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Phytochemical Analysis and Anti-Oxidant Activity of Gold Nanoparticles Synthesizing Plant - *Silybum marianum*

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A B S T R A C T

The use of nanoparticle in the field of medicine is increasing in the present world of technology. That too, green synthesis of nanoparticle has attracted scientist as a safe, easy and cheap mode of nanoparticle production in the developing and also in developed countries. In the present study, the gold nanoparticles were synthesized using the medicinal plant *Silybum marianum*. It is used from the ancient period for treating several liver disorders. The plant has been tested for its phytochemical constituents using standard procedures and was found to have flavonoids, alkaloids and anthocyanins. The synthesized gold nanoparticles was tested for its anti-oxidant activity using several assays such as DPPH free radical scavenging assay, Superoxide free radical scavenging activity, Anti-lipid peroxidation assay and Hydrogen Peroxidase scavenging activity. The antioxidant activity result showed that the extract of *Silybum marianum* has notable effect on suppressing oxidation and thus oxidants.

Introduction

Nanoparticle is a unique subset of the broad field called as nanotechnology. Nanoparticles are those unagglomerated particles ranging in size from 10 nm to 500 nm. These nanoparticles are gaining importance in the present day technology due to its use in various fields such as medicine, drug delivery, information, energy and environmental technologies [1, 2]. Nanoparticles can be classified into two types viz engineered nanoparticles and non-engineered nanoparticles. Engineered nanoparticles are those created or synthesized artificially such as silver nanoparticles, gold nanoparticles etc for their use in several techniques where as non-engineered nanoparticles are those that are freely available in the environment such as atmospheric nanoparticles that are produced during combustion, aerosols etc. Both engineered and non-engineered nanoparticles pose their uses in several industries. Several techniques have been developed to synthesize nanoparticles such as chemical mediated synthesis, gas and liquid phase process etc [2]. Despite these "Biosynthesis" or "Green synthesis of nanoparticle has been growing from last

decade to develop eco friendly technologies in material synthesis [3].

Biosynthesis or Green synthesis of nanoparticle has been considered as a perfect alternative for the chemical mediated synthesis due to their cheap cost and economically benign nature. Further the chemical and physical synthesis of nanoparticles was found to involve hazardous materials and leaves toxicity to nature [3]. Green synthesis using plant extracts is increasing when compared to synthesis mediated by microorganisms such as bacteria, algae and fungi because of their pathogenicity and elaborative cell culture maintaining process [1, 3]. The use of plants for the preparation of nanoparticles could be more advantageous, because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures. Several plants and their parts have been successfully used for the extracellular synthesis of metal nanoparticles such as *Elaeagnus latifolia* [4], *Tinospora crispa* [5], *Phyllanthus niruri* [6], *Amaranthus spinosus* [7], *Ananus comosus* [3], *Embilca officinalis* [8], *Tamarindus indica* [8], *Ficus microcarpa* [9] etc.

In the present study, green synthesis is mediated by the plant *Silybum marianum*. Silybum marianum is otherwise called as *Cardus mariamus* [11]. Its common name is Milk thistle in English and deve dikeni in Turkish [10, 11]. The plant is highly used for treating liver disorders. A roman nanturalist described milk thistle as "Excellent for carrying off bile". The plant contains several flavanolignans such as silybin, silymarin, silydianin and silychristine. Silymarin was found to be the highly present and effective compound in treating several liver disorders such as Amanita mushroom poisoning, Hepatitis, Alcoholic liver disease and cirrhosis, Hyperchloesterolemia, Psoriasis etc [10]. A study on the plant also revealed its hepatoprotective property [11]. In the present study, the phytochemical properties of the plant have been analyzed and its extract was used for the green synthesis of gold nanoparticles.

Materials and Methods

Sample Collection

Fresh leaves of *Silybum marianum* was collected from in and around Conoor, Tamilnadu, India. Primarily the leaves were washed with mercuric chloride and dried with water absorbent paper. Then they were cut into small pieces and dispensed in 100ml of sterile distilled water and boiled for one hour at 80º C. The extract was collected in separate conical flasks by typical filtration process [8].

Extraction

The collected leaves were washed with double distilled water and shadow dried before being grinded to fine powder and sieved to remove coarse particles. One gram leaf powder was mixed with 100 ml of methanol and the mixture was left in a shaking incubator operating at 200 rpm, 25°C for 24 h. The extract was then filtered and the filtrate was used for AuNPs synthesis [8].

Phytochemical Analysis of Plant Extracts

Phytochemical analysis of methanolic plant extracts have been done for glycosides, flavanoids, tannins, alkaloids, sugars, starches, saponins, proteins, aminoacids, terpenoids, vitamin C and phenolic

compounds using the standard procedure [12, 13].

Biosynthesis of Gold Nanoparticles

Choloroauric acid (HAuCl4) solutions of 10^{-3} M were prepared for the synthesis of gold nanoparticles. 0.2ml of leaf extract was added to 50ml of 10^{-3}M HAuCl4 solution [3].

DPPH Free Radical Scavenging Activity Assay

An aliquot of the samples was mixed with DPPH solution (5 mL, 23.6 µg/mL in ethanol), followed by incubation of 30 min. The absorbance of each sample was read at 517 nm. Ascorbic acid (0.9, 1.9, 3.9, 4.9, 6.9 µg/mL) was used as positive reference. The percentage of scavenged DPPH was calculated using equation 1:

$$\text{Percentage activity / inhibition} = \frac{100 \times (Ac - As)}{As} \text{ ---------> [Equation 1]}$$

Where *A*c is the absorbance of the control and *A*s is the absorbance of the sample. IC50 values calculated denote the concentration of the sample required to decrease the absorbance at 517 nm by 50% [14].

Superoxide Free Radical Scavenging Activity

The superoxide radical was detected by NBT reduction. The reaction mixture contained EDTA (0.1 M), 0.0015% NaCN, riboflavin (0.12 mM), NBT (1.5 mM) and various concentrations of extract and phosphate buffer (67 mM, pH 7.8) in a total volume of 3 ml. The tubes were uniformly illuminated for 15 min and optical density was measured at 530 nm before and after the illumination. The percentage inhibition was calculated by using equation 1 [15].

Anti-Lipid Peroxidation Assay

A modified thiobarbituric acid-reactive species assay was used to measure the lipid peroxide formed. Plant extract of 0.6ml of extract were added to a test tube and made up to 1ml with distilled water. 0.005ml of FeSO4 (0.07M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5ml of 20% acetic acid, 1.5ml of 0.8% (w/v) TBA in 1.1%sodium dodecyl sulphate and 0.5ml 20% TCA were added and the resulting mixture was vortexed and then heated at 95oC for 60 min. If the sample have high amount of anthocyanin then to eliminate this non-MDA interference, another set of samples were treated in the same way, incubating without TBA. After cooling, 5.0ml of butanol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532nm. Incubation of lipid peroxidation (%) by the extract was calculated according to the equation 1 [16].

H_2O_2 Radical Scavenging Assay

A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH 7.4) to which the extracts (1–10 µg/ml) were added to hydrogen peroxide solution (0.6 ml). After incubation for 10 mins, the absorbance of hydrogen peroxide was measured at 230 nm. Phosphate buffer without hydrogen peroxide was used as blank and it is compared with ascorbic acid as the reference compound. The percentage inhibition was calculated by using equation 1 [17] .

Results and Discussion

Synthesis of Gold Nanoparticles

The synthesis of gold nanoparticles can be found within an hour (50 minutes) when cherry red colour solution was obtained

Table.1 Phytochemical Composition of Aqueous and Methanolic Extracts of *Silybum marianum*

Phytochemical compounds	Aqueous extracts	Methanolic extracts
Sugars	Positive	Positive
Tannins	Negative	Positive
Flavanoids	Negative	Positive
Alkaloids	Positive	Positive
Terpenoids	Negative	Positive
Anthocyanins	Negative	Positive

Figure.1 DPPH Free Radical Scavenging Activity of Methanol Extracts of *Silybum marianum*

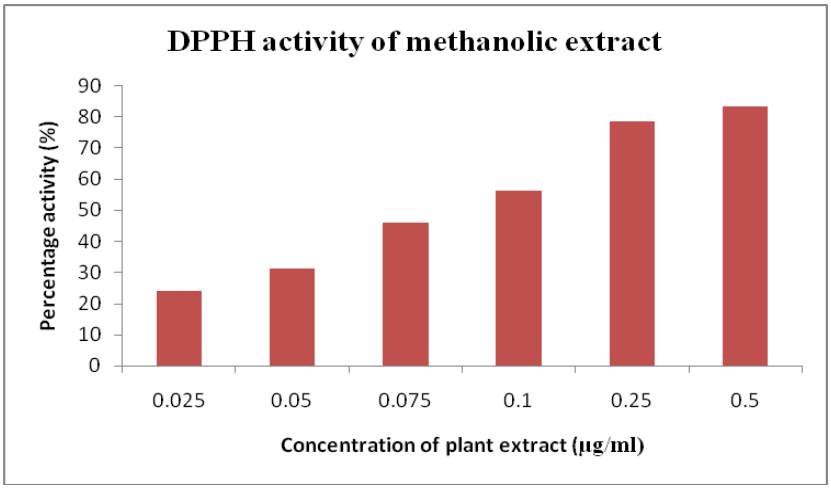


Figure.2 Superoxide Free Radical Scavenging Activity of Methanol Extracts of *Silybum marianum*

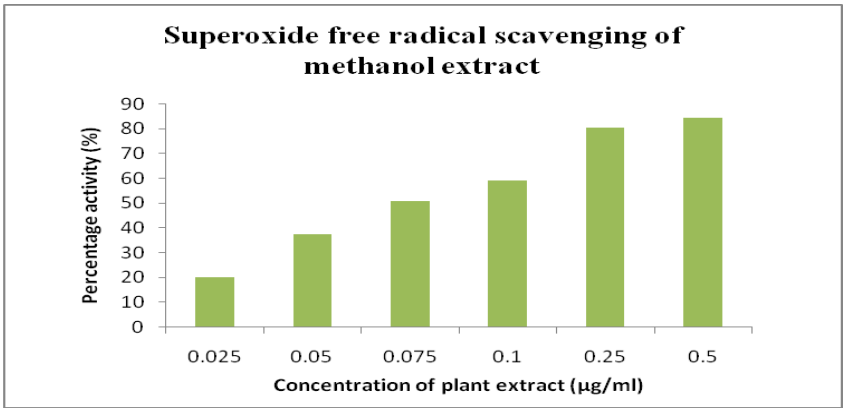


Figure.3 Anti Lipid Peroxidation Activity of Methanol Extracts of *Silybum marianum*

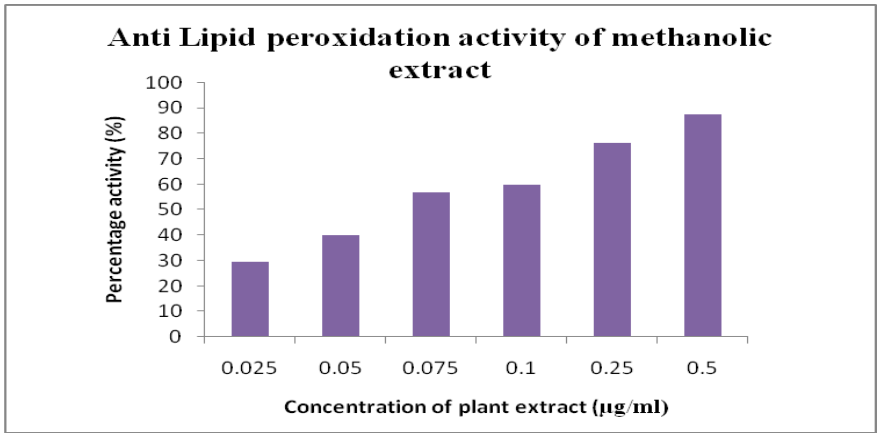
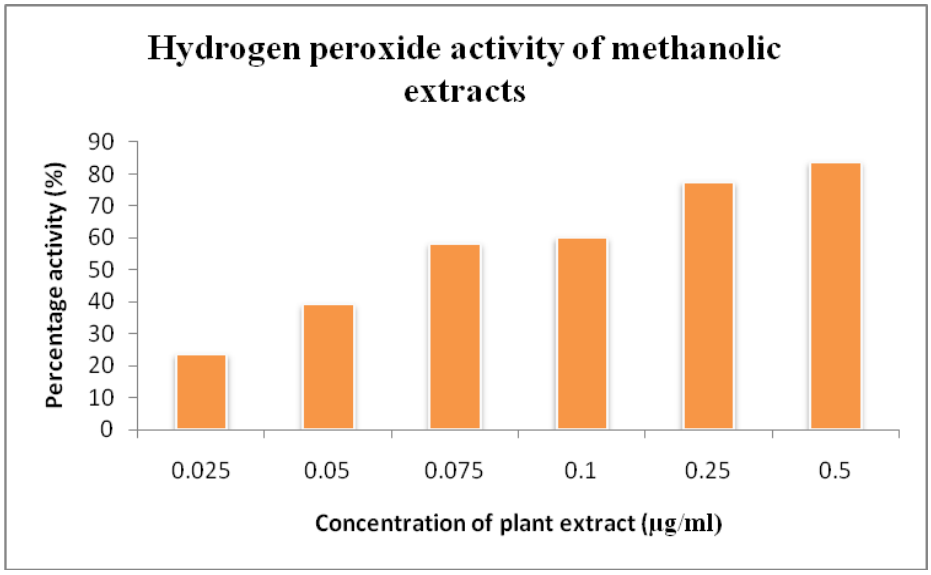


Figure.4 Hydrogen Peroxidase scavenging Activity of Methanol Extracts of *Silybum marianum*



Phytochemical Analysis of Plant Extracts

The aqueous and methanolic extracts of the plant *Silybum marianum* has been used to find the bioactive compounds present in the plant. The Phytochemical tests for sugars, tannins, flavanoids, alkaloids and terpenoids shows that the plant has more bioactive compound in its methanolic extract than the aqueous extract. Therefore the methanolic extract has been taken for further study. Table 1 shows the presence of compounds in methanolic and aqueous extracts.

Free Radical Scavenging Activity

The free radical scavenging activity of the methanolic extracts done by DPPH free radical scavenging activity, Superoxide radical scavenging activity, Anti-lipid peroxidation assay and Hydrogen peroxide assay was shown in Figure 1, 2, 3 and 4 respectively and was found to have notable antioxidant activity. It has also been noticed that there is increased activity with increase in concentration of the extract. Similar

results were noticed by several works done on antioxidant activity of plant extracts [15, 16, 17].

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