RESEARCH ARTICLE

Inhibitory power test of n-hexane extract of malaka leaves (*Phyllanthus emblica*) on growth of *Microsporum canis* in-vitro

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Abstract

Background and Aim: Malacca leaves are one of the medical plants that can be used in traditional medicine. Malacca leaves contain several active compounds that can be effective in inhibiting fungal growth. This study aims to investigate the inhibition of n-hexane Malacca leaves extract on *Microsporum canis* growth in vitro.

Materials and Methods: The method used in this study is Kirby-Bauer disc diffusion with 5 treatments, namely P1 (25% Malacca leaves n-hexane extract), P2 (50% Malacca leaves n-hexane extract), P3 (75% Malacca leaves n-hexane extract), P4 (control (+): ketoconazole) and P5 (control (-): carboxymethylcellulose (CMC) 1%) with 3 replications. The clear zone formed around the disc was measured using a caliper.

Results : The results showed that there was no clear zone formed around the disks of the n-hexane extract of Malacca leaves. It can be concluded that the n-hexane extract of Malacca leaves could not inhibit the growth of *Microsporum canis.*

Conclusion: The findings of this study indicate that the n-hexane extract of Malacca leaves, at concentrations of 25%, 50%, and 75%, does not exhibit antifungal activity against *Microsporum canis* in vitro, as evidenced by the absence of inhibition zones. Therefore, n-hexane may not be an effective solvent for extracting antifungal compounds from Malacca leaves against this fungal species.

Keywords: Antifungal, ketoconazole, maceration

Introduction

Indonesia is a tropical country characterized by relatively high air temperature and humidity. Skin conditions that sweat easily, humidity, and poor personal hygiene are variables that encourage fungal growth. Infections caused by fungi can attack the skin, hair, and nails (Puspitasari *et al*., 2019). Fungal skin infections are often the result of an unhygienic lifestyle, a tropical climate with high humidity, inadequate hygiene, or a very crowded environment that fosters fungal growth.

Cats are adaptable animals and can make very good companions for humans. Public awareness of skin disease disorders in cats is still limited. Skin disease is the most common disease in cats. In severe cases, cats may die as a result of their owners'

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negligence in proper maintenance and care. Some cat infections can be transferred easyly to humans (Kurniati *et al*., 2017).

As with all living beings, the health of cats should be carefully considered. The most common cat disease is skin disorders. Most fungal infections of the skin are caused by dermatophytes or fungi belonging to the *Trichophyton* and *Microsporum* families, manifesting as *Tinea pedis*, *Tinea unguium*, *Tinea cruris*, *Tinea capitis*, and *Tinea corporis*.

Dermatophytosis can be treated both topically and systemically. Ketoconazole is a commonly used therapy, but it has negative effects such as liver damage, especially when taken for more than 10 days. In contrast, numerous plant-based compounds have demonstrated potential as herbal medicines, offering fewer side effects than synthetic drugs. Consequently, herbal remedies are gaining popularity, not only due to their safety profile but also because they are generally more affordable (Melinda *et al*., 2019).

One of the characteristics of Indonesia is its vast diversity of plant species. This botanical richness is largely attributed to the country's tropical climate, which provides favorable conditions for the growth of a wide range of plants. Some of these plants are widely known as medicinal plants and have long been used in traditional healing practices. Medicinal plants are those that contain bioactive compounds with therapeutic potential. Part of plants such as roots, stems, leaves, flowers, and tubers connected by leaf axils are plant components that can be used as therapeutic substances (Darnita *et al*., 2020).

Traditional medicinal plants, modern medicinal plants, and prospective therapeutic plants are categorized as medicinal plants. Traditional medicinal plants are recognized as having therapeutic properties and have been utilized as raw materials for traditional medicines. Modern medicinal plants are plant varieties that have been scientifically validated to contain chemicals or bioactive substances with therapeutic characteristics, and their use can be justified medically. Prospective medicinal plants are types of plants that are suspected of having medicinal compounds or bioactive substances, but their use as therapeutic materials has not been scientifically-medically confirmed, and their traditional use has not been recognized (Lestari, 2006).

It is known that in the Aceh region, namely Aceh Besar Regency, there are plants that are used for traditional medicine. The plant in question is the Malacca plant (Asmilia *et al*., 2019). Medium-sized, with greenish stems and yellowish-green flowers, are the characteristics of the Malacca tree (Dhale, 2012).

Malacca plants are efficacious for treating diabetes, anti-inflammatory, analgesic, antipyretic, hepatoprotective, antidiarrheal, anticancer, antihyperlipidemia (Kaur *et al*., 2013). According to Ardiansyah *et al*. (2018), Malacca leaves contain alkaloids, flavonoids, tannins, saponins, terpenoids, benzenoids, phenolics, lignins, furanolactones, tertiary pene, and carbohydrates. According to Kumar *et al*. (2014), the ingredients contained in *P. emblica* are vitamin C, phenolic compounds, including tannins, proanthocyanidins, flavanols and compounds belonging to other phenolic groups.

Alfiah *et al*. (2015) stated that certain secondary metabolites can be used to limit fungal growth. The content in Malacca leaves is thought to inhibit fungal growth. Based on several statements above, this study was conducted to determine the inhibitory power of n-hexane extract of Malacca leaves on the growth of *M. canis*.

Materials and Methods

Sampling

The samples used were Malacca leaves obtained from Blang Bintang District, Aceh Besar Regency and *M. canis* mushroom preparations came from the Microbiology Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala.

Making N-Hexane Extract

The powder of the simplicia is extracted using a cold extraction technique, especially the maceration process. Malacca leaves that have been picked and separated from their branches are washed thoroughly with running water, then dried at room temperature for two weeks. The dried leaves are ground using a blender. The leaf powder is then macerated with n-hexane solvent in a tightly closed glass container. The maceration process is carried out for five days. After that, the macerate is separated using filter paper. then evaporated using a vacuum rotary evaporator. Then the evaporation results are stored in a glass container that is tightly closed with a rubber stopper.

Making Natural Resources Media

The preparation of Sabouraud Dextrose Agar (SDA) media begins by mixing 13 g of SDA powder with 200 mL of distilled water in a 250 mL Erlenmeyer flask. Then the SDA is sterilized using an autoclave at a temperature of 121°C.

Creating Control and Concentration

The negative control used was 1% CMC. According to Mpila *et al*. (2012), 1% CMC is made by dissolving 1 g of CMC powder with 100 ml of sterile distilled water and then stirring until homogeneous. The positive control used was Ketoconazole®. The concentrations of n-hexane extract of Malacca leaves used were 25%, 50% and 75%. According to Sugiarti *et al*. (2020), the preparation of the concentration begins with the preparation of the mother solution, namely by weighing 500 mg of n-hexane extract of Malacca leaves with 5 ml of 1% CMC.

Inhibitory Power Test of N-Hexane Extract of Malacca Leaves

The method used is Kirby-Bauer disc diffusion. This test was carried out using a Petri dish containing SDA media. The fungus was taken from peptone water containing *M. canis* fungal suspension with sterile cotton buds then spread evenly on the Petri dish containing SDA media and waited until the fungus diffused for ± 10 minutes. After that, sterile discs that had been soaked with each extract concentration (25%, 50%, 75%), negative control and positive control were placed. The negative control used was 1% CMC and the positive control used was Ketoconazole®. The media was incubated at room temperature. The diameter of the clear zone formed was measured using a caliper. The presence of a clear zone area around the disc indicates the inhibitory power of the n-hexane extract of Malacca leaves against *M. canis*. The test was carried out 3 times in replication.

Data analysis

The data obtained were analyzed descriptively and then presented in table format.

Results and Discussion

Extraction Results

The extraction result of 350 g of Malacca leaf simplicia with n-hexane solvent obtained a result of 5 mg. The n-hexane extract of Malacca leaves is brownish green in the form of a paste and has a distinctive aroma.

Microsporum canis Examination Results

Macroscopic observations include colony morphology, color, shape, size and the back of the colony. Microscopic observations were carried out using Lacto Phenol Cotton Blue (LPCB) staining (Indarjulianto *et al*., 2017). In Lacto Phenol Cotton Blue (LPCB) staining, Soedarmanto *et al*. (2014) reported that *M. canis* has large, round, long macroconidia, double-septate hyphae, and more than six cells. Based on the results of the study, both the macroscopic and microscopic characteristics of *M. canis* are presented in Figures 1 and 2.

Macroscopically, *M. canis* appears to spread like cotton with a flat topography and is yellowish white in color. Meanwhile, the results of microscopic observations indicated by arrow number 1 show double-septate hyphae.

Figure 1. Macroscopic observation results of *Microsporum canis*

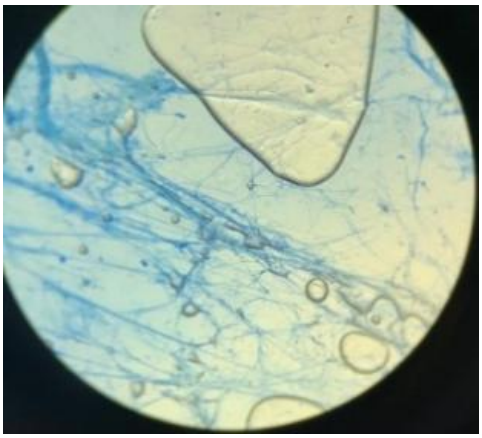

Figure 2. Results of microscopic observations of *Microsporum canis*

Inhibitory Power Test Results

The diameter of the inhibition test results can be seen in Table 1 below. Of the three concentrations (25%, 50% and 75%) of n-hexane extract of Malacca leaves on the

growth of *M. canis* could not inhibit the growth of *M. canis* because there was no clear zone formed. Ketoconazole® as a positive control could inhibit the growth of *M. canis* because of the clear zone formed with an average diameter of 18.09 mm.

Table 1. Diameter of inhibition zone of n-hexane extract of Malacca leaves against *M. canis*

Treatment	Inhibition zone (mm)		
	U1	U2	U3
25%	0	0	0
50%	0	0	0
75%	0	0	0
Negative control (-)	0	0	0
Positive control: Cetocanazole(+)	18.21	17.89	18.18

The inhibitory power test of n-hexane extract of Malacca leaves in this study used the Kirby-Bauer disc diffusion method (Wimpi *et al*., 2019). In Figure 3, the positive control results showed a clear zone around the disc with an average of 18.09 mm. The negative control did not show any clear zone formed around the disc. The results of the inhibitory power test using n-hexane extract of Malacca leaves with concentration variations of 25%, 50% and 75% were that no clear zone was formed around the disc, indicating no inhibitory power of n-hexane extract of Malacca leaves against *M. canis*.

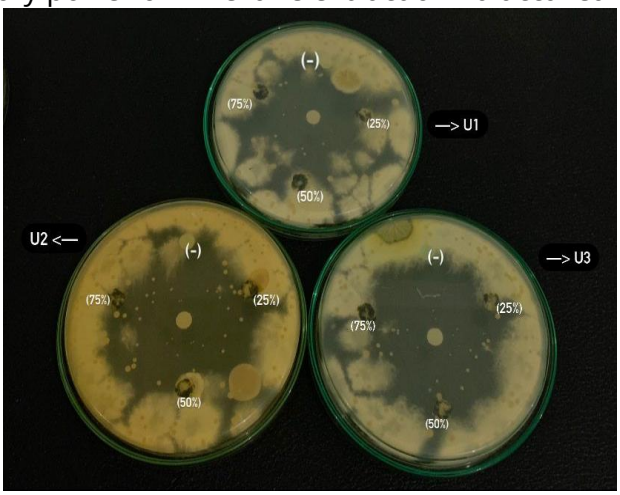

Figure 3. The inhibitory power test of n-hexane leaf extract Malacca against *Microsporum canis* on SDA media

According to Shalaby *et al*. (2016) and Soltys (2015), *M. canis* has thick and rough cell walls and a slow growth phase. This is one of the causes of the lack of inhibitory power. The fungal cell wall consists of many layers (2% chitin and 80% mannoprotein and glucan).

Mannoproteins express most of the outer surface. Ergosterol is a major component of the fungal plasma membrane. Ergosterol plays a vital role in maintaining cell integrity and defense mechanisms. The role of ergosterol is biosynthesis, uptake and release of materials, producing carbohydrate chains, transmitting signals from the environment, and as a storage place for cell wall enzymes. This factor affects how effective the drug permeability is into fungal cells, the thicker the cell wall the more difficult it is for a compound to enter the cell (Nigam, 2015).

According to Antoh & Timbun (2021), ketoconazole is an azole antifungal drug that is fungistatic. In fungi, ketoconazole inhibits the P450 enzyme and ergosterol production in the cell membrane. Dermatophytes and systemic fungi are treated with ketoconazole. The solvent n-hexane is a non-polar solvent that can damage leaf tissue and secondary metabolite compounds in the leaves can be extracted well. The polarity of the solvent has a significant impact on the test results. Polar chemicals dissolve in polar solvents, semi-polar compounds dissolve in semi-polar solvents, and non-polar compounds dissolve in non-polar solvents (Fitriah *et al*., 2017).

According to Alfiah *et al*. (2015), several secondary metabolite contents in Malacca leaves have antifungal properties that can inhibit fungal growth are saponins, alkaloids, terpenoids and tannins. Saponin compounds can disrupt the integrity of fungal cell membranes, resulting in the release of proteins, nucleic acids, and nucleotides. Saponins can also induce cell membrane damage. The production of nucleic acids, proteins, and membrane phospholipids can be inhibited by alkaloids. Terpenoids are lipophilic, allowing them to inhibit fungal growth by disrupting the process of making fungal membranes or cell walls, dissolving lipids in cell membranes, and affecting nutrient transfer, which can result in cell membrane deficiency and cell injury. Tannins are lipophilic chemicals that easily bind to cell walls and damage fungal cell walls.

According to Asmilia *et al*. (2020), the only secondary metabolite compounds contained in the n-hexane extract of Malacca leaves is steroids. Steroids and terpenoids can inhibit the formation of ergosterol and affect cell permeability which will result in lysis of the fungal cell membrane (Liu & Nes, 2009). This mechanism may help explain the lack of antifungal activity observed in the extract, as the thick and coarse cell walls of *Microsporum canis* make it more difficult for the active compounds to penetrate and exert their inhibitory effect.

Conclusion

The findings of this study indicate that the n-hexane extract of Malacca leaves, at concentrations of 25%, 50%, and 75%, does not exhibit antifungal activity against *Microsporum canis* in vitro, as evidenced by the absence of inhibition zones. Therefore, n-hexane may not be an effective solvent for extracting antifungal compounds from Malacca leaves against this fungal species. Further research using different solvents or extraction methods is recommended to explore the antifungal potential of Malacca leaves.

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