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SYNTHESIS OF NANOPARTICLES AND PHYTOCHEMICAL INVESTIGATION OF *CYPERUS ROTUNDUS L.*

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ABSTRACT Eco-friendly process for the synthesis of nanoparticles is one of the main steps in the area of bionanotechnology research. Here, the biological synthesis of gold and silver nanoparticles using plant extract. The synthesized nanoparticles is confirmed by colour changes and scanning electron microscopy(SEM).The high phenolic content of the hot water extract of *cyperus rotundus* having strong antioxidant properties helped in the reduction of silver cation to AgNPs and AuNPs synthesis does not use any toxic reagent and thus has a great potential for the use in biochemical applications and will play an important role in future opto-electronic and biomedical device applications. The data represented in our study contribute to a novel and unexplored area of nano materials as alternative medicine.

KEYWORDS : Nano particles; Cyperus rotundus; Phytochemical analysis; SEM.

INTRODUCTION

Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano science objects (Serjeev and Shabatina, 2008). The prefix nano indicates one billionth or 10 units. The nature of this unit determined by the word that follows. It is widely accepted in the context of nanoscience and nano technologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is widely agreed that nanoparticles are clusters of atoms in the size range of 1-100nm. Nanoparticles exhibit compeletely new or improved properties based on specific characteristics such as size, distribution and morphology (Williams, 2008).

The results of nanoscience are realized in nanotechnology as new materials and functional facilities. Nanotechnology is now creating a growing sense of excitement in life sciences especially biomedical devices and Biotechnology. (Serjeev and Shabatina, 2008).Frequently, nanometer size metallic particles show unique and considerably changed physical, chemical, and biological properties compared to their macro scaled counter parts, due to their high surface-to-volume ratio. Thus, these nanoparticles (NP) have been the subject of substantial research in recent years (Sharma *et al*.,2008; Iglesias-silva *et al*.,2007; Huang and yang, 2008).

Gold silver and platinum nanaparticles are widely applied to human contact areas such as shampoos, soaps, detergents, shoes, cosmetic products, and toothpaste as well as medical and pharmaceutical applications. The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans (Prabhu *et al*., 2010).

MATERIALS AND METHODS

Collection of plant materials

The dried rhizomes of the *Cyperus rotundus* were collected from local traditional market in Thanjavur. The rhizomes were rinsed with water thrice followed by distilled water to remove the fine dust materials and then, the rhizomes were dried under direct sunlight for 1 week to completely remove the moisture.

Preparation of rhizomes extract

The dried rhizomes were pulverized well with mortar and pestil to make a powder. Five grams of powder sample was mixed into 100ml of deionized water and the mixture was boiled for 10 min. After cooling the rhizome extract was filtered with Whatman No 1filter paper. The filtrate was stored at 4^0 C for further use.

Synthesis of silver nanoparticles

The 100ml of aqueous filterate extract of *Cyperus rotundus* was taken into 250ml of Erlenmeyer flask. Then the extract was mixed into silver nitrate ($AgNO_3$) to make the final volume concentration of 1mM solution. The reaction mixture was kept in to dark room condition until the colour change was arisen (24 hrs).The reaction solution colour changes have observed for the characterization of silver nanoparticles by SEM (Gopinath *et al*.,2012).

SEM analysis of Silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using JSM 6701F-6701 machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Synthesis of Gold nanoparticles

For the synthesis of Au-NPs (Gold nanoparticles), two boiling tubes were taken, one containing 10ml of 1Mm Hydrogen tetra chloroaurate ($HAuCl_4$) (Himedia, Mumbai) solution as control and the second flask containing 9ml of 1mM Hydrogen tetra chloroaurate solution and 1ml of plant extracts as test solution were incubated at room temperature for 1-2 hours. The gold nanoparticles were confirmed by colour changes (Song *et al*., 2009).

Phytochemical screening of *C.rotundus* rhizomes

Preparation of aqueous extract

The whole plant *Cyperus rotundus* was first washed well and dust was removed. Whole plant was washed several times with distilled water to remove the traces of impurities from the plant. The plant was dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of water under reduced pressure. The extract was stored in refrigerator until used. Chemicals test were carried out on the aqueous extract on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973,1984).

Test for Tannins:

About 0.5g of the dried powdered samples was boiled in 20ml of water in a test tubes and then filtered. A few drops of 0.1%ferric chloride was added and observed for brownish green or blue- black colouration.

Test for Flavonoids:

The following methods were used to determine the presence of flavonoids in the plant sample.

5ml of dilute ammonia solution were added to a portion of the aqueous filterate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on

standing. Few drops of 1% aluminium solution were added to a portion of each filterate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filterate was shaken with 1ml of dilute ammonia solution.

Test for Alkaloids:
Mayer's test:
To a few ml of the filterates , a drop of mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

Test for Terpenoids (Salkowski test):
Five ml of each extract was mixed in 2ml of choloform and concentrated $H2SO_4$ (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Phenols:
Fifty milligram of sample was dissolved in 5ml of distilled water. To this few drops of 5% ferric chloride was added. A dark green colour indicates the presence of phenolic compounds.

Test for Cardiac glycosides (keller – killani test):
Five ml of each extracts was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the inter face indicates a deoxy sugar characteristic of cardenoides. A violet ring may appear below the brown ring , while in the aceticacid layer, a greenish ring may form just gradually throughout thin layer.

Quantitative determination of phytochemicals:
Estimation of total phenol content:
Total phenol content was assayed according to the method of Singleton *et al*.,(1999).Test samples of different concentration (1-10mg) in 1ml ethanol were prepared and 0.25 ml of Folinciocalteau was added. After 2 min, 0.75 ml of 20% sodium carbonate was added and the volume made upto 5ml with distilled water. The mixture was vortexed, left for 2hrs and the absorbance was measured at 760 nm. The mixture without test solution was used as a blank. A standard curve of gallic acid was plotted for the calculation of polyphenolic content. The concentration of phenols was expressed in terms of mg gallic acid equivalent per gram dried leaves.

Flavonoid determination by the method of Bohm and Kocipai – Abyazan (1994):
5g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Tannin determination by Van-Burden and Robinson (1981) method:
500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferrocyanide .

The absorbance was measured at 120 nm within 10 min.

RESULTS AND DISCUSSION

The Phytochemical screening of *C. rotundus* rhizomes showed that the presence of flavonoides, phenolics, tannin, glycosides, terpenoids while alkaloids were absent.(Fig1) (Table 1).

Table1. Phytochemical screening of *C. rotundus*

Phytochemicals	colouration	Aqueous extract
Tannins	Brownish green	Present
Flavonoids	Yellow	Present
Glycosides	Greenish ring	Present
Alkaloids	-	Absent
Terpenoids	Reddish brown	Present
Phenols	Green	Present

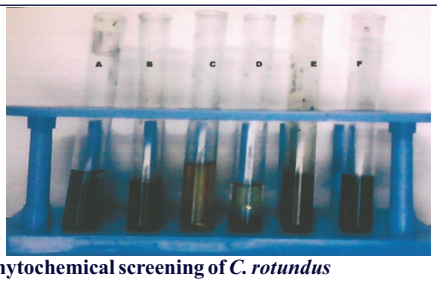

Fig 1. Phytochemical screening of *C. rotundus*

A - Test for Phenols
B - Test for Alkaloids
C - Test for Flavonoids

Phytochemical quantitative determination
In the phytochemical quantitative determination of the chemical constituency of *Cyperus rotundus* that 230+28 were observed in phenols whereas 167+24, 209+26 also observed in flavonoids and tannins respectively. The values were expressed as mean + SD for 5 times each sample.(Fig2) (Table2).

Table2. Phytochemical Quantitative determination of the chemical consistuency of *cyperus rotundus.*

Phytochemicals	Quantitative analysis (mg/5gram)
Phenols	230 ±28
Flavonoids	167 ±24
Tannins	209 ±26

Values were expressed as mean SD for 5 times each sample

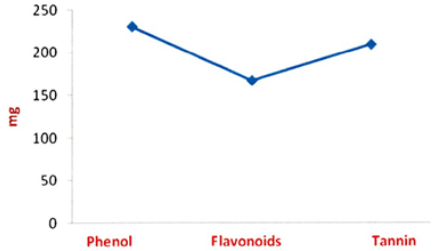

Fig2. Phytochemical Quantitative determination of the chemical constituents of *cyperus rotundus*

Synthesis of nanoparticles
The green synthesis of silver nanoparticles through plant extracts were carried out. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large – scale synthesis of other inorganic materials (nanomaterial's). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the rhizome extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish – brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles (Thirumurgan *et al*.,2010). The appearances of yellowish – brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Shankar *et al*., 2004).

Silver nanoparticles are being extensively synthesized using many different biological sources including fungi, bacteria and plants. (Shivaji *et al*., 2011; Saligram *et al*.,2009). Among them the plant mediated nanoparticles synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. In this present study, Cyperus *rotundus* extract was used for the synthesis of silver nanoparticles. The aqueous $AgNO_3$ solution turned on brown colour in 30 min with the addition of rhizome extract. (Fig 3a,b,c shows- $AgNO_3$,rhizome extract and AgNPs in the reaction solution probably as a result of excitation of surface Plasmon resonance (SPR) bands.(Mulvaney,1996).The control tubes ($AgNO_3$) showed no change in colour when incubated in a similar condition.SEM analysis

was carried out to understand the topology and the size of the AgNPs, which showed the synthesis of higher density polydispersed spherical Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the rhizome extract further confirmed the development of silver nano structures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 20-50nm as well the cubic, face-centered cubic structure of the nanoparticles(Fig 4).

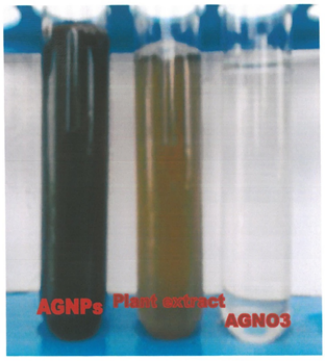

Fig3a. Photographs of AGNPS, Plant extract, and AGNO3

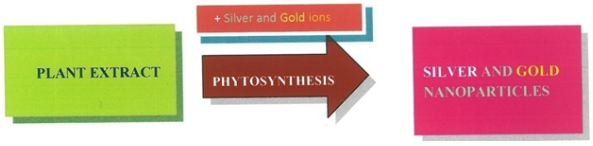

Fig3b. Flow chart representation of phyto nanopartical synthesis

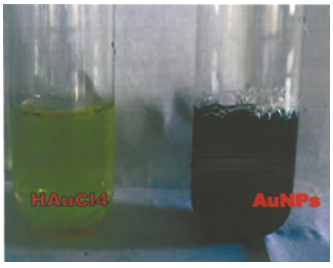

Fig3c. 1mM Hydrogen Tetra Chloro Aurate solution before adding extract

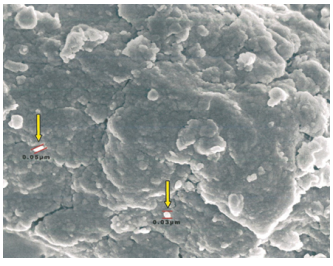

Fig4. High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed AgNPs ranged between 20-50 nm.

CONCLUSION

Nanotechnology is now creating a growing sense of excitement in the lifesciences especially biomedical devices and Biotechnology.Development of eco-friendly process for the synthesis of nanoparticles is one of the main steps in the area of bionanotechnology research.Here,the biological synthesis of gold and silver nanoparticles using plant extract.The synthesized nanoparticles is confirmed by not use any toxic reagent and thus has a great potential for the use in biochemical applications and will play an important role in future opto-electronic and biomedical device applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this research article.

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