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SYNTHESIS AND CHARACTERISATION OF AgNPs AND CuNPs USING LEAF EXTRACTS OF *Hibiscus cannabinus* (L.) AND *Colocasia esculenta* (L.) SCHOTT

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ABSTARCT

The field of Nanotechnology is making impact in all spheres of human life. The multifarious applications of nanoparticles are widened by reducing their size. The current project work explored green synthesis of Silver and Copper nanoparticles using plant extracts as reducing and capping agents, and also compared three methods of synthesis. The bio reduction of Silver Nitrate, Copper Acetate and Copper Sulphate was carried out using leaf extract of two plants, *Hibiscus cannabinus* (L.) and *Colocasia esculenta* (L.) Schott, in synthesis of Silver and Copper nanoparticles. Nanoparticles thus synthesised, were investigated employing UV/Vis spectroscopy, which confirmed the formation of nanoparticles. The Nanoparticles were further characterized by using Nanoparticle Tracking Analyzer (NTA) and FT-IR, where the characteristic peaks suggested nature of the phytochemicals present in these plants, which act as the biological reducing agents for nanoparticle production.

Keywords: Nanoparticles, Phytochemicals, UV/Vis spectroscopy, Nanoparticle tracking analyzer, FT-IR

1. INTRODUCTION

Nanotechnology is one of the promising fields of Science [1] which deals with design, synthesis of nano materials, manipulation and use of materials at the nano scale level [2] i.e in the range of 10^{-9}, which is one billionth of a metre [3]. Nanoparticles (NPs) are said to be the building blocks of nanotechnology [5] and are of tremendous importance because of their exceptionally minute dimension and

unique properties [1]. They have range of applications in the field of medicine, electronics, food science, fuel cell, solar cells, automobiles, space, cosmetics, clothes, chemical industries, Sporting goods etc [3]. The activity of nanoparticles varies with their size and shape, which depends greatly upon the method employed for their synthesis.

Nanoparticles can be synthesised by using physical, chemical and biological methods. Physical methods like pyrolysis, evaporation-condensation, etc. have drawbacks such as defective surface formation, high cost of manufacturing, and large energy requirement. Chemical synthesis methods like chemical reduction, Tollen's method, sol gel technique, etc. involve the usage of toxic chemicals, formation of hazardous by-products, and need conditions such as high temperature and high pressure [4][6]. The biological synthesis of nanoparticles overcomes the limitations of these abovementioned conventional methods and thus has ability to replace the physical and chemical methods.

Biological sources such as bacteria, fungi and plant extracts can be employed for the synthesis of nanoparticles. The use of plant extracts for synthesis of nanoparticles is beneficial over microorganisms due to easy availability of plants, easy to handle, cost-effective, eco-friendly and a variety of secondary metabolites which help in reduction and act as natural capping agents [4][6]. The major drawbacks of microbe-mediated nanoparticle synthesis, is maintenance of cell cultures and isolation of microbes, which requires trained staff. Due to all these reasons, along with simple and rapid synthesis of nanoparticles using plants, make them preferred biological resources than microbes.

Nature has been a great source of plants and they are known to possess various therapeutic compounds which are being exploited since ancient time as a traditional medicine [4]. According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [7]. In the present study, aqueous extract of *Hibiscus cannabinus* (L.) and *Colocasia esculenta* (L.) Schott have been used for the synthesis of silver and copper nanoparticles by three different methods. *Hibiscus cannabinus* (L.) is a monsoon grown plant, available only during monsoon, commonly called as 'Ambadi' and *Colocasia esculenta* (L.) Schottis an annually growing herbaceous plant, available throughout the year, commonly called as 'Ran alu'.

The main objective of this project was to synthesize and characterize, silver and copper nanoparticles, using aqueous leaf extracts of *Hibiscus cannabinus* (L.) and

Colocasia esculenta (L.) Schott and to detect the presence of certain phytoconstituents which act as reducing agents in synthesis of these nanoparticles.

2. MATERIAL AND METHOD

2.1 Collection and identification of plant species:

The plants were collected from local markets in Mumbai. They were identified by their morphological characters, using standard plant taxonomy reference books. [8][9]

Table 1: Identification of the plant species used for the experiment

Scientific Name	***Hibiscus cannabinus* (L.)**	***Colocasiaesculenta* (L.) Schott**
Family	**Malvaceae**	**Araceae**
Common Name	**Ambaadi**	**Ran alu**

Preparation of plant extract:

Collected plants were washed with distilled water and were air dried for 4 days and oven dried at 60^{o}C till constant dry weights were obtained. The dried plant samples were made into a fine powder using a grinder and then stored in amber coloured glass bottles. 10g of powdered extract were mixed with 100ml distilled water and boiled at 75^{o}C for 15 minutes. The mixture was cooled and then filtered using Whatman filter paper No. 1. The aqueous extract (filtrate) was collected and then stored in the refrigerator till further analysis.

2.2 Phytochemical Analysis:

The coarse powder of selected plants were analysed for the presence of phytochemicals such as Tannins, Flavonoids, Saponins, Cardiac Glycosides, Alkaloids, Steroids and Terpenoids. The aqueous extracts prepared were also analysed for the presence of phytochemicals such as Tannins, Flavonoids, Saponins, Cardiac Glycosides, Anthraquinone, Glycosides, Carboxylic Acid, Phenols, Phlobatannins and Terpenoids using standard methods. [10]

2.3 Green Synthesis of Nanoparticles:

A. Synthesis of Ag nanoparticles: [11][12]

- Preparation of 1mM $AgNO_3$
- Addition of 10ml of aqueous extract in 90ml of 1mM $AgNO_3$ and stirred
- Kept in dark at RT
- Observed for colour change

B. Synthesis of Cu Nanoparticles: [13][14]

Method 1:

- Preparation of 0.5% Cu acetate solution
- Addition of 10ml of aqueous extract in 100ml of 0.5% Cu acetate solution and stirred
- Kept in dark at RT
- Observed for colour change

Method 2:

- Preparation of 1mM $CuSO_4$

- Addition of Aqueous extract in 1mM $CuSO_4$ solution in 1:1 proportion and stirred
- Kept in dark at RT
- Observed for colour change

Absorbance for both these extracts after experimentation, was checked using U.V. Spectrophotometer to confirm the synthesis of NPs. The NPs solution thus obtained were 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^{o}C and stored for further analysis.

2.4 UV Spectrophotometer [15]**:**

To monitor the synthesis of silver nanoparticles and copper nanoparticles, about 1 mL of the sample suspension was taken in quartz tube and diluted it with 2 ml of deionized water, and subsequent scan was run on UV-Vis spectra, between wavelengths of 200-1100nm in UV-visible spectrophotometer (Model-Shimadzu UV 1800, Germany) and was recorded at the interval of 24hrs.

2.5 NTA [16]**:**

Nanoparticle Tracking analysis was performed to determine the size of nanoparticles. Thesample was centrifuged, suspension was discarded and the pellet was re-dispersed in deionized water and stored. These stored nanoparticles were used for the analysis. Nanoparticles were dissolved in sterile distilled water and inserted into prism using sterile syringe and then allowed to analyse for the size of nanoparticles present in in the sample by using Nanosight UK-LM20 instrument.

2.6 FT-IR [16][17]**:**

Fourier Transformed Infrared Spectroscopy was carried out to determine presence of possible biomolecules, which may be responsible for being the capping and reducing agent during nanoparticle synthesis, in the plant extracts under study. This Analysis was performed at SAIF, IIT Bombay. About 2-3 drops of sample was mixed with KBr for moisture absorption and then the sample was processed for analysis.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis:

Plants have a broad variability of secondary metabolites that prevent oxidative damage to cellular components. It was been previously reported that plant extracts contain biomolecules such as tannins, flavonoids, saponins, alkaloids, steroids, terpenes and phenols, which are used as a reductant to react with metal ions and reduce it for the formation of NPs in the solution [19]. Phytochemical analysis were performed for both dry powder and aqueous extract. It was observed that dry powder of *Hibiscus cannabinus* and *Colocasia esculenta* showed presence of tannins, flavonoids, saponins, alkaloids, steroids and triterpenoids. The aqueous

extract (10% w/v) showed the presence of flavonoids, saponins, phenols and terpenoids in both the plants whereas tannins and cardiac glycoside was found to be present only in *Hibiscus cannabinus*. Thus there was a difference found in type and also quantity of phytoconstituents present in dry powders and aqueous extracts of two plant species under study as observed in the Tables (2) and (3) below.

3.2 UV Analysis:

Nanoparticles were synthesized according to method described above, For $AgNO_3$ method the colloidal solution turned pale brown to yellowish brown due to reduction of silver ions. This indicated formation of silver nanoparticles [19]. For Cu Acetate method, the colloidal solution turned brownish to sea green and for $CuSO_4$ method the colloidal solution turned brown to dark brown due to reduction of copper ions. This indicated the formation of copper nanoparticles. Further the synthesis of nanoparticles was confirmed using UV-Vis spectroscopy. UV-Vis spectroscopy is generally used to monitor the reduction of pure metal ions and nanoparticles in aqueous suspensions. The absorption peaks of the reaction mixtures ranging from 354-408nm for $AgNO_3$, 320-368nm for Cu acetate and 416-424nm for $CuSO_4$ as shown in Table (4), for the two plants species used.

3.3 NTA[16]:

Nanoparticle Tracking Analysis 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. In the current study, size of nanoparticles were analysed using NTA. The average size of the nanoparticles was found to be below 100nm except for silver nanoparticles synthesized using *Hibiscus cannabinus*. The Cu Acetate method was found to be the most efficient one, as it produced nanoparticles of smallest size, during experiment as shown in Table (5).

3.4 FT-IR [11,20,21]**:**

The possible biomolecules which may have acted as a reducing agent for nanoparticle synthesis using plant extract was confirmed by FT-IR analysis. The composition of aqueous leaf extract shows abundance of flavonoids, cardiac glycosides and phenols in *Hibiscus cannabinus* whereas flavonoids and terpenoids in *Colocasia esculenta*. Figure 1 shows the FTIR spectrum of silver nanoparticles and copper nanoparticles synthesised using leaf extracts of *Hibiscus cannabinus* and *Colocasia esculenta*. The spectra recorded for AgNPs using *Hibiscus cannabinus* showed four different peaks 3449, 1639, 1028 and 656 cm^{-1}. These peaks are assigned to OH stretching of hydroxyl group compounds, C=O stretching of carbonyl group or else C=C

ring stretching, C-O bending and C-H bending respectively. Similar peaks were observed using *Colocasia esculenta,* except a peak at 2066 which indicated the presence of C-H stretching vibration. CuNPs synthesised using *Hibiscus cannabinus* by copper acetate method showed prominent peaks at 3448, 1630 and 683 cm^{-1} in its IR spectra whereas by copper sulphate method peaks were observed at 3429, 2082, 1643 and 695 cm^{-1}. The broad band's at 3448 cm^{-1} and 3429 cm^{-1} indicated the presence of hydroxyl group compounds. The band at 1630 cm^{-1} and 1643cm^{-1} showed C=O stretching respectively. Surface hydroxyl groups present in the plant extract of the bioactive molecule primarily reduce Ag and Cu ions. These results suggest the role of flavonoids present in the leaf extracts, in the reduction of metal ions and synthesis of nanoparticles.

Table 2: Phytochemical evaluation of dry powder of plants under study

Sr No.	Test	*Hibiscus cannabinus*	*Colocasiaesculenta*
1.	TANNINS	+++	++
2.	FLAVONOIDS	++	++
3.	SAPONINS	+	+
4.	CARDIAC GLYCOSIDES	+	–
5.	ALKALOIDS	+	+
6.	STEROIDS	++	++
7.	TERPENOIDS	+	+++

Table 3: Phytochemical evaluation of Aqueous Extract of plants under study

Sr No.	Test	*Hibiscus cannabinus*	*Colocasiaesculenta*
1.	TANNINS	+	–
2.	FLAVONOIDS	++	++
3.	SAPONINS	+	+
4.	CARDIAC GLYCOSIDES	++	–
5.	GLYCOSIDES	–	+
6.	PHENOLS	++	+
7.	TERPENOIDS	+	++

+++ : Strongly positive , ++: Moderately positive, + : Weakly positive , -: Negative

Table 4: Maximum absorbance of reaction mixtures after 24hrs

Methods	*Hibiscus cannabinus*	*Colocasiaesculenta*
	Maximum absorbance (nm)	
$AgNO_3$	408	354
$Cu(CH_3COO)_2$	368	320
$CuSO_4$	424	416

Table 5: Average size of nanoparticles formed

Methods	*Hibiscus cannabinus*	*Colocasia esculenta*
	Average size (nm)	
$AgNO_3$	265	65
$Cu(CH_3COO)_2$	35	44
$CuSO_4$	97	57

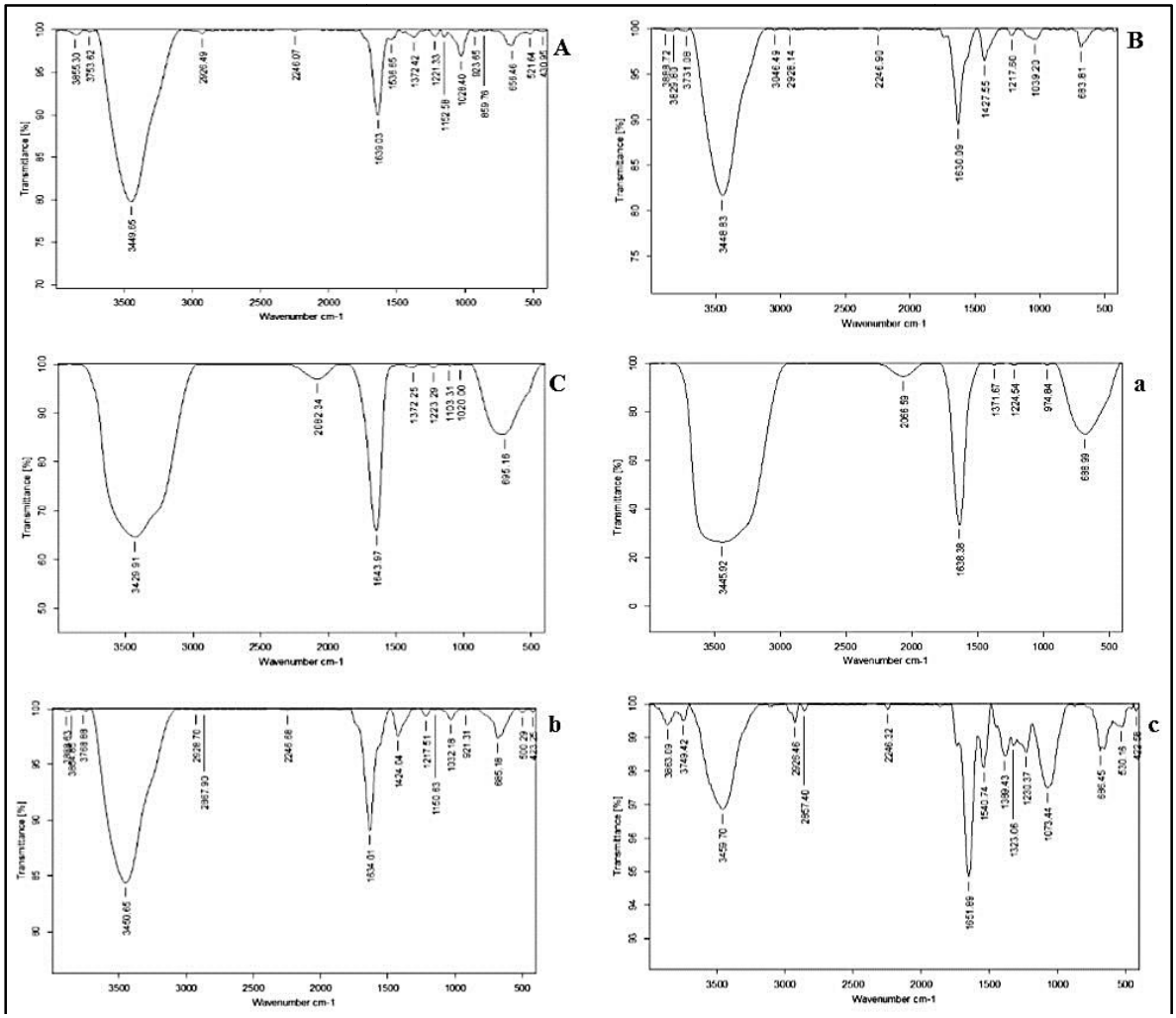

Figure 1: FTIR spectrum of AgNPs and CuNPs wherein (A, B and C) –*Hibiscus cannabinus*, (a,b and c) –*Colocasia esculenta*.
A and a – $AgNO_3$ method, B and b – $Cu(CH_3COO)_2$method, C and c – $CuSO_4$ method.

4. CONCLUSION:

In this study, we reported cost effective, eco-friendly, green synthesis of silver and copper nanoparticles using leaf extracts of *Hibiscus cannabinus* and *Colocasia esculenta*, two commonly found leafy vegetables. These nanoparticles could be synthesised by three different methods, using Silver nitrate, Copper acetate and copper sulphate. They were characterised by techniques such as UV spectroscopy and NTA. The leaf extracts of *Hibiscus cannabinus* and *Colocasia esculenta* were screened for phytochemicals present in and their presence was confirmed by FT-IR analysis. Our study shows that aqueous extract of both these plants can be employed for rapid synthesis of AgNPs and

CuNPs. The sizes of resulting nanoparticles were found to be below 100nm which is the essential characteristic during the application of nanoparticles. Presence of phytochemicals such as phenols, flavonoids and terpenoids, help in the reduction of Ag and Cu metal ions, which led to formation of nanoparticles. Thus, current findings of this research work, help in establishing baseline data for synthesis of small size nanoparticles, using leaf extracts of two leafy vegetables, *Hibiscus cannabinus* and *Colocasia esculenta*. Further studies are required to analyse morphological characteristics of nanoparticles using SEM and TEM. The synergistic effect of AgNPs and CuNPs synthesized using leaf extracts, can also be studied for their antimicrobial effect, thus exploring their role in antimicrobial formulations.

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