Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(1): 266–269

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Short Communication

Synergistic Effect of Plant Extracts against *Malassezia furfur*

Abhijit Sahasrabudhe*[1]

Received: 23 Nov 2020 | Revised accepted: 19 Jan 2021 | Published online: 05 Feb 2021

***Key words*:** Dandruff, *Malassezia furfur*, Inhibition, Fruit extracts, Synergistic effect

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 [1]. 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.

Dandruff can occur due to any reason such as dirty scalp, over use of hair styling products, product build up (shampoo, wax, gel etc.) on the scalp, excessive oil production etc. However, the most common causes of dandruff are;

- Change in the climatic condition
- Dry skin
- Skin conditions, such as psoriasis, eczema, or seborrheic dermatitis
- Reaction to a hair product or shampoo

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 [2]. Lipid dependant *Malassezia* yeasts are commonly found on human skin in particular in the upper part of the body, where sebum secretion is highest [3]. 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.

***Abhijit Sahasrabudhe**
abhijitvs@gmail.com

[1]Research Laboratory, Department of Botany, D.S PM's K. V. Pendharkar College of Arts, Science and Commerce, Dombivli (E), Thane – 421 203, Maharashtra, India

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 [4]. *M. furfur* 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 [5]. *M. furfur* is the most likely initiating organism by virtue of its high lipase activity, and that an *M. furfur* 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 (Fig 1). Therefore, effective treatment is the need of the hour for people suffering from dandruff formation [6].

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. Some studies also suggest the involvement of staphylococci bacteria in dandruff disease pathogenesis [7].

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 constituents that over the years have been exploited in traditional medicine for the treatment of various alignments [8].

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 [9]. This hotspot of biodiversity is a treasure house of genetic resources of many plant species.

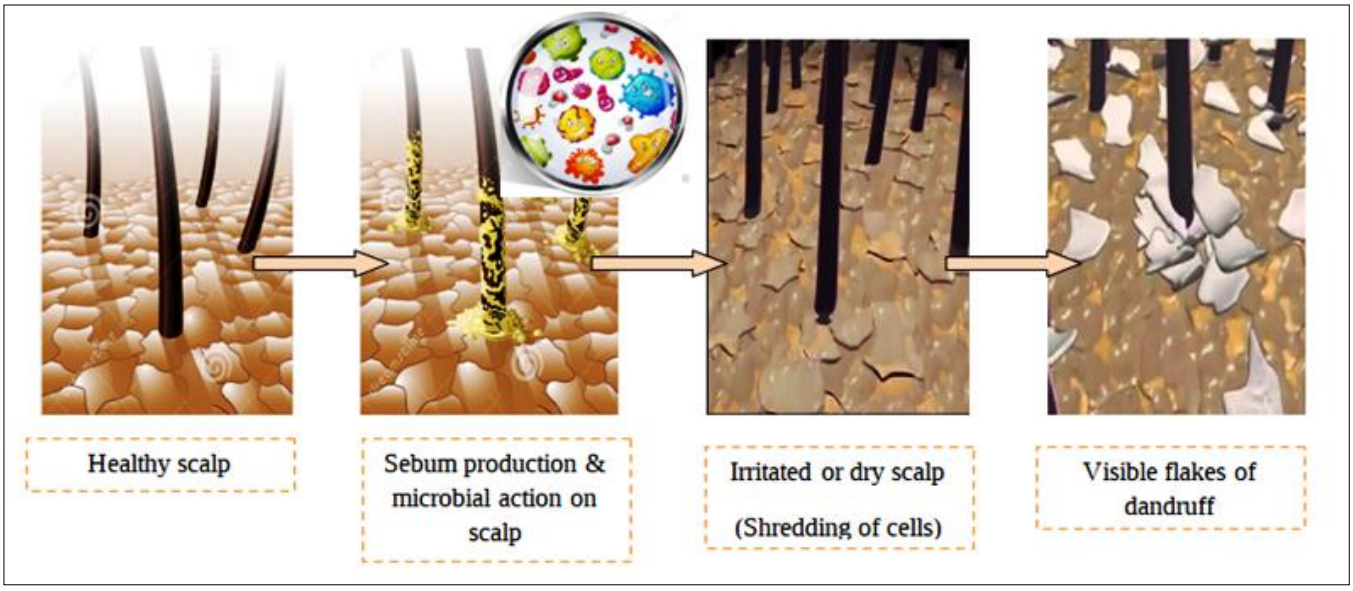

Fig 1 Mechanism of action of fungus on scalp

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 [10]. 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 [11]. Preliminary work on antidandruff activity of *Garcinia indica* showed promising results [12]. Taking this into consideration it was decided to screen synergistic effect of fruit extracts of various plants against *M. furfur*.

The ripe fruits of *G. indica*, *Terminalia chebula* and *Terminalia bellarica* were collected from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra.

Microorganism used: The test organism used in this study was *Malassezia furfur* (MTCC- 1374). The culture was purchased from the Microbial Type Culture Collection, Chandigarh, India.

Reagents and chemicals: This work was carried out in Research Laboratory of Department of Botany, K. V. Pendharkar College, Dombivli. Organic solvents used were of analytical grades (Merck and Qualigen). Sabouraud's Dextrose agar (M286) was purchased from Hi Media, Mumbai.

Preparation of powdered extracts: Shade dried fruit materials were powdered. 2gms of each powder is dissolved soaked in 100 ml of distilled water with addition of 1 ml of concentrated HCl (v/v). These extracts were air dried in evaporating dish with muslin cloth covering and once dried completely stored in air tight containers for further analysis.

Separation of ethyl acetate fraction: One gram of powdered extract was dissolved in 50 ml of D/W. To this 50 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 the fractions were air dried and used for antidandruff study.

Antifungal susceptibility testing by well diffusion method

Antifungal Susceptibility Testing: *Malassezia furfur* strain (MTCC-1374) was grown on Sabouraud's dextrose agar supplemented with 2% corn oil for one week at 30°C in an incubator. *M. furfur c*ulture was then further maintained on the same medium with subcultures being carried out every alternate week. Loopful colonies of the organism were transferred to 100 ml of sabouraud's dextrose broth and maintained for seven days at 30°C on a shaker till the culture became 70% confluent. The broth culture of *M. furfur* 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 into all three extracts with various concentrations ranging from 25, 50, 75 and 100%. *Emblica officinalis* (Amla) fruit extract was used as the positive control in the same concentrations as other tested extracts. The replicates were maintained. These plates were incubated at 30°C and the zone of inhibition was observed after seven days. Control was maintained with filter paper discs dipped in sterile distilled water.

Malassezia furfur is pleomorphic yeast like fungus. It is also referred to as *P. orbicularae* and *P. ovale* depending on the morphology of the cells. When the yeast like cells are rounded and budding form with narrow neck then are called as *P. orbicularae* while on the other hand when yeast cells are oval with broad neck are called as *P. ovale.* 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 [13]. 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 [14].

It was reported that *Terminalia chebula* and *Terminalia bellerica* exhibited a significant inhibition activity against *Malassezia furfur*. 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 [15].

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 [16]. In another study, screening with four plants (*Aloe vera*, *Eucalyptus globulus, Phyllanthus embilca* and *Wrightia tinctoria*), *E. globulus* (30 ± 1.14) and *Aloe vera* (29 ± 0.94) were found to be very much effective against this dandruff causing fungus [17].

Table 1 Antifungal activity of ethyl acetate fraction of various plants

Ethyl acetate fraction	Concentration of fraction (%) Zone of inhibition (mm)			
	25	50	75	100
Garcinia indica	2 ± 0.2	5.6 ± 0.7	12.2 ± 0.6	26.4 ± 0.1
Terminalia chebula	7 ± 0.5	9.6 ± 1.2	20..4 ± 0.2	24.5 ± 0.3
Terminalia bellarica	5 ± 0.2	10.3 ± 0.9	18.2 ± 1.2	25.5 ± 0.98
Emblica officinalis	6 ± 0.44	11.5 ± 0.2	18.5 ± 0.4	22.5 ± 0.2

Table 2 Antifungal activity of combined plant extracts

Combination of crude extracts	Zone of inhibition (mm)
Garcinia indica + Terminalia chebula	20.6 ± 0.6
Terminalia chebula +Emblica officinalis	22..6 ± 0.7
Terminalia bellarica + Garcinia indica	26.4 ± 1.4
Emblica officinalis + Garcinia indica	28.4 ± 1.4

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. In earlier studies, ethyl acetate fraction separated from fruit rinds of *G.indica* has shown significant inhibition activity against *M. furfur.* In the present study synergistic effect of ethyl acetate fractions separated from three different fruits were examined in combination against the growth of *Malassezia furfur*. It was observed that *G. indica* at lower concentration is not effective against fungus as compared to other fruit extracts (Table 1). A marked difference in inhibition activity was observed when concentration was in the range of 25 to 75%. At 50%, zone of inhibition for *G. indica* was 5.6 ± 0.7 mm while others it was in the range of 9 to 12 mm. Up to 75% concentration there was an increase in zone of inhibition for others as compared to *G. indica*. *T. chebula* had the maximum zone of inhibition (20.4 ± 0.2 mm). Surprisingly at 100% concentration of ethyl acetate fraction, *Garcinia indica* showed maximum zone (26.4 ± 0.1 mm) as compared to others. Four different combinations from these four extracts were made and checked for their synergistic activity against *Malassezia furfur*. *T. chebula* and *G. indica* showed less activity (20.6 ± 0.6 mm) while combination of *G. indica* with *E. officinalis* showed maximum zone of inhibition (28.4 ± 1.4 mm) as compared to other combinations.

SUMMARY

Dandruff is a common disorder affecting the scalp. The genus *Malassezia* is the main causative agent of dandruff. 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. Earlier studies showed fruit extracts of *Garcinia indica* (Kokam) have significant effect against fungus growth. In the present study an attempt was made to know the combine effect of different fruit extracts against dandruff causing organism *Malassezia furfur*. It was observed that ethyl acetate fraction of *G. indica* was effective at 100% concentration as compared to other extracts. Combination of *G. indica* and *E. officinalis* showed maximum zone of inhibition (28.4 ± 1.4 mm). Fruit extracts showed good activity against dandruff causing organism *Malassezia furfur*. From the results, we conclude that plant extracts have antifungal activity and could be safely used for treating dandruff. Further studies can be made on the active molecules of plant extracts responsible for antidandruff activity.

Acknowledgement: The author thanks Principal and Head, Department of Botany of K. V. Pendharkar College for providing the laboratory facilities.

LITERATURE CITED

1. Ravichandran G, Shivaram, Kolhapur SA. 2004. Evaluation of the clinical efficacy and safety of “Antidandruff Shampoo” in treatment of Dandruff. *The Antiseptic* 201(1): 5-8.
2. Mistry Z, More B, Shah G. 2016. Anti-dandruff activity of synthetic and herbal shampoos on dandruff causing isolate: Malassezia. *Int. J. Appl. Res.* 2(7): 80-85.
3. Ranjith M, Gokul SS, Ranganathan S, Shivramkrishanan M, Natarajan V, Rasool S. 2002. Role of ABO blood group in the infection rate of dandruff caused by *Pityrosporum ovale*. *I.J.D* 47(1): 21-23.
4. Gupta A, Batra R, Bluhm R. 2004. Skin diseases associated with *Malassezia furfur. Journal of American Academic Dermatology* 51(5): 785-798.

5. DeAngelis Y, Saunders C, Johnstone K, Reeeder N, Coleman C. 2007. Isolation and expression of a *Malassezia globosa* lipase gene, LIPI. *Jr. of Invest. Dermatol.* 127: 2138-2146.
6. Nazzaro- Porro M, Passi S. 1976. Growth requirements and lipid metabolism of *Pityrosporum obiculare*. *Jr. Invest. Dermatol.* 66: 178-182.
7. Leong C, Schmid B, Glatz G, Bosshard P. 2019. In vitro efficacy of antifungal agents alone and in shampoo formulation against dandruff-associated Malassezia spp. and Staphylococcus spp. *Int. Jr. Cosm. Sci*. 41(3): 221-227.
8. Srinivasan K, Natarajan D, Dheen M. 2006. Anti-bacterial activity of selected medicinal plants, Ham. *Medicine* 2: 5-8.
9. Myers N, Mittermeier R, Mittermeier CG, da Fonseca GAB, Kents J. 2000.Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
10. Sahasrabudhe A, Deodhar M. 2010. Anti-hyaluronidase, anti-elastase activity of *Garcinia indica*. *Int. Jr. Bot*. 6(3): 299-303.
11. Varalakshmi K, Sangeetha C, Shabeena A, Sunitha S, Vapika J. 2011. Antimicrobial and cytotoxic effects of *Garcinia indica*. *W.J.A.S* 7(2): 193-196.
12. Sahasrabudhe A, Phanse M. 2015. Culturing of *Malassezia furfur* and its growth inhibiting activity of *Garcinia indica. I. J. P*. 2(8): 409-413.
13. Tim CT, Lamb A. 2005. Antimicrobial activity of flavonoids. *Int. Jr. Antimicrob. Agents* 26: 343-356.
14. Hammer K, Carson C, Riley T. 1999. Anti-microbial activity of essential oils and other plant extracts. *Jr. Am. Acad. Dermatol*. 51(5): 785-798.
15. Balkrishnan K, Narayanswamy N, Mathews S, Gaurang K. 2011. Evaluation of some medicinal plants for their dandruff control properties. *Int. Jr. Pharma and Bio Sciences* 2(4): 38-45.
16. Krishnamurthy J, Rangananthan S. 2000. Anti *Pityrosporum ovale* activity of herbal drug combination of *Wrightia tinctoria* and *Hibiscus rosa sinensis*. *I.J.D* 45: 125-126.
17. Prabhu M. 2012. Anti-fungal activity of selected plant extracts against *Malassezia globose. Int. Jr. Adv. Sci. Res.* 2(5): 162-168.