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Analysis on the Natural Remedies to Cure Dandruff/Skin Disease-causing Fungus - *Malassezia furfur*.

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Abstract

A comparative study was conducted on the levels of effectiveness, of natural remedies such as cow's urine, lemon, boiled rice water, Neem extract etc. in curing dandruff and inhibiting the growth of the causative fungus *Malassezia*. Various plating techniques were applied using modified Oil - Potato Dextrose Agar as the nutrient medium for the fungal growth. Cow's urine was found to be more stable and effective in inhibiting the fungus, along with boiled rice-water. Lemon juice was highly effective though over a shorter period.

Keywords: *Malassezia*, Dandruff, Skin-disease, Rice-water, Neem, lemon, Cow's urine.

Introduction

Malassezia furfur (MTCC. 1765), a type of fungus (formerly known as *Pityrosporum*), belongs to the *malassezia* species, which is responsible for skin diseases including hair-dandruff (Gupta, 2004).

Malassezia is a lipophilic, dimorphic and yeast-like fungus which occurs in the human skin. Being an opportunistic pathogen, it causes diseases such as dandruff, Pityriasis Versicolor, Seborrheic dermatitis, etc. (Vijayakumar, 2006). Ancient literature in Ayurveda states that there are natural remedies to cure the diseases caused by this fungal infection. These natural remedies include effective methods such as treatment with rice-water, Neem extract, Lemon juice extract, cow's urine extract etc. (Sonica, 2011).

Literature on Ayurveda states that rice-water is capable of inhibiting the growth of dandruff-causing fungi (Shruti, 2011). As per the recent researches, boiled rice water kept aside for a day permits the growth of *Bacillus cereus* in it. It in turn produces the antibiotics Zwittermicin A and Kanosamine which inhibit the growth of plant pathogen, oomycetes, and certain fungi (Silo-Suh, 1994). This principle is being exploited as a cure against *Malassezia*.

Neem is a supplement that appears to have antibacterial, antifungal, and anti-inflammatory effects. The leaves contain nimbin, nimbinene, desacetylnimbinase, nimbandial, nimbolide and quercetin. At present, research has already proven the excellent benefits of Neem leaves extract as a cure for various skin conditions which includes skin ulcers, wounds, head lice etc. (Agrawal, 2001; Biswas, *et al.*, 2002). The anti-fungal activity of Neem is the main principle being exploited as its cure against *Malassezia*.

The juice of the lemon is about 5% to 6% citric acid. It is considered also as an anti microbial spectrum against bacterial infections and fungi. The acidic effect of the juice has high potential to inhibit fungal growth (Glinksy, 2009; Guess, *et al.*, 2003). This principle is being exploited as an efficient cure against *Malassezia*.

Ayurveda already has immense literature that states the effectiveness of cow's urine (Sanskrit: *Gomutra*) in treating disease (Sanskrit: *Panchgavya* – cowpathy or a treatment based on cow's products) (Dhama, 2005). Research states cow's urine to contain contains nitrogen, sulphur, phosphate, sodium, manganese, carbolic acid, iron, silicon, chlorine, magnesium, melci, citric, titric, succenic, calcium salts, Vitamin A, B, C, D, E, minerals, lactose, enzymes, creatinine, hormones, urea and gold acids. Urea, a key chemical in urine, is known to kill fungi and bacteria. This is the principle behind *Gomutra* being an effective fungicide (Pathak, 2003; Achliya, *et al.*, 2004); especially the urine micturated first, early morning would be more sterile and have more macro and micronutrients along with other enzyme/urea content (Pescheck-Bohmer, 1999) that could be more effective as a fungicide.

Materials and Methods

Malassezia furfur culture (MTCC.1755), boiled rice water from two rice varieties – red parboiled rice (*Rosematta* Rice) and white rice (*Ponni* rice), extract from neem leaves, extract from lemon juice, and cow's urine sample (Figure 1).

Tests

Preparation Methods

For preparing rice water extracts, both varieties of rice (red, white) were taken in sufficient quantities in a pressure cooker, and mixed with double the amount of water, in the proportion 1:2. Once the rice was cooked and cooled, the boiled rice water for both varieties was drained into separate closed container and kept aside for an overnight. This was then used as the test material. For preparing Neem extracts, the fresh leaves from Neem tree were taken, washed well, cut to smaller pieces and mixed with small quantity of water. The resultant was crushed into a thin paste used pestle and mortar. This was then centrifuged at 6000rpm and the supernatant

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Figure 1. 1. White Rice, 2. Red Rice, 3. Cow's Urine, 4. Neem Leaves and extract, 5. Lemon and juice extract



Figure 2a. *Malassezia* culture grown on modified PDA in petridishes and conical flasks.



Figure 2b. *Malassezia* culture grown on modified PDA in testtube slant

was used as test material. For preparing lemon juice, fresh juice was extracted from lemons, without the seeds and stored in a closed container; this being used as the test material. For preparing cow's urine sample, fresh urine sample was collected from the cattle breed in Palghat district of Kerala. This was cleared from any other particulates and used as the test material. (It was ensured that urine sample collected was the first urine micturated early morning once the cattle were awakened).

For preparing the *Malassezia* Culture, the MTCC culture sample was pure-cultured and sub-cultured to obtain pure strains of *Malassezia furfur* culture. Sub-cultures were prepared using techniques of both streak and slant culture (Figure 2a, 2b).

Modified Oil-PDA (Chua, *et al.*, 2005) was selected as the media for culture. Mixed correct proportions of Potato Dextrose Agar with water, and autoclaved the same. Once autoclaved, before it cooled off,

added a pinch of ampicillin powder to disallow any bacterial growth. Then plated the agar media and allowed the same to cool and solidify; overlaid the agar plates with olive oil. Then introduced the freeze-dried fungal culture sample from MTCC, on the plates. The plates were left undisturbed for 5-6 days for growth of *Malassezia*.

Anti-Fungal Tests

Three techniques were used for the anti-fungal tests: The pour-plate test, the spread-plate test and blotting paper disc- diffusion technique-test (Tambekar, 2010). In the pour-plate technique, 1ml of – boiled rice water from each variety, Neem extract, lemon juice, cow's urine sample – were pour-plated with the PDA in autoclaved petridishes. A fine layer of olive oil emulsion was overlaid on the solidified agar, on to which pure culture of *Malassezia* fungi were introduced. These plates were kept aside undisturbed for 4-5 days to observe the growth pattern. In the spread-plate technique, the PDA was pour-plated in autoclaved petridishes. 500 micro-litres to 1ml of – boiled rice water from each variety, Neem extract, lemon juice, cow's urine sample – were introduced on the solidified agar surfaces, and evenly spread using glass rod. A fine layer of olive oil emulsion was overlaid as a superficial layer, onto which pure culture of *Malassezia* fungi were introduced. These plates were kept aside undisturbed for 4-5 days to observe the growth pattern.

In the blotting paper disc-diffusion technique, the PDA was pour-plated in autoclaved petridishes. A fine layer of olive oil emulsion was overlaid as a superficial layer, onto which pure culture of *Malassezia* fungi were introduced and evenly spread using autoclaved cotton swabs. Equal-sized discs were cut out from Whatman filter paper, dipped in samples of boiled rice water (of each variety), Neem extract, lemon juice, cow's urine sample – and placed on the solidified agar surfaces above the culture spread. These plates were kept aside undisturbed for 4-5 days to observe the growth pattern.

Note: For each of the above technique, control plates of pure culture of *Malassezia* fungi were maintained separately.

Results and Discussion

All the three techniques used for the anti-fungal tests with the natural remedies were found effective in controlling the growth of *Malassezia* fungi (Figure 3, 4, 5, 6). Each natural therapeutic was found effective to a certain level in controlling / inhibiting the growth of the dandruff-causing fungi. Comparative studies ascertained the fact that each remedy had its own characteristic level in inhibiting the growth (Table 1).

Cow's urine extract was highly stable and capable in controlling/ inhibiting the growth of *Malassezia* fungi for a longer time frame, and the inhibitory/ containing action was steady. Rice water extract was stably capable of controlling/ inhibiting the growth of *Malassezia* fungi for a longer time frame, though the inhibitory/ containing action was slow considering other remedies.

Neem leaves extract was capable of controlling/ inhibiting the growth of *Malassezia* fungi, and was effective in timely action, though the results proved not to be stable over a longer timeframe. Lemon Juice extract was most effective in inhibiting the fungus within a shorter timeframe, though the results proved not to be stable over a longer time frame.

Remedy	Technique	Result Observed	Inhibitory Rate (with days)	Stability (Rate/days)	Percentile of Effectiveness / Inference
Rice Water extract	Pour-plate	Stable control of Malassezia growth	Steady control over 3-4 days period, reduced on day 5.	High	85-90% effective The <i>Bacillus cereus</i> growth within rice water is capable of inhibiting Malassezia growth at a steady rate, though not highest of stability.
	Spread-plate	Stable control of Malassezia growth	Steady control over 3-4days period, reduced on day 5.	High	
	Disc-diffusion	Stable/clear inhibitory zone around the disc	Stable zone over 3-4 days period, vague/ less resistant on day 5.	High	
Neem Leaves extract	Pour-plate	Slow/ medium control of Malassezia growth	Medium Control over initial 2 days, reduced action over next 3 days	Low	55-60% effective Neem leaf extract is less effective in inhibiting Malassezia growth and has the least stability.
	Spread-plate	Slow/ medium control of Malassezia growth	Medium Control over initial 2 days, reduced action over next 3 days	Low	
	Disc-diffusion	Small inhibitory zone around the disc	Small inhibitory zone first 2 days, vague/ less-resistant over next 3 days.	Low	
Lemon Juice extract	Pour-plate	Effective inhibition of Malassezia growth	Fastest inhibition rate over first 1 day, reduced over next 2-3 days, least on day 5.	Medium	70-75% effective The citric acid present within the Lemon juice is most effective in inhibiting Malassezia growth over a short period of time and has the medium stability.
	Spread-plate	Effective inhibition of Malassezia growth	Fastest inhibition rate over first 1 day, reduced over next 2-3 days, least on day 5.	Medium	
	Disc-diffusion	Clear inhibitory zone around the disc; lesser time frame	Clear/ effective inhibitory zone day 1, vague over 2-3 days,least resistance over 4-5 days.	Medium	
Cow's urine extract	Pour-plate	Stable control of Malassezia growth	Steady control over 4-5 days period	High	90-95% effective The enzyme/ urea/ nutrient content within cow's urine extract make it highly effective in inhibiting Malassezia growth and have the highest stability. It was noticed though that more quantity of cow's urine proved more effective. There was more inhibition activity in pour-plate than spread-plate and disc-diffusion technique.
	Spread-plate	Stable control of Malassezia growth	Steady control over 3-4days period	High-Medium	
	Disc-diffusion	Stable inhibitory zone around the disc	Relatively fine-stable zone over 4-5 days period.	Medium	

Table 1. Comparative studies for all natural remedies.

Conclusion

From the comparative studies done, it was concluded that Cow's urine was most effective and highest of stability in inhibiting/ controlling the growth of Malassezia fungi. Rice water extract was capable of inhibiting/ controlling the growth too, though not at the highest of

stability levels. Lemon juice extract was the most effective in inhibiting the fungi over a very short span of time, however the citric acid effects were short-lived and of medium stability. Neem extract had the least of stabilities and inhibitory action among the natural remedies experimented. Previous studies have also proven that natural remedies such as herbal solutions can prove effective in inhibiting or controlling

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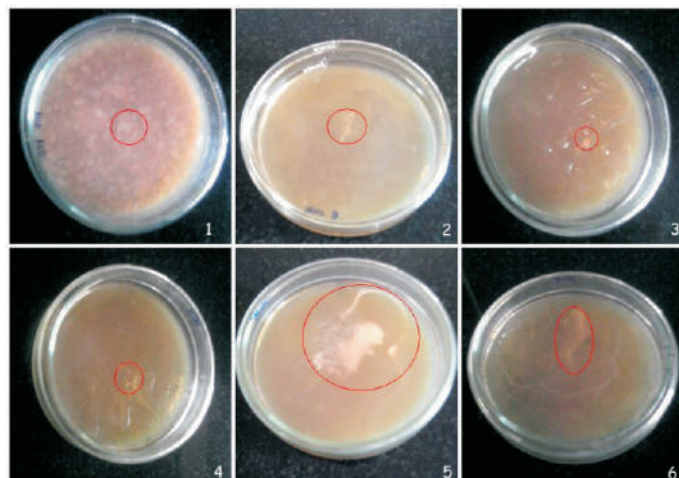


Figure 3. Malassezia growth on plates layered with 1. Red Rice, 2. White Rice, 3. Lemon Extract, 4. Cow's Urine, 5. Control, 6. Neem Extract

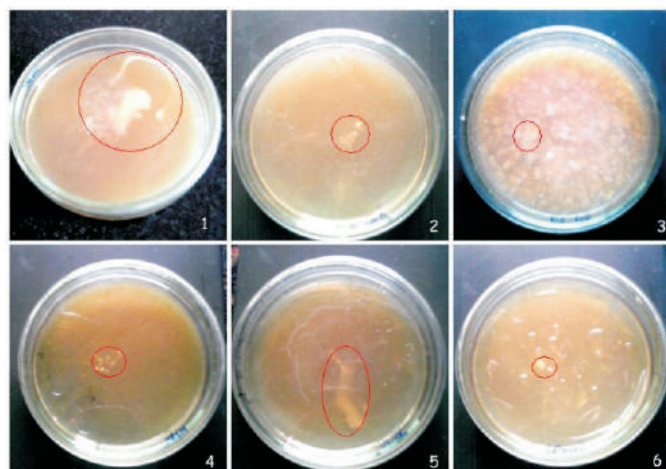


Figure 4. Malassezia growth on plates layered with 1. Control, 2. White Rice, 3. Red Rice, 4. Neem Extract, 5. Cow's Urine, 6. Lemon Extract

Purified cow's urine being used as an effective anti-bacterial/ anti-fungal therapeutic since decades, as stated in Ayurveda literature and proven in recent studies on fungal inhibition (Kekuda, *et al.*, 2007), would be most effective in controlling dandruff-causing fungal growth with minimal side effects. Boiled rice water would also be equally effective without side effects. Lemon juice would be effective; however the citric acid content has been proven to be a causative of hair graying. Neem extract can be used in combination with the above remedies in controlling the fungal growth. However Malassezia fungi are capable of growing back in the scalp once the above remedies are not constantly used. This has also been proven similar for the usage of modern-age shampoos. So usage of the natural remedies can cause less side effects compared to the chemical constituents present in the shampoos (Duvauchelle, 2010), however they need to be constantly used; atleast once in a week to ensure scalp free of Malassezia growth.

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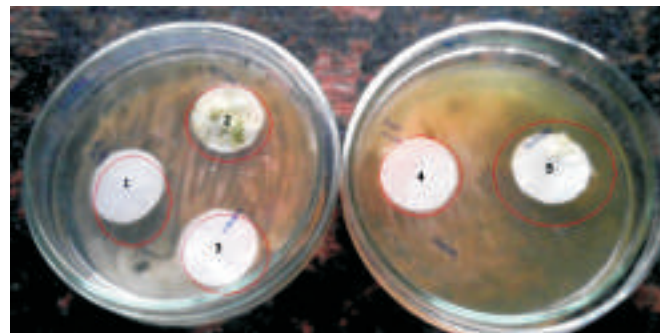


Figure 5. Malassezia growth after 1 day on plates disc-layered with 1. Red Rice, 2. Neem Extract, 3. Cow's Urine, 4. White Rice, 5. Lemon Extract

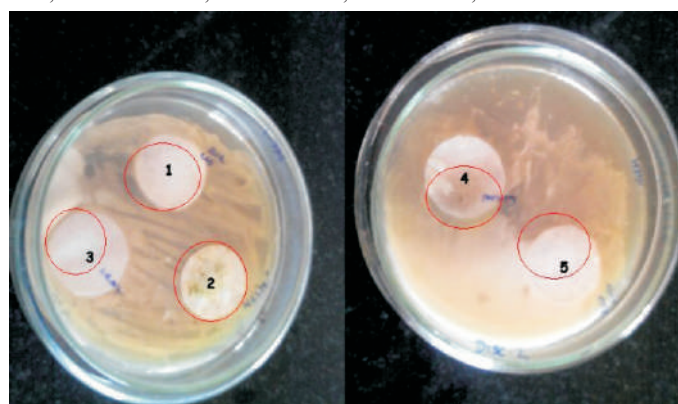


Figure 6. Malassezia growth after 2-3 days on plates disc-layered with 1. Red Rice, 2. Neem Extract, 3. Cow's Urine, 4. White Rice, 5. Lemon Extract

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