

**IN VITRO EVALUATION OF ANTIFUNGAL ACTIVITIES OF *Zingiber officinale* (GINGER) AND *Allium sativum* (GARLIC) EXTRACTS ON FUNGAL ISOLATES FROM TINEA CAPITIS**

**ABSTRACT**

**Aim:** To evaluate the *in-vitro* antifungal activities of garlic and ginger extracts against fungal isolates from *Tinea capitis*.

**Study Design:** This was cross-sectional research.

**Place and Duration of Study:** Scalp scrapping samples were collected from village primary school children with *Tinea capitis* in Ebonyi state, Nigeria, while fresh garlic and ginger samples were purchased from Ogige market in Nsukka, Enugu state, Nigeria. The analyses were done in Microbiology laboratory, University of Nigeria, Nsukka from June to September, 2023.

**Methodology:** Forty samples of scalp scrapings collected from the head were cultured on Sabouraud dextrose agar. The isolates were identified using biochemical tests and head perforation test. Methanol and water were used in the extraction of the phytoconstituents of the plant samples. Qualitative phytochemical analysis was carried out on the plant extracts. The antifungal activities of the extracts, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were also determined.

**Results:** The phytochemical analysis indicated that both plant extracts contain saponins, flavonoids, glycosides, tannins, terpenoids, alkaloids and phenols. Two fungi species isolated were identified as *Trichophyton* spp and *Microsporum* spp. At dilutions of 400, 200, 100 and 50mg/ml, the methanolic extracts of the garlic had inhibition zone diameters (IZD) of 35, 30, 25 and 20 mm respectively on the *Trichophyton* spp whereas ginger had IZD of 25, 20, 15 and 13 mm respectively. On the other hand, the methanol extracts of ginger and garlic gave IZDs of 25, 20, 14, 12mm and 36, 28, 25, 21mm respectively on *Microsporum* spp. The methanol extracts of both plants were found to be more effective than their aqueous extracts. However, the garlic extracts had better antifungal effects on the two isolates than the ginger extracts. The combined extracts had better antifungal activities; at the various dilutions of 400, 200, 100 and 50mg/ml, it gave IZDs of 38, 32, 28 and 23mm on the *Trichophyton* spp compared to the single extracts.

**CONCLUSION:** Both *Allium sativum* and *Zingiber officinale* were similarly active against *Tinea capitis* isolates. However, garlic was found to be more active than ginger, but have synergistic effect on each other. This study underscores the antifungal efficacies of *A. sativum* and *Z. officinale* as potential sources of novel anti-ringworm drugs especially when combined together.

Keywords: *Tinea capitis*, *Ginger extract*, *Garlic extract*, *Phytochemicals*, *Inhibition Zone Diameter*, *Minimum Inhibitory Concentration* and *Minimum Fungicidal Concentration*.

UNDER PEER REVIEW

## 1. INTRODUCTION

Fungi are saprophytic microorganisms that can be found in soil, decaying vegetation, air, aquatic habitats and plant saps. Fungi are found as parts of the microbiota in plants and animals including man [1]. Dermatophytosis, specifically Tinea capitis is caused by a group of fungi known as dermatophytes which are divided into three genera: *Trichophyton*, *Microsporum* and *Epidermophyton*, but *Epidermophyton* does not cause Tinea capitis [2]. They are very common and prevalent in the environment and have easy modes of transmission [3].

Dermatophytosis are chronic and prevalent among populations mainly due to the emergence of antimicrobial (antifungal) resistant species which occurred as result of prolonged use of the antifungal drugs [4]. It is a public health problem that can lead to poor attendance among school children in low- and middle-income countries like Nigeria [5].

Tinea capitis is a ringworm of the head caused by either *Trichophyton* or *Microsporumspp*[6]. It is a common contagious infection of the scalp and hair which occur mainly in children [7]. Identification of the species of the dermatophyte involved is necessary to make most suitable therapeutic choice. The prevalence of the etiologic fungi varies in different geographic locations throughout the world [8]. In Europe, Tinea capitis is mainly caused by anthropophilic *Trichophyton* species (*T. soudanense*, *T. tonsurans*) and less often by *Microsporum* species (*M. canis-zoophilic*; *M. audouinii*-anthropophilic) [2, 9]. The affected part is associated with loss of hair, clear ringed region with scales and kerion may occur at times [4].

From ancient time, medicinal plants have been in use because of their medicinal values and flavor impact on foods. Both crude extracts and dry powder samples from medicinal and aromatic plants species are used to develop and prepare alternative medicine and food additives [10]. Garlic, ginger and moringa have medicinal and culinary values [11]. Garlic (*Allium sativum*) belongs to the family Alliaceae. Garlic is high in a Sulphur compound called Allicin which conveys health benefits. Its close relative is onion [12]. Garlic has commonly been used for therapeutic purposes particularly as an antioxidant [13]. Allicin is the major biologically active organosulphur compound in garlic, poorly miscible in water, with the characteristic odour like freshly crushed garlic. It is readily obtained from alliin by the enzymatic activity of alliinase [14]. Alliin, an oxygenated sulphur amino acid present in the garlic clove, is the stable precursor of allicin [15, 16]. Ginger (*Zingiber officinale*) is a member of the family *Zingiberaceae*, with more than 45 genera, and 800 species [17]. Ginger is really a world domestic remedy. It is used in India and other places like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products. In Nigeria, it is included in soups and drinks to give a rich, hot and spicy flavor [18].

Ginger and garlic contain phytochemicals that can kill or inhibit the growth and other activities of the dermatophytes (*Trichophyton* species and *Microsporum* species) that cause Tinea capitis. They contain

therapeutic phytochemicals that enable them to fight against pathogenic infections like fungal infections and others [19, 20]. These plants have been used for millennia, for both culinary purposes and for the treatment of other illnesses, including rheumatism, muscle aches, constipation, indigestion, nausea and vomiting, hypertension, dementia, and fever [21].

The plants are ecologically friendly to humans and their extracts have been found to contain phytochemicals which are inhibitory to the growth and activities of pathogenic fungi especially the drug resistant species. They can be used independently or synergistically in order to achieve a better result [22]. Generally, prolonged use of conventional antifungal drugs in treating infections has been associated with different issues such as antimicrobial (including antifungal) drug resistance, low efficacy and negative side effects. These issues therefore promoted this research into discovering natural medicines/herbs or plants with the potency to treat these infections. The extracts and dry powder samples from medicinal and aromatic plants species are used to develop and prepare alternative medicine and food additives. The aim of this study is to evaluate the antifungal activities of methanolic extracts of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) on fungal isolates from Tinea capitis. The objectives include the isolation of fungi from Tinea capitis samples, extraction of bioactive compounds from both plants and determination of antifungal activities of the extracts, MIC and MFC on the fungal isolates.

## **2. MATERIALS AND METHODS**

### **2.1 Sample Collection**

Forty different samples of scalp scrapings from children with clinically suspected cases of ringworm infection (Tinea capitis), whose parents gave their consent after getting permission from the school authorities, and who had not used any antifungal drugs for the last one month were collected. The lesions and scalp scales of the study participants were carefully examined under bright sunlight, the affected area was properly cleaned with 70% ethanol. The scalp scales/crusts were collected by gently scrapping of the scales together with the hairs across the affected area using new razor blades, one for each patient, to avoid cross infections. The scrapings were put into sterile containers, separately and transported to the laboratory.

### **2.2 Microscopic Examination of the Specimen**

A drop of 20% potassium hydroxide (KOH) solution was put on a sterile glass slide. A small amount of the specimen was transferred to the KOH solution and covered with a cover slip. The mount was slightly warmed to enable the KOH dissolve the keratin in the sample, thus exposing the fungal elements, after which it was left for 30 minutes before viewing under X40 objective lens of the light microscope to

observe and identify the morphology; hyphae with branching, rod-shaped filaments of uniform width with lines of separation (septa) were seen.

### **2.3 Cultivation and Sub-Culturing of The Isolates**

The scalp scrapings/scales were inoculated onto Sabouraud Dextrose Agar (SDA) medium (containing chloramphenicol to avoid the growth of bacteria contaminants) by dropping them at different points. The culture plates were incubated at 28°C for 21 days and were checked for growth, at intervals of 4 days. Then, different colonies formed on the plates were picked and sub-cultured on a freshly prepared Sabouraud Dextrose Agar containing chloramphenicol antibiotic. Pure isolates from the subculture plates were inoculated onto SDA slants, incubated at 28°C for 7 days, and then stored by refrigeration at 4°C for further use.

### **2.4 Slide Culture Technique**

After allowing the plates of the prepared SDA to solidify, each of the agar was cut into small blocks of uniform area. The agar block was placed on a glass slide which was then placed on a U-tube glass rod that was inside the petri-dish containing about 1ml of water needed to provide a humid environment for the fungi to grow during incubation. The four sides of the agar block were inoculated with pure isolates under aseptic conditions, cover slip was placed on the agar block and incubated at 28°C for 4 days.

### **2.5 Identification of Fungal Organisms**

#### **2.5.1 Microscopic identification of pure isolates from slide culture**

After the four-day incubation, the cover slip was carefully removed from the agar block on the glass slide using a pair of forceps. A drop of 70% ethanol was dropped on the areas of the glass slide and the cover slip where growths were observed to remove water bubbles. A drop of lactophenol blue was placed on a new glass slide and the cover slip containing the fungal growths was carefully placed on the glass slide. It was then viewed under the microscope for morphological identification using X40 objective lens.

#### **2.5.2 Microscopic identification of pure isolates from culture plates using lactophenol blue**

A drop of lactophenol blue was placed on a clean sterile glass slide. A small piece of the fungal culture was picked with a sterilized inoculation needle and placed on the lactophenol blue drop. The mount was well spread using a small sterile pin and then covered with a clean sterile cover slip. The cover slip was gently pressed against the slide to make the fungi absorb the lactophenol fluid very well. Excess fluids were removed carefully. It was viewed under a microscope using X40 objective lens [23].

**2.5.3 Biochemical tests:** Urease test was carried out [24].

Hair perforation test was performed [25].

## **2.6 Ginger and Garlic Extracts Preparation**

### **2.6.1 Sample collection and preparation**

Fresh samples of ginger rhizomes and garlic cloves were purchased from Ogige market at Nsukka in Enugu State of Nigeria and taken to the Department of Plant Science and Biotechnology at the University of Nigeria, Nsukka for authentication.

They were washed and air-dried at room temperature for 24h. The outer layer was carefully peeled off, while the inner part was sliced into small bits, dried and then ground. A 100g of each of the powdered samples was carefully weighed and was divided into two portions of equal weight for the preparation of methanolic extracts by Soxhlet extraction and aqueous extracts by cold maceration procedures.

### **2.6.2 Preparation of methanolic extracts of the samples (ginger and garlic) by Soxhlet extraction**

A 50g of each of the powdered samples was carefully packed separately into the Soxhlet column up to two third of its entire volume. About 250ml of methanol was measured into the round bottomed flask and the Soxhlet column was inserted. Methanol was introduced into the Soxhlet column until it just began to siphon into the flask through the siphon arm. Reflux condenser was inserted into the Soxhlet column and rubber hoses were connected from the condenser to the water circulator. The round bottomed flask was placed in a heating mantle, and the mantle was connected to power supply. The initial colours of the extracts were noted and the extraction continued, amidst solvent refluxing, till the solvent on the siphon arm became fairly colorless. The setup was disconnected, and the extracts were collected and concentrated using a rotary evaporator. The extracts were concentrated using a rotary evaporator. The concentrated extracts were each stored in a 100ml wide mouthed plastic container at 4°C, labeled and kept carefully until used [26].

### **2.6.3 Preparation of aqueous extracts of the samples by cold maceration**

Aqueous extracts of the samples (ginger and garlic) were prepared by cold maceration [26, 27] with slight modifications. 50g each of the powdered samples was separately transferred into 300ml of distilled water and vigorously mixed intermittently to effect rapid equilibration between intracellular and extracellular fluids, thereby enhancing proper percolation of the water into the particles for exhaustive extraction. After 24 hours, the suspensions were each poured separately into a muslin cloth which was strained to express the water extractable into another container. This process was repeated 3 times in 300ml quantity of distilled water until the filtrates became colorless. The filtrates were concentrated with the rotary evaporator, then the concentrated extract was labelled accordingly. The concentrated extracts were stored in a 100ml clean wide mouth lidded plastic container, labelled and kept in a refrigerator at 4°C for further use.

## **2.7 Qualitative Phytochemical Analysis**

Qualitative phytochemical tests were carried to detect the presence of alkaloid, terpenoid, saponin, tannin, glycoside and phenol [26,28].

## **2.8 Antifungal Sensitivity Test**

### **2.8.1 Standardization of inoculum**

The fungal isolates were adjusted to 0.5 McFarland standard turbidity,  $1 \times 10^6$  spores/ml by adding sterile peptone water and shaking with sterile beads to break the hyphae. In preparation of the 0.5 McFarland standard, 0.05ml of Barium chloride ( $\text{BaCl}_2$ ) (1.17% w/v  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) was added to 9.95ml of 0.18M  $\text{H}_2\text{SO}_4$  (1.0% w/v) with constant stirring. The standard was compared against a white background with a contrasting black line [18].

### **2.8.2 Preparation of stock solutions of the extracts and making of serial dilutions**

800mg of each of the methanolic extracts was weighed appropriately into two different conical flasks labelled "ginger" and "garlic". 1ml of dimethyl sulfur oxide (DMSO) and 1 ml of distilled water were added to the different extracts in the tubes and both were mixed thoroughly to achieve homogeneity. The flasks were each labelled 400g/ml dilution. The solutions of the extracts were diluted serially to obtain various concentrations using double dilution technique as described by Idemudia *et al*, [27] to obtain 400mg/ml, 200mg/ml, 100mg/ml and 50mg/ml.

Antimicrobial susceptibility test was carried out by agar well diffusion method on SDA containing chloramphenicol. Fungal suspensions prepared from 72h culture in agar plates were diluted to concentration equivalent to  $1.0 \times 10^6$  CFU/mL (0.5 McFarland standards). The inoculum (0.1ml) standard prepared was spread on the set SDA using a swab stick to give monolayer of the fungal organisms all over the agar surface in each of the four plates of SDA, one for each of the extracts and were allowed to stand for 1 hr. A sterile cork borer of 7mm in diameter was used to create 4 wells on each of the evenly inoculated plates and 0.1 ml of each of the serially diluted extracts (400, 200, 100 and 50 mg/ml dilutions) was aseptically dispensed into each of the respective wells with proper labeling and left for 30 minutes to allow for proper diffusion of the antimicrobial extracts. Another plate of SDA for the control (fluconazole) was prepared and seeded with inoculum [29]. The plates were then incubated at the temperature of 28°C for 72h and the zones of inhibition were measured in millimeters (mm) using a meter rule.

### **2.8.3 Minimum inhibitory concentration (MIC) of the methanol extracts**

The MIC of the extracts against tested dermatophytes was determined by broth macro-dilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications. One ml of prepared liquid Sabouraud medium (broth) containing chloramphenicol antibiotic (to avoid the growth of bacteria contaminants) was added to each of 4 sterile test tubes. One ml of each of the serially diluted methanolic extracts suspensions was added to the tubes accordingly. The final concentrations of each plant solvent extracts were 400mg/ml, 200mg/ml, 100mg/ml

and 50mg/ml. 0.1ml of inoculum was added to the tubes labelled 400, 200, 100 and 50mg/ml and the contents were mixed. The tubes were incubated at 28°C for 72 hours. The plants exhibited very effective anti-dermatophyte activity in the extracts.

#### **2.8.4 Minimum fungicidal concentration (MFC) determination of the methanolic extracts**

To determine minimum fungicidal concentration (MFC) values after reading the corresponding MIC values, 100µl (0.1ml) samples from all optically clear tubes (with complete growth inhibition) plus the last tube showing growth, were sub-cultured on SDA. The plates were incubated at 28°C for a minimum of 3 days, until growth was clearly visible in the control samples, and MFC values were determined as the lowest concentration of extracts for which there was no visible growth[18].

### **3. RESULTS AND DISCUSSION**

#### **3.1 Microscopic Examination of the Specimen before Culture**

The microscopic examination of the fungal specimens using 40X objective lens showed the hyphal structures of the organisms which seem to be the pencil structure, suspected to be of *Trichophyton* spp or *Microsporum* spp.

##### **3.1.1 The fungal isolates on cultures plates**

From the experiments carried out, five fungal organisms were isolated and named T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>. T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> had white color, flat and cottony in appearance while T<sub>2</sub> was brownish in colour, raised and heaped in appearance. T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> have the same growth characteristics and were all suspected to be *Trichophyton* spp while T<sub>2</sub> with different growth characteristics was suspected to be *Microsporum* spp. Results of cultural morphology of the isolates is shown in table 1.

**Table 1: Growth characteristics of the isolates**

Isolates	Colour	Elevation	Appearance	Possible organisms
T <sub>1</sub>	White	Flat	Cottony	<i>Trichophyton</i> spp

T <sub>2</sub>	Brown	Raised	Heaped	<i>Microsporumspp</i>
T <sub>3</sub>	White	Flat	Cottony	<i>Trichophyton</i> spp
T <sub>4</sub>	White	Flat	Cottony	<i>Trichophyton</i> spp
T <sub>5</sub>	White	Flat	Cottony	<i>Trichophyton</i> spp

### **3.1.2 Microscopic examination of fungal culture using lactophenol cotton blue**

The results of lactophenol cotton blue of the isolates showed filamentous appearance of the hyphal elements. Similarly, hair perforation tests produced wedge-shaped perforations on the hair specimens, indicating positive results. From the microscopic examination of lactophenol cotton blue stain of the isolates also, T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were the same isolates while T<sub>2</sub> was different. Table 2 shows urease test and hair perforation test results of the fungal isolates. All the fungal isolates were positive to the hair perforation test and urease test. A colour change from yellow to pink, indicative of enzyme, urease was observed (table 2).

**Table 2: Hair perforation and Urease tests results of the fungal isolates**

<b>Isolates</b>	<b>Urease Test</b>	<b>Hair perforation test results</b>
T <sub>1</sub>	Positive	Positive
T <sub>2</sub>	Positive	Positive
T <sub>3</sub>	Positive	Positive
T <sub>4</sub>	Positive	Positive
T <sub>5</sub>	Positive	Positive

### **3.2 Phytochemical Analysis of the Methanolic Plant Extracts**

Qualitative phytochemical analysis carried out on the garlic and ginger methanol extracts showed the presence of saponins, flavonoids, glycosides, tannins, terpenoids, alkaloids and phenols as bioactive phytochemicals. In garlic extract, saponins and flavonoids were present, tannins and alkaloids were highly present, phenols were very highly present while glycosides and terpenoids were absent. In ginger extract, glycosides were present, flavonoids and phenols were highly present, terpenoids were very highly present while saponins, tannins and alkaloids were absent (table 3).

**Table 3: Phytoconstituents of garlic and ginger extracts**

Phytochemicals	Garlic extract	Ginger extract
Saponins	+	-
Flavonoids	+	++
Glycosides	-	+
Tannins	++	-
Terpenoids	-	+++
Alkaloids	++	-
Phenols	+++	++

**Key:**

+ = Present

++ = Highly present

+++ = very highly present

- = Absent

**3.3 *In vitro* Sensitivity Test of Garlic and Ginger against *Tinea capitis* Isolates**

Inhibitory activities of the methanolic and aqueous extracts of garlic and ginger on test isolates were observed. At equal concentrations of 400, 200, 100 and 50 mg/ml, the inhibition zone diameters (IZD) of the control (fluconazole) were 30, 25, 23 and 20 mm; the IZD of methanolic extracts of garlic were 35, 30, 25 and 20 mm; IZD of garlic aqueous extracts were 20, 15, 10 and 8 mm. Similarly, the IZD of ginger methanolic extract were 25, 20, 15 and 13 mm while ginger aqueous extract IZD were 15, 10, 8 and 0 mm respectively. The zones of inhibition of the extracts were almost the same for the two isolates. These results are represented in tables 4 and 5.

**Table 4: Zones of inhibition in milliliter of the extracts and control on the *Trichophyton* spp**

Conc. of extracts (mg/ml)	Garlic methanol extract	Garlic aqueous extracts	Ginger methanol extracts	Ginger aqueous extracts	Fluconazole (Control)
400	35	20	25	15	30
200	30	15	20	10	25
100	25	10	15	8	23
50	20	8	13	0	20

**Table 5: Zones of inhibition in milliliter of the extracts and control on the *Microsporumspp***

Conc. of extracts (mg/ml)	Garlic methanol extract	Garlic aqueous extracts	Ginger methanol extracts	Ginger aqueous extracts	Fluconazole (Control)
400	36	20	25	13	31
200	28	14	20	10	25
100	25	10	14	6	22
50	21	10	12	0	20

The combined aqueous extracts of garlic and ginger (1:1) showed a higher antifungal activity against the two isolates. Likewise, the combined methanol extracts gave wider inhibition zone diameter on the isolates compared to the IZD of the extracts singly as shown in table 6. This result shows the synergistic effect of the two extracts.

**Table 6: Zones of inhibition in milliliter of the combined extracts and control on the isolates**

Conc. of extracts (mg/ml)	Combined Aqueous extracts (on <i>Trichophyton</i> spp)	Combined Methanol extracts (on <i>Trichophyton</i> spp)	Combined Aqueous extracts (on <i>Microsporumspp</i> )	Combined Methanol extracts (on <i>Microsporumspp</i> )
400	24	38	22	27
200	18	32	16	23
100	14	28	13	16
50	11	23	12	13

### 3.4 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Garlic and Ginger Extracts (Singly and in Combination)

The MIC for the methanolic extracts of the garlic was 100mg/ml while that of its aqueous extracts was 200mg/ml. At these concentrations, there was reduced turbidity and increased transparency of the broth in the test tubes after 4 days incubation at 28 °C. The MFC for the methanolic extracts of garlic was 200mg/ml while that of its aqueous extracts was greater than 400mg/ml as shown in table 7.

The MIC for the methanolic extracts of the ginger was 200mg/ml while that of its aqueous extracts was greater than 400mg/ml. The MFC for the methanolic extracts was 400mg/ml while that of its aqueous cold maceration extracts was greater than 400mg/ml as shown in table. The combined extract was found to be more effective than the extracts singly. The MIC and MFC of the combined methanol extracts on

both isolates were 50mg/ml and 100mg/ml respectively compared to 100mg/ml and 200mg/ml for the single methanol extracts. Table 7 shows the MIC and MFC values for the extracts, singly and in combination.

**Table 7: MIC and MFC of the garlic, ginger and combined extracts on the culture plates**

Isolates	Garlic MIC mg/ml	Garlic MFC mg/ml	Ginger MIC mg/ml	Ginger MFC mg/ml	Comb. MIC Mg/ml	Comb. MFC Mg/ml
<i>Trichophyton spp</i>						
<b>Methanolic extracts</b>					50	100
	100	200	200	400		
<b>Aqueous extracts</b>	200	>400	>400	>400	100	200
<i>Microsporon spp</i>						
<b>Methanolic extracts</b>	100	200	200	400	50	100
<b>Aqueous extracts</b>	200	>400	>400	>400	100	200

## DISCUSSION

Tinea capitis is highly prevalent in rural areas with poor socio-economic status involving mainly primary school children between 4-15 years old and who probably had low body immunity and also could not maintain good personal hygiene and diets [8, 30]. This was confirmed in villages located in Ebonyi state of Nigeria where the experimental clinical samples were collected.

The direct microscopy of the clinical specimen from Tinea capitis mounted on 20% KOH prior to culture showed hyphae with rod shapes of uniform widths with lines of separation (septate). The works of Samia *et al* [25] and Barry [31] gave support to this result.

The urease test showed colour change from yellow to pink, indicating the ability of the organisms to metabolize urea into carbon (iv) oxide (CO<sub>2</sub>) and ammonia (NH<sub>3</sub>) by secreting and utilizing the enzyme, urease. This positive result was in consonance with the previous report by Jeremy [24]. In the hair perforation test, positive results were also observed as in the reports of Widad and Thekra [32]. There

was confirmation of perpendicular pits found along the hair shafts when viewed under the X40 objective lens. The positive result from hair perforation test further showed the ability of the fungi to secrete keratinase, an enzyme used to digest the keratinized hair by *Trichophyton* spp or *Microsporum* spp. Jeremy [24], confirmed these Isolates to be *Microsporum* spp or *Trichophyton* spp based on the test results.

In this study, it was observed that the inhibition zone diameter increased with increase in the concentration of the extracts. The concentration of 400mg/ml had the largest zone of inhibition compared to 200mg/ml, 100mg/ml and 50mg/ml concentrations in all the extracts. This result agrees with the report of Mercy *et al* [33] who also demonstrated that the zone of inhibition increased with increase in concentration of the extract.

The combined aqueous extracts of garlic and ginger (1:1) and that of methanol extract in this study gave wider inhibition zone diameter on the isolates compared to the IZD of the extracts singly against the two isolates. For instance, the combined methanol extracts at 200mg/ml gave IZDs of 32mm and 23mm against *Trichophyton* spp and *Microsporum* spp respectively while the single extracts gave 20mm on the two isolates. The combined extract was also found to be more effective on the *Trichophyton* spp than the *Microsporum* spp. The MIC and MFC of the combined methanol extracts on both isolates were 50mg/ml and 100mg/ml respectively compared to 100mg/ml and 200mg/ml for the single methanol extracts. These results correspond with the findings of Nguyen *et al* [34] and Alvin *et al* [35].

The sensitivity tests in this present study indicated that at equal concentrations, the methanol extracts of the garlic and ginger extracts exhibited higher activities than the aqueous cold extracts. The observed higher activity could be attributed to the higher activity potential of organic compounds found in the methanolic extracts than in the aqueous extracts, as well as the more efficient solubility of the active compounds in the organic solvents [36, 37]. This is in agreement with the work of Gomaa and Hashish [38], who observed that methanolic extracts of *Z. officinale* produced higher antimicrobial activity than the aqueous extract on the test organisms. For instance, this study recorded IZDs of 35 and 20mm for the methanol and aqueous extracts of garlic respectively at the concentration of 400mg/ml on *Trichophyton* spp. This result agrees with Anazodo *et al.*, [39], which reported that ethanol, aqueous, chloroform and acetone extracts of garlic at the same concentration, gave IZDs of 22, 18, 16 and 0mm respectively on *Trichophyton* spp. These results support the fact that the presence and antimicrobial effectiveness of the bioactive components of garlic and ginger differ based on the method of preparation, extraction and extraction solvent [40].

However, the methanolic extract of the garlic (with the inhibition zones diameters- IZD: 35, 30, 25 and 20mm) was more active than that of ginger (with corresponding IZD of 25, 20, 15 and 13mm) at the same concentrations and as indicated by the higher zones of inhibition, MIC and MFC values of the garlic extracts. Similarly, the garlic methanol extracts were more effective than the control (fluconazole) with IZD

of 30, 25, 23 and 20 mm respectively at the same concentrations. This greater efficacy of the garlic extracts may be due to the presence and potency of tannins and alkaloids not found in the control. The high efficacy of the ginger extract against several antifungal diseases is noteworthy, and is attributed to the presence of gingerols and ginger diols [18].

#### 4. CONCLUSION

Tinea capitis is a head ringworm (fungal infection) caused mainly by *Trichophyton spp* and *Microsporum spp.*, which inhabit keratinized parts of the body such as the hair, nails, skin surface, animal hoofs and feathers and fomites (ie, inanimate objects). They can be transmitted from person to person, from animal to person, from fomites to person and vice versa and this is mediated by low body immunity and poor socio-economic status of the people. This study recorded higher efficacy of *A. sativum* against Tinea capitis than the conventional chemotherapeutic agent, fluconazole particularly the methanol extracts. It also underscores the antifungal activities of *Z. officinale* and then suggest their (garlic and ginger) use as components of anti-tinea capitis drugs as well as other microbial infections.

#### CONSENT

Before the sampling, informed consent forms were filled by the parents of the participants.

#### Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

#### REFERENCES

1. Prescott, Harley, and Klein. (2008). Microbiology. 7th edition. McGrawHill Publishers, London. 629-640. [https://www.academia.edu/29333589/Prescott\\_Harley\\_and\\_Klein\\_Microbiology\\_7th\\_2008\\_PASH\\_A\\_BHAI\\_CIVIL\\_DUAL\\_pdf](https://www.academia.edu/29333589/Prescott_Harley_and_Klein_Microbiology_7th_2008_PASH_A_BHAI_CIVIL_DUAL_pdf)
2. Algimantas, P. and Jurgita, Š. (2013). Distribution and Species Composition of Causative Agents of Dermatophytoses in Lithuania. *Clinical Article* 21(2):99-104. *Acta Dermatovenerol Croat Dermatophytoses in Lithuania 2013*;21(2):99-104 Žaliujų Ežerų Str. 49 LT-08406 Vilnius Lithuania <https://hrcak.srce.hr/clanak/157172>
3. Segal, E. and Elad, D. (2021). Human and Zoonotic Dermatophytoses: Epidemiological Aspects. *Frontier Microbiology*. 12, 713532. <https://doi.org/10.3389/fmicb.2021.713532>
4. Magreth, E., Macha, Mariam, R. Makange and Gerald Misinzo (2020). Prevalence and identification of fungi associated with Tinea capitis in school children of Morogoro municipality,

- Tanzania. *African Journal of Medical and Health Sciences*,**19**(7): 103-109. <http://www.academicjournals.org/AJMHS> DOI: 10.5897/AJMHS2020.0108
5. Chikoi, R., Nyawale, H. A. and Mghanga, F. P. (2018). Magnitude and Associated Risk Factors of Superficial Skin Fungal Infection Among Primary School Children in Southern Tanzania Study design and setting. *Cureus***10**(7):1-12. <https://doi.org/10.7759/cureus.2993>.
  6. Shy, R. (2019). Tinea corporis and Tinea capitis. *Article Skin Disorders***28**:5. <https://doi.org/10.1542/pir.28-5-164>
  7. Kelly, B. P. (2012). Superficial fungal infections. *Pediatrics Review***33**:22-37. PMID: **22474120**DOI: [10.1542/pir.33-4-e22](https://doi.org/10.1542/pir.33-4-e22)
  8. Zhi, H., Shen, H., Zhong, Y., Sang, Bo, Lv, W. and Li, Q. (2021) Tinea capitis in children: A single-institution retrospective review from 2011 to 2019. *Mycoses*,**64**(5):550-554. <https://doi.org/10.1111/myc.13243>
  9. Rafael Isa Isa, Beatriz Yáñez Amaya, Mariel Isa Pimentel, Roberto Arenas, Antonella Tosti, and Ana Cecilia Cruz (2014). Dermoscopy in tinea capitis: a prospective study on 43 patients, **42** (1-3): 18-22. [www.medigraphic.com/medicinacutanea](http://www.medigraphic.com/medicinacutanea)
  10. Baydar, H., Sağdıç, O., Özkan, G. and Karadoğan, T. (2004). Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*,**15**:169–172. [https://doi.org/10.1016/S0956-7135\(03\)00028-8](https://doi.org/10.1016/S0956-7135(03)00028-8)
  11. Imo, C., Arowora, K. A., Awache, I. and Abdullahi, Z. R. (2016). Was Haematological Effects of Ethanolic Leaf, Seed and Fruit Extracts of *Datura metel* on Male Albino Rats. *FUW Trends in Science and Technology Journal***1**(2): 509-512. *FUW Trends in Science & Technology Journal*, [www.ftstjournal.com](http://www.ftstjournal.com)  
e-ISSN: 24085162; p-ISSN: 20485170; October, 2016 Vol. 1 No. 2 pp 509 – 512
  12. Block, E. (2010). *Garlic and Other Alliums: The Lore and the Science*. Royal Society of Chemistry, 2010; ISBN 0-85404-190-7. <https://cir.nii.ac.jp/crid/1574231875962961408>
  13. Bar, M., Binduga, U. E. and Szychowski, K. A. (2022). 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**(7), 1345. <https://doi.org/10.3390/antiox11071345>
  14. Aigerim Bazaraliyeva, Dias Moldashov, AknurTurgumbayeva, Elmira Kartbayeva, Assem Kalykova, Lazzat Sarsenova, Raushan Issayeva and Al Farabi Kazakh (2022). *Chemical and biological properties of bioactive compounds from garlic (Allium sativum)*,**69**(4): 955-964. <https://doi.org/10.3897/>
  15. Subroto, E., Cahyana, Y., Mahani, F., Lembong, E., Wulandari, E., Kumiaty, D., Saputra, R.A. and Raturachman, F. (2021). Bioactive Compounds in Garlic (*Alhum sativum*) as a Source of Antioxidants and the Potential to Improve the Immune System. *Food Research*.<http://www.myfoodresearch.com>.
  16. Sudip, K. M., Agnidipta, D., Suddhasattya, D., Ujjwal, S., Sankhadip, B., Anindya, B., Neerupma, D., Swati, M. and Mohamed, A.R. (2019). Bioactivities of Allicin and

Related Organosulfur compounds from Garlic. *Egyptian Journal of Chemistry*, **62** (1): 1-11. DOI: [10.21608/ejchem.2019.15787.1954](https://doi.org/10.21608/ejchem.2019.15787.1954)

17. Foster, S. (2011). Ginger (*Zingiber officinale*). Your food is your medicine. Available from: <http://www.stevenfoster.com/education/monograph/ginger.html>.
18. Mohammed, Y., Mukhtar, M. D and Orah, S. A. (2017). Synergistic activity of garlic, ginger and moringa on ear, nose and throat associated fungi and vancomycin resistant cocci at Aminu kano teaching hospital, kano, Nigeria. *World journal of pharmaceutical and medical research*, **3**(4), 01-10. ISSN 0975-2366 DOI: <https://doi.org/10.31838/ijpr/2020.12.03.412>
19. Kumar, A., Goyal, R., Kumar, S., Jain S., Jain, N. (2015). Estrogenic and Anti-Alzheimer's studies of *Zingiber officinalis* as well as *Amomum subulatum* Roxb.: the success story of dry techniques. *Medical Chemical Research* **24**(3): 1089-1097. <https://link.springer.com/article/10.1007/s00044-014-1166-y>
20. Shivakumar, Singh P. and Vidyasagar, G. M. (2015). Antifungal Screening of 61 Traditional Medicinal Plants of 305 Extracts Against Dermatophytic Fungi Trichophyton Tonsurans. *International Journal of Pharmacy and Pharmaceutical Sciences* **7**(3):186-190. <https://d1wqtxts1xzle7.cloudfront.net/38675748/ANTIFUNGAL>
21. Chinedu, I. and Jivini, S. Z. (2019). Medical Properties of Ginger and Garlic: A review. *Current Trends in Biomedical Engineering and Biosciences*, **18**(2): 555-585. DOI: 10.19080/CTBEB.2019.18.555985. <https://www.researchgate.net/profile/Chinedu>
22. Ridwan, O. A., Esther Ogbale, Akogmu, E. I., Oyiza, S. and Modinat, D. A. (2021). Aqueous Extracts of Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) Bulbs: Phytochemical Screening and *In vitro* Antitrypanosomal Effect. *An International Scientific Journal*, **37**: 135-150. [www.worldnewsnaturalsciences.com](http://www.worldnewsnaturalsciences.com)
23. Ridzuan, P. M., Proveen, K. and Kamarulzaman, S-A. (2021). Isolation of Dermatophytes from Infected Stray Dogs in Selangor, Malaysia, *Journal of Epidemiology*. **9**(2): 123-129. DOI: [10.20473/jbe.V9I22021.123-129](https://doi.org/10.20473/jbe.V9I22021.123-129)
24. Jeremy, R. Caddell (2001). Differentiating the Dermatophytes. *Journal on Clinical Laboratory Science*, **15**(1):13. (fax). [Caddell.Jeremy@Mayo.edu](mailto:Caddell.Jeremy@Mayo.edu). <https://doi.org/10.1093/jac/dkz344>
25. Samia Afreen Khan, S. M. Shamsuzzaman, A. K., Shahidur Rahman., M., Nesar Ahmed Khan Ashekin, Rasel Mahmu, Rabaya Sharmin, Mohammad Rahmat Ullah Siddiqu, Md Alauddin Khan, Sharmin Sultana, and Fahmida Haque. (2021). *Isolation and Identification of Dermatophytes Causing Dermatophytosis at a Tertiary Care Hospital in Bangladesh* **5** (3): 437-451. ISSN: 2572-5017 DOI: 10.26502/acbr.50170178 <https://www.fortuneonline.org/articles/isolation-and-identification-of-dermatophytes-causing-dermatophytosis-at-a-tertiary-care-hospital-in-bangladesh.html>
26. Hetavi, J. Thakor, Yesha, S. Rathi, and Nisha, S. Nayak (2023). Phytochemical Screening of Ginger (*Zingiber officinale*), a Medicinal Plant. *Scholars International Journal of Traditional and*

DOI:10.36348/sijtc.2023.v06i04.002[https://www.researchgate.net/profile/Nisha-](https://www.researchgate.net/profile/Nisha-Nayak/publication/371116479_Phytochemical_Screening_of_Ginger_Zingiber_officinale_a_Medicinal_Plant/links/649f1494c41fb852dd429fe6/Phytochemical-Screening-of-Ginger-Zingiber-officinale-a-Medicinal-Plant.pdf)

[Nayak/publication/371116479\\_Phytochemical\\_Screening\\_of\\_Ginger\\_Zingiber\\_officinale\\_a\\_Medicinal\\_Plant/links/649f1494c41fb852dd429fe6/Phytochemical-Screening-of-Ginger-Zingiber-officinale-a-Medicinal-Plant.pdf](https://www.researchgate.net/profile/Nisha-Nayak/publication/371116479_Phytochemical_Screening_of_Ginger_Zingiber_officinale_a_Medicinal_Plant/links/649f1494c41fb852dd429fe6/Phytochemical-Screening-of-Ginger-Zingiber-officinale-a-Medicinal-Plant.pdf)

27. Idemudia, I. B., Ozoaduche, C. L. and Ekhaise, F. O. (2022). Antifungal Properties of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) on Fungi Isolated from Postgraduate Student Hostels' Bathrooms, University of Benin, Benin City. *Journal of Science and Technology Research*,4(1) 120-121. <https://doi.org/10.37933/nipes/4.1.2022.10>
28. Jigna, P., Sumitra, V. and Chanda (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkey Journal of Biology*,31: 53-58. Available at: <https://journals.tubitak.gov.tr/biology/vol31/iss1/9>
29. Clinical and Laboratory Standards Institute (2011). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. *CLSI publication M100-S17*. Wayne, Pa. 2011. DOI: [10.4236/inframatics.2013.22002](https://doi.org/10.4236/inframatics.2013.22002)
30. Khosravi, A. R., Shokri H. and Vahedi, G. (2016). Factors in etiology and predisposition of adult tinea capitis and review of published literature. *Mycopathologia*,181(1):371-378. <https://doi.org/10.1007/s11046-016-0004-9>
31. Barry. L. Hainer (2003). Dermatophyte Infections. *Journal of Mycology*, 67(1):101-8. <https://pubmed.ncbi.nlm.nih.gov/12537173/>
32. Widad, Ismail Thallaj and Thekra Ahmed Humada (2015). Susceptibility of Trichophyton mentagrophytes Against Two Antifungals: Griseofulvin and Terbinafine. *Journal for Agricultural Science*,15(2). Journal Tikrit Univ. For Agri. Sci. Vol. (15) No.(2) – 2015 ISSN-1813-1646 <https://www.iasj.net/iasj/download/8780cdb91c7fb290>
33. Mercy, K. A., Ijeoma, I., & Emmanuel, K. J. (2014). Anti-dermatophytic Activity of garlic (*Allium sativum*) extracts on some Dermatophytic fungi. *International Letters of Natural Sciences*. 19. Available online at [www.ilns.pl](http://www.ilns.pl) 19 (2014) 34-40 ISSN 2300-9675
34. Nguyen, M. H., Tran, T. N. M., and Vu, N. B. D. (2022). Antifungal activity of essential oil-encapsulated lipid nanoemulsions formulations against leaf spot disease on tomato caused by *Alternaria alternata*. *Archives of Phytopathology and Plant Protection*. 55(2), 235-257. <https://doi.org/10.1080/03235408.2021.2015887>
35. Alvin, W. Karani, Dancan, M. Wakoli, Micah Lagat, ZablónMalago, and Julia J. Ouma. (2022) Antifungal Activity and Synergism of *Allium sativum*, *Zingiber officinale*, And Citrus Limon Peel Essential Oils Against *Tinea capitis*. *African Journal of Pharmacy and Alternative Medicine*. 2(1):2023. <https://doi.org/10.58460/ajpam.v2i1.37>.
36. de Boer, H. J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I. and Levenfors, J. J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology*96: 461-469. <https://doi.org/10.1016/j.jep.2004.09.035>

37. Doughari, J. H., Elmahmood, A. M. and Manzara, S. (2007). Studies on the antibacterial activity of root extracts of *Carica papaya* L. *African Journal of Microbiology Research*, **1**: 37-41. <http://www.academicjournals.org/ajmr>
38. Gomaa, N. F. and Hashish, M. H. (2003). The inhibitory effect of garlic (*Allium sativum*) on the growth of some microorganisms. *Journal of Egyptian Public Health Association*, **78**(5): 361-372. PMID: 17219900 <https://europepmc.org/article/med/17219900>
39. Anazodo C. A., Abana C. C., Agu K. C., Victor-Aduloju A. T., Okoli F. A., Ifediegwu M. C., Awari V. G. and Chidozie C. P. (2024) In Vitro Antifungal Efficacy of *Allium Cepa* And *Allium Sativum*: A Comparative Study with Commercial Drugs. *Research and Analysis Journals* 7(08): 33-39, 2024e-ISSN: 2589-9228, p-ISSN: 2589-921x
40. Silvana, Z., Ellizaliabetta, B. and Guilia, C. (2023). Garlic (*Allium sativum*) as an Ally in the Treatment of Inflammatory Bowel Disease. *Current Issues in Molecular Biology*,**45**(4): 685-698.<https://doi.org/10.3390/cimb45010046>

UNDER PEER REVIEW