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A New Frontier Drug Development in Nanomedicine and Its Anti-urolithiatic Activity of *Kalanchoe pinnata*

F. JANEETA PRIYA[1]*, A. LEEMAROSE[2], S. VIDHYA[3], A. ARPUTHARAJ[4], S. AKSHANA[5] and U. F. RAMEEZ FATHIMA[6]

[1,2,3,5,6,]PG and Research Department of Chemistry,Holy Cross College, Affiliated to Bharathidasan University, Tiruchirappalli-6200 02, Tamil Nadu, India.
[4]Department of Electronics, St. Joseph's College, Affiliated to Bharathidasan University, Tiruchirappalli-6200 02, Tamil Nadu, India.
*Corresponding author E-mail: janeetapriya@gmail.com

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ABSTRACT

Latterly, the green synthesis of metallic nanoparticles has stock pile and terminal interest over the last decade due to their idiosyncratic properties that make them felicitous in various fields of science and technology. In this study the silver nanoparticles can be manufacture by using the leaf extract of *Kalanchoe pinnata* plant using microwave radiation method. The nanoparticles that are synthesized by plants are non-toxic and eco-friendly and it is characterized by using UV-Visible, FT-IR , X-ray diffraction (XRD) and TEM. The crystalline natures of the synthesized silver nanoparticles were identified by XRD and its shape was confirmed by TEM analysis. Anti-urolithiatic activity of synthesized silver nanoparticles was accomplished which reduces the growth of kidney stone which was appraisal by using the *In vitro* growth of urinary calculi.

Keywords: *Kalanchoe pinnata*, UV-Vis, FTIR, TEM, XRD, Anti-urolithiatic activity.

INTRODUCTION

Kidney stone is a most common disease for urinary tract infections. It is the type of disorder which affects the 12% of the population in the world[1-3]. At the end stage it leads to the renal failure. The contraption of the stone formation involves the complex process such as nucleation, super saturation, growth, aggregation and retention process which are associated with the physiochemical events. Contemporarily, there is no adequate drug to cure the prevention of reoccurrences of kidney stone. Recent studies investigated the work on the silver nanoparticles which are incorporated with the plant extract shows the medicinal applications and especially the anti-urolithiatic activity. The leaves of the *Kalanchoe pinnata* plant naturally containing the various anti-oxidant properties. The leaf powder of this plant was taken for curing the burning in urination, blocked urination and leprosy[4]. The compounds present in the plants can acts as anticancer agents. *Kalanchoe pinnata* is far better than aspirin; the aqueous extract of this plant shows the antinoceptive effect and produces the hormone such as prostaglandin, histamine. Currently, organic and inorganic nanoparticles can attractively to the field for diagnosis of kidney stones[5-7]. The silver nanoparticles were synthesized from the aqueous solution of silver nitrate by using the leaf extract of *Kalanchoe pinnata* plant[8].

EXPERIMENTAL

Collection of plant materials

The healing plant used in this study is the leaves of *Kalanchoe pinnata* plant which was collected from the Gandhi market area of Tiruchirappalli district, Tamil Nadu. The collected leaves were thoroughly washed with running water and shade dried for 10-15 days after that with the help of mortar and pestle the leaves were grinded; the powdered plant sample is utilized for the synthesis of silver nanoparticles.

Preparation of the extract

The *Kalanchoe pinnata* plant leaf extract was prepared by using water as a solvent. By using solvent extraction process the plant extract was prepared. Then hot percolation method was carried out and the filtrate can be collected and it might be used for further studies[9].

Quantitative analysis of *Kalanchoe pinnata* leaf

To find out the quantity of the major phyto constituents like flavanoids, tannin, saponin, alkaloid, phenol and terpinoids were present carried out by using stranded procedure.

Synthesis of silver nanoparticle

First an aqueous solution 5 Mm of silver nitrate is prepared using water as solvent and it is taken in a beaker and then 1 mL of leaf extract was added to required amount of $AgNO_3$ solution. After half an hour the colour changes from colour less to dark brown, which indicates the formation of AgNPs. The colloidal solution was taken for further analysis.

Characterization of silver nanoparticles

UV-visible analysis

The synthesized silver nanoparticles were carried out with the UV-Visible spectrophotometer from the range of 300-800nm. The band can be obtained in the colloidal silver nanoparticles is 400-450nm due to the excitation of surface plasma vibration.

FT-IR analysis

FT-IR measurements are used to identify the functional groups present in the plant extract and also used to find out the stability of the silver nanoparticles. The specific functional groups present in their corresponding frequency were observed.

XRD

This measurement is used to affirm the crystalline nature of silver nanoparticles[11]. From this also measure the purity of the substance. According to (JCPDS, File n04-0783) the swatch shows the face centered cubic structure for silver.

TEM

This analysis is used to measure the dimensions, structure and exterior morphology were identified with the help of this technique. In this technique, image can be formed with the help of interaction of electrons transmitted through the specimen.

In vitro growth of urinary calculi

Growth of CHPD crystals

About 1M solution of H_3PO_4 was mixed with Na_2SiO_3 solution of density 1.04 g/cm^3 at pH 9.4 and it was left aside for 2-3 days so that the pH of the solutions was maintained. After gelatin formation process, the liquid shows above a solid residue of 1M solution of $CaCl_2$ was poured onto the set gel in different glass test tubes, and then it was stopper with airtight stopples. The experiments were maintained at room temperature (37^{o}C).

To examine the hypothetical activity of the extract as inhibitors of CHPD crystal development in gel technology. To find out the efficiency of leaf of *Kalanchoe pinnata* on the growth of CHPD Crystals, various concentrations of synthesized silver nanoparticles are added. After thirty days the development of crystals are analyzed and calculated[12-15].

RESULTS AND DISCUSSION

Quantitative analysis

This analysis revealed the amount of phytochemical constituents present in the aqueous extract of *Kalanchoe pinnata* leaves. The various quantities of phytochemical present in the extract were shown in Table 1.

Table 1: Quantitative analysis of *Kalanchoe pinnata* leaves

S. No	Phytochemical constituents	*Kalanchoe pinnata* (mg/g)
1	Alkaloids	0.007
2	Saponin	0.006
3	Flavanoid	0.012
4	Phenol	0.01
5	Tannin	0.005
6	Terpenoids	0.005

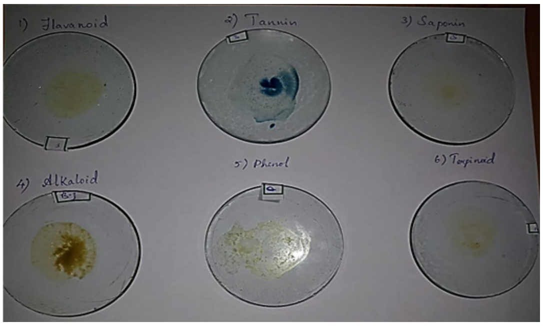

Fig. 1. Visual observation of phytochemical constituent present in *Kalanchoe pinnata*

Federico used HPLC-ESI/MS method to derive the phenolic compounds from the leaves of *Kalanchoe pinnata* and then leaves of *Kalanchoe pinnata* contain high level of flavonoids. Flavonoids used to prevent the oxidative cell damage, have strong anti-cancer activity. It contains high concentration of flavonoids followed by alkaloids, glycosides and steroids have been reported to be present in the aqueous leaf extracts (Figure 1).

Visual observation

In this experimental method, the addition of 50 µl aqueous extract of *Kalanchoe pinnata* is taken in a glass vial to that 1 ml of 5mM $AgNO_3$ solution is added drop by drop after 2 h the colour of the solution slightly changes from colorless to brown as shown in Fig. 2. The solution is centrifuged using cooling centrifuge at 15000 rpm then it is stored in the refrigerator for further analysis.

Fig. 2 (a) Silver nitrate solution, (b) synthesized silver nanoparticles

Characterization techniques

UV-visible spectroscopy

To determine the optical properties of a solution, absorbance spectroscopy is used. The broad absorption peak of synthesized silver nanoparicles was observed at 439nm was shown in Fig. 3. The phytochemical constituent present in *Kalanchoe pinnata* leaves extract was responsible for the reduction of silver nitrate to silver nanoparticles.

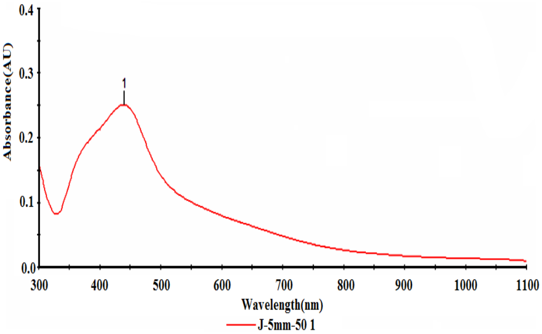

Fig. 3. UV-Vis spectrum of synthesized silver nanoparticles

FT-IR analysis

FT-IR measurements are taken to identify the functional group involved in this process. The FT-IR Spectra of synthesized silver nanoparticles were shown in Fig. 4. The corresponding peaks observed in the synthesized silver Nanoparticles are shown in the Table 2.

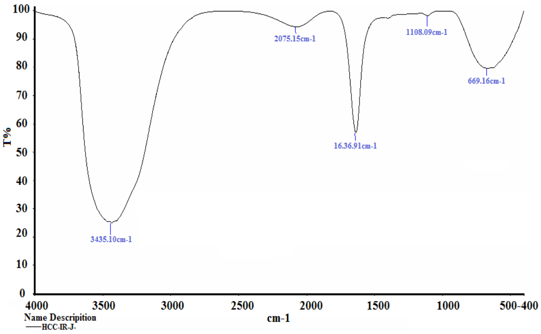

Fig. 4. FT-IR spectral data of synthesized AgNPs

Table 2; FT-IR spectral data of synthesized silver nanoparticles

Functional group	Band	Frequency, cm^{-1}
Primary amine	medium band	3435 cm^{-1} corresponds to N-H stretching vibrations
Isothiocyanate	strong band	2075 cm^{-1} corresponds to N=C=S stretching vibrations
Alkene	medium band	1636 cm^{-1} corresponds to C=C stretching vibrations
Amine	medium band	1108 cm^{-1} corresponds to C-N stretching vibrations
Halo compound	strong band	669 cm^{-1} corresponds to C-Br stretching vibrations

Transmission electron microscope

This study is used to identify dimensions, structure and exterior morphology of the synthesized nanoparticles. From this analysis, the synthesized AgNPs are well scattered and mostly spherical and some are having the structures of irregular in shape as shown in Fig. 5. The synthesized nanoparticles are homogeneous and spherical having 200nm and 100nm range.

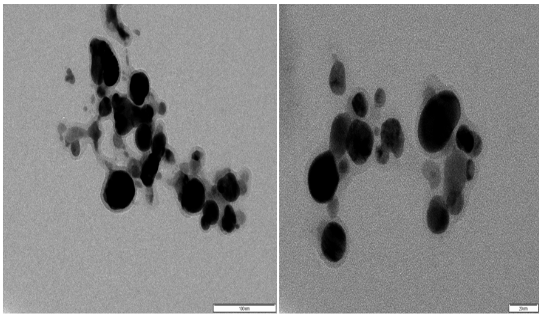

Fig. 5. TEM image of synthesized silver nanoparticle using *Kalanchoe pinnata*

XRD spectrum of silver nanoparticles

X-Ray Diffraction analysis used to find out the crystalline character of AgNPs. The analysis and XRD data are given in Table 3. Fig. 6 represents XRD analysis of synthesized silver nanoparticle using *Kalanchoe pinnata*. The peak corresponds at 38.4024, 44.8685 following diffraction faces are (111), (200) respectively. Unassigned peaks are as present in the graph this may due to extract contains some phytochemicals which may be capping the surface of nanoparticles.

Table 3: XRD analysis of synthesized silver nanoparticle using *Kalanchoe pinnata*

Position [°2Th.]	Heights[cts]	FWHM degree °2Theta	d-spacing[Å]	Rel. Int. [%]
17.1629	28.03	0.1476	5.16660	8.20
27.9614	170.73	0.2460	3.19103	49.92
32.3356	341.99	0.2460	2.76866	100.00
38.4024	28.41	0.5904	2.34408	8.31
44.8685	126.28	0.1968	2.02015	36.92
46.3896	143.71	0.2460	1.95740	42.02
54.9414	35.31	0.2952	1.67125	10.33
57.6203	44.36	0.1476	1.59976	12.97
65.1721	176.29	0.1476	1.43146	51.55
76.8331	23.92	0.2952	1.24070	6.99
78.2896	273.67	0.1800	1.22021	80.02
78.5638	126.02	0.1200	1.21966	36.85

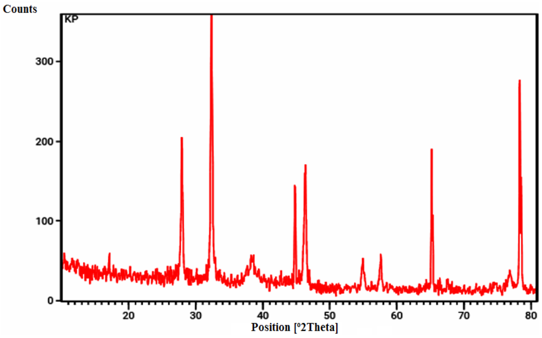

Fig. 6. XRD analysis of synthesized silver nanoparticle using *Kalanchoe pinnata*

Effect of *Kalanchoe pinnataon* CHPD crystals

The analysis of *Kalanchoe pinnata* extract on nucleation and crystallization of CHPD crystals is determined by finding out the quantity of crystals formed. The generations of Liesegang (5-10 rings) rings which have induce crystals growth (Fig. 7). In this technique, CHPD crystals development was reduced and framework of the crystals converted from hydroxyapatite in brushite crystals because of its inhibitory ability of nanoparticles at *In vitro* actions.

The formation of the control CHPD and combined with *Kalanchoe pinnata* CHPD crystals was shown below in Fig. 7. The amount of inhibition are shown in the Table 4.

Characterization of CHPD crystals

The Fourier transfer infra red spectral analysis of Calcium Hydrogen Phosphate dihydrate in presence and absence of the methanolic extract of sample shown below.

Table 4: inhibition of crystal growth

Crystals	Supernatant solution (groups and treatments)	Weight of crystal	Percentage inhibition of crystal
CHPD	I	132	0%
	II	72.6	45%
	III	51.6	60.9%
	IV	44.5	66.2%
	V	34	74.2%
	VI	26	80.3%
	VII	20	84.8%

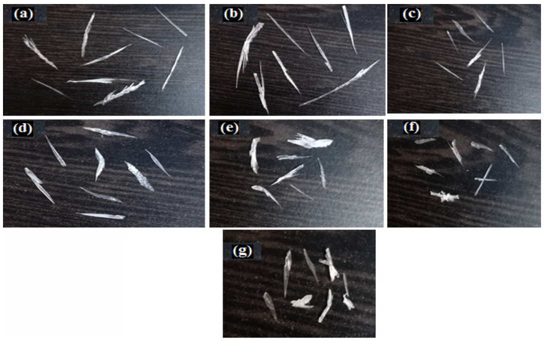

Fig. 7. The effect of leaves extract of *Kalanchoe pinnata* on Growth of Calcium Hydrogen Phosphate dihydrate crystals in the gel technique : Fig. 7(a) represents the Control which is taken for the analysis, Fig. 7(b) shows the distilled water taken, Fig. 7(c), 7(d), 7(e), 7(f), 7(g) represents the various concentrations (1%, 2%, 3%, 4% and 5%) of aqueous extract of synthesized silver nanoparticle synthesised from the leaves of *Kalanchoe pinnata*

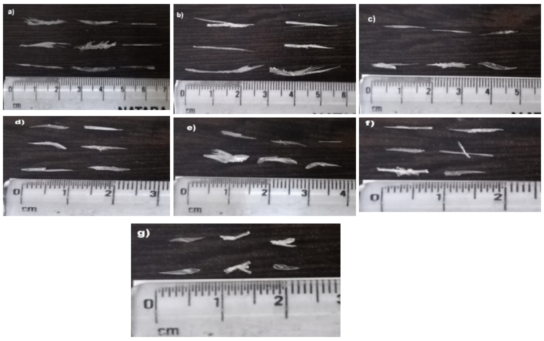

Fig. 8. Measurement of harvested Calcium Hydrogen Phosphate dihydrate crystals obtained from the leaves of *Kalanchoe pinnata* in the gel technique : Fig. 8(a) represents the Control which is taken for the analysis, Fig. 8(b) shows the distilled water taken, Fig. 8(c), 8(d), 8(e), 8(f), 8(g) represents the various concentrations (1%, 2%, 3%, 4% and 5%) of aqueous extract of synthesized silver nanoparticle synthesised from the leaves of *Kalanchoe pinnata*

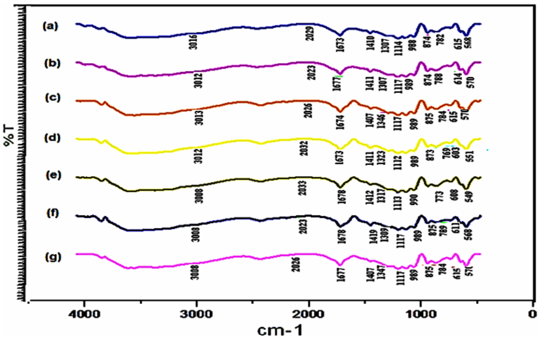

Fig. 9. The Fourier transfer infra red spectral analysis of Calcium Hydrogen Phosphate Dihydrate in the gel technique (a) without any additive (b) along the distilled water Fig.9(c), 9(d), 9(e), 9(f),9(g), represents the various concentrations (1%, 2%, 3%, 4% and 5%) of aqueous extract of synthesized silver nanoparticle synthesised from the leaves of *Kalanchoe pinnata*

Table 5: The FTIR spectral analysis of CHPD in the gel technique

Frequencies	Band
3016 cm^{-1}	intermolecular H bonded OH
2029 cm^{-1}	the peak is due to HPO_4^{2}
1673 cm^{-1}	H-O-H bending
1410 cm^{-1}	P=O combined with stretching vibrations
1114 cm^{-1}	P=O stretching vibrations
988, 874 cm^{-1}	P-O-P asymmetric stretching vibrations
615 cm^{-1}	owing to (H-O-) P=O
3012 cm^{-1}	intermolecular and weakly H bonded OH
1677 cm^{-1}	H-O-H bending
2023 cm^{-1}	week absorption of HPO_4^{2-}
1407 cm^{-1}	P=O associated stretching vibrations
1117 cm^{-1}	P=O stretching vibrations.
874 cm^{-1}	P-O-P asymmetric stretching vibrations
788 cm^{-1}	due to (H-O-)P=O
578 cm^{-1}	strong absorption peak at acid phosphate
3013 cm^{-1}	absorption of OH ions
2026 cm^{-1}	PO4 stretching vibrations
989, 875 and 784 cm^{-1}	P-O-P asymmetric stretching vibrations
615,570 cm^{-1}	acid phosphate
2023 cm^{-1}	owing to PO_4 stretching vibrations
989, 873 and 769 cm^{-1}	P-O-P asymmetric stretching vibrations
603,551cm^{-1}	Owing to acid phosphate.

CONCLUSION

By using the green method, synthesis of therapeutically used nanoparticles should be produced which is more effective and manufactured at larger scale. There may be a lot of upgrade treatments are available to treat the kidney stone. It is one among the study to medicaments the kidney stone by using naturally available plants with the help of nanoparticles synthesis and it is proved by carrying out certain studies are discuss above. This study concluded that the synthesis of silver nanoparticles using plant extract is energy efficient and bio-hazardous chemical synthesis. Our present study is to contribute for green chemistry particularly and also the green synthesis metal of nanoparticles and analyze it's therapeutic applications.

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Conflicts of Interest

The authors declare no conflict of interest.

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