

IN VITRO ANTIMICROBIAL ACTIVITIES OF *EUPHORBIA HETEROPHYLLA* AND *ZINGIBER OFFICINALE* ON SELECTED CLINICAL ISOLATES

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ABSTRACT

Euphorbia heterophylla and *Zingiber officinale* have been used to treat many microbial infections. The study assessed the *in vitro* antimicrobial potency of *E. heterophylla* and *Z. officinale* against clinical microbial isolates. The antimicrobial activities of aqueous and methanol extracts of *E. heterophylla* and *Z. officinale* were assessed against three clinical isolates: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. This was done by measuring their zones of inhibition, minimum inhibitory concentrations (MICs), and minimum bactericidal and fungicidal concentrations (MBCs and MFCs). The zones of inhibition against all the test microorganisms by almost all the extracts at a concentration of 10 mg/ml ranged from 12 to 29 mm. The highest zone of inhibition for *S. aureus* was obtained with the hot methanol *Z. officinale* extract, while that for *P. aeruginosa* was from the cold aqueous *Z. officinale* extract. *C. albicans* was most inhibited by hot aqueous *Z. officinale* extract. The cold and hot methanol extracts of *Z. officinale* did not affect *C. albicans*. The MIC ranged from 6.5 to 50 mg/ml. The MBC and MFC ranged from 25 to 100 mg/ml. These medicinal plants exhibited positive antimicrobial activity against clinical isolates, indicating their potential for use in antimicrobial drug development.

Keywords: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration

INTRODUCTION

Medicinal plants have been used for many decades and are crucial for providing basic health care to the general populace. They are used by more than 65% of communities worldwide, as extracts or decoctions by people of different regions (Solarov *et al.*, 2022; Orji *et al.*, 2025). They comprise natural, biologically active compounds with health-promoting potential, such as saponins, tannins, flavonoids, terpenoids, and alkaloids, which are responsible for their curative effects (Kone *et al.*, 2020; Orji *et al.*, 2025).

Euphorbia heterophylla is a medicinal plant that has been sparsely researched (Kone *et al.*, 2020). The plant grows as a weed, mainly in tropical and sub-tropical climates. In Nigeria, it is mainly found in cassava farms during the planting season. Known as Mexican fire plant, Spurge weed, or Milk weed the world over, the local tribes in Nigeria have different names for it: nono-kunchiya in Hausa, egele or aka-ito in Igbo, and *adimeru* in Yoruba (Oyedum *et al.*, 2021). The leaf, stem, and root extracts are used as purgatives and to

treat gonorrhea, malaria, and skin infections. *E. heterophylla* is also utilized against a wide range of health conditions like inflammation, parasitic infections, migraine, and respiratory disorders like asthma, among others (Kone *et al.*, 2020; Oyedum *et al.*, 2021).

Zingiber officinale (Ginger) is a pungent, spicy herbal rhizome that has been extensively used worldwide. It is a widely consumed root crop, cherished for its antibacterial properties for many years (Weil, 2005). This rhizome is used in different forms: as a powder or a liquid, as tea, or as a mixture. It is also a well-recognized spice used for cooking, flavoring, and herbal treatments (Hussain *et al.*, 2022). *Z. officinale* is noted for its curative effect over some unrelated health challenges like constipation, arthritis, dementia, cramps, vomiting, rheumatism, sprains, coughs, muscular aches and pains, hypertension, bloating, stings and bites, fever, and infections (Bode & Dong, 2011; Hussain *et al.*, 2022). *Z. officinale* has direct anti-microbial, anti-inflammatory, antiemetic, immune-protective, and hepato-protective activities and thus, is used to treat bacterial infections (Hussain *et al.*, 2022; Verma & Bisen, 2022). *Z. officinale* is of the family of Zingiberaceae, whose member plants have strong therapeutic properties because they possess potent secondary metabolites: 6-shogaol, 6-dehydrogingerols, 6-, 10- and 12-gingerols, gingerdiones and gigerdiols, among others, which explain their potent pharmacological capabilities (Olajide & Adetuyi, 2023).

A steady increase in demand for naturally occurring therapeutic resources, such as plants, used either singly or in combination as decoctions, has been noted. This is because the use of synthetic chemicals in pharmaceuticals has consistently been associated with adverse effects on the body (Eapen *et al.*, 2024). When plants are used as therapeutics, they exhibit few or no side effects while still possessing strong curative potential due to their pharmacologically active substances (George, 2011; Atanasov *et al.*, 2015; Orji *et al.*, 2025). This research aimed to explore the effects of these medicinal plant extracts on selected clinical isolates and the potential synergy when used in combination.

MATERIALS AND METHODS

Plant leaves and rhizome collection and preparation

Fresh leaves of *E. heterophylla* were collected in May from the premises of Federal University, Oye-Ekiti, Ekiti State, Nigeria. Fresh rhizomes of *Zingiber officinale* were obtained from *Oja-oba* market at Oye-Ekiti, Ekiti State, Nigeria. The plants were

taxonomically authenticated and properly air-dried for 288-336 hours at ambient temperature. They were then pulverized into powder and stored in an impregnable container.

Sample extraction

Cold methanol and aqueous extraction of *Euphorbia* and *Zingiber*

This was done using the method described by Ekundayo and Ekekwe (2013). Fifty grams of the pulverized samples were percolated in 500 mL each of 96% methanol and distilled water in separate conical flasks. The flasks were stirred manually for 24 hours. The extracts were filtered through Whatman No. 1 filter paper and collected in a clean beaker. This was concentrated to dryness in a rotary evaporator set to 50°C, then in a desiccator. Stock extracts were dissolved in Dimethyl Sulfoxide (DMSO).

Hot methanol and aqueous extraction of *Euphorbia* and *Zingiber*

Fifty grams of the pulverized samples were percolated in 500 ml of methanol and 500 ml of distilled water, each contained in a 1000 ml conical flask. The conical flasks were fixed directly on heating mantles, and the solutions were allowed to boil for 6 hours at 70°C. It was allowed to cool, filtered, and the filtrate evaporated to dryness until a gelatinous form was obtained.

Collection and Identification of Clinical Isolates

Clinical isolates: *Staphylococcus aureus*, a representative Gram-positive bacterium, *Pseudomonas aeruginosa*, a representative Gram-negative bacterium, and *Candida albicans*, a fungus, were obtained from the Drug Discovery Unit, Microbiology Department, Federal University, Oye-Ekiti, Ekiti State, and stored on double-strength nutrient agar and Sabouraud Dextrose agar media in stock bottles at 4°C, as recorded in Nwokeoma et al. (2022).

Phytochemical Screening: Qualitative phytochemical analysis of *Euphorbia heterophylla* and *Zingiber officinale* extracts was carried out to test for the presence of the following metabolites: tannins, alkaloids, saponins, flavonoids, phenols, sterols, glycosides, and Anthraquinones according to the methods of Ighodalo et al. (2012).

Assay for antimicrobial activity of extracts

The antimicrobial activity of the extracts on the clinical isolates was

determined using the Agar-well diffusion method. Mueller Hinton Agar (MHA) was prepared for bacteria, and Yeast Extract Agar (YEA) for the fungus, in line with the manufacturer's instructions. Plates were prepared for each clinical isolate. Isolate cultures were standardized using McFarland's turbidity standard by harvesting and diluting the cultures with sterile normal saline, ensuring the absorbance reached 580 nm at the spectrophotometer, yielding bacterial counts of 1.5×10^8 cfu/ml and 1×10^7 cells/ml for the yeast. The solidified agar plates were then inoculated with one loopful of broth culture of the test organisms/clinical isolates. The plates were thoroughly streaked with inoculum prior to boring holes in the agar using a sterile cork borer (5 mm in diameter). A single extract concentration (10 mg/ml) was introduced into the wells (0.5 ml each). Distilled water was introduced into control wells. The incubated plates were left for 18-24 hours. Clear inhibition zones around the wells of the extracts were measured (in mm).

Minimum Inhibitory Concentration (MIC): Different concentrations of the extract were prepared in sterile Mueller-Hinton broth. To 9 ml of the different concentrations, 50 µl of overnight broth cultures of the test bacteria and fungus were added, after standardizing them to McFarland turbidity standards. Control tubes had sterile distilled water instead. The concentrations used were 100mg/ml, 50mg/ml, 25.0mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml. Tubes were closed and incubated aerobically at 37 °C for 24 hours. The MIC readings were compared with the control turbidity.

Minimum Bactericidal Concentration (MBC): The contents of the MIC tubes with no visible growth were grown on sterile Mueller-Hinton agar and incubated for 24 hours. The MBC was the lowest extract concentration that prevented bacterial colony growth when plated on the medium.

Minimum Fungicidal Concentration (MFC): The MFC was determined using Sabouraud Dextrose agar (SDA) medium, as described for MBC above.

Antibiotic Sensitivity Test (AST): This was carried out using commercial antibiotic discs on solidified agar plates inoculated with the isolates. After incubation, clear zones around the discs, indicative of inhibition, were measured.

RESULTS

Table I: Qualitative phytochemicals in *Euphorbia heterophylla* and *Zingiber officinale* extracts.

Phytochemicals	Cold methanol <i>E. heterophylla</i> extract, CMEE	Hot methanol <i>E. heterophylla</i> extract, HMEE	Cold methanol <i>Z. officinale</i> extract, CMZE	Hot methanol <i>Z. officinale</i> extract, HMZE	Cold aqueous <i>Z. officinale</i> extract, CAZE	Hot aqueous <i>Z. officinale</i> extract, HAZE
Tannins	+	-	++	++	++	++
Alkaloids	+	+	+	++	+	++
Saponins	+	+	-	-	-	-
Flavonoids	+	++	++	++	++	++
Phenol	+	++	+	++	+	++
Sterols	-	+	-	+	-	+
Glycosides	+	+	-	+	-	+
Anthraquinones	+	+	+	+	+	+

KEY

1. Cold Methanol *E. Heterophylla* Extract (CMEE)
2. Hot Methanol *E. Heterophylla* Extract (HMEE)
3. Cold Methanol *Z. officinale* Extract (CMZE)
4. Hot Methanol *Z. officinale* Extract (HMZE)
5. Cold Aqueous *Z. officinale* Extract (CAZE)
6. Hot Aqueous *Z. officinale* Extract (HAZE)
7. + (Detected)
8. ++ (Detected in abundant quantity)
- (Not detected)

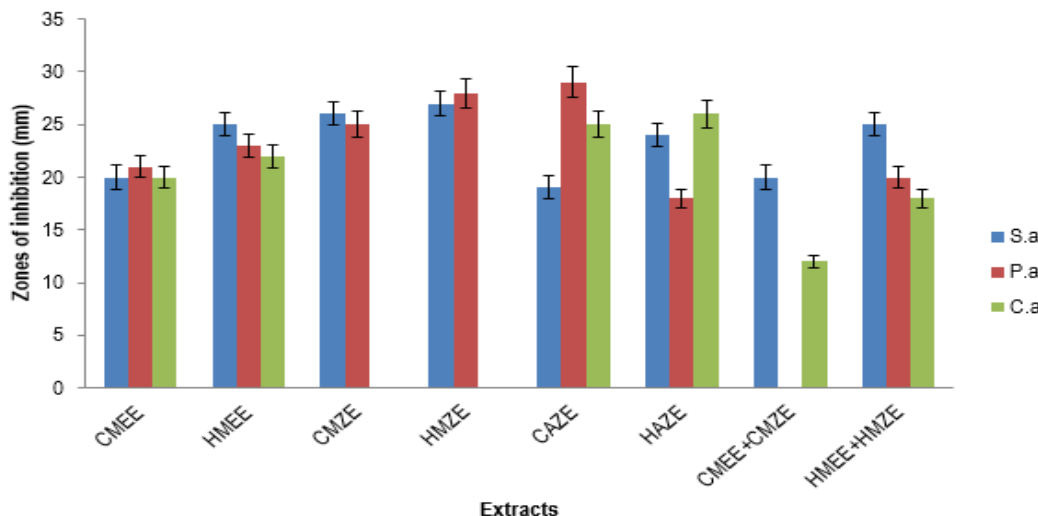


Figure 1: Zones of Inhibition produced by the isolates of the extracts on Muller-Hinton media

KEY

- 1 Cold Methanol *E. Heterophylla* Extract (CMEE)
- 2 Hot Methanol *E. Heterophylla* Extract (HMEE)
- 3 Cold Methanol *Z. officinale* Extract (CMZE)
- 4 Hot Methanol *Z. officinale* Extract (HMZE)
- 5 Cold Aqueous *Z. officinale* Extract (CAZE)
- 6 Hot Aqueous *Z. officinale* Extract (HAZE)
- 7 *Staphylococcus aureus* – S. a.
- 8 *Pseudomonas aeruginosa* – P. a.
- 9 *Candida albicans* – C. a.

Table II: Minimum Inhibitory Concentration of *Euphorbia heterophylla* and *Zingiber officinale* extracts against the clinical isolates

TEST ORGANISMS	CONCENTRATION OF EXTRACTS (mg/ml)					
	CMEE	HMEE	CMZE	HMZE	CAZE	HAZE
<i>Staphylococcus aureus</i>	50	12.5	6.25	6.25	25	25
<i>Pseudomonas aeruginosa</i>	50	12.5	6.25	6.25	25	25
<i>Candida albicans</i>	50	12.5	6.25	6.25	25	25

KEY:

1. Cold Methanol *E. Heterophylla* Extract (CMEE)
2. Hot Methanol *E. Heterophylla* Extract (HMEE)
3. Cold Methanol *Z. officinale* Extract (CMZE)
4. Hot Methanol *Z. officinale* Extract (HMZE)
5. Cold Aqueous *Z. officinale* Extract (CAZE)
6. Hot Aqueous *Z. officinale* Extract (HAZE)

Table III: Minimum Bactericidal and Fungicidal Concentration of *Euphorbia heterophylla* and *Zingiber officinale* against the Test Microbes

TEST ORGANISMS	CONCENTRATION OF EXTRACTS (mg/ml)					
	CMEE	HMEE	CMZE	HMZE	CAZE	HAZE
<i>Staphylococcus aureus</i>	100	25	25	25	50	25
<i>Pseudomonas aeruginosa</i>	100	25	25	25	50	25
<i>Candida albicans</i>	100	25	25	25	50	25

KEY

1. Cold Methanol *E. heterophylla* Extract (CMEE)
2. Hot Methanol *E. heterophylla* Extract (HMEE)
3. Cold Methanol *Z. officinale* Extract (CMZE)
4. Hot Methanol *Z. officinale* Extract (HMZE)
5. Cold Aqueous *Z. officinale* Extract (CAZE)
6. Hot Aqueous *Z. officinale* Extract (HAZE)

Table IV (A, B, C): Antibiotic Sensitivity Profile of the Clinical Isolates

A) For a Gram-positive bacterium

Clinical isolates	ZONES OF INHIBITION (mm)									
	PEF	GN	APX	Z	AM	R	CPX	S	SXT	E
<i>S. aureus</i>	15±0.01	NI	NI	NI	NI	12±0.01	16±0.01	NI	NI	10±0.01

B) For a Gram-negative bacterium

Clinical isolates	ZONES OF INHIBITION (mm)									
	SXT	CH	SP	OFX	AM	AU	GN	PEF	CPX	S
<i>P. aeruginosa</i>	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

C) For fungi

Clinical isolate	ZONES OF INHIBITION (mm)	
	KETOCONAZOLE	NYSTATIN
<i>C. albicans</i>	NI	30.00± 0.01

KEY:

NI = No inhibition

PEF= Pefloxacin (10µg), GN= Gentamycin (10µg), APX= Ampiclox (30µg), Z= Zinnacet (20µg), AM= Amoxicillin (30µg), R= Rocephin (30µg), CPX= Ciprofloxacin (10µg), S= Streptomycin (30µg), SXT= Septrin (30µg), E= Erythromycin (19µg), CH= Chloramphenicol (30µg), SP= Sparfloxacin (10µg), OFX= Tanvid (10µg), AU= Augumentin (10µg), Ketoconazole (10 mcg) and Nystatin (50 mcg)

DISCUSSION

Herbal plants play important roles in preventing, palliating, and treating diseases. Their role in treating diseases is evident from their use in key arms of medicine, including conventional medicine (Okeniyie et al., 2007; Ojo et al., 2025a).

Alkaloids, saponins, flavonoids, phenols, glycosides, and anthraquinones were detected in both the cold and hot methanol extracts of *E. heterophylla* (Table I). This is similar to the work of Kone et al. (2020), which reported the presence of flavonoids and alkaloids in their ethanoic extracts of *E. heterophylla*. Likewise,

Ughachukwu et al. (2014) reported the presence of alkaloids, saponins, sterols, and tannins in the aqueous extract of *E. heterophylla*. In contrast to this study, Kone et al. (2020) showed the absence of glycosides and saponins. Sterols were not present in the cold methanol extract, while tannins were absent in the hot methanol extracts.

All extracts from *Z. officinale* contained tannins, alkaloids, flavonoids, phenols, and anthraquinones (Table I). Yousf et al. (2021), in a similar study, demonstrated the presence of phenols and flavonoids in ethanol extracts of *Z. officinale*. Saponins were

absent in all *Z. officinale* extracts, while all the cold extracts of *Z. officinale* had no sterols or glycosides.

The phytochemical content of each sample differed, even when samples were from the same medicinal plant. This is likely due to the extraction method and the different solvents used. Falleh *et al.* (2008) reasoned that variations in phytochemicals were partly due to differences in extraction processes.

As shown in Figure 1, the highest zones of inhibition for *S. aureus* (27 mm) were obtained with hot methanol *Z. officinale* extract, HMZE. In comparison, that for *P. aeruginosa* (29 mm) was obtained with the cold aqueous *Z. officinale* extract, CAZE. The highest zone for *C. albicans* was produced by hot aqueous *Z. officinale*, HAZE (26 mm). In similar reports, Mohammed *et al.* (2019) observed zones of inhibition ranging from 14 to 25 mm of aqueous extract of *Z. officinale* against *S. aureus*, while Rahman *et al.* (2020) also derived zones of inhibition between 8 and 19 mm for ethanol extract of *Z. officinale* for the same bacterium.

The cold and hot methanol extracts from *E. heterophylla* yielded only moderate inhibition against all isolates: *S. aureus* (20 and 25mm), *P. aeruginosa* (21 and 23mm), and *C. albicans* (20 and 22mm), compared to extracts from *Z. officinale*. These results showed that the extracts in this study were more effective than the methanol extracts of *E. heterophylla* reported by Kone *et al.* (2020), which showed no antimicrobial activity against similar isolates.

Methanol extracts from both medicinal plants in this study were shown to inhibit the test isolates at varying rates (except the cold and hot methanol extracts of *Z. officinale* against *C. albicans*). This was supported by the work of Gunasekaran *et al.* (2019), who reported substantial antimicrobial activity of methanol extracts of *Gymnema sylvestre* against *S. aureus*, *P. aeruginosa*, and *F. oxysporum*. Arya *et al.* (2025) noted that solvent polarity plays a crucial role in optimizing the extraction of bioactive fractions and evaluating the effects of antimicrobial compounds.

It was observed that both cold and hot methanol extracts of *Z. officinale* had no antifungal effect on *C. albicans*. Even when cold methanol from *Z. officinale* extract was combined with cold methanol from *E. heterophylla* extract, the value was very low (12 mm), compared to the effect of cold methanol from *E. heterophylla* extract used alone. In a contrasting report, the *Z. officinale* ethanol extract in the study by Aghazadeh *et al.* (2016) exhibited distinct antifungal activity against *C. albicans* and *C. krusei*, likely due to the use of different solvents and fungal strains (Falleh *et al.*, 2008; Mostafa *et al.*, 2018).

The combined extracts from similar solvents showed no synergy in their antimicrobial activities. The cold methanol combination produced small inhibitory zones against *S. aureus* and *C. albicans* (at the lowest concentration in this work), but none against *P. aeruginosa*. The study by Bakamga-Via *et al.* (2016) reported a contrary finding: synergism against *S. aureus* was observed when their ethanol extracts were combined. The absence of synergism (termed antagonism) means that the two extracts had a smaller combined effect than when applied individually (Vaou *et al.*, 2022). The reason for this could be that the active antimicrobial constituent(s) of the individual extracts were masked by ingredients in the combined mix (Caesar & Cech, 2019).

The MICs for the isolates were similar across extracts (Table II). The MIC was 6.25 mg/ml for all test isolates in both cold and hot methanol extracts of *Z. officinale*. The concentration increased to 12.5 mg/ml for the hot methanol extract of *E. heterophylla*, followed by the cold and hot aqueous extracts of *Z. officinale*. The extract with the highest inhibitory concentration, 50 mg/ml, was cold

methanol from *E. heterophylla*, indicating it had the least effect on the test microbes. This was similar to the results obtained from the antimicrobial zones of inhibition in this study, where the effect on the bacterial isolates was low. The MIC for *Z. officinale* ethanol extract from the study by Rahman *et al.* (2020) against *S. aureus* was 400 µg/ml, while the concentrations from the research by Aghazadeh *et al.* (2016) were 40 mg/ml for *S. aureus*, 20 mg/ml for *P. aeruginosa*, and 10 mg/ml for *C. albicans* from their *Z. officinale* ethanol extract.

The lowest point MBC of 25mg/ml was attained for all the hot extracts and the cold methanol *Z. officinale* extract (Table III). This concentration increased to 50mg/ml for cold aqueous *Z. officinale* extract and 100mg/ml for cold methanol *E. heterophylla* extract.

In Adegoke *et al.* (2014)'s study, the *E. heterophylla* methanol extract showed MIC and MBC values of 12.5 mg/ml and 25 mg/ml against *S. aureus*, which were within the ranges obtained in this study. Igweet *et al.* (2024) reported MIC and MBC values of 50 mg/ml for the ethanol and aqueous extracts of *Z. officinale* against *S. aureus*. In the study by Kone *et al.* (2020), cold- and hot-water *E. heterophylla* extracts exhibited good antibacterial potential against *P. aeruginosa*, with a mean MIC of 12.5mg/ml. In contrast, methanol extracts showed no noticeable antibacterial activity against the bacterium.

The results of this study clearly indicate the antibacterial and antifungal activities of the extracts used (Table III). Herbs from the Euphorbiaceae family, such as *E. heterophylla*, are widely used as ethno-medicinal plants because they have demonstrated potent curative activities for various ailments and shown high antimicrobial activity against disease-causing Gram-positive and Gram-negative bacteria and fungi. The extensive use of members of this family is due to the presence of many active constituents in the plants (Islam, 2019; Benjamaa *et al.*, 2022).

Similarly, *Z. officinale* is globally recognized as an ethno-botanical herb, used as a food additive and for its medicinal properties in herbal treatment (Hussain *et al.*, 2022). The presence of potent aromatic and therapeutic constituents, as well as secondary metabolites, confirms its potential to confer the acclaimed health benefits. Little wonder about its effectiveness against pathogenic microbes (Kone *et al.*, 2020; Patel *et al.*, 2022; Olajide&Adetuyi, 2023).

When the isolates were assessed with conventional antibiotics (Table IV), *S. aureus* was sensitive to pefloxacin, rocephin, ciprofloxacin, and erythromycin. *P. aeruginosa* was insensitive to all the antibiotics, while *C. Candida albicans* was sensitive to only nystatin. In the studies reported by Ughachukwu *et al.* (2014) and Nwokeoma *et al.* (2022), *S. aureus* was sensitive to ciprofloxacin, consistent with the results of this work. Mohammed *et al.* (2019) found that *S. aureus* was sensitive to gentamicin and streptomycin. In contrast, Ojo *et al.* (2025b) reported *S. aureus* as sensitive to gentamicin and septrin (co-trimazole), which is contrary to the results of this study. The articles by Mohammed *et al.* (2019) and Ughachukwu *et al.* (2014) also showed that *P. aeruginosa* was sensitive to several antibiotics, such as gentamicin, Imipenem, ampicillin, streptomycin, and ofloxacin, unlike in this study. The variation in resistivity or sensitivity to different antibiotics could result from the diversity of strains in the test microbes. The development of antibiotic resistance in microbes via different mechanisms, such as the production of inactivating enzymes and mutations, thereby altering/increasing their tolerance to different antibiotics (Ojo *et al.*, 2018; Uddin *et al.*, 2021).

Conclusion

The study revealed the *in vitro* antibacterial and antifungal activities of *Euphorbia heterophylla* and *Zingiber officinale* against selected common clinical pathogens: *S. aureus*, *P. aeruginosa*, and *C. albicans*. From the results showing zones of inhibition, except for hot methanol *Z. officinale* extract, which did not inhibit *C. albicans*, and the combination of cold methanol *E. heterophylla* extract and cold methanol *Z. officinale* extract, which did not inhibit *P. aeruginosa*, all the extracts were able to inhibit the growth of the test isolates with more than a 10 mm zone. Similarly, the MICs at 6.25-50 mg/ml and the MBC/MFCs at 25-100 mg/ml indicated that the extracts had qualitatively modest antimicrobial activity against the selected isolates. These results suggest the potential use of these medicinal plant extracts as therapeutic agents once refined. Further research is needed to isolate the likely active ingredients from these extracts and test their specificity to these and other pathogenic microorganisms.

Conflict of Interest

The authors declare that there is no conflict of interest.

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