

ANTIMICROBIAL ACTIVITY OF NATURAL HERBAL PRODUCTS AGAINST DANDRUFF CAUSING FUNGUS AND BACTERIA

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ABSTRACT

Dandruff is a common chronic scalp disorder which is marked by flaking of the skin on the scalp. People with seborrheic dermatitis are very prone to dandruff which occurs due to an overgrowth of yeast like fungus called *Malassezia*. Seborrheic dermatitis affects many areas of the skin, including the backs of ears, the breastbone, eyebrows, and the sides of the nose. The patient will have red, greasy skin covered with flaky white or yellow scales. The drugstore remedies may contain shampoos with zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, sulphur and coal tar derivative extract. The

antidandruff shampoo only slow down the scalp flaking and have their own disadvantages. This work includes how natural herbal products like lemon juice, neem leaves extract, onion juice, and curd (yoghurt) are useful as antifungal and antibacterial agent which inhibit the growth of *Malassezia* fungi and *Staphylococcus aureus* which causes the dandruff.

KEYWORDS: Dandruff, Seborrheic dermatitis, *Malassezia*, *Staphylococcus aureus*, natural herbal products etc.

INTRODUCTION

Dandruff is a common scalp disorder that has occurred for centuries and has a prevalence of nearly 50% in the worldwide population (Sommer B., et al., 2015). The visual perception of individually distinguishable flakes on the scalp, in the hair, or on the clotting is considered an abnormal condition frequently referred to as dandruff, seborrheic dermatitis (Gupta, A.K., et al., 2003 & Schwartz J.R., et al., 2010). Dandruff is characterized as a hyperproliferation of the scalp epidermis accompanied with scalp itching and redness (Chhavi S., et al., 2011). Seborrhoeic dermatitis is a skin disorder which affects the sebaceous gland rich area of skin. It targets the scalp, followed by face.

Dandruff can be caused by number of things, including dry skin, oily skin, too much or too little hair washing, diet, stress, sensitivity to hair products and skin conditions such as pityriasis, seborrheic dermatitis or eczema. However, the real cause of dandruff is actually yeast like fungus, *Malassezia globosa*, which lives on your scalp, feeding on skin oils. The overgrowth of yeast can cause dandruff with is shedded skin. This happens because the *Malassezia* fungus which uses enzyme called lipases to metabolize the oils; to create a by-product called oleic acid (pro-inflammatory free fatty acids)(Ravichandran G., et al., 2004). This fatty acid gets penetrated into top layer of scalp and causes inflammation and increased skin cell flaking in susceptible people (Ravichandran G. et al., 2004; Cornah J., et al., 1988).

Malassezia (formerly known as *Pityrosporum*) is a genus of fungi (Shuster S., 1984). *Malassezia* is naturally found on the skin surfaces of many animals, including humans. In occasional opportunistic infection, some species can cause hyper pigmentation or hypo pigmentation on trunk and other location in humans. The vast majority of recent data supports a direct casual link between *Malassezia* fungi and dandruff (Schwartz J.R.). However, another microorganism community composed of bacteria also inhabits the human scalp, and includes facultative anaerobic bacteria, such as *P.acnes*, and aerobic bacteria, such as *Staphylococcus* (Grice E., & Segre J.A. 2011; Findley K. et al., 2013).

Staphylococci are widespread in nature, although they are mainly found on the scalp, skin, skin glands and mucus membranes of mammals (Samuel Baron 2010; Findley K. et al., 2013). They are a group of bacteria that can cause a number of diseases as a result of infection of various tissues of the body. Over thirty different types of staphylococci can infect humans, but most infection is cause by *Staphylococcus aureus*. Staphylococci can be found normally in the nose and on the skin (and less commonly in other locations) of around 25%-30% of healthy adults and in 25% of hospital workers.

Currently available dandruff treatment include therapeutic use of zinc pyrithione (Potluri A., et al., 2013), salicylic acid, imidazole derivatives, glycolic acid, steroids, and sulphur and coal tar derivative(Manikar and Jolly 2000).These agents show certain limitations, either due to poor clinical efficacy or due to the compliance issues. Furthermore these drugs are unable to prevent recurrence. The antidandruff shampoo only slow down the scalp flaking and have their own disadvantages like loss of hair, increased scaling, itching, nausea, headache, vomiting, photosensitivity. Therefore the use of natural and herbal products is the need of hours and these remedies are more acceptable in the market because of safe and fewer side

effect. They have some special properties such as hair fortifying and anti hair loss effects in addition to their less side effects, and lower cost (Sharma S., et al., 2016).

Natural herbal products like lemon juice, onion, neem leaves extract, and curd (yoghurt) are natural antifungal and antibacterial agent which inhibit the growth of *Malassezia* fungi and *Staphylococcus* which are mainly the cause dandruff.

Lemon (*C. Limon*) is the trusted ingredient to fight most of the dreaded enemies of hair growth-dandruff and hair fall which is rich in vitamin C and helps to restore the pH balance of the skin (Potluri A., et al., 2013). It is use to treat flaky dandruff(Reichling et al., 2009).

Onion (*Allium cepa*) is highly valued for its therapeutic properties and recognized as an important medicinal plant for many years ago. Onion juice has antimicrobial (Dorsch, 1996; Arunachalam 1980) and antifungal effect (Conner and Beuchat, 1984). It is rich in protein, carbohydrates, sodium, potassium and phosphorous (Lampe J., 1999), and sulphur which improves blood circulation and provides sufficient nourishment to hair follicles. It has antifungal properties and contains catalase which is an antioxidant that can prevent premature graying. It possesses many other biological activities including antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic etc. (Corzo-Martinez et al., 2007).

Neem (*A.indica*) belongs to family Meliaceae. Leaves of neem mainly yield quercetin (flavonoid) and nimboesterol (beta sitosterol) as well as number of liminoids (limbin and its derivatives) (<http://www.frienviis.nic.in>). Sufficient support is available in the ancient Indian literature regarding the antidandruff activity of the neem plant (Kirtikar and Basu 1995).

Curd (yoghurt) contains vitamin B₂, B₁₂; proteins etc. which is beneficial for hair. Curd helps fortify and moisturize hair including dandruff treatment (Milind P., and Malik J., 2014). The antibacterial agent present in curd reduces irritation and itchiness of the scalp.

METHODS AND MATERIAL

A) (I) Cultivation of fungi of dandruff

Dandruff was grown on potato dextrose agar and it was incubated for 2-3 days at room temperature.

(II) Direct microscopy of fungi

Fungal culture was taken on slide containing a drop of 10% potassium hydroxide with methylene blue and covered with cover slip. The slide was then heated over the flame to remove air bubbles, and observed under 10 X and 45X objective lenses of microscope. Direct microscopy showed the typical mixture of globose blastoconidia and pseudo- mycelia coupled with mycelia in the form of 'spaghetti' indicated the most identifiable difference of *M.globosa* from the other species of *Malassezia*.

(III) Catalase test

Catalase test was carried out to ascertain the presence of *M.globosa*. An isolated colony of fungal growth was added with sterile glass rod in 3 ml of 3% hydrogen peroxide solution. Active bubbles signifying the release of oxygen from hydrogen peroxide was observed indicate the positive test.

B) (I) Isolation of *Staphylococcus aureus*

The culture of *S.aureus* was isolated on nutrient agar by inoculation. An isolated colony was then subcultured.

II) Gram staining

A morphological characteristic was confirmed by microscopic observation under 45 X objective lens after gram staining.

C) Antimicrobial activity

Antimicrobial activity of lemon juice, neem leaves juice, onion juice, curd and marketed shampoo was tested against dandruff and *staphylococcus aureus* using Muller Hingtone agar (MHA) by well diffusion method. Bacterial lawn of 0.1 ml each of fungal and bacterial culture was made on separate solidified MHA plates. A well of was made in the centre with the help of sterilized borer, 0.5 ml each of lemon juice, neem leaves juice, onion juice, curd, and marketed shampoo was poured in the wells of separate MHA plates. All the fungal culture plates were incubated at room temperature for 2-4 days. Whereas bacterial culture plates were incubated at 37⁰C for 24 hours. After incubation the plates was observed for zone of inhibition.

RESULTS AND DISCUSSION

The results of antifungal and antibacterial activity of lemon juice, neem leaves extract, onion juice, curd and marketed shampoo against *Malassezia* and *Staphylococcus* is shown in table no.1. This results shows that use of natural remedies against antifungal test was found to be effective in controlling the growth of *Malassezia* fungi. Each natural therapeutic agent was found effective to certain level in controlling/inhibiting the growth of dandruff causing fungi and bacteria. Comparative study ascertained the fact that each remedy had its own characteristic level in inhibiting the growth.



Figure 1: Antifungal activity of *Malassezia* fungi against lemon juice, neem extract onion juice and curd (result was not stable over a longer time frame).

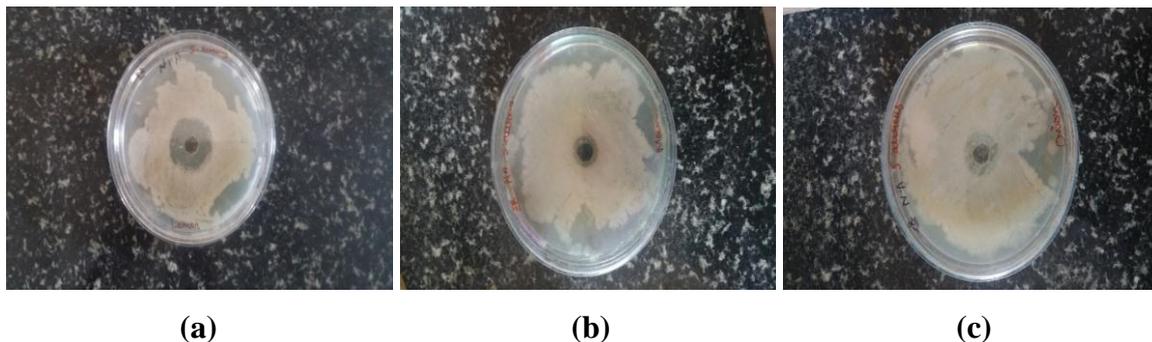


Figure 2: Antimicrobial activity of Lemon juice (a), neem leaves extract (b) onion juice (c) against *Staphylococcus aureus*.

Table no 1: Antifungal and antimicrobial activity on lemon juice, neem leaves extract, onion juice, curd and marketed shampoo against *Malassezia* and *Staphylococcus*.

Sr. no	Extracts (0.5 ml)	Zone of Inhibition (mm)	
		<i>Malassezia</i>	<i>Staphylococcus aureus</i>
1	Lemon Juice	18	20
2	Neem leaves	13	09
3	Onion juice	20	18
4	Curd (Yoghurt)	18	00
5	Marketed shampoo	15	18

Lemon juice extract was more effective in inhibiting the fungus within shorter timeframe, though the result proved not to be stable over a longer time frame. **G.Ravichandran *et.al* (2004)** was used the polyherbal formulation with extract of citrus lemon and oil Melaleuca leucondendron to treat the dandruff (**Ravichandran G., et al., 2004**).

Neem leaves extract was capable in controlling/inhibiting the growth of *Malassezia* fungi, as well as *Staphylococcus aureus*, and was effective in timely action, though the results proved not to be stable over a longer timeframe. Neems leaves extract have high antifungal, antibacterial properties (**Anand N., 2010**). Krishnamurthy *et.al* used plant extract from neem to study the antidandruff efficacy of the oil (**Krishnamoorthy J.R.et al., 2010**). Quercetin (a polyphenolic flavonoid) present in neem leaves is known to have antibacterial and antifungal properties (<http://www.friervis.nic.in>).

Onion juice extract was more effective in inhibiting the fungal growth. Onion juice is useful against hair loss because of its high sulphur content. Sulphur is known as the healing mineral since it has the ability to stimulate blood circulation and reduce skin inflammation.**Sharquie *et.al* (2002)** tested the effectiveness of topical crude onion juice in the treatment of patchy alopecia areata in comparison with tap water (**Sharquie K.E., and Al-Obaidi H.K., 2002**). It was also effective against tested bacteria. **Mohamed Eltaweel (2013)** studied effectiveness onion extract against bacteria.

Curd (yoghurt) was highly stable and capable in controlling/inhibiting the growth of tested fungi for a longer time frame. It has not shown any activity against *Staphylococcus aureus*. Curd is rich in B vitamins and protein which moisturizes the hair and represses the dandruff (<http://readanddigest.com>).

For the treatment of dandruff there are lots synthetic shampoos in market with different chemical compositions. Natural herbal products i.e. lemon juice, neems leaves extract, onion juice and curd, have antifungal and antibacterial properties. They do not have any health hazards, and also provides nourishment to the hair. The single /in combination use of these natural products in herbal shampoos, or its natural use will also minimize the chemical load in the environment due to chemical shampoos industries to some extent.

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