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BIOSYNTHESIS OF SILVER NANO PARTICLES FROM *LEUCAS ASPERA* (WILLD.) LINK AND ITS ANTI-INFLAMMATORY POTENTIAL AGAINST CARRAGEEN INDUCED PAW EDEMA IN RATS

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ABSTRACT: Synthesis of metal nanoparticles has become an important branch of nanotechnology and there is an increasing commercial demand for nanoparticles due to their wide applications. The present study was carried to synthesize silver nanoparticles (AgNPs) using the ethanolic extract of the *Leucas aspera* and evaluate its *in-vitro* anti inflammatory activity. The synthesis was done by using the ethanolic solution of *L. aspra* extract and AgNO3. The AgNPs were characterized using UV-Spectroscopic analysis, Scanning Electron Microscopy (SEM), Fourier Transform Infra Red Spectroscopy (FTIR) analysis. UV-Vis spectral analysis shows a maximum absorption peak at 421.00 nm. The FTIR spectra of AgNPs exhibited prominent peaks at 3697cm-1,1761cm-1,1390cm-1, 831cm-1 which are associated with OH stretching, C=C stretching, CH stretching respectively. The SEM analysis of the synthesized AgNPs clearly showed the clustered and irregular shapes, mostly aggregated and having the size of 25-80 nm. The synthesized AgNPs exhibited an enhanced anti inflammatory activity due to the synergistic effect of phytoconstituents in *L. aspera* extract and the silver ions.

INDRODUCTION: Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated based on their use in traditional medicine. Medicinal plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is known as secondary metabolites. Some of these secondary metabolites are produced for self-defense. Over the last 20 years, a large number of secondary metabolites from different plant were used in different country. The medicinally active plant compounds are usually their secondary metabolites like terpenoids, quinones, flavonoids, tannins etc.

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In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. One of the plants is *Leucas aspera* that is well known medicinal herb belonging to the family Lamiaceae (Labiatae). It is commonly known as 'Thumbai' and distributed throughout India. At some places it is also known as 'Dhronpushpi'.

It is used as antipyretic, stimulant, expectorant, aperient, diaphoretic, antirheumatic and insecticidal. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites [1, 2]. The *L. aspera* has the property to counteract the effect of nitric oxide formation due to the presence of tannins and flavonoids, considerable interest in preventing the ill effects of excessive nitric oxide generation. The possibility of *L. aspera* extract for having anti-inflammatory response could not have been overruled.

In nanotechnology, a nanoparticle is defined as a material with dimensions and tolerance limits of 0.1-100 nm that behaves as a whole unit in terms of its transport, properties and unique characteristics [2]. Among the various nanoparticles available, silver nanoparticles are gaining more importance due to their diversified biological properties and potential applications. Silver has been used since ancient times for the treatment of wounds and inflammation and nanoparticles of silver have been developed which have potent anti-inflammatory [3]. Silver nanoparticles can be synthesized by various physical and chemical methods, however they are costly, cumbersome and toxic to the environment.

The use of biological systems as potential nanofactories have been widely explored as they are economical and eco friendly. Plant extracts contain phyto-chemicals which aid in the reduction of the silver ions [4]. The added advantage of using plants is phytoconstituents which act as capping agents, thereby conferring the silver nanoparticles with additional pharmacological properties [5]. Hence, by keeping the above concept in mind, the present study was undertaken to synthesize silver particles using the ethanolic extract of the *L. aspera* to evaluate the silver nanoparticles for anti-inflammatory activity using standard *in-vitro* methods. Thus by synthesizing silver nanoparticles using *L. aspera* extract, the potential advantage of phytomedicine and nanomedicine can be combined to result in more enhanced and synergistic anti-inflammatory effect.

METERIALS AND METHODS:

Collection and authentication of experimental plant: Fresh, mature, *L. aspera* were collected from Kandhari, Thiruvarur District, Tamil Nadu. The plants were identified and authenticated by a taxonomist and an exemplar specimen was deposited at the Department of Botany, St. Joseph's College, Tiruchirappalli, India According to Mukerjee [6], the herbarium number of the plant is RVR008.

Preparation of extraction: The coarse powder plant material was extracted with ethanol by using soxhlet apparatus. The solvent were removed under reduced pressure to get crude extract. Standard methods were used for preliminary phytochemical screening of the extract, which was performed to know the phytoconstituents in the extract [7].

Preparation of 1mM silver nitrate aqueous solution: An accurately weighed 0.017g of silver nitrate was dissolved in 100 mL of double distilled water and stored in amber colour bottle for further use.

Synthesis of ethanolic leaf extraction of *L. aspera* silver nano particles: The synthesis of silver nanoparticle was performed by Rajasekar *et al.*, 2013 [8]. 5 mL of the ethanolic leaf extract of *L. aspera* was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50 mL of 1 mM $AgNO_3$ solution was added drop wise with constant stirring of 120 rpm at 50-60 ºC. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 5 hours was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the ethanolic extract of *L. aspera* to generate extremely stable silver nanoparticles.

Characterization techniques:

UV-VIS Spectroscopy: The silver nanoparticles were characterized in a Shimadzu-1800 UV-VIS Spectrophotometer. The optical properties (absorbance) of the sample were evaluated at the wavelength range of 300-600 nm. The double distilled water used as a blank reference.

Scanning Electron Microscope (SEM): Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the scanning electron microscopic grid were allowed to dry by putting it under a mercury lamp for 5 min.

Fourier Transform Infra-Red Spectroscopy (FTIR): To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, after complete reduction, silver nanoparticles were concentrated by repeated centrifugation (3 times) of the reaction mixture at 15,000 rpm for 20 min. The supernatant was replaced by distilled water each time.

Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by ALPHA FT-IR Spectrometer (from Bruker, Germany) for the detection of different functional groups by showing peaks from the region of 4000 cm^{-1} to 500 cm^{-1}.

Animals: Wistar rats 7-8 week old, weighing 150-200 grams were used for the present study. To maintain the animal house under the standard condition of temperature (24 ± 2 °C) and relative humidity (30-70) with a 12:12 light: dark cycle. The animals were fed with stranded pellet diet and water. The animal handling was performed according to good laboratory practice (GLP). Ethical clearance was obtained from institutional animal ethical committee (CPCSEA/265/2015) and conducted according to Indian national science academy guidelines for the use and care of experiments.

The Animals were divided into six groups of six animals each:
Group 1: Normal Control
Group 2: Negative Control (Carrageen 1% w/v)
Group 3: Positive Control (Indomethacin 10 mg/kg)
Group 4: Ethanolic Extract of *L. aspera* (200 mg/kg)
Group 5: Ethanolic Extract *L. aspera* (400 mg/kg)
Group 6: AgNPs in *L. aspera* (100mg/kg)

Acute toxicity study: The acute toxicity study was carried out as per the guidelines set by OECD-423 [9], received from Committee for the purpose of control and supervision of experiments on animals, Ministry of social justice and empowerment, Government of India. The ethanolic extract and Synthesis of AgNPs in *L. aspera* was orally administered to adult Wistar albino rats. The groups were continuously observed for mortality and behavioral changes during the first 24 hours and then daily for a fortnight.

Carrageen induced inflammation in wistar rats: Paw edema was induced in male Wistar rats (110–130 grams, 3 months old) by injection of 100 μL carrageenan 1% (λ-carrageenan, type IV) in the right hind foot pad [10]. The left hind foot pad was injected with the same volume of saline solution. The extract and colloidal suspension were orally administered daily, 4 days prior to injection of carrageenan, in a volume not exceeding 0.5 mL.

Evaluation of anti-inflammatory activity: Ethanolic and AgNPs of *L. aspera* was tested for anti-inflammatory activity against carrageenan induced paw-edema in rats. Both the extracts having anti-inflammatory activity against the carragenan induce paw edema in rats. The reductions of paw edema of rats are compared with the standard drug *i.e*. indomethacin.

RESULT AND DISCUSSION: The Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [11]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [12]. This study reveals that ethanolic extract of *L. aspera* exhibited the presence of alkaloids, terpenoids, flavonoids, phenol, tannins, phytoserol, carbohydrates and saponins. In phytochemical analysis the major compounds of alkaloids, phenols, tannins and etc., are rich in medicine and constitute most of the valuable drugs. They have physiological effect on animals [13].

The use of nano-herbal-technology to synthesize compounds with improved anti inflammatory properties is an area of current research by many scientists. In our study, we report the non toxic, practical and environmentally benevolent approach for the synthesis of silver nanoparticles using the ethanolic extract of *L. aspera* plant with potent anti inflammatory activity. The productions of cytokines are key events in the regulation of an inflammatory response and recent attention has been focused on the effect of the synthesized nanoparticles as selective cytokine inhibitory agents [14]. The development of green processes for the production of nanoparticles is evolving into a significant branch of nanotechnology [15].

Nanotechnology is expected to be the basis of many technological innovations in the 21st century. The synthesis of nanoparticles is a promising research field due to the possible applications for the extension of novel technologies [16].

Biological methods of nanoparticles synthesis using plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications in the field of medicine and agriculture [17].

The Biosynthesis of silver nanoparticles through plant extracts were carried out. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface Plasmon vibration in silver nanoparticles. The appearances of yellowish- brown colour in the reaction vessels suggest the formation of silver nanoparticles (**Fig. 1**).

FIG. 1: VISIBLE OBSERVATION OF AGNPS FROM *L. ASPERA*

The UV absorption peak of silver nanoparticles range from 400 nm - 450 nm [18]. **Fig. 2** shows the UV absorption peaks of *L. aspera.* UV-Vis spectra shows the peaks approximately at 421.00nm, clearly indicating the formation of spherical AgNPs in the plants extracts. The occurrence of the peak at 421 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface plasmon present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field [19].

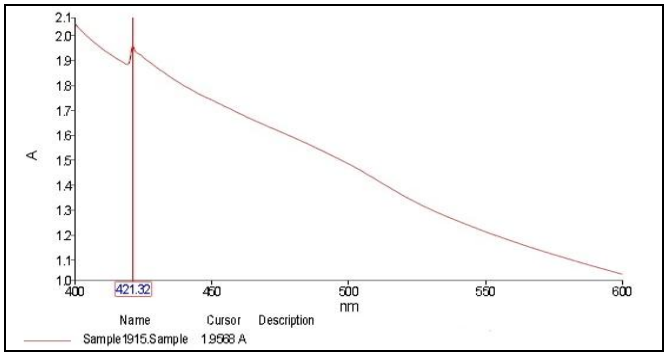

FIG. 2: UV-VIS SPECTROSCOPY OF AgNPs SYNTHESIZED *L. ASPERA*

The silver nanoparticles are cubical, rectangular, triangular and spherical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilizes the nanoparticles [20]. The measured sizes of the agglomerated nanoparticles were in the range 287.5 - 293.2 nm. However, the average size of an individual particle is estimated to be 70 nm. In the present study SEM analysis provides the morphology and size details of the nanoparticles. The high density AgNPs synthesized by the plant extract of *L. aspera* confirms the presence of AgNPs of size ranging from 20-35nm (**Fig. 3**). Particle size, size distribution and shape of silver nanoparticles are the important parameters that govern the properties and hence it has wide applications in medicinal fields.

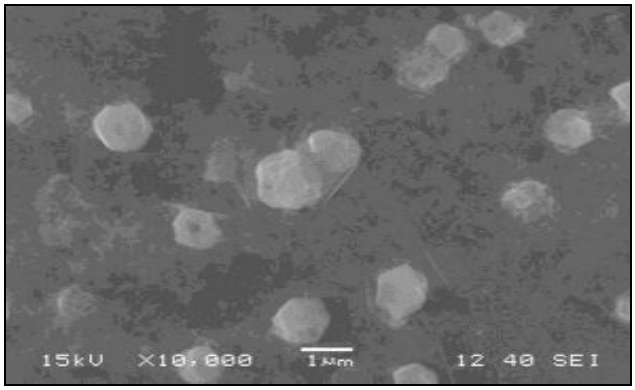

FIG. 3: SCANNING ELECTRON MICROSCOPE ANALYSIS OF AGNPS IN *L. ASPERA*

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules [21]. The inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing,

stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the plant extracts. **Fig. 4** shows the FTIR spectrum of *L. aspera* mediaited synthesized AgNPs, the silver nitrate salt and dried *L. aspera* extract, in $AgNO_3$ peaks were observed at 3697cm^{-1}, 1761cm^{-1}, 1390cm^{-1}, 831cm^{-1} which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. The Biological synthesis of AgNPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects [22]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogen. Silver sulfadazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids. The silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model [23]. In carrageenan induced paw edema in rats, oral administration of ethanolic extracts of *L. aspera* showed inhibition of paw edema at 3 hours after carrageenan injection and was compared with standard Indomethacin (**Table 1**).

TABLE 1: EVALUATION OF ANTI-INFLAMMATORY EFFECT OF *L. ASPERA* ETHANOLIC EXTRACT ON CARRAGEEN INDUCED PAW EDEMA IN CONTROL AND EXPERIMENTAL ANIMALS IN EACH GROUP (N=6)

Groups	Treatment	Mean paw edema	% edema
1	Control	-	-
2	Carrageenan alone	0.63±0.08	-
3	Carrageenan +*L. apera* extract 200mg/kg	0.45±0.02	53.3±1.61*
4	Carrageenan +*L. apera* extract 400mg/kg	0.42±0.02	49.05±5.74**
5	AgNPs in *L. aspera* (100mg/kg)	0.39±0.18*	47.05±5.74**
6	Indomethacin (5 mg/kg)	0.37±0.05**	44.12±2.11**

Values are expressed mean ± SD for 6 animals in each group.
Values not sharing a common superscript significantly differ at P < 0.05.

The orally administered ethanolic extracts of *L. aspera* (100 and 200 mg/kg) and silver nanoparticles of *L. aspera* (100 mg/kg) showed significant inhibition. The anti-inflammatory effect induced by Indomethacin progressively increased and reached a maximum of 44.12% after 3hrs (p<0.001). Silver nanoparticles of *L. aspera* (100 mg/kg), showed better inhibition of edema than dose at 100 mg/kg. Sub-plantar injection of carrageenan in rats showed a time dependent increase in paw thickness which was observed at 1 hour and was maximal at 3 hours in the control group (**Fig. 5**).

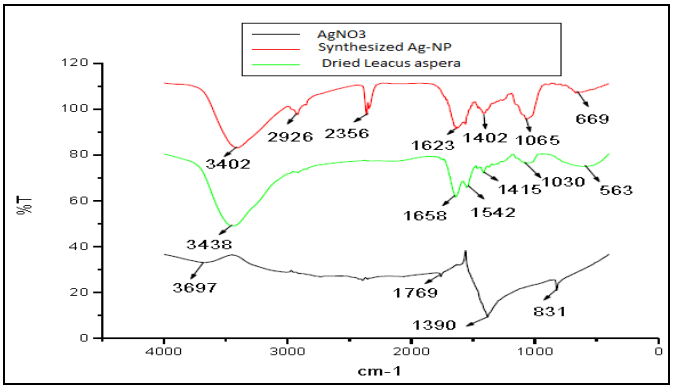

FIG. 4: FTIR ANALYSIS OF AGNPS SYNTHESIZED *L. ASPERA*

A) Control paw edema, B) Carrageenan induced paw edema, C) 3 hours Treated with AgNPs of *L. aspera*

FIG. 5: ANTI-INFLAMMATORY ACTIVITY OF CARRAGEENAN INDUCED PAW EDEMA IN RATS CONTROL AND EXPERIMENTAL ANIMALS

CONCLUSION: The medicinal plant could be used as an excellent and resourceful green material for the rapid and consistent synthesis of silver nanoparticles. Biological synthesis of nanoparticles has upsurge in the field of nano biotechnology to create novel materials that are ecofriendly, cost effective and stable. These nanoparticles have a great importance in the areas of medicine and agriculture. In the presence study carrageenan induced paw edema in rats, oral administration of

ethanolic extracts of *L. aspera* showed inhibition of paw edema at 3 hours after carrageenan injection and was compared with standard drug Indomethacin. Silver nanoparticle synthesized *L. aspera* extract showed excellent anti-inflammatory activity than crude extract. The findings could be targeted for the promising potential applications including drug formulation and biomedical applications in future.

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CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest.

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