

SEPERATION OF SILVER NANO PARTICLES, ANANLYSIS OF IR-SPECTROSCOPY AND EFFECTS OF ANTIFUNGAL FOR *NELUMBO NECIFERA* SAMPLE

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ABSTRACT

The present paper deals with the separation of silver nanoparticles and analysis of IR-spectroscopy for *Nelumbo nucifera*. *Nelumbo nucifera* is flower for used in traditional systems of medicine. Natural products, especially plants have been used for the treatment of various diseases for thousands of years. Separation of Silver nanoparticles from *Nelumbo nucifera* flower. silver nanoparticles tested for antifungal activity and analysis of IR-spectroscopy. Result are compared studies to compound of *Nelumbo nucifera*. silver nanoparticles gave a maximum zone of inhibition. That samples analysis of IR – spectrum. The results identify the different functional groups. This IR- results alkene group (457987) present in all samples.

KEYWORD: silver nanoparticles, IR – spectrum analysis, Antifungal activity.

INTRODUCTION

Nelumbo nucifera is a monogeneric plant belongs to family Nelumbonaceae, commonly known as sacred Indian lotus, rose of India, sacred water lily or East Indian lotus. *Nelumbo nucifera* is a perennial ornamental water plant grown in Asian countries for its edible rhizomes and seeds. The species *nucifera* is the most important (commercially and culturally), it is critical to describe the American species. 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.^[18] Silver nanoparticles (NPs).

Recent studies revealed the separation of silver NPs and effects of silver NPs on some species of fungi particularly *candida* genus. The lotus is “friendly” to the body. The effects are always positive and never negative. Regardless of one’s place on the evolutionary ladder, the lotus always leads higher evolution.

MATERIALS AND METHODS

Collection of plant samples

The plant *Nelumbo nucifera* was collected from moist regions of Tiruchirappalli 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

Separation of silver nanoparticles from Flower

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.

Separation of compound using Column Chromatography

Column chromatography is used to purify liquids by separating an organic solvent from a mixture of solvent.

The leaf 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.

A 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

IR Spectrum Analyses

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600 cm⁻¹.

Procedure

FTIR spectrum of the natural *Nelumbo nucifera*, compound obtain from column chromatography, AgNPs from crude was done using Shimadzu IR Affinity 1 instrument.

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.

RESULTS AND DISCUSSION

Silver nanoparticles

The colour of the reaction medium gradually started changing to dark brown, which is due to the excitation of the surface Plasmon resonance during reduction reaction (Ahmad *et al*, 2003).

In the present study colour change of the aqueous flower extract by the addition of 1 mM AgNO₃ after different the reaction periods Zero hr, 12 hr and 24 hr were depicted in (figure-1). The verification of the presence of the silver nanoparticles was done by observing the visible in the UV – illuminator analysis result (Figure-1).

Infrared spectrum analysis

In present study Table –1 and Figure - II, III and IV showed that the compared with all the three figures, the Alkenes (1635.64) present for three samples.

Antifungal activity

In the present study 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 & III and Fig – V & VI) 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.

Table 1: Infrared spectrum analysis compared with all the three tables.

| S.NO | Peak Value | Stretching | Interpretation |
|------|------------|---------------|----------------|
| 1. | 1635.64 | C=CStretching | Alkenes |

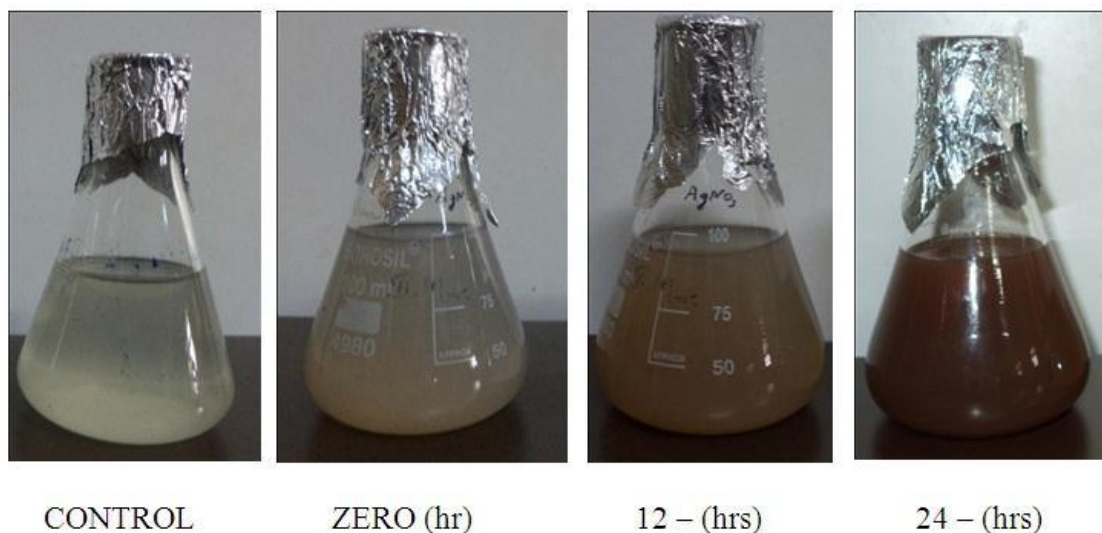


Figure 1: Synthesis of Silver nanoparticles from *Nelumbo nucifera* crude extract.

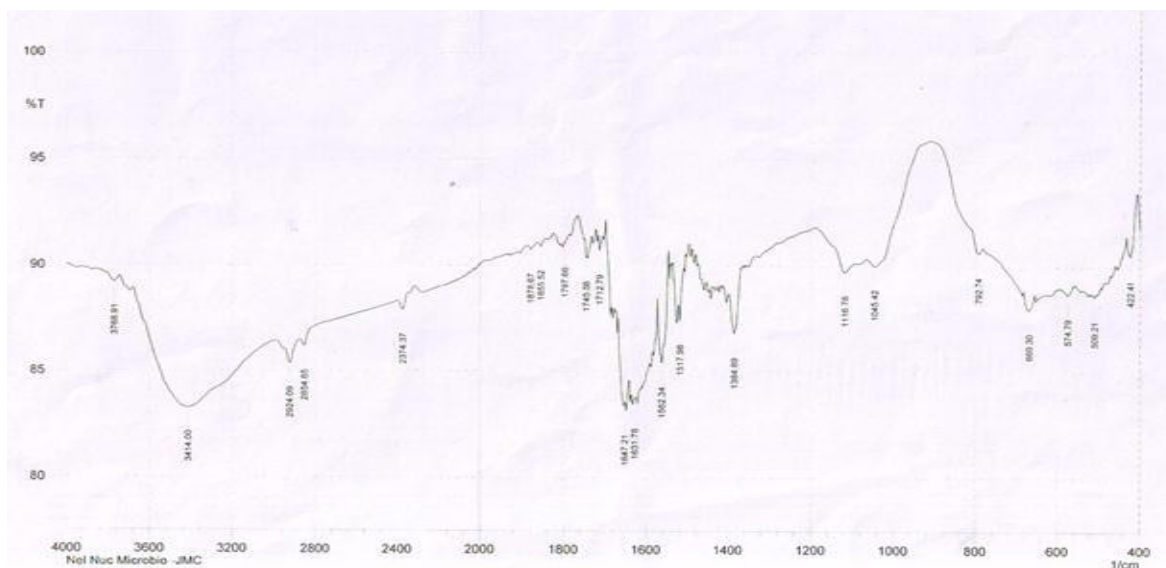


Figure II. Infrared spectrum analysis by *Nelumbo nucifera* flower powder (crude).

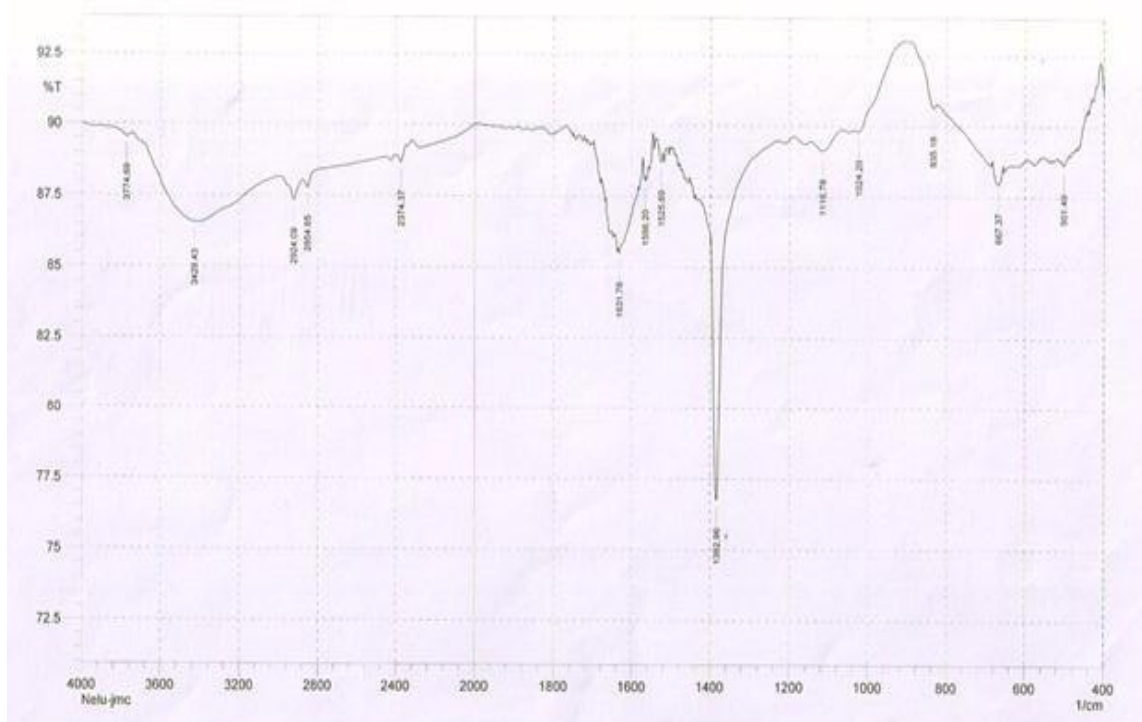


Figure III. Infrared spectrum analysis by Silver nanoparticles in *Nelumbo nucifera* flower.

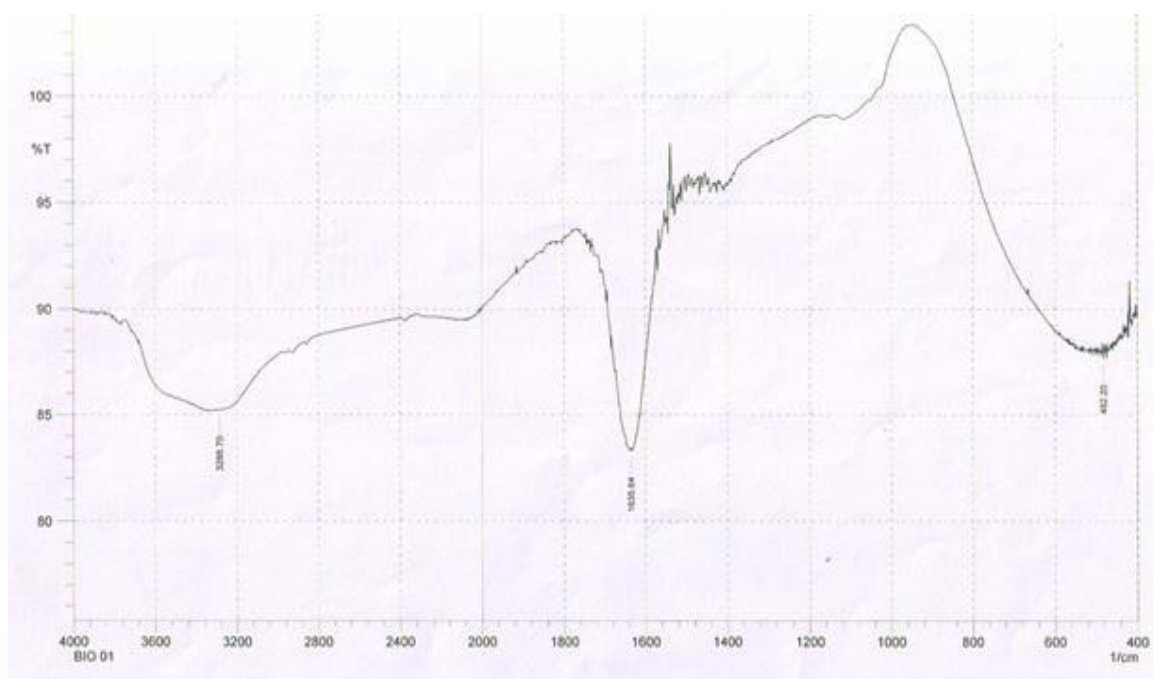


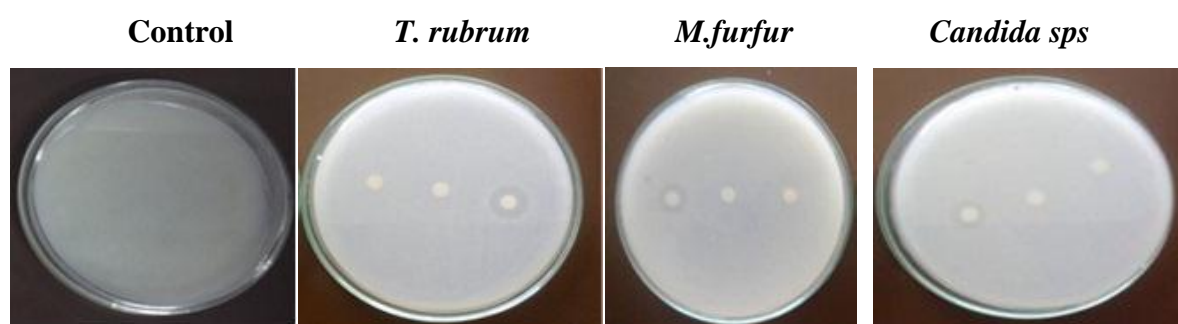
Figure IV. Infrared spectrum analysis by Compound obtained column chromatography.

TABLE – II: Zone of inhibition by column separated compound of *Nelumbo nucifera* flower powder 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. | <i>Nelumbo nucifera</i> flower powder | <i>T.rubrum</i> | 64 | 18 | 19 | 0.555 |
| 2. | | <i>M. furfur</i> | 64 | 18 | 15 | 0.502 |
| 3. | | <i>Candida sps</i> | 64 | 18 | 16 | 0.222 |

Table value X^2 (0.05) = 3.481.

Chi square value significance at 5% level.



64 μg concentration.

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

Table III. Zone of inhibition by silver nanoparticles of *Nelumbo nucifera* 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. | <i>Nelumbo nucifera</i> flower powder | <i>T.rubrum</i> | 64 | 18 | 20 | 0.222 |
| 2. | | <i>M. furfur</i> | 64 | 18 | 17 | 0.555 |
| 3. | | <i>Candida sps</i> | 64 | 1 | 17 | 0.222 |

Table value X^2 (0.05) = 3.481.

Chi square value significance at 5% level.

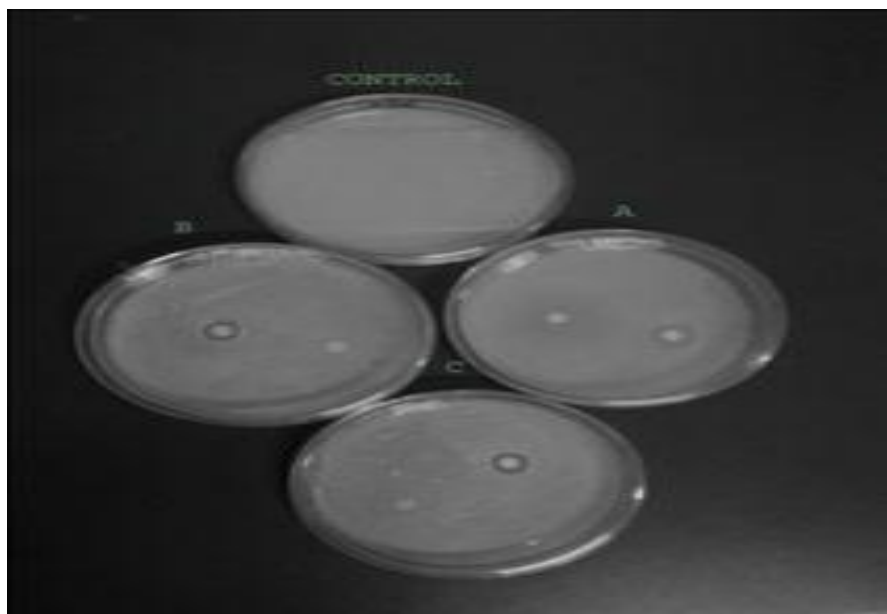


Fig VI: Zone of inhibition by silver nanoparticles.

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

DISCUSSION AND CONCLUSION

In the earlier study Saengkhae *et al*^[12,13] first found that the clarification activity *Nelumbo nucifera* flower has considerable reputation as a potent adjunct in the treatment of various ailments such as cancer, hypertension, diarrhoea, fever, weakness, infection and body heat imbalance.

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 suitable to inhibit the dandruff organisms.

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