ORIGINAL ARTICLE

In vitro effectiveness of the avocado peel extract (*Persea americana* Mill) as an antifungal against *Trichophyton rubrum*, the cause of dermatophytosis

Ira Armayanti, Meidina K. Wardani, Ariyati Yosi

Department of Dermatology and Venereology, Faculty of Medicine Universitas Sumatera Utara, Medan, Indonesia

ABSTRACT

Aim To determine the effectiveness of avocado peel extract (*Persea americana* Mill) as an antifungal against *T. rubrum*, which causes dermatophytosis.

Methods *In vitro* laboratory experimental study using a post-test-only control group design analysed active compounds of avocado peel and continued with the antifungal activity test. In each group divided according to the concentration of 0% (negative control), 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, and positive control (2% ketoconazole) antifungal activity test was carried out using the fungus *T. rubrum* ATCC 28188 for five repetitions.

Results The avocado peel extract contained phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids, and glycosides. Antifungal activity test showed a significant difference with the highest mean inhibition zone diameter of *T. rubrum*, which was demonstrated at a concentration of 75%.

Conclusion Avocado peel extract is useful to inhibit growth of *Trichophyton rubrum* in dose dependent.

Key words: avocado peel, dermatophytosis, *Persea americana* Mill., *Trichophyton rubrum*

Corresponding author:
Ira Armayanti
Department of Dermatology and Venereology, Faculty of Medicine Universitas Sumatera Utara, Medan, Indonesia
Phone: +62 61 821 1633;
E-mail: ira.armayanti07@gmaill.com
ORCID ID: https://orcid.org/0009-0005-1095-593X

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INTRODUCTION

Avocado (*Persea americana* Mill) is a plant that grows a lot in Indonesia, especially in the highlands where the climate is cool with high rainfall. Indonesia has seven varieties of avocados: round green, long green, round red, long red, mega gagauan, mega murapi, and mega paninggahan (1). The production of avocados from year to year tends to increase in Indonesia so that avocados are easy to obtain and often consumed by the community at quite affordable prices (2,3). The content of bioactive compounds from various avocado waste materials in producing phytochemicals have great potential to be used as food ingredients, cosmetics, or alternative treatments (4–6). In fact, avocado waste has interesting biological properties for the health sector, such as antiprotozoal, antibacterial and antifungal (2,7,8).

A study by Al Khikani et al. in Iraq in 2022, who reviewed 2,530 articles, explained that no area is clean from dermatophytosis infection worldwide (9). The study by Dalimunthe et al. found that dermatophytosis was in the first place out of the top three most common diagnoses in the Department of Dermatology and Venereology, University of North Sumatra Hospital in 2018, based on 1,899 outpatient visits (10). Research has shown that complex interactions between causative agents, host factors, predisposing factors, and the environment play a role in increasing the prevalence of dermatophytosis. It is known that the species *T. rubrum* is the most common cause found both in the world and Indonesia (11–13).

Dermatophytosis is a chronic recurrent disease, and many are resistant to antifungal drugs, which can lead to reduced quality of life and discomfort for patients (5). Increased prevalence plays an essential role in morbidity, mortality, and health care in the community. The drugs currently available to treat fungal infections have serious drawbacks, such as developing fungal resistance and toxic side effects (5,14).

Many antifungal preparations are recommended for treating dermatophytosis but are insufficient to ensure a complete cure. Until now, clinicians have been still trying to find the most effective drugs, one of which is using natural ingredients as inhibitors or antifungal agents as an alternative treatment (5,15). Several studies on the antifungal effectiveness of avocados against *C. albicans* species have yielded promising results. The survey by Mendrofa et al., which used avocado peel extract at a concentration of 25%, had an inhibition zone of 14.73 mm. This result was close to the positive control that used 2% ketoconazole cream, which was 20.40 mm (15).

Studies about this extract as an antifungal against *T. rubrum* have not been conducted yet.

The aim of this study was to assess the effectiveness of the avocado peel extract as an antifungal against *T. rubrum* that causes dermatophytosis using topical 2% ketoconazole as a positive control.

MATERIALS AND METHODS

Materials and study design

In vitro laboratory experimental study using a post-test-only control group design was conducted at the Pharmacy Laboratory and Microbiology Laboratory at Faculty of Medicine Universitas Sumatera Utara from October to December 2022. The study was approved by the Universitas Sumatera Utara Ethics Committee.

Methods

Fresh avocado peel (not rotten) with a homogeneous level of maturity comes from an avocado plantation in Kampung Padang, Serdang Berdagai Village, Medan. The extract was prepared by maceration using ethanol solvent at the Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara Medan, Indonesia. First, the fruit skin was separated from the fruit flesh, dried at 40 °C, and crushed with a blender to become a simplicial powder. Then, the powder was soaked in 96% ethanol with a ratio of 2:3 for five days and evaporated at 40–50 °C to produce avocado peel extract. The extract was dissolved in dimethyl sulfoxide (DMSO) into several concentrations: 0% (negative control), 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, and 2% ketoconazole (positive control).

The concentration of the avocado peel solution extract, which had been determined, was then followed by an antifungal activity test using the paper disc diffusion method (Kirby Bauer) (16) against the fungus *T. rubrum* ATCC 28188, which was cultured on Saboraud dextrose agar (SDA) medium for five repetitions according to the

Frederer (16). Medanense Herbarium (MEDA) Department of Biology, Faculty of Mathematics and Natural Sciences Universitas Sumatera Utara identified the avocado skin. The identification results stated that the plants used in this study were kingdom *Plantae*, *Spermatophyta* division, *Dicotyledonous* class, ordo *Laurales*, family *Lauraceae*, genus *Persea*, species *Persea americana* Mill, and the local name was avocado peel.

Statistical analysis

Data collected were analysed to determine the difference in inhibition zone diameter within each concentration with one-way Anova test and post-hoc using Tamhane test.

RESULTS

Laboratory examination results of the content of active compounds from avocado peel extract (APE) showed seven active ingredients from the avocado peel extract: phenolics, tannins, flavonoids, saponins, alkaloids, terpenoids, and glycosides.

Testing the antifungal activity of a pure solution extract from the avocado peel (*Persea americana* Mill) with several predetermined concentrations showed that the largest inhibition zone diameter was demonstrated at the concentration of 75% with an average of 20.36 mm (SD=0.85mm). The minor diameter of the inhibition zone was shown by the avocado peel extract with a concentration of 12.5% with an average of 10.72 mm (SD=0.54 mm). The measurement results in the negative control found no inhibition zone or inhibition zone diameter = 0, whereas in the treatment using 2% ketoconazole cream as a positive control, the average inhibition zone was obtained with a diameter of 29.77 mm (SD=0.77 mm) (Table 1).

Figure 1 showed the error bar diameter of inhibition zone of *T. rubrum* in different concentration of the avocado peel extract.

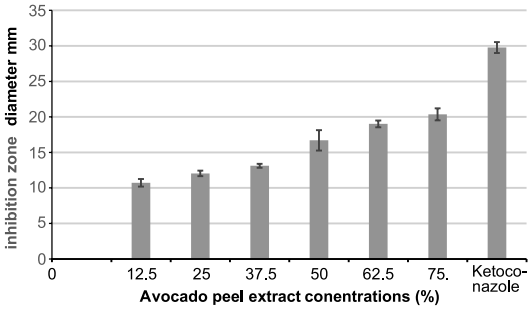

Figure 1. Error bar diameter of the inhibition zone of the fungus *Trichophyton rubrum* based on the adverse control treatment, 12.5%, 25%, 50%, 62.5%, 75% avocado peel extract (APE), and 2% ketoconazole

The effective concentration of a compound based on the Ministry of Health of the Republic of Indonesia in 1995 looked at antimicrobial activity with an inhibition zone diameter ranging from 14 mm to 16 mm (17).

Based on the treatment of the avocado peel extract, the effective concentration of inhibition as an antifungal was obtained on *T. rubrum* with an extract concentration from 50%. Inhibition zone was increased with the increase of the avocado peel extract concentration. The results of this study indicated that the highest mean inhibition zone diameter of *T. rubrum* was shown by the avocado peel extract (APE) with the concentration of 75%. Although 2% ketoconazole showed the highest inhibition zone with difference of 9.41 mm from avocado peel extract of 75%, APE 75% can also be considered an effective antifungal on *T. rubrum*.

DISCUSSION

The results of this study regarding the active compound in the avocado peel extract follow several previous studies. Research by Adebowale in Nigeria in 2021 found active compounds, namely tannins, saponins, alkaloids, flavonoids, and glycosides in the avocado peel extract,

Table 1. Differences in the diameter of the inhibition zone of *Trichophyton rubrum* based on the pure solution extract treatment

Treatment	Mean (SD) of inhibition zone (mm)	p	Post-hoc						
			APE 12.5%	APE 25%	APE 37.5%	APE 50%	APE 62.5%	APE 75%	Keto-conazol
Avocado pee extract concentration (APE) (%)									
0	0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12.5	10.72 (0.54)			0.072	0.003	0.008	<0.001	<0.001	<0.001
25%	12.04 (0.39)				0.003	0.008	<0.001	<0.001	<0.001
37.5	13.12 (0.28)					0.113	<0.001	<0.001	<0.001
50	16.7 (1.43)						0.415	0.057	<0.001
62.5	19.02 (0.48)							0.441	<0.001
75	20.36 (0.85)								<0.001
Ketoconazol 2%	29.77 (0.77)								

which showed antimicrobial effectiveness (18). Research conducted by Rosero et al. in 2019 also found several active compounds, namely phenolics, flavonoids, and catechins, in avocado peels phytochemical analysis (19). Avocado peel extracts were also found to contain many bioactive compounds such as flavonoids, phenolics, hydroxycinnamic acid, hydroxybenzoic acid, proanthocyanidins, procyanidins, carotenoids, and organic acids in a study by Lopez et al (20). Research in Indonesia by Wulandari et al. and Mendrofa et al. also found that the avocado peel extract contains secondary metabolites in the form of alkaloids, flavonoids, saponins, tannins, and polyphenols (15,21,22).

Geographical location, environmental temperature of an area, season, and availability of water, ultraviolet radiation, soil fertility, and additional nutrients for plants are related to the genetic content of fruit varieties. These factors will also directly affect the range of secondary metabolites of these plants. This explains the differences in the scope of secondary metabolites from plants of the same type but originating from different regions (23,24).

The results of this study indicated that the highest average diameter of the inhibition zone of the fungus *T. rubrum* was indicated by the avocado peel extract with the concentration of 75%. The ability of avocado peel extract with the concentration of 75% can inhibit the growth of the fungus *T. rubrum* and can pose as an alternative to 2% ketoconazole cream. The results of existing research regarding the effectiveness of the avocado peel extract as an antifungal were carried out on *Candida albicans* species by Mendrofa et al. gave the appropriate results where an increase in the diameter of the inhibition zone was directly proportional to the rise in the concentration of the extract: the extract concentration of 25% gave the inhibition zone diameter of 14.73 mm, and the concentration of 50% 15.73 mm, at the concentration of 75%, the inhibition zone diameter of 16.16 mm, and the concentration of 100% obtained the inhibition zone diameter of 17.66 mm (15). Based on the concentration of 75% avocado peel extract in Mendrofa et al. study gave the inhibition zone diameter of 16.66 mm against *Candida albicans* species. In contrast, the results of antifungal effectiveness against *T. rubrum* species obtained in our study at the same concentration were better, with zone diameter resistance of 20.36 mm.

The formation of an inhibition zone around the disc paper or the suitable method shows that this extract contains compounds that act as antifungals, such as using 2% ketoconazole cream (21). The potential of the avocado peel extract as a natural resource with many active components is very promising to be used as one alternative antifungal therapy against dermatophytosis, especially *T. rubrum* species. The increase in the fungal inhibition zone's diameter was directly proportional to the rise in the concentration of the avocado peel extract tested in this study. Synergistic effects, antagonistic effects between the various active compounds present, as well as the interactions of the extracts are things that need to be considered (25). Studies on the analysis of active compounds that have potential antifungal effects are essential for treating dermatophytosis and discovering new cellular targets for the treatment of fungal infections (26).

In conclusion, avocado peel extract (*Persea americana* Mill) showed an excellent antifungal effect on *T. rubrum*, and the inhibitory activity increased with extract concentration. The ability of the avocado peel extract to inhibit the growth of the *T. rubrum* fungus is inferior to 2% ketoconazole. Still, it can be an alternative therapy that needs to be considered. Therefore, further clinical trials on the avocado peel extract (*Persea americana* Mill) should demonstrate its efficacy in dermatophytosis patients.

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Competing interests: None to declare.

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