International Journal of Research in Advent Technology, Vol.7, No.2, February 2019
E-ISSN: 2321-9637
Available online at www.ijrat.org

Synthesis of Plant Mediated Metal Nanoparticle and Assessment of Its Antibacterial Activity

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Abstract- A laboratory experiment was conducted in order to examine the effect of phytochemicals in the form of nanoparticle as an anti-bacterial agent. This study focuses on effectiveness of silver nanoparticle in the treatment of diseases caused by bacterial infection. For this, the phytochemicals present in the plant *Abelmoschus esculentus* was analysed and they were estimated. The justified amount of phytochemicals present in this plant gave an interest to produce nanoparticle using $AgNo_3$ in order to enhance its anti-bacterial property as they exhibit larger surface area to volume ratio. Then, the green synthesised silver nanoparticle was confirmed and the anti- bacterial activity also performed by disc diffusion method. This study reveals that the plant the *Abelmoschus esculentus* has good antibacterial activity which shows better result when they were in a nanoscale than in bulk scale.

Keywords: Abelmoschus esculentus, HPLC, silver nanoparticle, UV spectrophotometer, anti-bacterial activity

1. INTRODUCTION

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of various diseases[7]. These medicinal properties of the plant extract can be enhanced with nano silver and it could play vital role in treatment of many diseases (Akhil Gupta et al., 2011; Shreesh Kumar Ojha and Dharamvir Singh Arya 2011).

Now- a-days, biosynthesis of nanoparticle by the plants extract is also currently under exploitation. The development of biologically inspired experimental processes for the synthesis of nanoparticle is evolved into an important branch of nanotechnology (Murphy, 2008). The silver nanoparticles (nano-Ag) have proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms [8]. The bacteriocidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity. Besides, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content.

Thus, the present study focuses on the evaluation of phytochemicals present in *Abelmoschus esculentus* (L) Moench and their enhanced antimicrobial activity along with silver when they converted into nanoparticle.

2. MATERIALS AND METHODS

Sample Collection and Authentication of Plant material

The fruits of*Abelmoschusesculentus* were collected from Melur Village in Madurai District. Authentication of plant was carried out.

Extraction of mucilage (RishabhaMalviya, 2011)

*Abelmoschusesculentus*fruits were used for isolation of mucilage. Fruits were washed with water to remove dirt. The seeds were removed and finely chopped and crushed into a mixer. The crushed fruit material was soaked in warm water for 4 h, boiled for 2 h and kept aside for 2 h for release of mucilage into water. The material was squeezed in a muslin bag to remove the mark from the filtrate. The filtrate was used for further study.

Phytochemical Analysis (Harbone, 1981)

(i) Qualitative Analysis

Alkaloids,Tannins, Saponins, Steroids, Flavanoids, Glycosides and Amino acids were analyzed qualitatively.

(ii) Confirmation of Phytochemicals by HPLC

Results of biochemical analysis were further tested through HPLC analysis to confirm the presence of phytochemicals in the extract.

iii) Quantitative analysis

Once the presence of phytochemicals were confirmed, their amount in the plant extract was also analyzed by various standard methods. Such as Flavonoids-Libermamn by Burchard *et al.*, Saponins by Brunner 1984, Vitamin C by Iodine method,Tannins by Van Burden and Robinson1981 .

Green Synthesis of Silver Nanoparticle(Govindaraju K *et al., 2010)*

To 5ml of plant extract, 100ml of 1mM AgNo3 in 250 ml Erlenmeyer flask. Flasks were kept in a shaker at 200 rpm for 10minutes [pH of the solution was maintained as slightly acidic (6.5-6.8)] incubated at room temperature for7hours. The colour change was observed.

Confirmation of synthesized Silver Nanoparticle
(i) UV-Visible spectral analysis (Klulkarni*et al.*, 2011)
To 0.2ml of the suspension, 2ml of double distilled water was added and measured at 200 nm in UV-VIS Spectrophotometer.

3. ANTIBACTERIAL ACTIVITY OF *ABELMOSCHUS ESCULENTUS* (Mishra *et al.*, 2010)

Disc diffusion method (Kirby Bauer method)
1.3g of nutrient agar was mixed with the double distilled water and allowed to sterilize by autoclaving it under 120psi pressure. The autoclaved agar was poured into sterile petriplates (Control, *E.Coli and Bacillus subtillus*) and kept for solidification[1]. After few minutes, the bacterial cultures such as *E.coli*, *Bacillus subtillus*, (3 hours old) was inoculated by using spread plate method and let it free for 3-4 minutes, then discs which were dipped into both Abelmoschus esculentus extract and plant mediated silver nanoparticle solution were placed on the agar plates. They were incubated at 37°C. The zones of inhibitions by plant extract as well as silver nanoparticle in both bacterial cultures were observed.

Determination of minimum inhibitory concentration (Eloff J.N, 1998)
Antibacterial activity of both plant extract and silvern nanoparticle was also studied using minimum inhibitory concentration test. About 50µl of mucilage was serially diluted with PBS saline with a initial concentration of 200ml/ml,400ml/ml,600ml/ml in a 96well micro titer plate and 50µl of 3hour old culture was added to each well separately. The microplate was covered and incubated at 37°C for 18 hours. Then, 40µl of 0.2mg/ml of methyltetrazolium was added to each well and incubated for minutes. The appearance of violet colour in the wells indicates the growth of bacterial culture. The lowest concentration of extract that completely inhibited the growth was considered as the MIC values.

4. RESULT AND DISCUSSION

Table: 1 Qualitative Analysis of Phytochemicals

TEST	RESULT
Steroids	-
Flavonoids	+
Vitamin C	+
Alkaloids	-
Glycosides	-
Tannins	+
Saponins	+

When the plant extract was examined for the presence of phytochemicals, the results showed that the phtochemicals such as flavanoids, tannins saponins and vitamin C were present.

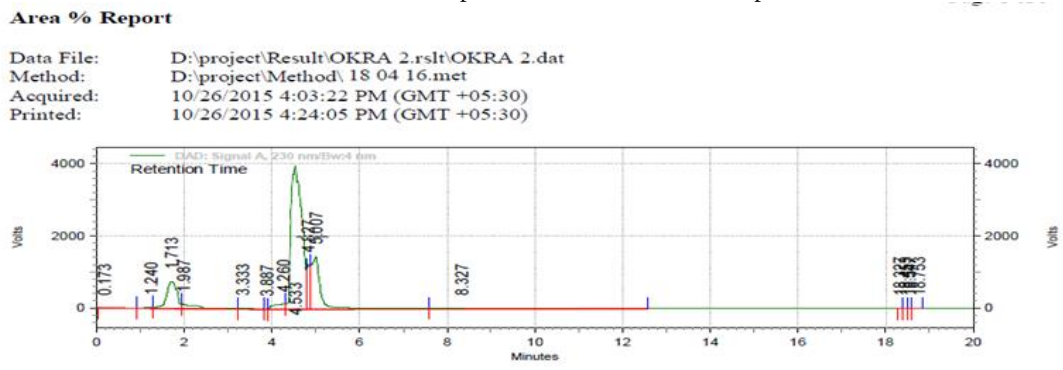


Fig: 1 Confirmation of Phytochemicals by HPLC

The chromatogram of HPLC at 250nm showed the presence of two peaks for the plant extract. Thus the presence of secondary metabolite in the plant extract was confirmed by HPLC method.

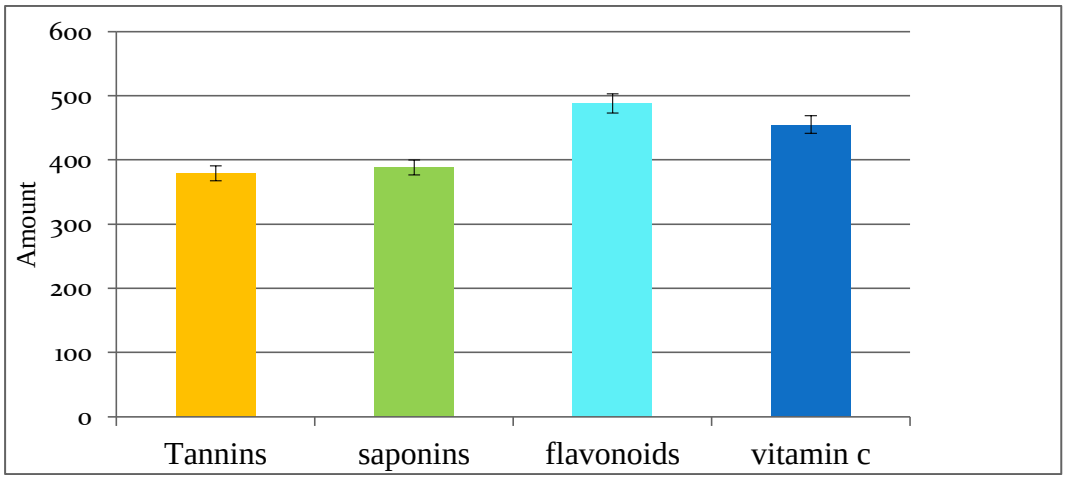


Fig: 2 Quantitative Analysis of Phytochemicals

The quantitative analysis of phytochemicals shows that the flavonoids are in higher level than tannins and saponins which are approximately equal in their amounts. The amount of Vitamin C is higher than tannin and saponin but lesser than flavonoids.

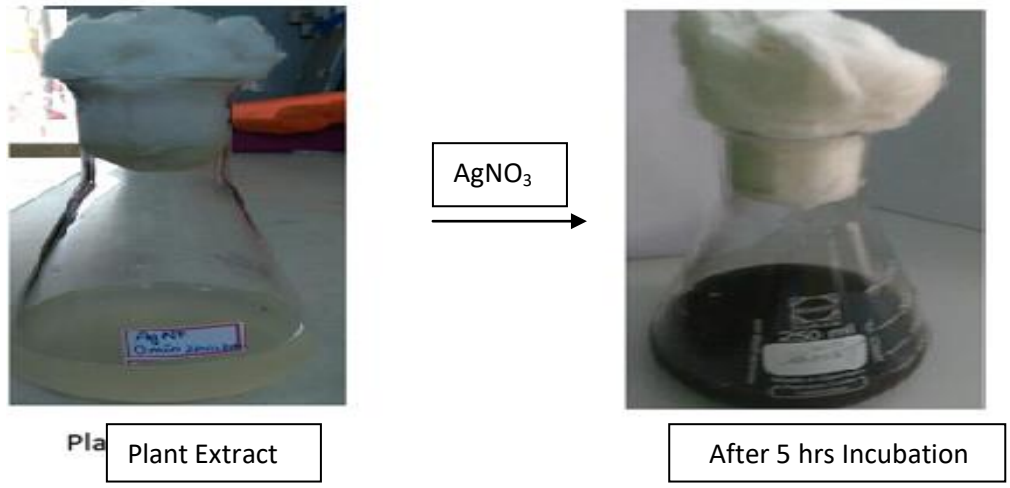


Fig: 3 Geen synthesis of silver nanoparticle

Before the addition of $AgNO_3$ the colour of the plant extract was pale yellow but after the treatment with $AgNO_3$, it has been changed in to dark brown which indicates the formation of silver nanoparticle.

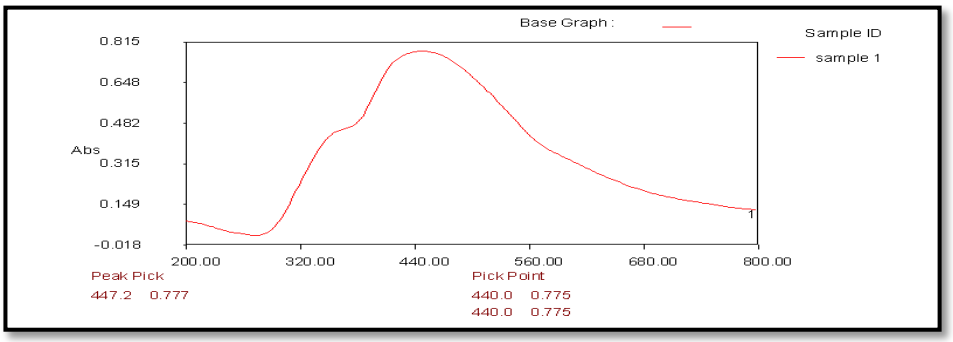


Fig: 4 UV-Visible spectroscopy

UV- Visible absorption peak of synthesized nanoparticle was observed approximately at 360 to 420nm. The spectrum shows the formation of spherical AgNP of the plant extract.

Plate.1: *Bacillus subtillus* against AgNPs

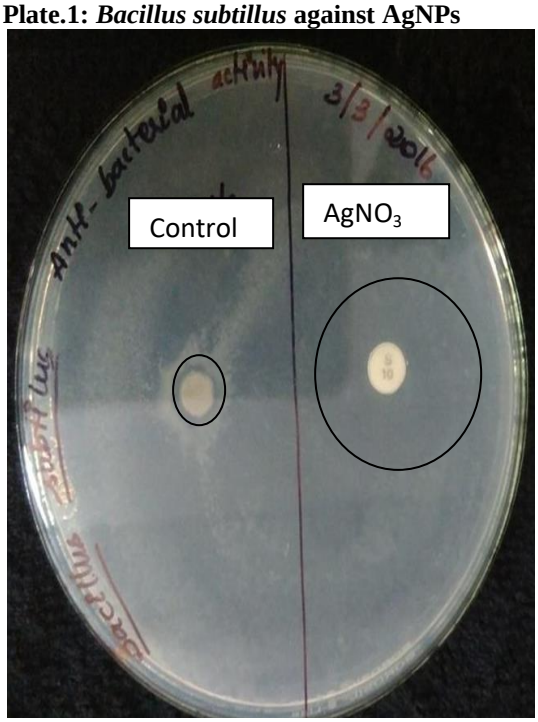


Plate.2: *E.coli* against AgNPs

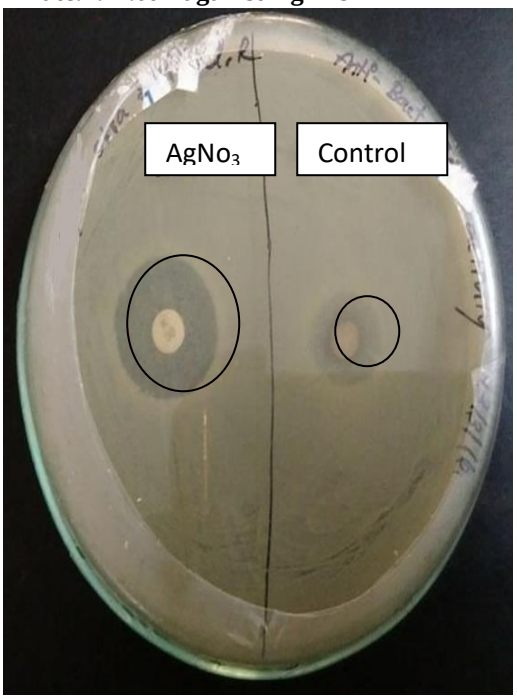
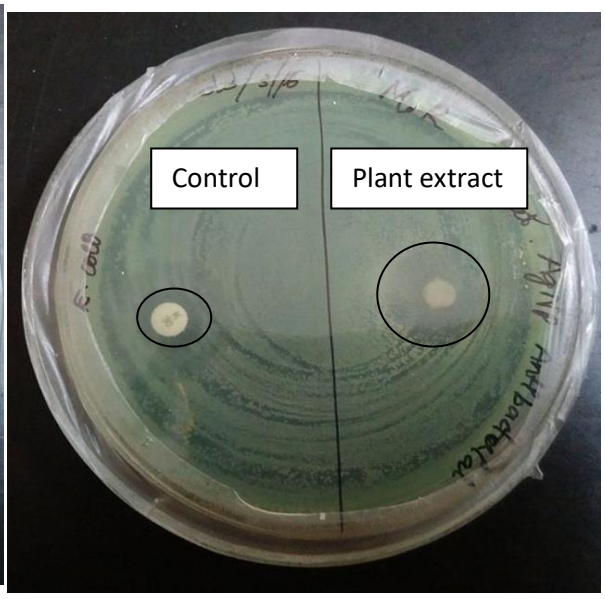


Plate.3:*E.coli* against Plant extract

4: *Bacillus subtills* against Plant extract



The anti-bacterial activity of plant (*Abelmoschus esculentus* (L) Moench) extract and its silver nanoparticles were examined by disc diffusion method against bacterial strains such as *Bacillus subtilis and E.coli.* Both bacerias were showed zone of inhibitions against plant extract and the nanoparticle synthesised from it. The competent zones of inhibitions were observed in silver nanoparticle synthesized from the plant.

5. CONCLUSION

The present study concluded that the plant *Abelmoschus esculentus* has anti-bacterial activity. At the same time, when the plant extract was converted into nanoparticle, its anti-bacterial efficiency was enhanced. Hence, the study suggests that the medicinal properties of the plant will give an improvised result if they were in nano size.

ACKNOWLEDGEMENT

I am grateful to Ms. Rekha who helped for this study.

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