PATNA WOMEN'S COLLEGE 1940

IRIS
Journal for Young Scientists
ISSN 2278 – 618X **(Print)**
ISSN 2278 – 6384 **(Online)**

http://www.patnawomenscollege.in/journal

Evaluation of Antioxidant & Antimicrobial activity of synthesized silver nanoparticles using *Phyllanthus niruri*

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Received : November 2017
Accepted : March 2018
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Abstract: *The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to allopathic drugs and are being used to develop Pharma drugs. Phyllanthus niruri has medicinal properties for the effective management of several aliments including Hepatitis. The present investigation was aimed to focus on the screening of phytochemical constituents. An environmental friendly approach is employed to synthesize silver nanoparticles. The biomolecules found in plants induce the reduction of Ag+ ions from silver nitrate to silver nanoparticles (AgNPs). UV-visible spectrum of the aqueous medium containing silver ions demonstrated a peak at 280nm and 580nm corresponding to the Plasmon absorbance of silver nanoparticles. Fourier Transform Infra-Red (FT-IR) spectroscopy was done to find the functional groups present. Antioxidant activities were done using Hydrogen Peroxide assay and antibacterial activity of Phyllanthus niruri AgNP extract. Different extracts of P niruri has the medicinally useful secondary metabolites and also act as antibacterial agent on bacterial (E.coli and S.aureus) strains. Green synthesis of silver nanoparticles exhibits an important eco friendly and useful to the environment. UV-visible spectrophotometer used to predict that where the synthesis has been done or not. Whereas the organic functional groups were determined by FT-IR with different wave number and it has determined by the functional group data analysis.*

Keywords: *P niruri, Phytochemical screening, Antioxidant activity, Silver nanoparticles synthesis, Green synthesis, Antibacterial activity.*

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Introduction :

Phyllanthus niruri L. (Euphorbiaceae), is a small herb, found in most tropical and subtropical regions. The use of environmentally benign materials like plant leaf extract, bacteria for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for and biomedical applications as they do not use toxic chemicals in the synthesis protocols. In this plant phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plant such as bioactive substance like flavonoid, alkaloid, tannin, antioxidant and phenolic compounds for have a beneficial effect on health. They were known to show the medicinal activity as well as physiological activity. Biomolecules of plant origin appear as alternatives for the control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis (Mathias et al.,2000).

The most effectively studied nanoparticles today are those made form noble metals, in particular Ag, Pt, Au and Pd. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape, and controlled disparity (Garderb Toressgay et al., 2003). Literature reveals a promising medical application of silver nanoparticles synthesized using herbal extracts have been reported to have good anti-bacterial and antioxidant properties.

Materials and Methods :

The present research work was conducted in the department of Botany, Patna Women's College, Patna during the period of July to October, 2017. The phytochemical analysis to detect the important chemical content present in the plant. The presence certain chemical compounds which resist the certain microorganism like bacteria by silver nanoparticles synthesized leaf extract and to reveal the plant antioxidant property.

The fresh leaves of Phyllanthus niruri was collected from the campus of Patna women's college. The leaf was collected in bulk and dried under room temperature for 2 week. The dry plant material was grind in a grinder. The coarse powder was be stored in air dry container.

From plant material 2.5g powder was extract with 50 ml of ethanol, methanol and dw. The extract was filtered through What man no.1 filter paper and then used for phytochemical test. The methanolic, ethanolic and aqueous extract of powder leaf of P. niruri was subject to preliminary testing for detection of major groups (Harborne et al., 1998).

The extract was taken about 3-5ml in separate test tube and then a few drops of 0.1% ferric chloride was added. Observed brownish green or a blue black coloration.

3ml of extract would taken in test tube separately with the Mayer's reagent (1.27g of Mercuric chloride and 2g of potassium iodide) in 100ml of distilled water. Reddish brown precipitate indicated presence of alkaloid in the sample.

2ml of extract was vigorously shaked with 5 ml of distil water in test tube. Observed the Persistent frothing indicate the presence of saponin.

To portion of the dissolved extract a few drop H_2SO_4 solution were added. A green or blue colour indicated the presence of phenolic nucleus.

1ml of extract was take in the test tube and 1ml of chloroform and 1-2 drop of H_2S0_4 was added carefully to from a layer reddish brown colour of the interface indicates the presence of terpenoid.

5 g of powdered sample was dissolved in 100 ml of distilled water and then was boiled at 60°C in water bath for 5min. The resulting crude extract was filtered through what man no. 1 filter Paper. The filtrate was stored in refrigerator at 4°C.

1mM of $AgNO_3$ (0.05g) was added to 300m1 of distilled water and the solution was stirred continuously until $AgNO_3$ is dissolved. This 1mM $AgNO_3$ solution was stored in brown bottle at 4°C for further experimentation.

50 ml of fresh leaf extract was added into aqueous solution of 1mM $AgNO_3$. To prepare smaller particles, then after mixture was centrifuged at the rate of 12000rpm for 15min, the supernatant collected for further processing.

The nanoparticles thus synthesized was subjected to various characterization techniques such as UV- vis spectra & FT-IR.

The bio reduction of Ag+ in aqueous solution was monitored by measuring the UV-vis spectrum of the reaction medium at different time interval and at different nanometer (280-580). UV-vis spectra were recorded at 2min, 30min, 1hr, 24hrs.

FT-IR spectra was recorded over the spectral range of 4000-400 cm^{-1}. 1t is an easy way to identify the presence of certain functional group in a molecule.

Two bacterial strains was used such as Staphylococcus aureus (gram positive cocci) and Escherichia coli (gram negative bacilli) were arranged from Patna Medical College and Hospital. The effect of the P. niruri extract on the bacterial strain was assayed by disc diffusion method (Jha et al., 2009).

Preparation of nutrient agar and broth. 100µl of synthesized silver nanoparticles extract were pour on the disc. That disc placed on the media and kept in incubator for 48hours.

1ml of extract was rapidly mixed with 2ml of 10mM phosphate buffered (0.1,Ph 7.4) Hydrogen peroxide solution. The absorbance was measured at 516 nm in UV-vis Spectroscopy (Bunghez et al., 2012).

The percentage of inhibition of H_2O_2 was calculated using the following formula =

$$\% \text{ inhibition } H_2O_2 = [AO]-[A1]/[AO]*100.$$

Where, (AO- Absorbance of control ; A1- Absorbance of sample)

Results and Discussion :

Phytochemical screening revealed the presence of tannin, alkaloid, saponin, flavonoid, terpenoid, protein, phenol (Table 1). These may contribute to the effect such as antimicrobial, antipyretic, anti-inflammatory, antispasmodic, cathartic and expectorant activities and also astringent properties, hastens the healing of wound.

Table 1. Phytochemical screening of *P.niruri* in different solvent extract

PHYTOCHEMICAL	AQUEOUS	METHANOL	ETHANOL
Phenolic compound	+ve	-ve	-ve
Saponin	+ve	-ve	-ve
Flavonoid	-ve	+ve	+ve
Terpenoid	+ve	+ve	+ve
Alkaloid	+ve	+ve	+ve
Tannin	+ve	+ve	+ve
Protein	-ve	+ve	+ve

It is generally recognized that UV-vis spectroscopy could be used to examine a size and shape-controlled nano particles in aqueous suspension (Wiley BJ et. al., 2006). Characterization of silver nanoparticles on UV-Vis spectroscopy were taken in 2 different nanometer as well as at different interval of time (at 2 min, 30 min, 1 hour and 24 hour).

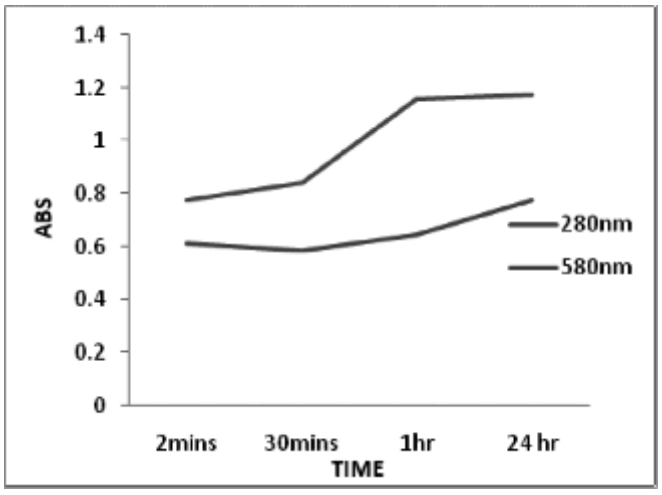


Fig. 1. Graph showing absorbance of silver nanoparticles at different time interval and wavelength.

The result in Figure 1 represent that the lowest reading (0.778 nm) come from at 2 min interval of time at 280 nm where as highest reading (1.175) comes at 24 hour interval of time at 280 nm. At 580 nm at different interval of time, the result is in between of these two value.

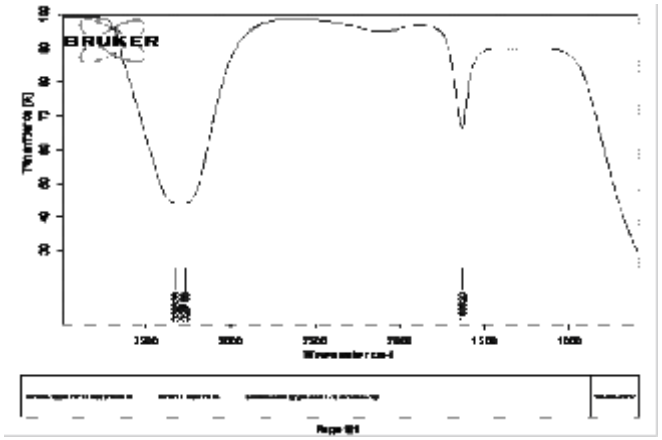


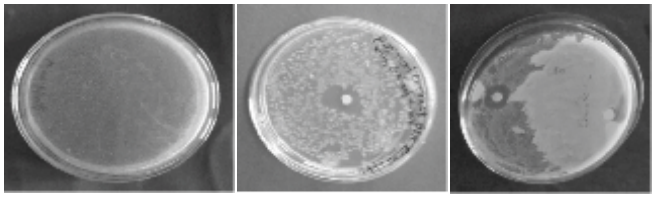
Fig. 2. Graph Absorbance peak showing different functional groups in Silver synthesized extract by FT-IR.

From the above Fig. 2, it can be concluded that the peak value of FT-IR for the Functional group (Alkenyl and Amine) at different peak value 1635.20, 3267.66, 3323.36.

Table 3. FT-IR peak values for *P.niruri.*

Peak value of FT-IR	Functional group
1635.20	Alkenyl C=C Stretch
3267.66	Alkenyl C-H Stretch
3323.36	Amine N-H Stretch

In the above Table 3 the functional group were Alkenyl group C=C Stretch, C–H Stretch and in Amine group N–H Stretch were present.



(a) (b) (c)

Fig. 3. Zone of inhibition

(a) control

(b) *E.coli* in SNP extract

(c) *S.aureus* in SNP extract

In Fig. 3 it can be seen SN extract of ***P. niruri.*** SNP extract is effective. Zone of inhibition was very clearly seen.

Table 4. This table shows that the leaf extract of *Phyllanthus niruri* was ineffective against the pathogenic bacteria *E.coli* and *S.aureus*. However, the SNP synthesized extract was effective *S.aureus*.

S. No.	Inoculum	Pathogenic strain	Zone of inhibition
			Silver nanoparticles
1.	Bacteria	***Escherichia coli***	± 12 mm
2.	Bacteria	***Staphylococcus aureus***	± 14 mm

The zone of inhibition was ±12mm for *E.coli* and ±14mm in *S.aureus* (Table 4). Synthesized nanoparticles extract showed better antimicrobial activity in gram +ve bacteria *S.aureus* in comparison to gram –ve bacteria *E.coli*.

In SNP extract the zone of inhibition was ±12mm in ***E.coli*** and ±14mm in ***S. aureus***. ***S. aureus*** was more effective against ***E.coli.***

Table 5. Shows the results of *P. niruri* antioxidant activity

S. No.	Sample	Absorbance of control	Absorbance of sample	% of inhibition
1.	Aqueous extract	1.288	0.441	53.882%
2.	Silver nanoparticles extract	1.288	0.594	65.761%

The absorbance of control was 1.288 (Table 5). The absorbance of sample in aqueous extract was 0.441 and % of inhibition was 53.882%. The absorbance of sample in SNP extract was 0.594 and % of inhibition was 65.761%. So the SNP extract was more effective than aqueous extract.

Conclusion :

From this study, we conclude that *P. niruri* showed differences in their phytochemical content in all the three extracts i.e (ethanol, methanol, & aqueous). In phytochemical test– Terpenoid, alkaloid and tannin are present in all three solvent.

But in Flavonoid and protein is present in ethanolic and methanolic solvent, where as phenol and saponin it is present in aqueous solvent only.

Synthesis silver nanoparticles leaf extract by reducing the respective silver ion solution observed by UV–vis spectroscopy and in FT–IR spectroscopy We analyzed the functional group which show the formation of silver nanoparticles.

In antimicrobial the leaf extract synthesized silver nanoparticles which show best result in *S. aureus* as comparison to *E.coli*.

In antioxidant silver nanoparticles extract showed best result followed by aqueous extract, the formation of nanoparticles and composition.

In all aspects, the natural plants are far more beneficial and harmless in side effects aspects, than the other from of medications. Wether in concern of health medication or food items, natural plants are proven to be the best of all other form of medications in long run use.

Acknowledgement :

We express our great sense and gratitude to our Former Principal, Dr. Sister. Marie Jessie A.C, for giving us the opportunity to do this research work. We also thankful to Dr. Pinky Prasad (H.O.D) of Botany, our supervisor Ms. Isha Gaurav, Department of Botany.

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