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Full Length Original Research Paper

A study on synthesis of silver Nanoparticles from *Murraya koengii* leaf and it's Antifungal activity

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Abstract: The herbal plant *Murraya koengii* is currently used in medicinal practices for treating various diseases. The present study was carried out to synthesize silver nanoparticles from the leaves of *Murraya koengii* and its antifungal activity was determined against four micro organisms. From this present study, it was found that the silver nanoparticle which was synthesized from plant extract shows highest activity against *Trichoderma* and *Rhizopus* and mild activity against *Aspergillius niger* and *Aspergillus flavus*. The highest zone of inhibition was observed in *Trichoderma*. But the silver nanaoparticle was more active against *Rhizopus* than the standard antibiotic Amphotericin and plant extract.

Keywords: Silver Nanoparticle, *Murraya koengii*

Introduction

Nanotechnology refers to the research and technology development at atomic, molecular, and macromolecular scales, which leads to the controlled manipulation and study of structures and devices with length scales in the range of 1—100 nanometers. In the last two decades, the research of nanotechnology has grown explosively with over three hundred thousands publications in the field of nanoscience according to Web of Science [1]. Nanoparticles play an important role in drug delivery, diagnostics Imaging, sensing, gene delivery, artificial implants and tissue engineering[2]. Chemical reduction is the most frequently applied method for the preparation of silver nano particles ($AgNO_3$) as stable, colloidal dispersion in water or organic solvents, commonly used reductants are borohydride, citrate, ascorbate and elemental hydrogen. The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameter of several nanometers [3]. Micromolar doses (1 to 10 μM) of silver ions are sufficient to kill bacteria in water [4],while silver can be toxic at high doses to mammals and freshwater and marine organisms probably compromising the growth and shape of animal cells by disrupting a variety of biological functions. Such micromolar concentrations of silver have no harmful effects on humans [5]. Therefore, silver has been widely used for the development of many biological and pharmaceutical processes, products, and appliances such as coating materials for medical devices [6], orthopedic or dental graft materials [7,8], topical aids for wound repair [9], water sanitization [10], textile products [11], and even washing machines [12].

Murraya koenigii (L.) is a small spreading shrub in the family of Rutaceae. *Murraya koenigii* or curry

leaf tree, a native of India and Sri Lanka, is a small tree with very pungent aromatic leaves. The leaves are used in curries.

MATERIALS AND METHODS

Collection of plant material

Murraya koenigii was collected from Malappuram district of kerala.

Preparation of plant extract

Murraya koenigii leaf was thoroughly washed in distilled water, dried, cut in to fine pieces and were powdered. 20gm of the dried powder were weighed and dispensed in to 100ml distilled water. Then the leaf extract was collected in a separate conical flask by kept in a shaker for 48 hrs and the extract was again filtered through Whatmann's No.1 filter paper.

Preparation of silver nanoparticle

1mM silver nitrate solution was prepared and used for the synthesis of silver nanoparticles. 10ml of the plant extract was added in to 90ml of aqueous solution of 1mM silver nitrate for the reduction in to Ag^+ ions. Immediately after the addition of silver nitrate the colour change of the leaf extract from green to dark brown was noted periodically. Then the extract was incubated at room temperature for further incubation till 28 hrs. The colour change from green to dark brown. After incubation, the silver nanoparticles were synthesized from the leaf and centrifuged at 10000 rpm for 20 minutes and the pellet was used SEM analysis and antifungal activity.

SEM analysis of silver nanoparticles

Scanning Electron Microscope (SEM) analysis was done using Hitachi S-4500 SEM machine. This film of the sample was prepared on a carbon coated copper grid by just dropping. A very small amount of the sample the grid, extra solution was removed using a blotting paper a film on and then the SEM grid were allowed to dry by putting it under mercury lamp for 5 minutes.

Antifungal activity

Four different pathogens were used to test the comparative antifungal activity of herbal nanoparticle. The clinical isolates like 4 fungi called *Aspergillus flavus, Aspergillus niger, Trichoderma* and *Rhizopus* were used. The medium is composed of the following component**:** Sodium chloride: 5 gm, Peptic digest of animal: 5 gm, Beef extract: 1 gm, Yeast extract: 2gm, Agar: 15 gm, Distilled water: 1000ml

1.0 gm of nutrient agar was weighed correctly and dissolved in 250 ml of distilled water. pH was adjusted to 7.2 and was autoclaved at 121˚C for 20 minute. 20 ml of molten agar medium was poured in to the sterile Petri plates and allowed to solidify.

RESULTS AND DISCUSSION

1. Biosynthesis of silver nanoparticles

Silver reduction is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles [13]. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. As the *murraya koengii* leaf extract was mixed with aqueous solution of the silver nitrate, it started to the change the colour from yellow to brown due

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to reduction of silver ion; which indicated the formation of the silver nanoparticles.

The flasks were observed periodically for change in colour from yellow to different shades of yellow and brown. The appearance of yellowish dark brown colour confirms the existence of silver nanoparticles. The results are depicted in table 1 and figure 1(a) shows the photographs of yellow colour of the extract (b,c) and (d) photographs shows the brown shade with silver nitrate at different time intervals.

TIME	COLOUR CHANGE
0Hr	Yellow
1Hr	Dirty orange
2Hr	Brown
3Hr	Brown
24Hr	Dark brown
28Hr	Dark brown

Table 1: Periodic colour change of the reaction mixture (*Murraya koengii*+$AgNO_3$)

Figure 1: Photographs of the reaction mixture at different time intervals

2. UV Spectroscopy

The Ag nanoparticles were characterized by UV-Visible spectroscopy. The bio reduction of Ag^+ to AgO was monitored by measuring the UV-Vis spectrum of reaction mixture (Silver nitrate + plant leaf extract) at different time intervals within the range of 420nm in the UV-Vis spectrophotometer [14].

Table 2: UV-Vis reading of reaction mixture

TIME	UV READINGS (420nm)
0 Hr	0.20
1 Hr	0.78
2 Hrs	1.49
3 Hrs	1.60
4 Hrs	1.72
16 Hrs	1.76
24 Hr	1.85
28 Hrs	1.90

3. SEM analysis

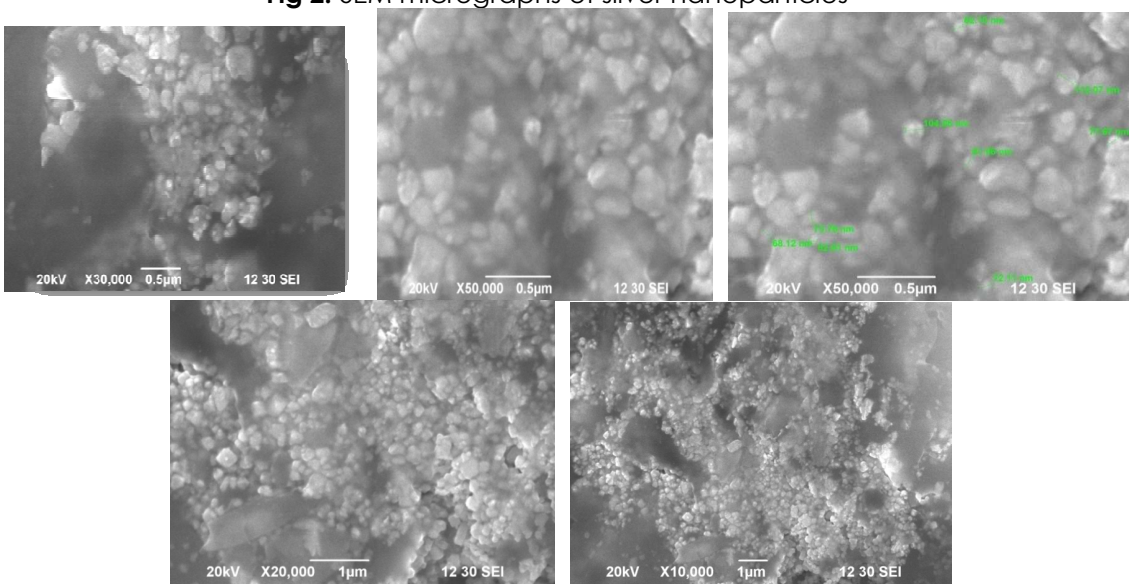
The silver nanoparticle solution thus obtained was centrifuged at 12,000 rpm for 15mins, after which the pellet was redispersed in deionized water to get rid of any uncoordinated biological molecules. The purified pellets were then freeze were then dried, powdered and used for SEM analysis.

To gain further insight in to the features of the silver nanoparticles, analysis of the sample was performed using SEM techniques. Scanning electron microscopy provided further insight in to the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameter of prepared nanoparticles in the solution was about 61.06-112.07.

Figure 2 show the scanning electron micrographs of silver nanoparticles obtained from the proposed bio reduction methods at various magnifications.

The SEM micrographs of nanoparticle obtained in the filtrate showed that silver nanoparticles are well distributed without aggregation in solution with an average size of about 61.06 - 112.07.

Fig 2: SEM micrographs of silver nanoparticles



4. Antifungal activity

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological msynthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects [15]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria and fungi of selected species.

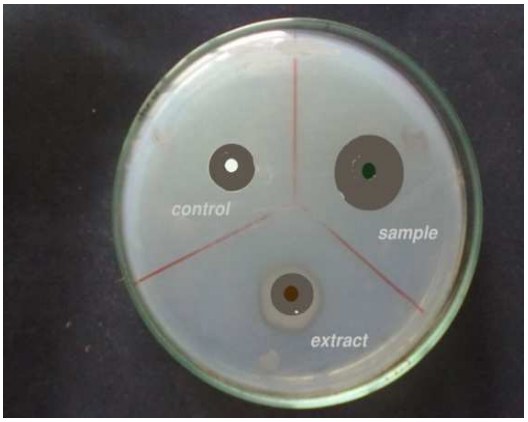
Nanoparticle synthesis by green route is found highly toxic against pathogenic bacteria. Antifungal effects of Ag nanoparticles obeyed a dual action mechanism of antifungal activity, i.e., the fungal effect of the polymer subunits.

Table 3: Effect of *Murraya koenigii L* silver nanoprticles on human Pathogen

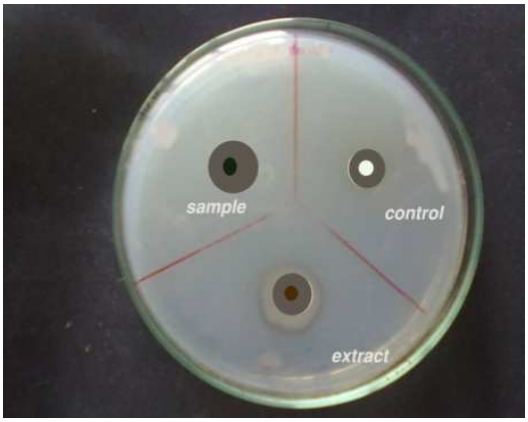
Name of the test organism	Zone of inhibition (mm) of various sample		
	STANDARD (Amphotericin)	EXTRACT (*Murraya koenigii*)	NANO PARTICLES (Silver) (1mMAgNO_3)
Aspergillus niger	3	4	9
Aspergillus flavus	3	4	8
Trichoderma	9	14	16
Rhizopus	8	7	10

Table 3 depicts the measurement of zone inhibition of silver nanoparticles against fungi. The present study clearly indicates that *Murraya koenigii L* possess antifungal activity.

Fig 3: Figure shows that the antifungal activities of nanoparticles, plant extract and Amphotericin antibiotic.



Aspergillus Niger



Aspergillus flavus

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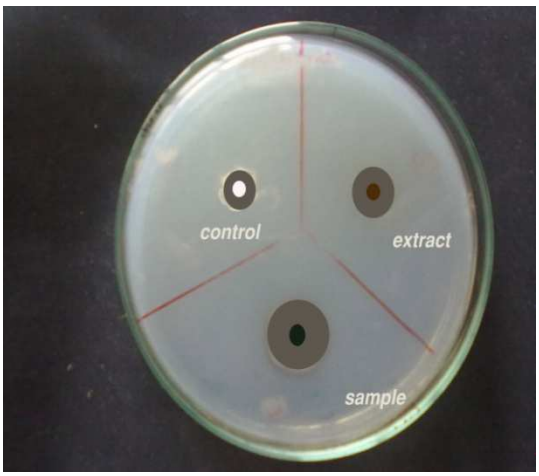
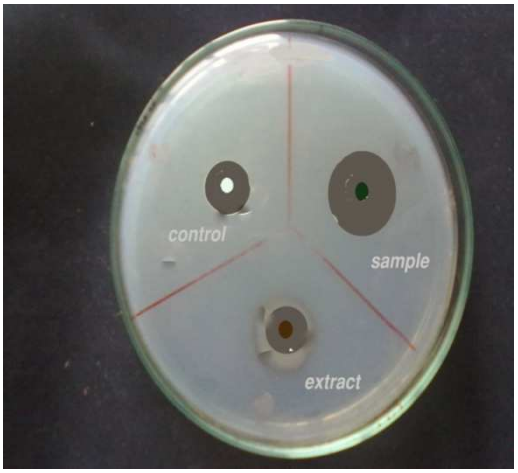


Trichoderma



Rhizopus

Sample: Silver nanoparticle **Extract:** *Murraya koengii* **Control:** Amphotericin

Conclusion:

The present study included the bio-reduction of silver ions through *Murraya koengii* plant extract and testing for its antifungal activity. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the change of colour of plant extract. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis spectroscopy. The results indicated that silver nanoparticles have good antifungal activity against different microorganisms. It is confirmed that silver nanoparticles are capable of rendering high antifungal efficacy and hence has a great potential in the preparation of drugs used against fungal diseases

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