

**ANTIMYCOTIC ACTIVITY OF NONI JUICE AGAINST SEBORRHOEIC DERMATITIS****SAINI JASDEEP KAUR, MHATRE BHAKTI AND MARAR THANKAMANI****School of Biotechnology & Bioinformatics, D.Y. Patil University, Sector 15, CBD Belapur, Navi Mumbai, Maharashtra, India-400614.***ABSTRACT**

Morinda citrifolia, commonly known as Noni, has been proven to be effective in curing a number of health problems and diseases. There have been several investigations in the past few decades demonstrating activity of *Morinda citrifolia* L in psoriasis. It has been found to possess anti-microbial properties as well. In order to determine its anti-mycotic activity, commercially available Noni juice was used in-vitro against *Malassezia furfur*, lipophilic yeast causing seborrhoeic dermatitis and other superficial skin infections. According to the anti-mycotic activity assay carried out using well diffusion method, the zone of an inhibition of Noni juice was compared to the zone of inhibition of few natural products and standard antidandruff shampoo. It was determined that Noni juice showed comparatively higher anti-mycotic activity, thus proving as an effective alternative against seborrhoeic dermatitis.

KEYWORDS: Noni juice, anti-mycotic, seborrhoeic dermatitis, *Malassezia furfur*, selenium sulfide, well diffusion.

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INTRODUCTION

Dandruff, a clinical condition caused by *Malassezia* species is of great cosmetic concern all over the world¹. It is characterized by white to whitish yellow, dry, loose scaling and mild pruritis of the scalp. Dandruff may gradually progress through redness, irritation and increasing scaling of the scalp to seborrhoeic dermatitis and both conditions are considered to be the same disorder of different severity². *Malassezia* species are yeasts that comprise part of the micro flora of human skin. Especially, they are abundant in regions supplied with sebaceous glands because of their lipid requirement for growth. Several skin problems like pityriasis versicolor, folliculitis, atopic dermatitis including dandruff are caused by *Malassezia* species under suitable environmental conditions. Taxonomic revision has divided the genus *Malassezia* into seven different species- *M. globosa*, *M. restricta*, *M. obtuse*, *M. sloofia*, *M. sympodialis*, *M. furfur* and *M. Pachydermatits*³. Among these, *M. furfur* is one of the main causative agents of dandruff. Currently available treatment options for the management of dandruff include the therapeutic use of zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, sulphur and tar derivatives. Of all the synthetic agents, ketoconazole (an imidazole derivative) has become the leading contender among various treatment options of dandruff^{1, 3}. It is a broad-spectrum antimycotic agent active against *Malassezia* and it is also effective in many dermatomycoses, including pityriasis versicolor². However, these agents have certain limitations, either due to poor clinical efficacy or due to the compliance issues. Furthermore, these drugs are unable to prevent recurrence, which is the most common problem³. Such complications have prompted the search for novel and effective antifungal agents of natural origin. *Morinda citrifolia* fruits have been traditionally used by Polynesians and Indians for over 2000 years in the

treatment of a wide range of diseases and disorders. The juice can be used as an alternative form of medicine for treating ailments such as arthritis, diabetes, high blood pressure, muscle aches, menstrual difficulties, senility, poor digestion, atherosclerosis, heart diseases, AIDS, cancers, blood vessel problems, and even drug addiction. It has been reported that fruit, leaf and root extracts have a wide variety of pharmacological activities such as analgesic, anti-inflammatory, anti-oxidant, immunomodulator, anti-tumor, hepato-protective, blood pressure lowering and vasodialator, cardio protective, antifungal, wound healing and anti-osteoporotic activity.⁴ *Morinda citrifolia* contains a number of vitamins, minerals, enzymes, and alkaloids. Xeronine, a very essential alkaloid is involved in the functioning of all body cells and allowing the proteins to carry out their fundamental duties. It is said to be the body's supreme pain killer and a sedative as it works with endomorphins to ease pain and causes sense euphoria.⁵ The potential of essential oils as antimicrobial agents is well established. Essential oils are mixtures of volatile secondary metabolites isolated from different parts of aromatic plants⁶. The aim of this study was to evaluate the effect of Noni juice, against a pathogenic fungi *Malassezia furfur* and develop a new formulation based on validated antimicrobial activity of alone or in combination with *Morinda citrifolia* L(Noni).

MATERIALS AND METHODS

The fungal culture was isolated from the collected dandruff flakes. The isolate was maintained in Sabouraud's dextrose agar (SDA).⁷ The morphology of the lipophilic yeast cells were studied by Gram staining and Nigrosin stained, smears of the isolates from Sabouraud's dextrose agar and modified Dixon's media after 3-4 days of incubation at 37°C.⁷ Sabouraud's dextrose broth, Sabouraud's dextrose agar were used to grow and maintain the isolated fungal culture.

Table 1
Natural products and their source used in the experiment

Natural products	Family	Part used
<i>Morinda citrifolia</i> (Noni Juice)	Rubiaceae	fruit
<i>Citrus limon</i>	Rutaceae	Fruit
<i>Cinnamomum camphora</i>	Lauraceae	Commercial product
<i>Trigonella foenum-graecum</i>	Fabaceae	Seeds
<i>Syzygium aromaticum</i>	Myrtaceae	Bud
<i>Allium sativum</i>	Amaryllidaceae	Clove
<i>Menthaspicata</i>	Lamiaceae	Leaves
<i>Brassica nigra</i>	Brassicaceae	Seeds
<i>Aloe vera</i>	Xanthorrhoeaceae	Leaves
<i>Vignaradiata</i>	Fabaceae	Seeds
<i>Azadirachta indica</i>	Meliaceae	Leaves
<i>Piper nigrum</i>	Piperaceae	Seeds
Vinegar (Acetic acid)		

Preparation of Natural samples

Commercially available Noni juice was used for the investigation of antifungal activity. Crude extracts were prepared from all the natural samples. *Citrus limon* was squeezed and fresh juice was obtained. The seeds of *Trigonella foenum-graecum*, *Brassica nigra* and *Piper nigrum*; the buds of *Syzygium aromaticum*; the cloves of *Allium sativum* and the leaves of *Mentha*

spicata were crushed using motor and pestel and distilled water was added to the powdered forms of these three samples. The crystalline cube of *Cinnamomum camphora* was crushed and mixed with Coconut oil. *Aloe vera* leaves were cut and the gel was extracted for further use. The seeds of *Vigna radiata* and the leaves of *Azadirachta indica* were soaked separately in sterile D/W overnight and the water of

both these samples were used for their antifungal property.⁹

Antimycotic activity Assay using Well Diffusion Method

For plating, Sabouraud's dextrose agar agar was prepared, autoclaved and dispensed into sterilized petri plates. After solidification of the agar, 100 μ L of *Malassezia furfur* inoculum from broth culture was added in the centre of the petri plate using a micropipette and spread evenly on the agar using a sterilized glass spreader. Four wells were bored into the agar at four corners of the plate, using well borer of 8mm, taking care that the wells did not lie in close proximity to the edges of the petri-plate or to each other³. Each of the four wells represented the standard viz. Selsun (Selenium sulfide) shampoo, and three different natural samples respectively (Fig1). Using a micropipette 50 μ L of samples were added to the three sample wells. In the well representing the standard 50 μ L of Selsun shampoo was added. The plates are placed in the refrigerator for 30 min and then transferred to an incubator set at 37⁰C. After 24 hrs of

incubation, the zone of inhibition was measured (diameter in mm).The experiment was conducted in triplicates for each sample.³

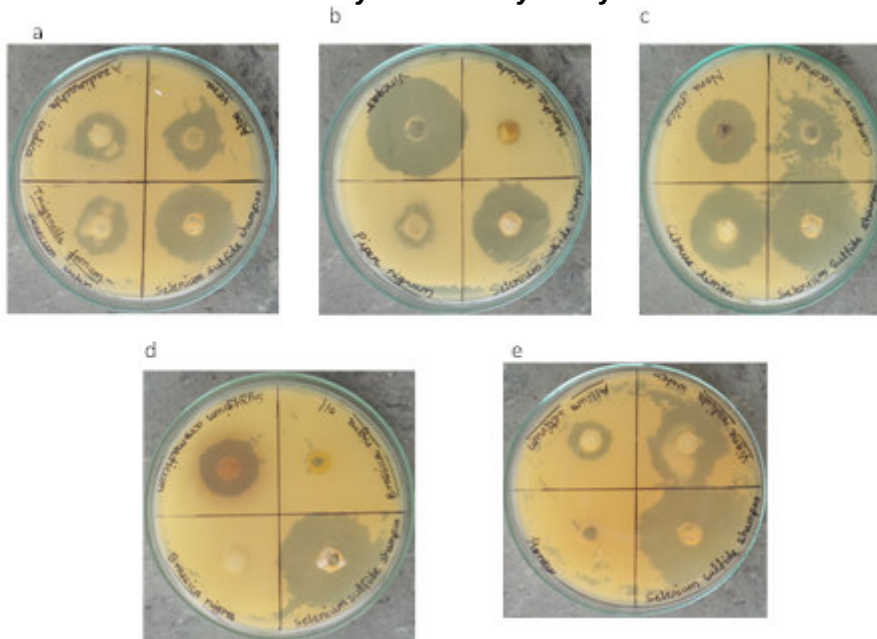
RESULTS AND DISCUSSION

The antifungal activity of certain bioactive compounds 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. In addition, medicinal plant oils are the promising sources of antifungal drugs. Based on these facts, the present study emphasizes the importance of plants essential oils as an alternative antimalassezia agent against pathogenic fungi causing dandruff Medicinal plants are increasingly of interest as antimicrobial agents and have been widely used in traditional medicine. The results of our study on antimycotic activity of different plant extracts and standard products used as anti dandruff agents are summarised in Table 2.

Table 2
Zone of inhibition of the natural products studied with their respective concentrations

Natural samples	Concentration	Zone of Inhibition (diameter in mm) and standard Deviation
Morinda citrifolia (Noni Juice)	Concentrated	29.0 \pm 0.12
Citrus limon	Crude extract	29.5 \pm 0.67
Cinnamomum camphora	1 cube/1ml coconut oil	20.5 \pm 0.78
Trigonella foenum-graecum	Concentrated	21.5 \pm 0.47
Syzygium aromaticum	100mg/1ml d/w	20.0 \pm 0.53
Allium sativum	100mg/1ml d/w	15.5 \pm 0.86
Mentha spicata	Concentrated	0
Brassica nigra	100mg/1ml d/w	0
Brassica nigra oil	Concentrated	0
Aloe vera	Crude extract	19.5 \pm 0.34
Vigna radiate	Concentrated	21.0 \pm 0.23
Azadirachta indica	Concentrated	19.5 \pm 0.34
Piper nigrum	100mg/1ml d/w	14.5 \pm 1.3
Vinegar (Acetic acid)	Concentrated	34.0 \pm 0.67
Honey	Concentrated	0
Selsun Shampoo (standard)	Concentrated	30.0 \pm 0.05

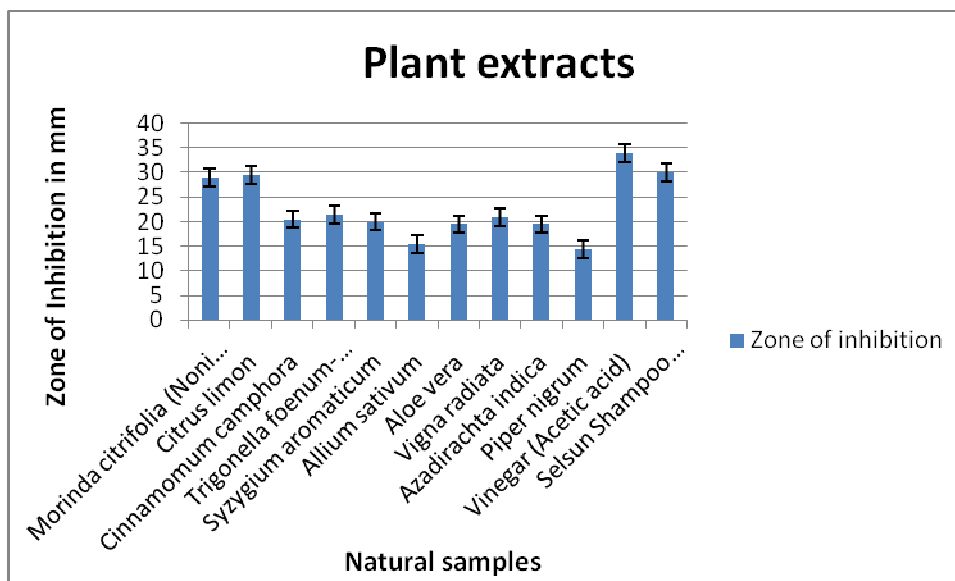
Figure 1
Antimycotic activity assay



- a. Zone of inhibition of *Trigonella foenum-graecum*, *Azadirachta indica* and *Aloe vera*
- b. Zone of inhibition of *Piper nigrum* and Vinegar (Acetic acid)
- c. Zone of inhibition of *Citrus limon*, Noni juice and Camphor cube mixed in Coconut oil
- d. Zone of inhibition of *Syzygium aromaticum*
- e. Zone of inhibition of *Allium sativum* and *Vigna radiata*

According to the antifungal activity assayed, vinegar and *Citrus limon* showed higher antifungal activity with the zone of inhibition of 34 mm and 29.5 mm respectively. This may be due to their high acidic content present in them. Noni juice was also successful in showing better results as compared to other products used with the zone of inhibition of 29 mm. Selenium sulfide (Selsun shampoo) showed an inhibition of 30 mm. Our results revealed that out of the 14 products assayed, 6 showed positive anti mycotic activity. The activity of *Morinda citrifolia* (Noni juice) was comparable to that of commercially available antidandruff shampoo and has potency against *Malassezia furfur* (Fig 2).

Figure 2
Graph1. Zone of inhibition in diameter mm against the natural products



CONCLUSION

Seborrhoeic dermatitis is a superficial skin infection caused by the human micro flora namely *Malassezia furfur*, a yeast that thrives on sebaceous glands-rich

areas. It depends on lipid for its growth, proliferation and metabolism. A variety of anti-mycotic agents have been utilized against *M. furfur* in vitro such as pyrithione zinc, selenium sulfide and ketoconazole, etc. However, these synthetic antifungal agents have

limitations due to poor efficiency and recurrences. Hence a lot of research has been carried out involving the application of plant products of medicinal origin. The present study has succeeded in demonstrating the anti-mycotic activity of Noni juice against *Malassezia*

furfur using well diffusion method. Based on the observations and results, it can be concluded that Noni greatly inhibits the growth of *M. furfur* and therefore, can be effectively used as an alternative to synthetic anti-mycotic agents.

REFERENCES

1. Arora P., Nanda A. Screening of plant essential oil against antifungal activity against *Malassezia furfur*. Int J Pharm Pharm Sci, 5(2):37-39,(2013).
2. Naveen S. In-vitro evaluation of herbal and chemical agents in the management of dandruff. J Micro and Biotech Res, 2 (6):916-921,(2012).
3. Sarika K.M., Aparajita V., Herbal extracts and their antifungal activity against *Malassezia furfur*. Int J Pharm Bio Sci, 4(3):969 – 974,(2013).
4. Brett J. W., Stephen K. P., Shixin D., Afa K.. P. Antimicrobial Activity of an Iridoid Rich Extract from *Morinda citrifolia* Fruit. Current Res J of Biol Sci, 4(1): 52-54,(2012).
5. Usha., Sangeetha., Sashidharan., Palaniswamy M., Antimicrobial Activity of a Rarely Known Species, *Morinda citrifolia* L., Ethnobotanical Leaflets, 14: 306-311, (2010).
6. Selvam P., Raj K., Vimisha V., Hari Krishnan R., Saria K.S., Umalekshmi R. Antimicrobial Activity of Fruit Extracts of *Morinda citrifolia*. J of App Chem Res, 10: 61-63. (2009)
7. Ram K., Bachani P., Kothari V. *In vitro* antimicrobial activity of certain plant products seed extracts against multidrug resistant *Propionibacterium acnes*, *Malassezia furfur*, and a flatoxin producing *Aspergillus flavus*. Res in Pharma, 2(3) : 22-31, (2012).
8. Jeong-Hyun .L., Jae-Sug L. Chemical Composition and Antifungal Activity of Plant Essential Oils against *Malassezia furfur*. Kor. J. Microbiol. Biotechnol, 38 (3): 315-318, (2010).
9. Anitha A., Sreedevi P., Arunkumar D. *In vitro* Evaluation of the indigenous Medicinal Plants for their Antidandruff Hair oil Preparation. Global J of Pharmacol, (7(4):429-435,(2013)