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Synthesis of Silver Nanoparticles using Leaf Aqueous Extract of Ocimum basilicum (L.)

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Abstract: The present study reports the synthesis of silver nanoparticles using aqueous *Ocimum basilicum*(L.) leaf extract and silver nitrate solution. The synthesized silver nanoparticles were analysed by UV-Spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM),X- Ray Diffraction Spectroscopy (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The particle size was found to be of an average size of 88nm. The silver nanoparticles were synthesized by different methods and sonication method was found to yield nanoparticles rapidly and easily.
Keywords: silver nanoparticles, *Ocimum basilicum*, sonication, SEM, XRD.

Introduction

Nano structured materials have been fascinating in recent years since they show valuable and unusual remarkable properties, compared to conventional polycrystalline materials. Nanoparticles play an important role in all fields. In medical field it is used to treat skin cancer and reduce bleeding in trauma patients[1]. There are different types of nanoparticles like those of metals, fibers, etc. among these silver nanoparticles have found many applications. Fabric containing bamboo-charcoal nanoparticles is used in masks owing to its antimicrobial properties[2]. Silver nanoparticles are used for deactivating HIV at low concentrations with less toxicity. It is also used in plasmonics, medicine, catalysis, photonics and optics (www.sooperaticles.com). Silver nanoparticles are used as antiviral agents[3], as antimicrobials[4], anti-proliferative agents[5] and many more. There are several methods of synthesize of nanoparticles. Most common are using chemical compounds. The chemical molecules may be toxic, costly and difficult to procure. Hence green methods of synthesis are currently envisaged.

Plant extracts are rich sources of secondary metabolites. Most of the extracts contain several metabolites that can easily reduce silver nitrate to silver nanoparticles. *Ocimum basilicum (L.)* is also known as sweet basil and King of spices. It is a popular Chinese medicinal herb of the family Labiatae. The whole plant materials and essential oils have been used extensively in food, perfumes, dental and oral products [6]. The leaves and flowering parts of these plants are antiplasmodic, aromatic, carminative, digestive, stomachic and stimulant. They are administered orally in the treatment of fever, poor digestion, nausea, depression and exhaustion (www.http.science.com). *Ocimum sanctum* has been used in the synthesis of nanoparticles. There are no reports in the use of *Ocimum basilicum* in the biosynthesis of silver nanoparticles.

Different components such as Caffeic acid, p-Coumaric acid, Myrcene, Rutin, Trytophan are present in the leaves of *Ocimum basilicum (L.).* These are used as remedies for disorders such as viral, ocular, respiratory and hepatic infections. *Ocimum basilicum (L.)* is one of the most important of all *Ocimum* species which are all together commonly referred to as Basil[7]. The essential oil of these plant is used as a food additive and in cosmetics[8].There are literally thousands of *Ocimum basilicum(L.) are available* which are classified based on plant morphology, pigmentation, and chemical composition of the essential oil[9].

Considering the significance of silver nanoparticles and the nonexistence of use of the extracts of *Ocimum basilicum (L.)* in the reduction of silver salts to nanosilver, this work is intended to biosynthesize nanoparticles using *Ocimum basilicum(L.)* extracts.

Experimental

All reagents used were of analytical grade. Double distilled Millipore water was used in study. The leaves of *Ocimum basilicum* were procured from a local plant dealer in Coimbatore. Silver nitrate solution (3mM) was used throughout the study.

Phytochemical Screening of the Plant Extract

The aqueous extract of *Ocimum basilicum* was screened for presence of phytochemicals like terpenoids, flavonoids, alkaloids etc. using standard colour tests[10].

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesised from silver nitrate using aqueous leaf extract of *Ocimum basilicum* (L.) (OB) as reducing agent. Different conditions and different methods were employed for the synthesis of silver nanoparticles for optimising the time of synthesis and yield of silver nanoparticles.The following parameters were varied for optimising the process of synthesis of silver nanoparticles.

- Room temperature
- Variation of concentration of silver nitrate
- Variation of pH
- Higher temperature
- Sonication method

Silver nanoparticles were synthesized by the addition of 5ml of silver nitrate solution to different volumes (1ml, 2m, 3ml, 4ml, 5ml) of plant extract. The colour changed to reddish brown after 30 minutes at different intervals of time under room temperature at constant pH (6).The formation of silver nanoparticles was monitored by UV studies. The study was conducted in a similar manner at 75˚C at constant pH 6 to find the effect of temperature on the ease of formation of silver nanoparticles. The effect of variation of volume of silver nitrate was with varying volumes (1ml, 2ml, 3ml, 4ml, 5ml) of silver nitrate solution at room temperature and constant pH.

In the variation of pH studies, 1ml of aqueous extract is treated with 5ml of silver nitrate solution at room temperature and different pH (7, 8, 9, 10 and 11). In the sonication method ultrasonic bath PCI Ultrasonics 1.5 L (H) was used. 5ml of 3Mm of silver nitrate solution was added to different volumes of aqueous plant extract such as (1ml, 2ml, 3ml, 4ml, 5ml) and sonicated till the formation of nanoparticles as evidenced by the colour change to reddish brown.

Further confirmation of the formation of silver nanoparticles was made using analytical and spectroscopic techniques mentioned below.

Characterisation of Silver Nanoparticles

The silver nanoparticles formed were characterized by using SEM, FTIR, XRD, UV-Visible Spectroscopy.

XRD- Study

A drop of synthesized silver nanoparticles was coated on a glass plate, and the coated film was characterized using X' Pert Powder PANALYTICAL X-Ray Diffraction Spectrometer. Debye-Scherrer's equation was used to determine the particles size of the silver nanoparticles from the 2θ values of the X-ray diffraction peaks.

Debye – Scherrer's Equation

$D = K\lambda / \beta \cos\theta$

where K= constant, λ = wavelength of the X-rays, β = full width half maximum of the XRD peak (radians), θ = Bragg's angle of the XRD peak.

SEM and FTIR characterisation of synthesized nanosilver particles

The synthesized silver nanoparticles were fabricated on a glass plate. The morphology and size of the silver nanoparticles was investigated by using TESCAN make Scanning Electron Microscope provided with Vega TC Software, Further Secondary Electron Sputtering at an applied potential of 20 Kv was adopted prior to recording SEM. FTIR was recorded for the synthesized silver nanoparticles in a Shimadzu Spectrophotometer.

Results And Discussion

Phytochemical Screening of the Aqueous Extract of *Ocimum basilicum*

Standard phytochemical tests were conducted to find the presence of metabolites like alkaloids, flavonoids, terpenoids etc in the aqueous extract of *Ocimum basilicum.* The results of the phytochemical screening are presented in (Table 1). It is well evident from the table that the aqueous extract was rich in metabolites like Alkaloids, Saponins, Terpenoids, Phenols, Tannins, Anthraquinones, Anthocyanins and Amino acids. These metabolites are commonly known to reduce silver nitrate to silver nanoparticles.

Synthesis of Silver Nanoparticles using the Aqueous Extract Of *Ocimum basilicum* at Room Temperature

The results of formation of nanosilver with varying concentrations of OB at room temperature are given in table 2. The colour change from brown to reddish brown was noted in 10 minutes. The formation of silver nanoparticles was confirmed by recording UV spectra of the solution and observing the bands obtained due to the Surface Plasmon Resonance (SPR) (480 nm) of silver nanoparticles. The UV spectrum shows that the bands were formed in the range of 440-445cm^{-1}.This confirms the formation of silver nanoparticles at room temperature. This study shows that 5ml of silver nitrate is easily reduced by 1ml of aqueous plant extract in 10 minutes whereas 1:1 solution of OB extract and silver nitrate gives silver nanoparticles in 35 minutes.

Effect of Concentration Variation of Silver Nitrate

The time taken for the formation of silver nanoparticles in the variation of volume of silver nitrate is shown in the table 3. The colour change from brown to reddish brown was noted in 21 minutes. The UV spectrum shows that the bands were formed in the range of 435-445 cm^{1}. This confirms the formation of silver nanoparticles by concentration variation method. This study shows that 5ml of silver nitrate is easily reduced by 1ml of aqueous plant extract in 21 minutes whereas 1:1 solution of OB extract and silver nitrate gives silver nanoparticles in 75 minutes. On increasing the concentration of silver nitrate with fixed volumes of plant extract the time taken for formation of nano particles is less. As the proportion of silver nitrate was increased, the formation of silver nanoparticles was on an increase. These results are similar to those obtained in the Sunlight induced rapid synthesis and kinetics of silver nanoparticles using leaf extract of *Achyranthes aspera* L[11].

Effect of pH Variation

Analysis of the formation of silver nanoparticles at varying pH of the solution (table 4) shows that in acidic medium there is formation of silver nanoparticles whereas in basic medium the silver nanoparticles was formed quickly within 1 minute. As the pH of the solution increases easier is the reduction of silver nitrate by aqueous leaf extract of OB. This may be probably due to the availability of more H+ from the metabolites at higher pH which enables quicker reduction of silver nitrate and hence the oxidation of the metabolites. The stability of the nanoparticles at alkaline pH may be due to the optimal activity of the enzymes and capping proteins[12] present in plant extracts.

Synthesis of Silver Nanoparticles using Aqueous Extract of *Ocimum basilicum* at High Temperature and by Sonication Method (Table 5)

The formation of silver nanoparticles was noted in 5 minutes at 75^0C and as the concentration of OB extract is increased the time required for formation of silver nanoparticles was also found to increase. Generally the rate of reaction increases with increase in temperature and also, with increase in temperature the activity of enzymes in plants also increases. The availability of capping agents for the reduction of silver nitrate is also more. These might be probably the factors attributing to the observations.

The reduction of aqueous silver ions into silver nanoparticles by sonication method was monitored and it was found that the silver nanoparticles were easily formed from 1ml of aqueous OB extract and 5ml of silver nitrate. The silver nanoparticles formed were stable at room temperature without flocculation of particles (Fig 1). Analysis of the formation of silver nanoparticles at various concentration of aqueous OB by sonication method shows that the concentration of aqueous OB (1ml) produces nanoparticles in 6 minutes whereas 1:1 solution of OB and silver nitrate gives nanosilver in 25 minutes.During sonication ultrasonic waves are generated in a liquid suspension which results in cluster breakdown or further agglomeration, as well as other effects including chemical reactions. This causes the gradual colour change from light brown to reddish brown[13]. The UV spectra of a representative sample of nanosilver prepared in given in fig.2.

Table 1 : Phytochemical Screening of aqueous leaf extract of *Ocimum basilicum*

Phytochemicals	**OB Leaf Extract**	**Phytochemicals**	**OB Leaf Extract**
Alkaloids	+	Phenols	+
Flavonoids	-	Anthroquinones	+
Tannin	+	Glycosides	-
Saponin	+	Proteins	-
Carbohydrates	-	Amino acid	-
Quinone	+	Anthocyanins	+
Terpenoids	+	Sterols	-

Table 2: Synthesis of silver nanoparticles using aqueous extract of *Ocimum basilicum* at room temperature

Concentration of OB extract (ml)	**Concentration of silver nitrate (ml)**	**P^H**	**Time of formation of silver nanoparticles (Min)**
1	5	6.07	10
2	5	6.10	17
3	5	6.13	25
4	5	6.18	28

Table 3: Synthesis of silver nanoparticles aqueous extract of *Ocimum basilicum* by concentration variation method

Concentration of OB extract (ml)	Concentration of silver nitrate (ml)	P^H	Time of formation of silver nanoparticles (Min)
1	1	6.07	75
1	2	6.10	58
1	3	6.13	51
1	4	6.18	33
1	5	6.20	21

Table 4: Synthesis of silver nanoparticles aqueous extract of *Ocimum basilicum* by pH variation method

Concentration of plant extract (ml)	Concentration of silver nitrate (ml)	P^H	Time of formation of silver nanoparticles (Min)
1	5	7.24	5
1	5	8.22	4
1	5	9.18	3
1	5	10.05	2
1	5	11.06	1

Table 5: Synthesis of silver nanoparticles using aqueous OB extract at different conditions

Concentration of aqueous OB (ml)	Concentration of silver nitrate (ml)	P^H	Time of formation of silver nanoparticles (Min)	
			at High Temperature	by sonication method
1	5	6.07	5	6
2	5	6.10	8	9
3	5	6.13	12	10
4	5	6.18	15	15
5	5	6.20	18	25

Figure 1: OB sample and silver nanoparticles formed from OB extract

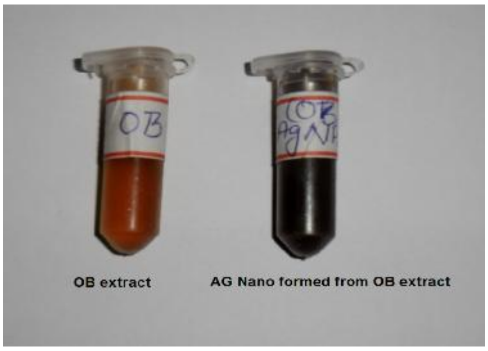
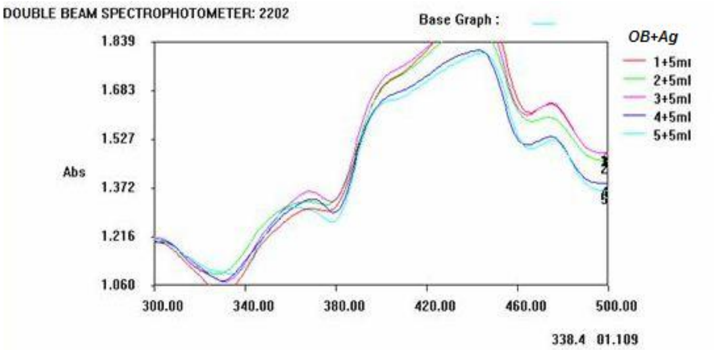


Figure 2 : The UV- Visible Spectra of silver nanoparticles synthesised using aqueous extract of *Ocimum basilicum*



Characterisation of the Silver Nanoparticles Formed using the Aqueous Extract of *Ocimum basilicum*

Scanning Electron Microscope (SEM)

SEM analysis is used to provide information about the morphology and size of the synthesised silver nanoparticles. The silver nanoparticles were investigated by using TESCAN make Scanning Electron Microscope provided with Vega TC software. The SEM micrograph (Fig 3.) reveals the size of silver nanoparticles as 120nm.

X- Ray Diffraction Analysis

X- Ray diffraction is a very important method to characterize the structure of crystalline materials and used for the lattice parameters analysis of single crystals, or the phase, texture or even stress analysis of sample. X- ray diffraction of the silver nanoparticles formed from aqueous OB extract showed a diffraction peak at 33 corresponding to nanosilver (Fig.4.). According to JCPDS standards of XRD of silver nanoparticles the most intense peaks are related to 2theta values of 38.2, 44.3, 62.8, 70.3 **(**JCPDS values.com).The size of the nanoparticles was calculated by Debye Scherer's equation using FMWHs obtained from the diffraction peaks. The calculated value for the size of the silver nanoparticles is about 88nm.

FTIR Analysis

FTIR analysis was used to characterize the nature of capping ligands that stabilises the silver nanoparticles formed by bio reduction process .The FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the silver ions into silver nanoparticles.

The peaks at 3347 cm^{-1} indicate the presence of O-H stretching, the peaks at 3327 cm-1 that of N-H stretching, 1641 cm-1 due to C=C stretching and the peak at 1513 cm-1 may be due to the presence of N-O asymmetric nitro compounds in the plant extract(fig 5).

Figure 3: SEM Micrograph of silver nanoparticles formed from OB extract

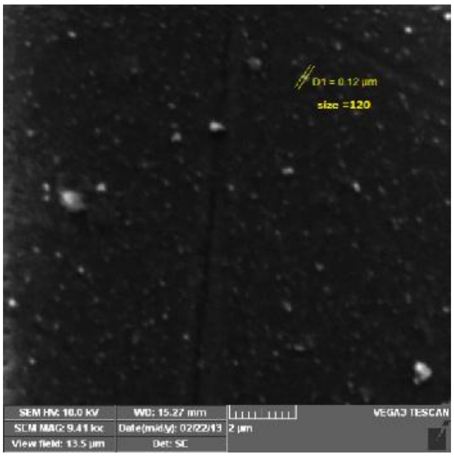


Figure 4: XRD Spectra of silver nanoparticles formed from OB extract

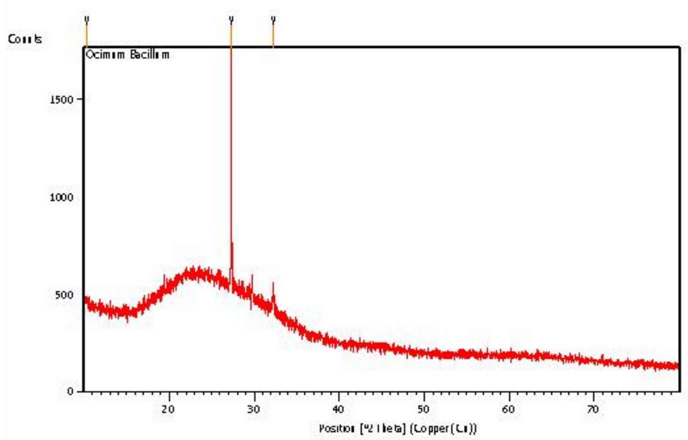
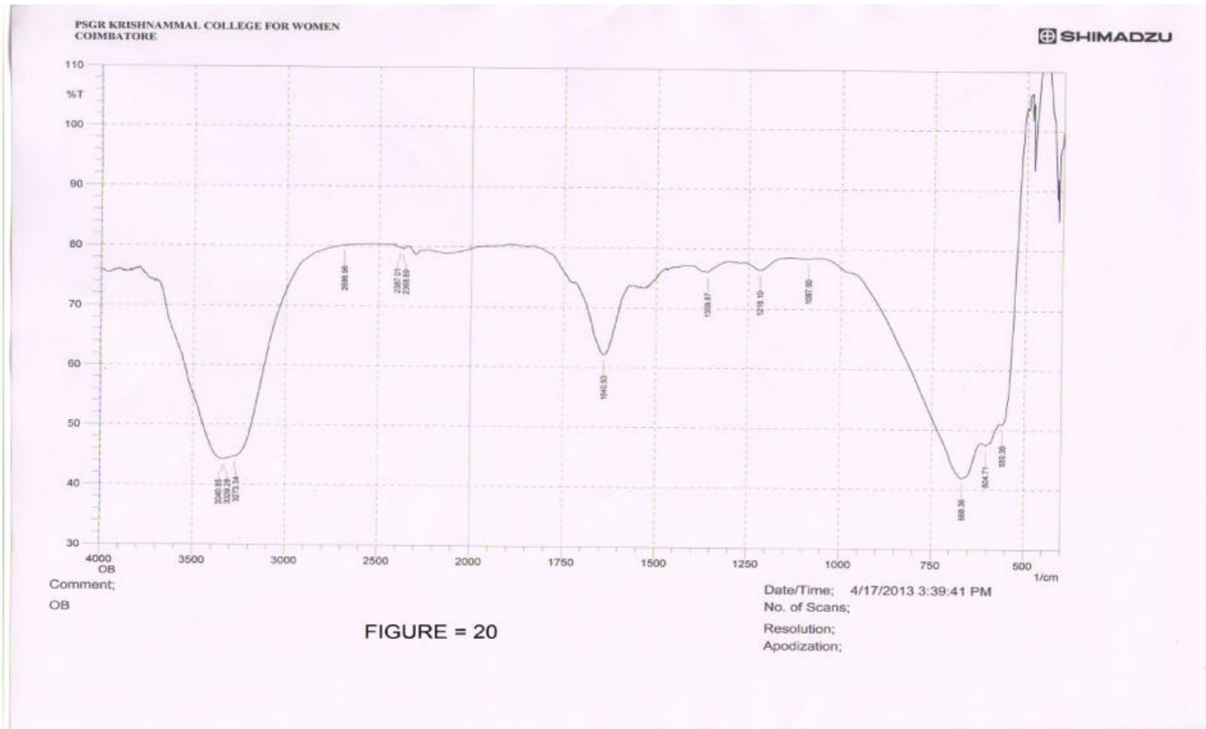


Figure -5 FTIR Spectra of silver nanoparticles formed from aqueous OB extract



Conclusion

In the present investigation it was noted that silver nanoparticles synthesized using aqueous OB extract exhibited reddish brown colour in aqueous solution due to excitation of Surface Plasmon Vibrations. The UV absorption spectra of biosynthesis nanoparticles in all methods gave absorption maximum at 450cm^{-1}.

The phytochemical screening of the aqueous extract of *Ocimum basilicum* revealed the presence of phytochemicals like Alkaloids, Flavonoids, saponins, carbohydrates, terpenoids, sterols, phenols, Tannins, Anthraquinones, Anthocyanins, Amino acids and proteins. The presence of such metabolites are indicative of their role in the reduction of silver nitrate to silver nanoparticles.Synthesis of silver nanoparticles using aqueous *Ocimum basilicum* extract at different conditions shows that sonication method formed nanosilver in 6minutes, whereas a 1:1 ratio of silver nitrate and plant extract formed silver nanoparticles in 25minutes. The SEM results reveals that average size of silver nanoparticles synthesized from OB extract was found as spherical shape and 120nm in size. The bioreduction of aqueous silver ions by the plant extract of *Ocimum basilicum* leaf extract is a good source for green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic feasibility, etc.

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