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# Evaluation of Antifungal Activity of Pure versus Nano-Encapsulated Essential Oils against *Candida albicans*

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**KEYWORDS**

Essential oil, encapsulation, lavender oil, ylang-ylang oil, antifungal, Monarda oil

**ABSTRACT:**

The aim of the present study is to encapsulate the combination of lavender and ylang-ylang oil in the nano-range and evaluated for their anti-fungal activity against the species Candida albicans. The study also included the evaluation of eighteen different essential oils for its anti-fungal potential in pure form. Different essential oils (Monarda oil, Lemongrass oil, Bay leaf oil, Eucalyptus oil, Nagarmotha oil, etc.,) extracted through hydro-distillation method. Solvent evaporation technique employed in the process of nano-encapsulation of lavender oil and ylang-ylang oil. Then both the oils characterize for different parameters like particle size, zeta potential and evaluated for their anti-fungal activity. Nano-encapsulated oil after the characterization incorporated into the carbopol gel for the activity evaluation. Litesizer 500 determined the zeta potential and size in nano-range. The maximum particle's diameter was found in the range of 100 nm to 1000 nm and another peak indicates the particles in the range of 1000 to 10000 nm. The mean zeta potential of the nano-encapsulated particles was found in the range of -19.5 mV with standard deviation of 0.7 mV. In the study, the gel containing nano-encapsulated essential oils i.e., lavender and ylang-ylang oil in combination was showed the activity against the Candida albicans. This may be the case that the encapsulation process slow down its release and also lowers down its activity also as compared to the pure oil. Monarda oil showed the highest zone of inhibition followed by palmarosa oil and chamomile oil.

## INTRODUCTION

In place of synthetic medications, a variety of therapeutic and aromatic plants, herbs, and species have been used as a major source of natural antibodies to treat bacterial infections. Herbal remedies and the essential oil derived from them because of its potent bioactive chemicals have been employed extensively in this context[1]. Essential oils have been shown to be effective in treating intestinal disorders, respiratory disorders, skin conditions[2], and urinary tract infections[3]. But due to their instability, essential oils get easily affected by changes in light, temperature, oxygen content, and humidity. The utilization of essential oils in various sectors is hampered by these chemicals' high

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volatility and reactivity. The encapsulation technology is frequently used to control the release of these chemicals while preserving their biological and functional properties in order to get around these restrictions[4].

The term "encapsulation" refers to the technique of keeping a substance inside another material, such as an edible capsule that contains essential oil. Based on their size, the capsules can be categorized into three groups: macro, which are more than 5000µm in size; micro, which are between 0.2 and 5000µm in size; and nano, which are less than 0.2µm. The process of encapsulation reduces the amount of time that the substance that makes up the nucleus interacts with the environment; it also slows down the rate at which the core compounds evaporate or transfer into the environment; it makes handling the encapsulated material easier; it permits release control; it lessens odd flavors and odors; and it permits the encapsulated compound to disperse evenly in a food product[5].

The aim of the study is to evaluate the anti-candidal activity of different essential oils and encapsulate the combination of lavender and ylang-ylang oil in the nano-range and evaluated for their anti-fungal activity against the species *Candida albicans*.

## 1. MATERIAL AND METHODS

### 1.1 Materials

Different plants like *Cinnamomum camphora* (Camphor), *Chrysopogon zizanioides* (Vitever), *Cymbopogon citrates* (Lemon grass), *Matricaria chamomilla* (Chamomile), *Cymbopogon martini* (Palmarosa), *Syzygium aromaticum* (Clove), *Tagetes erecta* (Marigold), *Cymbopogon nardus* (Citronella), *Cedrus deodara* (*Cedarwood*), *Mentha piperita* (Peppermint), *Laurus nobilis* (Bay leaf), *Monarda citriodora* (Monarda), and *Cyperus rotundus* (Nagarmotha) were collected from the Uttar Pradesh region. The essential oils of *Citrus aurantium* (Neroli), *Jasminum officinale* (Jasmine), *Lavandula angustigolia* (Lavender oil), *Rosamarinus officinalis* (Rosemary oil) and *Canaga odorata* (ylang-ylang) oil procured from the authentic sources. The polymer i.e., Sodium alginate, Acetone as solvent, Tween 80 (surfactant), and distilled water used for the encapsulation process. Carbopol 934 was used as the gelling agent for the preparation of gel. Sabouraud dextrose agar (SDA), Fungal Culture (*Candida albicans),* Amphotericin B-(Amphocare) (5mg/ml), and Ketoconazole disc was procured from the authentic sources.

### 1.2 Methods

#### 1.2.1 Extraction of different essential oils by hydro-distillation method

One of the oldest methods for extracting the essential oils from the different parts of the plants is hydro-distillation. Accurately weigh part of the plant from which the oil used to get extracted placed in the RBF (round bottom-flask) of Clevenger apparatus along with the distilled water. The process involves the evaporation of volatile components at low to high temperature. The vapors loaded with volatile components passed through the coolant and get condensed in condenser. The process gets continued for about 5-6 hours for complete separation. Separated oil get collected in the burette, later collected for the yield determination and antifungal evaluation[6].

% yield = weight of collected oil/ weight of plant material × 100………(i)

#### 2.2.2 Procedure for preparation of nano-encapsulated essential oil

The nano-encapsulated essential oil was prepared by the procedure described after doing some modifications in the specified procedure7. The combination of essential oils i.e., lavender oil and ylang-ylang oil was used as the lipid phase in the concentration of 1ml each. 2 ml of combined oil was dissolved homogenously in 20 ml of acetone. The aqueous phase was prepared by dissolving the 0.5 gm of sodium alginate in 1% Tween 80 solution. The lipid phase was slowly added to the aqueous phase with continuous shaking for 5 minutes and further sonicate for 20 minutes. The prepared encapsulated particles was analyze for its particle size.

#### 2.2.3 Particle size analysis of encapsulated oils and determination of their zeta potential

The encapsulated oils in combination form analyze for its particle size by the instrument Litesizer 500 at the temperature of 25 °C and Polydispersity index. Poly-dispersity index is a measure of population size dispersion, with values typically falling between 0.0 and 1.0. Polydispersity index values of 0.2 and less are appropriate for polymer-based nanoconstructs, as

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evidenced by the literature[8]. The solvent used for the analysis was water with the refractive index of 1.3303. The zeta potential was also determined by the instrument Litesizer 500 at the temperature of 25 °C with adjusted voltage of 200 V.

**2.2.4 Preparation of gel containing encapsulated oil**

Carbopol 934P was utilized for the preparation of gel. The distilled water in a definite amount was added to the carbopol with continuous mixing so that the gel gets completely swell. The stirring was done for about 1-2 hours for the homogenous mixing of the polymer. The prepared encapsulated particle of combined essential oils was added at the last and mix homogenously[9].

**2.2.5 Evaluation for antifungal activity of nano-gel**

The Antifungal activity was determined by following Kirby-Bauer method (Zone Inhibition Method). The agar plates were inoculated by spreading with 100 µl of Fungal culture, *C. albicans* and followed by making the wells containing 10 µl of different concentration (0 to 100 mg/ml). One well in each plate was filled with solvent alone which served as vehicle control and Amphotericin B well (25µg) were taken as standard or positive control. The plates of *C. albicans* were incubated (Basil Scientific Corp. India- Incubator) at 37 °C for twenty four hours. The clear zones created around the well were measured and recorded. The amount loaded was in the range of 0-1000µg/well[10].

**2.2.6 Evaluation of anti-candidal activity of different essential oils**

Petri plates containing SDA (Himedia) were inoculated aseptically ,using a sterile inoculating loop or needle or swab, 2-3 colonies of *Candida albicans* were picked and through swabbing technique, streaking was done 3 times over the entire agar surface, so the streak lines overlap, by rotating the plate approx. 60 degrees each time, so that the inoculums gets evenly distributed and the plates were kept in the laminar air flow for 30 min[11].

To check anticandidal activity of volatile oils, Agar-well diffusion Assay and Disk Diffusion method were used simultaneously to measure the zone of inhibition. Aseptically, well of diameter 9 mm were punched in the agar plates using a sterile cork borer. Into each well, 1 mL of the EO samples was dropped using a pipette. Using the forceps, the appropriate antifungal impregnated disks i.e. Ketoconazole disks were dispensed one at a time, onto the agar surface, at some distance from the well. Gently the disk was pressed on the agar surface, to ensure complete contact between disk and the agar surface; since there was quick drug diffusion after a disk comes into touch with the agar surface. After that 50 microlitre of oil was dropped into the prepared well for the activity evaluation. Then the plates were incubated at appropriate temperature (usually 35 degree celsius) for 24-48 hours[12].

## 2. RESULTS AND DISCUSSIONS

### 2.1 Results for yield value and organoleptic analysis

The yield values of different essential oils were calculated by using the above formula. After the extraction organoleptic analysis was performed by visual observations further data is mentioned in Table 1.

Table 1: Yield value and organoleptic analysis of different essential oils.

| S. No. | Essential oils | Part used for extraction | Yield value (%) | Odor | Color |
|---|---|---|---|---|---|
| 1. | *Cinnamomum camphora* | Leaves | 1.2 | Pungent and camphoraceous aroma | Transparent |
| 2. | *Chrysopogon zizanioides* | Roots | 1.1 | Woody and smoky | Brown |
| 3. | *Cymbopogon citrates* | Aerial part | 2 | Citrusy like lemon | Slightly yellowish |

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| | | | | | |
|---|---|---|---|---|---|
| 4. | *Matricaria chamomilla* | Flower | 0.8 | Herbaceous and sweet | Navy Blue |
| 5. | *Cymbopogon martini* | Aerial part | 1.7 | Floral and sweet | Transparent |
| 6. | *Syzygium aromaticum* | Flower buds | 1.4 | Spicy and pungent | Light brown |
| 7. | *Tagetes erecta* | Aerial part | 0.5 | Slightly pungent | Light brown |
| 9. | *Cymbopogon nardus* | Aerial part | 1.4 | Slightly sweet | Tranparent |
| 10. | Cedrus deodara | Wood chipos | 0.8 | Woody | Transparent |
| 11. | *Mentha piperita* | Leaves | 2.2 | Strong, cool and refreshing | Slightly yellowish |
| 12. | *Laurus nobilis* | Leaves | 1.8 | Pungent | Light yellowish |
| 13. | *Monarda citriodora* | Aerial parts | 2.3 | Sweet | Brown |
| 14. | *Cyperus rotundus* | Rhizomes | 1.6 | Woody and earthy | Brown |
| 15. | *Citrus aurantium* | Procured | ----- | Slightly sweet with spicy tone | Transparent |
| 16. | *Jasminum officinale* | Procured | ----- | Sweet smell | Transparent |
| 17. | *Rosamarinus officinalis* | Procured | ----- | Sweet and sharp | Transparent |
| 18. | *Canaga odorata* | Procured | ----- | Sweet smell | Transparent |

**3.2 Results for particle size analysis for encapsulated essential oils and their zeta potential**

In the analysis of particle size distribution by Litesizer 500 the maximum particle's diameter was found in the range of 100 nm to 1000 nm and another peak indicates the particles in the range of 1000 to 10000 nm (Fig 1). The range of particles was 186.2 nm for peak 1 particles which was in between the 100-1000nm (Table 2). Polydispersity index of the particles was found to be 29.2%. The mean zeta potential was found in the range of -19.5 mV with standard deviation of 0.7 mV (Fig 2). The stability of a colloidal particle is determined by the interaction energy between each particle, which is determined by the ζ-potential. High ζ-potential emulsions (positive or negative) are electrically stable, but low ζ-potential emulsions eventually coagulate or flocculate. The ζ-potential measurement for nano/microcapsules may be strongly influenced by particle size[13]. The conductivity of the particles was determined to be about 0.520 mS/cm with transmittance of 79.7%.

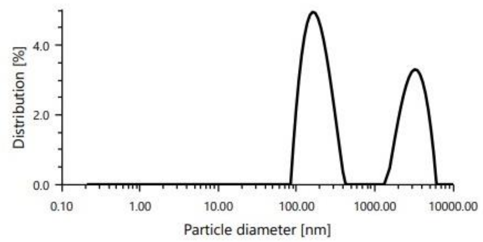


**Fig 1: Particle size distribution (intensity) peak**

**Table 2: Particle size distribution peak (intensity)**

| S. No. | Peak name | Size (nm) | Area (%) | Standard deviation (nm) |
|---|---|---|---|---|
| 1. | Peak 1 | 186.30 | 61.58 | 56.60 |
| 2. | Peak 2 | 3268 | 38.42 | 1044.6 |

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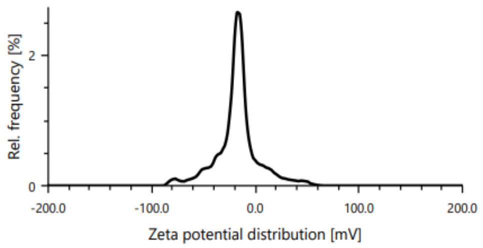


**Fig 2: Graph showing zeta potential distribution**

### 3.3 Results for antifungal activity against *Candida albicans*

**Antifungal activity of nano-gel and pure essential oils against *Candida albicans***

The antifungal activity of prepared gel with combination of nano-encapsulated oils was evaluated against the species *Candida albicans* and the graph was plotted having X-axis showing amount (µg/well) and Y-axis signifies the zone size (in mm). The evaluation was done in triplicate form. The amount used in the study was in the concentration of 0, 62.5, 125, 250, 500 and 1000 µg/well (Table 3). Amphotericin B was used as the standard drug.

The Amphotericin B was found to be effective against the strain, also used as the standard drug in the study. The gel containing encapsulated essential oil in the nano range was effective against the *Candida albicans*. The activity of gel in the concentration of 125 and 250 µg/well was same while the increasing concentration does not showed much variation in activity (Fig 3).

**Table 3: Antifungal evaluation against Candida albicans**

| S. No. | Amount (µg/well) | Plate A | Plate B | Plate C | Average | Standard deviation | SEM |
|---|---|---|---|---|---|---|---|
| 1. | Positive control | 11 | 10 | 11 | 10.6667 | 0.57735 | 0.33333 |
| 2. | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3. | 62.5 | 9 | 8 | 8 | 8.33333 | 0.57735 | 0.33333 |
| 4. | 125 | 8 | 8 | 8 | 8 | 0 | 0 |
| 5. | 250 | 8 | 8 | 8 | 8 | 0 | 0 |
| 6. | 500 | 7 | 8 | 7 | 7.33333 | 0.57735 | 0.33333 |
| 7. | 1000 | 6 | 7 | 6 | 6.33333 | 0.57735 | 0.33333 |

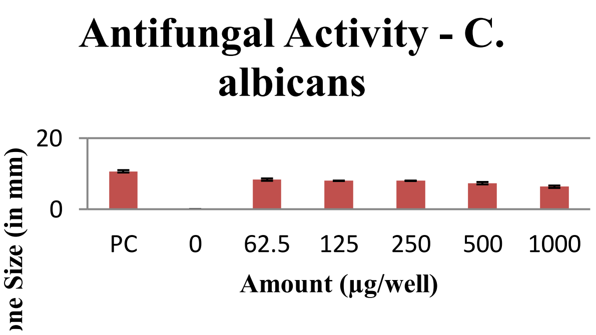


**Fig 3: Antifungal activity of gel containing nano-encapsulated oil against *Candida albicans***

Pure essential oils showed the effective results when evaluated against the fungal strain (Table 4). Monarda oil showed the highest zone of inhibition i.e., 26 mm followed by clove oil i.e, 25 mm and then palmarosa oil i.e., 24.33 mm. Citronella oil and Niroli oil showed same zone of inhibition i.e., 20.66 mm while bay leaf oil and vitever oil showed same results (Zone of inhibition was 20.33 mm). Other oils like camphor oil, nagarmotha oil, peppermint oil, marigold oil also showed the good results. The least activity showed by Cedarwood oil with minimum zone of inhibition of about 10 mm.

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**Table 4: Anticandidal activity of different essential oils**

| S. No. | Essential oil | Standard (Ketoconazole) Zone of Inhibition (mm) | Zone of Inhibition (mm) Trail I | Zone of Inhibition (mm) Trail II | Zone of Inhibition (mm) Trail III | Mean |
|---|---|---|---|---|---|---|
| 1. | Camphor oil | 27 | 21 | 16 | 19 | 18.66 |
| 2. | Jasmine oil | 20 | 14 | 15 | 17 | 15.33 |
| 3. | Citronella oil | 25 | 19 | 22 | 21 | 20.66 |
| 4. | Rosemary oil | 26 | 22 | 24 | 17 | 21 |
| 5. | Vitever oil | 27 | 24 | 18 | 19 | 20.33 |
| 6. | Cedarwood oil | 26 | 10 | 10 | 12 | 10.66 |
| 7. | Nagarmotha oil | 28 | 19 | 17 | 18 | 18 |
| 8. | Chamomile oil | 27 | 23 | 20 | 22 | 21.66 |
| 9. | Palmarosa oil | 19 | 22 | 25 | 26 | 24.33 |
| 10. | Niroli oil | 20 | 21 | 18 | 23 | 20.66 |
| 11. | Marigold oil | 23 | 18 | 17 | 14 | 16.33 |
| 12. | Peppermint oil | 20 | 19 | 17 | 24 | 20 |
| 13. | Bay leaf oil | 24 | 29 | 22 | 22 | 24.33 |
| 14. | Clove oil | 25 | 27 | 22 | 26 | 25 |
| 15. | Lavender oil | 22 | 18 | 16 | 19 | 17.66 |
| 16. | Monarda oil | 23 | 30 | 24 | 24 | 26 |
| 17. | Lemongrass oil | 20 | 21 | 19 | 19 | 19.66 |
| 18. | Ylang-Ylang oil | 18 | 16 | 19 | 19 | 18 |

## CONCLUSION

The increasing prevalence of antifungal resistance in *Candida albicans* underscores the need for continued research and concerted efforts to preserve the effectiveness of current antifungal therapies and develop innovative strategies to combat resistant infections. Overall, the multifaceted mechanisms of action of essential oils as reviewed by different researchers against fungal cells highlight their potential as alternative or adjunctive antifungal agents. The encapsulation of essential oils is a possible approach for slow down the release of essential oils. In the study the gel containing nano-encapsulated essential oils i.e., lavender and ylang-ylang oil in combination was showed the activity against the *Candida albicans*. This may be the case that the encapsulation process slow down its release and also lowers down its activity also as compared to the pure oil which was suggested in various literatures. Excellent antifungal action against Candida spp. has also been demonstrated by *Lavandula angustifolia*, or lavender essential oil which is mentioned in many literatures[14]. Though EOs has been shown in multiple trials to be effective as a complementary treatment for

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fungal infections, it should be highlighted that these characteristics are heavily reliant on the EO's own composition. The usage of different plant sections or the time of year for harvesting might cause major variations in these compositions are heavily reliant on the EO's own composition. The usage of different plant sections or the time of year for harvesting might cause major variations in these compositions. Monarda oil, Clove oil and Palmarosa oil showed the effectiveness against the *Candida albicans* in pure form. Essential oils are generally recognized as safe when used appropriately, and their natural origin makes them an attractive alternative to synthetic chemicals. They are less likely to cause severe side effects and can be used in various formulations, including topical applications and inhalations. Continued research into the specific bioactive components of essential oils and their interactions with fungal cells will further elucidate their therapeutic potential in combating fungal infections.

**CONFLICT OF INTEREST:**

The authors have no conflicts of interest regarding this investigation**.**

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**REFERENCES**

1. Mishra P, Prabahar AE, Verma A, Verma AK. Extraction & characterization of essential oils having mosquito repellent activity. *International Journal of Mosquito Research*. 2023;10(6):134-137.
2. Mishra P, Kumar J, Verma A, Prabahar AE, Verma AK. Linalool based herbal mosquito repellant. *International Journal of Mosquito Research*. 2023;10(3):15-23.
3. Puvača N, Milenković J, Coghill TG, et al. Antimicrobial Activity of Selected Essential Oils against Selected Pathogenic Bacteria: In Vitro Study. *Antibiotics*. 2021;10(5):546. doi:10.3390/antibiotics10050546
4. Sousa VI, Parente JF, Marques JF, Forte MA, Tavares CJ. Microencapsulation of essential oils: a review. *Polymers*. 2022;14(9):1730. doi:10.3390/polym14091730
5. Gandra EA, Radünz M, Helbig E, Borges CD, Gandra TKV. A Mini-Review on encapsulation of essential oils. *Journal of Analytical & Pharmaceutical Research*. 2018;7(1). doi:10.15406/japlr.2018.07.00205
6. Olascuaga-Castillo KA, Castillo-Medina O, Villacorta-Zavaleta M, et al. Extraction of essential oils by hydrodistillation of four aromatic species: Conditioning, extraction conditions, yield and chemical composition. *Scientia Agropecuaria*. 2024;15(3):385-408. doi:10.17268/sci.agropecu.2024.029
7. Cofelice M, Cuomo F, Chiralt A. Alginate films encapsulating lemongrass essential oil as affected by spray calcium application. *Colloids and Interfaces*. 2019;3(3):58. doi:10.3390/colloids3030058
8. Aguilar-Pérez KM, Medina DI, Parra-Saldívar R, Iqbal HMN. Nano-Size characterization and antifungal evaluation of essential oil Molecules-Loaded nanoliposomes. *Molecules*. 2022;27(17):5728. doi:10.3390/molecules27175728
9. Pattnaik S, Priyadarshini S, Niyogi P, Maharana L. Formulation and Evaluation of Microencapsulated Levofloxacin Gel. *Research & Reviews: A Journal of Drug Formulation, Development and Production.* 2016;3(1):39-47. https://doi.org/10.37591/rrjodfdp.v3i1.338
10. Kottferová L, Čonková E. In Vitro Antifungal Activity of Selected Essential Oils Against Trichophyton mentagrophytes. *Folia Veterinaria*. 2023;67(2):33-41. doi:10.2478/fv-2023-0015
11. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. *Clinical Microbiology Reviews*. 2020;33(3). doi:10.1128/cmr.00069-19
12. Mekonnen A, Yitayew B, Tesema A, Taddese S. In VitroAntimicrobial Activity of Essential Oil ofThymus schimperi,Matricaria

chamomilla,Eucalyptus globulus, andRosmarinus officinalis. *International Journal of Microbiology*. 2016;2016:1-8. doi:10.1155/2016/9545693

13. Feng Y, Kilker SR, Lee Y. Surface charge (zeta-potential) of nanoencapsulated food ingredients. In: *Elsevier eBooks*. ; 2020:213-241. doi:10.1016/b978-0-12-815667-4.00007-9

14. Rashed AA, Rathi DNG, Nasir NAHA, Rahman AZA. Antifungal properties of essential oils and their compounds for application in skin fungal infections: Conventional and nonconventional approaches. *Molecules*. 2021;26(4):1093. doi:10.3390/molecules26041093