Antifungal Effectiveness Of Extract Combinations Ethanol From Noni Leaves (Morinda Citriofolia L) And Tamarind Leaves (Tamarindus Indica L) Against Pityrosporum Ovale And Malassezia Furfur

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Abstract. The tropical climate in Indonesia is one of the causes of hair becoming smelly and damp. Scalp health also has an influence, especially frequent dandruff, which makes a person uncomfortable because it causes itching and irritation. Traditional medicine is still often used in Indonesia, the truth of the efficacy of traditional medicine must be supported by scientific studies. Therefore, it is necessary to carry out scientific testing on noni plants (Morinda citrifolia L) and tamarind plants (Tamarindus indica L). This research aimed to determine the antifungal effectiveness of a combination of ethanol extract from noni leaves and ethanol extract from tamarind leaves against the fungi Pityrosporum ovale and Malassezia furfur. Extraction using the maceration method and fungal testing using the spread plate method and data analysis using One Way Anova and T-Test. There is a difference between the two mushrooms with a One Way Anova test result of 0.000 (<0.05). T-Test test results for noni (0.001), tamarind (0.000), 1:1 combination (0.007), 1:2 combination (0.014), and 2:1 combination (0.348). The ethanol extract of noni leaves and the ethanol extract of tamarind leaves in combination can inhibit the growth of Pityrosporum ovale and Malassezia furfur fungi.
Keywords: [noni, tamarind, Pityrosporum ovale, Malassezia furfur.]

INTRODUCTION

Indonesia, which has a tropical climate, is a challenge for everyone so that their hair does not easily become smelly, damp, or limp. But not only hair, scalp health must also be maintained, because these two things are interrelated. Especially on the scalp, the presence of dandruff makes a person uncomfortable, because dandruff causes itching and irritation (Hidana & Fauziyyah, 2016). A dandruff sufferer often scratches the scalp, which results in the release of epidental keratin, which then falls onto the hair shaft and becomes visible if it sticks to clothes. Wounds can also occur and result in secondary infections due to the presence of other bacteria (BPOM, 2009).

Among the causes of infection, namely fungi. The specific characteristics of fungi are that they have a cell nucleus, produce spores, do not have chlorophyll, can reproduce sexually and asexually and fungi are included in eukaryotic microorganisms. Mycosis is a human disease caused by fungi. One of the causes of mycosis is the fungus Pityrosporum ovale and Malassezia furfur (Simanjuntak & Butar-butar, 2019).

*Pityrosporum ovale*is a single-celled fungus and is a member of the genus Malassezia sp, and belongs to the Cryptococcaceae family. In conditions of hair with excess oil glands, this fungus grows abundantly, which is actually a normal flora on the scalp, and it is thought that this fungus is the main cause of dandruff (Anwar et al., 2015). Noni leaves and tamarind leaves contain active compounds such as saponins, polyphenols, tannins, alkaloids, flavonoids, and terpenoids which are thought to be factors that cause inhibited growth of the fungus C. albicans (Fakhrurrazi, Hakim & Keumala., 2016).

METHODS

Types of research

This research uses an experimental type of research to determine the potential of the combination of noni leaf ethanol extract and tamarind leaf ethanol extract in inhibiting the growth of Pityrosporum

ovale and Malassezia furfur fungi and determining the Minimum Inhibitory Concentration (MIC). This research was conducted using the Completely Randomized Design (CRD) method.

Tools and materials

The tools used in this research were blender, oven, autoclave, Laminar Air Flow, hotplate magnetic stirrer (WINA Instrument Type: 208), analytical balance, water bath, moisture balance, glassware, micropipette and tip, incubator, test tube, tube rack reaction, Petri dish, Bunsen burner, tweezers, microscope, stir bar, flannel cloth, Drigalsky spatula, ose needle, vernier caliper, vortex tool (Thermo Scientific), rotary evaporator (RE 100-Pro), 40 mesh sieve, Whatman paper no. 1.

The materials used in this research were noni leaves taken from Kudus Regency, Central Java, and tamarind leaves taken from, Pityrosporum ovale fungus culture, Malassezia furfur fungus culture obtained from the Karyadi Hospital Laboratory collection in Semarang, 70% ethanol, DMSO (Dimethyl Sulfoxide) solvent, ketoconazole, distilled water, CYG (Casein Yeast Glucose) liquid media, PDA (Potato Dextrose Agar) media, Mg, concentrated HCl, FeCl3 1%.

RESULTS AND DISCUSSION

Results

Table 1. Phytochemical Screening Test Results

Identification	Extract Noni leaves		Tamarind Leaf Extract	
	Information	Results	Information	Results
Flavonoids	Formed Orange	+	Formed Orange	+
Tannin	Formedgreen color Black	+	Formedgreen color black	+
Saponins	Forms stable foam	+	Forms stable foam	+

Description: Positive for containing this compound (+).

Table 2. Results of Pityrosporum ovale Inhibition Zone diameter

Concentration	Inhibition Zone Diameter (mm) ± Standard Deviation
Combination M : AJ 100 : 200mg/ml	7.38 ± 1.2a
Combination M : AJ 150 : 150mg/ml	8.1 ± 1.1a
Noni 100mg/ml	8.1 ± 1.2a
Combination M : AJ 200 : 100mg/ml	8.34 ± 1.0a
Tamarind 100mg/ml	9.88 ± 0.3b
DMSO 4%	-
Sterile distilled water	-
Ketoconazole 20µg/ml	11.66 ± 0.7c

Note: numbers that have the same letter indicate there is no real difference at an error rate of 5% (α = 5%), Noni (M), Tamarind (AJ).

Table 3. Results of Malassezia furfur inhibition zone diameter

Concentration	Inhibition Zone Diameter (mm) ± Standard Deviation
Combination M : AJ 200 : 100mg/ml	8.94 ± 0.9a
Combination M : AJ 100 : 200mg/ml	9.66 ± 1.1a

Combination M : AJ 150 : 150mg/ml	11.32 ± 1.5b
Noni 100mg/ml	12.7 ± 1.6b
Tamarind 100mg/ml	16.52 ± 0.9c
DMSO 4%	-
Sterile distilled water	-
Ketoconazole 20µg/ml	11.74 ± 0.7b

Note: Numbers that have the same letter indicate there is no real difference at an error rate of 5% (α = 5%), Noni (M), Tamarind (AJ).

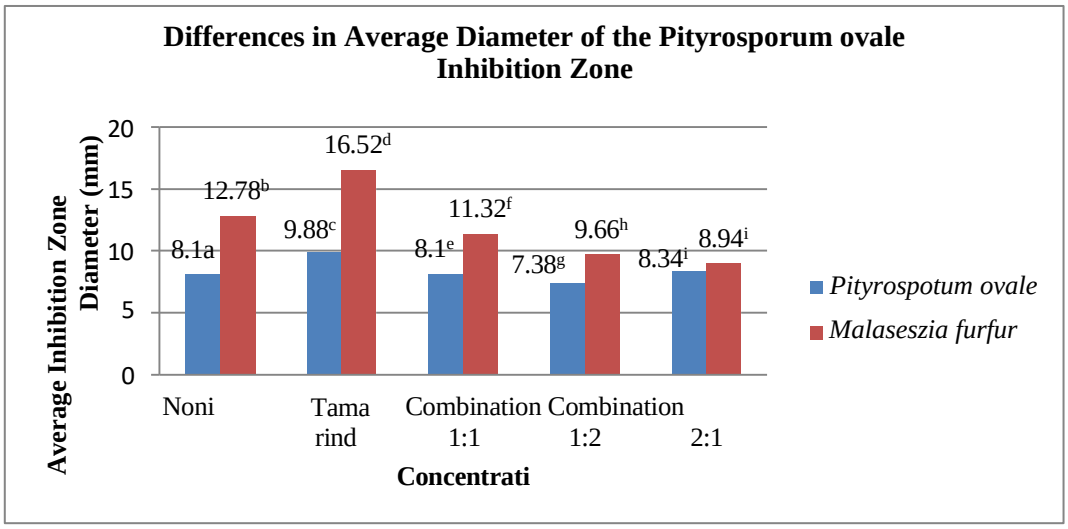

Figure 1.
Inhibition Zone Diameter Bar Chart
*Pityrosporum ovale*and Malassezia furfur

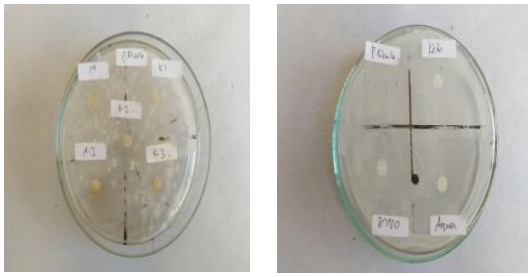

Figure 2.
Zone of inhibition against the fungus Pityrosporum ovale

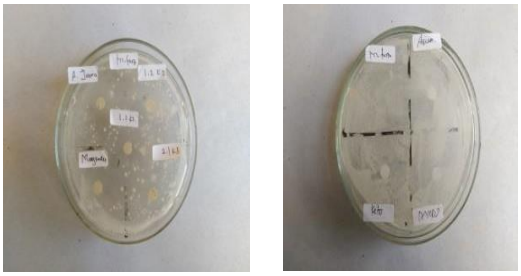

Figure 3.
Zone of inhibition against the fungus Malassezia furfur

Discussion

The thick extract of noni leaves is dark green-black with a sticky texture, easy to wash, and has a distinctive smell, 35 grams with a yield of 17.5%. The thick extract of tamarind leaves is faded dark green in color with a sticky texture, dissolves easily, and has a distinctive odor, 31.2 grams with a yield of 15.6%. The phytochemical screening test carried out to determine the presence of secondary metabolites using qualitative methods showed that the thick extract of noni leaves and tamarind leaves positively contained flavonoids, tannins, and saponins.

The antifungal activity of the ethanol extract of noni leaves and the ethanol extract of tamarind leaves is thought to be due to the presence of secondary metabolite compounds that have antifungal activity such as flavonoids, tannins, and saponins. This is based on research by (Tasleem et al., 2014) who isolated these secondary metabolite compounds from several plants. The results of this research show that alkaloids, phenols, flavonoids, saponins, and terpenoids have antifungal activity.

Flavonoids are the largest group of compounds in nature which are known as antioxidants and have antibacterial and antifungal effects because they contain phenol groups. Flavonoids containing phenolic groups can also coagulate proteins and reduce the surface tension of microbial cells (Waluyo, 2007). Tannins have antioxidant and antiseptic activity. Tannins are plasmolytics that can shrink cell walls or cell membranes thereby disrupting cell permeability

Alone. As a result of disrupted permeability, cells cannot carry out living activities so their growth is stunted or even dies. Tannins also can inhibit enzymes that play a role in stimulating cell division (Robinson, 1995).

Saponins have a high level of toxicity to fungi. The mechanism of action of saponins as antifungals is related to the interaction of saponins with membrane sterols. Saponin compounds contribute as antifungals by reducing the surface tension of the sterol membrane of the fungal cell wall so that its permeability increases. Increased permeability causes more concentrated intracellular fluid to be drawn out of the cell so that nutrients, metabolic substances, enzymes, and proteins in the cell escape and the fungus dies (Septiadi, Pringgenies & Radjasa, 2013).

The diameter of the fungal growth inhibition zone according to Rios et al., (1988) is divided into very strong (>20 mm), strong (11-20 mm), medium (6-10 mm), and weak (≤ 5 mm). In this study, the results of the inhibition zone diffusion test on Pityrosporum ovale single extract of noni were in the medium category, a single extract of tamarind was in the medium category, and the combination was 1:1; 1:2; and 2:1 is included in the medium category with an inhibition zone diameter ranging from 6-10 mm. In the results of the inhibition zone diffusion test on Malessesia furfur, the single extract of noni was in the strong category, and the single extract of tamarind leaves was in a strong category. And a 1:1 combination; 1:2; and 2:1 respectively fall into the strong, medium, and moderate categories with an inhibition zone diameter ranging from 7-17 mm.

According to Natheer et al., (2012) stated that the substance used as a negative control is a solvent used as a diluent for the compound to be tested. In this research, the solvent used to dissolve the sample was DMSO. So the negative control used is DMSO. The aim is as a comparison that the solvent used does not affect the antifungal test results of the compound to be tested. Meanwhile, the positive control uses 200 mg ketoconazole with a concentration of 20 µg/ml which aims to compare samples with antifungals that have potential as antifungal agents.

The results of the One Way Anova test on noni leaf extract and tamarind leaf extract with their combination on the fungi Pityrosporum ovale and Malassezia furfur with the results seen from the significance value of 0.000 (<0.05), it can be seen that there are differences between the two fungi.

Followed by the Post Hoc LSD test to find out which groups have significant differences. Post Hoc LSD test results can be seen in Tables 2 and

3. The final test was carried out using a T-test to determine the difference in the concentration inhibition zone of noni leaf extract and tamarind leaf extract between the Pityrosporum ovale and Malassezia furfur fungi. The T-Test test results showed that there was a significant difference in the noni concentration of 100 mg/ml; tamarind 100mg/mL; combination 1:1 150:150 mg/ml; the 1:2 combination of 100:200 mg/ml against each of the Pityrosporum ovale and Malassezia furfur fungi, while the 2:1 combination of 200:100 mg/ml did not have a significant difference against each of the Pityrosporum ovale and Malassezia furfur fungi. The T-test test results can be seen in Figure 1.

CONCLUSION

1. There is a difference in antifungal activity between the ethanol extract of noni leaves and the ethanol extract of tamarind leaves in combination against the fungi Pityrosporum ovale and Malassezia furfur.
2. The optimal combination of extracts to inhibit Pityrosporum ovale is a combination (2:1) with an inhibitory zone diameter of 8.34 mm. Meanwhile, for the Malassezia furfur fungus, the optimal combination is (1:1) with an inhibitory zone diameter of 11.32 mm.
3. There is a difference in inhibitory power between the combination of ethanol extract

of noni leaves and ethanol extract of tamarind leaves against the Pityrosporum ovale fungus and the combination of ethanol extract of noni leaves and ethanol extract of tamarind leaves against the fungus Malassezia furfur.

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