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BIOLOGICAL SYNTHESIS OF GOLD NANOPARTICLE FROM *VITEX TRIFOLIA* MEDICINAL PLANT AND THEIR ANTIMICROBIAL PROPERTIES

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ABSTRACT

There is an increasing commercial demand for nanoparticles due to their wide applicability in various markets, including medicine, catalysis, electronics, chemistry and energy. In this report, a simple and ecofriendly chemical reaction for the synthesis of gold nanoparticles from *Vitex Trifolia*(Lamiaceae Family) has been developed. Phytochemical and antibacterial activity of *Vitex Trifolia* leaf with the help of five different extract like petroleum ether, acetone, ethyl acetate, chloroform and distilled water. In this aqueus leaf extracts conforms the presence of various phytochemicals. To evaluate the antibacterial activities of these aqueous extracts were determined by disc diffusion method. From these five extract ethyl acetate shows strong antimicrobial effect on *E.coli, Acenatobactor, Proteus, Staphylococcus, Klebsiella*. None of leaf extract no more activity in *Pseudomonas.* Shade dried leaves of Vitex Trifolia was used for the synthesis of gold nanoparticles. To compare the antimicrobial activity of gold nanoparticles with the different leaf extracts.

KEYWORDS: Phytochemicals, Vitex Trifolia, antibacterial activity, gold nanoparticles *E.coli, Acenatobactor, Proteus, Staphylococcus, Klebsiella, Pseudomonas.*

INTRODUCTION

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracelluarly using plants or their extracts in a controlled manner according to

their size, shape and dispersity In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. Today, nano-metal particles, especially gold, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of chemistry, electronics, medicine and biotechnology at the nanoscale.

The medicinal plants are those that provide people with medicines to treat illness, maintain and promote health. The phytochemicals are the wide variety of compounds produced by plants manipulated wisely in the pharmacognostic drug development and treatment of the major ailments .Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products.

The genus Vitex contains 270 species all over the world with diverse medicinal active constituents and properties. These species are predominantly trees and shrubs, found in tropical and subtropical regions. Other species with fruits, seeds, roots are also important as traditional medicines. In India, some of Vitex species viz. *Vitex negundo, Vitex glabrata, Vitex leucoxylon, Vitex penduncularis, Vitex pinnata* and *Vitex trifolia* L. are found. Many of these vitex species and their active principals are studied for Pharmacognostical investigation and phytochemical screening**,** biological, pharmacological evaluation.

MATERIAL AND METHOD

Collection of plant material

The fully matured leaves of *Vitex Trifolia* were collected from pamgarh in district of Bilaspur, Chhattisgarh, India. The leaves were thoroughly washed and shade dried for 10 days.

Extract preparation

The *Vitex Trifolia* leaves after shade dried for a period of 10 days were blended and made into fine coarse powder. 10 gm of powdered leaf material was taken and 100 ml each solvent-petroleum ether, acetone, ethyl acetate, chloroform and distilled water. The initial weight of beaker is noted and the extraction was run in soxhlet apparatus continuously the 2 hours and extracts were collected in a beaker. The solvent is allowed to evaporation was achieved. The final weight – initial weight gives the weight of the leaves extract.

Preliminary Phytochemical Screening

Test for carbohydrate

Take about 2 ml of plant extract of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H_2SO_4 slowly along the side of the test tube. A red violet (purple) ring is formed at the junction of two layers.

Test for protein

1ml of 40% naoh solution and 1 to 2 drops of 1% cuso4 solution was added to 2ml each extract. A violet color indicated the presence of peptide linkage of the molecule.

Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of conc.h2so4 a yellow coloration was observed which confirms the presence of flavonoids and it disappears on standing.

Test for Terpenoids

5 ml of each extract was added to 2 ml of chloroform and 3 ml of conc. H2so4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

Test for Tannins

The extract with 20ml of distilled water was agitated in a graduated cylinder for 15min. The formation of 1cm layer of foam indicated the presence of saponins.

Antimicrobial Effect of plant extracts

The antibacterial activity of Vitex Trifolia plant extract with gold nanoparticles was evaluated by disc diffusion method. Nutrient agar media was prepared and poured into the petri plates and allowed to solidify. Then it was inoculated with a swab of culture and spread through out the medium uniformly with a sterile cotton sweb.A sterile filter paper disc was prepared and dipped with plant leaf extract (Acetone, Petoleum ether, Ethyl acetate, Chloroform, Distilled water). and then placed on the surface of agar plates. All the plates were incubated at 37oc for 24h. After incubation measuring the diameter of zone of inhibition around the well.

Synthesis of Gold nanoparticles

1mM AuNo3 solution was prepared and stored in amber color bottle. 5ml of leaf extract was taken in conical flask separately and to this 50ml of 1mM AuNo3 solution was addes drop

wise with constant stirring at 50-60 oc and observed the colour change. The colour change of the solution was checked periodically then the conical flask was incubated at room temperature for 48 hours. The colour change of the leaf extract from light to dark indicated the gold nanoparticles synthesis from leaves of *Vitex Trifolia* leaves.

RESULT AND DISCUSSION

For the present study carried out on the plant samples revealed the presence of medically active metabolites. The phytochemical characteristics of the methanol and acetone extract of Vitex Trifolia leaf was investigated for the presence of chemical constituents such as carbohydrates, proteins, flavonoids, terpenoids, tannins by phytochemical screening test **(Table-1)**. The petroleum ether extract positive for carbohydrate, protein, terpenoids, tannins and negative for flavonoids. The acetone extract positive for carbohydrate, protein, flavonoid, terpenoids and tannins. The ethyl acetate extract positive for flavonoids, protein and negative for carbohydrate, terpenoids and tannins. The chloroform extract positive for terpenoids and protein and negative for carbohydrates, tannins and flavonoids. The ditilled water extract positive for terpenoids and tannins, protein and negative for carbohydrates and flavonoids. *Vitex Trifolia* leaf extracts were used to investigate antibacterial activity of six micro organisms isolates namely *Klebsialla, Pseudomonas, Staphylococcus, Streptococcus, E.coli, Proteus*. Among the five extracts acetone leaf extract of *Vitex Trifolia* showed high antimicrobial activity against all the tested micro organisms except pseudomonas. The result showed Vitex Trifolia exhibited high activity against E.coli sp, moderate activity against Acenatobactor sp and low activity against in Proteus. But no more activity in Pseudomonas sp. **(Table-2,Fig-1).**

Table-1 Phytochemical Screening of *Vitex Trifolia*

S.No.	Name of the test	Petroleum ether	Acetone	Ethyl acetate	Chloroform	Distilled water
1	Carbohydrate	+		-	_	_
2	Protein	+	+	+	+	+
3	Flavonoids	_	+	+	_	_
4	Terpenoids	+	+	_	+	+
5	Tannins	+	+	_	_	+

Here "+" means presence " _" means absence

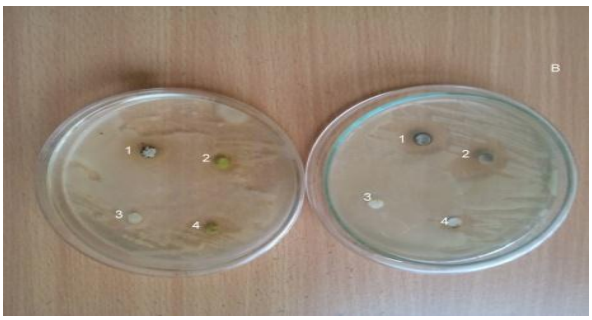
Table-2 Zone of inhibition on different Extracts of *Vitex Trifolia* leaf

S.No.	Micro organism	Petroleum ether	Acetone	Ethyl acetate	Chloroform	Distilled water
1	Staphylococcus	0.3	-	0.5	0.2	0.3
2	E.coli	1.2	-	1.6	1	0.2
3	Klebsiella	1.5	-	0.9	0.6	1
4	Pseudomonas	-	1	-	0.7	0.9
5	Proteus	-	-	0.5	0.4	-
6	Acenatobactor	1.3	-	1	1	-

Zone of inhibition in (mm).

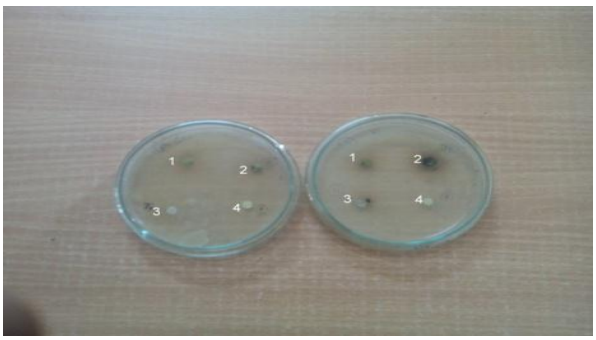
Before adding

AuNo3 show highest inhibition zone againt *E.coli*



After adding

AuNo3 show moderate inhibition zone againt *Acenatobactor*



nanoparticle

AgNo3 show low inhibition zone against *Proteus*

nanoparticle

AgNo3 no more inhibition zone againt *Pseudomonas*

Fig:- (1) Ethyl acetate, (2) Acetone, (3) Petroleum ether, (4) Chloroform

Fig:- Silver Nanoparticle synthesis from *Vitex Trifolia*

Before Synthesis- Light Green

After synthesis- Dark Green

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