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Original Research Article

Development of a Pharmaceutical Formulation Containing Clove (*Syzygium aromaticum*) Extract for the Management of Oral Candidiasis

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Abstract

***Introduction*:** Oral candidiasis is a common fungal infection caused by various yeast species, including *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata*. Clove, a well-known plant in traditional medicine, has been used as an antiseptic remedy for diverse infections, including those affecting the oral cavity induced by yeasts and bacteria. The emergence of resistance to numerous antifungal agents among *Candida* species necessitates exploring alternative treatments. Hence, this study aims to evaluate the antimicrobial activities of clove extract and develop a suitable pharmaceutical formulation containing clove extract for managing oral candidiasis. ***Method*:** The antimicrobial culture and sensitivity test were conducted using the agar-well diffusion assay, evaluating different concentrations of clove extract against *Candida albicans*. The minimum effective concentration was then formulated into a gel dosage form, and its antimicrobial activity was assessed, comparing it with miconazole oral gel. ***Result*:** The results showed that the prepared clove extract gel had antifungal effects on *Candida albicans* that were similar to those of the miconazole oral gel. The zone of inhibition for the clove extract gel was measured at 25 mm, while the miconazole gel showed a zone of inhibition of 27 mm. ***Conclusion*:** The results of this study show that clove extract gel may be an effective antifungal agent, especially against *Candida albicans*. This suggests that it may be a promising herbal alternative to conventional medicines for treating oral candidiasis.

Keywords: Clove extract, Pharmaceutical formulation, Oral gel, Oral candidiasis.

1. INTRODUCTION

A variety of microorganisms that live in the oral cavity are crucial to sustaining oral health (Zhang *et al.*, 2018). The oral microbiome is composed of bacteria, fungi, viruses, and other microorganisms that interact with each other and with the host to maintain a healthy balance, which is essential for maintaining oral health by preventing colonization of pathogenic microorganisms and promoting the development of a healthy immune system (Sharma *et al.*, 2018; Zaura *et al.*, 2014).

Oral candidiasis is one of the most common fungal infections in the oral cavity and is often caused by yeast species such as *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata* (Dangi *et al.*, 2010; Muadcheingka & Tantivitayakul, 2015). They generally exist on the skin and within the body without harmful effects, but if the environment in the mouth, throat, or esophagus changes in a manner that encourages their growth and leads to an oral candidiasis infection (Telles *et al.*, 2017).

Increasing microbial resistance to conventional antimicrobial treatments has led to a rising interest in researching plant-based natural active ingredients with antimicrobial properties (Abdellatif *et al.*, 2022). Historically, many cultures have utilized plants to treat a range of diseases, including microbial infections. Plants have represented a good alternative and potential source of new antimicrobial agents and have been used for centuries in traditional medicine in many countries to treat various diseases, including microbial infections (Khan & Ahmad, 2019). These natural resources house a plethora of bioactive compounds capable of combating a

wide array of microbes, including bacteria, fungi, and viruses (Cowan, 1999; Vaou *et al.*, 2021).

The benefits of using plant-derived antimicrobial agents over synthetic drugs include fewer side effects, lower costs, easy availability, and generally safe usage (Anand *et al.*, 2019; Swamy, 2020). The presence of intrinsic and developed resistance against many antifungal agents has been extensively documented among several *Candida* species, and this opens the doors for the development of alternative drugs and remedies that could be more efficient than those already in use (Ben-Ami & Kontoyiannis, 2021).

Clove (*Syzygium aromaticum*) is an aromatic flower that belongs to the family Myrtaceae. Clove has been used in traditional medicine as an antiseptic for many infections, including those of the oral cavity caused by many yeasts and bacteria (Diego & Wanderley, 2014; Idowu *et al.*, 2021). The goal of this study was to find out how clove extract works against yeast and to come up with an advanced pharmaceutical formulation that includes clove extract in a good drug delivery system that can be used in the mouth to treat oral candidiasis.

2. METHODOLOGY

Plant Materials Acquisition and Authentication

The pods of the clove tree (*Syzygium aromaticum*) were obtained from the local market in Omdurman, Sudan. The plant pods were identified and authenticated by the medicinal and aromatic plants and traditional medicine research institute. A voucher specimen was deposited in the herbarium of the Pharmacognosy Department, College of Pharmacy, Karary University, Sudan.

Bacterial and Fungal Cultures:

Clinical isolates of *Candida albicans* (CLMSA0002243) were used to test the antifungal effects of the clove extract. *Candida albicans* is the most common species found in both infected and healthy oral environments. The isolates were obtained from the Microbiology Department in the Central Lab of the Medical Services Administration Hospital in Khartoum.

Extraction and Fractionation:

The pods of the clove tree were subjected to drying at 40 °C and subsequently ground. The extraction process involved using 97% ethanol in a flask for 24 hours. The solvent was then drained, and a second extraction with 97% ethanol was carried out for another 24 hours. The obtained extracts were pooled, filtered using a cotton filter, and concentrated at 65 °C using a rotary evaporator (Biobase, China). To maintain its integrity, the extract was stored in airtight containers, placed in the dark, and refrigerated to prevent any chemical degradation or contamination.

Screening for Antifungal Activities of Clove Extract:

The agar well diffusion method was employed to determine the antifungal potential of the clove extract. A 6 mm well was loaded with 25 µL of the clove extract (20 mg/ml). Pure colonies of each microbial culture were meticulously selected from 48–72-hour-old cultures grown on Sabouraud dextrose agar. These selected colonies were suspended in sterile normal saline solution. According to the method described by Ramadan and his co-authors, 25 microliters of each microbial suspension (1–5 x 10^6 cells/ml) were uniformly spread on the surface of Sabouraud dextrose agar plates. Following inoculation, the plates were incubated at 37 °C for 48 hours to facilitate fungal growth. To determine the minimum effective concentration for developing the targeted pharmaceutical formulation, four different concentrations (25%, 50%, 75%, and 100%) of the clove extract were tested (Ramadan *et al.*, 2020). The inhibition zones were measured in millimeters, and the results were recorded as the mean ± SD of triplicate experiments. A positive control, Amphotericin B (5 mg), was utilized for comparison, and cultured species producing halos that were 13 mm or larger were considered sensitive.

Development of Clove Extract Oral Gel Pharmaceutical Formulation:

The clove extract oral gel formulation was prepared as follows: 15 ml of glycerol was mixed with five grams of carboxymethylcellulose (CMC), which acts as a gelling agent. Subsequently, 25 ml of vanillin as a flavoring agent and sucrose syrup as a sweetening agent were gradually added while mixing. Additionally, 0.5 g of benzoic acid was included as a preservative. A total volume of 25 ml of clove extract emulsified with polysorbate 20 (0.2%) was then added, and the formulation's total volume was increased to 100 ml using distilled water.

Assessment of clove extract oral gel homogeneity through Measurement of Mean Particle Size

The particle size, zeta potential, and polydispersity index (PDI) of the prepared samples were measured using dynamic light scattering (DLS) with a Zetasizer Nano ZS apparatus (Malvern Panalytical Ltd., Malvern, UK). To avoid multiple scattering effects, the samples were diluted, and two ml samples were placed into disposable polystyrene cuvettes at 20 °C. The measurements were performed in triplicate, and the PDI, a dimensionless measure of the width of the size distribution, was calculated from the cumulant analysis.

pH determination of clove extract oral gel

The pH measurements of the formulations were performed in triplicate using a calibrated digital pH meter (Denver Instruments, Bohemia, NY, USA) at 25 °C.

Evaluation of Clove Extract Oral Gel Effectiveness:

To assess the antifungal effectiveness of the developed clove extract oral gel compared to miconazole oral gel, freshly subcultured *C. albicans* isolates were suspended in 10 mL of sterile saline to achieve a turbidity of 0.5 McFarland standard. Sabouraud dextrose agar plates were inoculated with *C. albicans* suspension using a sterile swab. The miconazole oral gel was obtained from the local Sudanese market for comparison purposes.

The standard agar-well diffusion method was used, with preparations being diluted with distilled water to achieve a concentration of 25% w/v to simulate clinical effectiveness in oral formulations diluted by saliva. Each well was filled with 25 µL of the diluted gel solutions, and sterile distilled water was added as a negative control. Following incubation at 35 °C for 48 hours, the antifungal activity was determined by measuring the diameter of the zones of inhibition (mm). All plates were prepared in triplicate, and the results were expressed as mean ± SD.

3. RESULTS

Selection of the Gelling Agent and Determination of Extract pH:

The pH of the clove extract was evaluated to identify an appropriate gelling agent for the oral formulation. The test revealed an acidic pH value of 2.7 on the pH meter. Accordingly, carboxymethylcellulose (CMC), which is compatible with the acidic pH, was chosen as the gelling agent for the clove extract gel.

Selection of the Minimum Effective Concentration of Clove Extract against *C. albicans*:

The anticandidal activity of various concentrations of clove extract against *C. albicans* was assessed using the agar-well diffusion method. The results, displayed in Table 1 and Figure 1, demonstrate the zones of inhibition in millimeters for each concentration.

Table 1: Anticandidal Effect of Different Clove Extract Concentrations against *C. albicans*

Extract Concentration (%)	Zone of inhibition in mm
25	25 ± 1.0
50	25 ± 1.4
75	27 ± 1.4
100	29 ± 1.1

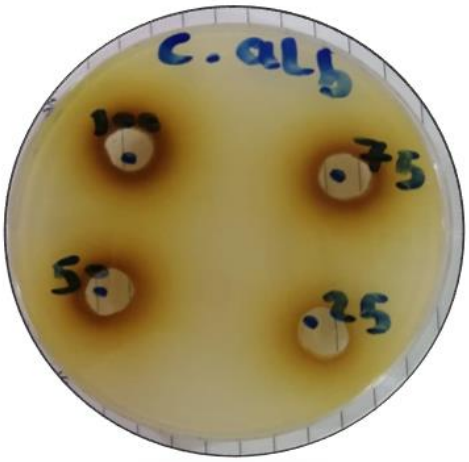

Figure 1: Photographic representation of the zone of inhibition of clove extract against *Candida albicans* on a petri dish

In terms of the negative control, DMSO (1%) showed no antimicrobial activity against *C. albicans.* Based on the preliminary antimicrobial results, the minimum effective concentration of the clove extract was found to be 25%, which was chosen for the development of the gel formulation.

Assessment of clove extract oral gel homogeneity through Measurement of Mean Particle Size

The developed clove extract gel formulation demonstrated excellent stability and uniformity. The results obtained from measurements of triplicate samples of the prepared gel showed a PDI equal to 0.15 0.03, which indicates a monodispersed population distribution of particle sizes.

Determination of the pH of the Developed Clove Extract Oral Gel:

A pH test was conducted to confirm the suitability of the gel formulation for the oral cavity's pH range (6.7–7.3). The findings indicated that the developed formulation exhibited a pH value of 6.5, which aligns well with the requirements for oral cavity formulations.

Anticandidal Activity of the Developed Formulation compared to Miconazole Oral Gel:

A test was done to see how well the clove extract gel worked against *C. albicans* compared to miconazole oral gel, which is a commercially available anti-fungal oral gel. The results showed that the developed formulation had a promising effect against yeast when compared to the oral miconazole gel (Table 2 and Figure 2). These results suggest that the developed clove extract gel holds promise as an effective anti-fungal agent, comparable to the commercial miconazole oral gel, and may serve as a potential alternative treatment for oral candidiasis.

Table 2: Antimicrobial Activity of Clove Extract Formulated Gel and Miconazole Oral Gel against *Candida albicans*

Tested oral gel formulation	Activity against *C. albicans* is represented as a zone of inhibition in mm.
Clove extract formulated gel	25 ± 1.0
Miconazole oral gel	27 ± 1.0

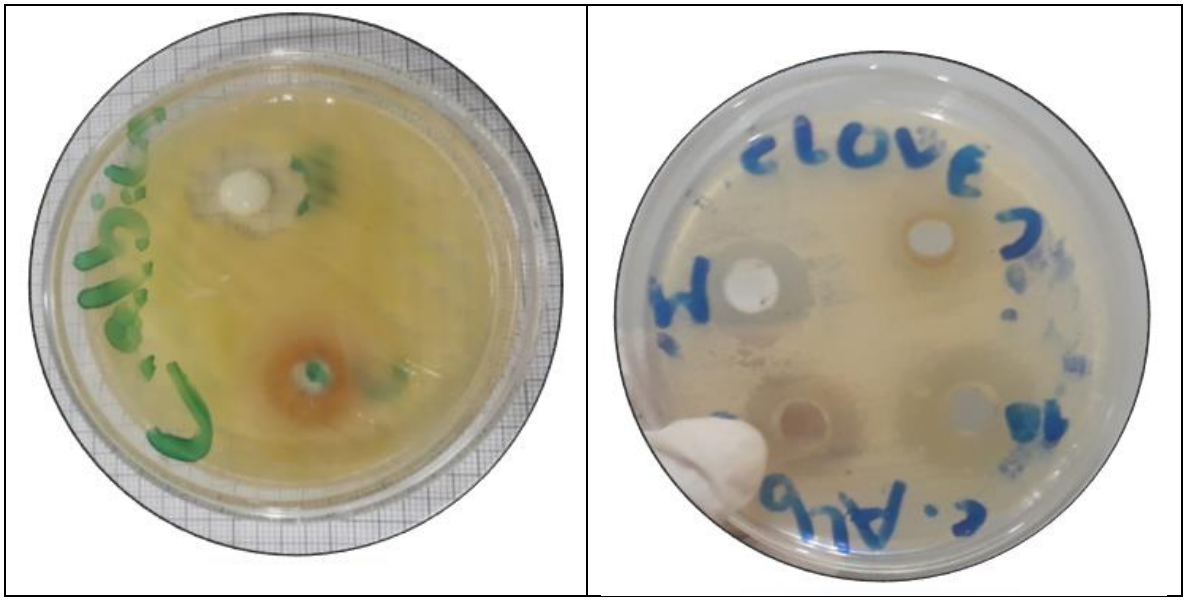

Figure 2: Photographic representation of inhibition zones of clove extract and miconazole oral gel formulations against *Candida albicans* on a petri dish

4. DISCUSSION

Clove (*Syzygium aromaticum*) is often used as a spice, flavoring, or scent in consumer goods like toothpaste, soaps, or cosmetics (Sachan *et al.*, 2018). Clove has been selected in this study due to its widespread use in traditional medicine around the world for managing oral infections, thanks to its high essential oil content and accessibility.

The present study's objective was to create a pharmaceutical formulation using clove extract for the treatment of oral candidiasis. We conducted an *in vitro* test to determine its antimicrobial action against *C. albicans*, and we compared this new formulation with an existing oral antifungal gel that contains miconazole.

Antifungal medications, predominantly azoles and polyenes, are the standard treatment for fungal infections (Segal & Elad, 2018). However, azoles often lead to side effects, and there is an increasing prevalence of drug-resistant strains, which presents ongoing clinical challenges (Revie *et al.*, 2018).

Candida albicans is the most common member of the human gut microbiota and is estimated to be present in 40–60% of the general population (Pérez, 2021). They may be present as transient or permanent colonizers in the oral cavity and in other parts of the gastrointestinal tract, where they are capable of causing diseases (Nobile & Johnson, 2015).

In a comparison with miconazole gel, the developed clove extract gel displayed promising antimicrobial action against *C. albicans*. Surprisingly, its effectiveness was on par with the miconazole gel.

The clove extract primarily contains eugenol, which has shown antimicrobial activity against several strains of pathogenic microorganisms, including fungi (Pavithra, 2014). It impedes ergosterol biosynthesis and disturbs the integrity and functionality of the cell membrane, leading to an impairment in membrane-bound enzymes involved in cell wall synthesis (de Oliveira Pereira *et al.*, 2013; Didehdar *et al.*, 2022). This damage increases permeability, eventually resulting in cell death. Eugenol is also reported to inhibit the rates of germination and elongation of *C. albicans*, along with

slowing down cell growth and proliferation, causing treated *Candida* cells to become deflated (Latifah-Munirah *et al.*, 2015).

Local drug delivery systems have long been used, especially in treating diseases of the oral cavity (Sankar *et al.*, 2011). Although some oral infections, such as candidiasis, are often extremely responsive to local therapy, the mouth often presents various difficulties because of limitations due to saliva and the mouth's functions, leading to a short retention time of dosage forms, thereby reducing therapeutic efficacy (Lou *et al.*, 2023). Advanced drug delivery systems like nano-emulsions and gels are thought to be a solution, especially for hydrophobic compounds like clove extract (Lou *et al.*, 2023; Nguyen & Hiorth, 2015; Rehman & Zulfakar, 2014).

Making the clove extract into a gel has many benefits, such as better drug delivery through the skin and percutaneously. It also avoids problems with drug absorption in the stomach caused by a low pH. Gels can also evade enzymatic activity and drug interactions with food and beverages. They offer an alternative route for drug administration, avoiding the first-pass effect that occurs after gastrointestinal absorption(Tangri & Madhav, 2011).

5. CONCLUSION

In terms of how well clove extract oral gel works to treat oral candidiasis compared to miconazole oral gel, the results of this study suggest that the new formulation is promising to treat oral candidiasis and could be a better alternative therapy. However, further research is needed to investigate the active components in vivo using different disease models, develop their application for the pharmaceutical industry, and figure out the exact molecular mechanisms and targets for stopping microbial growth. The findings from this study enhance our understanding of the great importance of natural products. The main limitation of this study was the absence of clinical trials. The results of this research have a number of significant ramifications for pharmaceutical formulation development in the future, particularly as they relate to the treatment of oral candidiasis. Therefore, it will help open up new avenues for herbal remedies with fewer side effects than modern drugs and encourage the use of herbal medicines to fight microbial resistance with fewer side effects than modern drugs.

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CONFLICT OF INTEREST

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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