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GREEN SYNTHESIZED SILVER NANOPARTICLES USING TUBERS OF EULOPHIA SPECIES-PART I: OPTIMIZATION STUDY USING UV SPECTROPHOTOMETER

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ABSTRACT

The phytochemicals present in the plant extract readily act as reducing agents for the efficient conversion of metal salts or ions into metal or metal oxide nanoparticles. Flourishing applications of novel nanomaterials in biomedical and pharmaceutical fields encourage the biogenic synthesis of nanoparticles with reduced toxicity. As green synthesis of nanoparticles is simple mechanism, vast available, low cost, non-toxic, eco-friendly natured and one-pot synthesis method, it gains importance in the medical field, agriculture, and bioremediation. The various biological activities of *Eulophia* tubers are reported but no reports available on silver nanoparticles biosynthesis potential of *Eulophia* tuber extract. In this study, the phytoconstituents of tuber aqueous extract responsible for biosynthesis were analysed qualitatively. The process parameters for AgNP biosynthesis were optimized and studied using UV–Visible spectrophotometry. The increase in absorbance and the colour change, of the solution with time, quantity of extracts and various concentrations of $AgNO_3$ were noted. The absorption optima of silver nanoparticles of *E. herbacea* and *E. ochreata* tuber aqueous extracts were obtained at wavelength 437 nm and 413 nm respectively at 1 ml extract quantity each for 5 h incubation at 60°C. The phytochemicals present in the tuber extract played a significant role in the bio-reduction and stabilization of the synthesized AgNPs.

KEYWORDS: Green synthesis, Silver Nanoparticles (AgNPs), Bio-reduction, Stabilization, Phytochemicals.

INTRODUCTION

Plants especially orchids are the richest source of a variety of potent drugs, which prevent and cure diseases where the synthetic drugs fail. The Genus *Eulophia* is perennial terrestrial orchid with fleshy tubers, rarely pseudo bulbs. The Genus includes about 230 species mostly terrestrial and distributed worldwide; many of them are attractive (**Fig 1**) and showy.[1] The plants have green leaves which are not always visible at flowering. Ethno botanical studies are carried out in ethically different groups of Andhra Pradesh, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttarakhand, Utter Pradesh, West Bengal, Zharkhand states of India and out of India also. The rural and tribal communities such as Bhillas, Pardhi, Pawara, Tadvi and Wanjari etc. inhabiting in Maharashtra have distinct traditions, beliefs, dialects, way of life and knowledge of local flora. They are intimately associated with the forests. These people depend on plants for their routine requirement. Pawara tribals of Toranmal region, Nandurbar, Maharashtra eat raw tubers of *E. ochreata* for rejuvenating and aphrodisiac properties and tuber sap is also applied externally for curing rheumatism.[1] *E. herbacea* is used to prepare a drink called Salep. According to the folklore, 'Salep' of Eulophia tuber is used as an aphrodisiac drug. Dried tubers of Eulophia are used to make a nutritious beverage by treating the powdered preparation with hot water as tonic, known as salep. Decoction of tuber is used on spermatorrhoea, urinary complaints, and menses.[2] In various states of India, rural and tribal communities use ethno-botanical potential of different *Eulophia* species for various ailments especially fertility, aphrodisiac, skin protective, vermifuge, antiscrofulous, anti-bellyache, anti-rheumatic antifutigue, wound healing and antitumour activity.[1] The tubers of *Eulophia* species are used for their rejuvenating, aphrodisiac and antirheumatic properties. In Ayurvedic medicine, *Eulophia* is `prescribed for the treatment of impotency, gynaecological problems, decreased sperm count and blood purification.[3] The tuberous roots or rhizomes of *Eulophia* species are rich in bioactive[1] substances which would be used as phytosomes to have better efficacy as therapeutic drugs. The various biological activities of *Eulophia* tubers reported are: anabolic,[2,4] anthelmintic,[5] antidiarrheal,[6] antimicrobial,[5] anti-inflammatory,[7] antioxidant,[3,7,8] hypolipidomic,[9] reproductive,[2,4] spermatogenic and estrogenic,[10] fertility,[2,4] and insect repellent.[11]

The bio-reduction capabilities of *Eulophia* species tuber extract has not been reported yet for biosynthesis of AgNPs. *Eulophia herbacea* Lindl. and *Eulophia ochreata* (family Orchidaceae) also known as kukkadkand or kutrikand (**Fig 1A**) and amarkand or

singadyakand (**Fig 1B**) respectively and are terrestrial herbs with fleshy tubers rarely pseudobulbs. Qualitative and quantitative phytochemical tests have shown that steroids, carbohydrates, amino acids, phenols, mucilage, tannins, and triterpenoids present in the tubers (**Fig 2A, 2B**). Patil et. al., have already reported various activities of *E. herbacea* and *E. ochreata* tubers such as anabolic, reproductive (Spermatogenic and estrogenic) and antioxidant activities. These orchids also show multiple activities such as anti-cancer, nutritional, anti-hyperlipidemic, anti-arthritic, anti-inflammatory, antimicrobial and immunomodulator. However, no reports available on AgNPs biosynthesis potential of *E. herbacea* and *E. ochreata* tubers.

Fig. 1A*:* Photoplate of *E. herbacea.*

Fig. 1B: Photoplate of *E. ochreata.*

Fig. 2A: Tubers of *E. herbacea.*

Fig. 2B: Tubers of *E. ochreata.*

Green synthesis of nanoparticles is easily available, eco-friendly, cost-effective, simple, and one-pot synthesis method. It has special importance in the medical field, agriculture and bioremediation. Moreover, this process is reproducible and easily scaled up.[12] The phytochemicals present in the plant extract readily act as reducing agents for the efficient conversion of metal salts or ions into metal/metal oxide nanoparticles.[13]

Silver is well-known for its medicinal as well as antimicrobial properties.[14,15] However, the antimicrobial effect of silver ions and their salts is limited and of short life. These limitations can be overcome by using silver nanoforms, which are inert, stable and especially efficient antimicrobial agents.[16,17]

In this study, the phytoconstituents of tuber extract responsible for biosynthesis were analysed qualitatively. The process parameters for AgNP biosynthesis were optimized and studied using UV–Visible spectrophotometry.

MATERIAL AND METHODS

Plant material Collection and Extract preparation

The tubers of E. *herbacea* and *E. ochreata* were collected and also purchased from local market of Vani (District Nashik) and Bhimashankar (District Pune) respectively. The tubers were identified and authenticated by an expert taxonomist. Tubers of both the *Eulophia* species were washed, sliced into thin, small pieces and shade dried for 2-3 weeks. Shade dried tubers were ground to make coarse powder of uniform particle size. Aqueous extract of tuber powder (2 gm) was produced by suspending it in 100 ml double distilled water and heat at 80°C for 6 hrs with continuous stirring. The extracts were filtered through Whatman filter paper No.1. Qualitative phytochemical screening of extracts was done using various methods described by Harborne and Sazada[18,19] (**Table 1**).

Table 1: Phytochemical screening results of *E. herbacea* and *E. ochreata* tuber extract.

Phytochemicals	Chemical Tests	*E. herbacea* tuber aqueous extract	*E. ochreata* tuber aqueous extract
Carbohydrate	Molisch's Test	+++	++
	Fehling test	++	++
	Benedict's test	++	++
Flavonoids	Shinoda test	+	+
	Zinc HCl test	+	+
Glycoside	Borntrager's test	-	-
	Legal's test	+	+
Steroids	Salkowski's test-	-	-
Saponin	Froth formation	+	+
Tannin	Ferric chloride test	-	-
	Gelatine test	-	-
Polyphenol	Folin Ciocalteu Reagent test	+	+
Triterpenes	Conc. HCl test	+	+
Proteins	Biuret test	-	-
	Millon's test	-	-
	Ninhydrin test	+	-
Alkaloids	Mayer's test	-	-
	Wagner's test	-	-
	Dragondorff's test	-	-

−, Absent; +, present at low concentration; ++, present at moderate concentration; +++, present at high concentration

Green synthesis of AgNPs

The aqueous extract of *E. herbacea* and *E. herbacea* tubers were used separately as reducing agents in the biosynthesis of AgNPs. One ml of each aqueous extract was added to 10 ml of 1

mM $AgNO_3$ in an Erlenmeyer flask. The reaction mixture was kept at 60°C for 5 h with constant stirring. The reaction was monitored by absorbance scanning (200–800 nm) after incubation using a UV–Visible spectrophotometer (UV 1700, Shimadzu, Japan). After incubation, the test samples were centrifuged at 10,000 rpm for 20 min at room temperature. The process of centrifugation and redispersion in double-distilled water was repeated thrice to ensure better separation of nanoparticles. The pellets of nanoparticles were dried and the powdered residues of both the test plants were used for further analysis.[20]

Optimization of AgNPs

The AgNP green synthesis was optimized using varying concentrations of $AgNO_3$ and tuber extracts with respect to time and temperature. In brief, different amounts of the aqueous extracts of *E. herbacea* and *E. ochreata* tubers (0.1 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml) and different concentrations of $AgNO_3$ (0.1 mM, 0.5 mM, 1 mM, 2 mM) were used, keeping all the other experimental parameters constant. The samples were scanned in the UV–Visible range up to 5 h.

RESULTS AND DISCUSSION

The main purpose of this work is to explore the use of bio material, i.e. the tubers of *E. herbacea* and *E. ochreata*, in the synthesis of AgNPs. AgNPs were synthesized by bio-reduction of $AgNO_3$ using an aqueous extract of *E. herbacea* and *E. ochreata* tubers. Process parameters such as the concentration of $AgNO_3$, the quantity of the tuber extract, temperature of reaction mixture and reaction time were optimized. The synthesized AgNPs were characterized using UV-visible spectrophotometer.

Green synthesis of AgNPs

Visual observation and UV–visible spectroscopy study

A colourless solution turns brown is the visual observation that confirms the reduction of the silver salt and the synthesis of AgNPs. The synthesis of the AgNPs (bio-reduction of Ag^+ to Ag^0) was monitored by studying UV–visible absorption spectra of the test samples with respect to time.

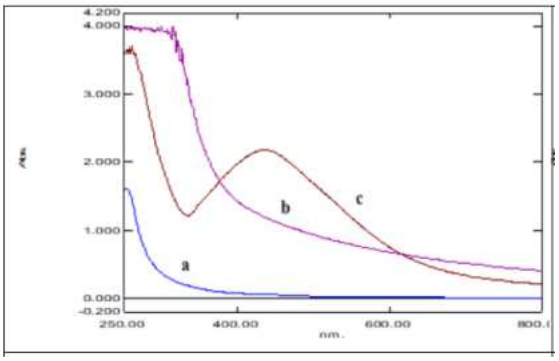

Fig. 1: UV-Visible absorption spectra of a. $AgNO_3$ solution b. *Eulophia herbacea* tuber extract c. synthesized AgNPs with absorption maxima at 437 nm.

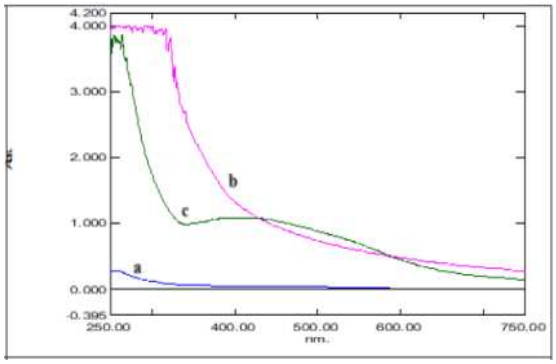

Fig. 2: UV-Visible absorption spectra of a. $AgNO_3$ solution b. *Eulophia ochreata* tuber extract c. synthesized AgNPs with absorption maxima at 413 nm.

The increase in absorbance and the colour change of the solution with time were noted. The AgNPs absorbance of *E. herbacea* and *E. ochreata* was measured at wavelength 437 nm and 413 nm respectively (**Fig 1 and 2**). The UV–Visible absorption spectra of the $AgNO_3$ solution and the *E. herbacea, E. ochreata* tuber extracts do not show any absorption at 437 nm or 413 nm (**Fig 1 and 2**). The brown coloration was due to surface plasma resonance vibration excitation of the AgNPs.[21] The AgNP solution had a yellowish-brown colour with a surface plasma resonance (SPR) absorption maximum at 437 nm and 413 nm respectively, which is the spectral characteristic of AgNPs.[22] This is due to adsorption of phyto-constituents of the tuber extract that may act as capping and stabilizing agents of nanoparticles. This confirms the capability of the aqueous extract of the tuber of *E. herbacea* and *E. ochreata* to reduce silver ions to zero-valent silver in the nano form.

Optimized synthesis of AgNPs

AgNPs were synthesized by adding 1 ml of *E. herbacea* and *E. ochreata* aqueous tuber extract (2% w/v) separately to 1 mM $AgNO_3$ (10 ml), the reaction mixture was kept for at 60°C for incubation up to 5 h (**Fig 1** and **2**). This method requires lesser time as compared to earlier reports on AgNPs synthesis using plant extracts.[23-26] Different phytoconstituents were found in the tuber extracts (**Table 1**).

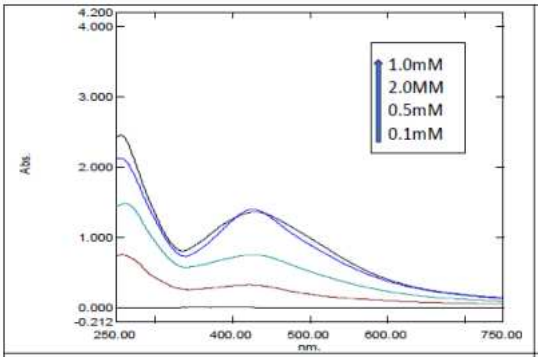

Fig. 3: UV–Visible spectra of AgNPs (60°C) with different concentrations of $AgNO_3$ with *E. herbecea* tuber extract.

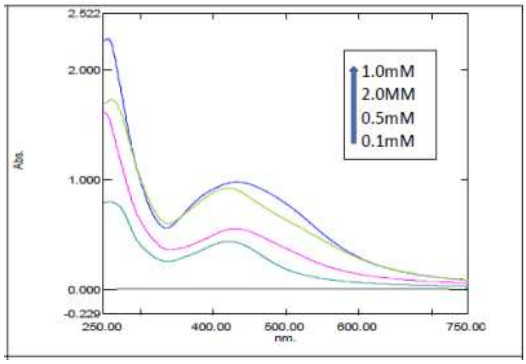

Fig. 4: UV–Visible spectra of AgNPs (60°C) with different concentrations of $AgNO_3$ with *E. ochreata* tuber extract.

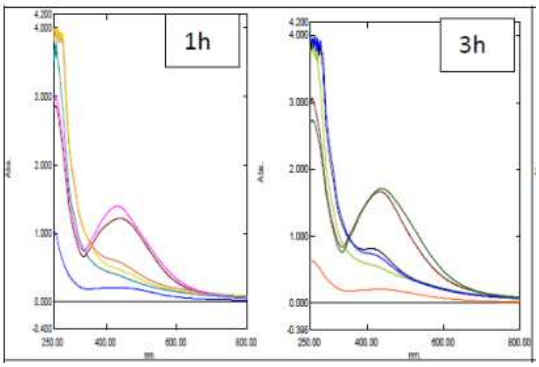

Fig. 5a,b: UV–Visible spectra of AgNPS (60°C) of different quantity of *E. herbecea* tuber Aqueous extract with 1 mM $AgNO_3$ at 1hr and 3h respectively.

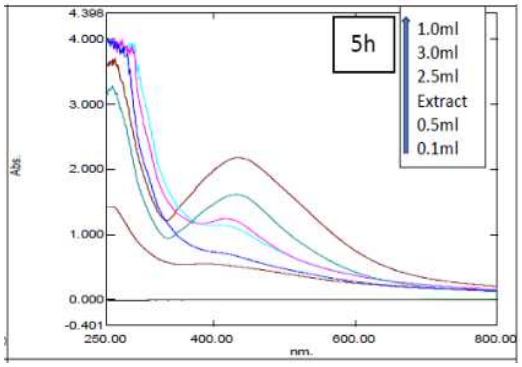

Fig. 5c: UV–Visible spectra of AgNPs (60°C) of different quantity of *E. herbecea* tuber Aqueous extract solution with 1 mM $AgNO_3$ at 5h.

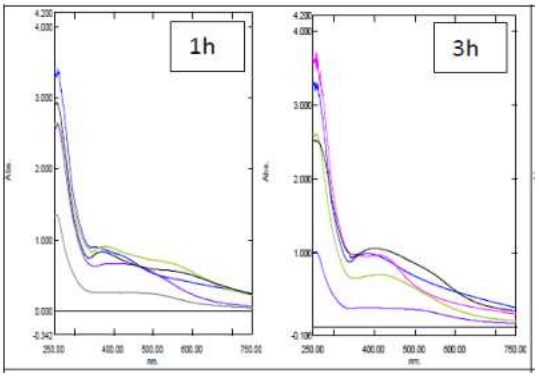

Fig. 6a, b: UV–Visible spectra of AgNPs (60°C) of different quantity of *E. ochreata* tuber Aq. extract with 1 mM $AgNO_3$ at 1hr and 3h respectively.

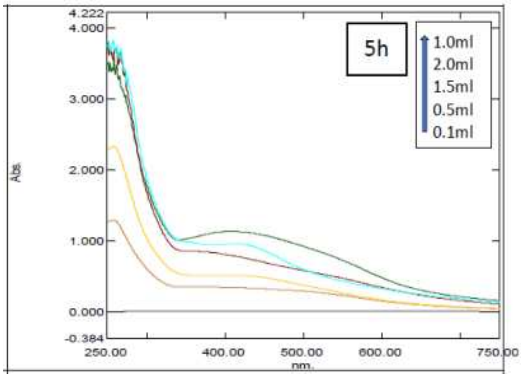

Fig. 6c: UV–Visible spectra of AgNPs (60°C) of different quantity of *E. ochreata* tuber Aqueous extract solution with 1 mM $AgNO_3$ at 5h.

Different concentrations of $AgNO_3$ with the extract of *E herbacea* and *E. ochreata* were scanned in the UV–visible region to optimize the $AgNO_3$ concentration (**Fig 3 and 4**). The UV–visible spectrum of the resulting AgNP solution was recorded at intervals of 1 h up to 5 h at different temperatures (**Fig 5a-c** and **Fig 6a-c**). The maximum absorption of 1ml aqueous

extract of both tubers was obtained at about 5 h at 60°C temperature (**Fig 5c** and **6c**). The deviation in the absorption was studied as a function of the concentration of $AgNO_3$ in the UV–Visible region. With high concentrations of $AgNO_3$ (1.5 mM, 2 mM) and high extract quantities (1.5 ml, 2.0 ml 3.0 ml), the resulting solution had broad peaks of lower intensities. This may be due to agglomeration of nanoparticles, which results to slow rates of bio-reduction.[20,27] The concentration of $AgNO_3$ (0.1 - 1.0 mM) has a significant increasing effect on the synthesis of AgNPs but at 2.0 mM $AgNO_3$ the effect was reverse. With a tuber extract quantity of 0.1 – 1.0 ml, an increase in absorption of the resulting nanoparticle solution was observed but at higher quantity (2.0 ml) the solution shows broad peaks with lower intensities. This may be due to the different phytoconstituents present in the tuber extract (**Table 1**). The phytoconstituents present in the plant extract directly or indirectly influence the reaction kinetics of the AgNP biosynthesis.[27] The phytochemicals are responsible for the fast reduction of metal salts.[14] Carbohydrates, flavonoids, saponins, tannins-phenol, and proteins were among the phytoconstituents present in the *E. herbacea* and *E. ochreata* extract (**Table 1**), which is consistent with previous reports. The progress of the AgNP synthesis (bio-reduction of Ag^+ to Ag^0) was monitored by studying UV–visible spectra with respect to time.

CONCLUSION

Simple, cost-effective and eco-friendly, reproducible and easily scaled up green synthesis of AgNPs using *E. herbacea* and *E. ochreata* tubers extracts were reported. The phytochemicals present in the tuber extract played a significant role in the bio-reduction and stabilization of the synthesized AgNPs.

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