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Preparation and Characterisation of Silver Nanoparticles using *Cissus Quadrangularis* Extract and its *in vitro* Anti-Arthritic Activity

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ABSTRACT: Silver nanoparticles synthesis by green synthesis process has achieved an essential role in different fields. Silver nanoparticles synthesis using a green synthesis process has been done by the extracts of a special plant named "*Cissus quadrangularis*". The synthesis of silver nanoparticles has been done by the use of FTIR and UV-V Spectroscopy. The main objective of using a green synthesis process as it is non-toxic in nature.

Keywords: Silver nanoparticles, Nanotechnology, *Cissus quadrangularis*, Anti-arthritic activity, Physicochemical.

INTRODUCTION

Nanotechnology is a part of science that becomes popular due to its advanced potential with the help of nanomaterial in suitable shapes and sizes in different fields such as in agriculture and medicine. The advancements in the field of nanotechnology has significantly contributed in the evolution of its associating fields that consists of synthesis and applications of nanoparticles in field of medicine and pharmaceutics (Kaur *et al.,* 2021). Nanoparticle synthesis has captured worldwide attention due to its physicochemical properties. Different techniques have been used for the synthesis of nanoparticles. These chemical and physical mediated methods are expensive, dangerous and toxic to nature. This serious matter can be defeated by using economic, eco-friendly and no-toxic greener methods. Hence, the method of green synthesis has been used for nanomaterial's synthesis with the use of microorganisms and plants. Silver nanoparticle synthesis with the help of several extracts of plants such as "*Cocos nucifera*", "*Carica papaya*", "*Allium cepa*", "*Basella*" and "*Ficus carica*" has been effective. A vining plant "*Cissus quadrangularis*" can be seen in Africa and India and belongs to the "*Vitaceae family*". Silver nanoparticles synthesis of the extract of the "*Cissus quadrangularis*" stem resulted in the activity of antibacterial.

Cissus quadragularis

"*Cissus quadragularis* or *Linn*" is a type of succulent or vine creeper that cultivates to an average of 1.5 metres and has the appearance of sectioned quadrangular. It is filled with vitamin- C type and has the activity of antioxidants (Sundaran *et al.,* 2020). This plant has several biomedical characteristics and is famous for its anti-osteoporotic activity.

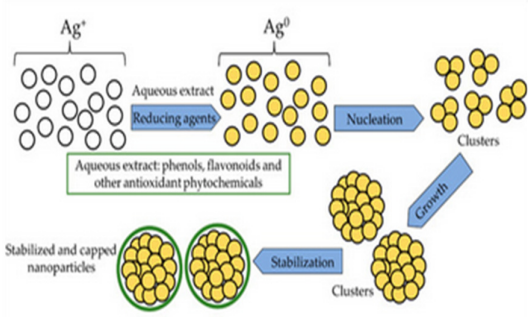

Fig. 1. Green synthesis of Silver Nanoparticles through *Cissus quadragularis* (Source: Mahendran *et al.,* 2022).

This plant is also used in many ayurvedic treatments as for bone healing, digestion, asthma and weight loss in a traditional way. The plant is also used in rehydration of oral; every part of this plant has different uses. Extract of methanolic from this plant has shown promising results in the activity of anti-cancer versus leukaemia cells (HL-60 cells) synthesizing of silver nanoparticles by different materials of biological in intracellular and extracellular methods in context to use of microbial cells (Mahendran *et al.,* 2022). Proteins and enzymes of biomolecules work as reducing agents, in this way of nanoparticle synthesis is known as "*green synthesis*". Hydroalcoholic extract enriched with polyphenol with good antioxidant activity (Verma *et al.,* 2020).

Silver nanoparticle

Silver nanoparticle is the most fascinating and important nanomaterials between different nanoparticles of metallic that are included in the applications of biomedical. It plays a major role in nanotechnology and nanoscience mainly in nanomedicine. The action of biological mechanisms of silver nanoparticles includes the extraction of silver ions, membrane structure destruction and oxygen species reactive generation. A different type of silver particle smaller silver nanoparticles and particles of silver Ångstrom conclude better activity of biological and less toxic level compared to silver nanoparticles.

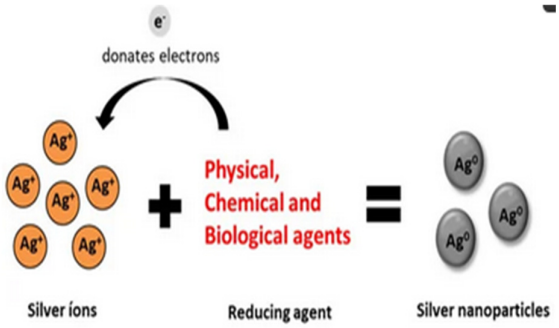

Fig. 2. Reaction of oxidation-reduction in the silver nanoparticles formation (Source: Le *et al.,* 2021).

Synthesis of silver nanoparticles includes three different methods; the methods are biological, chemical and physical synthesis methods. Silver nanoparticles are identified with antimicrobial activity and a wide spectrum; they can efficiently kill different pathogens even at low temperatures and concentrations (Le *et al.,* 2021). It has been seen that nanoparticles of silver have different activities such as anthelmintic activity and nematicidal activity. Recent studies regarding silver nanoparticles suggest that the toxicity of these particles in mammals is in vivo and in cells is in vitro that cautious about its usage.

MATERIALS AND METHODS

A. Phytochemical analysis and preparation of extract from the Cissus quadrangularis

Fresh leaves and stems of this plant were collected first then crushed and washed it. The proper extract was formed by mixing "***Cissus quadrangularis"*** 25 gm leaves and stems in deionized water in a conical flask of 250 ml. After that this proper was boiled for nearly 10 minutes until this extract diminish to 200 ml Then the extract was filtered with filter paper of Whatman. This exact solution was used in the silver nanoparticles synthesis or CqNp's. The screening of photochemical was completed for the "*Cissus quadrangularis*" aqueous extract using the method of Harborne.

B. Silver nanoparticles synthesis (CqNp's)

$AgNO_3$ solution (1mM) of aqueous was made and used in the process of biosynthesis of silver nanoparticles. A plant extract of about 35 ml was mixed into 245 ml of $AgNO_3$. The confirmation of silver nanoparticles was done by changing in the colour of the mixture solution. This colour change in the extract solution was done by the use of irradiation of microwave (Ahmad *et al.,* 2019).

In this way, the nanoparticles of silver were prepared with the help of extract from the "*Cissus quadrangularis*".

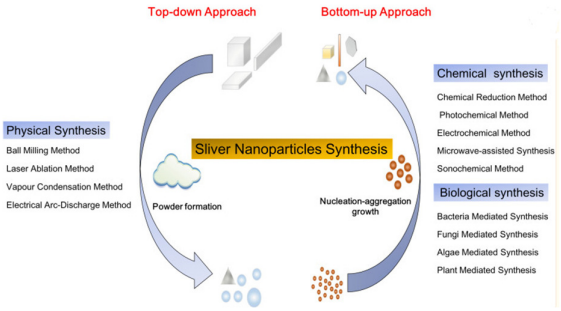

Fig. 3. Synthesis of Silver Nanoparticles (Source: Ahmad *et al.,* 2019).

C. Characterization of physiochemical of silver nanoparticles

Jasco spectrophotometer or UV-V spectrophotometer is used to find out the property of optical of the silver nanoparticles. After the change of colour in the prepared solution the UV-V spectrophotometer was executed between 200 nm to 700 nm (Pragathiswaran *et al.,* 2021). The spectrometer of FTIR was used to execute the functional group detections of the silver nanoparticle synthesis. The powdered and dried solutions were at 65 °C characterized as ranging between 4000 to 400 cm^{-1}. Scanning of field emission in microscopy of electron was used to understand the characteristics of the silver nanoparticle synthesis. "*SEM or Scanning Electron Microscopy"* was done to understand morphology of silver nanoparticles with the use of JEOL JM 5600 prepared with a voltage of 20 kV and 5nm to 6 nm wavelength. Analysis of XRD was done using Shimadzu x-ray-7000 to verify the crystalline or amorphous quality of the sample. The specific composition of elemental can be recognised and this specific system is simply connected and worked as a complete matter with instruments of electron microscopic.

D. In-vitro Anti-arthritic activity

In the systemization of silver nanoparticles the study of in-vitro was done by the activity of Anti-arthritic (Thirumalai *et al.,* 2020). This particular study was done by two methods, they are the method of albumin denaturation and the method of BSA denaturation.

E. The method of albumin denaturation

Saline of phosphate buffers (PBS, Ph 6.4) and albumin of the egg was mixed into 1 millilitre of different samples concentration within 100 µg to 500 µg without blank. Then it was heated and incubated at a temperature 70°C (Usoltsev *et al.,* 2019). After that, the solution absorption was determined at 660 nm and lastly, the denaturation of the protein of the study sample was premeditated.

F. The method of BSA denaturation

Different sample concentrations from 100 µg to 500 µg BSA (Bovine Serum Albumin) at 0.5 %. And the buffer of phosphate was determined in the test tube and nurtured in an incubator for at least 20 minutes at a temperature of 37 °C (Revathi *et al.,* 2019) and lastly, the absorption was determined at 255nm.

RESULTS

From the above points it has been found that the production of silver nanoparticles from the extract of "*Cissus quadrangularis*" is environment friendly. The process is known as the green synthesis that is also well for the environment. There are many methods of production of silver nanoparticle, yet the formation of silver nanoparticles with the help of green synthesis technique is more acceptable. It has been found that the processes of green synthesis are needed to avoid harmful and unwanted production of products. In this green synthesis technique of production of silver nanoparticles extract of "*Cissus quadrangularis*" is widely and broadly used. It has been profound that green synthesis technique is safer than any other techniques.

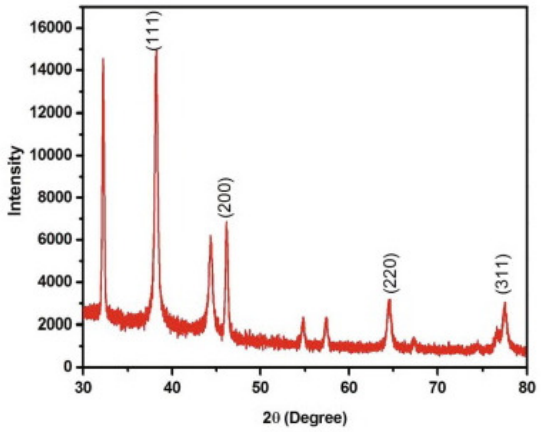

Fig. 4. XRD pattern of silver nanaoparticle from the extract of *Cissus quadrangularis* (Source: Mahendran *et al.,* 2022).

Different mechanics such as XRD, UV-spectrophotometer, and x-rays have been used (Mahendran *et al.,* 2022). The solution of $AgNO_3$ is also used in order to prepare the extraction of the process. There are proper measurements have to follow up to get better results of production of this silver nanoparticle.

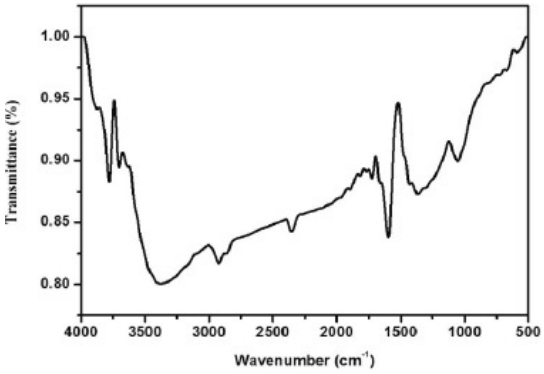

Fig. 5. FTIR spectrum of silver nanoparticle from *Cissus quadrangularis* extract (Source: Pragathiswaran *et al.,* 2021).

In-vitro anti-arthritic activity, ***BSA denaturation methods, albumin denaturation*** methods and FTIR spectrum of silver nanoparticle obtained from the plant extract have been used here (Pragathiswaran *et al.,* 2021). A proper temperature maintains helps to achieve the final product. Significant phytochemicals that are obtained from this plant help character reorganization of silver nanoparticles.

DISCUSSION

The technique of green synthesis is used to prepare the synthesis of nanoparticles through the extraction of the vining plant "*Cissus quadrangularis"*. There are various methods for synthesizing of silver nanoparticles and they are biological methods, physical methods and chemical methods. Other than these all methods the green synthesis technique is used due to its environment-friendly nature. The foremost mentioned methods are all toxic in nature and not environment-friendly this is the reason to use this green synthesis technique. The synthesis of silver nanoparticles can be understood by the changing of colour in the experiment in reducing agent from white colour to brown colour (Chirumamilla *et al.,* 2022). The specific phytochemicals that are present in this plant are recognized for their various uses in food science and pharmacology. The inclusion of phytochemicals resulted in anti arthritic activity which characteristics of silver nanoparticles. Spectroscopic of UV-visible analyzation in this study helps to better understand the phytoconstituents and analytes. Results obtained analyse the synthesis of silver nanoparticles from the extract of "*Cissus quadrangularis"* and verify the crystalline characteristics of nanoparticles of silver. The sizes of the crystallite of the nanoparticles of silver are measured by the formula of Debye-Scherrer.

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

In this formula λ presents the x-ray of wavelength, β presents the peak of diffraction of "*Full Width at Half Maximum (FWHM)"* and lastly, θ presents the reflection peak of Braggs (El-Kader *et al*., 2019). Images of nanoparticles of silver by scanning electron microscopy resulted in the shape being spherical of nanoparticles of polydispersed adjoining agglomeration. Nanoparticles' agglomeration happened due to the occurrence of extracts of the "*Cissus quadrangularis"* plant.

CONCLUSION

From the above points it can be concluded that in modern technology the use of nanoparticles of silver is several. In order to safely synthesis of silver nanoparticles green synthesis technology used is crucial, that do not harm the environment. Different methods are used in the silver nanoparticles synthesis and invitro anti arthritic activity method of albumin denaturation and the method of BSA denaturation. Different spectrophotometers such as UV spectrophotometers and FTIR spectrophotometers are also used to analyze the silver nanoparticles synthesis. The formula of Debye-Scherrer is also used in this study to measure the mean size of the crystallite of silver nanoparticles. It has been seen that the mean of the crystallite of silver nanoparticles was about 24nm. Extract of aqueous of "*Cissus quadrangularis"* plant

used as reducing and stabilizing agents in this study for the silver nanoparticle synthesis. The silver nanoparticles have an important specialization in the activity of anti-arthritic. The final outcome that is captured from this study will help in the way to increase the activity of silver nanoparticles obtained by the green synthesis process from the extract of "*Cissus quadrangularis"*.

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