Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(6): 43-47
© 2016 JMPS
Received: 10-09-2016
Accepted: 11-10-2016

Priya Dwivedi
B. Pharm Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Sayajipura, Vadodara, Gujarat, India

Jaya Dwivedi
B. Pharm Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Sayajipura, Vadodara, Gujarat, India

Dhara Patel
Assistant Professor of Quality Assurance, Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Sayajipura, Vadodara, Gujarat, India

Sharav Desai
Assistant Professor of Microbiology, Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Sayajipura, Vadodara, Gujarat, India

Dhanajay Meshram
Principal of Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Sayajipura, Vadodara, Gujarat, India

Correspondence
Priya Dwivedi
B. Pharm Pioneer Pharmacy Degree College, Nr. Ajwa Crossing, Sayajipura, Vadodara, Gujarat, India

Phytochemical analysis and assessment of *in vitro* urolithiatic activity of colocasia leaves

Priya Dwivedi, Jaya Dwivedi, Dhara Patel, Sharav Desai and Dhanajay Meshram

Abstract
Phytochemicals are responsible for medicinal activity of plant species. They have ability for curing various ailment and possesses potential anti-inflammatory, anti-bacterial, anti-oxidant and anti-fungal properties. Natural products from medicinal plants, either as pure compounds or as extracted out, provide opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to raising demand for chemical diversity nowadays in screening programs, seeking therapeutic drugs from herbal products, these are quite interesting throughout the world. Botanicals and herbal preparations for medicinal purpose contain various types of bioactive compounds. *Colocasia esculenta* commonly known as taro belonging to family Araceae are herbaceous perennial plants with a large corm on or just below the ground surface. Continuous research have revealed many chemical constituents isolated from this plant to exhibit several biological activities such as antimicrobial activity, fever, anti-inflammatory, stomach disorder, conjunctivitis, and loss of appetite for children. The present study aims at studying the anti urolithiatic activity of methanolic and chloroform extracts of the leaves of *Colocasia esculenta using* artificial kidney membrane. From the above study, it was found that it does show the urolithiatic study.

Keywords: Phytochemicals**,** Natural products, methanolic extracts, chloroform extracts *Colocasia esculenta*, anti urolithiatic activity

Introduction
The major problem faced by most of the population in India is related to kidney. Kidney is the major excretory organ in human beings. The most common disease associated with it is urolithiasis defined as the formation of a stone in the kidneys or urinary tracts for many reasons. In India, more than 10 lakhs patients suffer from stone disease and at least 1/1000 of Indian population require hospitalization because of kidney stone disease. Recently, urinary stone formation affects 10-12% of the population in industrialized countries at age 20 to 40 years [1-7]. Kidney stone mainly contains calciumin form of calcium oxalate monohydrate, calcium oxalate dihydrate the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%. In most of the cases the commonly occurring stones are calcium oxalate type. The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes nucleation, crystal growth, crystal aggregation and crystal retention. The stone formation requires supersaturated urine. Supersaturation also depends on urinary pH, ionic strength, solute concentration and complexations [8]. In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Endoscopic stone removal and extracorporeal shock wave lithotripsy are expensive and recurrence is quite common with these procedures [9]. Thus a drug for the prevention of this disease or its recurrence would be of great interest. Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the *in vitro* or *in vivo* models [10-11]. A large number of Indian medicinal plants are being routinely used by practitioners of Ayurvedic system of medicine in the treatment of urinary stone disease [12]. Many plants have also been reported all over the world which are able to inhibit kidney stones [13-14]. *Colocasia esculenta* are herbaceous perennial plants with a largecorm on or just below the ground surface. The leaves are large to very large, 20–150 cm (7.9–59.1 in) long, with a sagittate shape. The elephant's- ear

plant gets its name from the leaves, which are shaped like a large ear or shield. The plant reproduces mostly by means of rhizomes (tubers, corms) but it also produces clusters of two to five fragrant inflorescences in the leaf axil. Like other members of the family, the plant contains an irritant which causes intense discomfort to the lips, mouth and throat. This acridity is caused in part by microscopic needle like raphides of calcium oxalate monohydrate and in part by another chemical, probably a protease [15]. The acridity helps to naturally deter herbivores from eating it. It must be processed by cooking, soaking or fermenting - sometimes along with an acid (lime or tamarind) before being eaten [16]. The species *Colocasia esculenta* is invasive into wetlands along the American Gulf coast, where it threatens to displace native wetland plants.

Fig 1: *Colocasia esculenta* leaves

Material and Methods

Collection of Plant Materials and chemicals

Leaves of *Colocasia esculenta* were collected from the local market of Vadodara. The leaves were washed under the normal tap water to remove dust and other unwanted particles and rinsed the leaves and dried it completely under room temperature.

All the chemicals were procured from Aatur Rasayan laboratory, Vadodara.

Extraction and Isolation

Fresh leaves (10-20 gm) of *colocasia* were dried at room temperature (32 – 35 °C) to constant weight over a period of 7 days. The dried leaves were converted into powdered using a mixer. 16.4 g of the powdered leaves were extracted in 500 ml beaker with 90% methanol (methanolic extraction) and chloroform extracts. The beaker was covered appropriately with disk, and allowed to stand at room temperature for 2 days with occasional manual agitation of the flask using a sterile glass rod at every 24 hour. The extracts were filtered out using sterile whatman filter paper. Methanolic extracts were kept on a water bath for evaporating the methanol and the residue was dried in a desiccator. In case of chloroform extracts, it was directly filtered. These extracts was used in further process. The prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents like alkaloids, flavonoids, steroid, volatile oil, glycoside, reducing sugar, tannins and saponins. This experiment was carried out in 2016 in Pioneer Pharmacy Degree College, Vadodara.

Alkaloids

1) Mayer's Reagent (KI + Hg_2Cl_2 solutions)
2) Dragondorff's reagent (excess of KI + $BiNO_3$ solutions)
3) Wagner's reagent (I_2 + KI solutions)
4) Hager's reagent (Picric acid)

Flavonoids

1) **Ammonia Test:** Filter Paper strips were dipped in the alcoholic solution of the extract and ammoniated.
2) **Shinoda/Pew Test:** To 1 ml of the extract, a piece of metallic magnesium was added, followed by the addition of 2 drops of hydrochloric acid.

Tannins

1) 2ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution was added to each extract.

Reducing Sugars

1) To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.

Volatile Oil

1) 2ml of Extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl.

The result of phytochemical assessment is reported in Table 1

Evaluation for Anti-urolithiatic Activity

To know the role of plant extract in dissolving the already formed stones nucleus in renal system an artificial calcium oxalate crystals were prepared in the laboratory by standard method [17-19]. Also semi permeable membrane was prepared from egg using standard methods [20-21].

Step-1: Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation

1.47gm of calcium chloride dihydrate was dissolved in 100ml distilled water and 1.34gm of sodium oxalate was dissolved in 100 ml of 2N H_2SO_4. Both were mixed equally in a beaker to precipitate out calcium oxalate with stirring. Equimolar solution of calcium chloride dehydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10 ml of (2N H_2SO_4), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium

phosphate. Both precipitates freed from traces of H_2SO_4 by ammonia solution. Washed the precipitates with distilled water and dried at 60 ^{0}C for 4 hours.

Step-2: Preparation of semi-permeable membrane from farm eggs

The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Apex of eggs was punctured by a glass rod in order to squeeze out the entire content. Empty eggs were washed thoroughly with distilled water and placed in a beaker consisting 2 M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, placed it in ammonia solution for neutralization of acid traces in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step-3: Estimation of Calcium oxalate by Titrimetry

The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semipermeable membrane of egg as shown in the model designed given below (Figure 1 a-b). This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. First group served as blank containing only1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drug, *i.e*. cystone. The 3rd and 4th groups along with 1 mg of calcium oxalate contain methanolic, and chloroform extracts. The conical flasks of all groups were kept in an incubator preheated to 37 ^{o}C for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N KMnO4 till a light pink colour end point obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts.

Each ml of 0.9494 N $KMnO_4$ equivalent to 0.1898mg of Calcium oxalate.

(a) (b)

Fig 2: Experimental *in vitro* anti urolithiatic model (a) Egg membrane for urolithiasis activity (b) Titration result showing pink color as end color

Result

The present study reveals that Colocasia plant shows the presence of phytochemical constituents like alkaloids, flavonoids, proteins, tannins, and anthraquinones in solvent extracts as shown in

Table 1: Summary for phytochemicals tests and observations

Tests	Reagents	Observation	Chloroform	Methanolic
Alkaloids	Dragendroff's test:	Orange brown ppt is formed	Slightly occurs	Sharply occurs
	Mayer's test:	Its gives ppt	Slightly occurs	Sharply occurs
	Hager's test:	Gives yellow ppt	Slightly occurs	Sharply occurs
	Wagner,s test:	Gives reddish brown ppt	Slightly occurs	Sharply occurs
Glycosides and reducing sugars	Molish's test:	Violet ring is formed at the junction of two layers	Slightly occurs	Sharply occurs
	Benedict's test:	Solution appears like green colour	Slightly occurs	Sharply occurs
Flavonoids	Shinoda test:	Pink colour observed	Slightly occurs	Sharply occurs
	Add lead acetate solution	Yellow colour ppt is formed	Slightly occurs	Sharply occurs
Volatile Oils		Odour	Slightly occurs	Sharply occurs
Tannis	Lead acetate solution	White ppt	Sharply occurs	Sharply occurs
	Gelatin solution	White ppt	Sharply occurs	Sharply occurs

Table 2: Summary of phytochemical constituents in *Colocasia esculenta*

Solvents used for extraction	Alkaloid	Flavonoids	Saponin	Sterol	Tannin	Glycoside	Reducing sugar	Volatile oil
Chloroform	+	+	--	--	+++	+	+	+
Methanolic	+++	+++	--	--	+++	+++	+++	+++

Here, =+ indicates that slightly present, +++ sharply present, - absent

On basis of this fraction we performed *in vitro* Anti-urolithiatic activity by comparing different extracts of *Colocasia* with standard. % Dissolution of Calcium oxalate is given below:

Table 3: % Dissolution of calcium oxalate

Sr. No.	Groups	% Dissolution of calcium oxalate
1	Blank	0
2	Positive control	58.410
3	Chloroform extract	0.3398
4	Methanolic extract	0.2998

Table 4: Descriptive statistics for individual extracts with concentrations

Extract	Concentration	N	Mean%	Std deviation	Std error.	95% confidence interval for mean		minimum	maximum
						Lower bond	Upper bond		
standard	10	3	58.391	0.243	0.143	56.801	59.014	57.250	58.690
	20	3	60.390	0.227	0.135	59.926	61.594	61.230	61.230
	30	3	65.410	0.155	0.122	65.010	66.790	65.230	66.550
	40	3	65.193	0.160	0.089	64.77	66.156	62.330	66.320
methanolic	10	3	43.330	0.210	0.025	42.115	43.169	43.210	43.600
	20	3	45.500	0.750	0.230	45.113	45.123	45.310	44.620
	30	3	49.120	0.560	0.042	49.432	49.654	49.350	49.650
	40	3	49.222	0.112	0.035	48.293	49.564	49.320	49.230
Chloroform	10	3	47.300	0.178	0.062	42.632	47.832	47.231	48.230
	20	3	50.339	0.043	0.098	49.352	49.761	49.230	48.260
	30	3	50.700	0.221	0.028	50.234	50.597	50.230	50.230
	40	3	50.590	0.222	0.059	49.808	50.293	50.120	50.600

* mean is for % dissolution of calcium oxalate crystals

Discussion

The present study showed the presence of various chemical constituents like alkaloids, glycoside, flavonoid, volatile oil, tannins in Colocasia leaves. This study evaluated the anti urolithiatic activity of extracts of Colocasia esculenta leaves. This work was performed by using *in vitro* urolithiatic model for calculating percentage dissolution of kidney stone. This study has given primary evidence for Colocasia esculenta as the plant which possesses anti urolithiatic activity.

Conclusion

The present study conclusively demonstrates that Colocasia leaves a good source of various phytochemicals like alkaloids, flavonoids, glycosides, proteins, phenolic groups and reducing sugars. This study evaluates the antiurolithiatic activity of extracts of colocasia leaves. The work was done by using *in vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone. More specifically, we can conclude from the results that methanolic and chloroform extracts both shows good anti-urolithiatic activity. But methanolic extract showed better activity as compared to chloroform extract. The authors of the above work recommended the plant extract for the further studies by *in vivo* model study.

Acknowledgment

The authors are thankful to the management of Om Gayatri Charitable Trust for providing with the requirements to carry out the research work. Also to the Pioneer Pharmacy College for their help and support.

References

1. Hesse A, Brandle E, Wilbert D, Kohrmann KU, Alken P. Study on the prevalence and incidence of urolithiasis in Germany comparing the years 1979 vs. 2000. European Urology 2003; 44:709-713.
2. Taylor EN, Stampfer MJ, Curhan GC. Diabetes mellitus and the risk of nephrolithiasis. Kidney International 2005; 68:1230-1235.
3. Coward RJ, Peters CJ, Duffy PG. Epidemiology of paediatric renal stone disease in the UK. Archives of Diseases in Childhood 2003; 88:962-965.
4. Devuyst O, Pirson Y. Genetics of hypercalciuric stone forming diseases. Kidney International 2007; 72:1065-1072.
5. Knoll T. Stone disease. European Urology Supplements 2007; 6:717-722.
6. Tiselius HG. Epidemiology and medical management of stone disease. BJU International 2003; 91:758-767.
7. Moe OW. Kidney stones pathophysiology and medical management. Lancet 2006; 367:333-344.
8. Worcester EM, Coe FL. Nephrolithiasis. Prim Care 2008; 35:369-391.
9. Hadjzadeh M, Khoei A, Hadjzadeh Z, Parizady M. Ethanolic extract of *Nigella sativa* L. seeds on ethylene glycol-induced kidney calculi in rats. Urology Journal. 4:86-90.
10. Baumann JM. Stone prevention: why so little progress? Urological Research 1998; 26:77-81.
11. Leonetti F, Lechevallier E, Dussol B, Berland Y. Lithiase Urinaire-La Revue du Praticien 1996; 26:1557-1567.
12. Raats ICJ, Van den Born J, Berden JHM. Glomerular heparin sulfate alterations: mechanisms and relevance for proteinuria. Kidney International 2000; 57:385-400.
13. Obligado SH, Goldfarb DS. The association of nephrolithiasis with hypertension and obesity: a review. American Journal Hypertension 2008; 21:257-264.
14. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. JAMA 2005; 293:455-462.
15. Prajapati P. Phytochemical and pharmacological review on *colocasia esculenta* plant. International journal of nutrition, pharmacology, neurological disease. 2011; 1:90-96.
16. Patricia RP, Joab T. Crude extract from taro (*Colocasia esculenta*) as a natural source of bioactive proteins able to stimulate haematopoietic cells in two murine models. Journal of functional foods. 2015; 18:333-343.

17. Jha R, Ramani P. Phytochemical analysis and *in vitro* urolithiatic activity of *Peltophorum pterocarpum* leaves (DC) Baker. Journal of medicinal plants studies. 2016; 4(3):18-22.
18. Vijigiri D, Sharma PP. Herbal formulations used in treatment of kidney stone by native folklore of Nizamabad District, Andhra Pradesh, India. Bioscience Discovery 2013; 4(2):250-253.
19. Ghorpade DS, Modi AJ. *In vitro* antimicrobial activity of the crude extracts of *Colocasia esculenta* leaves. International journal of pharmaceutical sciences and research. 2010; 88-91.
20. Bradbury J, Howard Nion, Roger W. The acridity of raphides from the edible aroids journal of science of food and agriculture. 1998; 76(4):608-616.
21. Ramanath RV, Matthews PJ, Eyzajuirre P. The global diversity of taro: ethnobotany and conservation. Rome (Italy). Bioversity international 2010, 202.