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ANTIFUNGAL ACTIVITY OF *NELUMBO NUCIFERA* EXTRACT AGAINST THE DERMATOPHYTES

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ABSTRACT

Nelumbo nucifera flower used in traditional systems of medicine, Natural products, especially plants have been used in the treatment of varies diseases for thousands of years. The present work deals with the antifungal activity of *Nelumbo nucifera* flower against the silver nanoparticles. Dandruff is a major public health burden in both developed and developing countries. *Nelumbo nucifera* (lotus) plant derived agents are being used for the treatment of dandruff. Aqueous extract of lotus flower *Nelumbo nucifera* (lotus) was studied for antifungal properties against the dermatophytes, such as *Trichophyton, Malassezia furfur*, and *candida*. Three different types of compounds were separated to different time intervals (C-I, C-II and C-III) by using column chromatography and tested for disc diffusion test. The compound – III gave the maximum zone of inhibition. The results were compared with silver nanoparticles (Ag-NPs). The antifungal activity was significantly increased in the Ag-NPs.

INTRODUCTION

Nelumbo nucifera is a monogeneric plant belongs to the family Nelumbonaceae, commonly known as sacred Indian lotus, *Nelumbo nucifera* is a perennial ornamental water plant grown in Asian countries for its edible rhizomes and seeds.

Dermatophytosis is a superficial fungal infection in keratinized substrates and caused by a group of filamentous fungi called dermatophytes. Among these fungi, *Trichophyton rubrum (T. rubrum)* is known to account for as many as 69.5% of all dermatophyte infections [2, 3, 4]. Silver or silver ions have long been known to have strong inhibitory and bactericidal effect as well as a broad spectrum of antimicrobial activities. It is expected that the high specific surface area and high fraction of surface atoms of silver nano particles will lead to high antimicrobial activity compared to bulk silver metal [8]. Silver nanoparticles (NPs). Recent studies revealed the effects of silver NPs on some species of fungai particularly *candida* genus. However, only few studies have been performed for the mention effects on dermatophytes fungai such as *Trichophyton rubrum* [1, 5] .To the best of our knowledge, there is no study carried out for other dermatophyte pathogens such as Malassezia *furfur, and Tinea capitis*. In this study, we investigate the effects of compound I, II, and III against the fungal pathogen. The significant compound are identified and compared with silver nanoparticles.

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MATERIALS AND METHODS

Collection of the plant

The plant *Nelumbonucifera* was collected from moist regions of Tirichirappalli District and identified by local flora. The flower were separated from the collected plant and dried under shade. After drying, it was powdered and used for our studies.

Continuous extraction – using column apparatus

Column chromatography is used to purify liquids by separating an organic solvent from a mixture of solvent. The flower extract was prepared by grinding the mixture in mortar pistol containing 22 ml of acetone, 3ml petroleum

ether and calcium carbonate. The pigments was filtered and mixed with 20 ml petroleum ether and 20ml of 10% aqueous sodium chloride solution. The separating funnel was shaken carefully and the lower layer was allowed to drain in to the beaker plug of cotton is placed to the bottom of the column so that silica and soil won't fall out. Slurry of silica was prepared and poured into the column carefully. It is allowed to settle and sand is added. The sample was added using a pasture's pipette carefully above the sand. The eluent is added on top of the sand .The mobile phase slowly flows down through the silica gel column by gravity leaving behind zone of colour and a component was eluted from the column.

Experimental procedures involved in the synthesis of silver nanoparticles using plant extract

10 g of *Nelumbo nucifera* flower were boiled in 100ml of distilled water contained in the conical flask. The resulting filtrate (12ml) was taken and treated with 88ml of aqueous 1 mM AgNO3 solution and incubated in dark condition, at room temperature. Appearance of brownish yellow coloured solution indicates the formation of AgNPs

Disc diffusion method

Antifungal activity of aqueous were evaluated according to Potato Dextrose Agar was poured onto sterile petri dishes of 90 mm diameter. The agar was allowed to set at ambient temperature. The antifungal activity of the extracts was tested against *Trichophyton rubrum, Malassezia furfur, and Candida sps.* Fresh fungal culture was spread on surface of the PDA plate with the swab.Five millimeter discs containing different concentrations of *Nelumbo nucifera* and silver nanoparticles extract (16, 32 and 64 µg/mL) were placed on cultured fungi on agar plates and incubated at room temperature for 7 days. At the end of incubation the diameter of the zone of inhibition was measured.

Chi – Square test (X^2)

In this study chi – square test (X2) was applied (9). The purpose of chi – square test (X^2) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002).

Results and Discussion

In the present study (Table I and Fig - I) showed that compound III were separated from *Nelumbo nucifera* extract by using column chromatography by different time intervals (C – I :30 mins, C – II : 2 hrs and C – III : 4 hrs). Antifungal activity of flower extract of *Nelumbo nucifera* were evaluated at three different concentration (16, 32 and 64 µg/mL), against three fungal strain (Trichophyton *rubrum Malassezia furfur, and Tinea capitis)* by using disc diffusion method. At 64 µg concentration were gave the maximum zone of inhibition *Trichophyton rubrum* (19mm) *Malassezia furfur* (15mm) *and Tinea capitis* (16 mm). These results were compared with the activity of Ag –NPs. The Ag-NPs (Table II and Fig - II) gave the maximum zone of inhibition like *Trichophyton rubrum* (20mm) *Malassezia furfur* (18mm) *and Candida sps* (16 mm). These results were compared with the standard fungal antibiotic (18 – 20 mm). The chi – square value obtained has 1.279 (compound III) and 0.999 (Ag-NPs). Which was less that the calculated table value X^2 (0.05) = 3.481 at 5%, level of significance. The bove results lead to the conclusion that the data is consistent with the Hypothesis and Diameter of zone of inhibition obtained from observed data showed similarities with experimental data [9]. The present study concludes that the silver nanoparticles gave the significant zone of inhibition.

The previous studies showed that observed during reduction reaction of the reaction medium shows clear conclusion of the presence of silver nanoparticles. The color of the reaction medium gradually stared changing to dark brown, which is due to the excitation of the surface Plasmon resonance during reduction. In present study is identification of silver nanoparticles due to the color change (Fig – III)

Many studies have shown the antimicrobial effects of nano- Ag [6, 7, 8] but the effects of nano-Ag against fungal pathogens of the skin including clinical isolates of *Trichophyton rubrum Malassezia furfur, and Tinea capitis.* Species are mostly unknown. The primary significance of this study is the observation that nano-Ag could inhibit the growth of dermatophytes, which cause superficial fungal infections. To our knowledge, this is the first study to apply nano-Ag successfully to dermatophytes. Secondly, the fact that preparation method of nano-Ag described here is cost-effective is also of importance. Therefore, it could be expected that nano-Ag may have potential as an anti-infective agent for human disease caused by dermatophytes.

Table 1. Zone of inhibition by column separated compound of *Nelumbo nucifera* flower powder against Dermatophytes

S.NO	Antifungal agent	Name of the fungi	µg	Standard (mm)	Observed (mm)	$X^2=(O-E)^2$ / E
1	*Nelumbo nucifera*	*T.rubrum*	64	18	19	0.555
2	flower powder	*M. furfur*	64	18	15	0.502
3		*Candida sps*	64	18	16	0.222

Table value X^2 (0.05) = 3.481
Chi square value significance at 5% level

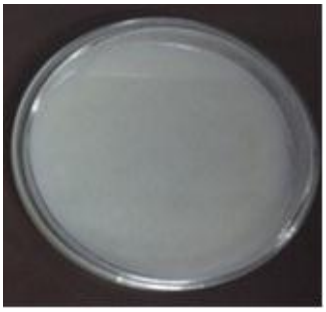
Table 2. Zone of inhibition by silver nanoparticles of *Nelumbo nucifer*a flower against Dermatophytes

S.NO	Antifungal agent	Name of the fungi	µg	Zone of the inhibition in diameter		$X^2=(O-E)^2$
				Standard (mm)	Observed (mm)	E
1.	Nelumbo nucifera silver nanoparticles	*T.rubrum*	64	18	20	0.222
2.		*M. furfur*	64	18	17	0.555
3.		*Candida sps*	64	18	17	0.222

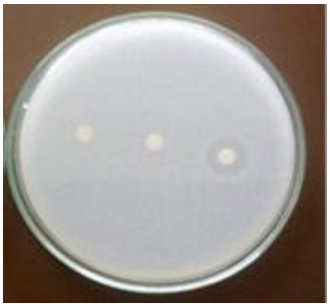
Table value X^2 (0.05) = 3.481
Chi square value significance at 5% level

Fig 1. Zone of inhibition by silver nanoparticles A - *Trichophyton rubrum* B - *Malassezia furfur,* C - *Tinea capitis*

Control



T. rubrum



64µg concentration

M.furfur

64µg concentration

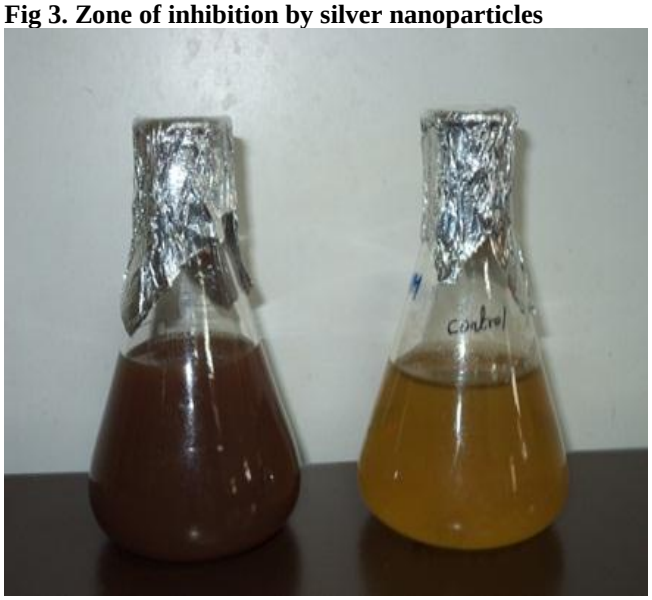
Candida sps

64µg concentration

Fig 2. Zone of inhibition by silver nanoparticles

A - *Trichophyton rubrum*
B - *Malassezia furfur*
C - *Tinea capitis*

Fig 3. Zone of inhibition by silver nanoparticles



Ag-NPs CONTROL
Due to the colour change

CONCLUSION

The present work concludes that 64µg concentration of *Nelumbo nucifera* powder more suitable to inhibit the dermatophytes such as *Malassezia furfur, Trichophyton rubrum* and *candida sps.* From the above preliminary study comparing with silver nanoparticles, we conclude that the silver nanoparticles from aqueous extract of *Nelumbo nucifera* proved to be one of the herbal remedies for dermatophytes. We recommend that the *Nlumbo nucifera* is more suitble to inhibit the dandruff organisms.

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