GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *Origanum majorana* LEAF EXTRACTS

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Abstract: Nanoparticles are used in various fields of science and has potential applications which is classified based on its morphology and size. *Origanum majorana* is a herb whose tender and mature leaves have been used for the synthesis of silver nanoparticles. A battery of phytochemical assays were performed to understand the secondary metabolites present in them. The antioxidant potential and haemolytic properties were determined. The particles were subjected to UV visible spectroscopy and SEM techniques to understand the morphology, size and characters of the particle. It was observed that both mature and tender nanoparticle leaf extracts did not show significant differences thought their antioxidant profiles were slightly different; however, the functional groups proved to the same.

Keywords: nanoparticles, silver nitrate, antioxidants, UV spectroscopy, SEM

1. Introduction: Nanoparticle is about 1 to 100 nanometers in size and is the study and application of extremely small things that can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Nanoparticle research is an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields.[1] Synthesis of nanoparticles is a crucial area of research, probing for a nature-friendly manner for current science. Countless methodologies are emerged to synthesize noble metal nanoparticles of specific shape and size depending upon requirement.[2]

Nanoparticles can be classified into different types according to the size, morphology, physical and chemical properties. Some of them are carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles. Nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, bio sensing, imaging, drug detective, Nano device fabrication and in medicine.[3]

1.1*Origanum Majorana:* *M. hortensis* is a Mediterranean perineal herb of Lamiacea family. Leaves are smooth, simple, petiolated, ovate to oblong-ovate, 0.5–1.5 cm (0.2–0.6 inches) long, 0.2–0.8 cm (0.1–0.3 inches) wide, with obtuse apex, entire margin, symmetrical but tapering base, and reticulate venation. The texture is extremely smooth due to the presence of numerous hairs. Commonly it is called sweet majoram

and used to add flavour in culinary purpose. It has been proved to have high potential as an antioxidant.[4] *M. hortensis* belongs to family Lamiaceae, finds its place in religious ceremonies and has several uses in therapeutic remedies, insecticidal effect due to terpenes, and carries several traditional uses.[5]

1.2 Methods of Green synthesis of nanoparticles:

There are several methods for green synthesis of nanoparticles. They are Polysaccharide method, Tollens method, Irradiation method, Biological method, Poly oxometalates method. In this study biological method is more focused to compare with other methods.[6] The synthesis of nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications.[7] Synthesizing nanoparticles via biological entities acting as biological factories offers a clean, non-toxic and environment- friendly method of synthesizing nanoparticles with the wide range of sizes, shapes, and composition and physiochemical properties.[8]

II. Materials and Methods:

2.1 Collection of Plant Sample:

The plant was collected in and around Coimbatore. Identification of the plant specimens were done at Botanical Survey of India, TNAU campus, Coimbatore as ***Origanum majorana*** (Voucher Number: BSI/SRC/5/23/2018/Tech./2266). The collected plant materials were split into two sample. They were tender and mature leaves. The upper half part of the plant has tender leaves and lower part of the plant has matured leaves. Both tender and mature leaves has different concentration of phytochemicals. Hence, the present study was aimed to check the activities of silver nanoparticles from both leaves. The samples were washed with sterile double distilled water (DDW) and air dried. The fresh leaves were stored in refrigerator and used for Green synthesis of silver nanoparticles.

2.2 Preparation of Leaf extract (Aqueous leaf extract):

20 g of leaves were washed with distilled water to remove the dust particles and then air dried. The dried ***O. majorana*** leaves were cut into small pieces and boiled with 100 ml of distilled water at 80˚C for 1 hour. After boiling, the brown coloured extract was separated by filtration and it was used for the reduction of silver nitrate to silver nanoparticles.

2.3 Phytochemical Analysis of *O. majorana*: Phytochemicals are the chemicals that are present naturally in plants. Phytochemicals play a vital protective role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. The phytochemicals cure diseases without causing any harm or side effects to human beings these can also be considered as “man- friendly medicines”.

Test for Phenols:

- **Ferric Chloride test:** To 2mL of extract few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.
- **Lead acetate test:** To 2mL of extract 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for Flavonoids:

- **Lead acetate test:** To 2mL of extract 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for Alkaloids:

- **Wagner's test:** A few drops of Wagner's reagent are added to 2 mL of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.
- **Hager's test**: Initially, take 2 mL of extract, stir well with few mL of dilute hydrochloric acid and then filter it. To a few mL of filtrate, add 1 or 2 mL of Hager's reagent (saturated aqueous solution of picric acid). A prominent yellow precipitate indicates the test as positive.

Test for Tannins

- **Ferric Chloride test:** To 2mL of extract few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

Test for Saponins:

The extract is diluted with distilled water. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

Synthesis of Silver Nanoparticles:

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. The synthesis of silver nanoparticles was done by mixing *O. majorana* extract of both tender and mature leaves and 1mM Silver nitrate solution respectively and incubated in water bath for 10 minutes at 60º C. The silver nanoparticles produced by *O. majorana* leaf extract was centrifuged at 6000 rpm for 20 minutes. The supernatant was discarded and the collected pellet was dried in hot air oven for 24 hours at 100ºC.

Antioxidant Assays:

DPPH Assay (2, 2-Diphenyl-1- picrylhydrazyl): The free radical scavenging activity of the synthesized silver nanoparticles was determined using DPPH (1, 1-diphenyl-2picrylhydrazyl). DPPH solution (0.004% w/v) was prepared in 95% methanol. Sample was mixed with distilled water to prepare the stock solution (1 mg/ml). To 0.5 ml of the prepared DPPH solution silver nanoparticles in varying concentrations (20-60 µg/ml) were added. The mixture was left at room temperature for 30

min and the absorbance was measured at 517 nm. Then the scavenging ability was calculated using the following equation:

% DPPH radical scavenging =100 x (control OD- sample OD)/Control OD

Antioxidant Activity of Vitamin C: Into a serious of test tubes 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the working standard solution was pipetted out. In three other test tubes, 0.1ml, 0.2ml and 0.3 ml of the given sample were taken (raw extract of the fruit). Another set of 0.1 ml, 0.2 ml and 0.3 ml of the given sample were taken (boiled extract of the fruit). The volume in all the tubes was made to 3ml with distilled water. 3ml of water served as the blank. 1ml of DNPH solution was added to each tube followed by 1-2 drops of Thiourea. The tubes were heated in a boiling water bath for 20 minutes. The contents were mixed thoroughly and incubated at 37 degree Celsius for 3hrs. After incubation, the orange-red osazone crystals formed were dissolved by adding 7ml of 80% sulphuric acid and measured at 540nm.

Total antioxidant Activity: The total antioxidant activity was evaluated by Phospho molybdenum method. 1.0 ml of AgNPs was mixed with 1.0 ml of the standard reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank. The total antioxidant capacity was expressed as milligram of Ascorbic Acid Equivalence (AAE) per gram of extract.

UV- Visible spectroscopy analysis: UV-visible absorption spectroscopy was used to monitor the quantitative formation of silver nanoparticles. It is a valuable tool for structural characterization of silver nanoparticles and is a fundamental technique to ascertain the formation of stable metal nanoparticles in aqueous medium based on Surface Plasmon Resonances (SPRs) that shift to longer wavelengths with increasing particle size. The colour change in reaction mixture was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling at different duration of aliquots and subsequently measuring UV- Visible spectra of the solution. UV- Vis spectra of these aliquots were monitored as a function of time of reaction on UV-Vis spectrophotometer at 420 nm.

III Results and Discussion:

Leaf extract preparation:

The leaf extracts of both mature and tender leaves were prepared at 80ºC in water bath and the brown colour extracts were obtained.

Phytochemical analysis of *O. majorana* leaves: The phytochemical analysis of *O. majorana* were performed and reported in Table 1. The results revealed the presence of medicinally active compounds like phenols, alkaloids, saponins and reducing sugar. The alkaloids present are used as analgesic, antipyretic and anaesthetic. Phenols play an important role in cancer prevention and treatment. They prevent apoptosis

by arresting cell cycle. They are also responsible for regulating carcinogen metabolism, inhibiting DNA binding and cell adhesion, migration, proliferation and blocking signalling pathways[1]. The presence of saponins has been reported to resist microbial pathogens and have detergent like properties. In recently proven the tender and mature leaves of *O. majorana* were exposed to all the phytochemical screening where both the samples showed the presence of all the 6 classes of phytochemicals namely, alkaloids, phenols, flavonoids, saponins, steroids and tannins.[9]

Table 1: Phytochemical analysis of *O. majorana* nanoparticle (mature and tender)

Phytochemicals		**Mature leaf extract**	**Tender leaf extract**
Phenols	**Ferric chloride test**	+	+
	Lead acetate test	+	+
Alkaloids	**Wagner's test**	+	+
	Hager's test	+	+
Flavonoids		+	+
Tannins		+	+
Saponins		+	+

Synthesis of Silver nanoparticles:

Aqueous silver ions were reduced to silver nanoparticles after addition of *O. majorana* leaf extract followed by incubation of the mixture in water bath for 10 minutes. The maximum reaction was indicated by the formation of reddish brown colour in the reaction mixture (Figure 1). It indicates the formation of silver nanoparticles. Present findings showed resemblance to the results already reported in the case of extract of *Origanum vulgare*.[10] They reported that when the extract of their respective test plants were challenged with silver nitrate (1mM) they turned reddish brown colour and it formed silver nanoparticles as shown in the figure 1 placed in the Eppendorf. Similar results and colour was observed for the tender plant leaves too.

Before further proceeding with the studies of the leaf extract, they were subjected to a battery of phytochemical analysis to comprehend if the metabolites which are present in the whole leaf extract was retained in the synthesized nanoparticle or not. However, it is conventional to find all the phytochemicals present in the nanoparticle if it is positive in the whole leaf of the plant. Palaniswamy and Raghunathan (2018) have worked with the whole extract of *M. hortensis* and proved the presence of phenols, flavonoids, alkaloids, saponins and tannin.[9]

UV-Visible Spectrophotometer:

The synthesized Silver nanoparticles of both the samples were analysed in UV-Visible spectrophotometer time period of 10 minutes. The absorption spectrum of AgNPs of mature and tender samples formed in the reaction had a peak at 450nm and 454nm respectively. It confirms the presence of silver nanoparticles. The stable Silver nanoparticles were synthesized and confirmed by using UV- Visible spectrophotometer. In the previous studies shown the confirmation of silver nanoparticle synthesized by using *Cassia fistula* leaf extracts (400nm)[1] and *Vitex Negundo* leaf extract contained two peaks, one at 422 nm and the other at 447 nm at UV- Visible spectrophotometer.[10,11]

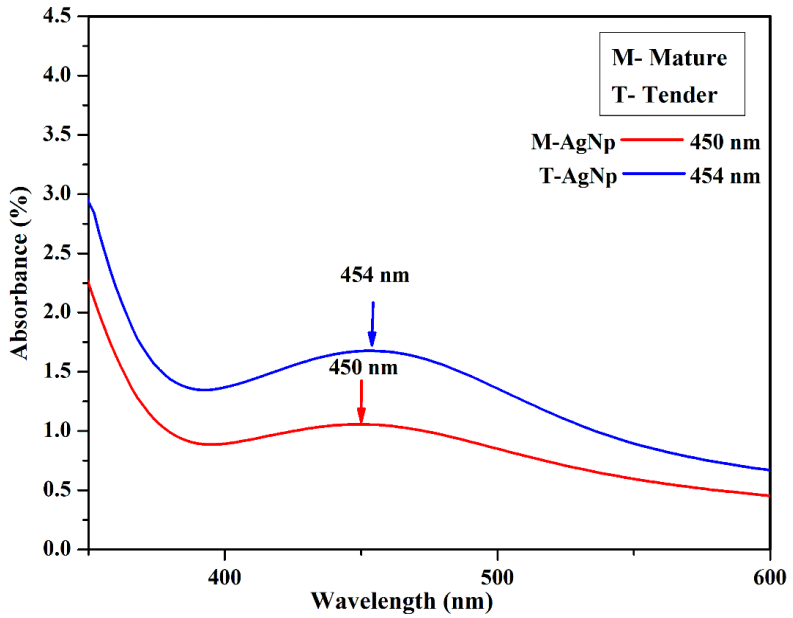

Figure 1: Absorbance of AgNPs (Mature and Tender samples)

Scanning Electron Microscope (SEM):

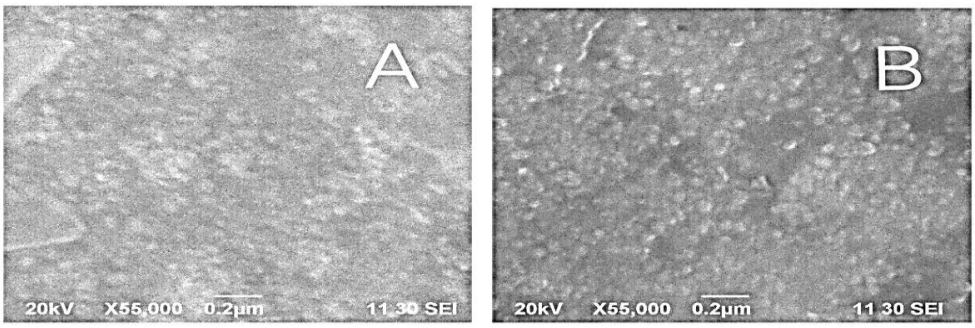

Figure 2: SEM image (A) nanoparticles of mature leaves (B) nanoparticles of tender leaves.

The nanoparticles of mature and tender leaf extract of *O. majorana* was subjected to SEM analysis which was used to determine the shape of the particles. Figure 5 showed the representative SEM images recorded from drop-coated films of the silver nanoparticles synthesized by treating silver nitrate solution with *O. majorana* leaf extracts (mature and tender leaves). The silver nanoparticles formed were predominantly spherical with uniform shape as seen in the image. Figure 5a shows the mature spherical and uniform shape of silver nanoparticles produced from the leaf extract and similar image was obtained for the tender leaves also as seen in Figure 5b. It is known that the shape of metal nanoparticles considerably change their optical and electronic properties. There was no difference in the morphology of silver nanoparticles of both mature and tender leaves sample. Similar results were determined for silver nanoparticles of mature and tender leaves. A study conducted by Ahmad *et al.*, (2015) showed SEM imaging analysis of *Skimmia laureola* leaf extract of silver nanoparticles showed that the surface morphology of nanoparticles was relatively spherical shape with size 40 nm.[12] Another study also indicated the silver nanoparticles on SEM analysis showed its images of relatively spherical shaped nanoparticle which mostly exists with the concentration of 5:95 aqueous dilution with 10^{-3}M $AgNO_3$.[13]

Antioxidant assays:

DPPH (2, 2-Diphenyl-1- picrylhydrazyl) Assay:

DPPH assay was compared with vitamin C as standard and free radical scavenging activity was calculated it was noted that with increasing concentration of leaf extract in both mature and tender samples the scavenging activity also escalated. The silver nanoparticles of tender leave sample showed greater levels of scavenging (63.2%, 86.4% and 88.9%) when compared with silver nanoparticles of mature leaf (64.6%, 67.3% and 77.2%). These values were compared with vitamin C which showed 81% for scavenging activity which was greater than the mature and tender leaves of silver nanoparticles. In the previous studies it was determined that ethanolic extract of leaves & flowers of *Ageratum* plant species from which nanoparticles were synthesized, it showed better antioxidant potential by DPPH radical scavenging method when compare to standard ascorbic acid.[14] In another study it showed that the capped silver nanoparticles were found to be potent free radical scavenger when compared to standard TROLOX. Free radical scavenging activity of silver nanoparticles at higher concentration (100 µg/mL) is comparable to TROLOX. [15]

Table 2: DPPH Assay of Silver Nanoparticles (Mature and Tender samples)

Sample	Conc. (mL)	Free Radical Scavenging (%)
Standard (Vitamin C)	0.1	81%
AgNP (Mature Leaf)	0.1	64.6 %
	0.3	67.3 %
	0.5	77.2 %
AgNP (Tender Leaf)	0.1	63.2 %
	0.3	86.4 %
	0.4	88.9 %

Antioxidant activity of Vitamin C and Total Antioxidant Assay:

Among the antioxidant activities both enzymic and non enzymic play a significant role. An example of non enzymic antioxidant is vitamin C. Hence vitamin C assay and total antioxidant levels were determined to understand the efficiency of silver nanoparticles of mature and tender leaves of *O. majorana*.

Scavenging of vitamin C in the silver nanoparticles of tender leaves showed double the levels of scavenging in the different concentration of 0.1mL, 0.3mL and 0.5mL (13µg, 38µg and 107.5µg) when compared with silver nanoparticles of mature leaf (16µg, 36µg and 52.5µg). In the previous study showed the maximum percentage inhibition of free radical production was observed in AgNPs of *Cassia tora* which was 51 % and it was lower than that of standard vitamin C at 200 µg /ml (91 %) which proves that vitamin C is a potent scavenger of free radicals.[16]

Conversely the total antioxidant assay showed that the silver nanoparticles of mature leaves sample (16µg, 40µg and 56.8µg) had higher activity when compared to tender leaves sample of silver nanoparticles (10µg, 34.2µg and 53.68µg). A study conducted in 2017 by Madhanraj *et al*. showed that among mushroom mycelial extracts, it has been found that the scavenging effect of *Pleurotus flabellatus* has the highest value of 28.33 mg AAE/g of extract in case of AuNPs and *Pleurotus ostreatus* has the highest value of 17.78 mg AAE equivalent / g of extract in case of AgNPs. Another study also described that the *Berginia ciliata* (BC) AgNPs and *Berginia ciliata* extract were assessed for the antioxidant activity where BCAgNPs showed higher total antioxidant activity compared to extract of *Berginia ciliate* which showed that the nanoparticles and crude extract exhibited a total antioxidant activity of 60.48 ± 2.2 and 38.8 ± 1.08 AAE/g respectively.[17]

Table 3: Antioxidant activity of Vitamin C and Total Antioxidant Assay of Silver Nanoparticles (Mature and Tender samples)

Sample	Conc. (mL)	Vitamin C (µg)	Total Antioxidant (µg)
AgNP (Mature Leaf)	0.1	16	16
	0.2	36	40
	0.3	52.5	56.8
AgNP (Tender Leaf)	0.1	13	10
	0.2	38	34.2
	0.3	107.5	53.68

IV Conclusion: Hence, the silver nanoparticles synthesized from the mature and tender leaves can be used for the production of drugs as it shows good antioxidant potential and the structural characterization can help in the drug designing for further analysis. Hence, a novel drug can be designed from the *O. majorana* extract.

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