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Antioxidant and Cytotoxic Activity of Red Sandal Mediated Silver Nanoparticles: An *In-vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Nanoparticles, or matter ranging between 1 and 100 nm size, are of immense value in nowadays technological ventures, in all fields. Besides, if made of biodegradable materials, the preparation supports sustained drug release for a long time. Green chemistry is thus incorporated in nanotechnology. Red Sandalwood, commonly known as Raktachandan, is a tree that is native and endemic to India. The aim of this study is to evaluate the antioxidant and cytotoxic activity of red sandal mediated silver nanoparticles (AgNP).
Materials and Methods: This study involves the synthesis of silver nanoparticles (AgNP) incorporating red sandal. Here the preparation of red sandal incorporated AgNP was followed by a test for its antioxidant activity using DPPH assay by incubating the red sandal mediated silver nanoparticles solution for 30 mins, later the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 MN. BHT was employed as control and the percentage of inhibition was determined and cytotoxicity using brine shrimps. To that 10 nauplii were added slowly to each concentration. Then the red sandal mediated silver nanoparticles were

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added according to the concentration level. The plates were incubated for 24 hours. After 24hrs, noted for the number of live nauplii's present.
Results: The red sandal mediated silver nanoparticle has considerably efficient antioxidant activity at high concentrations (50 µL). The red sandal mediated silver nanoparticle has considerably efficient cytotoxic activity at low concentrations (5 µL).
Conclusion: From the present study, it can be concluded that red sandal mediated silver nanoparticles have a considerably moderate cytotoxic and antioxidant activity at high concentrations.

Keywords: Red sandal; medicinal plant; antioxidant; cytotoxicity; green synthesis.

1. INTRODUCTION

Nowadays, the development of efficient green chemistry methods for synthesis of metal nanoparticles has become a major focus of researchers. It was investigated in order to find an eco-friendly technique for production of well-characterized nanoparticles. Among all other organisms, plants seem to be the best and are suitable for biosynthesis of nanoparticles [1]. Plant-mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants [2]. Nanotechnology involves the synthesis and application of materials having one of the dimensions in the range of 1–100 nm [3]. Materials in the nano dimensions (1–100 nm) have a remarkable difference in the properties compared to the same material in bulk.

Bio-molecules from various plant components and microbial species have been used as potential agents for the synthesis of silver nanoparticles (AgNPs) [4]. In recent years, silver nanoparticles (AgNps) have received enormous attention due to its extraordinary defense activity against a wide range of microorganisms and also due to the appearance of drug resistance against commonly used antibiotics [5]. The AgNPs had antibiotic activity against Gram-positive bacteria like *Staphylococcus aureus* and Bacillus cereus and Gram-negative bacteria like Escherichia coli and *Pseudomonas aeruginosa* and fungi like *Candida glabrata*, *Candida albicans*, and *Cryptococcus neoformans* so it is a agent for site-specific medication [6].

In this study red sandal is used, it is a valuable tree associated with Indian culture. A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system [7]. Red Sandalwood (*Pterocarpus santalinus* L.) belonging to the Family Fabaceae, is one of the most valuable medicinal plant species [8]. Red Sandalwood, commonly known as Raktachandan, is a tree that is native and endemic to India [9]. Red sandals have been reported as antioxidative, antidiabetic, antimicrobial, anticancer, anti-inflammatory properties as well as protective effects on the respiratory system, liver, nervous system and gastric mucosa [10]. In the traditional system of medicine, this red sandal is attributed various medicinal properties and has been used in treating eye diseases, mental aberrations, and ulcers [11]. Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects [12]. Many plant extracts have shown antioxidant properties but generally nowadays there is still a demand to find more information concerning the antioxidant potential of plant species [13]. Benzofuran compounds isolated from heartwood of red sandal showed cytotoxicity against Ca9-22 cancer cells with a lethal dose value [14]. Therefore it would be of interest to know the antioxidants and cytotoxic activity of red sandal mediated silver nanoparticles. Hence the aim of the study was to assess the antioxidant and cytotoxic activity of red sandal mediated silver nanoparticles.

2. MATERIALS AND METHODS

2.1 Synthesis of AgNP

To 100ml water, add 1g of red sandal powder and the mixture was heated for 15 – 20 min and filtered. Then 1 mM silver was added to the heated mixture and placed in the orbital shaker. The solution was later placed on the magnetic stirrer for nanoparticle synthesis. The colour change was observed visually and photographs were recorded (Figs. 1 and 2).

Fig. 1. Synthesis of nanoparticle

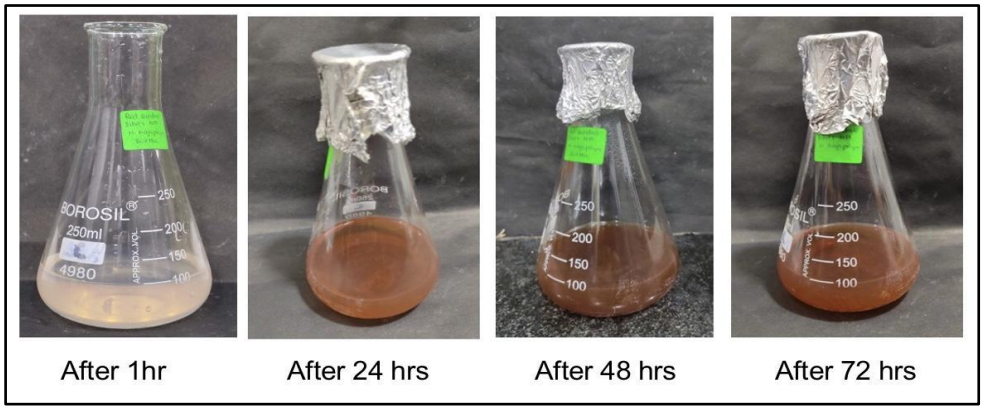

Fig. 2. Visual observation of the red sandal mediated silver nanoparticles

2.2 Assessment of Antioxidant Activity

DPPH assay was used to test the antioxidant activity of red sandal mediated silver nanoparticles (Fig. 3). 10 µL, 20 µL, 30 µL, 40 µL and 5 0µL of red sandal mediated silver nanoparticles was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µL of 50mM Tris HCL buffer (pH 7.4) incubated for 30minutes . Later the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 MN. BHT was employed as control. The percentage of inhibition was determined from the following equation.

% of inhibition = (Absorbance of control- Absorbance of test sample x 100) / Absorbance of control

2.3 Assessment of Cytotoxic Activity

2 g of iodine free salt was weighed and dissolved in 200 ml of distilled water. 6 wells of ELISA plate were taken and 10-12 ml of saline water was filled. To that 10 nauplii were added slowly to each well 5 µL, 10 µL, 25 µL, 40 µL, 80 µL and control. Then the red sandal mediated silver nanoparticles were added according to the concentration level. The plates were incubated

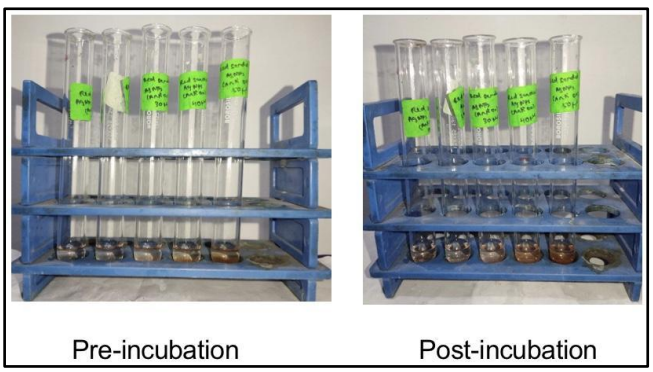

Fig. 3. DPPH assay used to assess the antioxidant activity

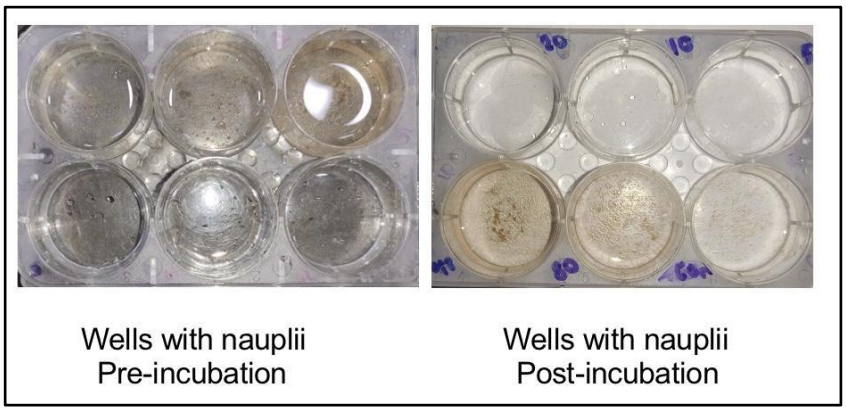

Fig. 4. Wells with nauplii for assessment of cytotoxic activity

for 24 hours. After 24hrs, the Elisa plate was observed and noted for the number of live nauplii's present (Fig. 4).

3. RESULTS

The antioxidant activity of red sandal mediated silver nanoparticles was demonstrated using DPPH assay. The role of antioxidants is to scavenge free radicals. Descriptive statistics was used. The [Fig. 5] graph depicts antioxidant activity of red sandal mediated silver nanoparticles. The results indicated a considerably efficient activity at high concentrations of red sandal mediated silver nanoparticles (50 µL). The test for cytotoxic properties was assessed using brine shrimps. Ten nauplii were placed in each of 6 wells with one standard and the remaining with nanoparticle concentrations 5 µL, 10 µL, 25 µL, 40 µL, and 80 µL . The results indicated a considerably efficient activity at low concentration (5 µL) [Fig. 6].

Table 1 depicts the antioxidant activity of red sandal mediated silver nanoparticles. At 10µL Standard value is 76.56 and AgNP value is 42, at 20µL standard value is 78.52 and AgNP value is 42.4, at 30µL standard value is 85.63 and AgNP value is 44.7, at 40µL standard value is 88.68 and AgNP value is 52, at 50µL standard value is 93.15 and AgNP value is 52.7. Table 2 depicts the cytotoxic activity of red sandal mediated silver nanoparticles. At 5µL 10 nauplii were alive in day 1 and day 2, at 10 µL 10 nauplii were alive in day 1 whereas only 9 nauplii were alive in day 2, at 25 µL 10 nauplii were alive in day 1 whereas only 7 nauplii were alive in day 2, at 40 µL 10 nauplii were alive in day 1 whereas only 7 nauplii were alive in day 2, at 80 µL 10 nauplii were alive in day 1 whereas only 7 nauplii were alive in day 2 and in control 10 nauplii were alive in day 1 and day 2.

Table 1. Depicts the antioxidant activity of red sandal mediated silver nanoparticles

Concentration(µL)	Standard	AgNP
10 µL	76.56	42
20 µL	78.52	42.4
30 µL	85.63	44.7
40 µL	88.68	52
50 µL	93.15	52.7

Table 2. Depicts the cytotoxic activity of red sandal mediated silver nanoparticles

Concentration(µL)	Day 1	Day 2
5 µL	10	10
10 µL	10	9
25 µL	10	7
40 µL	10	7
80 µL	10	7
Control	10	10

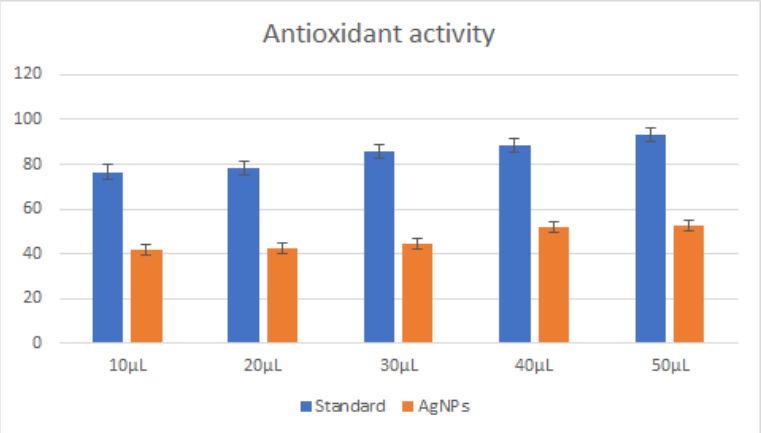

Fig. 5. Depicts the antioxidant activity of red sandal mediated silver nanoparticles. The x axis is a concentration in µL while the Y axis is representative of the percentage of inhibition. Blue denotes the standard and orange denotes the AgNP. The red sandal mediated silver nanoparticle has considerably efficient antioxidant activity at high concentrations (50 µL)

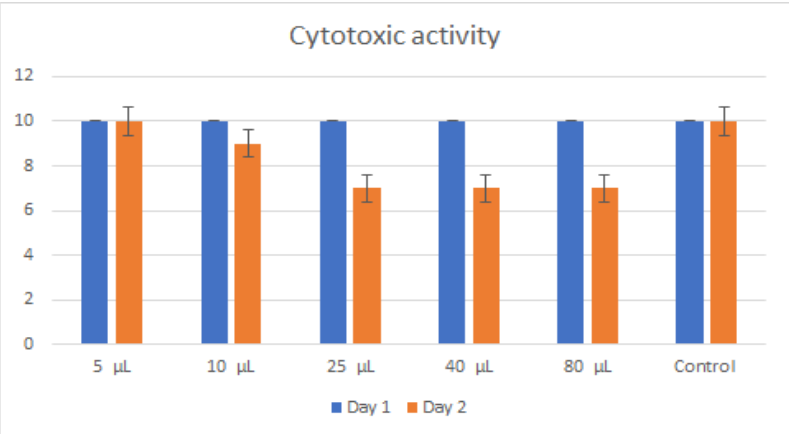

Fig. 6. Depicts the cytotoxic activity of red sandal mediated silver nanoparticles. The x axis is a concentration in µL while the Y axis is representative of the number of nauplii. Blue denotes day 1 and orange denotes day 2. The red sandal mediated silver nanoparticle has considerably efficient cytotoxic activity at low concentrations (5 µL)

4. DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications [15–34]. Pterocarpus santalinoides is one of the important natural materials yielding red colour for a variety of applications. The common name of Pterocarpus santalinoides is red sandal wood. The plant has significant importance in traditional medicine for its ethnomedicinal value. The plant had been investigated for pharmacological activities as it has high medicinal value.

For the evaluation of antioxidant capacity, the highest percentage of inhibition at 50 µl was 52.7 which was presented in the form of a graph in comparison with the percentage inhibition of the standard (BHT) used. This can be concluded that with increase in concentration, the percentage inhibition of Red sandal mediated silver nanoparticles is increased but it remains less than the percentage inhibition of standard ascorbic acid. Hence red sandal mediated silver nanoparticles possess better antioxidant capacity but it is less effective when compared to the antioxidant property of the standard used. The synonymous study carried by Mona Hamelian et.al performed the similar DPPH assay with BTH as standard and established the same result which adds evidence to our study and exists as a supporting study [35]. Another supporting study by Enemali MO et al., also performed DPPH assay for antioxidant determination. Total

phenolic content was estimated ensuring the significant antioxidant capacity in red sandal and thus adds evidence to our present study [36].

The cytotoxic activity of red sandal mediated silver nanoparticles shows that all the introduced shrimps were alive in the control whereas in the well of 5µl of nanoparticles, 10 shrimps were alive. However in the well with a concentration of 80 µl of nanoparticles only 7 shrimps were alive after 24 hours. Thus it is evident that there is no significant cytotoxicity in red sandal mediated silver nanoparticles. Similar study by Kumar P et.al showed that AgNPs have shown better cytotoxic effects which is synonymous with our study [37]. The Red sandal mediated silver nanoparticles (AgNP) are used in many biomedical and pharmacological applications. The limitations of the study was that the study focused only *in vitro* set up. To utilize it for human use, further *in vivo* studies have to be conducted.

5. CONCLUSION

From the present study, it can be concluded that red sandal mediated silver nanoparticles have a considerably moderate cytotoxic and antioxidant activity at high concentrations [38-51]. This can be used for further investigations in employing them as less biotoxic alternatives to already existing chemically synthesised biomaterials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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