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**ORIGINAL ARTICLE**

# Comparison between the Anti-*Candida* Activity of Fenugreek and Ginger rhizome Extracts and their Synergism with Fluconazole and Nystatin

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***Key words:***
***Candida; ginger rhizome; fenugreek ; nystatin; fluconazole***

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## ABSTRACT

***Background:*** *Oropharyngeal candidiasis is an alarming sign that may indicates a serious systemic disorder, especially in immunocompromised individuals who face the drastic side effects of the in market antifungal drugs. This increases the need for developing effective and safe natural products.* ***Objectives:*** *to evaluate the in vitro activities of both Fenugreek and Ginger rhizome extracts and to compare their effects with nystatin and fluconazole antifungals against Candida species. As far as we know, this is the first study to compare between the anti-candida activity of both extracts.* ***Methodology:*** *Different species of Candida were isolated from patients suffering from oropharyngeal candidiasis. The antifungal effects of methanolic extracts of Fenugreek and Ginger rhizome were tested using disc diffusion method. Each extract was added to both nystatin and fluconazole discs to measure the presence of synergistic effects.* ***Results:*** *Highly significant synergism was detected between both extracts and fluconazole. Ginger rhizome extract was more potent than Fenugreek extract.* ***Conclusion:*** *When used in combination with fluconazole, Fenugreek and Ginger rhizome extracts are promising, safe and effective anti-Candida agents.*

## INTRODUCTION

The commonest fungal infection in the human mouth is known to be oropharyngeal candidiasis[1]. It is referred to as an opportunistic affection of the buccopharyngeal mucosa inflicted by *Candida* species. Mainly caused by *C. albicans*, followed by other species such as *C. tropicalis, C. glabrata,* and *C. krusei*[2]. It is an important indicator of suppression in the immune system, such as leukemia, diabetes mellitus, and acquired immunodeficiency syndrome (AIDS), however it may sometimes occur in healthy individuals having bad oral hygiene, or others using prolonged courses of broad-spectrum antibiotics[3].

Oropharyngeal candidiasis involves very annoying symptoms including soreness of esophageal and buccal mucosa, difficulty in the act of swallowing, and in speech[4]. Moreover, it has the ability to disseminate causing systemic candidiasis all over the body with a mortality rate of about 71% to 79% [5]. It is much more difficult to treat such infections in immunocompromised patients, since reports of resistance to available antifungal agents in those patients are increasing[6]. Another problem in those patients is the relapses that commonly occur after stopping treatment in those patients.In severely immunocompromised patients relapse rates have reached high level (30%–50%)[2].

The recommended first line treatment for most cases of oropharyngeal candidiasis is using topical antifungal drugs leaving systemic drugs to the severe cases only[3]. The most widely prescribed topical antifungal for treating oropharyngeal candidiasis is Nystatin[7,8]. However, it has many side effects, including the annoying bad taste that results in nausea and vomiting[9]. Also, it can be used as an oral rinse form that has high levels of sucrose, that form is contraindicated in cases of diabetes and teeth decay[10]. Systemic triazoles such as fluconazole are very effective in severe infections or cases of intolerance to topical drugs[6]. Fluconazole is defined as a fungistatic drug used often to treat systemic mycoses due to its high efficacy and broad activity[11,12].

Generally, local antifungal agents do not often reach the recommended therapeutic levels in the mouth[13]. Hence, for systemic antifungals like fluconazole, higher doses are needed usually to reach the oral tissues in an effective concentration, this can result in serious complications especially in old ages such as hepatotoxicity. Drug interactions and drug resistance are additional limitations that are frequently encountered with fluconazole[14]. These limitations associated with the use of conventional antifungal drugs placed a need to study herbal medicines that may provide new therapeutic agents with adequate biological activity and nearly no side effects, such as Fenugreek and Ginger rhizome[15].

fenugreek [Trigonella foenum-graecum Linn (Fabaceae)] has often attracted many scientists across the world to study it. It was native to Eastern Europe and areas of Asia but nowadays it is widely cultivated for its seeds and leaves. The seeds of fenugreek have been used in the past as a demulcent, carminative, laxative, expectorant, and stomachic factor. Many active components such as vitamins, amino acids, fatty acids

have been found in the mature fenugreek seed, also saponins such as disogenin, neogitogenin, gitogenin, homoorientin, trigogenin, saponaretin, and neogigogenin, flavonoids, fibers, fixed oils, polysaccharides, and some alkaloids, that is, choline and trigonelline [16]. It was found that extracts prepared from all parts of fenugreek plant showed potent activity against mycelial growth of fungi, and the methanol extract had the main antifungal activity[17].

Ginger (Zingiber officinale Rosc) plant has extensively been used as a spice all over the world. In the past, ginger has been used to treat cold, digestive disorder, fever, pain, inflammation. Moreover, its extract was proven to possess pharmacological effects, including antidiarrheal, anticonvulsion, antidiabetic, antibacterial, antinausea, anti-inflammatory, and lipid decreasing effects. The antimicrobial activity recorded in prior studies showed a minimal inhibition concentration (MIC) value of 0.78- 3.12 mg/mL[18].

The aim of this study was to measure the *in vitro* antifungal effects of Fenugreek and Ginger rhizome extracts in a comparison and in synergism with fluconazole and nystatin on various *Candida* species isolated from patients with oropharyngeal candidiasis. To the best of our knowledge, this is the first study to compare between the *in vitro* anti-*Candida* activity of Fenugreek and Ginger rhizome extracts.

## METHODOLOGY

**Preparation of plant extracts:**

The seeds of fenugreek [Trigonella foenum-graecum Linn (Fabaceae)] were thoroughly washed under running filtered tap water then dried. An amount of (2Kg) Air-dried fenugreek seeds were very finely ground then soaked in petroleum ether 60- 80 (1.0lit.) at a temperature of (28°C) for 3 days, then filtered. After repeating this process twice, the filtrate was evaporated to dryness at 50°C, producing reddish-brown syrup (7.5gm). Next, soaking the residue in methanol (1.2 lit) at room temperature for 3 days followed by filtration was done. This process also was repeated twice then the filtrate was evaporated to dryness at 50 °C to give yellowish brown syrup (35gm)[19].

Ginger (Zingiber officinale Rosc) rhizome was cut into smaller parts, sun dried, and methanolic extract was prepared by using 50% v/ v methanol, 50% v/v water for 45 min. Next, the methanol extract was subjected to rotary evaporation to eliminate water molecules[20].

**Fungal isolation:**

This study included 60 patients who were admitted to Tropical Department, Tanta University Hospitals, suffering from hepatic diseases complicated by oropharyngeal candidiasis which was diagnosed clinically. Each of the participant gave an informed consent.

The oropharyngeal thrush in each patient was gently rubbed using a sterile cotton swab. The collected samples were transmitted without delay to the Microbiology and Immunology Department of Faculty of Medicine, Tanta University. The swabs were immediately cultured on preprepared Sabouraud dextrose agar (Oxoid, UK) plates and incubated aerobically at 37°C for 24-48 hours. Isolated colonies were identified to the genus and species levels using colony morphology, Gram-stained smears, germ tube testing and confirmation was done by subculturing on chromogenic *Candida* agar (CHROMagar™Candida, Paris, France). According to manufacturer guidelines, *C. albicans* grew as green colonies, *C. tropicalis* grew as blue colonies, while *C. krusei* gave pink fuzzy colonies (Fig. 1).

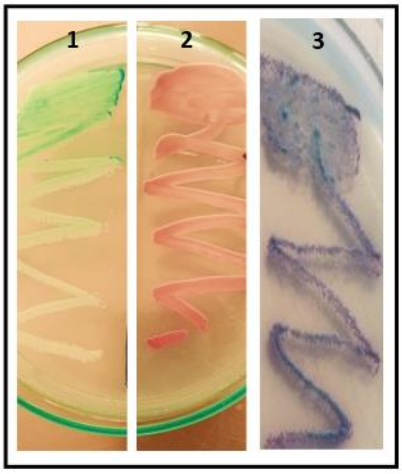


**Fig. 1: Color identification of isolated *Candida* species using CHROM agar. 1:** Green colonies of *C. albicans*; **2:** Pink colonies of *C. krusei*; **3:** Blue colonies of *C. tropicalis.*

**Antifungal susceptibility tests by disc diffusion method:**

Each *Candida* isolate was adjusted to 0.5 McFarland then spread homogenously using a sterile cotton swab onto a Mueller-Hinton agar (Oxoid, UK) plate supplemented by 2% glucose, based on the Clinical and Laboratory Standards Institute (CLSI) guidelines[21]. The discs containing the tested materials were then applied onto the cultivated plates and incubated for 24-48h at 35°C. The used discs were fluconazole 10 µg (Oxoid, UK), nystatin 100 units (Oxoid, UK), and sterile filter paper discs impregnated with 10 µ of either Fenugreek or Ginger rhizome extracts.

For testing the synergism, 10µ of each extract was added separately to fluconazole then nystatin discs. one absolute methanol disc and one empty disc were used as negative controls. Zones of inhibition that appeared around discs were measured using a ruler in millimeter.

**Statistical analysis:**

Collected data of the study were analyzed with the help of SPSS 26. Mean ± standard deviation was used to present the diameter of the compared zones of inhibition. The test used to compare between the measured diameters for zones of inhibition was Student's T-test. *P*-value < 0.05 was considered statistically significant and < 0.001 was considered a high significance.

**Ethical approval:**

The Faculty of Medicine at Tanta University in Egypt's Institutional Review Board gave the study their approval. (Approval code: 35946/10/22).

## RESULTS

A total of 61 *Candida* species were isolated from 60 samples, as 2 *Candida* species (*C. albicans* and *C. tropicalis*) were isolated from one sample. The largest number of samples yielded *C. albicans* (57/61, 93.44%), followed by *C. tropicalis* (3/61, 4.92%), then *C. krusei* (1/61, 1.64%). The results of the antifungal potency of the tested extracts against *C. albicans* and *C. tropicalis* are presented in one table in this study, as both have the same differentiating breakpoints. *C. albicans* and *C. tropicalis* breakpoints for fluconazole are (sensitive if ≥17 mm; resistant if ≤ 13 mm) based on CLSI[21]. On the other hand, *C. krusei* is known to intrinsically resist the effect of fluconazole[21], therefore testing it for synergistic activity was ruled out. CLSI didn't provide interpretative breakpoint values for nystatin.

The mean of the inhibition zones of fenugreek and ginger rhizome extracts against *C. albicans* and *C. tropicalis* isolates were 11.67 ± 0.48 mm and 12.33 ± 0.75 mm, respectively (Fig.2).

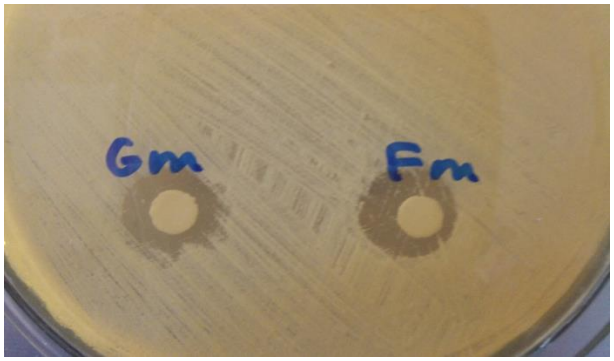


**Fig. 2: Zones of inhibition of both ginger rhizome and fenugreek extracts when tested on *Candida albicans*. GM:** Zone inhibition of ginger rhizome methanolic extract; **FM:** Zone inhibition of fenugreek methanolic extract

The diameters of zones of inhibition for fenugreek and ginger rhizome extracts were noticed to be smaller than those for nystatin and fluconazole (20.17 ± 0.38 and 33.00 ± 0.58, respectively). However, There was a significant increase in these diameters by synergistic effect when ginger rhizome and fenugreek extracts were added to fluconazole. On the Contrary both extracts showed decreased zones when added to nystatin (Fig.3) (Table 1).

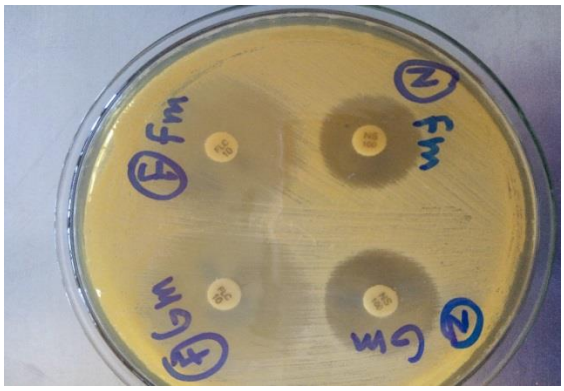


**Fig. 3: Inhibition zones of both ginger rhizome and fenugreek when added to fluconazole and nystatin.**
**NFM:** Zone inhibition of fenugreek with nystatin;
**NGM:** Zone inhibition of ginger rhizome with nystatin.
**FFM:** Zone inhibition of fenugreek with fluconazole;
**FGM:** Zone inhibition of ginger rhizome with fluconazole

**Table 1: The inhibition zone diameters shown by different tested components against both *C. albicans* and *C. tropicalis* (60 isolates):**

| **Mean ± SD of the inhibitory zones in millimeter** | | ***P*-value** |
|---|---|---|
| **Nystatin alone** | **Fenugreek + Nystatin** | 0.001* |
| **20.17 ± 0.38** | **19.83 ± 0.38** | |
| **Nystatin alone** | **Ginger rhizome + Nystatin** | 0.001* |
| **20.17 ± 0.38** | **19.67 ± 0.48** | |
| **Fluconazole alone** | **Fenugreek + Fluconazole** | 0.001* |
| **33.00 ± 0.58** | **35.67 ± 0.48** | |
| **Fluconazole alone** | **Ginger rhizome+ Fluconazole** | 0.001* |
| **33.00 ± 0.58** | **35.83 ± 0.38** | |

* Statistically significant at $P < 0.05$.
** Statistically highly significant at $P < 0.001$.

Table 2 shows that ginger rhizome extract showed more potency than fenugreek extract against *Candida* isolates ($P = 0.001$), and also shows a significant synergism between both extracts as compared with the potency of each extract tested alone ($P = 0.001$).

**Table 2: The potency of each of Fenugreek and ginger rhizome extracts against both *C. albicans* and *C. tropicalis* (60 isolates) alone and in combination:**

| Mean ± SD of the inhibitory zones in millimeter | | *P*-value |
|---|---|---|
| **Fenugreek alone** | **Ginger rhizome alone** | 0.001* |
| **11.67 ± 0.48** | **12.33 ± 0.75** | |
| **Fenugreek alone** | **Fenugreek + Ginger rhizome** | 0.001* |
| **11.67 ± 0.48** | **14.50 ± 0.50** | |
| **Ginger rhizome alone** | **Fenugreek + Ginger rhizome** | 0.001* |
| **12.33 ± 0.75** | **14.50 ± 0.50** | |

* Statistically significant at $P < 0.05$.
** Statistically highly significant at $P < 0.001$.

Regarding the single *C. krusei* isolate, diameters of inhibition zones of nystatin, fenugreek and ginger rhizome extracts were 15 mm, 7 mm, and 9 mm, respectively. The diameters noticed when nystatin combined with fenugreek and ginger rhizome extracts were (14 mm and 15 mm, respectively). There were no inhibition zones detected around the methanol disc or the blank filter paper disc (negative controls).

## DISCUSSION

In the present study, there was a significant anticandidal activity detected by ginger rhizome extract against *C. tropicalis* and *C. albicans* ($P = 0.001$). Other studies supported the anticandidal effect of ginger rhizome as supreetha.S. et al.[22] who proved that ginger rhizome powder in the form of ethanolic extract had noticeable inhibitory effects against *Candida albicans*.

The results of the present study are comparable with those of other studies concluding the presence of antifungal agents in the Ginger rhizome extract. The gingerol and shagelol were identified to be the most effective agents [23]. The present study also showed a significant synergism between ginger rhizome extract and fluconazole when both used against *candida albicans* and *candida tropicalis*. Our results agreed with those proved by Khan. et al.[24] who demonstrated that using fluconazole alone in treating vulvovaginal candidiasis was not effective because of the preformed biofilm. However, when used in combination with methanolic extract of Ginger they both completely eradicated that biofilm which suggested that ginger methanolic extract increased the susceptibility of *candida albicans* biofilm to fluconazole. In addition to that they also noticed that the co-administration of ginger extract with fluconazole reduced the renal toxicity induced by fluconazole when used alone in treated mice[24].

Regarding the potency of fenugreek *against candida albicans and tropicalis*, the current study showed a significant anticandidal activity. That finding agreed with the results detected by El Nour. et al.[25] who showed that petroleum ether extract of fenugreek seeds had potent antifungal effect against both *Aspergillus niger* and *Candida albicans*.

That finding also agreed with the results detected by Varadarajan S. et al.[26] who proved that fenugreek extract had antifungal activity against fluconazole resistant *Candida albicans.*

The present study showed a significant synergistic activity when fenugreek was added to fluconazole against *candida albicans and tropicalis* which is to the best of author's knowledge wasn't studied before, as most of the preceding studies tested fenugreek extract on fluconazole resistant strains of *Candida albicans*[26,27].

Regarding the synergism between either ginger rhizome or fenugreek and nystatin, the present study showed absence of synergism. Also to the best of our knowledge no previous studies tested the synergistic activity of them with nystatin. Only a number of studies compared between the antifungal activity of ginger and nystatin showing nystatin to have a more potent effect which agreed with the results detected in the present study [28,29].

On the other hand, the current study proved a significant synergism between fenugreek and ginger rhizome extracts when used together against *Candida albicans and tropicalis* isolates which is also as far as the author knows hasn't been studied in preceding research.

Finally, it is necessary to mention a few points on the safety or adverse effects of fenugreek and ginger rhizome extracts. Several preceding studies have been performed on fenugreek extract, but no clinically harmful side effects have been reported except for allergy, abdominal upset, hypoglycemia, increased prothrombin time and potential uterine stimulation in pregnancy[30]. As regards ginger, several studies tested its safety in human and showed no harmful adverse effects reported [31].

## CONCLUSION

In conclusion, our *in vitro* research highlights the effect of fenugreek and ginger rhizome extracts as potent antifungal agents in cases of oropharyngeal candidiasis, giving much more synergistic inhibition than the sole use of fluconazole. Also, ginger rhizome extract showed a powerful activity against *Candida* than fenugreek extract.

**Conflicts of interest:**

The author declares that there is no conflicts of interest (financial or non-financial) related to the present work done in this manuscript. This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

**Authors contribution:**

Sara Youssef Maxwell performed the study design, Mona Abd El-Aziz Gadallah collected the samples, Sara Youssef Maxwell and Mona Abd El-Aziz Gadallah contributed to laboratory experiments, data interpretation, Sara Youssef Maxwell drafted and revised the manuscript.

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