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REVIEW

#### ANTIFUNGAL ACTIVITY OF KOKAM LEAF EXTRACT

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#### ABSTRACT

Dandruff is a common disorder affecting the scalp. The genus Malassezia is the main causative agent of dandruff. This fungus lives and feeds on human skin, causing the itching and flaking associated with the condition. Out of 17 different species, Malassezia furfur and Malassezia globosa are the main cause of dandruff. In recent years plant based products are widely used as therapeutic weapon to cure human disorders. The plant Garcinia indica (Kokam) belonging to family Clusiaceae native to India is one of such plants which has shown many therapeutic uses. The present study shows the anti-dandruff activity of leaf extract of G.indica against Melassezia globosa Out of two screened fractions (ethyl acetate and water fraction), ethyl acetate fraction showed zone of inhibition  $9\pm 0.34$  mm and  $12.3\pm 0.12$  mm at 80 and 100 % concentration respectively

Key words: Dandruff, Malassezia globosa, leaf extract, inhibition, Garcinia indica

#### **INTRODUCTION:**

Dandruff (pityriasis, capitis, seborrheic dermatitis confined to scalp) is a disease that has been around for centuries despite of several treatment options. It is a common scalp disorder affecting almost half of the pubertal population of both genders but most prevalent in male population between age group of 20 to 60 years <sup>1</sup>. It is the major cosmetic problem which causes a great public health concern both in the developed as well as developing countries. Dandruff is characterized by slight to moderate scaling of the scalp with varying degrees of sensations of dryness. Characteristics flaking and scaling of the scalp suggest impairment in the desquamation process. In most of the dandruff affected people, hair fall is a very common problem.

Dandruff and dry scalp are mostly used interchangeably by almost everyone because of similar symptoms. Dry scalp lacks moisture which causes dryness and itchy scalp followed by the shedding of small flakes of dead scalp cells due to scratching. The causes of the dry scalp can be dehydration in the body, poor diet or environmental conditions. Dandruff occurs due to the overproduction of the sebum and excessive action of yeast-like fungus known as *Malassezia*. This yeast feeds on the excessive oil sebum and on dead scalp cells resulting in the faster renewal process and further leads to the frequent shedding of scalp cells which fall off in the form of visible flakes. *Malassezia* (formerly called as *Pityrosporum*), yeast like lipophilic basidiomyctous fungi is considered to be the chief cause of dandruff problem which is present as scalp commensal <sup>2</sup> Lipid dependant *Malassezia* yeasts are commonly found on human skin in particular in the upper part of the body, where sebum secretion is highest <sup>3</sup>. Though dandruff is associated with scalp, flakes may also appear on face, nose and eyebrows as well as on the skin behind the ears and neck. Due to impact of male hormone testosterone, the sebaceous glands are stimulated to secrete more sebum which enhances the microbial growth and also associated formation of dandruff on scalp.

#### **ACTION OF THE FUNGUS:**

Though there are seven different species of *Malassezia* found, till date the species *M.globusa*, *M.restricta* and *M.furfur* have been mostly related with dandruff in human beings <sup>4</sup>. *M.globosa* is an important causal factor for dandruff. Synthesis of lipase by species *Malassezia* hydrolyzes triglycerides which then release oleic acid that attracts neutrophils towards them. As a result neutrophils release the reactive oxygen species and cytokines that aggravate scalp by causing the dermal inflammation and tissue damage<sup>5</sup>. *M.globosa* is the most likely initiating organism by virtue of its high lipase activity, and that an *M.globosa* lipase is expressed on human scalp. As a result the corneocytes present in the epidermis clump together to form large flakes on the skin which causes irritation and uneasiness <sup>6</sup>. Therefore, effective treatment is the need of the hour for people suffering from dandruff formation.

In the current scenario, many synthetic chemical substances are used for treating dandruff. The main active agents present in it are imidazole derivatives such as ketoconazole and other compounds such as selenium sulphide, zinc pyrithione, piroctone olamine, cipropirox olamine and many others. They act by removing the scalp thereby reducing *Malassezia* species adherence to corneocytes and inhibit its further growth. Pharmacological properties of medicinal plants may be used as leads in developing novel therapeutic agents. Today herbal products and extracts are widely used to control various human diseases. Medicinal plants are providing an efficient local aid to the health care and disease free life. They contain physiological active

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constituents that over the years have been exploited in traditional medicine for the treatment of various alignments<sup>7</sup>. India is rich in biodiversity and has a wide spectrum of habitats from tropical rainforests to alpine vegetation and from temperate forests to coastal wetlands. About one third of the country's recorded flora is endemic and is concentrated mainly in the North-East, Western Ghats, and North-West Himalaya. Western Ghats of India are known for their valuable biodiversity and has been considered as one amongst the top most important eight hotspots in the world<sup>8</sup>. This hotspot of biodiversity is a treasure house of genetic resources of many plant species. Garcinia indica (family- Clusiaceae) is one such tree species endemic to tropical rain forests of Western Ghats of India. Its fruits are a rich source of Hydroxycitric Acid (HCA), an important biologically active plant metabolite used as anti-obesity and anti-cholesterol drug. The fruits are also used to prepare a pleasant attractive beverage which has bilious action. The fat extracted from the seeds is used in cosmetics as emollient. A lot of work has been carried out on various aspects of extracts separated from fruit rinds of *G.indica*. Fruit rind extracts have shown good anti hyaluronidase and anti elastase properties <sup>9</sup>. Researchers demonstrated anti microbial and cytotoxic effects of fruit rinds of G.indica. Garcinol and Hydroxycitric Acid (HCA) present in G.indica have showed significant anti oxidant and anti hyperlipidemic activity <sup>10</sup>. But there are very few reports on anti dandruff activity of *Garcinia indica*. Taking this into consideration it was decided to screen various fractions of leaf extract of G.indica for their anti dandruff ability against *M.globosa*.

#### MATERIALS AND METHODS:

- 1. Microorganism used: The test organism used in this study *Malassezia globosa* was of clinical origin
- 2. Reagents and chemicals: Organic solvents used were of analytical grades (Merck and Qualigen). Sabouraud's Dextrose agar (M286) was purchased from Hi Media, Mumbai.
- 3. Preparation of leaf extracts:
- **a. Preparation of Methanolic Extract:** The methanolic extract (ME) was prepared by immersing (10 gms) of fresh leaves of *G.indica* in 100 ml of acidified methanol (1% Concentrated HCl). The extract was poured in the evaporating dish and allowed to dry at room temperature to obtain 4 gm solid (ME).
- **b.** Separation of ethyl acetate fraction: One gram of ME was dissolved in 25 ml of D/W. To this 25 ml of ethyl acetate was added and two fractions were allowed to separate in a separating funnel for at least one hour. Ethyl acetate fraction (EAF) and water fraction (WF) were separated. Both fractions were air dried to obtain 0.2 gm EAF, and 0.5gm WF. These fractions were used to study anti dandruff activity.
- 4. **Preparation of positive control:** *Terminalia bellarica* fruit extract was used as the positive control.

#### ANTIFUNGAL SUSCEPTIBILITY TESTING BY WELL DIFFUSION METHOD

*Malassezia globosa* of clinical origin was grown on Sabouraud's Dextrose Agar supplemented with 1% corn oil for a period of one week at  $32^{\circ}$ C in an incubator. *M.globosa* culture was then further maintained on the same medium with subcultures being carried out every alternate week. Loopful colonies of organism was transferred to 100 ml of Sabouraud's dextrose broth and maintained for a period of seven days at  $32^{\circ}$ C on an shaker till the culture became 70% confluent. The broth culture of *M.globosa* was swabbed over the Sabouraud's dextrose agar by using sterile cotton buds. Sterile 5 mm diameter Whatman no. 32 filter paper discs were dipped in to all three extracts with various concentrations ranging from 20,40. 60, 80 and 100%. *Terminalia bellarica* fruit extract was used as the positive control in the same concentrations like other tested extracts. The replicates were maintained. These plates were incubated at  $32^{\circ}$ C and the zone of inhibition was observed after seven days. Control was maintained with filter paper discs dipped in sterile distilled water.

#### **RESULT AND DISCUSSION:**

*Malassezia globosa* is pleomorphic yeast like fungus. It is also referred to as *P. orbicularae* and *P. ovale* depending on the morphology of the cells. However in recent years the name Malassezia furfur is widely accepted for yeast like cells produced by *P.orbicularae*. It is also well known that the optimum requirement of physicochemical parameters varies depending on the species and the habitat in which they grow. Antifungal activity of certain bioactive compounds extracted from medicinal plants has attracted a lot of attention within the scientific community largely as a result of the growing problem of multidrug resistance among pathogenic fungi <sup>11</sup>. In addition to this medicinal plant extracts are the promising sources of antifungal drugs, even though they have relatively mild effect against human pathogenic fungi when compared with the commercial synthetic drugs <sup>12</sup> *Garcinia indica* commonly known as Kokam plant has already gained a lot of attention due to its various anti inflammatory, anti oxidant, free radical scavenging activities. Anti dandruff activity of two fractions separated from leaf extract of *G.indica* against *Malassezia globosa* at various concentrations (20, 40,

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60, 80 and 100%) were examined. It was observed that parent methanolic extract exhibited a week inhibition zone of 4.3 mm only at 100% concentration while at lower concentrations the activity was negligible. Out of the two fractions separated from ME, ethyl acetate fraction showed good inhibition at higher concentration i.e.  $9\pm0.34$  mm and  $12.3\pm0.12$  mm at 80 and 100% respectively (**Table 1**). It was observed that the water fraction was not effective in inhibiting the growth of *Malassezia globosa*.

Fraction Name	Concentrati		(%) and Zone m)	of inhibition	
	20	40	60	80	100
ME	1.2	1.9±0.2	2.0±0.4	2.3±0.9	4.3
 EAF	3±1.5	5±0.9	7.3±0.3	9±0.34	12.3±0.12
WF	0.8	2.4	3.2±0.3	5±0.6	7.6±1.3
Tb	5.6±0.3	9.3	12.4±1.2	16.2±0.8	25.6±1.2

#### Table 1: Effect of various concentrations of three fractions against M. globosa

## ME: METHANOLIC EXTRACT, EAF: ETHYL ACETATE FRACTION, WF: WATER FRACTION AND TB: TERMINALIA BELLARICA

It was reported that *Terminalia chebula* and *Terminalia bellerica* exhibited a significant inhibition activity against *Malassezia globosa*. They also showed that *Lantana camara* which was less effective against the fungus, but if used in combination with *Terminalia chebula* showed good synergistic effects against the fungus <sup>13</sup> Anti *Pityrosporum* activity of herbal drug, a combination of *Wrightia tinctoria* and *Hibiscus rosasinensis* was tested *invitro* against the isolates of *Pityrosporum ovale* recovered from dandruff <sup>14</sup>. In another study, screening with four plants (*Aloe vera, Eucalyptus globulus, Phyllanthus embilca* and *Wrightia tinctoria*), E. *globulus* (30  $\pm$  1.14) and *Aloe vera* (29  $\pm$  0.94) were found to be very much effective against this dandruff causing fungus <sup>15</sup>. In the present study, among the various tested fractions, only ethyl acetate fraction at higher concentrations showed inhibition against *Malassezia globosa* while water fraction was not that effective. Comparing with positive control (*Terminalia bellarica*) leaf extracts of *G.indica* were not that effective against the fungus.

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# Recent Developments in Nitrogen- and Phosphorous-Based Flame Retardants for Polyurethanes

Anil M. Palve\*, Jagruti S. Suroshe, and Ram K. Gupta\*

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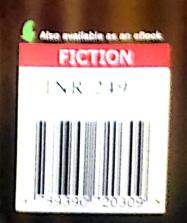
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