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Antifungal Properties of Fruit and Vegetable Seeds against *Malassezia*

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Abstract: *Malassezia is a common dandruff-causing fungus which is the reason behind daily life problems and embarrassment in the social scenario. In this study, Malassezia from the scalp of 10 individuals was isolated and its presence was confirmed through various biochemical and morphological tests. It was followed by selection and preparation of different seed extracts of local fruits and vegetables to check the growth of Malassezia by observing the zone of inhibition. The seed extracts of Coccinia (Ivy gourd), Citrullus (Watermelon) and, Momordica (Bitter gourd) exhibited great anti-fungal activity against Malassezia. The purpose of this research was to understand that natural sources can be preferred over chemical alternatives as they are safer and affordable. The capabilities of the various seed extracts were analysed which can be of potential use as a future organic potion for the control of Malassezia.*

Keywords: *Malassezia, seed extracts, Zone of inhibition.*

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Introduction:

Malassezia, the genus which presently consists of 17 species and in the recent past, has been assigned its class, Malasseziomycetes. They are Basidiomycetous yeasts that are lipophilic in nature and mostly inhabit humans and other mammals, especially on the skin and scalp causing disorders like dandruff (Grice and Dawson, 2017), (Theelen et al., 2018). Presently, available options to curb the problem of dandruff comprises antidandruff shampoos, serums, and conditioners which are loaded with chemicals like Salicylic acid, Sulphur, Zinc pyrithione, and Selenium sulphide but, these have limited implications due to reduced efficiency or side effects (Chandrani et al., 2012). Fungal Pathogens like *Malassezia* can be more

efficiently controlled with the support of novel natural antifungal compounds, specifically those derived from seeds and stems of plants. (Marin et al., 2011). This study has been intended to screen seed extracts of *Phyllanthus* (Indian gooseberry), *Ocimum* (holy basil), *Citrullus* (watermelon), *Citrus* (orange), *Citrus* (lemon), *Momordica* (bitter gourd), *Trichosanthes* (pointed gourd), *Coccinia* (Ivy gourd), *Abelmoschus* (okra) and *Vigna* (Broad Bean) to analyse their potential against *Malassezia.* These seeds have been chosen considering their availability in the local market and a potential source of anti-fungal properties.

Method:

Seed sample collection : The seeds of *Phyllanthus* (Indian gooseberry), *Ocimum* (holy basil), *Citrullus* (watermelon), *Citrus* (orange), *Citrus* (lemon), *Momordica* (bitter gourd), *Trichosanthes* (pointed gourd), *Coccinia*(Ivy groud), *Abelmoschus* (okra) and *Vigna* (Broad Bean) were collected from the local vegetable market and seed store.

***Malassezia* Sample Collection**: A total of ten samples were collected from the students of Patna Women's College. The scalp was visually observed for locating infection sites and Dandruff flakes were collected with the help of a sterile toothpick and sterile cotton swab on ten separate glass slides in October 2021.

Preparation of Culture Media, inoculation, and incubation: Potato Dextrose media, Sabouraud Dextrose Agar media and Modified Sabouraud Dextrose Agar (SDA) media with olive oil along with antibiotic (Chloramphenicol) were prepared. The dandruff samples collected were streaked with a cotton swab and incubated at 30° C for 48-72 hours. Modified SDA media was found to be the most suitable for the culture of *Malassezia*.

Identification of isolated colonies:

(a) Colony characterization: White to cream-colored small colonies with a shiny smooth to a slightly wrinkled surface were selected for further identification.

(b) Morphological identification: Cotton Lactophenol blue staining was done to perform microscopic examination for all the selected isolates to observe the oval or bottle-shaped microorganisms as shown in (Fig. 1).

(c) Catalase test: A drop of 6% hydrogen peroxide was placed on a glass slide and the sample was transferred to it by a sterile loop and mixed well. The production of gas bubbles indicates a positive catalase reaction and its absence indicates negative catalase test (Fig. 2) (Kindo et al., 2004).

(d) Urease test: Urea agar slant was prepared and a loopful cell from the culture was streaked and incubated at 32° for 24 hours. Urease test with bright pink to violet color is considered positive and no change in color is a negative test (Fig. 3) (Killermann and Boekhout, 2010).

Sub-culture of *Malassezia* : The colonies tested positive were sub-cultured on modified SDA media to obtain pure cultures and then further inoculated into SDA broth.

Preparation of seed extracts : The seeds were washed with distilled water and dried in hot air oven for an hour. It was then triturated individually using a motor and pestle. One gram of each seed sample was mixed in 5ml of water. Samples were filtered and centrifuged at 3000 rpm for 10 minutes and the supernatant was used. The seed extracts were then filtered using a syringe membrane filter.

Antifungal activity of seed extracts : The Cup plate method was used to investigate the antifungal activity of individual seed extracts on *Malassezia.* SDA media with antibiotic was coated with a drop of olive oil and then the inoculum from the SDA broth was spread uniformly over the agar surface. Bores of 5mm in diameter were punched aseptically round the margin of the plate, equidistantly (approximately 3cm apart). In each of these wells, 75 microliters of individual and combination of seed extract were placed carefully. The plates were incubated at 30° C for 48 hours (Pingili et al., 2016).

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

Result and Discussion:

Morphological examination showed oval or bottle-shaped microorganisms under microscope as shown in Fig. 1. Further, catalase test was done and production of gas bubbles was observed in the selected colonies indicating positive catalase test as shown in Fig. 2. Urease test was performed to confirm *Malassezia*. Bright pink colour was seen indicating positive urease test (Fig. 3).

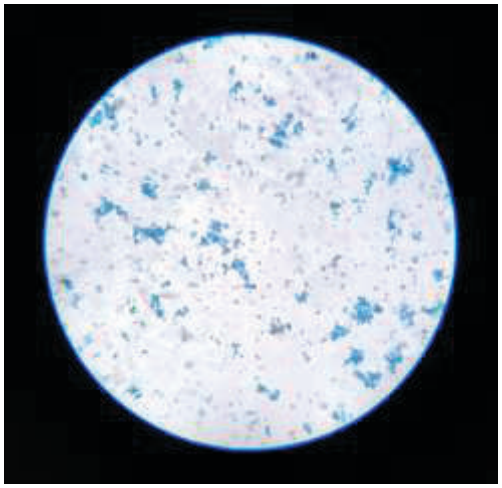

Fig. 1. *Malassezia* under a microscope

Fig. 2. Appearance of bubbles showing positive Catalase test

Fig. 3. Bright pink colour exhibiting positive Urease test

Result of cup-plate method to determine zone of inhibition: After the incubation of 48 hours, the results of the antifungal properties of the seed extracts against *Malassezia* was observed as the zone of inhibition (in mm).It was found that the seeds of *Phyllanthus* (Indian gooseberry) and *Coccinia* (Ivy gourd) have maximum antifungal property against *Malassezia* among the other tested seed extracts. The seeds of *Citrullus* (Watermelon), *Momordica* (Bitter gourd), *Citrus* (Orange), *Citrus* (Lemon), *Trichosanthes* (Pointed gourd), and *Vigna* (Broad Bean) also showed great antifungal property against *Malassezia.* The seeds of *Ocimum* (Holy basil) and *Abelmoschus* (Okra) showed considerably lesser zone of inhibition and thus indicated minimum antifungal property against *Malassezia* (Fig. 4). The Antifungal activity of the

individual seed extracts against *Malassezia* is given in a tabulated form in Table 1.

The combination of seed extracts of *Citrus* (orange) and *Coccinia* (Ivy gourd); and *Momordica* (bitter gourd) and *Abelmoschus* (okra) showed an increase in antifungal property against *Malassezia*. When the seed extracts of *Citrullus* (watermelon) and *Phyllanthus* (Indian gooseberry); *Citrus* (lemon) and *Citrullus* (watermelon); and *Trichosanthes* (pointed gourd) and *Vigna* (Broad Bean) were tested in combination a decrease in the zone of inhibition was observed (Fig. 5) (Table 2).

Table 1. Zone of inhibition of seed extracts

Sl. No.	Name of Seed Extract	Zone of Inhibition (Diameter in mm)
1.	*Coccinia* (Ivy gourd)	20
2.	*Phyllanthus* (Indian gooseberry)	19
3.	*Citrullus* (Watermelon)	15
4.	*Momordica* (Bitter gourd)	14
5.	*Citrus* (Orange)	13
6.	*Citrus* (Lemon)	13
7.	*Trichosanthes* (Pointed gourd)	13
8.	*Vigna* (Broad Bean)	12
9.	*Ocimum* (Holy basil)	8
10.	*Abelmoschus* (Okra)	7

Table 2. Zone of inhibition of combination of seed extracts

Sl. No.	Combination of Seed Extracts	Zone of Inhibition (Diameter in mm)
1.	*Citrus* (orange) + *Coccinia*(Ivy gourd)	25
2.	*Momordica* (bitter gourd) + *Abelmoschus* (okra)	17
3.	*Citrus* (lemon) + *Citrullus* (watermelon)	13
4.	*Trichosanthes* (pointed gourd) + *Vigna* (Broad Bean)	12
5.	*Citrullus*(watermelon) + *Phyllanthus* (Indian gooseberry)	12

Fig. 4. Zone of inhibition of individual seed extracts (A) 1-Phyllanthus (Indian gooseberry) 2-Ocimum (holy basil) 3-Citrullus (watermelon) 4-Citrus (orange) 5-Citrus (lemon)

(B) 6-Momordica (bitter gourd) 7-Trichosanthes (pointed gourd) 8-Coccinia (Ivy groud) 9-Abelmoschus (okra) 10-Vigna (Broad Bean)

Fig. 5. Zone of inhibition of combination seed extracts. 4+8-Citrus (Orange)+Coccinia(Ivy gourd); 6+9-Momordica (Bitter gourd) + Abelmoschus (Okra); 7+10-Trichosanthes (Pointed gourd) + Vigna (Broad Bean); 1+5-Phyllanthus(Indian gooseberry)+Citrus (Lemon); 1+3-Phyllanthus (Indian gooseberry)+Citrullus (Watermelon)

Different media were tested for the growth of *Malassezia* as it is a difficult fungus to culture and maintain. Modified SDA media was chosen as the best one (Begum et al., 2019). The chances of contamination were high so aseptic environment was maintained throughout the process. The seeds were selected on the basis of availability and usage in common households. Keeping in mind, the ease of utilising the seed extracts, crude preparation was opted which was tested against *Malassezia* using the cup-plate method. Antifungal properties of various plant extracts like lemon, Amla, shikakai, henna, aloevera, fenugreek, & reetha against *Malassezia* have shown similar results and support our studies. Antifungal properties of different combinations from these plant extracts and their synergistic activity against *Malassezia furfur* have also been reported earlier. Lemon juice & henna extract and lemon & amla combinations showed best antifungal activity against *Malassezia furfur* (Pingili et al., 2016). Out of the selected seed extracts, surprisingly, *Coccinia* (Ivy gourd or Kundri), *Citrullus* (Watermelon), *Trichosanthes* (Pointed gourd or Parwal) and *Momordica* (Bitter gourd or Karela), showed amazing antifungal properties against *Malassezia*. These vegetable seeds are also commonly available in the local market and can be comfortably used as an effective measure against dandruff. This study was needed because not all vegetable or fruit seeds have been tested against *Malassezia* and there is always a need for novel findings to control the problem of dandruff.

Conclusion:

Malassezia is the sole cause of many skin and hair-related disorders and people mostly rely on chemical methods for the purpose of treating dandruff but the current study is mainly for determining the potential of seed extracts so that they can be easily used as an effective alternative. In the study, seed extracts–*Coccinia* (Ivy gourd or Kundri), *Phyllanthus* (Indian gooseberry), *Citrullus* (Watermelon), and *Momordica* (Bitter gourd) have shown great anti-fungal properties against *Malassezia.* When tested in some random combination they showed a greater zone of inhibition in few of the combined extracts. A concoction of these seed extracts can be used as serum, oils, or elixirs in the future.

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