

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

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ABSTRACT

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 aqueous 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 acetone shows strong antibacterial effect on *Klebsiella*, *E.coli*, *Staphylococcus*, *Proteus*, *Acenatobactor*. None of leaf extract no more activity in *Pseudomonas* widely used in the field of medicine. Shade dried leaves of *Vitex Trifolia* was used for the synthesis of silver nanoparticles. To compare the antimicrobial activity of silver nanoparticles with the different leaf extracts.

KEYWORDS: Phytochemicals, *Vitex Trifolia*, antibacterial activity, silver nanoparticles.

INTRODUCTION

Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C give many references of medicinal plants. One of the remotest works in traditional herbal medicine is “*Virikshayurveda*”, compiled

even before the beginning of Christian era. “*Rig Veda*”, one of the oldest available literatures written around 2000 B.C. (Bentley and Trimen, 1980).

Vitex Trifolia (Lamiaceae Family) is a relatively small tree found primarily in countries the border on the pacific and Indian oceans. Due to its colourful flowers and use in traditional medicines, it is also cultivated in garden. One of the alternate name for the tree is “three leaflet Vitex” which stems from the fact that the leaves are not in one piece, but rather in three, or sometimes five, little leaflets stuck together at their bases, of which the middle leaf is the longest.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Iyengar, 1985; Chopra et al., 1992; Harborne and Baxter, 1995). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor et al., 1995; Ratnakar and Murthy, 1995; Silva et al., 1996; David, 1997; Saxena, 1997; Nimri et al., 1999; Saxena and Sharma, 1999).

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 extracellularly 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 Silver, 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 most effectively studied nanoparticles today are those made noble metals, in particular Ag, Pt, Au and Pd. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Metal nanoparticles have tremendous applications in area of catalysis, opto-electronic, diagnostic biological probes and display devices. Among the various inorganic metal attention for

various reasons that silver is an effective antimicrobial agent. The application of silver nanoparticles in metal industry as topical ointment to prevent infection against burn and open wounds.

Green synthesis provides advancement over chemical and physical method as it is cost effective, environmentally friendly; easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

MATERIAL AND METHOD

Collection of plant material

The fully matured leaves of *Vitex Trifolia* were collected from pangarh 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 for 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% $CuSO_4$ 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. H_2SO_4 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 silver 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 swab. A sterile filter paper disc was prepared and dipped with plant leaf extract (Methanol and Acetone) and then placed on the surface of agar plates. All the plates were incubated at 37°C for 24h. After incubation measuring the diameter of zone of inhibition around the well.

Synthesis of Silver nanoparticles

1mM AgNO₃ 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 AgNO₃ solution was added drop wise with constant stirring at 50-60 °C 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 silver 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 distilled 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 *Klebsiella*, *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 *Klebsiella* sp, moderate activity against *E.coli* sp and low activity in *Proteus* sp. 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, + = presence

- = 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	0.5	0.3	0.4	-	0.4
2	E.coli	-	1.2	0.4	0.4	0.5
3	Klebsiella	-	1.9	0.3	0.5	1.5
4	Pseudomonas	1.1	-	0.5	0.9	0.9
5	Proteus	-	0.2	0.2	0.3	-
6	Acenatobactor	-	1.3	0.6	0.7	-

Zone of inhibition in (mm)

Before adding

After adding

nanoparticle

nanoparticle



AgNO₃ show highest inhibition zone against *Klebsiella*



AgNO₃ show moderate inhibition zone against *E. coli*



AgNO₃ show low inhibition zone against *Proteus*



AgNO₃ no more inhibition zone against *Pseudomonas*

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



Fig 2:- Silver Nanoparticle synthesis from *Vitex Trifolia*

Before Synthesis- Light Brown

After synthesis- Dark Brown

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