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INHIBITORY EFFECT OF ESSENTIAL OILS ON EXTRACELLULAR LIPASE ACTIVITY OF *MALASSEZIA GLOBOSA*

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ABSTRACT

Pityriasis capitis is a common scalp disorder caused by a fungus *Malassezia globosa*. The mechanism of *Pityriasis capitis* includes *Malassezia*-induced fatty acid metabolism, particularly lipase-mediated breakdown of sebaceous lipids and release of irritating free fatty acids. We report that extracellular lipase activity was detected in *Malassezia globosa*. The presence of lipase enzyme was performed in specific media on Petri dishes for formation of a zone.In this article, the effect of *Cymbopogon citratus* and *Zingiber officinale* essential oils on the extracellular lipase activity of *Malassezia globosa* had been studied by titrametric *method*. At the end of titration 4 µmol fatty acid/ml of reaction mixture was released in the presence of *Cymbopogon citratus* oil and 7µmol of fatty acid/ml of reaction mixture was liberated in the presence of *Zingiber officinale* oil. It was found that both essential oils strongly inhibit the lipase activity of M. *globosa* at a lower concentration.

Keywords: ***Zingiber officinale, M. globosa, Cymbopogon citratus*, lipases**

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INTRODUCTION

Pityriasis capitis is a common scalp problem that occurs at pre-pubertal age of any gender and ethnicity[1]. It is a condition characterized by flaking of skin (most commonly scalp skin) resulting from rapid turnover and release of skin cells. *Pityriasis capitis* is reliant on three factors: sebum production, microbial metabolism (specifically *Malassezia*) and susceptibility of individual. It is caused by a fungus *Malassezia globosa*.The genus *Malassezia* includes a group of lipophilic fungi whose natural habitat is the skin of humans and other warm blooded animals. *Malassezia* species are dimorphic, existing in both yeast and mycelial phases. *Malassezia globosa* initiates *Pityriasis capitis* formation due to high lipase activity. Due to its inability to metabolize its own lipids, *Malassezia* species rely on the lipids present in sebum. Sebum is degraded to free fatty acids from triglycerides and saturated fatty acids are consumed. Unsaturated fatty acids are left behind: penetration of unsaturated fatty acids results in inflammation, irritation and scalp flaking[2].An extracellular lipase and phospholipase activities of several *Malassezia* species was analyzed and compared under common growth conditions. M. *globosa* had shown the highest lipase activity of all of the *Malassezia* species. The results indicated that *Malassezia* species are capable of utilizing lipids well in contrast to the other lipid-dependent species of the genus. Data suggested that M. *globosa* is an important pathogenic species in several human skin diseases despite its slow rate of growth [3] .In this paper we report the secretion of extracellular lipase and inhibitory effect of *Cymbopogon citratus* oil and *Zingiber officinale* oil on lipase activity of *Malassezia globosa*.

MATERIALS AND METHOD

Isolation of organism

The fungus *Malassezia globosa* was isolated from the scalp of students suffering from Pityriasis capitis. Cultures were obtained on mDixon's agar which is composed of malt extract (3.6%), mycological peptone (0.6%), agar agar type I (1%), bile salts (2%), tween 40 (1%), glycerol (0.2%), oleic acid (0.2%), The scrapings collected in mDixon's broth, was used as stock. 9 test tubes with distilled water were sterilized in an autoclave at 121°C for 15 minutes. The test tubes were then used to carry out serial dilution of the stock. mDixon's agar plate was prepared. 1mL of sample from the dilution $10^{-6,}$ was used to spread plate on the agar plate using a pre-sterilized L-rod. The plates were then incubated at 32°C for 72 hrs. Biochemical characterization was carried out according to the method described by Kanaeko[4].

Essential oils

Cymbopogon citratus and *Zingiber officinale* oil; both the oils were obtained from the local market with concentration 77.42mg/μL and 80.53mg/μL respectively.

Detection of extracellular lipase

A semi-quantitative analysis of the lipase activity of the isolates on the solid agar medium could be obtained by measuring the diameter of the zone of precipitate and relating this to the colony size. To detect the production of lipolytic enzymes, the medium described by Sierra[5] was used. Cultures were inoculated on Sorbitan monolaurate agar medium and observed for 10 to 15 days for the formation of an opaque zone of precipitation[6, 7].

Crude lipase preparation

In order to remove fungus cells and spores, the culture was filtered through filter paper (Whatman No.1) and then the filtrate was centrifuged at 10,000 rpm at 4 °C for 15 min. The supernatant was collected to perform lipolytic activity assays. The crude extract was stored at –20 °C until used[8].

Determination of lipase activity

Sierra medium (Sierra 1957) supplemented with 1% (wt/vol polyoxyethylene sorbitan

monolaurate (Tween 80) but which did not contain agar was used as the broth medium for measuring lipase activity. This medium was dispensed in 50mL volume into 100mL flat-bottom flasks before sterilizing. Triplicate flasks were put up for studying the effect of *Cymbopogon citratus* oil and *Zingiber officinale* oil on the test organism. 400mg/L concentration of *Zingiber officinale* oil and 100mg/L concentration of *Cymbopogon citratus* oil was added to the broth (growth inhibition was found at this concentration).The inoculums for each plate comprised an agar disc (8mm in diameter) obtain (with a cork borer) from a 4 day-old culture of the test isolate on mDixon plate. The inoculated flasks were finally incubated at 32 ^{o}C for 4 days. Growth curve analysis had shown peak growth of *Malassezia globosa* on forth day. On the same day lipase activity of the culture was maximum; therefore for lipase assay four day old culture was taken.The quantity of lipase in the culture of each flask was assayed titrimetrically by a modified method using olive oil as a subsrate[9]. Olive oil (10%v/v) was emulsified with gum Arabica (10%w/v) in 200mL of 50mM sodium phosphate buffer pH8. 5mL enzyme was added to initiate lipolysis on the emulsion substrate, started timer with continuous stirring. At five suitable reaction intervals (e.g., 5, 10, 15, 20, and 25 min), 5 mL reaction mixture was removed and transferred each subsample to a separate flask containing 10 mL of 95% (v/v) ethanol and 2 to 3 drops of 1% (w/v) thymolphthalein indicator. Control was fungal culture without an essential oil. Contents were swirled immediately to stop the reaction. The contents of each flask were titrated with 0.05 N NaOH using a burette until a light blue colour appeared[10]. The experiment was performed in triplicates for each sample and mean was taken.

Effect of essential oils on Malassezia globosa

Lipase activity of *Malassezia globosa* control sample was compared with that of lipase activity of *Malassezia globosa* in the presence of *Zingiber officinale* and *Cymbopogon citratus* oil. It was observed that there was a decrease in lipase activity of the organism. At the end of

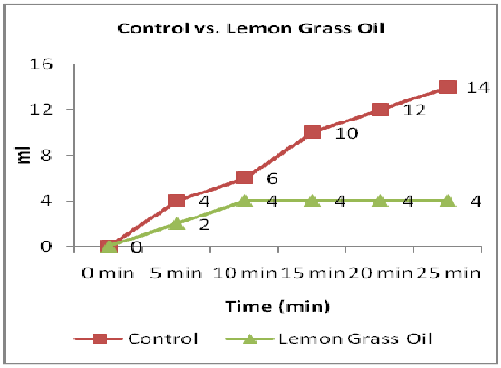
RESULTS AND DISCUSSION

Lipase production by all the isolates of *Malassezia globosa* tested was slow and the visible precipitate of calcium salts of lauric acid was observed after 5 - 7 days of incubation. The ratios of the diameter of the zone of precipitate to the colony size ranged from 7 to 10mm (Fig. 1).Assay of the lipase activity of *Malassezia globosa* was made every 24 hours for the first 2 days did not give sufficient enzyme activity. There was a steady increase in lipase activity from the second day i.e. 15 µmol fatty acid/ml reaching a peak on the fourth day i.e. 35 µmol fatty acid/ml of reaction mixture. The experiment was performed on the fourth day. The amount of fatty acids was calculated by the quantity of fatty acids liberated in each subsample based on the equivalents of NaOH used to reach the titration point, accounting for any contribution from the reagent, using the following equation µmol fatty acid/ml subsample= [(ml NaOH for sample-ml NaOH for blank) x Nx 1000] / 5ml Where N is the normality of the NaOH.

titration, 7µmol of fatty acid/ml of reaction mixture was liberated by *Zingiber officinale* (graph 2 and table2) sample and 5 µmol fatty acid/ml of reaction mixture was released by *Cymbopogon citratus* **(**graph 1 and table1**)** while14 µmol of fatty acid/ml of reaction mixture was released in control. This result indicates that both essential oils strongly inhibit the growth of the organism.

Figure1

Graph1



Graph2

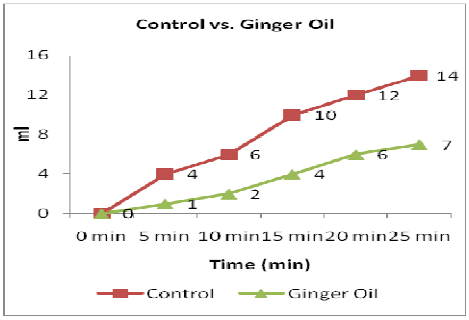


Table 1

Effect of Cymbopogon citratus on lipase activity of Malassezia globosa

Sample No.	TIME									
	5min		10min		15min		20min		25min	
	NaOH used in mL	µmol fatty acid/ml	NaOH used in mL	µmol fatty acid/ml	NaOH used in mL	µmol fatty acid/ml	NaOH used in mL	µmol fatty acid/ml	NaOH used in mL	µmol fatty acid/ml
1	2.1	2	2.3	4	2.3	4	2.3	4	2.3	4
2	2.1	2	2.2	3	2.3	4	2.1	2	2.3	4
3	2.1	2	2.4	5	2.3	4	2.6	6	2.3	4
mean	2.1	2	2.3	4	2.3	4	2.3	4	2.3	4

Table 2
Effect of Zingiber officinale on lipase activity of Malassezia globosa

	TIME									
Sample No.	**5 min**		**10 min**		**15 min**		**20 min**		**25min**	
	NaOH used in mL	**µmol fatty acid/ml**	**NaOH used in mL**	**µmol fatty acid/ml**	**NaOH used in mL**	**µmol fatty acid/ml**	**NaOH used in mL**	**µmol fatty acid/ml**	**NaOH used in mL**	**µmol fatty acid/ml**
1	2	1	2.1	2	2.3	4	2.4	5	2.6	7
2	2	1	2	1	2.1	2	2.5	6	2.8	9
3	2	1	2.2	3	2.5	6	2.6	7	2.4	5
mean	2	1	2.1	2	2.3	4	2.5	6	2.6	7

DISCUSSION

In our article, an extracellular lipase was detected in sorbitan monolaurate medium by forming a precipitate around the organism. In a study, a total of 123 isolates of 14 species of dermatophytes and yeasts were screened for the activity of five extracellular enzymes including elastase, keratinase, protease (gelatinase), lipase and phospholipase, by using sorbitan monolaurate medium. Lipase were produced by all dermatophytes and non-dermatophyte isolates. Most lipase activity was encountered for *Malassezia* furfur[11]. The capability of M. canis and T. mentagrophytes isolated from rabbits both with and without lesions was assessed for producing different enzymes. The results showed that T. mentagrophytes and M. canis from rabbits produce different enzymes. However, lipases were linked to the appearance of lesions in M. canis[6].The researchers are dealing with this topic to point out the importance of lipolytic enzymes that enable the fungi to obtain necessary nutrients from human skin secretions. The papers available differed from one another in such topics as culture medium, time of culture, technique for lipase assay. To evaluate enzymatic activity, we used a suspension of four day fungal culture in mDixon's broth because cell growth was found to be maximum on that day but some study reports, fifteen day cultures of *Malassezia* sp. on liquid Dixon medium to evaluate enzymatic activity[12]. In another article suspensions from 7-10 day cultures on solid LNA medium (Leeming and Notman agar) was used[13]. There are few reports in the literature concerning lipase assay detected using titration method in fungi isolated from the skin[6, 14.] *Malassezia globosa* has high lipase activity and due to an inability to metabolize its own lipids, it relies on the lipids present in the medium[2]. Lipase activity of *Malassezia* furfur was detected with alpha-naphthyl palmitate as a substrate. The enzyme activity was strongly activated by a lipase activator, sodium taurocholate (STC) and induced hyphae production. These results suggest that *Malassezia* lipase plays an important role in cell growth[14].In our research; we used 400mg/L concentration of *Zingiber officinale* oil and 100mg/L concentration of Cympogon citratus oil for lipase activity inhibition study. At the end of titration, 7µmol of fatty acid/ml of reaction mixture was liberated by *Zingiber officinale* sample and 5 µmol fatty acid/ml of reaction mixture was released by *Cymbopogon citratus* while14 µmol of fatty acid/ml of reaction mixture was released in control. An antifungal potential of essential oils on human pathogenic fungi Aspergillus fumigates and A. niger was determined. 100 µg/ml concentration of *Cymbopogon citratus* oil and 100 µg/ml concentration of *Zingiber officinale* oil were taken for inhibition studies. MIC results had shown that *Cymbopogon citratus* inhibited both the fungi at 0.25%(v/v) and *Zingiber officinale* inhibited at 2%(v/v)[15].In another study, an anti-*Malassezia*l activity of lemongrass oil and citral standard on M. furfur

by broth dilution assay was observed. The inhibitory and fungicidal effects of lemongrass oil occurred at the same concentration (6.25 µg/ml) [16]. While both the above mentioned articles merely observed that the inhibitory effect of essential oils on occurred dermatophytes growth, the reason behind the same was not explored.However, we have observed in the process of preparing this paper that the reason for growth inhibition of *Malassezia globosa* has been due to extracellular lipase inhibition by *Cymbopogon citratus* and Zingiber officinal oils.

CONCLUSION

Essential oils are widely used in folk medicine and cosmetic industry, but only in recent years they have been recognized as a potential antimicrobial agent. *Cymbopogon citratus* and *Zingiber officinale* oils had shown potent fungicidal activity at lower concentrations; hence it can be used on scalp for treating Pityriasis capitis, as there will be less chance of inducing any kind of inflammation or other harmful effects to skin.

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