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DESIGN, SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICALS (ANDROGRAPHIS ECHIOIDES)

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ABSTRACT

An environmentally friendly method of producing silver nanoparticles (AgNPs) with the ethanolic extract of Andrographis echioides. During manufacture, silver nanoparticles were found using UV and other analytical techniques. The produced AgNPs were evaluated for their in-vitro anti-inflammatory and anti-oxidant properties utilizing a protein denaturation assay with diclofenac sodium as standard and ascorbic acid (vitamin C) as standard in DPPH. Maximum absorption at 400–45nm indicates AgNP generation, and biosynthesis validates the color shift from colorless to reddish brown. When compared to standard under identical conditions, AgNPs have notable anti-inflammatory and antioxidant properties. These in vitro studies demonstrate the need for more studies on phytochemicals in order to increase their biological activity.

KEYWORDS: Andrographis echioides, antioxidant, DPPH assay, anti-inflammatory, silver nanoparticle AgNps, UV.

INTRODUCTION

Andrographis echioides is an Acanthaceae family plant; it is used for many medicinal purposes in South Asia, like India and China. This medicinal plant was extracted by different solvents, and its medicinal properties were identified by various techniques. Based on the literature, this plant possesses pharmacological properties like antimicrobial activity, anti-inflammatory, diuretic, anthelmintic, analgesic, antipyretic, hepatoprotective activities, and antioxidant effect. It contains plenty of phytochemical constituents such as glycosides,

flavonoids, flavones, steroids, tannins, carbohydrate, glycosides, and alkaloids (Mathivanan, D., and Suseem, S.R., 2015). Andrographis paniculata (Burm. f.) Wall. ex Nees, a renowned herb medicine in China, is broadly utilized in traditional Chinese medicine (TCM) for the treatment of cold and fever, sore throat, sore tongue, and snake bite with its excellent functions of clearing heat and toxin, cooling blood, and detumescence from times immemorial. Modern pharmacological research corroborates that andrographolide, the major ingredient in this traditional herb, is the fundamental material basis for its efficacy. As the main component of Andrographis paniculata (Burm. f.) Wall. ex Nees, andrographolide reveals numerous therapeutic actions, such as antiinflammatory, antioxidant, anticancer, antimicrobial, antihyperglycemic, and so on. (Zeng, B., et al., 2022). A. paniculata, known on the Indian subcontinent as Chirayetah and Kalmegh in Urdu and Hindi languages, respectively, is an annual plant, 1-3 ft high, that is one of the most commonly used plants in the traditional systems of Unani and Ayurvedic medicines. It is called Creat in English and is known as the “king of bitters. Akbar, S. (2011). Padma et al. (2012) evaluated the anthelmintic activity of ethyl acetate, methanol, and an aqueous extract of the whole plant of Andrographis echioides against Pheretima posthuma. The results revealed that the test extracts of A. echioides exhibited significant anthelmintic activity at a concentration of 50 mg/mL. The use of A. echioides as an anthelmintic has been confirmed, and further studies are suggested to isolate the active principles responsible for the promising activity. In this study, the methanolic extract of A. echioides was investigated for its hepatoprotective and antioxidant effects against acetaminophen-induced hepatotoxicity in Wistar albino rats as studied by Basu et al. (2009). Petroleum ether, chloroform, acetone, and methanol extracts of A. echioides leaves and stems were screened for their preliminary phytochemical analysis. The antimicrobial activity of the crude extracts was evaluated by Sermakkani et al. (2011) against Candida albicans. Diuretic activity of petroleum ether and chloroform extract of Andrographis echioides leaves was studied by Raama Murthy et al. (2012). The chloroform extract of A. echioides exhibited significant diuretic activity, as evidenced by increased total urine volume and the urine concentration of Na+, K+, and Cl-. The result of the work indicates that the plant can be used for future work and is effective against free radicalmediated disease. Anti-ulcer activity of an ethanol extract of Andrographis echioides was evaluated by Ramasubramania Raja et al. (2014). The extracts have shown potential anti-ulcer activity in all the tested models. Sankaran Rajkumar et al. (2012) studied the synergistic Leaves of Andrographis echioides are subjected to Soxhlet extraction using ethanol as solvent. The plant alcoholic extract was examined against 4th instar larvae of A. aegypti with

gradually increasing concentrations from 50 to 250 mg/L using WHO protocol. From the results, it can be concluded that the synergistic effect of A. echioides is a more powerful arsenal for control of A. aegypti. Raama Murthy et al. (2012) reported the presence of glycosides, flavonoids, flavones, steroids, tannins, carbohydrate, glycosides, alkaloids, proteins, amino acids, and saponins in petroleum ether and chloroform extract. Sermakkani et al. (2011) reported that petroleum ether, chloroform, acetone, and methanol extracts contain alkaloids, flavonoids, glycosides, steroids, phenols, tannins, and saponins. Based on the above facts, the present work investigates the biosynthesis of silver nanoparticles from the extract of Andrographis echioides by ecofriendly method, explores in vitro antioxidant activity by DPPH assay, and examines the anti-inflammatory activity by protein denaturation. Novel medicinal plants are enriched with potential bioactive molecules and exhibit broad-spectrum pharmacological activity.

Fig 1: Andrographis Echioides Plant and Flower.

MATERIAL AND METHODS

COLLECTION OF SAMPLES

Andrographis echioides leaves were collected around the KGCOP and RI Medicinal Garden, Tamilnadu for this investigation.

CHEMICALS AND REAGENTS

Silver nitrate (AgNO3), methanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid was purchased from Sigma Aldrich. All other reagents and chemicals used in this research were of analytical grade

METHOD OF PREPARATION OF SAMPLES

Leaves were grinned using of mortar and pestle. Grinned aerial part were stored at 24 hrs and concentrated the extract. Bio Reduction of AgNO3 by chemical constituents of extract was observed by color change from colorless to reddish brown in color.

PLANT EXTRACT PREPARATION

A 500 ml conical flask containing 100 ml of distilled water was filled with 10 g of dried plant leaf power. The mixture mentioned above was heated to 60ºC for 20 minutes. Whatman No. 1 filter paper was used to filter the boiling plant extract. Nanoparticle production was carried out using the filtrate.

SILVER NANOPARTICLE SYNTHESIS

90ml of a 1mM silver nitrate solution was combined with 10ml of filtered plant extract. For almost twenty-four hours, it was maintained at 100–120 degrees Celsius in a dark environment with magnetic stirring. Changes in hue were noticed. There is a greater chance that the silver ions will combine with the secondary metabolites included in the plant extract. The development of silver nanoparticles was shown by the yellow solution turning dark brown.

EVALUATION OF SILVER NANOPARTICLE PROPERTIES

The green produced silver nanoparticle was measured between 190 and 1100 nm using a UV visible spectrophotometer at Lambda 35. Using the KBr pellet technique and Shimadzu 8400 FTIR Spectroscopy (Perkin Elmer Spectrum), the presence of functional groups in the produced silver nanoparticle from A. echioides leaf extract was detected at a range of 4000–400 cm. FESEM was used to analyze the −1 morphology of SNP, and EDX verified the presence of silver.

Using the Particle Size Analyzer technique, the average size of the leaf extract was examined. Synthesized nanoparticles' antimicrobial activity was screened.

XRD EXAMINATION

The XRD technique was used to determine the AgNPs structure. Dried silver nanoparticles were applied to an XRD grid (XPERT-PRO) for X-ray diffraction (XRD) investigations, and diffraction was measured in the 2θ range between 0˚ and 80˚. The operating voltage and current for the spectral data were 40 kV and 40 mA, respectively.

FTIR

Using the KBr pellet technique and Fourier Transform Infra-Red (FTIR) spectroscopy (Nicolet is5, Thermo Scientific), the physiologically active substances that cause silver reduction, formation, and capping were found. At a resolution of 4 cm, an FTIR spectrum reading was performed in the 500–4000 cm-1 region.

VISIBLE UV SPECTROSCOPY

Visual observation of the color shift from watery to brown served as preliminary confirmation of the nanoparticle production.

UV-visible spectroscopy further verified the decrease of Ag ions in the solution combination. The produced AgNPs' spectral data were captured at a resolution of 1 nm between 300 and 700 nm**.**

Pharmacological Activities of Andrographis echioides

Andrographis echioides, a member of the Acanthaceae family, is traditionally used in Ayurvedic and Siddha medicine. Various pharmacological studies have highlighted its medicinal properties. Below are some of its key pharmacological activities:

1. Antimicrobial Activity

Several studies suggest that A. echioides exhibits antibacterial and antifungal properties. It is effective against pathogens such as Escherichia coli, Staphylococcus aureus, and Candida albicans, likely due to the presence of flavonoids, alkaloids, and terpenoids.

2. Anti-inflammatory Activity

The plant is known for its anti-inflammatory effects, which are attributed to its bioactive compounds that modulate inflammatory mediators. It may be useful in managing conditions like arthritis and inflammatory bowel diseases.

3. Antioxidant Activity

Extracts of A. echioides have been shown to possess strong antioxidant properties. The presence of phenolic and flavonoid compounds helps neutralise free radicals, potentially preventing oxidative stress-related disorders such as neurodegenerative diseases and cardiovascular conditions.

4. Hepatoprotective Activity

Preliminary studies indicate that A. echioides may have hepatoprotective effects, possibly reducing liver toxicity caused by drugs or environmental toxins. The plant's antioxidant components contribute to liver protection.

5. Anti-diabetic Activity

Some research suggests that A. echioides may help regulate blood glucose levels by enhancing insulin sensitivity or inhibiting carbohydrate digestion. This makes it a potential herbal remedy for diabetes management.

6. Anti-cancer Activity

The bioactive compounds in A. echioides have been reported to exhibit cytotoxic effects against certain cancer cell lines. Further studies are needed to validate its potential as an anti-cancer agent.

7. Wound Healing Properties

Traditional medicine uses A. echioides for wound healing due to its antimicrobial and antiinflammatory effects, promoting faster tissue regeneration.

PHARMACOLOGICAL EVALUATION

PREPARATION OF SILVER NANOPARTICLE

The fresh Andrographis echioides (AEET)extract solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flask along with 100 mL of sterilized double distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered through Whatman filter paper no 1 and stored at -15 °C and could be used within 1 week. The filtrate was treated with aqueous 1 mM AgNO3 solution in an Erlenmeyer flask and incubated at room temperature. As a result, a brown-yellow solution was formed, indicating the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of plant parts to generate extremely stable silver nanoparticles in water. After read the absorbance with different nanometer 400 – 600nm.

In vitro DTET AgNps Antioxidant activity

Andrographis echioides (AEET AgNps) aqueous extract investigated for in vitro antioxidant activity by DPPH, ABTS, FRAP and NO for the estimation of anti-oxidant potential of

Andrographis echioides (AEET AgNps) aqueous extract. Andrographis echioides (AEET AgNps) me also tested by DPPH assay.

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

Antioxidant activity in the sample Andrographis echioides were estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (BrandWilliams et al., 1995). 100µL of SC extract was taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. Reading the plate on Elisa plate reader at 490nm. Standard ascorbic acid was used as reference. All the analysis was performed in triplicates and the average values were taken.

RADICAL SCAVENGING ACTIVITY WAS CALCULATED BY THE FOLLOWING EQUATION

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100.

IN-VITRO AEET AGNPS ANTI-INFLAMMATORY ACTIVITY - INHIBITION OF ALBUMIN DENATURATION

The reaction mixture was prepared separately by mixing 0.5ml aqueous extract of AEET AgNps and its compounds A, B, and C (1mg/ml) with 0.45 ml aqueous solution of bovine albumin fraction (5%). The pH (6.3) of the solution was adjusted using a small amount of 0.1N HCl at 37 ˚C for 20 min, then heat to 57˚C for 30 min. Cool the solution and transfer it to the 96 well plates and measure the absorbance at 660nm. Standard was used as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml distilled water. The percentage of inhibition of albumin denaturation was calculated by the following formula, Percentage of inhibition (%) = [(A control – A sample) / A control] x 100 Where A control – Absorbance of reaction mixture except drug. A sample – absorbance of the reaction mixture with the Sample.

RESULT AND DISCUSSION

Ethanolic extract of Andrographis echioides has shown anti-inflammatory, antioxidant properties, and biosynthesis of silver nanoparticles. Andrographis echioides synthesized AgNPs were initially confirmed by UV-visible spectrophotometer absorbance (SPR band) at

456 nm (0.563 OD) (Table 1) (Fig 1). The SEM image (morphology) of the AgNPs shows spherical in shape. AE ET AgNPs size range from 20 to 100 nm (Figs. 2 and 3).

Andrographis echioides AET extract has shown in vitro antioxidant activity by DPPH assay in the current investigation. At AEET AgNps, the inhibition percentages are 57.1% (Table 2), compared to the standard ascorbic acid. As a result, even a low concentration of antioxidant activity is good compared to the standard ascorbic acid vitamin C. A previous paper reported that the methanol extract exhibited the highest DPPH scavenging activity and the best antioxidant activity with an EC50 value of 51.98 mg/mL. Among the different extracts, methanol was more effective in all the antioxidant assays, i.e., the DPPH radical scavenging assay and the superoxide scavenging assay (Murugan, A., et al. 2017). The inhibition of the albumin denaturation method was used to measure the anti-inflammatory activity in vitro. In comparison to standard diclofenac sodium, neither the crude extract.

DTET nor its separated constituents exhibit any appreciable anti-inflammatory efficacy. When compared to standard diclofenac sodium, the aqueous extracts of AEET exhibit moderate anti-inflammatory efficacy. AEET had a 41% (Table 3). While we were carrying out different concentrations in a dose-dependent way and comparing them with the standard, many of them showed the antioxidant and anti-inflammatory properties of AEET. A previous paper reported that Basu et al. (2009) evaluated the anti-inflammatory, analgesic, and antipyretic activity of ether, chloroform, and ethyl acetate extracts of Andrographis echioides in rats and mice. The results suggest that different extracts of A. echioide produce anti-inflammatory effects that could be due to the effect of one or a combination of the bioactive components in each extract. Overall investigation results, such as AEET AgNps, had good antioxidant and moderate anti-inflammatory activity. The silver nanoparticle was prepared from an ethanolic extract of ANDROGRAPHIS ECHIOIDES, and it was verified by UV analysis and color observation. The biosynthesis of AE ET AgNPs was characterized by FTIR and SEM analysis. The ethanolic extract AE ET prepared silver nanoparticle had good anti-oxidant activity by DPPH assay and good anti-inflammatory activity.

Table 1: FTIR Analysis.

S. NO	Nm	Maximum absorbance(n=3)
1	430	0.25
2	500	0.27
3	520	0.26
4	620	0.23

5	680	0.20
		Max 400-450 nm.

Table 2: Invitro antioxidant activity.

S. NO	COD	SOD	%Inhibition	Average
1	1	0.45	55	
2	1	0.32	68	57.1%
3	1	0.52	48	
Standard vitamin C Ascorbic acid				
1	0.34	0.04	88.24	
2	0.34	0.04	88.24	87.64%
3	0.34	0.05	85.29	

Table 3: Invitro Anti-inflammatory activity by protein Denaturation.

AGET AgNPs				
S.NO	COD	SOD	%Inhibition	Average
1	0.61	0.23	62	
2	0.72	0.44	38.8	41%
3	0.67	0.52	22.3	
Standard Diclofenac				
1	0.36	0.04	88.89	
2	0.36	0.04	88.89	87.96%
3	0.36	0.05	86.11	

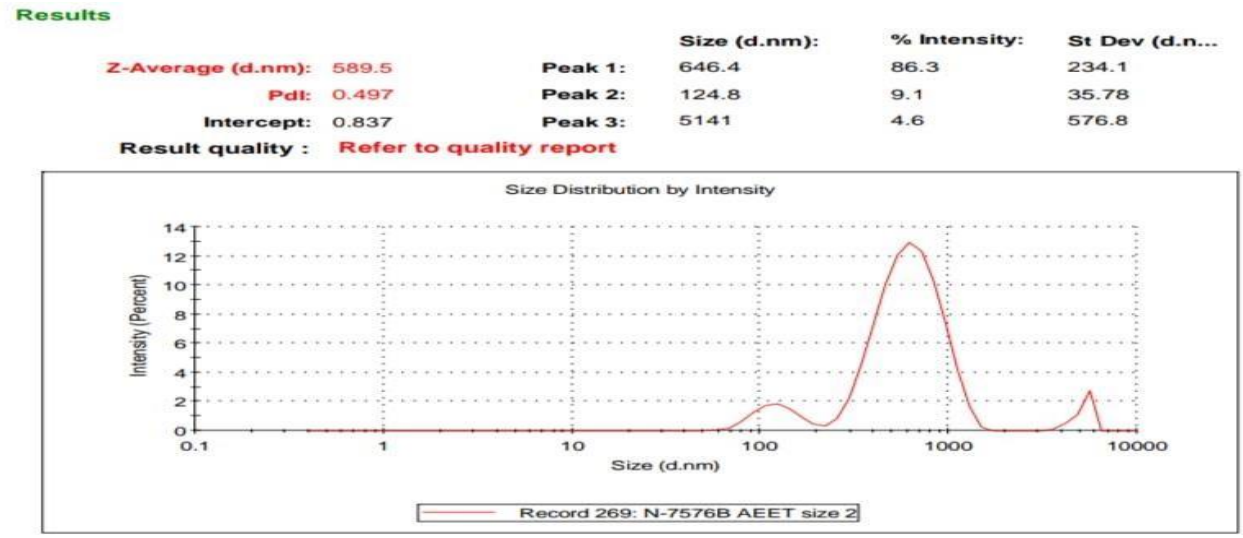

Fig 1: Size Distribution Report,

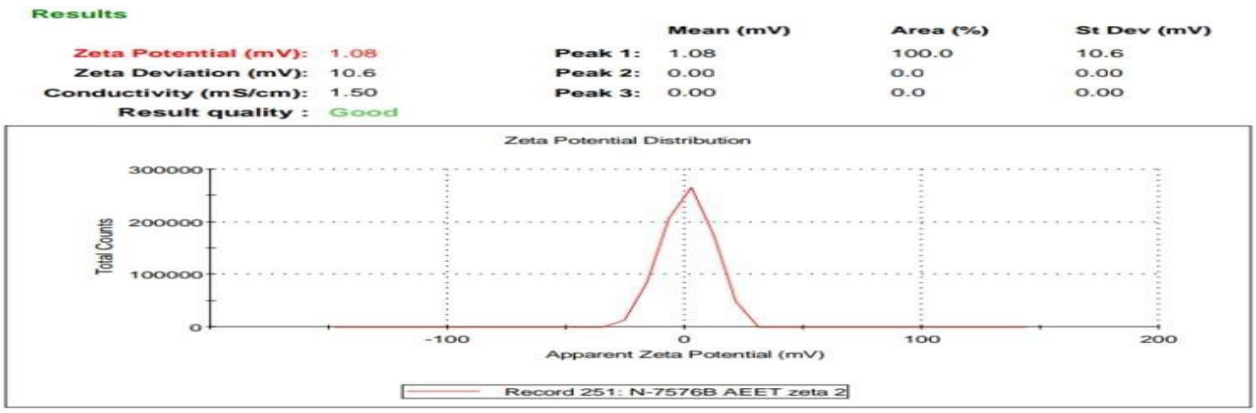

Fig 2: Zeta Potential report.

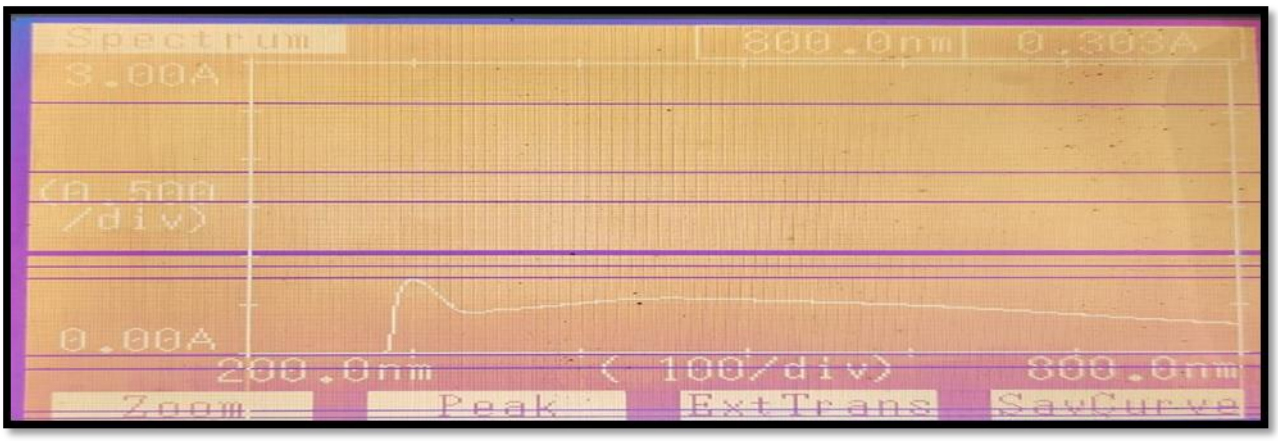

Fig 3: UV Analysis.

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