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Phytochemical analysis and *in vitro* urolithiatic activity of *Peltophorum pterocarpum* leaves (DC) Baker

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Abstract
Phytochemicals are responsible for medicinal activity of plant species. Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. *Peltophorum pterocarpum*, family Leguminosae is a tree natural to tropical South-Eastern Asia and was brought to Nigeria by immigrants. Continuous research have revealed many chemical constituents isolated from different parts of the this tree to exhibit several biological activities such as antimicrobial activity, antioxidant activity, cytotoxic activity, antiglycemic activity, aldose reductase inhibition activity, cardiotonic activity and choline esterase inhibitory activity. The present study aims at studying the Anti urolithiatic activity of methanolic and aqueous extracts of the leaves of *Peltophorum pterocarpum.* Results obtained from in-vitro, in- vivo and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. been**.** In this experiment aqueous and methanolic extracts of *Peltophorum pterocarpum* and standard for dissolving kidney stones-calcium oxalate by an in-vitro model. To check their potential to dissolve experimentally prepared kidney stones- calcium oxalate by an in-vitro model for *Peltophorum pterocarpum* leaves and cystone as a standard compound collected from market. Phenolic compound isolated from the benzene and aqueous, flavanoids and steroids from aqueous fraction of the seed. Aqueous fractions showed highest dissolution of stones as compare to others. Aqueous fraction was more effective in dissolving calcium oxalate.

Keywords: Phytochemicals**,** Natural products, methanolic Extracts, *Peltophorum pterocarpum*, Anti urolithiatic activity

1. Introduction
The problems related with kidney are the major problem for human beings throughout the world because kidney is the major excretory organ in animals and humans. Urolithiasis is characterized by the formation of a stone in the kidneys or urinary tracts. In India, approximately 5 -7 million patients suffer from stone disease and at least 1/1000 of Indian population needs hospitalization due to kidney stone disease. Currently urinary stone formation affects 10% to 12% of the population in industrialized countries and the peak incidence seems to be at ages 20 to 40 years [1-7]. Calcium-containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate are 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 prohibitively costly 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]. The *peltophorum pterocarpum* is a family of leguminose commonly found throught India and Shri Lanka [15]. The tree is widely grown in tropical regions as ornamental flowers. It is a deciduous tree growing to 15–25 m (rarely up to 50 m) tall, with a trunk diameter of up to 1 m. The leaves are bipinnate, 30-60 cm long, with 16- 20 pinnae, each pinna with 20-40 oval leaflets 8-25 mm long and 4-10 mm broad. The flowers are yellow, 2.5-4 cm in diameter, produced in large compound racemes up to 20 cm long. The fruit is a pod 5-10 cm long and 2.5 cm broad, red at first, ripening black, and containing one to four seeds. Trees begin to flower after about four years [15]. The plant is native to tropical southeastern Asia and northern Australasia, in Sri Lanka, Thailand, Vietnam, Indonesia, Malaysia, Papua New Guinea, Philippines and the islands of the coast of Northern Territory, Australia [16-18]. The plant is also found in different regions of India including Birbhum District, West Bengal. The wood of the plant is wide variety of uses, including cabinet-making 4 and the foliage is used as a fodder crop. *Peltophorum pterocarpum (DC.) Baker* is a deciduous tree commonly used for ornamental purpose and as an avenue tree [19]. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm and its flower extract is known to be a good sleep inducer and used in insomnia treatment [20]. Its bark is used as medicine for dysentery, as eye lotion, embrocation for pains and sores [21-27]. The traditional healers use the leaves in the form of decoction for treating skin disorders. Stem infusion of *Peltophorum pterocarpum Baker* used in dysentery, for gargles, tooth powder and muscular pain [28]. Flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores [29]. The study have been undertaken to evaluate *Peltophorum pterocarpum Baker* different leaves extracts and cystone as a standard for their possible potential to dissolve experimental kidney stone using a modified *in vitro* model [30-33] to isolate the chemical constituent responsible for the activity.

Materials and methods

Collection of Plant Materials

The experiment was conducted in the year 2015 in the college laboratory. Leaves of peltophorum pterocarpum were collected from the college herbal garden. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried.

Extraction and Isolation

Fresh leaves (20-30 gm) of peltophorum pterocarpum were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 5 days. The dried leaves were ground into powdered using a mortar and pestle. 25 g of the powdered leaves were extracted in 500 ml conical flasks with 90% methanol (methanolic extraction). The conical flask was plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5 days with occasional manual agitation of the flask using a sterile glass rod at every 24 hour. The extracts were separately filtered using sterile Whatman no. 1 filter paper. Methanolic extracts was concentrated on a water bath and residue was dried in a desiccator. This extract was used in further process. All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents. Phytochemical analysis of extract for qualitative detection of alkaloids, flavonoids, steroid, volatile oil, glycoside, reducing sugar, tannins and saponins was performed by the extract [35].

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.

Saponins

1) Frothing Test: 3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.

Steroids

1) Salkowski Test: 5 drops of concentrated H_2SO_4 were added to 1ml of each extract in a separate test tube.

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.

Glycosides

1) 25ml of dilute H_2SO_4 was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5ml of Fehling solution added.

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.

Evaluation for Anti-urolithiatic Activity

Behind this activity the idea was to know the role of plant extract in dissolving the already formed stones nucleus in renal system. For this artificial calcium oxalate crystal were prepare in the laboratory by standard method [30]. Also semi permeable membrane was prepared from egg using standard methods [30].

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 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 10,20,30,40 mg of the extract, packed it together in semipermeable membrane of egg as shown in the model designed given below (Figure 1 a-c). 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 10,20,30,40 mg of standard drug, *i.e*. cystone. The 3rd and 4th groups along with 1 mg of calcium oxalate containing, aqueous and methanolic 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 substracted 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 KMnO4 equivalent to 0.1898mg of Calcium oxalate.

Results

The present study reveals that peltophorum pterocarpum plant shows the presence or absence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, volatile oils in solvent extracts as shown in Table 1.

Table 1: Phytochemical constituent Present in various Extracts

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

On basis of this fraction we performed *in vitro* Anti-urolithiatic activity (Experimental *in vitro* model figure 1) by comparing different extracts of *peltophorum pterocarpum* with standard cystone. % Dissolution of calcium oxalate (table no 2 and figure 2) and Table no.3 it is also clear that positive correlation exists between individual extracts and concentration used in the study.

(a) (b)

Fig 1: Experimental *in vitro* anti urolithiatic model (a) Egg membrane for urolithiasis activity (b) Titration result showing pink color as end color

Table 2: Descriptive Statistics for individual extracts with concentration.

Extract	Concentration	N	Mean %*	Std. Deviation	Std. Error	95% Confidence Interval For Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Standard	10	3	58.410	0.243	0.140	57.806	59.014	58.250	58.690
	20	3	61.390	0.227	0.131	60.826	61.954	61.230	61.650
	30	3	66.400	0.157	0.091	66.010	66.790	66.230	66.540
	40	3	66.193	0.170	0.098	65.771	66.616	66.000	66.320
Methanolic	10	3	43.347	0.230	0.133	42.775	43.919	43.150	43.600
	20	3	45.517	0.075	0.043	45.330	45.703	45.430	45.560
	30	3	49.500	0.061	0.035	49.349	49.651	49.430	49.540
	40	3	49.230	0.110	0.064	48.957	49.503	49.120	49.340
Aqueous	10	3	47.383	0.181	0.105	46.933	47.834	47.250	47.590
	20	3	49.287	0.049	0.028	49.164	49.409	49.230	49.320
	30	3	50.340	0.101	0.059	50.088	50.592	50.250	50.450
	40	3	50.370	0.226	0.131	49.808	50.932	50.120	50.560

*Mean is for % dissolution of calcium oxalate crystals.

Table 3: Correlation between extract and concentration used in the study.

		Concentration	Standard	Methanolic	Aqueous
Concentration	Pearson Correlation	1	.940**	.935**	.917**
	N	12	12	12	12
Standard	Pearson Correlation	.940**	1	.997**	.956**
	N	12	12	12	12
Methanolic	Pearson Correlation	.935**	.997**	1	.951**
	Sig. (1-tailed)	.000	.000		.000
	N	12	12	12	12
Aqueous	Pearson Correlation	.917**	.956**	.951**	1
	N	12	12	12	12

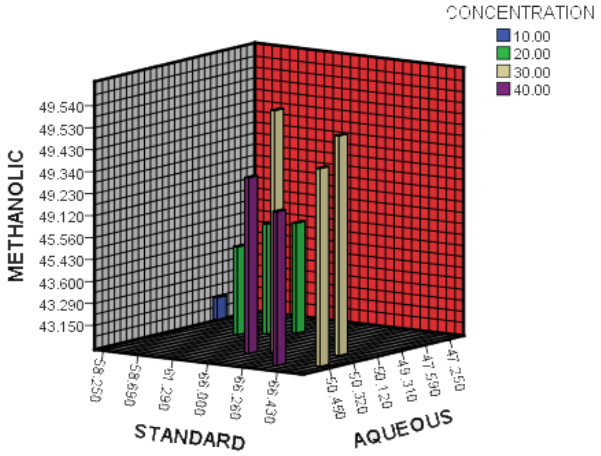

Fig 2: Individual Extracts Showing % Dissolution of Calcium oxalate crystals with different concentration.

This study has given primary evidence for peltophorum pterocarpum as the plants which possess lithotriptic property. This *in vitro* study has given lead data, and shown aqueous extracts is promising for further studies in this regard.

Conclusion

The present study conclusively demonstrates that peltophorum pterocarpum a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids. This study evaluate that antiurolithiatic activity of extracts of peltophorum pterocarpum leaves. The work was performed by using *in vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone. This study has given primary evidence for peltophorum pterocarpum as the plants which possess lithotriptic property. More specifically we can conclude from the results that methanolic and aqueous extracts both showing good anti urolithiasis activity. From the Table no.3 it is also clear that positive correlation exists between individual extracts and concentration used in the study. Out of four concentration used we can observe that activity increase as we increase the concentration and at one point further no increase in the activity observed. The plant used in the above study also showing good activity when it was compared with the standard drug cystone. The aqueous extract found to be more potent in terms of activity compare to methanolic and the authors of the above work recommend the plant extract for the further studies by conducting invivo model.

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