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Research Article

In vitro Antibacterial Activity of PEG Formulations of Crude Extracts of *Cleome viscosa*, *Tamarindus indica* and *Euphorbia hirta*

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Abstract

Background: In rural settings, some medicinal plant extracts serve as alternative agents for wound treatment. Topical application of such extracts would be greatly enhanced if formulations such as ointment-based preparations were available. **Methodology:** This study sought to investigate the antibacterial activity of polyethylene glycol ointment formulations of crude extracts of *Cleome viscosa*, *Tamarindus indica* and *Euphorbia hirta* against *Pseudomonas aeruginosa* and *Escherichia coli*. Antibacterial activities were studied at concentrations of 25, 50, 100 and 200 μg mL^{-1} for the test extracts and 25, 50, 100 and 200 μg g^{-1} for the polyethylene glycol formulations. **Results:** Generally, the crude extracts as well as their formulations showed increasing levels of inhibition with increasing concentrations. Polyethylene glycol formulations of *Euphobia hirta* and *Cleome viscosa* crude extracts exhibited significant potencies against growth of *Pseudomonas aeruginosa* and *Escherichia coli*. **Conclusion:** Both *Euphobia hirta* and *Cleome viscosa* crude extract-polyethylene glycol ointments have therefore emerged as potentially effective formulations against *Escherichia coli* and *P. aeruginosa* wound infections.

Key words: Plant extract, ointment, polyethylene glycol, *E. coli*, *P. aeruginosa*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wounds are typically contaminated by microorganisms, commonly fungi and bacteria. Bacteria frequently isolated from wound infections include *Escherichia coli* and *Pseudomonas aeruginosa*[1,2]. The use of crude extracts of plants parts with known antimicrobial properties have always been of great significance in the therapeutic management of many ailments due to infectious pathogens, including those associated with wounds[3,4].

Medicinal plants have been tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities. The majority of people in developing countries rely on traditional medicines, most of which are plant-based[5]. The primary benefits of using plant-based medicines are that they generally offer a wide range of therapeutic benefits and more affordable treatment. In Ghana, many plants used medicinally in traditional settings are reported to have scientifically documented bioactivities, including antimicrobial properties[6-8].

Cleome viscosa has been found to be useful in treatment of liver diseases, chronic joint pain and in mental disorders[5] and the pungent seeds can be used as mustard substitute in curries. The seed oil is used for cooking, the leaves are in a poultice for application to wounds and ulcers and both the leaves and young shoots are used as vegetables. The paste of the roots is applied externally in treatment of earaches[9]. *Tamarindus indica* parts are variously useful[10], either nutritionally or medicinally. Analyses of the fruit pulp found it to be rich in calcium, phosphorus, iron, thiamine and riboflavin and a good source of niacin. Ascorbic acid content was found to be low except in the peel of young green fruits. *Tamarindus indica* leaves and flowers are useful as mordant in the dyeing and tanning industry[11]. The stem bark of *T. indica* has also been reported to contain tannins, saponins, sesquiterpenes, alkaloids and phlobatannins[12]. In Angola, *E. hirta* it is widely used against diarrhea and dysentery, especially amoebic dysentery[13].

In administering extracts from medicinal plants onto external wounds, it is important to ensure that the extract is duly delivered to the site of action in appropriate doses and that the delivery systems do not irritate the skin[14]. For such topical applications especially, it is well known that the delivery vehicle significantly affects the permeability of the drug. In this study, plant extracts that were tested were formulated into ointments using a polyethylene glycol (PEG) base. Relative to the poultices traditionally prepared for application of these plants onto wounds, formulation into ointment will allow very easy and convenient topical application. The PEG ointment has good texture and is an appropriate ointment base for tropical and subtropical weather conditions[15]. The aim of this study was to assess the antibacterial activities of ointment formulations of crude extracts of *C. viscosa*, *T. indica* and *E. hirta* against isolates of *P. aeruginosa* and *E. coli*.

MATERIALS AND METHODS

Source of test bacteria: Clinical isolates of *Escherichia coli* and *Pseudomonas aeruginosa* were collected from incision type wounds at the Medical Microbiology Laboratory of the Tamale Teaching Hospital in the Northern Region of Ghana, in May 2014. The isolates were sub-cultured to verify purity on chocolate agar (OXOID, Basingstoke, Hampshire, England) and stored between 2 and 8°C in nutrient broth (OXOID, Basingstoke, Hampshire, England).

Plant material: Aerial parts of the *Euphorbia hirta* plant and leaves of *Cleome viscosa* were collected from Tono irrigation farms in Navrongo. The stem bark of *Tamarindus indica* was collected from Navrongo Campus of the University for Development Studies in the Upper East Region of Ghana in October, 2013. The plant material were authenticated by Dr. Isaac Sackey of the Department of Applied Biology in the Faculty of Applied Sciences, University for Development Studies, Navrongo Campus.

Size reduction: The aerial part of *Euphorbia hirta* and the leaves of *Cleome viscosa* were washed with clean water and shade dried for 2 weeks. The stem bark of *Tamarindus indica* was air-dried at room temperature for 1 week and oven dried at 35°C to constant weight. The dried samples were taken through size reduction using a surface-sterilized mortar and pestle to obtain uniform powdered samples that were used for the extraction process.

Aqueous and ethanolic or methanolic extracts of *Cleome viscosa*, *Euphobia hirta* and *Tamarindus indica*: A 50 g portion of the dried powdered sample of *Cleome viscosa* was macerated in 150 mL of methanol at room temperature. The extract was filtered after 48 h and concentrated under reduced pressure to obtain a yield of 33% w/w. Another extraction of the powdered sample using the same procedure as before with distilled water as solvent, yielded 11.4% w/w of crude extract.

Of the dried powdered sample of *E. hirta*, 50 g were macerated in 150 mL of ethanol. The mixture was filtered after 48 h through sterile cotton. The filtrate was then concentrated under reduced pressure using a rotary evaporator at 45 to obtain a crude extract with yield of 7.8% w/w of the initial 50 g portion. The same process for the aqueous extract gave a yield of 10.4% w/w of crude extract.

For the powdered *Tamarindus indica*, 50 g of material were macerated in 150 mL of ethanol and allowed to stand while shaking intermittently. The mixture was filtered after 48 h and concentrated by rotary evaporation at a temperature not exceeding 60 to obtain crude extract with a yield of 7.2% w/w. Following the same procedure, aqueous extraction of *Tamarindus indica* gave a yield of 5.19% w/w.

The concentrated extracts were kept in a desiccator to remove possible traces of solvent present and stored in air tight containers at 4 for subsequent use.

Preparation of crude extracts for direct antibacterial testing: Crude extracts of the 3 plants were weighed into separate beakers and dispersed in DMSO to derive various concentrations of 25, 50, 100 and 200 μg mL^{-1} for each extract. These concentrations were then used for the antibacterial studies[14].

Preparation of PEG ointment: Polyethylene glycol 4000 (PEG-4000) and polyethylene glycol 400 (PEG-400), 20 g each were weighed into a beaker and melted on a water bath at 70-75°C until liquefied to make 50% PEG-4000 and 50% PEG-400[12,14]. The mixture was stirred continuously with a glass rod in a bowl of water at room temperature until it congealed.

Preparation of crude extract-PEG ointment formulations: Portions of the crude extracts of *T. indica*, *E. hirta* and *C. viscosa* were separately formulated with PEG ointment for testing of antibacterial activities. Equal quantities (25, 50, 100 and 200 mg) of each crude extract were weighed into appropriately labeled beakers: $T.I_1$–$T.I_4$ for *T. indica*, $E.H_1$–$E.H_4$ for *E. hirta* and $C.V_1$-$C.V_4$ for *C. viscosa*. The PEG ointment, 1.0 g was then added to each beaker and warmed to 40 while stirring continuously with a glass rod. The mixtures were allowed to cool at room temperature to produce crude extract-PEG ointment formulations at varying concentrations of 25, 50, 100 and 200 μg g^{-1}, respectively of each plant extract. Formulations were re-melted at 40 just prior to testing.

Agar diffusion bioassay: For testing antibacterial activity, the modified agar well diffusion method was employed. Colonies of a pure culture of each test organism were suspended in 100 mL of sterile peptone water to get a turbidity of 0.5 McFarland standard. Aliquots of 0.1 mL of each suspension were surface-spread on different Mueller Hinton Agar plates. Wells of 5 mm diameters were made on the culture plates with a sterile cork-borer at wide enough intervals and corresponding with the number of concentrations of each formulation and the positive and negative controls. For each test extract and its PEG formulation, 0.1 mL of each concentration was drawn into a labeled well. The same volumes of the negative control (98% DMSO) and positive control (tetracycline at 30 μg mL^{-1}) were also introduced into a well each on the same plate (data not shown). Plates were left to stand for up to 30 min and then incubated in inverted positions at 37 for 48 h. Inhibition zone diameters were then measured and recorded in millimeters. The experiment was done in triplicate to check for reproducibility.

Statistical analysis: Means and standard mean errors were calculated for the zones of inhibition recorded. These means were analyzed for significant differences at p-value 0.05 using one-way ANOVA.

RESULTS AND DISCUSSION

The antibacterial activity of test and formulated extracts of *Cleome viscosa*, *Euphorbia hirta* and *Tamarindus indica* were studied at concentrations of 25, 50 100 and 200 μg mL^{-1} for the test extracts and 25, 50, 100 and 200 mg g^{-1} for the PEG formulations. Antibacterial activities were indicated by the diameters of zones of inhibition of bacterial growth for both the test extracts and extract-PEG ointment formulations. Generally, the crude extracts as well as their formulations showed significant inhibition of the test bacteria with levels of inhibition increasing with extract concentrations. The inhibition zones recorded were comparable to those reported by Donkor *et al.*[16] for activity of some plant extracts against *E. coli* and *P. aeruginosa*. Significant differences reported were determined at p-value of 0.05.

Except for the *C. viscosa* aqueous extract-PEG formulation, all *C. viscosa* preparations tested exhibited various levels of inhibition against *E. coli* (Fig. 1). Aqueous crude extract of *C. viscosa* in DMSO was not potent against *P. aeruginosa* but its formulation exhibited potency against *P. aeruginosa*, showing up to 11 mm inhibition at 200 μg mL^{-1} (Fig. 2). Saradha and Rao[17] however, reported activity at higher concentrations of aqueous extract of *C. viscosa* aerial parts against *P. aeruginosa* but not *E. coli*. In this study, both crude and the formulated methanol extracts of *C. viscosa* showed

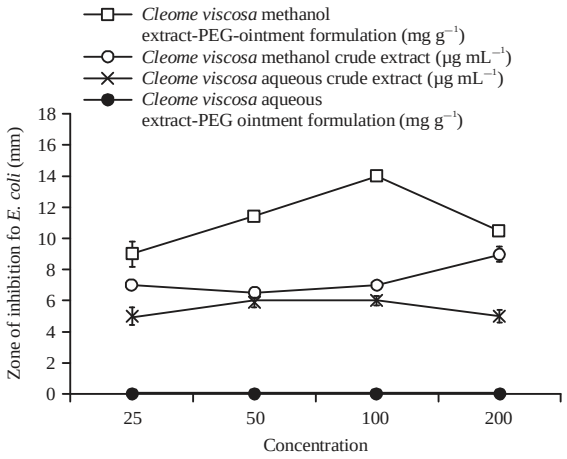

Fig. 1: Representative antibacterial activity of formulated and unformulated aqueous and methanolic extracts of *Cleome viscosa* against *E. coli*. The data shown represent the average of 3 wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within the range of the presented data

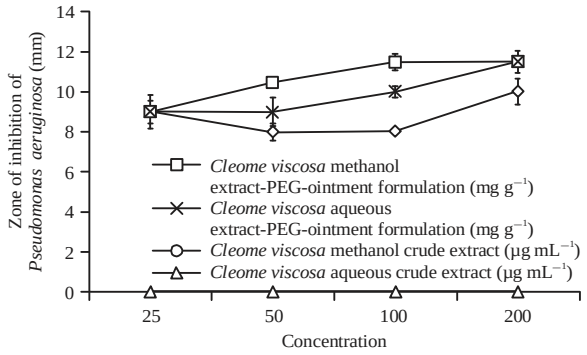

Fig. 2: Representative antibacterial activity of formulated and unformulated aqueous and methanolic extracts of *Cleome viscosa* against *Pseudomonas aeruginosa*. The data shown represent the average of 3 wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within the range of the presented data

activity against both *E. coli* and *P. aeruginosa*, with inhibition being highest at 100 µg mL^{-1} of the formulation. This is in line with reports by Donkor *et al.*[16], Jane and Patil[18] and Panduraju *et al.*[19]. There was no significant difference between inhibitions at 25 and 200 µg mL^{-1} of the crude methanolic extract (Fig. 1, 2).

Euphorbia hirta showed inhibition for only the aqueous crude extract against *E. coli*. The aqueous extract-PEG formulation and ethanolic extract and its formulation were not

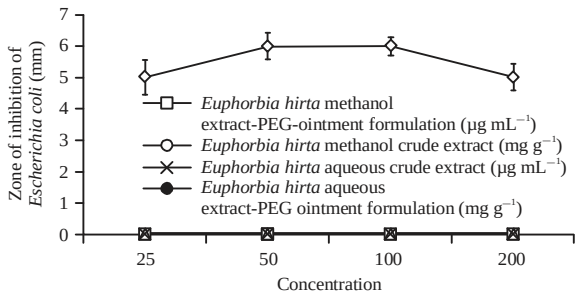

Fig. 3: Representative antibacterial activity of formulated and unformulated aqueous and ethanolic extracts of *Euphobia hirta* against *Escherichia coli*. The data shown represent the average of 3 wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within the range of the presented data

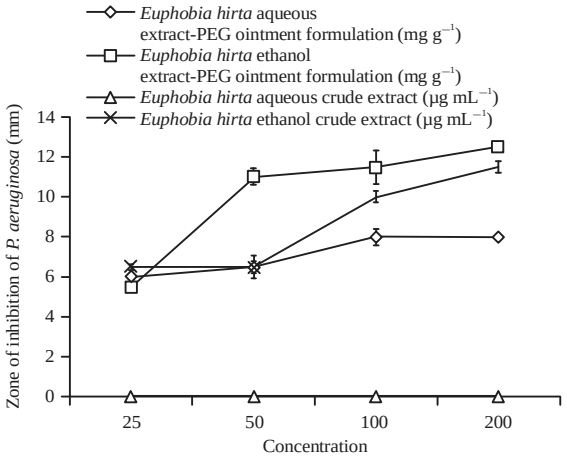

Fig. 4: Representative antibacterial activity of formulated and unformulated aqueous and ethanolic extracts of *Euphobia hirta* against *Pseudomonas aeruginosa*. The data shown represent the average of 3 wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within the range of the presented data

potent against *E. coli* (Fig. 3). Both the aqueous crude extract of *E. hirta* and its extract-PEG formulation showed potency against *P. aeruginosa* with maximum inhibition of the formulated sample occurring at 200 µg mL^{-1} (Fig. 4). The crude ethanolic extract showed no inhibition against *P. aeruginosa* at all concentrations tested, but the ethanolic extract-PEG formulation showed inhibitory activity against *P. aeruginosa*, also with a maximum at 200 µg mL^{-1} (Fig. 4). Crude ethanolic extract of *E. hirta* has however, been previously reported to have bioactivity at similar concentrations against both *E. coli* and *P. aeruginosa*[6,18,19].

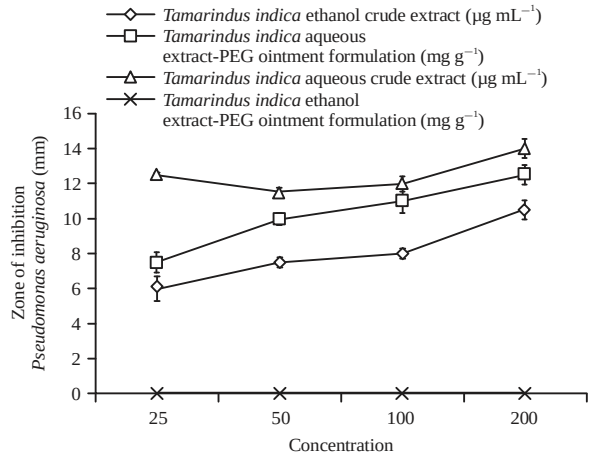

Fig. 5: Representative antibacterial activity of formulated and unformulated aqueous and ethanolic extracts of *Tamarindus indica* against *Pseudomonas aeruginosa*. The data shown represent the average of 3 wells treated on the same day. The experiment was repeated 3 times and day-to-day variation was found to be within the range of the presented data

In this study, aqueous crude extract of *T. indica* inhibited both *E. coli* (data not shown) and *P. aeruginosa* (Fig. 5), whilst the aqueous extract-PEG formulation only inhibited *P. aeruginosa*. This is contrary to the report by Escalona-Arranz *et al.*[20] indicates lack of activity of aqueous extract of *T. indica* leaves against both *E. coli* and *P. aeruginosa*. However, Doughari[12] also reported activities of both aqueous and ethanolic extracts of *T. indica* leaves against tested strains of both bacterial species. The ethanolic crude extracts were potent against both bacteria whilst the formulated ethanolic extract showed no inhibition against either (Fig. 5).

As suggested by Escalona-Arranz *et al.*[20], differences between the observed bioactivities of the plant extracts used in this study and data from other reports suggest the possibility of variations between the physiologies and accumulated metabolites of the different varieties of *C. viscosa*, *E. hirta* and *T. indica* used in the studies.

CONCLUSION

Generally, the crude extracts of *Cleome viscosa, Euphorbia hirta* and *Tamarindus indica* showed antibacterial activity against both *E. coli* and *P. aeruginosa*. This study confirms that the plants *are* potential sources of natural antibacterial agents to be used against wound infections. It has also been shown that ointment formulations of appropriate extracts of the plants with PEG could be explored for application in wound treatment. Further study on separation and purification of the bioactive compounds will contribute to utilization of the bioactive agents globally.

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