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A Comparative Study of Green Synthesis of Silver and Copper Nanoparticles using *Smithia sensitiva (Dabzell)*, *Cassia tora* (L.) and *Colocasia esculenta* (L.)

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ABSTRACT

Green synthesis of nanoparticles is becoming increasingly popular due to its advantages over physical or chemical methods. This research is carried out to compare the synthesis of nanoparticles employing three different methods and using some leafy vegetables as the source of bioreductants during the synthesis. Biological reduction of silver nitrate, copper acetate and copper sulphate was carried out using leaf extracts of some plants during the synthesis of silver and copper nanoparticles. The presence of established biological reducing agents such as flavonoids, terpenoides detected by phytochemical tests, confer the ability upon these plant extracts to act as reducing and capping agents during formation of the silver and copper nanoparticles. Synthesis of nanoparticles was confirmed by UV-Vis Spectroscopy method, using wavelength between 200-1100nm. The analysis revealed well capped nanoparticles with characteristic peaks. Particle size was detected using nanoparticle tracking analysis. It revealed nanoparticles of sizes from 27- 149nm.

Key words: *nanoparticles, bioreductant, phytochemical, UV-Vis spectroscopy, nanoparticle tracking analysis.*

INTRODUCTION

Nanotechnology has become one of the active fields of research in all the areas of science in today's modern times[1].It is an emerging field of science and concerned with manufacturing and manipulation of nano size materials using different methods. It is used to indicate one billionth of a meter represented as 10^{-9} [2]. Nanotechnology is gaining attention not only due to its dimension and properties but also due to their range of applications spanning from aeronautics to medical sciences[3].

Nanoparticles can be synthesised using physical, chemical and biological methods. Both physical and chemical methods are expensive and it involves use of toxic, hazardous chemicals as well[4]. Biologically nanoparticles can be synthesised using bacteria, fungi and plants.

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Biological methods are known for their green synthesis approach towards nanoparticle synthesis[5]. These methods have the ability to replace physical and chemical methods, as they are economically and ecologically sustainable[6]. Since plants are known for their therapeutic value, biologically synthesized nanoparticles, have found applications in the fields like bio-engineering, bio-sensors, catalyst, nanofabrics, medicine, drug delivery, cosmetics, paints, packaging, etc[7].

The current research aims at synthesizing nanoparticles using following edible plants found around Mumbai region, by using three different protocols and study their structural property such as size. Aqueous extracts of *Smithia sensitive (Dabzell)*, *Cassia tora* (L.) and *Colocasia esculenta* (L.) *(Schott)* have been used for the synthesis of silver and copper nanoparticles in this project.

MATERIALS AND METHODS

Collection and identification of plant species

The following plants were collected from various markets of Mumbai and identification of plant species was carried out using standard taxonomic references[8,9,10].

Table 1: Identification of the leafy vegetables used for the experiment

	Plant 1	Plant 2	Plant 3
Scientific Name	*Cassia tora* (L.)	*Colocasia esculenta* (L.) *(Schott)*	*Smithia sensitive (Dabzell)*
Family	Fabaceae	Araceae	Fabaceae
Common Name	Takla	Ran alu	Kawla

Preparation of leaf extract

Fresh leaves of plants were collected and washed with distilled water thoroughly and were shade dried for about 4 days, followed by oven drying to remove its moisture content. The dried samples were ground into a fine powder. 10g of plant powder was dispensed in 100 ml of distilled water and boiled for 15 min. at 75ºC using water bath[11]. The extracts obtained were filtered through Whatman no. 1 filter paper and the filtrates were collected and stored at 4°C for further use[3,12].

Phytochemical analysis

Phytochemical analysis of the powdered samples as well as aqueous extracts was carried out by employing standard conventional protocols. The samples were analysed to detect the presence of tannins, flavonoids, saponins, cardiac glycosides, triterpenoids, alkaloids, steroids, carotenoids, terpenoids, anthraquinone glycosides, glycosides and phenols, using standard protocol[13].

Synthesis of silver and copper nanoparticles

Table 2: Methodology for synthesis of nanoparticle

$AgNO_3$ Method [11,14]	$Cu(CH_3COO)_2$ Method[15]	$CuSO_4$ Method[16]
1. Preparation of 1mM $AgNO_3$ 2. Addition of 10ml of aqueous extract in 90ml of 1mM $AgNO_3$ and stirred 3. Kept in dark at RT for 24 hours 4. Observed for colour change	1. Preparation of 0.5% Cu acetate solution 2. Addition of 10ml of aqueous extract in 100ml of 0.5% Cu acetate solution and stirred 3. Kept in dark at RT for 24 hours 4. Observed for colour change	1. Preparation of 1mM $CuSO_4$ 2. Addition of Aqueous extract in 1mM $CuSO_4$ solution in 1:1 proportion and stirred 3. Kept in dark at RT for 24 hours 4. Observed for colour change

The nanoparticles solution thus obtained was purified by repeated centrifugation at 5,000 rpm for 30min followed by re-dispersion of the pellet in de-ionized water. Then the nanoparticles were dried in oven at 60°C and stored at 4°C, in amber coloured bottles, till further analysis.

UV-Vis spectra analysis

About 1 mL of the sample suspension was taken in quartz tube followed by dilution of the sample with 2 ml of distilled water to monitor the synthesis of nanoparticle, and subsequent scan in UV-Vis spectra, between wave lengths of 200-1100nm in UV-visible spectrophotometer (Model-Shimadzu UV 1800, Germany). UV-Vis spectra were recorded at the interval of 24hrsfor maximum absorption[17].

Nanoparticle tracking analysis (NTA)

NTAanalysis was performed with Nano sight UK-LM20 instrument. Nanoparticles are dissolved in very little amount of sterile distilled water and inserted into prism using sterile syringe and then allowed to analyse the size of nanoparticles present in it. NTA uses laser light as a source to illuminate metal particles. NTA image analysis software is used for tracking the Brownian motion of the particles and recording the size of nanoparticles[18].

OBSERVATIONS

Phytochemical analysis

Table 3: Phytochemicals commonly found in both dry powdered samples and aqueous plant extracts

Sr.No.	Test	Sample Form	*Plant1 C.tora*	*Plant2 C.esculenta*	*Plant3 S.sensitiva*
1.	TANNINS	Dry	+++	+++	++
		Aqueous	++	+	–
2.	FLAVONOIDS	Dry	++	++	++
		Aqueous	+	++	+
3.	SAPONINS	Dry	+	–	+
		Aqueous	++	–	+
4.	CARDIAC GLYCOSIDES	Dry	+	+	–
		Aqueous	++	+	–

Table 4: Additional phytochemicals present in dry powdered sample and aqueous plant extracts

Sr.No.	Test	Sample Form	*Plant1 C.tora*	*Plant2 C.esculenta*	*Plant3 S.sensitiva*
1.	TRITERPENOIDS	Dry Plant sample	++	+++	+
2.	ALKALOIDS		–	+	–
3.	STEROIDS		+	++	+
4.	CARTENOIDS		+	–	+
1.	TERPENOIDS	Aqueous Plant sample	++	++	++
2.	ANTHRAQUINONE GLYCOSIDES		–	–	+
3.	GLYCOSIDES		+	+	–
4.	PHENOLS		–	+	–

Key: Strongly positive: +++, moderately positive: ++, weakly positive: +, negative: –

UV-Vis spectroscopy

Table 5: Maximum absorbance of reaction mixtures with plant extracts

Methods	Plant 1 *Cassia tora*	Plant 2 *Colocasia esculenta*	Plant 3 *Smithia sensitiva*
	Maximum absorbance (nm)		
$AgNO_3$	408	354	368
$Cu(CH_3COO)_2$	416	320	320
$CuSO_4$	508	416	440

From the above table, it is observed that experimental samples prepared using three different methods, exhibited absorption patterns at different wavelengths with maximum absorption as mentioned in the above table (5), indicating formation of nano particles[18].

NTA analysis: The size of nano particles was analysed using NTA

Table 6: Size of nanoparticles formed and the concentration per frame

Method	Plant 1 *Cassia tora*		Plant 2 *Colocasia esculenta*		Plant 3 *Smithia sensitiva*	
	Nano particles: Average size(nm)and Concentration per frame					
$AgNO_3$	149	9.74	65	34.59	44	43.03
$Cu(CH_3COO)_2$	27	85.10	44	23.94	50	75.23
$CuSO_4$	69	46.77	57	34.64	136	78.80

RESULTS AND DISCUSSION

Phytochemical analysis

The role of bio molecules in the synthesis of nano particles is well established by several workers[7,22]. Phytochemicals such as polyphenols, ascorbic acid, flavonoids, sterols, triterpenes, alkaloids, alcoholic compounds, polysaccharides, saponins, and proteins/enzymes which act as bio reductant, react with metal ions and are used as scaffolds to direct the formation of metal nanoparticles in the solution[15].

Phytochemical analysis was performed using two sets of samples, dry powdered samples and aqueous extracts of the three plants under investigation. Since the nanoparticle synthesis involved the use of aqueous plant extracts, the presence of phytochemicals, reported to be the bio reductants and had to be confirmed in the aqueous state of samples. Phytochemicals such as tannins, flavonoids, saponins and cardiac glycosides were found in both the plant samples i.e. dry powdered as well as the aqueous extracts.In addition to these, dry powdered samples showed presence of triterpenoids, alkaloids, steroids and carotenoids, whereas terpenoids, anthraquinone glycosides, glycosides and phenols were the additional phytochemicals that were detected in aqueous extracts. Thus it can be noted that the dry powdered sample and aqueous extracts showed presence of phytochemicals, as mentioned in the tables (3,4),which can positively influence the synthesis of nano particles.

UV-Vis Spectroscopy

Synthesis of nano particles can be visualized by colour change in the experimental sample as compared with the control sample where no colour change was observed, after incubation period which indicates formation of nano particles. The colour change in silver nano particle was from light yellow to darker yellow to brown. This was similar to the observations reported by previous investigators[22], where as the colour change for synthesis of copper nano particles ranged from pale yellow to yellowish green to pale brown. The time period for change in colour differed method wise as well as plant wise. Formation of stable silver and other metal nano particles during the synthesis reaction was further confirmed using UV-Vis spectroscopy. It is generally recognized that UV-Vis spectroscopy could be used to monitor the reduction of pure metal ions and examine size- and shape-controlled nanoparticles in aqueous suspensions. The UV-Vis spectrum of the reaction mixtures showed absorption peaks at around different ranges, ranging from 354 - 408nm for $AgNO_3$, 320-416nm for Cu acetate and 416-508nm for $CuSO_4$ as shown in table (5), for the three plants species used. Broadening of peaks indicated polydispersed nano particles.

NTA analysis

NTA analysis reveals information regarding size of nano particles synthesised. It also reveals information regarding concentration and distribution of nano particles[18]. Earlier reports on size of nanoparticles synthesized from plant extracts range from as small as 2 nm in *Azadirecta indica* to 150 in *Mentha piperita*[7]. In the current investigations, the average size of nanoparticles was found to be within the range of 27-149nm with minimum size obtained in Cu acetate method as shown in table (6). Concentration of nanoparticles per frame recorded by NTA was inversely proportional to its size in all the samples except one.

One of the reasons for selecting these specific plants was that, these leafy vegetables which are said to have certain health benefits[19,20,21], are fast disappearing from urban cuisines. If their therapeutic significance and nano particle applications can be scientifically established, the local farmers could be prompted to cultivate them on a larger scale that would bring in additional income and improve their livelihood.

CONCLUSION

On the basis of the results mentioned above, it can be concluded that the three plant species under investigation i.e. *Cassia tora, Colocasia esculenta and Smithia sensitive* could be employed in the synthesis of nano particles. The phytochemical analysis of these leafy vegetables, revealed the presence of many bio molecules having potential antimicrobial activity and which also act as bio reductants during the formation of nanoparticles. This increases the potential use of these nanoparticles, in antimicrobial preparations, in view of previous reports on the above plants extracts having antimicrobial efficacy[23]. A comparative analysis of nanoparticle synthesis, using three different methods i.e. $AgNO_3$, $Cu(CH_3COO)_2$ and $CuSO_4$, established the fact that nanoparticle synthesis does take place by all three methods with $Cu(CH_3COO)_2$ giving better results than other two methods. The nanoparticle tracking analysis revealed the sizes of the nanoparticles formed, ranging from 27 nm -149 nm. Though the size range of nanoparticles obtained using the three plants mentioned in the above research, is reported for the first time; smaller size is one of the most essential characteristic from the point of view of applications of nanoparticles. Thus improving the methodology for further reduction in size would be the next step before working on its applications. Study of structural characteristics and functional groups of the nanoparticles formed, can be done by SEM and FTIR analysis.

Thus nanoparticles, obtained by using green synthesis method, are gaining importance in nanotechnology due to their easy raw material availability, time efficiency, environmental and economic feasibility and a promise of applications in various biological fields.

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