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INVESTIGATION ON THE FREE RADICAL SCAVENGING ACTIVITY OF BIOGENIC SILVER NANOPARTICLE

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ABSTRACT

Nanomedicines are a recent offshoot of the application of nanotechnology to medical and pharmaceutical challenges. The advent of nanoparticles has opened up new avenues in many different fields**.** The biomedical properties of silver nanoparticles were premeditated as free radical scavenging activity. Mangrove *Excoecaria agallocha* synthesized silver nanoparticles serve as strong hydroxyl, superoxide, nitric oxide and DPPH radical scavenger.

KEYWORDS: ***Exocaria agallocha,*** **silver nanoparticles, radical scavenger, nitric oxide, DPPH**

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INTRODUCTION

Nanotechnology endeavors are aimed at manipulating atoms, molecules and nanosize particles in a precise and controlled manner in order to build materials with a fundamentally new organization and novel properties. The fundamentals of nanotechnology lie in the fact that the properties of substances dramatically change when their size is reduced to the nanometer range. When a bulk material is divided into small size particles with one or more dimension (length, width or thickness) in the nanometer range and the individual particles exhibit unexpected properties which are different from those of the bulk materials. It is known that atoms and molecules possess totally different behavior than those of bulk materials; while the properties of the latter are governed by classical mechanics.Nanotechnology is an emerging interdisciplinary technology that has been booming in many areas during the recent decades, including mechanics, electronics, optics, medicine, plastics, energy and aerospace. Its profound societal impact has been considered at the huge momentum to usher in a second industrial revolution[1.] Nanotechnology has now started leaving the confines of laboratories and conquering new applications to change our lives. These unique properties are derived due to variations in specific characteristics such as size, distribution and structure of the particles. Due to the incredible properties, nanoparticles have become significant in recent years. The fast growing field of nanotechnology presents great potential to influence various sectors in the areas of energy, environment, agriculture, healthcare and consumer goods. Therefore, it has built great expectations not only in the academic community but also among the investors, governments and industries. According to estimates, the worldwide nanoproduct market is estimated to reach $ 1 trillion by the year 2015 [2.] To compete with this tremendous demand, the synthesis of nanomaterials of specific composition, shape and size is a burgeoning area of research in the field of nanotechnology.From time immemorial, nature has made noble metals part of our daily life. Recently there has been considerable interest in the development of techniques for the controlled synthesis of metal nanoparticles of well-defined size, shape and composition, as they find applications in the biomedical field and areas such as optics and electronics [3-5]. Recently, utilization of biological resources for nanoparticle synthesis has become an attractive process in concerned with its ecofriendly, biocompatible nature. Free radicals are produced in the body which are detrimental in attacking lipids, DNA and Proteins. To neutralize and scavenge the free radicals, antioxidants play an important role [6.] The present investigation discloses mangrove *Excoecaria agallocha* synthesized silver nanoparticle's free radical scavenging activity.

MATERIALS AND METHODS

(i) Green synthesis of Silver nanoparticles [7]

Biological synthesis of silver nanoparticles using a mangrove extract of *Excoecaria agallocha* has been achieved as reported earlier. Briefly, the leaves of *Excoecaroa agallocha* were collected from mangrove forest, Pichavaram Tamilnadu, India. The leaves were washed with deionized water and then shade dried and powdered using electronic blender. Different concentrations of *Excoecaria agallocha* and $AgNO_3$ solution (49:1, 48:2, 47:3, 46:4, 45:5) was subjected respectively. The reduction of silver ions to silver nanoparticles was completed within 35 min and forms brownish yellow color which indicates the formation silver nanoparticles.

(ii) DPPH radical quenching assay [8]

The antioxidant property of silver nanoparticles was estimated by observing the capacity of quenching synthetic, stable DPPH radical into non-radical form. 1ml of silver solution (20-200 µg/ml) was mixed with 1ml of the freshly prepared DPPH solution in methanol (0.15mm). After addition of methanol (3ml) the mixture was incubated in dark at room temperature for 30 min and observance of the mixture was then

recorded at 517 NM using a UV-Vis Spectrophotometer. The radical scavenging activity (RSA) was indicating in percentage of inhibition using the following equation. DPPH scavenging effect (%) = Ao – A1 / Ao X 100. Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the silver nanoparticles. Catechin was used as a positive control.

(iii) Superoxide radical scavenging assay [9]

Superoxide radical quenching ability of silvernanoparticles was appraised by inhibition of nitro blue tetrazolium (NBT) reduction with some modification, each 3ml of reaction mixture contained 50mm phosphate buffer (pH 7.8), 13 mM methionine, 2mm riboflavin, 100 mM EDTA, 75 mM NBT and 1ml sample solution. After the formation of blue formazon, the increase in absorbance at 560nm was recorded. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with the reaction mixture was kept in the dark and served as blanks. Ascorbic acid was used as the positive control.

Scavenging % = [1- A sample – (A blank /Acontrol] x 100

(iv) Nitric oxide scavenging activity [10]

The antiradical activity was estimated by spectrophotometrically in an EL 808 IU Ultra microplate Reader. Briefly, nitric oxide radicals was generated from 100 µl of 20 nm sodium nitroprusside were incubated with 100 µl (0.625, 0.125, 0.25, 0.5, 1 ml) of silver nanoparticles for 60 min at room temperature. Catechin, a NO scavenger was used as a positive control.

(v) Hydroxyl radical scavenging assay [11]

Hydroxyl radical inhibition of nanoparticle was quantified by the method of [11] with some modifications, the hydroxyl radicals were generated using 3ml sodium phosphate buffer replaced H_2O_2. The solutions were incubated 37º C for 1h, and the presence of the hydroxyl radical was detected by monitoring absorbance at 510 nm. Catechin was used as the positive control.

Scavenging % = [1- A sample – (A blank/ A control) x 100

RESULTS AND DISCUSSION

Greener approaches on synthesizing nanomaterials have gained enormous scientific and technological focus to get rid of the hazards and problems arising out of the use of chemical synthesis technology.The DPPH is a stable and well characterized synthetic solid radical for evaluation of antioxidant potential of compound / material. In the present study DPPH reducing ability of silvernanoparticles was quantified spectrophotometrically. The percent DPPH radical inhibition is indicated in Fig.1. Inhibition was found to be high in a concentration dependent manner of silver nanoparticles synthesized using *Excoecaria agallocha.*

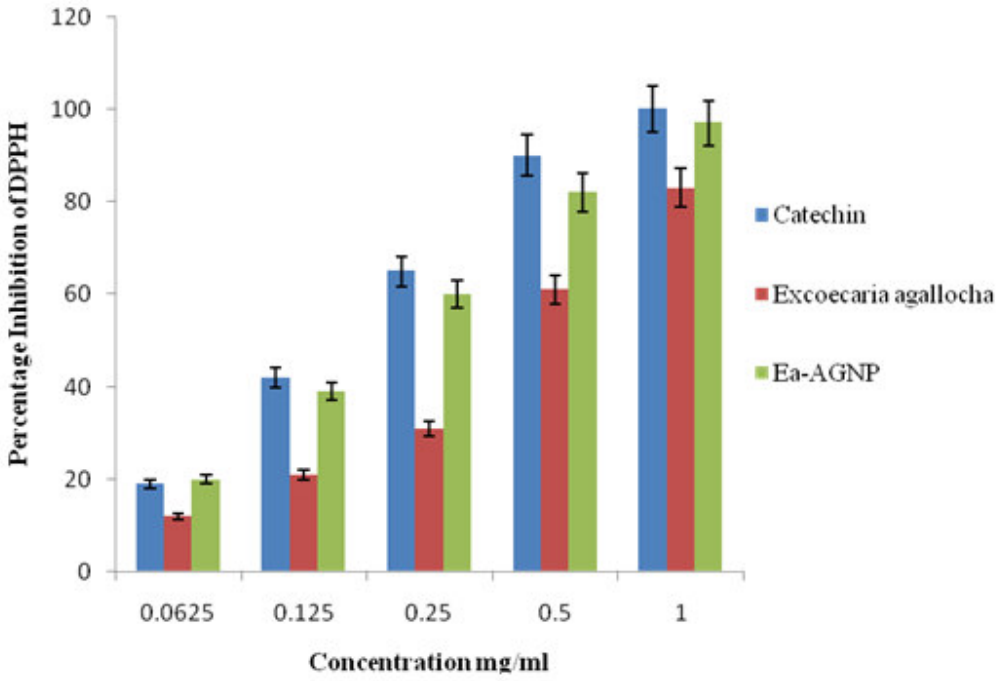

Figure 1
DPPH Radical Scavenging Activity of Catechin, Excoecaria agallocha and Ea-AGNP.

The superoxide comparatively stable radical generated in living system, the super oxide radical quenching ability of silver nanoparticle is quantified spectrophotometrically. The silver nanoparticles superoxide radical inhibition is 94% (Fig. 2). The superoxide radical inhibition has been reported for platinum and selenium nanoparticles [12-13]. The observed silver nanoparticles superoxide radical inhibition may be due to auto oxidation and catalytic activity of silver nanoparticles.

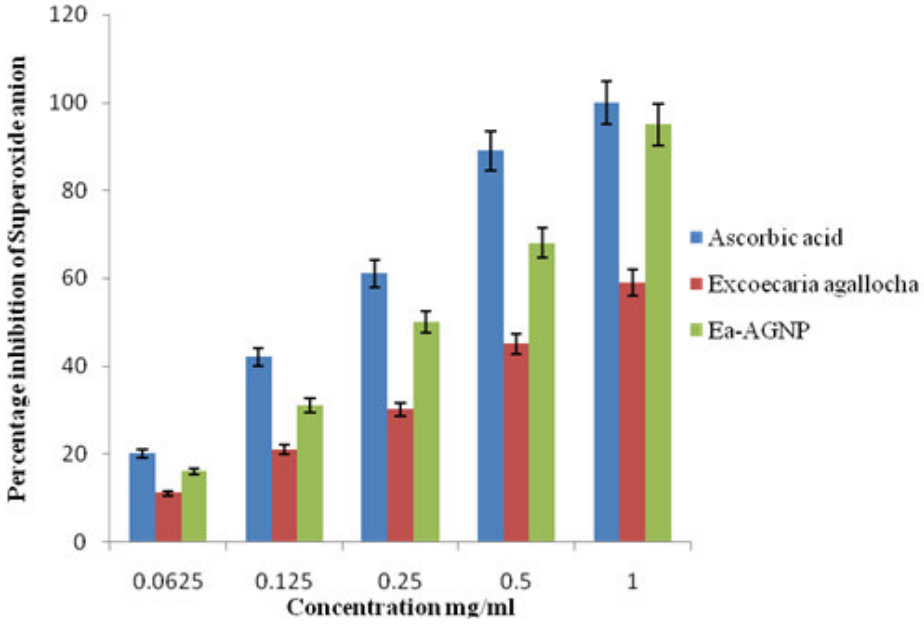

Figure 2
Superoxide radical scavenging activity of Ascorbic acid, Excoecaria agallocha and Ea-AGNP.

The scavenging activity of the nanoparticles against nitric oxide was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in the reacting mixture[14]. The significant decrease in the concentration of nitric oxide radical was comparable to the standard catechin (Fig. 3). It has been reported that the cerium nanoparticles showed good nitric oxide scavenging activity by an internal electron transfer mechanism. [15-16]

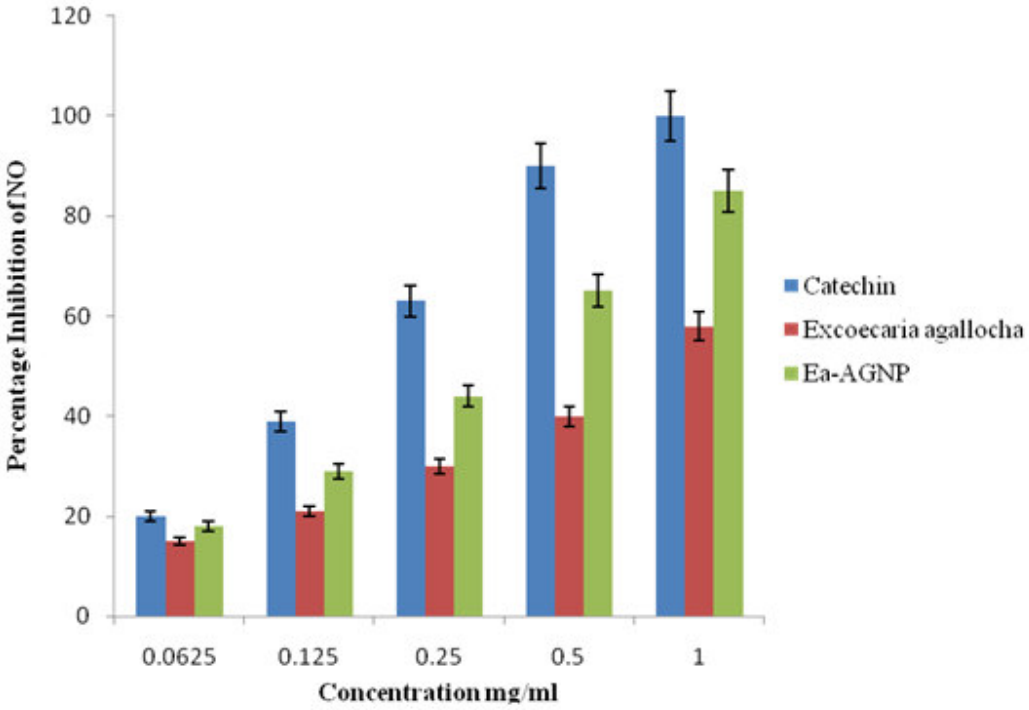

Figure 3
The Nitric Oxide radical scavenging activity obtained in the studies with Excoecaria agallocha, Ea-AGNP and Catechin.

Hydroxyl radical scavenging activity of silver nanoparticles are shown in Fig.4. The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins[17]. In the present study, the hydroxyl radical-scavenging effect of silver nanoparticles shows activity in a dose-dependent manner. Hence, the newly synthesized silver nanoparticles can be considered as a good scavenger of hydroxyl radicals.

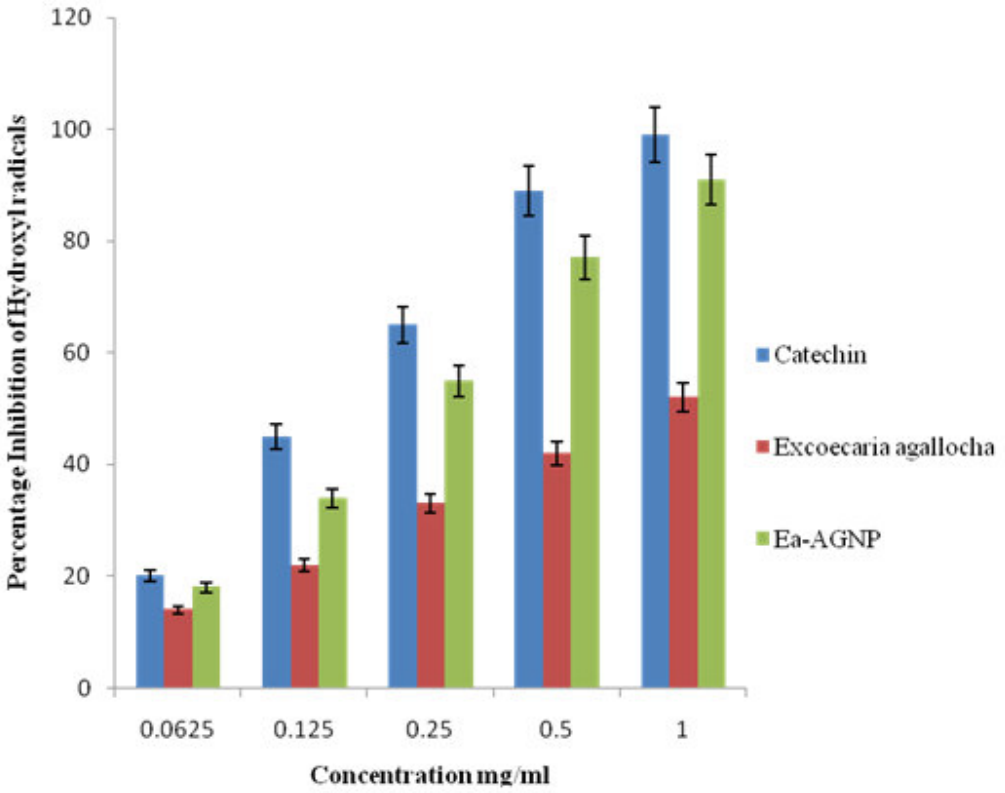

Figure 4
Hydroxyl Radical Scavenging Activity of Catechin, Excoecaria agallocha and Ea-AGNP.

The IC_{50} values for different assays such as DPPH, Superoxide, Nitric Oxide and Hydroxyl radical with standard, mangrove and gold nanoparticles are presented in Fig – 5. The inhibition obtained for gold nanoparticles of *Excoecaria agallocha* are nearly equal to the values obtained for standard suggesting that the plant with gold nanoparticles act as good antioxidants.

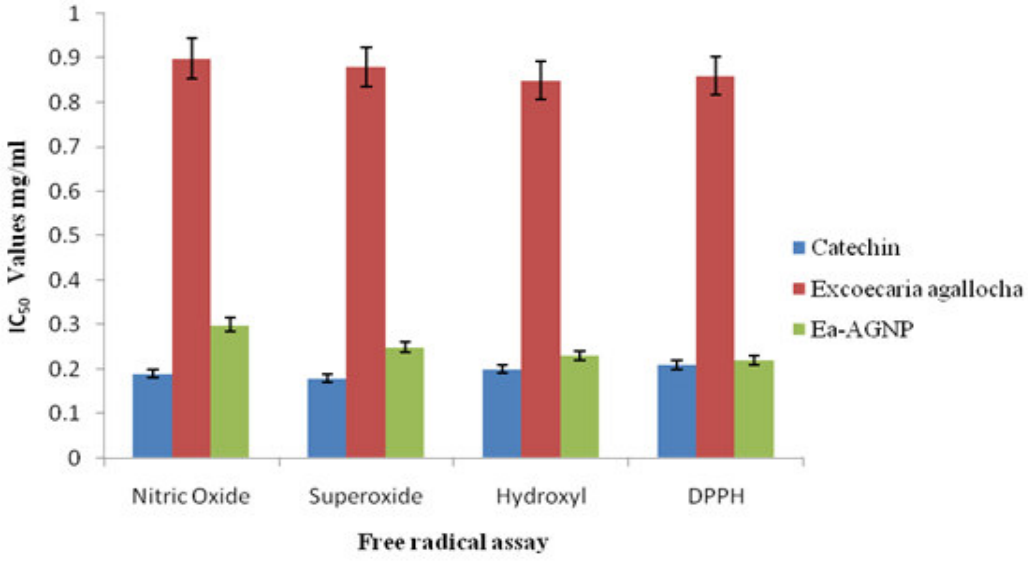

Figure 5
IC_{50} value of Catechin, Excoecaria agallocha and Ea-AGNP.

CONCLUSION

The present investigation suggests that ethopharmacological approach on the synthesis of silver nanoparticles will greatly facilitate on the creation of nanomedicine.

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