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Research Article

BIO-PROSPECTING THE ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF SILVER NANOPARTICLES SYNTHESIZED USING *TERMINALIA CHEBULA* SEED EXTRACT

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ABSTRACT

Objective: To synthesis silver nanoparticles using *Terminalia chebula Retz* seed extract and also to evaluate their antibacterial and anticancer activity.

Methods: Crude extracts were prepared from *T. chebula* seeds dissolved in different organic solvents such as water, chloroform, acetone, benzene and ethanol following standard protocols. Antibacterial activity was assessed using agar well diffusion method. Cytotoxic activity was performed by MTT assay using the seed extracts of *T. chebula* against HEP-2 cell lines. The qualitative and quantitative analysis performed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Synthesis of silver nanoparticles was analyzed by using UV-Vis spectrophotometer, further confirmation was done by SEM and TEM analysis.

Results: The seeds extract of *T. chebula* exhibited high antibacterial activity. Cytotoxic assay was performed using the seed extracts of *T. chebula* against HEP-2 cell lines, 40µg concentration showed LD50 growth inhibition. GC-MS analysis showed the total numbers of compounds identified in the seed extract were 47. Silver nanoparticles exhibit yellowish brown color in the aqueous solution.

Conclusion: This study shows that the active principle of *T. chebula* seeds against microbes are different from the active principles which are cytotoxic to cancer cells. The results of this study thus prove that *T. chebula* seeds can be employed as a source of natural antibacterial that can serve as an alternative to conventional medicines.

Keywords: *Terminalia chebula,* Silver nanoparticles, GC-MS, Antibacterial activity, MTT.

INTRODUCTION

Nanoparticle synthesis and the study of their size and properties are of fundamental importance in the advancement of recent research. It is found that the optical, electronic, magnetic, and catalytic properties of metal nanoparticles depend on their size, shape and chemical surroundings. In nanoparticle synthesis it is very important to control not only the particle size but also the particle shape and morphology as well. The synthesis of silver nanoparticles by chemical route is an easy, simple and convenient route for preparing metal particles in nanometer range. The prepared silver nanoparticles have been dispersed in chloroform and then examined using X-ray diffraction (XRD), Transmission Electron Microscope (TEM) and UV/Vis absorption spectroscopy. Perhaps the most important factor in this process is that the silver nanoparticles prepared by this process are stable for months [1].

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects [2]. However, the last few years have seen a major increase in their use in the developed world [3]. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [4]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances [5]. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [6].

T. chebula is a medicinally important plant that belongs to the family *Combretaceae*. The whole plant contains a number of medicinally important compounds such astannic acid, chebulinic acid, gallic acid, anthraquinone, sennoside, chebulin, terchebin, palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, and behenicacid [7]. In traditional medicine, this plant is being widely used for the treatment for diuretic, anti-helmintic, anti-diabetic and anti-hepatotoxic. Recent pharmacological investigation of the seed extract of this plant revealed antibacterial, hepatoprotective, cardioprotective, antimutagenic, anticarcinogenic, cytoprotective, radio protective, antioxidant, antianaphylactic and immunomodulatory [8]. *T. chebula*, one of the components of triphala, was shown to be a potent hyaluronidase and collagenase inhibitor that prevented degradation of cartilage. This study will investigate the synthesis of silver nano particles and bioactivity of *T. chebula* seed extract. This investigation will also be useful in identifying the bioactive compounds of *T. chebula* extracted from the seeds, which may be responsible for the therapeutic properties.

MATERIALS AND METHODS

Source of the seeds

T. chebula seed powder was obtained from Himalaya drug company, Makali, Bangalore, in September 2012.

Seed powder and preparation of the Extract

T. chebula seed powder was used to make the aqueous extract. Seed powder weighing 25g were thoroughly washed in distilled water, dried seed powder were crushed using100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters.

Antibacterial activity

Preparation of seed extracts

Apparently healthy plant seeds were thoroughly washed in tap water and dried in room temperature. The dried 15g of seeds was powdered and soaked separately in 30 ml chloroform, acetone, ethanol, benzene, water and amyl alcohol. This mixture is filtered into a petri plate using Whatman's No.1 filter paper and it is set for

evaporation. The dried sample is evaluated for its antibacterial activity.

Innoculum

The test microorganisms *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* were obtained from culture repository of Best Biotech culture collection, Bangalore, India. The organisms were inoculated onto MHB (Muller Hinton Broth), (0.5% Peptone, 0.5% Sodium chloride, 0.15% Yeast extract; pH 7.4) and incubated at 37 ˚C for overnight. The bacterial cells were harvested by centrifuging at 5000 rpm for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer [9].

Determination of anti-bacterial activity

The antibacterial activity of the seed extracts was determined using agar well diffusion method following published procedure with slight modification [10]. Muller Hinton agar (MHA) was inoculated with microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with seed extracts and control wells containing solvents (negative control) in the plate [11]. The plates were incubated at 37 ˚C for 18h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition in the plate. The resultant clear zones were measured in mm. The antibacterial activity of seed extracts were indicated by clear zones of growth inhibition.

Cell culture

HEp-2 cell line was obtained from National Center for Cell Sciences (NCCS), Pune, India. The cells were grown at 37°C under a humidified, 5% CO_2 atmosphere in DMEM medium supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 units/ml of penicillin and 50µg/ml of streptomycin.

Chemicals and reagents

DMEM medium, FBS, penicillin/streptomycin and trypsin/EDTA were purchased from Life Technologies, Inc. (GrandIsland, NY, USA). Dimethyl sulfoxide (DMSO), 3-(4, 5-dimethyl thizol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), propidium iodide (PI) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Preparation of *T. chebula* seed extract

The extract was prepared by dissolving the powder in phosphate buffered saline. The extract of *T. chebula* seed with different concentrations was used for the experiment.

Treatment schedule

Group I – HEp-2 cells without any treatment serve as control.

Group II- HEp-2 cells with final concentration of 250 µg /ml of *T. chebula* seed extract.

Group III- HEp-2 cells with final concentration of 500 µg /ml of *T. chebula* seed extract.

Group IV- HEp-2 cells with final concentration of 1000 µg /ml of *T. chebula* seed extract.

MTT Assay

Cell viability was evaluated by observing the ability of viable cells to reduce yellow colored MTT to purple colored formazan. MTT was dissolved in DMEM and added to the culture at a final concentration of 0.5 mg/ml. After an additional 2 h of incubation, the media was carefully removed and 100 µL DMSO was added to each well. The OD was measured on a plate reader at 570 nm.

GC/MS analysis

The GC-MS analysis was done using QP-5000 (Textile committee, Banglore). The sample was injected into the GC-MS on a 30m glass capillary column with a film thickness of 0.25 uM (30m X 0.2 mm ID coated with UCON HB 2000) and GC temperature program was 40-250 °C at 4 °C/min (10 min. initial hold). The gas chromatography (Schimadzu GC 15A) was equipped with FID detector connected to an integrator. The mass spectra were recorded in electron ionization (El) mode at 70 eV. The scan repetition rate was 0.5 s over a mass range of 39-650 atomic mass units (amu).

Identification of compounds

The identification was accomplished using computer search user-generated reference libraries (NIST), incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the bases of its mass spectral fragmentation. Reference compounds were co-chromatographed.

Synthesis of Silver Nanoparticles

1mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of aqueous *T. chebula* seed extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into Ag+ ions and kept at room temperature for 5 hours.

UV-Vis Spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on thegrid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

TEM analysis of silver nanoparticles

Morphology and size of the silver nanoparticles were investigated by TEM images. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid and drying in the lamp.

Statistical analysis

The resultant clear zones around the discs were measured in mm. The antibacterial activity of seed extracts were indicated by clear zones of growth inhibition. Data were statistically analyzed using Mean ± SE and significance was determined at 5% level [12].

RESULTS

Antibacterial activity of different solvent extracts

The antibacterial activity of the *T. chebula* seeds was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated**.** The results showed that the organic solvent extracts possessed strong antibacterial activity. In ethanol and acetone the highest antibacterial activity was retained in 50µl and 100µl concentration of seed extracts.

We found that ethanol and acetone extracts of seeds were successful in killing the bacteria in a dose dependent manner. At 50µl concentration, the ethanol extract showed pronounced inhibition against all the tested organisms, the maximum inhibition was observed against *Pseudomonas aeruginosa* (9.9.±0.33 mm), *Klebsiella pneumoniae* (9.4±0.23 mm) and *Staphylococcus aureus* (7.3±0.30 mm) and moderate inhibition was observed against *Salmonella typhi* (7.2±0.29 mm) and *Escherichia coli* (6.1±0.14 mm). The other solvent extracts did not actively inhibit the growth of the bacteria at 50 µl concentration except acetone against *Pseudomonas aeruginosa* (8.4±0.28 mm) (Table 1). The growth of *Pseudomonas aeruginosa* was inhibited by all the seed extracts at 100 µl concentration and maximum inhibition was observed with ethanol extract as

(16.3±0.35 mm) zone of inhibition, which was higher than the zone of inhibition caused by the standard drug chloromphenicol (17.4±0.37 mm). Similarly ethanol seed extract produced maximum inhibition (15.1±0.16 mm) to the growth of *Klebsiella pneumoniae* than chloromphenicol (13.5±0.18 mm) (Table 1). Whereas other extracts showed less inhibitory activity than chloromphenicol. *Pseudomonas aeruginosa* was inhibited by all the seed extracts and the maximum inhibition was observed with ethanol, acetone and chloroform extracts, whereas benzene and water extract showed least inhibition at 100µl concentration (Table 2).

We found that organic extracts of the seeds were successfully in inhibiting the bacteria in a dose dependent manner. Besides the 50µl concentration of seed extracts, the 100µl concentration of seed extracts was found to possess maximum inhibition.

Table 1: Antimicrobial activity of seed extract of *T. chebula* 50µl concentration against various microorganisms

Extact	Zone of inhibition in (mm) (Mean ± SE)				
	P.aeruginosa	***K.pneumoniae***	***E.coli***	***S.typhi***	***S.aureus***
Water	6.5±0.16	5.2±0.21	3.3±0.13	4.0±0.41	3.2±0.31
Chloroform	6.2±0.25	3.4±0.23	2.4±0.28	1.6±0.28	4.2±0.32
Acetone	8.4±0.28	2.3±0.27	6.4±0.30	4.3±0.41	3.2±0.25
Benzene	4.2±0.25	3.4±0.23	4.2±0.23	2.3±0.35	3.0±0.33
Ethanol	9.9±0.33	9.4±0.23	6.1±0.14	7.2±0.29	7.3±0.30
Chloromphenicol	8.3±0.23	8.1±0.23	6.0±0.14	7.1±0.29	7.0±0.30

The negative control wells were exposed with the neat solvent and the positive control was chloromphenicol (50 µg ml^{-1}). Each value represents the Mean ± SE of five replicates per treatment in three repeated experiments.

Table 2: Antimicrobial activity of seed extract of *T. chebula*100µl concentration against various microorganisms

Extract	Zone of inhibition in (mm) (Mean ± SD)				
	P.aerogenosa	***K.pneumonia***	***E.coli***	***S.typhi***	***S.aureus***
Water	9.2±0.16	13.2±0.39	5.3±0.33	7.3±0.33	6.45±0.32
Chloroform	11.4±0.24	10.2±0.21	4.5±0.32	3.4±0.30	7.1±0.31
Acetone	14.0±0.20	9.2±0.17	5.4±0.29	6.2±0.26	7.4±0.30
Benzene	9.4±0.28	7.3±0.27	6.1±0.56	5.08±0.23	5.26±0.28
Ethanol	16.3±0.35	15.1±0.16	11.2±0.30	13.2±0.33	13.9±0.25
Chloromphenicol	17.4±0.37	13.5±0.18	10.1±0.33	12.2±0.37	13.0±0.23

The negative control wells were exposed with the neat solvent and the positive control was chloromphenicol (50 µg ml^{-1}). Each value represents the Mean ± SE of five replicates per treatment in three repeated experiments.

Cytotoxicity studies using Hep-2 cells

HEp-2 cells were treated with different concentrations of *T. chebula* seed extract (final concentration of control, 250, 500, 1000 µg/ml). Among the three concentrations tested, the 1000µg/ml showed the LD50 growth inhibition (Figure 2). The dose dependent increase in the cytotoxicity was observed in different concentration of the *T. chebula* seed extract.

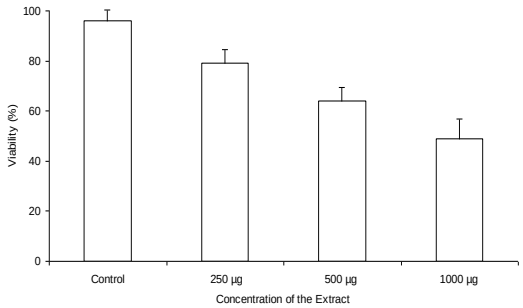
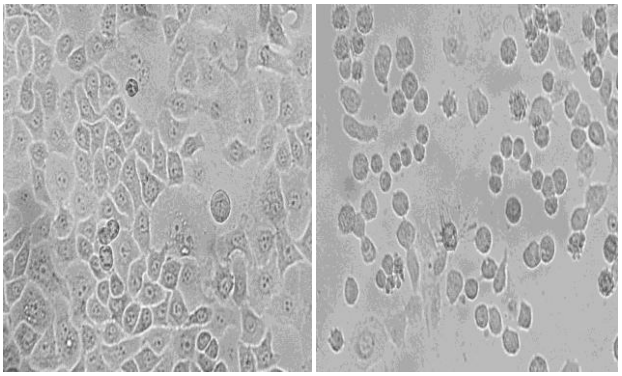


Fig. 2: Cytotoxicity in HEp-2 cells induced by exposure to different concentration of *T. chebula* as determined by MTT reduction assay.

The cytopathic effect of *T. chebula* seed extract on HEp-2 cells was analyzed using an optical microscope (Figure 3). Untreated cells appeared elongated, attached smoothly on the culture surface. Some cells were grouped together to form colonies. Following treatments with extract for 24hrs, the cells became rounded and lost cell contacts. In particular, surface morphological changes leading to cell detachment were observed. LD 50 was determined to be 900µg/ml.

The columns represent the percentage of viability in HEp-2 cells. Cell viability is shown as mean ± SD derived from at least three separate experiments done in triplicate. The values are represented as mean ± SD. ***p < 0.0001 Vs. Control.



(a). Control (b). Treated cells

Fig. 3: Microscopic examination of morphological changes in HEp-2 cells treated with *T. chebula seed extract*

Phytoconstituents of *T. chebula* by GC-MS

The qualitative and quantitative comparison of *T. chebula* seed extract
is reported in (Table 3). *T. chebula* seed extracted from alcohol were subjected for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The total numbers of compounds identified in the seed extract were 47. Few of them were, Z,Z-6,28-Heptatriactontadien-2-one (21.09%), Phytol (15.91%), Squalene (10.06%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (8.25%), 3-Eicosyne (6.99%), Noxiptiline (3.21%), Hexanedioic acid, bis (2-ethylhexyl) ester (2.64%), n-Hexadecanoic acid (2.12%), Decanoic acid, ethyl ester (0.79%), (Z)6,(Z)9-Pentadecadien-1-ol (0.72%), 2-Propenoic acid, 2-(dimethylamino)ethyl ester (1.58%), Undecanoic acid, hydroxy-, lactone (0.81%), 1-Cyclohexylnonene (0.44%), Phenoxathiin-2,8-diamine-10,10-dioxide (1.16%), 1,14-Tetradecanediol (0.46%), 1,2-Benzenedicarboxylic acid and disooctyl ester (1.29%).

Table 3: Phytoconstituents of *T. chebula* seed by GC-MS analysis

S. No.	R.T	Name	Formula	MW	Peak area %
1.	2.99	Butane, 1,1-diethoxy-	C8H18O2	146	0.46
2.	3.14	S)-(+)-2-Amino-3-methyl-1-butanol	C5H13NO	103	trace
3.	3.95	Ethyl pipecolinate	C8H15NO2	157	0.12
4.	4.07	Glycerin	C3H8O3	92	1.09
5.	4.26	Pentanoic acid	C5H10O2	102	0.16
6.	4.58	Cyclopropanecarboxylic acid, 1-amino-	C4H7NO2	101	0.94
7.	5.51	Benzeneacetaldehyde	C8H8O	120	0.27
8.	6.15	Glycine, N-(2-methyl-1-oxo-2-butenyl)-, methyl ester, (E)-	C8H13NO3	171	1.04
9.	6.45	2,5-Pyrrolidinedione	C4H5NO2	99	0.20
10.	7.67	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4	144	0.03
11.	8.00	4-Methoxycarbonyl-4-butanolide	C6H8O4	144	0.05
12.	9.48	Conhydrin	C8H17NO	143	0.78
13.	14.97	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	C15H24	204	0.11
14.	15.19	1,6-Anhydro-á-D-glucopyranose (levoglucosan)	C6H10O5	162	6.20
15.	15.26	3-Heptanol, 2-methyl-	C8H18O	130	0.31
16.	15.77	1R-à-Pinene	C10H16	136	0.84
17.	16.37	Decane, 6-ethyl-2-methyl-	C13H28	184	1.03
18.	16.66	Flamenol	C7H8O3	140	0.14
19.	17.03	2,5-Pyrrolidinedione, 3-ethyl-1,3-dimethyl-	C8H13NO2	155	0.03
20.	17.61	n-Decanoic acid	C10H20O2	172	0.17
21.	18.68	Hexane, 3,4-bis(1,1-dimethylethyl)-2,2,5,5-tetramethyl-	C18H38	254	0.05
22.	20.25	8-Dodecen-1-ol, (Z)-	C12H24O	184	0.06
23.	20.34	Bicyclo[2.2.2]octane, 1-methyl-4-(methylsulfonyl)-	10H18O2S	202	0.04
24.	20.42	1-Decene, 8-methyl-	C11H22	154	0.08
25.	20.90	Hexadecane	C16H34	226	1.55
26.	20.96	Z-2-Dodecenol	C12H24O	184	0.37
27.	21.07	Cyclohexanecarboxylic acid, 4-propyl-, 4-butoxyphenyl ester	C20H30O3	318	0.08
28.	22.02	3-Dodecanol, 3,7,11-trimethyl-	C15H32O	228	0.12
29.	22.82	2,4,6-Trimethyl-1-nonene	C12H24	168	0.07
30.	23.84	Z,Z-6,28-Heptatriactontadien-2-one	C37H70O	530	21.09
31.	23.99	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	278	8.25
32.	24.31	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	3.63
33.	24.69	3-Eicosyne	C20H38	278	6.99
34.	26.09	n-Hexadecanoic acid	C16H32O2	256	2.12
35.	26.63	Decanoic acid, ethyl ester	C12H24O2	200	0.79
36.	29.02	Phytol	C20H40O	296	15.91
37.	29.83	(Z)6,(Z)9-Pentadecadien-1-ol	C15H28O	224	0.72
38.	31.81	2-Propenoic acid, 2-(dimethylamino)ethyl ester	C7H13NO2	143	1.58
39.	31.96	Undecanoic acid, hydroxy-, lactone	C11H20O2	184	0.81
40.	32.58	1-Cyclohexylnonene	C15H28	208	0.44
41.	33.04	Phenoxathiin-2,8-diamine-10,10-dioxide	C12H10N2O3S	262	1.16
42.	33.64	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4	370	2.64
43.	34.73	Noxiptiline	C19H22N2O	294	3.21
44.	34.90	3-Methyl-2-(2-oxopropyl)furan	C8H10O2	138	2.45
45.	35.86	1,14-Tetradecanediol	C14H30O2	230	0.46
46.	36.00	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	1.29
47.	41.71	Squalene	C30H50	410	10.06

Silver nanoparticles

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. As the *T. chebula* seed extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion (Figure 1 a & b) which indicated formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. Figure 4 shows the UV-Vis spectra recorded from the reaction medium after one week. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance miler to our results peak at 429 nm, broadening of peak indicated that the particles are polydispersed (Figure 5 and 6).

DISCUSSION

Among the tested different organic solvents for the extraction from seeds of *T. chebula* has shown potent antibacterial effects against all the tested bacteria. The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. The seed extracts of *T. chebula* showed significant activity against all the bacteria tested contradiction to many other studies reported on *T. chebula* [13, 14, 15, 16]. In a study conducted on the biological activity of *Menthapiperita and Trigonella foenum-graecum* against the pathogenic bacteria similar results to our study were reported [17, 18, 19].

a. After adding $AgNO_3$ b. One week after the addition of $AgNO_3$

Fig. 1: *Terminalia chebula* seed extract

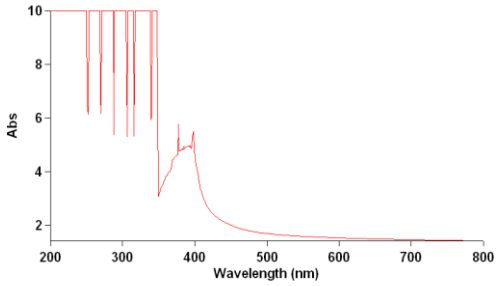


Fig. 4: UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with 10% *Terminalia chebula* seed extract after one week

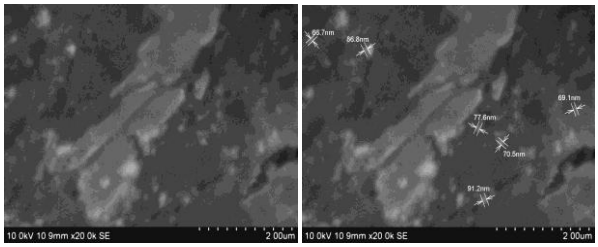


Fig. 5: SEM image of silver nanoparticles with measurements

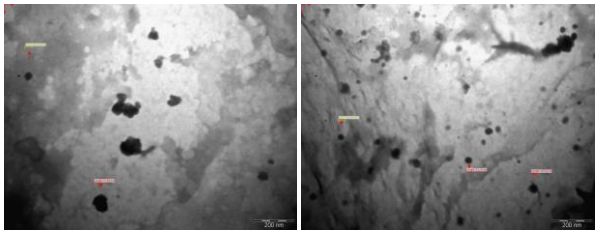


Fig. 6: TEM image of silver nanoparticles with measurements

Based on these studies we used five different solvent extracts of *T. chebula that* showed activity against all bacteria at all dosages. The seed extracts of *T. chebula* exhibited antibacterial activity only in chloroform, acetone and ethanol against the bacteria tested in agar well diffusion method at 50µl and 100 µl concentration similar to the study reported earlier [11]. We observed maximum activity at 100µl concentration against *Pseudomonas aeruginosa* than *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi* and *Escherichia coli.* The present work was similar to [17, 19] shows that the compounds from *T. chebula* possess potent antimicrobial activity and suggesting that the *T. chebula* seed extracts should contain the effective active constituents responsible for eliminating the bacterial pathogens. Finally it can be concluded that the active chemical compounds present in *T. chebula* should certainly find place in treatment of various bacterial infections. The results from the present study are very encouraging and indicate this plant seeds should be studied more extensively to explore its potential in the treatment of many infectious diseases. Carvacrol has reduced cell growth in Hep-2 cells, mouse myoblast cells and caused DNA damage in human lymphocyte. Morphological analysis suggested the involvement of apoptosis in the majority of cases [20]. Thymol has reduced the number of viable cells in Hep-2 cells, dental pulp fibroblasts and guinea pig neutrophils, and also caused DNA damage in human lymphocytes. In contrast, lower doses of thymol and carvacrol significantly reduced oxidative DNA damage in human lymphocytes. Besides, it has been shown that Caco-2 cells are able to glucuronidate various aglycones, including thymol. Intestinal glucuronidation represents the metabolic line of defense against ingested toxic xenobiotics, and it could also affect the cytotoxicity of thymol [21]. Thymol (5-methyl-2-(1-methylethyl)-phenol), found in thyme [22], has been commercially available as part of a mouthwash for more than hundred years. Thymol is active against *E. coli*, *S. aureus*, *L. monocytogenes*, *C. jejuni*, *S. enterica*, but also against insects. They are used as herbal drugs to treat dyspepsia, acute or chronic gastritis and diarrhoea. Their antimicrobial activity has also attracted great attention from many researchers [23, 24, 25]. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions were similar to a study conducted on papaya [1, 24, 26, 27].

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REFERENCES

1. Arshad F, Prakash Singh Chauhan MD, Praveen, K, Jameelshaik. Extraction of silver nanoparticles from the leaf extracts of *Clerodendruminerme*. J. Appl. Biomed, 2010; 43-49.
2. Amin A, Aysha A, Shamaa Al-Falasi, Sayel A, Daoud. Chemopreventive activities of *Trigonellafoenumgraecum* (Fenugreek) against breast cancer. Cell BiologyInternational, 2005; 687-694.
3. Ateb DA, Erdourul OT. Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol, 2003; 27: 157-162.
4. Elizabeth M, Adrien S, Johnson D, Warnock, W. Comparison of e-test and broth micro dilution methods for antifungal drug susceptibility testing of molds. Clin. Microbiol, 1999; 37: 1480-1483.
5. Kamal Rai Aneja, Radhika Joshi. Evaluation of antimicrobial properties of fruit extracts of *Terminaliachebula* against dental caries pathogens. Jundishapur Journal of Microbiology, 2011; 2(3): 105-111.
6. Varaprasad B, Varahala RV, Somasekhar Penumajji, K, Chandrasekhar Naidu. *Excoecaria agallocha* L. Antimicrobial properties against important pathogenic microorganism. International Journal of Chem Tech Research, 2009; 1(4): 865-867.
7. Archana S, Suchitra Meena S, Nachiketa Barman. Efficacy of ethyl acetate and ether extract of *Terminaliachebula* Retz against some human pathogenic strains.International Journal of Pharmtech Research, 2011; 3: 24-727.
8. Sumithira PS, Dhivya Mangala, Sophie AM, Padma Latha C. Antiviral and antioxidant activities of two medicinal plants. Int J Curr Sci, 2012; 256-261.
9. Owais M, Sharad KS, Shehbaz A, Saleemuddin M. Antibacterial efficacy of Withania somnifera (Ashwagandha) and indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine, 2005; 12(3): 229-235.
10. Perez C, Paul M, Bezique P. An Antibiotic assay by the agar well diffusion method. Alta Biomed Group Experiences, 1990; 15: 113.
11. Valsaraj R, Pushpangadan P, Smith UW, Adsersen A, Nyman U. Antimicrobial screening of seleed medicinal plants from India. J. Ethnophamacol, 1997; 58: 75-83.
12. Nandagopal S. Dhanalakshmi D.P. Ganesh Kumar A. and Sujithe.Phytochemical and antibacterial studies of fenugreek *Trigonella foenum-graecum* L. - a multipurpose medicinal plant. Journal of Pharmacy Research, 2012; 5(1): 413-415.
13. Ammar S, Michael H, Pirkko Ha Rko Nen, Kalevi P. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminaliachebula Retz* Fruit. Journal of ethnopharmacology, 2002; 81: 327-336.
14. Elizabeth KM. Antimicrobial activity of *Terminaliachebula*on *Pseudomonas aeruginosa*isolated from nasal secretions of chronic sinus patient. J. Hum, 2002;13(4): 311-314.
15. Elamparithi D, Boominathan M. Comparative studies of antibacterial effect of fraction and crude extract of *Terminaliachebula*on two pathogenic bacteria. Journal of Ethnopharmacology, 2007; 2249-6807.
16. 16.Golam Mustafa G, Mahdia R, Manjurul Karim M. Antimicrobial activity of *Terminalia chebula*. Int. J. Med. Arom. Plants, 2011; 1: 175-179.
17. Bupesh G, Nandagopal S, Ganesh Kumar G, Suresh Kumar P, Saravana Murali K. Evaluation of antibacterial activity from leaf extracts of Menthapiperita L, Acta. Agriculturae Slovenica, 2007; 88: 73-79.

18. Nandagopal S, Dhanalakshmi DP, Ganesh Kumar A, Sujithe. Phytochemical and antibacterial studies of fenugreek *Trigonellafoenum-graecum* L.-A multipurpose medicinal plant, Journal of Pharmacy Research, 2011; 5(1): 413-415.
19. Nandagopal S, Dhanalakshmi DP, Ganesh Kumar A, Sujithe. Phytochemical and antibacterial studies of fenugreek *Trigonellafoenum-graecum* L.-A multipurpose medicinal plant. Journal of Pharmacy Research, 2011; 5(1): 413-415.
20. Gosslao A, Chen KY. Nutraceuticals, apoptosis and disease prevention. Nutrition, 2004; 20: 95-102.
21. Abid M, Huss B, Rambour S. Transgenic Chicory (*Cichorium intybus* L.). Biotechnology in Agriculture and Forestry, 2001; 47: 102-123.
22. Pina Vaz C, Goncalves Rodrigues A, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro L, Cavaleiro C, Goncalves MJ, Martinez-de-Oliveira J. Antifungal activity of Thymus oils and their major compounds. J. European Acad. Dermatology, 2004; 18: 73-78.
23. Friedman M, Buick R, Elliott CT. Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus*. J. Food Protection, 2004; 67: 1774-1778.
24. 24.Jain D, Kumar Daima H, Kachhwahas S, Kothari L. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Journal of Nanomaterials and Biostructures, 2009; 4(3): 557-563.
25. Jinhuizhang, Li li, Sung-hoonkim, Ann E. Hagerman, Junxuanlu. Antibacterial activity of *Terminaliachebula* fruit extract. African Journal of Microbiology Research, 2010; 3(4): 180-184.
26. Mehrdad F, Khalil A. Biological and green synthesis of silver nanoparticles. Turkish J. Eng. Env. Sci, 2010; 34: 281-287.
27. Maribel G, Guzman, Jean Dille, Stephan Godet. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity, World Academy of Science. Engineering and Technology, 2008; 43-45.