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Antifungal Properties of Plant Extracts on Dandruff Causing Fungi *Malassezia furfur*

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Abstract: *Dandruff is a common scalp disorder that affects millions of people of the world. It is caused by the excessive growth of a fungus called Malassezia furfur, which feed on the sebum and dead skin cells of the scalp. Dandruff can cause itching, flaking, and inflammation of the scalp, and can also affect the quality of life and self-esteem of the sufferers. Many commercial anti-dandruff shampoos are available in the market, but they may have side effects such as dryness, irritation, or allergic reactions. Therefore, there is a need to find natural and safe alternatives for the treatment and prevention of dandruff.*

In this study, the anti-dandruff activity of seven plant extracts were tested. Coriander (Coriandrum sativum),fenugreek (Trigonella foenum-graecum), ginger (Zingiber officinale), cinnamon (Cinnamomum verum), amla (Emblica officinalis Gaertn), sweet potato (Ipomoea batatas) and extract of coconut.These are widely used in traditional medicine for various skin diseases and have anti-inflammatory, anti-microbial, and anti-oxidant properties. Alcoholic extracts of the plant parts were taken using methanol as a solvent. And then the extracts were tested against Malassezia furfur using the agar well diffusion method and disc diffusion method.

Results showed that all plant extracts had significant anti-dandruff activity against Malassezia furfur.

Keywords: *Malassezia furfur, zone of inhibition.*

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Introduction:

Dandruff is a common scalp disorder that affects millions of people over the world. There are 14 species of *Malassezia* yeast that infect humans and animals.

These are some lipophilic fungi of *Malassezia* species which are the causative organism for dandruff which can grow more in sebum and is part of skin flora of warm-blooded animals (Parihar et al., 2019).

It is caused by the overgrowth of a fungus called *Malassezia furfur*, which feeds on the sebum and dead skin cells of the scalp. Dandruff can cause itching and inflammation of the scalp and can also affect the quality of life and self-esteem of the sufferers. Many commercial

anti-dandruff shampoos are now available in the market, but they may have few side effects such as dryness, irritation, or allergic reactions. Therefore, there is a need to find natural and safe alternatives for the treatment and prevention of dandruff.

One of the promising approaches to treat dandruff is to use plants and herbal formulations that possess anti-dandruff properties. Plants have been used for centuries in traditional medicine for various skin diseases and have anti-inflammatory, anti-microbial, and antioxidant properties. Studies have shown that some plant extracts can inhibit the growth of *Malassezia furfur* and reduce the symptoms of dandruff.

Materials and Methods:

Plant sample collection: The different plant parts like fruit of amla *(Emblica officinalis Gaertn),* rhizome of ginger *(Zingiber officinale),* root of sweet potato *(Ipomoea batatas),* seed of coriander *(Coriandrum sativum),* seed of fenugreek *(Trigonella foenum-graecum*), stem of cinnamon *(Cinnamomum verum)* were collected from the local market.

Malassezia Sample collection: Dandruff was collected from affected scalp of the individuals. Flakes or scales were collected from the hair with a sterile comb and scrapping approximately one inch area using blunt scalpel.

Preparation of plant extracts: The different plant parts like fruit of amla *(Emblica officinalis Gaertn),* rhizome of ginger *(Zingiber officinale),* root of sweet potato *(Ipomoea batatas),* seed of coriander *(Coriandrum sativum),* seed of fenugreek *(Trigonella foenum-graecum*) and stem of cinnamon *(Cinnamomum verum)* were collected from the local market. All plant parts were cleaned with distilled water and dried under hot air oven for 15min. Then they were ground with the help of mortar and pestle. Some distilled water was added to dilute the mixture and was filtered to remove solid plant residues. The filtrate was centrifuged to obtain supernatant (Pingili et al., 2016).

Preparation of culture media inoculation and incubation: The culture media used was Sabouraud Dextrose Agar (SDA) which was prepared according to manufacturer's specification and sterilized in an autoclave machine at 121°C at 15 psi pressure for nearly 15mins (Obasi et al.,2018).

The collected samples were cultured on SDA which was incorporated with chloramphenicol to discourage the growth of bacterial contaminants. Small amounts of the samples collected were introduced into petri dishes containing the media using sterile forceps. The petri dishes were labelled correctly in a particular order and were incubated at 30°C for nearly 7days (Santhi et al.,2022).

Identification of fungi

Direct microscopy: A drop of 10% KOH was added onto a clean slide which contained the smear of sample and covered using a coverslip. The sample was then heated over a burner and then the slide was observed under 40X objective lens (Mistry and Gaurav, 2016).

Biochemical Tests :

Catalase Test: Catalase test was carried out to confirm the presence of *Malassezia* species as it is catalase positive. A pure culture of the fungi was selected, and a small amount of the fungal growth was transferred to a clean, dry slide or glass surface. By using a dropper or micropipette, a small amount of 3% hydrogen peroxide was added directly into the fungal growth on the slide. The immediate production of bubbles on the fungal growth was observed. Bubbling indicates the presence of catalase, as the enzyme breaks down hydrogen peroxide into water and oxygen (Kindo et al., 2004).

Urease test: Some strains of *Malassezia furfur* may be urease-positive, causing a change in the colour of the medium due to the breakdown of urea.

Antifungal activity of plant extracts: The antifungal activity of different extracts on *Malassezia furfur* was investigated in which the agar was taken into a petri dish and allowed to cool for some time then the media was coated with a drop of olive oil and then the organism was spread uniformly over the agar surface. Wells were punched aseptically with cork borer at equal distance of 3 cm. In each of these wells 50 microlitres of extracted solutions were placed carefully. The plates were allowed to diffuse for 30 minutes and incubated at 37˚C for nearly 72 hrs (Pingili et al., 2016). In all cases

zones of inhibition can be observed, the diameter of the zones giving a brief comparison of activities of different anti-microbial substances. After incubation zone of inhibitions were measured using a meter rule.

Determination of zone of inhibition: Zone of inhibition was observed and recorded in millimeters.

Results and Discussion:

At first, the fungi were observed under microscope. After that catalase test was done and gas bubbles were observed in the fungal colonies which gave positive catalase test. Urease test was done to confirm *Malassezia furfur*. Bright pink colour was seen which gave positive urease test in **Fig. 1.**

Fig.1. Bright pink color showing positive Urease test

Result of cup-plate method to determine zone of inhibition: After the incubation of 24 hours, the results of the antifungal properties of the plant extracts against *Malassezia* was observed as the zone of inhibition (in mm). It was found that the plant extracts of *Emblica officinalis Gaertn* (Amla) have maximum antifungal property against *Malassezia*. The plant extracts of *Ipomoea batatas* (sweet potato), *Coriandrum sativum* (coriander) and extract of coconut showed lesser zone of inhibition. The plant extracts of *Trigonella foenum-graecum* (Fenugreek), *Zingiber officinale* (ginger) and *Cinnamomum verum* (Cinnamon) showed minimum antifungal property against *Malassezia* (Fig. 2A and 2B). The zone of inhibition of different plant extracts are given in tabulated form in Table 1.

Result of disc diffusion method to determine zone of inhibition: In this method it was observed that *Emblica officinalis Gaertn* (Amla), extract of coconut and *Trigonella foenum- graecum* (Fenugreek) showed maximum antifungal property as compared to other tested plant extracts (Fig. 3A and 3B). The zone of inhibition of plant extracts through disc diffusion method is shown in tabulated form in Table 2.

Table 1. Zone of inhibition of plant extracts (cup plate method)

S. No.	Name of plant extracts	Zone of inhibition (diameter in mm)
1.	*Emblica officinalis Gaertn* (Amla)	13
2.	*Ipomoea batatas* (Sweet potato)	9
3.	*Coriandrum sativum* (Coriander)	8
4.	Coconut oil	8
5.	*Trigonella foenum graecum* (Fenugreek)	7
6.	*Zingiber officinale* (Ginger)	7
7.	*Cinnamomum verum* (Cinnamon)	7

Table 2. Zone of inhibition of plant extracts (disc diffusion method)

S. No.	Name of plant extracts	Zone of inhibition (diameter in mm)
1.	*Emblica officinalis Gaertn* (Amla)	8
2.	*Coriandrum sativum* (Coriander)	8
3.	Coconut oil	8
4.	*Ipomoea batatas* (Sweet potato)	7
5.	*Cinnamomum verum* (Cinnamon)	7
6.	*Zingiber officinale* (Ginger)	7
7.	*Trigonella foenum graecum* (Fenugreek)	6

Fig. 2A. Zone of inhibition of plant extracts

1. **Ipomoea batatas (Sweet potato)**
2. **Trigonella foenum-graecum (Fenugreek)**
3. **Coriandrum sativum (Coriander)**
4. **Cinnamomum verum (Cinnamon)**

Fig.2B. 5 – Control 6 – *Emblica officinalis Gaertn* (Amla) 7 – Coconut oil 8 – *Zingiber officinale* (ginger)

Fig.3A. Zone of inhibition of plant extracts

1. **I-*Ipomoea batatas* (Sweet potato)**
2. ***Trigonella foenum graecum* (Fenugreek)**
3. ***Coriandrum sativum* (Coriander)**
4. ***Cinnamomum verum* (Cinnamon)**

Fig. 3.B. 5 – Control 6 – *Emblica officinalis Gaertn* (Amla) 7 – Coconut oil 8 – *Zingiber officinale* (Ginger)

Dandruff is a common type of disease which is caused by *Malassezia* species one of the most common types is of *Malassezia furfur*. *Malassezia furfur* is a pleomorphic yeast like fungus (Parihar et al., 2019). Its lipolytic activity induces hydrolysis of human sebum triglycerides into free fatty acids that cause both hair loss and scalp (Naga Padma et al., 2015). Different media were taken to examine the growth of *Malassezia* as it is a difficult fungus to culture. The chances of getting infection were very high so a sterile condition was maintained throughout the process. The plant extracts were selected on the basis of their availability in the local markets. The antifungal effect of plant extracts was tested against *Malassezia* using the cup-plate and disc diffusion method. Antifungal activities of different plant extracts such as amla, henna, fenugreek and aloe Vera against *Malassezia* have shown similar results and support our studies. In one of the studies *Emblica officinalis Gaertn* (Amla) fruit extract was tested which showed antifungal activity, as they rapidly inhibited the growth of *Malassezia furfur* on SDA medium (Vijayakumar et al.,2006). Out of the selected plant extracts, surprisingly, *Emblica officinalis Gaertn* (Amla), *Ipomoea batatas* (Sweet potato), *Trigonella foenum-graecum* (Fenugreek) and *Coriandrum sativum* (Coriander) showed amazing antifungal activities against *Malassezia*. This study was required because not all plant extracts were tested against *Malassezia* and

there is always a need for new discoveries which can be helpful for controlling dandruff.

Conclusions:

The observed antifungal efficiency of plant extracts against *Malassezia furfur* suggests a great potential for combining botanical compounds into antifungal strategies. These extracts, rich in bioactive compounds, exhibit an approach in inhibiting growth. Further, the natural origin of these extracts used along with the growing preference for sustainable and eco-friendly alternatives in the medicine field. By focusing on the power of the antifungal agents not only addresses the need for effective treatments but also contributes to the ongoing exploration of environmental issues and their solutions.

The findings underscore the potential of plant extracts as a valuable resource in the development of great antifungal therapies, offering a great opportunity for future investigations and applications in health Sector.

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