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Original Research

Evaluation of the antifungal effects of mint, pomegranate, and coriander on Candida glabrata that is fluconazole-resistant

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ABSTRACT:
Introduction: The management of candidiasis has been significantly impacted by the antifungal resistance displayed by several species of Candida. For their management, better, safer therapeutic options are now required. There is a lengthy history of knowledge about the diverse therapeutic properties of phytochemical compounds.The purpose of this study was to investigate the antifungal effects of Coriandrum sativum, Mentha piperita, and Punica granatum in Candida glabrata and assess the efficacy and resistance of fluconazole. **Materials and Procedures:** Extracts are introduced at repeated dilutions and incubated after the organism was inoculated into a particular medium in order to determine the zone of inhibition. **Results:** In fluconazole-resistant C. glabrata, all three extracts demonstrated statistically significant and superior antifungal efficacy.The extracts demonstrated greater antifungal activity against resistant C. glabrata strains, but more research is required to determine whether the efficiency of these extracts in human beings is compromised by any systemic or local physiological variables.
Keywords: Fluconazole, herbal extracts, mint, Candida glabrata, candidiasis, coriander, antifungal resistance

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INTRODUCTION

Self-treatment has more negative effects than positive effects, and the desire to self-diagnose and reliance on online resources for medical issues have resulted in unethical usage of numerous pharmacotherapeutic substances. The increased use of steroidal drugs and antimicrobials, which cause immunosuppression and disrupt the balance of the microflora, contributing to the rise in the development of antimicrobial resistant organisms in recent decades. When we discuss an opportunistic infection, the term candidiasis, which is brought on by fungi of the genus Candida, immediately comes to mind. These organisms have faced the problem of antifungal resistance to the bulk of the antifungal medications used to treat them in the last ten years. Although these organisms can exist in healthy people without harming them, in those who are vulnerable to them, they can cause localised or, in rare instances, disseminated candidiasis. In the early 1990s, Candida glabrata was thought to be a nonpathogenic commensal of the oral cavity and other mucosal surfaces. Increased use of broad-spectrum antibiotics, immunosuppressants, and cancer therapy resulted in the host's immunity being reduced, which created an ideal environment for the organism's development. Currently, it is recognised that these organisms can cause candidiasis in immunocompromised people, such as hospitalised patients, people with diabetes, AIDS, and cancer patients. The second most frequent cause of

superficial and systemic candidal infections, particularly nosocomial infections, is C. glabrata, which has a built-in resistance to azoles and other standard antifungal medications. [3] It has a stronger capacity for colonisation dental appliances like dentures and a colonising frequency that is even higher than Candida albicans. This organism can create biofilms, especially the yeast form, which disperses and helps the disease spread, and it can resist high levels of oxidative stress, making it a successful human pathogen. [4]

The treatment of candidal infections is negatively impacted by the similar look of the fungal cellular structure in candidal species to that of human cells due to their eukaryotic status. Only a small number of chemotherapeutic medications are effective in managing Candida, despite extensive research on its pathophysiology, route of invasion, and virulence factors. The susceptibility profile of the fungi, the side effects, and the patient's tolerance all serve to further restrict the use of these substances. [5] Due to the lack of fresh classes of pharmacotherapeutic drugs or targets, several pharmacological targets are being tested in an effort to overcome these drawbacks. Since C. glabrata has a greater minimum inhibitory concentration than Candida albicans, several resistant strains have also been found. [6,7] Due to the establishment of resistance strains, the number of instances of candidiasis that are resistant to treatment has increased as a result of the unethical use of azoles and other antifungal drugs in the management and prevention of candidiasis. [6] For a variety of reasons, fluconazole is the medication of choice for people who are immunocompromised as well as those who are susceptible to or at high risk of contracting these diseases. Fluconazole's target location, the ERG11 gene, has recently been the site of mutation in some strains, which has resulted in azole drug resistance. [6] One promising therapeutic approach to halt and overcome multidrug resistance in candidiasis is the combined administration of both synthetic pharmacotherapeutic drugs and natural phytochemical compounds. [8] The use of herbal extracts and their use in the treatment of these infections will give us a more affordable and straightforward alternative, which may even turn out to be a better alternative to these chemotherapeutic agents. It is always preferable to find the answers to our questions in the wisdom offered by our ancestors. These substances, which can be found in organic or herbal plants like coriander, mint, and pomegranate, may help the body's susceptibility to medications return to what it once was. They can also be taken on their own as therapeutic substances. The cost savings and ease of preparation at home without the need for expensive equipment are additional benefits of using natural agents. These natural agents have fewer negative effects than synthetic pharmaceuticals. The presence of the secondary metabolites created by these extracts is responsible for all of these benefits.

The current study aimed to investigate the antifungal effects of Coriandrum sativum, Mentha piperita, and Punica granatum in C. glabrata and assess the efficacy and resistance of fluconazole.

METHOD AND MATERIALS

HOW TO EXTRACT A HERBAL INGREDIENT

Use an extractor to combine appropriately sized M. piperita, C. sativum, and P. granatum powder or pieces. Add alcohol, almost three times as much as the raw material, and heat under a reflux for 3–4 hours at a temperature between 80°C and 85°C. Filter the extract using a suitable-sized filter with a pore size of at least 10 microns. Three further extractions of the marc are performed, each time sifting the extract into a different vessel. The resulting filtrate is subsequently put in storage.

HOW TO MEASURE THE RATE OF MICROBIAL GROWTH

HiCromecandidal differential agar media is the specialised culture medium into which the organism is injected, and it is then incubated for 24-48 hours. After being incubated in the culture media for 24-48 hours, the presence of a yellowish creamy, smooth, elevated, and separable colony on the agar surface served as a sign that the organism was growing. Then, the organisms' presence was verified using the KOH and periodic acid-Schiff (PAS) stains.

HOW TO EVALUATE THE EFFECTIVENESS OF FLUCONAZOLE AND CANDIDA GLABRATA RESISTANCE

By adding fluconazole antifungal disc to the culture media and looking for zones of inhibition after 20–24 hours of incubation, the resistance of C. glabrata species to fluconazole will be determined. If insufficient growth is seen, the culture will then be incubated again for 48 hours. Even after 48 hours, the resistant species will still not exhibit a zone of inhibition, and these strains are isolated for for research.

HOW TO MEASURE THE EFFECTIVENESS OF MINT, POMEGRANATE, AND CORIANDER AGAINST CANDIDA GLABRATA

In order to determine the herbal extracts' antifungal activity by calculating the zone of inhibition, standard quantities of the extracts (25, 50, 75, 100, 125, 150, 175 and 200 l) were added to the culture and incubated with the fungal strains. After being incubated in the culture media for 24-48 hours, the presence of a yellowish creamy, smooth, elevated, and separable colony on the agar surface served as a sign that the organism was growing. KOH and PAS stains were used to confirm the organisms' existence.

STATISTIC EVALUATION

After being obtained, the raw data were analysed statistically. Due to the fact that the raw data did not follow normal distributions, nonparametric statistical analysis was conducted to determine the statistical significance. In order to compare different groups in the study, Pearson's correlation test was used. The test allowed for a comparison of the effectiveness of several extract groups against C. glabrata both individually and in combination with fluconazole.

RESULTS

By analysing the zone of inhibition and minimum inhibitory concentration for these extracts against the organisms, the study sought to determine the anticandidal effects of C. sativum, M. piperita, and P. granatum extracts on fluconazole-resistant C. glabrata.

Herbal extracts among the natural extracts failed to create an inhibitory zone at a concentration of 25 and 75 l. The diameter of the zone of inhibition increased concurrently from a concentration of 100 l to 200 l. Of the three extracts examined, C. sativum showed the widest zone of inhibition and the lowest minimal inhibitory concentration.

The study used a fluconazole-resistant strain of C. glabrata, although all of the extracts used in the study had less potent antifungal effects than the fungus. Despite the strains' resistance, fluconazole created a zone of inhibition at a concentration of 250 mg that was comparable to the 200 l concentration of the herbal extracts. These extracts are regarded as statistically significant because the examination of the values between the study group and the positive control group revealed P 0.001 in all of them. The antifungal effects of pomegranate, mint, and coriander herbal extracts against fluconazole-resistant C. glabrata differ significantly from those of fluconazole. The three separate extracts are compared among themselves, and because the P value is more than 0.001, they are deemed statistically insignificant. It demonstrates that the antifungal activity of the herbal extracts is not significantly different from one another, i.e., they are almost equally powerful. The correlation values were also very near to 1, indicating a positive link [Tables 1–5].

Table 1: Zone of inhibition against *Candida glabrata* by *Punica granatum* (mm)

Concentration of the extract (µl)	Zone of inhibition (mm)		
	Punica granatum	***Coriandrum sativum***	***Mentha piperita***
25	0	0	0
50	0	0	0
75	0	0	0
100	03	17	11
125	14	15	17
150	14	18	19
175	20	22	22
200	23	25	22
Positive control (fluconazole) - 250 mg		26	
Negative control (ethanol) - 200 mg		0	

Table 2: Comparison of antifungal property of Coriandrum sativum and Mentha piperita against fluconazole-resistant Candida glabrata

Natural extract used	Zone of inhibition (mm)	Concentration (µl)	Correlation value	*P*	Significance
Coriandrum sativum	26	200	0.956	0.866	Insignificant
Mentha piperita	23				

Table 3: Comparison of the antifungal property of fluconazole and Punica granatum, Coriandrum sativum and Mentha piperita against fluconazole-resistant Candida glabrata

Antifungal agents/extract	Zone of inhibition	Concentration	*P*	Significance
Fluconazole	25	250 mg	0.000	Significant
Punica granatum	20	200 µl		
Fluconazole	26	250 mg	0.001	Significant

Antifungal agents/extract	Zone of inhibition	Concentration	*P*	Significance
Coriandrum sativum	26	200 µl		
Fluconazole	26	250 mg	0.000	Significant
Mentha piperita	24	200 µl		

Table 4: Comparison of antifungal property of *Punica granatum* and *Coriandrum sativum* against fluconazole-resistant *Candida glabrata*

Natural extract used	Zone of inhibition (mm)	Concentration (µl)	Correlation value	*P*	Significance
Punica granatum	21	200	0.95	0.85	Insignificant
Coriandrum sativum	26				

Table 5: Comparison of antifungal property of Punica granatum and Mentha piperita against fluconazole-resistant Candida glabrata

Natural extract used	Zone of inhibition (mm)	Concentration (µl)	Correlation value	*P*	Significance
Punica granatum	21	200	0.90	0.87	Insignificant
Mentha piperita	23				

DISCUSSION

In the past ten years, one of the largest therapeutic concerns in the control of numerous infectious diseases has been the emergence of antimicrobial resistance. Due to the improper use of antimicrobials, a number of resistant microbes have emerged, which have given birth to increasingly severe diseases. Although an attempt was made to comprehend these illnesses better, no precise way for managing or reversing them has yet been discovered, forcing the hunt for new, safer options. [9,10] The primary therapeutic techniques for any disease in the past have been phytochemical agents or herbal remedies. Knowing these substances' harmful consequences has once again focused our search for herbal remedies to cure a variety of ailments. These herbal remedies were used to treat cancer, speed up wound healing, and cure infections. Due to the negative impacts of synthetic pharmacotherapeutic techniques and the advent of resistant strains of many infectious diseases, these natural medicines are regaining their lost therapy efficacy. There is therapeutic activity in the leaves, fruits, or roots of some herbal plants. They have a wide range of antibacterial, antifungal, and antioxidative potential due to the large number of bioactive compounds they contain. [11,12]

We performed an in vitro study to determine the antifungal activity of three herbal extracts, namely C. sativum, M. piperita, and P. granatum, against fluconazole-resistant C. glabrata. The effectiveness of the extracts was then compared with fluconazole and among themselves. Few studies have been conducted to evaluate the effectiveness of antifungal medications against C. glabrata. Our investigation of the antifungal activity of coriander, mint, and pomegranate against fluconazole-resistant C. glabrata is the first of its kind. All three extracts shown equivalent antifungal activity in the current study without acting in a statistically different manner. However, compared to fluconazole, every extract was much better.

One of the often used culinary spices is coriander, or C. sativum, which contains bioactive chemical compounds like linalool, cedrene, cymene, and pinene. These phytochemical components provide them a variety of therapeutic properties, including antidiabetic, antibacterial, antifungal, and anticarcinogenic properties. It has been discovered that one of the bioactive compounds in linalool and coriander plays a remarkable function in controlling these biological effects. [11,13] According to Msaada et al. (2014), coriander contains significant amounts of flavonoids, tannins, and polyphenols, which give it powerful antioxidant qualities. [14] According to Chaudhary et al., who conducted a study to assess the antibacterial properties of coriander, the herb has strong antibacterial properties and significantly inhibits the growth of bacteria like Staphylococcus, Streptococcus, Klebsiella, Lactobacillus, and Salmonella. The extract also has a high concentration of flavonoids, alkaloids, glycosides, and amino acids but not reducing sugars. [15] Mint has antibacterial effect against Gram-positive bacteria, according to research by Liu et al. (2016). Bacillus cereus, Gram-negative bacteria, and Staphylococcus aureus Escherichia coli, Pseudomonas aeruginosa, and antifungal activity against C. albicans were detected, although human embryonic kidney cell lines displayed very modest cytotoxicity. [16] In the current investigation, fluconazole-resistant species of C. glabrata responded better to C. sativum's antifungal activity than fluconazole did. Coriander

visually contrasted with the other extracts in that a distinct zone of inhibition was seen around the colonies, indicating complete lysis of the organisms, which was not present in the other two extracts.

Pomegranate seeds, peels, and skins are typically discarded, but recently, it has been discovered that they contain biological compounds that have medicinal potential. It has also been demonstrated to have estrogen-like effects. [17] The most important elements of these specific plant extracts, which are thought to be responsible for these therapeutic qualities, are punicalagin and ellagic acid. [18] Patel et al. concluded that pomegranate suppresses both the Gram-negative and Gram-positive organisms as well as Candida in a study where they examined whether pomegranate exhibits antibacterial activities on Gram-positive and Gram-negative bacteria as well as antifungal activity on C. albicans. There are two ways in which the bioactive compounds in pomegranates exert their antifungal activities. The first method involves breaking down the bacterial cell membrane by bonding to it. Second, these molecules prevent the membrane-integrated enzyme molecules from working, which causes apoptosis to be triggered. [19] By comparing various pomegranate genotypes and analysing the inhibition of mycelial growth, Rongai et al. (2019) determined that the wild genotypes CREA-FRU6, CREAFRU11, and CREAM-FRU76 have strong antifungal action. [18] In a study by Rizwan et al. testing the anticandidal activity of pomegranate peels and Darehald root bark, it was concluded that both extracts outperformed fluconazole and voriconazole in terms of antifungal activity. [20] Pomegranate showed statistically significant antifungal action that was superior than fluconazole in the current investigation, but lacking robust antifungal action against C. glabrata at lower dosages. Similar findings were reported by Mbatha (2018) from his research on pomegranate skin, and he came to the conclusion that pomegranate also suppresses C. albicans germ tube production at subtherapeutic levels in addition to having fungicidal effects. [21] In contrast to Nicole et al(2014) .'s findings, which claimed that pomegranate extract lacked antifungal ability against Candida species, the results of the current investigation were contrary to their findings. In contrast, at greater doses, pomegranate in our study had comparable antifungal effects to coriander and mint.

Mint is a culinary and cosmetic ingredient that is both antimutagenic and chemopreventive. The antiparasitic, antibacterial, analgesic, and bug-repelling effects of mint have long been established. Menthol and pulegone are the two major ingredients. Mint has anticandidal activity, according to a study by Deyab et al. comparing it to apple cider vinegar on Candida-related denture stomatitis. The molecules attach to the cell membrane, causing disruption, which reduces the cell's production of ergosterol and gives mint its fungicidal properties. [22] Githaiga et al. conducted an in vitro investigation to assess the antibacterial qualities of mint and an oil distillation to assess the constituents that make up mint essential. They said that mint had powerful antibacterial qualities and that tannins, alkaloids, and other key ingredients made up the majority of it. [23] In contrast to C. sativum's clear zone, mint created a greenish zone of inhibition around the colonies in our assay, demonstrating better antifungal activity than fluconazole. According to Oktay Erdogan et al., who evaluated the antifungal activity of mint, lavender, and thyme against Verticillium dahliaeKleb and observed that mint and thyme showed similar antifungal activity in a dose-dependent manner, the current study was in line with their findings. [24] The findings of this study are consistent with those of Wenji et alstudy .'s from 2019, which found that mint leaves have strong antifungal activity against C. albicans at a concentration of 80%. [25] The study offered a safe, natural substitute for the pharmacotherapeutic medicines now in use, which are known to have a number of negative effects. These plant-based chemicals are abundant in our nation and are a part of our culture, making them less expensive and more readily available to both rich and poor people. Additionally, because they are natural agents, predisposed individuals can utilise them as a preventative step without worrying about negative side effects. The biggest drawback of the study is that, because it was conducted in vitro, we were unable to determine how systemic variables or systemic disease might have affected the mode of action of these drugs. The majority of the initial illnesses involved were either bacterial infections or systemic ailments like diabetes and HIV since candidiasis most frequently develops as a superadded infection. It is unknown if these herbal remedies could interact negatively or favourably with any other medications taken for various reasons. The use of unrefined herbal extracts was another flaw in the current investigation. Furthermore, because the study was conducted in vitro, it was not possible to assess how other bodily or oral factors can affect a drug's effectiveness.

Finding additional antifungal targets, particularly for the treatment of candidal infections, which have the highest resistance to all antifungal medications now in use, is urgently needed to combat infections.[14,15]

CONCLUSION

The goal of the current study was to assess C. sativum, M. piperita, and P. granatum's antifungal efficacy against C. glabrata. All of the herbal extracts employed in the study displayed considerable statistically significant antifungal efficacy against C. glabrata. Thus, without any negative side effects, these drugs can be a cheaper, safer alternative to the current therapeutic systems. Additionally, these medicines outperformed fluconazole. These extracts

can be used alone or in a multi-drug combination with fluconazole to manage the condition with a lower dosage. To determine whether any systemic or local factors in the body are interfering with the efficacy of these extracts in treating patients, more research is required. Additionally, considering that the current study was an in vitro study and that this infection is more common in individuals with diabetes, immunosuppression, and acquired immunodeficiency syndrome, drug interactions with drugs used for other purposes should also be assessed.

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