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IN-VITRO ANTIUROLITHIATIC POTENTIAL OF LEAVES OF *EUPHORBIA HIRTA* L. AGAINST CALCIUM OXALATE KIDNEY STONES

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ABSTRACT: Plant *Euphorbia hirta* L. is a common weed plant that belongs to the family Euphorbiaceae characterized by the presence of milky latex in leaves and stems. Phytochemical screening of leaves of *Euphorbia hirta* L. confirmed the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, tannin and terpenoids. FT-IR confirms the formation of calcium oxalate crystals with major peaks at 3331.07, 1608.63, 1317.38, and 775.38, which corresponds to asymmetric O-H bending, C=O stretching, C-O stretching and C-H bending. Nucleation and aggregation assay has been done to investigate the *in-vitro* antiurolithiatic potential of leaves of *Euphorbia hirta* L., and significant % inhibition has been observed for nucleation and aggregation of calcium oxalate kidney stones. Maximum % inhibition has been observed at 1000 µg/ml concentration with 55.81% and 73.17% inhibition, respectively. % inhibition of nucleation and aggregation increases with the increasing concentration of plant *E. hirta.* GC- MS confirmed the presence of different active compounds.

INTRODUCTION: Urolithiasis is the third most prevailing and painful disorder of global concern. It is the process of kidney stone formation in the urinary tract. Supersaturation of the urine with crystal-forming substances and imbalance between promoters and inhibitors are two major causes of kidney stone formation. Nucleation is the first step in kidney stone formation in which the smallest unit of crystal *i.e.* "nuclei" or "nidus" of calcium oxalate stones formed [1, 2]. When the nuclei of calcium oxalate started binding to each other and formed larger particles, a process called aggregation. Strong intermolecular forces of crystals not allowed nuclei to get separated easily, and now these crystals are large enough to behave like a stone [1, 2]. Kidney stones vary in composition and hence can be of different types like calcium oxalate, calcium phosphate, uric acid, and mixed (magnesium, ammonium, calcium, and phosphate), but calcium oxalate stones are most abundant [3]. Many plants have been used in the treatment of kidney stones, and various plants are reported to have antiurolithiatic activity [4, 5]. Plant *Euphorbia hirta* L. commonly known as dhudhi, asthma weed and dugdhika, belongs to family Euphorbiaceae with many activities reported in the literature. Present study has been designed to bring light on the urolithiatic potential of leaves of *Euphorbia hirta* L.

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MATERIALS AND METHODS:

Collection of Plant and Preparation of Extract: Plant *E. hirta* has been collected from the garden of

the Department of Botany, the University of Delhi in the month of July-August and identified using "Illustration to The Flora of Delhi, Page 193 and Figure 193". Leaves of the plant were shade dried and powdered using a mixer grinder and extracted in ethanol by the soxhlet method.

Phytochemical Screening:

TABLE 1: METHODOLOGY FOR PHYTOCHEMICAL SCREENING

S. no.	Secondary metabolite	Name of test	Methodology	Observations
1	Alkaloids	Wagner test	2ml extract + 1% HCl + steam + 1ml Wagner's reagent drop by drop	Brownish red Precipitate[6]
2	Cardiac glycosides	Kellar-Killiani test	2ml extract + 2 ml of chloroform + H_2SO_4 to form a layer	Brown ring at interphase[7]
3	Flavanoids	NaOH test	Extract + dilute NaOH, + dilute HCl	Yellow solution on NaOH turns colorless on HCl[7]
4	Phenolic compounds	Lead acetate test	Extract + few drops of 10% lead acetate solution	Formation of white precipitate[8]
5	Saponin	Frothing test	0.5ml extract + 5ml distilled water and shake well	Persistence of frothing[6]
6	Tannin	Braemer's test	10% alcoholic $FeCl_3$ + 2-3ml of methanolic extract (1:1)	Dark blue or greenish grey coloration[6]
7	Terpenoids	Salkowski test	5ml extract + 2ml Chloroform + 3ml conc. H_2SO_4	Reddish Brown color of interface[6]
8	Anthroquinone	Ammonia test	1 ml dilute (10 %) ammonia + 2ml extract	A pink-red color in the ammoniacal (lower) layer[7]
9	Phlobatannin	HCl test	Extract boiled with 2 ml of 1% hydrochloric acid	Formation of red precipitate[8]
10	Starch	Iodine test	2ml extract + 2ml iodine solution	Formation of Blue color[9]

Nucleation Assay: The method used was similar to that described by Hennequin *et al*., [10] with some minor modifications. Solutions of calcium chloride and sodium oxalate were prepared at a final concentration of 3 mmol/L and 0.5 mmol/L, respectively, in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 5.5.1.9 ml of calcium chloride solution was mixed with 200 µL of the herb extract at different concentrations and incubated for 30 min at 37 ºC in a water bath. Crystallization was started by adding 1.9 ml of sodium oxalate solution. The OD of the solution was monitored at 620 nm for 420 sec.

% Inhibition = {(Abs. Control - Abs. Sample) / Abs. Control} × 100

Aggregation Assay: The method used was similar to that described by Hess *et al.*, [11] with some minor modifications. `Seed' CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50mmol/L. Both solutions were equilibrated to 60 ºC in a water bath for 1 h and then cooled to 37 ºC overnight. The crystals were harvested by centrifugation and then evaporated at 37 ºC. COM crystals were used at a final concentration of 0.8mg/mL, buffered with Tris 0.05mol/L and NaCl 0.15mol/L at pH 5.7. 1 ml extract was taken in a test tube to which 3 ml COM crystal solution was added and incubated 37º ºC, and readings were recorded at a different time interval of 30, 60, 90 and 120 min.

% Inhibition = {(Slope Control - Slope Sample) / Slope Control} × 100

RESULTS AND DISCUSSION:

Phytochemical Screening: Phytochemical screening of leaves of *Euphorbia hirta* L. confirms the presence of different phytochemical groups **Table 2**.

TABLE 2: PHYTOCHEMICAL SCREENING

S. no.	Secondary metabolite	Name of test	Results
1	Alkaloids	Wagner test	+
2	Cardiac glycosides	Kellar- Killiani test	+
3	Flavanoids	NaOH test	+
4	Phenolic compounds	Lead acetate test	+
5	Saponin	Frothing test	-
6	Tannin	Braemer's test	+
7	Terpenoids	Salkowski test	+
8	Anthroquinone	Ammonia test	-
9	Phlobatannin	HCl test	-
10	Starch	Iodine test	-

Nucleation Assay and Aggregation Assay: Plant *Euphorbia hirta* L. showed significant % inhibition against nucleation of calcium oxalate stones with a maximum of 55.81% inhibition at 1000 µg/ml concentration **Fig. 1A**. Plant *Euphorbia hirta* L. showed significant % inhibition for aggregation of calcium oxalate nidus or crystals to form stone-like structure with maximum 73.17% inhibition at 1000 µg/ml concentration **Fig. 1B**. Due to nucleation of calcium oxalate nuclei, absorption at 620 nm increases but gradually absorption starts falling down due to aggregation of these nuclei to form stones [10], as observed with the leaves of *Euphorbia hirta* L. **Fig. 1C**.

FT-IR spectra **Fig. 2** confirms the formation of calcium oxalate crystals as per aggregation assay protocol as it shows major peaks at 3331.07, 1608.63, 1317.38 and 775.38 which corresponds to asymmetric O-H bending, C=O stretching, C-O stretching and C-H bending [13]. In aggregation assay maximum negative slope recorded at 1000 µg/ml concentration with equation y = -0.011x + 0.434 (R^2=0.993) and y=-0.041x+0.359 (R^2=0.982) for control. y=-0.013x+0.407(R^2=0.993), y=-0.024x + 0.350(R^2=0.992), y=-0.032x+0.317(R^2=0.994) and y=-0.033x+0.316(R^2 = 0.993) at 750, 500, 250, 100 µg/ml concentration respectively.

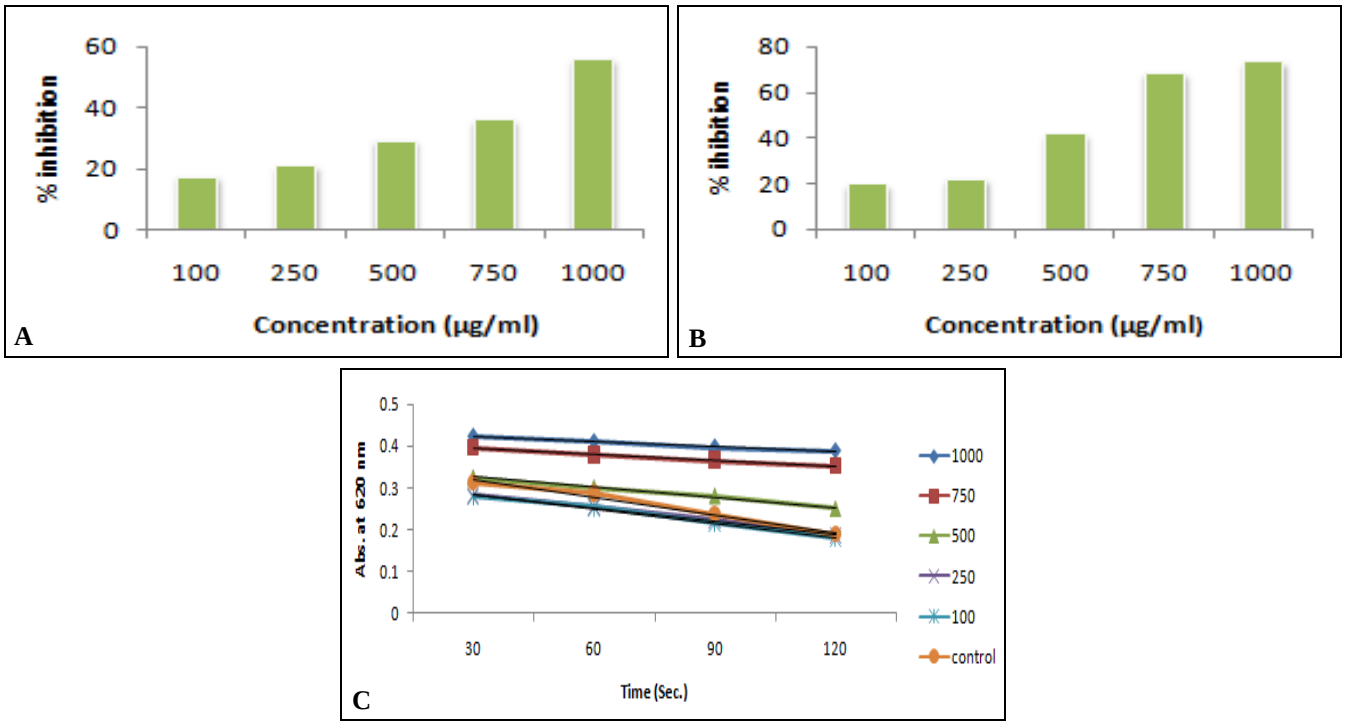

FIG. 1: NUCLEATION ASSAY (A) AND AGGREGATION ASSAY (B AND C) OF PLANT *EUPHORBIA HIRTA* L.

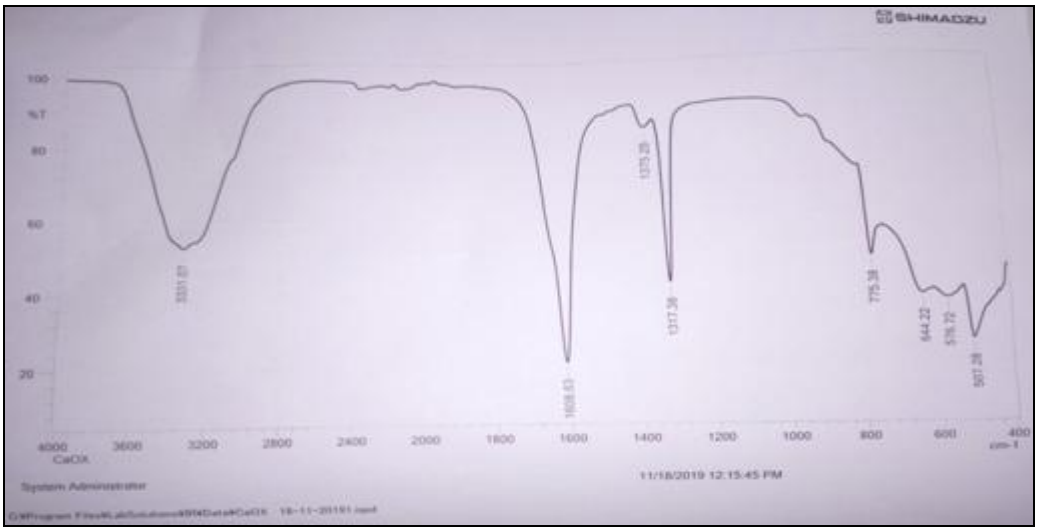

FIG. 2: FT-IR SPECTRA OF CALCIUM OXALATE CRYSTALS

Aryal *et al.,* [14] studied nucleation and aggregation activity of *Achyranthes aspera*, *Lawsonia inermis*, *Ficus benghalensis*, *Raphnus sativus* and *Macrotyloma uniflorum* and found maximum nucleation and aggregation activity in *R. sativus i.e*. 55.21% and 61.6% inhibition, respectively. 55.81% inhibition for nucleation is the significant figure as many plants like *Hirniaria hirsuta* favors the nucleation of crystals having a significant inhibitory effect for aggregation [15]. Antiurolithiatic potential of plant *E. hirta* has been studied by Pauzi *et al.*, with 95.78%, 76.38%, 38.95%, and 28.42% inhibition for aggregation of calcium oxalate stones of hexane extract, water extract, ethyl acetate extract, and methanol extract, respectively [16], which is comparable to our study with maximum % inhibition of 73.17%.

CONCLUSION: Plant *E. hirta* shows significant antiurolithiatic potential against calcium oxalate kidney stones and hence can be considered as a better herbal alternative for the treatment of kidney stones. It can be further studied by *in-vivo* methods for more clarity as per its clinical significance and also its role as an herbal drug in human health and medicine.

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CONFLICTS OF INTEREST: Nil

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