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Research Article

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## Synthesis and Characterization of silver nanoparticles using *Phyllanthus acidus* leaf extract and its anti-inflammatory activity against human blood cells

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### ABSTRACT

An attempt was made to explore the ethno botanical, economical and biological importance of *Phyllanthus acidus*. The plant is used for 28 types of remedies like cathartic, emetic, coughs, hypertension, asthma, skin diseases etc and as a food in the raw form. In India, it is found in different states including Tamilnadu and South Indian states as home garden ornaments. Synthesis and characterization of nanoparticles is under exploration due to its wide medical applications and various research interests in nanotechnology. In the current study, the leaf extract of *Phyllanthus acidus* (Family: Euphorbiaceae) is used for the synthesis of silver nanoparticles. The leaf extract is mixed with AgNO<sub>3</sub> and incubated. The synthesized silver nanoparticles were confirmed by color transformation. It was characterized by UV, FTIR, XRD and TEM. The silver nanoparticles synthesized were generally found in size 1-100 nm. The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The average size of synthesized silver nanoparticles is found to be 27.9nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis (28.6nm). In totality, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in Anti-inflammatory activity of Pharmaceutical research to obtain better result of plant as shown by our study. The anti-inflammatory activity of silver nanoparticles was tested against human blood cells which confirms that the plant mediated synthesis of silver nanoparticles have a significant anti-inflammatory activity against human blood cells.

**Keywords:** *Phyllanthus acidus*, Silver Nanoparticle, UV-Vis Spectroscopy, FTIR, TEM, XRD, Cytotoxicity, Human Blood Cells.

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## 1. Introduction

The field of Nanoscience has blossomed over the last twenty years and the need for nanotechnology will only increase, as miniaturization becomes more important in areas such as computing, sensors, and biomedical applications. Advances in this field largely depend on the ability to synthesize nanoparticles of various nano material, based on their sizes, and shapes, as well as their efficiency to assemble them into complex architectures (David D et al., 2005). Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research towards a multitude of potential uses for Nanomaterial (Saifuddin N et al., 2009).

*Phyllanthus acidus* Skeels, an important medicinal plant belonging to the genus *Phyllanthus* (Euphorbiaceae), is widely cultivated in worldwide, and its extracts have been used for treating alcoholism. *Phyllanthus acidus*, known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, country gooseberry, star gooseberry, starberry, West India gooseberry, damsel, grosella (in Puerto Rico), jimbilin (in Jamaica), damsel (in Grenada), karamay (in the Northern Philippines), cermai (in Indonesia and Malaysia), Goanbili (in Maldives) or simply gooseberry tree, is one of the trees with edible small yellow berries fruit in the Phyllanthaceae family. Despite its name, the plant does not resemble the gooseberry, except for the acidity of its fruits. It is mostly cultivated for ornamentation.

The medicinal activities of *Phyllanthus* species are antipyretic, analgesic, anti-inflammatory, anti-hepatotoxic and antiviral [1-4]. Fruits of the two well-known species, *P. acidus* L. and *P. emblica* L. contain high contents of vitamin C and have been used for used for improving eyesight and memory. It prevents action against Diabetes and reliefs from cough [5]. Another species of the family, *P. amarasis* an important herbal medicine due to its effective antiviral activities especially towards the hepatitis B virus [6-8]. Traditionally, *P. acidus* has been used in the treatment of several ailments including inflammatory and oxidative stress-related disorders such as gastric trouble (Jules and Paull, 2008) [9]. Hence, in the present study, a silver nanoparticle was synthesized using aqueous extract of *P. acidus* and confirmed by color transformation using UV-visible spectrophotometry. The sizes of the nanoparticles were analyzed by XRD and TEM analysis. The stability of nanoparticles was studied by FTIR and more significantly anti-inflammatory activity against human blood cells was also analyzed.

## 2. Materials and Methods

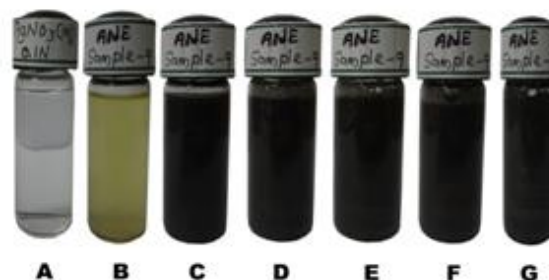
**Collection of leaf:** Fresh leaves of *Phyllanthus acidus* were collected from Trichy, during the month of May and identified by Dr. John Britto, The Director, Rabinat Herbarium and Center for Molecular Systematics, St. Joseph's College (Campus), Trichirappalli-2, Tamilnadu, India. (Plant authentication no: PN006).

### Preparation of leaf extract

The fresh and young leaf samples of *Phyllanthus acidus* was collected and washed thoroughly with sterile double distilled water (DDW). Twenty grams of sterilized leaf samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW. After that the mixture was boiled for 5 mins and then filtered. The extract was stored in 4 °C.

### Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml of *Phyllanthus acidus* leaf extract was added to 100 ml of 0.1N AgNO<sub>3</sub> aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (100 rpm) at 50° C and reaction was carried out for a period of 12 hrs. Then the mixture is kept in microwave oven for exposure of heat. The mixture was completely dried after a period of 20 minutes and hence nanoparticles in form of powders were obtained.



**Figure 1:** Optical photograph of *Phyllanthus acidus* A- 0.1 N AgNO<sub>3</sub> solution B- Leaf extract C- Leaf extract + AgNO<sub>3</sub> D- Leaf extract + AgNO<sub>3</sub> (After 30mins) E- Leaf extract+AgNO<sub>3</sub>(After 1hr) F-Leaf extract+AgNO<sub>3</sub>(After 2 hrs) G- Leaf extract + AgNO<sub>3</sub>(After 24 hrs)

### UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + leaf extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of solid and subsequently measuring UV-visible spectra of the solid sample. UV-visible spectra of sample were monitored as a function of time of reaction on The UV-visible spectroscopy and the investigations was carried out using PERKIN ELMER (Lambda 35 model) spectrometer in the range of 190 nm to 1100 nm.

### FT-IR measurement

The Fourier transform infrared (FTIR) investigations were carried out using PERKIN ELMER (Spectrum RXI) spectrometer in the range of 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup>. The functional groups were identified using the peak assignments.

### XRD measurement

The sample was drop- coated onto Nickel plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was

prepared. The particle size and nature of the silver nanoparticle was determined using X-ray diffraction (XRD). This was carried out using Rigaku miniflex-3 model with 30kv, 30mA with Cuk radiations at 2 angle.

**TEM analysis**

Sample is dispersed with acetone and exposed in ultrasonics for 5 minutes. Take a drop of a solution from the samples and drop it on the grid, leave until it dries. After drying the samples is inserted into TEM instruments using model Tecnai T20 Making in FEI, Netherlands operating at 200KeV Tungsten Filament.

**Anti-Inflammatory Activity**

**The human red blood cell (HRBC) membrane stabilization method**

The method as prescribed (Gopalkrishnan et al., 2009; Sakat et al., 2010) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection calculated using the following formula,

$$\text{Percentage of Protection (\%)} = (100 - \text{OD of drug treated sample} / \text{OD of Control}) \times 100$$

**Albumin denaturation method**

The method as prescribed (Sakat et al., 2010) was followed with modifications. The reaction mixture consists of test extracts and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the sample, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows,

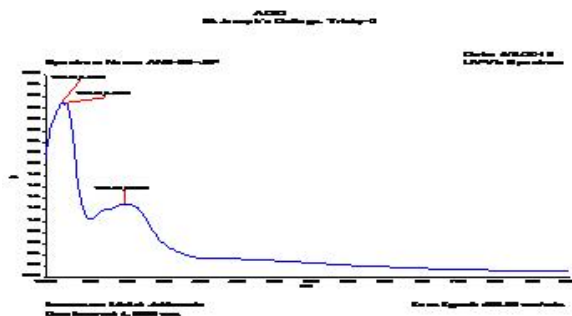
$$\text{Percentage of inhibition (\%)} = (\text{OD of Control} - \text{OD of Sample} / \text{OD of Control}) \times 100$$

**3. Results and Discussion**

**UV-Visible Spectroscopy Analysis**

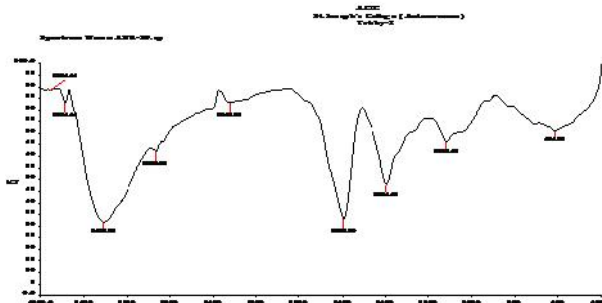
UV-Vis spectroscopy analysis showed the absorbance band of silver nanoparticles synthesized using *Phyllanthus acidus* leaf extract at 217.13 nm which confirms the presence of poly-unsaturated and aromatic compound (Isoquinoline) (Advanced strategies in food analysis,UV/VIS spectrometry International Journal of Medicine and Pharmaceutical Research

by Richard Koplík)



**Figure 2:** UV-Visible spectrum of synthesized silver nanoparticles using leaf extracts of *Phyllanthus acidus*

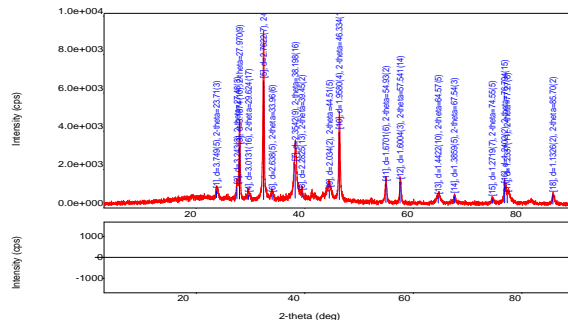
**FT-IR measurement**



**Figure 3:** FT-IR spectrum of synthesized silver nanoparticles using leaf extracts of *Phyllanthus acidus*.

The *Phyllanthus acidus* related functional groups were identified using the peak assignments. A strong peak at 3914.44 cm<sup>-1</sup> and 3776.64 cm<sup>-1</sup> was assigned to the OH stretching in Phenol group, The strong peak at 3422.79 cm<sup>-1</sup> was assigned to O-H stretching and H bonded phenol or alcohol group, The medium peak at 2928.08 cm<sup>-1</sup> was assigned to OH stretching in carboxylic acid group, The medium peak at 2243.81 cm<sup>-1</sup> was assigned to -C (triple bond) C- H stretching in alkenes group, The medium peak at 1595.70 cm<sup>-1</sup> was assigned to N-H bend stretching in primary amine group, The broad peak at 1394.47 cm<sup>-1</sup> was assigned to C- H stretching in alkenes group, The medium peak at 1118.42 cm<sup>-1</sup> was assigned to C-N stretching in aliphatic amines and The medium peak at 614.98 cm<sup>-1</sup> was assigned to C-Br stretching in alkyl halides are observed.

**XRD measurement**



**Figure 4:** XRD spectrum of synthesized silver nanoparticles using leaf extracts of *Phyllanthus acidus*  
**Determination of crystalline size**

Average crystallite size of silver was calculated using the Scherrer's formula,

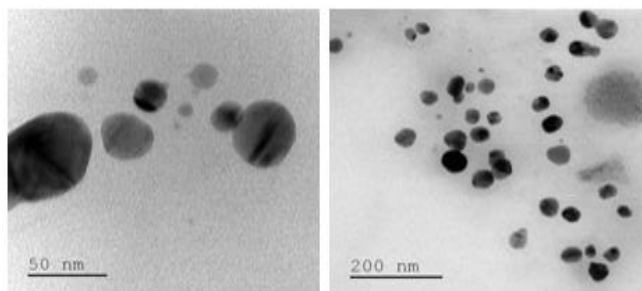
$$D = k / \cos$$

D-Average crystallite size:

K- Constant: - X- ray Wavelength: - Angular FWHM of the XRD peak at the diffraction angle: - Diffraction angle.

Applying XRD data in Scherrer's formula, the average size of the particle is approximately found to be 18.08nm.

**TEM analysis**



**Figure 5:** TEM image of synthesized silver nanoparticles using leaf extracts of *Phyllanthus acidus*

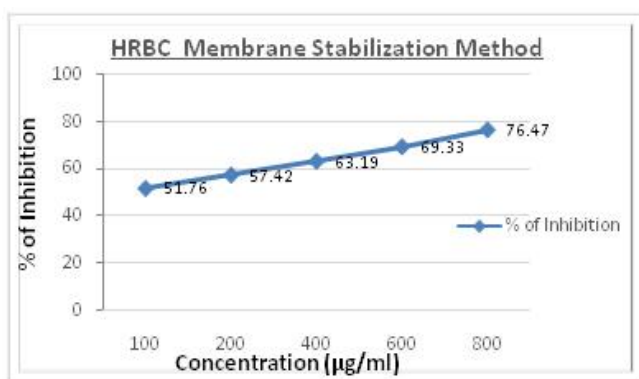
The figure shows the TEM image obtained by the reaction of *Phyllanthus acidus* leaf extract and 0.1N silver nitrate solution separately. The average size of Ag-NPs using *Phyllanthus acidus* leaf extract was found to be 18.14 nm.

**Anti-Inflammatory Activity**

Anti-inflammatory studies like human red blood cell (HRBC), membrane stabilization, inhibition of albumin denaturation indicate the anti-inflammatory activity. The medical use of *Phyllanthus acidus* has a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases.

**Table 1:** Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs of *Phyllanthus acidus*

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M
1	100	51.76 ± 0.27
2	200	57.42 ± 0.86
3	400	63.19 ± 0.34
4	600	69.33 ± 1.82
5	800	76.47 ± 1.93

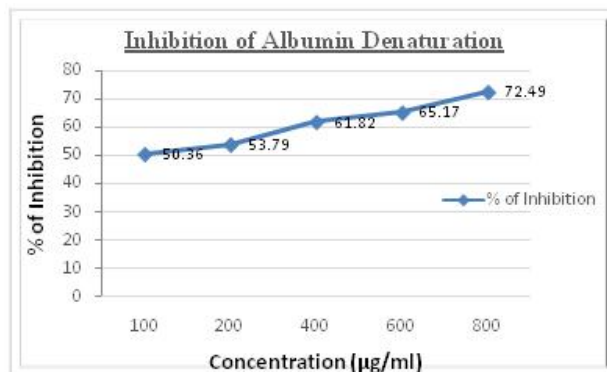


**Figure 5:** Graphical representation of Anti-inflammatory activity of human red blood cell (HRBC) by using AgNPs

of *Phyllanthus acidus*

**Table 2:** Anti-inflammatory activity of Albumin denaturation method by using AgNPs of *Phyllanthus acidus*.

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean±S.E.M
1	100	50.36 ± 0.81
2	200	53.79 ± 0.36
3	400	61.82 ± 0.49
4	600	65.17 ± 1.19
5	800	72.49 ± 1.57



**Figure 6:** Graphical representation of Anti-inflammatory activity of Albumin denaturation method by using AgNPs of *Phyllanthus acidus*

**Discussion:**

The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by products. Thus, there is an increasing demand for green nanotechnology” (GarimaSinghalet al., 2011) [10]. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants (Mukherjee et al., 2011 [11]; Spring et al., 1995) [12].

Inflammation involving the natural and adaptive immune systems is a normal response to infection. However, when allowed to Continue or unchecked, inflammation may result in autoimmune or anti inflammatory disorders, neurodegenerative disease, or cancer. A variety of safe and effective anti-inflammatory agents are available, including aspirin and other nonsteroidal anti-inflammatories, with many more drugs under development. In particular, the new era of anti-inflammatory agents includes “biological” such as anti cytokine therapies and small molecules that block the activity of kinases. Other anti-inflammatories currently in use or under development include statins, histone deacetylase inhibitors, PPAR agonists, and small RNAs (Charles, 2010)[13]. In Indian System of Medicine, the rhizome of *Cyperusrotundus* plant is recommended for use in different clinical conditions including fever and arthritis. The rhizomes are cooling, nerving tonic and diuretic and

traditionally used to treat diarrhoea, leprosy, bronchitis and blood disorders. The rhizome is reported to possess analgesic, anti-inflammatory and antipyretic activity (Sharma et al., 2010 [14], Nagulendran et al., 2007 [15], Guldur, 2010 [16]).

#### 4. Conclusion

In conclusion, the bio-reduction of aqueous silver ions by the leaf extract of the *Phyllanthus acidus* has been demonstrated. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles and the synthesized nanoparticles are quite stable in solution. The sizes of silver nanoparticles were determined by using XRD & TEM analysis. Transmission Electron Micrograph of Silver nanoparticles finds the size of the nanoparticles. Result of TEM micrograph of silver nanoparticles at 50nm shows that synthesized silver nanoparticles has the average size of 18.14 nm. By applying XRD data in Scherrer's formula, the average size of silver nanoparticles is found to be 18.08nm, which is approximately similar as the size obtained in TEM analysis (18.14). In addition to that anti-inflammatory studies like human red blood cell (HRBC), membrane stabilization, and inhibition of albumin denaturation indicate the anti-inflammatory activity. The medical use of *Phyllanthus acidus* has a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. Use of plants in synthesis of nanoparticles is quite novel leading to truly 'green Environment' route. This green environment approach towards the synthesis of nanoparticles has many advantages such as, process scaling up, economic viability and safe way to produce nanoparticles.

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