AEGLE MARMELOS FRUIT EXTRACTS AS NATURAL THERAPEUTICS AGAINST UROLITHIASIS: ANTIOXIDANT AND CRYSTALLIZATION INHIBITION STUDIES

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ABSTRACT

Urolithiasis, commonly known as kidney stone disease, is a global health concern with increasing prevalence, recurrence, and association with systemic complications such as chronic kidney disease and metabolic disorders. Current therapeutic approaches, including surgical interventions and allopathic medications, often present limitations such as invasiveness, side effects, and recurrence. Hence, there is a growing interest in exploring plant-based remedies with fewer adverse effects. The present study investigates the antiurolithiatic and antioxidant potential of *Aegle marmelos* fruit extracts through phytochemical screening and in vitro evaluation. Successive extraction using solvents of increasing polarity yielded diverse phytoconstituents, with the ethanolic extract showing the richest profile of bioactive compounds including alkaloids, flavonoids, phenolics, saponins, tannins, and terpenoids. In vitro antiurolithiatic assays—nucleation, aggregation, crystal growth inhibition, and calcium oxalate dissolution—demonstrated significant, dose-dependent inhibitory activity of the extract, with results approaching those of the standard drug Cystone at higher concentrations. Additionally, the extract exhibited moderate to strong antioxidant activity in DPPH, nitric oxide, and hydrogen peroxide scavenging assays, validating its role in mitigating oxidative stress implicated in stone formation. These findings support the traditional medicinal use of *Aegle marmelos* and highlight its therapeutic potential in preventing and managing urolithiasis. Further in vivo and clinical studies are warranted to establish its efficacy, safety, and mechanism of action for development as a phytopharmaceutical candidate.

Keywords

Aegle marmelos; Urolithiasis; Calcium oxalate; Antiurolithiatic activity; Antioxidant; Phytochemical screening; Kidney stones.

INTRODUCTION:

Kidney diseases represent a major global health burden, with conditions ranging from acute kidney injury to chronic kidney disease (CKD). Among these, **urolithiasis (kidney stone disease)** is one of the most prevalent urological disorders. Kidney stones, also termed

nephrolithiasis, are crystalline deposits formed in the urinary tract due to urinary supersaturation and imbalances between stone-promoting and stone-inhibiting factors. They affect both men and women worldwide, with risk factors including genetics, diet, obesity, diabetes, hypertension, and environmental influences such as climate and water intake.

Epidemiological data reveal an alarming increase in the incidence of nephrolithiasis globally, particularly in tropical regions forming the so-called "stone belt." Calcium oxalate (CaOx) stones constitute nearly 80% of all urinary calculi, with other types including uric acid, struvite, cystine, and drug-induced stones. The pathogenesis involves **supersaturation, crystallization, nucleation, growth, and aggregation of crystals**, often exacerbated by oxidative stress and metabolic abnormalities.

Although current treatments such as **shock wave lithotripsy, ureteroscopy, percutaneous nephrolithotomy, and pharmacotherapy** are effective, they are often invasive, expensive, and associated with high recurrence rates. Hence, there is growing interest in exploring **plant-based therapies** as safer and cost-effective alternatives.

Aegle marmelos (commonly known as Bael) has been traditionally used in Ayurveda for its diverse pharmacological properties, including antioxidant, anti-inflammatory, and nephroprotective effects. Its rich phytochemical profile—comprising flavonoids, phenolics, alkaloids, saponins, and tannins—suggests potential in inhibiting kidney stone formation by preventing crystallization, reducing oxidative stress, and promoting dissolution of preformed stones.

The present research investigates the **antiurolithiatic and antioxidant potential of Aegle marmelos fruit extracts**, with an emphasis on phytochemical screening, in vitro crystallization inhibition assays, and free radical scavenging studies. This work aims to provide a scientific basis for its traditional use and to establish its potential as a natural therapeutic agent in the management of urolithiasis.

NEPHROLITHIASIS

Kidney stones (KS) are a common urological disease entailing the formation and occasional passage of crystal agglomerates in the urinary tract. It is also called nephrolithiasis or urolithiasis from the Greek words nephros, for kidney, uro-, for urinary, and lithos, for stone. Kidney stones first appear in ancient Mesopotamian medical texts between 3200 and 1200 BC [7]. Nephrolithiasis describes a syndrome characterized by the development of solid crystalline masses within the urinary space of the kidney. There is significant diversity in its pathogenesis, risk factors, clinical course, and treatments. The factors that predispose individuals to kidney stone formation can be genetic, metabolic, and environmental. Nephrolithiasis is now recognized as a marker for systemic disease and a predictor of metabolic and cardiovascular complications [8]. Globally, approximately 80% of kidney stones are composed of calcium

oxalate (CaOx) mixed with calcium phosphate (CaP). Stones composed of uric acid, struvite and cystine are also common and account for approximately 9%, 10% and 1% of stones, respectively [9]

MATERIAL AND METHODS

All the other chemicals used in this study were of analytical grade and available commercially.

1. In vitro method for Anti urolithiasis:

- crystal nucleation assay
- Aggregation assay
- Growth Assay
- Calcium Oxalate Assay by Titrimetric Method

2. In vitro antioxidant studies

- DPPH radical scavenging assay
- Determination of Nitric Oxide Scavenging Assay
- Hydrogen peroxide scavenging assay

METHODOLOGY

Selection of Medicinal Plants:

Based on an assessment of the relevant literatures, Aegle marmelos was chosen for screening the anti urolithiasis activity

Preparation of plant material

The collected fruits was cleaned, washed with distilled water, dried under sun shade in a dark room, and powdered by using a mechanical mixer. After size reduction fruits was sieved under sieve No. 40 and sieve No. 60, stored in an airtight container at room temperature .

Extraction of the plant material

200 g of finely fruit powder was extracted with soxhlet using different solvents (nonpolar to polar solvents) successively with Petroleum ether, Chloroform, Ethyl acetate and Ethanol. After extraction the extracts was separately concentrated by distillation and dried at room temperature until they got to viscous solid mass. The obtained crude extracts was weighed and stored at 40 for the further analysis.

Aqueous Extract

The finely powdered fruit powder was extracted by cold maceration using water for 15 days. The extract was concentrated by surface evaporation followed by vacuum drying. Dry powder was weighed and stored in air-tight containers for further studies.

Calculation of Percentage Yield:

The percentage yield was calculated by using the formula and is given below.

$$\text{Percentage yield} = \frac{\text{weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

Results and Discussion

During the phytochemical analysis of *Aegle marmelos* fruit, solvents with increasing polarity—petroleum ether, chloroform, ethyl acetate, and ethanol—were used successively in a Soxhlet apparatus. This approach allowed separation of phytoconstituents based on their solubility in each solvent. The extraction yielded different quantities of crude extracts, indicating the solubility pattern and relative abundance of phytochemicals in the plant material.

The successive extractive values reflect the distribution of phytoconstituents in the plant matrix, as influenced by the solvent's polarity. Non-polar compounds such as fats and oils were primarily extracted in petroleum ether, while moderately polar and polar compounds like flavonoids, phenolics, alkaloids, and glycosides were more prevalent in the ethyl acetate and ethanol extracts.

The following table summarizes the percentage yield of each extract:

Table :1 Properties of different extracts from the fruits of Aegle marmelos

S. No.	Solvent Used	Polarity	Color of Extract	Weight of Extract (g)	% Yield
1	Petroleum Ether	Non-polar	Pale yellow	3.5 g	1.75%
2	Chloroform	Slightly polar	Greenish-brown	5.2 g	2.6%
3	Ethyl Acetate	Moderately polar	Brown	7.8 g	3.9%
4	Ethanol	Polar	Dark brown	10.6 g	5.3%
5	Aqueous (Maceration)	Highly polar	Deep brown	9.4 g	4.7%

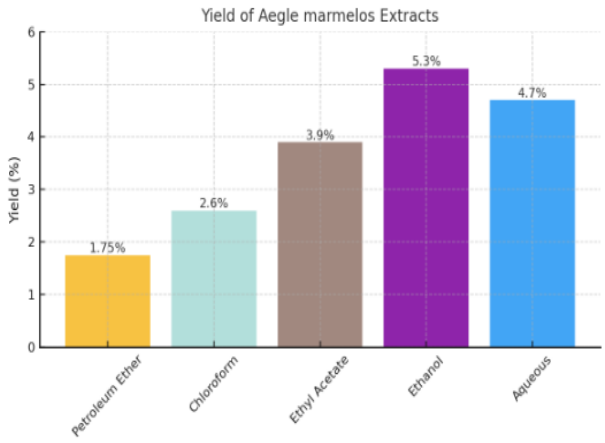


Fig: 2 Percentage yield of different extracts of Aegle marmelos

Phytochemical screening of Aegle marmelos :

Any plant that includes compounds with therapeutic properties or compounds that can be utilized as building blocks for semi-synthetic pharmaceuticals is considered a medicinal plant. These phytochemicals, which are non-nutrients found in plants, act as defence mechanisms for the plants against microbial diseases or pest infestations. Thus, the phytochemical components of the various extracts of Aegle marmelos was screened.

In vitro method for Anti Urolithiasis

1. Nucleation assay:

The effects of different extract on CaOx crystallization was studied by nucleation assay. The solutions of calcium chloride (.005 M) and sodium oxalate (.0075 M) were prepared in Tris-Hcl (0.5 M) and NaCl (0.15 M) buffer at pH 6.5. Different dilutions (100–1000 µg/mL) of cystone and extracts was prepared in distilled water. One milliliter of each dilution (100- 1000 µg/mL) of extracts and cystone were mixed with calcium chloride (3 ml) and then sodium oxalate solution (3 ml) was added in each dilution. These dilutions was incubated in oven at 37°C for half an hour then cooled down to room temperature. Optical density was measured using spectrophotometer (620 nm) and the percent inhibition of crystal nucleation was calculated from following formula

$$\text{Percent Inhibition} = [1 - (OD(\text{Test})/OD(\text{Control}))] \times 100$$

Where

OD (test) is the optical density of cystone and OD (control) is the optical density of the negative control

Results:

In the nucleation assay, addition of Sodium oxalate solution to the reaction mixture consisting of Calcium chloride resulted in the formation of numerous CaOx crystals.

Table: 3 Inhibition of Calcium Oxalate Crystal Nucleation

S. No.	Concentration (µg/mL)	% Inhibition (Aegle marmelos)	% Inhibition (Cystone)
1	100	18.46%	26.15%
2	200	25.00%	33.50%
3	400	32.30%	41.70%
4	800	40.10%	49.80%
5	1000	44.62%	52.31%

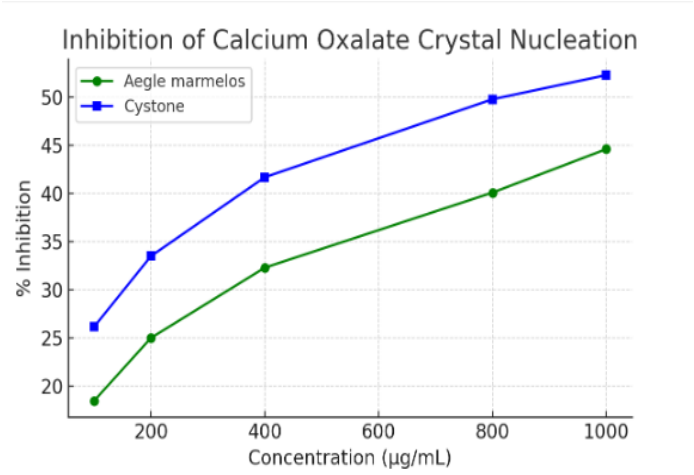


Fig: 6 % inhibition of calcium oxalate crystal nucleation at different concentrations

DISCUSSION:

The nucleation assay was conducted to evaluate the inhibitory potential of *Aegle marmelos* fruit extract on the formation of calcium oxalate crystals, which play a critical role in the development of kidney stones (urolithiasis). The inhibition efficiency was compared with a standard antiurolithiatic drug, **Cystone**, across various concentrations (100–1000 µg/mL).

The results indicated that *Aegle marmelos* extract exhibited **dose-dependent inhibition** of calcium oxalate crystal formation. At the lowest concentration of 100 µg/mL, the extract showed a modest inhibition of **18.46%**, which progressively increased with higher concentrations, reaching a maximum of **44.62%** at 1000 µg/mL. In comparison, Cystone showed higher inhibition at all concentrations, with **26.15%** at 100 µg/mL and **52.31%** at 1000 µg/mL.

The observed inhibitory effect of *Aegle marmelos* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, saponins, phenols, and terpenoids. These compounds are known to interfere with the nucleation and aggregation phases of calcium oxalate crystallization by:

- Chelating calcium ions,
- Modifying crystal morphology,
- Inhibiting crystal growth and adhesion.

Although the extract was slightly less potent than Cystone, the results demonstrate a **promising antiurolithiatic potential** of *Aegle marmelos*, especially at higher concentrations. This supports its traditional use in the management of urinary disorders and provides a scientific basis for further **in vivo and clinical evaluation**.

2. Aggregation assay

Calcium chloride solution 50 mmol/L and sodium oxalate of 50 mmol/L added together to prepare calcium oxalate monohydrate crystals. In a water bath for 1 h the solution was be equilibrated to 60°C and cooled to 37°C overnight. By centrifugation the crystals will harvested and at 37°C it was evaporated. Then buffering of calcium oxalate crystals with 0.5 ml of 0.15 mol/L NaCl solution and 0.5 ml of 0.05 mol/L Tris buffer at pH 6.5 to a final concentration of 1 mg/ml. In the absence or presence of plant extracts and cystone the experiments was conducted at 37°C at different concentrations (100, 200, 400, 800, and 1000 µg/ml) with constant stirring. The optical density was be measured at 620 nm in a spectrophotometer after stopping the stirring and the percentage inhibition of aggregation will be determined using the same formula as in turbidity method. By comparing the turbidity in the presence of the plant extracts with that obtained in the control, the percentage aggregation inhibition rate (Ir) was be calculated using following formula

$$Ir = (1 - Turbidity_{sample}/Turbidity_{control}) \times 100$$

Where, Ir is the percentage aggregation inhibition rate.

Table: 4 Inhibition of Calcium Oxalate Crystal by aggregation assay

S. No.	Sample	Concentration (µg/mL)	OD (Sample)	OD (Control)	% Inhibition (Ir)
1	Cystone	100	0.470	0.660	28.79%
2	Cystone	200	0.400	0.660	39.39%
3	Cystone	400	0.350	0.660	46.97%
4	Cystone	800	0.300	0.660	54.55%
5	Cystone	1000	0.280	0.660	57.58%
6	*Aegle marmelos*	100	0.520	0.660	21.21%
7	*Aegle marmelos*	200	0.460	0.660	30.30%
8	*Aegle marmelos*	400	0.390	0.660	40.91%
9	*Aegle marmelos*	800	0.340	0.660	48.48%
10	*Aegle marmelos*	1000	0.310	0.660	53.03%

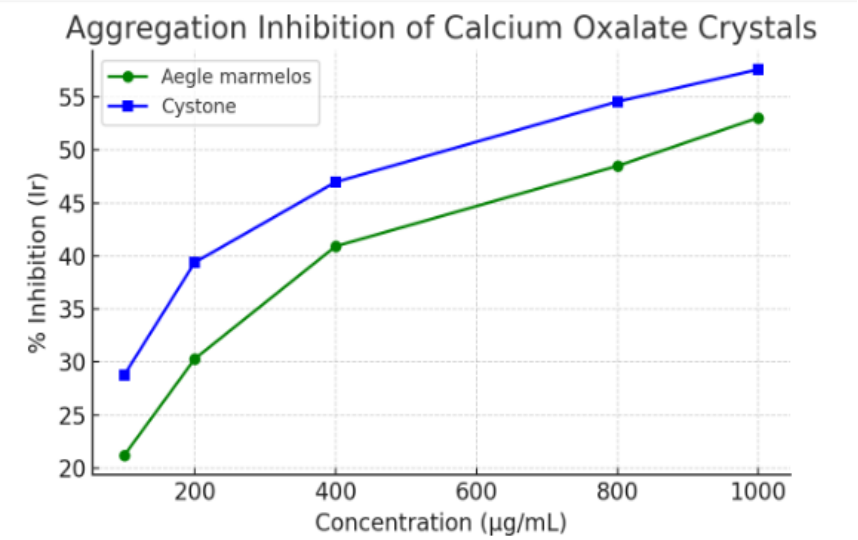


Fig: 7 % inhibition of calcium oxalate crystal aggregation by *Aegle marmelos* extract and Cystone

The **aggregation assay** evaluates the ability of *Aegle marmelos* fruit extract to inhibit the **aggregation of calcium oxalate (CaOx) crystals**, which is a critical phase in the pathogenesis of kidney stones. This assay simulates conditions under which smaller crystals aggregate to form larger, more harmful uroliths. The effectiveness of the extract was assessed by comparing the turbidity (optical density) of samples to a control at 620 nm, with **Cystone** as the reference standard.

The results demonstrated a **dose-dependent inhibition** of crystal aggregation for both the **standard drug (Cystone)** and *Aegle marmelos* extract. As the concentration increased, the optical density decreased, indicating a reduction in crystal aggregation:

- **Cystone** showed a strong inhibitory effect, with **28.79%** inhibition at 100 µg/mL and reaching **57.58%** at 1000 µg/mL.
- *Aegle marmelos* also showed substantial activity, beginning at **21.21%** inhibition at 100 µg/mL and increasing to **53.03%** at 1000 µg/mL.

3. Growth Assay

The relative percentage inhibition will be evaluated in presence and absence of extracts using calcium oxalate crystal growth assay method. 1 ml of 4mM sodium oxalate and 1 ml of 4mM calcium chloride each will be added to a 1.5 ml solution of

10mM sodium chloride and then buffered with 10mM Trisat pH 7.2. Calcium oxalate monohydrate crystal slurry (1.5mg/ml acetate buffer) about 30 µl will be added to the obtained solution.Utilization of oxalate begins at once after COM slurry addition and observed for 30 minutes for vanishing of absorbance at 214nm. The standard drug will be individually added into the reaction mixture. The standard drug (cystone 1000 µg/ml) and extracts will be taken in different concentrations (extracts 400, 800, 1000 µg/ml respectively). If extracts inhibits calcium oxalate crystal growth the depletion of free oxalate ions will be decreases. The relative inhibitory activity will be determined by the following formula

Percentage Relative inhibitory activity= ((C-S)/C)x100

Where, C - rate of reduction of free oxalate without any extract S- rate of reduction of free oxalate with drug extract.

Table:5 Inhibition of Calcium Oxalate Crystal Growth

S. No.	**Sample**	**Concentration (µg/mL)**	**Rate of Oxalate Reduction (C or S)**	**% Relative Inhibitory Activity**
1	Control (C)	–	0.130	–
2	Cystone	1000	0.060	53.84%
3	*Aegle marmelos*	400	0.085	34.61%
4	*Aegle marmelos*	800	0.072	44.61%
5	*Aegle marmelos*	1000	0.065	50.00%

The **crystal growth assay** was conducted to evaluate the ability of *Aegle marmelos* fruit extract to inhibit the **growth phase** of calcium oxalate (CaOx) crystal formation—a key stage in the development and progression of urolithiasis (kidney stones). In this assay, the reduction in free

oxalate ions was monitored over time by measuring the absorbance at 214 nm. A **lower rate of oxalate depletion** indicates **inhibition of crystal growth**, as oxalate remains unbound due to interference by the test sample.

The control (without any drug or extract) showed a high oxalate consumption rate of **0.130,** indicating active CaOx crystal growth. The standard drug **Cystone (1000 µg/mL)** significantly reduced oxalate consumption to **0.060**, resulting in **53.84% relative inhibitory activity**.

Aegle marmelos extract showed a **clear concentration-dependent inhibition**:

- At **400 µg/mL**, it reduced the growth rate to **0.085**, yielding **34.61% inhibition**.
- At **800 µg/mL**, inhibition increased to **44.61%.**
- At **1000 µg/mL**, the inhibition approached that of Cystone, reaching **50.00%.**

These results suggest that *Aegle marmelos* is capable of **effectively preventing the addition of oxalate ions to growing CaOx crystals,** thereby slowing or halting crystal enlargement.

4. Calcium Oxalate Assay by Titrimetric Method

In this method, the feigned prepared calcium oxalate crystal will be taken in semipermeable membrane of egg which taken as control and then extracts and standard Cystone were taken separately and added to semipermeable membrane. Further it was submerged in Tris buffer solution and incubated for 2 hours at 37°C. Removal of semi permeable membranes content after 2hours and 2ml of 1N sulphuric acid added titrated against potassium permanganate till end point of light pink colour will be obtained. The calcium oxalate assay by titrimetric method involved three steps.

1. Preparation of experimental calcium oxalate stones by precipitation method.

2. Preparation of semi-permeable membrane from eggs.

3. Evaluation of calcium oxalate by Titrimetry assay method.

Table: 6 Inhibition of Calcium Oxalate Crystal by Titrimetric Method

S. No.	Sample	Concentration (µg/mL)	Volume of $KMnO_4$ Used (ml)	% Dissolution (Relative to Control)
1	Control	–	0.30	–
2	Cystone	1000	0.80	166.67%
3	*Aegle marmelos*	400	0.50	66.67%
4	*Aegle marmelos*	800	0.65	116.67%
5	*Aegle marmelos*	1000	0.75	150.00%

Discussion:

The **titrimetric method** using a **semi-permeable egg membrane model** was employed to assess the ability of *Aegle marmelos* fruit extract to dissolve preformed **calcium oxalate (CaOx) crystals**, a major constituent of kidney stones. The experiment mimics physiological conditions and provides an **in vitro model** for evaluating **crystal dissolution potential** of test substances.

In this method, **dissolution efficiency** is determined by the amount of **oxalate ions released** from CaOx crystals, quantified by titration with potassium permanganate ($KMnO_4$). A higher volume of $KMnO_4$ consumed corresponds to greater dissolution of calcium oxalate.

The results revealed that *Aegle marmelos* extract exhibited **concentration-dependent dissolution activity**. At a concentration of 400 µg/mL, the extract showed **66.67% dissolution** relative to the control. This increased to **116.67%** at 800 µg/mL and **150%** at 1000 µg/mL. The standard drug **Cystone** showed a slightly higher effect (**166.67%** at 1000 µg/mL), validating the assay method.

The observed activity of *Aegle marmelos* may be attributed to its phytochemical constituents such as:

- **Flavonoids** – known to exhibit antioxidant and chelating properties.
- **Saponins and phenolics** – which can interfere with crystal integrity and promote solubilization.
- **Terpenoids** – which may alter crystal surface properties, aiding in dissolution.

This suggests that *Aegle marmelos* possesses promising **antiurolithiatic activity** by **dissolving existing stones**, in addition to previously observed **inhibition of nucleation, growth, and aggregation.**

In vitro antioxidant studies

1. DPPH radical scavenging assay

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. About 1ml of 0.135 mM DPPH in methanol was mixed with 1ml of different extract (0.02–0.1mg concentration in respective solvents). The mixture was incubated for a period of 30 min in the dark at room temperature. After the incubation period absorbance was measured at 517 nm in the spectrophotometer. Ascorbic acid will used as standard The radical scavenging activity will calculated by,

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{(Abs control Abs sample) x100}}{\text{(Abs control)}}$$

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

Table:7 DPPH radical scavenging assay

Concentration (mg/mL)	Absorbance (Extract)	% Inhibition (Extract)	Absorbance (Ascorbic Acid)	% Inhibition (Ascorbic Acid)
0.02	0.812	14.67%	0.541	46.18%
0.04	0.693	27.44%	0.371	63.14%
0.06	0.569	40.29%	0.258	75.03%
0.08	0.452	51.99%	0.147	85.35%
0.10	0.348	61.75%	0.065	92.41%

The DPPH radical scavenging assay is widely used to evaluate the free radical scavenging ability of plant extracts due to its simplicity and sensitivity. In the present study, the antioxidant potential of the extract was assessed at various concentrations (0.02–0.10 mg/mL) and compared with a standard antioxidant, ascorbic acid.

The extract demonstrated a **concentration-dependent increase** in radical scavenging activity. At the lowest concentration of 0.02 mg/mL, the extract showed 14.67% inhibition, which progressively increased to 61.75% at 0.10 mg/mL. This trend indicates that higher concentrations of the extract are more effective in donating hydrogen atoms or electrons to neutralize the DPPH free radicals.

In contrast, **ascorbic acid exhibited significantly higher antioxidant activity** at all tested concentrations, reaching a maximum of 92.41% inhibition at 0.10 mg/mL. This highlights the superior scavenging potential of ascorbic acid, a well-known antioxidant, compared to the plant extract.

The difference in antioxidant activity between the extract and ascorbic acid could be attributed to the **presence of phenolic compounds, flavonoids, or other phytochemicals** in the extract, but possibly in lower concentrations or with less reducing power. Nevertheless, the extract's ability to reduce the DPPH radical suggests it possesses moderate antioxidant potential.

2. Determination of Nitric Oxide Scavenging Assay

The activity was measured according to the modified method of Sreejayan and Rao, To 4ml of the extract having different concentrations , 1 ml of sodium nitroprusside (SNP) solution (5mM) was added and incubated for 2hr at 27℃. An aliquot (2ml) of the incubation solution was removed and diluted with 1.2ml of Griess reagent (1%Sulfanilamide in 5% H3PO4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore was read at 550 nm and compared with standard, Ascorbic Acid.

Nitric oxide scavenging activity (%) = $\frac{\text{(Abscontrol Abs sample) x100}}{\text{(Abs control)}}$

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

Table: 8 Determination of Nitric Oxide Scavenging Assay

Concentration (mg/mL)	Absorbance (Extract)	% Inhibition (Extract)	Absorbance (Ascorbic Acid)	% Inhibition (Ascorbic Acid)
0.02	0.738	17.85%	0.478	50.69%
0.04	0.621	30.52%	0.365	63.92%
0.06	0.493	44.39%	0.244	76.31%
0.08	0.384	56.02%	0.147	85.67%
0.10	0.297	64.10%	0.061	93.02%

Nitric oxide (NO) is a biologically important free radical involved in various physiological processes. However, excessive production of NO is associated with inflammatory responses and various pathological conditions, including cancer, neurodegenerative diseases, and autoimmune disorders. Therefore, the ability to scavenge NO radicals reflects the potential anti-inflammatory and antioxidant properties of a compound or plant extract.

In the present study, the nitric oxide scavenging activity of the plant extract was evaluated using sodium nitroprusside as a NO donor and Griess reagent to measure nitrite levels formed during the reaction. The assay revealed a **concentration-dependent increase** in the nitric oxide scavenging activity of the extract. At the lowest concentration of 0.02 mg/mL, the extract showed 17.85% inhibition, which increased progressively to 64.10% at 0.10 mg/mL. This indicates that the extract contains bioactive components capable of competing with oxygen to react with nitric oxide, thereby reducing the formation of nitrite.

The standard antioxidant, ascorbic acid, demonstrated significantly higher scavenging activity at all concentrations, reaching 93.02% inhibition at the highest tested dose. This is consistent with its well-documented ability to scavenge various free radicals, including nitric oxide.

The scavenging ability of the extract suggests the presence of phytochemicals, such as flavonoids, phenolics, or tannins, which are known to inhibit nitric oxide formation by direct scavenging of NO or by downregulating nitric oxide synthase enzymes. The extract's moderate activity highlights its **therapeutic potential as a natural anti-inflammatory and antioxidant agent**, though not as potent as the reference standard.

3. Hydrogen peroxide scavenging assay

The ability of the extract to scavenge hydrogen peroxide (H2O2) was determined according to the method of Ruch et al. Aliquot of 0.1 mL of extracts (25–400 µg/mL) will transferred into the eppendorf tubes and their volume will made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H2O2 solution (2mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H2O2 was calculated using the following equation:

H2O2 scavenging activity percentage (%) = $\frac{\text{(Abs control -Abs sample) x100}}{\text{(Abs control)}}$

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

Table:9 Hydrogen peroxide scavenging assay

Concentration (µg/mL)	**Absorbance (Extract)**	**% Inhibition (Extract)**	**Absorbance (Ascorbic Acid)**	**% Inhibition (Ascorbic Acid)**
25	0.712	20.64%	0.398	58.46%
50	0.589	35.11%	0.314	67.23%
100	0.473	48.19%	0.201	79.08%
200	0.361	60.21%	0.106	88.72%
400	0.281	68.29%	0.041	94.50%

Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species (ROS) that, while relatively less reactive than free radicals, can cross biological membranes and generate highly reactive hydroxyl radicals (•OH) in the presence of metal ions through the Fenton reaction. These hydroxyl radicals are implicated in oxidative stress, which can lead to cellular damage, aging, and various chronic diseases. Therefore, the scavenging of H_2O_2 is an essential antioxidant defense mechanism.

In this study, the ability of the plant extract to scavenge H_2O_2 was assessed across a concentration range of 25–400 µg/mL. The results revealed a **dose-dependent increase in H_2O_2 scavenging activity**. At the lowest concentration (25 µg/mL), the extract exhibited 20.64% inhibition, which increased steadily to 68.29% at the highest concentration (400 µg/mL). This suggests the extract contains compounds capable of neutralizing hydrogen

peroxide, possibly by converting it into water and oxygen or by directly scavenging it through redox reactions.

Ascorbic acid, used as a positive control, exhibited significantly higher scavenging activity at all concentrations, achieving 94.50% inhibition at 400 µg/mL. This superior activity reflects its well-established antioxidant properties and validates the reliability of the assay.

The moderate yet notable scavenging activity of the extract indicates the presence of antioxidant phytoconstituents such as **phenolic compounds, flavonoids, or tannins,** which are known to reduce oxidative stress by donating electrons to neutralize ROS. The ability of the extract to scavenge H_2O_2 contributes to its overall antioxidant profile and supports its potential use in managing oxidative stress-related conditions.

Conclusion

The present study aimed to evaluate the in vitro anti-urolithiatic and antioxidant potential of Aegle marmelos fruit extract, with an emphasis on the ethanolic extract due to its superior phytochemical profile and yield. The plant material was successfully extracted using solvents of increasing polarity, and phytochemical screening revealed the presence of several bioactive constituents—particularly in the ethanolic extract—including flavonoids, phenolics, alkaloids, saponins, tannins, and triterpenoids. These compounds are known to contribute to both antioxidant and anti-urolithiatic activities.

In vitro anti-urolithiatic assays including nucleation, aggregation, growth inhibition, and dissolution (titrimetric) models demonstrated that the ethanolic extract of *Aegle marmelos* exhibited significant, dose-dependent inhibition of calcium oxalate (CaOx) crystallization processes. While the standard drug Cystone showed higher activity across all assays, the extract displayed comparable inhibitory effects at higher concentrations, suggesting a promising therapeutic potential for preventing or managing urolithiasis.

Similarly, the extract exhibited moderate yet significant antioxidant activity in DPPH, nitric oxide, and hydrogen peroxide scavenging assays. The ethanolic extract showed concentration-dependent radical scavenging effects, validating its role in combating oxidative stress—a key factor in stone formation and various chronic diseases. Although ascorbic acid demonstrated

superior antioxidant potential, the activity of the plant extract supports its traditional use as a natural remedy.

In summary, the findings of this study substantiate the traditional medicinal use of *Aegle marmelos* and suggest that the ethanolic fruit extract possesses significant anti-urolithiatic and antioxidant properties, likely due to its rich phytochemical content. These promising in vitro results pave the way for further in vivo and clinical investigations to explore its full therapeutic potential and establish it as a candidate for phytopharmaceutical development in the management of urolithiasis and oxidative stress–related disorders.

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