INHIBITORY EFFECT OF FORMULATED LEMONGRASS SHAMPOO ON *MALASSEZIA FURFUR*: A YEAST ASSOCIATED WITH DANDRUFF

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Abstract. Lemongrass (*Cymbopogon citratus* Stapf) has been used in cooking and in many traditional medicines; the essential oil contains citral as a major constituent. This study evaluated the antifungal activity of lemongrass oil against *Malassezia furfur*, an opportunistic yeast associated with dandruff, by using a broth dilution assay. From the minimum fungicidal concentration (MFC) obtained, the oil was then incorporated at different percentages into shampoo formulations. The formulated shampoos were kept at room temperature (28º-30ºC) and under accelerated condition (45ºC). At the end of the first and sixth weeks, after preparation, all formulations were tested again and the appearance was recorded. Selection of an appropriate formula was based on antifungal activity against *M. furfur*, the physical appearance, the chemical properties and stability of the formula. Two percent lemongrass oil shampoo provided the required qualities necessary for commercial use. After being kept for 6 weeks at 28º-30ºC and 45ºC, this formulated shampoo gave MFCs against *M. furfur* of 75 µl/ml and 18.75 µl/ml, respectively.

Keywords: *Cymbopogon citratus,* dandruff, herbal shampoo, lemongrass, *Malassezia furfur*

INTRODUCTION

Malassezia furfur is a common saprophytic, lipophilic yeast found on sebaceous areas of human skin: face, scalp, and upper trunk. This unicellular fungus is associated with several skin disorders that predominantly involve the superficial layers of skin and exacerbates many dermatologic diseases: tinea versicolor, seborrheic dermatitis, folliculitis and atopic dermatitis (Levin, 2009). Other species of *Malassezia*, *M. globosa* and *M. restricta*, have also been reported in human dandruff (Gupta *et al*, 2004). Dandruff is a superficial disorder of the stratum corneum of the scalp, with cell hyper-proliferation cause flaking, itching and redness. The clinical appearance and symptoms are similar to seborrheic dermatitis, only the latter is more severe. The symptoms develop when the lipophilic yeast *Malassezia* takes up free fatty acids (FFAs) from sebaceous triglycerides. The FFAs penetrate the stratum corneum breach the scalp skin barrier and lead to transepidermal water loss attributed to dandruff (Dawson *et al*, 2005). The treatment of dandruff includes application of topical antifungal or other

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products. Since recurrences occur commonly, prophylaxis using products for skin and hair to maintain good healthy skin and a healthy appearance is needed.

Lemongrass (*Cymbopogon citratus* Stapf), from the family Gramineae, is an important Asian culinary herb that has been included in a wide range of herbal products, household items and traditional medicines. The principal constituent of lemongrass essential oil is citral (3,7-dimethyl-2,6-octadienal), a mixture of isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral A) and neural (*cis*-citral, citral B) with a small quantity of geraniol, geranylacetate and monoterpene olefins (Simonsen and Owen, 1953; Katsukawa *et al*, 2010). These constituents comprise 75-85% of lemongrass oil and are a pale yellow liquid with a strong lemon-like odor (FAO, 1967; Formacek and Kubeczka, 1982). Because of these properties, citral is widely used as an essential raw material in pharmaceuticals, perfumery and cosmetics industries. Many studies have evaluated its biological properties, such as antifungal activity against plant and human pathogens (Yousef *et al*, 1978; Asthana *et al*, 1992; Shadab *et al*, 1992; Rodov *et al*, 1995; Adegoke and Odesola, 1996; Schwiertz *et al*, 2006; Tzortzaki and Costas, 2007; Tyagi and Malik, 2010a), bactericidal effects (Onawunmi *et al*, 1984; Cimanga *et al*, 2002; Wannissorn *et al*, 2005; Lertsatitthanakorn *et al*, 2006; Schwiertz *et al*, 2006; Naik *et al*, 2010; Tyagi and Malik, 2010a) and insecticidal properties (Rice and Coats, 1994; Saddiq and Khayyat, 2010). The WHO Model Formulary for 2008 (Stuart *et al*, 2009) lists it as a topical dermatological medicine used in many proprietary shampoos with antifungal and keratolytic properties against seborrheic dermatitis. Thus, the integration of herbals or traditional medicinal plants with available scientific evidence into daily use products has been reasonably encouraged.

This study focused on determining the effective ingredient of the herb and the appropriate procedure to include that active ingredient into a shampoo against *Malassezia furfur*.

MATERIALS AND METHODS

Plant materials

Lemongrass leaf sheaths were purchased from a market in Bangkok, Thailand, in June 2008. Identification of lemongrass was made by Prof W Gritsanapan, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. Specimens were kept at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University.

Volatile oil hydrodistillation

Fresh lemongrass leaf sheaths were cut into small pieces, put in a round bottom flask and distilled water was added (1:10). This was connected to a hydrodistillation instrument. The mixture of oil and water obtained was separated by a separating funnel; the collected volatile oil was kept in a tightly closed container with light protection until used.

Lemongrass oil and citral

Zero point one milliliter each of lemongrass oil and citral standard were mixed in 1 ml of hexane, and spotted onto a thin layer chromatography aluminium sheet of siliga gel GF_{254}. The mobile phase was toluene:ethyl acetate 97:3. The developed plate was observed under UV 254 nm and sprayed with vanillin sulfuric acid reagent (5% sulfuric acid in ethanol and 1% vanillin in ethanol).

Anti-malassezia activity

Quantitative evaluation: broth dilution assay. The principle of the test was modified

from reference method M27-A3 (CLSI, 2008) of the Clinical and Laboratory Standards Institutes (CLSI). An inoculum of *M. furfur* was prepared from a mature pure culture on Sabouraud dextrose agar (SDA; Pronadisa, Madrid, Spain) using Sabouraud dextrose broth (SDB; Pronadisa, Madrid, Spain) to give $3x10^3$-10^4 cfu/ml. A twofold serial dilution of oil/shampoo was made using SDB and dimethyl sulfoxide (DMSO). A serial dilution of DMSO at similar concentrations was used as control. A culture control was also included. All were incubated at 28º-30ºC. The end point of the test was recorded at the same time as the culture control became positive. Subculture test concentrations yielded the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC).

Formulation of shampoo containing lemongrass oil

Lemongrass oil was added to shampoo formulations at different concentrations. Other ingredients added to improve the quality and give the most appropriate characteristics to the shampoo were Lolane complex (LC), EDTA, DI water, Texapon N 28, Dehyton K, sodium lauryl sarcosinate, comperlan KD and Glydant plus. Twenty-five percent citric acid and sodium chloride were used to adjust the pH and viscosity, respectively.

To develop an anti-dandruff shampoo formula, lemongrass oil was added to different shampoo bases designated MU001 and MU002 at 1, 2, 3, 4 and 5% w/w. These formulations were left at room temperature for 1 and 3 weeks and the formulas with an acceptable appearance were modified into MU003 and MU004 at the same concentrations of lemongrass oil and kept at room temperature (28º-30ºC) or high temperature (45ºC) for 6 weeks. Further evaluation of anti-malassezia activity by these formula was done after the first and sixth weeks.

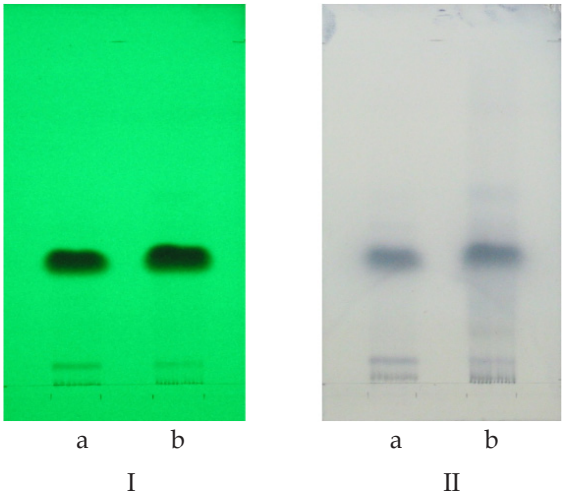

Fig 1–Comparison of thin layer chromatography of citral reference standard and lemongrass oil employed in test sample. I, TLC plate under UV 254 nm; II, TLC plate sprayed with vanillin-sulfuric acid reagent; a, Citral reference standard; b, Lemongrass oil sample.

Efficiency of formulated shampoo against *M. furfur*

The accepted stable formulations were tested for anti-malassezia activity by broth dilution assay to test the effectiveness the shampoos left at normal room temperature (28º-30ºC) and at high temperature (45ºC).

RESULTS

Lemongrass oil and citral standard

The lemongrass oil used in the shampoo formulations was evaluated for its active ingredients using thin layer chromatography (TLC) at an Rf value of 0.38, similar to the citral reference standard. A purple band appeared after spraying with vanillin-sulfuric acid, indicating the presence of terpenes (Fig 1).

Table 1
Anti-malassezia activities of lemongrass oil extract and citral standard against *M. furfur.*

Test sample	Anti-malassezia activity (µg/ml)	
	MIC	MFC
Lemongrass oil extract	6.25	6.25
Citral standard	3.13	12.50

Anti-malassezia activity of lemongrass oil and citral standard

The activity of lemongrass oil and citral standard on *M. furfur* was investigated by broth dilution assay. The inhibitory and fungicidal effects of lemongrass oil occurred at the same concentration (6.25 µg/ml), half way between the inhibitory and fungicidal concentrations of the citral standard (Table 1).

Table 2
Formulations of lemongrass oil shampoo.

Formulation[a]	Storage condition		Quality[b]			
	Week[c]	Temp	Thickness	Color	Separation into layers	MFCs (µl/ml)
MU001	0	28º-30ºC	ac	ac	ac	[d]
	1	28º-30ºC	ac	±	±	-
MU002	0	28º-30ºC	±	ac	ac	-
	1	28º-30ºC	ac	ac	un	-
MU003	0	28º-30ºC	ac	ac	ac	37.50-300.00
	6	28º-30ºC	ac	ac	ac	18.75-75.00
		45ºC	ac	ac	ac	9.38-37.50

[a] Formulations MU003-B, MU003-X, MU004 and MU004-X were not included in the table.
[b] Satisfied result, ac, acceptable; un, unacceptable; ±, partially acceptable
[c] 0: refers to a freshly prepared sample
[d] Not done

Table 3
MFCs of different percentages of lemongrass oil shampoo MU003 against *M. furfur.*

Duration in week(s)	Temp	MFC (µl/ml) of shampoo at different percentages of lemongrass oil				
		1%	2%	3%	4%	5%
0	28º-30ºC	300.0	75.0	75.0	37.5	37.5
6	28º-30ºC	75.0	75.0	37.5	18.8	18.8
	45ºC	37.5	18.8	18.8	18.8	9.4

MFC, minimum fungicidal concentration

Development of shampoo containing lemongrass oil. After the preliminary shampoo containing lemongrass oil was formulated, it was further developed by adding or taking out of some components. The shampoo bases MU001 and MU002 consisted of different percentages of surfactant (C. Betaines), foam booster and thickener [cocamide MEA, hydroxy ethylcellulose (HEC), NaCl] and various concentrations of lemongrass oil (1, 2, 3, 4 and 5% w/w). After being left at room temperature for one week, an acceptable combination of MU001 was modified by adding HEC to the shampoo base of MU003 and MU004 and stored at 28º-30ºC and 45ºC for 6 weeks. Subformulae MU003-B, MU003-X, MU004, MU004-X were also stored likewise before testing for anti-malassezia activity.

The MU003 formula with 2% lemongrass oil demonstrated the best stability under test conditions with good physical appearance: clear solution, no separated layer, moderate thickness and acceptable odor (Table 2). Anti-malassezia activities were determined under test conditions. After 6 weeks, MU003 formula with lemongrass oil demonstrated increasing antifungal activity with increasing percentages of lemongrass oil (Table 3).

DISCUSSION

Medicinal plants and crude herbal drugs have been included in traditional medicines and household remedies for a long time. Not all herbal preparations have been scientifically tested. In an attempt to determine the benefits of various herbal extracts, we evaluated the effect of lemongrass oil against *M. furfur*, a yeast associated with dandruff.

Thin layer chromatography confirmed the constituents of lemongrass oil used in this study were similar to the citral reference standard using its Rf value and the similar color of the spots after spraying with vanillin-sulfuric acid reagent, which revealed the presence of terpenes. Further studies of the chemical stability of the formulae by quantitative analysis of active ingredients using HPLC/TLC densitometry should be carried out.

In order to develop a shampoo formula, the selection was based on the physical stability under room temperature and high temperature (45ºC) to reflect 2 years of storage. Other properties included clear solution, moderate thickness, and acceptable odor. In addition, lemongrass oil containing citral is also used as a natural bio-active component.

The 2% lemongrass oil shampoo formula MU003 was the most appropriate formula with required properties and anti-malassezia activity. This shampoo formula did not deteriorate over time and exhibited anti-malassezia activity. The MFC after one week at room temperature was 75 µg/ml and at 45ºC was 18.75 µg/ml. This may be a result of losing water in the formula. The 2% concentration of lemongrass oil was selected because the smell, consistency and stability of the shampoo were better than the others.

ACKNOWLEDGEMENTS

We would like to thank The Industrial and Research Projects for Undergraduate Students (IRPUS) for their support and SC Artistry Co Ltd for advice on development of shampoo for formular.

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