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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITIES OF SILVER NANOPARTICLES SYNTHESIZED FROM *CISSUS QUADRANGULARIS* PLANT EXTRACT.

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Abstract

Cissus quadrangularis is one among the most frequently used medicinal plants in India which can be found throughout the country. The present investigation was focused with much attention to investigate the phytochemical constituents, antimicrobial activity of *Cissus quadrangularis* silver nanoparticles. The synthesized nanoparticles of the plant *Cissus quadrangularis* were characterized by UV-VIS spectrophotometer and FTIR .The antimicrobial activity of *Cissus quadrangularis* nanoparticles was performed with disc diffusion method. The biosynthesis of silver nanoparticles (AgNPs) was initially detected by the color change of the solution from light yellow to honey brown. UV-Vis spectra of synthesized AgNPs which give a sharp band at around 430 nm and confirmed the synthesis of AgNPs. The FTIR analysis of synthesized nanoparticles from *Cissus quadranguaris* revealed prominent peaks at 1631.52 cm^{-1}, 2147.12 cm^{-1} and 3370.56 cm^{-1} corresponds to N-H bending of primary amines. The absorption peaks obtained at 2146 cm^{1} assigned to –C≡C- stretching of alkynes. The phytochemical screening of the *Cissus quadrangularis* nanoparticles revealed the presence of flavonoids, proteins, alkaloids, phenols, carbohydrates and terpenoids. The antimicrobial activity of *C.quadrangularis* was found to be very effective against *S.aureus* with a maximum zone of inhibition with 26 mm in diameter. The silver nanoparticles obtained from *Cissus quadrangularis* exhibited significant antimicrobial activities without any serious side effects. These findings clearly demonstrated that the bioactive metabolites present in *Cissus quadrangularis* can be used for the treatment of disease.

Introduction:-

Ayurveda and Chinese medicinal systems are the most acceptable traditional system which has a considerable amount of research on pharmacogenomics, chemistry, pharmacology and clinical therapeutics (Kiranjor, S and Kunwarjeet, P , 2010) . It is evident that several plants have been used in traditional ayurvedic medicine for treatment and management of distinct inflammatory disorders and wound healing activities (Gacche, RN *et al*., 2011). *Cissus quadrangularis*(CQ), scattered all over India particularly in tropical regions, usually called as

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Vajravalli in Sanskrit, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandai in Tamil and Nalleru in Telugu in Indian languages and in English it is called as Edible-stemmed Vine. Based on morphological characters three different variants have been identified, they are square – stemmed, round-stemmed and flat stemmed. Frequently available varieties are square stemmed and round-stemmed. These are rich in source of ascorbic acid, carotene, anabolic steroid substance and calcium. The whole plant is considered to be edible while each part of the plant pharmacologically contributes to some activity. *Cissus quadrangularis Linn* has potent fracture healing property, antimicrobial, antiulcer, antioxidative, antiosteoporotic, cholinergic activity as well as beneficial effects on cardiovascular diseases (*Jainu, M and Devi, CS, 2003).* The efficacy against different activities varies for different extract (Jainu, M and Devi, CS, 2005). In Indian traditional medicine *Cissus quadrangularis* is used as a component of a plaster for treating swelling and bone fractures (Annie shirwackar *et al*., 2004). The biogenic synthesis of metal nanoparticles reduces the hazards through the elimination/minimization of generated waste and the implementation of sustainable processes. Biological methods of nanoparticles synthesis using microorganisms (Klaus, T *et al*., 1999; Konishi, Y *et a*l., 2007) and plant or plant extracts have been studied as possible eco-friendly alternatives to chemical methods. Using plants for nanoparticle synthesis can be advantageous over other biological process of maintaining cell cultures and can be scaled up for large- scale nanoparticle synthesis (Shankar SS *et al*., 2004). In recent year, the biosynthesis method using plant extracts has received more attention than chemical and physical methods and even more than the use of microbes, for the nanoparticle metal synthesis due to the absence of any requirement to maintain an aseptic environment. Nanoparticles have attracted considerable attention because of their various applications. Silver nanoparticles are reported to possess anti-bacterial (Sathishkumar M *et al*., 2009). There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones (Parimaladevi.B *et al*., 2004). Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms (Rawat,R.B.S. and Uniyal , 2003)

Hence in the present investigation, much focus of attention was given by extracting *Cissus quadrangularis* to validate its medicinal importance by synthesizing nanoparticles of *Cissus quadrangularis* against antimicrobial activity.

Materials and methods:-

The present project was carried out in the department of PG Biochemistry at V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India. The preliminary work (silver nanoparticle synthesis and antimicrobial activity) was done in V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India. The pharmacological activity of mice study was carried out in the Venture Institute of Biotechnology and Bioinformatics Research, Madurai,Tamilnadu,India.

Collection of samples:-

The plant *Cissus quadrangularis* was collected from Madurai District, Tamil Nadu, India. The plant was identified and authenticated by a botanist Assistant Professor Dr.B.Karunaiselvi, Department of Botany, V.V.Vanniaperumal College for women, Virudhunagar, Tamilnadu, India. The stem part of the plant was washed several times with distilled water and kept for drying under shade. After drying, it was powdered using a domestic grinder. The plant powdered was then stored in an airtight container.

Preparation of the extract:-

10 g of plant powder were weighed and was extracted with 80 ml of petroleum ether and 20 ml of chloroform using soxhlet apparatus with temperature maintained for extraction was 55^0C. The duration of soxhlet extraction was 3 to 4 hours. The extracts were concentrated by pouring them into clean round bottom flask and were allowed for evaporation of solvents by using distillation apparatus. Then the concentrated crude extract was stored at 4^0C in airtight bottle until further use.

Preparation of stock solution of plant extract:-

400mg of extract was taken and dissolved in 40 ml of double distilled water and filtered using Whatmann filter paper No 1. It was stored at room temperature for further experiments as stock solution.

Synthesis of silver nanoparticles (AgNPs):-

In a typical synthesis of silver (Ag) nanoparticles, the stock solution (40 ml) was added to 10 ml of 10mM silver nitrate aqueous solution and kept at room temperature. The experiment was done in triplicate for reproducibility.

After 1 hour the colour of the solution changed from colorless to honey brown [Fig -1] indicating the formation of silver nanoparticles this is confirmed by UV-Visible spectroscopy.

UV-Vis Spectra Analysis:-
The bio reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectra of the reaction medium. UV–Visible spectral analysis was carried out with a SYSTRONICS UV-Vis Spectrophotometer 119. UV–Visible absorption spectrophotometer with a resolution of 1nm between 400nm and 520 nm. A small aliquot of 1ml of the nanoparticles is diluted 10 times with Millipore water to avoid errors due to high optical density of the solution.

Fourier Transform infrared (FT-IR) spectroscopy analysis:-
FTIR measurements, the silver nanoparticles solution was centrifuged at 10,000rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with potassium bromide pellets and analyzed. The pellet was immediately put into the sample holder and FT-IR spectra were recorded in the range 4000-400 cm^{-1}.

Preliminary Phytochemical Analysis of AgNps of *Cissus quadrangularis:-*
The sequentially extracted nanoparticles were used for the qualitative analysis for the identification of phytoconstituents (Harborne JB, 1998).

Anti Microbial Activity:-
Microbial strains:-
Authentic pure cultures of bacteria namely *Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Staphylococcus aureus,* and *Pseudomonas fluorescence* obtained from Venture Institute of Biotechnology and Bioinformatics Research, Madurai, Tamilnadu, India. Overnight cultures of the above mentioned strains were used for this study.

Agar Disc Diffusion Method:-
The nanoparticles were screened against five bacterial pathogens. Antibacterial activity was carried out using disc diffusion method. The bacterial strains were inoculated in Nutrient broth and incubated for 24 hours before used in antibacterial assay. Sterile Muller Hinton Agar (Hi – Media) plates were prepared and allowed to set. The cultures to be screened were swabbed on tap of the solidified media. Disc impregnated with the nanoparticles were placed on the swabbed plate. Petroleum ether and Chloroform solvent combination was used as a control. Ampicillin (antibiotic) was used as standard. The plates were incubated at 37ºc for 24 hours. After incubation, the inhibition zone was measured. Assay was carried out in triplicates and control plates were maintained. Zone of inhibition was measured from the edge of the disc to the clear zone in millimeter (mm).

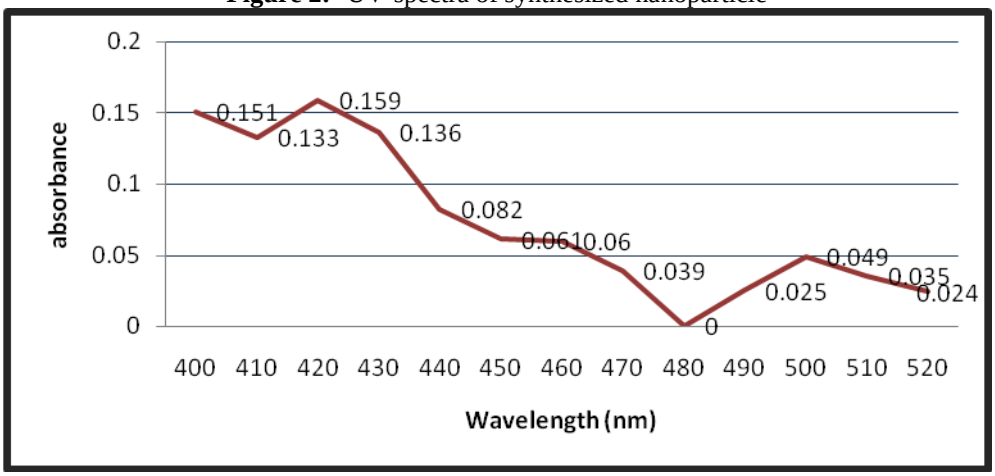
Result:-
In the present investigation, synthesis of $AgNO_3$ and plant mediated AgNPs was carried out. The biosynthesis of NPs was initially detected by the colour change of the solution from light yellow to honey brown. The synthesized NPs were well characterized.
Figure -1: Plant extract color changed from colorless to honey brown of synthesized nanoparticle of plant extract

a) Plant extracts **b) plant extract with silver nitrate**

UV-Vis Spectroscopy:-
The synthesized nanoparticles were characterized by UV- Vis Spectroscopy [Fig.2]. UV-Vis spectral analysis was carried in the range of 400 – 520 nm to observe the appearance of specific peak of plant mediated silver

nanoparticles. UV-Vis spectra of synthesized AgNPs, which give a sharp band at around 430 nm and confirmed the synthesis of NPs.

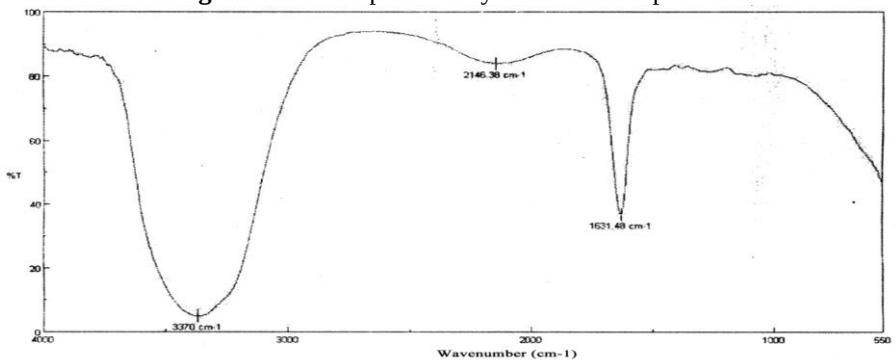
Figure 2:- UV-spectra of synthesized nanoparticle



FITR Analysis:-

Assessment of synthesized nanoparticles in *Cissus quadrangularis* were characterized by FTIR revealed prominent peak at1631.52 cm^{-1} and another peak with an intensive band at 2147.12 cm^{-1} followed by 3370.56 cm^{-1} indicate N-H bending of amines. Similarly, the FTIR results indicated the presence of phenols, which play an important role in the biosynthesis of nanoparticles. The peaks obtained at 2146 cm^{-1} denote stretching of alkynes [Fig-3].

Figure 3:- FTIR spectra of synthesized nanoparticle



FTIR Spectra of nanoparticle synthesized plant of *Cissus quadrangularis* showing intensive peaks of Amines, Alkynes, Amides, Phenols and Alcohol

Phytochemical Analysis:-

The results on phytochemical screening of the *Cissus quadrangularis nanoparticles* were tabulated in Table-1. It revealed the presence of flavonoids, proteins, alkaloids, phenols, carbohydrates and terpenoids. Alkaloids and flavonoids have been associated with medicinal uses and it is one of the common biological properties with antibacterial activity.

Table 1:- Phytochemical Analysis Of The Nanoparticles Synthesized Plant Extract *Cissus Quadrangularis*

S.No	Phytochemical constituents	Nanoparticles of *Cissus quadrangularis*
1	Flavonoid	Positive
2	Protein	Positive
3	Tannin	Positive
4	Alkaloid	Positive
5	Phenol	Positive

6	Carbohydrate	Positive
7	Quinone	Positive
8	Terpenoid	Positive
9	Glycoside	Positive
10	Anthraquinone	Negative

Antimicrobial Activity:-

The antimicrobial activity was performed with five bacterial pathogens *Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Staphylococcus aureus* and *Pseudomonas fluorescence* using well diffusion method [Fig-4 and Table-2]. *Cissus quadrangularis* was found to be very effective against *Stahylococcus aureus* which exhibited a maximum zone of inhibition with 28 mm in diameter. *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* both pathogens revealed a zone of inhibition with 26 mm in diameter.

Figure 4:- Antimicrobial activity of synthesized nanoparticle against selected pathogens.

Table 2:- Antimicrobial Activity Of *Cissusquadrangularis* Nanoparticles Against Selected Pathogens

Microorganism	**ZONE OF INHIBITION IN DIAMETER (in mm)**		
	Control	**Standard**	**Extract**
Escherichia coli	0	16	12
Pseudomonas aeruginosa	0	15	26
Shigella flexneri	0	12	12
Staphylococcus aureus	0	18	28
Pseudomonas fluorescence	0	15	26

Discussion:-

The bio-reduction of silver ions to silver nanoparticles in the solution was monitored by UV-Visibe spectroscopy. In the present study, UV-Visible spectra recorded the silver nanoparticles at 400 – 450 nm. Our results coincide with the findings of Gowghami Marquis *et al.*, 2016. FTIR is a valuable tool for measuring many chemical constituents in plants and it is used to reveal some qualitative aspects regarding the organic compounds. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products (Kristin Lammers *et al*.,2009). In the present investigation, synthesized nanoparticles of the plant *Cissus quadrangularis* were characterized by FTIR analysis. The green chemistry approach towards the synthesis of silver nanoparticle was studied and it has many advantages such as, eco-friendly rapid approach and economic viability application of this eco-friendly nanoparticle antibacterial was performed. In the present study phytochemical analysis of the *Cissus*

quadrangularis nanoparticles was carried out to investigate antimicrobial activity. The antibacterial activity tested with *Cissus quadrangularis* nanoparticles against *Staphylococcus aureus* revealed 28mm in diameter which is considered to be the best result with the plant extract. It is followed by *Pseudomonas fluorescence and Pseudomonas aeruginosa* exhibiting a zone of inhibition of 26 mm in diameter. The plant extract showed a remarkable activity with the above organisms. Our results coincides with the findings of Garima *et al.,* 2009 where in they reported that *Cissus quadrangularis* extracts was found to be inhibitory against both gram positive and gram negative bacterial pathogens.Since ancient ages, plants have served human beings as a natural source of treatments and therapies. Amongst them, medicinal herbs have gain attention because of its wide use and less side effects. Previous study made by Raquel, 2007 had reported that steroids present in plant extracts have antibacterial activity. Alkaloids have been associated with medicinal uses for centuries and one of the common biological properties is their antibacterial property (Okwu, DE , 2004). Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the invitro antibacterial assay (Tona, L *et a*l., 1998). Many reports are available on the antibacterial properties of plants (Rio, JL and Reeio, MC , 2005; Kumaraswamy, Y *et al*,2002; Bylka,2004; Ayshwarya.M and Sudha Rameshwari,2015; Karthigaiselvi and Sudha Rameshwari,2016). The minerals and phytochemical constituents may be responsible for the antimicrobial activities of the plants (Prema sampathkumar and Shalini S, 2012). In the present study, antibacterial activity of petroleum ether – chloroform extracts of *Cissus quadrangularis* was studied. The extracts revealed remarkable inhibitory activity against gram positive and gram negative bacterial pathogens. Treatment of diseases possesses challenging problems due to emerging infectious disease and increasing number of multidrug resistance microbial pathogens. In spite of large number of antibiotics and drugs used a substantial need for new class of potential compound as drug is obtained from plant extract mandatory. Plant extract play an important group in designing a new class of structural antibiotics of medicinal importance with new mechanism of action. Similar results were reported in Chloroform extract of *Glinus lotides linn* showed good antibacterial activity against *Staphylococcus aureus* (Sudha Rameshwari.K *et al*.,2013).The silver nanoparticles obtained from *Cissus quadrangularis* exhibited significant antimicrobial activities without any serious side effects. These findings clearly demonstrated that the bioactive metabolites present in *Cissus quadrangularis* can be used for the treatment of disease.

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