Antifungal efficacy of three medicinal plants
*Glycyrrhiza glabra*, *Ficus religiosa*, and *Plantago major* against oral *Candida albicans*: A comparative analysis

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**ABSTRACT**

Introduction: From ancient times, plants with medicinal values are being tested and used in the treatment of various infectious diseases.

Aims and Objectives: The present *in vitro* study was designed to assess the antifungal activity of three commonly available medicinal plants *Glycyrrhiza glabra*, *Ficus religiosa*, and *Plantago major* on inhibiting oral *Candida albicans* in comparison to standard antifungal agents.

Materials and Methods: Bark of *G. glabra*, stem of *F. religiosa*, and husk of *P. major* were collected, crushed into fine powder, and dissolved in 67% ethanol. Extracts were subjected to antifungal efficacy test against oral *C. albicans* (ATCC 66027) using Kirby–Bauer disc diffusion method. Mean zone of inhibition (ZOI) was measured by HI antibiotic zone scale. One-way ANOVA using Tukey’s post hoc and *t*-test were applied for statistical analysis.

Results: *G. glabra* was found to be most effective among the three with highest mean ZOI measuring 19.8 ± 0.83, 19.4 ± 0.54, and 18.2 ± 1.09 at 24, 48, and 72 h, respectively. Tukey’s post hoc test showed statistically nonsignificant difference between antifungal activity of *F. religiosa* and *P. major* with itraconazole 10 mcg.

Conclusion: *G. glabra*, *F. religiosa*, and *P. major* showed acceptable potency against *C. albicans* (ATCC 66027) comparable to that of synthetic antifungal agents. However, further studies should be undertaken to affirm the same and test their efficacy in different concentrations and clinical utility.

**Key words:** Antifungals, *Ficus religiosa*, *Glycyrrhiza glabra*, medicinal plants, phytochemicals

In the past few decades, a dramatic increase in fungal infections incidence has been noted across the world owing to the appearance of resistant fungi to different fungicides used in medicinal practice.1] Newer drug research targeting fungal species are the most neglected ones, this can be evident from the fact that “gold standard drug” for antifungal therapy remains same since 1956. Very few antifungal agents are known till now, and their continuous and indiscriminate use have led to the development of resistance by fungal species, some shows ineffectiveness toward fungal disease.2] These drugs not only show ineffectiveness due to resistance by fungal species but also show undesirable side effects or are very toxic, shows recurrence or drug interactions causing severe problems.[3‑6]

In recent decades, it is observed that fungal infections in humans and animals are due to infections by opportunistic *Candida* fungi, and the diseases caused due to these organisms not only impart challenges for clinicians to treat these infections but also cause a financial burden to the patients suffering from these diseases.[6‑8] More than twenty species of *Candida* are known till now and among them,
Candida albicans is common opportunistic fungus associated with candidal infections. In conditions such as compromised host immune system, these C. albicans species and related species may overcome host immune response, can become pathogenic, causing systemic, vaginal, or oral candidiasis. In humans, it causes oral thrush. C. albicans infections impart a wide variety of problems in patient treatment ranging from limited options of effective antifungal agents, toxicity, and resistance of Candida to commonly used antifungals to relapse of Candida infections. This has led to increased interest of clinicians and researchers to discover new drugs, plant, and herbal products to treat these fungal diseases with less severe adverse effects and relatively less economic burden, compared to chemical drugs.

The use of medicinal plants and herbs as medicines to treat various diseases could be traced as far back as the beginning of ancient human civilization. “Rigveda,” an ancient Hindu culture literature gives the earliest evidence of the use of medicinal plants to cure diseases of humankind. Medicinal plants are not only renewable in nature but also offers a wide variety of phytochemicals which are said to have significant antimicrobial and antifungal activities. G. glabra (Mulethi), Ficus religiosa (peepal tree), and P. major (isabgol) are examples of some abundantly used plants in ancient times as traditional medicines to treat bacterial and fungal infections but very less is known about their exact medicinal property in today’s world.

Considering current trends in occurrence of fungal disease and the need to discover new plant and herbal products with potential medicinal properties; this in vitro study was designed with the aim to compare the antifungal efficacy of extracts of three medicinal plants G. glabra, F. religiosa, and P. major against oral C. albicans (ATCC 66027) and comparing it with known standard antifungal agents.”

MATERIALS AND METHODS

Collection of plant material
The parts of the three medicinal plants, i.e. bark of G. glabra, stem of F. religiosa, and P. major were obtained from outlet of Chhattisgarh State Minor Forest Produce Federation (Sanjeevani, Raipur, CG) and were identified by a botanist.

Preparation of medicinal plant extracts
Digestion a form of maceration in which gentle heat is used during the process of extraction was carried out to obtain the extracts of the medicinal plants, for which the plant parts were first washed with distilled water, air dried at room temperature, and then preweighted 50 g were dissolved in 67% ethanol following crushing into fine powder with the help of a mechanical grinder. The obtained extracts were stored till further use in sterile, tinted glass bottles which were previously washed and dried with distilled water.

Preparation of anti-fungal medicinal plant extract sensitivity testing disc
Preparation of discs containing medicinal plant extract for sensitivity testing involving, pouring of 5 ml medicinal plants extracts into three sterile Eppendorf separately followed by labeling for ease of identification. Sterile Whatman No. 1 paper was punched into 5 mm diameter disc size. Ten such discs were placed into each Eppendorf and were allowed to soak for 48 h for proper absorption. After 48 h, the discs were transferred to sterile Petri dishes and were air dried at room temperature and were stored in the same Petri dishes till further use.

Antifungal assay
The C. albicans (ATCC 66027) obtained from (Hi-Media Laboratories Pvt. Ltd; Mumbai, India) was used to test the antifungal efficacy of the three medicinal plant extracts, using Kirby–Bauer disc diffusion technique described by Bauer et al. (1966). Growth of the freshly subcultured isolates was suspended in 10 ml of sterile saline to obtained a turbidity of 0.5 McFarland. Premeasured 25 ml of Sabouraud dextrose agar was poured into sterile Petri dishes (Borosil®) of size 90 mm to prepare 5 such plates for lawn culture.

Lawn culture of the C. albicans was obtained by aseptic swabbing of suspension on dried Sabouraud dextrose agar plates in a Sterile Lamellar Airflow Chamber equipped with ultraviolet light and high-efficiency particulate air filter. Five such plates were prepared for antifungal assay.

Prepared medicinal plants extract discs along with three other commercially available standard antifungal disc ( clotrimazole 10 mcg, fluconazole 10 mcg, and itraconazole 10 mcg) and a control disc (99.9% ethanol) were placed on the Sabouraud dextrose agar plates and were incubated at 37° for 24, 48, and 72 h, respectively. The antifungal activity of medicinal plant extract was then compared with known standard antifungal agents using measurement of diameter of zone of inhibition (ZOI; mm).

Statistical analysis
Collected data then were tabulated in Microsoft Office Excel and analyzed using IBM SPSS software vs. 22 for windows (New York, USA). Since the data were continuous type, parametric tests were used for analysis. Mean ( ) and standard deviation were calculated. One-way analysis of variance test was used for multiple group comparisons, followed by Tukey’s post hoc for group-wise comparisons, and P < 0.05 was considered statistically significant.

RESULTS
For each of the three medicinal extracts, the results were analyzed at 24, 48, and 72 h using mean ZOI and were compared with standard antifungal agents. The evaluation
of mean ZOI of the tested medicinal extracts and fungicides showed that all three medicinal extracts had an effect of varying degree on C. albicans (ATCC 66027) [Tables 1 and 2]. Results showed that among all the three medicinal extracts used, G. glabra showed maximum efficacy at all the three-time intervals.

G. glabra at 24 h showed lesser activity as compared to clotrimazole (10 mcg), but the difference in activity was statistically nonsignificant. However, when compared to fluconazole (10 mcg) and itraconazole (10 mcg), G. glabra proved to be superior at all the three-time intervals with statistically significant difference in activity [Tables 1 and 2]. Antifungal efficacy of F. religiosa showed statistically nonsignificant difference in activity in comparison to itraconazole (10 mcg) at 24 and 48 h, respectively [Tables 1 and 2]. While at 72 h, the efficacy of F. religiosa was higher as compared to itraconazole (10 mcg) and the difference was statistically significant ($P = 0.038$). P. major showed efficacy similar to that of synthetic antifungal agent itraconazole (10 mcg) at all the three-time intervals with statistically nonsignificant difference in activity ($P = 0.992, 0.999$, and $0.999$, respectively, Table 2).

### DISCUSSION

Our study revealed that the extract of G. glabra exhibits a stronger inhibitory property on C. albicans in comparison to the other extracts and also in comparison to synthetic antifungal agents such as fluconazole (10 mcg) and itraconazole (10 mcg), but lesser efficacy as compared to clotrimazole (10 mcg). F. religiosa and P. major showed efficacy similar to that of synthetic antifungal agent itraconazole (10 mcg). Superior antifungal efficacy exhibited by G. glabra and similar efficacy shown by F. religiosa and P. major as compared to synthetic antifungal shows promising results to be used as a potent naturally occurring ingredients in antifungal mouthwashes, and component of slowly dissolving lozenges.

This assay showed that the inhibition zone decreased at 48 and 72 h as compared to 24 h. It was also revealed that there was a statistically significant difference in the inhibitory zones at 24 and 48 h with maximum inhibition at 24 h, irrespective of the concentration. It is established fact that C. albicans will be in log phase during 24 h and reaches stationary phase at 48 h, and probably this is the best explanation for the observations of the present study showing 24 h ZOIs to be better than 48 h.$^{17-19}$ Since the study is first of its kind using medicinal plants which have not been used previously, the results cannot be compared with any other studies.

Furthermore, no efforts were taken to determine the minimum inhibitory concentration of medicinal plants at which they start showing their antifungal efficacy and measures to determine their side effects in humans as this is an in vitro study. Hence, it is recommended to conduct further studies to determine appropriate minimum inhibitory concentration so that these medicinal plants can be utilized for beneficence with least side effects.

To the best of our knowledge, only a few studies have been done on antifungal effects of Indian medicinal plants against oral C. albicans; it is better that the effect of these herbal extracts on other strains of oral Candida be studied. The bioactive compounds responsible for antifungal activities of these three plants need to be identified and isolated, and it should also be taken into account that results obtained from in vitro assays may not necessarily be reproducible in vivo due to the metabolic processes in the test subjects. Because of the antifungal effects of these medicinal plants

### Table 1: Antifungal activity of the extracts against Candida albicans (ATCC 66027)

| Sample          | Mean zone of inhibition (in mm)±SD |
|-----------------|-----------------------------------|
|                 | 24      | 48      | 72      |
| G. glabra       | 19.8±0.83 | 19.4±0.54 | 18.2±1.09 |
| F. religiosa    | 11.4±0.54 | 11.0±0.70 | 10.6±0.54 |
| P. major        | 10.6±0.89 | 9.8±0.44  | 8.8±1.09  |
| Fluconazole (10 mcg) | 15.8±2.16 | 14.8±1.64 | 14.0±1.58 |
| Itraconazole (10 mcg) | 10.2±0.83 | 9.6±0.54  | 8.6±0.54  |
| Clotrimazole (10 mcg) | 22.4±2.54 | 21.8±2.09 | 20.6±0.54 |

SD=Standard deviation, G. glabra=Glycyrrhiza glabra, F. religiosa=Ficus religiosa, P. major=Plantago major

### Table 2: Intergroup comparison of antifungal efficacy using Tukey’s post hoc

| Time duration (h) | Medicinal plant extracts | Comparison with standard anti-fungal agents | $P$ |
|-------------------|--------------------------|--------------------------------------------|-----|
| 24                | G. glabra                | Fluconazole (10 mcg)                        | 0.000 |
|                   |                          | Itraconazole (10 mcg)                       | 0.000 |
|                   |                          | Clotrimazole (10 mcg)                       | 0.130* |
|                   | F. religiosa             | Fluconazole (10 mcg)                        | 0.001 |
|                   |                          | Itraconazole (10 mcg)                       | 0.547* |
|                   |                          | Clotrimazole (10 mcg)                       | 0.001 |
|                   | P. major                 | Fluconazole (10 mcg)                        | 0.001 |
|                   |                          | Itraconazole (10 mcg)                       | 0.992* |
|                   |                          | Clotrimazole (10 mcg)                       | 0.001 |
| 48                | G. glabra                | Fluconazole (10 mcg)                        | 0.000 |
|                   |                          | Itraconazole (10 mcg)                       | 0.000 |
|                   |                          | Clotrimazole (10 mcg)                       | 0.003 |
|                   | F. religiosa             | Fluconazole (10 mcg)                        | 0.001 |
|                   |                          | Itraconazole (10 mcg)                       | 0.163* |
|                   |                          | Clotrimazole (10 mcg)                       | 0.001 |
|                   | P. major                 | Fluconazole (10 mcg)                        | 0.001 |
|                   |                          | Itraconazole (10 mcg)                       | 0.999* |
|                   |                          | Clotrimazole (10 mcg)                       | 0.001 |
| 72                | G. glabra                | Fluconazole (10 mcg)                        | 0.000 |
|                   |                          | Itraconazole (10 mcg)                       | 0.000 |
|                   |                          | Clotrimazole (10 mcg)                       | 0.009 |
|                   | F. religiosa             | Fluconazole (10 mcg)                        | 0.001 |
|                   |                          | Itraconazole (10 mcg)                       | 0.038 |
|                   |                          | Clotrimazole (10 mcg)                       | 0.001 |
|                   | P. major                 | Fluconazole (10 mcg)                        | 0.001 |
|                   |                          | Itraconazole (10 mcg)                       | 0.999* |
|                   |                          | Clotrimazole (10 mcg)                       | 0.001 |

*The mean difference was considered statistically nonsignificant at the level (>0.05). G. glabra=Glycyrrhiza glabra, F. religiosa=Ficus religiosa, P. major=Plantago major
extracts, which have minimal side effects in comparison with chemical drugs, more in vivo and in vitro investigations about the oral cavity flora may be recommended. It is, therefore, suggested that the extracts which showed antifungal activity during this study be tested in vivo.

This was a preliminary study to evaluate the antifungal ability of these plant extracts against C. albicans (ATCC 66027). Superior antifungal efficacy exhibited by G. glabra and similar efficacy shown by F. religiosa and P. major as compared to synthetic antifungal shows promising results to be used as potent naturally occurring ingredients in antifungals mouthwashes, and component of slowly dissolving lozenges for effective inhibiting the growth of oral fungi in vivo. The observations in the present investigation can facilitate in forming the basis for further phytochemical studies to isolate active compounds, elucidate the structures, evaluate them against a wider range of C. albicans strains and in vivo models and may also be tested for their safety and efficacy to find new therapeutic principles against oral C. albicans.

CONCLUSION

It is concluded that G. glabra, F. religiosa, and P. major do possess antifungal properties against infectious fungal diseases caused by C. albicans and this activity is largely dependent on the extract used. The possible loss of some of the volatile components present in the extracts of G. glabra during drying and evaporation might have produced results lower and different from those obtained with extract of F. religiosa and P. major at 72 h. However, further studies are required to gain more clarity as to the specificity and biochemical mechanisms responsible for the antifungal properties of these three plants in different concentrations and on other strains of C. albicans.

New milestone in the development of pharmaceutical products can be achieved by discovering bioactive natural products from these medicinal plants that address unfulfilled therapeutic needs against these fungal infections. Further investigations are warranted to determine whether oral preparations with antifungal effects may be fabricated from these plants and tested against different strains of oral C. albicans. Since G. glabra, F. religiosa, and P. major are easily available and well tolerated, it can be incorporated into medications for topical antifungal therapy. However, further studies for its incorporation into oral preparations, safety, and cost-effectiveness have to be conducted.

Acknowledgment

We will like to acknowledge Dr. Heena Sahni, Dr. Amit Reche, Dr. Rishree Dubey, and Dr. Chandan Kumar Matsyapal, Department of Public Health Dentistry, Rungta College of Dental Science and Research, Bhilai, Chhattisgarh, for their excellent moral support. Without them, this research would not have come to light.

Financial support and sponsorship

The study was supported by Rungta College of Dental Science and Research, Bhilai, Chhattisgarh.

Conflicts of interest

There are no conflicts of interest.

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