Trop J Nat Prod Res, October 2020; 4(10):831-837 **ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

**Tropical Journal of Natural Product Research**

Available online at https://www.tjnpr.org

*Original Research Article*

# Evaluation of *Zingiber officinale* Rosc. and *Ocimum basilicum* L. Essential Oils-Loaded Gel Base for the Treatment of Oral Candidiasis

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ARTICLE INFO

*Article history:*
Received 10 September 2020
Revised 04 October 2020
Accepted 24 October 2020
Published online 02 November 2020



ABSTRACT

Oral candidiasis is an opportunistic infection that affects the oral cavity and could worsen the severity of an underlying disease. This research is focused on the formulation of an oral herbal gel from essential oils of *Zingiber officinale* Rosc. (ZO) and *Ocimum basilicum* L. (OB) for the potential use in the management of oral candidiasis. Antifungal activities of the oil extracts were evaluated against ten clinical isolates of *Candida albicans* using the agar well diffusion method and measurement of minimum inhibitory/fungicidal concentration (MIC/MFC). The oils were tested individually and subsequently combined at predetermined ratios. The oils were formulated into carbopol-based gels, the antifungal activity and physico-chemical properties of the gels were assessed. Nystatin was used as a positive control. **S**ensitivity test showed that *Candida albicans* isolates were sensitive to each of the essential oil extracts at a concentration of 10%v/v. The essential oil extracts combination of 25:75 (ZO:OB) yielded the highest zones of inhibition which ranged from 28.00 ± 0.65 to 39.00 ± 1.45 mm. The results were higher than that obtained for the nystatin control (26.00 ± 1.20 to 30.00 ± 1.05 mm). Five batches of gels incorporated with the essential oils exhibited good physicochemical characteristics. However, the herbal gels exhibited lower antifungal activity than the essential oils against *Candida albicans* isolates. These outcomes demonstrate the antifungal activity of the herbal gels and thus its potential for use in the management of oral candidiasis.

***Keywords***: *Zingiber officinale*, *Ocimum basilicum*, Carbopol gels, Antifungal activity, Dental care, Oral candidiasis.

## Introduction

Oral candidiasis is an opportunistic infection of the oral cavity and as the name implies, it is caused by *Candida species*. The rate of incidence occurs in approximately 5-7% of infants, prevalence in HIV is between 9-31% and approximately 20% in patients with cancer.[1] Predisposing factors such as salivary gland dysfunction, use of dental prostheses, smoking, indiscriminate use of antibiotics, endocrine disorders, nutritional deficiencies, poor oral hygiene, age and immune disorders such as HIV can cause oral candidiasis.[2] The immune system of the host typically controls the proliferation of *Candida albicans*; but, *C. albicans* cause severe opportunistic infections in patients with diabetes mellitus, immuno-compromised patients on chemotherapy, immunosuppressive therapeutic agents and patients living with HIV.[3]

The drug of choice in candidiasis for non-immunosuppressed adults is either nystatin suspension or clotrimazole douche. However, the numerable side effects and the growing incidence of drug-resistant fungal diseases pose a unique task to antifungal drug discovery.[4]

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Citation: Mustapha AT, Jaiyeola ET, Olufadi-Ahmed HY, Ayotunde HT, Ghazali YO. Evaluation of *Zingiber officinale* Rosc. and *Ocimum basilicum* L. Essential Oils-Loaded Gel Base for the Treatment of Oral Candidiasis. Trop J Nat Prod Res. 2020; 4(10):831-837. doi.org/10.26538/tjnpr/v4i10.28

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Plants have been used continually in the management of diseases, globally. Essential oils which contain volatile mixture of organic compounds can represent one of the most promising natural products for fungal inhibition.[5] Many kinds of essential oils obtained from different plants or herbs exhibit intense antifungal properties such as those extracted from *Coriandrum sativum* and *Ocimum sanctum*,[6] demonstrated an interesting activity as biofilm inhibitors against *C. albicans*. Many of the clinically used antifungals for the treatment of candidiasis are largely effective but their use have demonstrated drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of resistant strains.[7] New and effective strategies for the treatment of candidiasis and other fungal diseases in immunocompromised patient is therefore pertinent. The availability and relative safety of herbal extracts in the management of *Candida* and other fungal infections as shown in previous studies provides a platform for the formulation of stable herbal gels from the oil extracts of *Ocimum basilicum and Zingiber officinale* for the management of oral candidiasis.[8]

In recent years, several plants have been investigated for the treatment of oral candidiasis. *Zingiber officinale* Rosc. (Zingiberaceae) known as ginger, is widely used as a spice and medicinal traditional medicine. Ginger oil possesses antibacterial, antifungal, anti-inflammatory, anti-ulcer, immunomodulatory, respiratory and gastrointestinal activities.[9,10] El-Baroty and co-workers reported that essential oil from ginger (*Z. officinale)* possessed high antifungal activity against *Candida* and other fungi specie.[8] *Ocimum basilicum* L. (Lamiaceae) known as sweet basil is a rich source of flavour compounds and volatile oils containing a variety of compounds which possess antimicrobial activity.[11] The essential oil of *O. basilicum* was

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

tested against bacterial strains *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and the yeast *Candida albicans*. It has been reported to have antiviral, larvicidal, antinociceptive, and antifungal activity.[12] Raut and Karuppayil reviewed and reported articles which covered studies on the inhibitory activities of essential oils against many fungal pathogens.[13] The essential oils extracted from tea tree, laurel, anise, basil, bergamot, lavender, mint, oregano, grapefruit, rosemary, winter savory and ginger against strains of *C. albicans* isolates have been studied.[14]

Ginger essential oil was reported to have β-zingiberene (12.2%), 1,8-cineole+limonene+*β*-phellandrene (10.5%), geraniol (15%), neral (8.9), *β*-bisabolene (5.6%) and *β*-sesquiphellandrene (6.5%) which reduced cytoplasmic content of fungi and interrupted membrane integrity.[15] While major constituents for *Ocimum basilicum* were *α*-terpineol (59.78%) and *β*-caryophyllene (10.54%).[16]

The possible solution to curb antimicrobial resistance or enhance antimicrobial activity against resistant strains of organism may lie in the use of plants and their products.[17] The renewed interest in the use of medicinal plants in the management of fungal diseases coupled with the increased prevalence of oral candidiasis, has necessitated a need for the development of effective strategies for treatment. In recent times, the plant natural sources are required in the discovery of new antifungals, especially those that may inhibit fungi-specific pathways or act on novel cellular targets. This is due to an obvious rise in drug resistance to antifungals generally and more frequent use of antifungals for treatment and/or prophylaxis.[18] Hinged on this premise, this research is directed at exploring therapeutic solutions from natural plant materials for the management of oral candidiasis with potential application in dentistry. The results of this work will support the research for new alternatives or complementary therapies against oral candidiasis.

## Materials and Methods

*Materials*

Sabouraud dextrose agar (SDA) and sabouraud dextrose broth (Biomalk, India), dimethyl sulphur oxide (DMSO) (Guangdong Chemical Reagent, China), normal saline (Dana Pharmaceuticals Limited, Nigeria), ethanol (Guangdong Chemical reagent, China), methyl paraben, propylene glycol and triethanolamine (BDH Chemicals Ltd Poole England), nystatin suspension (Krismediks Nigeria Limited), and carbopol 940 (Guangdong Chemical Reagent, China).

*Microorganisms*

Ten identified clinical isolates of *C. albicans* oral swabs obtained from University of Ilorin Teaching Hospital during routine hospital laboratory procedure were cultured in sabouraud dextrose agar slants and stored at a temperature of 4°C.

*Collection and treatment of plant materials*

Leaves of *Ocimum basilicum* and Rhizomes of *Zingiber offficinale* were collected in March 2019, from a location within Ilorin, Kwara State. Authentication was done at the herbarium of the Department of Plant Biology, University of Ilorin and voucher numbers of UILH/001/1102 and UILH/001/976 were assigned, respectively, and samples were deposited for future references.

*Essential oils from plants*

Fresh leaf samples of *O. basilicum* (1000 g) was subjected to steam distillation in a Clevenger-type apparatus for a minimum of 3 h. Fresh rhizomes of *Z. Officinale* (1000 g) was subjected to steam distillation in a Clevenger-type apparatus for 6 h. This was carried out according to the steam hydro-distillation method described by Idang and co-workers with slight modifications.[19] The essential oils were stored in airtight containers in a refrigerator at 4°C.

*Characterization of the essential oils*

The essential oils of *O. basilicum* and *Z. officinale* were examined for physical characteristics such as their colour, odour, solubility in ethanol and water, specific gravity and percentage yield.[20]

*Ethical clearance*

The researchers have been certified by the Nigerian National Code for Health Research Ethics under the Collaborative Institutional Training Initiative Program of the West Africa Bioethics Training Program (Record ID: 24781406). They are therefore fit to conduct a study of this nature.

*Collection of clinical isolates*

Ten identified cultures of different strains of *C. albicans* from oral candida samples were collected from the University of Ilorin Teaching Hospital as part of routine laboratory procedure. The strains were confirmed using standard methods and subsequently sub-cultured on Sabouraud Dextrose Agar (SDA) plates and incubated at 28°C for 72 h. The grown organisms were made into slants and stored for use at 4°C.[21]

*Inoculation of agar plates*

Samples from the ten clinical isolates of *C. albicans* were transferred into bottles containing 0.9% NaCl solution (normal saline) and mixed adequately to disperse the yeast. The organisms were incubated for 24 hours at 28°C after which they were standardized to 0.5 McFarland turbidity to obtain an organism density of 1 X $10^{-6}$ cfu/mL. The standardized *C. albicans* were streaked on SDA plates using sterile cotton swabs.[21]

*Evaluation of antifungal activity of the essential oil extracts*

The essential oils were further prepared for further analysis as follows: *O. basilicum* and *Z. officinale* oils were dissolved individually in 30% v/v DMSO (1:5% v/v). Two concentrations of 10 and 20% v/v of each oil in 30% DMSO was first used to conduct a sensitivity test on each of the ten clinical isolates of *C. albicans*.

The agar well diffusion procedure was carried out according to the method described by Zhu and co-workers with slight modifications.[22] A sterile cork borer of 6 mm was used to make four holes on each plate. The base of each hole was sealed with molten SDA. The wells were filled with 1 mL of the oil extracts in DMSO. Control tests were carried out using 30% DMSO as negative control and nystatin suspension as positive control. The plates were incubated at 28°C for 48 h and the resulting zones of inhibition were measured along the two axes and the mean of the two measurements were calculated. These tests were carried out in triplicates for each clinical isolate.

Based on the result of this sensitivity test, the extract concentrations of 20%v/v *O. basilicum* and *Z. officinale* was selected for the antifungal activity test. For this test, combinations of these extracts *Z. officinale* and *O. basilicum* in the ratios; 75:25, 25:75 and 50:50 at a concentration of 20% v/v were tested against ten clinical isolates of *C. albicans* using the agar well method earlier described.

Zones of inhibition were measured after 72 hours for the fungal organisms using a vernier caliper along each axis and the mean was taken. Each of these tests were conducted in triplicates. nystatin (2 mL at 100,000 units/mL) was used as the positive control for the fungal tests while 30% DMSO solution as the negative control.

*Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) by broth dilution method*

The minimum inhibitory concentration (lowest concentration of oil where the absence of growth was observed) of each oil extract was determined by ten-fold tube dilution method. For both oil extracts, 20% v/v test concentrations were used, and 5 mL was added to tube 1 containing 5 mL of double-strength Sabouraud Dextrose Broth (SDB) and 5 mL of normal strength SDB was added to tubes 2 to 12. In tube 2, 5 mL of high concentrations was added. Tube 2 was mixed well and 5 mL was transferred aseptically to tube 3, mixed again until tube 9. Tube 9 was mixed well and 5 mL was discarded. A 48-hour culture of *Candida albicans* was previously diluted to achieve a turbidity of 1 McFarland. After standing on the bench, 0.5 mL of *Candida* culture

was added to 19.5 mL of SDB and mixed well to achieve a dilution of 1:200. A 0.5 mL of the newly diluted culture was added to tubes 1 to 10. It was mixed well, allowed to stand on the bench for 30 minutes and incubated at 28°C for 48 h. Tube 10 served as organism viability control, broth sterility control, test agent sterility control.[23]

To determine the MFC, aliquots (20 µL) of broth were taken from each tube that did not show growth after reading the MIC tubes and cultivated in Sabouraud Dextrose Agar plates, incubated at 28°C for 48 h. The MFC is defined as the lowest concentration at which the incubated organisms were completely killed. All experiments were carried out in triplicates.[24]

The MFC/MIC ratio was calculated to determine whether thymol has a fungistatic (MFC/MIC ≥ 4) or fungicidal activity (MFC/MIC < 4).[25]

*Formulation of oral herbal gels*

The gels were formulated with 1% w/w carbopol 940, incorporated with predetermined ratios (5% w/w) of *Z. officinale* and *O. basilicum* oils and labelled F1, F2, F3, F4 and F5 as presented in Table 1. Carbopol 940 (1 g) was dispersed in purified water in a beaker and stirred using a magnetic stirrer. In another beaker, the 5% combination of *Z. officinale* and *O. basilicum* oil (Z:O) was added to propylene glycol and adequately mixed. The two solutions were mixed in the beaker and made up with distilled water to 100 mL. The pH of the mixture was adjusted with few drops of triethanolamine. Propyl paraben and saccharin were used as preservative and sweetener respectively.[26]

*Characterization of herbal gels*

*Physical Characterization*

Organoleptic properties which include odour and colour and texture were evaluated for the herbal gels. Other physical evaluations such as homogeneity, ease of application, ease of removal on skin were conducted.

*Determination of pH*

The pH values of the herbal gels were measured by calibrated digital pH meter (Hanna, UK). The measurements were carried out in triplicates.

*Spreadability*

The spreadability of the herbal gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) as used by Behera and co-workers with slight modifications.[27] The horizontal plates weighed 125 g each,1 g of the formulated gel was weighed and placed carefully on the horizontal glass slide. The second glass slide was placed on top of the gel and the time taken for the gel to spread in one minute were recorded. The diameter of the gel was measured, and the mean of the diameters calculated. This test was done in triplicates for the formulated gels.

*Determination of viscosity*

Herbal gel samples were placed at room temperature for 30 min. Viscosities were determined using NDJ-5S Digital Display Viscometer (Rinch, China) with number 4 spindle. The viscosity was determined at 25°C and 60 rpm. The tests were done in triplicates and recorded.

*Determination of antifungal activities of formulated oral gels*

The solution of the gels was prepared, and the antifungal activity was tested by the cup-plate diffusion method.[28] The samples were sub-cultured for 24 h at 28°C after which they were standardized to 0.5 McFarland turbidity to obtain an organism density of 1 x $10^6$ cfu/mL. The standardized organism was streaked on SDA plates using cotton swab. A sterile cork borer of 6 mm was used to make four circular holes on each plate. The base of each hole was sealed with molten SDA. The wells were filled with solutions of each gel; F1, F2, F3, F4 and F5. The plates were allowed to stay on the bench for one hour and subsequently incubated at 28°C for 48 h. The resulting zones of inhibition were measured along the two axes and the mean of the two measurements were calculated. These tests were carried out in triplicates for each clinical isolate.

*Statistical analysis*

Results were presented as mean ± standard deviation for the zones of inhibition. Statistical comparison was done by one-way analysis of variance (ANOVA). The difference between the antifungal activities of the different mixing ratios of essential oils extracts and control nystatin was also evaluated. The statistical analysis was carried out with GraphPad prism version 7.0 software. Significant differences were set at $p < 0.05$.

## Results and Discussion

*Characterization of the essential oils*

Generally, gel-based formulations are biocompatible and have mucoadhesive properties that enable them attach to the mucosa of the mouth thereby acting locally and preventing systemic side effect.[29] Gel bases have high water content, low surface friction, better application on mucous membranes and burnt or injured tissues. Carbopol was used to extend the resident time of the oils in the oral cavity through its cross-linked structure and water insolubility. Thus, the polymer can extend release of the drug component. All the five batches of formulation (F1 to F5) were prepared by incorporating the essential oils extracts into carbopol 940 polymer gel base as presented in Table 1.

The physical properties of the *Z. officinale and O. basilicum* essential oils such as colour, odour, solubility in water and ethanol, specific gravity and yield are presented in Table 2. The result showed that *Z. officinale* was pale yellow and *O. basilicum* was yellowish green while the odour was pungent for *Z. officinale* and minty for *O. basilicum*. *Z. officinale* gave a yield of 1.40 ± 0.40% and a specific gravity of 0.85±0.04 while *O. basilicum* gave a lower yield of 0.5 ± 0.02% and specific gravity of 0.90 ± 0.04. The extracts were both insoluble in water. These results were comparable to earlier values reported by Akise and co-workers for *Zingiber officinale*.[20]

*Antifungal activity of Z. officinale and O. basilicum essential oils*

The clinical isolates of *Candida albicans* had different sensitivities to the essential oils. Based on the sensitivity of *Candida albicans* on the essential oil concentrations, the agar well sensitivity tests showed activity for *Z. officinale* and *O. basilicum* at 10%v/v in 30% DMSO. Table 3 represents the antifungal activity of the investigated oil extracts. The zones of inhibition for 20%v/v were higher than 10%v/v for the two essential oil extracts. The inhibitory effects of the essential oils increased with increasing concentration. The diameter of the zone of inhibition of the oil extracts (20%v/v) ranged from 23.00 ± 1.70 to 32.00 ± 1.67 mm for *Z. officinale* and 22.00 ± 1.57 to 26.00 ± 2.08 mm for *O. basilicum*. On this basis, 20%v/v of *Z. officinale* and 20%v/v *O. basilicum* (higher activity) were selected and at these concentrations *Zingiber officinale* and *Ocimum basilicum* were combined at different ratios and individually against *Candida albicans (Z. Officinale:O. basilicum)* at 100:0, 75:25, 50:50, 25:75, 0:100 as presented in Table 3 and 4. Nystatin positive control showed a range of 26.00 ± 1.19 to 31.00 ± 0.65 mm, zones of inhibition for the ten *Candida* isolates. The result was consistent with the work of Ahmad and co-workers which reported a progressive increase in the effect of methanol extract of *O. basilicum* against *C. albicans*.[30] Another study showed the antifungal effect of *Z. officinale* oil on biofilm formed by *C. albicans* at concentration between 0.625 to 5 mg/mL.[31]

Table 4 represents the antifungal activities of the combination of extracts. All the essential oils extract combinations tested showed improved activity as compared to individual essential oils extracts. The highest antifungal activity range of 28.00 ± 0.65 to 39.00 ± 1.45 mm zones of inhibition were observed at a combination ratio of 25:75 (ZO:OB). The antifungal activity of the combination of oil extract exhibited superior activity to nystatin which was in the range 26.00 ± 1.20 to 30.00 ± 1.05 mm. Significant differences ($p < 0.05$) were observed between the means of the antifungal activities of the essential oils extracts and the control nystatin.The result suggests the synergistic and enhanced effect of the combination of *Z. officinale* and *O. basilicum* essential oils extracts against *C. albicans*.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The MIC and MFC values for *Z. officinale* were 12.50 ± 0.74 and 25.00 ± 1.24%v/v respectively; and for *O basilicum* were 6.25 ± 0.42 and 12.50 ± 0.68%v/v as shown in Table 5. The MIC/MFC ratio (2) for the two essential oils extracts showed that they had fungicidal effect against *C. albicans* isolates.

*Characterization of herbal gels*

The appearance of all the formulation batches as represented in Table 6 were clear and transparent with good representing a fairly homogenous formulation and very good representing a perfectly homogenous formulation. All the gels were of good aesthetics and had characteristic smells representative of the essential oils extracts present.

Saliva acts as a buffer neutralizing and maintaining pH in the mouth. Saliva has a pH ranging from 6.2 to 7.6, a reduction towards acidity or an increase to alkalinity may interfere with the normal flora of the mouth thereby promoting their overgrowth.[32] The pH of the formulated gels as represented in Table 7, ranged from 6.31 ± 0.31 to 6.64 ± 0.37. This result is an indication that the formulated herbal gels will not irritate the mouth as they have pH consistent with the saliva and in addition precludes the growth of Candida as these organisms have been observed to grow more in acidic environment.[33] The gels had spreading diameter ranging from 100 ± 0.25 to 80 ± 0.53 mm as represented in Table 7, which indicates their ability to locally spread around the affected area when applied. Their viscosity ranging from 15.3 ± 1.2 to 15.7 ± 1.4 Pas also indicates the ability of the gel to remain in the oral cavity when applied.

*Antifungal activity of formulated herbal gels*

The herbal gels exhibited a lower antifungal activity as compared to the activity of combination of essential oil extracts. The gel formulations, F4 exhibited the highest zone of inhibition against *C. albicans* as represented in Table 8. The diameter of the zone of inhibition was lower than the antifungal activity of the oil extracts using the agar-well diffusion method. Incorporation of the essential oils into the gel decreased their antifungal activity. This might have been due to the network of polymers that formed the gel. These results demonstrated the antifungal potentials of the gel formulations from these oil extracts against *C. albicans* commonly associated with oral candidiasis.

**Table 1:** Composition of herbal gels

| Ingredients (g) | F1 (1:0) | F2 (3:1) | F3 (1:1) | F4 (1:3) | F5 (0:1) |
|---|---|---|---|---|---|
| *Z. officinale* oil | 5.0 | 3.75 | 2.5 | 1.25 | 0.0 |
| *O. basilicum* oil | 0.0 | 1.25 | 2.5 | 3.75 | 5.0 |
| Methyl paraben | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Propylene glycol | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Carbopol 940 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Saccharin | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Distilled water to | 100.0 | 100.0 | 100.0 | 100 .0 | 100.0 |

**Table 1:** Physical Properties of *Z. officinale* and *O. basilicum* Essential Oils

| Essential Oil | Colour | Odour | Solubility in water | Solubility in ethanol | Specific gravity | Yield (%) |
|---|---|---|---|---|---|---|
| *Z. officinale* | Pale yellow | Pungent | Insoluble | Insoluble | 0.85 ± 0.04 | 1.40 ± 0.14 |
| *O. basilicum* | Yellowish green | Minty | Insoluble | Insoluble | 0.90 ± 0.04 | 0.50 ± 0.02 |

Data presented as Mean ± SEM (n = 3).

**Table 3:** Antifungal activity of *Zingiber officinale* (ZO) and *Ocimum basilicum* (OB) oil extracts against 10 clinical isolates of *C. albicans*

| *C. albicans* isolates | Zones of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 10% oil extract 100:0 (ZO) | 20% oil extract 100:0 (ZO) | 10% oil extract 0:100 (OB) | 20% oil extract 0:100 (OB) | Nystatin | 30%DMSO |
| 1 | 22.00 ± 0.34$^{b}$ | 28.00 ± 0.76$^{a}$ | 18.00 ± 0.76$^{b}$ | 23.00 ± 1.15$^{b}$ | 27.00 ± 0.54$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 2 | 23.00 ± 0.70$^{b}$ | 23.00 ± 1.70$^{b}$ | 15.00 ± 1.70$^{b}$ | 25.00 ± 1.73$^{b}$ | 29.00 ± 0.79$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 3 | 21.00 ± 1.48$^{b}$ | 29.00 ± 1.78$^{b}$ | 13.00 ± 1.68$^{b}$ | 26.00 ± 2.08$^{b}$ | 31.00 ± 0.65$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 4 | 20.00 ± 1.19$^{a}$ | 31.00 ± 1.58$^{c}$ | 14.00 ± 1.67$^{b}$ | 25.00 ± 1.78$^{a}$ | 25.00 ± 1.23$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 5 | 19.00 ± 0.46$^{b}$ | 30.00 ± 0.59$^{c}$ | 14.00 ± 1.06$^{b}$ | 24.00 ± 2.31$^{b}$ | 26.00 ± 1.19$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 6 | 19.00 ± 0.58$^{b}$ | 24.00 ± 0.98$^{b}$ | 16.00 ± 1.02$^{b}$ | 25.00 ± 0.00$^{b}$ | 29.00 ± 0.55$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 7 | 21.00 ± 1.75$^{b}$ | 26.00 ± 1.38$^{b}$ | 15.00 ± 1.67$^{b}$ | 22.00 ± 1.57$^{b}$ | 31.00 ± 0.49$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 8 | 22.00 ± 1.36$^{a}$ | 32.00 ± 1.67$^{c}$ | 16.00 ± 1.73$^{b}$ | 24.00 ± 1.48$^{b}$ | 30.00 ± 0.62$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 9 | 18.00 ± 1.21$^{a}$ | 31.00 ± 0.61$^{c}$ | 15.00 ± 1.12$^{b}$ | 23.00 ± 1.53$^{b}$ | 27.00 ± 0.57$^{a}$ | 0.00 ± 0.00$^{b}$ |
| 10 | 18.00 ± 1.05$^{b}$ | 32.00 ± 1.65$^{c}$ | 18.00 ± 1.57$^{b}$ | 25.00 ± 1.65$^{a}$ | 26.00 ± 0.89$^{a}$ | 0.00 ± 0.00$^{b}$ |

Data presented as Mean ± SEM (n = 3). Values carrying superscripts different from the control nystatin, for each essential oil extracts tested against each isolate are significantly different ($p < 0.05$).

**ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

**Table 4:** Antifungal activity of a combination of *Zingiber officinale and Ocimum basilicum* (ZO:OB) oil extracts against 10 clinical isolates of *C. albicans*

| ***C. albicans* isolates** | **Zone of inhibition (mm)** | | | | |
|---|---|---|---|---|---|
| | **Extract ratio 75:25** | **Extract ratio 50:50** | **Extract ratio 25:75** | **Nystatin** | **30%DMSO** |
| 1 | 24.00 ± 1.50[b] | 28.00 ± 1.00[a] | 38.00 ± 1.64[c] | 28.00 ± 1.01[a] | 0.00 ± 0.00[b] |
| 2 | 25.00 ± 1.50[b] | 27.00 ± 0.81[a] | 30.00 ± 1.50[c] | 27.00 ± 1.43[a] | 0.00 ± 0.00[b] |
| 3 | 23.00 ± 1.67[b] | 29.00 ± 1.08[c] | 39.00 ± 1.45[c] | 26.00 ± 1.23[a] | 0.00 ± 0.00[b] |
| 4 | 21.00 ± 2.59 | 27.00 ± 0.68 | 35.00 ± 1.60[c] | 30.00 ± 1.05[a] | 0.00 ± 0.00[b] |
| 5 | 24.00 ± 0.88[b] | 24.00 ± 0.54[b] | 31.00 ± 0.45[c] | 29.00 ± 1.03[a] | 0.00 ± 0.00[b] |
| 6 | 25.00 ± 0.75[b] | 25.00 ± 1.68[b] | 30.00 ± 0.69[c] | 27.00 ± 1.11[a] | 0.00 ± 0.00[b] |
| 7 | 27.00 ± 0.45[b] | 22.00 ± 2.68[b] | 35.00 ± 0.44[c] | 29.00 ± 1.63[a] | 0.00 ± 0.00[b] |
| 8 | 26.00 ± 0.68[a] | 28.00 ± 0.67[b] | 29.00 ± 0.37[b] | 26.00 ± 1.44[a] | 0.00 ± 0.00[b] |
| 9 | 25.00 ± 0.32[b] | 27.00 ± 1.68[a] | 28.00 ± 0.65[a] | 28.00 ± 1.50[a] | 0.00 ± 0.00[b] |
| 10 | 24.00 ± 0.76[b] | 23.00 ± 0.56[b] | 32.00 ± 0.59[c] | 26.00 ± 1.20[a] | 0.00 ± 0.00[b] |

Data presented as Mean ± SEM (n = 3). Data presented as Mean ± SEM (n = 3). Values carrying superscripts different from the control nystatin, for each mixing ratio of essential oils extracts tested against each isolate are significantly different ($p < 0.05$).

**Table 5:** Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentrations (MFC) of *Ocimum basilicum* and *Zingiber officinale* Oil Extracts against *Candida albicans*

| **Candida strains** | ***Ocimum basilicum* oil extract** | | | ***Zingiber officinale* oil extract** | | |
|---|---|---|---|---|---|---|
| | MIC range (%v/v) | MFC range (%v/v) | MFC/MIC ratio | MIC range (%v/v) | MFC range (%v/v) | MFC/MIC ratio |
| *C. albicans n = 10* | 6.25 ± 0.42 | 12.50 ± 0.68 | 2.00 | 12.50 ± 0.74 | 25.00 ± 1.24 | 2.00 |

**Table 6:** Physical characterizations of the herbal gels

| **Formulation** | **Colour** | **Odour** | **Appearance** | **Homogeneity** |
|---|---|---|---|---|
| F1 | White | Pungent, minty | Clear and Transparent | Good |
| F2 | White | Pungent, minty | Clear and Transparent | Very good |
| F3 | White | Pungent, minty | Clear and Transparent | Good |
| F4 | White | Pungent, minty | Clear and Transparent | Good |
| F5 | White | Pungent, minty | Clear and Transparent | Very good |

Good – fairly homogenous, Very good – Perfectly homogenous.

**Table 7:** pH, spreadability, and viscosity of herbal gels

| **Formulation** | **pH** | **Spreadability (mm)** | **Viscosity (Pas)** |
|---|---|---|---|
| F1 | 6.31± 0.31 | 100.00 ± 0.25 | 15.7± 1.4 |
| F2 | 6.64± 0.37 | 95.00 ±0.65 | 15.4± 1.2 |
| F3 | 6.61± 0.76 | 82.00 ±0.21 | 15.5± 1.3 |
| F4 | 6.40 ± 0.69 | 81.00 ±0.03 | 15.4± 1.1 |
| F5 | 6.59 ± 0.50 | 80.00 ±0.53 | 15.3± 1.2 |

Data presented as Mean ± SEM (n = 3).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 8:** Antifungal activities of the herbal gels

| *C. albicans* isolates | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | F1 (1:0) | F2 (3:1) | F3 (1:1) | F4 (1:3) | F5 (0:1) |
| 1 | 15.00 ± 0.76 | 23.00 ± 1.15 | 21.00 ± 0.00 | 24.00 ± 0.56 | 14.00 ± 1.55 |
| 2 | 16.00 ± 1.70 | 18.00 ± 1.73 | 20.00 ± 1.57 | 23.00 ± 0.23 | 13.00 ± 2.08 |
| 3 | 13.00 ± 1.68 | 19.00 ± 2.08 | 22.00 ± 1.18 | 25.00 ± 0.19 | 14.00 ± 1.11 |
| 4 | 15.00 ± 1.67 | 17.00 ± 1.78 | 18.00 ± 2.03 | 23.00 ± 0.31 | 16.00 ± 0.47 |
| 5 | 14.00 ± 1.06 | 21.00 ± 2.31 | 19.00 ± 1.52 | 22.00 ± 0.00 | 14.00 ± 1.45 |
| 6 | 16.00 ± 1.02 | 17.00 ± 0.00 | 21.00 ± 0.68 | 25.00 ± 0.71 | 15.00 ± 0.00 |
| 7 | 17.00 ± 1.67 | 16.00 ± 1.57 | 17.00 ± 1.00 | 21.00 ± 0.54 | 13.00± 0.76 |
| 8 | 12.00 ± 1.73 | 20.00 ± 1.48 | 19.00 ± 2.00 | 23.00 ± 0.65 | 16.00 ± 1.29 |
| 9 | 13.00 ± 1.12 | 23.00 ± 1.53 | 19.00 ± 1.65 | 19.00 ± 2.00 | 14.00 ± 0.85 |
| 10 | 15.00 ± 1.57 | 25.00 ± 1.65 | 20.00 ± 0.00 | 24.00 ± 0.50 | 12.00 ± 0.79 |

Data presented as Mean ± SEM (n = 3).

## Conclusion

The oil extracts of *Zingiber officinale* and *Ocimum basilicum* and a combination of the two extracts showed antifungal activity against the clinical isolates of Candida tested. Hence its potential in the local delivery of the oil extracts incorporated into carbopol gel, to the oral cavity as treatment for oral candidiasis. However, an exploitation of different buccal drug delivery system of the essential oils extracts might present a significant milestone in the management of oral candidiasis.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## Acknowledgements

The authors wish to appreciate the laboratory staff of the Department of Pharmaceutics and Industrial Pharmacy and the Department of Pharmaceutical Microbiology, University of Ilorin for their technical assistance during the study.

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