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Antifungal activity of garlic (*Allium sativum*) extract and honey against some selected fungi isolated from isolated from patients with skin diseases

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Abstract
The majority of clinically used antifungal agents have various drawbacks in terms of toxicity, efficacy and cost. There are published reports available in the literature regarding antifungal activity of honey and garlic. Present study was undertaken to determine the *in vitro* activity of honey and garlic against various kinds of fungi isolated from patients with skin diseases, at the Department of Microbiology of T.N. Medical College and B.Y.L Nair Charitable Hospital, Mumbai. Total 510 patients were selected. Identification of Malassezia, Yeasts, Fungi and other filamentous fungi was carried out by standard methods. All Malassezia isolates were inhibited by Honey. MIC of Honey for Malassezia species ranged between 27 –30 %. All Dermatophyte isolates were inhibited by Honey. And MIC ranged from 1% v/v to 30 % v/v wherein maximum number of isolates was inhibited at 9 % v/v. All the Candida isolates were inhibited by honey and the MIC range obtained was from 3 to 27 %. Maximum numbers of isolates of Candida were inhibited by 21 % of honey. All Malassezia isolates were inhibited by Garlic extract. The MIC of garlic extract was in between 1: 25 to 1:50 dilutions. Maximum number of isolates was inhibited at 1: 25 dilution. Overall garlic extract showed 100 % in-vitro effect towards Malassezia species. On the whole, aqueous garlic extract exhibited MICs in the range of 1:25 – 1: 300 dilutions to all dermatophytes. Maximum numbers of dermatophytes were inhibited at 1:25 and 1:50 dilutions. Maximum number of Candida and yeast isolates were inhibited at MIC of 1:100 dilutions for garlic extract and the range obtained was from 1: 25 to 1: 300. The findings of this study showed that honey and extracts of garlic had a marked significance in inhibiting the test organisms. As the findings of this study compared favorably with previous studies on antifungal activity of honey and garlic, they might be a promising source of drugs for treatment of fungal infections. Further work on this study may help to design a new drug against various fungal infections.

Keywords: Honey, garlic, antifungal activity, Malassezia, candida

1. Introduction
The majority of clinically used antifungal agents have various drawbacks in terms of toxicity, efficacy and cost. These antifungal drugs are toxic and may give rise to adverse side effects, which may harm the patient rather than treating them [1]. Also their frequent use has led to the emergence of resistant fungal strains. Hence there is a great need for novel antifungal agent selectively acting on new targets with fewer side effects [2]. Since the fungal pathogens are eukaryotes, the antifungal treatment given by the conventional drugs may affect the infected patients and these pathogens are known to develop resistance to antifungal drugs. Hence, cheap, affordable, eco-friendly plant extracts and natural compounds may possibly be used for the treatment as an alternative [3]. Some plant extracts and natural compounds have shown to have antifungal properties. These medicinal plants and natural compounds are good reservoirs of Chemotherapeutants with considerable potential.
There are published reports available in the literature regarding activity of honey and garlic in wound healing process and antibacterial as well as antifungal activity on broad spectrum of bacteria and fungi. An antifungal action of honey has been observed against some yeasts and species of *Aspergillus* and *Penicillium*, as well as common dermatophytes. An inhibitory action of honey is mainly due to osmotic effect of its high sugar content and an enzymatic action that produces hydrogen peroxide [4]. Recently, the potential antifungal effect of honey has attracted serious attention within the scientific community. Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose

oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [5]. The *in vitro* antifungal activity of honey was reported by Maria *et al.*, who observed that honey stops the growth of *C. albicans*, *Candida krusei* and *Cryptococcus neoformans* [6].

An aqueous extract of Garlic (*Allium sativum*) has shown to have antifungal effect against the fungal skin pathogens [3]. Historically; Garlic has been used for centuries to combat infectious diseases. It can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Therapeutic effect of garlic is possible because of its oil and water soluble Organo-sulfur compounds, which are responsible for the typical odor and flavor of garlic [7]. Occurrence of dermatophytic infection is a public health problem especially in children. This is because of the development of antifungal drug resistance of the pathogens and side effects exhibited by the drugs used for fungal diseases. Hence there is a great demand for safer alternative and effective chemotherapeutic agents. Use of medicinal herbs in the treatment of skin diseases including mycotic infection is an age old practice in many parts of the world [8]. A review of literature indicates that garlic was used as a folk medicine all over the world from ancient times [9]. A number of reports are available on antifungal, antibacterial and antiviral activities of garlic on different microorganisms. Gulsen G. and Erol A. in 2010 [10] published a mini review of the antimicrobial properties of garlic.

There may be some more medicinal plants and natural compounds, which may have similar antibacterial and antifungal properties, which needs to be studied and explored. Once the antifungal property of a given extract from plant and natural compounds is proved under *in vitro* conditions, in vivo trials can be carried out for the treatment of human skin diseases by external application or oral administration of the product [3]. Hence, the present study was undertaken to determine the *in vitro* activity of honey and garlic against various kinds of fungi isolated from patients with skin diseases, before carrying out in its in vivo studies.

2. Material and Methods

This study was carried out at the Department of Microbiology, after taking the permission from Institutional Ethics committee of T.N. Medical College and B.Y.L Nair Charitable Hospital, Mumbai.

2.1 Determination of Minimum Inhibitory Concentration (MIC) of honey against yeast and Moulds

Indian Jambhul honey was obtained from Phondaghat Pharmacy, Mumbai and checked for its sterility using 'Test of sterility' by Direct Inoculation method as stated in Indian Pharmacopoeia [11]. The unsterile honey was further sterilized by Gamma irradiation at 25 kGy at the ISOMED unit of BARC-Mumbai [12] before *in vitro* testing on isolates obtained.

Honey was diluted in distilled water in the gradient of 1-30% and used for determination of MIC.

2.2 Determination of MIC of garlic against yeast and Moulds

Fresh bulbs of garlic (*Allium sativa*), which were identified by botanist, were bought from a local market in Mumbai. Aqueous Garlic extract was prepared by the method described by D. B. Lauria *et al.* [13].

One hundred gram of superior quality garlic bulbs were dehusked, cleaned with sterile distilled water, were homogenized in 100 ml of distilled water in a warring blender for 10 min. The suspension was then centrifuged for 20 min at 2000 g, filtered through a whatman no. 1 filter paper. Extract was checked for sterility before its *in vitro* testing for fungal isolates. The filtrate was further diluted in the range 1:25, 1: 50 up to 1: 300 dilution.

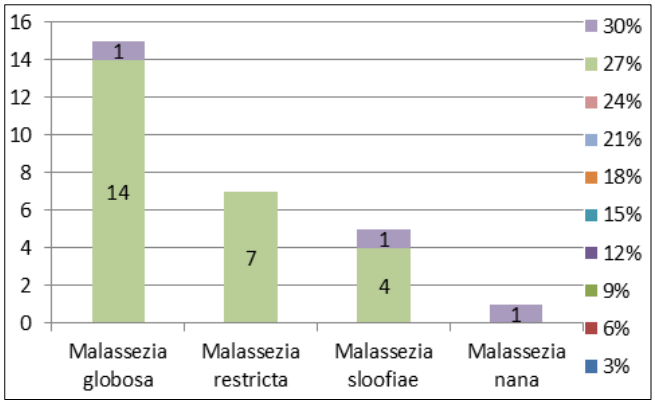
In vitro anti-fungal effects of honey and garlic on all fungal isolates was determined using Agar dilution method. Each dilution was added to the molten agar at 45 ^{o} C in a fixed ratio so that the appropriate drug concentration was achieved. The prepared agar plates for Yeasts and tubes for moulds were inoculated with 10ul of standardized inoculum. The drug medium and the growth medium were used as the controls [14, 15].

2.3 Tests Organisms

Total 510 patients attending Skin and Venereal Diseases of out Patient Department of B.Y.L Nair Charitable Hospital, Mumbai, clinically diagnosed as having skin infections, were selected and patient's infections were classified as superficial, cutaneous and subcutaneous. After thorough cleaning of diseased area with 70% alcohol, skin scrapings, nails, hair & skin and tissue biopsy samples were collected in sterile test tubes using sterile blade no.15.

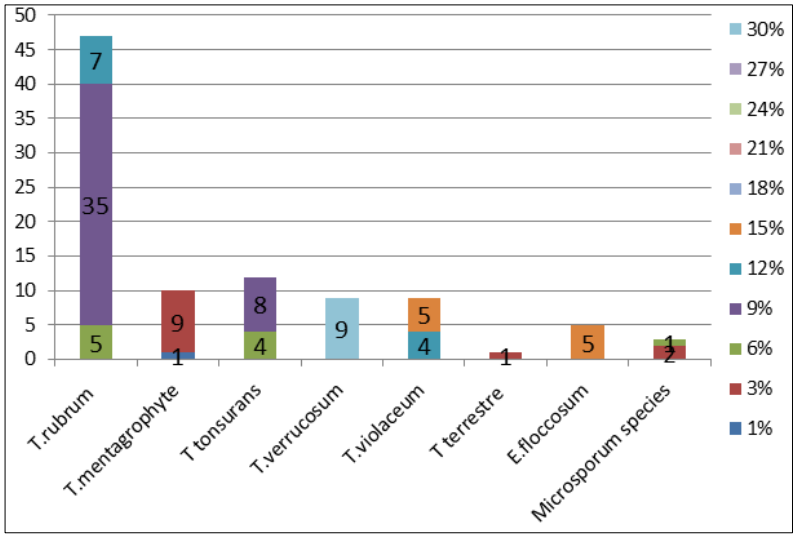
Direct microscopic examination of specimens [16, 17] was carried out by 20 % KOH mount so that the fungal elements like blastospores, septate or non-septate hyphae, arthrospores, "Spaghetti and meatballs" in case of *Malassezia* infection, ectothrix and endothrix infection of hair can be evaluated easily. By culture method, the growth was obtained and was identified by standard methods [18]. Further, identification of Malassezia [18] and Yeasts, Fungi & other filamentous fungi [19] was carried out by standard methods and slide culture was performed wherever necessary.

3. Results



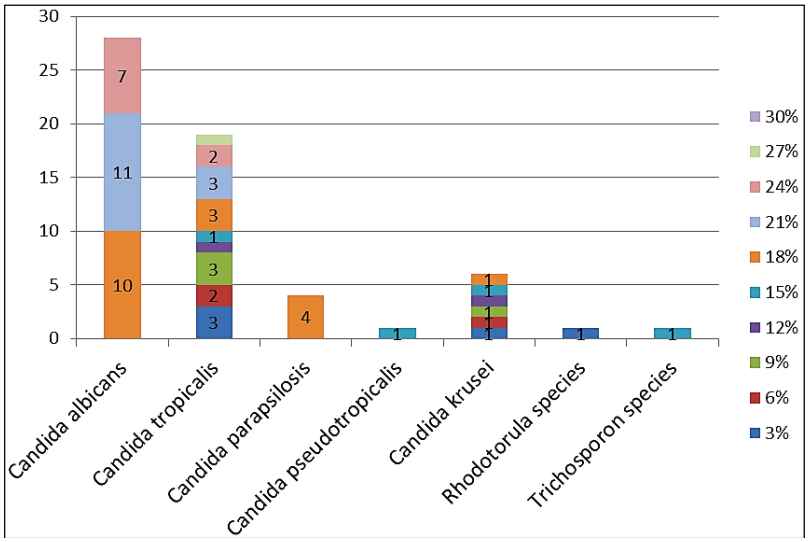
Graph 1: MIC of Honey in V/V % for different *Malassezia* species *n= 28*

All *Malassezia* isolates were inhibited by Honey. MIC of Honey for *Malassezia* species ranged between 27-30 %. Overall Indian Jambhul Honey showed 100 % *in-vitro* effect towards *Malassezia* species.



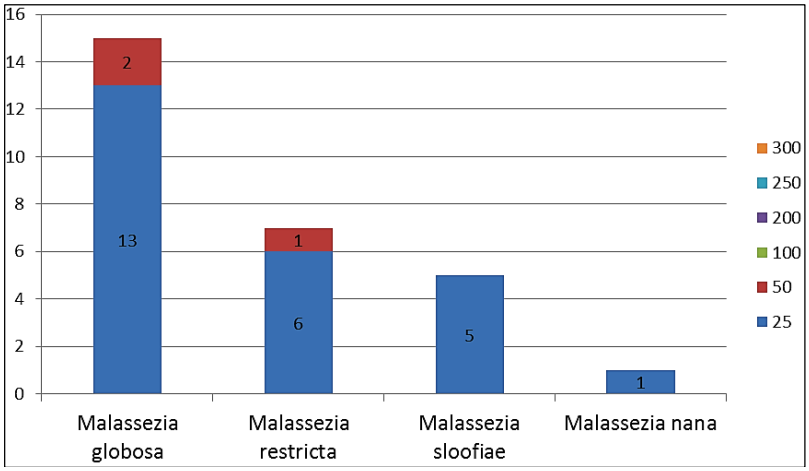
Graph 2: MIC of Honey in V/V % for different *Dermatophyte species. (n= 98)*

All *Dermatophyte isolates were* inhibited by Honey. MICs of Jambhul honey ranged from 1% v/v to 30 % v/v wherein maximum number of isolates was inhibited at 9 % v/v



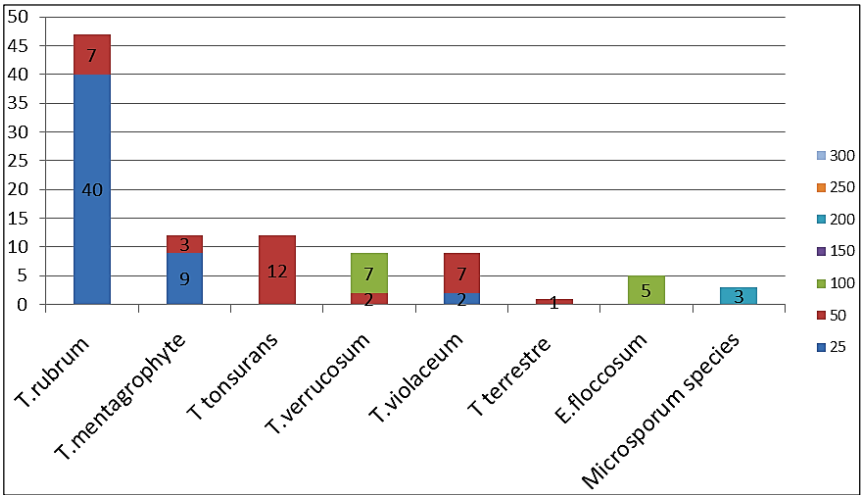
Graph 3: MIC of Honey in V/V % for different C*andida and* yeast *species (n=60)*

All the *Candida* isolates were inhibited by honey and the MIC range obtained was from 3 to 27 %. Maximum numbers of isolates of *Candida* were inhibited by 21 % of honey.



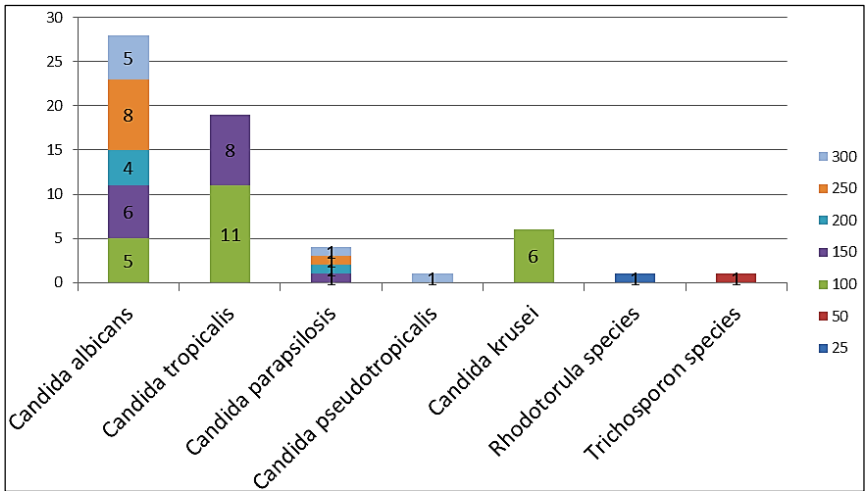
Graph 4: MIC of Garlic Extract in mg/ml for different *Malassezia species (n=28)*

All *Malassezia* isolates were inhibited by Garlic extract. The MIC of garlic extract was in between 1: 25 to 1:50 dilutions. Maximum number of isolates was inhibited at 1: 25 dilution. Overall garlic extract showed 100 % in-vitro effect towards *Malassezia* species.



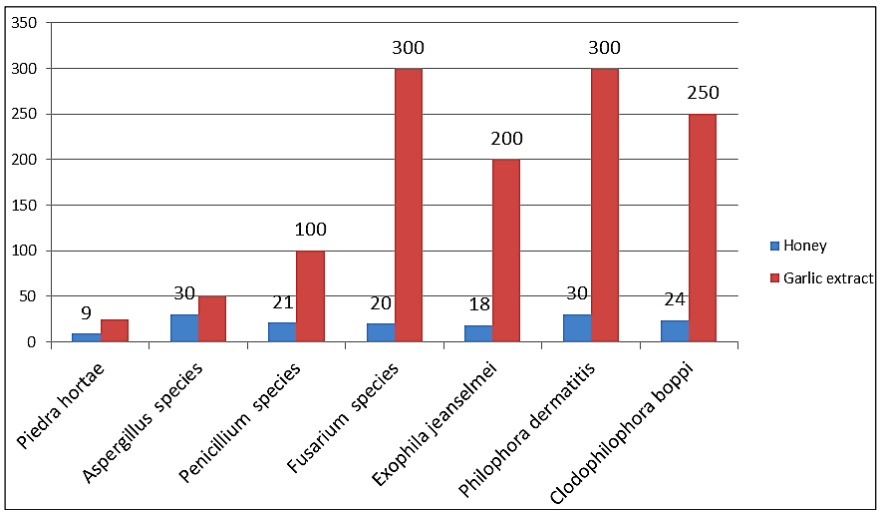
Graph 5: MIC of Garlic Extract in mg/ml for different *Dermatophyte species (n= 98)*

On the whole, aqueous garlic extract exhibited MICSs in the range of 1:25 – 1: 300 dilutions to all dermatophytes. Maximum numbers of dermatophytes were inhibited at 1:25 and 1:50 dilutions.



Graph 6: MIC of Garlic Extract in mg / ml for different *Candida and yeast species. (n=60)*

Maximum number of *Candida* and yeast isolates were inhibited at MIC of 1:100 dilutions for garlic extract and the range obtained was from 1: 25 to 1: 300.



Graph 7: MIC Endpoints of Honey and Garlic against NDMs

MIC values of honey against NDMs ranged from 1%--30%. While MIC values of garlic extract ranged from 1: 25 to 1: 300

4. Discussion

Anti-fungal activity of Jambhul Honey and aqueous Garlic extract was studied and their MIC was determined by Agar dilution method [14, 15] on the fungal isolates obtained.

Indian Jambhul Honey and Garlic extract showed 100 % *in-vitro* antifungal activity against Yeasts, *Malassezia*, dermatophytes and other filamentous fungi. Present study findings indicated effective antifungal action of honey and garlic on isolates from skin infections. There is a need to carry out further extensive *in-vitro* and in-*vivo* studies on experimental animals and then on patients to prove its role as a supportive therapy for fungal skin infections. This can be useful to reduce many different side effects produced in patients due to administration of conventional antifungal agents.

Honey has been reported to have antifungal activity, but not many species of fungi have been tested. In the present study, maximum numbers of dermatophytes were inhibited at 9-15 % (v/v) dilutions. Similar results were observed by Rademaker M. *et al.* [20]. Although the concentrations of honey needed to inhibit some of the dermatophytes were higher than needed to inhibit bacteria, less dilution of honey was likely with Tinea infection than with infected wounds, burns and ulcers where there would be serum exudation. Arrerat L. *et al.* [21] had reported the antifungal effect of honey on dermatophytes by agar diffusion method and had also determined MIC values by broth dilution method. Present study also demonstrated the antifungal effect of honey on various filamentous fungi isolated from skin infection. This showed honey to be a most potent agent to work on fungal isolates causing Tinea. However which type of honey is most effective and the practical usefulness of honey as a topical antifungal agent, would be known only if comparative clinical trials were conducted.

The results of investigation by Brady N.F. *et al.* [22] showed that the common dermatophytes were sensitive to the antimicrobial activity of honey, indicating that clinical evaluation of honey in the treatment of tineas was warranted. This would determine whether the hydrogen peroxide or the non-peroxide antifungal agent diffuses better into the skin.

In the present study, all Candida species were inhibited by Indian jambhul honey at 18-21 % v/v. In Koc AN *et al.* study [23], honey samples from different floral sources were evaluated for their ability to inhibit the growth of 40 yeast strains (*Candida albicans, C. krusei, C. glabrata and Trichosoporon* spp.). Broth micro dilution method was used to assess the activity of the honeys against yeasts at different concentrations ranging from 1.25-80% (v/v). All of the yeast strains tested was inhibited by honeys in this study. Broth micro dilution assay revealed that inhibition of growth depended on the type and concentration of honey as well as the test pathogen. Little or no antifungal activity was seen at honey concentrations <2%. Rhododendron and multifloral honeys had generally more inhibitory effect than eucalyptus and orange honeys ($P<0.05$). Fluconazole-resistant yeast strains were examined for their susceptibility to honeys. This study demonstrated that, in vitro, these honeys had antifungal activity at the high concentration of 80% (v/v) in these fluconazole-resistant strains. Further studies were now required to demonstrate if this antifungal activity had any clinical application.

Honey, an age-old remedy has been rediscovered in modern times. Its therapeutic potential has been credited to its anti-microbial, anti-inflammatory and anti-oxidant properties as well as boosting of the immune system. Moreover, the effectiveness of honey against antibiotic sensitive and resistant micro-organisms, the ease of administration for the treatment of wounds, lack of side effects in alleviating gastric pain and shortening the duration of diarrhea and its low likelihood of selecting for further resistant strains culminate to the fact that this agent may represent a satisfactory alternative and / or complementary means of chemoprophylaxis and or chemotherapy. Hence in the present era of multiple drug resistant organisms, honey can be as an effective, cheap, easily available, non-toxic, extremely useful, and reliable adjuvant without any adverse side effects in the treatment of fungal infections [4].

Aqueous Extract of garlic was said to have *in vitro* antifungal effect against yeast like fungi like *Candida, Cryptococcus, Rhodotorula, Torulopsis* and *Trichosporon*. Medicinal, insecticidal, antibacterial, antiprotozoal and antifungal properties had been ascribed to garlic [24]. In the present study; the effect of aqueous garlic extract on yeasts and filamentous fungi / dermatophytes was studied by agar dilution method. The ubiquitous opportunistic pathogen *C. albicans* was sensitive to garlic; resistance to the broad spectrum of active principles present was unlikely so that its anti-Candida effects may provide an important alternative route to chemotherapy. Garlic had been reported to affect the lipid constituents of the outer surface of *C. albicans* [25] and the fungicidal membrane of *Paracoccidiodes* spp. [26]. Venugopal PV *et al.* [27] from Madras had reported the anti-dermatophytic activity of aqueous garlic extract on clinical isolates of dermatophytes and compared them with the ketoconazole which correlated with the present study. Interest in herbal medicine is enjoying a renaissance at present. Garlic (Allium sativum) was an intriguing herb with a long history of medicinal use for a variety of diseases including ringworm infections. Garlic (A. sativum) could be used as an effective antidermatophytic agent. Further purification and extraction of the active principle of garlic would give a true antidermatophytic activity comparable to standard antifungal drugs.

Dermatophytic infection is a common infection that constitutes public health problem among children. Anti dermatophytic activity of aqueous, ethanolic and methanolic extracts of garlic was investigated against isolates of dermatophytic fungi obtained from sixty primary school children in Aba. The well in agar diffusion technique was used to determine the sensitivity patterns of the test organisms. The results were compared with the activity of a known antifungal drug nystatin. The result of the antifungal activity of garlic showed high but varied levels of antifungal effectiveness on the different species of the dermatophytes at four different concentrations of 12.5 %, 25 %, 50 % and 100 % used. Further purification and extraction of active principle of garlic would give an antidermatophytic activity comparable to standard antifungal drugs [28]. True activity of garlic can be compared if principal component of garlic is purified and extracted further. The potent *in vitro* activities of onion and garlic extracts against *Malassezia, Candida* and *dermatophytes* were reported by Ghahfarokhi M.S. *et al.* [29] from Iran to suggest a potential therapeutic efficacy of

these plant extracts in the treatment of related fungal disorders. Similarly, inhibition of keratinolytic activity on *T. mentagrophytes* by garlic was reported in another paper by Ghahfarokhi MS *et al*. This is the first report on keratinase inhibition by natural compound. Since fungal growth and keratinolytic activity are important factors in pathogenesis of the dermatophytes, their inhibition by garlic indicate that it may have potential value in treatment of human and animal dermatophytosis. Although, dermatophytes respond well to conventional antifungal agents, many patients usually cannot afford the cost of these agents and hence use local medicinal plants to treat the infections. Good *in-vitro* activities of some plant extracts against dermatophytes were demonstrated in western African countries such as Nigeria. Amer M *et al*. [30] has reported effective treatment of topical application of garlic extract on lesions produced in experimental animals.

The present *in vitro* study demonstrates the antifungal effect of garlic extract and stated that it could be used against the human skin pathogens. Once the in-vitro antifungal effect is proved, in-vivo trials can be carried out for the treatment of human skin diseases by topical application. The antimicrobial properties of garlic can be exploited as an effective alternative to those of more common pharmaceutical preparations. A broad range of effects, in particular the observed antifungal properties, is now being investigated as well as research on the breakdown products of organosulphur compounds for their specific inhibitory effects. The mechanisms of action of garlic constituents *in vivo* still require much research.

5. Conclusion

Thus it can be concluded that, medicinal plants and plant products like honey and garlic extract, are not only important to the millions of people for whom traditional medicine is the opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals. Therefore natural products, either as pure compounds or as standardized plant extracts, provide limitless opportunities for new drug leads because of the matched less availability of chemical diversity.

6. Recommendations

The findings of this study showed that honey and extracts of garlic had a marked significance in inhibiting the test organisms. As the findings of this study compared favorably with previous studies on antifungal activity of honey and garlic, they might be a promising source of drugs for treatment of fungal infections. Further work on this study may help to design a new drug against various fungal infections.

7. Acknowledgement

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