Journal of Pharmaceutical Research International

33(45A): 437-447, 2021; Article no.JPRI.73880
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Anti -Fungal Activity of Commercially Available Extracts of Garlic (*Allium sativum*), *Turmeric (Curcuma longa*), Amla (*Emblica officinalis*): An *in vitro* Study

Mohammad Abu Bakr Siddique[1], Ladke Vaibhav Sunil[2*], Shirol Pallavi D.[3], Kumbhar Gauri M.[2], Koshy Ajit V.[3], Shah Amisha[3] and Farhana Ashraf[3]

[1]Department of Oral Pathology & Microbiology, People's College of Dental Sciences and Research Centre, Bhopal, M.P., India.
[2]Interdisciplinary Research, Central Research Facility, Dr. D. Y. PATIL Medical College, Hospital and Research Centre, Pune, India.
[3]Department of Oral Pathology & Microbiology, M. A. Rangoonwala College of Dental Sciences & Research Center, Pune, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i45A32764
Editor(s):
(1) Dr. Asmaa Fathi Moustafa Hamouda, Jazan University, Saudi Arabia.
Reviewers:
(1) Miguel Angel Garcés Villala, Fundación Corazón de Jesús, Argentina.
(2) Anselmo Enrique Ferrer Hernandez, University of Havana, Cuba University, Cuba.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73880

Original Research Article

Received 06 July 2021
Accepted 16 September 2021
Published 02 October 2021

ABSTRACT

Background and Purpose: Many innovations are constantly emerging with the advances in modern medicine and also it is occurring in various traditional medicines as they are considered to be safe and economical in current increasing health care financial burden. Due to emerging of MDR (multiple drug resistance) microorganisms and reduce efficacy of Modern medicines researchers and clinicians are required to revisit the traditional and alternative medicines.
Material and Method: Extracts of garlic (*Allium sativum*), turmeric (*Curcuma longa*), amla (*Emblica officinalis*) and honey were prepared at concentration of 1mg/mL, 2mg/mL, 3mg/mL and were tested on different cancdidal species like Candida Albicans, Candida tropicalis, Candida *glabrata,*

**Corresponding author: E-mail: vaibhav.ladke@dpu.edu.in;*

Candida Parapsilosis and Candida Krusei and on Fluconazole as a positive control. Disc Diffusion Test for antifungal assay was performed.

Results: The anti-fungal effect was maximum at 3mg/mL concentration for all the herbal extracts. Zone of inhibition for fluconazole, was highest (35.00 ± 1.73). Among herbal extracts zone of inhibition for garlic was maximum (16.67 ± 0.88), followed by turmeric (12.67 ± 1.20), honey (11.00 ± 0.58), amla (9.33 ± 0.67). Similarly for each dose, comparing the mean zone of inhibition between antifungal groups Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in herbal extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

Conclusion: Among all the extracts garlic showed maximum efficiency. Also, the results obtained for herbal extracts showed that they were less efficient compared to fluconazole which was used as positive control.

Keywords: Candidiasis; medicinal plants; herbal extracts; anti-fungal; in-vitro.

1. INTRODUCTION

Microbial infections are very common and varied in nature which leads to many human pathologies.

Candida is one of the most common human mycotic pathogens [1] and represents the most important cause of opportunistic mycoses globally [2]. It is a common colonizer of muco-cutaneous areas. *Candida* is a useful and normal inhabitant of normal flora of skin, mouth, vagina, and bowel, which is also found on leaves, flowers, water and soil. The genus *Candida [C.]* comprises about 154 species. Out of which six are frequently seen in human infections. *C. albicans* being the most common and important species, other species like *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* are also seen as causative agents of *Candida* infections. However, recently there has been an increase of infections due to non-*albicans Candida* species., such as *C. glabrata* and *C. Krusei* [3,4]. Patients who are being treated with fluconazole as a prophylaxis seems to develop infections owing to fluconazole-resistant *C. krusei* and *C. glabrata* strains [5].

Various oral areas including the buccal mucosa, tongue, palate, subgingival microbiota , biofilm, carious lesions and prosthetic appliances shoe frequent sites for fungal isolation [6]. Various Studies [7, 6] have proposed a possible relation between Candidal species and periodontal disease, dentin /or root caries and mucosal diseases. According to these studies C. albicans has a comparable capacity of colonizing hydroxyapatite crystals like Streptococcus mutans by using diverse mechanisms.

Oral candidiasis is considered as “the disease of the diseased”. This can lead to a life-threatening infections in an immunocompromised patients. Numerous antifungal agents are available for oral candidal infections [8] whose efficacies vary depending on the candida species. As the isolates of *C. albicans* could reveal intrinsic (primary) resistance or secondary resistance to the drugs leading to failure during treatment [9]. The development of antifungal resistance is multifaceted and is subject to multiple host and microbial factors [10] Owing to the development of resistance in current fungal pathogens and the emergence of fungal pathogens that are basically resistant to the currently existing antifungal agents, it is imperative to explore the novel antifungal agents. The emergence and spread of resistant Candida species have been reported regularly. Hence better of how resistance is evolving within naturally susceptible species is key factor in developing novel, more active treatment approaches. To manage the wide spread problem of anti-microbial resistance, their alternatives such as medicinal plants or herbal extracts need to be explored in depth with evidence based research [11-13].

In developed and developing countries use of Medicinal plants has a long history in treating different human diseases [14,15]. Recently, various medicinal plants have got much attention owing to their uses and many benefits, like fewer side effects and less expensiveness [16,17]. Various plant-based products have been considered as an effective approach against fungal, bacterial, and parasitic infections [18,19].

Oral cavity being a complex part of human body consists of mixture of bacteria and other microorganisms that leading to mixed infections of the oral cavity, that requires medicines with broader spectrum of activity. The oral microbiota exhibit interbacterial coaggregations along with the yeasts such as *C. albicans* which helps in establishing and maintaining the same.

Various herbal extracts like ginger, babul, garlic, turmeric, amla, coriander, cumin, fennel bakul, neem , tulsi and honey have been studied and exhibited to have antibacterial, anti-viral and fungicidal effects. The use of many natural products as an antifungal agent are considered as fascinating alternatives to synthetic fungicides owing to their less adverse effect, well acceptable, easily available, and cost effective. Therefore these traditional treasures should be encouraged for the research and use [20]. There are numerous studies reported about anti-bacterial effects of herbal extracts, but very few are reported against commonly occurring opportunistic fungal infections.

Hence the present study was therefore conducted to evaluate the antifungal potential of garlic (*Allium sativum*), turmeric (*Curcuma longa*), amla (*Emblica officinalis*) and honey on common oral Candidal species like *C. albicans, C. tropicalis*, C. *glabrata*, *C. parapsilosis* and *C. krusei.*

2. MATERIALS AND METHODS

2.1 Chemical Substances

The crude powder of Fluconazole (FZ) as the control agent was purchased from Sigma-Aldrich, St Louis, MO, USA. Sabouraud dextrose agar (SDA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Also, potato dextrose agar (PDA) was obtained from Oxoid Ltd. (Basingstoke, Hampshire, UK). All other chemicals and solvents were of an analytical grade. Yeast Extract Peptone Dextrose (YEPD) agar plate., Muller Hinton (MH) agar plate, Dimethyl Sulfoxide(DMSO), Fluconazole disc in three concentration, Peptone Water, Methanol & Distilled water were used

2.2 Fungal Strains

The fungal strains investigated in the study were acquired from Institute of Microbial Technology, Chandigarh. C. albicans (MTCC 3958), *C. tropicalis* (9038), C. *glabrata (3814),* C. Parapsilosis (4448) and C. Krusei (9215) were obtained.

2.3 Preparation of Extract

The herbal extracts in powder form were dried in an oven at 40^0C for 4 hours and were used for extraction. Accurately weighed 50g of powdered samples were extracted with 500 ml methanol. The extracts were concentrated??? and kept for drying in incubator for 37^0C and then stored in room temperature at (20-40^0C). These extracts were dissolved in dimethyl sulfoxide (DMSO) to get three different concentrations (1mg/mL , 2mg/mL , 3mg/mL) to be tested.

2.4 Preparation of Culture Media

According to manufacturer's guidelines, sterile glass ampoule containing freeze dried form of the candidal species were opened under aseptic conditions and the contents were added to peptone water and were mixed. They were incubated at 37 ^{0}centigrade for 24 hours. The 30 ml of molten sterile agar was poured aseptically in four sterile petri plates and were allowed to solidify.

2.5 Procurement of Inoculum

Stock cultures were maintained at 4^0 C on the slant of nutrient agar. Active Cultures for experiments were prepared by transferring a loopful of cell organisms from the stock cultures to test tubes of nutrient broth for fungi .Streaking was done on YEPD agar plates and incubated for 24-48 hours at 37 ^{0}C in which the assay was performed by disc diffusion method.

2.6 Disc Diffusion Test for Antifungal Assay

Anti-fungal activity of the given sample was determined by disk diffusion method on Muller Hinton Agar (MHA) medium. Cultured colonies were picked from YEPD agar plates and were added in peptone water and was incubated for around 30 minutes. The MHA medium was poured in the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moisture with the fungal suspension. Four fungal suspensions were used in MHA plate and drained with excess suspension. The anti-fungal discs were placed on MHA plate with the help of sterile forceps and different concentration of each sample were loaded on discs. Fluconazole was used as positive control, and 50% DMSO and sterile distilled water were maintained simultaneously as negative controls in the same plate. Then all the plates were incubated in upright position at 37^0 C for 24-48 hours. The inhibition zones were measured on the underside of the plates, using Hi-media zone scale after 24 and 48 hours. The tests were repeated three times and the mean diameter of the zone of inhibition were determined [21] [Fig. 1. Zone of inhibition]

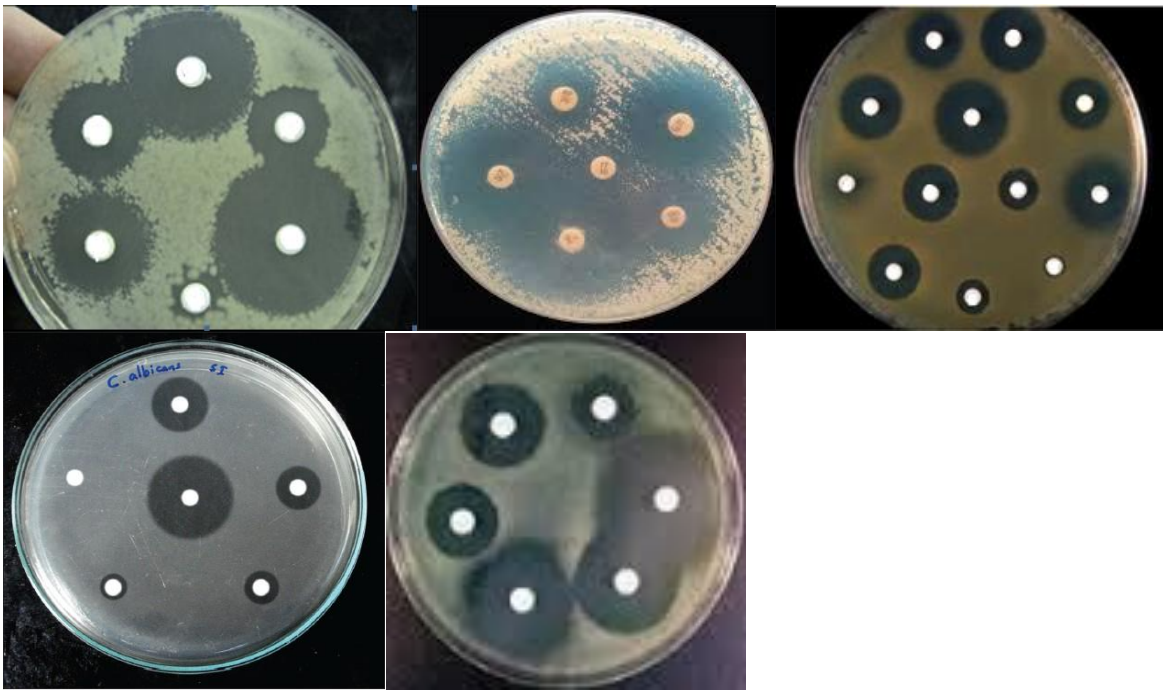

Fig. 1.
A. Zone of inhibition for Garlic.
B. Zone of inhibition for Turmeric
C. Zone of inhibition for Amla.
D. Zone of inhibition for Honey
E) Zone of inhibition for Fluconazole

3. OBSERVATIONS AND RESULTS

3.1 Statistical Analysis

Data were summarized as Mean ± SE (standard error of the mean). Groups were compared by two-way analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance by Levene's test. A two-tailed (α=2) p value less than 0.05 ($p<0.05$) was considered statistically significant. Analyses were performed on SPSS (Windows version 17.0).

For different Candidal species, the effects of herbal extracts versus fluconazole were as follows-

The antifungal activity (zone of inhibition) of different antifungal agents at different doses on different common Candidal species is summarized in Table 1, 2 and 3. The mean zone of inhibition of fluconazole was the highest. Among herbal extracts garlic showed higher mean zone of inhibition, followed by turmeric, honey and amla (Amla < Honey < Turmeric < garlic < fluconazole). Further, in all antifungal groups antifungal activity was highest in 3 mg/mL followed by 2 mg and 1 mg the least (1 mg < 2 mg < 3 mg).

Similarly, for each dose, comparing the mean zone of inhibition between antifungal groups, Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in herbal extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

Candida albicans

The antifungal activity (zone of inhibition) of different antifungal agents on *C. Albicans* is shown in Table S1. The mean zone of inhibition of fluconazole was the highest. Among herbal extracts garlic showed higher mean zone of inhibition, followed by turmeric, honey and amla (Amla < Honey < Turmeric < garlic < fluconazole). Further, in all antifungal groups antifungal activity was highest in 3 mg/mL followed by 2 mg and 1 mg the least (1 mg < 2 mg < 3 mg). (Fig. S2)

Table 1. Zone of inhibition (Mean ± SE, n=3) of different antifungal agents at 1 mg

Antifungal agents	*C. albicans*	*C. krusei*	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*
Garlic	9.00 ± 0.58	1.33 ± 0.88	1.33 ± 0.33	4.00 ± 1.73	6.00 ± 0.58
Turmeric	3.67 ± 1.45	0.67 ± 0.67	2.00 ± 1.00	2.67 ± 0.67	2.33 ± 0.88
Honey	1.67 ± 0.88	0.33 ± 0.33	0.67 ± 0.67	3.00 ± 0.58	1.00 ± 0.58
Amla	1.00 ± 0.58	0.00 ± 0.00	1.67 ± 0.33	0.33 ± 0.33	0.67 ± 0.67
Fluconazole	23.00 ± 2.08	13.67 ± 0.88	19.67 ± 1.45	17.00 ± 0.58	19.33 ± 1.86

Table 2. Zone of inhibition (Mean ± SE, n=3) of different antifungal agents at 2 mg

Antifungal agents	*C. albicans*	*C. krusei*	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*
Garlic	13.67 ± 0.88	7.67 ± 0.88	9.67 ± 0.88	10.67 ± 0.67	11.00 ± 1.73
Turmeric	10.00 ± 1.15	5.33 ± 0.88	8.67 ± 0.88	8.00 ± 1.15	8.00 ± 0.58
Honey	4.33 ± 0.33	4.67 ± 0.33	5.67 ± 0.88	6.00 ± 1.15	5.00 ± 0.58
Amla	4.67 ± 0.88	1.67 ± 0.88	4.67 ± 0.33	3.67 ± 0.33	2.67 ± 1.33
Fluconazole	30.00 ± 1.15	16.33 ± 0.88	21.67 ± 0.88	21.33 ± 0.88	27.33 ± 1.20

Table 3. Zone of inhibition (Mean ± SE, n=3) of different antifungal agents at 3 mg

Antifungal agents	*C. albicans*	*C. krusei*	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*
Garlic	16.67 ± 0.88	10.67 ± 1.20	14.67 ± 1.33	16.33 ± 1.20	15.67 ± 0.88
Turmeric	12.67 ± 1.20	10.33 ± 1.20	12.00 ± 0.58	12.67 ± 1.76	13.33 ± 1.20
Honey	11.00 ± 0.58	8.33 ± 0.88	10.00 ± 0.58	13.67 ± 0.88	12.00 ± 1.15
Amla	9.33 ± 0.67	7.00 ± 0.58	9.00 ± 0.58	9.33 ± 0.88	7.67 ± 0.88
Fluconazole	35.00 ± 1.73	18.67 ± 0.88	29.00 ± 1.53	27.00 ± 1.15	33.33 ± 1.20

Similarly, for each dose, comparing the mean zone of inhibition between antifungal groups (Table S2 and Fig. S3), Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in herbal extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

Candida tropicalis

The antifungal activity (zone of inhibition) of different antifungal groups on *C. Tropicalis* is summarized in Table S3. The mean zone of inhibition of fluconazole was the highest. Among herbal extracts garlic showed higher mean zone of inhibition, followed by, turmeric, honey and amla the least at all the doses (Amla < Honey < Turmeric < garlic < fluconazole).). Further, in all antifungal groups antifungal activity was highest in 3 mg/mL followed by 2 mg and 1 mg the least (1 mg < 2 mg < 3 mg). doses (Fig. S4).

For each dose, comparing the mean zone of inhibition between antifungal groups (Table S4 and Fig. S5), Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in commercially available extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

Candida glabrata

The antifungal activity (zone of inhibition) of different antifungal groups on *C. Glabrata* is summarized in Table S5. The mean zone of inhibition of fluconazole was the highest. Among herbal extracts garlic showed higher mean zone of inhibition, followed by, turmeric, honey and amla the least at all the doses (Amla < Honey < Turmeric < garlic < fluconazole). Further, in all antifungal groups antifungal activity was highest in 3 mg/mL followed by 2 mg and 1 mg the least (1 mg < 2 mg < 3 mg). (Fig. S6).

Similarly, for each dose, comparing the mean zone of inhibition between antifungal groups (Table S6 and Fig. S7), Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in all extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

Candida parapsilosis

The antifungal activity (zone of inhibition) of different antifungal groups on *C. Parapsilosis* is summarized in Table S7. The mean zone of inhibition of fluconazole was the highest. Among herbal extracts garlic showed higher mean zone of inhibition, followed by, turmeric, honey and

amla the least at all the doses (Amla < Honey < Turmeric < garlic < fluconazole). Further, in all antifungal groups antifungal activity was highest in 3 mg/mL followed by 2 mg and 1 mg the least (1 mg < 2 mg < 3 mg). (Fig. S8).

Further, for each dose, comparing the mean zone of inhibition between antifungal groups (Table S8 and Fig. S9), Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

Candida krusei

The antifungal activity (zone of inhibition) of different antifungal groups on *C. Krusei* is summarized in Table S9. The mean zone of inhibition of fluconazole was the highest. Among herbal extracts garlic showed higher mean zone of inhibition, followed by, turmeric, honey and amla the least at all the doses (Amla < Honey < Turmeric < garlic < fluconazole). Further, in all antifungal groups antifungal activity was highest in 3 mg/mL followed by 2 mg and 1 mg the least (1 mg < 2 mg < 3 mg). (Fig. S10),

For each dose, comparing the mean zone of inhibition between antifungal groups (Table S10 and Fig. S11), Tukey test showed significantly ($p<0.001$) different and lower zone of inhibition in extracts (garlic, turmeric, honey and amla) as compared to fluconazole at all the doses.

In the present study among herbal extracts garlic showed maximum zone of inhibition at 3 mg dosage against all types of Candidal species.

4. DISCUSSION

Very few currently available antifungal agents are being used to treat Fungal infections caused by Candida species [22-24].

Fluconazole and itraconazole have been used extensively as prophylactic and treatment for systemic fungal infections due to their satisfactory oral bioavailability and safety profiles [25-27]. Fluconazole resistance has been increasing among patients [28]. Oropharyngeal candidiasis in patients with HIV has shown azole-resistant [29]. Therefore, many medicinal plants are being researched.

In medicinal plants experiments regularly dried and powder sample is preferred [30] because they are fragile and have tendency to deteriorate faster than dried samples, Powdered form of samples are more homogenized and have smaller particle size which gives better surface contact with extraction solvents [31]. In the present study powdered form was used.

The comparison of anti-microbial activity of aqueous infusions and aqueous decoctions of *Emblica officinalis* (amla) and *Coriandrum sativum* (coriander) against gram positive bacteria and *Candida albicans* showed that amla exhibited potent anti-microbial activity against candida albicans. It was also found that coriander did not show any anti-microbial activity against *Candida albicans* [32].

Some studies [33, 34] showed anti-fungal property against candida albicans with punica *granatum, Acacia nilotica, Cuminum cyminum* and *Foeniculum vulgare* and it was found that punica granatum showed higher zone of inhibition against candida albicans. This was in accordance with the present study.

S. Doddanna et al. [35] found that alcoholic curry leaves showed maximum zone of inhibition on *Candida albicans* followed by aqueous tea leaves, alcoholic onion leaves, alcoholic tea leaves, alcoholic onion bulb, alcoholic aloe vera, and alcoholic mint leaves. In the present study we observed that among all the herbal extracts studied i.e. garlic, turmeric, amla, honey against candidal species such as the *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis and C. krusei,* garlic showed maximum efficiency against all the species when compared with other extracts.

Of In-Vitro Susceptibility of Clinical Fungal Isolates from Immunocompromised Patients demonstrated anti-fungal activity of Amphotericin B, ketoconazole, itraconazole, clotrimazole, fluconazole and also of natural herbs like neem, tulsi, garlic ginger, aloevera, turmeric. Among all the anti-fungal agents ketoconazole showed the maximum zone of inhibition [36].

The antifungal activity of the methanol extracts of 10 plants were evaluated where they concluded that Acacia rigidula (0.93-3.75µg/mL) and Quercus canbyi (0.93-7.5µg/mL) had antifungal activity against 7 strains with MIC values <8µg/mL in all cases [37].

It was reported in previous studies that the antifungal effects of herbal extracts against

candidal species were found to be less efficient when compared with fluconazole or any other anti-fungal drugs, [38-42]. This was in concordance with the results obtained in the present investigation wherein we found that the effects of garlic, turmeric, amla, honey on all the candidal species studied were less effective compared to efficacy of fluconazole.

Fluconazole (FLC), a kind of azoles, is the most frequently used antifungal drug for prevention and treatment of C. albicans infections due to the high efficacy and low toxicity. However, drug resistance to antifungals, especially to FLC among C. albicans species, increased sharply along with long-term use of it [43]. Fluconazole is generally well tolerated and is infrequently associated with adverse side effects. The most commonly observed clinical adverse events are nausea in 3.7%, headache 1.9%, skin rash 1.8%, vomiting 1.7%, abdominal pain 1.7%, diarrhoea 1.5%, and transient enzymes in <5% [44]. Serious hepatic toxicity is rare [45].

However, the repetitive and long-term use of fluconazole has favoured the appearance of resistant isolates [46]. Hence proving its usefulness only in short duration infections. In such cases herbal extracts prove to be better alternative with lesser side effects and there is no reported development of drug resistant strains.

Advantages of herbal medicines are effectiveness with chronic conditions (as many fungal infections), lower cost, wide spread availability which makes it more appropriate to use. Though herbal extracts are considered as safe, they may pose some side effects and interactions with other drugs with which one should be cautious before prescribing these.

Further studies are essential to prove beneficial effects of garlic and other extracts in vivo against candidal species before its consideration as therapeutic antifungal agent. Studies on larger population should be promoted.

5. CONCLUSION

The frequency of life-threatening infections caused by pathogenic microorganisms is becoming an alarming factor of morbidity and mortality in immuno-compromised patients in developing countries. Many pathogenic microorganisms are constantly developing resistance to these agents. Since many of the existing antifungal drugs have undesirable side effects or are very toxic, show drug-drug interactions or develop resistance affecting treatment planning. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these shortcomings. The herbal extracts are easily available and cheaper having added advantage of widespread availability, minimal side effects, cost effective and efficiency in long term usage. Considering the side effects and disadvantages of fluconazole, these herbal extracts mainly can be considered as a better alternative anti-fungal agent.

In the present study herbal extracts like garlic, turmeric, amla and honey exhibited efficacy against most common pathogens involved in candida infection such as the *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis* and *Ca. krusei.* The findings of this study may need further validation with larger sample size.

SUPPLEMENTARY MATERIALS

Available in this link https://journaljpri.com/index.php/JPRI/libraryFiles/downloadPublic/18

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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