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TO STUDY IN-VITRO UROLIATHIASIS ACTIVITY OF *DENDROPHTHOE FULCATA*

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ABSTRACT

The leaves of plant Dendrophthoefalcata were selected for present study,The plant was collected from rural area of Shirur.The fresh leaves are dried under sunlight up to 4-5 days.Then powdered with the help of electric grinder and extracted with Alcohol,Pet ether,Water for 24hrs by using Soxhlet apparatus successively with various solvent are removed under reduced pressure.Extract are concentrated to dryness at controlled temp.Dried powder drug are evaluated for amount of drug extracted during process of extraction and % of extraction of drug in various solvent are also calculated.Then preliminary phytochemical sceerining were performed and microscopic and characters were studied compound,microscopic characters also studied.Dried powdered drug contain only Flavonid.Galicacid ,Pentacyclictriterpenoid, saponinglycoside. Microscopic study also performed for fresh leaves using Sudan Red and Phloroglicenol:HCl

Keywords:- *Calcium oxalate, Crystallisation, Dendrophthoe falcate ,urolithiasis*

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INTRODUCTION

Urolithiasis is an extremely painful disease that afflicts the human population since ancient time. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease. Oxalate, the major stone-forming constituent, is known to induce lipid peroxidation which causes disruption of the cellular membrane integrity. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. This alters the membrane fluidity, permeability and thereby affects the ion transport across the cellular organelle.Calcium oxalate is one of the main constituents ofdeposits

in urinary tract. Crystallisation of calcium oxalateis of particular interest not only from the theoreticalpoint of view but also because of its biological importance.The exact mechanism of the initiation of the calciumoxalate stone formation is not completely understood.Factors leading to the nucleation, crystal growth andaggregation of various hydrates of calcium oxalate dependnot only on the excess of calcium and oxalateconcentrations but also on the presence of various foreign substances.

EXPERIMANTAL

Material and Method

Plant material:-

The leaves of plant Dendrophthoefalcata were selected for present study,The plant was collected during June-July from rural area of Shirur.The fresh leaves are dried under sunlight up to 4-5 days.Then powdered with the help of electric grinder and extracted with Alcohol,Petether,Water for 24hrs by using Soxhlet apparatus successively with various solvent are removed under reduced pressure.Extract are concentrated to dryness at controlled temp.

In-vitro Crystal Inhibition

Model:- 1] Crystallization assay in whole urine

Urine samples (collected over 24h) from a healthy subject were accumulated in a polypropylene bottle containing sodium azide as an antibacterial agent; the urine sample was refrigerated during collection. Aliquots of 2 ml of urine were transferred to tubs and allowed to warm to 37^0c;50µl of herb extract solutions at different cncentrations were added to the tubes. Tubes with no extract added were used controls. Finaly,50µl of 0.1mol/L sodium oxalate solution was added and the tubes incubated at 37^0c for 30 min. The solution optical density (OD) was then read at 620 nm, the samples filered through 0.22µm membranes and the filtrate processed for scanning electron microscopy. In a separate experiment, the herb extract was used after dialysis against distilled water overnight at 4^0c, using membranes with a threshold of 6-8kDa, and filtration through 0.45µm membranes.

2]Nucleation assay:-

The method used was simillarto that described by Hennequin et al., with some minor modification. Soluction of calcium chloride and sodium oxalate were prepared at a final concentration of 2mm/L,and 0.5mol/L respectively,in a buffer containing Tris 0.05mol/L and Nacl 0.15mol/L at PH 6.5. Both solution were filtered three times through an 0.22µm filter,950µL of calcium chloride solution was mixed with 100 µL of of the herb extract at different concentration.Crystallization was started by adding 950 µL sodium oxalate solution. The final solution was magnetically stirred at 800r.p.m. using a PTEE-coated stirring bar. The temperature was maintained at 37^0c. The OD of the solution was monitored at 620 nm.The rate of nucleation was estimated by comparing the induction time in presence of the extract with that of the control.

3] Aggregation assay:-

The method used was similar to that described by Hess et al. with some modifications 'Seed'CaOx monohydrate (com) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L, Both solutions were equilibrated to 60^0c in a water bath for 1 h and then cooled to 37^0c overnight. The Crystals were harvested by centrifugation and then evaporated at 37^0c. COM crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and Nacl 0.15mol/L pH 6.5 then experiments were conducted at 37^0c in the presence or absence of plant extract after stopping the stirring. The rate of aggregation was estimated by comaring the slope of turbidity in presence of extract with that obtain in control.

RESULTS AND DISCUSSION

Preliminary pharmacognostical screening of crude drug as well as pet ether, chloroform, ethanol and water extracts were done on *Dendropthoefalcata (*Loranthaceae*).DendropthoeFalcata* showed the presence of alkaloids, flavonoids, glycosides, reducing sugar, saponin, terpenoids, tannins and steroids. Physicochemical parameter evaluated in

this article helps in standardize and authenticate the species *Dendropthoefalcata*and possibly help to differentiate from its other species. Extracts was prepared from *Dendrophthoefalcata* at different concentration. Crystalisation was induced in whole normal human urine samples in the absence or presence of extract. The nucleation and aggregation of calcium oxalate crystals were measured separately using spectro-photometric methods. The nucleation rate was followed at 620 nm after mixing calcium chloride and sodium oxalate solution at 37^{o}c with stirring. The induction time in the presence of herb extract was compared with that of the control. The aggregation rate was followed at 620 nm in buffered solution containing calcium oxalate monohydrate crystals after stopping the stirring. The rate was evaluated by comparing the slope of turbidity in the presence of the extract with that of the control.

Result: In vitro Crystal Inhibition

Table 1: Crystallization assay in whole urine

Sr. No.	Concentration of Extract	Absorbance of D.F.	
		Ethanolic extract	Water extract
1	Blank	0.00	0.00
2	Control	0.0013	0.0084
3	100 ug/ml	0.022	0.0116
4	200 ug/ml	0.0314	0.0134
5	300 ug/ml	0.0407	0.0171
6	400 ug/ml	0.0522	0.0193
7	500 ug/ml	0.0496	0.0199

Table 2: Nucleation assay

Sr. No.	Concentration of Extract	Absorbance of D.F. Ethanolic extract
1	Blank	0.00
2	Control	0.3123
4	200 ug/ml	0.4446
5	300 ug/ml	0.5456
6	400 ug/ml	0.5670
7	500 ug/ml	0.6574

CONCLUSION

From phytochemical and in-vitro crystal inhibition study of plant *Dendrophthoe falcata* it was found that the leaves of plant contain chemical constituent like Flavonid,Pentacyclic triterpenoid,Saponin glycoside Galic acid,Cardic glycoside.The plant show in-vitro crystal inhibition action.It might be because of presence of saponin.So this experiment will be helpful in future for scientific evaluation constituent which show for urolithasis activity in-vivo.

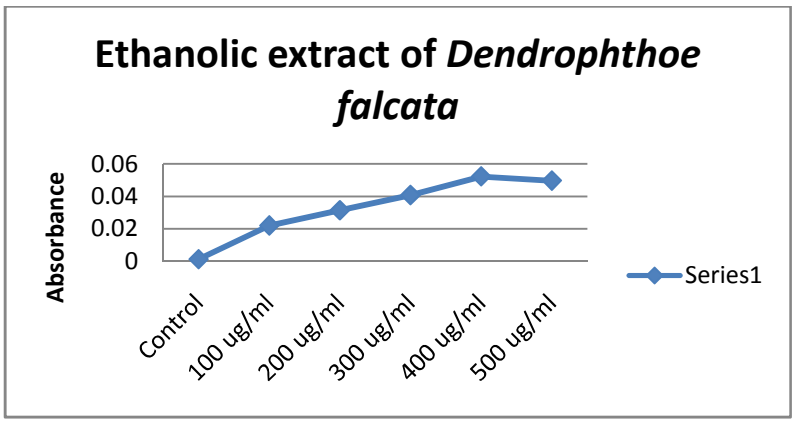

Fig:1 In vitro urolithiatic activity of Ethanolic extract of *D. falcata* by crystalization assay

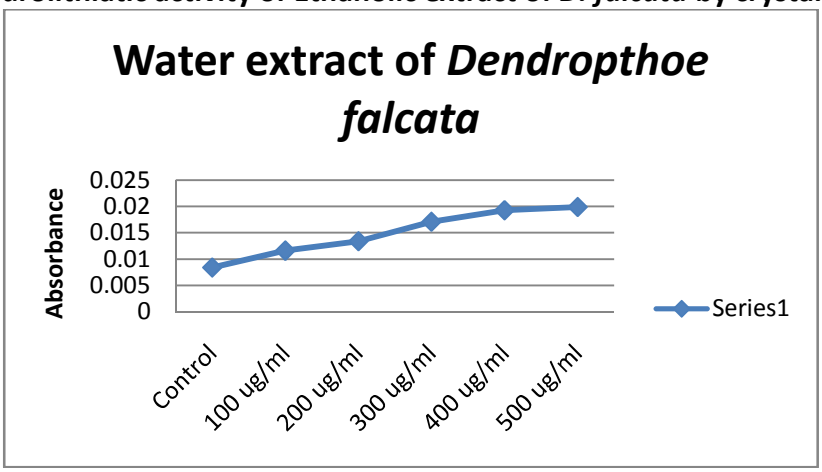

Fig:2 In vitro urolithiatic activity of water extract of *D. falcata* by crystalization assay

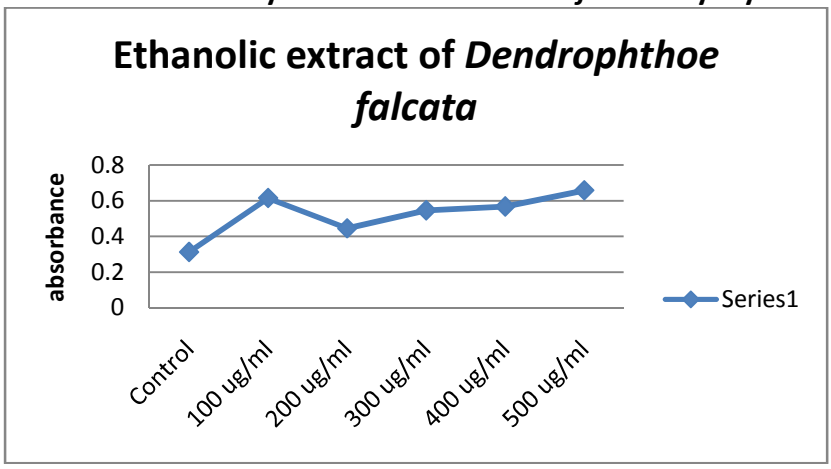

Fig:3 In vitro urolithiatic activity of Ethanolic extract of *D. falcata* by nucleation assay

Table-3: Pharmacognostic Screening of the entire plant powder of D. falcata

Sr. No.	Test	Result
1.	Foreign organic matter	-
2.	Ash value a. Total ash b. Acid Insoluble c. Water soluble	 5.5% 1% 1%
3.	Extractable matter	

	a. Water extract b. Alcohol extract	6.9% 11.5%
4.	LOD	8.7%
5.	Swelling factor	2 ml.
6.	Volatile oil	-
7.	Tannins	-
8.	Foaming Index	117.95
9.	Limit test for Arsenic	Passed
10.	Limit test for Lead	Passed

Table-4: Preliminary phytochemical screening of the entire plant powder of *D. falcata*

Test	Petroleum Ether	Chloroform	Ethanol	Water
Alkaloids				
1. Dragendroff test	-	-	-	-
2. Wagner's test	-	-	-	-
3. Mayer's test	-	-	-	-
4. Hanger's test	-	-	-	-
Carbohydrates				
1. Molish test	-	-	-	+
2. Fehling's test	-	-	-	+
3. Benedict's test	-	-	-	+
Protein & Amino acid				
1. Biuret test	-	-	-	-
2. Xanthoprotein test	-	-	-	-
3. Lead acetate test	-	-	-	-
4. Ninhydrine test	-	-	-	-
Steroids				
1. LibermannBurchard test	+	-	-	-
2. Salkowaski test	+	-	-	-
Glycoside				
1. Legal test	-	-	-	-
2. Baljet test	-	-	-	-
3. Borntrager test	-	-	-	-
4. Keller Killiani test	-	-	-	-
Saponins	-	-	+	+
Flavonoids				
1. Shinoda test	-	-	-	-
Phenolic compounds and tannins	-	-	+	+
Tritrepenoid	-	-	-	-
Fixed oil				
1. Spot test	+	-	-	-
2. Saponification test	+	-	-	-

+ Present, - absent

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