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Exertion of Gold Nanoparticles Synthesis in Extract of *Garcinia combogia* Leaves, Evaluation of its Total Phenolic Content and its Distinct Antioxidant Activity

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ABSTRACT

As the green nano synthesis is flourishing constantly it is indispensable to incorporate nanotechnology in medicine. Prelimiary studies in the leaves of Garcinia combogia have shown the presence of bioactive secondary metabolites. The present investigation discloses the synthesis gold nano particle using the leaves of Garcinia combogia and its antioxidant property. The total phenolic contents (TPC) were determined by the Folin- Ciocalteu method. The free radical scavenging activity of the synthesized nanoparticles was ascertained by the DPPH assay, Nitric oxide (NO) radical scavenging, Superoxide (SO) radical scavenging and Hydrogen Peroxide scavenging. The gold nano particles of Garcinia combogia exhibit apparent scavenging activity and excellent antioxidant activity. The phenolic compounds that exist in Garcinia combogia have the potential to function as antioxidants by scavenging or stabilizing the free radicals involved in oxidative processes.

Key words: *Garcinia combogia, nano particles, TPC. DPPH assay, NO, SO Scavenging*

INTRODUCTION

The field of nanotechnology has recently witnessed spectacular advances in the methodology of nanomaterial's fabrication and utilization of their exotic physicochemical and Optoelectronic properties[1] Green nanotechnology offers the opportunity to stop the adverse effects of the use of chemical methods[2]. Nowadays phytochemical mediated Metal nanoparticles have been employed and it has special chemical and physical properties. In particular, Bio reductions of gold nanoparticles are very important and it has potential application as antimicrobial agent and employed in research methods like biosensing[3,] cancer therapy[4] and others. A variety of Gold nanoparticles synthesis has been previously reported such as *Mangifera indica*[5] leaf. *Hibiscus rosasinensis*[6], *Murraya koenigii* and *Ocimum sanctum*, have been reported.

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Free radicals species like O_2^-, H_2O_2, Hydroxyl radical (OH), Organic hydroperoxide (ROOH) are generated by our body by various endogenous systems, owing to different physiochemical conditions and pathological states. The free radicals damage the cells and leads to pathological changes associated with aging.[7] The highly reactive oxygen species, are profecient of damaging biologically pertinent molecules such as DNA, proteins, carbohydrates, and lipids of the nucleus and cell membranes[8]. An antioxidant is a perdurable molecule that donates an electron to the extravagant free radical and neutralizes it, thus reducing its magnitude to damage. These antioxidants hinder or inhibit cellular damage predominantly through their free radical scavenging property.[9]

Plants are vital source of antioxidants in nature; they contain chemical compounds like flavonoids, phenols, and other compounds which show high antioxidant activity. Researches are being carried out to find natural antioxidants from plants [10,11]. Plants are safe and effective natural antioxidants, especially spices and herbs[12]. Polyphenols from food is important to prevent the oxidative stress due to over production of ROS [13,14].

In the current study, we have reported on the synthesis of AuNPs from *Garcinia cambogia* belongs to the family *Guttiferae (Clusiaceae).* It is a wild sub-tropical and tropical medicinal plant. Phytochemical analysis revealed that *G.cambogia* contains phenolic compounds, steroids, xanthins, benzophenone[15] tannins, gutiferrins, and Saponins. In the present study the phenolic content of synthesized gold nanoparticle of Garcinia combogia and its antioxidant property was evaluated.

MATERIALS AND METHODS

Preparation of the plant extract

The fresh leaves of *G. cambogia* were collected from the Western Ghats of Idukki district. It was washed thoroughly thrice with deionised water. After cleaning the plant was dried in shade at room temperature for one week and was crashed to give powder and stored in airtight amber bottles.

Gold nanoparticle synthesis

About One gram leaf powder of Garcinia combogia was fused with 50 ml of sterile distilled water. Ground in a blender and soon after the mixture was left in a shaking incubator performing at 200 rpm, 25°C for 24 hours. It is then filtered through mesh and centrifuged at 10,000 rpm for 10 min at 4°C by REMI cooling centrifuge to expel cell-free debris. The resulting supernatant was then filtered through a 0.2 µm filter paper and selected for the synthesis of gold nanoparticles.5 ml of leaf extract of Garcinia combogia were mixed with 4 ml aqueous solution of HAuCl4 (1 mM) and incubated at room temperature for 24hrs . The immediate change in color of the solution from pale yellow to violet color implied the preliminary confirmation for the formation of plant extract mediated synthesis of gold nanoparticles[16].

Total phenol

Total phenolic contents (TPC) were determined by following the Folin- Ciocalteu method with slight modifications[17]. About 1 mL of methanol extract and gold Nps extracts were added separately to a 25 ml volumetric flask filled with 9 ml distilled water. Folin-Ciocalteu phenol reagent (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 ml of Na CO solution was mixed up. The solution was instantly diluted to 25 ml with distilled water and mixed thoroughly. Thereafter it is then allowed to stand for 60 min before measurement .The absorbance was measured at 750 nm versus the prepared blank. Quantification was done on the basis of a standard curve with gallic acid. Results were expressed as gram of gallic acid equivalents (GAE) per 100 g dry weight.

DPPH Radical scavenging activity

The free radical scavenging capacity of the extract and the synthesised gold nanoparticles was determined using DPPH (2,2-diphenyl-l-picryl hydrazyl) Radical Scavenging Assay[18]. The antioxidant activity of the plant extract was estimated using the DPPH radical scavenging protocol. 2 ml of freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and synthesised gold nanoparticles was added in different concentration (20µg- 100 µg

/ml) and final volume was 3 ml. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 523 nm against the blank. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Where: A control- Absorbance without samples; A Test- Absorbance in the presence of the samples.

Reducing power activity

The reducing power can be determined by the method of Athukorala[19]. About 1ml of extract (plant extract and gold nps) solution of different concentrations (20 to 100 µg) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 0.1%). This was incubated at 50^0 C for 20 min. After the incubation, 2.5 mL of 10% trichloroacetic acid was added.2.5 mL of the reaction mixture was mixed with distilled water (2.5mL) and ferric chloride (0.5 mL, 0.1%). The solution absorbance was measured at 700 nm. Increasing absorbance of the reaction mixture indicates increasing reducing power. The same procedure was applied for ascorbic acid which acts as the standard. Increase in the absorbance indicates increase in reducing power.

The percentage of reducing power was calculated using the formula given below;

% of reducing power = control – test / control X 100

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity of gold nanoparticles was studied using slightly modified method[20]. In this test, H2O2 (40 mM) w prepared freshly in phosphate buffer saline (pH 7.4). 300 µl of test samples containing various concentrations of gold nanoparticles (20µg-100 µg /ml) was added to 600 µl of H2O2 (100 mM) and the total volume was made upto 1 ml with PBS. The absorbance was measured at 230 nm against the separate blanks using spectrophotometer. The percentage of inhibition was calculated by taking BHT as positive control.

Superoxide radical scavenging activity

The superoxide anion scavenging activity is measured as described by Robak and Gryglewski [21]. The purple formazan formed by nitrobluetetrazolium (NBT) by reacting with the superoxide radicals generated from phenazine meth sulfate–nicotinamide adenine dinucleotide (PMS/NADH) non-enzymatic system was measured spectrophotometrically. In this assay, the 1 ml reaction mixture contained phosphate buffer (100 mM, pH 7.4), NADH (468 µM), NBT (156 µM), PMS (60 µM) and different concentrations (10-50µg/ml) of goldnanoparticles.The mixture was incubated for 5 min at room temperature and then absorbance was measured at 560nm against appropriate blank to determine the quantity of formazan generated. Quercetin was used as positive control in this test. After incubation for 5 min at room temperature the absorbance at 560nm was measured against appropriate blank to determine the quantity of formazan generated. Quercetin was used as positive control in this test.

RESULT AND DISCUSSION

Total phenolic assay

Phenolic compounds are responsible for the antioxidative action. polyphenolic compounds inhibits on mutagenesis and carcinogenesis if

the diet contains approximately 1 gm of phenolic substance rich in fruits and vegetable. Moreover it plays a vital role in stabilization of lipid peroxidation.The hydroxyl group present in the phenolic compounds are responsible for the radical scavenging activity[22].In the present study the total phenol present in Garcinia combogia also indicate a positive relationship exhibiting antioxidant activity. It was observed that the Garcinia combogia gold nano particles showed the higher amount equivalent of 1.896mg gallic acid than the control plant extract 0.568mg. Thus the Garcinia combogia AuNps have the significant percentage of phenolic contents.

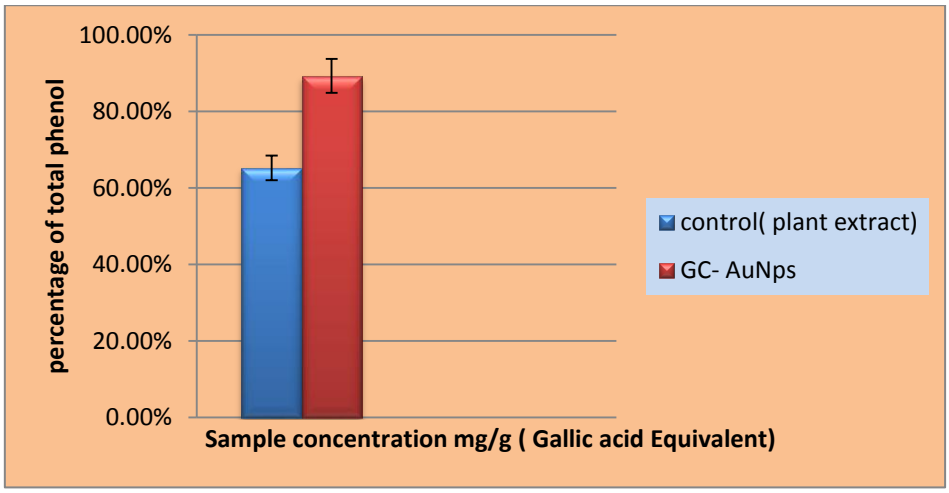

Fig. 1: Percentage of total Phenol, Garcinia combogia plant extract, GC-AuNPs

DPPH Radical scavenging

The 2,2-diphenyl-1-picrylhydrazyl is a dark-colored crystalline powder composed of stable free-radical molecules. And acts as a a trap ("scavenger") for other radicals. Therefore, during reduction reaction the addition of DPPH is used as an indicator of the radical nature of that reaction. DPPH has a strong absorption band centered at about 520 nm, its absorption get decreased upon reduction with an antioxidant[23.] Thus, the radical scavenging activity in the presence of a hydrogen-donating antioxidant can be monitored by a decrease in the absorbance of DPPH solution. Fig 2. represents the DPPH radical scavenging activity of Garcinia combogia plant extract and gold nano particles synthesized using Garcinia combogia. The gold nanoparticles of the plant had appreciable scavenging activity on the radical DPPH. The absolute DPPH test put forward that the plant gold nano particles have higher free radical scavenging activity compared to the plant extract.

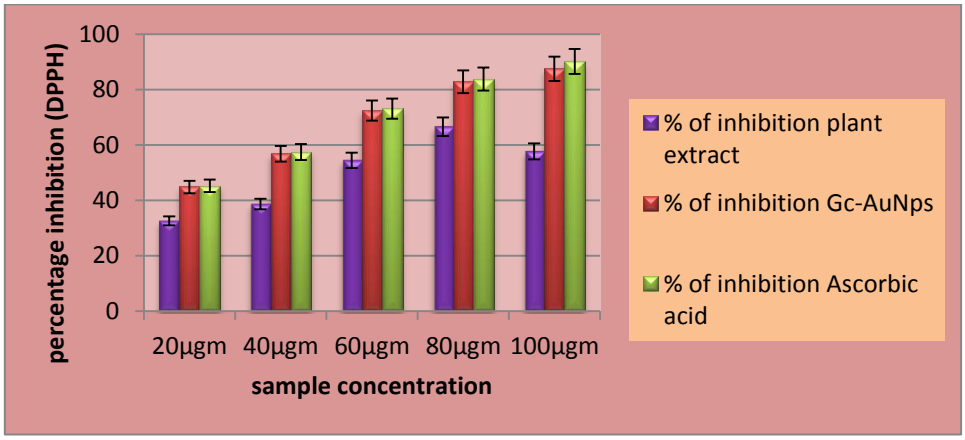

Fig.2 DPPH Radical Scavenging Activity of plant Garcinia combogia and Gc- AuNPs

Reducing power activity

Reducing power of the plant samples indicate that they are highly an electron donors and they are capable of reducing the oxidized intermediates of lipid per oxidation process. Thus the power of reducing in a plant sample represents its antioxidant activity. The reducing power of Garcinia combogia gold nano particles was potentially high similarly to the standard ascorbic acid. The higher concentration of the reaction mixture with increasing absorbance indicates higher reducing power when compared to the control plant extract.

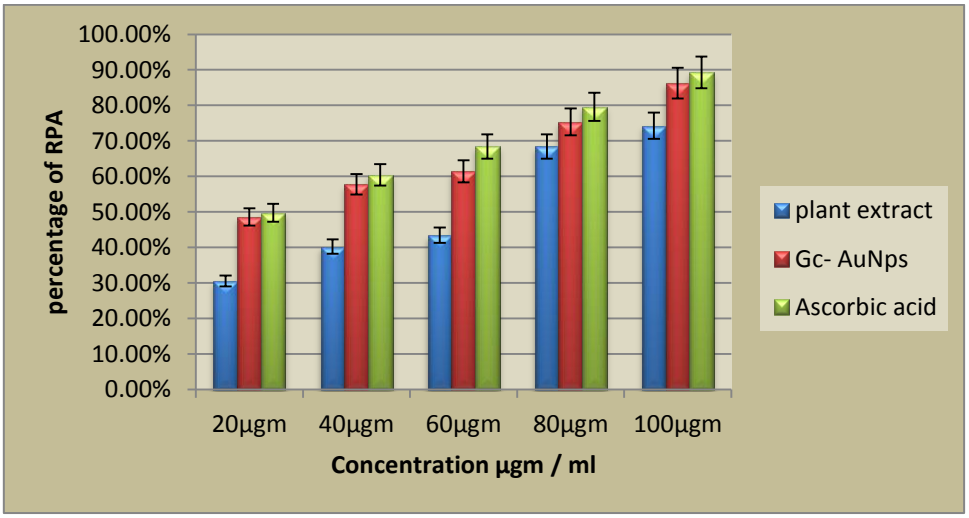

Fig. 3: Reducing Power Activity of Ascorbic acid, Garcinia combogia plantextract and GC-AuNps

Hydrogen peroxide scavenging assay:

H2O2 Scavenging activity of the plant compounds may be attributed due to transfer of electrons to H2 O2 and neutralize it into water. H2O2 inhibition activity assay is an important method for the determination of antioxidant activity[24]. The percentage inhibition of H2O2 by GCE and GC-AuNPs showed an inhibition efficiency of 73.58 % at 100 µg/mL while that for GC Extract is 60.67 % at 100 µg/ml. Thus GC-AuNPs exhibited higher anti-oxidant activity than the GC Aqueous extract owing to the attachment of large number of antioxidant moieties at the gold ion.

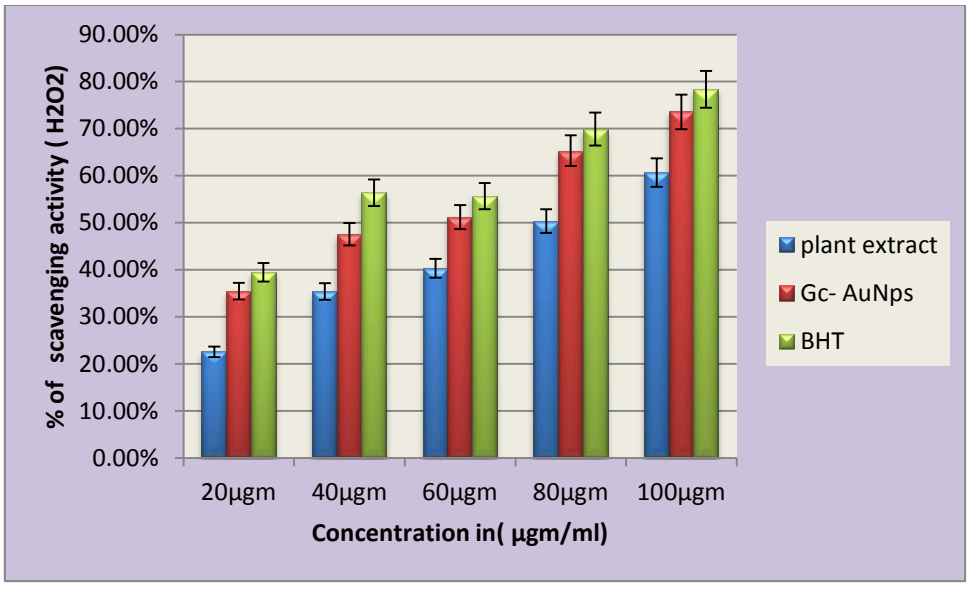

Fig. 4: The Hydrogen Peroxide radical scavenging activity with Garcinia Combogia plant extract, GC- AuNPs

Superoxide radical scavenging activity

Powerful and dangerous hydroxyl radicals as well as singlet Oxygen are generated by the Superoxide anion. Due to this oxidative stress are created inside the cellular components [25]. In the PMS/NADH-NBT system, the superoxide anion evolved from dissolved oxygen from PMS/NADH linking reaction reduces NBT. With antioxidants the absorbance decreases at 560 nm and this manifest to the absorption of superoxide anion in the reaction mixture. **Fig.5** exhibits the superoxide radical scavenging activity obtained in the studies with *Garcinia combogia*, the gold nanoparticles and quercetin. The *Garcinia combogia* and the gold nanoparticles had significant superoxide radical scavenging activity. The results also put forward that gold nanoparticles are highly active than *Garcinia combogia* alone, and are comparable to the reference drug, as good antioxidants with superoxide radical scavenging activity.

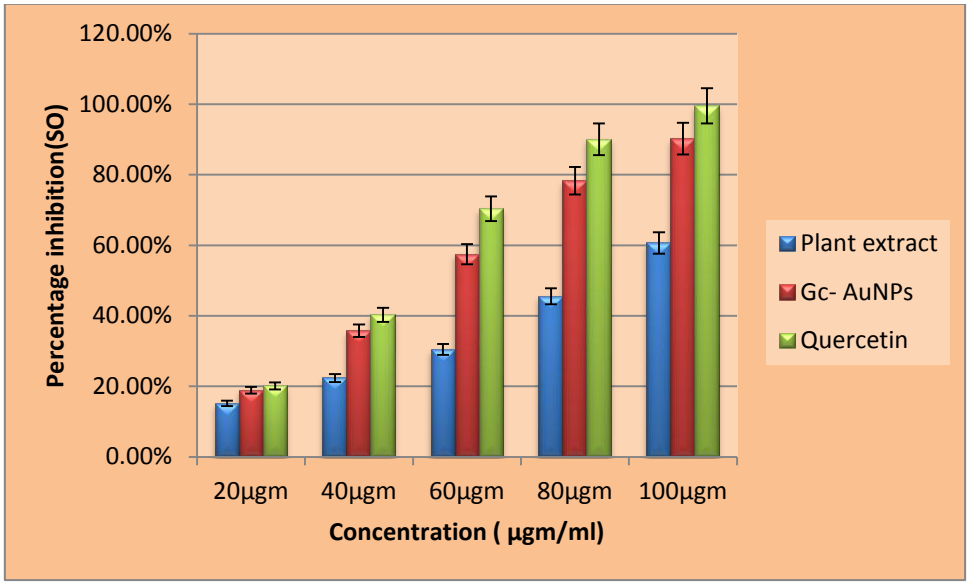

Fig. 5: Superoxide Radical Scavenging Activity of Quercetin, Garcinia combogia and the newly synthesized gold nanoparticles (GC-AuNPs)

CONCLUSION

In the present investigation, gold nanoparticles were synthesized from Garcinia combogia and the antioxidant properties of Garcinia combogia were studied. The total amount of phenol which is present in the plant gold nano particles were significantly high. This DPPH assay lay forward that the plant gold nano particles have effective free radical scavenging inhibition compared to the plant extract. Moreover the result of reducing power activity, Hydrogen peroxide scavenging assay ,Superoxide radical scavenging activity of Garcinia combogia gold nano particles were also potentially high compared to the Garcinia combogia alone. Since increased oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease. Supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress[26]. The results in the present report suggest that the gold nanoparticle of the plant extracts of Garcinia combogia exhibited potent antioxidant activity and it can be further` subjected to evaluation of their bio-efficacies, active constituents, and molecular and biological mechanisms in vitro as well as in vivo on anti-oxidation or cancer chemoprevention effects.

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