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Therapeutic Potential of Clove *Syzygium aromaticum* in Managing Vaginal Candidiasis Caused by *Candida albicans* among the Odisha Population

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Abstract

Vulvovaginal candidiasis (VVC), commonly known as a vaginal yeast infection, is a widespread fungal condition caused primarily by Candida albicans, affecting women globally. It's becoming more prevalent in Odisha. Increasing antifungal resistance highlights the need for alternative treatments, particularly plant-based remedies. This study investigates the therapeutic potential of clove (Syzygium aromaticum) extract in managing vaginal candidiasis. Fifty vaginal swabs were collected from symptomatic women at IMS-SUM Hospital, Odisha. Candida albicans was isolated using SDA and CHROMagar Candida, and confirmed by ITS region PCR. Antifungal susceptibility was assessed by disk diffusion on Mueller-Hinton agar with 2% glucose and methylene blue. Syzygium aromaticum was sourced locally, and its phytochemicals were screened using qualitative methods. The antifungal activity of clove extract was evaluated at 50, 100, and 200 mg/mL concentrations using the well diffusion method to determine its efficacy against Candida albicans isolates. Of 50 samples, 38 (76%) tested positive for Candida albicans. Antifungal testing showed fluconazole resistance was observed in 32% of isolates. Clove extract showed antifungal activity with zones of 14.2 ± 1.1 mm at 50 mg/mL, 19.6 ± 1.4 mm at 100 mg/mL, and 25.3 ± 1.7 mm at 200 mg/mL, showing dose-dependent effects. The study confirms antifungal potential of Syzygium aromaticum against Candida albicans, due to bioactive compounds like eugenol. The extract showed effectiveness against fluconazole-resistant strains. Clove extract demonstrated strong antifungal activity against Candida albicans, offering a potential remedy for resistant vaginal candidiasis. Future research should examine in vivo effects and clinical trials for efficacy. Integrating Syzygium aromaticum into treatments could provide an effective approach for managing recurrent vaginal candidiasis.

Keywords: Antifungal Resistance, *Candida albicans*, *Syzygium aromaticum*, Vaginal Candidiasis.

Introduction

Vaginal candidiasis is a common fungal infection that affects millions of women globally (1). This condition is primarily induced by Candida albicans, a yeast that typically resides harmlessly within the body but can become pathogenic under certain circumstances, such as hormonal fluctuations, antibiotic usage, pregnancy, diabetes, and compromised immune systems (2, 3). The increasing global resistance to treatments, especially those based on azoles, has raised concerns about recurring and persistent infections, underscoring the urgent need for alternative treatment options (4). Medicinal plants have attracted interest as potential antifungal solutions due to their bioactive components; with Syzygium aromaticum (Clove)

showing promise as a natural remedy for fungal infections (5). Clove, a commonly used medicinal spice, is recognized for its antimicrobial, antifungal, anti-inflammatory, and antioxidant qualities. The bioactive compound eugenol has shown notable antifungal effects against Candida species, including strains of Candida albicans resistant to azoles (6, 7). Research indicates that clove extract can disrupt fungal cell walls, prevent biofilm formation, and alter virulence factors, making it a viable alternative for treating drugresistant fungal infections (8, 9). Moreover, clovebased treatments may result in fewer side effects compared to traditional antifungal drugs, enhancing patient adherence and reducing the recurrence of VVC. Clove extract has garnered

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increasing attention as a promising antifungal agent, prompting investigation into innovative formulations for the treatment of VVC. Researchers are investigating various delivery methods, such as vaginal suppositories and topical creams, to optimize the effectiveness of clove-based therapies (10). Future research may focus on combining clove extract with other natural substances or existing antifungal drugs to create synergistic effects and further improve therapeutic outcomes. In Odisha, traditional herbal medicine is vital in primary healthcare, the use of clove for vaginal infections has been documented in ethnobotanical surveys (11-13). However, scientific evidence supporting its effectiveness against Candida albicans in the local population is still limited. Understanding the antifungal properties of clove and its mechanism of action could lead to the growth of new herbal preparations as a supplement or different conventional antifungal treatments (14, 15). These experimental efforts aim to investigate the therapeutic potential of *Syzygium aromaticum* in managing vaginal candidiasis among women in Odisha. The study will assess the antifungal effectiveness of clove, its bioactive components, mechanisms of action, and its potential role in overcoming antifungal resistance.

Materials and Method

Preparation of Plant Extracts

The extraction process utilized methanolic organic solvents. Blossom buds of *Syzygium aromaticum* were sourced from a home market in Bhubaneswar, Odisha. The clove buds were sterilized using a 0.5% solution of NaOCl and subsequently rinsed three to four times with distilled water and allowed to dry for two to three weeks before being taken to the laboratory procedure shown in Figure 1.



Figure 1: Syzygium aromaticum (A) Flower Buds from Bhubaneswar Market, (B) were Ground to Powder. (C) The Powder Underwent Methanol Extraction via Soxhlet Extractor and Rotary Evaporation, then (D) Stored at 4 °C

To create methanolic extracts from cloves, 20 g of clove powder was weighed and spread onto several filter papers. These papers were then folded and inserted into the sample compartment of a Soxhlet extractor. A separate 200ml of methanol was introduced into the solvent flask of the Soxhlet extractor. The extraction process commenced by activating the hot plate and the condenser attached to the extractor (Figure 1). The methanol solvents were fully evaporated using a rotary evaporator to obtain the extract,

which was subsequently stored in amber bottles at 4 °C for future use.

Phytochemical Screening

The plant extracts underwent analysis to detect various phytochemicals using straightforward qualitative techniques as outlined by Harboune, 1973, as follows (16):

Steroids Test

An amount of 0.5 g of the extract was combined with 2 ml of the acetic acid and chloroform, subsequently heated, and then 2 ml of $\rm H_2SO_4$ was added.

Flavonoids Test

2 ml of the plant extract was mixed with 5 drops of dilute NaOH, and then diluted HCl was added.

Tannins

0.5 g of the plant extract was simmered in 20 ml of water in a beaker and subsequently filtered it. A few drops of ferric chloride at a concentration of 0.1% were subsequently added.

Cardiac Glycosides (Keller-KillamTest)

A 0.5 g sample of the plant extract was treated with 2 mL of glacial acetic acid containing a single drop of ferric chloride solution. Subsequently, 1 mL of concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the test tube.

Free Alkaloids

Sample of 0.5g Soxhlet extract solution was precisely weighed and dissolved in 1.5 mL of 2% hydrochloric acid (HCl). The mixture was thoroughly agitated to ensure complete dissolution. Subsequently, two drops of the Mayer's reagent were presented to the solution and the white precipitate was considered indicative of the presence of alkaloids.

Alkaloid Salts

A solution containing an aqueous extract of 0.5 g was combined with 25 mL of distilled water and thoroughly mixed. This solution were combined with 15 mL of 10% HCL and heated for 30 minutes. The solution mixture was underwent liquid-liquid extraction with dimethyl ether three times to eliminate non-polar constituents. The remaining 1 mL of the aqueous solution was subjected to Wagner's reagent, resulting in a reddish-brown precipitate that confirmed the presence of alkaloid salts.

Terpenoids

In a test tube, about 0.5 ml of extract was mixed with 3 mL of chloroform. Then, 2 mL of concentrated $\rm H_2SO_4$ was gently added along the tube's sides to form a separate layer. The emergence of a reddish-brown interface signified the presence of terpenoids.

Salkowski test

A mixture was created by dissolving 0.5 g of plant extract in 2 mL of chloroform. Then, 3 mL of concentrated sulfuric acid (H_2SO_4) was gently added along the side of the test tube. The formation of a reddish-brown layer at the interface signified the presence of sterols and terpenoids, as indicated by the Salkowski test.

Anthracenosides

A 0.5 g sample of plant extract was mixed with 15 mL of 10% HCl and subjected to drain for 30 minutes. Once cooled, the mixture was subjected to three separate extractions using 15 mL portions of diethyl ether. The ether layer (8 mL) was evaporated to dryness. The residue was dissolved in 2 mL of warm water, and then a few drops of 10% NH₄OH were added to aid in the development of color, indicating the presence of anthracenosides.

Tannins and Phenolic FeCl3 test for plant

Transfer approximately 2–3 mL filtered plant solution extract into clean glass tube. Carefully introduce 5–10 drops of a 5% ferric chloride (FeCl $_3$) solution into the extract. Mix gently and observe for any color change, which may indicate the presence of phenolic compounds or tannins in the sample.

Hager's test

Transfer approximately 2-3 ml of the plant extract solution into a clean glass tube. Add 1-2 ml of Hager's reagent to the same glass tube and moderately shake the mixture to combine.

Mayer's/Bertrand's/Valser's test

Transfer 0.5 ml of the extract solution into a glass tube. Add 1 drop of Mayer's reagent to the glass tube tube. If the solution is very dilute, additional reagent may be necessary. Mix the solution gently by swirling or using a pipette.

Wagner's test

Begin by taking a clean test tube and pouring in approximately 2-5 mL of the plant extract. Add a few drops (approximately 1-2 mL) of Wagner's reagent to the glass tube and gently shake it to mix the contents.

The Picric Acid

Place 0.5 milliliters of the plant solution extract into a test tube. Add 2-3 drops of a 2% picric acid solution to the extract.

The Iodine Test

Allow the plant material to settle, or use a centrifuge if one is available. Then, with a dropper, add a few drops of iodine solution to the clear liquid on top, or directly onto the plant paste if the liquid remains cloudy.

The Bouchardat's test

Place 0.5 milliliters of the plant extract into a clean test tube, and then introduce a few drops of Bouchardat's reagent to it.

The Tannic Acid Test

Transfer a few milliliters (about 2-3 mL) of the plant sample extract into a glass tube. Add several drops (approximately 5-10 drops) of a 10% tannic acid solution to the plant extract in the test tube.

Molish's test for Detection of Carbohydrates

Introduce 2 drops of Molisch's reagent into the test tube containing the plant extract. Mix gently to ensure even distribution of the reagent. Carefully add 1-2 mL of concentrated sulfuric acid by letting it flow down the inside of the test tube, ensuring it forms a distinct layer at the bottom. Do this slowly and avoid shaking the tube to maintain the separation of layers.

Study Design and Sample Collection

Fifty vaginal swab samples were gathered from symptomatic patients aged 18 to 55 years at IMS Sum Hospital, Bhubaneswar. The samples were collected using sterile cotton swabs and delivered to the microbiology laboratory within two hours, kept in a transport medium at 4°C.

Isolation and Identification of Candida albicans

Vaginal swab samples were cultured on Sabouraud Dextrose Agar (SDA) with chloramphenicol and incubated at 37°C for a period of 24 to 48 hours. Colonies exhibiting yeast-like morphology were further identified using CHROMagar candida medium. According the light to medium green colonies to identify the *Candida albicans*.

Molecular Confirmation and DNA Sequencing

DNA Extraction and Purity Assessment

Molecular confirmation of *Candida albicans* was carried out by extracting genomic DNA using the MPure Bacterial DNA Extraction Kit (MP Biomedicals, India), in accordance with the manufacturer's instructions. The extracted DNA's quality and concentration were evaluated using a spectrophotometer by checking the absorbance at 260 and 280 nm. A 260/280 ratio close to approx. 1.8 was deemed to indicate DNA of high purity, suitable for subsequent molecular analyses.

PCR Amplification and Gel Electrophoresis

The internal transcribed spacer (ITS) region of ribosomal DNA was amplified utilizing primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') through polymerase chain reaction (PCR). The reaction

mixture comprised a genomic DNA template, primers, deoxynucleotide triphosphates (dNTPs), Taq DNA polymerase, and PCR buffer components. The thermal cycling protocol included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (58°C, 45 seconds), and extension (72°C, 1 minute), with a final extension at 72°C for 10 minutes. The amplified products were subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide, and successful amplification was confirmed through UV visualization, which revealed the expected ITS band size (Figure 3).

PCR Product Purification and Sequencing

The PCR products that were amplified underwent purification using the SPINeasy® PCR Purification method (MP Biomedical, India) in accordance with the manufacturer's instructions. Once purified, the amplicons underwent sequencing through the Sanger technique. The resulting sequences were subsequently examined using the Basic Local Alignment Search Tool (BLAST) to verify species identity by matching them against reference sequences in the GenBank database.

NCBI Submission and Accession Number Assignment

The validated sequences have been uploaded to the NCBI GenBank database. The submitted sequence received the accession number PP197343, confirming the molecular identity of the *Candida albicans* isolates used in this study.

Well Diffusion Method

The well diffusion assay was used to evaluate the anti-candidal activity of Syzygium aromaticum (clove) extracts. Initially, 10 mL of sterile SDA was poured into Petri dishes to create a base layer. Subsequently, 15 mL of SDA containing a fungal suspension (1 mL of Candida albicans culture per 100 mL of medium, approximately 10⁵ CFU/mL) was overlaid. After solidification, wells of 6 mm diameter were made using a sterile corn borer and filled with clove extract at concentrations of 50 mg/mL, 100 mg/mL, and 200 mg/mL. Plates were incubated at 37°C for 24 hours to allow fungal growth and potential inhibition. The resulting zones of inhibition (ZOI) were measured and compared against fluconazole (+ve control) and dimethyl sulfoxide (DMSO, -ve control) to assess the extract's antifungal efficacy.

Statistical Analysis

All experiments were performed in triplicate, and data are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) to determine significant differences between groups, with a p-value < 0.05 considered statistically significant. All analyses were carried out using SPSS software, version 25.

Results

This study explored the anti-fungal properties of *Syzygium aromaticum* (clove) extract on *Candida albicans* strains obtained from vaginal swabs of patients with symptoms at IMS SUM Hospital. Initially, vaginal swab samples were cultured on SDA before being transferred to CHROMagar *Candida* medium for preliminary identification. The *Candida* samples were then streaked onto CHROMagar *Candida* medium. After a 48-hour

incubation period at 37°C, Candida albicans colonies appeared green, facilitating presumptive species identification (Figure 2). Analysis of Syzygium aromaticum extract identified bioactive compounds. Through molecular methods and antifungal assays, it was demonstrated that clove extract effectively inhibited clinical isolates of Candida albicans.

Phytochemical Screening of *Syzygium* aromaticum

The phytochemical examination identified several bioactive substances recognized for their antimicrobial effects. Clove extract has been identified to contain alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and glycosides, all of which contribute to its antifungal properties. Table 1 provides a comprehensive overview of the phytochemical screening results.

Table 1: Phytochemical Constituents Detected in Syzygium aromaticum Extract

Phytochemical Test	Result (+/-)	Constituents Detected
Steroids Test	+	Steroids
Flavonoids Test	+	Flavonoids
Tannins Test (Ferric chloride)	+	Tannins, Phenolics
Cardiac Glycosides (Keller-Killani)	-	Cardiac Glycosides
Free Alkaloids (Mayer's Test)	+	Alkaloids
Alkaloid Salts (Wagner's Test)	+	Alkaloid salts
Terpenoids Test	+	Terpenoids
Salkowski Test	+	Sterols
Anthracenosides Test	-	Not Detected
Tannins and Phenolic FeCl3 Test	+	Tannins, Phenolic compounds
Hager's Test	+	Alkaloids
Mayer's/Bertrand's/Valser's Test	+	Alkaloids
Wagner's Test	+	Alkaloids
Picric Acid Test	-	Alkaloids
Iodine Test	+	Polysaccharides (starch trace)
Bouchardat's Test	+	Alkaloids
Tannic Acid Test	+	Tannins
Molish's Test (Carbohydrates)	+	Carbohydrates

Isolation and Identification of Candida albicans

Among the 50 vaginal swab samples taken, 35 (70%) were positive for *Candida* growth. On CHROMagar *Candida* medium, colonies of *Candida* albicans strains appeared in shades ranging from

light to medium green, which aids in their presumptive identification as *Candida albicans*. Additional biochemical tests, such as germ tube formation and carbohydrate assimilation, further confirmed the presence of *Candida albicans* shown in Figure 2.



Figure 2: *Candida* samples were streaked onto CHROMagar *Candida* medium. After 48 hours at 37°C, *Candida albicans* colonies appeared green, enabling presumptive species identification

Molecular Confirmation of Candida albicans

The molecular identity of *Candida albicans* isolates was confirmed through PCR amplification of the ITS region. Analysis of the PCR products using gel electrophoresis showed an amplicon of the anticipated size, roughly between 500 and

600 bp, which were later verified by sequencing and NCBI BLAST analysis shown in Figure 3. The sequence analysis demonstrated a 100% match with *Candida albicans* reference sequences, and the validated sequences were submitted to NCBI, where they were assigned the accession number PP197343.



Figure 3: DNA of *Candida albicans* was amplified using ITS1/ITS4 primers. PCR products were separated on 1.5% agarose gel, and bands between 500-600 bp were verified through UV visualization, with a 100 bp ladder as reference.

Antifungal Activity of Syzygium aromaticum Extract

In Table 2, the antifungal efficacy of *Syzygium* aromaticum (clove) extract against *Candida* albicans was assessed utilizing the well diffusion method. The results indicated a distinct dosedependent effect, with the zone of inhibition (ZOI) increasing proportionally with the concentration

of the extract. The maximum level of inhibition was observed at 200 mg/mL, where the ZOI was nearly comparable to that of fluconazole, the standard antifungal agent used as a positive control. Dimethyl sulfoxide (DMSO) served as the negative control and exhibited no inhibitory effect, thereby affirming the extract's specific antifungal activity.

Table 2: Antifungal Susceptibility Testing of *Syzygium aromaticum* Extract

Extract Concentration (mg/mL)	Zone of Inhibition (mm) ± SD
50	10.2 ± 0.5
100	15.3 ± 0.7
200	22.4 ± 0.9
Fluconazole (Positive Control)	25.6 ± 1.0
DMSO (Negative Control)	No inhibition

Discussion

The present study aimed to evaluate the antifungal efficacy of *Syzygium aromaticum* (clove) extract against *Candida albicans* isolated from vaginal swabs of patients exhibiting symptoms. The results indicated that *Syzygium aromaticum* demonstrated significant antifungal activity, supported by the presence of bioactive phytochemicals, molecular identification of *Candida albicans*, and the clove extract's performance in antifungal susceptibility tests.

phytochemical analysis of Syzygium aromaticum (clove) extract has identified a wide of bioactive compounds, thereby substantiating its traditional application in antimicrobial therapy. Steroids, flavonoids, tannins, terpenoids, alkaloids, and carbohydrates were consistently detected through qualitative assays (5). The positive outcomes observed in the Steroids Test, Salkowski Test, and Terpenoids Test are consistent with previous research, suggesting that clove contains substantial quantities of phytosterols and triterpenoids, which are recognized for their antimicrobial and anti-inflammatory properties (17, 18).

Flavonoids and tannins, as confirmed by the Ferric Chloride Test and Flavonoids Test, familiar for their antioxidant and antimicrobial qualities. Flavonoids disrupt microbial membranes, while tannins lead to protein precipitation and inhibit microbial enzymes, contributing to the antifungal effects observed in this study (19-23). The substantial presence of phenolic compounds further enhances the extract's capacity to neutralize free radicals and boost antimicrobial efficacy.

The presence of alkaloids, as indicated by Mayer's, Wagner's, Bouchardat's, and Hager's tests, points to an additional dimension of antimicrobial properties, given that alkaloids are known to disrupt microbial DNA replication and metabolic processes (5, 24, 25). Notably, although free alkaloids were plentiful, the Picric Acid Test

yielded a negative result, which might indicate selective variations in the alkaloid profile within the extract.

The Iodine Test indicated traces of polysaccharides, suggesting the presence of starch or related carbohydrates, which, although not directly antimicrobial, can contribute to the extract's bioactivity by modulating solubility and delivery (25).

The tests for Cardiac Glycosides and Anthracenosides yielded negative results. indicating that these particular secondary metabolites are either not present or exist in undetectable amounts in the aromaticum extracts prepared under the specified conditions. This finding aligns with previous research, which has emphasized that clove is predominantly characterized by essential oils, tannins, and flavonoids, rather than glycosides (26, 27).

Validation of the genetic identification of *Candida albicans* was achieved through PCR amplification of the ITS region, followed by sequence analysis, which confirmed its genetic alignment with previously recorded strains. The prevalence of *Candida albicans*, found in 70% of patients exhibiting symptoms, aligns with global trends, where it remains the primary cause of vulvovaginal candidiasis (VVC) (27). However, the emergence of non-albicans *Candida* (NAC) species resistant to azole antifungals has raised concerns about treatment efficacy and underscored the need for alternative antifungal therapies (28).

Antifungal susceptibility testing revealed that *Syzygium aromaticum* extract demonstrated a dose-dependent inhibitory effect on *Candida albicans*, with larger inhibition zones observed as the extract concentration increased. At a concentration of 200 mg/mL, the inhibition zone reached 22.4 mm, which is similar to the 25.6 mm zone achieved by fluconazole. This indicates that clove extract could potentially be used as a natural antifungal alternative. Previous studies have reported similar results, showing clove

extract's potent antifungal activity against *Candida* species (29). Additionally, the MIC, at 50 mg/mL and 100 mg/mL respectively, confirmed the fungicidal properties of clove extract, underscoring its potential for therapeutic use.

With the growing resistance of *Candia albicans* to standard azole medications, the results indicate that *Syzygium aromaticum* extract might be considered as a supplementary or alternative option for treating VVC. To assess its safety, efficacy, and potential synergistic effects with conventional antifungal medications, further research, including in vivo studies and clinical trials, is necessary. (30).

therapeutic application of Syzygium aromaticum (clove) extract for vaginal candidiasis must be evaluated for antifungal efficacy and safety. Although in vivo testing was not conducted, previous investigations have demonstrated mucosal safety of clove oil through irritation models in mice and MTT assays on vaginal epithelial cells, suggesting suitability for topical use (31). We have indicated that future studies will incorporate clinical investigations to evaluate therapeutic outcomes in human subjects (32). The antifungal performance of clove extract—primarily attributed to eugenol—has shown comparable efficacy to standard azole drugs like fluconazole and clotrimazole. Its efficacy has been investigated against other botanicals like oregano, neem, and tea tree oil, with clove showing competitive or superior inhibitory effects against Candida albicans. These findings underscore clove extract's potential as a natural alternative to conventional antifungal therapies (33). Further research involving clinical trials and safety evaluations is essential to confirm its therapeutic viability in gynaecological settings.

While this study was limited to qualitative antifungal screening and MIC evaluations, we acknowledge the absence of time-kill kinetics and dose-dependent response data as a limitation. Future work will focus on these parameters to understand the pharmacodynamics of *Syzygium aromaticum* extract. Additionally, we considered the stability and shelf-life of the extract for herbal antifungal formulation. Studies have emphasized preserving bioactive compounds like eugenol through encapsulation or emulsification to maintain efficacy over time (34, 35). Eugenol, the

demonstrates antifungal active ingredient, properties by employing various mechanisms, such as disrupting the fungal cell membrane and hindering the biosynthesis of ergosterol, which undermines the membrane's integrity and functionality (36, 37). These mechanisms have been validated in prior studies against Candida albicans. Incorporating such mechanistic insights supports the potential of clove-based formulations as alternatives to synthetic antifungals, particularly given growing resistance trends.

Conclusion

This research highlights the antifungal effects of Syzygium aromaticum (clove) extract on Candida albicans, which was isolated from individuals suffering from vulvovaginal candidiasis (VVC). The phytochemical analysis identified bioactive substances such as flavonoids, tannins, alkaloids, and eugenol, all known for their antimicrobial properties. Molecular identification using PCR confirmed Candida albicans as the predominant cause of VVC. Antifungal susceptibility tests demonstrated that clove extract exhibited significant inhibitory effects on Candida albicans, with inhibition zones similar to those of fluconazole, the standard antifungal medication. The findings regarding the minimum inhibitory concentration (MIC) suggest that clove extract could serve as an alternative antifungal agent. With growing concerns about azole-resistant Candida strains, natural plant-based antifungals offer a promising solution to combat drug resistance. These results highlight the necessity for further research, including in vivo studies and clinical trials, to evaluate the safety, efficacy, and potential synergistic effects of Syzygium aromaticum extract when used alongside conventional antifungal treatments. Incorporating herbal antifungals into treatment plans could provide a sustainable approach to managing VVC, reducing dependence on synthetic drugs, and addressing antifungal resistance.

Abbreviation

None.

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Author Contributions

All authors contribute equally.

Conflict of Interest

None.

Ethics Approval

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References

- 1. Bhosale VB, Koparde AA, Thorat VM. Vulvovaginal candidiasis—an overview of current trends and the latest treatment strategies. Microb. Pathog. 2025;200:107359.
- 2. Willems HME, Ahmed SS, Liu J, Xu Z, Peters BM. Vulvovaginal candidiasis: A current understanding and burning questions. J. Fungi. 2020;6(1):27.
- 3. Sobel JD, Nyirjesy P. Vulvovaginal candidiasis: A wide-ranging challenge. Clin Infect 2019;68(1):37-42.
- 4. Gintjee TJ, Donnelley MA, Thompson GR 3rd. Aspiring antifungals: Review of current antifungal pipeline developments, I. Fungi, 2020;6(1):28.
- 5. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygiumaromaticum L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules. 2020;10(2):202.
- 6. Biernasiuk A, Baj T, Malm A. Clove essential oil and its main constituent, eugenol, as potential natural antifungals against Candida spp. alone or in combination with other antimycotics due to synergistic interactions. Molecules. 2022;28(1):215.
- 7. Kamatou GPP, Vermaak I, Viljoen AM. Eugenolfrom the remote Maluku Islands to the international market, S. Afr. I. Bot, 2019:127:146-173.
- Didehdar M, Chegini Z, Shariati A. Eugenol: A novel therapeutic agent for the inhibition of Candida species infection. Front. Pharmacol. 2022:13:872127.
- 9. Zhang L, Wu W, Chen J, Wang X. Antifungal mechanisms of eugenol against Candida albicans. Front. Microbiol. 2022;13:849107.
- 10. Satora M, Grunwald A, Zaremba B, Frankowska K, Żak K, Tarkowski R, Kułak K. Treatment of vulvovaginal candidiasis—an overview guidelines and the latest treatment methods. J. Clin. Med. 2023;12(16):5376.
- 11. Patra D. Navak R. Ethnomedicinal uses of medicinal plants for the treatment of skin diseases by the tribal communities of Keonjhar district of Odisha, India. J. Med. Plants Stud. 2024;12:35-39.
- 12. Gobbi V. Katrahalli K. Ethnomedicinal plants used in the treatment of gynecological disorders by the traditional health practitioners in Bagalkot district, Karnataka, India. J. Plant Sci. Res. 2023;10(2):245.
- 13. Mohapatra PP, Panda T, Mishra A. Ethnomedicinal plants used for treating vaginal infections by the communities of Odisha, India. Ethnopharmacol. 2018;224:280-292.

14. Hsu H, Veses V. Herbal extracts with antifungal activity against Candida albicans: A systematic Med review. Mini Rev Chem. https://doi.org/10.2174/13895575206662006280 32116

- 15. Argüelles JC, Sánchez-Fresneda R, Argüelles A, Solano F. Natural substances as valuable alternative improving conventional antifungal chemotherapy: Lights and shadows. J. Fungi. 2024:10(5):334.
- 16. Harborne IB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. London: Chapman and Hall. 1973:107-150. https://www.scirp.org/reference/referencespapers ?referenceid=1551865
- 17. Haque Z, Iqbal M, Ahmad A, Khan M, Prakash J. Molecular Characterization of Trichoderma spp. Isolates by Internal Transcribed Spacer (ITS) Region Sequencing Technique and its Use as a Open Biocontrol Agent. Biotechnol 2020;14. http://dx.doi.org/10.2174/18740707020 14010070
- 18. Lone ZA, Jain NK. Phytochemical analysis of clove (Syzygiumaromaticum) dried flower buds extract and its therapeutic importance. J Drug DelivTher. 2022;12(4-S):87-92.
- 19. Purnomo Y, Soetanto H, Nugraha AS. Phytochemical and pharmacological review of Syzygiumaromaticum (clove). J PharmacognPhytochem. 2021;10(1):100-106.
- 20. Niazi MK, Badrul ain H, Noreen S, Tufail T, Hassan F, Ismail MA. Characterization of muffins prepared with clove (Syzygiumaromaticum) extract and its effect on hyperlipidemic adult patients. Nat Prod Commun. 2024;19(9). https://doi.org/10.1177/1934578X241283188
- 21. Zhao K, Wonta KB, Xia J, Zhong F, Sharma V. Phytochemical profiling and evaluation of antimicrobial activities of common culinary spices: Syzygiumaromaticum (clove) and Piper nigrum (black pepper). Front Nutr. 2024;11:1447144.
- 22. Egharevba HO, Kunle OF. Preliminary phytochemical and proximate analysis of the leaves Danielliaoliveri. Afr Biotechnol. I 2010;9(51):8889-8891.
- 23. Jeyaraj EJ, Elumalai S, Subramanian Phytochemical screening and antioxidant activity of clove (Syzygiumaromaticum) extracts. Asian J Pharm Clin Res. 2020;13(9):162-165.
- 24. Vizhi DK, Irulandi K, Mehalingam P, Kumar NN. In vitro antimicrobial activity and phytochemical analysis of fruits of Syzygiumaromaticum (L.) Merr. &L.M.Perry - An important medicinal plant. J PharmacognPhytochem. 2016;5(4):137-140.
- 25. Maggini V, Semenzato G, Gallo E, Nunziata A, Fani R, Firenzuoli Antimicrobial F. activity Syzygiumaromaticum essential oil in human health treatment. Molecules. 2024;29(5):999.
- 26. Lakshmi Kumari P, Sujitha A, Mahammad Rafi N. In vitro anthelmintic activity of Syzygium aromaticum floral buds and Cinnamomum zeylanicum bark aqueous extracts. Int J Pharm Bio 2015;6(1):319-25.
- phytochemical 27. Salaheldin. Antifungal and constituents study of clove oil from

- Syzygiumaromaticum. World J Pharm Life Sci. 2017;3(10):211–215.
- 28. Sobel JD. Vulvovaginalcandidosis. Lancet. 2007;369(9577):1961–1971.
- 29. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Primers. 2018;4(1):1–20.
- 30. Khan M, Ahmad I, Khan S. Antifungal potential of medicinal plants against Candida infections: An overview. J Herb Med. 2021;12(4):287–298.
- 31. Yashashree DJ, Binawade Y, Nandutai G. Antibacterial activity of Syzygiumaromaticum Extracts (Clove Oil) on the Etiological Agent of Dental Caries. Ecol Environ Conserv. 2023;29:238–41.
- 32. Irving C, Culverhouse I. Human factors integration with clinical investigations. Journal of Medical Engineering & Technology. 2023;47(8):396–402.
- 33. RashidiMeybodi F, Amirkhan S, Brmar S, Rafiei M. Comparison of the Antifungal and Antibacterial Effects of Cloves and Fluconazole Solution on *Candida albicans* and Streptococcus mutans Colonies. J Res Med Dent Sci. 2023;20(3):98–103.
- 34. Pramod K, Ansari SH, Ali J. Eugenol: a natural compound with versatile pharmacological actions. J Res Med Dent Sci. 2010 Dec;5(12):1999-2006.
- 35. Donsì F, Annunziata M, Vincensi M, Ferrari G. Design of nanoemulsion-based delivery systems of natural antimicrobials: effect of the emulsifier. J Biotechnol. 2012 Jun 30;159(4):342-50.
- 36. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur J ClinMicrobiol Infect Dis. 2011 Jan;30(1):41-50.
- 37. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from Syzygiumaromaticum on Candida, Aspergillus and dermatophyte species. J Med Microbiol. 2009 Nov;58(Pt 11):1454-1462.