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"Green synthesis of silver nanoparticles using Annona muricata and Trigonella foenum graceum and its in vitro Antiurolithiatic activity"

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***Abstract*—** Green synthesis of silver nanoparticles mediated by methanolic and aqueous extract of leaves of *Annona muricata* and *Trigonella foenum graceum* was analyzed for its antiurolithiatic activity by Calcium oxalate crystallization in synthetic urine in *in-vitro* condition. Formation of calcium oxalate crystals were induced by addition of 0.01M sodium oxalate in the urine. The nanoparticles were taken in four different concentrations (1000, 2500, 5000 and 10000µg/ml) to study their effect on calcium oxalate crystals. The effect was studied by measuring their turbidity in presence and absence of nanoparticles at 620 nm in spectrophotometer. The nanoparticles were characterized by UV-VIS Spectrophotometry, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscope. The results suggest us, that silver nanoparticles synthesized by leaf extract of *Annona muricata* and *Trigonella foenum graceum* can be a novel drug established against urolithiasis.

***Keywords*— AgNPs, *Annona muricata*, *Trigonella foenum graceum*, Calcium oxalate, Sodium oxalate.**

1. INTRODUCTION

Humanoid race is continuously being defied by many terrible ailments and it is a difficult task to fight them in the present-day situation. Leading vigorous life is a challenge in an extremely inexpensive world, where public scarcely get a chance to upkeep about their régime or guarantee the excellence of the food they consume (Anu et al., 2019). The Social Revolution and comfortable life styles have made notable swing in the food habits of all public of world with increased dependence on junk foods and soft drinks. This condition has brought about a frightening situation with recurrent rise in the occurrences of life style diseases like diabetes mellitus, heart diseases, arthritis, cancer and urolithiasis, around the world. Among these urolithiases is oldest and widely reported diseases known to mankind. Kidney stone development is a multifaceted process that results from a series of some physico-chemical changes includes, super saturation, nucleation, growth, aggregation and retention of urinary stone constituents within the renal tubules. The crystallization of the calcium oxalate begins with increased urinary supersaturation, with the subsequent formation of the solid crystalline particles within the urinary tract. This is followed by nucleation, by which stone-forming salts in supersaturated urinary solution coalesce in to clusters that then increase in size by the addition of new constituents (Basavaraj et al., 2007). These crystals then grow and aggregate with other crystals in solution, and are ultimately retained and accumulated in the kidney (Kok et al., 1990). Renal injury promotes crystal retention and the development of a stone nidus on the renal papillary surface, and further supports crystal nucleation at lower supersaturation levels (Fasano and Khan 2001). Therefore, levels of urinary supersaturation correlate with the type of stone formed, and reducing supersaturation is effective in preventing stone recurrence. Therefore, if this progression of crystallization can be prevented, then lithiasis could also be prevented. Usually, the first sign of a kidney stone is dangerous pain, which happens when a stone intensely blocks the current of urine. The pain often starts unexpectedly when a stone passages in the urinary tract, producing irritation or obstruction. If the stone is too large, it often leads to bleeding and blood may appear in the urine. Effective kidney stone inhibition is reliant on the stone type and the finding of risk issues for stone formation.

Stones bigger than 5mm or stones that fail to pass; are treated by ESWEL and ureteroscopy (Knoll, 2002; Pearle *et al.*, 2001). However, these

techniques are very costly for common man as well as recurrence of kidney stone and numbers of side effects are associated with these procedures (Silberstein *et al*., 2008). When dietary alteration is insufficient, the next option is to initiate pharmacological treatment but this treatment also has side effects. In a nut-shell, exhaustive and comprehensive review of literature exposed that, reasonable treatment for lithiasis is warranted.

Nanotechnology is the science of particles ranging between 1-100 nanometer. They are of great importance, as the metals physio-chemical properties changes on reaching the nano size. Physical and chemical methods of synthesis require excessive chemicals and energy, which may be harmful for human purpose. Biological synthesis use extracts of plant and animal origin which are cost effective and environmental friendly. Of which silver nanoparticles are most effectively synthesized and most preferably used by researchers due to its anti-microbial property.

As per the indigenous system of medicine, the plant *Annona muricata* and *Trigonella foenum graceum* have been traditionally claimed as well as scientifically documented for their various pharmacological activities revealing their usefulness in various diseases and disorders.

The genus *Annona* belongs to Family Annonaceae, this evergreen commonly known as 'Shulramphal'. All the parts of the plants have medicinal uses. The fruit is used as Blood purifying component, Gastroprotective agent, Anti-inflammatory agent. Leaves are traditionally used to treat bronchitis biliary disorder, dysentery. Nanoparticles synthesis mediated by its leaves has not been reported by any researchers in urolithiatic activity.

The genus *Trigonella foenum-graceum* belongs to Family Fabaceae, this herb commonly known as 'Methi'. The use of this plant to treat hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. Even though these plants have been documented for different pharmacological activities, their effect on urolithiasis has yet not been explored. In regard to this, the present study is planned to Green synthesis of silver nanoparticle using *Annona muricata* and *Trigonella foenum graceum* leaves extracts to evaluate the Antilithiasis activity.

2. Materials and Methods

Collection of plant: The plants were collected from North Maharashtra Region in July 2017. The plant *Annona muricata* and *Trigonella foenum graceum* was identified by Dr. Tanveer Khan, Department of Botany, H. J. Thim College, Jalgaon and deposited a voucher specimen in the Department of Zoology.

Preparation of extract: Leaves of *Annona muricata* and *Trigonella foenum graceum* were separated and shed dried. Dried powdered plants materials were exhaustively extracted through Soxhlet apparatus with methanol (MeOH) and distilled water (Aq.). The solvent extracts so obtained were then filtered to remove any suspended impurities. Extracts were concentrated under reduced pressure and controlled temperature (55^0C to 60^0C). The aqueous and methanol extracts of plant were preserved in dry, cool condition in desiccator.

Synthesis and Characterization of AgNPs

Silver nitrate ($AgNO_3$) was used as a precursor for the synthesis of AgNPs. Silver nanoparticles were synthesized by adding 5 mL of the extracts of all concentrations to 1 mL of aqueous silver nitrate solution (1M) at room temperature. The mixtures were then kept for 10 minutes on rotary shaker and kept in the laboratory at room temperature. The change in color was continuously observed.

Characterization of AgNPs

Silver nanoparticles synthesized were confirmed and characterized by following methods; UV-Vis spectrum, FT-IR and colour change. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 24 h after diluting 0.5ml of the sample (each concentration) with 1.5 ml sterile D. W. UV-Vis spectral analysis was done by using UV-Vis Spectrophotometer. Fourier transformed infrared (FTIR) spectrum of the sample was recorded.

Preliminary phytochemical screening: The qualitative secondary metabolites investigation of MeOH and Aq was carried out to check the presence of various phytoconstituents (Harborne, 1984).

Experimental Protocol

Turbidity method (*In-vitro* Crystallization) (Srinivasa et al., 2013): The effect of extracts on CaOx manifestation was determined by the turbidity variations observed due to the manifestation in artificial urine on addition of 0.01M sodium oxalate solution in specific course of time. The precipitation of calcium oxalate at

37°C and pH 6.8 has been studied by the measurement of absorbance at 620 nm using UV-Visible spectrophotometer.

Preparation of artificial urine (Srinivasa et al., 2013): The artificial urine (AU) was prepared according to the method Burns and Finlayson (1980) with slight modification and use the following composition: sodium chloride 105.5mM, sodium phosphate 32.3mM, sodium citrate 3.21mM, magnesium sulfate 3.85mM, sodium sulfate 16.95mM, potassium chloride 63.7mM, calcium chloride 4.5mM, sodium oxalate 0.32mM, ammonium hydroxide 17.9mM, and ammonium chloride 0.0028mM. The AU was prepared fresh each time and pH adjusted to 6.0.

Study without inhibitor: 1.0 ml of AU and 0.5 ml of distilled water added together in a cell and blank reading was taken. 0.5 ml of 0.01M sodium oxalate was added, to the previous volume, and the measurement is immediately started for a period of ten minutes.

Study with inhibitor: Methanolic and Aqueous extract mediated nanoparticles were taken in concentration of 1000, 2500, 5000 and 10000µg/ml. Ashmayrina tablet (Bhardwaj, Pharmaceutical work, Indore) was used as standard drug. A mixture of 1 ml of AU and 0.1ml, 0.25ml, 0.5ml, 1ml of nanoparticles were added in the cell and blank reading was taken. Then 0.5 ml of 0.01M sodium oxalate solution was added and immediately the absorbance was measured for a period of 10 minutes at 620nm.The percentage of inhibition of calcium oxalate crystal formation was calculated by using the following formula:

$$\%\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Test}}{\text{Absorbance of control}}$$

Statistical analysis: All data statistically evaluated and were expressed as mean ± SE and the ANOVA were applied to determine the significance of the difference between the standard group and the experimental group.

3. Results

The phytochemical study of methanolic and aqueous extracts of both plants showed the occurrence of Alkaloids, Flavonoids, Tannins, Phenolic compounds, Saponins, Cardiotonic glycoside (Table 1). The results show the presence of phenols, saponins, and flavonoids responsible for bio-reduction of Ag^+ to Ag^0 and stability of AgNPs.

In- Vitro inhibition of Calcium oxalate crystallization by nanoparticles mediated by *A. muricata* and *Trigonella foenum-graceum* were determined by using turbidity method. All the test groups; after 5 and 10 min exhibits significant ($p<0.001$) inhibition of Calcium oxalate crystal formation. High concentration of nanoparticles mediated by methanolic and aqueous extracts of *Annona muricata* exhibits near about same percent inhibition. Nanoparticles mediated by methanolic extract of *Annona muricata* showed dose depended percent inhibition of Calcium oxalate crystal formation. However, lowest concentration (1000µg/ml) of nanoparticles mediated by aqueous extract reveals (68.56 ± 0.082 and 69.35 ± 0.082) high percent inhibition of Calcium oxalate crystals formation than methanolic at 5000µg/ml concentration (61.86 ± 0.080 and 62.15 ± 0.047) percent inhibition (Table 2).

The AgNps mediated by extracts of *Trigonella foenum-graceum* (MeOH and Aq) at the dose 10000 µg/ml after 5 min and 10 min showed high % of inhibition of Calcium oxalate crystal formation (92.12 ± 0.062, 92.44 ± 0.038 and 85.01 ± 0.112, 84.35 ± 0.057 respectively) (Table 2). Both concentrations AgNPs mediated by Methanolic and Aqueous showed significant % inhibition when compared to standard Ashmayrina. Lowest % of inhibition of Calcium oxalate crystal formation was observed at Aq 1000 µg/ml. MeOH at 2500 and Aq at the dose 10000 µg/ml have close % of inhibition of Calcium oxalate crystal formation.

4. Discussion

Urolithiasis is a complex process that results from a succession of several physico-chemical events (super saturation, nucleation, growth, aggregation and retention within renal tubules) (Khan, 1997). In the present study both test extracts of target plants inhibited the stone crystal formation, thus proved the presence of some active antiurolithiatic compounds work.

The results show the presence of phenols, saponins, and flavonoids responsible for bioreduction of Ag^+ to Ag^0 and stability of AgNPs. This finding is supported by the report of Pochapski *et al.* (2011) and Dada *et al.* (2018b). Saponins are known to have anti-crystallization properties by disaggregating the suspension of mucoproteins, the promoters of crystallization (Gurocak and Kupeli, 2006).

Antiurolithiatic activities also have been attributed to triterpenes, lupeol (Anand *et al*., 1994) and polyphenolic compound like quercetin (Park *et al*., 2008). It is therefore probable that the components that are present in abundance in the extract might exert their action directly on the calcium oxalate crystallization. Our results are also corroborated with the finding of Diana, (2013).Their results emerged from crystallographic studies provide additional evidence to validate the ethnobotanical information of the herbal collectors and traditional healers of Kottayam District of Kerala state. It is expected that, *in vitro* screening of various pure bioactive compounds followed by *in vivo* antiurolithiatic and toxicological evaluations may help to develop a highly efficacious herbal drug from the *A. muricate* and *T. foenum-graecum.*

5. Conclusion

The nanoparticles of methanolic and aqueous extract have inhibitory effect on CaOx crystallization and hence may be beneficial in the treatment of urolithiasis. There is a necessity to investigate in details to establish the use of plant as antiurolithiatic agent. Thus, it is expected that, *in vitro* screening of various pure bioactive compounds followed by *in vivo* antiurolithiatic and toxicological evaluations may help to develop a highly useful herbal drug from *Annona muricate* and *T. foenum-graecum.*

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7. REFERENCES

1. Anand R, Patnaik G.K, Kulshreshtha D.K, Dhawan B.N., (1994), Antiurolithiatic activity of lupeol, the active constituent isolated from *Crateva nurvala*. *Phytother Res* 8: 417-421.
2. Anu V., Sarath D. and Sneha, P. K., (2019), *In-Vitro* **Anti-Oxidant Studies of Macerated Ethanolic And Aqueous Extract of** *Hylocereus Undatus* **Fruits, International Journal of Pharmacognosy, 5:**319-322.
3. Diana K. J., (2013), Ethnobotanical and crystallographic studies of selected antiurolithiatic studies of selected antiurolithiatic medicinal plants. *Thesis*, School of Environmental Sciences Mahatma Gandhi University, Kottayam. Mahatma Gandhi University, Kottayam, Kerala.
4. Gurocak S, Kupeli B., (2006), Consumption of historical and current phytotherapeutic agents for urolithiasis - A critical review. *J Urol* 176: 450-455.
5. Harborne J.B., (1984), Phytochemical method – A guide to modern technique of plant analysis. Chapman and Hall, NEW YORK, 2nd edition, 85.
6. Khan S.R., (1997), Interactions between stone forming calcific crystals and macromolecules. *Urol Int*, 59 (2):59-71.
7. Knoll, T., (2002), Stone disease. *Eur Urol Suppl*, 6: 717–722.
8. Park H.K, Jeong B.C, Sung M, Park M. and Choi E.Y., (2008), Reduction of oxidative stress in cultured renal tubular cells and preventive effects on renal stone formation by the bioflavonoid quercetin. *J Urol*, 179: 1620-1626.
9. Silberstein, J., Lakin, C. M. and Kellogg P. J., (2008), Shock wave lithotripsy and renal hemorrhage. *Rev Urol*, 10: 236–241.
10. Basavaraj D. R., Biyani C. S., Browning A. J. and Cartledge J. J., (2007), The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. *EAU-EBU Update Series* 5: 126-136.
11. Kok D. J., Papapolous S. E. and Bijovet O. L., (1990), Crystal agglomeration is a major element in calcium oxalate urinary stone formation. *Kidney Int*, 37: 51-56.
12. Fasano J. M. and Khan S. R., (2001), Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an *in vitro* study. *Kidney Int*, 59: 169-178.
13. Srinivasa, Ashok Kumar Bagepalli, Lakshman Kuruba, Saleemulla Khan and Gopi Setty Saran, (2013), Antiurolithiatic Activity of Gokhsuradi Churan, an Ayurvedic Formulation By *In Vitro* Method, *Advanced Pharmaceutical Bulletin*, 3(2), 477-479.

14. Pochapski M.T., E.C. Fosquiera, L.A. Esmerino, E.B. dos Santos, P.V. Farago, F.A. Santos, F.C. Groppo, (2011), Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from Ipomoea batatas (L.) Lam, *Pharmacogn. Mag.*, 7(26):165-171.
15. Dada A.O., A.A. Inyinbor, I.E. Idu, O.M. Bello, A.P. Oluyori, T.A. Adelani – Akande, A.A. Okunola, O. Dada, (2018), Effect of operational parameters, characterization and anti-bacterial studies of green synthesis of Silver Nano particles, using *Tithonia diversifolia*, *Peer J*, 6.

Table 1: Phytochemical analysis of MeOH and Aq extract of leaves of *A. muricata* and *T. foenum-graecum*

Extracts / Phytochemicals	*Annona muricata*		*Trigonella foenum-graecum*	
	MeOH	**Aq**	**MeOH**	**Aq**
Alkaloids	+	-	+	+
Flavonoids	+	+	–	+
Tannins	+	+	+	–
Phenolic compounds	+	+	+	+
Saponins	+	+	+	+
Cardiotonic glycoside	-	+	+	+

+ Presence, - Absence

Table 2 Effect of different concentrations of MeOH and Aq on Calcium oxalate crystallization in artificial urine

Groups	**% inhibition of Calcium oxalate crystal formation**			
	Annona muricata		***Trigonella foenum-graecum***	
	After 5 min.	**After 10 min.**	**After 5 min.**	**After 10 min.**
Control	0.8438 ± 0.001	0.836 ± 0.0007	0.8438 ± 0.001	0.836 ± 0.0007
Standard	73.8 ± 2.103	76.43 ± 0.356	73.8 ± 2.103	76.43 ± 0.356
MeOH (1000µg/ml)	18.45 ± 0.105***	17.68 ± 0.055***	24.49 ± 0.131***	23.73 ± 0.084 ***
MeOH (2500µg/ml)	43.21 ± 0.079***	42.56 ± 0.054***	83.03 ± 0.094 ***	82.7 ± 0.042 ***
MeOH (5000µg/ml)	61.86 ± 0.080***	62.15 ± 0.047***	53.29 ± 0.072 ***	52.87 ± 0.039 ***
MeOH (10000µg/ml)	79.28 ± 0.224***	80.00 ± 0.044***	92.12 ± 0.062***	92.44 ± 0.038 ***
Aq (1000µg/ml)	68.56 ± 0.082***	69.35 ± 0.082***	9.046 ± 0.096**	9.56 ± 0.120 ***
Aq (2500 ug/ml)	74.74 ± 0.312***	75.29 ± 0.075***	12.52 ± 0.132 ***	13.21 ± 0.049 ***
Aq (5000 ug/ml)	79.38 ± 0.209***	81.41 ± 0.224***	36.77 ± 0.293 ***	35.91 ± 0.079 ***
Aq (10000 ug/ml)	81.25 ± 0.495***	80.55 ± 0.261***	85.01 ± 0.112 ***	84.35 ± 0.057 ***

Each Value expressed as mean +-SE, n=6,***P<0.001 *Vs* Standard

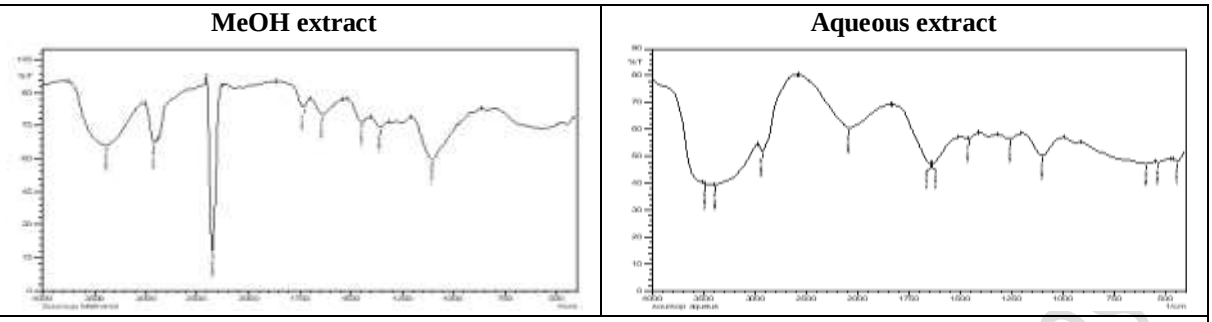


Fig. 1 FT-IR spectra of AgNPs of MeOH and Aqueous extract of leaves of *A .muricata*

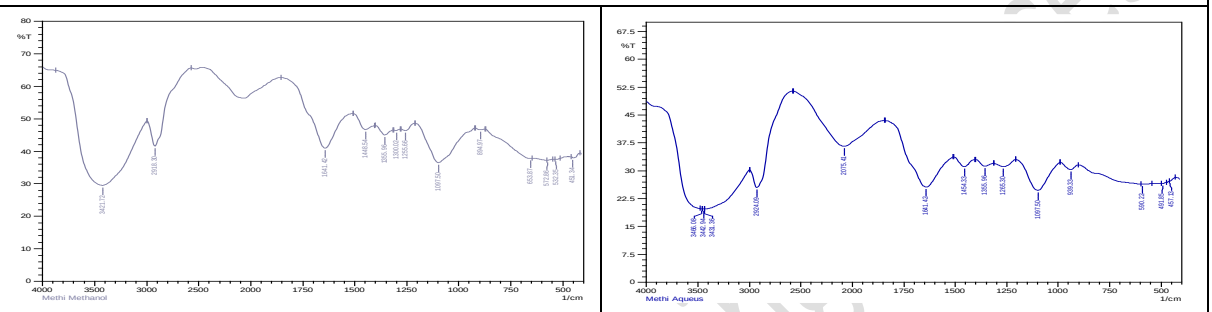
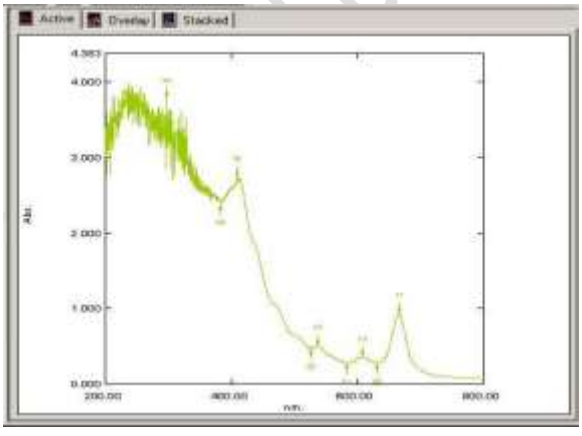


Fig. 2 FT-IR spectra of AgNPs of MeOH and aqueous extract of *T. foenum-graecum*

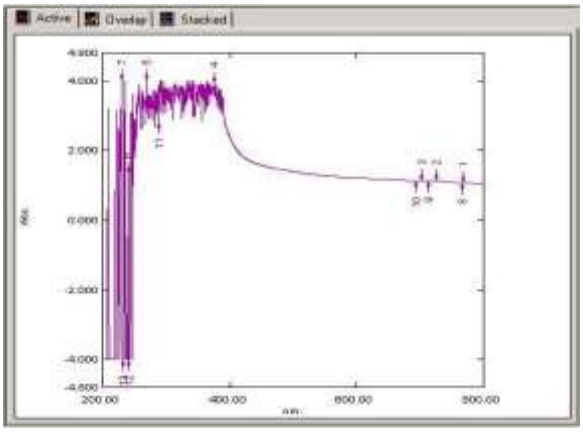
Table 2 FT-IR spectrum analysis of synthesized silver nanoparticles from extracts of *A. muricata* and ***T. foenum-graecum***

Sr. No.	Wave Number	Stretching	Interpretation
Silver nanoparticles mediated from aqueous extract of *A .muricata*			
1.	3370.36	Dimer OH or Ar OH H bonded	Caroxylic acids or Penols
2.	2916.37	CH stretch or dimer OH	Alkane or Carboxylic acids
3.	2349.30	Si-H Silane	Misc.
4.	1730.15	C=O stretch	Aldehyde , Ester
5.	1637.66	C=C stretch	Alkene
6.	1448.54	S=O Sulphate ester	Misc.
7.	1366.96	S=O Sulphonyl chloride 1	Misc
8.	1103.28	C-O strech	Alcohol
Silver nanoparticles mediated from methanolic extract of *A .muricata*			
1.	3489.23	OH stretch	Alcohol
2.	3392.79	Dimer OH or Ar OH H bonded	Carboxylic acids or Phenols
3.	2929.87	-CH2-	Alkane
4.	2086.05	N=C in R NC=S	Misc.
5.	1645.28	C=C stretch or dienes or C=O stretch	Alkenes or Alkenes or Amides
6.	1635.64	C=O stretch	Amide
7.	1458.18	CH2 and CH3	Alkane
8.	1257.59	P=O Phosphoramide	Misc
9.	1097.50	C-O stretch	Alcohol
Silver nanoparticles mediated from aqueous extract of *T. foenum-graecum*			

1.	3460.08	O-H Stretch	Alcohol
2.	3442.94	NH Stretch	Amides
3.	3431.36	O-H Stretch	Alcohols
4.	2924.09	CH2	Alkanes
5.	2075.41	R-N=C=S	Misc.
6.	1641.42	C=C Stretch	Alkenes
7.	1454.33	CH2 and CH3	Alkanes
8.	1335.96	S=O Sulfone 1	Misc.
9.	1265.30	CH3	Alkanes
10.	1097.50	C-O Stretch	Alcohols
11.	939.33	=NOH (N-O)	Misc.
12.	590.22	C-Br	Alkyl halides
Silver nanoparticles mediated from methanolic extract of *T. foenum-graecum*			
1.	3421.72	O-H Stretching	Alcohol
2.	2918.30	CH stretch	Alkanes
3.	1641.42	C=C Stretch	Alkenes
4.	1448.54	S=O sulphate ester	Misc.
5.	1355.96	Arom. Nitrogen	Misc.
6.	1300.02	N-O Aromatic	Misc.
7.	1255.66	CH3	Alkanes
8.	1097.50	C-O Stretch	Alcohols
9.	894.97	=CH	Alkenes
10.	663.87	C-Br stretch	Alkyl halides
11.	572.86	C-Br stretch	Alkyl halides
12.	532.35	S-S Disulphide Asym.	Misc

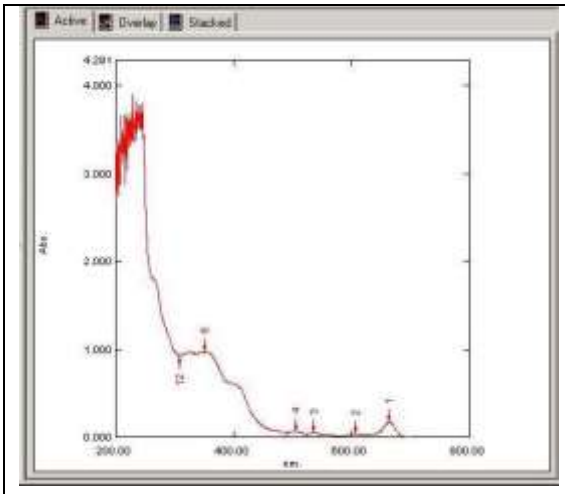


409.50 nm for Methanol extract

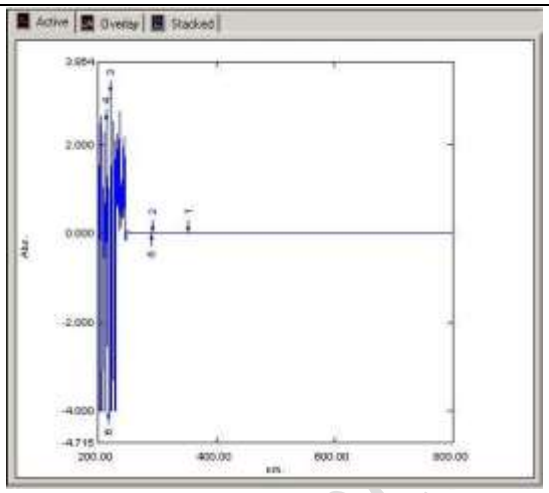


375.00 nm for Aqueous extract

Fig. 3 UV-Vis spectra of the formed AgNPs using MeOH and Aq extracts of *A. muricata*



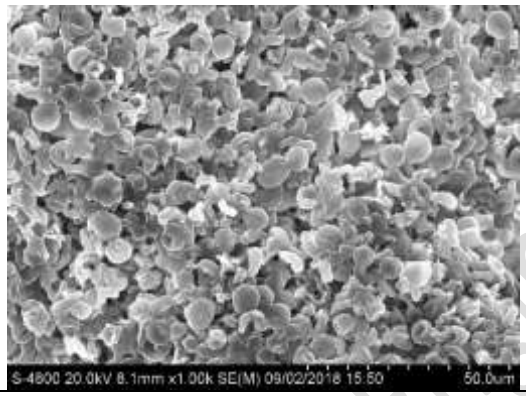
536.00 nm for Methanol extract



353.00 nm for Aqueous extract

Fig. 4 UV-Vis spectra of the AgNPs using MeOH and Aq extracts of *T. foenum-graecum*

Methanol extract



Aqueous extract

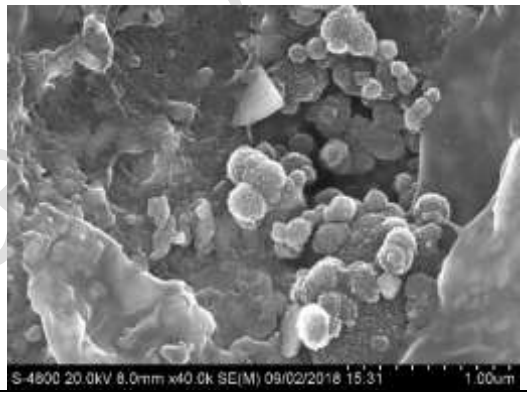
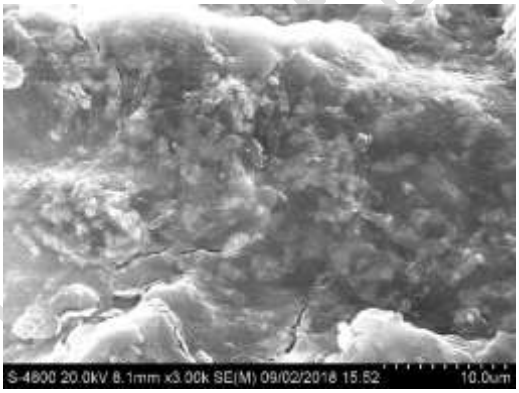
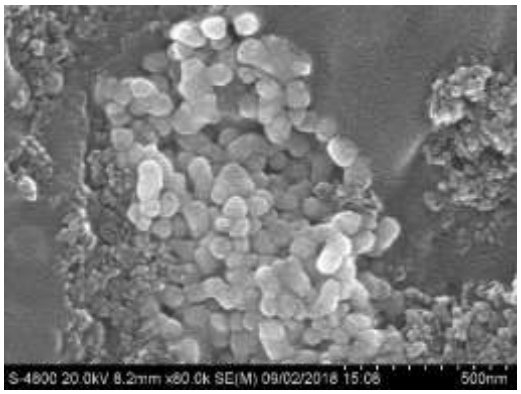


Fig. 5 TEM of the formed AgNPs using MeOH and Aq extracts of *A. muricata*



Methanol extract



Aqueous extract

Fig. 6 TEM image of AgNPs from methanol and aqueous extract of *T. foenum graecum* leaves