant strains of bacteria. There is, therefore, the necessity to develop some newer, safer, effective and above all, inexpensive antimicrobial agents to wrestle this problem. There are numerous reports in the scientific literature recounting the antimicrobial properties of crude extracts prepared from plants [6] and such reports had enticed the attention of scientists worldwide.

The importance of medical ethnobotanic research has been increasing since potential sources for drugs could disappear in the future as a result of the rapid loss of biodiversity

Many plant extracts have been used as herbal medicines for the treatment of many diseases. Extracts are administered as syrup as well as in the form of essential oils and creams. These extracts have been used from times immemorial as phytoremedy, traditionally all over the world. *E. hirta* is a medicinal, rhizomatous herb, which in East and West Africa extracts of the plant are used in the treatment of asthma and respiratory tract inflammations. It is also used for coughs, chronic bronchitis and other pulmonary disorders [7]. It is reported that extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promotion of wound healing [8].

Research indicates that E. hirta is known to be very popular herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility and venereal diseases [9]. Additionally, the plant is similarly used to treat infections of the skin and mucous membranes,

including warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, guinea-worm and as an antiseptic to treat wounds, sores and conjunctivitis [9]. The plant has a status as an analgesic to treat a severe headache, toothache, rheumatism, colic and pain during pregnancy. Other reports indicate its use as an antidote and pain reliever of scorpion stings and snakebites [9]. The use of the latex to expedite removal of thorns from the skin is also common. In Ghana, a number of plant-based products are claimed to be effective for the treatment of onchocerciasis [10]. Extracts from the plant of *E. hirta* have been found to have the potential to serve as anticancer agents [10].

The plant, *P. angulata* is an annual herb distributed in many countries located in tropical and subtropical regions of the world. The plant is extensively used in popular medicine as a treatment for an assortment of diseases and has antitumoral activity already demonstrated [11, 12]. *P. angulata* has a broad spectrum of biological activity including antibacterial, molluscicidal, antiprotozoal, anticancer and cytotoxic and immunomodulatory activities [13, 14]. In Ghana, it is called "*totototo*" among the Akans. The juice is used in the treatment of an earache, jaundice, fever, bladder disease and the fruit and other aerial parts are used in the treatment of boils, sores or wounds, constipation and digestive problems [15, 16].

The pathogen, S. aureus is a gram-positive bacterium and perhaps the most dangerous of all the many common staphylococcal bacteria [17]. The spread of these bacteria is by direct contact with an infected person, by using a contaminated object, or by inhaling infected droplets dispersed by sneezing or coughing. Skin infections are common, but the bacteria can spread through the bloodstream and infect distant organs. Skin infections may cause blisters, abscesses, redness and swelling in the infected area. Antibiotics are chosen based on whether they are likely to be effective against the strain causing the infection. People most likely to be carriers include those whose skin are repeatedly punctured or broken, such as individuals with diabetes mellitus and have to regularly inject insulin, those who inject illegal drugs, ones being treated with hemodialysis or chronic ambulatory peritoneal dialysis, individuals with skin infections, AIDS, or previous staphylococcal bloodstream infections [17].

There are many strains of *S. aureus* and some produce toxins that can cause the symptoms of staphylococcal food poisoning, toxic shock syndrome and scalded skin syndrome. Many strains have developed resistance to the effects of antibiotics, and if carriers take antibiotics, the antibiotics kill the strains that are not resistant, leaving mainly the resistant strains. These bacteria may then multiply, and if they cause infection, the infection is more difficult to treat. Whether the bacteria are resistant and which antibiotics they resist often depend on where people got the infection: in a hospital or other health care facility or outside of such a facility. Staphylococcal infections may be difficult to treat because many of the bacteria have developed resistance to antibiotics [17], hence, the need for alternative therapeutics which necessitated the relevance of this research. *P. aeruginosa* is also a gram-negative rod-shaped bacterium. These pathogens are widespread in nature, inhabiting soil, water, plants, and animals including humans. They are notorious in causing nosocomial infections such as pneumonia, urinary tract infections, respiratory system infections and infections of severe burns [18].

MATERIALS AND METHODS

Source of test bacteria

Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were collected from incision type wounds at the Kintampo Health Research Centre in the Brong Ahafo Region of Ghana, in June 2014. The isolates were sub-cultured to verify purity on chocolate agar (OXOID, Basingstoke, Hampshire, England) and stored between 2ºC and 8ºC in nutrient broth (OXOID, Basingstoke, Hampshire, England).

Plant material

Aerial parts (leaves, stems, and flowers) of the *E. hirta* plant and leaves of *P. angulate* were collected from Tono irrigation farms in Navrongo in the Upper East Region of Ghana in October 2013. The plant material was authenticated by Dr. Isaac Sackey of the Department of Applied Biology in the Faculty of Applied Sciences, University for Development Studies, Navrongo Campus

Sample preparation

The plant samples were air-dried at room temperature of (25-30 ^{o}C) for two weeks, after which they were ground into a uniform powder.

Preparation of ethanol extracts of the plants sample

Powdered plants samples, 60 g each of *E. hirta* and *P. angulata* were measured into round bottom flasks and both flooded with 95 % ethanol. The flasks were stoppered and left to stand for 24 h. The extracts were then filtered using sterilized cotton wool. The filtrates were concentrated under reduced pressure using rotary evaporator. The resultant materials were kept in sample vials and stored in a refrigerator for further analysis.

Aqueous extracts of the plants sample

Powdered samples, 60 g each was measured into beakers, and the volumes made up to 600 mL with distilled water; enough to cover the mixture. The mixtures were stirred for 10 min after which the plant materials were filtered through sterilized cotton wool. The filtrates were concentrated using rotary evaporator. The residues were then stored in the refrigerator for use in the next experiment.

Preparation of polyethylene glycol (PEG) ointment

A mass of 15 g each of Polyethylene glycol 4000 (PEG 4000) and Polyethylene glycol 400 (PEG 400) was weighed into a beaker and melted on a thermostatic water bath at 70 °C until liquefied to make 50 % PEG 4000 and 50 % PEG 400. It was stirred with a glass rod under tap water at room temperature until congealed. [19]

Combination of extracts of *Euphorbia hirta* and *Physalis angulate*

Equal amount of each of the concentrations (50, 100, 200 and 250) µg/ml of *E. hirta* were amalgamated with equal amount of each of the concentration of *P. angulata* to produce a mixture of crude extract of *E. hirta* and *P. angulata* at concentrations 50 µg/ml, 100 µg/ml, 200 µg/ml and 250 µg/ml. The mixtures were then vortexed for two minutes and used to test for antibacterial activities. The same process was used to test for the combination of the aqueous extracts of the two plants.

Plant crude extract-PEG ointment formulations

Employing a previous method of [19], *E. hirta* and *P. angulate* crude extracts were separately formulated together with PEG ointment to determine whether they would have a potential effect on the bacterial isolates. Equal quantities (25, 50, 100, and 200 µg) of each crude extract were weighed into appropriately labeled separate beakers; E. H_1–E. H_4 for *E. hirta* and P. A_1–P. A_4 for *P. angulata* PEG ointment. The extract-PEG, 1.0 g, was then added to each beaker and warmed at 40 °C while stirring continuously with a glass rod. The mixtures were allowed to cool at room temperature (25-30 ^{o}C) to produce a crude extract-PEG ointment formulation at varying concentrations of 25 µg/mg, 50 µg/mg, 100 µg/mg and 200 µg/mg respectively of each plant extract. The combination of the two plants was also formulated to test for their synergistic effect.

Agar diffusion bioassay

The modified agar well diffusion method was employed. Colonies of a pure culture of each test organism was suspended in 100 ml of sterile peptone water to get a turbidity of about 1 McFarland Standard. Aliquots of 0.1 ml of each suspension were separately surface-plated on different Mueller Hinton Agar (OXOID) plates. Wells of 5 mm diameters were made on the culture plates with a sterile cork borer at wide enough intervals. For each sample, 100 µl was drawn into a labeled well. The same volumes of the negative control (98% DMSO) and positive control (Ciprofloxacin at 30 µg/µl) were also introduced into a well each on the same plate. Plates were left to stand until complete diffusion of the formulations into the medium. Plates were incubated in inverted positions at 37 °C for 48 h after which they were observed for inhibitory activity depicted by zones of inhibition around the wells. Inhibition zone diameters were measured using a ruler and recorded in millimetres (mm). The experiments were repeated three times to check for reproducibility. The same procedure was used to test for the antibacterial activity of crude extract-ointment formulation.

Statistical analysis

Means and standard error of the mean were calculated for the zones of inhibition measured for the three sets of experiments in each case. These means were statistically compared using the one–way ANOVA to determine if they were significantly different at P<0.05.

RESULTS

The antibacterial activity of crude and formulated extracts of *P. angulate* and *E. hirta* were studied at concentrations of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml for both crude extracts and of 25 µg/mg, 50 µg/mg, 100 µg/mg and 200 µg/mg for the PEG ointment formulations. The test bacteria used were *P. aeruginosa* and *S. aureus* which are commonly associated with skin and wounds infections. The results showed the concentration-dependent inhibitory activity of the extracts against the test bacteria. Largely, most of the crude extracts showed increasing levels of inhibition at variable concentrations. Antibacterial activities were indicated by the diameters of zones of inhibition of bacterial growth for both the test extracts as well the extract-PEG ointment formulations.

Aqueous crude extract of *E. hirta* was potent against *P. aeruginosa* which was dose dependent with a maximum zone of inhibition of 20±0.06 mm at a concentration of 250 µg/ml. The crude ethanolic extract exhibited an opposite trend, with maximum zone found to be 24±0.08 mm at a concentration of 50 µg/ml (fig 1). Both the ethanolic and aqueous extract-PEG formulation did not exhibit a substantial level of potency against *P. aeruginosa* and *S. aureus* in this current work (Data not shown). Previous work conducted by [19], formulation of *cleome viscosa* crude extract-PEG ointment showed appreciable zones of inhibition against *P. aeruginosa.* In that work the extract-PEG formulation was warmed to 40 °C to liquefy before applying to the test organism.

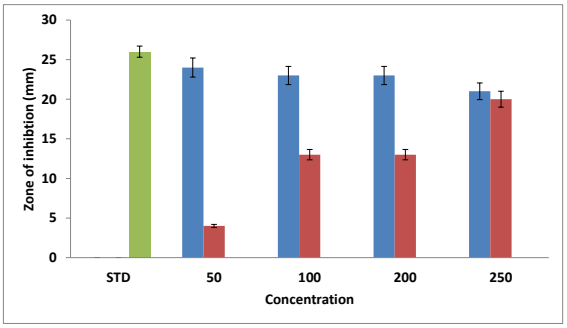

Fig. 1: Representative antibacterial activity of unformulated aqueous and crude ethanolic extracts of *Euphobia hirta* against *P. aeruginosa*
(■) *E. hirta aqueous* crude extracts (µg/ml), (■) *E. hirta* ethanolic crude extracts (µg/ml), (■) *Standard antibiotic* (Ciprofloxacin) (µg/g)

In the current work, the procedure was modified for the purpose of differentiating the effect of the two different processes. Here, the extract-PEG ointment formulation was applied to the test organism directly without liquefying. The comparison of the two separate experiments indicates that liquefying the extract-PEG ointment formulation seems to achieve better results due to the fact that the active drug stands a better chance of getting in contact with the test bacteria compared with just the direct application of the semi-solid ointment formulation to the test organism. This has been confirmed in our laboratory by a recent unpublished data collected on another formulation studies. In this study, *E. hirta* showed activity against *S. aureus* with maximum inhibition at 50 µg/ml for the ethanolic extracts and only concentration of 100 µg/ml showed inhibition against *S. aureus* with the aqueous extracts (fig. 2).

The current research indicates that crude aqueous extract of *P. angulata* inhibited *P. aeruginosa* in a dose-dependent fashion with a maximum zone of 13.00±0.03 at a concentration of 250 µg/ml. The crude ethanolic extract showed a linear correlation between the zones of inhibition and the concentrations used, with increased zones with respect to increased concentration of the extracts up to 200 µg/ml but showed a deviation at 250 µg/ml with a reduction in inhibition zone (fig. 3). Further, both the ethanolic and aqueous extract-PEG formulation of *P. angulate* did not significantly inhibit both bacteria, which might be attributable to the reasons suggested above.

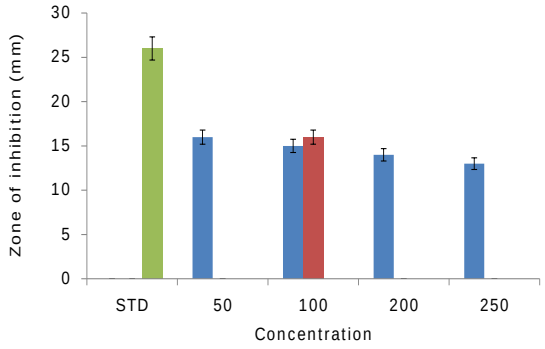

Fig. 2: Representative antibacterial activity of unformulated aqueous and ethanolic extracts of *E. hirta* against *S. aereus*
(■) *E. hirta aqueous* crude extracts (µg/ml); (■) *E. hirta* ethanolic crude extracts (mg/ml) (■) *Standard antibiotic* (Ciprofloxacin) (µg/g)

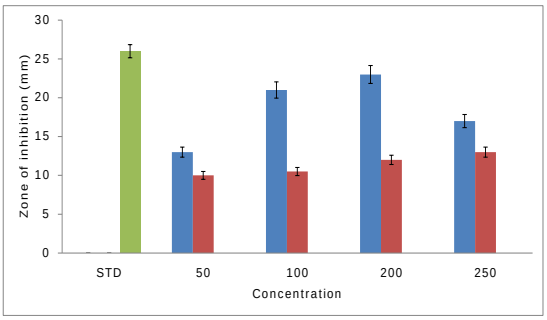

Fig. 3: Representative antibacterial activity of unformulated aqueous and ethanolic extracts of *P. angulata* against *P. aeruginosa*
(■) *P. angulata crude aqueous* extracts (µg/ml);(■) *P. angulata* ethanolic crude extracts (µg/ml); (■) *Standard antibiotic* (Ciprofloxacin) (µg/g)

The crude ethanolic extracts were potent against *S. aereus* with maximum zone occurring at 21.00±0.30 at a concentration of 250 µg/ml whilst the aqueous extracts showed no inhibition at all concentrations used in this research (fig. 4).

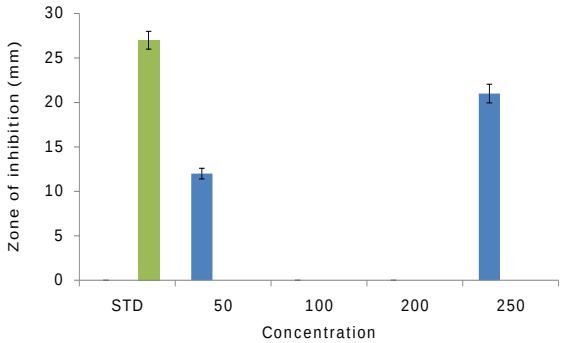

Fig. 4: Representative antibacterial activity of unformulated aqueous and ethanolic extracts of *P. angulata* against *S. aereus*
(■) *P. angulata crude aqueous* extracts (µg/ml); (■) *P. angulate* ethanolic crude extracts (µg/ml); (■) *Standard antibiotic* (Ciprofloxacin) (µg/g)

At low concentration of 50 µg/ml, the combination of ethanolic *E. hirta and P. angulata* inhibited *S. aureus* at a zone of inhibition of 2 mm (fig. 5). For aqueous combination, *P. aeruginosa* was inhibited with a zone of inhibition of 1 mm at a concentration of 50 µg/ml (fig. not shown). However, the formulation prepared by the combination of the crude extract-PEG formulations of both *E. hirta* and *P. angulata* used in this research did not show an appreciable level of potency against the bacteria at all concentrations employed.

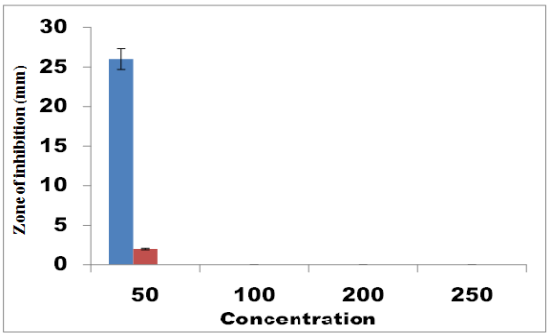

Fig. 5: Representative antibacterial activity of amalgamated aqueous and ethanolic extracts of *E. hirta and P. angulata* against *S. aereus*
(■) Combination of *E. hirta* and *P. angulata* crude ethanolic extracts (µg/ml), (■) *Standard antibiotic* (Ciprofloxacin) (µg/g)

DISCUSSION

In the present study, ethanolic extract of both *E. hirta* and *P. angulata* exhibited inhibitory activity against *S. aureus* and *P. aeruginosa* supporting their ethno pharmacological utilization of the medicinal species against wound and skin infections in Ghanaian communities. The level of potency observed against these two bacteria by *E. hirta and P. angulata* ethanolic extracts further validate the use of these plants in Ghanaian ethnotherapy. For the ethanolic extracts of the medicinal plant *E. hirta* against *P. aeruginosa and S. aureus,* inhibition was found to decrease as the concentration of the extracts decreased, whilst the ethanolic extracts of *P. angulata* against both bacteria demonstrated an inconsistent pattern of inhibition (fig. 6).

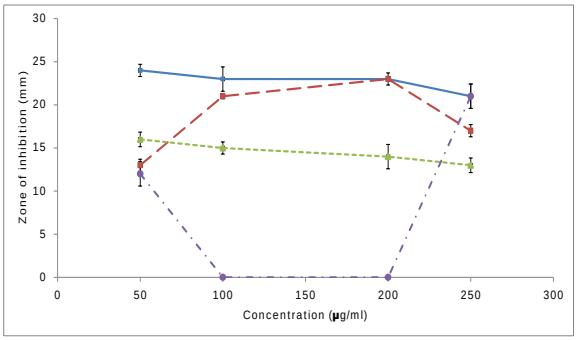

Fig. 6: Representative antibacterial activity of ethanolic extracts of *E. hirta and P. angulata* against *P. aeruginosa and S. aureus*
(■) *E. hirta crude ethanolic* extracts against *P. aeruginosa*; (■) *E. hirta* ethanolic crude extracts against *S. aereus*; (■) *P. angulata* ethanolic extracts against *P. aeruginosa* (■)*P. angulata* ethanolic extracts against *S. aureus*

A research report by Donkor *et al.* [20], indicated that anti-plasmodial activities of crude ethanolic extracts of *Moringa oleifera* presented similar trend as exhibited by the ethanolic extract of *E. hirta* in this current research. The observed biological activity calls upon further investigation to isolate the active phytochemicals responsible for the reported activity. The aqueous extracts of both plants showed a similar trend in activity against *P. aeruginosa* that is, increased activity with increasing concentration of extracts. It can be pointed out that, *E. hirta* crude extract was much more significant in terms of potency compared with that of *P. angulata* which gave a lower level of potency against the bacteria (fig. 7). Again, the synergistic experiments of both the ethanolic and aqueous extracts could not exhibit significant inhibition on both bacterial species

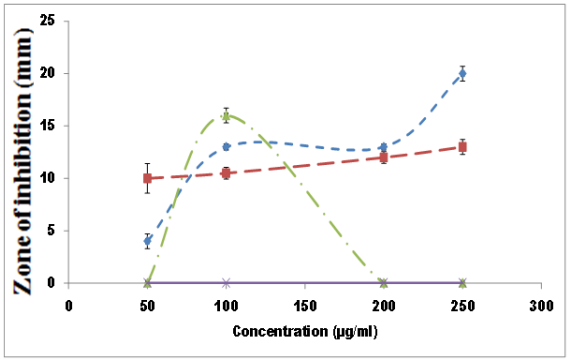

Fig. 7: Representative antibacterial activity of aqueous extracts of *E. hirta and P. angulata* against *P. aeruginosa and S. aureus*
(■) *E. hirta* aqueous crude extracts against *P. aeruginosa*; (■) *E. hirta* aqueous crude extracts against *S. aereus*; (■) *P. angulata* aqueous extracts against *P. aeruginosa* (■)*P. angulata* aqueous extracts against *S. aureus*

CONCLUSION

The study indicates that crude extracts of *E. hirta* and *P. angulate* are possible sources of natural antibacterial agents to be used against both skin and wound infections. The potency of Ciprofloxacin could be matched by the isolated bioactive compound(s) from the plants parts used. Largely, the extracts showed antibacterial action against both *S. aureus* and *P. aeruginosa*. These results have link with claims made by traditional practitioners for control of skin and wound infections and among the reasons traditional knowledge is considered reliable for the exploitation of herbal remedies. Future study on separation and purification of the bioactive compounds will contribute medicinally to application of these plants worldwide.

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CONFLICT OF INTERESTS

We have no conflict of interest to declare

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