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Biosynthesis and Characterization of Silver Nanoparticles from *Croton Bonplandianum Baill* and its Antioxidant Activity

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Abstract
Nanotechnology has become the significant area of research due to its wide variety of applications. Plants are rich resources of drugs in traditional and modern medicine and they can be eco-friendly alternative for the synthesis of nanoparticles. The biosynthesis of nanoparticles provides advancement over physical and chemical methods as it is environment friendly, safe and cost effective method. In the present study, the preliminary screening of the leaves of *Croton bonplandianum* reveals that leaves are rich source of secondary metabolites such as tannin, saponin, alkaloid, terpenoid, phenolic compounds, glycosides, flavonoid and proteins. The leaf extracts of *Croton bonplandianum* were assessed for the synthesis of silver nanoparticles and production of nanoparticles were characterized with the help of UV-visible absorption spectroscopy analysis. It was observed that the presence of phytochemicals in the leaf extracts of *Croton bonplandianum* were responsible for the reduction process and stabilization of the silver nanoparticles. The silver nanoparticles synthesized by using *Croton bonplandianum* leaves may be the significant source of therapeutic agent but further studies are required in this direction for the development of value added nano-products from *Croton bonplandianum* for the biomedical based industries.

Keywords: *Croton bonplandianum*, nanotechnology, phytochemicals, silver nanoparticles

Introduction
Nanotechnology has tremendous applications in the areas of renewable energy, environmental remediation, drug delivery and pharmaceutical industries. Nanoparticles are cluster of atoms ranging between 1-100 nm in size and these are fundamental building blocks of nanotechnology. The physical and chemical methods have been employed in the synthesis of silver nanoparticles but these methods are tedious and non-ecofriendly with low productivity (1). The chemicals used in the synthesis of nanoparticles are hazardous which leads to environmental pollution. Now-a-days biosynthesis of nanoparticles has been established as an alternative to physical and chemical methods of synthesis of nanoparticles as in the green synthesis of nanoparticles there is no involvement of high pressure, temperature and toxic chemicals (2). Silver nanoparticles have many distinctive properties such as conductivity, chemical stability, catalytic nature, antibacterial, antifungal and anti-inflammatory activities (3). According to Kulkarni et al. (4) synthesis of metal nanoparticles by utilization of plant extracts is a green method as the plants are widely distributed, easily available, safe to handle and they are rich source of secondary metabolites. Plants can be eco-friendly alternative for the synthesis of nanoparticles. Plants have been found the excellent source of natural reducing agents with high efficiency (5). *Croton bonplandianum* Baill. is commonly known as ban tulsi and jungle tulsi. *Croton bonplandianum* Baill. is an exotic weed which belongs to the family Euphorbiaceae and it grows as wild plant in wasteland, vacant site and roadsides. *Croton bonplandianum* is used to treat liver and skin diseases and also to cure the swelling of body. Leaves of *Croton bonplandianum* are medicinally used for the treatment of wounds, cholera, fever and high blood pressure. The seeds are used for the treatment of abdominal dropsy, constipation, jaundice and internal abscess (6). Earlier studies revealed that the leaves of higher plants such as *Catharanthus roseus* (7), *Rauvolfia tetraphylla*, *Enicostema hysopifolium* (8) and *Hyptis suaveolens* (9) have been extensively exploited for the synthesis of silver nanoparticles. However, no effort seems to have been made to study the synthesis of silver nanoparticles with the utilization of *Croton bonplandianum* weed. The present study was conducted to assess the phytochemical constituents and antioxidant capacity of *Croton bonplandianum* and the effect of leaf extract of *Croton bonplandianum* in reduction mechanism of silver

ions into silver nanoparticles. Hence, the present investigation can provide a new direction by the utilization of *Croton bonplandianum* weed in the formulation of cost effective, eco-friendly and value added therapeutic products.

Materials and Methods

The present investigation was conducted in the Plant Physiology Laboratory, Amity Institute of Biotechnology, Amity University, Noida, India. The fresh and healthy leaves of *Croton bonplandianum* 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 *Croton bonplandianum* 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, fluorescence analysis, screening of phytochemicals present in the leaves of *Croton bonplandianum* and antioxidant activity of the leaf extract were done by different biochemical tests.

A. Fluorescence analysis of leaf powder of *Croton bonplandianum*

The dry leaf powder of *Croton bonplandianum* was analyzed for its fluorescence activity with different chemical reagents such as nitric acid, hydrochloric acid, sodium hydroxide, potassium hydroxide and acetic acid.

B. Screening of phytochemical components

For the screening of phytochemical components, 100 grams of dried leaf powder of *Croton bonplandianum* 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 the leaves of *Croton bonplandianum* (10) by using the standard procedures described by Harborne (11) and Trease and Evans (12).

***1*. Test for tannin**

Approximately 0.5 g of dried leaf powder of *Croton bonplandianum* 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 indicated the presence of tannin in a given sample.

***2*. Test for saponin**

Two grams of the leaf powder of *Croton bonplandianum* 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 carbohydrates**

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 *Croton bonplandianum*.

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 respective alkaloids in the leaf extracts.

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 extracts of *Croton bonplandianum* 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

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

8. Test for flavonoids

One gram of dry leaf powder of *Croton bonplandianum* 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 *Croton bonplandianum.*

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 *Croton bonplandianum*. Mixed them properly and 2 ml of concentrated H_2SO_4 was also added. A blue-green color indicated the presence of steroids in the leaves of *Croton bonplandianum*.

C. Antioxidant activity of leaf extract of *Croton bonplandianum*

Antioxidant property of the leaf extract of *Croton bonplandianum* was analyzed by DPPH assay (13). 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:

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

D.* Biosynthesis of silver nanoparticles by leaf extracts of *Croton bonplandianum

Twenty five grams of fresh leaves of *Croton bonplandianum* 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 Whatman no. 1 filter paper (14). 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 for 12 hours. After 12 hours, the sample was analyzed for its maximum absorbance using UV-visible spectrophotometer.

Results and Discussion

The present study was conducted to assess the phytochemical constituents and antioxidant capacity of the leaves of *Croton bonplandianum* and effect of leaf extract of *Croton bonplandianum* in reduction mechanism of silver ions into silver nanoparticles. The dry leaf powder of *Croton bonplandianum* was analyzed for its fluorescence with different chemical reagents (Table-1).

Table 1. Fluorescence analysis of the leaves of *Croton bonplandianum*.

S.No.	Treatment	Sun light	UV- light
1.	Leaf powder	Green	No fluorescence
2.	Leaf powder mixed with 1 N HNO_3 in distilled water	Brownish yellow	Colourless
3.	Leaf powder mixed with 1 N HCl in distilled water	Colourless	Greenish yellow
4.	Leaf powder mixed with 1 N NaOH in distilled water	Brown	Yellow
5.	Leaf powder mixed with KOH	Yellow	White
6.	Leaf powder mixed with acetic acid	Colourless	Light yellow

The preliminary screening of the leaves of *Croton bonplandianum* revealed that leaves contain secondary metabolites such as tannin, saponin, alkaloid, terpenoid, phenolic compounds, glycosides, flavonoids, steroids, carbohydrates and proteins which may play significant role in the formation of nanoparticles (Table-2).

Table 2. Screening of phytochemical components present in the leaves of *Croton bonplandianum*

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

(+) sign indicates the presence of phytochemical components in the leaves of *Croton bonplandianum*

The antioxidant activity of the leaf extract of *Croton bonplandianum* was studied by using DPPH assay. The higher antioxidant activity was observed with the higher concentration of the leaf extracts of *Croton bonplandianum*. The oxidation reaction produces reactive oxygen species, which may start chain reactions that can damage biomolecules but antioxidants can terminate these chain reactions by removing free radical intermediates. The basic function of antioxidant molecules is to help in preventing the oxidative stress and to help in protecting the cells by scavenging the free radicals and they may play important role in the treatment of various diseases (15). *Croton bonplandianum* is rich in secondary metabolites such as alkaloids, terpenoids, phenolic compounds, flavonoids and steroids (16). The data of the present study clearly indicate that leaf extracts of *Croton bonplandianum* have the potential to act as an antioxidant and it can be used as a source of useful drug due to the presence of various phytochemicals (Figure-1).

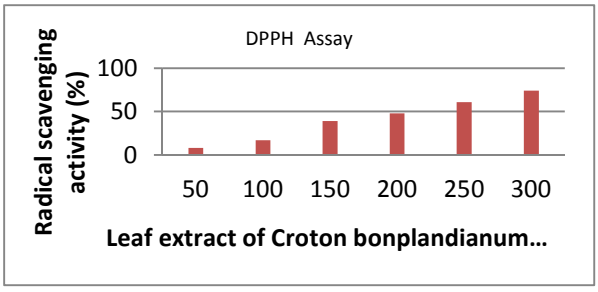

Fig. 1: Antioxidant activity of the leaf extract of *Croton bonplandianum* analyzed by DPPH assay.

The leaf extracts of *Croton bonplandianum* were also analyzed for the synthesis of silver nanoparticles which were characterized with the help of UV-visible absorption spectroscopy. In the present study when the leaf extract of *Croton bonplandianum* was mixed in the aqueous solution of the silver nitrate, it started to change in colour from green to light brown. According to Parashar et al. (17) the change in colour was due to the excitation of surface plasmon vibrations, which indicated the formation of silver nanoparticles. The formation of silver nanoparticles was visually authenticated by the appearance of light brown colour and reaction was completed within 12 hours of incubation period. This change in colour from green to light brown may be due to the reduction of silver nitrate into silver nanoparticles (18).

The leaf extracts of *Croton bonplandianum* was found to show the peak at 415 nm which confirmed the reduction of silver nitrate to silver nanoparticle. The peak was observed at 415 nm (λmax) which corresponds to the absorbance of silver nanoparticles until the reduction process completes (Figure - 2). According to Ganesan et al. (19) secondary metabolites such as alkaloids, terpenoids, phenolic compounds and flavonoids are responsible for the formation and stabilization of the silver nanoparticles. The phytochemicals present in the leaf extract of *Croton bonplandianum* may act as the surface active stabilizing molecules for the synthesis of silver nanoparticles.

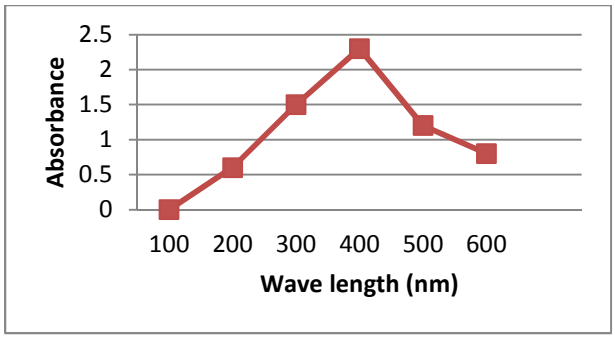

Fig. 2: UV- visible absorption spectra of silver nanoparticles synthesized by leaf extract of *Croton bonplandianum* recorded after 12 hours of incubation period.

Conclusion

The present study reveals the reduction of Ag^{+} ions into silver nanoparticles by using the aqueous leaf extracts of *Croton bonplandianum*. Hence, the biosynthesis of silver nanoparticles with the leaf extracts of *Croton bonplandianum* is simple, cost effective and eco-friendly method. The present study confirms that *Croton bonplandianum* weed is an excellent source for the synthesis of silver nanoparticles but further clinical trails are required for its use as therapeutic agent against diseases.

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