Plant Archives Vol. 20, Supplement 2, 2020 pp. 4295-4299 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EVALUATION OF *IN VITRO* ANTIUROLITHIATIC ACTIVITY OF *THUNBERGIA ERECTA*

V. Chandel*, A. Choubey and S.Asati
*Madhyanchal Professional University (MPU) Educational Institute, Bhadbhada Road, Ratibad,
Madhya Pradesh 462044 India
*Corresponding Author : VinodChandel
Madhyanchal Professional University (MPU)-Educational Institute,
Bhadbhada Road, Ratibad, Madhya Pradesh 462044
E-mail: vinodchandel1429@gmail.com

Abstract

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. The aim of the present study was to evaluate qualitative, quantitative phytochemical analysis and *in vitro* antiurolithiatic activity of a methanolic extract from the leaves of *Thunbergia erecta* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. The antiurolithiatic assay was performed by nucleation method. Phytochemical analysis revealed the presence of carbohydrates, terpenoids, flavonoids, alkaloids, glycosides and steroids. The total phenolics content of leaves of methanolic extract was (174.66 mg/gm), followed by flavonoids (282.33mg/gm). Methanolic extract exhibited concentration-dependent inhibition of CaOx crystal formation. The *in vitro* assay revealed significant inhibition of crystal formation (65.38%), in the group treated with 100% extract. *Thunbergia erecta* extract possesses significant antiurolithiatic activity against CaOx urolithiasis *in vitro* which could be attributed to its saponins, tannins, flavonoids and polyphenolic content.
***Keywords*:** *Thunbergia erecta,* Antiurolithiatic activity, Phytochemical analysis, nucleation method

Introduction

Urolithiasis refers to the formation of calculi anywhere in the urinary tract afflicting the kidneys, ureters, bladder or urethra (Vijaya *et al.,* 2013). It is the third most prevalent urological disorder after urinary tract infection and prostate conditions (Al-Jebouri & Atalah 2012). Approximately 12% of the world population is affected by the incidence of urinary stones with a comparatively higher frequency of recurrence in males (70-81%) than in females (47-60%) (Joy *et al.,* 2102). Depending upon the chemical nature, there are various types of stones such as calcium oxalate monohydrate, calcium oxalate dihydrate, basic calcium phosphate (brushite), magnesium ammonium phosphate (struvite), uric acid and cystone stones (Nagal & Singla 2013). Manifestations include colicky pain, vomiting, dysuria, haematuria, pyuria and oliguria (Tiwari *et al.,* 2012). These calculi arise as an outcome of super saturation of urine with stone forming constituents resulting in crystallization followed by crystal nucleation, aggregation and growth leading to their adherence to the renal tubules (Agarwal *et al.,* 2013). Numerous surgical strategies exist in scenario for management of urinary calculi such as shock wave lithotripsy, ureteroscopic lithotripsy, digital endoscopy, percutaneous nephrolithotomy and robotic surgery(Rosa *et. al.,* 2013). However, many of these techniques bring along with them several drawbacks such as renal casualties in the long run, hypertension and repeated episodes of stones (Began *et al.,* 1991). Several allopathic interventions are available but are increasingly being overtaken by herbal therapeutics on account of their safety, minimal side effects, comparatively greater efficacy in dissolving stones and preventing the chances of recurrence (Alok *et al.,* 2013). Moreover, the applications of medicinal plants in combating urinary stones have been extensively mentioned in the ancient medical literature as well as Ayurvedic system of medicine in addition to their relevance as folk remedies (Chitme *et al.,* 2010). *Thunbergia erecta* (Syn: *Meyeniaerecta,* Acanthaceae) is also known as King's Mantle, Blue Bell and Bush Clock Vine (Manokari & Shekhawat, 2017). It is woody, straight shrub which has 2-2.5 m altitude. It contains flower that blooms throughout the year and has blue to purple color range (Kitajima *et al.,* 2010). *Thunbergia* is native to the tropical part of Madagascar, Australia, Africa, India and South Asia. Leaves, roots and stems of *Thunbergia* species have the traditional history to be used for the treatment of inflammation and pyrexia (Jeeva *et al.,* 2011). *Thunbergia* species have been reported to show antibacterial effects against both gram positive and gram negative bacteria (*Oonsivilai et al.,* 2008). *Thunbergia* species also possess analgesic, antipyretic, anthelmintic, antidiabetic, cytotoxic, antioxidant, hepatoprotective (*Oonsivilai et al.,* 2008 & Wonkchalee *et al.,* 2008), antitumor and antinociceptive activities (Jetawattana *et al.,* 2015). Chemical ingredients separated from *Thunbergia* genus include alkaloids, glucosides, naphthalene, coumaroylmalic acid, iridoidglucosides, grandifloric acid, benzyl beta glucopyranoside, delphinidin, apigenin and phenolic compounds such as tannin, feruloylmalic, flavonoids, phenolic acids, rosmarinic acid (Kanchanapoom *et al.,* 2002 &Areekul *et al.,* 2009). Therefore, current investigation was carried out for the screening of the antilithic potential of *Thunbergia erecta* against calcium oxalate crystallization in an in vitro setting.

Materials and Methods

Plant material

The leaves of *Thunbergia erecta* were collected from Pinnacle Biomedical Research Institute (PBRI), Near, Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Depot Chouraha, Bhopal, Madhya Pradesh 462003, Indiain the month of July 2019.The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number 195/Saif./Sci./Clg/Bpl was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction

Cold maceration method

In present study, plant materials was extracted by using cold maceration method; the leaves of were collected, washed and rinsed properly. About 3kg of the powder was extracted with different organic solvents petroleum ether and methanol and allow standing for 4-5days each. The extract was filtered using Whattman no.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated & amp; excessive moisture was removed and extract was collected in air tight container. Each extract was dissolved by Dimethyl sulfoxide (DMSO) and sterilized using 0.22 µm syringe filters (Axiva, Scichem Biotech) for further use (Nayak *et al.,* 2019).

Qualitative analysis of phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in methanolicextracts of *Thunbergia erecta*. The above phytoconstituents were tested as per the standard method (Kokate*et. al.,* 2006).

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phyto constituents present in plant extracts. For this TPC and TFC are determined. Methanolic leaves extracts of *Thunbergia erecta* plant material of subjected to estimate the presence of TPC and TFC by standard procedure (Parkhe *et al.,* 2018).

Total phenolic content estimation

The total phenolic contents were determined by using Folin-Ciocalteu reagent. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100µg/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The total phenolic contents were expressed as mg/g gallic acid equivalent.

Total flavonoid content estimation

The total flavonoid contents were determined using the aluminum chloride assay. An aliquot (0.5 ml) of extracts were taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminum trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used as blank. The total flavonoid content was expressed in mg of rutin equivalents per gram of extract.

In-vitro anti urolithiasis (Nucleation assay)

Preparation of synthetic urine

We chose the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used. Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 m mol/1) and B: Ca Cl_2 $2H_2O$ (10 m mol/l). The two solutions were prepared stock NaCl 9 g to obtain the ionic force like the Indoor environments. The formation and growth of the COM crystals of oxalate from artificial urine at different concentration was the object of our investigation. Artificial urine is prepared by mixing and stirring two equal volumes of 50 ml of solutions A and B at constant temperature (37°C) in capped vessels to give final artificial urine. Mixture agitation was maintained to prevent sedimentation (Beghalia *et al.,* 2008).

Simulation of the sedimentary crystal formation

The crystal size development was monitored in sample drops every five minutes by polarized microscope. A drop of sample was put on hemacytometer counting chamber and observed sample under microscope at time after 30 min. calculated number of crystals and catches of sight with a camera. A series of experiments corresponding to the physiological concentrations of 25, 50, 75, and 100% of plants extracts were used. The follow-up of the crystal size development by microscope was carried out at time after 30 min of formation of crystals and catches of sight with a camera. Calculated the percentage of Inhibition (I %) was based on the formula (Hennequin *et al.,* 1993).

$$\mathbf{I\% = [(TSI - TAI) / TSI] * 100}$$

TSI- represents the number of calcium oxalate monohydrate crystals without inhibitor.

TAI- represents the number of calcium oxalate monohydrate crystals after addition of inhibitor.

Result and Discussion

Phytochemical analysis of methanolic extract of *Thunbergia erecta* leaves showed the presence of

carbohydrate, alkaloids, flavonoids, phenolics, tannin, saponins, and triterpenoids table 1.Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and the TPC in methanolic extract was found to be 174.66 mg/gm equivalent to gallic acid table 2. TFC was then calculated with respect to rutin taken as standard. The TFC in methanolic extract was found to be 282.33 mg/gm equivalent to rutin table 3.A large number of people in this world are suffering from urinary stone problem. Calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) containing stones are commonly found in kidney. Herbal extracts may contain substances that inhibit the growth of CaOx crystals. This property of plants may be important in preventing kidney stone formation; CaOx crystals induced by urinary macromolecules are less tightly bound to epithelial cell surfaces, which are then excreted with urine (Wesson *et al.,* 1998). The extract may also contain substances that inhibit CaOx crystal aggregation; the agglomeration of particles is a critical step in urinary stone formation, as larger crystals are less likely to pass spontaneously in the urinary tract (Kok & Khan, 1994). If the extract keeps CaOx particles dispersed in solution, they are more easily eliminated. The formation and growth of the calcium oxalate monohydrate crystals from artificial urine at different concentrations were studied. Stone formation was the due to super saturation of urine with some urinary salts such as calcium oxalate. The number of calcium oxalate monohydrate crystals was found to be maximum in control. Different percentages of plant extract were tested in order to assess the inhibiting potential of plant extract for oxalate crystallization. In the presence of different percentages of plant extract, the size (length and the width) of the crystals were reduced. It was observed that the plant used in this study inhibited the crystal development with maximum number of crystals found at 25 % physiological extract concentration while at 100 % physiological concentration of extract, the crystal formation was found minimal. Results show that the decrease in number of crystal as well as % inhibition of the formation of calcium oxalate monohydrate crystals was directly proportional to the increase in percentage of plant extract, with minimum inhibition of 30.77% at 25 % physiological concentrations of methanolic extract of *Thunbergia erecta*, while maximum inhibition of 65.38% at 100 % physiological concentrations of methanolic extract of *Thunbergia erecta* as compared to standard as depicted in Table 4.

Conclusion

Findings of the present study clearly demonstrate that the methanolic extract of *Thunbergia erecta* can inhibit the nucleation of CaOx crystallisation *in vitro*. However, the mechanism of action of the extract in animal models of lithiasis needs to be investigated. As the observed activity of the plant extract might be due to other phytochemicals present in it, further characterization and isolation of the major active components from the plant extract are required.

Table 1 : Result of phytochemical screening of *Thunbergia erecta*Petroleum ether extract

S. No.	Experiment	Results	
		Pet. Ether Extract	Methanolic extract
	Test for Carbohydrates		
1	Molisch's Test	-ve	+ve
2	Fehling's Test	-ve	+ve
3	Benedict's Test	-ve	+ve
	Test for Protein & Amino acids		
4	Biuret's Test	-ve	-ve
5	Ninhydrin Test	-ve	-ve
	Test for Glycosides		
6	BorntragerTest	-ve	+ve
7	Killer killaniTest	-ve	+ve
	Test for Alkaloids		
8	Mayer's Test	-ve	+ve
9	Hager's Test	-ve	+ve
10	Wagner's Test	-ve	+ve
	Test for Saponins		
11	Froth Test	-ve	+ve
	Test for Flavonoids		
12	Lead acetate	-ve	+ve
13	Alkaline reagent test	-ve	+ve
	Test for Triterpenoids and Steroids		
14	Libermann-Burchard Test	-ve	-ve
15	Salkowski Test	-ve	-ve
	Test for Tannin and Phenolic Compounds		
16	Ferric Chloride Test	-ve	+ve
17	Gelatin Test	-ve	+ve
18	Lead Acetate Test	-ve	+ve

Table 2 : Total Phenolic content in *Thunbergia erecta*methanolic extract

S.No.	Absorbance	Concentration	Total phenolic content in mg/gm equivalent of Gallic acid
1	0.412	1mg/ml	174.66 mg/g equivalent of Gallic acid
2	0.411	1mg/ml	
3	0.414	1mg/ml	

Table 3 : Total Flavonoid content in *Thunbergia erecta*methanolic extract

S.No.	Absorbance	Concentration	Total flavonoid content in mg/gm equivalent of Rutin
1	0.373	1mg/ml	282.33 mg/g equivalent of Gallic acid
2	0.374	1mg/ml	
3	0.376	1mg/ml	

Table 4 : Effect of different concentrations of standard and methanolicextract of *Thunbergia erecta*on COM crystal nucleation

S. No.	Sample	Standard		Methanolic extract of *Thunbergia erecta*	
		COM/mm^3	% Inhibition	COM/mm^3	% Inhibition
1	Control	325	-	325	-
2	25 %	187.5	42.31	225	30.77
3	50 %	150	53.85	187.5	42.31
4	75 %	100	69.23	137.5	57.69
5	100 %	62.5	80.77	112.5	65.38

References

Agarwal, K.P.; Narula S.; Kakkar M.; Tandon C. (2013) Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. Biomed Res Int; 292-953.

Al-Jebouri M.M.; Atalah N. (2012). A study on the interrelationship between renal calculi, hormonal abnormalities and urinary tract infections in Iraqi patients. Open J Urol; 2: 6-10.

Alok, S.; Jain, S.K.; Verma, A.; Kumar, M. and Sabharwal M. (2013) Pathophysiology of kidney, gall bladder and urinary stones treatment with herbal and allopathic medicine: a review. Asian Pac J Trop Dis; 3: 496-504.

Areekul S. *et al.* (2009). Wild plant knowledge used in northern Thailand III. Bangkok: Amarin Printing and Publishing Public Company Limited.

Beghalia, M.; Ghalem, S.; Allali H.; *et al.* (2008). Inhibition of calcium oxalate monohydrate crystal growth using Algerian medicinal plants. Journal of Medicinal Plants Research, 2(3): 066-070.

Begun, F.P.; Knoll, C.E.; Gottlieb, M.; Lawson, R.K. (1991) Chronic effects of focused electrohydraulic shock-waves on renal function and hypertension. J Urol; 145: 635-9.

Chitme, H.R.; Alok, S.; Jain, S.K. and Sabharwal, M. (2010) Herbal treatment for urinary stones. Int J Pharm Sci Res; 1: 24-31.

Hennequin, C.; Lalanne, V.; Daudon M.; *et al.* (1993). A new approach to studying inhibitors of calcium oxalate crystal growth. Urology Research, ; 21(2):101-8.

Jeeva, S. *et al.* (2011) Preliminary phytochemical and antibacterial studies on flowers of selected medicinal plants. International Journal of Medicinal and Aromatic Plants.; 1(2): 107-14.

Jetawattana, S. *et al.* (2015). Radical intermediate generation and cell cycle arrest by an aqueous extract of *Thunbergia laurifolia* Linn. In human breast cancer cells. Asian Pacific Journal of Cancer Prevention.; 16(10): 4357-61.

Joy, J.M.; Prathyusha, S.; Mohanalakshmi, S.; Praveen Kumar, A.V.S.; Ashok Kumar, C.K. (2012). Potent herbal wealth with litholytic activity: a review. IJIDD; 2: 66-75.

Kanchanapoom, T.; Kasai, R.; Yamasaki, K. (2002). Iridoidglucosides from *Thunbergia laurifolia*. Phytochemistry.; 60(8): 769-71.

Kitajima, E.W.; Rodrigues, J.C. and Freitas-Astua, J. (2010). An annotated list of ornamentals naturally found infected by Brevipalpus mite-transmitted viruses. Scientia Agricola.; 67(3): 348-371.

Kok, D.J. and Khan, S.R. (1994). Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney International, 46: 847-854.

Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. (2006). Pharmacognosy; 23 ed.; Nirali prakashan.; 493-497.

Manokari, M. and Shekhawat, M.S. (2017). Biosynthesis and characterization of zinc oxide nanoparticles using *Thunbergia erecta* (Benth.) T. Anders plant extracts. Archives of Agriculture and Environmental Science; 2(3): 148-51.

Nagal, A. and Singla, R.K. (2013). Herbal resources with antiurolithiatic effects: a review. Indo Global J Pharm Sci; 3: 6-14.

Nayak, A.; Garg, M.; Jain, S.; Khan, M.A.; Jain, D.K.; Khan, N. (2019). Anti-Urolithiatic and Invitro-Invivo Anti-Oxidant Effects of Methanolic Extract of Thunbergialaurifolia on Ethylene Glycol-Induced Kidney Calculi in Rats. Sch. Acad J Pharm; 8 (3): 94–104.

Oonsivilai, R.; Ferruzzi, M.G. and Ningsanond, S. (2008). Antioxidant activity and cytotoxicity of Rang Chuet (*Thunbergialaurifolia* Lindl.) extracts. Asian Journal of Food and Agro-Industry.; 1(02): 116-28.

Parkhe, G.; Jain P. and Jain, D.K. (2018) Total phenolic content, total flavonoid & anti-bacterial activity of hydroalcoholic acacia catechu (l.f.) Willd roots extracts for treatment of mouth ulcer. Pharmacologyonline; 2: 227-233.

Rosa, M.; Usai, P.; Miano, R.; Kim, F.J.; Agrò, E.F. and Bove, P. (2013). Recent findings and new technologies in nephrolithiasis: a review of the recent literature. BMC Urol; 13: 1-11.

Tiwari, A.; Soni, V.; Londhe, V.; Bhandarkar, A.; Bandawane, D. and Nipate, S. (2012). An overview on potent indigenous herbs for urinary tract infirmity: urolithiasis. Asian J Pharm Clin Res; 5: 7-12.

Vijaya, T.; Kumar, M.S.; Ramarao, N.V.; Babu, A.N.; Ramarao, N. (2013) Urolithiasis and its causes- short review. J Phytopharmacol; 2: 1-6.

Wesson, J.A.; Worcester, E.M.; Wiessner, J.H. *et al.* (1998) Control of calcium oxalate crystal structure and cell adherence by urinary macromolecules. Kidney International, 53: 952-957.

Wonkchalee O. *et al.* (2012). Anti-inflammatory, antioxidant and hepatoprotective effects of *Thunbergia laurifolia* Linn. On experimental opisthorchiasis. Parasitology research; 111(1): 353-359.