Vol. 35 / No. 2 / August 2023
Sinta 2 (Decree No: 200/M/KPT/2020)
Online ISSN : 2549-4082 ; Print ISSN : 1978-4279
Available online at https://e-journal.unair.ac.id/BIKK

Berkala Ilmu Kesehatan Kulit dan Kelamin

Original Article

Periodical of Dermatology and Venereology

Antifungal Activity of Rosemary (*Rosmarinus officinalis* L.) Emulsion Gel Compared to Nystatin on *Candida albicans* Stored Isolate from HIV/AIDS Patients with Oral Candidiasis

Maya Wardiana[1], Astindari[1], Evy Ervianti[1], Afif Nurul Hidayati[1,2], Diah Mira Indramaya[1], Pepy Dwi Endraswari[2,3], Budi Utomo[4], Dwi Murtiastutik[1]

[1]*Department of Dermatology and Venereology, Dr. Soetomo General Academic Hospital, Surabaya*
[2] *Universitas Airlangga Teaching Hospital, Surabaya, Indonesia*
[3]*Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya*
[4]*Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*

ABSTRACT

Background: Oral candidiasis (OC), frequently caused by *Candida albicans*, is the most common opportunistic infection in HIV/AIDS patients. Topical treatment for OC is limited. In vitro study revealed rosemary (*Rosmarinus officinalis* L.) essential oil has an antifungal effect. However, the essential oil is volatile and is not recommended to be applied to the skin and mucosa; therefore, emulsion gel (emulgel) is made. **Purpose:** This study was conducted to evaluate the antifungal activity of rosemary emulgel 6.25%, 25%, 37.5%, and 50% compared to nystatin. **Methods:** This study is an in vitro test using the disk diffusion method to d etermine the antifungal activity shown by the inhibitory zones of rosemary emulgel at 6.25%, 25%, 37.5%, and 50% compared to nystatin to stored isolates of *C. albicans* from HIV/AIDS patients with OC. **Result:** Rosemary emulgel 6.25% dan 25% did not show antifungal activity because no inhibitory zone was shown. The inhibitory zone diameter provided by rosemary emulgel 37.5%; 50%; and nystatin against *C. albicans* isolates were 3.17±3.763 mm; 7.00±4.107 mm; and 30.13±5.319 mm respectively. Significant differences in antifungal activity were shown by the inhibitory zone diameter provided between rosemary emulgel 37.5%, 50%, and nystatin (p<0,05). **Conclusion:** Rosemary emulgel 37.5% dan 50% had antifungal activity showed by the formation of inhibitory zone against *Candida* species isolates in disk diffusion method even though it was weaker compared to nystatin as a standard antifungal.

Keywords: oral candidiasis, nystatin, rosemary, *Rosmarinus officinalis* L., HIV/AIDS.

Correspondence: Dwi Murtiastutik, Department of Dermatology and Venerology Faculty of Medicine, Universitas Airlangga / Dr. Soetomo General Academic Hospital, Surabaya, Jl. Mayjen Prof. Dr. Moestopo No. 6-8 Surabaya 60131, Indonesia. Phone: (031) 5501609, e-mail: dwi.murtiastutik@fk.unair.ac.id

| Article info |
Submited: 25-08-2022, Accepted: 22-02-2023, Published: 31-07-2023

BACKGROUND

Oral candidiasis (OC) is the most common opportunistic infection in patients with Human Immunodeficiency Virus (HIV). A study of new HIV/acquired immunodeficiency syndrome (AIDS) patients in Prof. dr. R. D. Kandou General Teaching Hospital, Manado, Indonesia, from 2014 until 2016 showed OC is the most common mucocutaneous disorder happening in 90,85% of patients.[1] Oral candidiasis (OC) lesions can be painful and cause difficulty in eating, a condition that reduces the patient's quality of life.[2] *Candida albicans* is the species that is predominantly found in OC lesions. Ninety-five percent of OC are caused by *C. albicans* albeit an increase in infections caused by non-*albicans Candida* species has been observed in the last decade.[3,4]

Nystatin is still the mainstay of topical antifungal treatment; however, it has some adverse effects such as poor taste and gastrointestinal reactions, including vomiting, nausea, diarrhea, anorexia, and abdominal pain.[5] Research to find new antifungal drugs, especially from natural ingredients, is still being developed. Rosemary (*Rosmarinus officinalis* L.) is an

DOI : 10.20473/bikk.V35.2.2023.88-92

aromatic plant that has been known to have an antifungal effect. A study to evaluate the antifungal activity of rosemary essential oil to *Candida* species from HIV/AIDS patients with OC showed the minimum inhibitory concentration is 6.25%, while the minimum fungicidal concentration is 25%.[6] The downside of essential oil is its low volatility and the potential for skin irritation in direct application.[7,8] Emulsion gel (emulgel) can help resolve this problem. Emulgel is a combination of emulsion and gel. A gel is a semisolid dosage form with several advantages compared to cream or ointment in terms of the higher aqueous component, which allows for increased drug solubility and easier drug migration across the vehicle. However, gel has limitation in delivering hydrophobic substances. Emulgel was presented to facilitate hydrophobic drugs to benefit from the unique feature of gel.[9]

The study about rosemary antifungal activity, especially in emulgel form, is still limited. This study aimed to evaluate the antifungal activity of rosemary emulgel compared to nystatin as the standard topical treatment on *C. albicans* stored isolated from HIV/AIDS patients with OC.

METHODS

The research design of this study was an experimental laboratory to evaluate the antifungal activity of rosemary (*Rosmarinus officinalis* L.) emulgel compared to nystatin. Estimation of a sample size to assess the relationship between the type of antifungal and the diameter of the inhibitory zone were counted using the mean difference formula between 2 unpaired groups. There were 9 *Candida albicans* isolates taken from the oral cavity of confirmed HIV/AIDS patients hospitalized at the Infectious Disease Intermediate Treatment Unit Dr. Soetomo General Academic Hospital Surabaya from April to July 2019, which were reactivated. The inclusion criteria were HIV/AIDS patients with OC who had been treated with antifungals before. While the exclusion criteria were patients with candidemia. The rosemary emulgel concentration used was based on the former study, which were 6.25%, 25%, and two other concentrations, which were 37.5% and 50%. The antifungal activity was evaluated using the disk diffusion method. Nystatin disk 100 IU was used as the comparison.

The stored isolates were taken from the cryo tubes in Microbiology Department Laboratory, Medical Faculty, Universitas Airlangga. It was reactivated using Sabouraud dextrose agar (SDA) as the media and incubated at 35^0 C for 24-48 hours. Activated stored isolates were prepared as inoculums for the antifungal activity test by forming suspension in semisolid media with turbidity equal to 0.5 McFarland scale. *Candida albicans* suspensions were then blotted onto Muller Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue. Paper discs containing 20 µl of rosemary emulgel 6.25%, 25%, 37.5%, 50%, and nystatin with a potency of 100 IU as a positive control and base emulgel as negative control were placed on Muller Hinton agar and the agar was dried at room temperature for 15 minutes after which it was incubated at 35^0 C for 48 hours, to observe whether there was a clear zone around the disc paper. The diameter of the inhibition zone was measured according to the clear zone using a caliper in millimeters (mm).

The data were entered into a data collection sheet and analyzed with SPSS (Statistical Package for Social Sciences) version 21. Non-parametric statistical methods (Mann-Whitney Test) were used when the data were not normally distributed or homogenous. A *p*-value less than 0.05 was considered statistically significant. This research has obtained ethical approval from the Ethics Committee of Dr. Soetomo General Academic Hospital Surabaya (0567/LOE/301.4.2/ VIII/2021).

RESULT

This study showed that the mean inhibitory zone of nystatin disk 100 IU in *C. albicans* isolates was 30.13±5.319 mm. Rosemary (*Rosmarinus officinalis* L.) emulgel 6.25% and 25% did not show antifungal activity in all *C. albicans* isolates. (Table 1).

Table 1. Mean diameter of the inhibitory zone of rosemary (*Rosmarinus officinalis* L.) emulsion gel (emulgel) and nystatin in *Candida albicans* isolates

Anti-fungal	*Candida albicans* (n=9)
Rosemary emulgel 6.25%	0.00
Rosemary emulgel 25%	0.00
Rosemary emulgel 37.5%	3.17±3.763
Rosemary emulgel 50%	7.00±4.107
Nystatin disk 100 IU	30.13±5.319

The comparative analysis of the mean inhibitory zone between rosemary (*Rosmarinus officinalis* L.) emulgel 37.5%, 50%, and nystatin on the growth of *C. albicans* isolates were established. This study showed that the mean inhibitory zone of nystatin was larger than rosemary emulgel 37,5% or 50% and was statistically significant with the *p*-value <0.05 (Table 2). Rosemary emulgel 50% also showed larger

inhibitory zone than rosemary emulgel 37.5% and was statistically significant with the *p*-value <0.05 (Table 2).

Table 2. Comparison of the mean diameter of the inhibitory zone of rosemary (*Rosmarinus officinalis* L.) emulsion gel (emulgel) and nystatin in *Candida albicans* isolates

Anti-fungal	*Candida albicans* (n=9)	
	Inhibition zone (mm) ± Standard Deviation (SD)	*p*-value
Rosemary emulgel 37.5%	3,17±3,763	0.001
Nystatin 100 IU	30,13±5,319	
Rosemary emulgel 50%	7,00±4,107	0.001
Nystatin 100 IU	30,13±5,319	
Rosemary emulgel 37.5%	3,17±3,763	0.026
Rosemary emulgel 50%	7,00±4,107	

DISCUSSION

There was no inhibitory zone formed by rosemary (*Rosmarinus officinalis* L.) emulgel 6.25% and 25% in all *C. albicans* isolates. This was a conflicting result compared to a study by Anjuwita et al. in which using the microdilution method, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of rosemary essential oil were obtained and it were 6.25% and 25%.[6] This study used the same rosemary essential oil as the study mentioned before to maintain reproducibility. The conflicting result can be caused by the different methods used (microdilution in the study by Anjuwita et al. and disk diffusion in this study). In the microdilution method, the antimicrobe tested will interact directly with the microbe in the medium while in the disk diffusion method, a disk containing antimicrobe that will diffuse through the medium.[10] A study compared disk diffusion and microdilution methods to evaluate the antifungal activity of natural compounds stated that the disk diffusion method was less sensitive.[11]

Inhibitory zone started to form in rosemary emulgel at 37.5% and 50%. The result in this study showed a better result compared to a study by Primasari et al. That study tested the antifungal activity of rosemary essential oil against *C. albicans* isolates using the disk diffusion method. The mean inhibitory zone found in that study was 1.75±3.64 mm for *C. albicans.*[12] This difference in results can be caused by differences in the dosage forms used, namely the essential oil in that study and the emulgel in this study. The zone of inhibition can be affected by the volatility of the antifungal agent tested, the size of the disc, the amount of antifungal added to the disc, the adsorption of the disc, the type of agar and volume of the agar, and the type of antifungal used.[13] Essential oils have high volatility so there is a possibility of vaporization during storage or before diffusion on the disc paper which can reduce its effectiveness. The manufacture of emulgel preparations also makes the emulsion formulation more stable.[9] The diameter of the rosemary emulgel inhibition zone was larger in this study than the diameter of the rosemary essential oil inhibition zone in the previous study, indicating that emulgel is a promising dosage form in the development of natural ingredients as topical antifungals.

This study used stored clinical strains from patients with HIV/AIDS. A different source of pathogens can affect the antifungal activity test because they can have different virulence factors. Research by Alnuaimi et al. compared the ability of laboratory and clinical strains to form biofilms, which are important virulence factors. In that study, laboratory and clinical strains showed differences in the ability to form biofilms.[14] Maheronnaghsh et al. tried to investigate several virulence factor activities of *Candida* species derived from OC lesions of patients with cancer. The virulence factors assessed were the ability to produce phospholipase, esterase, hemolysin, and proteinase. The percentage of *C. albicans* capable of producing phospholipase, esterase, hemolysin, and proteinase was higher than that of non-*albicans Candida* species.[15] Different sources of clinical isolates can also cause different virulence factors. Villar-Vidal et al. conducted a study to evaluate variations in biofilm formation between blood and oral *C. albicans* and *C. dubliniensis* isolates. Isolates of *C. albicans* and *C. dubliniensis* from blood showed higher biofilm production than isolates from oral.[16] This study helps provide information on the antifungal activity of rosemary emulgel on *C. albicans* derived from HIV/AIDS patients with OC.

Rosemary emulgel produced inhibitory zones for the growth of *C. albicans* at concentrations of 37.5% and 50%, although they were smaller than nystatin. Gas chromatography mass spectrometry (GC-MS) test on rosemary essential oil which was used as an active ingredient in this study had been carried out in research by Anjuwita et al. with the results that the essential oil contains 45.12% 1,8-cineol, 12.78% α-pinene, 10.79% camphor and 3.50% borneol.[6] The typical components of rosemary are 1,8-cineol, α-pinene, and camphor. These components can have relatively stable ratios that define each plant essential oil chemotype. There are three rosemary chemotypes, namely cineol, verbenon, and camphor.[17] Rosemary essential oil in this study corresponded to the cineol

chemotype with a 1,8-cineol content of more than 40%. Based on research by Matsuzaki et al. the cineol chemotype showed the highest antifungal activity of the three existing chemotypes, but the antifungal activity against *C. albicans* in this study was low with 1,8-cineol alone. Another study tried to compare the antifungal activity of rosemary essential oil, 1,8-cineol and α-pinene. In that study, rosemary essential oil had better antifungal activity than 1,8-cineol or α-pinene alone.[18] The highest antifungal activity for rosemary components was in α-pinene but it was reported that α-pinene has enantiomers, namely (+)α-pinene which has biological activity and (-)α-pinene which has no biological activity. The enantiomeric distribution depends on the geographical origin of the plant.[17] Unfortunately, the enantiomer of α-pinene in this study is not known, so it cannot be explained further about the antifungal activity of the rosemary component in this study.

Comparative analysis of the diameter of the inhibitory zone of rosemary emulgel and nystatin on the growth of *C. albicans* isolates species showed that the diameter of the nystatin inhibitory zone was larger than the diameter of the inhibition zone of rosemary emulgel with a significant difference (p <0.05). Rosemary emulgel 50% also showed a larger diameter of inhibition zone than rosemary emulgel 37.5% with a significant difference (p <0.05). Research by Shebi et al. which evaluated the antifungal effect of rosemary essential oil on *C. albicans* using fluconazole and amphotericin B as a positive control showed that at a dose of 25 microliters, rosemary showed antifungal activity but with a smaller inhibition zone than fluconazole and amphotericin B.[19] Research by Gauch et al. who examined the antifungal activity of rosemary essential oil against *C. albicans*, *C. dubliniensis*, *C. parapsilosis* and *C. krusei* showed that *C. albicans* was the most susceptible species compared to other *Candida* species which showed the lowest MIC.[20] Rosemary has antifungal activity as indicated by the inhibition of growth of *C. albicans* based on existing research, although it is still below the standard drug.

Rosemary emulgel was able to inhibit *C. albicans* growth as indicated by the formation of an inhibitory zone. However, it was still lower than nystatin. Rosemary emulgel still cannot replace nystatin as a topical therapy. Nevertheless, research can be conducted to evaluate the potency of rosemary emulgel as an adjuvant therapy or prophylactic of OC.

REFERENCES

1. Niode NJ, Sondakh R, Sengkey T, Nugroho A. Kelainan mukokutan dan infeksi menular seksual pada pasien HIV-AIDS di RSUP Prof. DR. R. D. Kandou, Manado. Media Dermato Venereologica Indonesiana 2018;45(2)60–5.
2. Clark-Ordóñez I, Callejas-Negrete OA, Aréchiga-Carvajal ET, Mouriño-Pérez RR. Candida species diversity and antifungal susceptibility patterns in oral samples of HIV/AIDS patients in Baja California, Mexico. Med Mycol 2017;55(3):285–94.
3. Spalanzani RN, Mattos K, Marques LI, Barros PFD, Pereira PIP, Paniago AMM, et al. Clinical and laboratorial features of oral candidiasis in HIV-positive patients. Rev Soc Bras Med Trop 2018;51(3):352–6.
4. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral candidiasis: A disease of opportunity. J Fungi 2020;6(1):1–28.
5. Lyu X, Zhao C, Yan ZM, Hua H. Efficacy of nystatin for the treatment of oral candidiasis: A systematic review and meta-analysis. Drug Des Devel Ther 2016;10:1161–71.
6. Anjuwita BY, Zulkarnain I, Listiawan MY, Ervianti E, Rahmadewi R, Endraswari PD, et al. Antifungal activity of *Rosmarinus Officinalis* essential oil and nystatin on store isolate of *Candida* species from HIV/AIDS patients with oral candidiasis. Berk Ilmu Kesehat Kulit dan Kelamin 2020;32(3):167–73.
7. Asfiyah S, Supaya. Modifikasi Deanstark upaya efisiensi proses distilasi uap minyak biji pala dalam praktikum kimia organik. Indones J Lab 2020;2(2):10–5.
8. Gandhi J, Suthar D, Patel H, Shelat P, Parejiya P. Development and characterization of microemulsion based topical gel of essential oil of clove (*Syzygium aromaticum*) for superficial fungal infections. Adv Tradit Med 2021;21(3):519–34.
9. Iyer MS, Gujjari AK, Paranthaman S, Lila ASA, Almansour K, Alshammari F, et al. Development and evaluation of clove and cinnamon supercritical fluid extracts-loaded emulgel for antifungal activity in denture stomatitis. Gels 2022;8(33):1–20.
10. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016;6(2):71–9.
11. Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VS, Mendes-Giannini MJS. Comparative study of disk diffusion and microdilution methods for evaluation of

antifungal activity of natural compounds against medical yeasts *Candida* sp. and *Cryptococcus* sp. Rev Ciencias Farm Basica e Apl 2007;28(1):25–34.

12. Primasari PI, Murtiastutik D, Endraswari PD, Prakoeswa CRS, Ervianti E. Comparison of in vitro testing antifungal activity between rosemary essentials oil and fluconazol on candida species isolate from HIV/AIDS patients with candidiasis oral. Berk Ilmu Kesehat Kulit dan Kelamin 2020;32(3):182–88.
13. Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VDS, Gianinni MJSM. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp. and *Cryptococcus* sp. Brazilian J Microbiol 2007;38(3):391–7.
14. Alnuaimi AD, O'Brien-Simpson NM, Reynolds EC, Mccullough MJ. Clinical isolates and laboratory reference *Candida* species and strains have varying abilities to form biofilms. FEMS Yeast Res 2013;13(7):689–99.
15. Maheronnaghsh M, Fatahinia M, Dehghan P, Mahmoudabadi AZ, Kheirkhah M. Comparison of virulence factors of different Candida species isolated from the oral cavity of cancer patients and normal individuals. Jundishapur J Microbiol 2019;12(5).
16. Villar-Vidal M, Marcos-Arias C, Eraso E, Quindós G. Variation in biofilm formation among blood and oral isolates of *Candida albicans* and *Candida dubliniensis*. Enferm Infecc Microbiol Clin 2011;29(9):660–5.
17. Matsuzaki Y, Tsujisawa T, Nishihara T, Nakamura M, Kakinoki Y. Antifungal activity of chemotype essential oils from rosemary against *Candida albicans.* Open J Stomatol 2013;03(02):176–82.
18. Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, et al. Chemical composition and antimicrobial activity of the essential oil of rosemary. Environ Toxicol Pharmacol 2011;32(1):63–8.
19. Shebi S, Geetha R, Thangavelu L. Evaluation of the anti-mycotic activity of rosemary oil against *Candida albicans*. Int J Res Pharm Sci 2019;10(2):1228–32.
20. Gauch LMR, Pedrosa SS, Esteves RA, Silveira-Gomes F, Gurgel ESC, Arruda AC, et al. Antifungal activity of *Rosmarinus officinalis* Linn. essential oil against *Candida albicans, Candida dubliniensis, Candida parapsilosis* and *Candida krusei*. Rev Pan-Amazônica Saúde 2014;5(1):61–6.