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Formulation and evaluation of herbal tea bag containing *Costus igneus* nak for diuretic and hypolipidemic activity

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Abstract
Costus igneus Nak. is a perennial herb, belongs to Costaceae used in the treatment of bone related issues such as Fracture, Pain, Inflammation, Osteoporosis, Rheumatoid and Osteoarthritis. The Preliminary Phytochemical screening indicates the presence of alkaloids, flavonoids, tannins, saponins and phenolic compound. The aim of this study is to investigate the Phytochemical and Pharmacological evaluation of *Costus iguanas* Nak. The Pharmacological studies performed by using calcium oxalate method and Membrane stabilization assay. The ethanolic extract of different concentrations (500, 250, 100, 50 and 10 µg/ml) were tested using cystone as positive control and tested for membrane stabilization assay using Cystone as standard. Results showed that the Ethanol extract of *Costus igneus* at concentration 500mg/ml effective against lipid lowering and proved as an effective diuretic.

Keywords: Herbal tea bag, *Costus igneus*, diuretic and hypolipidemic activity

Introduction
Herbal tea, also called tisane. It has increased popularity due to its biological properties and certainly can be a complement to modern medicine. Dried leaves, seeds, grasses, flowers, nuts or any other botanical components originating from plant species other than the commonly consumed tea species, *Camellia sinensis*, are consumed in this beverage. Herbal tea is made using a combination of herbs in addition to those brewed in hot water. Herbal remedies have been created by ancient cultures, such as Ayurveda and Traditional Chinese Medicine (TCM), to cure a variety of illnesses. The herbs were mixed based on the similarities of health benefits for individual species. The current market has shown that most herbal-based products have shifted from using a single herb to polyherbs, which are believed to exert more pharmacological effects compared to a single herb [1].
Tea is a prevalent and focal point for cultural and social gathering. It is a preparation which boosts up immunity, keeps active, rejuvenates cells it relieves stress, fatigueless, tiredness and anxiety. The aim of present study is to prepare herbal tea in combination of medicinal herbs like *Costus igneus*, Cardamom, Beetroot, stevia with the possibility to have maximum therapeutic benefits and suitable consumption. The medicinal herbs selected reported for various activities such as Diuretic, hypolipidemic activity, anti-influenza, immunostimulant, anti- bacterial, bioavailability enhancer, vitamin C supplement, sweetener, flavouring and colorant respectively. The decoction of tea powder mixture containing the above medicinal herbs evaluated for qualitative and quantitative estimations for carbohydrate, ascorbic acid, protein, tannins, and phenolic acid. The Diuretic and Hypolipidemic activity have also been performed [2-6].

Plant Selection
We have selected *Costus igneus* Nak based on a literature survey for the preparation of herbal tea bags.

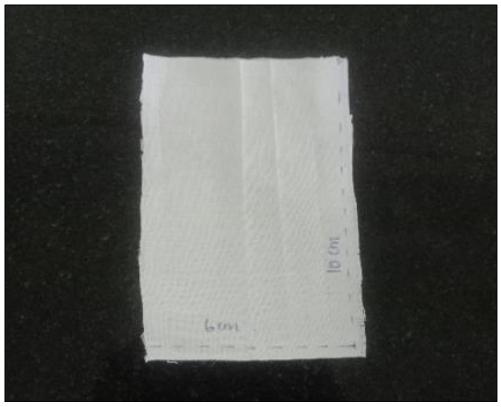
Plant Collection and Authentication
Fresh leaves of *Costus igneus* Nak, were collected from Dr.Shantha Herbal Garden, Thanjavur during the month September 2024 and authenticated by Dr. S. Soosairaj, Ph.D., Department of Botany, St. Joseph´ s College Thiruchirapalli, Tamilnadu with specimen number 8036. Specimen kept in our Laboratory for future reference.

Preparation Of Herbal Tea Bags

Empty tea bags were prepared and add coarse powdered dry leaves and rhizomes placed in empty cotton tea bags and adding a dried natural additives like flavouring agent, colouring agent and sweetening agent.

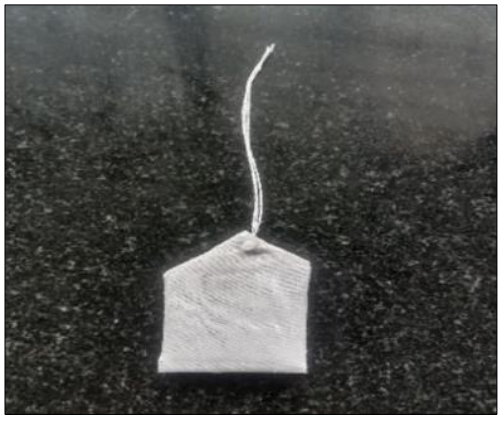
0.1g of stevia leaves, 0.2g of beetroot, 0.1g of cardamom were added to a tea bags.

- **Step: 1:** Preparation of empty tea bags: Dimension of cloth - 1m of cotton cloth was bought from textile shop and dimensions were marked and make in the form of empty tea bags
- **Step: 2:** Stitching of empty tea bags - The cloth dimensions are measured as 6cm breadth and 10cm length. Stich the cloth by using scissors, needle, thread and tie it.
- **Step: 3:** Filling the tea bag - Fill the coarsely powdered plant material into the empty tea bag and fill the other additives into it.



Step 1: Preparation of empty tea bags

Step 2: Stitching of empty tea bags



Step 3: Filling the tea bag

Phytochemical Screening: The aqueous extract of *Costus igneus* are subjected into the preliminary phytochemical screening using standard test procedures

The cold water-beaker: The tea bag will sink and diffuse when it is put in the beaker. Although it will take longer time before it reaches the bottom than if it would have been hot water The whole diffusion process will also occur slower because of the cold water collected in the beaker

The hot water-beaker: The tea bag will start the diffusion as soon as it has been put in the beaker. It will sink faster than the "cold water-beaker because the heat in the water makes the whole process speed up.

Materials & Methods

2 Tea bags, 2 Beakers with 200ml of water in each Teakettle

200ml of water was collected and put in the teakettle. Another 200ml of water was taken, only this time it was poured into a beaker standing on a table. The at the time hot water in the teakettle was put in a beaker as well, next to the other one already on the table. Both of the teabags were put in the water at the same time. The hot water- beaker's tea bag started to diffuse immediately as it reached the water. Within a couple of seconds, it had already reached the bottom. As the water turned brown, a fruity smell emitted from the beaker. The tea bag in the cold water-beaker did not sink or diffuse immediately as it hit the water. Instead, the bag was laying on the surface for a little while before it slowly sank towards the bottom. The colour of the water was still very transparent and a smell was not detected [7, 8].

Organoleptic Evaluation

Organoleptic evaluation of tea bags is a sensory analysis that assesses the tea's colour, aroma, taste, and texture. It uses the senses of sight, smell, taste, and touch.

Evaluation of tea bag pH

pH level of tea bags can be measured with a digital pH meter. The pH level of tea can vary depending on the type of tea.

Average pH levels of different teas

- **Black tea:** Average pH level of 4.9-5.5
- **Green tea:** Average pH level of 7-10
- **Chamomile, mint, and fennel tea:** Average pH level of 6-7
- **Lemon tea:** Average pH level of 3
- **Oolong tea:** Average pH level of 5.5-7
- **White tea:** Average pH level of 8-10

The pH of the tea in herbal tea bag of *Costus igneus* was obtained as 7.5.

Wettability Test

- **Preparation of tea bags:** Obtain a set of identical tea bags with the materials want to compare (e.g., different brands, different fabric compositions).
- **Weight of each bags:** Accurately weigh each tea bag to ensure consistent starting mass.
- **Immersion in water:** Place each tea bag in a container filled with a known volume of water at room temperature.
- **Time recording:** Start a timer as soon as the tea bag touches the water and note the time when the tea bag appears fully saturated and releases its contents visibly.

- **Repeating the test:** Repeat the test with multiple tea bags for each sample to ensure reliable results. The time taken for wetting the herbal tea bag in 2-3minutes (9)

Pharmacological Activity: *In vitro* Antiurolithiatic Activity

Materials Required

Calcium oxalate, Calcium chloride, Ammonium dihydrogen phosphate, and H_2SO_4 was purchased from Merck, USA.

Calcium oxalate method

The experimental kidney stones of calcium oxalate (CaOx) crystals were determined by the method of Mazni Abu Zarin *et al*., with a slight modification. The CaOx monohydrate crystals were prepared by mixing equal concentrations of calcium chloride (50 mmol/L) and sodium oxalate (50 mmol/L). Both solutions were equilibrated in a water bath for 1 h at 60 °C for the formation of CaOx monohydrate crystals. The crystals were cooled to 37 °C prior to evaporation. The CaOx monohydrate crystals were prepared at a final concentration of 0.8 mg/mL in a Tris buffer (Tris 0.05 mol/L and NaCl 0.15 mol/L) at pH 6.5. A different concentration of CQ sample (500, 250, 100, 50 and 10 µg/ml) were added to the CaOx monohydrate crystals solution and incubated at 37 °C for 24 hrs. Cystone was used as positive control (10 mg/ml). The aggregation activity was estimated by the turbidity using a microplate reader (Thermo fisher scientific, USA) in the presence of the extract compared to the control by measurement at 620 nm. The experiment was done in triplicate. The percentages inhibition of aggregation by the plant extract was calculated as below:

% of inhibition = OD control - OD test = OD control x 100

Oil Red O Staining

Lipid droplets (LDs) are dynamic, ubiquitously present lipid-storage organelles, predominantly present in the adipocytes. Triglycerides, neutral lipids, and cholesterol esters stored in LDs are the largest sources of energy. The presence of excess LDs in adipocytes results in obesity and obesity-linked pathologies such as dyslipidemia and diabetes type 2.1, 2 The Lipid (Oil Red O) Staining Kit is suitable for selective staining and detection of neutral lipids in cultured cells.

Materials Required

DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and Oil Red O stain were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plates and wash beakers were from Tarson (India).

Oil red O stock and working solutions

Oil red O powder was obtained from Sigma-Aldrich (O0625). A stock solution was prepared by dissolving 0.2 g in 40 ml 2-propanol (0.5% w/v). This stock solution was stored at room temperature. The working solution was obtained by diluting the stock solution 2:3 with distilled water, yielding a concentration of 0.2% oil red O in 40% 2-propanol. The working solution was prepared freshly for each experiment and filtered once immediately before use.

Procedure

Cell culture

3T3-L1 (Murine Fibroblast cells) were purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37 °C.

Cells treatment

Briefly, the cultured 3T3-L1 were harvested by trypsinization and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×105 cells/ml cells/well (200 µL) into the 24-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 hours at 37 °C. The wells were washed with sterile PBS and treated with different concentrations of CI sample in a serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37 °C in a humidified 5% CO2 incubator for 24 h. After the incubation period, the cells proceeded with Oil red O staining method.

Oil red O staining procedure

After removing the supernatant from the culture plates, cells were washed once with phosphate-buffered saline (PBS). After washing with PBS 0.1M pH 7.4, the cells were fixed for 20 min with 4% formalin in PBS 0.05M. Cells were washed with sterile double distilled water and subsequently with 60% isopropanol for 2 min and stained with a filtered 0.35% Oil Red O solution in 60% isopropanol for 10 min at room temperature. Then, cells were washed with sterile double distilled water. Slides were treated with Dako paramount aqueous mounting medium ready to use and then was applied to the coverslip. The images were taken using Olympus light microscope. To quantify staining, Oil Red-O was extracted from the cells with isopropanol containing 4% Nonidet P-40, and optical density was then measured at a wavelength of 520 nm [10-15].

Results

Preliminary Phytochemical Screening

The Aqueous extract of *Costus igneus* showed the presence of alkaloids, glycoside, anthraquinone steroids, flavonoids, phenols and saponins.

Table 1: Phytochemical analysis of *CI*

SI. No.	Phytochemicals	Aqueous Extract
1.	Flavonoids	+
2.	Tannins	+
3.	Alkaloids	+
4.	Terpenoids	+
5.	Saponins	+
6.	Anthraquinones	+
7.	Glycosides	+
8.	Reducing sugar	+
9.	Steroids	+
10.	Proteins	+

Fig 1: Phytochemical screening

Pharmacological evaluation
Anti urolithic activity
OD value at 620 nm
Control Mean OD value - 1.052

Table 2: OD value at 620 nm for Aqueous extract of *CI*

S. No.	Tested sample concentration (µg/ml)	OD value at 620 nm (in triplicates)		
1	Control	1.6	1.648	1.636
2	500 µg/ml	0.898	0.881	0.810
3	250 µg/ml	0.987	0.952	0.964
4	100 µg/ml	1.058	1.026	1.021
5	50 µg/ml	1.052	1.017	1.012
6	10 µg/ml	1.043	1.052	1.065
7	Cystone	0.189	0.178	0.165

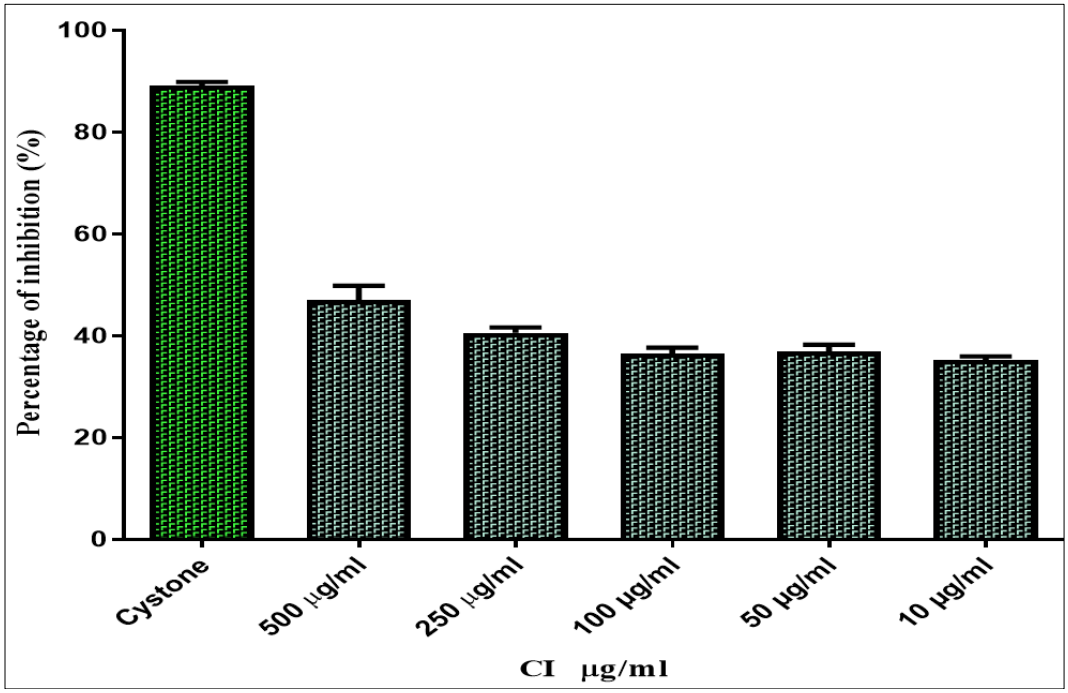


Fig 2: Percentage of inhibition of Aqueous extract of *CI*

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		2.412
HillSlope		-5.372
IC50		258.5
Std. Error		
LogIC50		0.02871
HillSlope		4.301
95% Confidence Intervals		
LogIC50		2.350 to 2.474
HillSlope		-14.66 to 3.920
IC50		224.1 to 298.1
Goodness of Fit		
Degrees of Freedom		13
R square		0.8730
Absolute Sum of Squares		2772
Sy.x		14.60
Number of points		
Analyzed	3	15

Fig 3: IC_{50} Value of Aqueous extract of *CI* / 258.5µg/ml

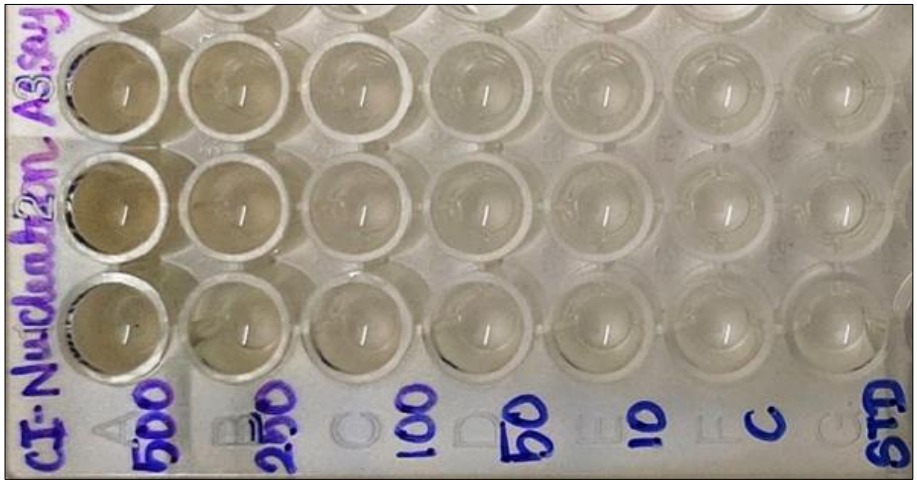


Fig 4: Nucleation assay of aqueous extract of *CI*

Calcium oxalate crystals were treated with various concentrations of the *CI* sample

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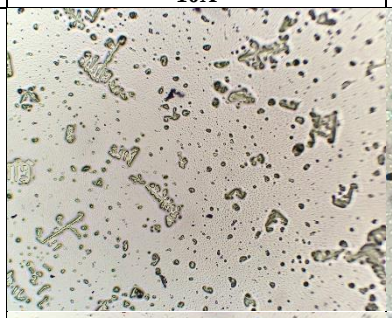
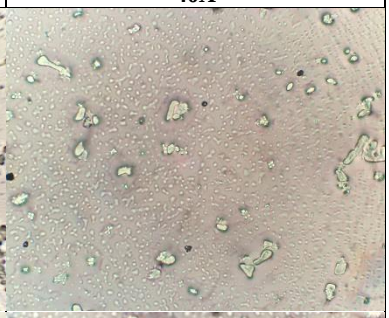
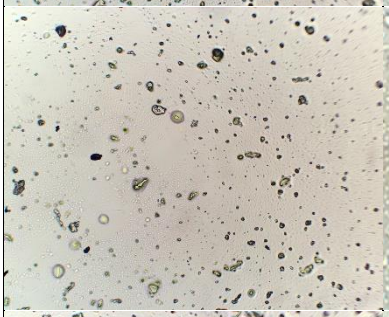
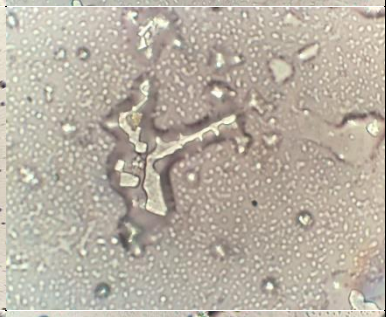
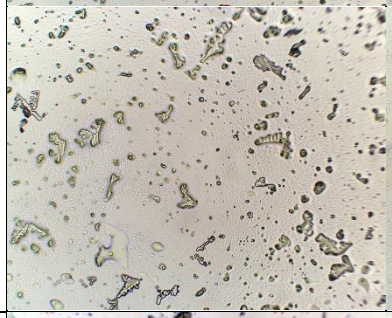
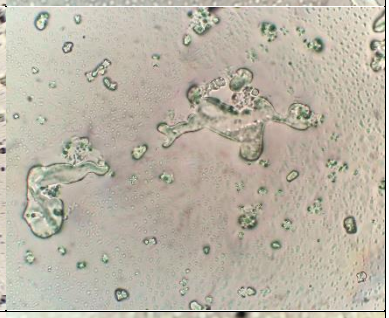
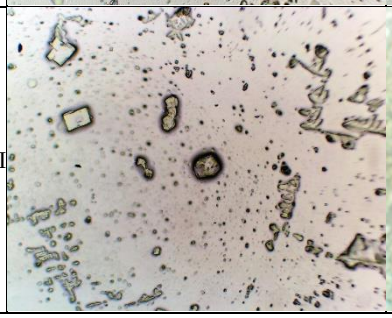
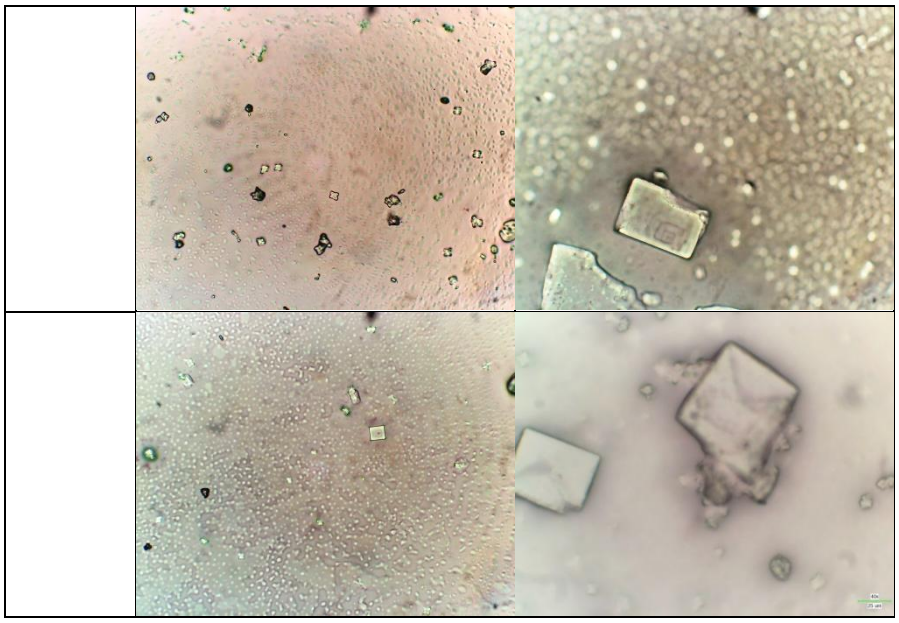
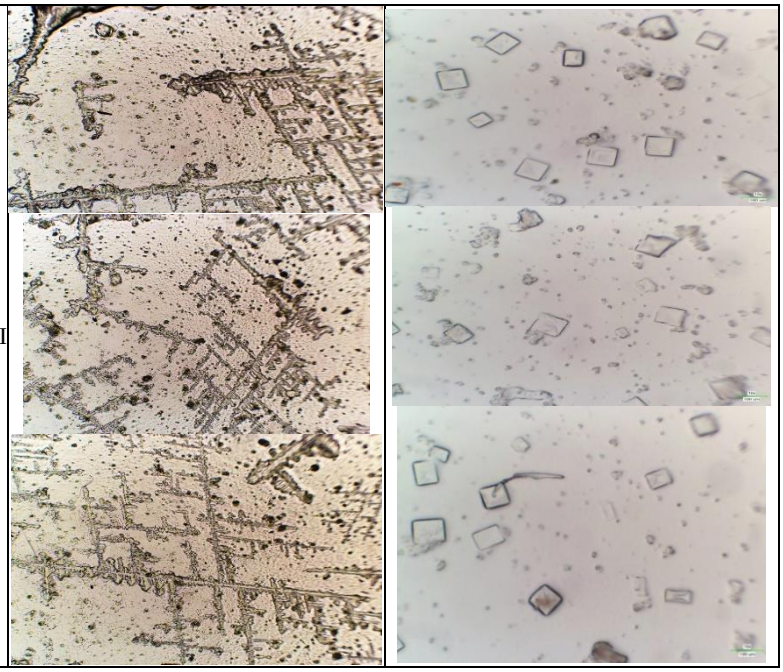
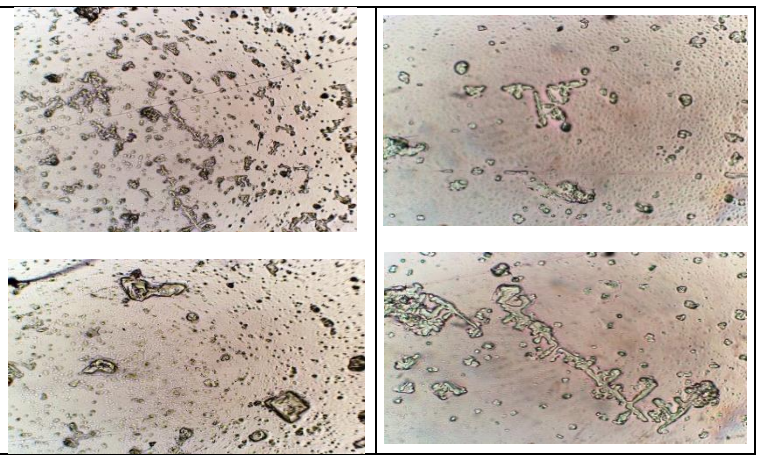
Control		
		
		
500 µg/ml CI		

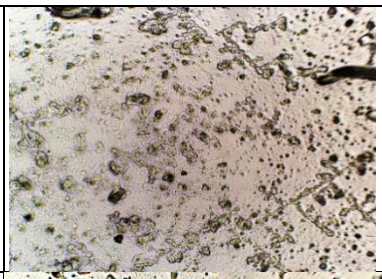
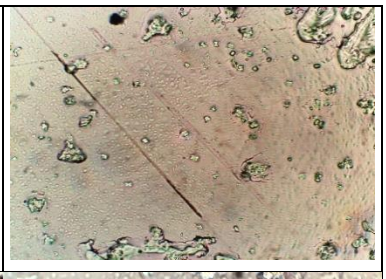
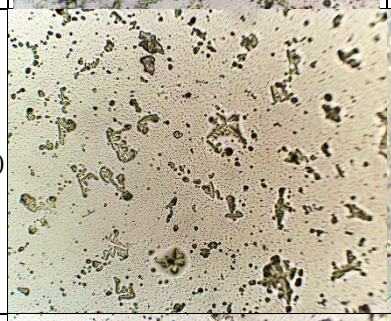
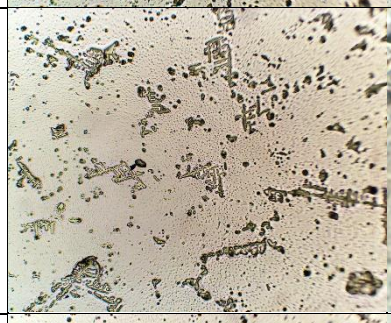
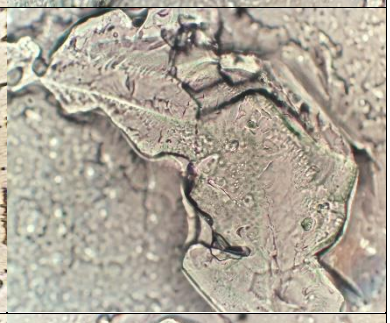
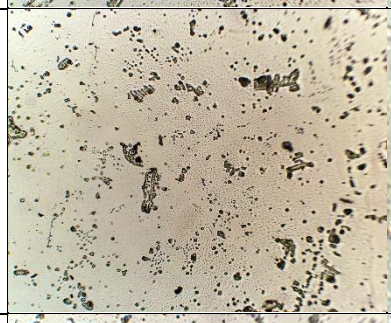
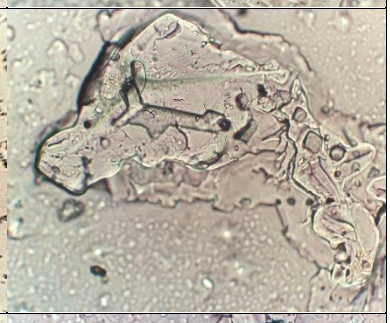
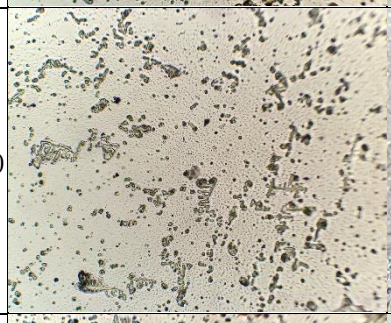
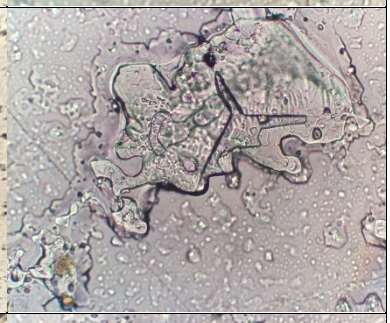
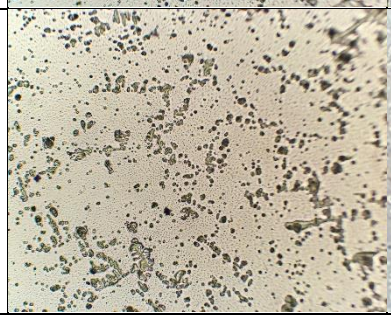
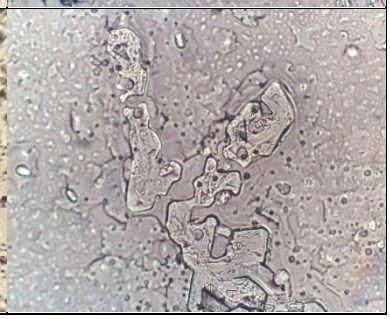


250µg/ml CI



100 µg/ml(CI)



		
50 µg/ml (CI)		
		
		
10 µg/ml (CI)		
		

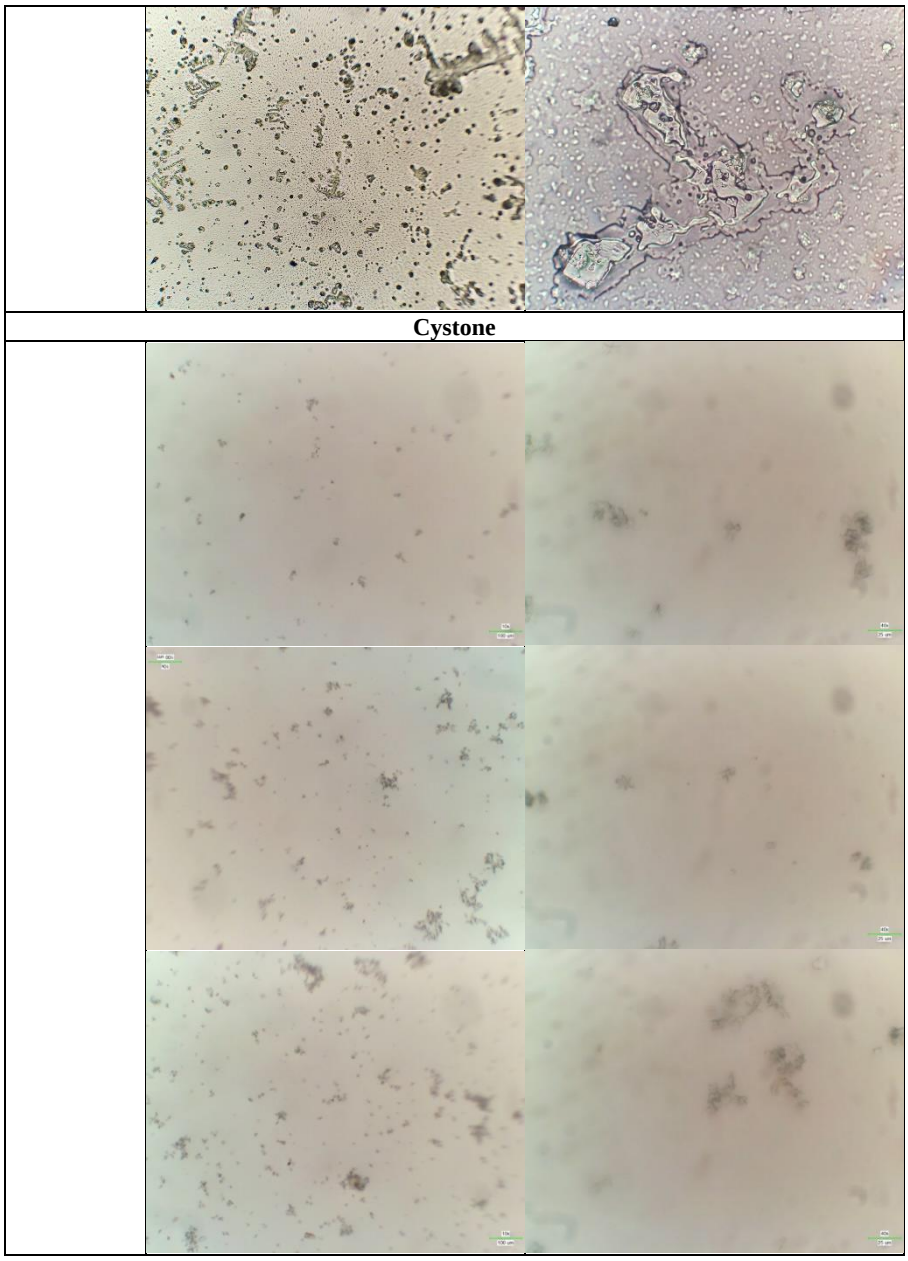
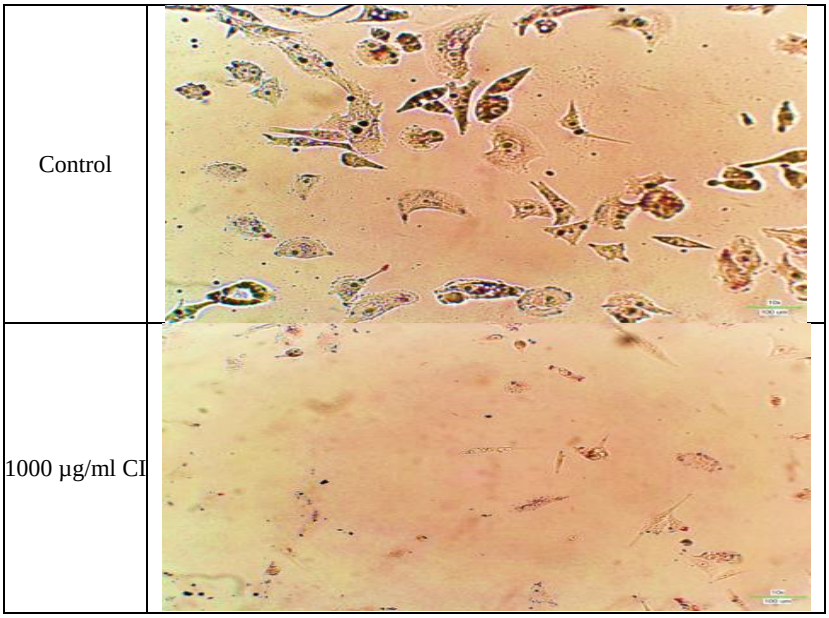


Fig 5: Calcium oxalate crystals were treated with various concentrations of the *CI* sample

Oil Red O staining



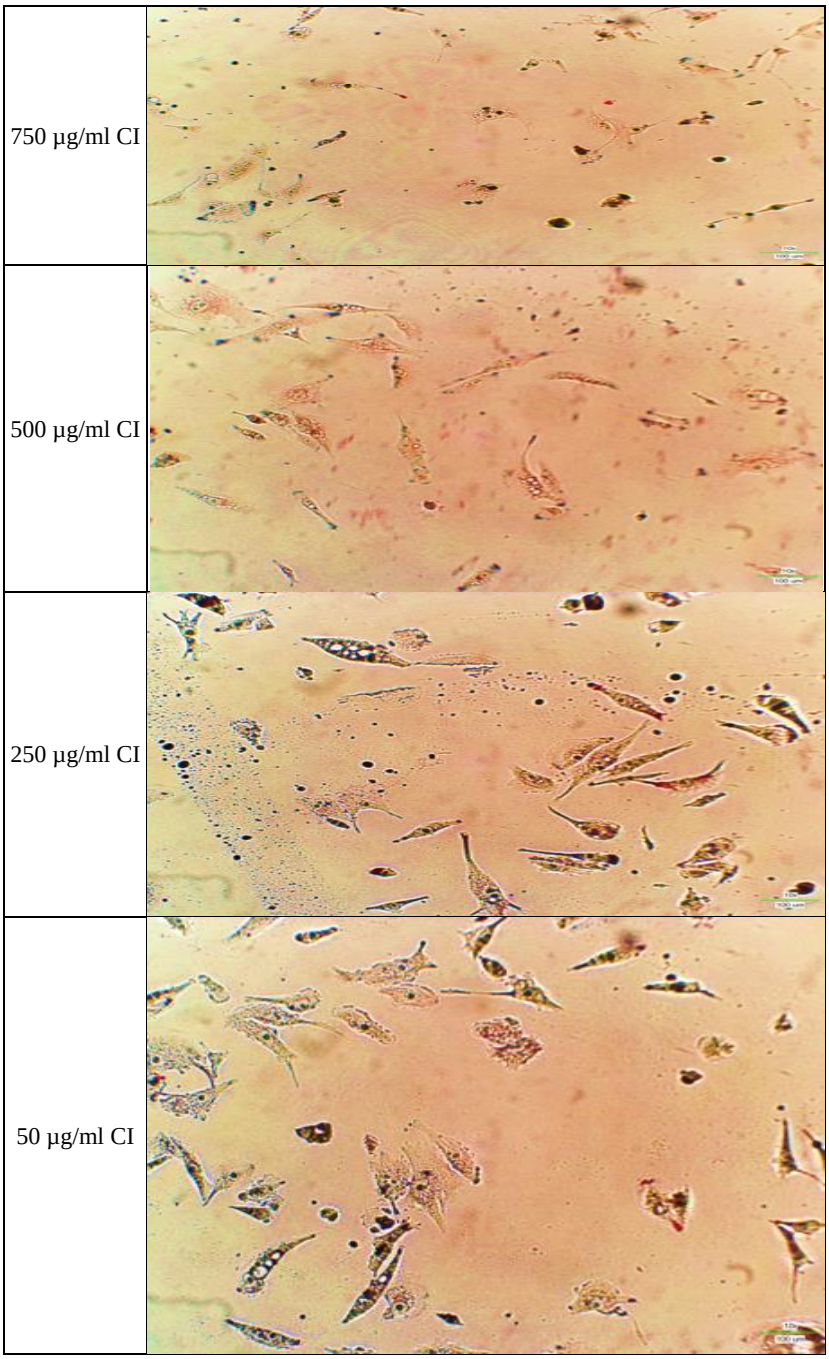


Fig 6: Oil Red O staining of Aqueous extract of *CI*

OD value at 520 nm

Table 3: OD value at 520 nm of Aqueous extract of *CI*

S. No.	Tested sample concentration (µg/ml)	OD value at 520 nm(in triplicates)		
1.	Control	1.113	1.125	1.107
2.	1000 µg/ml	0.297	0.311	0.282
3.	750 µg/ml	0.321	0.337	0.353
4.	500 µg/ml	0.546	0.553	0.571
5.	250 µg/ml	0.762	0.722	0.698
6.	50 µg/ml	0.872	0.912	0.924

Table 4: Mean value of Aqueous extract of *CI*

S. No.	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	1000 µg/ml	26.684	27.644	25.474	26.601
3.	750 µg/ml	28.841	29.955	31.888	30.228
4.	500 µg/ml	49.056	49.155	51.580	49.931
5.	250 µg/ml	68.463	64.177	63.053	65.231
6.	50 µg/ml	78.346	81.066	83.468	80.960

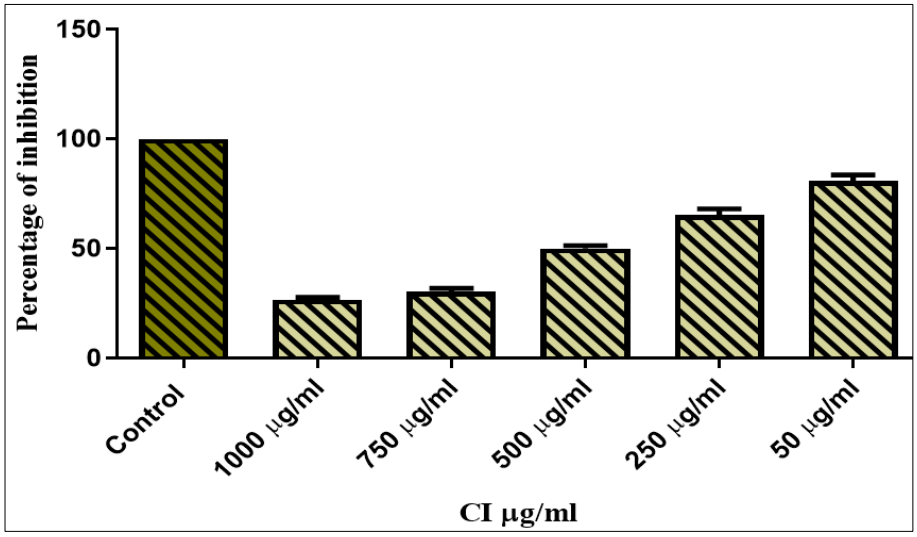


Fig 7: Percentage of inhibition Aqueous extract *CI*

Discussion

The Plant *Costus igenus* is famous for its therapeutic usefulness as hypolipidemic drug and as a diuretic. The herb commonly known as Insulin Plant having the phytoconstituents like flavonoids, alkaloids, terpenoids, and saponins. As a potent antioxidant, the phytochemicals play a major role in disease prevention and treatment. The aqueous extract of *Costus igenus* at the dose of 500µg/ml effectively reduces the lipid level and its proves that it may incorporate in any formulation intended for treating any hyperlipidaemic conditions.

The Aqueous extract of *Costus igenus* at the dose 500µg/ml showed maximum activity for both diuresis and reducing the lipid levels. Such activity might be due to synergistic role of therapeutic phytochemicals present in it. This finding will helpful to include this plant in various formulations or as extract to treat various ailments based on the literature.

Conclusion

Based on the above results, the plant extract *Costus igenus* Nak found to possess significant diuretics and hypolipidemic property. As this extract contains groups of phytoconstituents that may synergistically support the effectiveness of the therapeutic claim based on the literature.

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