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IN VITRO EVALUATION OF ANTILITHIC AND ANTI-INFLAMMATORY POTENTIALS OF CERTAIN EDIBLE PLANTS POPULAR AS ETHENOMEDICINES IN MANAGEMENT OF KIDNEY STONES

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ABSTRACT

This study evaluates the anti-lithic and anti-inflammatory properties of four natural substances-Indian gooseberry (*Phyllanthus emblica),* pomegranate (*Punica granatum*), coriander (*Coriandrum sativum)* and horse gram (*Macrotyloma uniflorum)*-with the aim of identifying potential plant-based therapies for kidney stones and associated inflammation. Aqueous extracts of each sample were analysed using nucleation and aggregation assays to assess antilithic activity, complexometric titration for calcium ion estimation, and the egg albumin assay for anti-inflammatory potential. Results from the nucleation assay showed that coriander had the strongest antilithic effect, while horse gram was most effective in the aggregation assay. Indian gooseberry displayed the highest calcium-regulating ability over 48 hours, suggesting prolonged efficacy in stone prevention. The egg albumin assay revealed Indian gooseberry as the most potent anti-inflammatory agent. Statistically significant differences ($p<0.05$) were observed across all samples. The findings highlight the therapeutic promise of these natural substances, particularly horse gram and Indian gooseberry, in managing kidney stones and inflammation.

KEYWORDS

Antilithic, Nucleation assay, Aggregation assay, Anti-inflammatory and Spectrophotometer.

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INTRODUCTON

Calcium rich foods in our digestive tract combines with oxalate in our digestive tract and forms a complex which is excreted out from the body. Excess oxalate or lack of enough calcium results in absorption of oxalate in blood and accumulation in urine resulting in formation of kidney stones. These hard deposits formed inside the kidneys are also known as renal calculi. Around 75-90% of kidney stones are composed mainly of calcium-containing compounds such as calcium oxalate and calcium

phosphate[1]. Other variations include uric acid and struvite stones[2]. These stones cause pain, urinary blockages, and can lead to severe complications if untreated. Since calcium is the major component in most stones, controlling its deposition is crucia[3]. Antilithic properties refer to the ability of a substance to prevent the formation, growth, or aggregation of stones, or to help in dissolving already-formed stones in the urinary tract[4]. Kidney stones can also trigger inflammation in the urinary system. As the stone moves or gets stuck, it irritates the inner lining, causing swelling, redness and pain. Managing inflammation is important because it supports tissue healing and reduces discomfort, making the removal or passing of stones easier. Over centuries, many traditional remedies based on natural ingredients have been used to treat kidney stones[5] and associated inflammation[6]. These remedies are often safer and have fewer side effects compared to chemical medicines[7]. The goal of this research is to develop a natural product based alternative that can complement the modern medical system. Natural treatments can work together with conventional therapies to improve patient care in a safer and holistic manner[8]. In this study, following four samples were selected for evaluation:

Indian gooseberry (*Phyllanthus emblica*)

Is rich in ascorbic acid (Vitamin C), tannins and flavonoids such as quercetin and kaempferol[9]. These compounds provide strong antioxidant and anti-inflammatory properties, helping to reduce oxidative stress and support overall cellular health. Additionally, it contains ellagic acid and gallic acid, which contribute to its therapeutic effects on the urinary system and immune function. It boosts immunity and protects against infections also enhances skin health and promotes hair growth. It supports liver function and aids indetoxification[10].

Pomegranate (*Punica granatum*)

Is rich in bioactive compounds such as ellagic acid, punicalagins, anthocyanins and flavonoids[11]. These compounds contribute to its strong antioxidant and anti-inflammatory properties, helping to reduce oxidative stress and inflammation. Additionally, its high potassium content supports urinary health and prevents calcium aggregation, which is beneficial in managing kidney stones. It improves heart health by reducing cholesterol level and also enhances skin hydration and promotes a radiant complexion[12].

Coriander (*Coriandrum sativum*)

Contains essential oils like linalool and geraniol[13], which contribute to its anti-inflammatory and antioxidant properties. It is also rich in flavonoids, phenolic acids, and vitamins such as A, C and K, which support overall health and detoxification processes. It improves digestion and reduces bloating. Supports healthy blood sugar levels[14]. Acts as a natural detoxifier for the body.

Horse gram (*Macrotyloma uniflorum*)

Is rich in phenolic acids, Phytic acid, flavonoids and proteins, which contribute to its antioxidant and anti-inflammatory properties[15]. It contains essential minerals like calcium, phosphorus, and iron, supporting bone health and metabolic functions. Additionally, its high fibre content aids in digestion and detoxification. It helps in weight management[16] and improves digestion also supports urinary health and prevents kidney stone formation. Provides steady energy and boosts overall stamina.

In this study evaluation of anti-inflammatory and anti-lithic properties was carried out using well-established analytical methods to ensure accuracy and reliability. The anti-lithic potential of the samples was assessed through the following assays:

Nucleation Assay

Aggregation Assay

Calcium Ion (Ca2+) Analysis

In addition, the anti-inflammatory activity of the samples was analysed using the Egg Albumin Assay, which is a recognized method for measuring the inhibition of protein denaturation, a key indicator of inflammatory processes. These assays provided valuable insights into the effectiveness of the natural samples in managing kidney stones and inflammation.

MATERIALS AND METHODS

Collection of samples and chemicals samples

Fresh and good quality pomegranate and Indian gooseberry (Amla) were sourced from local markets in Bauria, Howrah market. Similarly, fresh and good quality horse gram (Macrotyloma uniflorum)

and coriander (Coriandrum sativum) were also collected from the local markets in Bauria for use in this research. Similarly a fresh and good quality hen egg was brought from a local poultry.

Chemicals

Calcium chloride ($CaCl_2$), sodium oxalate ($Na_2C_2O_4$), ammonium chloride (NH_4Cl), ammonia buffer (pH 10), magnesium chloride ($MgCl_2$), Eriochrome black T indicator, Patton-Reeder's indicator mixture, EDTA (ethylenediaminetetraacetic acid) solution (~M/50), methyl red indicator, 10% NaOH solution, distilled/deionized water were purchased from Merck Specialties Private Limited.

Apparatus

SYSTRONICS Spectrophotometer 106 was used in this experiment.

Preparation of samples

Calcium chloride dihydrate, weighing 1.47 grams, was dissolved in 100mL of distilled water. Separately, 1.34 grams of sodium oxalate was dissolved in 100mL of 1 molar sulfuric acid. These two solutions were then combined and thoroughly stirred, allowing calcium oxalate crystals to precipitate. The remaining sulfuric acid in the mixture was neutralized using an ammonia solution. The crystals were subsequently rinsed with distilled water and dried at 60°C for four hours. The synthetic kidney stones were then prepared for further experimentation, such as testing with artificial urine[17].

Preparation of Aqueous Extracts

About 17 grams of each of the following samples- Indian gooseberry (Amla), pomegranate, coriander, and horse gram- were weighed. Each sample was then dissolved in 50mL of water to prepare the aqueous extracts. The ratios of sample mass to water volume are as follows:

Indian Gooseberry (Amla): 17grams of Indian gooseberry to 50mL of water (ratio = 17:50 or 0.34:1).

Pomegranate: 17 grams of pomegranate to 50mL of water (ratio = 17:50 or 0.34:1).

Coriander: 17 grams of coriander to 50mL of water (ratio = 17:50 or 0.34:1).

Horsegram: 17 grams of horsegram to 50mL of water (ratio = 17:50 or 0.34:1).

For Indian gooseberry, pomegranate, and coriander, the samples were ground using a mortar and pestle to form homogeneous aqueous extracts. In the case of horsegram, the sample was soaked directly in 50mL of water for overnight from each of the 0.34:1 ratio aqueous extract 6 test tube containing 3mL of aqueous extract of the sample were made.

EXPERIMENTAL PROCEDURE

Nucleation Assay

The nucleation assay was performed according to the method described Sujatha *et al*[18], with slight modifications. Calcium chloride (4mmol/L) and sodium oxalate (7.5mmol/L) solutions were prepared in Tris buffer (0.05mol/L Tris and 0.15mol/L NaCl, pH 6.5). In a 96-well plate, 95µL of the calcium chloride solution was mixed with 10µL of plant extract (2mg/mL), followed by 95µL of sodium oxalate solution to initiate crystallization. The plate was incubated at 37°C for 30 minutes. Absorbance was then recorded at 620nm to assess crystal formation[5,17,19]. Two extract samples were tested and each was measured in duplicate.

Aggregation Assay

The calcium oxalate (CaOx) crystal aggregation assay was conducted following the method reported by Hess *et al*[20] and Saha and Verma[21], with minor modifications. Calcium oxalate monohydrate (COM) crystals were prepared by mixing equal concentrations (50mmol/L) of calcium chloride and sodium oxalate, followed by incubation at 60°C for 1 hour. The resulting crystal suspension was cooled and brought to 37°C before use. A volume of 100µL of COM suspension was mixed with 10µL of extract (0.8mg/mL in Tris buffer, pH 6.5) and incubated for 30 minutes at 37°C. The extent of aggregation was evaluated by measuring absorbance at 620nm[19,22]. Two extract samples were analysed in duplicate[23].

Complexometric Estimation of Ca^{2+}

Procedure

The sample was dissolved in distilled water and transferred to a 100-milliliter volumetric flask. A

25-milliliter aliquot of the diluted sample solution was pipetted into a conical flask. Five millilitres of NH_4Cl-NH_3 buffer solution (pH 10) and approximately 50 milligrams of Eriochrome Black T indicator were added[24]. The mixture was titrated with standard (~M/50) EDTA solution until the colour changed from wine red to pure blue. The volume of EDTA used (V_3) was recorded to calculate calcium concentration[25].

Preparation of 1% Egg Albumin Solution

A 1% (w/v) egg albumin solution was prepared using the clear albumin portion extracted from fresh hen's eggs or commercially available powdered albumin. For preparation using fresh eggs, approximately 1mL of the translucent egg white was carefully separated and diluted in 100 mL of cold distilled water. The mixture was stirred thoroughly to ensure homogeneity. It was ensured that the dilution was carried out in cold conditions to prevent premature coagulation of the protein[26].

Egg Albumin Assay

The anti-inflammatory potential of the test samples was assessed by evaluating their capacity to inhibit heat-induced denaturation of egg albumin proteins. Each reaction mixture consisted of 0.2mL of 1-2% egg albumin solution, 2mL of the test sample or standard drug (Diclofenac sodium) at various concentrations and 2.8mL of phosphate-buffered saline (pH 7.4), yielding a total volume of 5mL. The control mixture was prepared by replacing the test sample or standard with 2 mL of triple-distilled water, while maintaining the same concentrations of egg albumin and buffer. All reaction mixtures were incubated at 37 ± 2°C for 30 minutes, followed by heating in a water bath at 70 ± 2°C for 15 minutes to induce protein denaturation. After the mixtures were cooled to room temperature, their absorbance was recorded at 280 nm using a UV-Visible spectrophotometer[26-29].

STATISTICAL ANALYSIS

All the experiments have been done in triplicate and data from three different experiments were subjected to analysis of variance (ANOVA) ($P<0.05$)[30].

RESULTS AND DISCUSSION

From the experimental analysis, it was observed that with the increase in absorbance, the antilithic activity tends to decrease. Specifically, among the tested samples for antilithic activity in the aggregation assay, horse gram exhibited the strongest antilithic activity, as evidenced by the lowest absorbance value. In contrast, pomegranate and coriander displayed moderate antilithic activity, with absorbance values higher than horse gram but lower than that of Indian gooseberry, which demonstrated the least antilithic activity with the highest absorbance. Gallic acid has shown effectiveness in suppressing crystal aggression, as evaluated through the aggregation assay. An increase in gallic acid content corresponded with a noticeable reduction in absorbance values, indicating enhanced antilithic activity. This relationship was supported by a moderate negative correlation ($r = -0.68$) between gallic acid concentration and aggression assay absorbance, suggesting gallic acid's role in minimizing crystal–crystal interaction.

The statistical analysis using ANOVA indicated a significant difference in the antilithic activity across the samples, with a p-value < 0.05, confirming that the variations in absorbance are statistically significant and directly correlate with the observed differences in antilithic effectiveness.

From the experimental analysis of the nucleation assay, it was observed that Coriander exhibited the lowest absorbance, indicating the strongest antilithic activity among the tested samples. Conversely, pomegranate showed the highest absorbance, which correlates with the weakest antilithic activity. Both horse gram and Indian gooseberry displayed moderate absorbance values, with horse gram showing lower absorbance than Indian gooseberry, suggesting a relatively stronger antilithic effect for Horse gram compared to Indian gooseberry. Quercetin is known to inhibit the nucleation of crystals and with increasing quercetin content, a noticeable decrease in nucleation assay absorbance was recorded. This decline in absorbance reflects a reduction in crystal formation, indicating stronger antilithic potential. A correlation analysis further

supported this observation, revealing a significant ($P<0.05$) negative correlation ($r = -0.77$) between quercetin concentration and nucleation activity. This suggests that quercetin plays a key role in suppressing the early stages of crystal development.

The statistical analysis using ANOVA revealed significant differences in absorbance values across the samples, with a p-value < 0.05, confirming that the variations in absorbance are statistically significant and associated with the observed differences in antilithic activity.

Calcium estimation by complexometric titration

The calcium estimation results reveal that Indian Gooseberry has the highest calcium concentration (1.67 millimolar), suggesting its strong long-term effect in regulating calcium levels and potentially preventing kidney stone formation. This could be due to its prolonged activity over 48 hours. Horse Gram showed a moderate calcium concentration (1.33 millimolar), indicating its potential for antilithic activity, though not as strong as Indian Gooseberry. Both Pomegranate and Coriander had lower calcium concentrations (0.667 millimolar), implying more moderate effects. The lower calcium levels in these samples might reflect their less pronounced ability to prevent stone formation, with Pomegranate's quercetin content potentially inhibiting nucleation. These findings highlight the varying efficacy of the samples in regulating calcium, with Indian Gooseberry showing the most significant ($P<0.05$) potential for preventing stone formation over time.

From the experimental analysis of the egg albumin assay for anti-inflammatory properties, it was observed that Indian gooseberry exhibited the highest concentration of egg albumin, indicating the strongest anti-inflammatory activity among the tested samples. In contrast, Coriander showed the lowest concentration, which correlated with the weakest anti-inflammatory effect. Pomegranate demonstrated the second highest concentration, followed by Horse gram, which exhibited the third highest concentration, suggesting moderate anti-inflammatory activity compared to the other samples. Quercetin is also effective in reducing inflammation, as assessed through the egg albumin assay. With increasing quercetin content, a significant ($P<0.05$) decrease in absorbance was observed, indicating stronger anti-inflammatory activity. This trend was further supported by a high negative correlation ($r = -0.85$) between quercetin concentration and absorbance, highlighting quercetin's role in mitigating inflammatory responses.

The statistical analysis using ANOVA showed a significant difference in the concentrations of egg albumin across the samples, with a p-value < 0.05, confirming that the variations in egg albumin concentration are statistically significant ($P<0.05$) and correlate with the observed anti-inflammatory effects.

Table No.1: Aggregation assay

S.No	Aggregation assay Sample name	Absorbance
1	Horse gram	0.039±0.001
2	Pomegranate	0.132±0.02
3	Coriander	0.25±0.011
4	Indian gooseberry	0.423±0.025

Table No.2: Nucleation assay

S.No	Nucleation assay Sample name	Absorbance
1	Coriander	0.004±0.00
2	Horse gram	0.046±0.01
3	Indian gooseberry	0.064±0.02
4	Pomegranate	0.092±0.01

Table No.3: Egg albumin assay

S.No	Egg albumin assay Sample name	Concentration
1	Indian gooseberry	551.75±1.00
2	Pomegranate	429.101±0.8
3	Horsegram	415.078±0.99
4	Coriander	371.196±0.98

Table No.4: Correlation

S.No	Correlation table	Percentage
1	nucleation assay and quercitrin	77%
2	aggregation assay and gallic acid	68%
3	egg albumin and quercitrin	85%

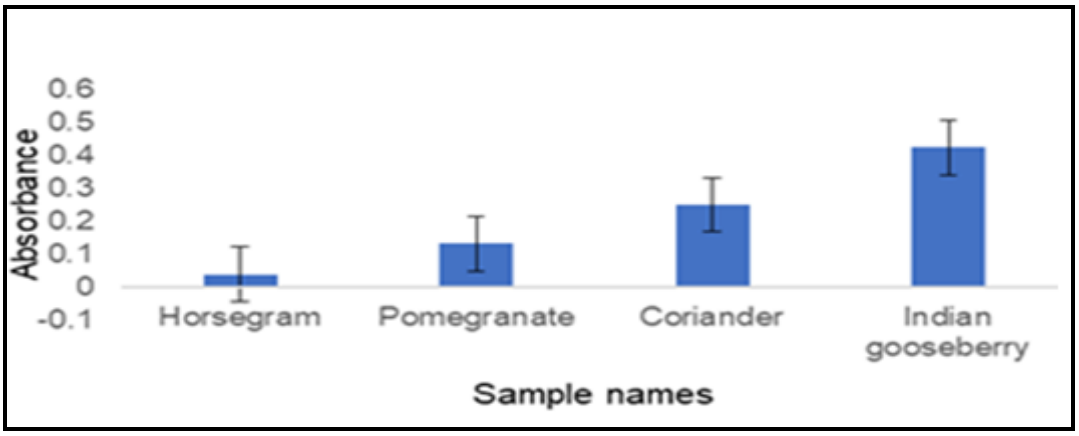

Figure No.1: Aggregation assay

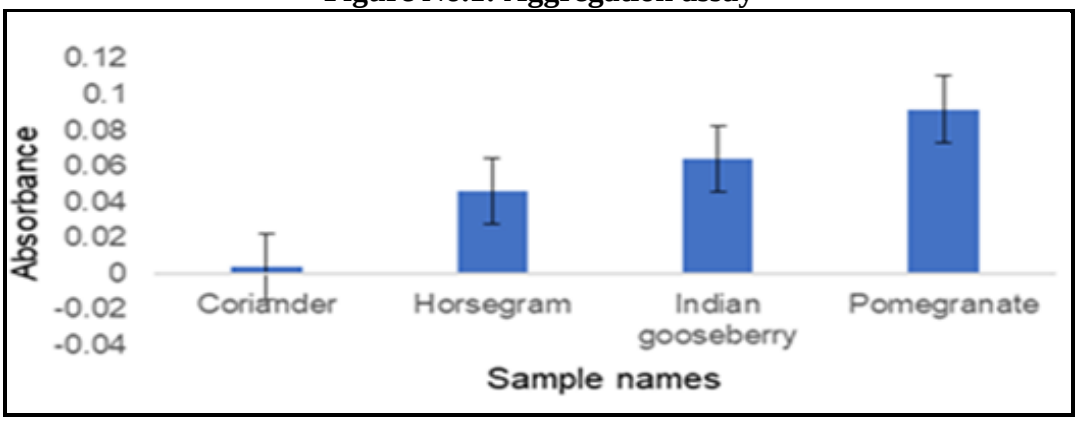

Figure No.2: Neucleation assay

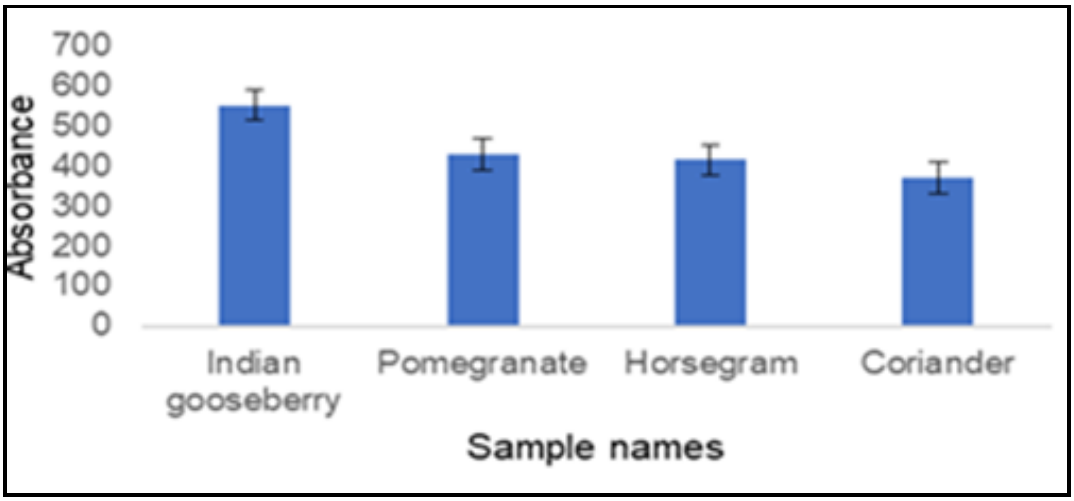

Figure No.3: Egg albumin assay

CONCLUSION

The comparative evaluation of the selected plant extracts revealed distinct differences in their antilithic and anti-inflammatory activities. In the aggregation assay, horse gram exhibited the most pronounced antilithic effect, as indicated by the lowest absorbance value, followed by pomegranate, coriander and Indian gooseberry. The nucleation assay showed coriander to be the most effective in preventing initial crystal formation, with horse gram and Indian gooseberry displaying moderate activity, while pomegranate was the least effective in this context. Calcium estimation highlighted Indian gooseberry′s potential for long-term regulation of calcium levels, suggesting its sustained impact on inhibiting stone formation. In terms of anti-inflammatory potential, the egg albumin assay demonstrated Indian gooseberry as the most active sample, followed by pomegranate, horse gram, and coriander. Overall, the findings suggest that while each extract exhibits specific strengths, horse gram and Indian gooseberry are particularly promising due to their consistent performance across multiple parameters. These results support the potential use of these natural extracts as complementary agents in managing kidney stones and related inflammatory conditions.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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