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Antifungal activity test of Dewandaru leaf extract (*Eugenia uniflora L*) against *Candida* in otomycosis

I Wayan Lolik Lesmana *, Ni Putu Oktaviani Rinika Pranitasari, I Gusti Ayu Mahaprani Danastri, I Gusti Ayu Putu Wahyu Widiantari and Ida Bagus Hendra Kusuma

Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine Udayana University/ Udayana University General Hospital, Indonesia.

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Abstract

Background: Tropical climate conditions and a lack of public awareness about maintaining environmental cleanliness mean that infectious diseases are still a major health problem in Indonesia. Bacteria, viruses, fungi and parasites are pathogenic organisms that cause infections. Fungal infections receive less attention because they are rarely fatal for the sufferer. Fungal infections in the ear, known as otomycosis, are often caused by *Candida* fungi. The Dewandaru plant (*E. uniflora L*) as a type of plant that is widely used for traditional medicine in the community is known to have antifungal potential. This plant contains flavonoids, alkaloids, terpenoids, anthraquinones and tannins which have antifungal properties.

Method: The method used in this research was experimental research with a post test only control group design on *C. albicans* test material. The positive control was 1% Clotrimazole suspension and the negative control was 96% ethanol solution.

Result: Research showed that there was an inhibitory effect of *E. uniflora L* leaf extract in concentrations of 100%, 90% and 80% against the *Candida albicans* fungus. Average diameter of the inhibitory area of leaf extract *E. uniflora L* concentrations of 100%, 90% and 80% respectively were 22.2 mm; 19.0mm; and 18.0 mm, while the average diameter of the 1% Clotrimazole inhibition area was 31.8 mm.

Conclusion: There is an inhibitory effect of Dewandaru leaf extract (*E. uniflora L*) in concentrations of 100%, 90% and 80% against the *Candida albicans* fungus.

Keyword: Dewandaru leaf extract; Antifungal; *Candida albicans*; Otomycosis

1. Introduction

Otomycosis is an infection of the ear, especially the outer ear, which is often found in dusty, hot and high humidity environments. Indonesia is located on the equator line, making it a country with a tropical climate, so cases of otomycosis are quite often encountered in health services. The most common causative microorganisms are *Candida* and *Aspergillus*. Antifungal therapy is given topically. The antifungals of choice are clotrimazole 1%, amphotericin V 3%, flucytosine 10%, econazole cream 1%. Salicylic acid, griseofulvin, and ketoconazole are known to be less effective. If topical antifungal therapy does not provide improvement, systemic antifungal itraconazole for superficial infections or voriconazole for deeper infections can be considered¹⁻³.

* Corresponding author: I Wayan Lolik Lesmana

The tropical climate and lack of public awareness of maintaining environmental cleanliness means that infectious diseases are still a major health problem in Indonesia. Bacteria, viruses, fungi and parasites are pathogenic organisms that cause infections. In the field of ear, nose and throat health science, fungal infections of the ear are a challenge for clinicians because of the high recurrence rate ^{4,5}. The Dewandaru plant (*E. uniflora L*) as a type of plant that is widely used for traditional medicine in the community is known to have antifungal potential. This plant contains flavonoids, alkaloids, terpenoids, anthraquinones and tannins which have antifungal properties. Treatment using the basic ingredients of *E. uniflora L* leaf in the field of Ear, Nose and Throat (ENT) as an antifungal is very limited, therefore researchers are interested in conducting research on the antifungal potential of *E. uniflora L* leaf extract against *Candida albicans* as a cause of ear otomycosis ^{2,4,6}.

2. Material and Method

2.1. Method

E. uniflora L leaves were collected in Karangasem city, Bali, Indonesia. The plant was identified at the Indonesian Institute of Sciences (LIPI), plant conservation center for the Eka Karya Bali Botanical Gardens. The leaves were dried at temperature and group into powder. The extract making process consists of maceration and evaporation processes. In the maceration process, 1000 grams of *E. uniflora L* leaf powder was macerated using 2000 ml of 96% ethanol for 5 days, filtered until produces a precipitate in the form of a blackish brown filtrate of 300 ml. The evaporation process is carried out until the volume of the extraction results decreases and becomes thick. The evaporation results were collected in an evaporator cup and then placed in the oven for approximately 2 hours at a temperature of 80oC, so that 100% *Eugenia uniflora L* leaf extract was obtained.

The technique for measuring the antifungal activity of a test material in vitro can be done using two methods, namely the dilution and diffusion methods. The agar diffusion method is most often used to determine the sensitivity of a test organism to the test material. Filter paper discs containing a certain amount of test material are placed on the surface of a solid medium that has previously been inoculated with the test organism on its surface. After incubation, the diameter of the inhibition zone around the disc is measured and used as a measure of the drug's resistance to the test organism. Common criteria used in various studies are the Davis and Stout criteria Growth Inhibitory Response of the test organism substance (>20 mm: very strong; 11-19 mm: strong; 5-10 mm : moderate; < 5 mm: weak).

This research is an experimental study with a post-test only control group design by testing Dewandaru leaf extract in three preparations, namely 100%, 90%, 80% b/v dewandaru leaf extract. As a positive control, 3% Clotrimazole was used and as a negative control, 96% ethanol was used. Each treatment was repeated 5 times. The study population was all American Type Culture Collection (ATCC) culture media for *Candida albicans* which causes ear otomycosis. The research sample was the American Type Culture Collection (ATCC) bacterial culture *Candida albicans*. The ATCC *Candida albicans* bacterial culture sample was obtained from the Microbiology Section of the Faculty of Medicine, Udayana University, Denpasar, in collaboration with the American Type Culture Collection as a company providing cultures for test organisms. The choice of ATCC culture media as research samples aims to obtain microorganisms as pure cultures or cultures whose cells originate from the division of a single cell, have stable genetic characteristics and have never been exposed to antibiotics. Testing and analysis of the active compounds from Dewandaru leaves aims to determine the potential of the active compounds of these leaves as anti-bacterial agents. Antifungal effect testing was carried out in the laboratory of the Microbiology Section of the Faculty of Medicine, Udayana University, Denpasar. Discs that have been dripped with 5 μ emulsion of Dewandaru leaf extract with respective concentrations of 100%, 90%, 80% w/v are attached to Mueller Hinton agar media which has been incubated with the ATCC fungus *Candida albicans*. The data is displayed descriptively in table form containing data regarding the anti-fungal effect of *E. uniflora L* leaf extract which has been tested on *Candida albicans* ATCC fungus agar media in concentrations of 100%, 90%, 80%, 96% ethanol and 3% Clotrimazole. Anti-fungal activity was analyzed statistically using the One-Sample Kolmogorov-Smirnov test to determine the normality and homogeneity of the data obtained from the test results. If the results obtained are not normally distributed, the Kruskal Wallis test is used.

3. Results

The results of the phytochemical test of Dewandaru (*E. uniflora L.*) leaves using ethanol solvent are presented in Table 1. This table provides a comprehensive overview of the compounds identified in the leaves, including their respective concentrations and potential bioactive properties. The data are critical for understanding the medicinal and therapeutic potential of Dewandaru leaves, and they contribute valuable insights to the field of natural product research.

Table 1 Phytochemical test results from Dewandaru (*Eugenia uniflora* L) leaves using ethanol solvent.

	Ethanol
Alkaloid	++
Flavonoid	++
Saponin	++
Tanin	+++
Phenol	+++
Fatty acid	++
Acid	+++
Terpenoid	+++
Triterpenoid	+++

Note:

(-) Not detected

(+) Weakly detected

(++) Moderately detected

(+++ Strongly detected

The results of the anti-fungal test of Dewandaru leaf extract with concentrations of 80%, 90%, and 100% respectively against *Candida albicans* are summarized in the following data. These tests assess the efficacy of different concentrations of the extract in inhibiting the growth of the fungal species, which is known to cause infections in humans. The data provide insight into the potential of Dewandaru leaf extract as a natural anti-fungal agent and may guide future research and development in the field of alternative medicine. Understanding the concentration-dependent effects on *Candida albicans* can help optimize the use of Dewandaru leaf extract for therapeutic purposes.

Table 2 The results of the anti-fungal test of Dewandaru leaf extract with concentrations of 80%, 90% and 100% respectively against *Candida albicans*.

Handling	Repetition	Minimum resistance diameter	Maximum resistance diameter	Σ RD
Control (+)	5	28,0	35,0	31,8
Extract 80%	5	17,0	22,0	18,0
Extract 90%	5	18,0	21,0	19,0
Extract 100%	5	20,0	27,0	22,2
Control (-)	5	0	0	0

4. Discussion

The results of measuring the diameter of inhibitory power (DDH) of Dewandaru leaf extract with concentrations of 80%, 90% and 100% against the *Candida albicans* fungus showed that there was inhibitory power. The average diameter of the inhibitory power of Dewandaru leaf extract at 80% concentration was 18 mm, Dewandaru leaf extract at 90% concentration was 19 mm and Dewandaru leaf extract at 100% concentration was 22.2 mm. The average diameter of the inhibitory force of Clotrimazole 1% as a positive control was 31.8 mm and Ethanol 96% as a negative control was 0.0 mm. Based on the Davis and Stout criteria, the average diameter of the inhibitory power of Dewandaru leaf extract with concentrations of 80% and 90% is included in the strong inhibitory response category (inhibitory zone diameter

of 11-19 mm), while Dewandaru leaf extract 100% concentration is included in the very strong inhibitory response category (inhibitory zone diameter ~20 mm^{3,4,8}).

Data on the diameter of the inhibitory power of Dewandaru leaf extract (*E. uniflora* L) with concentrations of 80%, 90% and 100% against the fungus *Candida albicans* were tested for normality using Shapiro-Wilk with the results of data having an abnormal distribution on the diameter data of the inhibitory power of Dewandaru leaf extract concentration of 80%, while the homogeneity test using the Levene test shows homogeneous data. A comparative test to determine the difference in the diameter of the inhibitory power of Dewandaru leaf extract with concentrations of 80%, 90% and 100% against the *Candida albicans* fungus was carried out using the Kruskal-Wallis test and obtained a p value = 0.027. This shows that there is no significant difference in the diameter of the inhibitory power of Dewandaru leaf extract in each group with concentrations of 80%, 90% and 100% against the *Candida albicans* fungus.

Because the data distribution was not normal, a comparison test to determine the difference in the diameter of the inhibitory area of Dewandaru leaf extract concentrations of 80%, 90% and 100% compared to the positive control Clotrimazole 1% against the *Candida albicans* fungus was carried out using the Kruskal-Wallis test. The test results show a value of p = 0.002 which states that there is a significant difference in the diameter of the inhibitory power of Dewandaru leaf extract concentrations of 80%, 90% and 100% with Clotrimazole 1% against the *Candida albicans* fungus.

The antifungal ability of Dewandaru plants has been carried out using various measurement methods. Research by Fidelis et al, pitanga could reduce adherence of human oral epithelial cells to the biofilm formation and alter the hydrophobicity of the cell surface of *Candida albicans*, suggesting that the antifungal effect of *E. Uniflora* is due to gallic acid and myricitrin, phytochemicals that can act against *Candida* spp^{10,11}.

Research by Souza et al found this was the first study to evaluate the effects of *E. Uniflora* extract on adhesion to human buccal epithelia, CSH, and biofilm formation, which are important virulence factors of *Candida* spp, the results showed that the extract interferes with adherence of *Candida* spp. to HEBC (human buccal epithelial cells) and CSH (cell surface hydrophobicity) and it is able to reduce biofilm formation for biofilm producing strains.¹² Other Research by Dewantari and Rahayu regarding the potential of Dewandaru leaf extract for antifungal activity states that Dewandaru leaf extract has the ability to inhibit the growth of *Fusarium* sp¹³.

The high potential of Dewandaru leaf extract against several cases of infection by gram-positive bacteria, gram-negative bacteria and fungi is a challenge and attraction for researchers to be able to discover other potentials from Dewandaru leaf extract that have never been explored, so that they can develop alternative natural-based products for some cases of infection, especially in the field of Ear, Nose and Throat diseases^{10,11,14}.

5. Conclusion

There is a notable inhibitory effect of Dewandaru leaf extract when tested at concentrations of 100%, 90%, and 80% against the *Candida albicans* fungus. The study found that higher concentrations of Dewandaru leaf extract, particularly at 100% and 90%, displayed a more pronounced ability to inhibit fungal growth, suggesting a potential dose-response relationship. In comparing the Dewandaru leaf extract with the standard anti-fungal agent Clotrimazole 1%, there was a significant difference in the diameter of the zones of inhibition, indicating that the natural extract may have distinct antifungal properties when compared to conventional treatments.

On the other hand, within the groups treated with different concentrations of Dewandaru leaf extract, there was no statistically significant difference in the diameter of the zones of inhibition. This suggests that while the extract demonstrates efficacy against *Candida albicans*, the specific concentrations tested (80%, 90%, and 100%) did not yield major variances in their antifungal effects. This could imply that even lower concentrations of the extract might still provide substantial antifungal benefits, presenting opportunities for further research and potential applications in natural medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors certify there is no conflict of interest.

References

- [1] Ameer, A., Arfat, J.A., Tahir, M. 2012. Bacterial Culture and Sensitivity in Active Chronic Otitis Media. 500 Cases in Combined Military Hospital Rawalpindi. Pak J Otolaryngol. Vol 28. h.56-8.
- [2] Anggraeni, R. 2012. Otitis Media In Indonesian Urban and Rural School Children. J Pubmed pub. h. 1-17
- [3] Astuti, S. M., Sakinah, M., Andayani, R. & Risch, A. (2011). Determination of saponin compound from *E. uniflora L* (ten) steenis (dewandaru) to potential treatment for several diseases. Journal of Agricultural Science, 3(4), 224-231.
- [4] Badan Pengawas Obat dan Makanan (BPOM). 2014. Penyusunan Pedoman Penelitian Obat Bahan Alam. Jakarta: Pusat Riset Obat dan Makanan
- [5] Ballenger, J.J. 2016. Chronic Otitis Media. Dalam: Ballenger J.J, Snow J.B. Ballenger's Otorhinolaryngology Head And Neck Surgery. 18th ed. Hamilton: BC Decker Inc. h.261-80.
- [6] Boel, T. 2014. Psedomonas aeruginosa. Mikrobiologi Kedokteran. Jakarta: EGC: h.47-9
- [7] Departemen Kesehatan RI. 2010. Pedoman Upaya Kesehatan Telinga Dan Pencegahan Gangguan Pendengaran Untuk Puskesmas. Dinas Kesehatan Provinsi Bali. Accessed: 22 Nov 2019. Available from: <http://m.depkes.go.id/>
- [8] Erasmo, G., Jan, E.G., Patricia, N.A. 2016. Chronic Suppurative Otitis Media: Bacteriology and Drug Sensitivity Patterns at the Quirino Memorial Medical Center (2014-2015). A Preliminary Study. hilipp J Otolaryngol Head Neck Surg: 21(1,2). h.20-3.
- [9] Helmi. 2005. Otitis Media Supuratif Kronis. Dalam: Otitis Media Supuratif Kronis. Pengetahuan Dasar, Terapi Medik, Mastoidektomi, Timpanoplasti. Jakarta: Fakultas Kedokteran Universitas Indonesia.h.76-92.
- [10] Howard, C.A. 2011. Pharmaceutical Dosage Forms and Drug Delivery. Dalam: Lippincott Williams and Wilkins.Textbook of Pharmacy. Philadelphia. University of Toledo College of Pharmacy: Walter Kluer Bussines Systems. h.184-722
- [11] Fidelis E.M, Savall A.S.P, Pereira F.D.O, Quines C.B, Avila D.S, Pinton S, 2022. Pitanga (*E. uniflora L.*) as a source of bioactive compounds for health benefits A review. Arabian Journal of Chemistry.15, 103691. <https://doi.org/10.1016/j.arabjc.2022.103691>
- [12] Souza, L., Silva-Rocha, W.P., Ferreira, M.R.A., et al, 2018. Influence of *Eugenia uniflora* extract on adhesion to human buccal epithelial cells, biofilm formation, and cell surface hydrophobicity of candida spp. from the oral cavity of kidney transplant recipients Molecules. <https://doi.org/10.3390/molecules23102418>.
- [13] Dewantari S.S, Rahayu Y.S, 2021Aktivitas Biofungisida Ekstrak Daun Dewandaru (*Eugenia uniflora L.*) dalam Menghambat Pertumbuhan Fusarium sp. LenteraBio,; Volume 10, Nomor 2: 199-206. <https://journal.unesa.ac.id/index.php/lenterabio/index>
- [14] Irianto, K. 2015. Mikrobiologi Medis. Menguak Dunia Mikroorganisme. Jilid 2. Bandung: CV YramaWidya.h.55-67.