

Silver Nanocomposites from *Aeglemarmelos* and its Potential Applications

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Abstract: *The green synthesis of nanoparticles has gained a significant role among the research now a days. This study was undertaken to investigate the phytochemical analysis of medicinally significant plant Aeglemarmelos and also investigates the total antioxidant activity, antidiabetic, anticancerous and antimicrobial activity of the leaf extract. The combination of nanocomposite with D-Ribose drug is a notable investigation, which was proved by efficacy study. This overall study highlights the consequence of synthesised and traditional Bael leaf (A.marmelos).*

Keywords: Bioactive compounds, green synthesis, cytotoxicity, antimicrobial activity, drug conjugation

1. Introduction

Nanotechnology has become the important and fascinating aspect in the modern scientific field. The nanoparticles are the one which possess a significant role in the wide variety of potential applications in biomedical, optical and electronic fields. The nanoparticles are regarded as nanometrics whose size spans between 1 and 100nm. These particles are simply defined as the microscopic particle which acts as a bridge between bulk materials and atomic or molecular structures.

In ancient times, the stained glass makers used tiny amounts of silver and gold in glass. They knew that by putting varying tiny amount of nanoparticles, they could produce the red and yellow colours in the stained glass windows. Today's scientists have found that a small amount of a nanoparticle have the ability to change the materials physical appearance [1].

Nanoparticles are broadly divided into two groups. They are organic and inorganic nanoparticles. Dendrimers, liposomes, compact and hybrid nanoparticles comes under the organic nanoparticles whereas gold nanoparticles, silver nanoparticles, silica and quantum dots etc. are inorganic nanoparticles which possess certain physical properties which mainly includes size dependent optical, magnetic, electronic and catalytic properties. Dendrimers arise from two Greek words "Dendron" meaning tree and "Meros" meaning part. As the name suggests, the dendrimers is morphologically characterised by branched structure grown from two or more core. Dendrimers are hyper branched, globular, monodisperse, three dimensional nanoscale synthetic polymers [2].

In the recent years, development and use of the green synthesis method employing natural capping, stabilising and reducing agent to prepare silver nanoparticle with desired size and morphology become a major focal point for researchers [3]. Silver nanoparticles are of vast attention in the field of nanotechnology, because it can synthesis without any toxic, harsh and expensive chemical substance. Pure

natural constituents could be used to bioreduce and stabilise silver nanoparticle [4].

Aeglemarmelos is an indigenous plant of India, have been used as a natural source of medicinal compounds since thousands of years and the tree belongs to rutaceae family. The different parts of *Aeglemarmelos* are using for various therapeutic purpose such as in the treatment of anaemia, fracture, asthma, wound healing, swollen joints, jaundice, diarrhoea, high blood pressure and typhoid troubles during pregnancy [5]. Leaf extract from *Aeglemarmelos* have served as green reactants in silver nanoparticles synthesis. They exhibit unique properties (eg; size, shape, depending optical, electrical and magnetic properties [6]. These unique properties of silver can be incorporated into cosmetic products, antimicrobial application, biosensor materials, composite fibres and electronic components [7].

Indian subcontinent consists of wide variety of flora which is Ayurveda. Recently many plants are gaining importance due to their unique constituents and their versatile applicability in the various developing field of research and development. The present study was done to identify the bioactive compounds in qualitative (phytochemical analysis) and quantitative methods (antioxidant activity). Followed by synthesising, composite preparation and its antimicrobial, anti diabetic and anticancer study. Further the composite was used for drug conjugation to increase the efficacy study.

2. Materials and Methods

2.1 Collection of sample

The leaves of *Aeglemarmelos* plant was collected near Marudhamalai, Coimbatore district, Tamilnadu, India. The collected leaves were selected without any fungal disease.

2.2 Preparation of sample

The medicinal plant *Aeglemarmelos* collected and the fresh-healthy leaves were washed under tap water to remove the dirt, and followed by distilled water. The leaves are then dried under shade of sunlight for 3- 5 days. 10 grams of

leaves were weighed and crushed using mortar and pestle and transferred to 250ml conical flask containing 100ml of distilled water and incubated in shaking incubator for overnight. The extracts were filtered using Whatmann's No.1 filter paper to get the clear extract and which was used for further study.

2.3 Phytochemical analysis

The qualitative phytochemical analysis of the leaf extracts were performed with *Aeglemarmelos* plant using the protocol of [8].

2.4 Antioxidant activity

The antioxidants which constitute the plant materials helps in converting the free radicals to less reactive species and hence it acts as a radical scavengers. Dietary sources like fruits, vegetables contain a wide variety of free radical scavenging antioxidants. Traditional herbal medicines were main source of antioxidants for people in ancient times. The present study for the identification of radical scavenging activity, different types of antioxidant activity of total antioxidant activity and FRAP was done and is given below;

2.4.1. Total Antioxidant Activity

Phosphomolybdenum method was used to identify the total antioxidant activity of *Aeglemarmelos* extract. To the 0.5ml of sample (*Aeglemarmelos* leaf extract) was mixed with equal amount of antioxidant mixture (0.6M H₂SO₄, 28mM sodium phosphate, 4mM ammonium molybdate), and incubated at 50°C for 90 minutes in a water bath and it is allowed to cool to take the OD at 695nm using spectrophotometer (ELICO SL 159).

2.4.2. FRAP Assay

Ferric reducing antioxidant power assay was used to measure the FRAP activity. In this assay, the antioxidants are used as areductants in a redox linked calorimetric reaction in which colourless ferric probe complex is converted into coloured ferrous probe complex. It is a high through put adaptable assay which can detect antioxidants capacities as low as 0.2 millimolar Fe²⁺ equivalents. 0.5ml of sample along with 0.5 ml of phosphate buffer solution and 0.5 ml of 1% potassium ferric cyanide was added and mixed thoroughly. After mixing incubated at 50°C in a water bath for 20 minutes. The sample was allowed to mix 0.5 ml of distilled water and 0.5 ml of 1% ferric chloride solution and the optical density was measured using spectrophotometer (ELICO SL 159) at 700nm.

2.5 Antidiabetic Activity

Diabetics is a chronic disease which is characterised by the lack of insulin production. It is associated with the hyperglycaemia. The present investigation includes an evaluation of an invitroantidiabetic activity of *Aeglemarmelos* leaf extracts, In vitro antidiabetic activity of *Aeglemarmelos* were detected by using alpha amylase activity. To the 1ml of sample, 0.5ml of 0.1% starch solution in 16molar sodium acetate buffer was added and mixed. After mixing 0.5 ml of alpha amylase solution and sodium potassium tartarate (0.5ml), 3-5 dinitrosalicylic acid

was also added and incubated in an alkaline condition 25 degree solution for 10 minutes. Optical density is measured at 540nm using spectrophotometer (ELICO SL 159).

$$\% \text{ of inhibition} = (\text{Control} - \text{Sample} / \text{Control}) \times 100$$

2.6 Synthesising of Nanoparticles

Aeglemarmelos leaf extract was used to synthesise the silver nanoparticle. 1mM of silver nitrate solution was prepared by using standard formula and used for the study. The filtered sample was mixed with equal amount of the silver nitrate solution and incubated in room temperature for 24-48hrs in dark condition.

2.7 Characterisation of Nanoparticle

2.7.1 Visual identification and UV- Visible study

For the synthesised nanoparticle, primary completion of bio reduction in Ag⁺ ions in aqueous solution was done by checking the color change followed by scanned under UV-Visible spectrophotometer (ELICO SL 159) between the wavelength of 300-600nm having a resolution of 2nm, after 24 hrs of incubation.

2.8 Antimicrobial Activity

Antimicrobial activity of the *Aeglemarmelos* leaf extract and the nanoparticle was done the protocol of [9] using well diffusion method, for antibacterial activity *E.coli*, *salmonella typhi* and for antifungal activity *A.niger* and *A.flavus* were used.

2.9 Anticancer Activity

The anticancer activity was done by using the protocol of [10], different concentration of synthesised nanoparticles (10µl, 20µl, 30µl) was examined against HeLa Cell Lines by using MTT assay method.

$$\% \text{ cell death} = \frac{\text{control OD} - \text{treated OD}}{\text{control OD}}$$

2.10 Preparation of Nano Composites and Drug Conjugation with D- Ribose

To the plant nanoparticle solution, 2% of sodium alginate was added and allowed to heat in water bath for 5-10 minutes for the complete mixing, and added 50µl of 25% (W/V) glutaraldehyde solution, the mixture was incubated for overnight. Optical density was measured at the interval 5.0nm of 200-900 nm using spectrophotometer (LABTRONICS Model LT 291).

Drug delivery systems have attained a significant attention in the development of novel medical devices. Drug delivery system is safe when compared to unmodified drugs. The present study was done to conjugate with d ribose by dissolving 0.5ml of composite and 0.001 gram of drug, the mixture was incubated for 24 hours. Optical density was measured at 254nm using spectrophotometer.

2.11 UV- Visible and Efficacy Study

UV- Visible confirmation was done in the nanometer of 200-900nm. The efficacy of the drug was determined by

well diffusion method. Mueller hinton agar was prepared and sterilised, the solidified agar was used for the swabbing of 50 µl *E.coli*. Wells were made with corkborer contains control (composites), composite and drug, drug respectively.

3. Results and Discussions

3.1 Phytochemical analysis

The present study was experimented using aqueous extract of *Aeglemarmelosto* identify the presence of phytochemical

constituents of the leaf sample. The extract revealed the presence of various compounds such as alkaloids, terpenoids, phenol, saponin, flavonoids and protein and which is showing the absence of steroids, quinines and sugar. The result were coinciding the investigation of [11], which showing the presence of same compounds in the aqueous and methanolic extract of sample (*Aeglemarmelos*). The quantitative phytochemical estimation specified the leaves contained the presence of, flavanoids, tannin, alkaloids, saponin and phenol[12].



Figure 1: Colour changes in phytochemical analysis(steroid test, sugar test, saponin test, terpenoids test, protein test, phenol test, quinine test, Meyers test, flavonoid test) result was given in table 1.

Table 1: The qualitative analysis of leaf extract (*Aeglemarmelos*).

Phytochemical constituents	Aqueous extracts
Alkaloidss	Present
Terpenoids	Present
Phenol	Present
Sugar	Absent
Saponin	Present
Flavanoids	Present
Quinines	Absent
Protein	Present
steroids	Absent

3.2 Antioxidant Activity

Antioxidants are man made or natural substance that may delay or prevent the free radical catalyzed reactions, Oxidative stress is developed due to various environmental exposure, during normal metabolic process or due to chemical factors which may ultimately lead to cell damage, As a result the free radicals are generated. In general antioxidants are classified based on their function or on other disease, depending on their function there are primary

antioxidants and secondary antioxidants. Antioxidants are the first line of defense against the damage of free radical. Total antioxidant activity is used for the identification of radical scavenging activity which is measured at 695nm using speactrophotometer and the mg/g total antioxidant was 158 mg/g. The quantitative FRAP analysis of antioxidant activity to reduce the fe3+ tripyridyl-s-triazine complex[12]. FRAP (ferric reducing ability of plasma) was also done to determine the scavenging activity of free radicals which was measured at 700nm and showing 172mg/g. done antioxidant activity of leaf extract of *Aeglesmarmeloscorrea ex roxb* also representing similar results [13].

3.3. Synthesising of Nanoparticles, visual identification and UV-Visible study

As the leaf extract was added to silver nitrate solution, it changes from yellow colour to brown colour which is indicating the presence of colloidal AgNP formation, similar changes were observed in the study of [16]. Hence it confirming the completion of reaction between *Aeglemarmelos*leaf extract and AgNO3. The UV- visible spectra analysis was showing the plasmon resonance of AgNPs in 456.5nm. The results were given in figure 2;

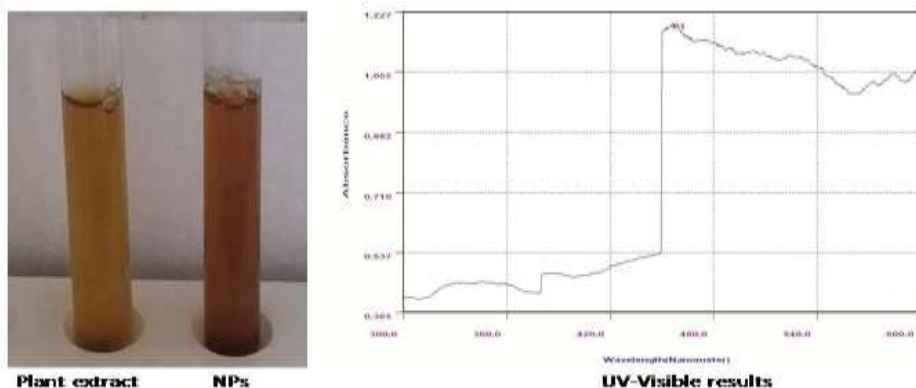


Figure 2: Synthesis of nanoparticle and uv visible reading.

3.4 Antimicrobial Activity

The pharmacological action of plant extract and synthesised nanoparticle was determined by antibacterial activity against *Salmonella typhi* and *E.coli*. Well diffusion method denoting high zone of inhibition against *E.coli* compared with *Salmonella typhi*. Anti fungal effect of silver nanoparticle against *A.niger* and *A.flavus*, *A.flavus* was shown better activity compared with *A.niger* [17]. In another study the *Aeglemarmelos* was found to be more efficient in controlling the growth of *A.niger*, with the zone of inhibition was observed in 17mm in hexane and 19mm in acetone at 10mg/ml concentration respectively in the study of [11]. The result were given below in figure (3) and table (2)



Figure 3: Antimicrobial activity of the synthesised *Aeglemarmelos*

Table 2: Antibacterial and Antifungal activity of nanoparticle

Samples used	Zone of inhibition in mm			
	Antibacterial activity		Antifungal activity	
	<i>E.coli</i>	<i>Salmonella typhi</i>	<i>A.niger</i>	<i>A.flavus</i>
Nanoparticle	9mm	3mm	2mm	3mm
Antibiotic	10mm	2mm	nil	nil
AgNO ₃	1mm	No zone	No zone	No zone
Plant extract	6mm	No zone	1mm	8mm

From the table compared with the plant extract, nanoparticle giving better zone of inhibition against bacteria and fungi. Aqueous extract of *Aeglemarmelos* observed highest activity against *S.epidermis* (14.3mm) followed by *S.aureus* and *K.pneumoniae* (10.6mm) in the study of [11]. The maximum zone of inhibition was observed at a concentration of 40mg/ml. Antibacterial activity which is of varying degree by *Aeglemarmelos* leaf extract against various tested bacterial species has been [18].

3.3 Antidiabetic Activity

Antidiabetic activity study is worth assessing the hypoglycemic effects of leaf extract-*Aeglemarmelos*, and

was quantified by using alpha amylase glucosidase activity. Percentage of inhibition showing 18.24% in invitro study. [14], investigated antidiabetic activity of leaf and callus extract in rabbit, inferred advisable result; [15] reported biochemical test for the identification of antidiabetic activity.

3.5 Anticancer Activity

There are different types of nanoparticle which are recently investigated to apply in biomedical with the emphasis on cancer study. This study was done with the synthesised nanoparticle to identify the anticancer activity against HeLa Cell Line using MTT assay method. Different concentration of the nanoparticle, 10 to 30µl was showing increasing % of cell death with increasing concentration. Higher cell death was occurred in the 30µl of the sample and lower % of cell death was occurred in the 10µl of the *Aeglemarmelos* nanoparticle.

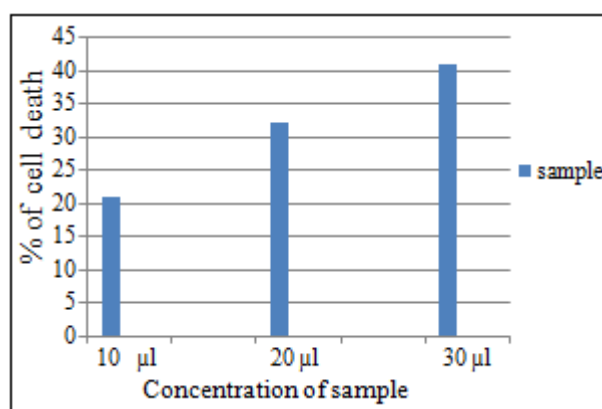


Figure 4: Anticancer activity of the *Aeglemarmelos* nanoparticle

3.6 UV-Visible result and efficacy study of drug conjugation

Drug conjugation was carried out by using D-Ribose – a building block of ATP which will help to restore depleted energy in sick hearts. Multifunctional drug carriers based nanoparticle deliver bioactive molecules to intent tissues by nano size or adsorption of bioactive compounds on the particle surface [19]. Combination of nanotechnology with herbal medicine will improve bioavailability and will help to decrease the toxicity. Nanotechnology is rapidly expanding the benefits of implication for industry cosmetics and especially in medical field [20]. Here the conjugation was done with the composited sample of the extract to enhance the medicinal property of the nano composite. The primary confirmation was done by UV-Visible study and the peak denoting the presence of bioactive compounds in the nano composites which may the reason to increase the medicinal properties. After conjugating with lower concentration, drug showing high zone of inhibition in the efficacy study which is given below in figure (5);

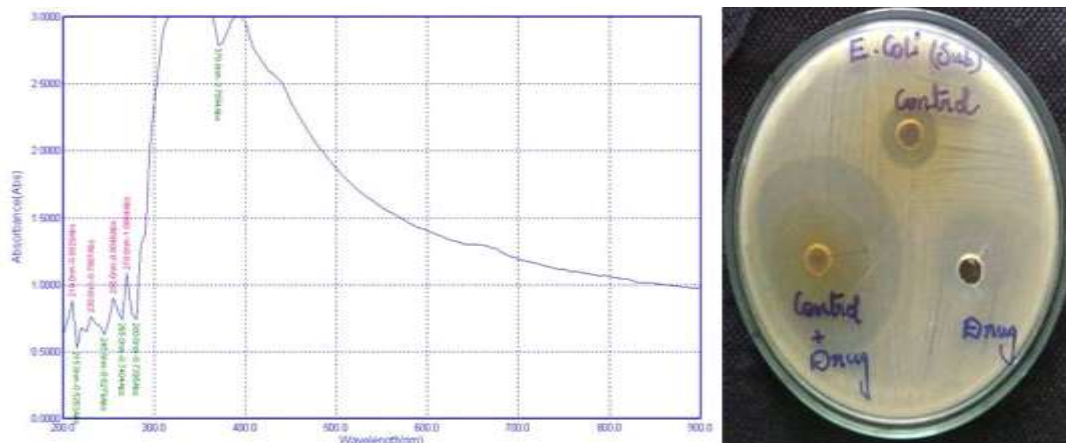


Figure 5: UV visible reading of nanocomposites and its efficacy study

From the figure, the conjugated sample showing advisable zone of 19mm, D-Ribose (drug) 4mm zone and plain composites (without drug) showing 5mm. [21] Assessed enhancement of antibacterial properties of silver nanoparticles- ceftriaxone conjugate through *Mukiamaderasapatana* leaf extract mediated synthesis against ATCC and MTCC culture by disc diffusion method and they obtained flawless results.

4. Conclusion

Synthesising of nanoparticle and nanocomposites is an emerging technique and showing better result in various field of medicinal, industrial, agricultural etc. Microorganisms are producing resistant capacity against the medicines and which causing lots of problem to human being. The present study giving an alternative source against the pathogens with nanoparticle and nanocomposites. Drug conjugated sample shows a better result compared with nanoparticle and nanocomposites. Anticancer activity against HeLa cell lines expressing higher percentage of cell death in synthesised nanoparticle of *Aeglemarmelos* because of the presence of various bioactive compounds.

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