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Original Article

Antifungal Potential of Honey against Dermatophytes: A Comprehensive Study on Isolates from Children and Farmers in Wukari, North East Nigeria

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Abstract

This study explores the antifungal properties of honey against dermatophytes isolated from children and farmers in Wukari, Nigeria, addressing the global public health concern of dermatophytosis. Dermatophytes such as *Trichophyton species, Epidermophyton floccosum*, and *Microsporum canis* were identified in skin, hair, and nail samples. Through agar well diffusion and minimum inhibitory concentration (MIC) methods, the study demonstrated concentration-dependent inhibitory effects of honey on these dermatophytes, with substantial zones of inhibition. Notably, *Trichophyton species* exhibiting a maximum zone diameter of 36 mm at 100% honey concentration followed by *Epidermophyton floccosum* (25 mm) and *Microsporum canis* (40 mm). MIC results at a 60% honey dilution showed very scanty growth for *Trichophyton species* and *Epidermophyton floccosum*, while *Microsporium canis* displayed no growth after both five and seven days of culture. The findings suggest honey's potential as an alternative antifungal agent against dermatophytes amid increasing antimicrobial resistance. Future research should focus on identifying honey's specific bioactive components and conducting clinical trials for practical application.

Keywords: Dermatophytosis, Honey, Antifungal activity, Antimicrobial resistance.

Introduction

Dermatophytosis, a prevalent global infectious disease, particularly affecting developing countries remains a global public health concern with varying prevalence rates [1]. Understanding its epidemiology and causative agents is crucial for effective prevention and treatment strategies. The aetiologic agents of Dermatophytosis are Dermatophytes, a closely related group of filamentous fungi. In the current taxonomy of dermatophytes, seven genera are acknowledged within this fungal group [2] including

Epidermophyton, Trichophyton, Arthroderma, Microsporum, Nannizzia, Paraphyton, and *Lophophyton*, with more than 50 species identified, among which *Epidermophyton, Microsporum*, and *Trichophyton* stand out as the most prevalent genera that infect humans [3].

Dermatophytes produces keratinases, which facilitates the degradation of the keratinized tissues like the skin, hair, and nails and subsequent invasion of cutaneous skin tissue [4]. Infections are typically cutaneous, confined to non-living, cornified skin layers. However, in chronic cases, fungi may penetrate deeper tissues, particularly in concurrent infections [5]. Clinical manifestations vary based on the etiological agent and affected anatomical sites. Skin infections often present as circular, erythematous, and pruritic lesions, while nail infections (*onychomycosis*) may lead to nail separation, thickening, or dystrophy [6]. Transmission of dermatophytosis occurs through direct contact with infected individuals or animals or indirectly through contaminated objects [7].

While not often fatal, these infections can significantly impact quality of life, causing dermal inflammation, cosmetic concerns, and social stigmatization, posing a public health challenge. Recent reports highlight a growing incidence of superficial mycosis, leading to frequent visits to dermatological clinics.

Prevalence varies worldwide due to social practices, migration, urbanization, living conditions, and climate [8]. Dermatophytosis, including ringworm and Tinea corporis, affects 20-25% of the global population, with 90% of fungal nail diseases attributed to dermatophytes [8]. Prevalence of Dermatophytosis varies in Nigeria, with reports ranging from 3.4% to 55% depending on the population and geographic location [9]. Ethiopia, being a tropical nation, faces a high prevalence of dermatophytosis [10]. The rise in dermatophytosis is linked to socioeconomic conditions, international travel, immigration, climate change, overcrowding, environmental hygiene, cultural practices, awareness, age, hygiene, lifestyle and immunosuppressive drug use [11].

Honey, a natural sweet substance produced by honeybees, is derived from flower nectar or plant secretions [13]. It contains carbohydrates, mainly fructose and glucose, along with various oligosaccharides. Honey possesses potent antimicrobial properties, demonstrated in studies against bacteria and fungi. While antibacterial effects are well-documented, fewer studies have explored honey's action against fungi [14].

Antimicrobial resistance of Dermatophytes to conventional antibiotics is on the rise [15]. Honey has shown antifungal activity against *Candida albicans, Aspergillus baumannii,* and *Penicillium chrysogenum* [16]. As conventional antifungal treatments have limitations, there is growing interest in natural compounds. Honey, with its broad-spectrum antibacterial and antifungal potential, is considered as a safe candidate for antifungal applications [17]. The composition of honey is influenced by plant varieties, environmental conditions, and climatic factors, affecting its antifungal activity. Phenolic compounds, especially flavonoids, contribute to honey's biological activity [19].

The three most important generals of dermatophytes (*Trichophyton, Microsporum,* and *Epidermophyton*), are commonly associated with soil [20]. Children between 4 and 16 years are more susceptible to dermatophytosis due to increased contact with soil and insufficient exposure to fungi-inhibiting fatty acids [21]. Likewise, rice farmers, due to their occupational exposure to soil, are particularly vulnerable to

dermatophytosis [22]. Their contact with irritant agents like mud, manure, fertilizers, and dust, along with constant immersion in water during agricultural activities, increases their risk of developing skin, hair, and nail dermatophytosis [23]. Hence, this study aims to investigate the antifungal activity of honey Dermatophytes isolated from children and farmers in Wukari metropolis, Southern Taraba, North East Nigeria.

Materials and Methods

Research Design

Upon obtaining ethical approval from the research ethics committee of the Federal University Wukari, this experimental research investigating the efficacy of honey on fungal samples isolated from skin, hair samples from the scalp and nail samples were conducted in Wukari metropolis, Southern Taraba, North East Nigeria. Wukari is the base of the Wukari Federation, a traditional state. It has an area of 4,308 km^2 and a population of 241,546 at the 2006 census. The occupation of the inhabitants of the area is basically farming. Although some are civil servants while others are involved in one form of trade or the other [24].

Population of the Study

A total number of fifteen (15) persons consisting of 10 children aged between 6 and 11 years from Ebenezer Primary School and 5 farmers aged between 18 and 35 years, all in Wukari Town volunteered to be included in this experimental research.

Sample Collection of the Test Organism

Skin, hair scalp, and nail samples of participants were obtained by scraping the surface of the margin of the lesion using a sterile blunt scalpel blade and placed in a sterile container and labeled as SKS (Skin sample) 1-15, SHS (Scalp hair sample) 1-15, and NAS (Nail sample) 1-15. Prior to the collection of the samples, the affected area was clean each with 70% ethanol.

Honey Sample Collection and Preparation

Honey was purchased in the open market in Wukari and aseptically collected in sterile bottles containing sterile water to achieve 60% v/v, 70% v/v, 80% v/v, 90% v/v, and 100% v/v honey solution [25]. The solution was stored at a room temperature, of 25 °C.

Identification of Fungal Isolate

Specimen were aseptically inoculated using spread plate technique on already prepared Sabouraud Dextrose Agar (SDA) using standard microbiological procedure as recommended by Senanayake [26]. Culture plates were incubated at room temperature (25 °C) for 1-3 weeks to for fungal growth to occur. Observable colonies with mycelia growth were subject to further analysis for identification.

Antimicrobial Sensitivity (Agar Well Diffusion)

The agar well diffusion method was employed for the antifungal assay [27]. The honey concentrates were evaluated for sterility by inoculating the various concentrates on Potato Dextrose Agar (PDA) with no test organism. A fungal suspension was made from the mycelial growth and was inoculated already prepared SDA using pour plate techniques.

Using a sterile cork borer, six equidistant wells of 6 mm in diameter were made at different side on the plate. About 60 µL of the different concentration (60% v/v, 70% v/v, 80%

v/v, 90% v/v, and 100% v/v) of the honey solution were separately placed on the individual well with 1mL sterile syringe. The sixth well was inoculated with sterile water as negative control. The plate was allowed to stay for 15 minutes, for pre-diffusion to take place before incubating for 24-72 hours at room temperature [27]. Observable zones of inhibition were recorded.

Minimum Inhibitory Concentration (MIC)

The agar dilution method was used [28]. 60% dilution was prepared and 2 ml was transferred into sterile petri dishes. Sterilized agar was poured in the plates containing the honey and allowed to set. The media was allowed to dry before streaking with the isolates. The plates were incubated at room temperature for five to seven days after which they were examined for the presence or absence of growth [28]. The MIC was taken as the lowest concentration that will prevent the fungal growth to occur.

Results

Table 1 presents the biochemical analysis of dermatophytes isolates. The result from this investigation as shown in Table 2, indicates that three (3) dermatophytes *Trichophyton species, Epidermophyton floccosum, and Microsporium canis* were isolated and identified from both hair and nail samples. The epidemiological analysis of the cultured samples revealed varying prevalence patterns of dermatophyte isolates, as listed in Table 3.

Trichophyton species demonstrated widespread prevalence across multiple samples, being notably present in SKS 1, SKS 2, SKS 5, SKS 6, SHS 1, SHS 2, SHS 4, SHS 5, NAS 1, NAS 4, NAS 5, and NAS 6. *Epidermophyton floccosum* exhibited prevalence in SKS 1, SKS 4, SKS 5, SKS 6, SHS 1, SHS 2, SHS 4, SHS 5, NAS 2, and NAS 6. Microsporium canis, while less prevalent, was observed in SHS 1, SHS 3, SHS 5, NAS 1, NAS 2, NAS 4, and NAS 5. Negative results were noted in samples SKS 3, SKS 7, SHS 6, SHS 7, and NAS 3. These findings provide a comprehensive overview of the distribution of dermatophyte species in the sampled cases, emphasizing the varying prevalence of *Trichophyton species*, followed by *Epidermophyton floccosum*, and less frequently, Microsporium canis.

Table 4 indicates the results of the antimicrobial sensitivity test of honey concentrates on the dermatophyte. Furthermore, the result showed a concentration dependent increase in susceptibility of dermatophytes. *Microporium canis* has the highest diameter zones of inhibition of 40 mm at 100% v/v and least zone of inhibition being 9.0 mm and Table 5 shows the minimum inhibitory concentration (MIC) after five (5) and seven (7) days of culture, with *Microporium canis* having no growth after five to seven days of culture. Table 1 below is the biochemical characteristics of isolates.

Table 1 Biochemical characteristics of isolates

Biochemical Test	Result for Dermatophytes
Sugar Fermentation	Negative
Enzyme Production	Positive
Nitrate Reduction	Negative
Urease Test	Negative

Negative means that the dermatophyte strain tested does not ferment sugars, does not reduce nitrate, and does not produce urease and positive means the dermatophyte strain tested

does produce enzymes (likely including keratinases).

Table 2 Fungal isolates with their colonial appearance and microscopic appearance

S/N	Dermatophyte isolate	Colonial appearance	Microscopic appearance
1	*Trichophyton species*.	Flat, white to cream colonies and powdery in texture. Reverse is cream to dark brown.	Macroconidia are cylindrical and thin while microconidia are predominantly round.
2	*Epidermophyton floccosum*	Yellow, tiny colonies which is powder in texture. Reverse is pale brown.	Macroconidia are oval shaped forming tree-like structures.
3	*Microsporium canis*	Colonies are flat with few radial folds; the colonies are pale to white in color with a yellow to colorless edge. Reverse is bright yellow to colorless at the edge.	Macroconidia are spindle shaped, rough, with apical end which is curved to one side.

Table 3 Occurrence of dermatophyte isolates in cultured samples

Sample	*Trichophyton species*.	*Epidermophyton floccosum*	*Microsporium canis*
SKS 1	+	+	–
SKS 2	+	–	–
SKS 3	–	+	–
SKS 4	+	+	–
SKS 5	+	+	–
SHS 1	+	–	+
SHS 2	+	–	+
SHS 3	–	–	+
SHS 4	+	–	–
SHS 5	+	–	+
NAS 1	+	–	–
NAS 2	–	+	–
NAS 3	+	+	–
NAS 4	+	+	–
NAS 5	+	–	–

SKS = Skin sample, SHS = Scalp hair sample, NAS = Nail sample, Positive = + Negative = -

Table 4 Zones diameter of inhibition (mm) at different dilution of honey

Dermatophyte isolate	0% (Control)	60%	70%	80%	90%	100%
***Trichophyton species*.**	0 mm	5 mm	11 mm	20 mm	25 mm	36 mm
Epidermophyton floccosum	0 mm	3 mm	6 mm	15 mm	21 mm	25 mm
Microsporium canis	0 mm	9 mm	10 mm	25 mm	34 mm	40 mm

Table 5 The minimum inhibitory concentration (MIC) at 60%v/v dilution of honey

Dermatophyte isolate	Observation after five days of culture	Observation after seven days of culture
***Trichophyton species*.**	Very scanty growth	Very scanty growth
Epidermophyton floccosum	Very scanty growth	scanty growth
Microsporium canis	No growth	No growth

Discussion

The results collectively underscore the promising anti-dermatophytic potential of honey, as evidenced by the distinct inhibitory effects observed against the isolates Dermatophytes (*Trichophyton species, Epidermophyton floccosum,* and *Microsporum canis*). Each isolate exhibited a similar pattern, emphasizing a concentration-dependent response that correlated with increased honey dilutions. This finding is similar to that of [24] who observed antifungal activity of honey against select Dermatophytes and Candida albicans.

Various zones of inhibition were observed for all concentrates of honey on all isolates. The absence of inhibitory effects in the control established a baseline for growth. The observed trends across all dermatophyte isolates highlight the concentration-dependent antimicrobial efficacy of honey [29]. The progressive increase in zones of inhibition signifies a positive correlation between honey concentration and anti-dermatophytic activity. This aligns with previous study by Imarenezor *et al*. [30], indicating concentration dependent antibacterial properties of honey on *Staphylococcus aureus, Escherichia coli,* and *Streptococcus pyogenes* isolates from wound samples. Lower zones of inhibition on isolates were observed at reduced concentrations of the antifungal agent. At 60% v/v concentration, 5 mm, 3 mm, and 9 mm were zones of inhibition observed for *Trichophyton species., Epidermophyton floccosum, and Microsporum canis,* respectively. This MIC results at a 60% honey dilution indicate that honey has an inhibitory effect on the growth of *Dermatophytes*, with very scanty growth observed for these fungi. This suggests that honey at a 60% v/v dilution is effective in suppressing the growth of these dermatophyte fungi, further highlighting its potential as a natural antifungal agent. The substantial 36 mm, 25 mm, and 40 mm zone of inhibition at 100% v/v dilution particularly highlights the potency of honey *Trichophyton species., Epidermophyton floccosum, and Microsporum canis* respectively, offering robust support for the hypothesis of its antifungal properties. This occurrence has been previously highlighted by [31] by observing that honey and aqueous propolis extract demonstrate antifungal properties against *Trichophyton rubrum, T. mentagrophytes, Microsporum gypseum, Epidermophyton floccosum, and Candida albicans*. These collective findings contribute to our understanding of honey's antimicrobial properties and its potential applications in the management of fungal infections

The investigation into the antifungal effects of honey on dermatophytic isolates reveals notable differences in susceptibility among *Microsporum canis, Trichophyton species*, and *Epidermophyton floccosum* [32]. The robust and concentration-dependent antifungal impact observed against *Microsporum canis* suggests heightened sensitivity to honey's antimicrobial components, highlighted by a substantial 40mm inhibition zone at 100% v/v dilution. In contrast, *Trichophyton species*

and *Epidermophyton floccosum* exhibited a less pronounced response (36 mm and 25 mm, respectively), indicating potential variations in resistance or reduced susceptibility to honey. Factors influencing this variability may include inherent differences in cell wall composition, metabolic pathways, or specific resistance mechanisms among the isolates. This finding is not unconnected to that of [33] which highlighted reduced antimicrobial activity of *Lawsonia inermis, Securidaca longipedunculata* and *Enantia chlorantha* on *Dermatophytes* owing to their cell wall composition, metabolic pathways.

The clinical implications are significant, especially concerning *Microsporum canis*-associated dermatophytosis, where honey could serve as a targeted intervention [34]. *Trichophyton species* are characterized by complex cell wall structures, potentially acting as barriers to antimicrobial agents [35]. Enzymatic defenses, such as keratinases, contribute to their resistance. Similarly, *Epidermophyton floccosum* displays robust cell walls and produces specific enzymes, factors that likely contribute to its resistance mechanisms [36]. This explains the reduced sensitivity of the organisms to the antifungal agent (honey). On the other hand, *Microsporum canis*, while sharing some characteristics with *Trichophyton species, and Epidermophyton floccosum*, may differ in cell wall composition and metabolic adaptations, contributing to its unique resistance profile. Genetic variations, virulence factors, and specific antimicrobial resistance mechanisms further add to the distinctiveness of each dermatophyte species. *Trichophyton species* are additionally characterized by adhesins and allergens, while *Microsporum canis* exhibits urease production and distinctive conidia formation [37]. Understanding these variations is crucial for developing targeted antifungal strategies, as the intricacies of resistance factors shape the fungi's responses to external challenges, including natural substances like honey.

The antimicrobial efficacy of honey against dermatophyte isolates stems from a combination of factors [38]. The low pH of honey being between 3.2 and 4.5 creates an environment unfavorable for the growth and survival of microorganisms, including dermatophytes. In addition, honey's osmotic effects play a crucial role in enhancing its antimicrobial potentials [39]. The high sugar content of honey draws water out of microbial cells through osmosis, leading to dehydration and apoptosis [40]. Furthermore, despite having a relatively low osmotic pressure, the water activity of ripened honey which ranges from 0.562 aw to 0.62 aw is still sufficiently low to create an enabling environment for microbial growth including fungi [41]. This low osmotic pressure exerted by honey contributes to dehydration of microbial cells, hindering their metabolic processes and inhibiting growth. Furthermore, the production of hydrogen peroxide by honey is another key antimicrobial mechanism. This compound has potent oxidizing properties that can damage essential cellular components, contributing to the inhibition of dermatophyte growth [42]. Moreover, honey contains a diverse array of bioactive compounds, including *polyphenols, flavonoids*, and other phytochemicals. These compounds possess antimicrobial properties and can interact with the cellular structures of dermatophytes, disrupting their normal function. The collective action of these mechanisms makes honey a multifaceted antimicrobial agent. Its ability to create an inhospitable environment, induce osmotic stress, generate hydrogen peroxide, and deliver bioactive compounds contributes to the observed

inhibitory effects on dermatophyte isolates. This comprehensive understanding of honey's antimicrobial properties enhances its potential as a natural and effective therapeutic agent against fungal infections.

The discovered antifungal efficacy of honey against *Dermatophyte* infections presents promising prospects for potential therapeutic applications [43]. The observed effects suggest that honey could be explored as a natural and effective remedy for combating Dermatophytes [44]. However, to establish its clinical utility, further investigations are essential. Specifically, research endeavors should focus on identifying the specific bioactive components within honey responsible for the observed antifungal effects. Understanding these components would not only deepen our comprehension of honey's mechanism of action, but also facilitate the development of targeted interventions.

To validate and translate these findings into practical applications, rigorous clinical trials are warranted. These trials should assess the effectiveness and safety of honey in real-world settings, involving individuals with dermatophyte infections. Clinical data would provide crucial insights into the feasibility, optimal dosage, and potential side effects of honey-based interventions. In addition, comparative studies with existing antifungal treatments would help position honey within the spectrum of available therapeutic options. Ultimately, future directions should delve into the molecular and cellular interactions underlying honey's antifungal activity, expanding studies to include a broader spectrum of dermatophytes, and exploring potential synergies with existing antifungal agents for innovative therapeutic strategies.

Conclusion

In conclusion, while the current findings highlight the potential therapeutic role of honey in dermatophyte infections, the journey towards practical application involves further research, particularly in elucidating its bioactive components, and conducting robust clinical trials to validate its efficacy and safety in clinical settings.

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Competing Interests

The authors have declared that no competing interests exist in this study.

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