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Synthesis of Silver Nanoparticles by *Amaranthus spinosus* Leaves and its Antioxidant Activity

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Abstract—*The preliminary screening of the leaves of Amaranthus spinosus reveals that leaves are rich source of secondary metabolites such as alkaloid, terpenoid, phenolic compounds, glycosides, flavonoid and proteins. The phytochemicals present in the leaves of Amaranthus spinosus were responsible for the reduction process and stabilization of the silver nanoparticles. Further investigations are required for the development of drugs via biosynthesis of nanoparticles from Amaranthus spinosus.*

Keywords*: Amaranthus spinosus, phytochemicals, silver nanoparticles.*

1. INTRODUCTION

Nanoparticles are the fundamental building blocks of nanotechnology. Nanomedicine involves utilization of nanoparticles for the benefit of human health.Earlier physical and chemical methods have been utilized in the synthesis of silver nanoparticles but these methods are tedious, expensive andtime consuming with less productivity [9].The synthesis of nanoparticles by the chemicals leads to the environmental pollution. The biosynthesis of nanoparticles has been established as an alternative to physical and chemical methods as in the green synthesis of nanoparticles there is no involvement of high pressure, temperature and toxic chemicals [17].Silver nanoparticles have many distinctive properties such as chemical stability, catalytic nature, conductivity, antibacterial and antifungal activities [4].Biosynthesis of nanoparticles with plant biomass is a green method as plants are widely available, safe to handleand they are rich source of secondary metabolites [10].

*Amaranthus spinosus*is nutrient rich, underutilized pseudo cereal that can play an important role against hunger and malnutrition[11].*Amaranthus spinosus* belongs to the family Amaranthaceae. It adapts itself to harsh environmental conditions, produces huge biomass and resists drought, heat and pestattack [18]. The grainsof *Amaranthus spinosus*are excellent source of fiber, iron, calcium, amino acids and protein [13]. Amaranth grain is gluten-free and used as an ingredient in confectionary products [11].*Amaranthus spinosus* has also been used for the treatment of diarrhoea, dysentery, excessive menstrual flow and ulcer etc [1].The studies revealed that the leaves of *Croton bonplandianum*[8], *Rauvolfiatetraphylla*[21],*Hyptis suaveolens*[14]and*Amaranthus retroflexus*[3]have been used for the synthesis of silver nanoparticles. To the best of our knowledge, no study has been reported till date related with the biosynthesis of nanoparticles by *Amaranthus spinosus.* Hence, the present study was conducted to assess the phytochemical components and antioxidant potential of *Amaranthus spinosus*weed which is available in huge amount and can be used in the formulation of cost effective and safe therapeutic products.

2. MATERIALS AND METHODS

The present investigation was conducted in the Plant Physiology Laboratory, Amity Institute of Biotechnology, Amity University, Noida. The fresh and healthy leaves of *Amaranthus spinosus* were collected at the vegetative stage. Fresh leaves were removed, washed gently with tap water followed by quick rinsing in distilled water and drying with clean absorbent paper.

Experimental design

The fresh leaves of *Amaranthus spinosus* were kept in single layer on plastic tray under the shade for air drying for 72 hours. After air drying, leaves were powdered in a grinder and dry leaf powder was stored in sterilized polythene bags to avoid contamination. In the present study, screening of phytochemicals present in the leaves of *Amaranthus spinosus*and antioxidant activity of the leaf extract were analyzedby different biochemical tests.

A. Screening of phytochemical components

For the screening of phytochemical components, 100 grams of dried leaf powder of *Amaranthus spinosus* was mixed with 500 ml of methanol and mixture was kept on rotary shaker for 48 hours at 190-220 rpm. After 48 hours, mixture was filtered and supernatant was evaporated to the one-fourth of its original volume. The methanolic leaf extract obtained was used for the qualitative analysis of phytochemical components present in *Amaranthus spinosus*leaves[20]by using the standard procedures described by Harborne [6] and Trease and Evans [19].

1. Test for tannin

Approximately 0.5 g of dried leaf powder of*Amaranthus spinosus* was boiled with 20 ml of distilled water and after filtration few drops of 0.1% ferric chloride solution was added. A blue - black colour of the test solution showedthe presence of tannin in a given sample.

2. Test for saponin

Two grams of the leaf powder of *Amaranthus spinosus*was boiled with 20 ml of distilled water and after filtration, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. The foamy leather formation indicated the presence of saponin in the test solution.

3. Test for protein

Biuret reagent (4 drops of 40% NaOH + 2-3 drops of 1 % $CuSO_4$) was added in 0.5 ml of leaf extract. The test solution turned into violet colour showed the presence of proteins.

4. Test for carbohydrate

Leaf extract (0.5 ml) was mixed with equal volume of Fehling's reagent, heated on water bath for 10 minutes. Formation of red colour indicated the presence of carbohydrate in the leaves of *Amaranthus spinosus.*

5. Test for alkaloids

The methanolic leaf extract was evaporated to dryness in a boiling water bath and residue was dissolved in 2N HCl. The mixture was filtered and filtrate was treated with Mayers, Dragondroffs and Wagners reagents separately. The creamish, orange and brown coloured precipitate showed the presence of alkaloids in the*Amaranthus spinosus* leaves.

6. Keller - Kiliani test for glycosides

Few drops of glacial acetic acid and 2-3 drops of ferric chloride solution were added to 2 ml of leaf extractof *Amaranthus spinosus*along with 1 ml of concentrated sulfuric acid. Appearance of brown ring at the interface confirmed the presence of glycosides in the leaves.

7. Test for phenol

Leaf powder of*Amaranthus spinosus*(500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compound in the leaves.

8. Test for flavonoids

One gram of leaf powder of *Amaranthus spinosus*was boiled with 10 ml of distilled water and filtered. Few drops of 20% NaOH solution were added to 1 ml of cooled filtrate. The yellow colour which on addition of acid changed to colourless solution showed the presence of flavonoids in the leaves of*Amaranthus spinosus.*

9. Test for terpenoids

The leaf extract (5 ml) was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4. A reddish - brown colour of test solution showed that presence of terpenoids in the leaf extracts.

10. Test for steroids

Two ml of acetic anhydride was added to 0.5 ml of leaf extract of *Amaranthus spinosus.* Mixed them properly and 2 ml of concentrated $H_2SO_4$was also added. A blue-green colour showed the presence of steroids in the leaves of *Amaranthus spinosus.*

B. Antioxidant activity of leaf extract of *Amaranthus spinosus*

Antioxidant property of the leaf extract of*Amaranthus spinosus* was analyzed by DPPH assay [16]. One ml of 0.1mM DPPH in ethanol was prepared and to this solution different concentrations (50-300µg/µl) of leaf extracts, 1ml ethanol and 0.95 ml Tris HCl were added. The mixture was left for 30 minutes in room temperature and absorbance was measured at 517nm. The DPPH free radical scavenging activity was calculated by the given formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control (absorbance)} - \text{sample (absorbance)}}{\text{Control (absorbance)}} \times 100$$

C. Biosynthesis of silver nanoparticles by leaf extracts of *Amaranthus spinosus*

Fresh leaves of *Amaranthus spinosus*(25 grams)were thoroughly washed in distilled water, dried and cut into small pieces and boiled with 100 ml of distilled water up to 15 minutes and filtered with What man no. 1 filter paper [2]. The filtrate was centrifuged at 10,000 rpm for 10 minutes and supernatant was collected and stored at 4^0C in refrigerator. The filtrate was used as reducing and stabilizing agent for the preparation of silver nanoparticles. The filtrate (50 ml) was added to the aqueous solution of 1 mM $AgNO_3$ and mixture was incubated in dark for12 hours. After 12 hours, the sample was analysed for its maximum absorbance using UV-visible spectrophotometer.

3. RESULTS AND DISCUSSION

The present study was conducted to assess the phytochemical constituents and antioxidant potential of the leaves of *Amaranthus spinosus* and effect of leaf extract of *Amaranthus spinosus* in reduction mechanism of silver ions into silver nanoparticles. The preliminary screening of the leaves of

Amaranthus spinosus revealed that leaves contain secondary metabolites such as tannin, saponin, alkaloid, terpenoid, phenolic compounds, glycosides, flavonoids, steroids, carbohydrates and proteins which may play a significant role in the formation of nanoparticles (Table-1).

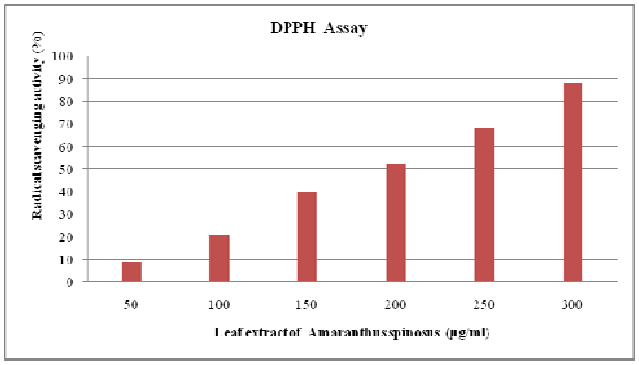
Table 1.Phytochemical components present in the leaves of *Amaranthus spinosus.*

S. No.	Phytochemical components	Leaves of *Amaranthus spinosus*
1.	Tannin	+
2.	Saponin	+
3.	Carbohydrate	+
4.	Protein	+
5.	Alkaloids	+
6.	Glycosides	+
7.	Flavonoids	+
8.	Terpenoids	+
9.	Steroids	+
10.	Phenolic compound	+

(+) sign indicates the presence of phytochemical components in the leaves of *Amaranthus spinosus*

For the assessment of antioxidant activity of the leaf extract of *Amaranthus spinosus*, DPPH assay was done. Antioxidant activity was significantly increased with the higher concentration of the leaf extracts of *Amaranthus spinosus*. The antioxidant molecules reduces oxidative stress by scavenging the free radicals and play important role in the treatment of various ailments [7]. *Amaranthus spinosus* is rich in secondary metabolites such as alkaloids, terpenoids, phenolic compounds, flavonoids and steroids etc. The results of the present study clearly showed that leaf extracts of *Amaranthus spinosus* have the potential to act as an antioxidant and it can be used as a potential source for drug formulation (Figure-1).

Figure 1. Antioxidant activity of the leaf extract of *Amaranthus spinosus* analyzed by DPPH assay.



The leaf extracts of *Amaranthus spinosus*were analyzed for the synthesis of silver nanoparticles which were characterized with the help of UV-visible absorption spectroscopy. In the present study when theleaf extract of *Amaranthus spinosus*was mixed in the aqueous solution of silver nitrate, it started to change in colour from green to pale yellow. The change in colour was due to the excitation of surface plasmon vibrations, which indicated the formation of silver nanoparticles[15]. The formation of silver nanoparticles was visually authenticated by the appearance of pale yellow colour and reaction was completed within 12 hours of incubation period. This change in colourfrom green to pale yellowmay be due to the reduction of silver nitrate into silver nanoparticles [12]**.**The leaf extracts of *Amaranthus spinosus*was found to show the peak at 435 nm which confirmed the reduction of silver nitrate to silver nanoparticle. The peak was observed at 435 nm (λmax) which corresponds to the absorbance of silver nanoparticles until the reduction process completes.The phytochemicals present in the leaf extract of *A. spinosus* mayact as the surface active stabilizing molecules for the synthesis of silver nanoparticles [5].

4. CONCLUSION

The biosynthesis of silver nanoparticles with the leaf extracts of *Amaranthus spinosus* is simple, cost effective and eco-friendly method. The present study reveals that *Amaranthus spinosus* weed is an excellent source for the synthesis of silver nanoparticles but further clinical trials are required for its medicinal uses against diseases.

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