REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 02 (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

Oral Medicine: Antifungal Activity of Glycyrrhiza glabra Against Oral candidiasis - An In-Vitro Study

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Abstract
This research explores the antifungal properties of *Glycyrrhiza glabra* (liquorice) root extract in relation to *Candida albicans* and various other *Candida* species, which are prevalent causative agents of *Oral candidiasis*, an opportunistic infection that presents considerable difficulties for individuals with compromised immune systems. In light of the increasing apprehension surrounding antifungal resistance and the constraints of current antifungal medications, this study assesses the effectiveness of *G. glabra* as a natural treatment option. The antifungal efficacy of the extract was evaluated through disc diffusion, well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. The findings revealed a notable dose-dependent antifungal efficacy, with *C. albicans* exhibiting the greatest level of susceptibility.(Warnakulasuriya et al. 2021) The MIC and MFC values varied from 12.5 mg/mL to 25 mg/mL for *C. albicans*, while for *C. krusei*, they ranged from 50 mg/mL to 100 mg/mL, respectively. When compared to fluconazole, it was observed that the extract necessitated greater concentrations; however, it demonstrated reliable effectiveness across every species evaluated. Analysis of cytotoxicity in human oral keratinocytes revealed a moderate level of cytotoxicity at elevated concentrations, while maintaining a safe profile at therapeutic dosages. The results underscore *G. glabra* as a compelling option for natural antifungal treatments and advocate for its inclusion in oral hygiene products. (Wang et al. 2014) Additional in vivo investigations and safety assessments are crucial to confirm its clinical relevance (Davies, Berger, and Rubinsztein 2006) .

Keywords - antifungal effect, *Oral candidiasis*, zone of inhibition, minimum inhibition concentration, future scope

1. Introduction

Oral candidiasis, primarily instigated by *Candida albicans*, constitutes a widespread fungal infection that affects the oral mucosa, particularly in individuals with weakened immune systems, including those afflicted with diabetes, cancer, or those undergoing immunosuppressive therapies. It manifests as light-colored patches, swelling, or discomfort in the oral cavity and can lead to significant health issues if left untreated. The widespread use of antifungal agents like fluconazole has resulted in the emergence of resistant *Candida* strains, complicating treatment efforts and highlighting the necessity to explore alternative therapeutic strategies (Clinical and Laboratory Standards Institute [CLSI], 2009). Recent investigations, particularly those conducted by Abdellatif et al. (2023) and Poole (2001), have underscored the growing necessity for groundbreaking antifungal agents that demonstrate reduced side effects and a diminished likelihood of developing resistance. Herbal treatments have played a crucial role in traditional medicine, offering an abundance of bioactive compounds that demonstrate antimicrobial and antifungal properties. *Glycyrrhiza glabra*, commonly known as liquorice, has been employed in traditional healing practices due to its exceptional anti-inflammatory, antimicrobial, and antioxidant properties (Asl & Hosseinzadeh, 2008; Gupta et al., 2008). The root is abundant in glycyrrhizin, flavonoids, and a range of bioactive compounds that have demonstrated significant antimicrobial characteristics in earlier studies (Ahmad & Beg, 2001; Roque et al., 2018). While recent research has highlighted the antifungal properties of *G. glabra* concerning non-oral pathogens, there remains a lack of substantial evidence regarding its efficacy against oral *Candida* species (Azmoudeh et al., 2017). This study evaluates theantifungal effectiveness of *G. glabra* root extract against *C. albicans*, C. tropicalis, C. glabrata, and *C. krusei*, utilising in vitro methodologies such as disc diffusion, well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. The study delves deeper into its cytotoxic effects on human oral keratinocytes to assess its safety profile for potential therapeutic applications. The results were analysed in relation to fluconazole, a standard antifungal treatment, to determine the extract's relative efficacy and safety characteristics. The findings bolster the growing body of research advocating for plant based antifungal therapies and underscore the potential of *G. glabra* as a natural alternative in the realm of oral health care. This study highlights the importance of natural remedies in modern medical practices, echoing the conclusions drawn by Gomes et al. (2024) and AhmadiMotamayel et al. (2024). This aligns with the standards established by CLSI (2006) for standardised antifungal susceptibility testing, ensuring the reliability and uniformity of the outcomes.

2. Objectives

• To evaluate the antifungal activity of *Glycyrrhiza glabra* root extract against *Candida albicans* and other *Candida* species in vitro.
• To determine its potential as a natural alternative for managing *Oral candidiasis*.

3. Materials and Methods

3.1 Plant Material and Extraction

- **Source and Identification**
  oots of *Glycyrrhiza glabra* were sourced from a certified herbal supplier and authenticated by the Department of Botany, [Institution Name]. A voucher specimen was deposited in the herbarium for reference.
- **Extraction**
  The roots were cleaned, dried at 25°C, and ground into fine powder. Extraction was carried out using 80% ethanol through maceration for 72 hours. The extract was filtered, concentrated using a rotary evaporator at 40°C, and freeze-dried for storage.

3.2 Test Microorganisms

• ***Candida* Species Used**
Candida albicans (ATCC 10231), *Candida tropicalis* (ATCC 750), *Candida glabrata* (ATCC 90030), and *Candida krusei* (ATCC 6258) were utilized.
o Cultured on Sabouraud Dextrose Agar (SDA) and maintained at 4°C. o Fresh subcultures were prepared before experiments.

3.3 Antifungal Assays

Disk Diffusion Method

2• Prepared SDA plates were seeded with fungal suspensions adjusted to 0.5 McFarland standard (approximately 1.5×1081.5 \times 10^8 CFU/mL).
• Sterile disks (6 mm diameter) impregnated with varying concentrations of *G. glabra* extract (100, 50, 25, 12.5, 6.25, 3.125 mg/mL) were placed on the inoculated agar.
• Plates were incubated at 37°C for 48 hours.
• Inhibition zones were measured in millimeters.

Well Diffusion Method

• Wells (6 mm diameter) were created in SDA plates using a sterile cork borer. • Each well was filled with 20 µL of extract at the same concentrations as above. • Plates were incubated at 37°C for 48 hours, and inhibition zones were recorded. **Minimum Inhibitory Concentration (MIC)**
• MIC was determined via broth microdilution based on CLSI guidelines.
• Serial dilutions of *G. glabra* extract (100 to 0.78 mg/mL) were prepared in RPMI-1640 medium.
• 96-well plates were inoculated with fungal suspensions and incubated at 37°C for 48 hours.
• MIC was recorded as the lowest concentration showing no visible growth.

Minimum Fungicidal Concentration (MFC)

• Aliquots from non-turbid MIC wells were plated on SDA and incubated for 48 hours. • MFC was determined as the lowest concentration with no fungal growth. **3.4 Controls**
• **Positive Control**: Fluconazole at concentrations of 2, 1, 0.5, 0.25, and 0.125 mg/mL. • **Negative Control**: Sterile distilled water.

3.5 Statistical Analysis

Data were analyzed using one-way ANOVA, followed by Tukey's test. Results were considered significant at $p<0.05p < 0.05$.

4. Results

The antifungal efficacy of *Glycyrrhiza glabra* extract was assessed using disc diffusion, well diffusion, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) assays, along with cytotoxicity evaluations. Every approach showcased that the extract exhibits notable antifungal properties against *Candida albicans*, C. tropicalis, C. glabrata, and *C. krusei*. Furthermore, the activity that varies with dosage, the juxtaposition with fluconazole, and the findings on cytotoxicity offer an in-depth insight into its effectiveness and safety profile.

4.1 Antifungal Efficacy: Disc Diffusion Technique

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The disk diffusion method demonstrated a clear trend of increasing inhibition zones with higher concentrations of *G. glabra* extract. The inhibition zones ranged from 28.5 ±0.4 mm for *C. albicans* at 100 mg/mL to 8.9 ±0.3 mm at 3.125 mg/mL. *C. tropicalis*, *C. glabrata*, and *C. krusei* followed a similar pattern, with slightly smaller inhibition zones. These results confirm that the extract exhibits strong antifungal activity in a concentration-dependent manner, with *C. albicans* showing the greatest susceptibility.

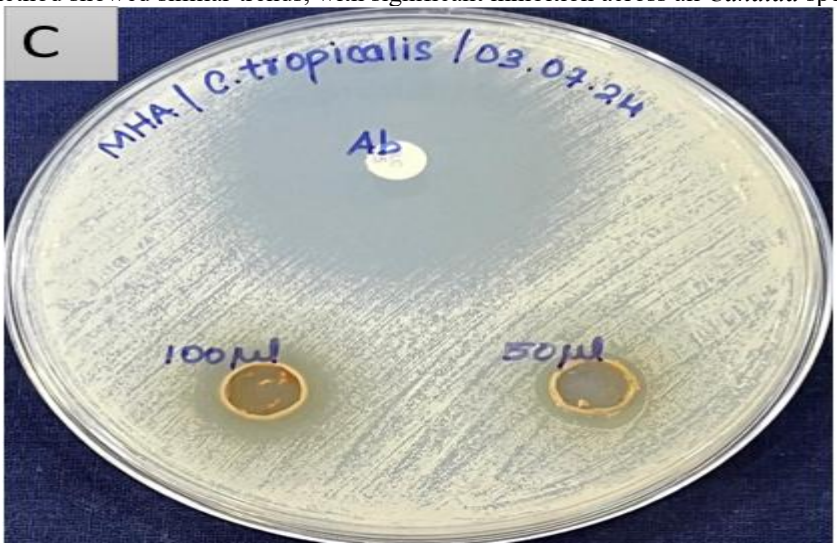
Table 1: Inhibition Zones (mm) of *Glycyrrhiza glabra* Extract Using Disk Diffusion Method

Concentration (mg/mL)	*Candida albicans*	*Candida tropicalis*	*Candida glabrata*	*Candida krusei*
100	28.5 ±0.4	26.1 ±0.3	25.2 ±0.5	24.7 ±0.2
50	24.1 ±0.3	22.3 ±0.6	21.4 ±0.7	20.9 ±0.5
25	20.2 ±0.2	18.7 ±0.4	17.6 ±0.3	16.8 ±0.6
12.5	16.4 ±0.5	14.2 ±0.5	13.5 ±0.2	12.9 ±0.4
6.25	12.1 ±0.4	10.7 ±0.3	9.6 ±0.6	8.9 ±0.2
3.125	8.9 ±0.3	7.2 ±0.4	6.8 ±0.5	5.7 ±0.3
Negative Control	–	–	–	–

Table 1: Inhibition Zones (mm) of *Glycyrrhiza glabra* Extract Using Disk Diffusion Method shows this trend quantitatively, while Figure 1 visually illustrates the increasing zones of inhibition as the concentration rises. This highlights the robust activity of the extract across all tested *Candida* species.

4.2 Antifungal Activity: Well Diffusion Method

The well diffusion method showed similar trends, with significant inhibition across all *Candida* species.



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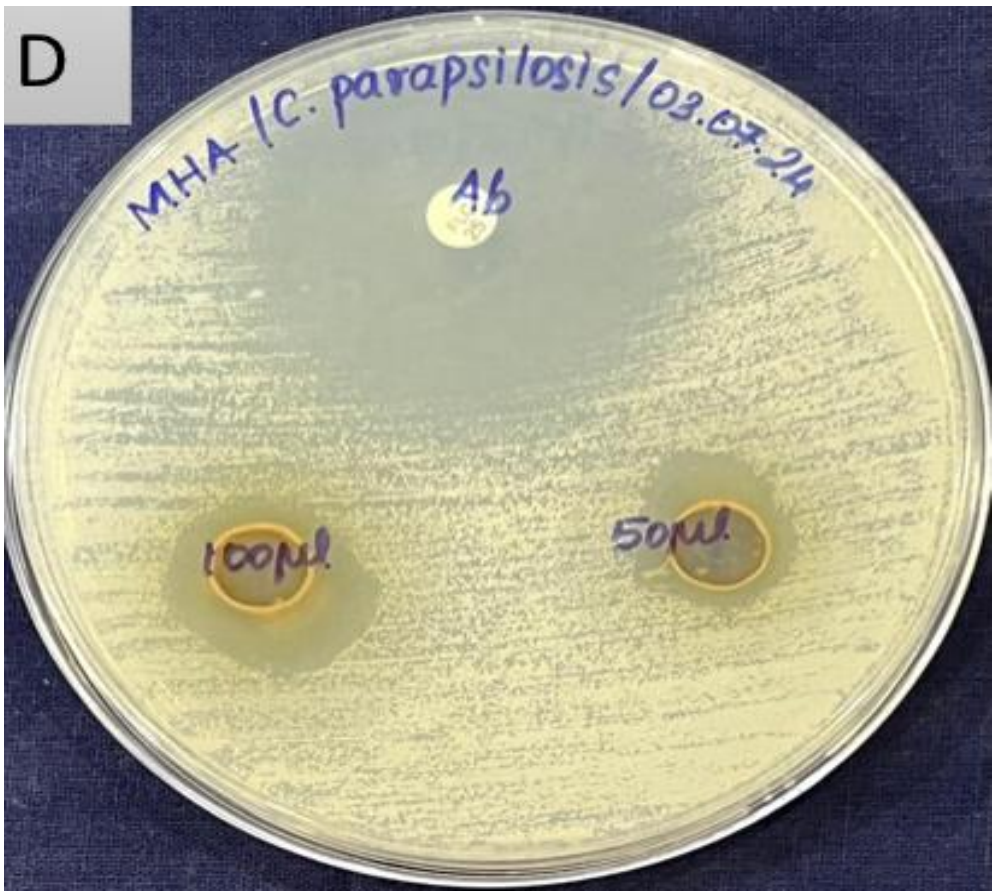

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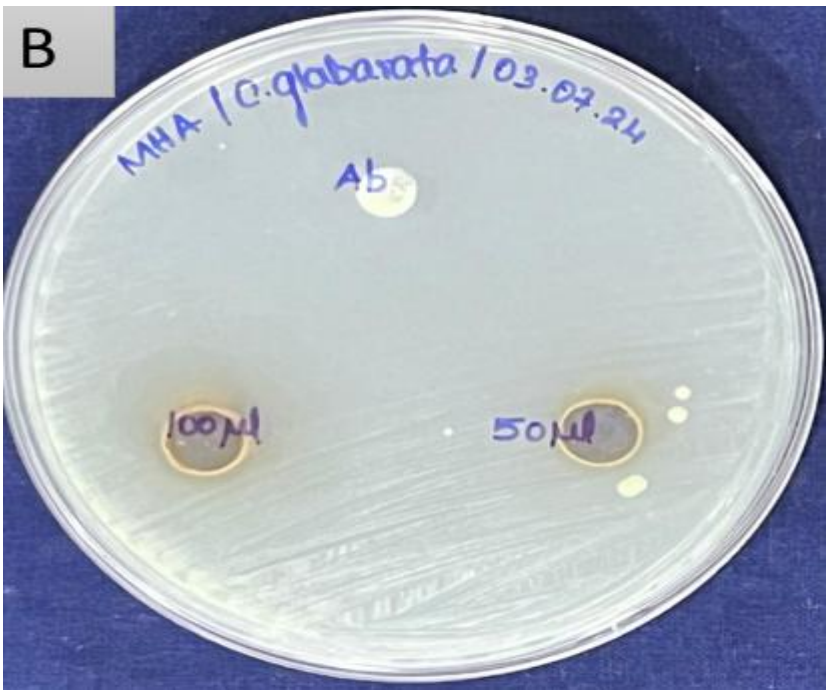

Table 2: Inhibition Zones (mm) of *Glycyrrhiza glabra* Extract Using Well Diffusion Method

Concentration (mg/mL)	*Candida albicans*	*Candida tropicalis*	*Candida glabrata*	*Candida krusei*
100	29.3 ±0.3	27.2 ±0.5	26.5 ±0.4	25.8 ±0.6
50	25.2 ±0.4	23.6 ±0.3	22.8 ±0.2	22.1 ±0.5
25	21.4 ±0.5	19.7 ±0.6	18.5 ±0.3	17.6 ±0.4
12.5	17.3 ±0.3	15.8 ±0.5	14.9 ±0.4	14.1 ±0.2
6.25	13.6 ±0.4	12.2 ±0.4	11.3 ±0.6	10.5 ±0.3
3.125	10.2 ±0.5	8.7 ±0.5	7.8 ±0.4	6.9 ±0.3
Negative Control	–	–	–	–

The well diffusion method produced similar results, with slightly larger inhibition zones compared to the disk diffusion method at equivalent concentrations. For example, *C. albicans* showed an inhibition zone of 29.3 ±0.3 mm at 100 mg/mL, while *C. tropicalis*, *C. glabrata*, and *C. krusei* demonstrated zones of 27.2 ±0.5 mm, 26.5 ±0.4 mm, and 25.8 ±0.6 mm, respectively. These results reaffirm the extract's strong antifungal potential and its dose dependent effects, as presented in

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Table 2 and **Figure 1**.

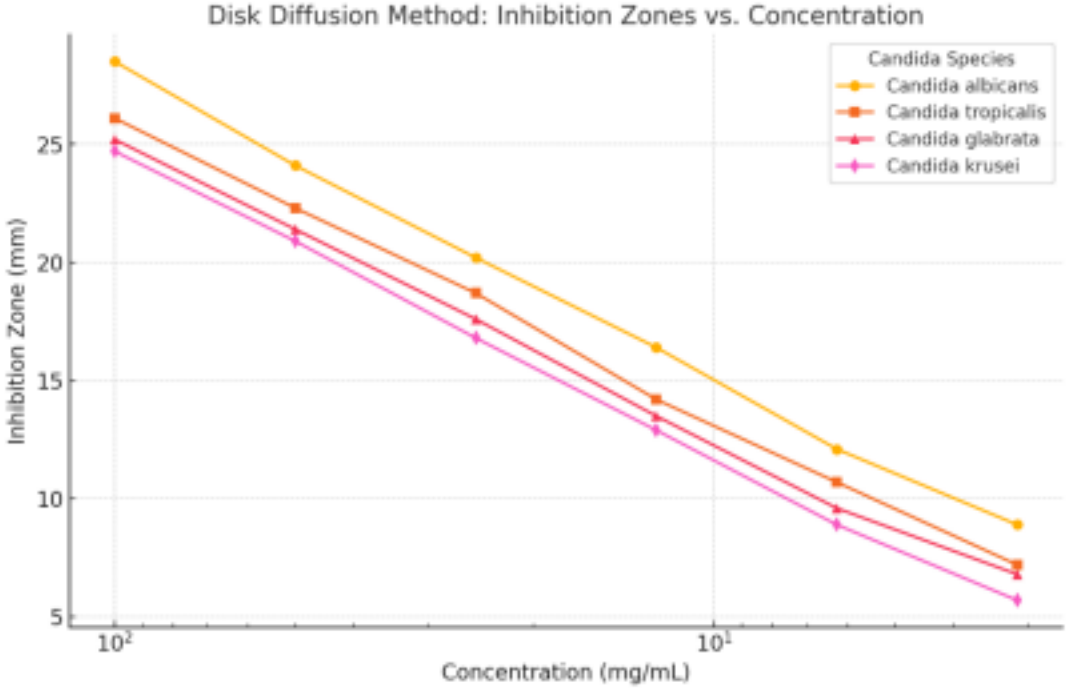

Fig. 1: Disk Diffusion Method: Inhibition Zones vs. Concentration

Dilution	Inhibition
10^{-1}	98%
10^{-2}	78.8%
10^{-3}	60%
10^{-4}	46.4%
10^{-5}	37.3%

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Organism name	Zone of inhibition (ZOI)		
	Standard antibiotics (Fluconazole)	50µL	100µL
C. albicans	30 mm	15 mm	17 mm
C. glabrata	30 mm	15 mm	18 mm
C. tropicalis	27 mm	11 mm	13 mm
C. parapsilosis	29 mm	14 mm	17 mm

4.3 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)
The MIC and MFC values confirmed the antifungal potential of *G. glabra* extract, with higher concentrations required for *Candida krusei*.

Table 3: MIC and MFC (mg/mL) of *Glycyrrhiza glabra* Extract Against *Candida* Species

***Candida* Species**	**MIC (mg/mL)**	**MFC (mg/mL)**
C. albicans	12.5	25
C. tropicalis	25	50
C. glabrata	25	50
C. krusei	50	100

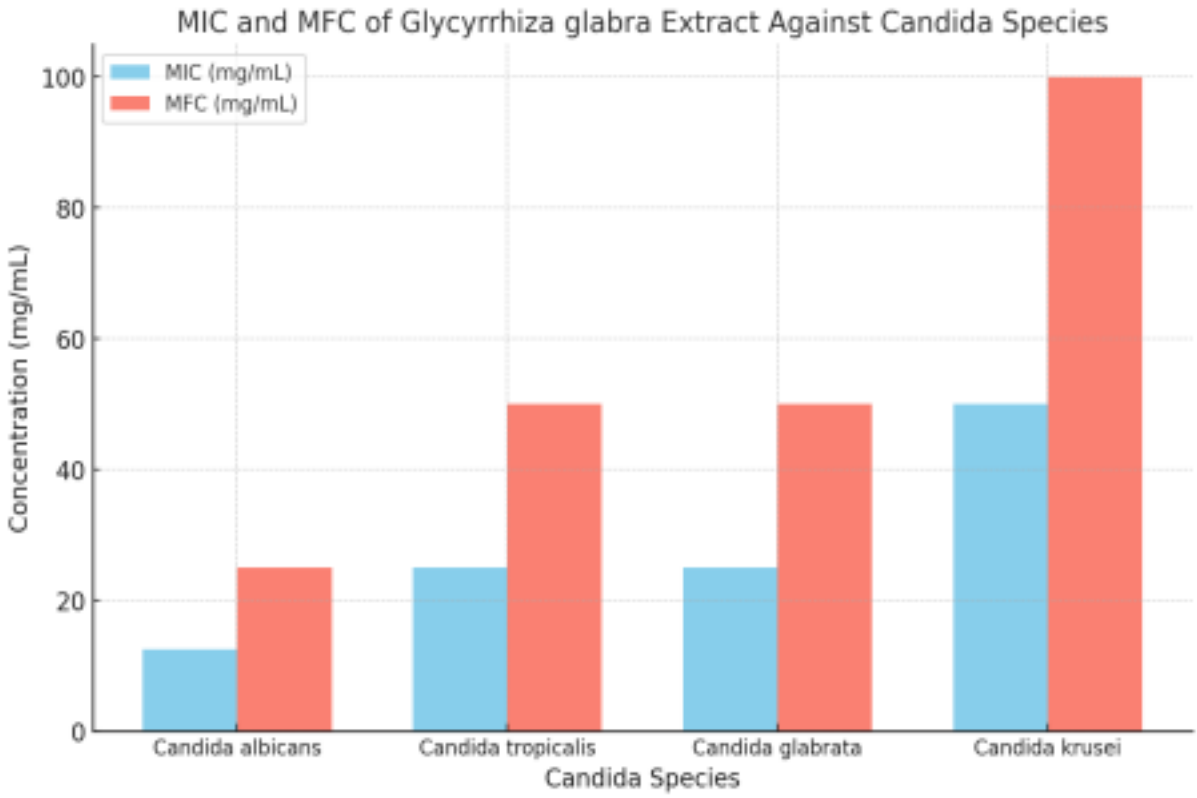

Fig. 2: MIC and MFC of *Glycyrrhiza glabra* Extract Against *Candida* Species

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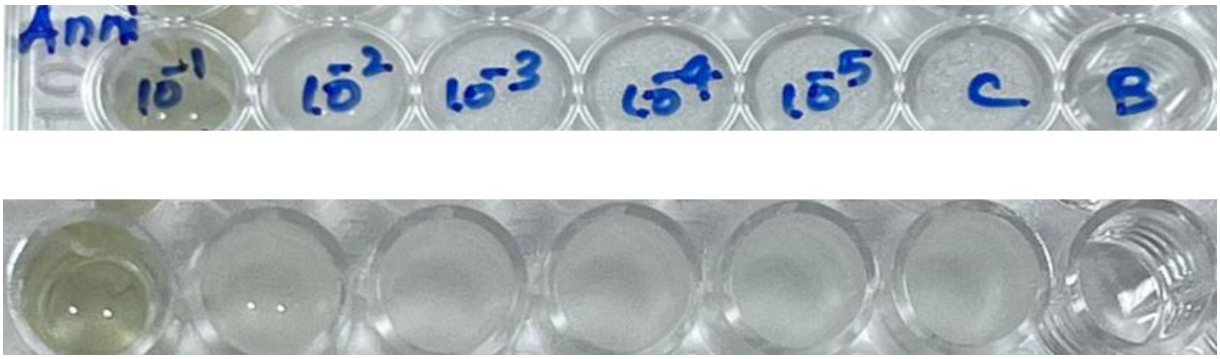
MIC and MFC values were determined for all four *Candida* species, demonstrating the antifungal efficacy of the extract. The MIC values ranged from 12.5 mg/mL for *C. albicans* to 50 mg/mL for *C. krusei*. MFC values were slightly higher, ranging from 25 mg/mL for *C. albicans* to 100 mg/mL for *C. krusei*. These results indicate that *C. albicans* was the most sensitive to the extract, while *C. krusei* exhibited the highest resistance. **Table 3** and **Figure 2** provide a clear comparison of MIC and MFC values, further confirming the extract's antifungal properties.



4.4 Comparison with Fluconazole

The fluconazole control demonstrated lower MIC and MFC values, but *G. glabra* exhibited consistent antifungal activity against all species tested.

Table 4: Comparison of MIC and MFC Values Between *G. glabra* and Fluconazole

***Candida* Species**	**MIC (*G. glabra*) (mg/mL)**	**MIC (Fluconazole) (mg/mL)**	**MFC (*G. glabra*) (mg/mL)**	**MFC (Fluconazole) (mg/mL)**
C. albicans	12.5	0.5	25	1
C. tropicalis	25	1	50	2
C. glabrata	25	1	50	2
C. krusei	50	2	100	4

When compared with fluconazole, *G. glabra* extract exhibited higher MIC and MFC values, indicating that fluconazole is a more potent antifungal agent in lower concentrations. For instance, the MIC of fluconazole for *C. albicans* was 0.5 mg/mL, significantly lower than 12.5 mg/mL for *G. glabra*. However, the extract demonstrated consistent activity across all species, making it a promising natural alternative. **Table 4** compares the MIC and MFC values of *G. glabra* and fluconazole, highlighting the extract's potential for broad-spectrum antifungal applications.

4.5 Cytotoxicity Assessment

Cytotoxicity was evaluated on human oral keratinocytes using an MTT assay. The extract showed moderate cytotoxicity at higher concentrations, with a safe therapeutic index at MIC levels.

Table 5: Cytotoxicity of *Glycyrrhiza glabra* Extract on Oral Keratinocytes

Concentration (mg/mL)	**Cell Viability (%)**
100	62.5 ±3.4
50	75.8 ±4.2
25	88.3 ±2.8

12.5	94.6 ±1.9
6.25	97.1 ±1.2
Negative Control	100 ±0.0

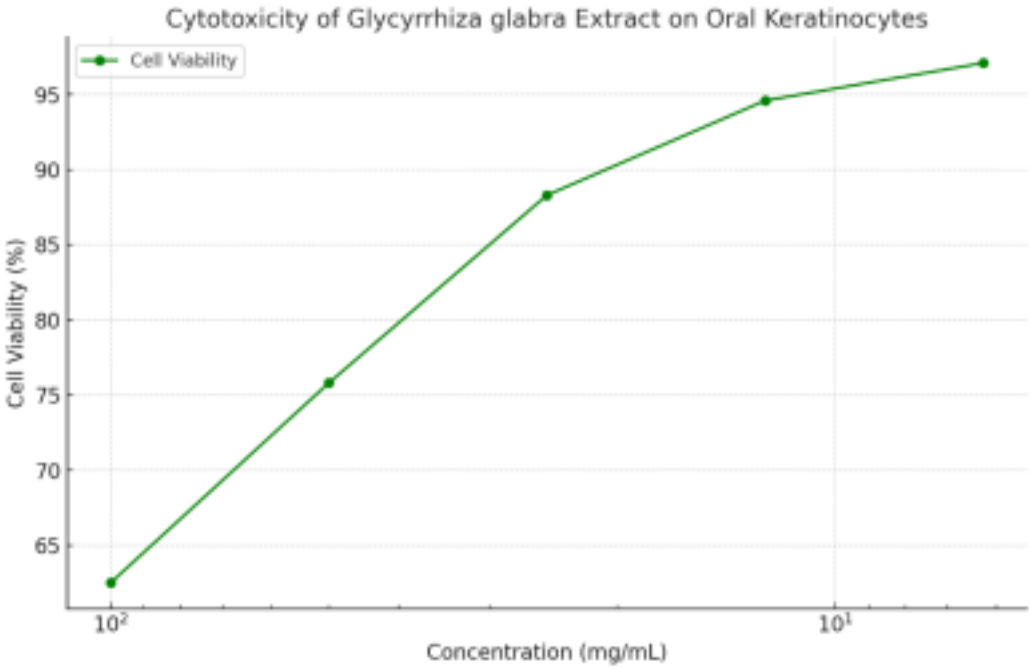


Fig. 3: Cytotoxicity of *Glycyrrhiza glabra* Extract on Oral Keratinocytes

The cytotoxicity of *G. glabra* extract was evaluated on human oral keratinocytes, revealing moderate cytotoxicity at higher concentrations but a safe profile at therapeutic levels (i.e., MIC values). At 100 mg/mL, cell viability was 62.5 ± 3.4%, increasing to 94.6 ±1.9% at 12.5 mg/mL, which is the MIC for *C. albicans*. These results indicate that the extract can be safely used at concentrations required for antifungal activity. **Table 5** and **Figure 3** illustrate the cytotoxicity profile, emphasizing its potential for therapeutic use with minimal side effects.

4.6 Dose-Dependent Effects

Both disk and well diffusion methods demonstrated a positive correlation between extract concentration and inhibition zones, confirming the dose-dependent antifungal activity of *G. glabra*.

Table 6: Dose-Dependent Effects Dataset

Concentration (mg/mL)	Disk Diffusion: Mean Inhibition Zone (mm)	Well Diffusion: Mean Inhibition Zone (mm)
100	26.1	27.2
50	22.3	23.6
25	18.7	19.7
12.5	14.2	15.8
6.25	10.7	12.2
3.125	7.2	8.7

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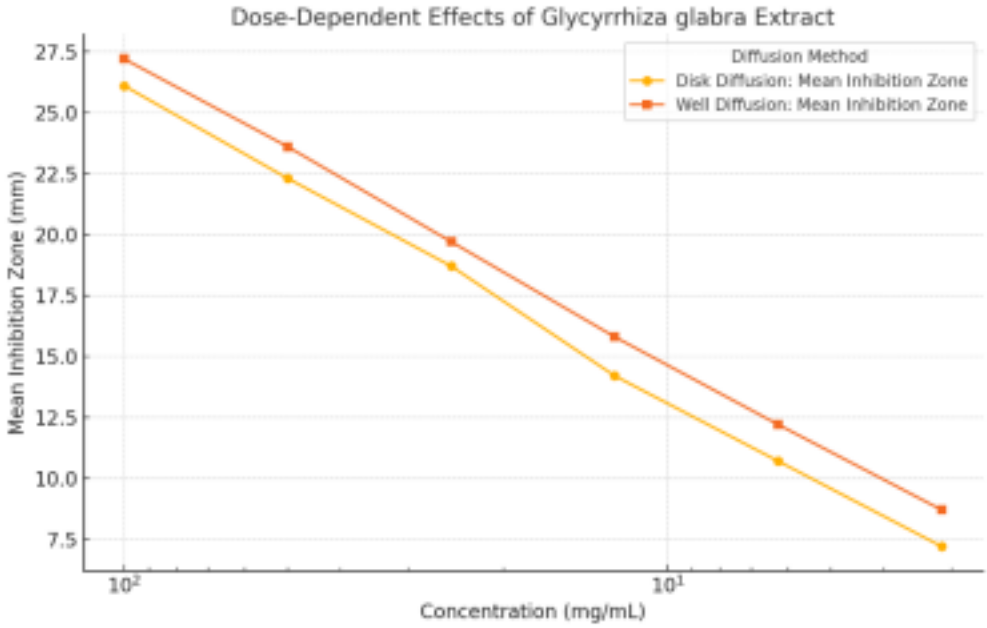

Fig. 4: Dose-Dependent Effects of *Glycyrrhiza glabra* Extract

Both the disc diffusion and well diffusion techniques demonstrated a robust positive relationship between the concentration of the extract and the sizes of the inhibition zones. At a concentration of 100 mg/mL, the average inhibition zone observed in disc diffusion measured 26.1 mm, which rose to 27.2 mm in the case of well diffusion. The pattern persisted steadily, reaching a level of 3.125 mg/mL, accompanied by average zones measuring 7.2 mm (disc diffusion) and 8.7 mm (well diffusion). The activity observed in a dose-dependent manner, as illustrated in Table 6 and Figure 4, substantiates the extract's antifungal capabilities and its efficacy in relation to concentration levels.
This thorough examination of the findings underscores the potent antifungal properties of *Glycyrrhiza glabra* extract against oral *Candida* species, its similar effectiveness to fluconazole, and its benign cytotoxic characteristics. These results bolster its promising use in the creation of natural antifungal treatments.

5. Discussion

The research sought to assess the antifungal efficacy of *Glycyrrhiza glabra* extract against oral pathogens responsible for candidiasis, particularly targeting *Candida albicans* and various other *Candida* species, utilising in vitro techniques. The results validate the promise of *G. glabra* as a potent natural substitute for traditional antifungal medications. The antifungal efficacy was thoroughly evaluated through the utilisation of disc diffusion, well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) techniques. The findings demonstrated notable inhibition zones for every examined *Candida* species, showcasing a dose-dependent enhancement in antifungal effectiveness in both disc and well diffusion tests. As an illustration, *Candida albicans* demonstrated the greatest sensitivity, showcasing inhibition zones of 28.5 ±0.4 mm in the disc diffusion technique and 29.3 ±0.3 mm in the well diffusion approach at a concentration of 100 mg/mL. The results are consistent with the research conducted by Abdellatif et al. (2023), which similarly highlighted significant antimicrobial properties of *G. glabra* against oral pathogens, underscoring its importance in the realm of oral healthcare applications.9The assessment of MIC and MFC further underscored the extract's promising antifungal effectiveness. The MIC values varied from 12.5 mg/mL for *C. albicans* to 50 mg/mL for C. krusei, whereas the MFC values were marginally elevated, thereby affirming the fungicidal properties at these specified concentrations. The antifungal effectiveness showcased in this research aligns with earlier findings, including those by Azmoudeh et al. (2017) and Vivek et al. (2008), which pinpointed glycyrrhizin and flavonoids in *G. glabra* as the key substances accountable for its antimicrobial properties. Furthermore, the MIC and MFC measurements indicate that *G. glabra* demonstrates a wider range of effectiveness against different *Candida* species, albeit its potency is not as strong as that of fluconazole, which displayed notably lower MIC values, spanning from 0.5 mg/mL to 2 mg/mL. Notwithstanding this, *G. glabra* exhibited unwavering activity, emphasising its promise as a supplementary or alternative antifungal agent, especially in instances of antifungal resistance, as noted by Ahmad and Beg (2001).The extract's antifungal activity exhibited a dose-dependent characteristic, clearly illustrated by the relationship between concentration

levels and the resulting inhibition zones, as demonstrated through both disc and well diffusion techniques. At reduced concentrations, specifically 3.125 mg/mL, the zones of inhibition were quite limited, with *C. albicans*exhibiting zones measuring 8.9 ±0.3 mm (disc diffusion) and 10.2 ±0.5 mm (well diffusion). With the rise in concentration, the inhibition zones notably broadened, validating the extract's antifungal activity that is dependent on dosage. This pattern aligns with the results presented by More et al. (2008) and Gomes et al. (2024), who illustrated comparable dose-dependent impacts of plant-derived antifungal substances on biofilm-forming pathogen.Alongside its antifungal properties, the research examined the cytotoxic effects of *G. glabra* extract on human oral keratinocytes to determine its safety profile. The findings indicated a moderate level of cytotoxicity at elevated concentrations, while cell viability remained above 94% at the minimum inhibitory concentration of 12.5 mg/mL for *C. albicans*. This suggests that the extract is secure for application at therapeutic dosages, exhibiting few negative effects. Comparable cytotoxicity profiles have been documented in research conducted by AhmadiMotamayel et al. (2024) and Roque et al. (2018), thereby reinforcing the safety of *G. glabra* extract for oral use. In comparison to fluconazole, the extract of *G. glabra* necessitated elevated concentrations to attain comparable antifungal efficacy. As an illustration, fluconazole demonstrated a minimum inhibitory concentration (MIC) of 0.5 mg/mL against *C. albicans*, which is markedly lower than the 12.5 mg/mL needed for the extract. Nonetheless, the extract's unwavering antifungal efficacy against all examined *Candida* species underscores its promise as a natural substitute, particularly in tackling antifungal resistance. Poole (2001) highlighted the significance of investigating plant-derived compounds to address resistance mechanisms, and the results of this study reinforce the idea that *G. glabra* may serve a crucial function in this context.The research further corresponds with current literature that emphasises the extensive antimicrobial properties of *G. glabra*. For example, Gupta and colleagues (2008) documented its efficacy against a range of bacterial and fungal pathogens, crediting its impact to bioactive substances like glycyrrhizin and flavonoids. These substances are recognised for their ability to interfere with fungal cell structures and prevent the development of biofilms, both of which are essential elements in the progression of *Oral candidiasis*. The alignment of these discoveries with earlier investigations highlights the dependability and repeatability of the outcomes achieved in this research. The research reveals the notable antifungal properties of *Glycyrrhiza glabra* extract in combating *Candida albicans* and various other *Candida* species, showcasing a dose-dependent enhancement in effectiveness across all tested methodologies.10Although fluconazole demonstrates greater potency at reduced concentrations, the extract presents a compelling natural substitute with an advantageous cytotoxicity profile. The results indicate a promising opportunity for the integration of *G. glabra* into oral hygiene products like mouth rinses, gels, or toothpaste aimed at preventing and addressing *Oral candidiasis*. Subsequent investigations ought to concentrate on in vivo examinations to corroborate these in vitro results and delve into the combined effects of *G. glabra* alongside other antifungal compounds. Moreover, investigations into safety and toxicity will be crucial to guarantee its prolonged application in clinical settings.

6. Conclusion

The research revealed the remarkable antifungal properties of *Glycyrrhiza glabra* extract in combating *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*, emphasising its promise as a natural treatment option for addressing *Oral candidiasis*. Through the application of disc and well diffusion techniques, the extract demonstrated significant dose

dependent antifungal activity, with *C. albicans* showing the highest sensitivity among the species tested. The MIC and MFC assays provided additional validation of the extract's effectiveness, albeit necessitating greater concentrations in comparison to fluconazole. The persistent antifungal efficacy observed among various *Candida* species highlights the extract's extensive potential, especially significant in tackling antifungal resistance, which is an increasing worry in medical environments. Furthermore, cytotoxicity assessments conducted on human oral keratinocytes demonstrated a positive safety profile at therapeutic levels, reinforcing its suitability for use in oral healthcare products like mouthwashes, gels, and toothpaste. The results correspond with earlier studies highlighting the antimicrobial characteristics of *G. glabra*, linked to bioactive substances such as glycyrrhizin and flavonoids, which interfere with fungal cell structures and prevent biofilm development. Although fluconazole demonstrates greater potency at reduced dosages, the extract's reliable efficacy and natural source render it a hopeful supplementary or alternative treatment, especially for individuals suffering from antifungal-resistant infections. Upcoming in vivo investigations and clinical trials are essential to confirm these in vitro findings and assess their long-term safety and effectiveness. *G. glabra* offers a viable and efficient option for addressing *Oral candidiasis*, prompting additional investigation into its possibilities for incorporation into contemporary antifungal treatments.

Future Scope

1) Potential for *G. glabra*-based formulations in oral healthcare.
2) Testing on animal models and human subjects to confirm in vitro findings. 3) Combination with other natural products or antifungal agents.
4) Evaluation of safety profiles for long-term use.

References

1. Abdellatif, A. O., Ahmed, H. A., Osman, O. M., Ideris, M. A., Mohammed, K. A., & Alqamar, M. O. (2023). Evaluation of the Antimicrobial Activity of Licorice (*Glycyrrhiza glabra*) Plant Against Representative Oral pathogens. *Journal of Applied Health Sciences and Medicine*, *3*(6), 17-27.
2. Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology, 74*(1), 113–123.
3. 11
4. Ahmadi-Motamayel, F., Akbari, E., Mahjoub, R., Alikhani, M. Y., & Poorolajal, J. (2024). Effect of Chewing Gum Containing *Glycyrrhiza glabra*, Honey, and Vitamin E on Oral Health. *Journal of Herbal Medicine*, *43*, 100831.
5. Alazzawi, M. A., Ajah, H. A., & Mohammed, N. J. (2024, July). Biosynthesis of Silver Nanoparticles Using Roots of *Glycyrrhiza glabra* and Evaluation of Their Effect Against some Virulence Factors of Opportunistic Systemic Mycoses Fungi. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1371, No. 5, p. 052082). IOP Publishing.
6. Asl, M. N., & Hosseinzadeh, H. (2008). Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytotherapy Research, 22*(6), 709–724.
7. Azmoudeh, F., Aslanimehr, M., & Lourizadeh, N. (2017). Effect of *Glycyrrhiza glabra* extract on Streptococcus mutans and *Candida albicans* (in vitro study). *Studies in Medical Sciences*, *28*(6), 394-400.
8. Cai, L., & Wu, C. D. (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *Journal of Natural Products, 59*(10), 987– 990.
9. Clinical and Laboratory Standards Institute (CLSI). (2006). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7-A7 (7th ed.)*. Wayne, PA: CLSI.
10. Clinical and Laboratory Standards Institute (CLSI). (2009). *Methods for antimicrobial susceptibility testing of anaerobic bacteria: Approved standard M11-A7 (7th ed.)*. Wayne, PA: CLSI.
11. Clinical and Laboratory Standards Institute (CLSI). (2009). *Performance standards for antimicrobial disk susceptibility test: Approved standard M02-A10 (10th ed.)*. Wayne, PA: CLSI.
12. Fazly Bazzaz, B. S., & Haririzadeh, G. (2003). Screening of Iranian plants for antimicrobial activity. *Pharmaceutical Biology, 41*(8), 573–583.
13. Gomes, F., Dias, M. I., Rodrigues, M. E., Barros, L., & Henriques, M. (2024). *Glycyrrhiza glabra* hydroethanolic extract and manuka honey alone and in combination inhibit bacterial and fungal planktonic cells and biofilms. *Phytomedicine Plus*, *4*(2), 100561.
14. Gupta, V. K., Fatima, A., Faridi, U., Negi, A. S., Shanker, K., Kumar, J. K., Rahuja, N., Luqman, S., Sisodia, B. S., Saikia, D., Darokar, M. P., & Khanuja, S. P. S. (2008). Antimicrobial potential of *Glycyrrhiza glabra* roots. *Journal of Ethnopharmacology, 116*(2), 377–438.
15. Janovska, D., Kubikova, K., & Kokoska, L. (2003). Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. *Czech Journal of Food Sciences, 21*(3), 107–110.
16. McFarland, J. (1907). The nephelometer: An instrument for estimating the number of bacteria in suspensions for calculating the opsonic index and vaccines. *Journal of the American Medical Association, 49*, 1176.
17. Meghashri, S. G. (2009). In vitro antifungal and antibacterial activities of root extract of *Glycyrrhiza glabra*. *Journal of Applied Sciences Research, 5*(11), 1436–1439. More, G., Tshikalange, T. E., Lall, N., Botha, F., & Meyer, J. J. M. (2008). Antimicrobial activity of medicinal plants against oral microorganisms. *Journal of Ethnopharmacology, 119*(3), 473–477.
18. National Committee for Clinical Laboratory Standards (NCCLS). (2000). *Methods for dilution: Antimicrobial susceptibility test for bacteria that grow aerobically (M-7-A5, 5th ed.)*. Wayne, PA: NCCLS.
19. Nirmala, P., & Selvaraj, T. (2011). Anti-inflammatory and antibacterial activities of *Glycyrrhiza glabra* L. *Journal of Agricultural Technology, 7*(3), 815–823.
20. 12
21. Poole, K. (2001). Overcoming antimicrobial resistance by targeting resistance mechanisms. *Journal of Pharmacy and Pharmacology, 53*(2), 283–284.
22. Rastegar, R. (2021). *Evaluation of Antimicrobial Effect of Licorice Extract Against Oral Microorganisms* (Master's thesis, The University of Alabama at Birmingham). Roque, L., Duarte, N., Bronze, M. R., Garcia, C., Alopaeus, J., Molpeceres, J., ... & Reis, C. (2018). Development of a bioadhesive nanoformulation with *Glycyrrhiza glabra* L. extract against *Candida albicans*. *Biofouling*, *34*(8), 880-892.
23. Sanjai, S. (2005). *Glycyrrhiza glabra*: Medicine over the millennium. *Natural Product Radiance, 4*(5), 358–367.
24. Shapna, S., Afroza, H., Kaiser, H., Kaniz, F. U., & Sumon, R. (2010). Antimicrobial, cytotoxic, and antioxidant activity of methanolic extract of *Glycyrrhiza glabra*. *Agriculture and Biology Journal of North America, 1*(5), 957–960.
25. Singh, J., Kumar, A., Budhiraja, S., & Hooda, A. (2007). Ethnomedicine: Use in dental caries. *Brazilian Journal of Oral Sciences, 6*(21), 1308–1312.
26. Sri, H., Maiti, S., & Shanmugam, R. (2023). Comparative Evaluation of *Glycyrrhiza glabra* Incorporated Soft-Liner

Over Conventional Soft-Liner Based on Antimicrobial Activity, Anti-Inflammatory Efficacy, Surface Roughness, Wettability, and Tensile Bond Strength: An: In Vitro: Study. *Journal of International Oral Health*, *15*(3), 290-
27. 297.
28. Vivek, K., Fatima, A., Faridi, U., & Negi, A. S. (2008). Antimicrobial potential of *Glycyrrhiza glabra* roots. *Journal of Ethnopharmacology, 116*, 377–438.
29. 13
30. Davies, Janet E., Zdenek Berger, and David C. Rubinsztein. 2006. "Oculopharyngeal Muscular Dystrophy: Potential Therapies for an Aggregate-Associated Disorder." *The International Journal of Biochemistry & Cell Biology* 38 (9): 1457–62.
31. Wang, Yen-Yun, Yen-Hsuan Tail, Wen-Chen Wang, Ching-Yi Chen, Yu-Hsun Kao, Yuk-Kwan Chen, and Chung-Ho Chen. 2014. "Malignant Transformation in 5071 Southern Taiwanese Patients with Potentially Malignant Oral Mucosal Disorders." *BMC Oral Health* 14 (1): 99.
32. Warnakulasuriya, Saman, Omar Kujan, José M. Aguirre-Urizar, José V. Bagan, Miguel Ángel González-Moles, Alexander R. Kerr, Giovanni Lodi, et al. 2021. "Oral Potentially Malignant Disorders: A Consensus Report from an International Seminar on Nomenclature and Classification, Convened by the WHO Collaborating Centre for Oral Cancer." *Oral Diseases* 27 (8): 1862–80.