International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 550-553

Received: 01-07-2019
Accepted: 03-08-2019

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Characterization of *Malassezia furfur* and its control by using plant extracts

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Abstract
Malassezia furfur (formerly known as *Pityrosporum ovale* in its hyphal form) is a species of fungus that is naturally found on the skin surfaces of humans and is associated with seborrhoeic dermatitis. *Malassezia furfur*, a lipophilic, dimorphic and yeast-like fungus, occurring in human skin as an opportunistic pathogen, causes diseases such as dandruff, pityriasis versicolar, seborrheic dermatitis, etc. Suitable media for culturing the organism were standardized. A modified medium for the culturing of *M. furfur* has been proposed. Growth of the fungus was also determined in the presence of different carbon sources under the influence of different temperature, pH and salinity. Plant extracts of 19 species were screened against the growth of the fungus by using disc diffusion method and the results are discussed.

Keywords: Biochemical characters, growth factors, medicinal plants

Introduction
Malassezia (formerly known as *Pityrosporum*) is a genus of fungi. *Malassezia* is naturally found on the skin surfaces of many animals, including humans. In occasional opportunistic infections, some species can cause hypopigmentation or hyperpigmentation on the trunk and other locations in humans. Allergy tests for this fungus are available. *Malassezia furfur* (formerly known as *Pityrosporum ovale* in its hyphal form) is a species of fungus that is naturally found on the skin surfaces of humans and is associated with seborrhoeic dermatitis. As an opportunistic pathogen, it has further been associated with dandruff, pityriasis versicolor and tinea circinata as well as catheter-related fungemia and pneumonia in patients receiving hematopoietic transplants. The fungus can also affect other animals, including dogs. Mycotic infection of the skin by the dermatophytes may be categorized into superficial and deep fungal infections. *Malassezia furfur* (*Pityrosporum ovale*), a lipophilic fungus, affects the hair and causes diseases called dandruff [1] and also called pityriasis versicolar, tinea circinata, seborrheic dermatitis [2]. Dandruff is a condition, which causes small white flakes of skin that separate and fall from the scalp. People who suffer from dandruff have over active sebaceous glands, which make their scalp oily [3]. It has been investigated and reported that there was no complete cure for this disease. The influence of the plant extracts of 19 species on the growth of *M. furfur* has been investigated and reported.

Materials and Methods
Collection and maintenance the culture
Pure culture of *M. furfur* (MTCC: 1374) was obtained from Institute of Microbial Technology, Chandigarh, India. The culture was maintained in Emmon's modified medium[4] (dextrose 40 g, peptone 10 g and agar 18 g with corn oil 2 ml/litre).

Morphological characteristics
Culture was stained with methylene blue and examined under the high power objective of the microscope, and the characters were recorded.

Biochemical tests
The organism was biochemically analysed by using gelatin hydrolysis test, litmus milk reaction, carbohydrates *viz*., dextrose, xylose, rhamnose, raffinose and mannital fermentation tests also performed and the results were recorded.

Effect of fatty substances on the growth of *M. furfur*
Six different fatty substances namely, corn oil, butter, olive oil, coconut oil, oleic acid and castor oil were mixed (2 ml) with both liquid and solid media of Sabouraud's dextrose medium, and without fatty substance medium also maintained. Growth rate of *M. furfur* was recorded.

Screening of suitable media
Since the Emmon's modified medium did not show well developed growth of the organism, eight different media namely, Czapek's dox medium, corn meal medium, rose bengal medium, nutrient medium, potato dextrose medium, malt extract medium, Sabouraud's dextrose medium and Sabouraud's maltose medium both solid and liquid media were screened for determining the suitable medium.

Effect of temperature on the growth of *M. furfur*
One ml of the pure culture broth of *M. furfur* was inoculated into each tube containing sterilized liquid Sabouraud's dextrose medium and incubated at 10 ± 2, 20 ± 2, 30 ± 2 and 40 ± 2 ºC for 7 days.

Effect of pH on the growth of *M. furfur*
pH of liquid Sabouraud's dextrose medium was adjusted to 4.10 by using 1N NaOH and 1N Orthophosphoric acid. one ml of pure culture of *M. furfur* was inoculated into the tubes containing the liquid medium adjusted with different pH, and incubated 30 ± 2 ºC for 7 days.

Effect of salinity: Liquid Sabouraud's dextrose medium has the salinity 20 ppt. It was adjusted to 40, 60, 80 and 100 ppt by using Sodium Chloride (NaCl). Pure culture of the organism was inoculated into each tube and incubated at 30 2°C for 7 days.

Effect of carbon sources

a. **Peptone:** Peptone was added to the liquid Sabouraud's dextrose medium in the concentration of 0, 5, 10, 15 and 20 g/litre. Pure culture of *M. furfur* grown in liquid medium was inoculated and incubated at 30 ± 2 °C for 7 days.
b. **Dextrose:** Similarly dextrose was added to the liquid medium in the concentration of 0, 20, 40, 60 and 80 g/litre. Pure culture of the organism, grown in liquid medium, was inoculated and incubated at 30 ± 2 °C for 7 days. The growth of the organism was determined by using spectrophotometer (Turbidity method).

Effect of plant extracts on the growth of *M. furfur*
Nineteen plant spp. (Table 1) were collected from in and around Karur District of Tamil Nadu and for their antimycotic activity against *M. furfur*. The plant parts (Table 1) were washed thoroughly in tap water followed by sterile distilled water and ground by using mortar and pestle. The crude extract was filtered through a nylon cloth and centrifuged at 5,000 rpm for 10 minutes. The supernatant was collected and used for the assay of antimycotic activity. This extract was considered as 100% and it was diluted to 25, 50 and 75% with sterile distilled water [7].

Antimycotic assay (Disc diffusion method)
The broth culture of *M. furfur* was swabbed over the Sabouraud's dextrose agar by using sterile cotton buds. Sterile 5mm diameter Whatman no. 32 filter paper discs were dipped in plant extracts and Clotrimazole (reference antibiotic) were placed equidistantly (3 cm apart) round the margin of the plates. Three replicates were maintained. The plates were incubated at 30 ± 2 °C and the zone of inhibition was observed after 7 days. Control was maintained with filter paper discs dipped in distilled water.

Results
Malassezia furfur (Robin) Baillon was developed as white to tan cream in colour and smooth pasty yeast like appearance over the medium (Fig. 1a). Microscopically, the cells are bottle shaped (Fig. 1b).

Effect of fatty substances
Among the six fatty substances tested, *M. furfur* grew well in Sabouraud's dextrose broth and agar medium containing butter followed by corn oil, olive oil, coconut oil, oleic acid and castor oil (Table 1 and Fig. 2).

Screening of suitable media
Among the different media tested, *M. furfur*, grew well in Sabouraud's dextrose agar and its broth contained 2% of butter (Fig. 1a) followed by Sabouraud's maltose medium,

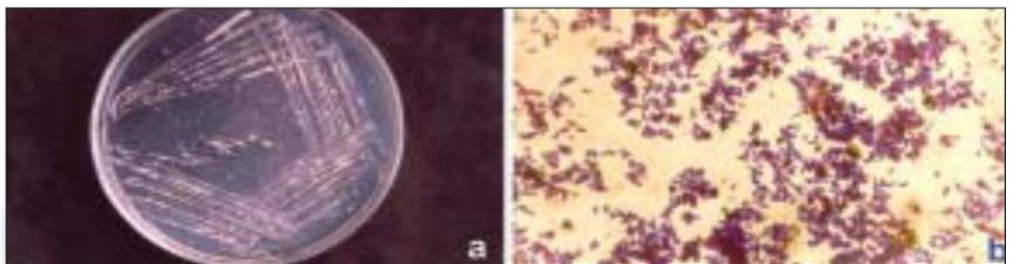

Fig 1: (a) Colony morphology and (b) Microscopic (450x) view of *Malassezia furfur*

Fig 2: Growth of *Malassezia furfur* on SDA medium with different fatty substances

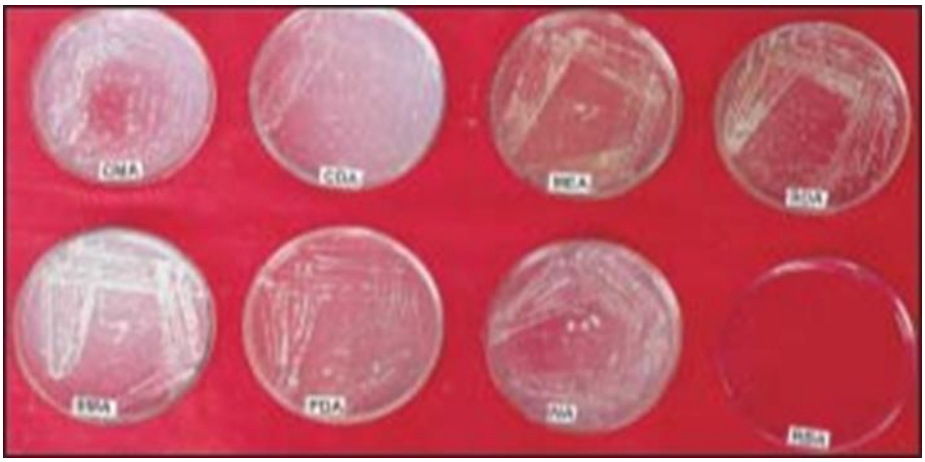

Fig 3: Growth of *Malassezia furfur* on different culturemedia with butter CMA - Corn meal agar; CDA - Czapek's dox agar; MEA -Malt extract agar. SDA - Sabouraud's dextrose agar; SMA -Sabouraud's maltose agar. PDA - Potato dextrose agar; NA -Nutrient agar and RBA - Rose bengal agar

Table 1: Antimycotic activity of some plant extracts against *Malassezia furfur*

S. No.	Plant name	Plant part	Concentration of extracts (%) and zone of inhibition (mm)				
			25	50		75	100
1.	*Acacia concinna* Lam.	Seed	-	-		-	-
2.	*Acalypha indica* L.	Leaf	-	-		-	-
3.	*Adhatoda vasica* nees	Leaf	-	-		-	-
4.	*Allium cepa* L.	Bulb	-	-		-	-
5.	*A. sativum* L.	Bulb	-	-		-	-
6.	*Aloe vera* Tourn.	Leaf sheath	5.3 ± 1.24	8.0 ± 0.8	11.7	± 1.24	29.7 ± 1.42
7.	*Azadiracta indica* Juss.	Leaf and oil	-	-		-	-
8.	*Citrullus colocynthis* Schard	Fruit	-	-		-	-
9.	*Citrus medica* Linn.	Fruit	-	-		-	-
10.	*Eucalyptus globulus* Labil	Oil	7.0 ± 0.81	14.7 ± 1.24	22.0	± 0.81	30.0 ± 1.63
11.	*Hibiscus rosasinensis* L.	Leaf and flower	-	-		-	-
12.	*Jatropa gladulifer* L.	Leaf (latex)	-	-		-	-
13.	*Laosonia inermis* L.	Leaf	-	-		-	-
14.	*Lippia nodiflora* L.	Leaf	-	-		-	-
15.	*Ocimum sanctum* L.	Leaf	-	-		-	-
16.	*Phyllanthus emblica* L.	Leaf	6.0 ± 0.81	9.0 ± 0.81	11.0	± 0.81	12.0 ± 1.63
17.	*Pongamia globera* Vent.	Seed	-	-		-	-
18.	*Wrightia tinctoria* Roxb.	Leaf	7.7 ± 1.24	10.3 ± 1.67	16.0	± 0.81	19.7 ± 1.7
19.	*Zingiber officinale* Rose	Rhizome	-	-		-	-
	Clotrimazole (antibiotic)		7.3 ± 0.81	14.3 ± 1.24	20.0	± 0.08	24.6 ± 0.94

potato dextrose medium, cornmeal medium, Czapek's dox medium, malt extract medium, nutrient medium and rose bengal medium. Influence of temperature, pH, salinity, carbon sources (peptone and dextrose) on the growth of *M. furfur* The present investigation establishes that dextrose at 40 g/ litre and peptone at 10 g/litre are optimum for the growth of *M. furfur* in the Sabouraud's dextrose agar supplemented with butter. It is also established that the optimum temperature 30 ± 2 °C, pH as 7-9 and salinity 40ppt for the growth of *M. furfur* (Figs. 4 a-f).

Effect of plant extracts

Among the nineteen plant extracts tested, the extracts of four plants namely *Aloe vera, Eucalyptus globulus, Phyllanthus emblica and Wrightia tinctoria* were more effective than other species. The volatile oil of *Eucalyptus globulus* significantly reduced the growth of *M. furfur* (Table 2).

Discussion

Malassezia furfur is a pleomorphic yeast like fungus. It is referred to as *Pityrosporum orbiculare* and *P. ovale* depending on the morphology of the cells. When the yeast like cells are rounded and budding from with narrow neck, they are called *P. orbiculare* and when the yeast like cells are oval and budding form with broad neck, they are called *P. ovale*. However, commonly in recent years the name *Malassezia furfur* is widely accepted for all forms of yeast like cells produced by *Pityrosporum orbiculare* [8]. Hence, in the present study the name *M. furfur* is used for the yeast like cells of the organism. It has been reported that the growth and morphology of *Candida albicans*, another yeast like fungus, was controlled by various physicochemical characteristics and the composition of the media [9, 10]. It is also well known that the optimum requirement of physicochemical parameters varies depending on the species and the habitat in which they grow. Optimization of the requirements of *Malassezia furfur* in the present study showed that the organism grew well at pH 7 to 9, temperature 30 ± 2 °C and the salinity at 40 ppt. Similarly, the carbon sources-dextrose and peptone were suitable at 40g and 10 g/litre respectively.

Table 2: Growth of *Malassezia furfur* on Sabouraud's dextrose medium in different fatty substances

Fatty substances	Growth of *M. furfur*
Butter	++++
Corn oil	+++
Olive oil	+++
Coconut oil	+++
Castor oil	++
Oleic acid	++
Without fat	+

= excellent growth, +++ = good growth, ++ = fair growth + = poor growth

Commonly Sabouraud's dextrose agar medium is used for the culturing of dermatophytes. Emmon's (1970) modified this medium by adding corn oil for the culturing of *M. furfur*. But, the present study clearly established that the growth of *M. furfur* was more favoured in the presence of butter than corn oil. Hence, it is suggested that Emmon's modified medium can be further modified by supplementing Sabouraud's dextrose agar medium with butter in the place of corn oil.

Antipityrosporum activity of herbal drug, a combination of *Wrightia tinctoria and Hibiscus rosasinensis* was tested *in vitro* against the isolates of *Pityrosporum ovale* recovered from dandruff. In the present investigation nineteen plant extracts were tested for the antimycotic activity against *M. furfur*. *Aloe vera, Eucalyptus globulus, Phyllanthus emblica and Wrightia tinctoria* leaf extracts and oil showed antifungal property as they progressively inhibited the growth of *M. furfur* on Sabouraud's destrose agar medium. *E. globulus* (30 ± 1.63) and *A. vera* (29 ± 1.14) were more effective than other species and antibiotic of Clotrimazole (24.6 ± 0.94) tested. Hence, the extractions of active principle from these plants and their assay against *M. furfur* have been suggested as future course work.

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