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PRELIMINARY STUDIES REGARDING THE ANTIMICROBIAL PROPERTIES OF SOME BIOPRODUCTS DERIVED FROM *Zingiber officinale* AND *Curcuma longa*

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Abstract

The study aimed to evaluate the antimicrobial activity of certain bioproducts obtained from turmeric and ginger rhizomes, respectively, four essential oils (EO) purchased from NJoy and Oleya and four bioproducts obtained through chloroform and ethanol extraction. The results showed that the EO of ginger (NJoy) exhibits moderate antimicrobial activity against E. coli and significant antimicrobial activity on S. aureus, C. albicans, and C. parapsilosis; the EO of turmeric (Oleya) exhibit moderate antimicrobial activity for E. coli and C. albicans. The bioproduct, derived from the extract of turmeric in chloroform, has a significant antimicrobial effect on C. albicans and C. parapsilopsis. The alcoholic extracts of turmeric and ginger exhibit local antimicrobial effects on E. coli and no antimicrobial activity for C. albicans, C. parapsilopsis, or S. aureus. In conclusion, the preliminary tests achieved in vitro indicate significant differences between the tested bioproducts, some of these showing high potential for specific antimicrobial applications,

***Key words**: Zigiber officinale, Curcuma longa, antimicrobial properties.*

INTRODUCTION

The rhizomes of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) have gained considerable attention over time due to their uses in traditional medicine (Daily et al., 2016). Turmeric rhizomes, known for their intense aroma and colour, are often used as a spice. Ginger rhizomes, with their mildly spicy taste, are utilised as a seasoning or in the formulation of various tea blends (Christine et al., 2021). Both spices not only enhance the flavour of food but also offer health benefits due to their antioxidant, antitumour, and antimicrobial properties. Although numerous scientific articles present the therapeutic potential of turmeric and ginger, their antimicrobial activities against specific pathogenic microorganisms, such as *S. aureus*, *E. coli*, *C. albicans* and *C. parapsilosis*, remain insufficiently studied. The incidence of infections caused by these microorganisms is significant. For instance, *Stapylococcus aureus* frequently causes skin and soft tissue infections (Carolus et al., 2019). *Escherichia coli*, a Gram-negative bacteria, is associated with urinary tract infections and gastrointestinal diseases (Kaper et al., 2004). *Candida albicans* and *Candida parapsilosis* are the opportunistic fungal species linked to invasive candidiasis (Pappas et al., 2018; Silva et al., 2012). The present study aimed to evaluate the antimicrobial activities of certain bioproducts derived from turmeric and ginger rhizomes, specifically essential oils obtained through hydrodistillation and bioproducts obtained by extraction in selective solvents such as ethanol or chloroform.

MATERIALS AND METHODS

Bioproducts of ginger and turmeric

The essential oils of turmeric (EO-2.1; EO-2.2) and ginger (EO-1.1; EO-1.2) were purchased from the Romanian market at a local medicinal plant store (Plafar, Bucharest, Romania). The chemical composition of the essential oils used in the studies was determined by gas chromatography coupled with mass spectrometry (GC-MS, CLARUS 500, PERKIN ELMER, Waltham, USA) and is presented in

Table 1. The extracts (bioproducts) were obtained from fresh ginger and turmeric rhizomes, purchased from the Romanian market (organic products grown under ecological agriculture in Peru and Brazil), through maceration in 96% ethanol (GE = ginger extract in ethanol; TE = turmeric extract in ethanol) and chloroform (GC = ginger extract in chloroform; TC = turmeric extract in chloroform), using a plant-to-solvent mass ratio of 1: 2. Fresh turmeric and ginger rhizomes were washed, peeled, and cut into small pieces, forming cubes of approximately 2.5 mm. These pieces were placed in a black glass bottle with the corresponding solvent. The four containers were stored in a dark place at room temperature for two weeks, with manual agitation every two days. After two weeks, the clear liquid phase was removed from the bottles and filtered through 150 mm filter paper (Prat Dumas France, Solantis, Bucharest, Romania). The ethanolic extract was used unmodified. For the chloroform extract, the solvent was evaporated using a rotary evaporator at 50°C, and the remaining residue was dissolved in a minimal amount of dimethyl sulfoxide. In each extract obtained, the total polyphenol content was assessed using the Folin-Ciocalteu method (Agbor et al., 2014), using the gallic acid as the reference. The coding and polyphenol content for each extract are presented in Table 1. All the extracts were stored in darkness in refrigerator at 5ºC.

Table 1. Characteristics of bioproducts derived from *Zingiber officinale* and *Curcuma longa*

Plants	Bioproduct type	Code	Polyphenols content (mg GAE/L ± ST. DEV.	Source	Observației
Zingiber officinale (Ginger rhizoms) Brazil, Peru	Essential Oil NJoy	E.O 1.1	-	Romanian market	Voucher specimen deposited in the ICCF herbarium with the identifier name: Ziof21
	Essential Oil Oleya	E.O 1.2	-		
	Alcoholic extract (etanol)	GE	91.00±1.82		
	Crude extract derived from chloroform extraction, condiționed in DMSO (dimethyl sulfoxide)	GC	398.00±7.56		
Curcuma longa (Turmeric rhizoms) Brazil, Peru	Essential Oil Oleya	E.O 2.1	-		Voucher specimen deposited in the ICCF herbarium with the identifier name: Culo21
	Essential Oil NJoy	E.O 2.2	-		
	Alcoholic extract (ethanol)	TE	1980.00±37.62		
	Crude extract obtained în chloroform, and conditioned in DMSO	TC	12.48±0.12		

Microorganisms

The antimicrobial activity of the essential oils and of extracts of turmeric and ginger was tested using the Kirby-Bauer diffusive disks methodology on the following microorganisms: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10231), and *Candida parapsilosis* (ATCC 22019), following the methodology outlined by Schroder et al. (2022) and Albisoru et al. (2024).

Methodology used for antimicrobial activity

For reactivation, the microorganisms were inoculated in Petri plates with TSA (Tryptone Soy Agar, Sigma Aldrich. Darmstadt, Germany) medium for bacteria and PDA (Potato Dextrose Agar, Sigma Aldrich, Darmstadt, Germany) medium for *Candida* sp. and incubated at 37°C for 24 and 48 hours, respectively. After that, a suspension of each microorganism was prepared in sterile saline serum (0.9% NaCl), containing 10^8 CFU/mL. This suspension was evenly spread across the surface of each Petri dish (Φ = 90 mm) using a sterile cotton swab. For bacteria, Mueller Hinton agarised (Sigma Aldrich, Darmstadt, Germany) plates were used, and for *Candida* sp., PDA medium was employed. On the surface of each Petri dish inoculated with the test microorganism, 3-5 sterile cellulose discs

(Φ = 6 mm) were placed and impregnated with 30 µL of a 1 % essential oil solution (diluted in sterile propylene glycol) or 30 µL of each extract. The resulting plates were incubated at 37°C for 24 hours for bacteria and 48 hours for yeasts, after which the inhibition zone diameters were measured. For comparison, standardised discs impregnated with a specific antibiotic for each tested microorganism were used: ampicillin (AMP25) for *S. aureus*, gentamicin (Gen10) for *E. coli*, and clotrimazole (CT10.8) for *Candida* sp. (Sigma Aldrich, Darmstadt, Germany BioRad Lab, Hercules, CA, USA) (Babeanu et al., 2022; Voicu et al., 2022; Fotina et al., 2024; Mohammed et al., 2023; Marchidan et al., 2023).

Statistical Analysis

The experimental data obtained were aquired from (3-5) determinations and were reported as the mean inhibition zone diameter (notation Φ), with the corresponding standard deviation (STDEV).

RESULTS AND DISCUSSIONS

Characterisation of the essential oils

The major compounds from the essential oils of turmeric and ginger from NJoy are shown in Figure 1.

Ar-turmerone represents the major compound found in turmeric oil, accounting for 44.61% of the mixture, followed by turmerone (23.91%) and curlone (19.92%).

The major compound from ginger essential oil is geraniol (40.43%), followed by nerol (21.86%) and geraniol acetate (7.15%).

The major compounds in the turmeric and ginger essential oils from Oleya are presented in Figure 2. As can be observed, the major compound in Oleya's turmeric oil is Ar-turmerone (37.35%).

In the essential oil of ginger, the main compounds found by GC MS analysis are zingiberene (47.31%) and cedrene (29.13%).

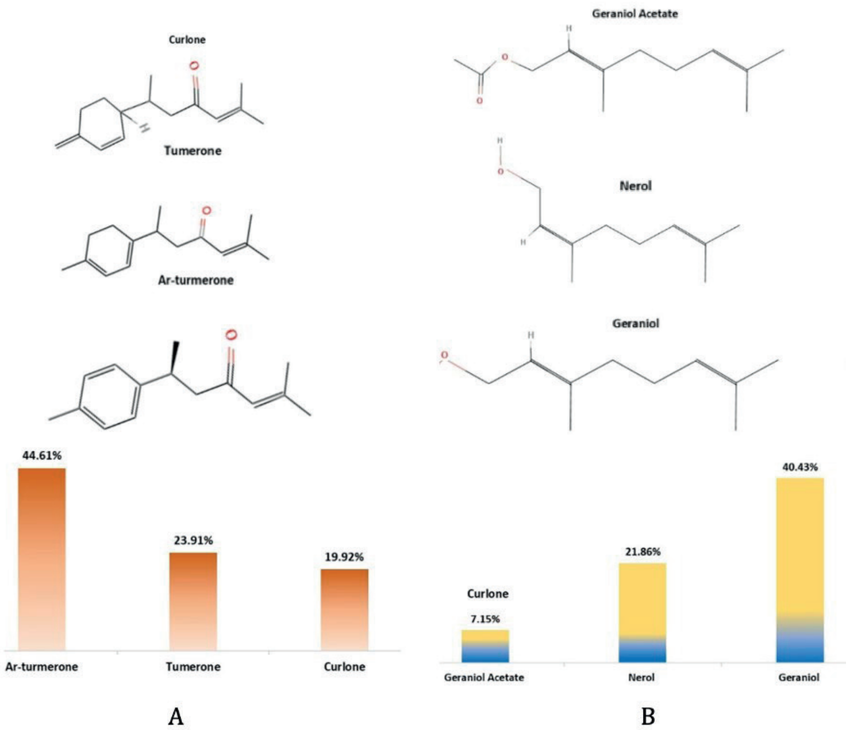

Figure 1. Major compounds present in NJoy turmeric (A) and ginger (B) essential oils

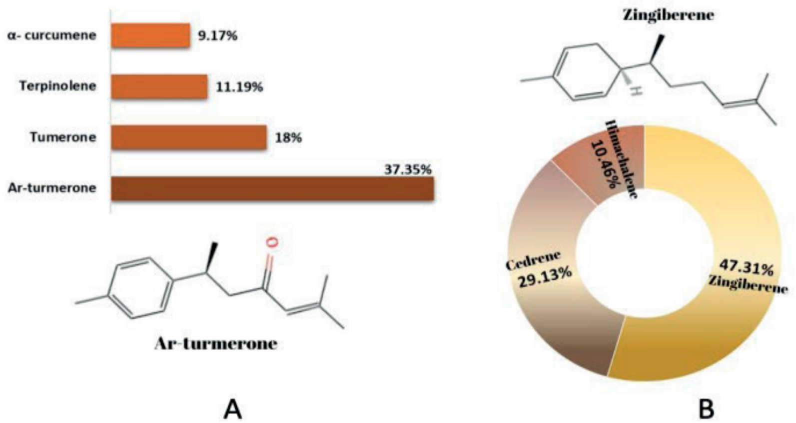

Figure 2. Major compounds present in Oleya's turmeric (A) and ginger (B) essential oils

Characterisation of the turmeric and ginger extracts

The main components of the bioproducts obtained by ethanol or chloroform extraction were quantified using the NIST data bases (Table 2) after the analysed samples were normalised. In the turmeric ethanolic extract, the major chemical compounds found by GC-MS analysis are curlone (35.43%) and Ar-turmerone (16.39%). In the ethanolic ginger extract, the major compounds are (1S,5S)-2-methyl-5-((R)-6-methylhept-5-en-2yl) bicyclo [3.1.0]hex-2-ene(7-epi-sesquithujene) (25.41%) 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-cyclohexene(β-sesquiphellandrene) (12,28%) şi 1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene (α-curcumene) (10.15%). In the Table 3 are presented the major compounds identified in the chloroform extracts obtained from fresh rhizomes of turmeric and ginger. The turmeric extract contains tumerone (32.9%), Ar-tumerone (16.9%), and curlone (16.1%). The ginger extract contains trans-α-bergamotene (21.2%), benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (15.2%), and (E)-β-farnesene (11%). Regarding the polyphenolic compound content, it was found that the extract obtained from turmeric rhizomes in ethanol contains the highest amount of polyphenols (1980±37.62 mg GAE/L), followed by the extract obtained from ginger rhizomes in chloroform (398±7.56 mg GAE/L) (Table 1).

Table 2. Major compounds in bioproducts obtained by extraction from turmeric and ginger rhizomes in ethanol

	Compounds	Relative concentration, %	Retention time, min
Turmeric	Tumerone	35.43	15.57
	Curlone	19.90	15.84
	Ar-turmerone	16.39	15.52
Ginger	(1S,5S)-2-methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene (7-epi-Sesquithujene)	25.41	14.15
	3-(1,5-Dimethyl-4-hexenyl)-6-methylene-[S-(R*,S*)]-cyclohexene (β-Sesquiphellandrene)	12.28	14.42
	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene (α-Curcumene)	10.15	14.04

Table 3. Major compounds in bioproducts obtained by extraction from turmeric and ginger rhizomes in chloroform

	Compounds	Relative concentrations, %	Retenţion time, min
Turmeric	Tumerone	32.9	15.54
	Ar Tumerone, izomer	16.9	15.61
	Curlone	16.1	16.09
Ginger	Trans, α- bergamotene	21.2	12.99
	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	15.2	12.83
	(E)-β -Farnesene	11.0	13.42

Antimicrobial Activity

The preliminary study results revealed the following:

a) For *E. coli*, the essential ginger oil from NJoy (E.O-1.1) and the essential turmeric oil from Oleya (E.O-2.1) demonstrated significant antimicrobial activity, with average inhibition zone diameters Φ = 18.33 mm and Φ = 13.67 mm, respectively. The other tested essential oils exhibited only local antimicrobial activity, characterised by average inhibition zone diameter below 7.67 mm. The bioproduct obtained from turmeric rhizomes in chloroform showed no antimicrobial activity (Figure 3).

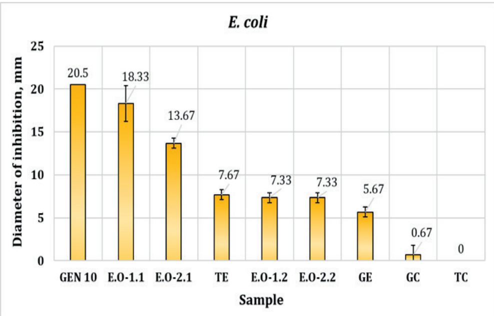

Figure 3. Antimicrobial activity of turmeric and ginger bioproducts on *E. coli*

b) In the case of *S. aureus*, the antimicrobial activity was lower. The essential ginger oil from NJoy (E.O-1.1) exhibited moderate antimicrobial activity, with an average inhibition zone diameter of 10.67 mm. The essential turmeric oil from Oleya (E.O-2.1), the essential ginger oil from Oleya (E.O-1.2), and the ethanolic ginger extract showed only local antimicrobial activity, with average inhibition zone diameters Φ=7.67 mm, Φ=6 mm, and respectively Φ=0.33 mm. The essential turmeric oil from NJoy (E.O-2.2), the ethanolic turmeric extract (TE), the turmeric extract derived from chloroform (TC), and the chloroform ginger extract (GC) do not exhibit antimicrobial activities (Figure 4).

c) For *C. albicans*, the essential ginger oil from NJoy (E.O-1.1) exhibited significant antimicrobial activity, with an average inhibition zone diameter Φ = 18 mm, followed by the essential turmeric oil from Oleya (E.O-2.1) with Φ = 13 mm, and the essential oil of ginger from Oleya (E.O-1.2) with Φ = 12.67 mm.

The turmeric extract derived from chloroform (TC), the essential oil of turmeric NJoy (E.O-2.2), and the chloroform ginger extract (GC) displayed only local antimicrobial activity, with average inhibition zone diameters Φ = 10 mm, Φ = 9.67 mm, and Φ = 3 mm, respectively. The ethanolic extract of turmeric and ginger does not exhibit antimicrobial activity (Figure 5).

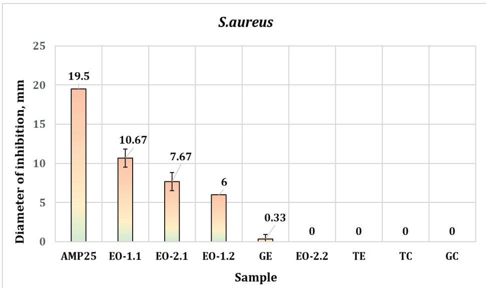

Figure 4. Antimicrobial activity of turmeric and ginger bioproducts on *S. aureus*.

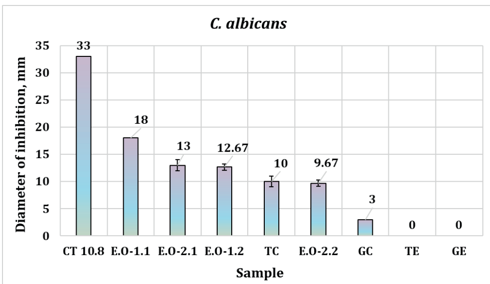

Figure 5: Antimicrobial activity of turmeric and ginger bioproducts on *C. albicans*

d) In the case of *Candida parapsilosis*, the essential ginger oil from NJoy (E.O-1.1) and the turmeric extract derived from chloroform exhibited significant antimicrobial activity, with average inhibition zone diameters Φ = 18.33 mm and Φ = 16.67 mm, respectively. The ethanolic turmeric extract showed only local antimicrobial activity, with an average inhibition zone diameter Φ = 2 mm. The other bioproducts do not exhibit antimicrobial activity for *Candida parapsilosis* (Figure 6).

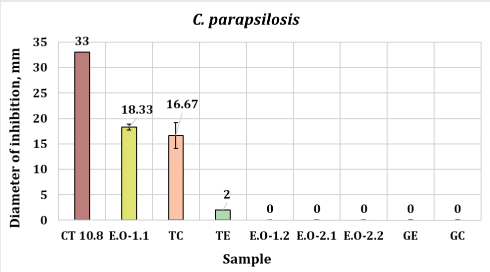

Figure 6. Antimicrobial activity of turmeric and ginger bioproducts on *C. parapsilosis.*

The analysis of turmeric essential oils highlighted the predominant presence of Ar-turmerone. These findings are supported by other researchers who have identified Ar-turmerone as the main compound in turmeric essential oil. For instance, Poudel et al. (2022) detected 65 constituents in the essential oil of *C. longa*, with Ar-turmerone being the dominant compound, reaching a concentration of 25.5% in the system. Another study by Pino et al., (2018) confirmed that Ar-turmerone was the most abundant compound (45.5%) in the analysed essential oil. For ginger essential oils, the composition varies between the two suppliers. The essential oil from NJoy contains geraniol as the major compound, followed by nerol. In contrast, the essential oil from Oleya has zingiberene as the dominant compound, followed by cedrene. Variations in the composition of essential oils from different sources have also been reported by Al-Dhahli et al., (2020). Studies performed on essential oils obtained from two different sources (Chinese ginger and Saudi ginger) revealed differences in their chemical composition. Chinese ginger essential oil had α-zingiberene as the dominant compound, whereas in Saudi ginger essential oil, the primary component was Ar-curcumene. The authors emphasise that such variations are common in essential oils and can be attributed to factors such as the genetic diversity of plants, climatic conditions, and extraction techniques. Regarding bioproducts obtained through solvent extractions, the turmeric rhizome extracts in ethanol and chloroform were found to contain tumerone and curlone as the predominant compounds. These results align with findings reported by Raje et al. (2015), who identified tumerone and curlone as the major constituents in turmeric extracts. In terms of antimicrobial activity against *Escherichia coli,* the essential ginger oil from NJoy (E.O-1.1) exhibited the highest antimicrobial activity, with an average inhibition zone diameter of 18.33 mm. This result is consistent with other studies (Silva et al., 2018; Imamović et al., 2021), where inhibition diameters of 19 mm and 23.67 mm, respectively, were reported for ginger essential oil on *E. coli*. The significant antimicrobial efficacy of NJoy ginger essential oil may be attributed to its high geraniol content, a finding also supported by studies conducted by Mączka et al., (2020). The mechanism of action of geraniol is based on its ability to dissolve lipids in the microbial membrane, leading to loss of cellular integrity and microbial cell death. Other researchers (Odo et al., 2023; Gonçalves et al., 2019) have reported a local antimicrobial activity for alcoholic extracts and turmeric essential oil against *E. coli,* with average inhibition zone diameters Φ = 7 mm and Φ = 8 mm, respectively. These findings are consistent with the results obtained in this study for biopreparations TE and E.O-2.2. For *S. aureus,* the essential ginger oil from NJoy (E.O-1.1) exhibited the highest antimicrobial activity, with an average inhibition zone diameter Φ = 10.67 mm, a value comparable to those reported by other studies (Sharma et al., 2016; Njobdi et al., 2018). The results suggest that most of the tested essential oils exhibit moderate antimicrobial activity against *S. aureus*. Exposure to the essential turmeric oil from Oleya (E.O-2.1) led to the appearance of an inhibition zone Φ = 7.67 mm, a value which indicates a local antimicrobial activity on *S. aureus*, similar to the value reported by Gonçalves and collaborators (Gonçalves et al., 2019) (Φ = 8.3 mm). This similarity may be attributed to the common presence of Ar-turmerone and α-curcumene in turmeric essential oils.

Exposure *Candida sp*., to the essential ginger oil (E.O-1.1), containing geraniol and nerol as major compounds, resulted in the appearance of significant antimicrobial activity. This observation is supported by other studies, which indicate the antifungal effect of these compounds. For instance, da Silva et al. (2024) demonstrated that geraniol exhibits significant antifungal activity on *Candida sp*., inhibiting microorganism growth at concentrations between 110 μg/mL and 883 μg/mL. Sharma et al. (2018) showed that the geraniol strongly inhibits *Candida sp.* growth, by preventing processes of biofilm formation, cell adhesion, and secretion of hydrolytic enzymes. Tian et al. (2017) evaluated the activity of nerol (a major compound in the essential oil used in this study, respectively E.O-1.1) and reported its involvement in both early and late apoptosis of *C. albicans* cells. Regarding the antimicrobial activity of biopreparations obtained through alcohol extraction, it was observed that these types of bioproducts generally do not exhibit antimicrobial activity for *C. parapsilosis*, *C. albicans*, or *S. aureus*. In the case of *Escherichia coli,* the alcoholic extracts obtained from ginger or turmeric rhizomes exhibit a local antimicrobial activity

CONCLUSIONS

Studies performmed regarding the antimicrobial properties of various bioproducts derived from turmeric and ginger rhizomes, revealed distinct activities. During the preliminary tests, it was found that the essential ginger oil from NJoy (E.O-1.1) exhibited moderate or significant antimicrobial activity against all tested microorganisms, namely: *E. coli* (moderate antimicrobial activity), *S. aureus* (significant antimicrobial activity), *C. albicans* (significant antimicrobial activity), *C. parapsilosis* (significant antimicrobial activity). The essential turmeric oil from Oleya (E.O-2.1) showed moderate antimicrobial activity on microorganisms such as *E. coli* and *C. albicans*. Among the bioproducts obtained by solvent extractions, the bioproduct obtained through extraction with chloroform and turmeric rhizomes, which contain 398 mg GAE/L, exhibit significant antimicrobial activity against *C. parapsilosis* and *C. albicans*. The bioproducts obtained by extraction from alcoholic media and turmeric or ginger rhizomes exhibit local antimicrobial activity on *E. coli* but do not exhibit antimicrobial activity on *C. albicans*, *C. parapsilosis*, or *S. aureus*.

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