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FORMULATION AND EVALUATION OF POLYHERBAL SYRUP FOR TREATMENT OF KIDNEY STONE.

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ABSTRACT

There are different types of syrup currently available to treat Kidney stone, but they can only be used for covering against foreign stimuli and particles. In order to treat or inhibit an infection, they provide no pharmacological benefit. The present study aimed to Formulation and evaluation of polyherbal syrup of treatment of kidney stone containing herbal drugs. We have developed a medicated herbal syrup containing *Kalanchoe Pinnata*, Ghokaru and *Tridax Procumbens linn* that will be helpful for treating kidney stone. Medicinal herbs have been used to treat kidney stone since ancient times. We all know Kalanchoe pinnata show the best antiurolithiatic activity. Indian farmers crush fresh leaves of Tridax procumbens and let the juice treat their kidney stone. *Kalanchoe pinnata* plays important role in the inhibition of calcium stone. We have selected herbal drugs because herbal medicines have always been deemed safer than any other medicine due to the absence of side effects comparable to those associated with allopathy. The presence of essential phytochemical components was observed as per earlier study report, during the medicinal herbs screening. In order to ensure that it is ready for use, the mixture of herbal drugs has been transferred into and syrup. The marketed cystone syrup is used for comparison study. The properties of herbal syrup in the field of organoleptic have been further evaluated. It was found that herbal preparations were free of gritiness, homogeneous mixture and with a smooth texture and the appearance of greenish colour. The egges semipermeable membrane method was used to perform the antiurolithiatic activity and it was observed that decalcification of eggs semipermeable membrane.

Keywords: - Antiurolithiatic activity, Herbal medicated syrup, kidney stone, Egg semipermeable membrane

INTRODUCTION

A kidney stone is a solid mass that develops in the kidneys due to the accumulation of minerals from urine (1) the incidence of nephrolithiasis (kidney stones) is raising worldwide, especially in women and with increasing age. Kidney stones are associated with chronic kidney disease(2) The most common type of kidney stone is calcium containing stones, represent about 80% of all cases, these typically contain calcium oxalate (CaOx) either alone or in combination with calciumphosphate. Calcium oxalate stones are of two forms, calcium oxalate monohydrate (COM) or calcium oxalate dihydrate (COD) (3) a high dietary calcium intake is strongly suspected of raising the risk that a kidney stone will form. Consequently, patients with calcium-containing stones are often advised to decrease their calcium intake. (4) Herbal drugs have created interest among the people because of its clinically proven effects like immunomodulation, adaptogenic

and antimutagenic. The excess intake of synthetic drugs results in higher incidence of adverse drug reactions which has motivated humans to return to nature for safe remedies (5) Tridax procumbens is a plant belonging to the Asteraceae family, which have worldwide distribution. Local people known it as ‒Ghamara‖ (Hindi), Gaddi Chemanthi (Telugu), Thata poo (Tamil), in English popularly called ‗coat buttons' and is dispensed for ‒Bhringraj‖ by some of the practitioners of Ayurveda. The phytochemical screening of T. procumbens revealed the presence of alkaloids, saponins, flavonoids (catechins and flavones), fumaric acid, carotenoids and tannins. It is rich in carotenoids, saponins, oleic acid and ions sodium, potassium and calcium. Its flower reported to berich in Luteolin, glucoluteolin, quercetin and isoquercetin. It is known for its number of pharmacological activities like anti-inflammatory, hepatoprotective activity, wound-healing, antidiabetic activity, immunomodulating property, dysentery, diarrhoea and promotes the growth of hair, and as well as antimicrobial activity against both gram-positive and gram-negative bacteria. Its leaf juice has antiseptic, insecticidal and parasiticidal properties, as a remedy against conjunctivitis. Traditionally, used to check haemorrhage from cuts, bruises and wounds, also as insect repellent too. It is also used as bioadsorbent for chromium (6) Kalanchoe pinnata (Lam.) Pers. (syn. Bryophyllum pinnatum; family Crassulaceae) is a popular plant used in traditional medicine in many temperate regions of the world and particularly in South America. In Guyana, the leaves are traditionally used as an anti-inflammatory and antiseptic to treat coughs, ulcers, and sores (7)

METHODOLOGY:

Collection of plant materials – The part of plants of *Kalanchoe pinnata*, *Gokhru* and *Tridax* Procumbens .Were freshly collected from local garden from Pandharpur, Maharashtra, India. The plant were identified and authenticated by, Department of Botany, KBP College Pandharpur, and India. The Plant materials washed and dried under the shed and converted into powder by mechanical grinding. Then powders were passed throughsieve no 16, and used for further study.

Phytochemical screening – In order to determine the presence or absence of primary and secondary metabolites, phytochemical screening of plant extracts has been carried out usinga standard procedure to verify the purity of herbal medicinal products. Phytochemical tests for steroids, saponins, Anthocyanins, Coumarins, Emodinants, Alkaloids, Proteins, Amino Acids, Diterpenes, and Phenol were carried out adopting standard procedures

Table 1: Phytochemical Screening tests

Kalanchoe pinnata	Ghokharu	Tidax Procumbens
1)Flavonoid:)H + Few drops of HCl+palntextract	1) Saponin ml extract +3ml dist water , shake and stand	1) Steroid extract + 0.5 ml of aceticanhydride+ 0.5ml+Chlorofor m + H2SO4

2)Tannin: 2ml extract +Alcohol +1 ml Fecl3	2) Tannin l extract +1 ml of 10%Fecl3	2) Tannin 1 ml distilled water + ml extract +1-2 drops of Fecl3
3) Saponin extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes.	3) Phenol : 2 ml Extract +5 % FeCl3	3) Saponin 2 ml of distilled water+1 ml of the xtract,shaken for few Minutes in a test tube.

Test for carbohydrate:-Small Quantity of extract drops of Molish reagent +1ml of Sulphuric acid stand for 2 min + Dist Water	rotein 2 ml extract + 1ml of 40% NaOH + 1 % Cuso4	4) Anthrocynin: ml extract +2 N HCL +NH3
5) Steroid ml extract +2 ml ethanol +1 mlof Conc.Sulphuric acid	ardiac Glycoside: 1 ml extract + 0.5 ml of GAA + 3 drops of ferric Chloride	5)Coumarin l of 10% NaOH +2 mlaq extract
6) Terpenoid 0.5 gm of plant extract +CHCl3+ Conc.Sulphuric acid	6)Terpenoid of plant extract +0.5 mlCHCl3+ Sulphuric acid	6)Emodins l NH4OH+3mlbenzene + extract
7)Quinones:- Few drops of plant extract +HCl	Test for carbohydrate:-Small Quantity of extract 3 drops of Molish reagent +1 ml of Sulphuricacid	7) Alkaloid : extract + 3ml 1%HCl(in steam bath) +Mayers reagent agner reagent added to mix
8)Glycoside:- GAA + FeCl3 + H2So4 +Extract	8) Flavonoid : ml extract + Few drops of Na2oH + 70%HCl (Drops)	8)Protein act + Con HNO3(drops)
		9)Phenol : Extract + FeCl3
		0)Diterpines:- Extract vater +10 drop of copper acetate

Preparation of Polyherbal syrup:-
Preparation of extract (8-11) –

Extract of kalnachoe pinnata - Fresh leaves of Kalanchoe pinnata were chopped into small pieces by hand and put into a conical flask. 100ml of distilled water was added to the conical flask and boiled for a while in order to maximize the extraction. After cooling it was filtered through Whatmann filter paper and as aqueous extract stock solution transferred to a suitable container.

Extract of tridex procunmben -

Utilizing 400 mL of methanol and 400 g of powdered leaves in a soxhlet device at 64°C temperature, all the components were extracted into the solvent to produce the methanolic extract. At a temperature of 45°C, a rotary evaporator was used to evaporate and concentratethe extract. For additional examination, dried extract was kept in a light- and airtight container and kept in the refrigerator at 4°C.

Extract of Gokharu -

Tribulus terrestris (83gms) were mixed with 4000ml of water and boiled until the total volume become one fourth of the initial volume. The decoction is filtered and the filtrate was taken to prepare the polyherbal syrup.

Preparation of calcium oxalate crystals by homogeneous precipitation method
In separate beakers, calcium oxalate precipitate was created by stirring together calcium chloride dihydrate (4.41g) and sodium oxalate (4.02g) solutions that had been previouslydissolved in distilled water and 2N sulphuric acid, respectively. By washing with ammonia solution and distilled water, respectively, excess sulfuric acid was eliminated. For four hours, it was dried at 60°C 20

Preparation of semi-permeable membranes from eggs

A glass rod was used to pierce the egg's apex and remove the entire contents. Empty egg shells were properly cleaned with distilled water before being placed in a beaker with 2M HCl for an overnight process that completely decalcified the shells. Membranes were then thoroughly cleaned with distilled water before being submerged for a period in an ammonia solution to neutralise any remaining acid residues. After that, they were cleaned with distilled water andkept in a refrigerator with a pH of 7–7.4

Formulation of Polyherbal syrup: - Sugar base was prepared by mixing 85 g of sucrose and 45 g of water, heated to boiling point. The liquid was strained and volume made up to 100 ml with distilled water. The preservatives were dissolved in few milliliter of boiled and cooled water and added to a sugar base extract of all herbs was dissolved in propylene glycol at 45–50°C and this glycerin and sorbitol were added. The remaining sweetening agents were added and mixed thoroughly. Adjust the pH between 5.5 and 6.5 with citric acid, if necessary. Then, volume was made up to 100 ml with boiled and cooled water

Table 2: Formulation chart of Herbal syrup

SN	Name of Ingredient	F1	F2	F3	F4	F5	F6
1	*Kalanchoe Pinnata*	1.	1.5	1.7%	1.9	1.9%	2.0
2	*Gokharu*	1	1.2	1.3%	1.4	1.5%	1.5
3	*Tridax Procumbens linn*	2.	2.5	2.7%	2.9	3.00	3.2
4	Sugar base	43	43.7	43.75%	43.	43.7	43.
5	Methyl Paraben	0.	0.01	0.0125 %	0.0	0.01	0.0
6	Propyl paraben	0.	0.12	0.125%	0.1	0.12	0.1
7	Propylene glycol	30	30%	30%	30	30%	30
8	Glycerin	12	12.5	12.5%	12.	12.5	12.
9	Sorbitol	6.	6.62	6.62%	6.6	6.65	6.6

Evaluation

Phytochemical screening study: -The herbal drugs namely *Kalanchoe Pinnata*, *Ghokharu and Tridax Procumbens.*

The general evaluation of herbal Formulation - The Prepared syrup was evaluated visually for colour, odour, and texture. The pH of herbal syrup was evaluated using pH meter. [16]

Preparation of semi-permeable membranes from eggs- A glass rod was used to pierce the egg's apex and remove the entire contents. Empty egg shells were properly cleaned with distilled water before being placed in a beaker with 2M HCl for an overnight process that completely decalcified the shells. Membranes were then thoroughly cleaned with distilled water before being submerged for a period in an ammonia solution to neutralise any remainingacid residues. After that, they were cleaned with distilled water and kept in a refrigerator with PH of 7-7.4 [17]

Fig.1 Egg decalcification

Method 2 :-

Group I: 1ml of calcium oxalate (1mg/ml) + 1ml of distilled water

Group II: 1ml of calcium oxalate (1mg/ml) + 1ml of Cystone solution (400mg/ml)

Group III: 1ml of calcium oxalate (1mg/ml) + Calculated quantity of all plant extract all groups were packed it together in egg semi permeable membrane tied With thread at one end and were suspended in a conical flask containing 150 ml 0.1 M Tris Buffer each. At another end of thread tied by a stick placed on the mouth of conical flask and Covered with aluminum foil. All groups were kept in an incubator, pre heated to 37^0C for 4 Hours, kept for three days. The entire content of each group was removed from sutured semi permeable membrane and was transferred into test tube individually. 4ml of 1N H2SO4 and 60-80 of 0.02M KMnO4 were added and kept aside for 2 hours. Colour change from dark pink to colourless was observed after 2 hours. Change of colour intensity was measured against 620nm spectrophotometrically. Concentration of undissolved calcium was determinedfrom standard calibration curve of calcium oxalate by using the measured absorbance

Nucleation assay (Turbidity method): the inhibitory activity of the extracts on the nucleation of calcium oxalate crystals was determined by a spectrophotometric assay12. Crystalliztion was initiated by adding 100ml of 4 mM calcium chloride and 100ml of 50 mMsodium oxalate solutions to 0.5ml of human normal urine, both prepared in a buffer containing 0.5ml of 0.05 mM Tris buffer and 0.5ml of 0.15mM NaCl solution at pH 6.5 and 37^0C and adjusted to volume by adding 1.5ml

of distilled water. The rate of nucleation was determined by comparing the induction time of crystals (time of appearance of crystals that reached a critical size and thus became optically detectable) in the presence of the extractand that of the control with no extract. The optical density (OD) was recorded at 620nm, and the percentage inhibition calculated as (1-OD (experimental)/OD (control))/100solution (0.1M). All of the conical flasks underwent a 7 hr incubation period at 37º C. After that, 2 mL of 1N sulfuric acid was added to the contents of the semi-permeable membrane in thetest tube. The resultant combination was titrated against the reference KMnO4 solution until the light pink colour was seen. To obtain the precise results, the entire process was carriedout three times. To assess the activity, the calcium oxalate crystal dissolution percentages were computed for each sample. [18]
Nucleation assay showed that the various concentration of polyhebal extract and Cystone against absorbance at 620nm

Aggregation assay:-
The rate of aggregation of the calcium oxalate crystals was determined by a spectrophotometric assay12 with slight modifications. The calcium oxalate monohydrate (COM) crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate of 50 mM each. Both solutions were then equilibrated. The solutions were then cooled to 37°C and then evaporated. The COM crystals were then dissolved with 0.5ml of 0.05mM Tris buffer and 0.5ml of 0.15mM NaCl solution at pH 6.5 to a final concentration of 1 mg/ml. Absorbance at 620 nm was recorded. The rate of aggregation was estimated by comparing the slope of turbidity in the presence of the extract against control.[19]

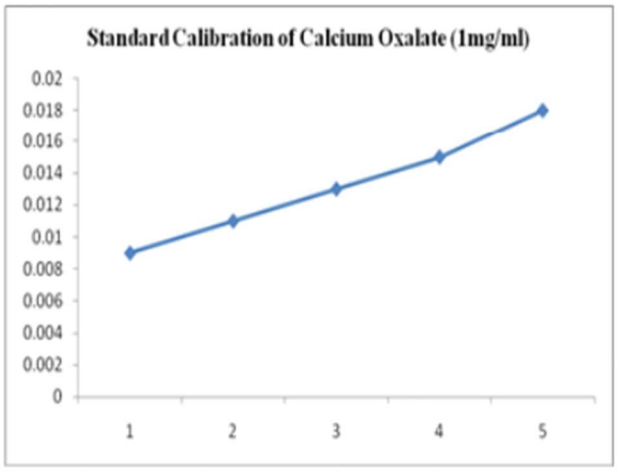


Fig. 1: Standard calibration of calcium oxalate

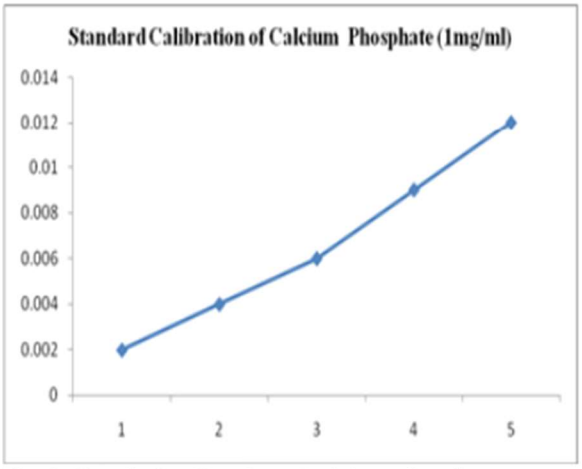
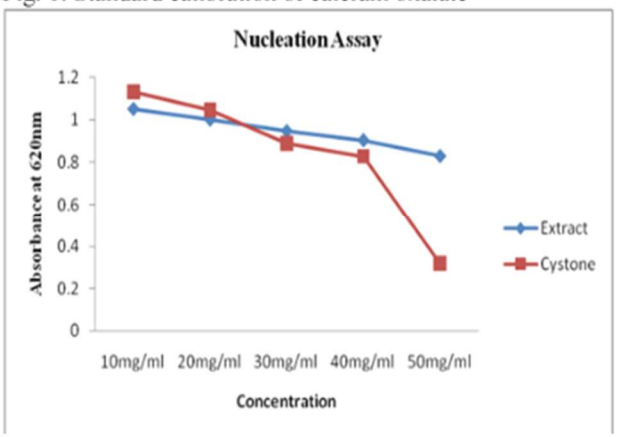


Fig. 2: Standard calibration of calcium phosphate



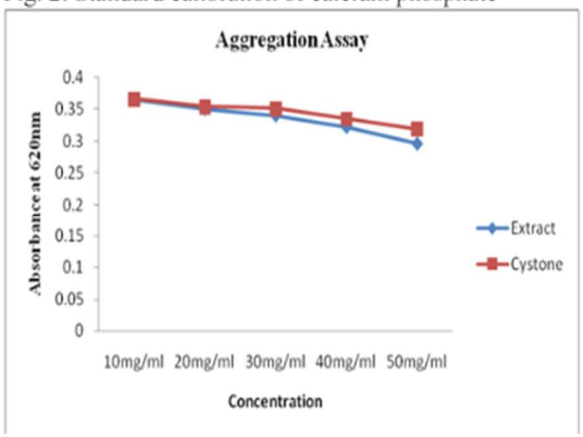


Table 3: Dissolution of calcium oxalate

Group	Mean ± SD	Weight of calcium reduced	Dissolution Percentage
Group-I	0.127± 0.0005	-	-
Group-II	0.056 ± 0.0005	0.072	57.82
Group-III	0.066 ± 0.072	0.063	47.66

Evaluations of herbal drugs for purity- The herbal medicated formulation containing syrup were prepared by using *Kalanchoe Pinnata Gokharu* and *Tridax Procumbens linn in* different

proportions. As specified in these standards, the results of a phytochemical test for all herbal medicinal drugs show that certain essential phytochemical constituents are present.

Table:-4 Phytochemical screening (12-15)

Sr.No	Test	*Kalanchoe Pinnata*	*Gokharu*	*Tridax Procumbens linn*
1.	Carbohydrate	(+)	(+)	(-)
2.	protein	(-)	(+)	(+)
3.	Glycoside	(+)	(-)	(-)
4.	Flavanoid	(-)	(+)	(-)
5.	Terpenoid	(-)	(+)	(-)
6.	Saponin	(+)	(+)	(+)
7.	Tannin	(-)	(+)	(+)
8.	Quinone	(+)	(-)	(-)
9.	Alkaloid	(-)	(-)	(+)
10.	Coumarin	(-)	(-)	(+)

(+ presence of chemical constituents; - absence of chemical constituents)

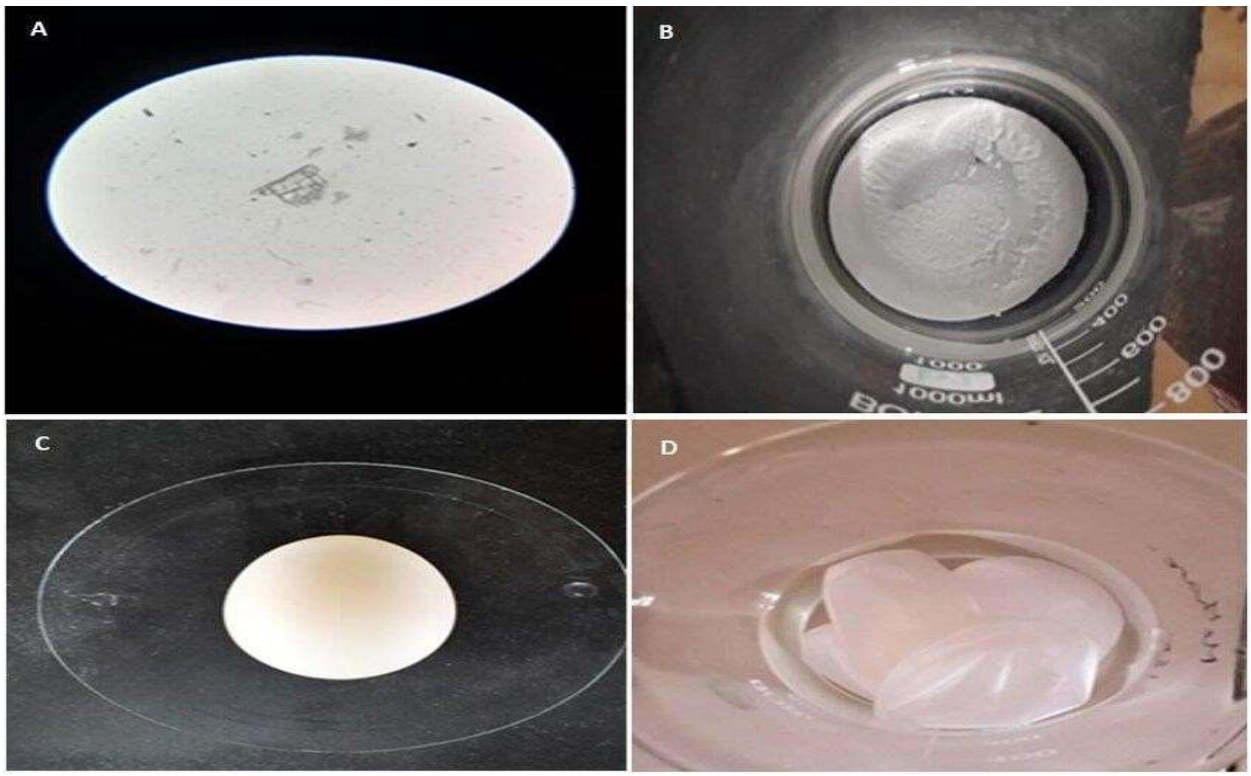
General evaluation of Polyherbal Syrup :-

In order to evaluate pharmaceutical syrup, the evaluation parameters are important tests. It has been established that the pH are satisfactory. The formulation has a pH of 6- 7, which corresponds to normal cystone syrup. It was found that an herbal preparation was free of grittiness, homogeneous mixture and with a smooth texture with greenish colour.

Table 5: General evaluation for Polyhebal Syrup.

S	Characters	F1	F2	F3	F4	F5	F6
1	Colour	Greenish	Gree	Gree	Gree	Gree	Greenish

2	Odour	Odourless	Odo	Odo	Odo	Odo	Odourless
3	Test	Sweet	Swe	Swe	Swe	Swe	Sweet
4	Texture	Smooth	Smo	Smo	Smo	Smo	Smooth
5	Appearance	Glossy	Glos	Glos	Glos	Glos	Glossy
6	pH	6-7	6-7	6-7	6-7	6-7	6-7



A) Microscopic view of calcium oxalate crystal, B) The homogenous precipitate of calciumoxalate, C) Decalcified egg, D) Semipermeable membrane

Polyherbal syrup for treatment of kidney stone evaluated by using eggs semipermeable method by this method semipermeable membrane shows decalcification of egg, so it is used to treat kidney stone .

CONCLUSION

The formulated herbal syrup has shown potential antiurolithiatic activity and could replace marketed syrup with respect to rapid treatment of kidney stone. The formulated herbal syrup has good antiurolithiatic activity. This is of first kind of syrup which will be pharmacologically active and has herbal base benefit too. The formulated herbal medicated syrup is safe and efficient product along with antiurolithiatic activity with ready to market potential

- The phytochemical screening study of herbal medicinal products reveals the presence of essential chemical constituents in accordance with the standard.
- The pH of the formulation was found to be 6-7, which is acceptable and does not cause any side effect.
- The decalcification of eggs semipermeable membrane was observed.

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