

ANTI-FUNGAL ACTIVITY OF *Persicaria Minor* EXTRACTS
TOWARD *Malassezia* AND *Aspergillus Niger*

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ABSTRACT

The research of natural resources to inhibit fungi has accelerated in recent years. Thus, the aim of this study is to evaluate the essential oil of *Persicaria minor* and its hot water extract on antifungal susceptibility testing towards human pathogenic yeast *Malassezia* and filamentous fungi *Aspergillus niger*. *Malassezia* was isolated from human scalp with the presence of dandruff and identified using macroscopic and microscopic morphology while *A. niger* was observed on its macroscopic morphology. The extraction yield was 0.10% and 2.0% for essential oil and hot water extract of *P. minor* respectively for each 100 g initially. The antifungal screening was conducted using disk diffusion method while minimum inhibitory concentration (MIC) was conducted using microdilution in 96-well microplate and minimum fungicidal concentration (MFC) was conducted on Potato dextrose agar (PDA) plate. *Malassezia* was resistance towards both *P. minor* extracts while there were susceptibility of *A.niger* towards essential oil at the range of concentration 12.5 $\mu\text{L}/\text{mL}$ to 100 $\mu\text{L}/\text{mL}$ with no inhibition from *P. minor* hot water extract. The MIC of essential oil towards *A. niger* was at 12.5 $\mu\text{L}/\text{mL}$ while MFC at 50 $\mu\text{l}/\text{ml}$.

ABSTRAK

Kebelakangan ini, penyelidikan terhadap sumber asli untuk menghalang kulat semakin meningkat. Oleh itu, tujuan kajian ini adalah untuk mengekstrak minyak pati *Persicaria minor* dan ekstrak air panas untuk menilai ujian kerentanan anti-kulat terhadap yis patogen manusia *Malassezia* dan kulat berfilamen *Aspergillus niger*. *Malassezia* telah diasingkan daripada kulit kepala manusia yang berkelemumur dan dikenal pasti menggunakan morfologi makroskopik dan mikroskopik manakala *A.niger* diperhatikan pada morfologi yang makroskopik. Hasil perahan adalah 0.10% dan 2.0% untuk minyak pati dan ekstrak air panas *P. minor* bagi setiap 100 g masing-masing pada mulanya. Pemeriksaan antikulat telah dijalankan menggunakan kaedah resapan cakera manakala perencatan minimum kepekatan (MIC) menggunakan kaedah pencairan mikro dalam 96-lubang plat mikro dan dan kepekatan kulat minimum (MFC) telah dijalankan ke atas kentang dekstroza agar plat. *Malassezia* tidak menunjukkan perencatan terhadap kedua-dua pengekstrakan *P. minor* manakala *A. niger* menunjukkan perencatan terhadap minyak pati *P.minor* pada julat kepekatan 12.5 µl/ml kepada 100 µl/ml dan tiada perencatan terhadap *P. minor* ekstrak air panas. Perencatan minimum kepekatan minyak (MIC) pati *P. minor* ke arah *A. Niger* adalah pada 12.5 µl/ml manakala kepekatan kulat minimum (MFC) di 50 µl/ml.

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LIST OF ABBREVIATIONS

mg/mL	-	Milligram per millilitre
µL/mL	-	Microlitre per millilitre
min	-	Minute
PDA	-	Potato Dextrose Agar
SDA	-	Sabouraud Dextrose Agar
DMSO	-	Dimethyl sulfoxide
MIC	-	Minimum Inhibitory Concentration
MFC	-	Minimum Fungicidal Concentration
Abs	-	Absorbance
rpm	-	Rotation per minute
v/v	-	Volume per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of study

The flakes on the hair scalp, or clothing is considered an abnormal condition usually referred to as dandruff especially in advertising (James *et al.*, 2012). Dandruff is characterized by an itching symptom which accumulates diffusely on the scalp in localized patches of loosely adherent flakes. It is a pathological skin condition and is indicated by flaking with minimal to absent inflammation (Georgios *et al.*, 2012).

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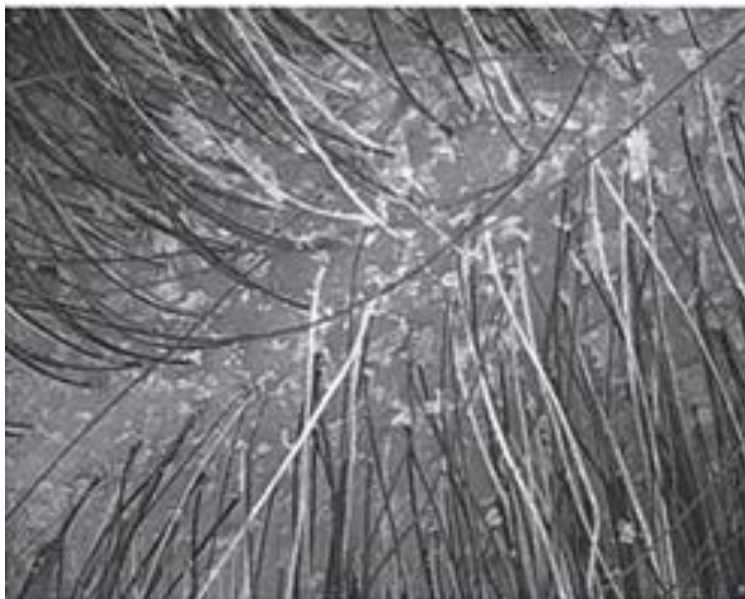


Figure 0.1: Topical presentation of dandruff (James *et al.*, 2012)

Dandruff has been blamed for many causes, including dry skin, oily skin, too much or too little hair washing, diet, stress and hygiene. Nevertheless the real cause of dandruff is actually a yeast-like fungus, which is known as *Malassezia* which lives on our scalp. The genus *Malassezia* consists of lipophilic yeasts which are known to be components of the microflora of human skin, many other mammals and birds are rarely isolated from the environment and these yeast-like fungus have the typical physiological property of using lipids as a source of carbon (Crespo *et al.*, 1999). Some *Malassezia* species may act as pathogens when exposed to certain changes in the skin's microclimate (Christina *et al.*, 2002).

Besides *Malassezia*, there is another fungus that will be examine later which is the *Aspergillus niger*. *A. niger* is a member of the genus *Aspergillus* which includes a set of fungi which is generally considered asexual. It differs from *Malassezia* which is a fungus affecting human beings and is ubiquitous on human skin, this *A.niger* is a type of fungus which is commonly known in food spoilage but has the ability to infect human beings. Although it is type of fungi that has been given the GRAS (generally recognized as safe) status by the US Food and Drug Administration, if inhaled in sufficient quantities, it can result in infections to human beings such as severe lung problems (Ruchi, 2012).

It is ubiquitous in soil and causes a disease called black mold in certain fruits and vegetables such as grapes, onions and peanuts and is a common food contaminant (Ruchi, 2012). This species is highly aerobic and most commonly found in mesophilic environments such as decaying vegetation, soil and plants (Meera *et al.*, 2012). Therefore, a solution to inhibit these fungi could lie in, a potential local plant *Persicaria minor* (Huds).

P. minor leaves is from one of the plants that has been traditionally used worldwide in medicine, cuisine, pharmacy, cosmetics and can be classified under the polygonaceae family and is a genus of *Persicaria* (Ridzuan *et al.*, 2013). There are various names for this plant in different countries. It is known as ‘daun kesom’ in Malaysia, Singapore and Brunei. It grows in tropical and subtropical zones which are warm and damp. It has dark green leaves while the stem is joined to each leaf through a stalk. According to Mohammad *et al.*, (2012) this species has a range of medicinal properties such as antimicrobial, anti-inflammatory, antitumor, and antioxidant properties. Thus, the aim of this study is to determine whether these species have antifungal activities through different types of extraction methods and their reaction towards different types of fungus.

1.2 Significance of study

Fungi are plant-like organisms which are beneficial and useful but some might act as a threat to human beings and some can contaminate foods and fruits. As stated earlier, dandruff is caused by the *Malassezia* fungus, and using non-medicated shampoos to simply wash away the flakes is not quite effective as they generate too quickly (James *et al.*, 2012). While *Aspergillus niger* is the most abundant species of *Aspergillus* in nature which causes the rotting of fruits, vegetables and other food products, resulting in substantial economic losses due to spoilage. Nowadays, finding alternative solutions by using natural sources has become more crucial because it

proves to be safe, natural and effective (Ridzuan *et al.*, 2013). In recent years, several studies have been focused on the screening of plant extracts to develop new antifungal compounds. Hence, an alternative method of treating dandruff and food spoilage from *A.niger* is currently being researched. The extracts of *Persicaria minor* will be evaluated to see if it has the ability to control these fungi as well as for its antifungal properties.

1.3 Objectives

1. To extract essential oils and hot water extracts of *Persicaria minor*
2. To isolate and identify the tropical *Malassezia* and *Aspergillus niger* by macroscopic and microscopic morphology
3. To evaluate antifungal activities of *Persicaria minor* towards *Malassezia* and *Aspergillus niger*

1.4 Scope of research

The herbs known as *Persicaria minor* was collected from the vicinity of Taman University, Johor Bahru and sent to the Forest Research Institute of Malaysia (FRIM) for species identification. The herbs were extracted using hydrodistillation and hot water extract methods to obtain essential oils and aqueous extracts. These extracts were then tested with two types of fungi which were *Malassezia* and *Aspergillus niger* to see if they had any inhibitory potential towards these fungi by using the disk diffusion method. In addition, the fungi were identified using macroscopic morphology, and microscopic morphology.

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