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Biosynthesis of silver nanoparticles using Nepenthes Spp. and its bactericidal effect

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ABSTRACT

Nanotechnology have emerged a new technology in nanomedicine applications to produce alternative medicines which can counter the antibiotic resistance to be implemented in healthcare area. The present study focus on to synthesize silver nanoparticles by the using the plant Nepenthes. sp. The synthesis of nanoparticles was observed by the change in the color of extract in to dark brown. Characterization of silver nanoparticles was done by UV-Visible spectrophometery and Fied Emission electron microscopy (FESEM) which showed the size around 20 – 30 nm . These nanoparticles showed excellent antibacterial activity against some common bacterial pathogens.

Key words: *Nepenthes* sp. FESEM, UV spectrophotometry, Bacterial pathogens

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INTRODUCTION

Nanotechnology has grown rapidly for the past few years and has been implemented in various areas including healthcare system [1]. Nano-size particles lesser than 100 nm in diameter are in demand because of its wide range in various applications [2].Silver and silver based compounds from ancient times has bactericidal effect so much focus has now been paid on the synthesis of silver nanoparticles to enhance its bactericidal activity Therefore, researchers have tried their best in conducting multiple research experiements to find out the inner potential of using silver nanoparticles to enhance their bactericidal property to counter the antibiotic resistance among pathogens[3,4].

Different approaches are now a days used for the biosynthesis of nanoparticles but using green source as a nan factories is advance because of less hazardous ,safe and not too much expensive.

The aim of the present study is to synthesize the safe and stable from the plant *nepenthes* spp. These silver nanoparticles were further characterized by using FESEM to determine size and topology of the nanoparticles. These nanoparticles showed good antibacterial against some selected pathogens.

MATERIALS AND METHODS

Collection of plant material.
The leaves of *Nepenthes* spp was collected from Sri Iskandar, Ipoh Perak. After that the leaves were dried, mashed and grind by using blender machine into powder form.

Aqueous Extraction
10 grams of dried powdered leaves of *Nepenthes* spp. was mixed with 200ml of distilled water in a 250ml conical flask. The mixture was further heated on the hot plate approximately 10 minutes until boiled and filtrated by using filter paper into conical flask. 50ml of the aqueous extracts were collected as sample.

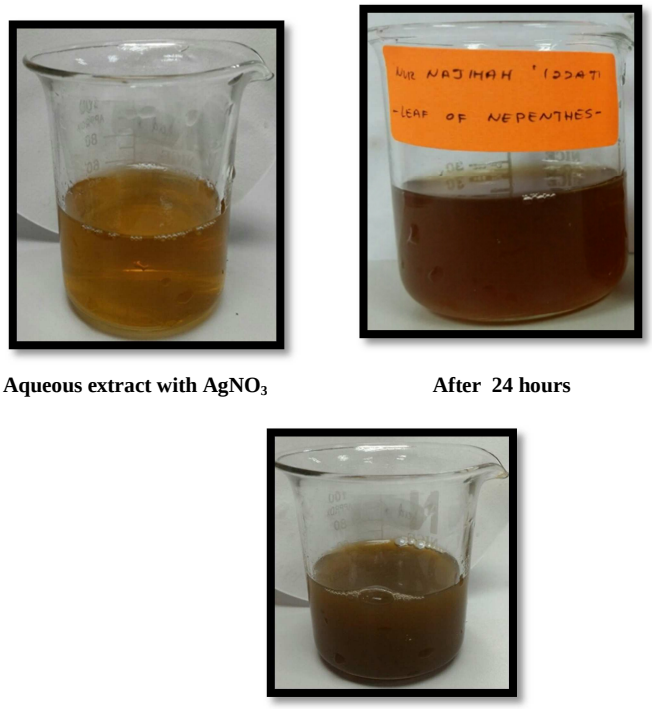
Biosynthesis of silver nanoparticles.
1mM of silver nitrate solution was freshly prepared by dissolving silver nitrate in the form of solid in 100ml of distilled water in a conical flask. Take 50 ml of aqueous extract and mixed it with silver nitrate solution. Conical flask was plugged with cotton and kept on rotary shaker at 155-160rpm for 72 hours. Colour changed from yellow into dark brownish colour which indicates the formation of silver nanoparticles.

Characterization of silver nanoparticles.
The reduction of silver ions in to silver nanoparticles was confirmed by UV-Visible spectroscopy. 1ml of silver nanoparticles solution was taken into the quartz cuvette and the wavelength was observed. These nanoparticles were further characterized by using FESEM .For SEM sample was prepared first sonication for around two minutes followed by centrifugation .Supernatant was discarded and pellet was dried for SEM analysis.

Antimicrobial activity test
The nutrient agar plates were prepared to check bactericidal effect of AgNPS and each of agar plate was swabbed with different pathogens viz *Escherichia coli, Salmonella enterica, Staphylococcus aureus* by using disc diffusion method.[5]. Each disc was impregnated with different concentrations of biosynthesized silver nanoparticles like 5 µl, 10 µl, 15 µl, 20 µl and 25 µl) which were placed on the solidified agar medium. These agar plates further were incubated for 24 hours at 37^{o}C for bacterial growth.

RESULTS AND DISCUSSION

The aqueous extract of stem *Nepenthes* spp. were used for the first time in the biosynthesis of silver nanoparticles. During observation, reaction between the solution with 1Mm silver nitrate yield a brownish colour and gradually increased in its colour intensity to dark brown when it was kept for 72 hours. The colour change at different time interval.as Fig1 [6,7]



Aqueous extract with $AgNO_3$ **After 24 hours**

After 72 hours.

Fig 1. Colour change observed in the leaves sample of Nepenthes spp. at different time intervals upon addition of $AgNO_3$

These biosynthesized silver nanoparticles showed the absorption at 380nm when evaluated by UV-Visible spectrophotometry which is due to the surface plasmon resonance of nanoparticles present insides the solution. Fig 2.

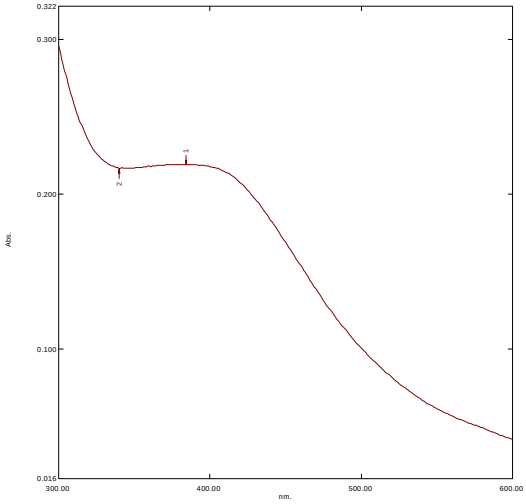


Fig 2: UV Visible spectrophotometric analysis

FESEM analysis showed that silver nanoparticles are spherical, well distributed and with average size 20 to 40 nm ,

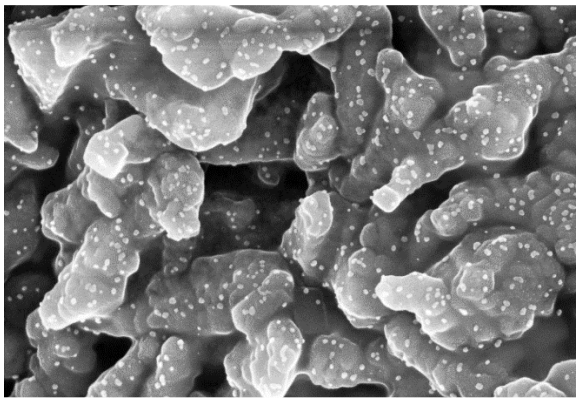


Fig 3: FESEM analysis

These biologically synthesized nanoparticles were check for antibacterial effect against some common human pathogens like *Escherichia coli, Salmonella enterica, and Staphylococcus aureus* by using disc diffusion method. Each disc was impregnated with different concentrations of nanoparticles from5 µl, 10 µl, 15 µl, 20 µl and 25 µl which were placed on the solidified agar medium while pure extract without addition of AgNPs was dropped on the control disc. The results were positive for every pathogens but among all, synthesized the highest zone of inhibition was showed against *B. Cereus* 22 mm followed by *S.,aureus* 20 mm then *E.coli* 14 mm.

These nanoparticles exhibit excellent antimicrobial property but exact mechanism of action of these nanoparticles not yet know but several researchers suggest that these silver nanoparticles attacks the necessary enzymes of DNA and causes the morphological changes in the DNA which leads to cell death.[8].

Table 1: Zone of Inhibition in millimetre (mM) upon different concentration of nanoparticles

Pathogens	**5 µl**	**10 µl**	**15 µl**	**20 µl**	**25 µl**
Escherichia Coli	7	10	12	13	14
Salmonella Enterica	8	12	13	14	18
Staphylococcus Aureus	14	16	17	19	20

CONCLUSION

Nepenthes spp. acts as a good source for the biosynthesis of silver nanoparticles. Silver nanoparticles synthesized from the *Nepenthes spp*. showed a good antibacterial activity against *Escherichia coli, Salmonella enterica*, and *Staphylococcus* Hence, because of this strong antibacterial effect possessed by silver nanoparticles, it may be helpful to counter the bacterial resistance

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