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Isolation of *Malassezia* Species from dandruff and its control by selected plant extracts

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ABSTRACT:
Dandruff - the shedding of dead skin cells – is a common scalp disorder affecting almost half of the population at the post pubertal age of any sex and ethnicity which often causes itching. Malasseziae is a lipophilic yeast like fungi comes under Basidiomycota usually cause dandruff. It is naturally found on skin surfaces of many animals including humans. Samples were taken from the scalp of students are inoculated on to SDA medium and incubated at 37 ^{o} C for 48-72 hrs. The colonies were identified by morphological studies and various biochemical tests as Malasseziae spp. The selected isolates were tested against different traditionally available plant extract (aqueous).All plant extract shows good activity against Malasseziae spp with Alovera as best maximum.
KEY WORDS*: Dandruff, Malasseziae, SDA*

I. INTRODUCTION

Skin is mechanically protective layers as well as cosmetically significant anatomical structure. The superficial cutaneous fungal infections involve its outer most covering including appendages like hair and nails. *Malazessiae* lipophilic yeast -like fungus inhabit in stratum corneum of human skin and cause dandruff in humans and animals. Dandruff is the shedding of dead skin cells from the scalp which is the common scalp disorder affecting almost half of the population at the post pubertal age and of any sex and ethnicity. The severity of dandruff may fluctuate with season and often worsen in winter. It often causes itching, and can cause social or self-esteem problems, indicating the treatment for both physiological and psychological reasons. Usually, all skin cells die and are replaced by new cells. For people with dandruff skin cells may mature and be shedding 2-7 days, as opposed to around a month in people without dandruff. The result is that dead skin cells are shed in large, oily clumps, which appears as white or grayish patches on the scalp, skin and cloths. *Malassezia* is a lipophilic yeast. It is naturally found on the skin surfaces of many animals, including humans. The investigations shows that the *Malassezia* species causing most skin disease in humans, including most common cause of dandruff and sebarrhoic dermatitis, in human can be up to 10 million. The main objective of this study is to isolate dandruff causing microorganisms from scalp or hair and its control by selected traditionally available plant extract.

II. MATERIALS AND METHODS

Collection of samples & Isolation of fungus

Samples were taken from the scalp of different students by using swabbing. While taking samples care should be taken that the patient should not take bath and the hair should be dried. The medium used for the study was sabouraud's dextrose agar (SDA). Before inoculation the swabs were dipped in SDB medium for enrichment and then streaked on to SDA. The plates were incubated at 37 ^{o} C for 48-72 hrs. After incubation the colonies obtained were stained for differentiation of organism.

Identification of selected fungal isolates

Different morphological and biochemical tests were done to identify the strain. The morphological studies and microscopic observations were done by simple staining reaction. The fungal isolates were biochemically characterized by various biochemical studies like Urease test, Catalase test, Sugar fermentation etc.

Anti-fungal activity of the plant extract

To demonstrate the antifungal activity traditionally available plants like Neem (*Azardirachta indica*), Alovera (*Aloe vera),* Henna (*Lawsonia inermis)* curryleaf (*Murraya koenigii*) selected and aqueous extracts were prepared by cold extraction method. Antifungal activity of the extract was tested by agar well diffusion method on SDA media. The crude extract was added into the well aseptically. The plates Incubated in upright position for 24 hours. The diameter of clear zones was measured.

III. RESULTS AND DISCUSSION

Different types of colonies were developed in the SDA agar plate. The morphology of colonies was noted (Table 1). The biochemical characters of isolates were recorded (Table 2,3) and antifungal activity test of different plant extract showed considerable effect (Table 4).

Samples	Colony characters	Inference
S1	Small, off-white, round, opaque, raised	Oval
S2	Large, white, round, opaque, flat	Round
S3	Small, offwhite, round, opaque, raised	Oval Budding
S4	small off-white, round, opaque, raised	Oval
S5	Large, white, round, opaque, convex	Oval Budding
S6	Large, white, round, opaque, flat	Round
S7	Small, off-white, round, opaque, raised	Oval
S8	off-white, round, opaque, raised	Oval
S9	off-white, round, opaque, flat	Oval Budding
S10	Small, off-white, round, opaque, raised	Round
S11	Small, off-white, round, opaque, raised	Oval Budding
S12	small, off-white, round, opaque, raised	Oval
S13	Large, white, round, opaque, flat	Oval
S14	Slight, off-white, round, opaque, raised	Oval Budding
S15	Slight, off-white, round, opaque, convex	Oval Budding
S16	Small, off-white, round, opaque, raised	Oval
S17	small, white, round, opaque, flat	Round
S18	Slight, off-white, round, opaque, raised	Oval

Table1: Morphological characteristics of fungal isolates

Biochemical characteristics of fungal isolates

Samples	Result
S1	+
S2	Negative
S3	+
S4	Negative
S5	+
S6	+
S7	Negative
S8	+
S9	+
S10	+
S11	+
S12	+
S13	Negative
S14	+
S 15	+
S16	Negative
S17	+
S18	Negative

Table 2: Urease test

Samples	Catalase test	Sugar fermentation			
		Glucose	Lactose	Sucrose	Mannitol
S1	+	+	-	+	-
S3	+	+	-	+	-
S5	+	+	-	+	-
S6	+	+	-	+	-
S8	+	+	-	+	-
S9	+	+	-	+	-
S10	+	+	-	+	-
S11	+	+	-	+	-
S12	+	+	-	+	-
S14	+	+	-	+	-
S15	+	+	-	+	-
S17	+	+	-	+	-

Table 3: Catalase and sugar fermentation tests

Crude extract of plants	Zone of inhibition (cm)
Neem (*Azardirachta indica*)	1.7
Alovera (*Aloe vera*)	1.9
Henna (*Lawsonia inermis*)	1.5
Curry leaf (*Murraya koenigii*)	1.3

Table 4: Antifungal activity of different plant extracts

Twenty-five samples were taken from hairs of different students using swab which is dipped saline and inoculated to SDA media and incubated for 48 hours at 37^0c. After proper incubation, different types of colonies developed on the SDA plates were observed. Based on the morphological and staining reactions fungal isolates were observed as oval, round and budding Yeast like cells. Then it were subjected to various biochemical tests. From the isolated 18 samples of yeast 12 were urease positive. Since all the *Malassezia* species are urease positive the samples which showed urease positive were selected and stored for further biochemical reactions such as catalase and sugar fermentation tests. From these reactions, the organisms obtained from the scalp were confirmed as *Malassezia*. The traditionally available different plant extracts were tested as remedy against these organisms. Among these, *Aloe vera* showed maximum antifungal activity while curry leaf *Murraya koenigii* with least.

IV. CONCLUSION

In addition to traditional methods of treatment, several commercially available products are also recommended for dandruff self-care, which include often usage of shampoo, cut back on styling products, hair gells etc. But as these products contain chemical components, it may cause side effects like hair fall, inflammation of hair follicle, skin irritation etc. These commercial products are expensive than traditional plant extracts. So, in all aspects traditional remedies are better when compared to other commercial products.

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