wjpmr, 2024, 10(11), 280-286

SJIF Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

FORMULATION OF POLYHERBAL SYRUP FOR ANTIUROLITHIATIC ACTIVITY

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Article Received on 14/09/2024 **Article Revised on 04/10/2024** **Article Accepted on 25/10/2024**

ABSTRACT

Background: Traditionally medicine plants are having the ability dissolving kidney stone which is very effective and save. The scientific study of these plants for their urolithiasis has been studied and further it is not forwarded to use as a medicine in the form of syrup **Objective:** The aim of our present study is to formulate polyherbal syrup for antiurolithiatic activity. Urolithiasis is the formation of urinary calculi at any part of the urinary tract. **Material and methods:** Aqueous extract of Bergenia ligulata (AEOBL), Boerhavia diffusa (AEOBD), Tribulus terrestris (AEOTT), Raphanus sativus (AEORS), and Cocos Nucifera (AEOCN) are prepared by maceration method. Phytochemical investigation of all the extract was made to detect the various phytoconstituents or the secondary metabolites. Further the bioactive constituents are confirmed by subjecting the extract thin layer chromatography. Evaluation of antiurolithiasis activities of the extract against calcium oxalate crystals by employing an in- vitro studies is performed by egg semipermeable membrane model, using cystone as a standard drug. **Results:** % dissolution of AEOBL, AEOBD, AEOTT, AEORS, AEOCN was found to be 75.33%, 75.33%, 75.33%, 66.96% respectively which compared with standard cystone 83.7%. **Conclusion:** The data obtained through this study, it is confirmed that these plants are really effective in the treatment of dissolving kidney stones. Polyherbal syrup was made by using these extract, simple syrup and preservatives. The formulation is subjected to various standardization parameters like, appearance, specific gravity, viscosity and ph.

KEYWORDS: Polyherbal syrup, Urolithiasis, Kidney stone, Evaluation.

INTRODUCTION

Tyler defines herbal medicines as "crude drugs of vegetable origin utilized for the treatment of diseased state often of a chronic nature or to attain or maintain a condition of improved health".[2] For primary healthcare, between 75 and 80 percent of the world's population still turns to herbal treatment, mostly in underdeveloped nations. This is mostly due to the widespread perception that herbal medications are inexpensive, readily available, and side effect-free. According to the WHO, the usage of herbal treatments throughout the world exceeds that of the conventional drugs by two to three times.[1] Although over 500 traditional societies in India use over 800 plant species for healing, only roughly 20,000 medicinal plant species have been recently identified in the country.

However, around 800 plant species are used by more than 500 traditional societies to treat a variety of illnesses.[3] About 121 plant based medicines were developed in the last century using traditional knowledge gathered from a variety of sources.[4]

Traditional medicine is most frequently used because it more closely aligns with the patient's ideology, allays fears regarding the side effects of chemical (synthetic) medicines, fulfills the need for more individualized treatment, and increases public access to health information. The predominant use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, illnesses.[5]

NEPHROLITHIASIS

Urinary calculi can occur at any stage of the urinary tract and are referred to as nephrolithiasis or urolithiasis. Renal stone disease is thought to affect 2% of people at some point in their lives, with a male-to-female ratio of 2:1.[7] The renal calculi are characterized by infection in urinary tract, haematuria, acute discomfort, hydronephrosis, and obstructive neuropathy.[6]

When urine elements that are ordinarily in solution—most commonly phosphate and oxalate salts—precipitate, calculi can develop in the bladder and kidneys. They are more common in males and after 30 years of age and commonly recur. The majority starts in the renal papillae or collecting tubules. After that, they go into the renal pelvis, where they could enlarge. Some grow to be too big to fit through the ureter, which can block urine flow and harm the kidneys. Others go to the

bladder, where they either are eliminated or enlarge and block the urethra.

Among the predisposing factors are

Dehydration: This results in a low volume of highly concentrated filtrate in the collecting tubules because it increases the reabsorption of water from the tubules without changing solute reabsorption.

Urine pH: Some compounds may precipitate when the normally acid filtrate becomes alkaline. This happens in certain infections and when the renal buffering system is compromised.

Infection: pus and necrotic material serve as foci for the possible deposit of solutes in the filtrate, and the resultant infection may change the pH of the urine.

Metabolic disorders: these comprise gout and hyperparathyroidism. Renal colic resulting from ureteric obstruction can occur when small calculi pass through or become lodged in a ureter, damaging the epithelium and causing haematuria, fibrosis, and stricture after healing. When a stone reaches the bladder, it may grow larger and potentially block the urethra or be discharged in urine. Proximal infection to obstruction, pyelonephritis, bilateral hydronephrosis, urine retention, and serious kidney injury are among the consequences.

Large calculi form frequently over several years, occupying the renal pelvis and the calyces. Urine stagnation is the result, which increases the risk of infection, hydronephrosis, and occasionally kidney cancers. Chronic renal failure could result from it.[8]

HERBAL DRUGS IN UROLITHIASIS

People's interest in using natural medicine for their basic healthcare requirements is growing as a result of their observations and beliefs about traditionally utilized medicinal herbs. Many nations and cultures have utilized a diverse array of medicinal herbs as urolithiasis preventive and treatment methods. The antiurolithiatic effect of currently available herbal medicines is mediated by a variety of multifaceted pharmacological actions, including the inhibition of the angiotensin converting enzyme, analgesic, anti-inflammatory, antioxidant, antispasmodic, astringent, diuretic, demulcent, litholitic, and lithotriptic effects, as well as changes in the concentrations of ions in urine, such as an increase in magnesium and citrate excretion.

Kidney stones have been treated by 503 species, 365 genera, and 119 families. The families Asteraceae, Fabaceae, Lamiaceae, Apiaceae, Rosaceae, and Poaceae are the most frequently mentioned. Boerhavia diffusa L., Bergenia Ligulata, Tribulus terrestris, Raphanus sativus L., Cocos nucifera L., Allium cepa L., Centella asiatica L., Coriandrum sativum L., Foeniculum vulgare Mill., Calendula officianalis L., Asparagus officianalis L., Ricinus communis L., Punica granatum L., Moringa oleifera Lam., Triticum aestivum L., Ocimum basilicum L., Glycyrrhiza glabra L., and Rosa indica L. are a few examples of antiurolithiatic plants. The chemicals flavonoids, polyphenols, glycosides, steroids, alkaloids, terpenoids, benzenoids, and tannins provide these plants their antiurolithic properties.[9,10]

COLLECTION OF HERBAL PLANTS

The leaves of Boerhavia diffusa Linn were collected from Vadakkencherry, and it is washed with running water to remove the dirt and soil. The powdered rhizome of Bergenia ligulata Linn, the dried seeds of Tribulus terrestris Linn, is obtained from commercial market. Cocos nucifera Linn was collected from Thrissur. The Raphanus sativus Linn were collected and cleaned with water.

AUTHENTICATION

The plant specimens included in the study such as *Boerhavia diffusa*, *Cocos nucifera* were authentified by the scientist in charge of Kerala Forest Research Institute, Peechi Kerala, India. (Forest Botany Dept.)

DRYING AND POWDERING

One of the most important steps in formulation is drying since it guards against microbial attack. Microbes grow in the presence of moisture content. The moisture in the plant portions is removed through appropriate drying. So the provided sections of the plant is chopped and dried in sun shade for weeks and it is milled until gets fine powder.

EXTRACTION

50 gm of each drug was accurately weighed and subjected to the extraction with water by simple maceration methods.

PHYTOCHEMICAL SCREENING

1) Molish Reaction: In a porcelain spot plate, a few milligrams of a medication containing carbohydrates is combined with two to three drops of a 15% solution of one naphthol in alcohol. Mix thoroughly after adding an equivalent amount of pure sulphuric acid. The rich violet colour indicates the presence of carbs.
2) Fehling's reaction: With carbohydrate a reddish brown hue is generated.
3) Barfoed test: Used to identify disaccharides from monosaccharides. Strong red precipitates are formed by barfoed reagent solutions including galactose, dextrose, and levulose; lactose and maltose may also precipitate to some extent, although sucrose stays negative for a longer period of time.
4) Selivanoff test: This test is used to identify ketose and aldose sugars. The test is unique to ketoses. When combined with the reagent, sucrose and fructose turn pink in 30 to 80 seconds.

Check for alkaloids

1) Mayer's reagent: It produces an alkaloids-based white or cream precipitate.
2) Dragendorff's reagent: Alkaloids combine with the reagent to generate an orange-colored precipitate.

3) Hager's reagent: It produces a distinctive precipitate that is crystalline and yellow.
4) Wagner's reagent: When combined with alkaloids, it produces a brown or reddish brown precipitate.

Check for glycosides

1) Keller-Killiani test: The medication is dissolved in glacial acetic acid and mixed with one or two drops of ferric chloride. Then, strong sulphuric acid is added to the test tube drop by drop. The presence of cardiac glycosides is indicated by the formation of a reddish brown tint at the junction.
2) Legal's test: Add 1 ml each of pyridine and sodium nitroprusside to an aqueous or alcoholic extract. Colour changes from pink to crimson.

Baljets test: Sodium picrate causes a thick piece to turn yellow to orange in hue.

Check for tannins

1) Goldbeaters skin test: Soak a tiny piece of skin in 2% Hcl, rinse it with purified water, and then immerse it in a tannin solution for five minutes. The skin portion is preserved in a ferrous sulphate solution after being cleaned with distilled water. Because tannins contain phenols, the skin turns dark or black.
2) Gelatin test: An aqueous solution of gelatin (1%), sodium chloride (10%), and tannin (0.5–1%) are added to a solution. Precipitation forms with a white, buff color.

Test for flavonoids

1) Alkaline reagent test: 2-3 drops of sodium hydroxide were applied to 2 ml of extract. When a few drops of diluted Hcl were added, the initially intense yellow colour eventually turned colourless, showing the presence of flavonoids.
2) Shinoda test: To test solution add 5 ml of alcohol (95%), few drops of concentrated Hcl and 0.5 g of magnesium turnings. The production of a pink tint shows the presence of flavonoids.
3) Zinc HCL test: When a solution containing zinc and HCL is heated, a pink to red hue is seen.

Check for saponins

1) Foam test: In a test tube, mix a small amount of the medication with a small amount of water. When foam starts to form, saponin glycosides are present.
2) Haemolytic test: spread a drop of drug extract and blood onto a slide, stir thoroughly, and see under a microscope. Blood cell haemolysis is a sign that saponin glycoside is present.[11,12,13,14]

THIN LAYER CHROMATOGRAPHY

It's a chromatographic method for dividing up mixtures. TLC is carried out on a glass, plastic, or aluminium foil sheet that has been lightly covered in an adsorbent substance, most often cellulose, silica gel, or aluminium oxide. The term "stationary phase" refers to this absorbent layer. A solvent or solvent combination (mobile phase) is dragged up the plate by capillary action after the sample has been put to it. Because various analytes ascend the TLC plate at different rates, separation is obtained.

TLC requires less effort, costs less, and requires a simpler approach to complete. It is often used in pharmaceutical analysis and for figuring out what contaminants are in a molecule. Usually, the samples just need a little preparation.

TLC is essential to many standard procedures in the fields of industrial chemistry, environmental toxicity, food chemistry, water and pesticide analysis, plant materials, and herbal analysis. Distance travelled by the solute / Distance travelled by the solvent equals the Rf value.

Rf value depends upon various aspects such as; kind of adsorbent, mobile phase, temperature, thickness of layer, developing tank, mass of sample, chromatographic process.

Stationary phase: In thin layer chromatography, a silica gel-coated thin glass plate serves as the stationary phase. The struggle between the solute and the mobile phase for binding sites on the stationary phase is the basis for compound separation. When two compounds with different polarities are combined, the more polar component interacts with the silica gel more strongly and can thus remove the mobile phase from the binding sites more effectively. Higher up the plate, less polar compound travels, increasing the Rf value.[15]

INVITRO STUDY FOR ANTIUROLITHIC ACTIVITY

Work done with cells, tissues, or other biological components extracted from the living organisms of interest is referred to as in vitro experiments. It gives a beginning point for obtaining insights regarding how a cell responds to a novel medicine in a controlled, isolated environment. Herbal aqueous extracts were used in conjunction with the titrimetric method to measure the in vitro antiurolithiasis activity. An analysis was conducted to determine the antiurolithiatic activity of herbal extracts against the synthetic kidney stones.

Preparation of synthetic kidney stone

The homogenous precipitation method described below was utilized to create the experimental kidney stones made of calcium oxalate (CaOx). 1.47 g of calcium chloride dihydrate and 1.34 g of sodium oxalate were dissolved in 100 mL of 2N H2SO4 and 100 mL of distilled water, respectively. In a beaker, both were mixed, and the calcium oxalate was precipitated by stirring. Ammonia solution was employed to liberate the crystals from H2SO4. Finally, the crystal was cleaned with distilled water and dried for 4 hours at 60 degree Celsius and stone has been prepared.

Preparation of semipermeable membrane from eggs

The exterior calcified shell was removed chemically by placing the eggs in 2mL HCL for overnight, which caused complete decalcification. After that, the egg is thoroughly cleaned with distilled water and the contents are gently extracted using a sharp pointer to make a hole in the top. After giving it a thorough wash with distilled water, it was wet for a while with an ammonia solution, and then it was rinsed with distilled water. refrigerated, with a pH of 7.3–7.4.The egg membrane is shown in **figure 1**

Figure 1: Preparation of egg semipermeable membrane.

Titrimetric method

Packed in a semipermeable membrane using suturing, weighed precisely 100 mg of calcium oxalate, extract, and standard cystone were as in the model **(figure 2)**. They were let to float in a 100 ml 0.1M TRIS buffer contained in a conical flask. As a negative control, one group merely had one milligram of calcium oxalate has displayed in figure no 2. Every group's conical flask will spend two hours—roughly seven or eight hours—in an incubator that has been preheated to thirty degrees Celsius. Each group's semi-permeable membrane will have its contents taken out and placed in a test tube. Titrate with 0.9494N KMnO4 and add 2 ml of 1N sulfuric acid until a pale pink endpoint is reached.1 milliliter of 0.9494N KMnO4, or 0.1898 milligrams of calcium oxalate.[16]

Figure 2: Calcium oxalate stone with aqueous extracts and standard and negative control.

FORMULATION OF SYRUP

How to Make Simple Syrup

Weighed is 66.7g of sucrose. After adding the distilled water, boil the mixture until the sucrose dissolves entirely. Finally, add distilled water to produce up to 100 milliliters.

Making Polyherbal Syrup

Five parts simple syrup are combined with one part of the prepared decoction (1:5 ratio). An appropriate amount of peppermint oil and methyl paraben are added to the combination above.

POLYHERBAL SYRUP EVALUATION

The evaluation and characterisation of herbal syrup were evaluated. The examination parameters were viscosity, pH, specific gravity, and visual appearances according to the IP standard.

Outward appearance Vibrancy

We looked at the herbal syrup's colour. The brightness on the white background allowed for the identification of the colour.

Odour and taste

The odour of the prepared syrup was tested and is identified.

To taste the final syrup, a pinch was placed on the tongue's taste bud.

Particles

The particle's presence in the formulation was examined. The mixture was moved into the clear container, where it was examined in comparison to the white and black platform. Regarding the particles or dust, the formulation was explicit.

pH

Using a digital pH meter, the measurement of pH was examined and noted. After measuring and pouring 10 ml of the syrup into the volumetric flask, the total volume was increased to 100 ml. The probe of pH meter was dipped into the solution and pH is recorded.

Specific gravity

1. Use nitric or chromic acid to thoroughly clean the bottle of particular graveness.
2. Give the bottle at least two or three thorough washes with fresh water.
3. Let the bottle air dry after washing it with acetone and organic detergent, if needed.
4. Weigh an empty dry bottle using a capillary tube breach.
5. After adding distilled water to the bottle, place the breach on it and clean away any fatty liquid by using towel paper on the side tube.
6. Weigh a water bottle with a cork using a rational balance.
7. Repeat steps 4 through 6 with the liquid under test, replacing water, after evacuating and drying as indicated.
8. Put the bottle with the cork n and weigh it along with the liquid being tested.

Weight of the liquid being tested / Weight of water equals the specific gravity of the syrup under test.

Viscosity

It is determined by Ostwald's viscometer. Use warm chromic acid to thoroughly clean the viscometer, and acetone or another organic solvent should be used if needed. Place the viscometer vertically on an appropriate stand. Up to mark G, fill the dried viscometer with water. Calculate how long it takes for water to flow from mark A to mark B, in seconds. Determine how long it takes the liquid to flow to mark B after rinsing the viscometer with test liquid and filling it to mark A.[17,18]

Calculation of viscosity

Viscosity = [(Density of test liquid × time required to flow test liquid) / (Density of water × Time required to flow water)] × viscosity of water

RESULTS

EXTRACTION

All the powdered drugs are subjected to extraction by maceration successfully and the percentage yield was calculated in **table 1**

Table 1: Percentage yield of the extract.

Name of drug	Wt. taken of dried crude drug	Wt. of extract Obtained	% yield
Pashenbhed	50g	7.72g	15.44%
Gokhru	50g	9.1g	18.2%
Punarnava	50g	7.65g	15.3%
Coconut embryo	50g	5.8g	11.6%
Radish	50g	5.65g	11.3%

PHYTOCHEMICAL SCREENING

All the extracts were subjected to the different qualitative chemical test to detect the presence of various secondary metabolites which is shown in **table 2**.

Table 2: Phytochemical screening.

Phytoconst ituent	Coconut Embryo (Aeocn)	Gokhru (Aeott)	Radish (Aeors)	Punarnava (Aeobd)	Pashanbhed (Aeobl)
Alkaloid	+	+	+	+	--
Carbohydrate	+	--	+	+	+
Glycoside	+	+	+	+	+
Flavonoid	+	+	+	+	+
Saponin	+	+	+	+	--

Phenolic compound	+	+	--	--	+
Phytosterol &triterpenoids	+	+	+	--	--
Amino acid	+	--	+	--	--
Tannins	--	+	+	+	+
Steroid	--	--	+	+	--

(+) Present (-) Absent
AEOCN- Aqueous extract ofCocos Nucifera
AEOTT - Aqueous extract ofTibullus terrestris
AEORS - Aqueous extract ofRaphanus sativus
AEOBD - Aqueous extract ofBoerhavia diffusa
AEOBL - Aqueous extract ofBergenia ligulata

TLC
All the extracts were subjected to TLC and its Rf value was calculated. The Rf value was compared with standard and it is identified as quercetin and its derivative of quercitrin which is the flavonoid glycosides has displayed in **table 3**.

Table 3: Thin layer chromatography.

SAMPLE	ADSORBENT	SOLVENT	DETECTION	Rf VALUE
AEOBL	Silica gel	n-butanol: glacial acetic acid: water	U V detector	0.83
AEOBD	Silica gel	n-butanol: glacial acetic acid: water	U V detector	0.81
AEOTT	Silica gel	n-butanol: glacialacetic acid: water	U V detector	0.86
AEOCN	Silica gel	n-butanol: glacialacetic acid: water	U V detector	0.96
AEORS	Silica gel	n-butanol: glacialacetic acid: water	U V detector	0.82

AEOBL-Aqueous extract of Bergenia ligulata, AEOBD-Aqueous extract of Boerhavia diffusa, AEOTT-aqueous extract of Tribulus terrestris, AEOCN-Aqueous extract of Cocos nucifera, AEORS-Aqueous extract of Raphanus sativus.

INVITRO STUDY
The efficacy of the extract was performed against calcium oxalate crystals to determine the antiurolithiatic activity. Hence all the extracts were shown the potent antiurolithiatic activity and the parameters are below which is highlighted in **table 4.**

Table 4: % Percentage dissolution of the extracts against calcium oxalate stone.

Groups	Vol. of KMnO4	Wt. of Ca estimated	Wt. of Ca reduced	% dissolution
Negative	0.12	0.0227	0	
Standard	0.02	0.0037	0.019	83.7%
AEOBL	0.03	0.0056	0.017	75.33%
AEOBD	0.03	0.0056	0.017	75.33%
AEOTT	0.03	0.0056	0.017	75.33%
AEORS	0.05	0.0094	0.013	58.59%
AEOCN	0.04	0.0075	0.015	66.96%

FORMULATION OF SYRUP
The polyherbal syrup was prepared by using extracts and simple syrup along with preservatives.The composition of syrup is shown in **table 5.**

Table 5: Composition of polyherbal formulation.

	Ingredients	Concentration	Quantity
1	AEOBL	1mg/1ml	5ml
2	AEOBD	1mg/1ml	5ml
3	AEOTT	1mg/1ml	5ml
4	AEORS	2mg/1ml	5ml
5.	AEOCN	2mg/1ml	5ml
6.	Methyl paraben	-	0.5mg
7.	Peppermint oil	-	q.s

AEOBL-Aqueous extract of Bergenia ligulata, AEOBD-Aqueous extract of Boerhavia diffusa, AEOTT-aqueous extract of Tribulus terrestris, AEOCN-Aqueous extract of Cocos nucifera, AEORS-Aqueous extract of Raphanus sativus.

EVALUATION OF POLYHERBAL SYRUP
The prepared syrup was evaluated to determine the quality. Following parameters were observed as per **table 6.**

Table 6: Parameter evaluated on polyherbal syrup.

Parameter	Observation
Colour	Light brown
Odour	Peppermint
Taste	Sweet
Particle observation	No settling of particle against dark and white background
pH	5.34
Viscosity	0.2630
Specific gravity	1.181 w/w.

DISCUSSION

Formulation of polyherbal syrup for antiurolithiasis involves several steps. First we choose the herbs which have antiurolithiatic properties like *Boerhavia diffusa, Bergina ligulata, Tribulus terrestris, Cocos nucifera and Raphanus sativus*. Extraction is performed by maceration method to obtain active constituents effectively. The phytochemical screening was performed for the identification of chemical constituent present in the herbs. Glycosides and flavonoids commonly present as the main constituent which is responsible to produce antiurolithic activity and moreover it is justified by performing TLC. The study was conducted as per "invitro evaluation of scoparia dulcis Linn for antiurolithiatic activity". The invitro evaluation of all the plant extracts was done against calcium oxalate crystals using egg semipermeable membrane with standard cystone and the efficacy was proved based on the calcium oxalate dissolution. Further we prepared polyherbal syrup for the effective treatment of kidney stone. This syrup has undergone various evaluation parameters to improve the quality of the syrup and it is within the limit.

CONCLUSION

In this study, it is concluded that the prepared polyherbal syrup contain similar antiurolithiatic activity compared to standard drug cystone. Hence it is the safest medicine to treat kidney stone due to its improved effectiveness and lack of side effects compared to synthetic medicines, and surgery. It is easily accessible, cost effective and more efficient when compared to synthetic medicine. Thus the prepared polyherbal formulation contains the potent active constituents which can be consumed in the form of decoction, juice, or extract so it favours the patient's needs. The formulation of polyherbal syrup for antiurolithiasis shows promising potential natural remedy for preventing and treating urinary stone. Through comprehensive research and experimentation, this study has demonstrated the effectiveness of combining various herbal extracts known for their lithiotriptic properties. Ultimately, the development of this syrup represents a significant advancement in the field of herbal medicine. Hence this drug is highly recommended for the treatment of kidney stone and this can also formulate in the form of syrup, tablet and other dosage forms in future.

ACKNOWLEDGEMENT

I sincierly thanks to Principal and management of sanjo college of pharmaceutical studies for their support and encouragement to make this study possible.

REFERENCES

1. Sanjoy Kumar Pal, Yogeshwer Shukla. Herbal Medicine: Current status and the future.
2. Tyler VE Herbs of Choice: The Therapeutic Use of Phytomedicals, Pharmaceutical Products, Binghampton, New York, 1994.
3. Kamboj VP: Herbal medicine. Cur. Sc., 2000; 78(1): 35-39.
4. R. Perumal Samy, P. Gopalakrishnakone. Current status of herbal and their future perspectives.
5. Sissi Wachtel-Galor and Iris F. F. Benzie. Herbal medicine : a growing field with a long tradition
6. B. Reuben, C. Narmadha. An excessive review of kidney stone symptoms, types and prediction methods in up to date, 12(01): 1-8.
7. Harshmohan. Textbook of pathology7th edition, 2015; 672.
8. Anne Waugh, Allison Grant Ross and Wilson Anatomy and physiology in health and illness.12th edition, 354.
9. Dr. Anand Vikash, Dr. Nalinaksh pankaj, A review of the punarnava (Boerhavia diffusa linn): A plant with many medicinal uses. EJPMR, 2023; 10(7): 493-497.
10. Harshad Suryawanshi, Mayuri Suryawanshi, Gaurav Patil. A Review on herbal treatment for kidney stones. IAJPS, 2020; 07(12): 102.
11. Dr. K.R. Khandelwal. Practical Pharmacognosy technique and experiments, 25.4.
12. C. K. Kokate Practical Pharmacognosy, 110.
13. Dr. Madhu. C. Divakar. Plant drug evaluation a laboratory guide. 3rd edition., 2014; 8.7.
14. Mohammed Ali. Textbook of Pharmacognosy second edition, 283.
15. Archana A. Bele and Anubha Khale.An overview on thin layer chromatography IJPSR, 2011; 2(2): 256-260.
16. Sumathy Raj a, Monica Shree Gnana Soundara Rajan a, Suganthi Ramasamy b, Rimal Isaac Rajamony Suthies Goldy c, Ramathilaga Ariyamuthu a, Monisha Sudhagar d, Sakthivel Gandhi e, Prakash Shoba f, Manikandan Gurusamy. An in vitro Anti- urolithiasis Activity of a Herbal Formulation: Spinacia oleracea L. and Coriandrum sativum L., 2024; 4(1).
17. Pawar Pranjal Nimba, Zoman Tejashree Sudam, Tarle Kanchan Bhausaheb, Wagh Rakesh Manik, Deore Pramod Anna. formulation and Evaluation of herbal cough syrup IJNRD, 2023; 8(5).
18. ManasaReddy. J, Prathyusha.K, Himabindhu. J, Ramanjaneyulu.K. Evaluation of invitro antiurolithiatic activity of mentha piperita. IJPSM, 2018; 3(8): 22-28.