

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLE FROM *Camelia sinensis* LEAF AND EVALUATING ITS ANTIMICROBIAL, ANTIOXIDANT ACTIVITY AGAINST UROPATHOGENIC *Escherichia coli* (UPEC)

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Abstract

Urinary tract infection (UTI) is one of the most common bacterial infections in everyday clinical practice. Herbal medicine was used to treat a variety of illnesses, including UTIs. In the present investigation, biosynthesis and characterization of silver nanoparticle of *Camelia sinensis* leaf was analyzed for antimicrobial and antioxidant property. The colonies identified as *E. coli* were positive for haemolysin, haemagglutination (MRHA) & cell surface hydrophobicity aggregated in concentration of 1.4M. *C. sinensis* leaves are rich in carbohydrates, proteins, phenols act as excellent antimicrobial and antioxidant agent. Quantification of antioxidant property observed in crude extract & synthesized silver of *C. sinensis* leaves using DPPH-free radical scavenging assay with IC₅₀ value of 91.8. Antimicrobial activity carried out for both crude extract & silver nanoparticles from *C. sinensis* significant antimicrobial activity observed for both crude extract & silver nanoparticles. The result revealed DMSO₄ extract of silver nanoparticles of *C. sinensis* was found to be effective with a maximum zone of inhibition at 200µl (2.2mm) when compare to crude extract even the aqueous and methanolic extract of silver nanoparticle was effective at 150µl. The findings of this study, suggest that the biosynthesized silver nanoparticles from *C. sinensis* leaves can be developed in to a promising drug candidate for biomedical applications.

Key words: Camellia sinensis, silver nanoparticle, hemolysin, antimicrobial.

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Introduction

Urinary tract infections commonly affect men and women of all ages, and vary dramatically in their presentation and sequelae. UTIs can occur in any population and at any age, but the risk of UTIs is greatest in women of childbearing age. According to a recent study, 40–50% of women experience at least one clinical episode throughout their lifetime. 50% to 90% of all simple urinary tract infections are caused by *E. Coli*, the most common urine pathogen. The bacteria that cause urinary tract infections generally ascend from the rectal reservoir. The pool for the start of a UTI is provided by *E.coli* that is present in the digestive system as commensals. Uropathogenic *E.coli* (UPEC) is specific serotypes of *E.coli* that have been consistently linked to uropathogenicity. There are several virulent factors in *E.coli* which help to differentiate pathogenic from non pathogenic. There are two UTI infections are Acute pyelonephritis and asymptomatic bacteriuria (ABU). Acute urinary tract infections are among

the most common bacterial infections in women presenting in primary care (Akortha et al., 2008). Uropathogenic *Escherichia coli* causes a systemic infection called acute pyelonephritis and asymptomatic bacteriuria. Especially green tea, have historically been attributed to a wide group of plant chemicals called polyphenols, which includes catechins. Epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) are the major catechins. The catechin epigallocatechin-3-gallate (EGCG) is the most potent and prevalent in green tea. An intricate polyphenol green tea's antibacterial activities are due to its polyphenols, primarily the flavonoids, particularly catechins. (Vuong et al. (2011) also reported that green tea extract is very effective in treating Urinary tract infections. Hsu et al. (2003) reported that the highest antimicrobial activity of green tea is due to the presence of catechins and polyphenes which damage bacterial cell membrane. The use of biological microorganisms or plant extracts in green synthesis techniques has become a

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popular and straightforward substitute for chemical synthesis. The green synthesis approach typically entails three primary steps: choosing a solvent medium, choosing an environmentally friendly reduction agent, and choosing non-toxic materials for nanoparticle stability.

MATERIALS AND METHODS

COLLECTION OF SAMPLE

The clinical materials for the present study were obtained from Annai Teresa Hospital in Chennai. A total of 100 urine samples from suspected urinary tract infected patients and 50 stool samples from healthy individuals were studied. Clean-catch midstream urine was collected as follows for microbiological examination. 5 grams of stool samples were collected in a wide-mouthed clean container.

IDENTIFICATION OF BACTERIAL ISOLATES

Isolates were first sub-cultured on MacConkey agar and incubated for 24 h at 37°C. Pink colonies of *E. coli* were then sub-cultured on nutrient agar and incubated for 24 h at 37°C. Discrete colonies of *E. coli* were then purified through successive streaking on nutrient agar. The primary culture was maintained on agar slants at 4°C. Working cultures for experiments were prepared by transferring a loopful of the test organism (*E. coli*) to a nutrient agar plate using a sterile wire loop and incubated at 37°C for 24 hrs and the growth was identified using standard techniques and biochemical tests were performed.

DETECTION OF VIRULENCE FACTORS

1. HEMOLYSIN

The method is according to Raksha et al. Hemolysin was detected by determining a zone of lysis around each colony on 5% sheep blood agar plates after overnight incubation.

2. HAEMAGGLUTINATION TEST

The test was performed by mixing one drop of carefully mixed bacterial culture with one drop of RBC suspension on a glass slide. UPEC serotypes O6 was used as MRHA positive control.

3. CELL SURFACE HYDROPHOBICITY

SALT AGGREGATION TEST

E. coli grown on MacConkey agar plate were inoculated into 1 mL of PBS pH 6.8 and turbidity were matched with McFarland tubes 6 and 7 to get a colony count of 5×10^9 [9] colonies/mL. Different molar concentrations of ammonium sulphate were prepared. The column formed in different molar concentrations of ammonium sulphate was observed under microscope.

PLANT SAMPLE COLLECTION AND PROCESSING

Camellia sinensis leaves were collected from Munnar tea plantation, Kerala. Collected leaves were washed with tap water then rinsed with

distilled water, dried, Cut into fine pieces and were crushed into fine powder and stored at 37 °C.

PREPARATION OF *Camellia sinensis* LEAVES EXTRACTED BY SOXHLET EXTRACTION METHOD

The dried plant material was pulverized into fine powder using a mixer and about 50g of powder material was extracted in soxhlet extraction apparatus with 250ml of Methanol and Aqueous solvents. These extracts obtained with each solvent were filtered; solvent has been poured into petridish and kept at room temperature. The sticky greenish-brown substances were scraped and collected a pure plant extract in an Eppendorf tube and some has been stored in liquid form in a centrifuge tube stored in refrigerator for prior use.

SCREENING OF PHYTOCHEMICAL COMPONENTS

TEST FOR ALKALOIDS

1. Mayer's test

Two drops of Meyer's reagent are added to a few ml of plant sample extract on the walls of the tube. The appearance of a creamy white precipitate indicates the presence of alkaloids.

2. Test for Phenols compounds

Ferric chloride test

3-4 drops of ferric chloride solution were added to the extract. The formation of a bluish-black color indicates the presence of phenols.

3. Test for Flavonoids

Alkaline reagent

The extracts were treated with a few drops of sodium hydroxide solution. The formation of deep yellow color, which becomes colorless when dilute acid is added, indicates the presence of flavonoids.

4. Test for Steroids

To 1ml of the test solution was added an equal volume of chloroform and 3 drops of concentrated sulfuric acid. The formation of a brown ring indicates the presence of steroids.

5. Test for Tannins

To 0.5 ml of the extracted solution was added 1 ml of water and 1-2 drops of ferric chloride solution. A blue discoloration was observed for gallus tannis and a black discolouration for catechol tannis.

6. Test for terpenoids

2ml of the extract was mixed with 2ml of chloroform in a test tube. To this, 3ml of concentrated sulfuric acid was added along the tube walls to form a layer and the red-brown contact surface confirmed the presence of terpenoids.

7. Test for Glycosides

Glacial acetic acid, a few drops of concentrated ferric chloride was added to the extract solution and a red-brown color was obtained at the interface of the two layers and a bluish-green color at the top, indicating the presence of glycosides.

8. Saponins

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Approximately 1ml of the test sample was separately diluted to 20ml with distilled water and stirred in a graduated cylinder for 3 minutes. A 1cm foam after 10 minutes indicates the presence of saponins.

QUANTIFICATION OF ANTIOXIDANT ACTIVITY

DPPH-(2, 2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

DPPH solution was prepared by adding DPPH (12.5mg) to 50ml of DMSO. The absorbance of this stock solution was measured at 317 nm. The DPPH solution was diluted with methanol to an absorbance of 0.98 and stored in an amber bottle ready for use. An aliquot of 100 μ L of *Camellia sinensis* leaf extract at different concentrations (10, 25, 50, 75, 100, 150 μ g/ml prepared in DMSO) was added to 1ml of DPPH solution from stock C1 to test tube 1 and discard the pipette or tip and The reaction mixture was incubated at 37°C in darkness for 30min. The decrease in absorbance was determined at 517nm. Ascorbic acid served as a standard. The reaction was carried three times. Scavenging activity was the calculated by using the equation was performed in triplicate. Scavenging activity was calculated by using the equation; where Q represents the DPPH scavenging activity. The results were noted.

BIOSYNTHESIS OF SILVER NANOPARTICLE

10 ml of water, methanol, and DMSO Extract of *Camellia sinensis* were taken. Added 90 ml of 1 Mm aqueous AgNO_3 solution in 250ml conical flask. Incubated with continuous stirring process at 28°C for 24 hours. Bio reduction of silver nitrate to silver nanoparticle can be confirmed. Visually, by the change of color to colourless to dark brown. Color change indicated the formation of silver nanoparticle by *Camellia sinensis* extract.

CHARACTERIZATION OF BIOSYNTHESIZED SILVER NANOPARTICLES

UV-VISIBLE SPECTROPHOTOMETRIC ANALYSIS OF SILVER NANOPARTICLES

The bio-reduction of Ag^+ ions in the solution was monitored by periodical sampling of aliquots and the UV-Visible spectra for these aliquots were monitored as a function at time of reaction in 250-900nm range operated at a resolution of 1nm. Distilled water was used as a blank (Sengottaiyan A et.al.,2016). The spectrum of the silver nanoparticles were plotted.

SILVER NANOPARTICLES IN THE INFRARED (FTIR) WITH FOURIER TRANSFORM

FT-IR spectral analysis was carried out to identify the possible bio molecules responsible for the reduction of Ag^+ ions and capping of AgNPs synthesized by *Camellia sinensis* extract. The chemical composition of silver nanoparticles were analyzed using FTIR spectrometer after bio reduction with silver nitrate. 2ml of bio synthesized liquid silver nanoparticles were scanned using infrared in the range of 400-4000 cm^{-1} . The results were noted.

SEM ANALYSIS OF AgNO_3 NPs

In order to get exact information about the crystal structure, surface morphology, particle size etc, the sample has been observed under SEM (Scanning Electron Microscope).

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Similar to the disk diffusion method, the surface of a Muller-Hinton agar plate is inoculated by spreading an appropriate amount of microbial inoculum over the entire surface of the agar.

A volume (100,150,200 μ l) of the *Camellia sinensis* crude extract solution and AgNPs synthesized extract solution at a desired concentration is introduced into the well. The agar plates are incubated. The antibacterial agent diffuses in the agar medium and inhibits the growth of the microorganism strain tested. The results were noted.

MICRODILUTION ANTIBACTERIAL ASSAY

MINIMUM INHIBITOR CONCENTRATION (MIC)

Put the content into 50ml conical flask, add 15ml sterile distilled water. Dissolve the *Camellia sinensis* powder in sterile distilled water so as to obtain a concentration of 1000 μ g/ml. In a well arrange a row of 10 sterile test tubes (15x170) and label them C1-C10, to all the test tubes in a rack add 2ml of Muller Hinton Broth. To 8.72 ml of Muller Hinton Broth in another test tube add 1.28ml of stock c label this as a stock C1 Transfer 2ml μ g/ml prepared in DMSO) was added to 1ml of DPPH solution from stock C1 to test tube 1 and discard the pipette or tip and continue the doubling dilutions up to 9th and every time using 7ml tips. Store them for incubating to observe the turbidity.

RESULTS

Of the 100 urine samples in which E.coli was isolated, 80 had significant bacteriuria with count 10^5 colonies/mL and 20 had probably significant bacteriurias with counts between 10^4 - 10^5 colonies/mL. PRELIMINARY TESTS AND GROWTH ON

MEDIAS

Gram stain : Gram negative rods, Motility : Motile, Catalase Positive, Oxidase : Negative, NA : large, thick grayish white moist smooth opaque translucent colonies, MAC : Lactose fermenting colonies.

BIOCHEMICAL REACTIONS

Organism : *Escherichia coli*, Indole : Positive, Methylene red Positive, Voges Proskauer : Negative, Citrate : Negative, TSI : A/AGas +ve No. H_2S .

TABLE 1 PHYTOCHEMICAL ANALYSIS FOR *Camellia sinensis* LEAF EXTRACT

S.N	Phytochemical test	Name test	Observation	Extract	Inference
1)	Alkaloids	Mayers	Formation of white creamy precipitate	Methanol	+
				Aqueous	-
2)	Flavonoid	Alkaline Reagent test	Formation of Intense Yellow Colour	Methanol	+
				Aqueous	+
3)	Phenols	Ferric Chloride test	Formation of Bluish Colour	Methanol	+
				Aqueous	+
4)	Tannins	Gelatin	Formation of white precipitate	Methanol	+
				Aqueous	+
5)	Terpenoids	Salkowski test	Formation of reddish brown colour	Methanol	+
				Aqueous	+
6)	Glycosides		Formation of yellow colour	Methanol	+
				Aqueous	+

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7)	Steroid test		Formati	Methan	+
			red colo	Aqueou	+
8)	Saponin		Formati	Methan	+
			Foam	Aqueou	-

TABLE 2: Antioxidant Activity from *Camellia sinensis* Leaf Extract Using DPPH Radical Scavenging Assay

S.NO	CONCENTRATION THE SAMP	INHIBITION% DPPH scavenging ef AgNPs sample
1	10µl	6.5%
2	25 µl	15.7%
3	50 µl	29.0%
4	100 µl	53.1%
5	150 µl	80.8%
6	IC ₅₀	91.8 µl

FIG-1: ANTIOXIDANT ACTIVITY OF *Camellia sinensis* USING DPPH SCAVENGING ASSAY

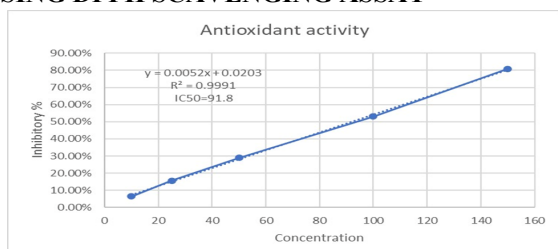


FIG-2 BIO SYNTHESIS OF *Camellia sinensis* SOLVER



NANOPARTICLES

A)water and methanolic extract of *Camellia sinensis*

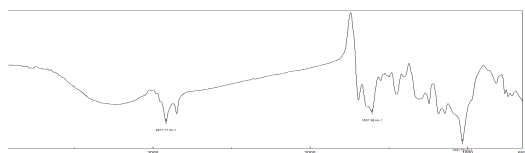
Camellia sinensis

TABLE 3 FTIR ANALYSIS OF *Camellia sinensis* LEAF EXTRACT

Methanol extract

Freque ncy Range	Absorp tion	Appeara nce	Grou p	compo und name
3000-2850cm ⁻¹	2917.77 cm ⁻¹	Medium	C-H stretch	Alkanes
1650-1580cm ⁻¹	1607.38 cm ⁻¹	Medium	N-H bend	1° amines
1250-1020 cm ⁻¹	1031.73 cm ⁻¹	Strong	C-N stretch	Aliphatic amines

FIG-3 FTIR SPECTRA OF CRUDE EXTRACT OF *Camelia sinensis* LEAF EXTRACT



The FT-IR spectra source showed the first band stretching at **2917.77cm⁻¹** indicating an C-H stretch of alkanes besides presence of N-H bending at **1607.38 cm⁻¹** and band at **1031.73cm⁻¹** corresponds to C-N stretch Aliphatic amines.

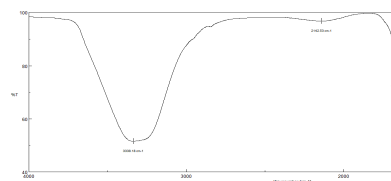


TABLE 4 FTIR ANALYSIS OF SILVER NANOPARTICLES SYNTHESIZED *Camellia sinensis* LEAF EXTRACT

B)DMSO extract

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FIG- 4 FTIR SPECTRA OF AgNPs SYNTHESIZED FROM *Camellia sinensis* LEAF EXTRACT



The FT-IR spectra for AgNO₃ nanoparticles extract shows that first band stretching at 3338.18 cm⁻¹ indicating an O-H stretch Alcohol, Phenols besides N-H bending there is a presence of 1° Amines at 1637.27cm⁻¹ and a band at 1016.3 cm⁻¹ corresponds to C-O stretch of Alcohols, carboxylic acid, Esters, Ethers.

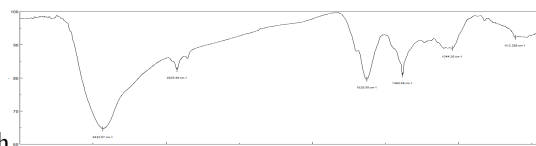
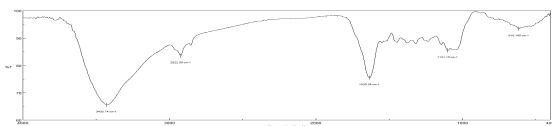
TABLE 5 FTIR ANALYSIS OF *Camellia sinensis* LEAF EXTRACT Water extract

Frequency Range	Absorption	Appearance	Group	compound name
3500-3200 cm ⁻¹	3338.18 cm ⁻¹	Strong, Broad	O-H stretch	Alcohols, phenols
1650-1580 cm ⁻¹	1637.27 cm ⁻¹	Medium	N-H bend	1° Amines
1320-1000 cm ⁻¹	1016.3 cm ⁻¹	Strong	C-O stretch	Alcohols, carboxylic acid, Esters, Ethers.

Frequency Range	Absorption	Appearance	Group	compound name	Frequency Range	Absorption	Appearance	Group	compound name
3500-3200 cm ⁻¹	3430.74 cm ⁻¹	Strong, Broad	O-H stretch, H-bonded	(Alcohols, phenols)	1600-1585 cm ⁻¹	1635.34 cm ⁻¹	Medium	C-C stretch (in ring)	Aromatics
3300-3200 cm ⁻¹	2922.59 cm ⁻¹	Medium	C-H stretch	Alkanes	1320-1290 cm ⁻¹	1101.15 cm ⁻¹	Strong	C-O stretch	Alcohols, carboxylic acid, Ester, Ethers.
					690-515 cm ⁻¹	616.145 cm ⁻¹	Medium	C-Br stretch	Alkyl halides

FIG-6 FTIR SPECTRA OF CRUDE EXTRACT OF *Camellia sinensis* LEAF EXTRACT

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FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticle synthesized. The FT-IR spectra source showed the first band stretching at **3430.74** cm⁻¹ indicating an O-H stretch, H-bonded of Alcohols, phenols besides presence of C-H stretch at **2922.59** cm⁻¹ and band at **616.145** cm⁻¹ corresponds to C-Br stretch Alkyl halides.

The FT-IR spectra for AgNO₃ nanoparticles extract shows that first band stretching at **3432.67** cm⁻¹ indicating an O-H stretch Alcohol, Phenols besides C-H stretch there is a presence of Alkanes at **2925.48** cm⁻¹ and a band at **612.288** cm⁻¹ corresponds to C-Br stretch of Alkyl halides.

TABLE 6 FTIR ANALYSIS OF SILVER NANOPARTICLES SYNTHESIZED *Camellia sinensis* LEAF EXTRACT

Frequency Range	Absorption	Appearance	Group	compound name
3500-3200 cm ⁻¹	3432.67 cm ⁻¹	Strong, Broad	O-H stretch, H-bonded	Alcohols, phenols
3000-2850 cm ⁻¹	2925.48 cm ⁻¹	Medium	C-H stretch	Alkanes
1650-1580 cm ⁻¹	1628.59 cm ⁻¹	Medium	N-H bend	1° Amines
1320-1000 cm ⁻¹	1383.68 cm ⁻¹	Strong	C-H stretch	Alcohols, Carboxylic acid, Esters, Ethers.
1250-1020 cm ⁻¹	1044.26 cm ⁻¹	Medium	C-N stretch	aliphatic amines
690-515 cm ⁻¹	612.288 cm ⁻¹	Medium	C-Br stretch	Alkyl halides

FIG-7 FTIR SPECTRA OF AgNPs SYNTHESIZED FROM *Camellia sinensis* LEAF EXTRACT

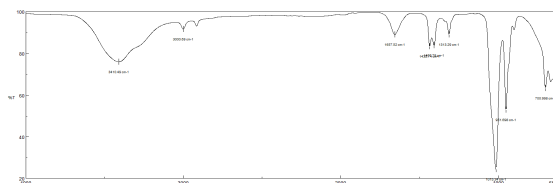
TABLE 7 FTIR ANALYSIS OF *Camellia sinensis* LEAF EXTRACT DMSO extract

Frequency Range	Absorption	Appearance	Group	compound name
3500-3200 cm ⁻¹	3410.49 cm ⁻¹	Strong, Broad	O-H stretch, H-bonded	alcohols, phenols
3100-3000 cm ⁻¹	3000.69 cm ⁻¹	Strong	C-H stretch	aromatics
1680-1640 cm ⁻¹	1657.52 cm ⁻¹	Medium	-C=C- stretch	alkenes
1550-1475 cm ⁻¹	1435.74 cm ⁻¹	Strong	N-O asymmetric stretch	nitro compound
1500-1400 cm ⁻¹	1407.78 cm ⁻¹	Medium	C-C stretch (in ring)	aromatics
1360-1290 cm ⁻¹	1313.29 cm ⁻¹	Medium	N-O symmetric stretch	nitro compound
1250-1020 cm ⁻¹	1015.34 cm ⁻¹	Medium	C-N stretch	aliphatic amines

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cm ⁻¹				
1000-650 cm ⁻¹	951.69	Strong	=C-H bend	alkenes
850-550 cm ⁻¹	700.98	Broad, Strong	C-Cl stretch	alkylhalides

FIG-8 FTIR SPECTRA OF CRUDE EXTRACT OF *Camellia sinensis* LEAF EXTRACT

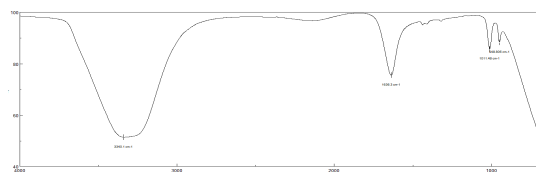


The FT-IR spectra showed the first band stretching at **3410.49 cm⁻¹** indicating an O-H stretch, H-bonded of Alcohols, phenols besides presence of C-H stretch at **3000.69 cm⁻¹** and band at **700.988 cm⁻¹** corresponds to C-Cl stretch alkylhalides.

TABLE 8: FTIR ANALYSIS OF SILVER NANOPARTICLES SYNTHESIZED *Camellia sinensis* LEAF EXTRACT

Frequency Range	Absorption	Appearance	Group	Compound name
3500-3200 cm ⁻¹	3340.1 cm ⁻¹	Strong, Broad	O-H stretch	alcohols, phenols
1650-1580 cm ⁻¹	1636.3 cm ⁻¹	Medium	N-H bend	1° amines
1320-1000 cm ⁻¹	1011.46 cm ⁻¹	Strong	C-N stretch	Alcohols, carboxylic acid, Esters, Ethers.
950-910 cm ⁻¹	948.806 cm ⁻¹	Medium	O-H bend	carboxylic acid

FIG-9 FTIR SPECTRA OF AgNPs SYNTHESIZED FROM *camellia sinensis* LEAF EXTRACT

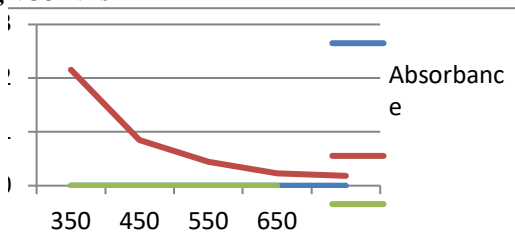


The FT-IR spectra for AgNO₃ nanoparticles extract shows that first band stretching at **3340.1 cm⁻¹** indicating an O-H stretch Alcohol, Phenols besides N-H bend there is a presence of 1° amines at **1636.3 cm⁻¹** and a band at **948.806 cm⁻¹** corresponds to O-H bend of carboxylic acid.

TABLE 9: CHARACTERIZATION OF DMSO AgNPs EXTRACT OF *Camellia sinensis* LEAF EXTRACT USING UV VISIBLE SPECTROSCOPY

Wave	Absorption
350	0.2157
450	0.0854
550	0.0442
650	0.0226
750	0.0182
850	0.0151
950	0.0040

FIG-10 UV-VISIBLE SPECTROSCOPY ANALYSIS OF AgNO₃ NPs



The UV-Visible spectrum showed a peak of Silver nanoparticles at 350nm which reflects the size & shape of AgNPs and the absorption spectrum obtained showed a strong surface Plasma resonance band maximum at 350nm a characteristic peak of silver nanoparticles. The absorption maxima were obtained around between 350nm to 450nm indicates the particles are poly dispersed.

FIG -11 SEM IMAGES INDICATES THE PRESENCE OF SILVER NANOPARTICLES SYNTHESIZED IN LEAF EXTRACT OF *Camellia Sinensis*.

TABLE 10:ANTIMICROBIAL SCREENING OF CRUDE *camellia sinensis* LEAF EXTRACT AGAINST UTI *E.COLI*

UTI <i>E.COLI</i>	Zone of inhibition(mm)			
	control	100µl	150µl	200µl
Aqueous extract	-	-	-	-
Methanolic extract	-	-	-	-
DMSO extract	-	0.4mm	0.6mm	0.8mm

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FIG-12 ANTIMICROBIAL SCREENING OF CRUDE *camellia sinensis* LEAF EXTRACT AGAINST UTI *E.COLI*

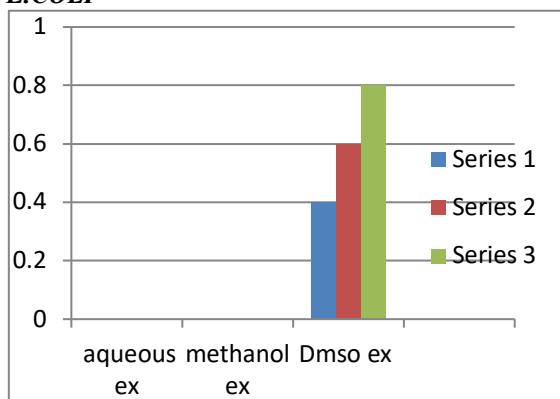


FIG-13 ANTIMICROBIAL SCREENING OF AgNPs SYNTHESIZED *camellia sinensis* LEAF EXTRACT AGAINST UTI *E.COLI*

Aqueous extract
extract



DMSO extract

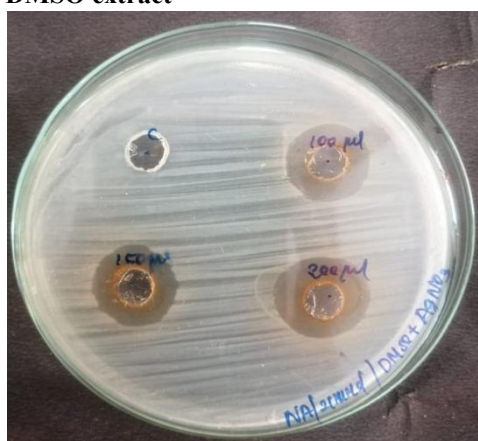
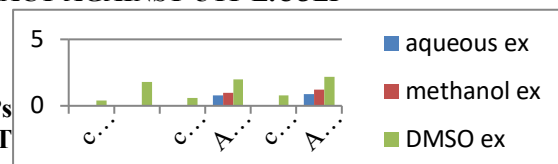


TABLE 11: ANTIMICROBIAL ACTIVITY –COMPARISON OF CRUDE AND AgNPs SYNTHESIZED *camellia sinensis* LEAF EXTRACT AGAINST UTI *E.COLI*

UTI <i>E.CO</i>	Zone of inhibition(						
	Con	Cru	AgN	Cru	AgN	Cru	AgN
		100µ	100µ	150µ	150µ	200µ	200µ

Aqueous ext	-	-	-	-	0.8m	-	0.9n
Methanolic extract	-	-	-	-	1mm	-	1.2n
DMSO extra	-	0.4r	1.8r	0.6m	2mm	0.8n	2.2n

FIG 14:ANTIMICROBIAL ACTIVITY –COMPARISION OF CRUDE AND AgNPs SYNTHESIZED *camellia sinensis* LEAF EXTRACT AGAINST UTI *E.COLI*



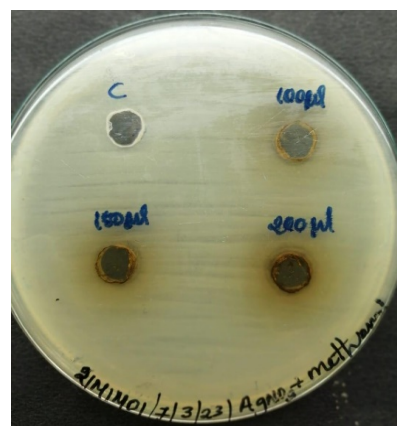
Methanolic

The result revealed DMSO extract of Silver nanoparticles of *Camellia sinensis* of found be effective with a maximum zone of Inhibition at 200µl (2.2mm)when compare to crude extract even the aqueous and methanolic extract of Silver nanoparticle was effective at 150µl were as no zone of inhibition at the concentration of 100 µl crude extract.

DISCUSSION

Urinary tract infection (UTI) is one of the most common bacterial infections

encountered in clinical practice in clinical practice in Europe and North America. It is estimated that 150 million cases of UTI occur on a global basis per year resulting in more than 4 billion pounds in direct healthcare expenditure. UTIs often recur in healthy young women, although traditionally classified as acute infections. Conventional wisdom assumes that the uropathogens that cause recurrent infections in these individuals originate from the stool or vaginal flora in the same way as the initial infection. Cell morphology and molecular biology studies have revealed that uropathogenic *E.coli* express several surface structures and secrete protein molecules, some of them being cytotoxic, peculiar to the strains of *E.coli* causing UTI (Steadman et al.,1998). Therefore, it is important to identify UPEC from non-UPEC isolates in urine samples. The result of the present study showed that a large number of urinary isolates from cases had more



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than one virulence marker. In this case-control study, we conclude that UPEC strains are associated with the action pathogenesis of UTI. These results were implied by Johnson JR.1991. The occurrence of multiple virulence factors in UPEC strains further strengthens the concept of association of UPEC with urinary pathogenicity. It showed that UPEC with multiple virulence factors were significantly more in cases than in controls. (Raksha *et al.*, 2003). Biochemical characterization revealed a total of 100 isolates of *E.coli* and all of these were obtained from cases that were similar to Warren JW *et al.*, 1997. In the present study, of the 100 isolates of *E.coli* 31 were haemolytic, 18 had hydrophobicity, 23 were MRHA positive and all the typical *E.coli* had one or other virulence markers indicating that their characterization contributes to their virulence. The cytolytic protein toxin secreted by most haemolytic *E.coli* strains is α -haemolysin. Though the nature of haemolysin, was not characterized it can be considered a cytotoxic necrotizing factor. The difference between cases and controls for the production of haemolysin was highly significant, this was similar to Johnson *et al.* Moreover, in the present study, 23 uropathogenic strains showed haemagglutination mediated by fimbriae, which was resistant to mannose, while almost all the fecal haemagglutination, strains were mannose sensitive as done by Sunanda *et al.*, 1999 which further showed MRHA can be mediated by fimbriae. The role of cell surface hydrophobicity (CSH) was studied. The clumps formed in different molar concentration of ammonium sulfate was observed and scored positive for hydrophobicity if they aggregated in the concentration of 1.4M (Raksha *et al.*, 2003). Hydrophobicity is described novel virulence mechanism by *E.coli*. In this study, 40 out of 100 isolates had one or more virulence factors. A comparison between urinary and stool *E.coli* isolates showed that haemolysin production and the ability to cause MRHA are important virulence factors. The mentioned virulence markers are reasonably easy and screening them in a clinical microbiology laboratory is also easy thus paving the way for screening the cases of pyelonephritis since it was shown cases of pyelonephritis were caused by uropathogenic *E.coli* with one or more virulence markers.

CONCLUSION

The overall view of the present study showed. Out of 100 isolates, urinary isolates, 40 could be labelled as UPEC. The capacity to cause MRHA was due to various adhesins mainly P fimbriae, and FIC fimbrial which was seen in pyelonephritis. Thus, Uropathogenic *E.coli* (UPEC) is considered as the primary cause of urinary infections and are thought to colonize in the gastrointestinal tract from where they migrate to the periurethral area & ascend up the urinary tract to cause infection.

Furthermore, it was concluded women suffer from recurrent UTI at a higher rate of Vaginal Colonization with UPEC. *Camellia sinensis* biosynthesized silver nanoparticle was found to be a very effective alternative for the treatment of urinary tract infections caused by Uropathogenic *Escherichia coli*.

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