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Phytochemical screening and biosynthesis of silver nanoparticles of selected medicinal plants used in Traditional Medicine

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Abstract
Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. In the present study, chief phytoconstituents of the four selected medicinal plants (*Hemigraphis alternate, Phyllanthus niruri, Tinospora cordifolia and Vitex negundo*) of different families were identified in order to relate their presence with bioactivities of the plants. Screening of the plants was performed using standard methods and resulted in the detection of the presence of tannins, flavonoids, phenolics, steroids, cardiac glycosides, coumarins, terpenes and alkaloids. Flavanoids were found in four of the selected plants and alkaloids were found in three of them except *Vitex negundo.* The biosynthesis of silver nanoparticles from 1 mM $AgNO_3$ solution using aqueous extracts of four plants were done and the surface plasma resonance was measured using UV-VIS spectrophotometry. These results validate the exploitation of the studied medicinal plants, for further isolation of active principles and their application in medical and Cosmetics industries.

Keywords: Bioactive principles, phytoconstituents, flavonoids, nanoparticles

Introduction
Plants have a limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12000 have been isolated, a number estimated to be less than 10% of the total. These substances are used by plants as defensive molecules. Some of which may involve in plant odour, pigmentation and flavour. However, these defensive molecules give plants their medicinal value which is appreciated by human beings because of their great importance in health care of individual and community [8]. The treatment and control of disease by the use of available medicinal plant in a locality will continue to play significant roles in medical health care implementation in the developing countries of world. The present work mainly focused on the medicinal plants such as Vitex negundo (Verbenaceae) which is credited with innumerable medicinal activities such as analgesic, anti- inflammatory, antioxidant, bronchial relaxant etc., Phyllanthus niruri (Euphorbiaceae) has medicinal value such as kidney stones, anti-liver damage, anti-tumor activity, anti-HIV, diabetes treatment etc., Tinospora cordifolia (Menispermaceae) which has notable medicinal properties such as anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial etc., and finally Hemigraphis alternata (Acanthaceae) which is used to treat diarrhoea, hemorrhoids, excessive menstruation and skin diseases [8]. And this work is there foreaimed at studying medicinal plants used locally for treatment of some disease for their phytochemical properties and antimicrobial activities.

Materials and Method
Preparation of Extract
The collected leaves of *Hemigraphis alternate* (Murikootti), *Phyllanthus niruri* (keezharnelli), *Tinospora cordifolia* (amruthu), *Vitex negundo* (karunochi) were washed and dried under shade. The coarse powder of the leaves (500 gm) was soaked in 500 ml of Chloroform and extracted in the cold for 3 days with occasional shaking. The solvent from the total extract was filtered and filtrate was dried under shade, it was used for phytochemical screening [2].

Phytochemical Screening

Steroid: 1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H2SO4 acid was added from the side of test tube. The upper layer turns red and H2SO4 layer showed yellow with green fluorescence. This indicates the presence of steroid [7].

Tannin

i. 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.
ii. 4ml extract was treated with 4 ml FeCl3 formation of green colour indicates that presence of condensed tannin.

Saponin: 5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min. formation of foam indicates Saponin.

Anthocyanin: 2 ml of aqueous extract is added to 2 ml of 2N HCl and NH3, the appearance of pink red turns blue violet indicates presence of Anthocyanin.

Coumarin: 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

Alkaloids: A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

- **Wagner test:** Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.
- **Hager's test:** Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

Proteins: Xanthoproteic test: Extract was treated with few drops of concentrated HNO3 formation of yellow indicates the presence of proteins.

Amino acids: Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

Phytosterol: Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H2SO4 and shakes, allow standing, appearance of golden red indicates the positive test.

Phenol: Ferric Chloride test: Test extract were treated with 4 drops of Alcoholic FeCl3 solution. Formation of bluish black colour indicate the presence of Phenol.

Phlobatannins: Deposition of red ppt when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of Phlobatannins.

Leucoanthocyanin: 5 ml of isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leuanthocyanin.

Cardial Glycosides: Keller-Killani Test: Plant extract treated with 2 ml glacial acetic acid containing a drop of FeCl3. A brown colour ring indicates the presence of positive test [2].

Flavonoid

- **Alkaline reagent test:** Extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.
- **NH4OH test:** 3 ml of extract were 10% NH4OH solution development of yellow fluorescence indicates positive test.
- **Mg turning test:** Extract were treated with Mg turning and add conc. HCl to this solution add 5ml of 95% ethanol, formation of crimson red colour indicates Flavonoid
- **Zn test:** 2 ml extract were treated with Zn dust and conc. HCl development of red colour indicates presence of Flavonoid [5].

Biosynthesis of Ag Nanoparticle from the Aqueous Extract

Five grams of each powder was weighed and taken into 250 ml conical flasks which contains 100 ml of sterile distilled water. The extracts were then boiled for ten minutes at 100 ºC. 5 ml of the each extract were taken separately and to this 50 ml of 1 mM silver nitrate solution was added drop wise with constant stirring at 50–60 ºC and observed the color change. Then the conical flasks were incubated at room temperature for 48 h. The color change of the solutions was checked periodically. A change from yellow to dark brown indicated the silver nanoparticles were synthesized by the samples. The contents were centrifuged at 10000 rpm for 10 min. The supernatants were used for the characteristics of the silver nanoparticles through UV-Vis spectrum [3].

Uv-Vis Spectra Analysis: The reduction of pure silver ions were monitored by measuring the UV-Vis spectrum of the reaction medium at 3 h after diluting small aliquots of the samples into distilled water. UV-Vis spectral analysis was carried out at 400–600 nm. The spectral analysis of 1 mM silver nitrate solution and the extracts without silver nitrate solution were carried out.

Antimicrobial Assays

Organisms Used

Urinary tract isolates like *Staphylococcus aureus* and *Pseudomonas sp*.

Antibacterial Assays The antibacterial efficacy of samples were studied and evaluated against the urinary tract isolates by MIC and well diffusion method [6, 9].

Testing Antioxidant Property.

Method for DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable 2, 2 diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams *et al* 1995 [1]. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol 6×10-5 M was prepared fresh daily before UV measurements. Three ml of this solution was mixed with 100 microgram/ml concentration of individual plant extracts as well as herbal preparation. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula.

- % Inhibition =1-(Ai-Aj) × 100

Ac

Where Ac = absorption of blank sample, Ai= absorption of test extract with DPPH solution, Aj= absorption of test extract with distilled water [1]

Results and Discussion

The phytochemical screening of *Hemigraphis alternata, Phyllanthus niruri, Tinospora cordifolia and Vitex negundo* showed that the leaves are rich in proteins, lipids, carbohydrates, phenols, tannins, flavonoids, saponins, alkaloids and anthocyanins. The results are summarized in Table-1. Proteins are found to be higher in all of them which are primary components of living organisms. Proteins are essential to maintaining the structure and function of all life and vital for growth and development. The presence of higher protein level in the plants points towards their possible increase in food value or that a protein based bioactive compound could also be isolated in future.

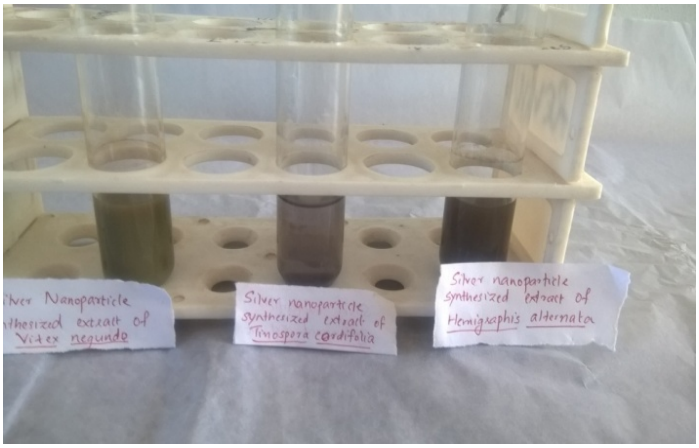
Phenol was present in only *Vitex negundo* Phenolics are secondary metabolites that are ubiquitously present in fruits. Many of the phenolics have been shown to contain higher levels of antioxidant activities Endogenous phenolics may also play a role in inhibiting the fruit browing process. Nutritional quality of fruit tissue is in part a function carbohydrate metabolism, colour, pigment, and flavonoid, phenolic content and anti-oxidative capacity. Antioxidants provide chemical protection for biological systems against harmful effects of reaction or process that cause excessive oxidation, protein and DNA damage and cell death. Several studies have indicated that antioxidants prevent the onset of degenerative illness such as certain cancers, cardiovascular and neuro degradative diseases, contracts, oxidative stress dysfunctions and aging [4].

Phytochemical Screening: The aqueous extract of the selected plants were prepared. The phytochemical analysis was carried out and was tabulated. Present study deals with qualitative analysis of leaves extract of *Hemigraphis alternata*, *Phyllanthus niruri*, *Tinospora cordifolia* and *Vitex negundo*. Table no. 1 shows the results of phytochemical analysis of leaves of four selected medicinal plants. Ethanol extract of leaves shows the presence of Steroid, Proteins, Amino acids, Tannin, Coumarins, Alkaloids, Diterpenes, Phenol, Phlobatannin and Flavonoids whereas Anthocyanin, Leucoanthocyanin, Saponin and Cardial Glycosides were absent. Flavanoids were found in four of the selected plants and alkaloids were found in three of them. Flavonoids are reported to possess many useful properties, including anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and anti-tumour activity.

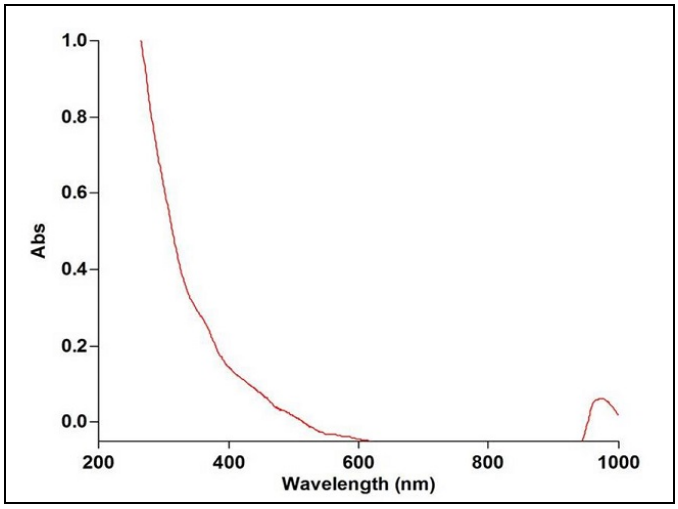
Phytochemicals		**Ethanol extract**			
		Hemigraphis alternata	***Phyllanthus niruri***	***Tinospora cordifolia***	***Vitex negundo***
Steroids		+	-	-	-
Tannin	Lead acetate	+	+	+	+
	Ferric chloride	+	+	+	+
Saponin		-	-	-	-
Anthocyanin		-	-	-	-
Caumarin		+	+	+	+
Alkaloids	Wagner's test	+	+	+	-
	Mayer's test	+	+	+	-
Proteins Xanthoproteic test		+	+	+	+
Amino acids Ninhydrin test		+	+	+	+
Diterpenes Copper acetate test		+	-	-	-
Phytosterols Salkowski's test		+	-	-	+
Phenol Ferric chloride test		-	-	-	+
Phlobatannins		+	-	-	-
Leucoanthocyanin		-	-	-	-
Cardial glycosides Keller killiani test		-	-	-	-
Flavanoids		+	+	+	+

1. Synthesizing Silver Nano Particles

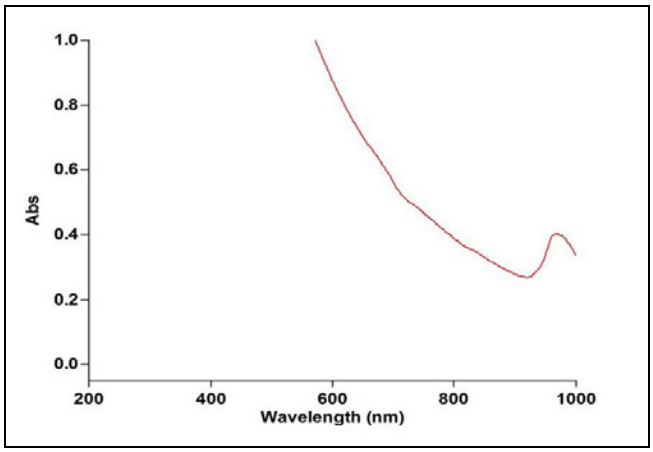
The silver nanoparticle extracts of the selected plants were prepared.



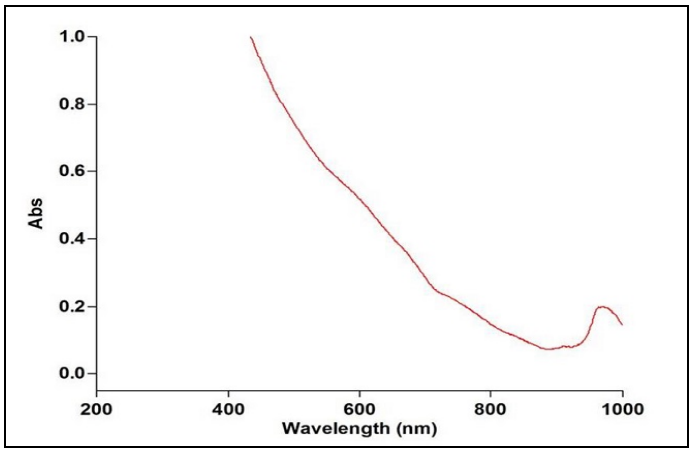
2. Uv-Vis Spectrophotometric Analysis



Hemigraphis alternate



Tinospora cordifolia



Vitex negundo

3. Estimating Antioxidant Property

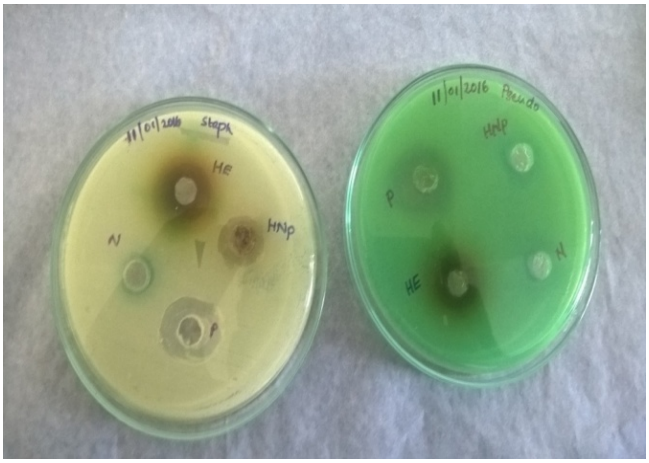
Radical activity% = 1-(Ai-Aj) *100% Ac

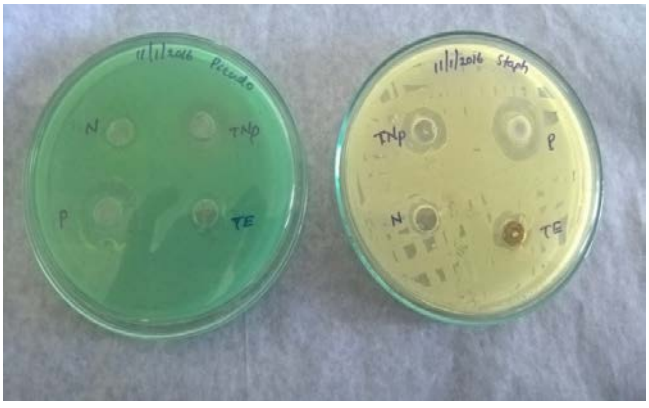
Plant species	Ai	Aj	Radical Activity
Hemigraphis alternata	1.14	0.57	65%
Vitex negundo	1.68	1.0	50.8%
Tinospora cordifolia	0.86	0.19	60.3%

4. Antimicrobial analysis by well Diffusion

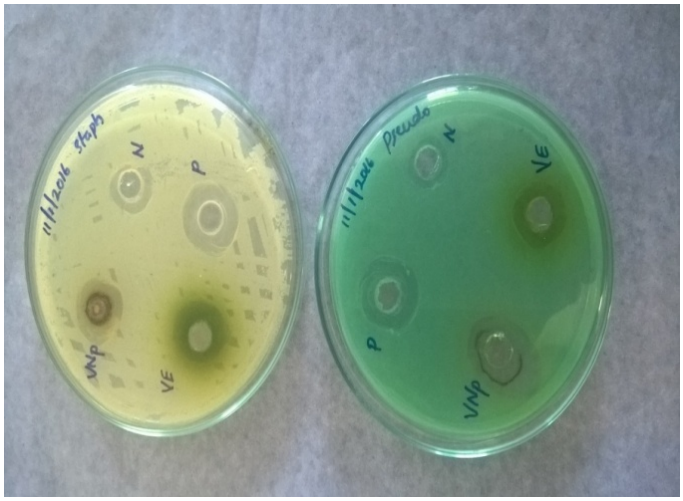
Name of species	Zone inhibition(mm)					
	Silver nanoparticle			Amoxycillin		
	Hemigraphis	***Tinospora***	***Vitex***	***Hemigraphis***	***Tinospora***	***Vitex***
Staphylococcus aureus	10	9	11	17	13	16
Pseudomonas aeruginosa	11	10.5	9.5	20	15	13

Largest zone inhibition was exhibited by *Hemigraphis alternata*





Tinospora cordifolia



Vitex negundo

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