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FORMULATION AND EVALUATION OF LAURUS NOBILIS LEAVES EXTRACT SYRUP FOR UROLITHIASIS

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Abstract- A renal calculus is most common problem in the urinary tract which could lead to the serious problem related to the kidney. This particular experiment was conducted to formulate and evaluate the herbal syrup by using the bay leaf extract incorporated in the simple syrup according to the prescribed standard of Indian Pharmacopeia. The formulation was prepared using the fresh leaves of the bay leaves macerated in the water, water: ethanol and ethanol. The final yield was calculated and evaluation performed using parameters using refractive index, specific gravity, pH, physical appearance, viscosity and in vitro nucleation assay. The results of the herbal syrup were comparable to the standard drug.

Keywords: **Renal Calculus, Herbal Syrup, Bay leaves, Kidney stone**

1. INTRODUCTION

Kidney stone diseases, which is also known as nephrolithiasis or Urolithiasis is the time when the formation of a hard piece of stone (kidney stone) is formed within the urinary tract. Kidney stones are typically formed in the kidney and then leave the body through the urine stream. Small stones can be able to pass through without causing any symptoms [1]. If the size of a stone increases to greater than five millimeters (0.2 in) the stone could result in obstruction to the urinary tract and cause intense and painful pain in the lower back and abdomen.. A stone can cause urine that is bloody and vomiting or painful urine [2]. A majority of people who suffer from kidney stone will suffer from another one within the next ten years. Nephrolithiasis can be characterized by the presence of kidney calculi (kidney stones) which develop within the renal pelvis. There are four kinds of kidney stones that are classified by the stone-forming substances [3].

1. Calcium,
2. Strucvite (composed of ammonium, magnesium as well as phosphate),
3. Uric acid is a major contributor to uric acid, as well as
4. Cysteine [4]

A variety of factors can contribute to stone formation, including the following such as: [5]

- Food-related factors (high protein organ meats and salt beet, rhubarb and spinach and black tea consumption).
- Diseases (Hyperparathyroidism, sarcoidosis, metabolic syndrome, diabetes mellitus).
- Medicines related to carbonic anhydrase inhibitors and triamterene, indinavir, as well as vitamin D or C.

If the urine is excessively saturated (when it is found that the solvent in urine is able to hold more solutes than it is able to contain in solution) by a combination of one or several Calculogenic (crystal-forming) substances a seed crystal could be formed through nucleation [6]. The process of heterogeneous nucleation (where the crystal has a substrate where a crystal is able to develop) occurs more quickly as compared to homogeneous nucleation (where the crystal has to grow within a fluid medium which has no solid surface) as it requires less energy [7]. As it binds to the cells that line the surface of the kidney papilla, a crystal seed may grow and form a well-organized mass. Based on the substance of the crystal the process of forming stones can happen faster when the pH of urine is unusually excessive or below five [8].

Super saturation in urine in relation to a compound that is calculogenic is influenced by pH. For instance, with an acidic pH 7.0 that means the solubility for uric acid within urine is approximately 158 mg/100ml. Reduced pH to 5.0 reduces in the amount of solubility of the acid to less than 8 mg/100ml [9]. The formation of uric acid stones requires a combination hyperuricosuria (high urinary uric-acid levels) as well as a low pH of urine but hyperuricosuria on its own isn't connected with the formation of uric acid stones in the case of urine pH that is acidic. The urine's super saturation is a required, but not the only requirement for the formation of urinary calculi. Super saturation is most likely to be the root reason for uric acid and cysteinestones; however the calcium-related stones (especially calcium oxalate stones) could have a more complicated root reason [10].

2. MATERIALS AND METHODS

2.1. Collection of Leaves[11]

Laurus Nobilis leaves were collected nearby nursery and kept under the sun for minimum 15 days to make the fine powder. These leaves were grinded and stored in the closed container. The powder was subjected to the further analysis i.e. extraction, phytochemical screening, extractive values. About 1 kg of the powder was prepared for the extraction.

2.2. Extraction [12]

The extraction was performed with the maceration process; the powder was kept in the water and ethanol simultaneously to make two different solutions for minimum three days. After three days the soaked powder was filtered out with the help of the filter paper and separated. And the concentrated material was evaporated using the rotatory evaporator. The residue was finally dried until the solvent evaporated (Final yield is mentioned in the Table-1). The residue was collected and again grinded to make the fine particles for the further process. The final powder was kept in the desiccator to avoid moisture.

2.3. Pre formulation of raw materials

2.3.1. Moisture Content [13]

To identify the moisture content in the crude drug, 2g of the sample was weighed in the glass petri plate and kept in the hot air oven for minimum 1 hour at 100°C. The final sample was weighed and moisture content was calculated by using the formula: (Table- 2)

MC = (w - d) / w * 100

Where: w= Wet Weight of the sample

D= Dry Weight of the sample

2.3.2. Ethanol Extractive Values [14]

These values were calculated by weighed 5g of the powdered sample and dissolved in the 100 mL of 95% of the ethanol alcohol in glass beaker with lid on for minim 24h. The solution was mixed and stirred at every 6 hour to avoid the clumping and sedimentation. The solution was filtered using the filter paper and put it on the glass petri plate and kept it on heating at 105°C for minimum 2h. The final residue was collected and weighed. Ethanolic values were calculated by using the formula. (Table- 2)

% Ethanol Extractive Values = Weight of residue/weight of the drug x 100

2.3.3. Water Extractive Values [15]

Water Extractive Values were determined using the chloroform water; 2.5 mL of the chloroform was dissolved in the 1L of the water and final solution prepared. 5g of the powdered sample was weighed and dissolved in the 100mL of the prepared chloroform water up to 24h. The flask was shaken every 6 hours and kept for the 24h. The final mixture was taken out on the petri plate and about 25mL of the water evaporated to the dryness. The mixture was kept for drying in the hot air over at 105°C for minimum 2h. The final residue was weighed using the formula. (Table-2)

% Water Extractive Values = Weight of residue/weight of the drug x 100

2.4. Phytochemical Screening [15]

Phytochemical screening was done to evaluate the phytochemicals present in the final powder which was extracted through the different solvents (water, water-ethanol and ethanol). Finally, the process was carried out as per the procedure of Basak, P., et al., (2018). (Table-3)

2.5. Formulation of Syrup [16]

The simple syrup was prepared using the standard mentioned in the Indianpharmacopoeia (66.67% w/v). 1 g, 2g and 3g of the drug was added to the drug base and evaluated for its parameters. (Table-4)

2.5.1. Formulation of Syrup Base[17]

To prepare the base of the syrup 666.7 g of the sucrose weighed and transferred it to in the 1L of the glass beaker and kept it on the heating mental and then 300 of the RO water was added with continuous stirring on heat. The temperature was maintained up to 80 degree Celsius. Once the sucrose dissolved in water completely, the remaining volume of the 700 mL of 1000mL was added and stirred. The final volume makes up to 1000mL and stored in the cool place.

2.5.2. Final Syrup Preparation[16,17]

The final syrup was added in two parts, first part was added of the drug and other part of the syrup was added in 5 parts (1:5). Preservatives (Methyl paraben) were added quantity sufficient

One part of the prepared decoction is mixed with five parts of simple syrup USP (1:5 ratios). Methyl paraben and peppermint oil is added to the above mixture quantity sufficient to.

2.6. Characterization of Herbal Syrup [18]

Herbal syrup was assessed for its characterization and evaluation. The evaluation parameters included the visual appearance, pH, Specific gravity, Refractive index and viscosity as per the prescribed standard in Indian Pharmacopeia.

2.6.1. Physical Appearance [19]

Color

The color of the herbal syrup was investigated for all the batches. The all three formulation carried the similar color (brown). The color was identified in the light against the white background. (Table-5)

Odour

The odour of the formulated syrup was investigated for all the formulations. The odour was identified as aromatic. (Table-5)

Taste

The syrup taste was at the pungent side. The taste for all the formulation was similar to all the formulation. But pungent taste slightly increased in the formulation no.3. (Table-5)

Particles

The formulation was investigated for the presence of the particles. All the formulation were transferred in to the transparent container and investigated against the white and black platform. None of the formulation showed the particles. All the formulation was clear in terms of the debris, particle or dust. (Table-5)

2.6.2. pH [20]

The measurement of the pH was investigated using the digital pH meter and recorded. 10 mL of the syrup of the each formulation was measured and transferred in to the volumetric flask and the final amount was made up to 100mL. The probe of pH meter was dipped in to the solution and pH recorded. pH was recorded three times for each formulation. (Table-6)

2.6.3. Specific Gravity [21]

To determine the specific gravity the pycnometer bottle was used. Bottle was cleaned thoroughly using RO water. The bottle was again washed with the help of the nitric acid to remove any of the particles. The bottle was kept for drying to remove the nitric acid. The bottle was weighed and recorded (w1).

To perform the next step the bottle was filled with the water and then stopper of the bottle was placed over it and excess water was removed using the tissue paper. The bottle was weighed along with the water and recorded (w2).

At the final step, the bottle was filled with the formulation syrup and put the stopper. The excess liquid was wiped with the tissue paper. The bottle filled with the syrup was weighed and recorded (w3). Specific gravity of the syrup was assessed using the formula: (Table-7)

Specific gravity of liquid = Weight of liquid under test/ Weight of water

2.6.4. Refractive Index [22]

Refractive Index was measured using an instrument Refractometer. The drop of the each formulation was placed on the glass and covered with the cover slip provided in the instrument. The refractive index is mentioned in the table-8.

2.6.5. Viscosity [23]

Viscosity was measured using the viscometer with spindle 2 at the 30 RPM. The 200 mL of the syrup was filled in the glass beaker and spindle dipped in solution. The reading was noted once the instrument stops running. The viscosity was measured at the room temperature. The evaluation was performed three times for each formulation. (Table-9)

3. In vitro Evaluation [24, 25]

In-vitro Urolithiatic activity by Nucleation assay

In vitro anti-Urolithiasis activity was performed using the Nucleation assay. This assay involved the nucleation of the calcium oxalate and the determination of the crystal formulation and evaluated using the spectrophotometer. 5mmol/l solution of the Calcium chloride and sodium oxalate are mixed in the Tris buffer and maintained the pH at the 6.5. Cystone drug was used as the standard drug and compared the efficacy with the herbal syrup.

Three concentrations of the formulation and standard drug were prepared 100, 200 and 300 microgram/ml. 1 mL of the each concentration of the each formulation was added in 3 mL of calcium chloride and sodium oxalate solutions.

The final solution was incubated BOD incubator at 37°C for 30min. After incubation the solution was measured at the 620nm.

The percentage inhibition of nucleation by standard drug and test sample is calculated using the following formula. (Table-10)

$$\% \text{ Inhibition} = OD_{control} - OD_{test} / 100$$

4. RESULTS AND DISCUSSION

The herbal formulation was prepared to see the effects on the renal calculus. The bay leaves were extracted and evaluate for its efficacy using an in vitro model. The herbal formulation was made using three concentration of the leaves extract 5, 7 and 10g each (Table-4). The simple syrup 66.67% was prepared and then added in to the herbal syrup. Each formulation was inspected for its pH, viscosity, Refractive index, specific gravity, and physical appearance.

The physical appearance appeared to be the similar for all the formulation except the taste. The F1 and F2 were less stringent in taste as compare to the F3. These formulations were tested for color, odour, and particles. All the formulation does have the same color, odour (table-5).

The pH was investigated for the prepared formulation and was satisfactory and in accordance with the standard. The obtained pH was in range of the 6.63- 6.47 which is under the range. The pH was obtained using the digital pH meter for all the formulations in triplicates (table-6).

The specific gravity defines the stabilization of the formulation under certain condition such as pressure and temperature. The specific gravity was measured using the glass bottle filled and observed the weight. It was found in the results that all the formulation falls in the range and comparable to the standard formulation (7).

Refractive Index was measured the ratio of light velocity of a specified wavelength in air to its velocity in the substance under evaluation. The RI was also found significant in all the formulation and comparable to the standard drug (8).

The viscosity of the formulated herbal syrup was identified using the viscometer, the solution was filled in the glass beaker and readings were recorded for all the formulations in triplicates. The readings were satisfactory in all the formulations (table-9).

Finally, the in vitro nucleation study was performed using the Calcium chloride and sodium oxalate and the optical density was recorded. The % inhibition was as similar to the standard drug. Hence, the herbal syrup formulated as per standard of the Indian Pharmacopeia performed well in all the parameters.

5. CONCLUSION

The herbal syrup prepared using the bay leaves extracts does possess the activity of anti-Urolithiasis. The herbal syrup was evaluated for parameters such as refractive index, specific gravity, pH, physical appearance, viscosity and in vitro nucleation. Prepared formulations worked well and produced the satisfactory results and comparable to the standard drug.

6. TABLES

Table-1: Percentage Yield

Sn.	% Yield	Result
1	Ethanol	5.01%
2	Water:Ethanol	4.21%
3	Water	7.05%

Table-2: Moisture Content, Ethanol Extractive Values and Water Extractive Values

Sn.	Extractive Values	Results
1	Water Soluble Extractive	11.70%
2	Alcohol Soluble Extractive	8.80%
3	Moisturecontent	11.8%

Table-3: Phytochemical Screening

Sn.	Test	Solvents		
		Water	Ethanol: Water	Ethanol
1	Alkaloid	+	+	+
2	Glycoside	+	+	+
3	Phenol	+	+	+
4	Tannin	+	+	+
5	Flavonoid	+	+	+
6	Sterol	+	+	+
7	Saponins	+	+	+
8	Anthraquinone	+	+	+
9	Terpenoids	+	+	+
10	Carbohydrate	+	+	+

Table-4: Formulation of Syrup

S. No	INGREDIENTS	Formulation 1	Formulation 2	Formulation 3
1	Bay leaf powder	5g	7g	10g
2	Methylparaben	5mL	5mL	5mL
3	Sucrose	6.6g	6.6g	6.6g
4	Purified Water	UPTO 100ML	UPTO 100ML	UPTO 100ML
5	Propylene glycol	5mL	5mL	5mL

Table-5: Physical Appearance

Sn.	Formulations	Color	Odour	Taste	Particles
1	**F1**	Brown	Aromatic	Slight Pungent	None
2		Brown	Aromatic	Slight Pungent	None
3		Brown	Aromatic	Slight Pungent	None
4	**F2**	Brown	Aromatic	Slight Pungent	None
5		Brown	Aromatic	Slight Pungent	None
6		Brown	Aromatic	Slight Pungent	None
7	**F3**	Brown	Aromatic	Pungent	None
8		Brown	Aromatic	Pungent	None
9		Brown	Aromatic	Pungent	None

Table-6: pH of Syrup

Sn.	Formulations	Triplicates	pH
1	F1	1	6.63±0.15
2		2	
3		3	
4	F2	4	6.77±0.15
5		5	
6		6	
7	F3	7	6.47±0.15
8		8	
9		9	

Table-7: Specific Gravity

Formulations	Triplicates	Specific Gravity
F1	1	0.76±0.04
	2	
	3	
F2	4	0.69±0.01
	5	
	6	
F3	7	0.71±0.02
	8	
	9	

Table-8:Refractive Index

Sn.	Formulations	Triplicates	Refractive Index
1	F1	1	1.28±0.03
2		2	
3		3	
4	F2	4	1.30±0.05
5		5	
6		6	
7	F3	7	1.30±0.02
8		8	
9		9	

Table-9:Viscosity

Sn.	Formulations	Triplicates	Viscosity (cps)
			30
1	F1	1	0.8796±0.01
2		2	
3		3	
4	F2	4	0.8814±0.02
5		5	
6		6	
7	F3	7	087.56±0.01
8		8	
9		9	

Table-10: In-vitro Urolithiatic activity by Nucleation assay

Sn.	Conc. (µg/ml)	% Inhibition (Average of triplicates)			
		Formulation			Standard Drug
		1	2	3	
1	100	34.22	35.26	36.91	37.24
2	200	34.55	35.91	37.21	37.95
3	300	36.25	36.55	37.89	38.02
4	400	37.25	37.96	39.62	40.21
5	500	38.65	38.96	41.95	42.26
6	600	40.51	41.23	43.86	44.07

7. REFRENCES

1. Howles, S. A., & Thakker, R. V. (2020). Genetics of kidney stone disease. Nature Reviews Urology, 17(7), 407-421.
2. Thongprayoon, C., Krambeck, A. E., & Rule, A. D. (2020). Determining the true burden of kidney stone disease. Nature Reviews Nephrology, 16(12), 736-746.
3. Geraghty, R. M., Cook, P., Walker, V., & Somani, B. K. (2020). Evaluation of the economic burden of kidney stone disease in the UK: a retrospective cohort study with a mean follow-up of 19 years. BJU international.
4. Black, K. M., Law, H., Aldoukhi, A., Deng, J., & Ghani, K. R. (2020). Deep learning computer vision algorithm for detecting kidney stone composition.
5. Mitchell, T., Kumar, P., Reddy, T., Wood, K. D., Knight, J., Assimos, D. G., & Holmes, R. P. (2019). Dietary oxalate and kidney stone formation. American Journal of Physiology-Renal Physiology, 316(3), F409-F413.
6. Thongboonkerd, V. (2019). Proteomics of crystal–cell interactions: a model for kidney stone research. Cells, 8(9), 1076.
7. Curry, J. N., Saurette, M., Askari, M., Pei, L., Filla, M. B., Beggs, M. R., ... & Alan, S. L. (2020). Claudin-2 deficiency associates with hypercalciuria in mice and human kidney stone disease. The Journal of clinical investigation, 130(4), 1948-1960.
8. Kazemi, Y., & Mirroshandel, S. A. (2018). A novel method for predicting kidney stone type using ensemble learning. Artificial intelligence in medicine, 84, 117-126.
9. Mitra, P., Pal, D. K., & Das, M. (2018). Does quality of drinking water matter in kidney stone disease: A study in West Bengal, India. Investigative and clinical urology, 59(3), 158-165.
10. Pucci, N. D., Marchini, G. S., Mazzucchi, E., Reis, S. T., Srougi, M., Evazian, D., & Nahas, W. C. (2018). Effect of phyllanthus niruri on metabolic parameters of patients with kidney stone: a perspective for disease prevention. International braz j urol, 44, 758-764.
11. Saleem, A., Islam, M., Saeed, H., & Iqtedar, M. (2021). In-vivo Evaluation of Anti-urolithiatic Activity of Different Extracts of Peel and Pulp of Cucumis melo L. in Mice Model of Kidney Stone Formation. Pakistan Journal of Zoology, 53(4).
12. Gautam, A., Singh, S., Baghel, D. S., Singh, G., Kumar, B., Singh, S. K., ... & Pandey, N. K. (2021). Protective effect of Polyherbal syrup and tablet against Ethylene glycol induced Urolithiasis in rats. Research Journal of Pharmacy and Technology, 14(1), 249-253.
13. Arawande, J. O., Akinnusotu, A., & Alademeyin, J. O. (2018). Extractive value and phytochemical screening of ginger (Zingiber officinale) and turmeric (Curcuma longa) using different solvents. Int. J. Trad. Nat. Med, 8(1), 13-22.
14. Wayal, S. R., & Gurav, S. S. (2019). Pharmacognostic and phytochemical investigation of potentially important plants of Western Ghats, India. Int J Pharm Sci Res, 10(6), 3101-3108.

15. Naik, A. V., & Sellappan, K. (2019). Physicochemical and Phytochemical Analysis of Different Plant Parts of Annona muricata L.(Annonaceae). Pharmaceutical methods, 10(2).
16. Raju, K., Rose, A. S., Rohini, B., Sahaja, P., Shylaja, G., & Simran, S. (2020). Formulation and evaluation of anti diabetic herbal syrup. vitro, 3, w2.
17. Khosrowpour, Z., Fahimi, S., Faizi, M., Shaaban, Z., Tansaz, M., & Sahranavard, S. (2022). Evaluation of Formulated Herbal Syrup (Containing Fennel, Anise, and Celery) on the Letrozole-Induced Polycystic Ovary Syndrome Model. Jundishapur Journal of Natural Pharmaceutical Products, (In Press).
18. Ali, S. Z., Rahman, K., & Sultana, A. (2022). Formulation Development, Characterization and Antimicrobial Activity Evaluation of Sugar-Based and Sugar-Free Syrup Prepared with the Ingredients of Jushanda-Nazla. Traditional and Integrative Medicine.
19. Dimiņš, F., & Augšpole, I. (2019). Total phenolic, antioxidant activities and flavonoid contents of herbal syrups. In Key Engineering Materials (Vol. 800, pp. 60-64). Trans Tech Publications Ltd.
20. Sonawane, S. V., Maru, A. D., & Sonawane, M. P. (2021). Development, Characterization and Evaluation of Ritonavir Nanosuspension Treatment of Antiretroviral Therapy. Research Journal of Pharmaceutical Dosage Forms and Technology, 13(4), 297-304.
21. Cynthia, C., & Averal, H. I. (2020). Phytochemical characterization and cytotoxicity analysis of ayurvedic formulation of drug. International Journal of Research in Pharmaceutical Sciences, 11(3), 3164-3170.
22. Firoozeei, T. S., Barekatain, M., Karimi, M., Zargaran, A., Akhondzadeh, S., & Rezaeizadeh, H. (2020). Lavender and dodder combined herbal syrup versus citalopram in major depressive disorder with anxious distress: A double-blind randomized trial. Journal of Integrative Medicine, 18(5), 409-415..
23. Chen, M., Xing, J., Pan, D., Peng, X., & Gao, P. (2020). Chinese herbal medicine mixture 919 syrup alleviates nonalcoholic fatty liver disease in rats by inhibiting the NF-κB pathway. Biomedicine & Pharmacotherapy, 128, 110286.
24. Tariq, A., Green, L., Jeynes, J. C. G., Soeller, C., & Wakefield, J. G. (2020). In vitro reconstitution of branching microtubule nucleation. Elife, 9, e49769.
25. Suvarna, Y., & Rahaman, S. A. (2020). In Vitro–In Vivo evaluation of antiurolithiatic activity of piperine from Piper nigrum. Research Journal of Pharmacy and Technology, 13(1), 63-68.