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FORMULATION AND EVALUATION OF *MADHUCA LONGIFOLIA* EXTRACT SYRUP FOR LITHIASIS

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ABSTRACT:
M. longifolia's natural habitat spans from the southernmost tip of the Konkan peninsula down to the westernmost tip of the Western Ghats. Previous studies suggests that syrup of *Madhuca longifolia* (mahua) leaves & flower extract has not been formulated yet. Lithiasis is the presence of calculi within the urinary tract. So, present study is focused on the formulation and estimation of *Madhuca longifolia* syrup for urolithiasis activity. The fresh leaves and flower of Madhuca longifolia were collected from the lucknow region in Uttar Pradesh. It was washed, dried under shade, and sieved for making dust-free and kept at room temperature or shade. Chemicals i.e., propylene glycol, glycerin, sucrose, sorbitol, sodium saccharin, methylparaben, and propylparaben were purchased from the local chemical supplier. The leaves and powder of *Madhuca longifolia* were weighed and extracted through Soxhlet process using methanol and water, separately. The syrup was formulated by adding mahua extract and different excipients. The parameters including organoleptic features, flavonoid estimation, pH determination, density, viscosity, solubility and stability. In results, both the formulation was observed as optimized in terms of their organoleptic features- greenish colour, pH (6.0), characteristic odour with bitter taste. Appearances were heterogenous. The % yield was 9.8% and 8.2% in methanolic flower extract and aqueous flower extract, respectively. It concludes that both formulation of mahua syrup consisting leaves and flowers have shown a remarkable organoleptic characteristic as well as density, solubility and stability. It also demonstrated the presence flavonoids that might be important constituent for the urolithiasis potential and other pharmacological effects of mahua. It may also be confirmed that its syrup production at bulk level would be reasonable in terms of cost. It will improve the use of herbal syrup of *Madhuca longifolia* with reduced side effects.

Keywords: *Madhuca longifolia*, flavonoid estimation, syrup, urolithiasis.

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INTRODUCTION

Urolithiasis is the third most common urinary tract condition, affecting between 2% and 4% of the population [1-2]. The presence of stones in the urinary tract is called urolithiasis [3]. Kidney stones arise when the kidneys are overloaded with calcium oxalate or calcium phosphate [4-6]. Without preventative treatment, recurrence rates are roughly 10% in the first year, 33% in the fifth year, and 50% in the tenth year, demonstrating the urgent need to create effective substitute treatments [7,8].

Most kidney stones are stuck inside the affected organ [9]. Urinary stones are the most common urinary tract ailment, affecting one in ten people worldwide and affecting human history as far back as 4000 B.C. Renal stone recurrence prevention is still a major health issue [10,11]. Better understanding of the mechanisms involved in stone formation is necessary for preventing future stone formation [12,13]. The likelihood of developing cardiovascular disease, diabetes, hypertension, and end-stage renal disease are all higher in people who have had kidney stones [14].

M. longifolia's natural habitat spans from the southernmost tip of the Konkan peninsula down to the westernmost tip of the Western Ghats. The height of an M. longifolia tree can reach up to 70 feet. The fruiting period of the tree begins between the ages of 8 and 15, and can last up to 60 years [15-18]. The anthers are 16, 2-seriate subsessile, the lips are 3-toothed, and the stems are strong and leathery. The leaves are lanceolate and narrower at both ends, glabrous, and noticeably nerved [19,20]. The flowers are tiny and white in colour, with a fleshy appearance. When ripe, the fruits have a greenish yellow colour and are oval in shape [21,22]. Sugars, vitamin A, ascorbic acid, thiamine, riboflavin, Ca, P, Fe, Mg, Cu, anthocyanins, betains, salts of malic and succinic acid [23], and many other vitamins and minerals [24] can be found in flowers [25].

Ethnopharmacological uses

The tannin content of bark is 17%. Rheumatism, ulcers, itches, bleeding, and spongy gums are all treated with bark [26-28]. Inflammation, sprains, and pruritus are all treated effectively with the bark [29]. The economic value of mahua seeds stems from its usefulness as a food source [30,31].

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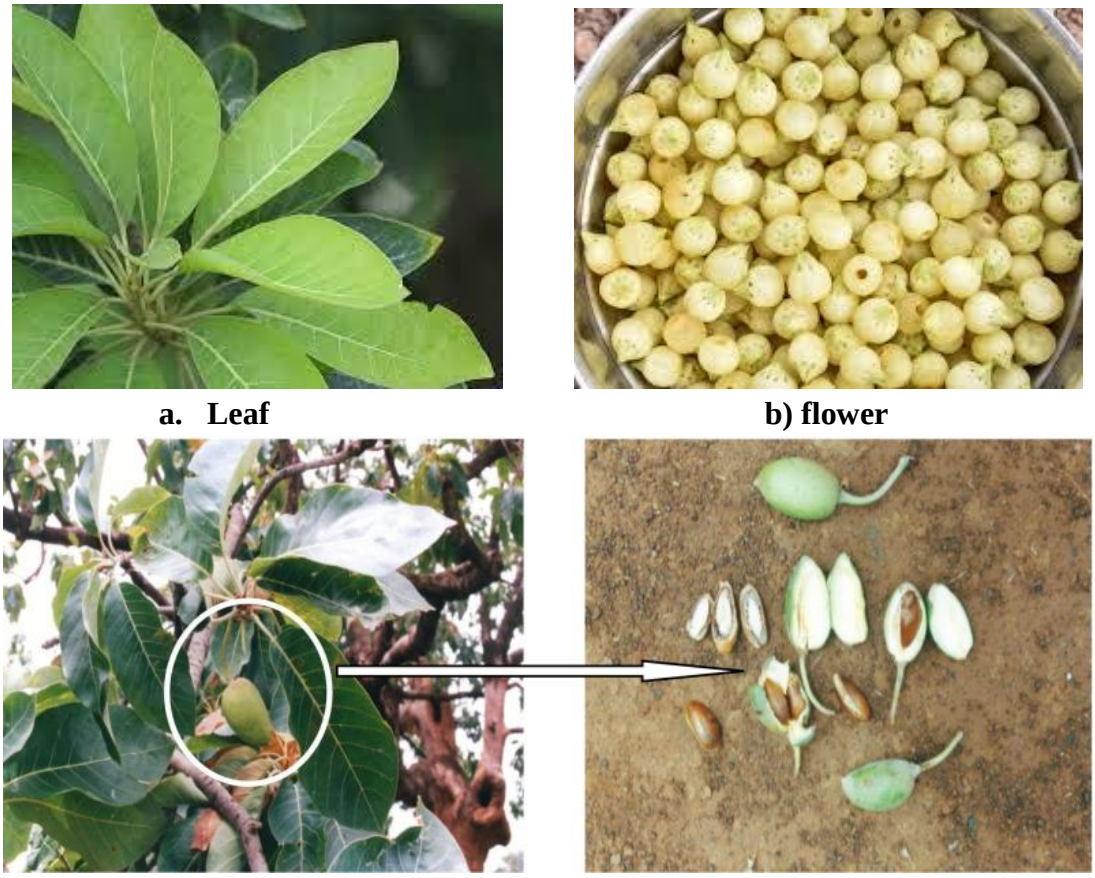

a. Leaf **b) flower**

b. fruit

Fig 1. Different parts of *Madhuca longifolia* plant [32]

Taxonomy

Kingdom : Plantae
Order : Ericaleae
Family : Sapotaceae
Genus : *Madhuca*
Species : *longifolia*

Chemical constituents

Isolated and identified bioactive components [33] in Madhuca leaves include-

- quercetin
- 3-O-lrhamnosid
- Stigmasterol

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- n-hexacosanol
- n-octacosanol
- carotene
- myricitin
- erthrodiol
- -sitosterol
- 3-caproxyolcan-12-en-28-ol
- 3-galactoside
- 3-O-arabinoside
- Xanthophylls

Previous studies suggests that syrup of *Madhuca longifolia* leaves & flower extract has not been formulated yet. So, present study is focused on the formulation and estimation of *Madhuca longifolia* syrup for urolithiasis activity.

MATERIALS AND METHODS

Experimental requirements

Madhuca longifolia was obtained from Lucknow region. Propylene glycol, glycerin, sucrose, sorbitol, sodium saccharin, methylparaben, and propylparaben were purchased from Sigma Aldrich Pvt Ltd India.

Collection, Identification & Authentication of plant

The fresh leaves and flower of *Madhuca longifolia* were collected from the Lucknow region in Uttar Pradesh. It was washed, dried under shade, and sieved for making dust-free and kept at room temperature or shade [34].

Extraction of plant

The leaves and powder of *Madhuca longifolia* were weighed and extracted through Soxhlet process using methanol and water. After, it was filtered with whatman filter paper to get the extract in homogenous manner. A rotating evaporator was used to dry the greenish, semisolid extract obtained under partial vacuum. The yield of the extract was calculated in terms of percentage [35].

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Methanolic leaves extract of Madhuca longifolia

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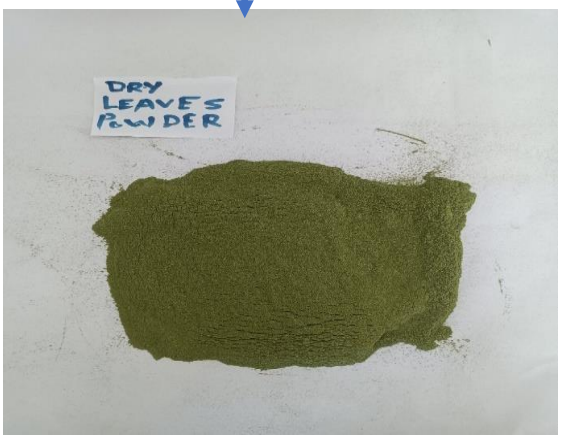

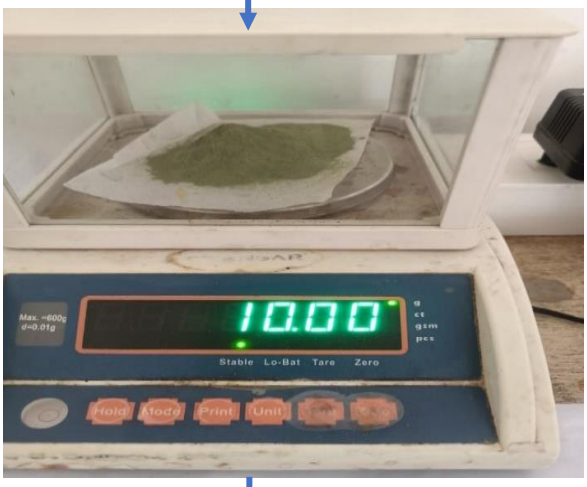

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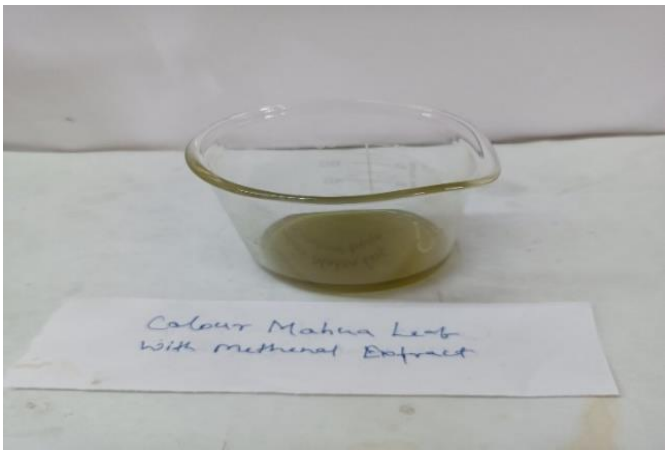

Fig 2. Methanolic leaves extraction process

Aqueous leaves extract of Madhuca longifolia

The process of extraction was same as followed above but here the solvent was taken as distilled water. Extraction completes in following steps-

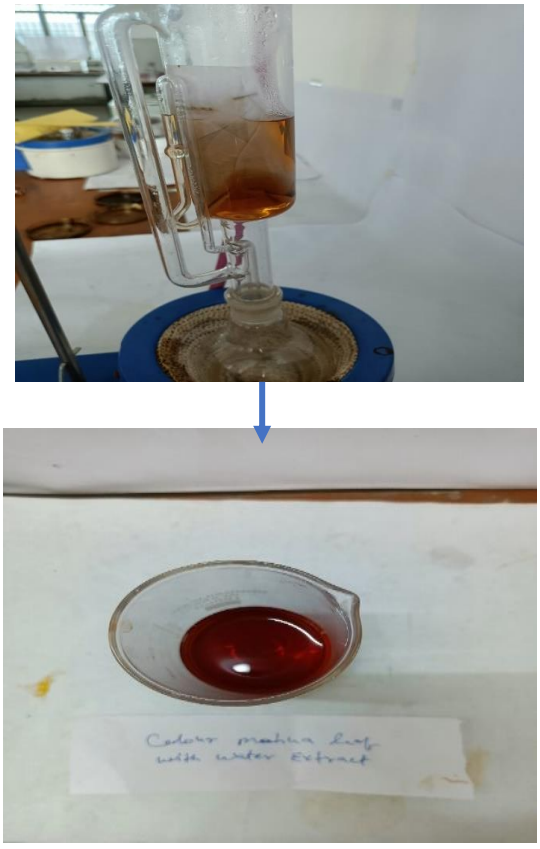

Fig 3. Aqueous leaves extraction process

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Methanolic and aqueous flower extract of Madhuca longifolia

Steps and procedure were same for flowers in methanol and water. It was also extracted in Soxhlet apparatus using distilled water.

The following steps involved for the extraction of flowers of mahua in methanolic solvent.

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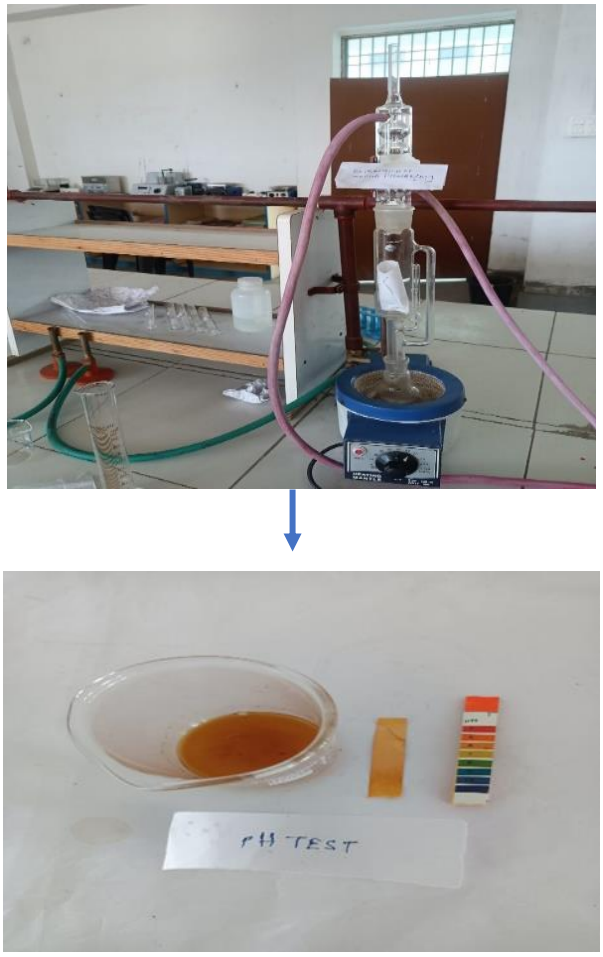

Fig 4. Aqueous leaves extraction process

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Formulation of syrup

The sugar foundation was made by boiling together 45 grammes of water and 45 grammes of sucrose. Distilled water was added to bring the total volume to 100 ml after the liquid was filtered. The preservatives were mixed into a sugar solution after being dissolved in a little amount of water that had been cooked and cooled. Glycerin and sorbitol were added to a solution of Madhuca longifolia extract powder soaked in propylene glycol at 45-50°C. All of the remaining sweeteners were added and blended together. If the pH is too high (above 6.5), use citric acid to lower it to 5.5. The remaining 25 ml were then reconstituted with cooled, boiling water [36].

Table 1. Formulation of *M. longifolia* syrup

Material	Quantity(g)	Material	Quantity(g)
	F1		F2
M. longifolia extract powder from leaf	1.0	*M. longifolia* extract powder from flower	1.0
Propylene glycol (Solubilizer)	6.0	Propylene glycol	6.0
Methyl paraben (Preservative)	0.025	Methyl paraben	0.025
Propyl paraben (Preservative)	0.0025	Propyl paraben	0.0025
Saccharin (Sweetener)	0.025	Saccharin	0.025
Sorbitol (Sweetener)	2.0	Sorbitol	2.0
Sugar base	8.75	Sugar base	8.75
Glycerine (Thickening agents)	0.5	Glycerine	0.5

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CHARACTERIZATION PARAMETERS OF SYRUP

Organoleptic property

The prepared syrup will be examined for their appearance, color, odor, and taste.

Flavonoids test

1 ml plant extract has been taken in to test tube +2ml of 1% sodium hydroxide (NaoH) solution has been added in the test tube. The presence of yellow color is the sign that it contains flavonoids.

Measurement of pH

The pH of the syrup is determined by using digital pH meter. The measurement of pH of each syrup is done in triplicate and average values were noted [37,38].

Solubility

To check the solubility of the prepared syrup [39,40].

Determination of density

The following steps are followed in determination of density [41,42]-

- The specific gravity bottle can be completely cleaned using chromic acid or nitric acid.
- Fill the bottle with distilled water and rinse it out at least twice.
- Third, if necessary, clean the bottle with acetone or another organic solvent and let it dry.
- Weigh the container with the capillary tube stopper in place (w1).
- Put the cap on the bottle and pour in the mystery liquid; then, using a tissue, remove any surplus liquid from the tube.
- Measure the density of an unidentified fluid using an analytical balance (w2).
- The weight of the mysterious liquid (w3) must be determined in grams.

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Viscosity

The viscosity was estimated by following steps of procedure-

- Use heated chromic acid or an organic solvent like acetone to thoroughly clean the Ostwald viscometer.
- Set up a vertical setup for the viscometer.
- Fill the dry viscometer with water to the G mark.
- How long, in seconds, does it take for water to travel from point A to point B?
- Perform step 3 at least three times to ensure a reliable reading.
- Measure the time it takes for the liquid to flow from mark A to mark B after rinsing the viscometer with the test liquid.

$$\text{Viscosity \%} = \frac{\text{Density of liquid} \times \text{Flow time for liquid}}{\text{Density of water} \times \text{Flow time for water}} \times \text{water viscosity}$$

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Stability study

Three months of storage at 40 2°C and 75 5% RH are used to evaluate the stability of the final syrup formulation. The samples were analysed at 0, 30, 60, and 90 days for things like colour, smell, and taste.

RESULTS AND DISCUSSION

A. Evaluation of methanolic and aqueous extract of mahua leaves

Organoleptic characteristics

A comparative study was done for the leaf extract in methanol and water solvent. The following table represents the comparison profile-

Table 2. Organoleptic characteristics

Features	Methanolic leaves extract	Aqueous leaves extract
Appearance	Heterogenous	Heterogenous
Colour	Greenish	Pale
Odor	Characteristic	Characteristic
Taste	Bitterness	Bitterness

Fig 5. greenish colour of syrup

Phytochemicals (Flavonoids) test

Both the extracts were determined for their flavonoids content as a crude that is highly potent and effective moiety in the cure of many medical conditions. Methanolic extract of mahua leaves were shown the presence of flavonoids but aqueous extract demonstrated the absence of flavonoids. The outcome of this test was given in following table-

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Table 3. Observation of flavonoids

Test	Methanolic leaves extract	Aqueous leaves extract
Flavonoids test	*Observation:* Presence of yellow colour indicates that the presence of flavonoids in the plant extract. **Inference:** Present	*Observation:* Presence of yellow color indicates that the presence of flavonoids in the plant extract- **Inference:** Not present

Estimation of pH, solubility and % yield

The extracts were observed for their pH, solubility and % yield. Methanolic extract of mahua leaves shown the pH, and % yield as 6.0 and 15.7%, respectively. While aqueous extract of mahua leaves shown the pH as 6.0 and % yield as 13%.

Table 4. Estimation of pH, solubility and % yield

Features	Methanolic leaves extract	Aqueous leaves extract
pH	6.0	6.0
Solubility	Soluble in methanol	Insoluble in water
% Yield	15.7%	13%
Density	0.87g	0.82g
viscosity	3.64cp	3.58cp

Fig 6. pH estimation

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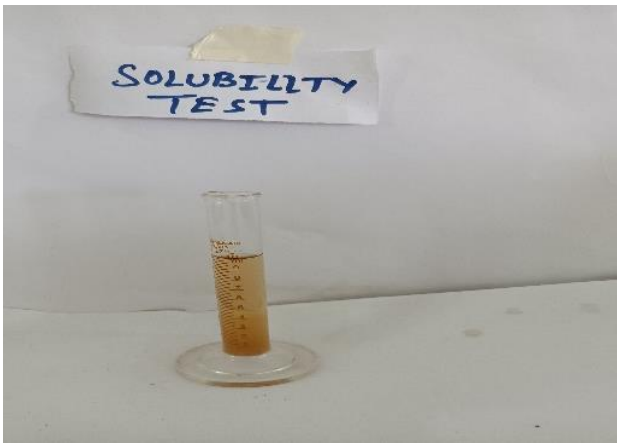

Fig 7. Solubility

Fig 8. Density determination

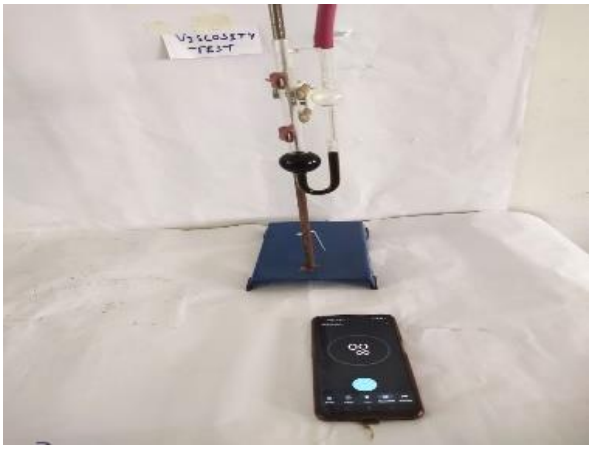

Fig 9. Viscosity estimation

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Stability

After 90 days, stability exhibited a significant unchanged behaviour. All the parameters were tested and found almost same only some changes were observed in colour it turned into brownish and pH with 6.5.

Table 5. Stability of methanolic and aqueous leaves extract of mahua

Features	Stability after 90 days	
	Methanolic leaves extract	Aqueous leaves extract
Physical appearance	Heterogenous	Heterogenous
Colour	Greenish	Brownish
Odour	Characteristic	Characteristic
Taste	Bitterness	Bitterness
pH	6.0	6.5

B. Evaluation of methanolic and aqueous extract of mahua flowers

Organoleptic characteristics

A comparative study was done for the flowers extract in methanol and aqueous solvent. Both the extracts were exhibited pale colour. While order was observed as characteristic. Flowers were found as bitter in taste. The following table represents the comparison profile-

Table 6. Organoleptic characteristics

Features	Methanolic flower extract	Aqueous flower extract
Appearance	Heterogenous	Heterogenous
Colour	Pale	Pale
Odor	Methanolic	Characteristic
Taste	Bitterness	Bitterness

Phytochemicals (Flavonoids) test

Both the extracts were determined for their flavonoids content as a crude that is highly potent and effective moiety in the cure of many medical conditions. Methanolic extract of mahua leaves were shown the presence of flavonoids but aqueous extract demonstrated the absence of flavonoids. The outcome of this test was given in following table-

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Table 7. Observation of flavonoids

Test	Methanolic flower extract	Aqueous flower extract
Flavonoids test	*Observation:* Presence of yellow colour indicates that the presence of flavonoids in the flower extract. **Inference:** Present	*Observation:* Presence of yellow colour indicates that the presence of flavonoids in the plant extract- **Inference:** Not present

Estimation of pH, solubility and % yield

The extracts were observed for their pH, solubility and % yield. Methanolic extract of mahua leaves shown the pH, and % yield as 6.0 and 15.7%, respectively. While aqueous extract of mahua leaves shown the pH as 6.0 and % yield as 13%.

Table 8. Estimation of pH, solubility and % yield

Features	Methanolic flower extract	Aqueous flower extract
pH	6.0	6.0
Solubility	Soluble in methanol	Insoluble in water
% Yield	9.8%	8.2%
Density	1.03g	1.01g
Viscosity	3.28cp	3.09cp

Stability

After 90 days, stability of methanolic and aqueous flower extract of mahua demonstrated a significant unchanged behaviour. All the parameters were tested and found almost same only some changes were observed in colour it turned into brownish and pH with 6.3.

Table 9. Stability of methanolic and aqueous flower extract of mahua

Features	Stability after 90 days	
	Methanolic flower extract	Aqueous flower extract
Physical appearance	Heterogenous	Heterogenous
Colour	Pale	Brownish
Odour	Methanolic	Methanolic
Taste	Bitterness	Bitterness
pH	6.0	6.3

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In results, both the formulation was observed as optimized in terms of their organoleptic features- greenish colour, pH (6.0), characteristic odour with bitter taste. Appearances were heterogenous. The % yield was **9**.8% and 8.2% in methanolic flower extract and aqueous flower extract, respectively.

CONCLUSION

Urolithiasis is becoming increasingly common, despite significant advances in the development of novel medicines for the management of urinary stones. There is still a lot we don't understand about what causes kidney stones. These appear to be important points of focus for creating a new approach to preventing kidney stone disease and medicines to treat kidney stones. In addition, better medications can be created by identifying novel therapy targets based on molecular and cellular abnormalities associated with stone formation. Furthermore, it will be crucial for stone-removing drugs to have a deeper comprehension of the mechanisms of urolithiasis related with stone inhibitors or promoters. Furthermore, it is hoped that new medications and strategies for managing urolithiasis may be discovered as a result of research into the pathophysiology, aetiology, and genetic basis of kidney stone development.

It concludes that both formulation of mahua syrup consisting leaves and flowers have shown a remarkable organoleptic characteristic as well as density, solubility, and stability. It also demonstrated the presence flavonoids that might be important constituent for the urolithiasis potential and other pharmacological effects of mahua. It may also be confirmed that its syrup production at bulk level would be reasonable in terms of cost. It would be great deal for efficacy and stability of formulated syrup. It will improve the use of herbal syrup of *Madhuca longifolia* with reduced side effects.

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All the authors contributed equally in the manuscript writing.

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CONFLICT OF INTEREST

Authors declared for none conflict of interest.

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