RESEARCH PAPER

Evaluation of Neuroprotective and Antiurolithiatic Activity of Ethanolic Stem Extracts of *Malvastrum Coromandelianum*

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ABSTRACT
This study assessed the neuroprotective and antiurolithiatic properties of the mixed ethanolic extract by comparing Soxhlet and microwave-assisted extraction (MAE) of *Malvastrum Coromandelianum* stem. Flavonoids, alkaloids, phenolics, and steroids were validated by phytochemical screening, and MAE demonstrated a greater extractive yield (17.7%) than Soxhlet (15.1%). At 100 µg/mL, the extract increased glutathione levels (5.91 pg/mL) and SH-SY5Y cell viability (83.1%). Additionally, it demonstrated notable nucleation inhibition (66.7%) and calcium oxalate crystal dissolution (61.5%). These results show encouraging neuroprotective and antiurolithiatic potential, which calls for additional in vivo research.
Keywords: Antiurolithiatic Activity, Calcium Oxalate, *Malvastrum Coromandelianum*, Microwave-Assisted Extraction, MTT Assay, Neuroprotective Activity; Nucleation Assay.

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1. INTRODUCTION:

Urolithiasis and neurodegenerative illnesses, such as Alzheimer's disease, are serious health issues linked to oxidative stress, inflammation, and gradual tissue destruction. [1-4] The need for safer, multitarget therapeutic agents are highlighted by the limited benefits of current medicines. [2,4] Bioactive phytochemicals found in medicinal plants, including flavonoids, phenolics, alkaloids, tannins, and terpenoids, have anti-inflammatory, cytoprotective, and antioxidant properties against urolithiasis and neurodegeneration. [5–11] *Malvastrum coromandelianum* has shown antioxidant, antibacterial, anti-inflammatory, analgesic, and hepatoprotective qualities and has long been used to treat fever, inflammation, wounds, gastrointestinal, urinary, and respiratory diseases. [12,13] Nevertheless, nothing is known about its neuroprotective and antiurolithiatic capabilities. Furthermore, the recovery of bioactive components is influenced by extraction techniques; microwave-assisted extraction (MAE) has advantages over traditional Soxhlet extraction. [14, 15] Therefore, this work compared Soxhlet and MAE for extracting *Malvastrum coromandelianum* stem and evaluated the combined extract for phytochemical composition, neuroprotective, and antiurolithiatic effects utilizing in vitro tests.

2. MATERIALS AND METHODS:

2.1 Plant material collection and authentication

Dr. Sanjay S. Sathe of the Department of Botany verified the authenticity of *Malvastrum coromandelianum* stems that were gathered from the Sangli district of Maharashtra, India. After being cleaned and shade-dried for ten to fifteen days, the stems were ground into a powder using a mechanical grinder, sieved to ensure a consistent particle size, and kept in airtight containers.

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2.2 Transverse section microscopy

Fresh stem transverse slices were produced, stained with phloroglucinol and strong hydrochloric acid, mounted in glycerin, and inspected under a compound microscope. A SAGLO Digital Camera was used to take photomicrographs at 100× magnification, and SAGLOsoft Version 2 software was used for processing. [16]

2.3 Powder microscopy

A powder microscopic analysis was performed on the dried stem powder. A compound microscope was used to analyze small amounts of the powder that had been separately treated with glacial acetic acid, Sudan red solution, and phloroglucinol-hydrochloric acid reagent. The powder was then put on glass slides and covered with cover slips. Photomicrographs were acquired with a SAGLO Digital Camera (SAGLO, China) and processed with SAGLOsoft Version 2 software. [17]

2.4 Preparation of extracts

a) Soxhlet extraction

Using a Soxhlet apparatus kept at 50 °C until the siphoning solvent turned colorless, 250 mL of ethanol was used to extract 20 grams of stem powder. After being concentrated and dried in a desiccator, the extract was kept in an airtight container with a label. [18]

b) Microwave-assisted extraction

Twenty grams of stem powder was extracted with ethanol using microwave-assisted extraction at 150 W for three consecutive 5-min cycles. After every cycle, new solvent was added to the marc, and Whatman No. 1 filter paper was used to filter the extract. After being concentrated and dried in a desiccator, the mixed filtrates were kept in airtight containers. [19,20]

c) Extractive yield

The dried extracts obtained by Soxhlet and microwave-assisted extraction (MAE) were carefully weighed using an analytical balance, [21] and the percentage extractive yield was determined using the formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Extract Obtained}}{\text{Weight of Crude Drug Taken}} \times 100$$

2.5 Preliminary phytochemical screening

The ethanolic extracts were qualitatively tested for flavonoids, alkaloids, tannins, phenolic chemicals, steroids, and cardiac glycosides using established phytochemical standard procedures based on distinctive color changes or precipitate formation. [22]

2.6 Preparation of combined stem extract

Equal amounts (1:1) of the Soxhlet and MAE extracts were blended to generate the combined stem extract, which was used for future in vitro biological experiments.

2.7 Determination of Biological Activity

2.7.1 Neuroprotective activity

a) Cell viability assay (MTT assay)

Neuroprotective efficacy was tested using SH-SY5Y human neuroblastoma cells grown in DMEM supplemented with 10% fetal bovine serum and antibiotics at 37 °C in 5% CO_2. Cells (1×10^3 cells/mL) were sown in 96-well plates and treated to 500 µM H_2O_2 for 90 min to produce oxidative stress. [23] The cells were then treated with varied doses of the mixed extract, followed by incubation with MTT solution (20 µL, 2 mg/mL) for 2 h. Formazan crystals were dissolved in 100 µL DMSO, and absorbance was measured at 570 nm. To evaluate the extract's cytoprotective activity against $H2O_2$-induced oxidative damage, cell viability was expressed in relation to untreated control cells. [24–26]

b) Intracellular glutathione (GSH) estimation assay

Intracellular reduced glutathione (GSH) levels were evaluated using a competitive ELISA-based technique to examine the antioxidant state of treated neuronal cells. Cell lysates were treated in antigen-coated microplate wells with anti-GSH antibodies, followed by washing, enzyme-linked secondary antibody incubation, and chromogenic substrate addition. Absorbance was measured at 450 nm using a microplate reader, and GSH concentrations were derived from a standard calibration curve. In this competitive assay, color intensity was inversely related to intracellular GSH levels, providing an indicator of cellular antioxidant capability and oxidative stress. [27, 28].

2.7.2 Antiurolithiatic activity

a) Preparation of calcium oxalate crystals

Calcium chloride dihydrate and sodium oxalate solutions were combined to create calcium oxalate crystals, which were then precipitated with diluted ammonia. After filtering, washing, and drying at 60 °C, the precipitate was kept in sealed containers. [29,30]

b) Preparation of semi-permeable membrane

Hydrochloric acid solution was used to decalcify fresh hen eggs overnight. Before being used, the resultant membranes were cleaned, neutralized with diluted ammonia, and chilled. [31,32]

c) Egg membrane dissolution assay

Calcium oxalate crystals were suspended in pure water as the negative control. Combined stem extracts (10–50 mg/mL) and Cystone were employed as test and standard, respectively. The solutions were suspended in 0.1 M Tris buffer, wrapped in semi-permeable egg membranes, and incubated for 24 hours at 37 °C. To measure the dissolution of calcium oxalate, the contents were acidified with 0.5 M sulfuric acid and titrated against 0.02 M potassium permanganate following incubation. [33–35]

d) Nucleation assay

To start the crystallization process, combined stem extracts (5–20 mg/mL) were combined with calcium chloride solution and then sodium oxalate. A JASCO V-550 UV–Visible spectrophotometer was used to detect absorbance at 620 nm following a 30-minute incubation period at 37 °C. [36–38] Crystal nucleation inhibition was assessed by comparing absorbance with the control using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance\_control} - \text{Absorbance\_sample})}{\text{Absorbance\_control}} \times 100$$

2.8 Statistical analysis

The data are shown as mean ± standard deviation (SD), and all studies were conducted in triplicate (n = 3). Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA) was used to compute mean values and standard deviations. Graphs were made using Microsoft Excel 2021.

3. RESULTS:

3.1 Transverse section microscopy

Fig. 1: T.S. of Stem of *Malvastrum coromandelianum*

The transverse section of the stem showed several characteristic dicotyledonous traits, including a single-layered epidermis, multilayered cortex, distinct endodermis, well-developed vascular bundles arranged in a ring, lignified xylem arteries, and a large parenchymatous pith. (Fig. 1)

3.2 Powder microscopy

Lignified fibers, xylem vessel fragments, cork cells, parenchymatous tissues, and granular inclusions were seen in glacial acetic acid-treated powder by powder microscopy (Fig. 2). While Sudan red staining revealed the presence of lipid particles and oil globules (Fig. 3), phloroglucinol-HCl staining verified lignified fibers, sclerenchymatous elements, and xylem vessels with secondary wall thickening (Fig. 4).

Fig. 2: Powder microscopy of stem treated with Glacial acetic acid

Fig. 3: Powder microscopy of stem treated with Sudan Red

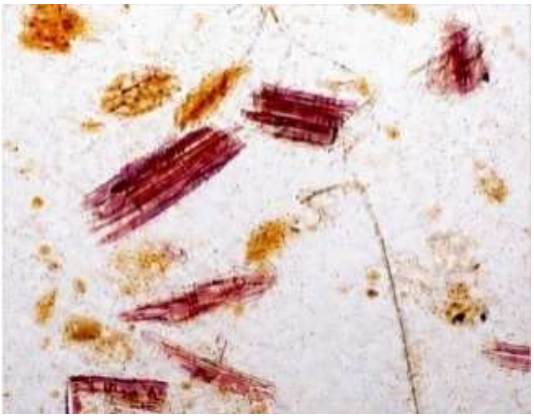

Fig. 4: Powder microscopy of stem treated with Phloroglucinol + HCl

3.3 Extractive yield

Soxhlet extraction and microwave-assisted extraction were applied to the dried stem powder. Microwave-assisted extraction produced a higher extractive yield than Soxhlet extraction (Table 1).

Table 1: Extractive yield of stem extracts of *Malvastrum coromandelianum*

Extraction Method	Weight of Sample (g)	Solvent Volume (mL)	Physical Nature	Color	Percentage Yield (% w/w)
Soxhlet Extraction	20	250	Semisolid	Green	15.1
Microwave-Assisted Extraction	20	250	Semisolid	Green	17.7

3.4 Phytochemical Analysis

Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, phenolic compounds, and steroids in both Soxhlet and microwave-assisted extracts, although cardiac glycosides were lacking (Table 2).

Table 2: Phytochemical screening of stem extracts of *Malvastrum coromandelianum*

Phytochemical Test	*Soxhlet Extract	*Microwave-assisted Extract
Flavonoids		
Lead acetate test	++++	+++
Shinoda test	+++	+++
Sodium hydroxide test	+	+
Sulfuric acid test	+++	+++
Alkaloids		
Dragendorff's test	+++	+++
Mayer's test	+++	+++
Wagner's test	++	++
Tannins & Phenolics		
Ferric chloride test	-	-
Bromine water test	-	-
Acetic acid test	-	-
Dilute nitric acid test	+++	+++
Potassium dichromate test	+++	+++
Steroids		
Salkowski test	+++	+++
Cardiac Glycosides		
Legal's test	-	-
Keller–Killiani test	-	-

*+++ = strongly present; ++ = moderately present; + = slightly present; - = absent

3.5 Biological Activity

3.5.1 Neuroprotective Activity

a) MTT assay

The mixed stem extract of *Malvastrum coromandelianum* showed concentration-dependent protection against H_2O_2-induced cytotoxicity in SH-SY5Y cells, with maximum cell viability seen at 100 µg/mL, while the H_2O_2-treated group showed considerably reduced viability (Table 3).

Table 3: Effects of combined stem extract against SH-SY-5Y by MTT assay

Concentration (µg/ml)	Absorbance (O D)			Mean ± SD (n=3)	Cell viability (%)
	R1	R2	R3		
Control	2.107	2.114	2.11	2.110±0.0028	100
Combined stem Extract 100	1.752	1.753	1.756	1.753±0.0017	83.1
40	1.523	1.526	1.52	1.523±0.002449	72.2
10	0.712	0.712	0.716	0.713±0.001886	33.8
H_2O_2 (500 mM)	0.175	0.181	0.167	0.174±0.00573	6.9

b) Intracellular GSH estimation

The mixed stem extract of *Malvastrum coromandelianum* considerably raised intracellular GSH levels in a concentration-dependent manner, with the greatest level seen at 100 µg/mL (Tables 4 and 5).

Table 4: Standard GSH calibration data

GSH	Pg/ml	Absorbance
Standard 8	100	1.965
Standard 7	50	1.687
Standard 6	25	1.442
Standard 5	12.5	0.985
Standard 4	6.25	0.658
Standard 3	3.12	0.468
Standard 2	1.57	0.385
Standard 1	0	0.325

Table 5: Effect of combined stem extract on intracellular GSH levels

Concentration of combined stem extract (µg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	Mean Absorbance ± SD	GSH (pg/mL)
10	0.385	0.383	0.388	0.385 ± 0.003	1.57
40	0.462	0.461	0.463	0.462 ± 0.001	3.79
100	0.651	0.651	0.650	0.651 ± 0.001	5.91

3.5.2 Antiurolithiatic activity

a) Egg Membrane Assay

The combined stem extract demonstrated concentration-dependent calcium oxalate dissolution in the egg membrane test. Maximum solubility was seen at 50 mg/mL, and the activity under the experimental conditions was comparable to that of the traditional drug Cystone. (Fig. 6, Table 6).

Table 6: Effect of combined stem extract on calcium oxalate dissolution

Sample	Concentration (mg/mL)	Titration value (mL)			Mean ± SD (n=3)	% Dissolution (%)
		R1	R2	R3		
Control	—	1.5	1.6	1.6	1.5 ± 0.1	0
Combined stem extract S1	10	1.0	0.9	1.1	1.0 ± 0.1	35.9
S2	20	0.9	0.8	1.0	0.9 ± 0.1	42.3
S3	30	0.8	0.7	0.9	0.8 ± 0.1	48.7
S4	40	0.8	0.6	0.7	0.7 ± 0.1	55.1
S5	50	0.7	0.6	0.5	0.6 ± 0.1	61.5
Std (Cystone)	10	0.6	0.5	0.7	0.6 ± 0.1	61.5

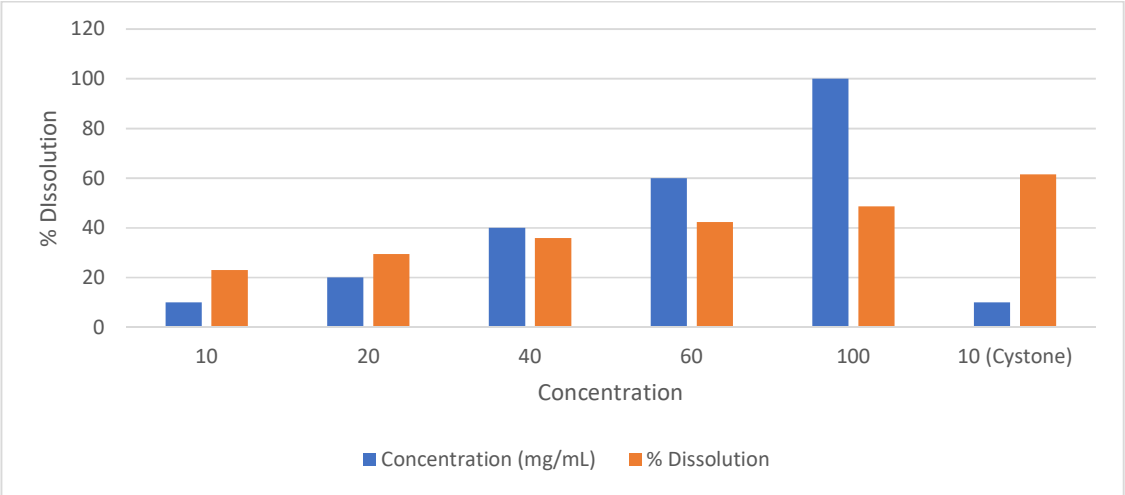

Fig. 5: Effect of combined extract on calcium oxalate dissolution

b) Nucleation assay

The calcium oxalate crystal nucleation was inhibited by the mixed stem extract in a concentration-dependent manner, peaking at 20 mg/mL (Table 7, Fig. 6).

Table 7: In vitro antiurolithiatic activity of combined stem extract by nucleation assay

Sample	Concentration (mg/mL)	Absorbance (O.D)			Mean ± SD (n=3)	% Inhibition (%)
		R1	R2	R3		
Control	—	0.30	0.31	0.29	0.30 ± 0.01	0
Extract S1	5	0.25	0.24	0.26	0.25 ± 0.01	16.7
S2	10	0.20	0.19	0.21	0.20 ± 0.01	33.3
S3	12	0.18	0.17	0.19	0.18 ± 0.01	40.0
S4	15	0.12	0.11	0.13	0.12 ± 0.01	60.0
S5	20	0.10	0.09	0.11	0.10 ± 0.01	66.7
Std (Cystone)	10	0.12	0.11	0.13	0.12 ± 0.01	60.0

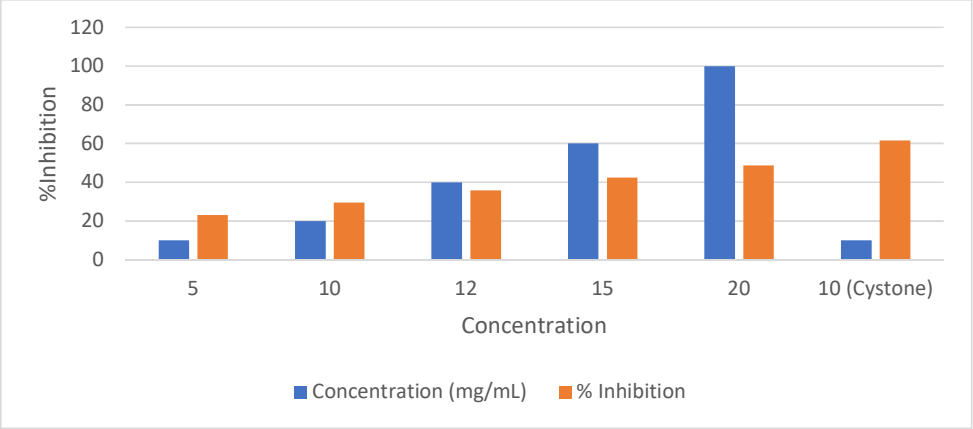

Fig 6: Nucleation inhibition activity of combined extract

4. DISCUSSION:

The present work examined the phytochemical content, extraction efficiency, and in vitro neuroprotective and antiurolithiatic properties of *Malvastrum coromandelianum* stem extracts. Microwave-assisted extraction produced a greater extractive yield than Soxhlet extraction, likely due to faster cell wall rupture and better release of intracellular phytoconstituents.

Preliminary phytochemical screening confirmed the presence of flavonoids, alkaloids, steroids, and phenolic chemicals in both extracts, validating the plant's traditional therapeutic use and its biological activities. The mixed stem extract displayed neuroprotective properties against hydrogen peroxide-induced oxidative stress in SH-SY5Y cells and dramatically increased intracellular glutathione levels, indicating enhanced antioxidant defense and protection against oxidative damage.

By preventing calcium oxalate crystal nucleation and encouraging crystal disintegration, the extract also demonstrated antiurolithiatic activity. Flavonoids, phenolics, and alkaloids, which are known to lower oxidative stress and prevent crystal formation and aggregation, may be responsible for these effects.

Nevertheless, the study was restricted to in vitro trials and qualitative phytochemical analysis; neither in vivo validation nor the quantification of active ingredients were included. The isolation and identification of bioactive substances, quantitative phytochemical analysis, clarification of molecular mechanisms, and in vivo investigations to verify safety and efficacy should be the main topics of future research.

Overall, the results point to *Malvastrum coromandelianum* stem extract as a possible source of bioactive chemicals with neuroprotective, antiurolithiatic, and antioxidant properties that call for more research.

5. CONCLUSION:

The current investigation indicated that *Malvastrum coromandelianum's* ethanolic stem extract displayed encouraging in vitro neuroprotective and antiurolithiatic capabilities. Phytochemical screening verified the existence of flavonoids, alkaloids, phenolic compounds, and steroids, and microwave-assisted extraction yielded a larger extractive yield than Soxhlet extraction. The extract inhibited the production and dissolution of calcium oxalate crystals, enhanced neuronal cell survival, and restored intracellular glutathione levels. These results enhance *Malvastrum coromandelianum's* therapeutic promise, but more in vivo and mechanistic research is needed to establish its safety and effectiveness.

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