RESEARCH PAPER

Formulation and Evaluation of Anti-Fungal Cream Containing Azadirachta indica Extracts Against Candida albicans and Trichophyton rubrum

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ABSTRACT

Fungal infections pose a significant challenge to public health, necessitating the development of effective and safe antifungal agents. Herbal remedies have garnered attention for their potential antimicrobial properties, including antifungal activity. In this study, we formulated antifungal cream containing Azadirachta indica extracts. The cream was prepared using a standardized method and evaluated for its antifungal efficacy against Candida albicans and Trichophyton rubrum using agar well diffusion and broth dilution methods. Additionally, physicochemical properties such as pH, homogeneity, consistency, and washability were assessed to ensure product quality and consistency. Overall, our findings suggest that the antifungal cream containing Azadirachta indica holds great potential as a natural alternative for the management of fungal infections.

Keywords: Antifungal cream, Azadirachta indica extracts, Candida albicans, Trichophyton rubrum

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INTRODUCTION

Fungal infections cause about 1.7 million deaths worldwide mainly in immunocompromised individuals with two or more pathological conditions. The incidence of infections caused by the genus *Candida* has steadily increased since the 1970s perhaps due to an increased risk of opportunistic infections, the improvement in clinical procedures that identify fungi causing nosocomial infections, as well as the development of antifungal resistance due to prolonged exposure to treatment (V.K. Chin *et al.,*2016**)**

There are currently more than 150 species of *Candida*, and approximately 20 are known to cause infections in humans. *Candida albicans* is the main causative agent of candidiasis and the primary fungal infection in adults and pediatric patients. In INDIA, it was reported that sepsis caused by *C. albicans* has a mortality rate of approximately 40%, which is higher than any other sepsis caused by bacteria or fungi Infections caused by the genus *Candida* are the main cause of nosocomial fungal infections especially in tertiary care hospitals (M. Dadar et al.2018). In particular, *C. auris* is considered an emerging serious global health threat by the Centers for Disease and Control Prevention (CDC) because of its multidrug resistance. However, *C. albicans* stands as the major fungal pathogen of humans. (J.C.O. Sardi *et al.,* 2013). T. rubrum is the most common cause of dermatophytosis; however, in some parts of the world, particularly the Indian subcontinent and also recently associated with emerging terbinafine resistance. T. rubrum can cause dry, annular scaly plaques on the feet (tinea pedis), corporis, cruris, and nails (onychomycosis), with relatively mild and gradually progressing lesions. (Sonego, B *et al*., 2024)

The neem tree *(Azadirachta indica)* has been thought to have miraculous health-promoting qualities. Truth be told, there is evidence that neem was used for medicinal purposes as far back as 4,500 years ago. Its use dates back to ancient India and surrounding countries, where it has long been revered as one of the most useful plants on the planet. The neem tree is still renowned as the "Village Dispensary" since all parts of it are recognized to have unique medicinal potential. Neem tree belongs to the family Meliaceae which is found in abundance in tropical and semitropical regions like India, Bangladesh, Pakistan, and Nepal. It is a fast-growing tree with 20–23 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound, imparipinnate, with each comprising 5–15 leaflets. Its fruits are green drupes which turn golden yellow on ripening in the months of June–August. (Lee *et al* 2015)

It has therapeutics implication in diseases cure and formulation based on the fact that neem is also used to treat various diseases. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol Quercetin and ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Mohit Solanki et al.,2019). The exact molecular mechanism in the prevention of pathogenesis is not understood entirely. It is considered that *Azadirachta indica* shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin (Govindachari T. R et al., 1998).

Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective herbal antifungal compounds has been used in the treatment of a variety of dermatological skin infections and candidiasis. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels and lotions however, oral antifungals are associated with adverse effects that can cause patients to discontinue treatment, which may be complicated by the presence of comorbid conditions. Lim (EH et al., 2014). Therefore, In the present study we formulate new antifungal cream using neem extracts for the treatment of dermatophyte and candida infections.

MATERIALS AND METHODS

Collection, authentication and preparation of the extract

Fresh Neem leaves (*Azadirachta indica*) (4Kg) were collected from vallam, Thanjavur. The plant materials were authenticated by Asso Prof Dr Anbu jeba sunilson, Department of Siddha medicine, Tamil University. Then leaves were shade dried for few days at room temperature and powdered with a grinder. Dried powder (150 gm) of *A. indica* leaves was mixed with 70% ethyl alcohol and kept at room temperature for 36 hr. The slurry was stirred intermittently for 2 hr and left overnight using mechanical stirrer. The mixture was then filtered and the filtrate was concentrated using water bath at 50°C and finally dried to form the extract which is kept for phytochemical screening (Akpuaka, A, 2013).

Preliminary Qualitative Phytochemical Analysis

Quality analysis done on the 75% ethanol extract of the leaf extract of *Azadirachta indica* and the presence of various phytochemical constituent such as alkaloids, glycosides, flavonoids, steroids, , terpenoids, tannis, saponins, was detected by using standard methods.

Collection of Fungal Strain

The fungal strains, *Candida albicans* and *Trichophyton rubrum* were obtained from Microbiology laboratory, Kavuery Hospital, Tiruchirappalli.

Maintenance of Inoculum

The stock culture of each strain was stored in Potato dextrose agar at 4˚C. To maintain the stock culture, the fungal were sub-cultured separately on prepared Potato dextrose agar media and maintained at 4˚C.

Standard drug

Fluconazole (150mg) have been used as standard antibiotic for fungal infections. It disrupts fungal cell membrane by inhibiting the synthesis of ergosterol

Determination of Invitro Anti-fungal of *Azadirachta indica* Leaf Extract against *Candida albicans* and *Trichophyton rubrum* by using Well Diffusion Technique

Agar well diffusion technique was used to identify the antifungal activity of plant extract of ***Azadirachta indica*** .The Muller Hinton agar media plate was labelled with the 75% ethanol extract standard and negative control. Firstly, the Mc Farland solution was prepared. A loop full of organism was taken from the

stock culture and was diluted into the prepared Mc Farland solution. The cloudiness of mixture was compared with the normal saline. This was done to ensure the accuracy of the concentration of the organism was prepared to be used for the streaking on the Muller Hinton agar plate in well plate method. Inoculation was done by spreading a volume of *Candida albicans* and *Trichophyton rubrum* over the entire agar surface by using a cotton swab. A well of 8mm diameter was made with a sterile cork aseptically on the agar plate. Then, A volume of standard volume in concentration of 150mg/ml and ethanol extract (500mg) was introduced into the well. The agar plate was incubated under 30˚C for5 to 7 days. The antifungal agent diffuses in the agar medium and inhibits the development of the fungal strain. The zone of inhibition was measured in millimetre. The experiment was done in triplicates (Kumar Yadav. et al.2022).

Determination of minimum inhibitory concentration (MIC) of *Azadirachta indica* against *Candida albicans* and *Trichophyton rubrum.*

Broth dilution method was used to determine the value of minimum inhibitory concentration. The extract was serially diluted with 1ml of Muller Hinton broth to give concentration of (1000mg/ml, 500mg/ml, 200mg/ml, 62.5 mg/ml, and 31.25 mg/ml). Each test tube containing plant extract was inoculated with 1ml of fungal strains. The test tubes were incubated at 30˚C for 5 to 7 days and the results were observed. The minimum inhibitory concentration that does not have any fungal growth was selected as MIC.

Formulation of the herbal antifungal cream

The formulation containing and *A. indica* was formulated by the following method: Different amount of ingredients was incorporated together in 2 phases i.e. oil phase and aqueous phase separately. The oil phase consists of liquid paraffin, bees wax, stearyl alcohol, tween-80 and stearic acid while the aqueous phase was composed of methyl paraben, sorbitol solution and potassium hydroxide. Both aqueous and oil phases were heated to 75 °C on a water bath separately. The aqueous phase was then added drop wise to the oil phase with continuous stirring and finally the herbal extracts of *A. indica* were incorporated in the emulsion. Gradually temperature was decreased with continuous stirring and emulsion was formed which was then stored in the air tight wide-mouth container.

Physical Evaluation of formulation

The physical properties of the cream formulation were evaluated by measuring pH, assessing homogeneity, consistency, and washability. pH was measured using a digital pH meter after dissolving the cream in distilled water. Homogeneity was assessed visually and by touch, while consistency was determined visually. Washability was checked manually after application to the skin. Finally, antifungal activity was evaluated by measuring the zone of inhibition.

Statistical Analysis

The data was expressed as mean ± S.E.M. The assessment for MIC study was performed in triplicate and the data was subjected to one way analysis of variance (ANOVA) using Dunnett 'T' test and p values < 0.05 was considered as significant

RESULTS

The colour, consistency and percentage of yield of ethnolic extract of *Azadirachta indica* were shown in the Table 1 and Fig 1

Table No 1 The nature and yield percentage

No	Extract	Colour	Consistency	% Of Yield
1	Ethanol	Dark green	Semisolid	57.84

Fig 1. Ethnolic extract of *Azadirachta indica*

Phytoconstituents Present in the Leaf Extract of *Azadirachta indica*

Qualitative phytochemical screening of Azadirachta indica (neem) leaf extract revealed the presence of several bioactive compounds. The ethanol extract tested positive for alkaloids, flavonoids, steroids, tannins, and saponins. However, terpenoids were not

detected in the extract. These findings suggest that neem leaves contain a variety of potentially beneficial phytochemicals.

Invitro antifungal activity of ethanol extract of *Azadirachta indica* against *Candida albicans*, and *Trichophyton rubrum.The results were shown in Table 2and Fig 2*

Test Organism	**Standard Drug (Fluconazole 150 mg/ml)**	**Ethanol Extract (500 mg/ml)**
CANDIDA ALBICANS	26±0.88	24±0.88*
TRICHOPHYTON RUBRUM	17±1.45	25±0.58**

n= 3; ***p< 0.001 = high significant, **p< 0.01 = significant and *p< 0.05 = less significant

Minimum Inhibitory Concentration (MIC) Activity of ethanol extract of *Azadirachta indica*

The MIC values of ethanol extracts of **ethanol extract of *Azadirachta indica*** were determined by using dilution method.. The ethanol extract showed inhibitory effect against *Candida albicans* the concentation of 125mg/ml..The results were shown in Table 3.

Test organism	**Different concentration of ethanol extract of *Azadirachta indica* (mg/ml)**					**Standard drug**
	500	**250**	**125**	**62.5**	**31.25**	**Fluconazole (150 mg/ml)**
C.albicans	0.52±0.15	0.45±0.01	0.67±0.02**	1.28±0.02	1.68±0.06	0.38±0.01
T.rubrum	0.13±0.01	0.44±0.01	0.63±0.01**	1.25±0.01	1.35±0.02	0.32±0.02

n= 3; ***p< 0.001 = high significant, **p< 0.01 = significant and *p< 0.05 = less significant

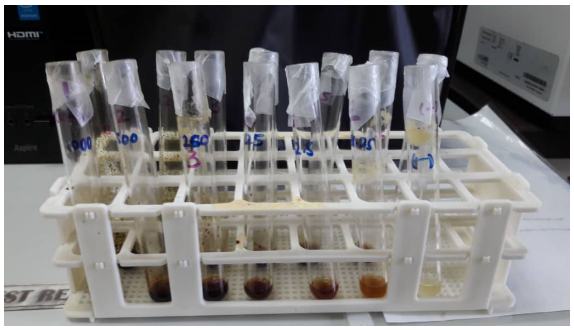

Fig 2. MIC test of different concentration of ethanol extract of *Azadirachta indica* against *Candida albicans and Trichophyton rubrum*

Evaluation of Formulated cream

The formulated cream was evaluated using various physicochemical parameters. The results were shown in Table: 4and Fig 3

Table 4 Physical evaluation of the cream

S NO	TEST	FORMULATED CREAM
1	Ph	6.57
2	Colour	Creamy Green
3	Consistency	Semi solid
4	Homogeneity	Homogenous
5	Washability	Good

Fig 3. Formulated *Azadirachta indica* cream

Antifungal activity of the formulation containing ethanolic extract of *Azadirachta indica* leaves

The results of antifungal activity revealed that the formulation of ethanolic extract of Azadirachta indica leaves exhibited significant antifungal activity. Both the standard sample and test sample were compared on the antifungal testing. The result showed good antifungal activity of formulated cream as shown in table 6 and fig 6

Test organisms	Zone of inhibition (mm)	
Candida albicans	Standard Flucanozole (150mg/ml)	Formulated cream containing *Azadirachta indica leaves*

	24±0.333***	20±0.333***
Trichophyton rubrum	22±0.333***	18±0.333***

Results presented the mean ± SEM value of n = 3; ***p<0.001=highly significant, **p<0.01 = significant *p<0.05=less significant

DISCUSSION

Invasive fungal infections, which include candidiasis, have improved in incidence international over the past two decades, and consequently, the use of antifungal drugs inclusive of azoles has extended (Borgers M et.al 2015). Antifungal drugs such as fluconazole and ketoconazole, have significant roles within the treatment of candidiasis, dermatophytosis and other invasive fungal infections but from time to time with using these marketers, clinically essential toxic effects including skin rash, nausea, pores, increased liver enzyme (for fluconazole) gynecomastia, adrenal insufficiency and hepatotoxicity (for ketoconazole) are visible. Overtime, beneath a few medical settings, the efficacy of azoles has decreased due to accelerated resistance to the antifungals (Ajello, L et.,al 2019)

Cold maceration was used as the method of extraction in the study. Ethanol used as the extraction solvents. These solvents were incorporated to extract the bioactive compounds from the leaf of the Azadirachta indica. Different solvents have different polarities which have ability to extract different hydrophobic and hydrophilic compounds in the samples. Ethanol is chosen because it is used for extraction various polar compounds and also certain group of non-polar compounds are fairly soluble in Ethanol. Other than that, it easily evaporates so it can be separated from the extract. Ethanol also brings out trace amounts of various substances from the plant (Aditi, G et.,al 2011) .

The phytochemical qualitative analysis revealed that alkaloid, protein, terpenoid, tannin, phytosterol, and saponin, were present in the ethanol extracts of of *Azadirachta indica* extract. In this study, the presence of alkaloid ,flavonoid, saponin, polyphenol and tannin in the extract exhibit the antifungal activity. Much of the protective effects of herbal plants have been attributed to their phytochemicals constituents alkaloids, flavonoids, glycosides, saponins exert multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, anti-viral and anti-cancer activities, anti-leprosy activities, antimicrobial activity (Singh V et al., 2014).

These research were conducted on antifungal activity of *Azadirachta indica* against *Candida albicans,* and *Trichophyton rubrum*. In the antifungal activity, the ethanol extract of *Azadirachta indica* showed efficient antifungal activity against *Candida albicans* (24±0.88 mm) and *Trichophyton rubrum* (25±0.58 mm) From this study we can conclude that, the ethanol extract of *Azadirachta indica* against *Candida albicans* are significant with p value of <0.05 while ethanol extract for *Trichophyton rubrum* are high significant with p value of <0.01. *Tricophyton rubrum* is known as dermatophyte, which are responsible for the superficial fungal infection while *Candida albicans* are yeast that causes opportunistic fungal infection that effects the immune system. The leaves of *Azadirachta indica* are used traditionally for the treatment of fungal infection. Quercetin and ß-sitosterol, polyphenolic flavonoids purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Govindachari T.R et al., 1998). Therefore, the leaves of *Azadirachta indica* can used against *Trichophyton rubrum and Candida* species.

The minimum inhibitory concentration (MIC) is regarded as the lowest concentration that needed to inhibit the fungal growth . In the present research, there is no growth for *Candida albicans* in methanol extract for the following concentration of 500mg/ml, 250mg/ml and 125mg/ml and it was identified by the absence of turbidity in Mueller Hilton broth. While no growth is observed at 500mg/ml, 250mg/ml, and 125mg/ml of methanol extract of *Trichophyton rubrum*. It was observed that the lower the extract concentration, the higher the visibility of fungal growth. The MIC should be done carefully as it can easily get affected by the environment, incubation condition and time of incubation (Al-Haj et al., 2009) The MIC value for methanol extract for both *Candida albicans* and *Trichophyton rubrum* is 125mg/ml. Thus, it revealed that the ethanol extract of plant *Azadirachta indica* exhibit inhibitory activity which can be detected by the absence of turbidity in the test tubes. From this study we can conclude that, the ethanol extract of *Azadirachta indica* against both *Candida albicans* (0.67±0.02) and *Trichophyton rubrum* (0.63±0.01) are highly significant with a p value of <0.01. This study also revealed that ethanol extract of *Azadirachta indica* shows significant antifungal activity against *Candida albicans* and *Trichophyton rubru*

A herbal formulation was prepared using *Azadirachta indica* extracts against *Candida albicans* and *Trichophyton rubrum.* A study of physical parameters were carried out and the results obtained were

satisfactory. The optimized formulation has pH 6.5, creamy green in colour, semi solid consistency and homogenous. From the above compiled data the study clearly shows that the formulation is showing good in-vitro anti-fungal activity against *Trichophyton rubrum* and *candida albicans.* The formulation of antifungal along with Neem extract exhibited enhanced rate of diffusion and antifungal activity (Mei X. Chen et al., (2016)

CONCLUSION

In conclusion, the ethanol extracts of *Azadirachta indica* leaves extract exhibited good antifungal activities and were capable of reducing growth of *Candida albicans* and *Trichophyton rubrum*. The in-vitro assessment of the plant extracts against the test organism and the phytochemical compounds present in the plants shows good inhibitory activity. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects. The herbal cream formulation of ethanol leaf extract of *Azadirachta indica* appeared to have more antifungal performance. Therefore, it is considered in future for clinical trials as a potential antifungal agent product for the treatment of fungal infection.

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