ORIGINAL RESEARCH ARTICLE

Antimicrobial potency of *Euphorbia heterophylla* against selected clinical isolates

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Abstract

Reports on the antimicrobial potentials of *Euphorbia heterophylla* are scanty globally. This study investigated the antimicrobial effects of *E. heterophylla* against microorganisms of clinical importance. Cold water, hot water, chloroform and methanol extracts of the leaf, stem and fruit of *E. heterophylla* were obtained. The phytochemical properties of the plant parts were determined, and antimicrobial analyses of extracts investigated against sixteen clinical isolates, in accordance with standard procedures. The microorganisms tested were nine clinical bacterial strains which included *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* NCIB 950, *Salmonella typhi*, *Escherichia coli*, *Escherichia coli* NCIB 86, *Staphylococcus aureus*, *Staphylococcus aureus* NCIB 8588, *Klebsiella pneumoniae* and *Serratia marcescens,* and seven fungal strains which were *Candida albicans, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Trichoderma viride, Trichophyton rubrum,* and *Malassezia furfur.* The qualitative and quantitative phytochemical analyses of extracts revealed the presence of steroids, alkaloids, flavonoids, tannins, terpenoids and carbohydrates at varying concentrations. *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* NCIB 950 and *Candida albicans* were sensitive to the cold water and hot water extracts of the plant's parts while chloroform and methanol extracts did not show antimicrobial activities against any of the organisms. The MIC of the extracts ranged from 6.25 – 25 mg/mL. This study revealed that *E. heterophylla* is a promising plant species that could be employed in the treatment of infections caused by *P. aeruginosa* and *C. albicans.*

Section: Biological

Key words: *Antimicrobial, microorganisms, clinical isolates, medicinal plants, phytochemical.*

INTRODUCTION

Medicinal plants have been employed since ancient times, not just as antioxidants and flavoring agents, but as well, for their antimicrobial activities (Curti *et al*., 2014; Diop *et al*., 2018). Several plants used in traditional medicine are rich sources of natural bioactive substances with health-promoting activities and no side effects. In the recent days, over 65% of the world population relies on traditional medicine for health care (Curti *et al*., 2014; Diop *et al*., 2018;

Saleh *et al*., 2019). A high demand has, also, risen for preservative-free cosmetics and antimicrobial extracts of plant origin, aimed to reduce the challenges of allergies connected to synthetic preservatives such as methylparabens (Herman *et al*., 2013).

E. heterophylla is also referred to as *E. geniculate*, *E. pronifolia*, *Poinsettia geniculata* and *P. heterophylla.* The plant species is a tropical annual weed possessing characteristic milky latex. The genus *Euphorbia* (*Euphorbia*ceae) are flowering plants, with 1836 established species (The Plant List, 2019), sub-divided into several subgenera. This genus is widely distributed and present in all temperate and tropical regions. This group of plants has variety of forms, from small ephemerals to various forms of herbaceous annuals or perennials, small trees, big shrubs, cactus-like succulents and cushion-forming subshrubs (Saleh *et al*., 2019). Out of 243 *Euphorbia* species evaluated by the IUCN Red List of Threatened species, 170 (70%) are going into extinction (categories vulnerable, endangered, and critically endangered) (IUCN Red List, 2019). Over 5% of *Euphorbia* species are employed in traditional medicine, majorly as emetic and purgative agents, to address digestive and respiratory disorders, migraine, skin and inflammatory conditions, intestinal parasites, gonorrhoea, wart etc. The roots, seeds, fruits, latex, stem, wood, barks, leaves, and whole plants of the *Euphorbia* species are usable (Özbilgin & Saltan, 2012; Ernst *et al*., 2015; Pascal *et al*., 2019).

Plate 1: *E. heterophylla's* leaves; **Plate 2: *E. heterophylla*'s stem;** **Plate 3: *E. heterophylla*'s fruits**

Euphorbia species possess curative properties due to the presence of various phytochemicals, which constitute their secondary metabolites (Mali & Panchal, 2017a; Pascal *et al*., 2019). They belong majorly to the flavonoids, and polyphenols classes which also exhibit various biological effects such as anti-inflammatory, multidrug resistance modulators, cytotoxic, mammalian mitochondrial respiratory chain inhibition, HIV-1 and bacterial infection inhibition (Geng *et al*., 2015). There is a lot of attention in *Euphorbia*-derived metabolites majorly because of the diterpeneingenol mebutate present in *E. peplus* L. (as well as in *E. lathyris* L., *E. nivulia* Buch.-Ham., *E. esula* L., *E. antiquorum* L., *E. serpens Kunth*, and *E. fischeriana Steud*.), and is the active ingredient of Picato®medicine employed in topical therapy against the precancerous skin condition actinic keratosis (Frezza *et al*., 2018; Seca & Pinto, 2018). Some *Euphorbia* compounds are, however, toxic, as a result of evolutionary strategy of plant defence against predators (e.g., herbivores), compounds that possess a caustic and irritating effect to the skin and promote tumours (Machado *et al*., 2016).

Euphorbia plants can be distinguished easily by their toxic and skin irritant milky latex and majorly inflorescences, designated as cyathia (Horn *et al*., 2012), while some species such as *E. milii* Des Moul., *E. tirucalli* L., and *E. lacteal* Roxb are generally used as ornamental plants (Saleh *et al*., 2019). The latex is the most valuable product obtained from *Euphorbia* species despite being toxic, it contains several biologically active natural compounds, such as triterpenoids. Latex is useful commercially in the production of invaluable material such paints and natural rubber (Saleh *et al*., 2019). *E. heterophylla* parts have been used in traditional medicine as laxative, anti-gonorrheal, migraine and wart cures. The plant lattices have been efficiently applied as fish poison, insecticide and ordeal poisons. A scanty research had been carried out on antimicrobial potentials of *E. heterophylla.* This study, therefore, investigated the antimicrobial potentials of *E. heterophylla* against microorganisms of clinical importance.

MATERIALS AND METHODS

Collection and Identification of *Euphorbia heterophylla*

The leaves, stem and fruits of *Euphorbia heterophylla* (Plates 1, 2 and 3, respectively) were freshly harvested from the botanical garden of the Federal University of Technology, Akure in July, 2018. The plant species was identified and authenticated by Prof. M.O. Soladoye, a Professor of Botany at Augustine University, Ilara Epe, Lagos, Nigeria.

Collection of Organisms

Six clinical bacterial isolates which were *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli* and *Klebsiella pneumonia* and five clinical fungal isolates which included *Aspergillus fumigatus, Malassezia furfur, Candida albicans, Trichoderma viride* and *Trichophyton rubrum* were obtained from the Microbiology Laboratory of Ondo State Specialist Hospital, Akure. Three typed bacterial cultures including *E. coli* NCIB 86, *S. aureus* NCIB 8588 and *P. aeruginosa* NCIB950 were obtained from Microbiology Department, Obafemi Awolowo University (OAU) Ile-Ife and two fungal pathogens - *A. flavus* BN22 and *A. parasiticus* BN48 - were obtained from International Institute of Tropical Agriculture (IITA), Ibadan.

Preparation of Crude Plant Extracts

Extraction of the leaves, stem and fruits of the *E. heterophylla* was carried out with chloroform, methanol (80 %), hot water and cold water as solvents. Using chloroform and methanol for extraction, the aerial parts of the plant (leaves, stem and fruits) were rinsed with cooled boiled water, drained and air dried for two weeks. These were separately milled into powdery forms using a clean mechanical blender and stored in sterile air tight plastic container. The containers were allowed to stay for 48 hours with occasional stirring and after which filtration was done. Freshly harvested aerial part (700 g) of *E. heterophylla* was macerated using mortar and pestle after it has been rinsed in sterile water. This was then boiled in two litres of distilled water for 30 minutes. It was allowed to cool and then filtered into sterile flasks using muslin cloth that had been doubly folded. The cold water extraction was done by weighing 700 g of freshly harvested and macerated plant parts and soaked in two litres of sterile distilled water for 48 hours with constant stirring. This was later filtered in sterile flask at the end of the 48 hours using sterile muslin cloth. These were allowed to settle to get concentrated extracts. Since the organic solvents were volatile, the filtrates were exposed so that the solvents could evaporate while the supernatants of the hot and cold water extracts were decanted. All the prepared extracts were

kept at 4 ^{o}C in a refrigerator for at least 24 hours before subsequent testing (Ugachuckwu *et al*., 2014).

Reconstitution and Sterilization of Extracts

The methanolic and chloroform extracts were reconstituted using 0.01 % Tween 20. This was done by dissolving 1 g of the extract in 10 ml 0.01 % Tween 20. The resultant solution was then filtered using sterile Millipore membrane filter (0.45 µm). The cold and hot water extracts were reconstituted by dissolving 1 g of the extract in 10 ml sterile distilled water and then filtered using sterile Millipore membrane filter (0.45 µm) (Hazra *et al*., 2019).

Screening for various phytochemical groups

Screening of phytochemicals in the plant extracts was determined as described by Evans (2009). Chemical tests were carried out on the extracts using standard procedures.

Alkaloids

To test for alkaloids, 50 g of the powdered extract was mixed with 250 ml of 1 % sulphuric acid. It was allowed for 3 mins and then filtered. Ten mils of the filtrate was shaken and added to Meyer's reagent. A whitish yellow precipitate indicated presence of alkaloids.

Flavonoids

A few drops of dilute hydrochloric acid and a small piece of magnesium was added into a test tube containing 2 mls extract of plant and boiled for a few minutes. Reddish pink or dirty brown colour was produced in the presence of flavonoids.

Saponins

A 2-ml portion of the crude extract was dissolved in 50 ml of sterile distilled water and vigorously shaken. There was a honey-comb formation for few minutes which indicated the presence of saponins.

Cardiac Glycosides

A2-ml proportion of the crude extract was dissolved in 1 ml of distilled water and aqueous sodium hydroxide solution added. A yellow color showed the presence of glycosides.

Terpenoids

A2-ml portion of the crude extract was mixed in 2 ml of chloroform; 3 ml of concentrated sulphuric acid was added carefully to form a layer. A reddish-brown color formed at the interface showed the presence of terpenoids.

Tannins

Powdered *E. heterophylla* aerial parts (0.2 g) were separately weighed into a conical flask and mixed with 50 mls of water, boiled in a water bath for 5 min. The mixture was filtered, without allowing to cool, with a filter paper and the filtrate collected in a beaker. Two mils of the filtrate were mixed with 10 mls of distilled water and then a drop of iron (III) chloride was added.

Steroids
Two mils of the crude extract were placed into a test tube in which 0.5 ml sulphuric acid, acetic anhydride and chloroform in similar amounts were added. A green coloration showed the presence of steroids.

Molisch's Test
Powdered sample (0.1 g) was weighed into a beaker and 20 ml of distilled water was added. The beaker was heated for 6 min in a water bath. Two mils of the filtrate were introduced into a test tube and 2 drops of alcoholic solution of α-naphthol added; concentrated sulphuric acid was added down the side into the test tube. A colour change of the reaction was noted.

Antimicrobial Potentials of *E. heterophylla*
Agar well diffusion technique was used to determine the *in vitro* antimicrobial activity of the crude extracts. One mil of the 18-hour old broth culture of the selected microorganisms, whose suspension had been adjusted to turbidity equivalent of 0.5 McFarland standards, was dispensed into sterile appropriately labelled Petri dishes. Molten sterile nutrient agar and potato dextrose agar were aseptically and separately poured into the plates which were gently rotated to obtain homogenous distribution of the bacteria and fungi, respectively. The agar plates were allowed to solidify, and the seeded plates were incubated for 2 hours to ensure adaptability. A cork borer of 6 mm in diameter was used to cut uniform wells in the agar plates. The wells were then filled with 0.5 ml of each of the extracts. The experiment was conducted in duplicates. All the plates were incubated at 37 ^{o}C for 24 hours for bacteria and at 25 ^{o}C for 48 hours for fungi. Zones of inhibition around the well were noted and measured in millimeters.

Minimal inhibitory concentration (MIC) of *E. heterophylla*
Minimal inhibitory concentration (MIC) of *E. heterophylla* was determined by the broth microdilution assay. The various plant extracts were separately diluted to the concentration of 75 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.125 % in a 96-well microtiter plate. Then, 100 µl each of bacterial and fungal suspensions were separately added to duplicate wells of each dilution and incubated at 37 °C for 24 hr, and 25 °C for 24 hr, respectively. DMSO treated wells and wells with no organism (media only) were included for controls.

Data Analysis
Data were collated and statistically analyzed by MedCalc statistical software; version 17.2 using one-way Analysis of Variance (ANOVA) and independent t-test. Data were presented as mean ± standard error (SE). The significance was determined at 95% level of confidence ($p \leq 0.05$).

RESULTS
Table 1 shows the phytochemical constituents of the leaf, stem and fruit of *E. heterophylla*. Cardiac glycosides and saponins were absent from the leaf, stem and fruit of the plant. Steroids were present in the leaf and more abundant in the stem and fruit of the plant. Alkaloids and tannins were also present in the fruit but more abundant in the leaf and stem. The leaf, stem and fruit were found to be rich in terpenoids and carbohydrates as these constituents were found to be highly abundant in *E. heterophylla.*

The antimicrobial potential of cold water, hot water, methanol and chloroform extracts of *E. heterophylla*'s leaf, stem and fruit against selected pathogenic microorganisms was shown in Table 2. The antimicrobial potency of the cold water, hot water, methanol and chloroform extracts of *E. heterophylla* was evaluated against 9 clinical bacterial strains which included *Pseudomonas aeruginosa, Pseudomonas aeruginosa* NCIB 950*, Salmonella typhi, Escherichia coli, Escherichia coli* NCIB 86*, Staphylococcus aureus, Staphylococcus aureus* NCIB 8588*, Klebsiella pneumoniae* and *Serratia marcescens,* and 7 fungal strains which were *Candida albicans, Aspergillus fumigatus, Aspergillus flavus, Aspergillus parasiticus, Trichoderma viride, Trichophyton rubrum* and *Malassezia furfur.* Among these organisms, *Pseudomonas aeruginosa, Pseudomonas aeruginosa* NCIB 950 and *Candida albicans* were sensitive, only to the cold water and hot water extracts of the plant's parts.

Pseudomonas aeruginosa strain showed inhibition zones of 18.00 $\pm$ 1.00, 16.00 $\pm$ 0.80 and 17.00 $\pm$ 1.00 mm, as exhibited by the cold-water extract of the leaf, stem and fruit, respectively while the organism showed inhibition zones of 16.00 $\pm$ 0.50, 13.50 $\pm$ 0.50 and 9.50 $\pm$ 0.50 mm to the hot water extract of leaf, stem and fruit, respectively. There were no statistical differences ($p \geq 0.05$) among susceptibility patterns exerted by cold water extracts of the leaf, stem and fruits of the plant against *P. aeruginosa* while the hot water extracts of the plant's parts showed significant differences form each other ($p \leq 0.05$). *Pseudomonas aeruginosa* NCIB 950 showed inhibition zones (mm) of 20.00 $\pm$ 1.00, 17.5 $\pm$ 0.80 and 19.00 $\pm$ 1.00 to the cold-water extract of leaf, stem and fruit, respectively, while to the hot water extract, inhibition zones of 9.00 $\pm$ 0.50, 7.00 $\pm$ 0.50 and 7.00 $\pm$ 0.50 mm, respectively, were shown. The susceptibility patterns exerted by cold water extracts of the leaf, stem and fruits of the plant against *P. aeruginosa* NCIB 950 showed no statistical differences ($p \geq 0.05$) as that exerted by hot water extracts of the plant's parts also showed no significant differences from each other ($p \geq 0.05$).

Furthermore, *Candida albicans* exhibited inhibition zones of 14.00 $\pm$ 0.80, 16.50 $\pm$ 1.00 and 11.50 $\pm$ 0.50 mm to the cold-water extract of leaf, stem and fruit, respectively while 7.50 $\pm$ 0.40, 10.50 $\pm$ 0.50 and 8.00 $\pm$ 0.50 mm, respectively were shown to the hot water extracts of the plant's parts. There were significant differences among sensitivity patterns exerted by the cold and hot water extracts of the leaf, stem and fruit of *E. heterophylla* against *Candida albicans.* The study showed that *Pseudomonas aeruginosa, Pseudomonas aeruginosa* NCIB 950 and *Candida albicans* were susceptible to cold and hot water extracts of the leaf, stem and fruit of *E. heterophylla* while no antimicrobial activities were exerted by the methanol and chloroform extracts of the plant's parts.

Fig.1 shows the minimum inhibitory concentrations (MICs) of cold-water extracts of *E. heterophylla*'s leaf, stem and fruit against sensitive clinical isolates. The MIC of the cold-water extracts of *E. heterophylla*'s leaf, stem and fruit against *P. aeruginosa* was 6.25 mg/mL while it ranged from 6.25 – 12.5 mg/mL in the case of *P. aeruginosa* NCIB 950 with fruit extract having the highest. The MICs of the plant's leaf, stem and fruit against *C. albicans* were 12.5, 12.5 and 25 mg/mL, respectively.

The minimum inhibitory concentrations (MICs) of hot water extracts of the leaf, stem and fruit of *E. heterophylla* against sensitive clinical isolates were shown in Fig. 2. The MICs of the hot water extract of the plant's leaf, stem and fruit against *P. aeruginosa* were 6.25, 12.5 and 12.5 mg/mL, respectively. The MIC of the hot water extracts of the three aerial parts of the plant

against *P. aeruginosa* NCIB 950 was 12.5 mg/mL while it was 25, 12.5 and 25 mg/mL for *C. albicans.*

Table I: Photochemical analysis of *E. heterophylla*

	E. heterophylla		
Component	**Leaf**	**Stem**	**Fruit**
Cardiac Glycosides	-	-	-
Steroids	+	++	++
Alkaloids	++	++	+
Flavanoids	+	+	+
Saponins	-	-	-
Tannins	++	++	+
Terpenoids	+++	+++	+++
Carbohydrate	+++	+++	+++

Keys:
- = absence of phytochemicals
+ = presence of phytochemicals
++ =abundance of phytochemicals
+++ = highly abundant

Table II: Antimicrobial potential of 100 % concentrations of cold water, hot water, methanol and chloroform extracts of *E. heterophylla*'s leaf, stem and fruit against selected pathogenic microorganisms

Microorganisms	Zones of inhibition (mm)											
	Cold water extract			Hot water extract			Methanol extract			Chloroform extract		
	Leaf	Stem	Fruit	Leaf	Stem	Fruit	Leaf	Stem	Fruit	Leaf	Stem	Fruit
Pseudomonas aeruginosa	^{a}18.00±1.00	^{a}16.00±0.80	^{a}17.00±1.00	^{a}16.00±0.50	^{c}13.50±0.50	^{b}9.50±0.50	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Pseudomonas aeruginosa NCIB 950	^{a}20.00±1.00	^{a}17.50±0.80	^{a}19.00±1.00	^{b}9.00±0.50	^{b}7.00±0.50	^{b}7.00±0.50	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Salmonella typhi	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Escherichia coli	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Escherichia coli NCIB 86	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Staphylococcus aureus	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Staphylococcus aureus NCIB 8588	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Klebsiella pneumonia	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Serratiamarcescens	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Candida albicans	^{c}14.00±0.80	^{a}16.50±1.00	^{d}11.50±0.50	^{b}7.50±0.40	^{d}10.50±0.50	^{b}8.00±0.50	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Aspergillus fumigates	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Aspergillus flavus	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Aspergillus parasiticus	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Trichoderma viride	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Trichophyton rubrum	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00
Malassezia furfur	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00	^{e}0.00

Data with same alphabetic superscript ($^{a-e}$) along same column and row showed no statistical difference

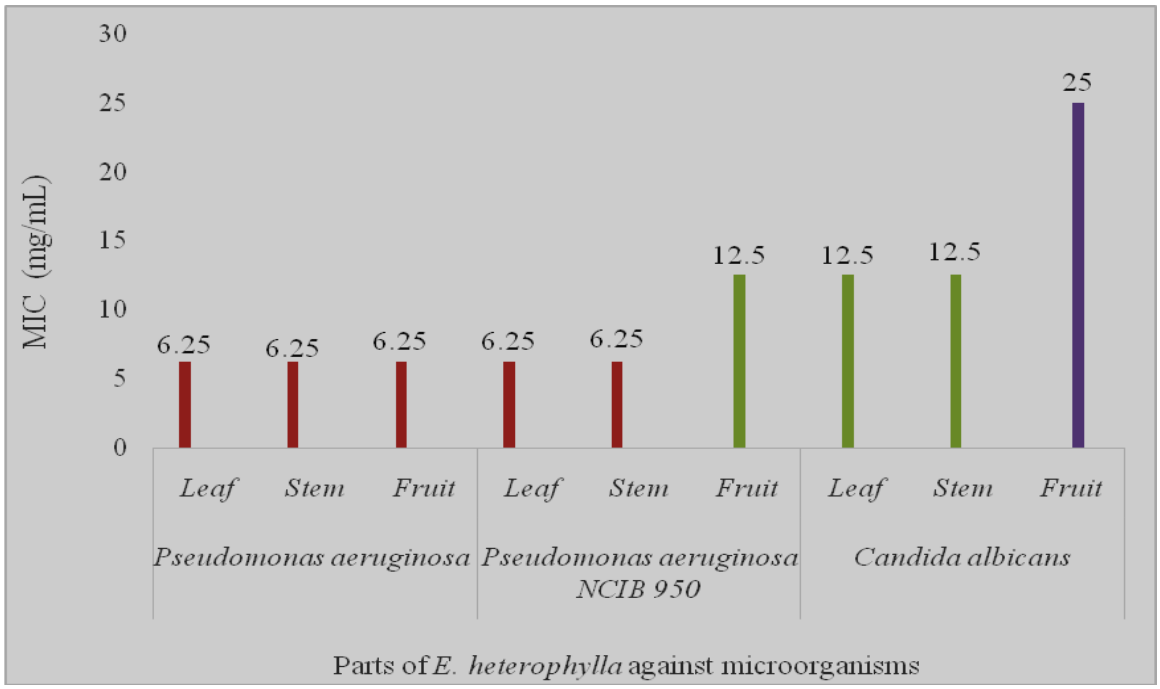

Figure 1: Minimum inhibitory concentrations (MICs) of the cold-water extracts of *E. heterophylla*'s leaf stem and fruit against sensitive clinical isolates

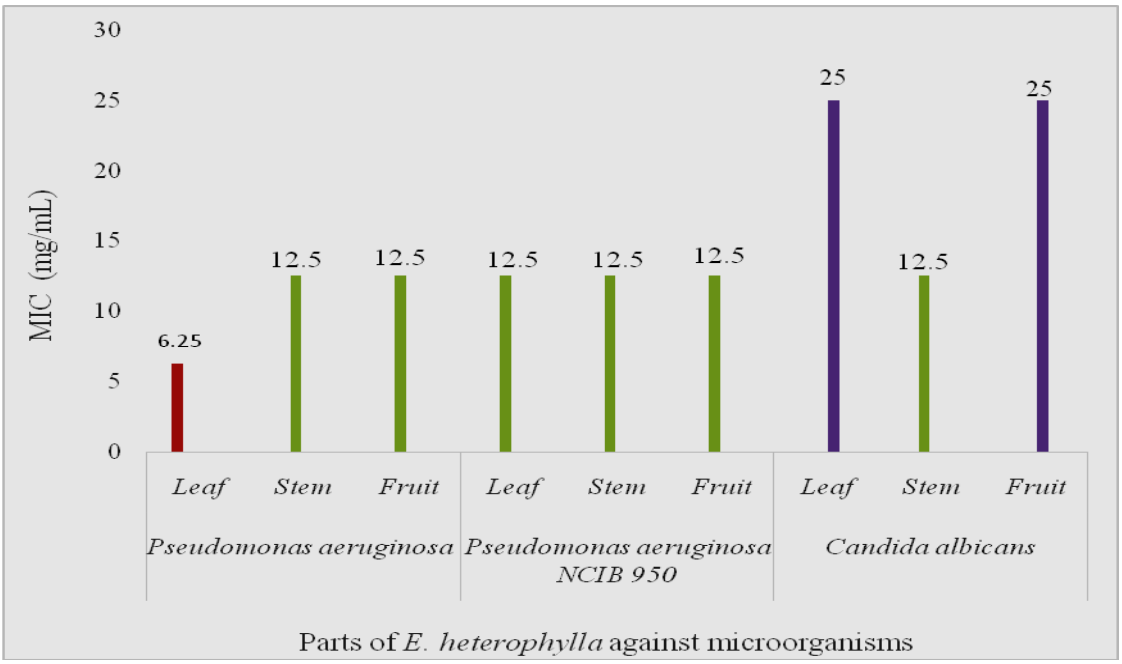

Figure 2: Minimum inhibitory concentrations (MICs) of hot water extracts of *E. heterophylla*'s leaf, stem and fruit against sensitive clinical isolates

DISCUSSION

Since consumers prefer healthy products free of synthetic raw materials, the continually growing interest in the natural and ecologically friendly antimicrobial agents is still being noted. Therefore, research on the antimicrobial activity involving *Euphorbia* species is relevant and, even if findings are negative, it is crucial they are reported to enrich available information and literature of these plant species.

The antimicrobial potency of *E. heterophylla* against *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* NCIB 950 and *Candida albicans* reported in this study was buttressed by the submissions of Sharanappa and Vidyasagar (2013), Mali and Panchal (2017b), Kumari (2018) and Siritapetawee *et al*. (2019) that plants belonging to the genus *Euphorbia* are of the great interest in the matter of their antimicrobial activity. Diop *et al*. (2018) also reported that the genus *Euphorbia* have been generally important in traditional medicine in the treatment of microbial infections. This study also agrees with the notion of Zengin *et al*. (2017) that *Euphorbia* plants are a promising source of phytochemicals employed in pharmacy and food industries.

The methanol and chloroform extracts of the leaf, stem and fruits of *E. heterophylla* showed no antimicrobial activity against the test organisms in this study. Ashraf *et al*. (2015) reported that the hexane extract of *E. royleana*, when compared with methanol and water extracts, had the highest phenolic and flavonoid contents and the best antimicrobial activity. The authors further reported that the plant species exhibited antimicrobial activities against *Aspergillus niger* and Gram-positive bacteria which contradicted the findings of this study. Although, the fact that different species of the plant were investigated in the studies could have been responsible for the variations in the results. Kumara-Swamy *et al*. (2011) reported that, irrespective of the prepared extracts and the test microorganisms, *E. neriifolia* exhibited low antibacterial and antifungal activity, making *Euphorbia* species less interesting, as a source of antimicrobial agent. In a similar study by Hlila *et al*. (2017), the chloroform extract of *E. paralias L.* stem was the most interesting extract as it exhibited similar activity against fungi, Gram-positive and Gram-negative bacteria (MIC= 15 µg/mL against *C. albicans, B. subtilis*, and *E. coli* strains), while the chloroform extract of leaves only exhibited activity against *C. albicans*, which is different from the finding of this study on *E. heterophylla.*

Chanda and Baravalia (2010) reported the spectrum of bacteria and fungi associated with skin infections (zones of growth inhibition ranged from 9.0 mm (*B. subtilis*) to 14.0 mm (*P. aeruginosa*). These results support the use of these species in traditional Indian medicine, as they could be used as an easily accessible source of natural antimicrobial agents (Saleh *et al*., 2019). It was also reported by Awaad *et al*. (2017) that the ethanolic extract of *E. hirta* aerial parts had the highest potency against all the microorganisms tested when compared with *E. granulate* and *E. helioscopia* ethanol extracts, exhibiting an antifungal activity similar to amphotericin B against *M. canis* in the same experimental conditions. The authors, however, demonstrated thatheptacosan-1-ol, isolated from the active extract, could be responsible for the antimicrobial activity of the *E. hirta* extract.

Similar to this study, Ogbulie *et al*. (2007) revealed that the ethanolic extracts of *E. hirta* leaves showed higher activity than the ethanol extract of aerial parts against same strains of bacteria.

This, however, differed from the study of Perumal *et al*. (2012) who reported that the aerial parts exhibited the strongest antimicrobial activity against *Salmonella typhi* with MIC value of 31 mg/mL, an activity higher than the chloramphenicol activity against the same strain. Furthermore, the authors reported that hexane extract was not active against all the tested microorganisms, except *P. vulgaris* against which a weak activity was exhibited (Perumal *et al*., 2012). Pisano *et al*. (2016) carried out studies on the antimicrobial activity of the aqueous and ethanolic extracts from leaves, stems, and flowers of *E. characias L* and reported that these extracts exhibited no activity against the organisms tested except *Bacillus subtilis* to which the leaf extract exhibited antimicrobial activity, which was in line with the findings of this present study where antimicrobial activities were exhibited against three of the sixteen microorganisms tested.

CONCLUSION

This study revealed that *E. heterophylla* is a promising plant species that could be employed in the treatment of infectious diseases caused by *P. aeruginosa* and *C. albicans.* More research should be channeled towards investigating the antimicrobial potentials of the *E. heterophylla* against different microorganisms in order to enrich the presently scanty literature on the antimicrobial activities of the plant species.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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