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ANTIDANDRUFF POTENTIAL OF *Kaempheria galanga* ETHANOLIC EXTRACTS FOR HAIR CREAM FORMULATION

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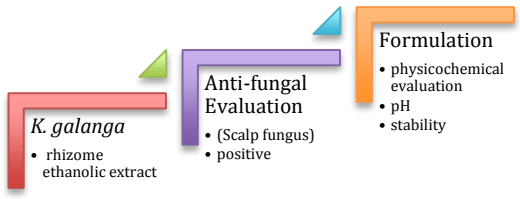
Graphical abstract



Abstract

The evaluation of *Kaempferia galanga*, *Citrus hystrix* and *Cinnamomum zeylanicum* ethanolic extracts on antifungal activities and zone of inhibition were conducted. The yield for *K. galanga*, *C. hystrix* and C. zeylanicum were 0.4, 0.7 and 0.43 % of raw dried samples respectively. All the extracts demonstrated 5 mg/mL MIC with *C. zeylanicum*, *K. galanga* and *C. hystrix* average holozones diameter of 14.5 ± 3.8, 12.0 ± 1.8 and 12.0 ± 0.8 mm after 3 days of incubation respectively with no effect on negative control. On the other hand, Zinc Pyrithione being more potent than imidazaole as a positive control with inhibition of 32.8 ± 2.2 and 21.8 ± 3.4 respectively. Based on the findings, the anti-fungal hair cream containing *K. galanga* ethanolic extract was formulated into oil-in-water cream and the physicochemical properties were evaluated. The cream demonstrated desirable characteristic with no separation between oil and water after vigorously shaken at 14,600 rpm for half an hour. Furthermore the viscosity and pH were 543.7 ± 19.2 and 5.46 ± 0.01 respectively. In conclusion, *K galanga* ethanolic extract has a potential to be used as an anti-fungal oil in water cream formulation.

Keywords: *K. galanga, C. Hystrix, C. zeylanicum,* antifungal, *Malassezia, Citrus hystrix, Cinnamomum zeylanicum*

Abstrak

Pencerapan antifungus melalui kaedah perencatan telaga penyebaran ektrak etanol *Kaempferia galanga*, *Citrus hystrix* dan *Cinnamomum zeylanicum* ke atas *Malassezia* spp. telah dilaksanakan. Kesemua ekstrak mempamirkan kepekatan perencatan minimum serendah 5 mg/mL dengan *C. zeylanicum, K. galanga* dan *C. hystrix* mempunyai holozon berdiameter purata 14.5 ± 3.8, 12.0 ± 1.8 dan 12.0 ± 0.8 mm selepas 3 hari inkubasi masing-masing manakala tiada perencatan oleh kawalan negatif. Zinc Pyrithione menunjukkan perencatan yang lebih tinggi berbanding imidazole sebagai kawalan positive dengan diameter purata holozon sepanjang 32.8 ± 2.2 dan 21.8 ± 3.4 masing-masing. Seterusnya, krim rambut anti-kelemumur yang mengandungi ekstrak ethanol *K. galanga* telah diformulasi berasaskan krim minyak di dalam cecair dan sifat fizikal-kimianya dianalisa. Krim menunjukkan ciri-ciri yang sesuai dengan tiada pemisahan antara minyak dan air apabila digoncangkan pada kelajuan 14 600 rpm selama setengah jam. Tambahan pula kelikatan dan pH krim rambut

tersebut adalah 543.7 ± 19.2 and 5.46 ± 0.01 masing-masing, di dalam lingkungan kesesuaiannya. Kesimpulannya ektrak etanol *K. galanga* berpotensi digunakan sebagai bahan aktif untuk krim anti kelemumur minyak di dalam cecair.

Kata kunci: *K. galanga, C. Hystrix, C. zeylanicum,* antifungal, *Malassezia, Citrus hystrix, Cinnamomum zeylanicum*

1.0 INTRODUCTION

Spices and herbs have been used by human civilisation for various purposes from food enhancer to preservation. *Kaempferia galanga (K. galanga), Citrus hystrix* (*C. hystrix)* and *Cinnamomum zeylanicum (C. zeylanicum)* are three important herbs in South East Asia not only used in dishes preparation but also in traditional medicine. *K. galanga, C. hystrix* and *C. zeylanicum* belongs to Zingiberaceae, Rutaceae and Lauraceae family respectively[1], [2], [3].

The rhizome of *K. galanga* has been used by indigenous traditional medical practitioners from ancient times in Asia as they contain volatile oil and other important compounds with medicinal values. This include to relief inflammatory related symptom such as nasal blockage and asthma while in cosmetic, the rhizome was used as precursor for perfumery [4, 5]. In addition, *C. hystrix* is more popular and have been used throughout the world in food flavouring. Like other Citrus species, they are found across continent in India, China, Brazil, Mexico, United State and Spain [6].

Similarly, a well-studied herb with various medicinal purposes is *C. zeylanicum*. They are famously found in China, India and Vietnam although the tree has been commercially grown throughout the tropics and subtropics. Major compositions of cinnamon which derived from the tree bark are (*E*)-cinnamaldehyde, benzaldehyde, and (*E*)-cinnamyl acetate [7]. In cooking, they are used as food flavouring mostly in curry and soup. The addition of cinnamon in tea or coffee to enhance the aroma may possess beneficial health due to their antioxidant properties [8]. The oil has been reported with strong antifungal activity against *C. albicans, C. tropicalis*, and *C. krusei* [9]. The mechanism is unknown but were believed in delaying conidia germination in filamentous fungus [10]. They were also reported to inhibit elastase and keratinase activities in *A. fumigatus* and *T. rubrum a* dermatophyte that cause skin diseases. [11], [12], [13]. Although the methanolic extract of Cinnamon have been scientifically demonstrated as antifungal activities against *Malassezia* spp. [14, 15], non were reported for *K. galanga* and the effect of ethanolic extract of these herbs against *Malassezia* spp. is unknown.

Dandruff caused by numerous host factors in conjunction with *Malassezia furfur* colony on the surface of a scalp. *Malassezia furfur* is yeast like lipophilic basidiomycetous fungus commonly found on sebum rich skin on the upper body, and have been link to the problem of hair fall [16, 17]. The symptoms are sex and age related. It is reported at 70 years of age, 38 % of women having female-pattern hair loss, with men are more severe where it can start as early as 20 to 45 year old [18].

Presently commercially available antidandruff hair shampoos contained synthetic compounds as an active ingredient to reduce or control the symptom of seporrhoeic dermatitis with a little usage of natural product [19, 16]. Although the synthetic based antidandruff shampoos are available, the market is still relatively small with imidazole and zinc pyrithione (zpt) being the active ingredients. Furthermore due to the consumer demand on natural derived product, these have open a new alternative to find a natural, sustainable and greener active agent.

2.0 METHODOLOGY

2.1 Collection and Preparation of Plant Material

The *K. galanga* rhizome, *C. hystrix fruits* and *C. zeylanicum bark* in this study were obtained locally in Johor Bahru, Malaysia. Crude extractions of plants were performed according to Kanjanapothi *et al*., [20] with some modifications. The amount of 20, 20 and 30 g of *K. galanga* rhizome, *C. hystrix* and *C. zeylanicum*, were air dried and ground respectively. The samples were then soaked in 200 mL of absolute ethanol for an hour. The extracts were then filtered and evaporated on rotary evaporator at 45 °C to obtain dried powder extracts. The powders were stored in air tight containers and kept in refrigerator at 4 °C prior for further testing. The yield of the evaporated dried extracts based on dry weight basis was calculated using the following formula:

$$\text{Yield (\%)} = (W_1) / (W_2) \times 100$$

Where W_1 was the weight of extract after evaporation from solvent and W_2 was the dry weight of the powder dried samples.

2.2 Chemicals, Solvents and Instruments

Chemicals and reagents were of analytical grade obtained from Sigma-Aldrich. Sample of organism was isolated from scalp of volunteer with dandruff by sampling using a sterile cotton swab [21]. The sample was maintained on Potato Dextrose Broth (Difco Ltd. UK) media at 34 °C for 48 hours. The sample was inoculated as per standard protocols over the surface of Potato Dextrose Agar (Oxoid Ltd. London) media which was incorporated with 0.05 % chloramphenicol and 50 µL of olive oil. The plates were incubated in room temperature at 34 °C for 2 weeks and inspected every 2-3 days to observe the presence of growth. The microscopic examination of fungal growth was observed after stained with methylene blue by using Cat Cam 300 Microscope. The sample characteristics of *Malassezia* spp. are thick-walled, round and budding yeast like cells. The isolates were subculture weekly to maintain a healthy fungal growth [22]. The organism was identified based on cultural screening and microscopic methods, see Figure 1 [23, 24].

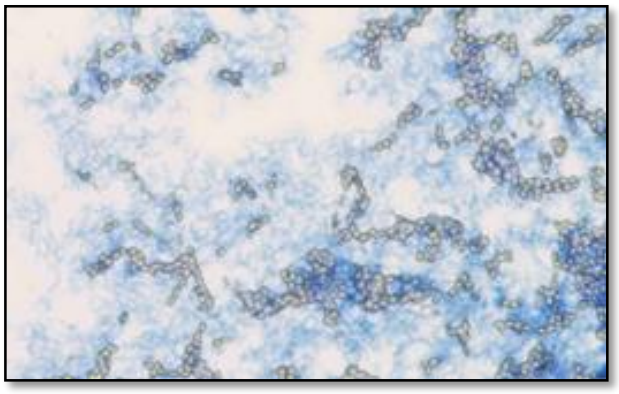


Figure 1 Scalp fungus stained using methylene blue under 400 X magnifications

2.3 Antifungal Assay

Agar-well diffusion method was conducted to evaluate the antifungal activities of ethanolic crude extracts of *K. galanga*, *C. hystrix* and *C. zeylanicum*. A 50 µL of vegetable oil was dispensed and evenly spread using a sterile glass spreader onto 20 to 25 mL of sterilized Potato Dextrose Agar plate. The plates were left to dry prior to the addition of 0.1 mL of active cultures. The cultures were grown earlier for 16 to 24 hours with 0.5 - 0.8 O.D. at A_{600} nm wavelength. A sterile 9 mm borer in diameter was then punched in the agar medium to create a well. Subsequently, wells were filled with 50 µL of the crude extracts. The addition of 50 µL sterile distilled water, 50 µL of ketoconazole containing imidazole and 50 µL of zpt were used in the assay as negative control, positive control, respectively. The plates were incubated at 34 °C for 3 days and the diameter of inhibition zone was observed every 24 hours [22].

2.4 Effect of Herb Extracts Concentration on Fungal Growth

Overnight cultures were seeded in 96-well plate filled with 50 µL of sterilized Potato Dextrose Broth. Subsequently, 100 µL of ethanolic extracts ranges from 5 to 100 mg/mL in concentration were loaded into the respective wells. 100 µL of zpt were used as a standard. The plates were incubated for 24 hours at 34 °C. At the end of incubation, drop plate technique was conducted by dropping 10 µL of the mixture from each well onto the sterilized Potato Dextrose Agar.

2.5 Cream Formulation

Oil in water base hair cream containing *K. galanga* ethanolic extract was prepared according to *Kumar et al.,* [25] with some modifications as shown in Table I. Both distilled water from Phase A and whole Phase B formula were heated up to 70 °C respectively. In Phase A preparation, the carbopol was added into the heated distilled water and instantly stirred prior to the addition of glycerin. Homogenizer stirrer was used to ensure mixing of the whole components were in stable condition. As the two precedent phases reached the indicated temperature, the heater was turn down immediately. Phase B was then transferred slowly into phase A beaker and homogenized. The hair cream was left to cool down at room temperature. As the temperature drop to 40 °C, phase C was then introduced and mixed.

Table 1 Composition of Ingredient and phases for hair cream formulation

Phase A	Phase B	Phase C
Distilled water	Glyceryl monostearate	Vitamin E
Carbopol	Refined corn oil	Propylene glycol
Glycerin	Olivoil emulsifier	Microkill cos
-	Stearic acid	*K. galanga* rhizome extract

2.6 Physicochemical Test on Hair Cream Containing *Kaempheria galanga* extract

Physicochemical test were conducted to ensure the addition of *K. galanga* extract is suitable and did not alter much of the require condition of the hair cream.

2.6.1 Organoleptic Properties

The organoleptic were conducted by untrained panelist. This section was conducted to ensure the cream formulated were accepted by potential user for personal use. The color and odor of hair cream

were evaluated physically based on hedonic scale [25]

2.6.2 Stability Test

The hair cream was placed in microcentrifuge tube and vigorously shaken at 14 600 rpm for half an hour. The stability studies were determined based on separation section of oil-in-water (O/W) cream.

2.6.3 Viscosity Test

Viscosity of the formulation was measured by Brookfield DV – II + Pro Viscometer at 5 rpm using spindle number 64.

2.6.4 pH

The pH of hair cream was measured by DELTA 320 pH meter.

3.0 RESULTS AND DISCUSSION

3.1 Extraction Yield and Antifungal Assay

The yield of *K. galanga* rhizome, *C. hystrix* and *C. zeylanicum* were found to be 4.0, 7.0 and 4.3% of raw dried samples, respectively. The isolated fungal were maintain as described earlier in materials and methods.

In this investigation, the ability of the three extracts to inhibit the growth of fungus culture were evaluated. ZPT and imidazole were used as positive control while sterile distilled water was used as negative control.

After 3 days of incubation, holozones were observed from both positive controls with ZPT being more potent than imidazole with 32.8 $\pm$ 2.2 and 21.8 $\pm$ 3.4 in mean of diameter respectively. Similarly all three ethanolic herbs extracts from *K. galanga*, *C. Hystrix* and *C. zeylanicum* tested show positive inhibition with mean of diameter holozone of 12.0 $\pm$ 1.8, 12.0 $\pm$ 0.8 and 14.5 $\pm$ 3.5 mm respectively to *Malassezia* growth. No inhibition zone were observed on negative control wells (See table 2).

The capability of these herbs to inhibit other dermatophyte such as *Candida albicans* has been reported previously. [26], [9].

Table 2 Antifungal activities of selected South East Asia herbs for three days. Data represent average mean diameter $\pm$ S.E.M of holozones by different sample (n=4)

Sample	Holozones diameter (mm)
dH2O	0 +0
K. galanga	12.0+1.8
C. hystrix	12.0+0.8
C. zeynalicum	14.5+3.5
ZPT	32.8+2.2
Imidazole	21.8+3.4

3.2 Effect of Concentration on Fungal Growth

The extracts were further tested in serial dilution concentration. In this investigation, the antifungal activity of *K. galanga*, *C. hystrix* and *C. zeylanicum* against *Malassezia* spp. at various concentrations between 5 to 100 mg/mL were examined along with the standard. All extracts tested show inhibitory effect as low as 5.0 mg/mL (see Table 3). However, the inhibitory concentration for all extracts may have lower value as reported by other researches but using different fungus [26, 27, 9]. Comparatively, our findings showed that the ethanolic extract of *K. galanga* *C. hystrix,* and *C. zeylanicum* exhibited antifungal activity against *Malassezia* spp. with expanding zone of inhibition at 5 % concentration. Although all samples shows positive rection, taking into consideration of the results obtained for *K. galanga* extract and the economic impact of potential needs and commercialization, it was selected to be an active ingredient for hair cream formulation.

Table 3 The effect of culinary herbs extracts concentration on fungal growth Hair Cream Formulation

Concentration of sample (mg/mL)	*K. galanga*			*C. Hystrix*			*C. zeylanicum*			ZPT	dH_2O
5.0	+	+	+	+	+	+	+	+	+		
10.0	+	+	+	+	+	+	+	+	+	ND	-
50.0	+	+	+	+	+	+	+	+	+	ND	-
100.0	+	+	+	+	+	+	+	+	+	+	-

+ denotes inhibition observed
- denotes non inhibition observed
ND denotes non test done

3.3 Hair Cream Formulation with *K. Galanga* Ethanolic Extract

Dandruff sufferer may require regular or long-term use of therapeutic agents. The study implies that ethanolic extract of *K. galanga* rhizome possessed anti-dandruff activities. Based on the data observed, hair cream comprising *K. galanga* rhizome extract was formulated to oil in water based cream [28]. Hair cream was chosen compared to shampoo with medicated properties based on the target. Cream having the advantages by providing a direct contact with the fungal population when compared to the applications of shampoo to the scalp. Furthermore the contact period between the cream and fungus are longer. The physicochemical properties of hair cream formulated were summarized in Table 4. The formulated hair cream has an appearance of white emulsion with pleasant *K. galanga* aromatic smell. It was physically stable as no separate section between oil and water phases observed. The lower the viscosity

of hair cream indicates the easily spreadable by small amount of shear however too low will reduce the contact period [29]. Finally, the pH obtained for hair cream was 5.46 ± 0.01 which is appropriate for human scalp as normal pH of human scalp ranges from 4 to 5 [30].

Table 4 Physicochemical properties of *K. galanga* hair cream

Organoleptic properties	Stability 14600 rpm for 30 min	Viscosity 5 rpm (cP)	pH
Cream, white emulsion, aromatic smell	No separation	543.7 ± 19.2	5.46 ± 0.01

4.0 CONCLUSION

In conclusion, all ethanolic plant extracts tested possess inhibition activity against *Malassezia* spp. with *K. galanga* being the best character for active ingredient formulation. The formulation of *K. galanga* oil-in-water (O/W) cream showed desired physicochemical characteristic as tested and can be considered for future application to control dandruff.

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