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The Antifungal Activity of n-Hexane Extract of *Eleutherine palmifolia* (L). Merr Bulbs Against *Candida albicans* and *Trichophyton mentagrophytes*

Masfria Masfria*, Mitra S. A. Tampubolon

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia

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***Correspondence:** Masfria Masfria. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia. E-mail: masfria@usu.ac.id

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Abstract

AIM: This research aimed to determine the characteristics of dried bulbs of *Eleutherine palmifolia*, the group of active chemical compounds contained in *n*-hexane extract of *Eleutherine palmifolia* bulbs and the antifungal activity of *n*-hexane extract of *Eleutherine palmifolia* bulbs against *Candida albicans* and *Trichophyton mentagrophytes.*

METHODS: The *Eleutherine palmifolia* bulbs were extracted by percolation method using *n*-hexane solvent. The extract was tested for its antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* by diffusion method using paper discs.

RESULTS: The characterisation of dried *Eleutherine palmifolia* bulbs were obtained water content 9.38%, water-soluble extract content 12.15%, ethanol-soluble extract content 14.48%, total ash content 0.91%, and acid insoluble ash content 0.70%. Determination of Phytochemical content showed alkaloid, flavonoid, tannin, saponin, glycoside, and steroid/triterpenoid compounds. The antifungal activity of *Eleutherine palmifolia* bulbs *n*-hexane extract (EPBHE) by concentration 200 mg/ml demonstrated the inhibition diameter of 19.48 and 42.20 mm for *Candida albicans* and *Trichophyton metagrophytes,* respectively.

CONCLUSION: The antifungal test indicates that *n*-hexane extract of *Eleutherine palmifolia* bulbs provides inhibitory power to *Candida albicans* and *Trichophyton mentagrophytes*.

Introduction

Infectious disease is one of the problems in the field of health, which, from time to time, continues to grow. It is a disease caused by pathogens that attack body tissues and cause damage. Infectious pathogens can be classified into five major groups: viruses, bacteria, fungi, protozoa, and metazoa, usually worms [1]. Infections caused by fungi are called mycosis. Mycosis with the highest incidence rates occurring in humans is candidiasis and dermatophytosis. Candidiasis is caused by *Candida* species that are part of normal microorganisms in humans whereas dermatophytosis is caused by dermatophyta fungi group, grouped in *Microsporum*, *Trichophyton*, *Epidermophyton*. One of the most common species of causes of candidiasis and dermatophytosis is *Candida albicans* and *Trichophytonmentagrophytes* [2].

Eleutherine palmifolia L. Merr is a typical plant of Borneo and has been used for generations of the Dayak community as a medicinal plant. Empirically, this plant is known can cure diabetes mellitus, hypertension, lowering cholesterol, treat skin diseases and ulcers, and abdominal pain after childbirth. The fact that there is in the local community is proof that this plant is a multifunctional medicinal plant that is very useful so that further research and development is needed for the benefit of society [3]. *Eleutherine palmifolia* bulbs have secondary metabolite components such as alkaloids, saponins, flavonoids, steroids/terpenoids, monoterpenoids/sesquiterpenes, tannins, polyphenols [4] and glycosides [3]. *Eleutherine palmifolia* also contains compounds of naphtoquinonens and their derivatives such as

elecanacine, eleutherine, eleutherol, eleuthernone [5,6]. Naphtoquinones are known as antimicrobial, antifungal, antiviral and antiparasitic. In addition, naphtoquinones have bioactivity as anticancer and antioxidants that are usually present in vacuole cells in the form of glycosides [7].

According to Wahyuni et al., (2016), *Eleutherine palmifolia* extract in a 70% ethanol solvent and distilled water have an inhibitory effect on *Candida albicans* [8]. Antifungi activity test was done by using wells method and obtained inhibitory power of each extract equal to 65.44% and 54.78%. Ethanol extract of *Eleutherine palmifolia* bulbs also had antibacterial activity against *Staphylococcus aureus,* which is one of the bacteria causing skin infection [4].

Based on the above, the researcher interest to conduct research of antifungal activity of n-hexane extract, *Eleutherine palmifolia* bulbs with various concentrations on *Candida albicans* and *Trichophyton mentagrophytes*.

Material and Methods

The materials used in this study were *Eleutherine palmifolia* bulbs(*Eleutherine palmifolia* (L.) Merr), qualified analysis grade ingredients (Merck): α-naphthol, amyl alcohol, anhydrous acetic acid, concentrated hydrochloric acid, concentrated nitric acid, ethanol, sodium hydroxide, iodine, isopropanol, potassium iodide, chloralhydrate, chlorofom, methanol, n-hexane, potato dextrose agar (PDA), mercury (II) chloride, saboraud dextrose broth (SDB), magnesium powder, lead (II) acetate, and toluene. Fungi used in this study were *Candida albicans* and *Trichophyton mentagrophytes*. The tools used in this study were glassware, blast furnace, aluminum foil, autoclave (Fisons), blender (Phillips), paper discs, Petri dishes, incubators (Fiber Scientific), ose needle, Laminar Air Flow Cabinet (Astec HLF 1200 L), Bunsen lamp, Refrigerator (Toshiba), Dryer closet, Oven (Memmert), Tweezers, Micropipettes (Eppendorf), Rotary Evaporator (Haake D), Spectrophotometer visible (Dynamica Hello Vis-10) and analytical balance (Metler Toledo).

Characterisation of the *Eleutherine palmifolia* bulbs was performed in macroscopic and microscopic examinations, determination of water content, determination of water-soluble extract, ethanol solubilisation, also the determination of total ash and acid-soluble ash contents.

The preparation of the extract was performed by percolation method using n-hexane solvent. An amount of 300 g of *Eleutherine palmifolia* dried powder was inserted into a closed vessel, poured with n-hexane solvent until all the powder was completely immersed and left for at least 3 hours. The mass was gradually put into the percolator. The solvent was carefully poured until the liquid begins to drip and there was a layer of liquid at above of the sample; then the percolator was covered and left for 24 hours, the liquid drip was let at a rate of 1 ml per minute. Percolation was stopped until the last few drops of percolation that were evaporated leaving no residuals. The obtained gap was concentrated with a rotary evaporator.

The antifungal effect of EPBHE was evaluated by diffusion method using the paper disc. Candida albicans and Trichophyton mentagrophytes were chosen as the tested fungi. The EPBHE with inoculated fungi in PDA medium was incubated at 22°C for 48 hours and 3 days, respectively, for *Candida albicans* and *Tricophyton mentagrophytes* [10], [11]. The diameter of the clear zone around the disc was measured as the inhibitory area.

Results

The *Eleutherine palmifolia* bulb was round elongated, purplish-red with a very slippery surface. Bulbs consist of 5-7 layers with each layer having different thickness with dark red colour and white meat, usually 4-8 cm long and 4-6 cm in diameter, odourless, and bitter taste. The location of the *Eleutherine palmifolia* leaves in pairs with the composition of leaves and the flowers were white. Leaf type paralleled to the edge of slippery leaves, and ribbon-shaped leaves form a line along the 15-20 cm and 3-5 cm wide. The macroscopic appearance of *Eleutherine palmifolia* is presented in Figure 1.

Figure 1: Eleutherine palmifolia

The characterisation of *Eleutherine palmifolia* bulbs dried powder included water content, water-

soluble extract content, ethanol-soluble extract content, total ash content, and acid insoluble ash content. The characteristics of *Eleutherine palmifolia* bulbs dried powder can be seen in Table 1.

Table 1: Characteristics of *Eleutherine palmifolia* Bulbs Dried Powder

Parameter	Result (%)
Water content	9.38%
Water-soluble extract content	12.15%
Ethanol soluble extract content	14.48%
Total ash content	0.91%
Acid insoluble ash content	0.70%

The water content of *Eleutherine palmifolia* dried powder was obtained 9.38%, and it met the standard water content of dried powder requirement that is not more than 10%, if the water content is more than 10% then the dried powder will be vulnerable to overgrown microorganisms [12]. The water-soluble extract and ethanol-soluble extract content in *Eleutherine palmifolia* bulb were 12.15% and 14.48%, respectively. It was fulfilling the requirement of Indonesian Materia Medica (IMM) which is not less than 4% and not less than 2%, respectively, for water-soluble extract and ethanol-soluble extract [9].

The examination of total ash content and acid-soluble ash content were conducted to provide an overview of the internal and external mineral content of the *Eleutherine palmifolia* bulbs dried powder. This is related to the purity and contamination of the sample. Based on the result in Table 1, it can be seen that total ash content was 0.91% which fulfilled the requirement of IMM that is not more than 1% and the acid insoluble ash content was 0.70%, fulfilled the requirement of IMM that is not more than 1.5% [9].

Table 2: Phytochemical Screening Results of *Eleutherine palmifolia* Bulbs

Compound	Dried powder	EPBHE
Alkaloid	+	-
Flavonoid	+	-
Tanin	+	-
Saponin	+	-
Glikosida	+	-
Steroid/Triterpenoid	+	+

Note EPBHE: *Eleutherine palmifolia* bulbs n-hexane extract; +: positive; -: negative.

The results obtained in Table 2 showed that the *Eleutherine palmifolia* bulb dried powder had a complete class of compounds, namely alkaloids, flavonoids, tannins, saponins, glycosides, and steroids/terpenoids. The other hand, the non-polar n-hexane extracts contained steroid/terpenoid compounds.

The average diameter of EPBHE towards *Candida albicans* and *Trichophyton mentagrophytes* can be seen in Table 3.

Table 3: The Antifungal activity of *Eleutherine palmifolia* bulbs n-hexane extract

Concentration (mg/ml)	Inhibitory zone diameter (mm)	
	Candida albicans	Trichophyton mentagrophytes
200	19.48	42.20
100	15.22	36.03
80	14.03	33.91
60	13.22	31.52
40	12.92	25.45
30	12.33	23.33
20	12.18	21.02
10	11.38	15.87
8	8.58	13.20
6	6.28	11.95
4	-	8.96
3	-	7.12
2	-	6.26

Discussion

Based on the results in Table 3, the inhibitory measurements showed that n-hexane extract of *Eleutherine palmifolia* bulbs gave antifungal activity on both fungi, whereby the n-hexane extract inhibited the growth of *Trichophyton mentagrophytes* more stronger compared to the *Candida albicans*. If the diameter of the inhibitory zone formed on the diffusion test is less than 9 mm, the inhibitory activity is categorised as weak inhibition. If the diameter is 9-12 mm, the activity is categorised as being moderate, and 18 mm or more is categorised as very strong inhibition [13].

The EPBHE at concentrations of 200 mg/ml against C. *albicans* and 20 mg/ml against *Trichophyton mentagrophytes* had demonstrated very strong inhibitory activity. Each activity showed a clear zone diameter of 19.48 mm and 21.02 mm, respectively. The minimum inhibitory concentration (MIC) of the EPBHE was 6 mg/ml and 2 mg/ml for *Candida albicans* and *Trichophyton mentagrophytes,* respectively.

The fact that the EPBHE could inhibit the growth of *Candida albicans* as well by some previous studies in which non-polar compounds were able to induce a change in the permeability of *Candida albicans* membrane by the interaction of the active site of the compound with the active site of the cell membrane. Such interactions produced membrane kinetic energy which results in a change in permeability and caused the cell membrane to become unstable then cause the cell death and mould [14], [15]. The non-polar terpenoid of secondary metabolite compounds was reported to be effective in inhibiting microorganisms extensively. This phenomenon is because terpenoids can damage the membrane of microorganisms with their lipophylic components [16]. It was suspected that the EPBHE could inhibit the growth of *Trichophyton mentagrophytes* as well. It can be concluded that the characteristics of *Eleutherine palmifolia* bulb dried powder has a water content of 9.38%, water-soluble content of 12.15%, ethanol-soluble content of 14.48%, ash content of 0.91% and an acid-soluble ash content of 0.70%. The n-hexane extract of *Eleutherine*

palmifolia bulbs contains steroids/triterpenoids compounds. The n-hexane extract of *Eleutherine palmifolia* bulbs has stronger antifungal activity against *Trichophyton mentagrophytes* compared to *Candida albicans*.

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