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A COMPARATIVE STUDY ON METAL ION REDUCING ABILITY, PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITY OF FLOWER, STEM AND LEAF PARTS OF TODALIA ASIATICA (Linn.) Lam.

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Abstract: Antibiotic resistance is the world's major public healthcare problem[1,2]. AgNPs particles play a vital role in nano biotechnology as a biomedicine against human pathogens[3,4]. In the present study aqueous extract of different parts viz.flower, stem and leaf of a plant Todalia asiatica (Linn.) Lam. was used to synthesize silver nanoparticles. The difference in the reduction capacity of the extract was compared with the phytoconstituents present in the extract. GC-MS analysis of all the three extracts were carried out to unfold the existence of individual phyto chemicals in the three different extracts to sort out the compounds that possibly could act as a reducing agent to reduce silver ions into silver. In addition to this anti-oxidant , FRAP and antibacterial activity of the three extracts were tested and the results are presented.

Index Terms - *Todalia asiatica* (Linn.) Lam., GC-MS analysis, AgNPs, Antioxidant activity, FRAP, Antibacterial, Green synthesis..

I. INTRODUCTION

It has been explored in the literature that plant parts like seed[5] leaf[6], bark[7], stem and fruit[8] extracts have been effectively used for synthesis of nanoparticle. The plant-mediated synthesis of nanoparticle is a simple, rapid, and cost effective[9] and a flexible process compared to any other physical and chemical methods developed so for[10]. All most all parts of the plant were utilized to synthesis metal nanoparticle in particular the silver nanoparticle. Various plant sources like Lemon grass[11],Azadirachta indica[12], Abutilon indicum (L.)[13] , Cinnamon zeylanicum[14] , Peel extract of Pomegranate[15] , *Tamarindus indica* leaf extract[16] , *Piper betle* leaf extract[17] , *Plumbago zeylanica* leaf extract[18] and Aloe vera leaf extract[19] were subjected to synthesise the AgNPs and were reported to possess anti-fungal , anti-inflammatory , and anti-viral activity . Due to the excellent antimicrobial properties, the silver nanoparticles are used in medicine , food packaging, food preservation , and cosmetics preparations. In the present study, we report the synthesis of silver nanoparticles using aqueous leaf, stem and bark extract of *Todalia asiatica* (Linn.) Lam. and their efficacy in FRAP, antioxidant and antibacterial properties.

2. MATERIALS AND METHODS

Preparation of leaf extracts from the stem flower and leaf part of *Todalia asiatica* (Linn.) Lam.

Fresh *Todalia asiatica* plant was collected from Western Ghats of Nilgiri district and were thoroughly washed with water to remove the dust on the surface of the materials. Stem flower and leaf part were segregated and dried. Five grams of each of the dried material were added to 50mL of double distilled water and heated to boil to prepare the extract. To the filtered clear decoction 5ml from each part, 100mg of silver nitrate dissolved in 1ml of water was mixed and shaken for five minutes. The nano particles developed in the three different parts of the plant extract were filtered and dried. The dried samples were used to take XRD and SEM for confirmation. FRAP and Free radical scavenging activity of the three different extract and antibacterial activities were also studied.

The formation of silver Nano particles was monitored with colour change and UV –Vis spectroscopy. The colour of the reaction mixture started changing to yellowish brown within 10 min and to reddish brown after 1h, indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag+ into silver nanoparticles Ag via the active molecules present in the *Toddalia asiatica* (L.)Lam. This colour is attributed to the excitation of SPR. As shown in Fig. 1, a characteristic and well-defined SPR band for silver nanoparticles was obtained at around λ 250nm.

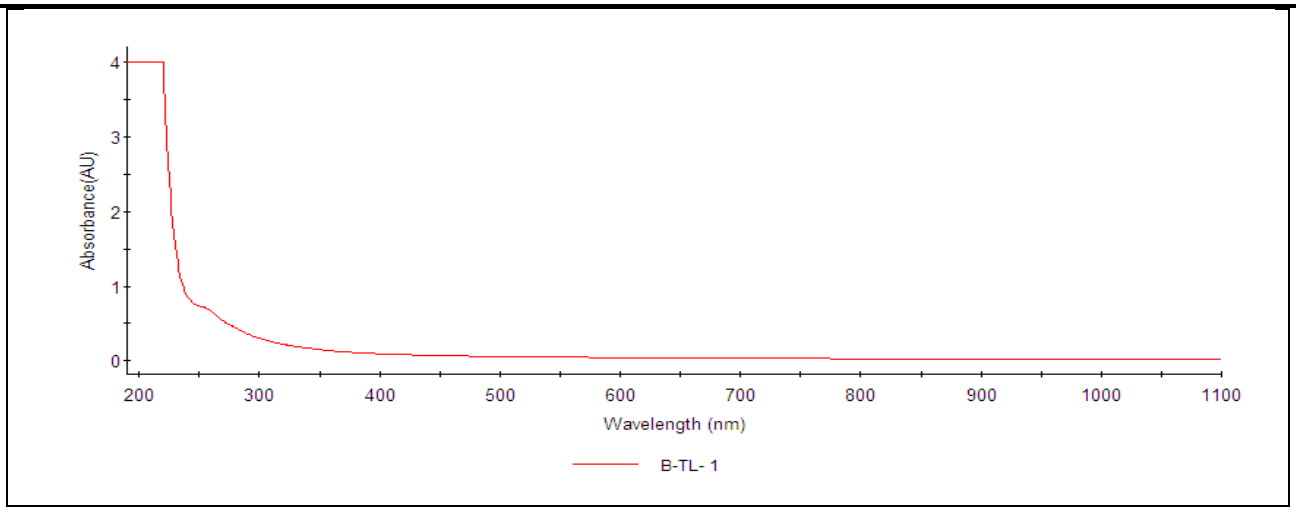


Fig.1 UV –visible spectra of Silver Nano particles

XRD analysis (Leaf part of *Todalia asiatica* **)**

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD pattern as shown in Fig.2 the four distinct diffraction peaks at 2Θ values of 32.41, 38.48, 46.47, 64.75, and 77. 68 can be indexed to the 111, 200, 220, and 311 reflection planes of face centred cubic structure of silver. In addition to the Bragg peaks representative of silver Nano crystals, additional peaks were also observed at 54.23, 58.14, 68.34, 71.23, and 73.12. These peaks are due to the organic compounds which were present in the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles. The XRD pattern obtained is consistent with earlier reports. The XRD results of nano particles arrived from flower Fig.3 and stem Fig.4 extracts are also presented to highlight their varying capacity in the reducing ability of the metal ions into metal.

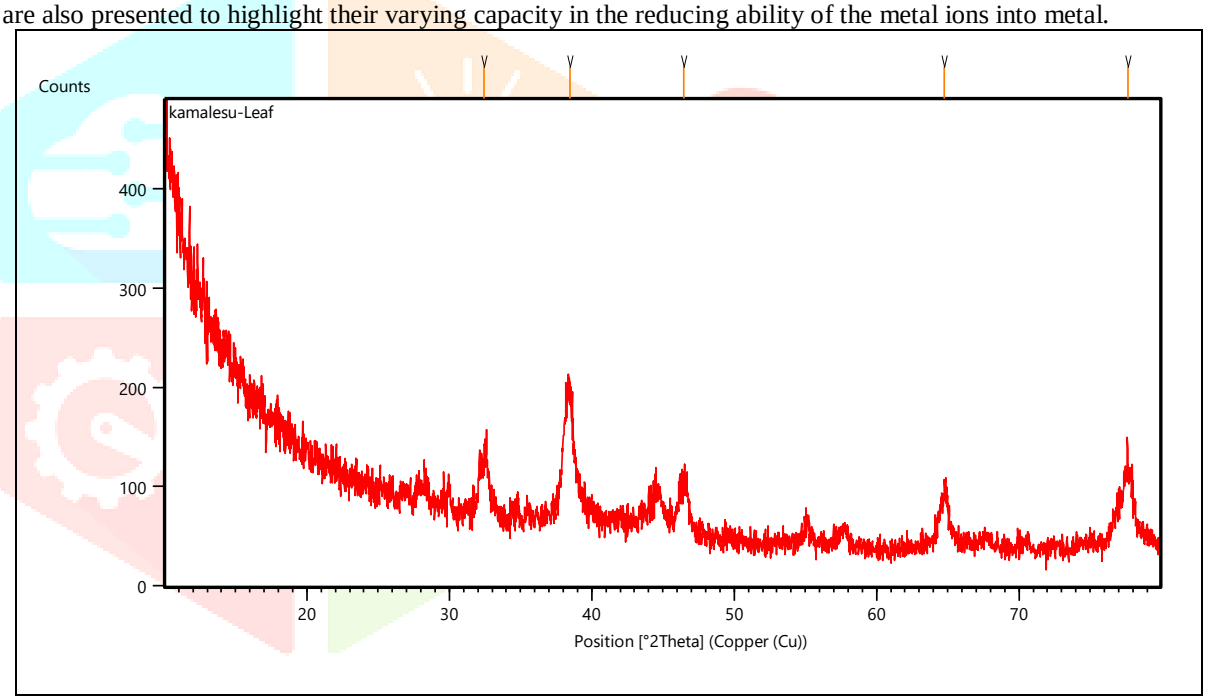


Fig.2. XRD pattern for silver Nano particles from leaf extract of *Todalia asiatica*

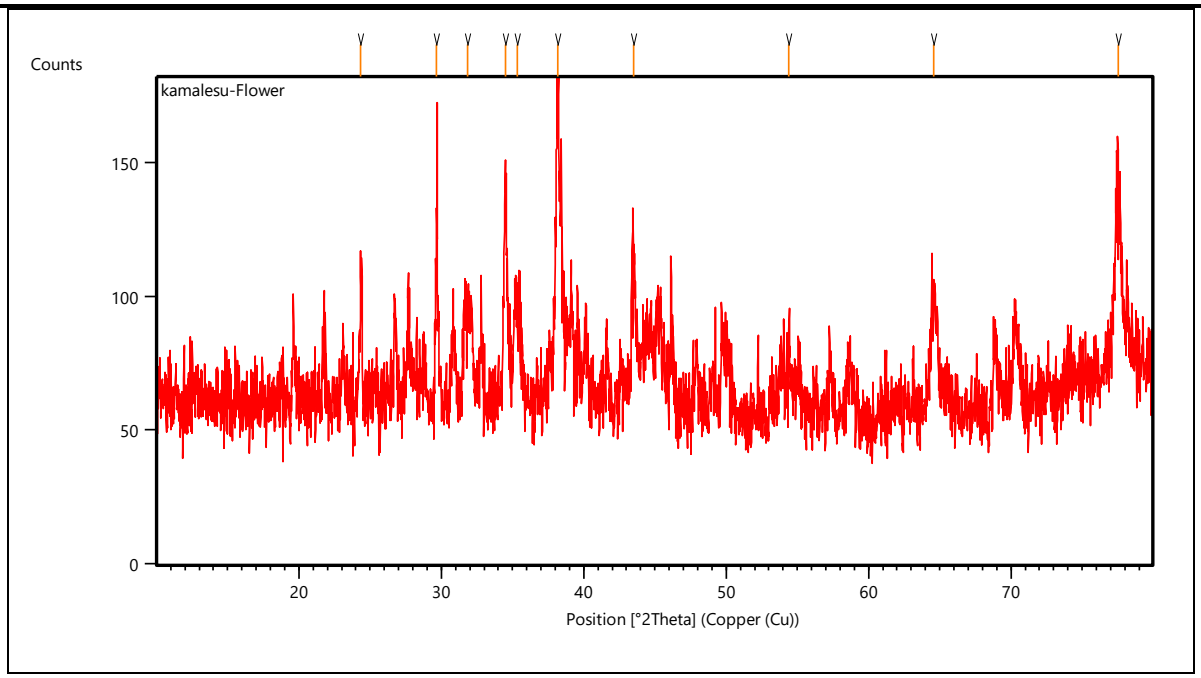


Fig.3. XRD pattern for silver Nano particles from flower extract of *Todalia asiatica*

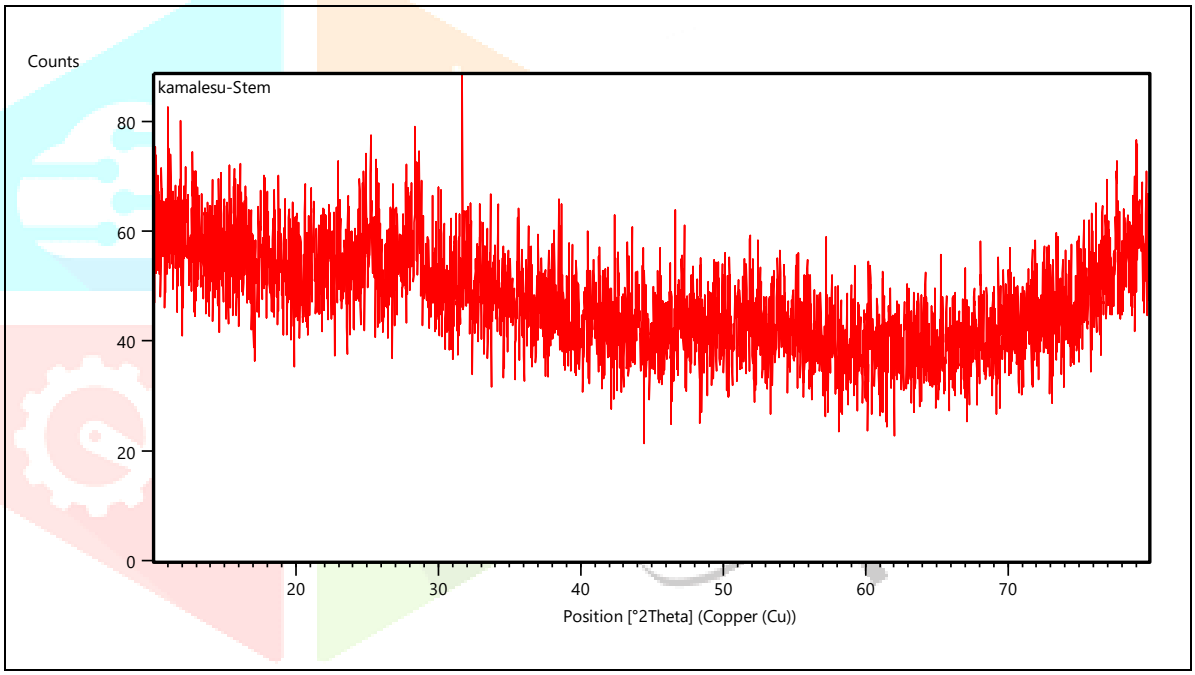


Fig.4. XRD pattern for silver Nano particles from stem extract of *Todalia asiatica*

SEM ANALYSIS

The shape of the synthesized silver nanoparticles was analysed by SEM Fig.5. The image obtained showed a uniform spherical shape of the Silver nanoparticles.

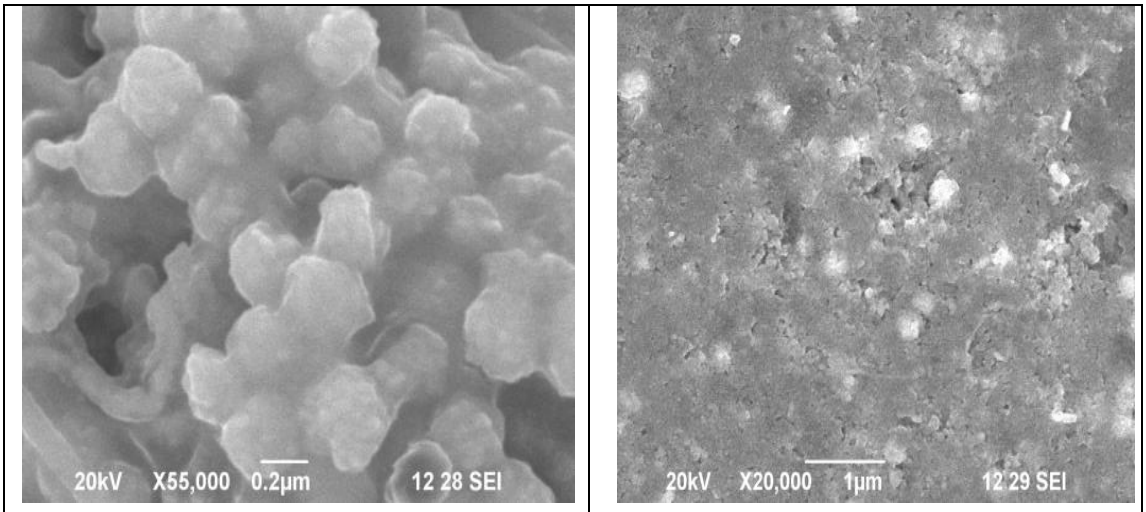


Fig.5 SEM image of the Silver Nano particles from Todalia asiatica leaf extract

Phytochemical examination of the leaf, flower and stem parts of *Toddalia asiatica* (L.)Lam.

A qualitative phytochemical screening Table.1 and also GC-MS analysis of aqueous extract of the three parts viz Leaf (Table.2) , Stem (Table.3) and Flower (Table.4) of *Toddalia asiatica* were carried out to establish the differences in the nature of phytoconstituents distribution within in the plant so as to correlate their capacity in the metal ion reducing propertyand the results are tabulated.

Table.1 phytochemical analysis of leaf, flower and stem parts of *Toddalia asiatica* (L.)Lam.

Phytochemical systems	**Quantity of chemical constituents present**		
	Leaf	**Flower**	**Stem**
Alkaloids			
1.Dragandorff's test	-ve	-ve	++ve
2.Mayer's test	-ve	-ve	-ve
3.Wagner's test	+ve	+ve	+ve
Phenolic compounds			
Lead acetate test	+++ve	++ve	+ve
Flavonoids			
1.Shinoda's test	+ve	-ve	-ve
2.Ferric chloride test	+ve	-ve	-ve
3.Sodium hydroxide test	+ve	+ve	+ve
Glycosides			
1.Killer killani test	-ve	+ve	++ve

2.Legal's test	-ve	-ve	-ve
Terpenoides			
Chloroform-H_2SO_4	-ve	-ve	-ve
Tannins			
Ferric chloride test	+++ve	-ve	++ve
Saponins			
Sodium carbonate test	++ve	+ve	-ve

Table.2 GC-MS result of leaf part of T*odalia asiatica*(L.)Lam.

4-hydroxy benzene sulfonic acid,
N-[Benzylidene]-2,2dimethylcyclopropanecarbonitrile
1,4-Benzenediol
5-[Hydroxymethyl]-2-furanecarrboxaldehyde
2,6-dimethoxy Phenol
4-hydroxy-3-methoxy Benz aldehyde
3-Methoxybenzoic acid,allyl ester
2-Propaneone,1-[4-hydroxy-3-methoxyphenyl]
4-hydroxy-3,5-dimethoxy Benz aldehyde,
2,4-Dihydroxyacetophenoneoxime
Pyrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-[phenyl methyl]
a-l-Galactopyranose,6-deoxycyclic1,2:3,4-bis[butylboronate]
Diazabicyclo[3,3,1]nonane9,9-dimethyl
9-Octadecenamide

Table.3 GC-MS result of Stem part of T*odalia asiatica*(L.)Lam.

Betulin
àlpha-cyperone
1-(Benzyloxy)-2-fluoro-2-phenyl-3-(p-toluenesulsulfonyloxy)propane
4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid
Isochiapin B
Lactaropallidin
Lupeol
Podocephalol
6-Octadecenoic acid, methyl ester
Solanesol
1-Tetradecanol (CAS)

Table.4 GC-MS result of Flower part of T*odalia asiatica*(L.)Lam.

Acetophenone
2- (Allyloxy)-1-Octadecyne
1,2- Dimethyl Benzene
1,2-Benzenedicarboxylic acid
1-Dodecanol
1-Eicosanol
Methanethioamide
Methyllaurate
Phytol
1-Undecyne

DPPH Free Radical Scavenging Activity

DPPH is used as a main substrate to evaluate antioxidant activity. DPPH assay is based on the measurement of ability of antioxidant towards DPPH radical. The method is based on a change in purple coloured ethanol solution of DPPH in presence of hydrogen donating antioxidants, by formation of yellow coloured non radical form. The scavenging ability of leaf (Table 5), fllower (Table 6) and stem (Table 7) parts of *Todalia asiatica* (L.) Lam.water extract was compared with Ascorbic acid and are presented.

The Percentage of inhibition can be calculated using the formula.
Inhibition (%) = 100- (Ao-A1/Ao) x 1
Where : Ao is the absorbance of control and A1 is the absorbance of test.

Table 5: Free radical scavenging activity of leaf part for *Todalia Asiatica*(L.)Lam.

Concentration of samples µg/ml	% of inhibition	Control % Ascorbic Acid
100	35	96
200	40	
300	57.5	
400	62.5	
500	75	

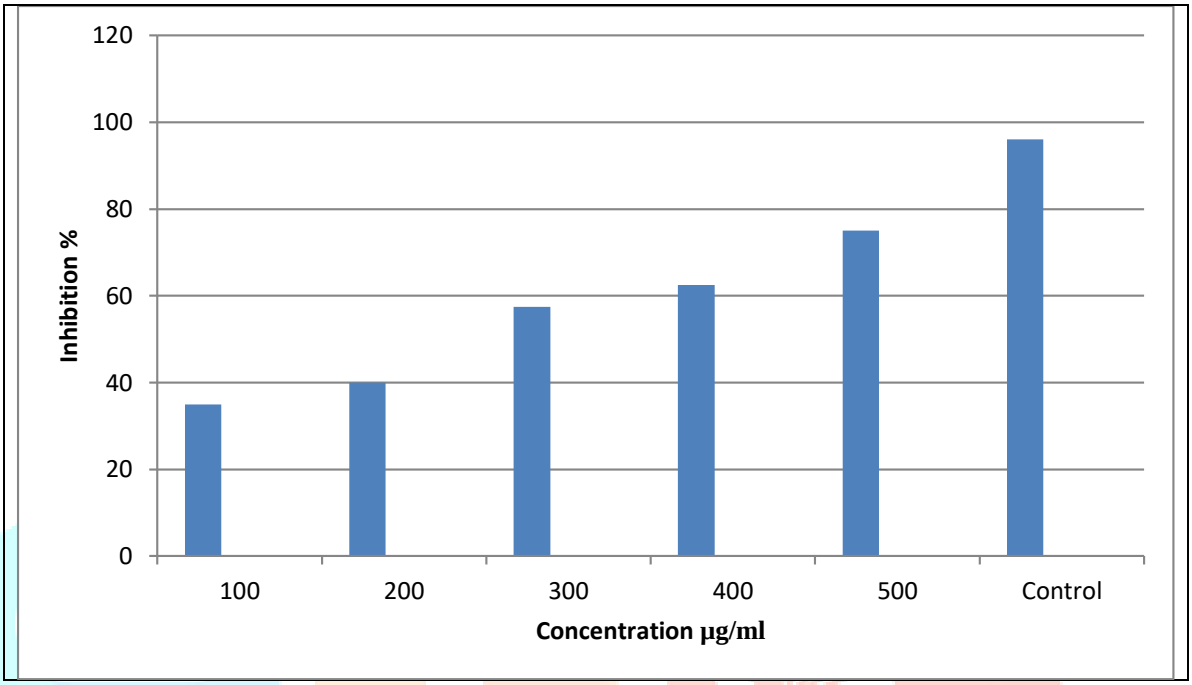


Table 6: Free radical scavenging activity of flower part of *Todalia Asiatica* (L.)Lam.

Concentration of samples µg/ml	% of inhibition	Control % Ascorbic Acid
100	32	96
200	38	
300	55.5	
400	60.5	
500	72	

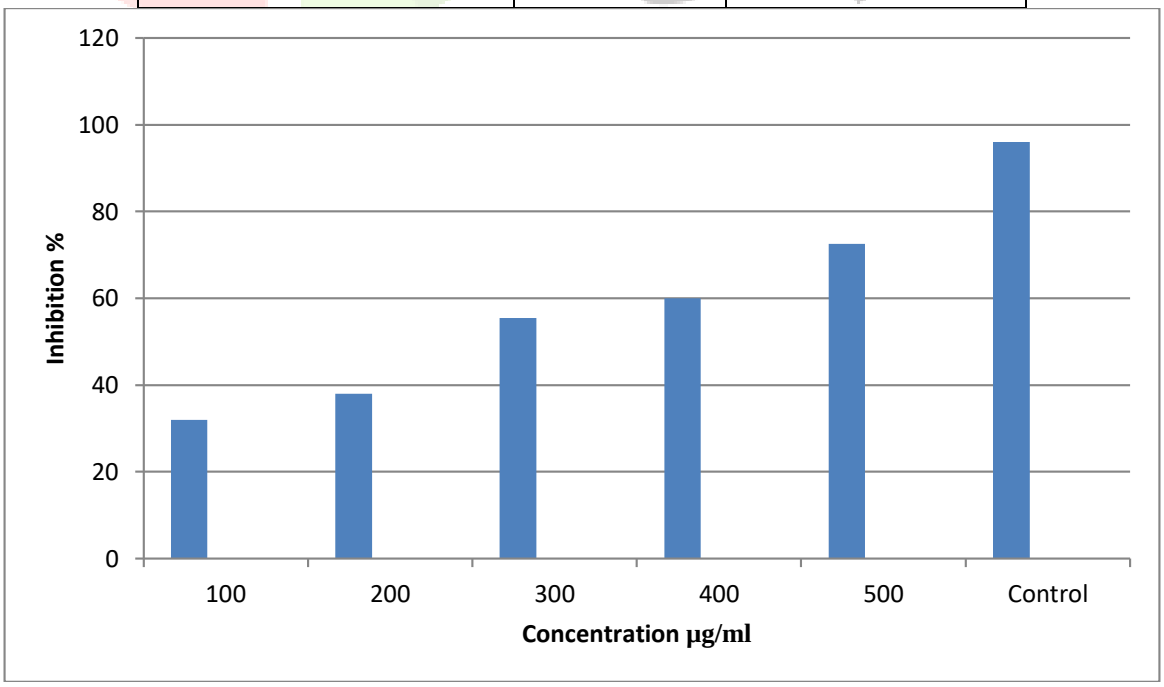
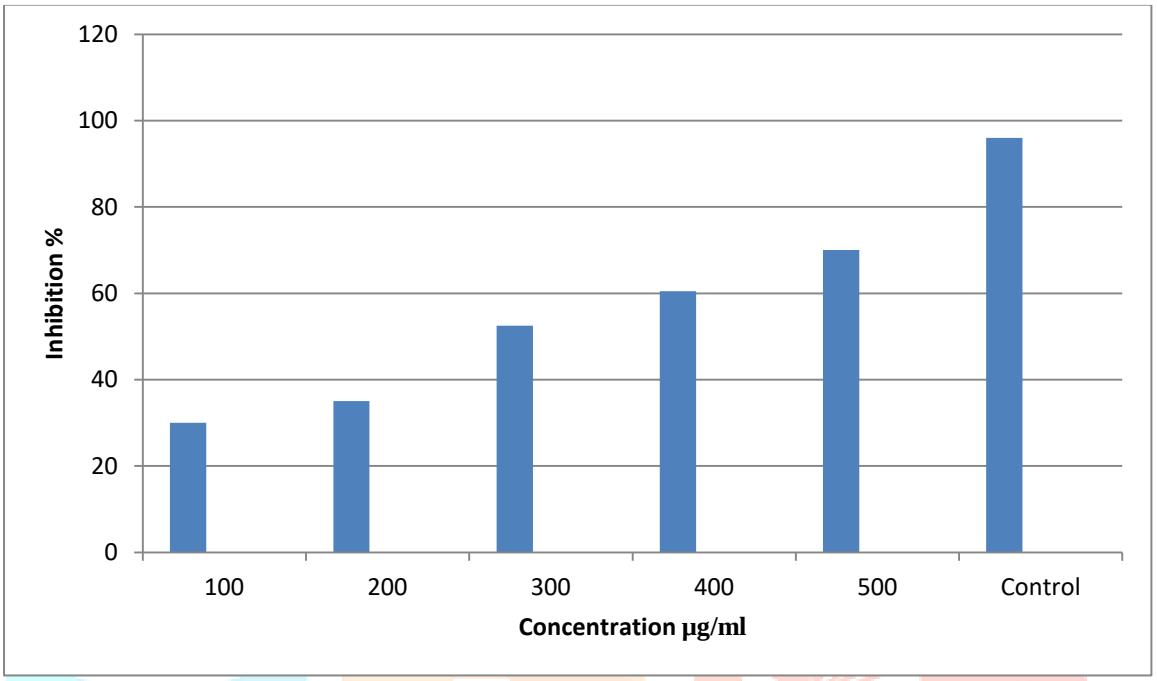


Table 7: Free radical scavenging activity of stem part of *Todalia Asiatica*(L.)Lam.

Concentration of samples µg/ml	% of inhibition	Control % Ascorbic Acid
100	30	96
200	37	
300	52	
400	60	
500	65	



Frap (Ferric Reducing Ability of Plasma)

Three patrs of the plant extract were taken in various concentrations like 0.1, 0.2, 0.3, 0.4 and 0.5ml with that 2.5ml of phosphate buffer and 2.5 ml of potassium ferricyanide is added to each. Ascorbic acid was used as a standard. Then all the samples and standard were heated to 50^0C for 20minutes. 2.5ml of trichloro acetic acid is added and centrifuged at 2000rpm for 10minutes. The supernatant 2.5ml was mixed with 2.5 ml of ferric chloride solution and the absorbance was measured at 700nm under UV visible spectrophotometer presented in Table 8-10.

Table 8. Frap of leaf part for *Todalia Asiatica*(L.)Lam.

Concentrtion of sample per µg/ml	FRAP scavenging activity Ethanol Fe^+/ µg	FRAP scavenging activity of control ascorbic acid Fe^+/ µg
100	0.55	0.82
200	0.62	0.84
300	0.78	0.88
400	0.83	0.90
500	0.92	0.95

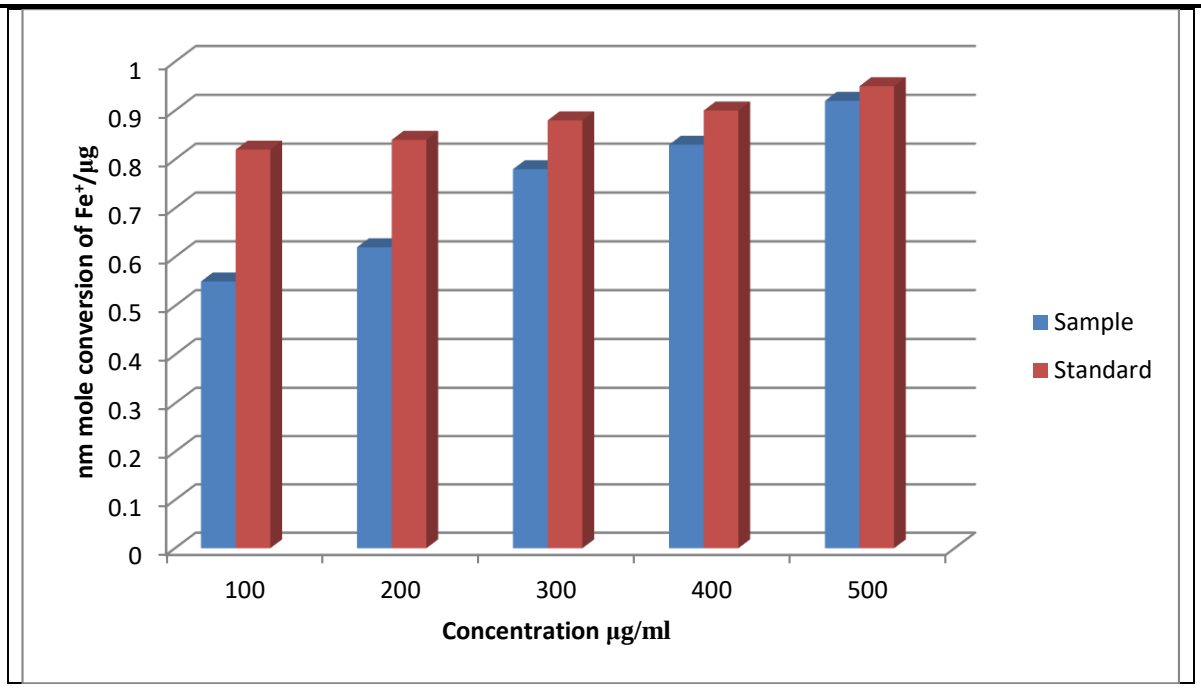


Table 9. Frap of Flower part for *Todalia Asiatica*(L.)Lam.

Concentrtion of sample per µg/ml	FRAP scavenging activity Ethanol Fe^{+}/ µg	FRAP scavenging activity of control ascorbic acid Fe^{+}/ µg
100	0.52	0.82
200	0.60	0.84
300	0.75	0.88
400	0.80	0.90
500	0.91	0.95

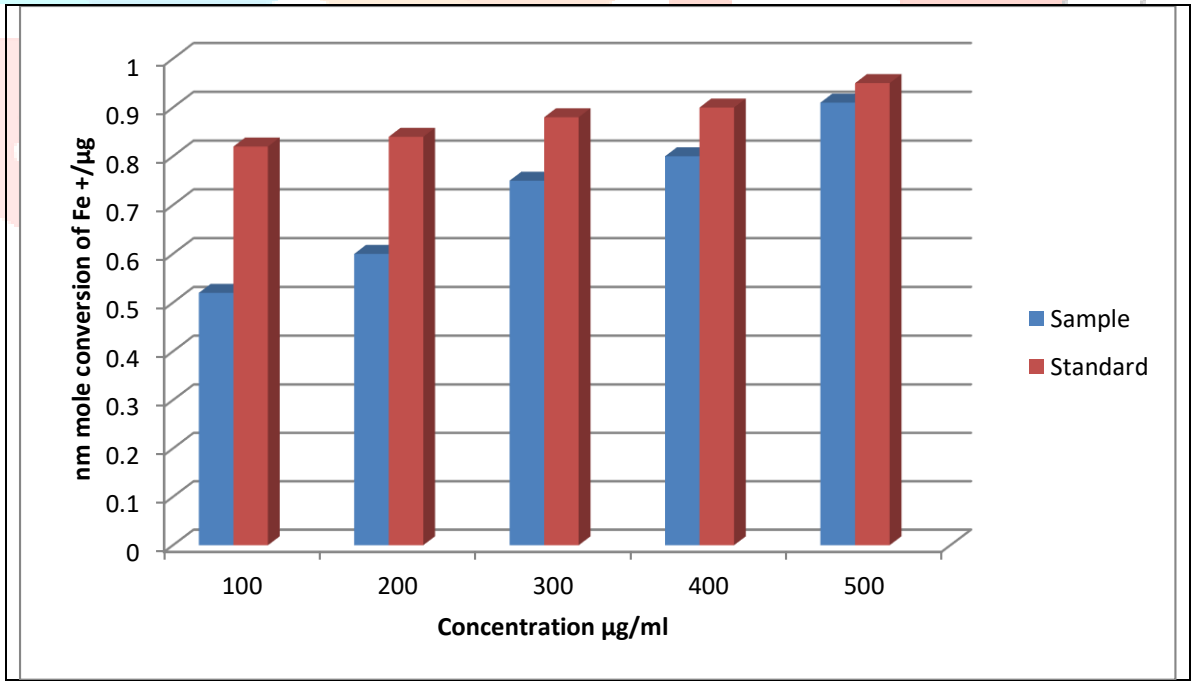
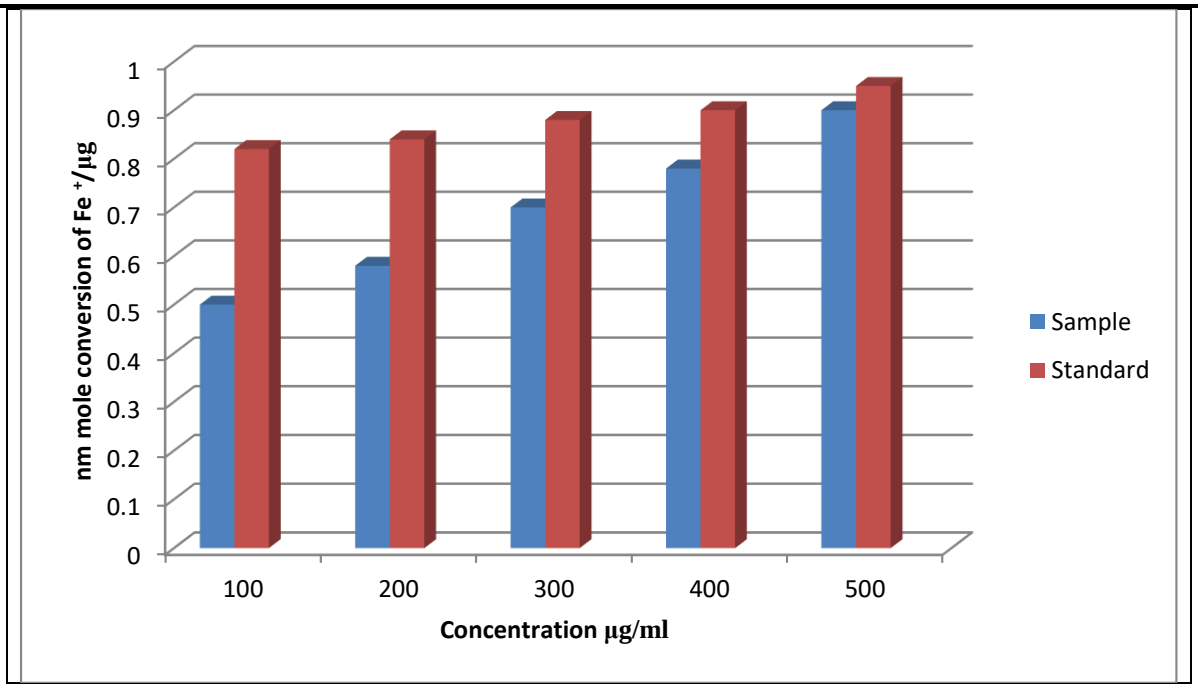


Table 10. Frap of Stem part for *Todalia Asiatica*(L.)Lam.

Concentrtion of sample per µg/ml	FRAP scavenging activity Ethanol Fe^{+}/ µg	FRAP scavenging activity of control ascorbic acid Fe^{+}/ µg
100	0.50	0.82
200	0.58	0.84
300	0.70	0.88
400	0.78	0.90
500	0.90	0.95



Superoxide Anion Scavenging Activity:

The superoxide anion scavenging activity is measured as described by Robak and Gryglewski(1988). The superoxide anion radicals are generated in 3.0 ml of Tris-HCJ buffer (16 mM.pH 8.0), containing 0.5 ml of NBT (o.3 mM), 0.5 ml NADH (0.036 mM) solution, 1.0 ml extract and 0.05 ml. Tris- HCI buffer (16 mM. pH 8.0). The reaction is started by adding 0.5 ml PMS solution(o.12 mM) to the mixture, incubated at 25 degree Celsius for 5 minutes and then the absorbance is measured at 560 mM) against a blank sample. Ascorbic acid is used as a positive control,

Formula: Inhibition (%)=(Ao-A1/Ao) x 100.

Antibacterial Assay

Bacterial strains such as *Bacillus subitilus, Escherchia coli, Psedumonas beteli, Psedumonas fluroscence, Salmonella paratyphi, Staphyllococcus aureus, Psedumonas putida.* were used for anti bacterial activity. The bacteria were incubated on a nutrient agar-slant for 48 hours at 37°C

The chloramphenicol and Tetracycline were used as standard antibiotics.The antibacterial activity was demonstrated using a modified method. All the tests were done by well diffusion method. The extracts of three different parts of the plant *Todalia asiatica* are impregnated with discs and placed on Nutrient Agar plates. These plates are already inoculated with 20 ml of Nutrient broth medium with Gram positive and Gram negative bacteria. Respective solvent without plant extracts served as negative control. Standard antibiotics were used as reference. Plates were incubated at 37 degree Celsius for 24 hours. The diameter of the inhibition zone around the leaf, flower and stem extracts were measured and compared with the standard antibiotics and the values are presented Table.11.

Table.11. Antimicrobial activity of Leaf, Stem and flower extracts of *Todalia Asiatica* (L.) Lam.

Name of the species	Zone of inhibition in mm				
Name of the species	Standard used	Leaf	Stem	Flower	Standard
Escherichia coli	Chloromphenical	20	11	10	25
Pseudomonas Fluorescence	Tetracycline	16	12	07	25
Pseudomonas putida	Chloromphenical	20	15	11	30
Pseudomonas beteli	Chloromphenical	13	-	05	20
Salmonella Paratyphi	Tetracycline	16	07	-	19
Bacillus subitilus	Tetracycline	12	08	-	17
Staphylococcus Aureus	Tetracycline	12	09	07	18

Result and Discussion

In the present study green synthesis of silver nanoparticles using aqueous extract of different parts leaf, flower, and stem of the plant *Todalia asiatica* **(**L.)Lam.was performed. Depends upon the amount of reducing and stabilizing agent present in the different parts of the plant conversion of Ag ions into silver nanoparticle was observed. Phytochemicals present in different parts of the plant was analyzed using qualitative analysis and GC-MS analysis. The percentage conversion of Ag^+ ion into silver nanoparticles was attempted to correlate with the diverse distribution of the active principles in the various parts of the plant. Due to complexity of the type of chemical moiety distribution in various parts of the plant, a conclusion on the compounds favoring the conversion is highly tedious to conclude.

The antioxidant activity of the aqueous extract of three parts of the plant was evaluated using *α,α*-Diphenyl-*β*- picryl-hydrazyl radical scavenging (DPPH) assay, Ferric reducing ability of plasma(FRAP) and Superoxide anion Scavenging activity of leaf ,flower and stem extract of *Todalia asiatica* **(**L.)Lam. is proportionally increasing with the increase in the concentration of the extract. The antibacterial activity of the three parts of the plant were also examined against the bacterial strains of *Escherchia coli, Psedumonas beteli, Psedumonas fluroscence, Salmonella paratyphi, Staphyllococcus aureus and Bacillus subitilus.* It was

observed that the plant extracts possesses moderate activity against all the strains except the flower extract inactiveness against*Salmonella paratyphi and Bacillus subitilus.*

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