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RESEARCH ARTICLE

EFFECT OF GERMINATED SEEDS OF FENUGREEK ON *Malassezia furfur* FROM HAIR DANDRUFF

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ABSTRACT

In the present study we compared the antifungal activity of different concentrations of fenugreek germinated seeds extract to check the right concentration effective against dandruff. Two methods namely Disk diffusion method, Pour Plating and Colony Counting method were used. Our study demonstrated that the extract 0.035g/ml (1ml of extract and 3 ml of water (1:4)) was found to be more effective in declining the growth of dandruff causing fungus *Malassezia furfur*. Concluding that, the use of fenugreek extract was functional in inhibiting the growth of microorganisms.

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INTRODUCTION

Pityriasis simplex capillitii (also known as "Dandruff") is the shedding of dead skin cells from the scalp (Ro and Dawson, 2005; Dixit *et al.*, 2005). Dandruff is sometimes caused by frequent exposure to extreme heat and cold. Dandruff is a common scalp problem in which you experience scalp itch and excessive flaking of the scalp skin. Although it is not a serious problem, it can remain for a long time and can make you feel socially uncomfortable and embarrassed. Fortunately the problem is not transmitted from person to person and can be controlled. As it is normal for skin cells to die and flake off, a small amount of flaking is normal and common. Some people, however, either chronically or as a result of certain triggers, experience an unusually large amount of flaking, which can also be accompanied by redness and irritation. If you are suffering from a mild bout of dandruff shampooing everyday with a soft shampoo can provide relief. In more severe cases, medicated shampoo can be of immense help. Symptoms of dandruff usually manifest as sprinklings of white, oily looking flakes of dead skin on your hair and shoulders accompanied by itching and flaking of the scalp. However, certain factors can worsen symptoms and can cause excessive skin scaling. They include: Certain weather conditions can make your skin dry, such as during winter when the air is very cold. Dry skin can lead to itchy flaky scalp (Elewski, 2005). Common older literature cites the fungus *Malassezia furfur* (previously known as *Pityrosporum ovale*) as the cause of dandruff. The organism has complex lipid requirements for growth, which also explains its occurrence on the skin. *Malassezia furfur* (*Pityrosporum ovale*), a lipophilic fungus, affects the hair and causes diseases called dandruff and also cause *Pityriasis*

versicolor, *Tinea circinata*, *Seborrheic dermatitis* (Elewski, 2005; Gupta *et al.*, 2004). While this fungus is found naturally on the skin surface of both healthy people and those with dandruff, it was later discovered that a scalp specific fungus, *Malassezia globosa*, is the responsible agent. This fungus metabolizes triglycerides present in sebum by the expression of lipase, resulting in a lipid by product oleic acid (OA). Penetration by OA of the top layer of the epidermis, the stratum corneum, results in an inflammatory response in susceptible persons which disturbs homeostasis and results in erratic cleavage of stratum (Gupta *et al.*, 2004). Simply increasing usage with normal shampooing will remove flakes. However, elimination of the fungus results in dramatic improvement. Regular shampooing with an anti-fungal product can reduce recurrence. Pyrithione zinc is highly effect against *Malassezia* species found on scalp. Reduction in the number of fungi reduces free fatty acid metabolism, in turn reducing scalp flaking and itch (Gupta *et al.*, 2004). Treatment can also be done by using natural plant seeds extracts or by any other natural and herbal method which reduces the chances of side effects to a large extend. This is the basic aim of our experiment that is to use a natural method to treat dandruff. Keeping this in mind we performed our experiment for checking out the antifungal activity of fenugreek seeds on dandruff (Krishnamurthy and Ranganathan, 2000; Peter *et al.*, 1968).

MATERIALS AND METHODS

Dandruff flakes were collected from various sources. Fenugreek seeds were purchased from local market of Greater Noida. 100 gm of Fenugreek seeds were washed and soaked in a wet muslin cloth. They were kept for 2 days for germination.

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After germination 10 gm seeds were taken and were crushed with 70 ml of distilled water in mortar and pestle. After complete crushing the mixture was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected.

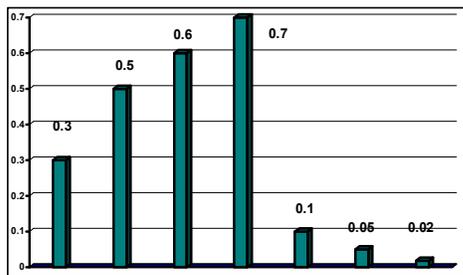
Potato Dextrose Agar (PDA) medium was used for growing fungus. PDA was prepared with addition of Butter. Dandruff was dissolved in Potato dextrose broth for its further use as inoculum. The inoculum obtained was serially diluted to 10^{-6} and 10^{-7} dilutions and 100 μ l of inoculum was inoculated onto the PDA plate which was spread using a L-Shaped spreader. The Petri plates were then sealed using a parafilm and incubated at 30 degree Celsius for 48 hours to get plates with uniform growth of fungus. Various dilutions of the pure Fenugreek extract with distilled water were prepared to check for the most effective dilution. 7 different dilutions were used (Pure Extract, 1:2, 1:4, 1:6, 1:8 and 1:10). Disk diffusion method was used to check the zone of inhibition for all the dilutions of extract.

RESULTS

After 48 hours of incubation of Petri plates, zone of inhibition was measured (Table 1). Different dilutions of Fenugreek extract gave different of zones of inhibition.

Table 1. Zone of inhibition with different dilutions of Methyl extract

| Conc. Of extract (gm/ml) | Zone of inhibition (cm) |
|--------------------------|--------------------------|
| Pure | 0.05 |
| Control | 0 |
| 0.07 | 0.1 |
| 0.035 | 0.7 |
| 0.02 | 0.6 |
| 0.0175 | 0.5 |
| 0.014 | 0.3 |



Y-axis (Zone of inhibition) : 1 unit = 0.1cm
X-axis (Conc. of Fenugreek seeds extract): 1 unit = 0.014 g/ml

Graph showing radius of zone of inhibition for different dilutions of fenugreek seeds extract

The use of fenugreek extract was functional in inhibiting the growth of microorganisms. On analyzing the zone of inhibition it was observed that extract concentration of 0.035g/ml was most effective because maximum zone was observed for it.

DISCUSSION

If the use of fenugreek extract is commercialized then its healthy benefits can easily be accomplished in a better way. This would help people to get rid of the various side effects of the chemical based shampoos along with the problem of dandruff. If we are able to do so then the process will become economical in the long run and the extra cost for chemical shampoos may be cut down. The use of fenugreek extract was functional in inhibiting the growth of microorganisms. In the control sample there was substantial increase in the number of colonies which means that without extract growth increases with time. But when extract was added number of colonies was gradually decreased and the difference was also seen in the two dilutions. The decline in growth was observed as decline in number of colonies. Different extract concentrations worked differently and the one with 1ml of extract and 3 ml of water (1:4) and also with 1ml of extract and 7 ml of water (1:8) were found to be more effective. On analyzing the observations for both colonies and zone of inhibition it was observed that extract concentration of 1:4 was most effective because minimum colonies and maximum zone was observed for it. The CFU/ml was minimum by 1:4 concentration of methyl extract. It is concluded that out of six concentrations tested 1:4 and 1:8 are much effective against the rest. But the minimum inhibitory concentration (MIC) of extract was concluded as 1:4.

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