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Original Research Article

ANTIFUNGAL ACTIVITY OF COLD AND HOT WATER EXTRACT OF SELECTED READY TO USE PLANT-BASED SPICES

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ABSTRACT

The rising challenge of antifungal resistance has spurred interest in natural plant-based alternatives. This study investigated the phytochemical composition and antifungal activity of hot- and cold-water extracts of five common spices (*Thymus vulgaris*, *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, and *Mentha* spp.) against *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. Phytochemical screening showed that hot-water extracts yielded a broader spectrum of metabolites, notably saponins and cardiac glycosides, compared to cold extracts. Results revealed that hot-water extracts exhibited stronger antifungal activity across most spices. Turmeric was most effective against *Penicillium* spp., producing inhibition zones of 23.40 ± 0.01 mm (cold extract) and 25.60 ± 0.20 mm (hot extract), with MFC values of 250 mg·mL^{-1} (cold extract) and 250 mg·mL^{-1} (hot extract) respectively. Thyme showed fungicidal effects against *Penicillium* spp. at 500 mg·mL^{-1} (cold extract) and 250 mg·mL^{-1} (hot extract), but no detectable activity against *Fusarium* spp. Garlic and ginger showed moderate but selective effects, with garlic active against *Aspergillus* spp. (inhibition zone 11.82 ± 0.02 mm; MFC 500 mg·mL^{-1}, hot extract only) and ginger fungicidal against *Penicillium* spp. (inhibition zone: 12.08±0.05 mm and 10.15 ± 0.18 mm at MFC 500 mg·mL^{-1}, for cold and hot extract respectively). Mint extracts displayed weak activity, restricted to *Aspergillus* spp. at (inhibitory zone: 10.60 ± 0.18, MFC at 500 mg·mL^{-1} (hot extract), with no detectable fungicidal effect against *Penicillium* or *Fusarium* spp.

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INTRODUCTION

Millions of people worldwide are impacted by fungal infections each year, which significantly increased morbidity, mortality, and the financial burden on society [1]. Despite the fact that these eukaryotic diseases infect billions of people globally and kill over 1.5 million annually, the influence of fungus on human health has been underestimated over time [2–5]. Aspergillus, Cryptococcus, Candida, or Pneumocystisspecies have been associated with around 90% of fungal infection-related mortality [6], with over 300,000 patients being diagnosed of aspergillosis each year and over 30 million people at risk due to corticosteroid or other therapies, Aspergillus organisms constitute an immediate hazard [7, 8]. In patients with impaired immune systems, opportunistic fungi including Candida, Aspergillus, and Cryptococcus species are especially problematic, with dermatophytic fungi reported to cause harm in both tropical and temperate climates [6, 9, 10]. There is an urgent need for safer, more effective, and affordable alternatives due to the rising incidence of antifungal resistance, the scarcity of effective antifungal drugs, and the unfavorable side effects connected to

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conventional treatments [11–13]. Report has been made of some of the adverse effect synthetic antifungal medication such as amphotericin B [12–14]. Natural plants, especially culinary spices and medicinal herbs, are now receiving more attention as possible sources of antifungal and antibacterial chemicals [11, 15–20]. Consumer expectations for foods with ingredients that may provide health benefits beyond basic nutrition, such as herbal ones, are gaining attentions currently. Spices have long been reported for their medicinal qualities in addition to their taste and ability to preserve food [4, 10, 21]. Their bioactive constituents such as phenolics, terpenoids, flavonoids, saponins, and organosulfur compounds have demonstrated broad-spectrum antimicrobial activity, including significant antifungal effects [22–26]. Emerging reports of antimicrobial properties of our traditional medicinal plant extracts suggesting an alternative for chemically synthesized molecules. Among plant-based spices, Allium sativum (garlic) [27–29], Mentha species (mint) [30, 31], and Thymus vulgaris (thyme) [32, 33] have received considerable scientific attention. Turmeric, rich in curcuminoids, has been reported to exhibits notable antifungal and anti-inflammatory properties [23, 34–37], ginger contains essential oil with reported antifungal and antioxidant activities as reported in [25]. Garlic is particularly renowned for its allicin content, which displays strong fungicidal action against yeasts and molds [6]. Mint essential oils, characterized by high menthol and menthone concentrations, has been reported to exert inhibitory effects on fungal growth and biofilm formation [38]. Thyme, through its major component thymol, has demonstrated robust antifungal activity against a wide range of pathogenic fungi [39]. Given the global rise in antifungal resistance and the demand for natural, accessible, and cost-effective remedies, evaluating the antifungal activities of readily available plant-based spices is both scientifically and socially relevant. This study investigates the antifungal potential of turmeric, ginger, garlic, mint, and thyme, with the aim of establishing their efficacy as alternative and complementary antifungal agents.

MATERIALS AND METHODS

Collection of Plant materials

The five spices that were used includes Turmeric (Curcuma Longa) stem, Ginger (*Zingiber Officinale*) rhizome, Garlic (*Allium Sativum*) bulb, Pepperminit (*Mentha piperita*) leaves and Thyme (*Thymus Vulgaris L.*) leaves. The spices were purchased from a local market in Amechi Idodo, Nkanu east, Enugu State, Nigeria. The leaves and rhizomes were washed several times with distilled water, dried, crushed and then pulverized separately and kept for extraction.

Extraction of the plant materials

The extraction was carried out following the method used in [24]. For cold-water extraction, 20 g of ground spice samples were soaked in 100 mL of sterile distilled water in a 250 mL Erlenmeyer flask, continuously agitated on a rotary shaker for 24hrs at room temperature. 100 g of plant paste was then homogenized with 100 mL of sterile distilled water and left to stand for 24 hours. For hot-water extraction, 100 g of each plant paste was infused with 100 mL of sterile distilled water in a 250 mL Erlenmeyer flask and incubated in a water bath at 90 °C for 1 h. The resulting mixtures were first filtered through Whatman No. 1 filter paper, followed by passage through a 0.2µm membrane filter to eliminate microbial contaminants.

Preparation of Nutrient Agar Solution

Nutrient agar medium (Oxoid Ltd., Basingstoke, United Kingdom) was prepared with composition of beef infusion (300 g/L), casein and hydrolysate (17.5g/L), agar (17g/L), starch (1.5mg/L) was prepared in accordance with following the manufacturers instruction and autoclaved at 21^0C for 15mins. 28g of agar powder was heated gently and dissolve completely in peptone water with 5.0g for each of peptone, tryptone and sodium chloride at a pH of 7.3±0.2. The medium was autoclaved at 15psi (121 ^{0}C) for 15minutes and allowed to cool at room temperature prior to dispense for use.

Test Organisms

For this investigation, isolates of fruit and vegetable rot fungi (*Aspergillus* species, *Fusarium* species, and *Penicillium* species) were employed. The isolates were obtained from the Department of Microbiology, Federal University of Technology Owerri, Imo State, Nigeria, as authenticated pure cultures. The cultures were maintained and sub-cultured on Potato Dextrose Agar (PDA) (Oxoid Ltd., Basingstoke, United Kingdom) and stored under refrigeration for fifteen (15) days prior to use. Microscopic examination was carried out periodically to confirm culture purity throughout the storage period.

Microscopic Examination

Sterilized forceps were used to cut adhesive tape into 1 cm squares, which were then applied to the mycelial surface and removed. A few lactophenol cotton blue (LPCB) droplets were put on a different slide. It was then covered with the adhesive tape that contained the fungal mycelia, mounted, and examined using the bright field light microscope's low power 10X and high power 45X objective lenses.

Preparation of McFarland's standard
A 0.5 McFarland standard was used. 1.0×10^5 CFU/mL was obtained by diluting *Candida albicans* with sterile water (CLSI, 2010).

Determination of antifungal activity of the extracts
The agar well diffusion techniques as described in Magaldi et al. [40] was adopted for this study to evaluate the antifungal activity of the ready-to-use plant-based spices. A standardized fungal inoculum, adjusted to the 0.5 McFarland turbidity standard, was dispensed onto the surface of pre-solidified and dried Sabouraud dextrose agar plates (Oxoid Ltd., Basingstoke, United Kingdom) using a sterile Pasteur pipette (0.2 mL per plate). The inoculum was uniformly distributed across the agar surface with the aid of a heat-sterilized glass spreader shaped like a hockey stick, ensuring even lawn growth. After allowing the plates to rest for approximately 5 minutes, two wells of 6 mm diameter were aseptically punched into each agar plate using a flame-sterilized cork borer, and the wells were appropriately labeled. Measured volumes of the plant extracts (0.2 mL per well) were then introduced into the wells, while ketoconazole (standard antifungal drug) was used as the reference control. The plates were left undisturbed for about 30 minutes to facilitate adequate diffusion of the test solutions into the medium. Subsequently, the inoculated plates were incubated at 28 °C for 72 hrs. Following incubation, antifungal activity was determined by recording the diameter (in millimeters) of the inhibition zones produced around each well, representing the extent of fungal growth suppression by the extracts.

Tests for minimum inhibitory concentrations (MIC)
For the MIC testing, about two milliliters (2 ml) of the extract were dissolved in four milliliters (4 ml) of peptone water to yield 500 mg/ml. Subsequently, two milliliters of the 500 mg/ml concentration were transferred to two milliliters of peptone water in a test tube and thoroughly homogenized in order to do two-fold serial dilutions from the 500 mg/ml concentration. Up until the eighth tube, two milliliters of the tube were transferred to two milliliters of peptone water in the next tube. 500 mg/ml, 250, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml, and 3.90 mg/ml were the concentrations that were subsequently attained. Following the acquisition of the various concentrations and dilutions, three drops of the test organisms' overnight broth cultures were added to each dilution. After that, the tubes were incubated for three days at 28^0C. The MIC, as defined in Ayodele et al. [41], was the lowest concentration of each extract that prevented the test organisms from growing.

Test for the minimum fungicidal concentration
Tubes showing no visible growth from the MIC test were sub cultured onto sterile Sabouraud dextrose agar plates and incubated at 28^0C for 3 days. The lowest concentration of the samples yielding no growth was recorded as the Minimum Fungicidal Concentration as the case may be.

Phytochemical screening of the ready-to-use plant-based spice extracts
Routine qualitative phytochemical screening for saponins, anthranoids, anthraquinones, phenols, alkaloids, tannins, phlobatannins, and cardiac glycosides was carried out using standard established methods involving characteristic color changes, frothing reactions, and precipitate formation as previously described by Ekeleme et al. [42].

Statistical Analysis
All data were expressed as mean ± standard deviation (SD) of replicate determinations. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA). Differences in antifungal activity among the extracts and control were analyzed using one-way analysis of variance (ANOVA), followed by post hoc multiple comparison tests. Statistical significance was determined at $p < 0.05$, where values with p-values less than 0.05 were considered statistically significant, while $p > 0.05$ indicated no significant difference.

RESULTS
Cultural morphology and microscopic characteristics of the fungal isolates used for the antifungal activity testing
Table 1 below showed the cultural morphology and microscopic characteristics of the fungal isolates used for the antifungal activity testing. Selection of these fungi was selected based on the infections they are known to cause in humans. They comprised; Penicillium species, Aspergillus species and Fusarium species.

Table 1: Cultural morphology and microscopic characteristics of the fungal isolates

Cultural Morphology	Microscopy	Possible Fungi
Whitish cottony broom-like colonies with greenish centre	Septate hypae with spores	*Penicillium species*
Whitish, enlarged fluffy with black centre colonies	Septate hypae	*Aspergilius Species*
Whitish cottony broom-like colonies	Septate hypae with spores	*Fusarium Species*

Qualitative phytochemical screening of cold-water extracts of ready-to-eat plant- based spices

Table 2 shows the qualitative phytochemical screening of the cold-water extracts of selected ready-to-eat spices. The analysis revealed the presence of various classes of bioactive compounds across the extracts. Saponins were only detected in turmeric, while anthranoids and anthraquinones were present in thyme, ginger, garlic, and turmeric but absent in mint. Phenols were absent in thyme but detected in ginger, garlic, turmeric, and mint. Alkaloids were identified in garlic and mint, whereas phlobatannins were found only in garlic. Tannins were observed in thyme, ginger, turmeric, and mint, but not in garlic. Cardiac glycosides were present in turmeric and mint, and absent in thyme, ginger, and garlic.

Table 2: Qualitative phytochemical screening of cold-water extracts of some ready-to-eat plant- based spices

Phytochemical constituent	Spices (cold water extracts)				
	Thyme	**Ginger**	**Garlic**	**Turmeric**	**Mint**
Saponins	-	-	-	+	-
Anthranoids	+	+	+	+	-
Phenols	-	+	+	+	+
Alkaloids	-	-	+	-	+
Phlobatannins	-	-	+	-	-
Tannins	+	+	-	+	+
Cardiac glycosides	-	-	-	+	+
Anthraquinones	+	+	+	+	-

Key: + = Presence of phytochemicals; - = Absence of phytochemicals

Qualitative phytochemical screening of hot water extracts of some ready-to-eat plant- based spices

Table 3 presents the qualitative phytochemical profile of the hot-water extracts of the selected spices. Saponins were detected in thyme, garlic, and turmeric, but absent in ginger and mint. Anthranoids and anthraquinones were consistently present in thyme, ginger, garlic, and turmeric, and absent in mint. Phenols were detected in all the spices except thyme. Alkaloids were found in garlic and mint, while phlobatannins were only present in garlic. Tannins were observed in thyme, ginger, turmeric, and mint, but absent in garlic. Cardiac glycosides were identified in ginger, turmeric, and mint, but absent in thyme and garlic.

Table 3: Qualitative phytochemical screening of hot water extracts of some ready-to-eat plant- based spices

Phytochemical constituent	Spices (Hot water extracts)				
	Thyme	**Ginger**	**Garlic**	**Turmeric**	**Mint**
Saponins	+	-	+	+	-
Anthranoids	+	+	+	+	-
Phenols	-	+	+	+	+
Alkaloids	-	-	+	-	+
Phlobatannins	-	-	+	-	-
Tannins	+	+	-	+	+
Cardiac glycosides	-	+	-	+	+
Anthraquinones	+	+	+	+	-

Key: + = Presence of phytochemicals; - = Absence of phytochemicals

Antifungal activity of cold-water extracts of some ready-to-use plant-based spices

Table 4 shows the antifungal activity of the cold-water extracts of the investigated spices against *Penicillium spp.*, *Aspergillus spp.*, and *Fusarium spp.* using the disc diffusion method. The control drug, ketoconazole (3.90 mg/disc), produced inhibition zones of 27.19 ± 0.45 mm, 29.33 ± 0.35 mm, and 26.10 ± 0.50 mm, respectively. Among the extracts, turmeric exhibited the highest inhibition against *Penicillium spp.* (23.40 ± 0.01 mm), while thyme demonstrated the greatest effect against *Fusarium spp.* (17.09 ± 0.15 mm). Ginger and garlic showed moderate inhibitory activity across the three fungi, with inhibition zones ranging from 10.01 ± 0.04 mm to 12.08 ± 0.05 mm. Mint leaf extract displayed inhibition only against *Aspergillus spp.* (12.21 ± 0.05 mm) and showed no activity against the other fungi tested.

Table 4: Antifungal activity of the cold-water extracts of some ready-to-use plant-based spices

Spices	Fungi organisms/zones of inhibition (mm)		
	Penicillium spp	**Aspergillus spp.**	**Fusarium spp.**
Control (Ketoconazole) at 3.90mg/disc	27.19 ± 0.45	29.33 ± 0.35	26.10 ± 0.50
Thyme	9.43±0.23	7.03±0.04	17.09±0.15
Turmeric	23.40±0.01	11.19±0.02	NI
Ginger	12.08±0.05	10.01±0.04	11.32±0.03
Garlic	12.00±0.04	11.82±0.02	10.03±0.01
Mint Leaf	NI	12.21±0.05	NI

Key: mm = Millimeter; NI = No inhibition; Each value is mean of 3 replicates ± standard error
CLSI = Clinical Laboratory Standard Institute; R = Resistant = 0-12mm; S = Susceptible 16mm and above)

Antifungal activity of hot water extracts of some ready-to-use plant-based spices

Table 5 presents the antifungal activity of the hot-water extracts of selected spices against *Penicillium spp.*, *Aspergillus spp.*, and *Fusarium spp.* Ketoconazole (3.90 mg/well) used as the control produced inhibition zones of 18 ± 0.25 mm, 15.13 ± 0.21 mm, and 11.0 ± 0.33 mm, respectively. Among the extracts, turmeric demonstrated the highest inhibition against *Penicillium spp.* (25.60 ± 0.20 mm) and also showed activity against *Aspergillus spp.* (15.30 ± 0.15 mm) and *Fusarium spp.* (12.40 ± 0.22 mm). Thyme exhibited notable activity against *Fusarium spp.* (19.10 ± 0.40 mm) and moderate inhibition of *Penicillium spp.* and *Aspergillus spp.* (14.20 ± 0.30 mm and 11.50 ± 0.25 mm, respectively). Ginger and garlic extracts showed weaker activity, with inhibition zones ranging from 8.40 ± 0.12 mm to 10.15 ± 0.18 mm across the tested fungi. Mint leaf extract inhibited only *Aspergillus spp.* (10.60 ± 0.18 mm) and showed no effect against *Penicillium spp.* or *Fusarium spp.*

Table 5: Antifungal activity of the hot-water extracts of some ready-to-use plant-based spices

Spices	Fungi organisms/zones of inhibition (mm		
	Penicillium spp	**Aspergillus spp.**	**Fusarium spp.**
Control (Ketoconazole) at 3.90mg/well	18 ± 0.25	15.13 ± 0.21	11.0 ± 0.33
Thyme	14.20 ± 0.30	11.50 ± 0.25	19.10 ± 0.40
Turmeric	25.60 ± 0.20	15.30 ± 0.15	12.40 ± 0.22
Ginger	10.15 ± 0.18	8.40 ± 0.12	9.50 ± 0.20
Garlic	9.80 ± 0.10	10.10 ± 0.15	8.70 ± 0.12
Mint Leaf	NI	10.60 ± 0.18	NI

Key: mm = Millimeter; NI = No inhibition; CLSI = Clinical Laboratory Standard Institute; R = Resistant = 0-12mm; S = Susceptible 16mm and above)

Minimum inhibitory concentrations of cold-water extracts of ready-to-use plant-based spices

Table 6 shows the minimum inhibitory concentrations (MICs) of the cold-water extracts of the investigated spices against *Penicillium spp.*, *Aspergillus spp.*, and *Fusarium spp.* Thyme inhibited *Penicillium spp.* and *Aspergillus spp.* at 125 mg/mL, and *Fusarium spp.* at 250 mg/mL. Turmeric displayed the lowest MIC against *Penicillium spp.* (62.5 mg/mL), inhibited *Aspergillus spp.* at 500 mg/mL, while no inhibition was determined

against *Fusarium spp.* Ginger inhibited *Penicillium spp.* and *Aspergillus spp.* at 125 mg/mL, and *Fusarium spp.* at 500 mg/mL. Garlic showed activity against *Penicillium spp.* at 125 mg/mL, while higher concentrations (500 mg/mL) were required to inhibit *Aspergillus spp.* and *Fusarium spp.* Mint leaf extract demonstrated inhibition only against *Aspergillus spp.* (500 mg/mL), while no MIC was recorded for the other fungi.

Table 6: Minimum inhibitory concentration (MIC) of the cold-water extracts of some ready-to-use plant-based spices

Spices	Fungi organisms/concentrations (mg/ml)		
	Penicillium spp	Aspergillus spp.	Fusarium spp.
Thyme	125	125	250
Turmeric	62.5	500	ND
Ginger	125	125	500
Garlic	125	500	500
Mint Leaf	ND	500	ND

Key: ND = Not determined mg/ml = Milligram per milliliter

Minimum inhibitory concentrations of hot water extracts of ready-to-use plant-based spices

Table 7 presents the minimum inhibitory concentrations (MICs) of the hot-water extracts of selected spices against *Penicillium spp.*, *Aspergillus spp.*, and *Fusarium spp.* Thyme inhibited *Penicillium spp.* at 125 mg/mL, and both *Aspergillus spp.* and *Fusarium spp.* at 250 mg/mL. Turmeric exhibited the strongest activity, with inhibition of *Penicillium spp.* at 62.5 mg/mL, *Aspergillus spp.* at 250 mg/mL, and *Fusarium spp.* at 125 mg/mL. Ginger required 250 mg/mL to inhibit all three fungal organisms. Garlic inhibited *Aspergillus spp.* at 250 mg/mL, but higher concentrations were needed for *Penicillium spp.* (500 mg/mL) and *Fusarium spp.* (500 mg/mL). Mint leaf extract inhibited only *Aspergillus spp.* at 500 mg/mL, with no detectable inhibition against the other test organisms.

Table 7: Minimum inhibitory concentration (MIC) of the hot water extracts of some ready-to-use plant-based spices

Spices	**Fungi organisms/concentrations (mg/ml)**		
	Penicillium spp	**Aspergillus spp.**	**Fusarium spp.**
Thyme	125	250	250
Turmeric	62.5	250	125
Ginger	250	250	250
Garlic	500	250	500
Mint Leaf	ND	500	ND

Key: ND = Not determined mg/ml = Milligram per milliliter

Minimum fungicidal concentrations (MFC) of cold-water extracts of ready-to-use plant-based spices

Table 8 presents the minimum fungicidal concentrations (MFCs) of the cold-water extracts of the investigated spices against *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. Thyme exhibited fungicidal activity against *Penicillium* spp. at 500 mg/mL, while no fungicidal effect was observed against *Aspergillus* spp. and *Fusarium* spp. Turmeric displayed fungicidal activity against *Penicillium* spp. at 250 mg/mL and *Fusarium* spp. at 500 mg/mL, but no effect was detected against *Aspergillus* spp. Ginger showed fungicidal activity against *Penicillium* spp. at 500 mg/mL, with no fungicidal effect recorded for *Aspergillus* spp. and *Fusarium* spp. Garlic extract demonstrated no fungicidal activity against any of the test fungi. Mint leaf extract did not exhibit fungicidal activity against any of the fungal organisms evaluated.

Minimum fungicidal concentrations (MFC) of hot water extracts of ready-to-use plant-based spices

Table 9 shows the minimum fungicidal concentrations (MFCs) of the hot-water extracts of the investigated spices against *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. Thyme extract exhibited fungicidal activity against *Penicillium* spp. at 250 mg/mL and *Aspergillus* spp. at 500 mg/mL, while no activity was observed against *Fusarium* spp. Turmeric demonstrated fungicidal effects against *Penicillium* spp. at 250 mg/mL and against both *Aspergillus* spp. and *Fusarium* spp. at 500 mg/mL. Ginger showed fungicidal activity only against *Penicillium* spp. at 500 mg/mL, with no detectable effect on *Aspergillus* spp. and *Fusarium* spp. Garlic displayed fungicidal activity against *Aspergillus* spp. at 500 mg/mL, but no fungicidal effect was observed against *Penicillium* spp. and *Fusarium* spp. Mint leaf extract showed no fungicidal activity against any of the fungal species tested.

Table 8: Minimum fungicidal concentration (MFC) of the cold-water extracts of some ready-to-use plant-based spices

Spices	Fungi organisms/concentrations (mg/ml)		
	Penicillium spp	Aspergillus spp.	Fusarium spp.
Thyme	500	ND	ND
Turmeric	250	ND	500
Ginger	500	ND	ND
Garlic	ND	ND	ND
Mint Leaf	ND	ND	ND

Key: ND = Not determined mg/ml = Milligram per milliliter

Table 9: Minimum fungicidal concentration (MFC) of the hot water extracts of some ready-to-use plant-based spices

Spices	Fungi organisms/concentrations (mg/ml)		
	Penicillium spp	Aspergillus spp.	Fusarium spp.
Thyme	250	500	ND
Turmeric	250	500	500
Ginger	500	ND	ND
Garlic	ND	500	ND
Mint Leaf	ND	ND	ND

Key: ND = Not determined mg/ml = Milligram per milliliter

DISCUSSION

Phytochemical constitutes of plants serves as defense mechanism against by many microorganisms. The therapeutic properties of medicinal plants are possibly due to the presence of various secondary metabolites [23, 30]. The result in Table 2 shows the presence of alkaloids, phenols, tannins, anthranoids, and anthraquinones in the cold-water extracts of the tested spices, while saponins and cardiac glycosides were only detected in turmeric and mint respectively These findings were supported by those of [43–45] who discovered the presence of alkaloids, phenols, tannins, saponins, cardiac glycosides and anthraquinone-like compounds in turmeric, garlic and ginger. The presence of these phytochemicals confirms the medicinal potentials of the selected plants. Alkaloids have been reported to possess diverse pharmacological effects including antimicrobial, antifungal, and antioxidant properties, making them important contributors to the biological activity of garlic and mint in this study [3, 46–48]. Phenols were observed in ginger, garlic, turmeric, and mint but absent in thyme, and their antimicrobial efficacy is widely attributed to their ability to cause protein precipitation and membrane disruption in microbial cells. A study by [49] showed essential oil from ginger, its effect in membrane disruption, and protein/nucleic acid leakage. This finding corresponds with that of [50] who discussed the phenolic compounds in ginger, turmeric and garlic. Previous studies have demonstrated that phenolic compounds in plant extracts inhibit fungal pathogens by binding to cell wall proteins and interfering with enzyme activity [50]. Tannins were found in thyme, ginger, turmeric, and mint but not in garlic, and their antimicrobial role has been reported in several research work, for its protein-precipitating and metal-ion-chelating ability, which reduces microbial growth and virulence [23]. It has been reported that tannins are capable of inhibiting the growth of fungi, bacteria, and viruses, largely by depriving them of essential proteins and enzymes required for survival [2, 51]. The detection of anthranoids and anthraquinones across most of the cold-water extracts except mint also supports the medicinal value of these spices, as these compounds are known to interfere with fungal cell wall synthesis and exhibit direct fungicidal activity [52, 53]. The result in Table 3 shows that the hot-water extracts exhibited a broader distribution of phytochemicals compared to the cold-water extracts. In this case, saponins were detected in thyme, garlic, and turmeric but absent in ginger and mint. Saponins are well recognized as potent antifungal agents, with their mechanism of action linked to their ability to complex with sterols in fungal cell membranes, leading to increased permeability and cell death[43, 54]. The presence of tannins in thyme, ginger, turmeric, and mint further strengthens the antimicrobial potential of these extracts, as discussed earlier. The detection of cardiac glycosides in ginger, turmeric, and mint, which were absent in the cold-water extracts of thyme and garlic, may reflect the effect of temperature on the solubilization of these compounds [55, 56]. Cardiac glycosides are reported to exhibit antimicrobial activity through interference with microbial respiratory processes and cell wall integrity [57]. The variations between cold- and hot-water extracts highlight the

influence of extraction temperature on phytochemical solubility. While hot-water extraction appeared to enhance the release of saponins and cardiac glycosides, certain cold extracts retained specific metabolites such as heat-sensitive compounds that might have been lost through thermal degradation. Similar findings have been reported by Cowan, (1999) who emphasized that extraction conditions significantly alter the phytochemical yield and consequently the antimicrobial efficacy of plant-based preparations.

Bio-control using plant extracts can be achieved by exploiting their anti-fungal properties. Table 4 shows the antifungal activity of cold-water extracts of commonly used spices. Result show that turmeric had significantly ($P<0.05$) largest inhibition zone against *Penicillium* spp. (23.40 ± 0.01 mm), substantially exceeding the activity of other extracts and significantly approaching the zone produced by ketoconazole ($P<0.05$). This strong antifungal activity of turmeric is consistent with that of [59] who showed that aqueous turmeric preparations can exhibit robust antifungal effects attributed to curcuminoids and associated phenolics. Thyme showed the greater significant effect ($P<0.05$) against *Fusarium* spp. (17.09 ± 0.15 mm), when also compared to the control drug. Ginger and garlic produced moderate but measurable zones across the three fungi; mint was largely inactive except against *Aspergillus* spp. These patterns echo the frequent observation in the literature that turmeric and thyme often provide the most consistent aqueous antifungal activity among culinary spices, whereas activity of garlic, ginger and mint can be more variable and strain-dependent [59]. Hot-water turmeric retained and even slightly increased activity against *Penicillium* (25.60 ± 0.20 mm) and gained measurable activity against *Fusarium* (12.40 ± 0.22 mm), while thyme's hot extract produced an especially large zone against *Fusarium* (19.10 ± 0.40 mm). This improvement in hot-extract potency for turmeric and thyme is consistent with extraction theory and empirical reports showing that heating often increases recovery of polar phenolics, tannins and other heat-stable antifungal constituents from plant matrices, thereby enhancing observed antimicrobial effects [55, 56]. Cowan's review revealed that extraction conditions (solvent polarity, temperature, time) have large effects on which phytochemicals are recovered and consequently on biological activity, and more recent comparative extraction studies confirm that hot aqueous extraction can increase phenolic yields and antimicrobial potency for certain spices [58]. Recent studies of the antifungal and antimicrobial activity of hydroethanolic and aqueous Curcuma extracts have been documented and have linked it to phenolic/curcuminoid content and oxidative stress induction in fungi [33, 45, 50, 59, 60]. Recent reviews and experimental studies attribute thyme's antimicrobial efficacy to thymol and related constituents and demonstrate strong antifungal and antibiofilm activity for thyme preparations [39]. Garlic and ginger showed moderate, more variable antifungal zones in this study, a result which is concordant with recent work showing garlic which constituent of allicin, is highly potent [5, 21, 25, 43, 44, 49, 50]. Li et al. (2022) highlighted allicin's membrane-disrupting, broad-spectrum fungicidal action and showed that processing and heating reduce allicin yield and bioactivity. This helps to explain why garlic's cold extracts performed relatively better. Ginger's active phenolics (gingerols and shogaols) and volatile terpenoids show has been reported to show antifungal and antibiofilm effects in many reports. Rahmani et al. (2014) reported that 6-gingerol and related constituents can inhibit fungal growth and biofilm formation, which supports the moderate activity observed in this study. Mint leaf extracts were largely inactive against *Penicillium* and *Fusarium* in both extraction conditions, showing measurable inhibition only versus *Aspergillus* spp. Several studies indicate that the antifungal activity of Mentha spp. is predominantly associated with the essential oil fraction (principally menthol and menthone), whereas simple aqueous extraction yields limited amounts of these volatile actives and therefore often shows weaker inhibition [9, 62, 63]. Fungal hyphae can collapse and their tips can burst due to crude plant extracts, which causes cytoplasmic material to flow out. This may result to a decrease in cytoplasm, an increase in vacuolization, and a buildup of osmophilic chemicals in the impacted hyphae [24]. Numerous factors, such as the plant's age, the extraction solvent, the extraction technique, and the time of plant material harvesting, have been reported to affect the active principles found in plants [64]. This variation in anti-fungal activity of cold and hot water extract in plants against the same fungi have been noted and certain fungus is resistant to one kind of extract, whereas another kind of the same plant's extract works best against the same fungi.

MIC is the lowest concentration of an antimicrobial agent that inhibits microbial growth in an appropriate medium after incubation [25]. Deviations in MIC are attributed to the chemical variation within the different extracts and the concentration of bioactive compounds within extracts due to the nature of the solvents. Variation in chemical characteristics and antimicrobial activity was noted when 3 different cultivars of Australian grown garlic were compared [1]. Tables 6 and 7 showed the minimum inhibitory concentrations (MIC) of the cold and hot-water extracts of the spices. Result revealed variations in antifungal potency depending on both the plant species and extraction method. In the

cold-water extracts, turmeric demonstrated the lowest MIC against *Penicillium spp.* (62.5 mg/ml), suggesting that it possessed the strongest inhibitory effect among the tested extracts. Ginger, garlic, and thyme also exhibited activity at moderate concentrations (125–500 mg/ml), while mint was generally less active, especially against *Penicillium spp.* and *Fusarium spp.* where no inhibitory effect was recorded. In comparison, the hot-water extracts (Table 7) showed that turmeric again stood out, exhibiting strong activity with MIC values as low as 62.5 mg/ml against *Penicillium spp.* and 125 mg/ml against *Fusarium spp.* Thyme also displayed enhanced inhibitory activity in the hot extracts, particularly against *Aspergillus spp.* and *Fusarium spp.*, when compared with its cold-water counterpart. These findings highlight the influence extraction temperature can have on solubility and bioavailability of antifungal metabolites as stated in [64].

The MFC of cold and hot water extract are presented in Table 8 and 9 respectively. Between cold- and hot-water extracts, the fungicidal activity of the assessed plant-based spices differed significantly, indicating the impact of extraction temperature on phytochemical solubilization and bioactivity. Heat facilitated the release or activation of antifungal components such phenolics, flavonoids, and volatile oils because hot-water extracts has been reported to show broader fungicidal effects at lower concentrations than cold-water extracts [24, 65, 66]. These results align with reports by Touba et al., (2012) who revealed that cold water extract of garlic amongst seven selected spices (cardamom, chilli, coriander, onion, garlic, ginger, and galangale) exhibited good anti-fungal activity against all three tested fungi (*Phoma exigua, Fusarium nygamai and Rhizoctonia solani*). However, hot water extracts, garlic and ginger showed the best anti-fungal activity. In this study, garlic and ginger showed reduced fungicidal efficiency in hot-water extracts compared to cold-water extracts. This could be attributed to the thermal degradation of heat-sensitive organosulfur compounds in garlic, such as allicin, and gingerols in ginger, which are known to be unstable at elevated temperatures [28]. Experimental work on *Allium sativum* has repeatedly shown that raw/less-processed extracts have stronger antifungal effects than heated ones, likely due to allicin's volatility and instability [28, 67].

CONCLUSION

This study revealed that extraction temperature strongly influences the diversity and abundance of bioactive metabolites, with hot-water extracts showing a broader spectrum of constituents compared to cold-water extracts. Turmeric stood out as the most effective, showing consistent fungicidal action against *Penicillium spp.*, *Aspergillus spp.*, and *Fusarium spp.* at concentrations ranging from 250–500 mg/mL across both extraction methods. Thyme exhibited fungicidal effects only in cold-water extracts, with activity against *Penicillium spp.* (500 mg/mL), while hot-water extracts required higher concentrations (250–500 mg/mL) and showed no detectable activity against *Fusarium spp.* Ginger and garlic displayed limited fungicidal potential, requiring concentrations of 250–500 mg/mL, with no detectable effects against some fungi in both cold and hot extracts. Mint leaf showed the narrowest spectrum, with activity confined to *Aspergillus spp.* (500 mg/mL in hot extracts), and no fungicidal effect observed against *Penicillium spp.* or *Fusarium spp.* under either extraction condition.

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AUTHORS' CONTRIBUTION

AEC and IO carried out the study design and experimental planning. AEC performed the laboratory experiments and data acquisition. IO provided supervision, technical guidance, and overall research oversight. AEC drafted the original manuscript, while IO reviewed and approved the final version for submission.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this work.

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REFERENCES

1. Phan A, Netzel G, Chhim P, Netzel M, Sultanbawa Y. Phytochemical characteristics and antimicrobial activity of Australian grown garlic (Allium sativum L.) cultivars. Foods, 8(9), 2019:358. DOI: 10.3390/foods8090358.
2. Pandey AK, Mishra AK, Mishra A. Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant Cinnamomum tamala. Cellular and Molecular Biology, 58, 2012:142-147.
3. Arora D, Rani A, Sharma A. A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacognosy Reviews, 7, 2013:179. DOI: 10.4103/0973-7847.120520.
4. Kumari K, Sachan AK, Kumar S, Singh D, Anupam Kr Sachan C. Medicinal uses of spices used in our traditional culture: Worldwide. Journal of Medicinal Plants Studies, 6, 2018:116-122.
5. Gebreyohannes G, Gebreyohannes M. Medicinal values of garlic: A review. International Journal of Medicine and Medical Sciences, 5, 2013:401-408. DOI: 10.5897/IJMMS2013.0960.
6. Li Z, Li Z, Yang J, Lu C, Li Y, Luo Y, Cong F, Shi R, Wang Z, Chen H, Li X, Yang J, Ye F. Allicin shows antifungal efficacy against Cryptococcus neoformans by blocking the fungal cell membrane. Frontiers in Microbiology, 13, 2022. DOI: 10.3389/fmicb.2022.1012516.
7. Bongomin F, Gago S, Oladele R, Denning D. Global and multi-national prevalence of fungal diseases—estimate precision. Journal of Fungi, 3, 2017:57. DOI: 10.3390/jof3040057.
8. Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. Medical Mycology, 51, 2013:361-370. DOI: 10.3109/13693786.2012.738312.
9. Stringaro A, Colone M, Angiolella L. Antioxidant, antifungal, antibiofilm, and cytotoxic activities of Mentha species essential oils. Medicines, 5, 2018:112. DOI: 10.3390/medicines5040112.
10. Abirami S, Edwin Raj B, Soundarya T, Kannan M, Sugapriya D, Al-Dayan N, Ahmed Mohammed A. Exploring antifungal activities of acetone extract of selected Indian medicinal plants against human dermal fungal pathogens. Saudi Journal of Biological Sciences, 28, 2021:2180-2187. DOI: 10.1016/j.sjbs.2021.01.046.
11. Achar PN, Quyen P, Adukwu EC, Sharma A, Msimanga HZ, Nagaraja H, Sreenivasa MY. Investigation of the antifungal and anti-aflatoxigenic potential of plant-based essential oils against Aspergillus flavus in peanuts. Journal of Fungi, 6, 2020:1-19. DOI: 10.3390/jof6040383.
12. Loução A, Jacomini D, Moroni J, Sanches A. Adverse reactions to amphotericin B in adults-data mining. Revista Brasileira de Farmácia Hospitalar e Serviços de Saúde, 2018:e091.006. DOI: 10.30968/rbfhss.2018.091.006.
13. Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: Side effects and toxicity. Revista Iberoamericana de Micología, 26, 2009:223-227. DOI: 10.1016/j.riam.2009.06.003.
14. Gill J, Sprenger HR, Ralph ED, Sharpe MD. Hepatotoxicity possibly caused by amphotericin B. Annals of Pharmacotherapy, 33, 1999:683-685. DOI: 10.1345/aph.18181.
15. Yuan X, Cao D, Xiang Y, Jiang X, Liu J, Bi K, Dong X, Wu T, Zhang Y. Antifungal activity of essential oils and their potential synergistic effect with amphotericin B. Scientific Reports, 14, 2024:31125. DOI: 10.1038/s41598-024-82380-0.
16. Pandey VK, Srivastava S, Ashish, Dash KK, Singh R, Dar AH, Singh T, Farooqui A, Shaikh AM, Kovacs B. Bioactive properties of clove (Syzygium aromaticum) essential oil nanoemulsion: A comprehensive review. Heliyon, 10, 2024:e22437. DOI: 10.1016/j.heliyon.2023.e22437.
17. Alves FS, Cruz JN, de Farias Ramos IN, do Nascimento Brandão DL, Queiroz RN, da Silva GV, da Silva GV, Dolabela MF, da Costa ML, Khayat AS, de Arimatéia Rodrigues do Rego J, do Socorro Barros Brasil D. Evaluation of antimicrobial activity and cytotoxicity effects of extracts of Piper nigrum L. and piperine. Separations, 10, 2022:21. DOI: 10.3390/separations10010021.
18. Chandini R, Saranya R, Mohideen K, Nandagopal P, Jayamani L, Jeyakumaran S. Anti-candidal effect of Ocimum sanctum: A systematic review on microbial studies. Cureus, 2022. DOI: 10.7759/cureus.24749.
19. Aljuhani S, Rizwana H, Aloufi AS, Alkahtani S, Albasher G, Almasoud H, Elsayim R. Antifungal activity of Carica papaya fruit extract against Microsporum canis: In vitro and in vivo study. Frontiers in Microbiology, 15, 2024. DOI: 10.3389/fmicb.2024.1399671.
20. Wylie MR, Merrell DS. The antimicrobial potential of the neem tree Azadirachta indica. Frontiers in

Pharmacology, 13, 2022. DOI: 10.3389/fphar.2022.891535.
21. Labu Z, Khalid Labu Z, Mostafizur Rahaman M. Proven health benefits of garlic-a review. 2019.
22. Charles DG, Izionworu VO. Antifungal activities of curry leaf (Murraya koengii) extract on some selected fungi. Chemistry and Material Research, 2014.
23. Hosea ZY, Kator L, Rhoda EH. Phytochemical properties and antimicrobial activities of aqueous extract of Curcuma longa (turmeric) rhizome extract. Asian Journal of Research in Crop Science, 2, 2018:1-8. DOI: 10.9734/ajrcs/2018/43142.
24. Touba EP, Zakaria M, Tahereh E. Anti-fungal activity of cold and hot water extracts of spices against fungal pathogens of Roselle (Hibiscus sabdariffa) in vitro. Microbial Pathogenesis, 52, 2012:125-129. DOI: 10.1016/j.micpath.2011.11.001.
25. Akullo JO, Kiage B, Nakimbugwe D, Kinyuru J. Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiber officinale) and garlic (Allium sativum). Heliyon, 8, 2022. DOI: 10.1016/j.heliyon.2022.e10457.
26. Jayaprakash A, Ebenezer P. Antifungal activity of curry leaf (Murraya koenigii) extract and an imidazole fungicide on two dermatophyte taxa. Journal of Academia and Industrial Research, 1, 2012:124.
27. Magryś A, Olender A, Tchórzewska D. Antibacterial properties of Allium sativum L. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Archives of Microbiology, 203, 2021:2257-2268. DOI: 10.1007/s00203-021-02248-z.
28. Bryan-Thomas J, McClear T, Omoregie S. Antimicrobial potential of unstressed and heat stressed Allium sativum. Saudi Journal of Biological Sciences, 30, 2023:103749. DOI: 10.1016/j.sjbs.2023.103749.
29. Alves RR, Andrade BRD de, Silva A da C, Silva MLRB da. Atividade antimicrobiana do extrato de (Allium sativum, Liliaceae) in natura e do extrato aquoso frente Candida albicans, Staphylococcus aureus e Streptococcus pyogenes. Research, Society and Development, 10, 2021:e10610716206. DOI: 10.33448/rsd-v10i7.16206.
30. Piras A, Porcedda S, Falconieri D, Maxia A, Gonçalves MJ, Cavaleiro C, Salgueiro L. Antifungal activity of essential oil from Mentha spicata L. and Mentha pulegium L. growing wild in Sardinia island (Italy). Natural Product Research, 35, 2021:993-999. DOI: 10.1080/14786419.2019.1610755.
31. Vakili-Ghartavol M, Arouiee H, Golmohammadzadeh S, Naseri M. Antifungal activity of Mentha × piperita L. essential oil. Acta Scientiarum Polonorum Hortorum Cultus, 21, 2022:143-152. DOI: 10.24326/asphc.2022.1.12.
32. He D, Wu X, Wu K, Chai X, Liang Y, Zhang X, Cha Q, Xie W. Synergistic activity of clove essential oil and thyme essential oil and their interaction against Malassezia furfur, Escherichia coli, Staphylococcus aureus. Lebensmittel-Wissenschaft and Technologie, 204, 2024:116431. DOI: 10.1016/j.lwt.2024.116431.
33. Fathy HM, Ahmed MN, Goda HA, Moselhy MA. Thyme essential oil potentials as a bactericidal and biofilm-preventive agent against prevalent bacterial pathogens. Scientific Reports, 15, 2025:31644. DOI: 10.1038/s41598-025-16485-5.
34. Marchi LB, de Castro Dornellas F, Polonio JC, Pamphile JA, Giriboni Monteiro AR, Gonçalves OH, Geraldo Perdoncini MRF. Antifungal activity of Curcuma longa L. (Zingiberaceae) against degrading filamentous fungi. Chemical Engineering Transactions, 75, 2019:319-324. DOI: 10.3303/CET1975054.
35. Oza K, Khumji JB, Maitreya B. Antifungal activity of turmeric (Curcuma longa) rhizome against different fungi. 2021.
36. Koyu H, Ozturk I, Tekintas Y, Yurtsever SG. Investigation of antimicrobial and antibiofilm activity of Curcuma longa L. extracts against methicillin-resistant Staphylococcus aureus isolates. Journal of Research in Pharmacy, 29, 2025:1122-1133. DOI: 10.12991/jrespharm.1694295.
37. El-Mougy N, Abdel-Kader M. Antifungal effect of powdered spices and their extracts on growth and activity of some fungi in relation to damping-off disease control. 2007.
38. Quintana Soares Lopes L, Fortes Guerim PH, Maldonado ME, Wagner R, Hadlich Xavier AC, Gutknecht da Silva JL, Bittencourt da Rosa Leal D, de Freitas Daudt N, Christ Vianna Santos R, Kolling Marquezan P. Chemical composition, cytotoxicity, antimicrobial, antibiofilm, and anti-quorum sensing potential of Mentha piperita essential oil against the oral pathogen Streptococcus mutans. Journal of Toxicology and Environmental Health Part A, 87, 2024:824-835. DOI: 10.1080/15287394.2024.2375731.

39. Vassiliou E, Awoleye O, Davis A, Mishra S. Anti-inflammatory and antimicrobial properties of thyme oil and its main constituents. International Journal of Molecular Sciences, 24, 2023:6936. DOI: 10.3390/ijms24086936.
40. Magaldi S, Mata-Essayag S, Hartung de Capriles C, Perez C, Colella MT, Olaizola C, Ontiveros Y. Well diffusion for antifungal susceptibility testing. International Journal of Infectious Diseases, 8, 2004:39-45. DOI: 10.1016/j.ijid.2003.03.002.
41. Ayodele SM, Onwubolu. Antifungal properties of some locally used spices in Nigeria against some rot fungi. African Journal of Plant Science, 3, 2009:139-141.
42. Ekeleme K, Tsaku P, Nkene I, Ufomadu U, Abimiku R, Oti V, Sidi M. Phytochemical analysis and antibacterial activity of Psidium guajava L. leaf extracts. GSC Biological and Pharmaceutical Sciences, 1, 2017:013-019. DOI: 10.30574/gscbps.2017.1.2.0024.
43. Akullo JO, Kiage-Mokua BN, Nakimbugwe D, Ng'ang'a J, Kinyuru J. Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic. Heliyon, 9, 2023:e18806. DOI: 10.1016/j.heliyon.2023.e18806.
44. Bar M, Binduga UE, Szychowski KA. Methods of isolation of active substances from garlic (Allium sativum L.) and its impact on the composition and biological properties of garlic extracts. Antioxidants, 11, 2022:1345. DOI: 10.3390/antiox11071345.
45. Jyotirmayee B, Mahalik G. A review on selected pharmacological activities of Curcuma longa L. International Journal of Food Properties, 25, 2022:1377-1398. DOI: 10.1080/10942912.2022.2082464.
46. Zhang X, Jin Y, Wu Y, Zhang C, Jin D, Zheng Q, Li Y. Anti-hyperglycemic and anti-hyperlipidemia effects of the alkaloid-rich extract from barks of Litsea glutinosa in ob/ob mice. Scientific Reports, 8, 2018:12646. DOI: 10.1038/s41598-018-30823-w.
47. Tiong S, Looi C, Hazni H, Arya A, Paydar M, Wong W, Cheah SC, Mustafa M, Awang K. Antidiabetic and antioxidant properties of alkaloids from Catharanthus roseus (L.) G. Don. Molecules, 18, 2013:9770-9784. DOI: 10.3390/molecules18089770.
48. Okonta JM, Aguwa CN. Evaluation of hypoglycemic activity of glycosides and alkaloids extracts of Picralima nitida Stapf (Apocynaceae) seed. International Journal of Pharmacology, 3, 2007:505-509. DOI: 10.3923/ijp.2007.505.509.
49. Gunasena MT, Rafi A, Mohd Zobir SA, Hussein MZ, Ali A, Kutawa AB, Abdul Wahab MA, Sulaiman MR, Adzmi F, Ahmad K. Phytochemicals profiling, antimicrobial activity and mechanism of action of essential oil extracted from ginger (Zingiber officinale Roscoe cv. Bentong) against Burkholderia glumae causative agent of bacterial panicle blight disease of rice. Plants, 11, 2022:1466. DOI: 10.3390/plants11111466.
50. Ajanaku CO, Ademosun OT, Atohengbe PO, Ajayi SO, Obafemi YD, Owolabi OA, Akinduti PA, Ajanaku KO. Functional bioactive compounds in ginger, turmeric, and garlic. Frontiers in Nutrition, 9, 2022. DOI: 10.3389/fnut.2022.1012023.
51. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6, 2016:71-79. DOI: 10.1016/j.jpha.2015.11.005.
52. Savarirajan D, Ramesh VM, Muthaiyan A. In vitro antidermatophytic activity of bioactive compounds from selected medicinal plants. Journal of Analytical Science and Technology, 12, 2021:53. DOI: 10.1186/s40543-021-00304-3.
53. Agarwal SK, Singh SS, Verma S, Kumar S. Antifungal activity of anthraquinone derivatives from Rheum emodi. Journal of Ethnopharmacology, 72, 2000:43-46. DOI: 10.1016/S0378-8741(00)00195-1.
54. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. Journal of Ethnopharmacology, 94, 2004:219-243. DOI: 10.1016/j.jep.2004.05.016.
55. Tian DM, Cheng HY, Jiang MM, Shen WZ, Tang JS, Yao XS. Cardiac glycosides from the seeds of Thevetia peruviana. Journal of Natural Products, 79, 2016:38-50. DOI: 10.1021/acs.jnatprod.5b00611.
56. Che Sulaiman IS, Basri M, Fard Masoumi HR, Chee WJ, Ashari SE, Ismail M. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. Chemical Central Journal, 11, 2017:54. DOI: 10.1186/s13065-017-0285-1.
57. Sumiyoshi M, Kimura Y. Enhancing effects of a chromone glycoside, eucryphin, isolated from Astilbe rhizomes on burn wound repair and its mechanism. Phytomedicine, 17, 2010:820-829. DOI: 10.1016/j.phymed.2010.01.010.
58. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12, 1999:564-582. DOI: 10.1128/CMR.12.4.564.

59. Cruz A, Sánchez-Hernández E, Teixeira A, Martín-Ramos P, Cunha A, Oliveira R. Antifungal and antioomycete activities of a Curcuma longa L. hydroethanolic extract rich in bisabolene sesquiterpenoids. Horticulturae, 10, 2024:124. DOI: 10.3390/horticulturae10020124.
60. Omorodion NJ, Ujoh EC. Antimicrobial activities of aqueous and ethanolic extracts of some natural spices (garlic, turmeric, thyme and onions) on some clinical isolates. GSC Biological and Pharmaceutical Sciences, 19, 2022:335-345. DOI: 10.30574/gscbps.2022.19.1.0094.
61. Rahmani AH, Shabrmi FM Al, Aly SM. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International Journal of Physiology, Pathophysiology and Pharmacology, 6, 2014:125-136.
62. Napiórkowska A, Khaneghah AM, Kurek MA. Essential oil nanoemulsions-a new strategy to extend the shelf life of smoothies. Foods, 13, 2024:1854. DOI: 10.3390/foods13121854.
63. Camele I, Gruľová D, Elshafie HS. Chemical composition and antimicrobial properties of Mentha × piperita cv. 'Kristinka' essential oil. Plants, 10, 2021:1567. DOI: 10.3390/plants10081567.
64. Okigbo RN, Ogbonnaya UO. Antifungal effects of two tropical plant leaf extracts (Ocimum gratissimum and Aframomum melegueta) on postharvest yam (Dioscorea species) rot. African Journal of Biotechnology, 5, 2006:727-731.
65. Ramirez-Rodrigues MM, Plaza ML, Azeredo A, Balaban MO, Marshall MR. Physicochemical and phytochemical properties of cold and hot water extraction from Hibiscus sabdariffa. Journal of Food Science, 76, 2011. DOI: 10.1111/j.1750-3841.2011.02091.x.
66. Mekam PN, Martini S, Nguefack J, Tagliazucchi D, Stefani E. Phenolic compounds profile of water and ethanol extracts of Euphorbia hirta L. leaves showing antioxidant and antifungal properties. South African Journal of Botany, 127, 2019:319-332. DOI: 10.1016/j.sajb.2019.11.001.
67. Li Z, Li Z, Yang J, Lu C, Li Y, Luo Y, Cong F, Shi R, Wang Z, Chen H, Li X, Yang J, Ye F. Allicin shows antifungal efficacy against Cryptococcus neoformans by blocking the fungal cell membrane. Frontiers in Microbiology, 13, 2022. DOI: 10.3389/fmicb.2022.1012516.