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A review on the antiurolithiatic effects of *Blumea balsamifera*: A comparative methodology analysis

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Abstract
Kidney stones, also known as urolithiasis, primarily due to calcium oxalate crystals, is a prevalent renal disorder that poses numerous health concerns. The existing methods, citrate treatment, lithotripsy, and others, although being efficacious, expose the patient to hazards of diseases, along with side effects. The high cost factor is another well-known aspect of present treatment. Thus, over time, people have shown keen interest in natural remedies, among which Blumea balsamifera, or soot flower, is one. This is used medically to treat kidney stones. The research proves that Blumea balsamifera extracts aid in inhibiting the formation, slowing growth, and preventing the agglutination of crystal clumps formed by calcium oxalate, which act as kidney stones. The extracts also possess the ability to convert calcium oxalate monohydrate (COM) to calcium oxalate dihydrate (COD), which is less stable compared to the previous form. This makes Blumea balsamifera extracts efficient, natural, and potent sources for the treatment of Anti-urolithiasis.

Keywords: Urolithiasis, kidney stones, calcium oxalate, calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD), blumea balsamifera, anti-urolithiasis

Introduction

Urolithiasis, the pathological development of kidney stones in the urinary system, has remained a major health concern globally owing to its high rate of recurrence, severe clinical presentation, and complications [1, 2]. Moreover, a high proportion of cases with urolithiasis present with calcium oxalate stones, where calcium oxalate monohydrate (COM) crystals are the most stable and adhesive form of these stones [3, 4]. Additionally, COM crystals have been identified as more retained in the renal tubules, causing obstruction, inflammation, and further development of pathologic stones [3]. However, calcium oxalate dihydrate (COD) crystals remain less stable, less adhesive, and more readily excreted from the kidneys owing to a weaker crystal lattice structure[4]. Therefore, a transition from COM to COD crystal development has been advocated as a fundamental therapeutic target in managing urolithiasis [3, 4]. However, traditional management strategies like lithotripsy and citrate therapy may be beneficial in fragmenting and dissolving stones chemically, but these do not necessarily ensure the prevention of recurrence and correction of urine chemical equilibrium [5]. This implies an imperative need to opt for other alternatives that can target dissolution as well as earlier crystallizations [1, 5].

The growing interest in plant-based interventions has motivated the screening of natural phytoconstituents that target calcium oxalate crystallization mechanisms [6, 7]. Blumea balsamifera is a widely used ethnomedicinal plant in Southeast Asia, which has long been recognized for its diuretic, anti-inflammatory, and renal detoxifying properties [6, 9]. The plant contains flavonoids, sterols, alkaloids, saponins, glycosides, and phenolic compounds, many of which have been reported to interfere with stone formation pathways [7, 8]. Phytochemical analysis has confirmed the predominance of sterols and alkaloids in its ethanolic extract, which lends credence to their possible intervention in altering crystal surface charge, growth kinetics, and aggregation potential [7, 8]. Its capability for altering crystal shape, reducing crystal mass, and modification of physicochemical conditions promoting supersaturation makes it a promising phytotherapeutic candidate [6, 8]. In contrast with synthetic agents acting via a single biochemical stage, the multi-compound profile of the plant allows the potential intervention along multiple formation points in the renal stone cycle [6, 8, 9].

Recent scientific studies confirm that Blumea balsamifera inhibits the early crystallization process by lowering nucleation frequency in supersaturated environments [10]. In various simulated urine systems, the extract was observed to reduce the thermodynamic relationship between supersaturation and nucleation rate, which signifies that its bioactive components affected the crystal nucleation process [11]. The reduction in nucleation activity carries clinical relevance because it characterizes the earliest point of origin of calculi, which will progress into solid deposits upon formation with enough duration [9]. Other results emphasize that at higher extract concentrations, the turbidity slope—a standard for measuring the intensity of crystal formations—significantly decreases, serving as an indication of the phytochemical components increasing the free energy barrier to form crystal nuclei [12]. These biochemical obstructions compromise the stability of initial crystal clusters and thus minimize the possibilities of clinically significant renal deposits [13].

Beyond nucleation, Blumea balsamifera has been associated with the reduction of crystal growth rate and surface adhesion, two of the most critical mechanisms responsible for pathological progression [13, 14]. Reducing crystal growth slows the enlargement of microcrystals, preventing them from reaching sizes capable of obstructing renal pathways [13]. Meanwhile, the inhibition of aggregation prevents smaller crystals from binding together into larger, more harmful masses [13, 14]. Quantification of the inhibition potential has shown that the extract demonstrates

measurable IC_{50} values across the three primary stages of stone formation: nucleation, growth, and aggregation. Notably, the extract exhibits the strongest response in aggregation inhibition, suggesting a significant advantage in preventing the large-scale structural development that leads to symptomatic stones [14]. These findings stress the therapeutic value of the plant as a multi-target intervention capable of disrupting the cycle at multiple biochemical checkpoints.

Another significant mechanism is the plant's facilitation of structural transformation between COM and COD crystals [11, 13]. The molecular structure of COM crystals is rigid and tightly packed, making them more resistant to dissolution, while the crystal lattice structure of CODs is open, reducing its adhesion to renal epithelial surfaces [4, 11]. Thus, conversion from COM to COD is clinically beneficial because it creates more effortlessly eliminable crystals through renal tubule [4, 11]. Such mechanisms are visually observed in both microscopy and XRD, which demonstrated that treatment with Blumea balsamifera promotes the adoption of crystallization profiles dominated by COD, improving the likelihood of spontaneous urinary elimination [13]. Supporting this mechanism, SEM analysis has shown that there was a decrease in surface aggregation, clearer geometric outlines, and decreased cluster density. These prove that the plant does not act simply by reducing the quantity of the crystals but also by changing the quality and clinical behavior of formed stones [11].

These scientific findings together validate the ethnomedicinal use of Blumea balsamifera for the relief of kidney stones and offer mechanistic insights into its antiurolithiatic action. Its demonstrated influence on nucleation, growth, aggregation, and COM-to-COD transition establishes its relevance as a therapeutic agent capable of preventing and managing stone formation. Although promising *in-vitro* and analytical studies have been performed, further research will have to include *in-vivo* animal studies, pharmacokinetic evaluation, dose standardization, and finally controlled clinical trials to establish its safety and therapeutic index. This makes the herb a commendable candidate for the integration of plant-based medicine into preventive or adjunctive urolithiasis care, amidst increasing interest in such medicine. Proper standardization, extraction optimization, and regulatory validation will be key in advancing it to clinical acceptance as a natural antiurolithiatic phytomedicine.

Plant Description: *Blumea balsamifera*

Synonyms

Sans. Kukundara; Kukkura-dru (Dog-bush). Hind. Kukronda; Kakoranda. Bom. - Bhamaruda. Ben. - Kukur-soka; Kuk-sungh. Mal. Sombong; Banga-chappa. Burm. - Pung-ma-theing. China. -Nagi [15].

Habitat

Tropical Himalayas, from Nepal to Sikkim, the western part of the Deccan plateau, and very abundantly in Burma. Blumea densi-flora is a small bushy plant found in various parts of Assam, the Khasia Hills, and Chittagong.

Parts Used

Leaves and sometimes the herb.

Preparation

Both species contain a volatile oil with the odour of wormwood, and a camphor known as Nagi camphor; it has the same physical properties as Borneo Camphor, but differs in optical properties. Decoction of dried herb; powder of leaves.

Action

Astringent and anthelmintic, sudorific, carminative, and expectorant.

USES

Externally, fresh juice of the leaves is dropped into the eyes in chronic purulent discharges. Internally, the decoction is given for worms, in dysentery, and chronic uterine discharges. It is particularly useful in the disease of the nose called "Ahwah" peculiar to Bengal, and accompanied by strong fever, heaviness in the head, and pains in the neck, shoulders, and loins. Powder of leaves is given internally in two drachm doses mixed with butter, and is also used as a snuff [15].

Scientific Classification

Kingdom: Plantae
Clade: Tracheophytes / Angiosperms
Order: Asterales
Family: Asteraceae
Genes: Blumea
Species: Blumea balsamifera

In the Philippines, where it is most known as Sambong, Blumea balsamifera is used in traditional herbal medicine for the common cold and diuretic. The genus Blumea is found in the 2 tropical and subtropical zones of Asia, especially the Indian subcontinent and Southeast Asia.

Plant description

Softly hairy, woody, strongly aromatic shrub, 1-4 meters high. Simple, alternate, broadly elongated leaves, 7-20 cm long, with roasted margin and appendaged base. Loose yellow flower heads scattered along much-branched leafy panicles [16].

Two types of dactylifera: peripheral ones, tiny and more numerous, with tubular corolla, and central flowers, few, large, with Campanulate corolla. Fruit dry. 1-seeded, 10 ribbed, hairy at the top.

Chemical constitution

1. Volatile Oil
2. Camphor
3. Limonene

4. Saponin
5. Lanin
6. Sesquiterpene

Geographical source

Southeast Asia: This includes countries like the Philippines, Malaysia, Thailand, Vietnam, and Myanmar.

China: It's found in southern regions like Hainan, Guizhou, Yunnan, and Guangdong provinces, as well as Taiwan.

Indian Subcontinent: It's also present in parts of India, Nepal, and Bangladesh.

Other regions: The plant is also found in Indonesia, Cambodia, Laos, and New Guinea [17].

Elements

Volatile oil, Flavonoids, Sterols, Phenylpropanoids [18].

Biological activity

Antitumor activity, Hepatoprotective effect, Superoxide radical scavenging activity,
Antioxidant activity, Anti-microbial, Anti-inflammation, Antiplasmodial, Anti Tyrosine,
Wound healing activity, Antiobesity activity, Platelet aggregation activity [19, 20, 21, 22, 23].

Methodology

1. Extraction technique

- **Soxhlet ethanolic extraction**

Blumea balsamifera leaves were cut dried at 40 °C for six days and crushed into a powder form. Extraction was conducted using a Soxhlet apparatus with ethanol as the extracting solvent. The extraction is performed at 80 °C with constant stirring for 6 hrs. A total of 7 g of crushed B. balsamifera leaves and 700 mL of 99.5% ethanol were used for every extraction. After extraction, ethanol was boiled-off in a water bath at 80 °C to obtain a crude extract [10].

- **Hydroalcoholic extraction**

The plant material was cleaned with distilled water and damaged leaves were
removed. The material was shade dried with a freeze drier and then ground into powder
for extraction. The extracted powder (200 g) was soaked with 80% ethanol with the ratio
1 to 10 (w/v) at room temperature for 72 hours and then filtered through Whatman filter
paper No. 1. After the mixture was evaporated at 50 °C to remove solvent, the crude
extract was dried with a freeze drier and stored at -20 °C [14].

2. Morphology Studies

The crystallization experiments consisted of incubating artificial urine samples, with and without plant extract, for three days at room temperature. Afterwards, the samples were observed under a light microscope using a hemocytometer to determine the size of the crystals. We photographed the samples at 100x and 400x magnification and analyzed the images with software [24].

3. Nucleation Studies

Turbidity testing, similar to the test described by Hess *et al*., was used to check the formation of crystals. Cloudiness is measured at 620 nm by means of a Perkin Elmer Lambda 850 UV-Vis spectrophotometer [25]. Turbidimetry was carried out using different supersaturation ratios (17.5, 20.0, 22.5 and 25.0) with various extract concentrations (0.0, 0.5, 1.0, 2.0, and 5.0 mg/mL) using a Perkin Elmer Lambda 850 UV-Vis spectrophotometer. Each condition was tested in triplicate. Artificial urine, extract solution, calcium chloride and sodium oxalate were sequentially added to each 1-mL cuvette. Distilled water was used instead of extract for the control samples (0.0 mg/mL extract).

4. Light Microscopy

We prepared calcium oxalate crystals by combining calcium chloride with sodium oxalate in pre-sterilized polypropylene cuvettes and allowing the mixture to stand at ambient temperature for 72 hours to achieve crystallization equilibrium [13]. We then gently stirred the suspensions and withdrew aliquots for microscopy. A sample was placed on a hemocytometer and examined under a light microscope, and micrographs were taken for examination of shape and structure [11]. Crystal size and morphology were quantified with image-analysis software. We assigned shape classes by comparing maximum and minimum Feret diameters to calculate shape ratios [26].

5. XRD and SEM Techniques

Crystals for structural and surface analysis were generated under supersaturated conditions by addition of water, extract, sodium oxalate, and calcium chloride, followed by sealing of the vessels to promote crystal growth over 72 hours. The material was harvested by centrifugation at 2504 g for 30 minutes, after which residual moisture was removed by drying the material in a desiccator for 72 hours. Dried crystals were stored in airtight containers until use [27]. Phase identification was performed using Powder X-Ray Diffraction to determine the crystal composition, confirming the patterns of COM/COD [28]. Surface topography and particle morphology were examined at the Department of Chemical Engineering, University of the Philippines Diliman, using a Hitachi S3400N SEM with optimized instrument parameters in terms of magnification and beam voltage that will optimize the resolution of the images taken [29].

6. IC50 Inhibition Assay

The CaOx crystals solution was prepared by combining calcium chloride with sodium oxalate at a concentration of 50 mM. The solution was heat-treated at a water bath at 60 °C for an hour; afterwards, it was slowly cooled down at 37 °C. The solution was left overnight to allow the CaOx crystal formation. The solution was not allowed to either grow or dissolve into the solvent. The CaOx crystals solution was collected by spinning at a speed of 14,000 rpm and dried at 37 °C. The CaOx crystal solution final working concentration was 1 mg/ml. The rock crystal solution was buffered with TRIS at a concentration of 0.05M and sodium at a concentration of 0.15M with a pH value at 6.5. A control solution without any extract was made. The solution was compared with a variety of inhibitors at varying concentrations. The varying inhibitors included the extract solution and sodium citrate. The absorbance at a wavelength of 620 nm was measured at a time duration of 30 minutes, 60 minutes, 90 minutes, 180 minutes, and a total of 360 minutes, respectively [30]. The percentage inhibition can be calculated using the formula:

$$\% \text{ Inhibition} = (1 - (Si/Sc)) \times 100$$

where Si is the slope of the trend line in the presence of inhibitors and Sc is the slope without inhibitors.

IC50 is the value used to determine the potency of the inhibitor in the sample. It shows the concentration of the sample in IC50 (mg/mL) required to suppress 50% of the free radicals, cells, or enzymes, with lower IC50 values indicating higher activity.
IC50 is calculated by subjecting the sample to various concentrations. When a linear graph is formed between the result and the concentration, the graph y = ax + b is produced by considering the y-coordinate as the % inhibition and the x-coordinate as the concentration. But in case of a non-linear graph, two points involving inhibition concentrations are selected on either side of 50%, and a linear graph is formed by a straight line passing through them to get the equation y = ax + b. Then by substituting y = 50%, the value of x is determined, which is IC50.

Table 1: Comparison of the Methodology

Study	Extraction method	Experimental model	Assessment parameter
Effect of Blumea balsamifera extract on the morphology and nucleation of calcium oxalate crystals in artificial urine[10].	Soxhlet ethanolic extraction	Artificial urine crystallization model	Nucleation Study
Inhibition of calcium oxalate crystallization causing kidney stones *in vitro* by an extract of Bluméa balsamifera[14]	Ethanolic extraction	CaOx morphology analysis using XRD & SEM	XRD & SEM
Effect of Blumeabalsamifera extract on the phase and morphology of calcium oxalate crystal[13]	Hydroalcoholic extraction	*In vitro* IC50 based Inhibition in 3 phases	IC50 inhibition assay

Result and discussion

1. Extraction technique

- **Soxhlet ethanolic extraction**

The Soxhlet extraction using ethanol, carried out under optimized heat, provided a concentrated extract containing high amounts of phytoconstituents, namely sterols, alkaloids, saponins, flavonoids, and glycosides. The constant solvent reflux ensured better penetration of solvents into the plant materials, ensuring that agents capable of binding to Calcium and Oxalate ions were extracted from the plants. This concentrated extract showed intense inhibition of crystal nuclei formation, as seen from its gentler turbidity slope and slower nuclei formation in the artificial urine sample. This was consistent with the high concentrations of heat-stable agents such as sterols and alkaloids [10].

- **Hydroalcoholic extraction**

The hydroalcoholic extract followed by freeze-drying led to a more stable and pure extract. The extract was more suitable for the quantification of the concentration-dependent inhibitory effect, as it enabled the determination ofIC50 for nucleation, growth, and aggregation. The data obtained indicated a higher inhibitory effect compared to the Soxhlet extract, and the inhibitory effect was greatly increased in the aggregation process. The freeze-dried extract was found to have an IC50 of 4.25 mg/mL for nucleation, 2.99 mg/mL for growth, and 2.56 mg/mL for aggregation [14].

Table 2: Comparative Outcome of Both Techniques

Parameter	Soxhlet ethanolic extraction	Hydroalcholic extraction
Heat Impact	Higher; may degrade light compounds	Minimal; preserves heat-sensitive molecules
Bioactive Complexity	Broad spectrum, full phytochemical range	More purified, controlled molecular concentration
Best Activity Stage	Early-stage nucleation inhibition	Concentration-dependent growth & aggregation inhibition
Practical Use	Suitable for crude therapeutic studies	Suitable for dose-response and formulation research

This comparison indicates that Soxhlet extraction is more effective for generating a broad phytochemical mixture that interrupts early crystallization, while freeze-dried hydroalcoholic extraction is better at delivering purified compounds with stronger controlled inhibition at later developmental stages of crystal formation. This reveals that the extraction technique does not merely prepare plant material—it defines the clinical behavior of the final extract. The findings confirm that extraction standardization is essential before clinical development, as therapeutic success depends on solvent selection, temperature control, and compound preservation.

2. Morphology Study

Figure 1. At supersaturation ratios of 17.5, 20, 22.5, or 25, there were no significant differences in the mean Feret diameter of calcium oxalate crystals (P = 0.1882, 0.3118, 0.3960, 0.1880). This is because the mean Feret diameter, measured using area, is greater than that measured using arithmetic mean due to the greater number of larger crystals. Both methods indicated that crystal size decreased from supersaturation ratio 17.5 to 20 and then increased from supersaturation ratio of 20 to 25, yet the difference is still statistically insignificant [31].
Figure 2. Crystal size was reduced at all levels of supersaturation by the extract. Two-factor ANOVA showed that crystal size was significantly affected by the exact concentration (P-value = 7.2 × 10^-6) but not by the supersaturation ratio (P-value = 0.7288). It is interesting to note that even a concentration of 0.5 mg/mL of the extract decreased crystal size. When the control sample was not included, the significance of extract concentration diminished to P-value = 0.3219.
The following Figure 3 shows that the extract caused a change in the crystal morphology from COM to calcium oxalate dihydrate (COD). Without the extract, the COM

level was 60% to 80%, whereas the level was 0% in the presence of the extract. The change in morphology indicates lower pathogenicity since the COD generally remains below the pathogenic level, becomes unstable at higher supersaturation, and gets expelled before attaining a pathogenic size. Such COD has been observed in other organic crystal growth modifiers like polyacrylates, poly-1-aspartate polyglutamate [32], Osteopontin[33], and Khella (Nigella) plants [34].

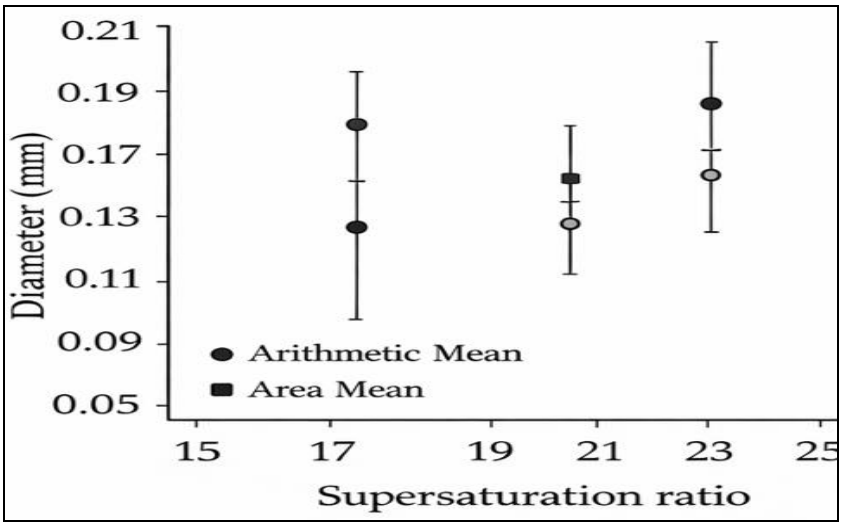

Fig 1:

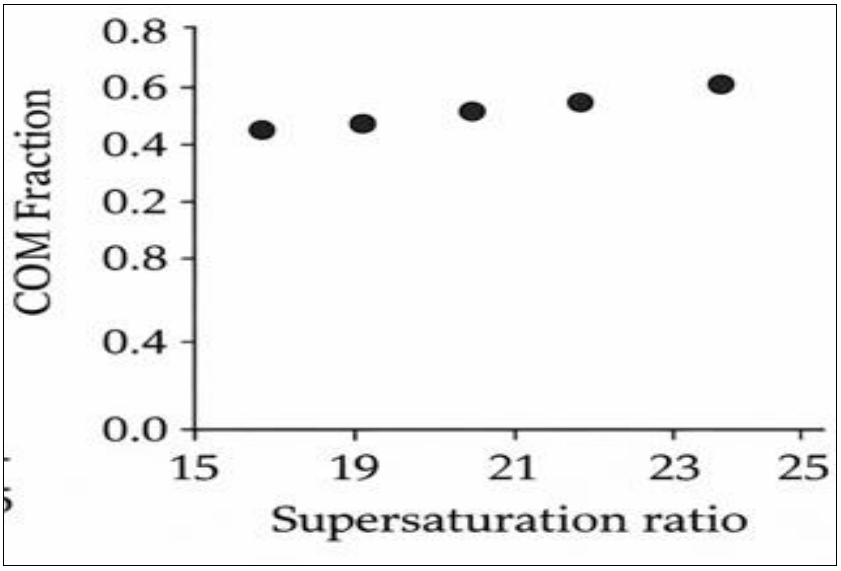

Fig 2:

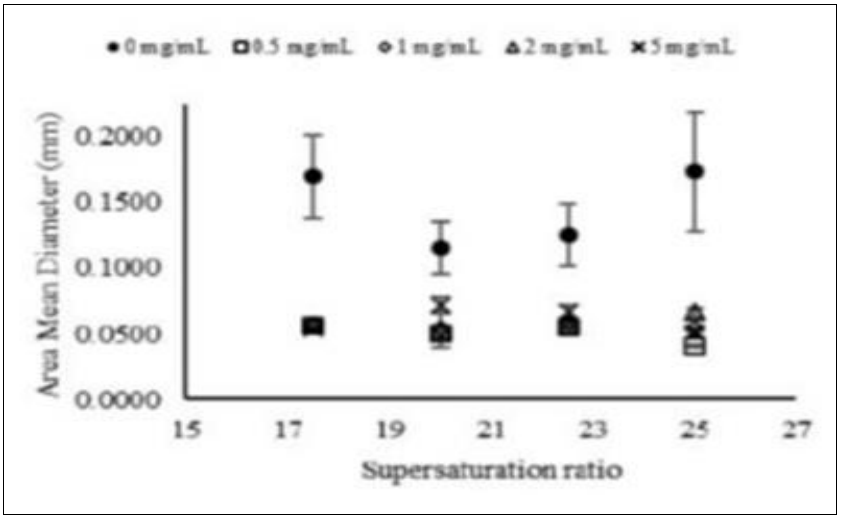

Fig 3:

3. Nucleation Studies

Figure 4 illustrates a typical graph for adsorption versus time; it consists of an initial region with low absorption corresponding to the observed literature. The turbidity slopes with the rates of turbidity increase were obtained from the linear part of the absorption graphs. Figure 5 shows how the turbidity slope increases as supersaturation increases. The correlation coefficient between turbidity slope and supersaturation is found to be 0.9906, which is in agreement with traditional nucleation theory, as nucleation rate increases at a greater supersaturation value [35].

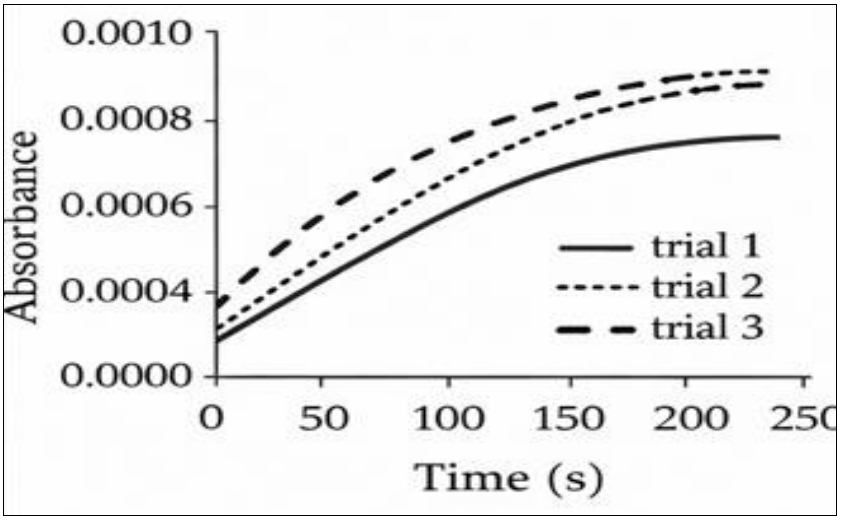

Fig 4:

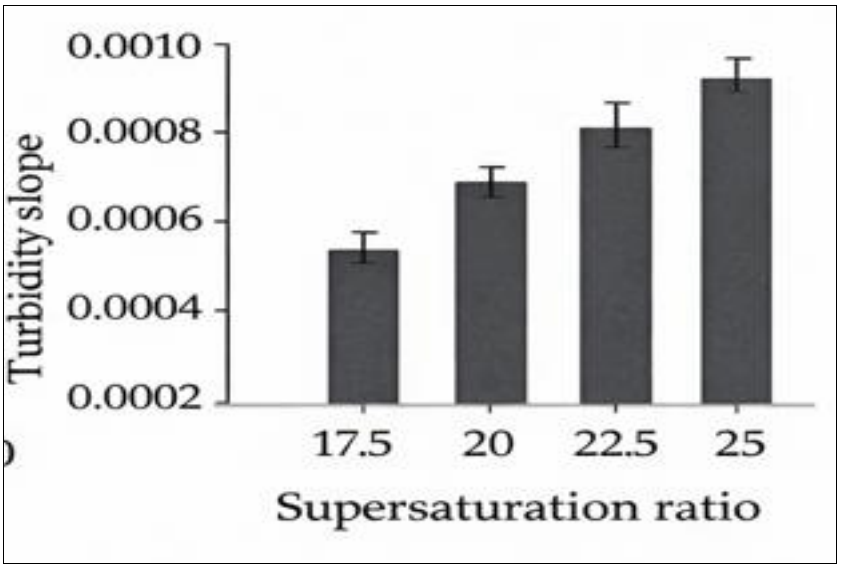

Fig 5:

But as the concentration of the extract solution of Blumea Balsamifera increases, the correlation coefficient is observed to decrease, as shown in Figure 6. As the concentration increases from 0.5 to 5 mg/mL, the value of R^2 corresponding to the correlation coefficient decreases from 0.99 to 0.8928, 0.5884, 0.3814, and 0.099.

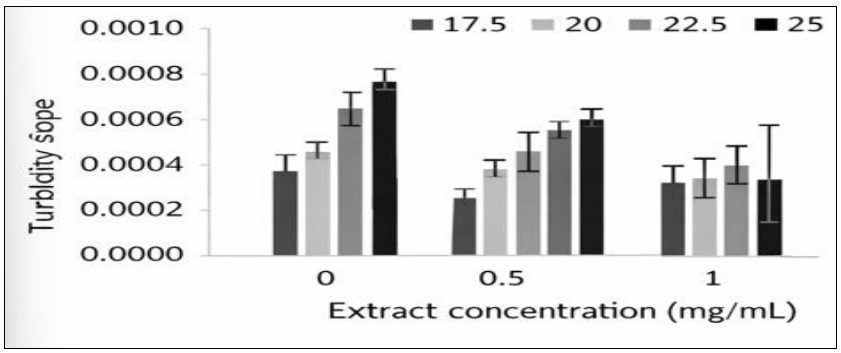

Fig 6:

4. Light Microscopy

These crystals are fewer in number but larger in size, with a distinct hexagonal profile. At more concentrated levels of supersaturation, the growth turns irregular. In comparison, Figure 7 shows that with Blumea balsamifera extract, more crystals form, but they are much smaller. The crystals in this case are mostly square-shaped, typical of calcium oxalate dihydrate (COD) and usually less hazardous. Images under the microscope were processed using ImageJ, brightening or normalizing contrast to accurately quantify crystal dimensions and morphology.

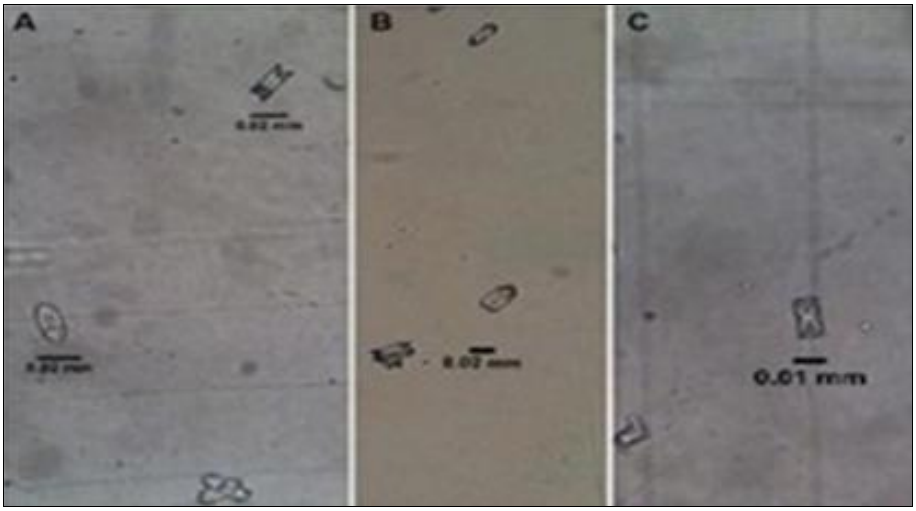

Fig 7:

- **Size of crystals**

Particle size was characterized by two measurements: Feret diameter, dF, and the equivalent circle diameter, deq. The crystal PSD was evaluated for both of these metrics. Figures 8 present PSD of crystals generated at a supersaturation of 20 with no extract added. By definition, the Feret diameter should always be greater than the equivalent circle diameter, except in the case of a perfectly circular particle.

This makes sense, especially for COM and COD crystals which are usually elongated so that diagonals are longer than the diameter of a circle of equivalent area. Hence the PSD calculated from dF extends to larger sizes than that from deq. Since the data may be summarized either as arithmetic means or area means for both Feret and equivalent circle diameters, there are four ways results can be reported. Figure 9 compares these methods. Area-mean diameters are always larger than arithmetic means because they weigh larger particles more heavily, which gives big crystals more prominence. As illustrated in Figure 10, the order is as follows: the largest crystal sizes occur for the area-mean of dF, followed by the arithmetic mean of dF, then the area-mean of deq, with the smallest values coming from the arithmetic mean of deq. Except for a sudden jump between 12.5 and 15, most crystal sizes decrease with an increase in supersaturation within a range of 10–20. The RSD generally ranges from 23.24% to 26.93%, but at supersaturation 20, it jumps up to 46.54%, reflecting greater variation in size. The crystal sizes become more homogeneous and markedly smaller when Blumea balsamifera extract is added.

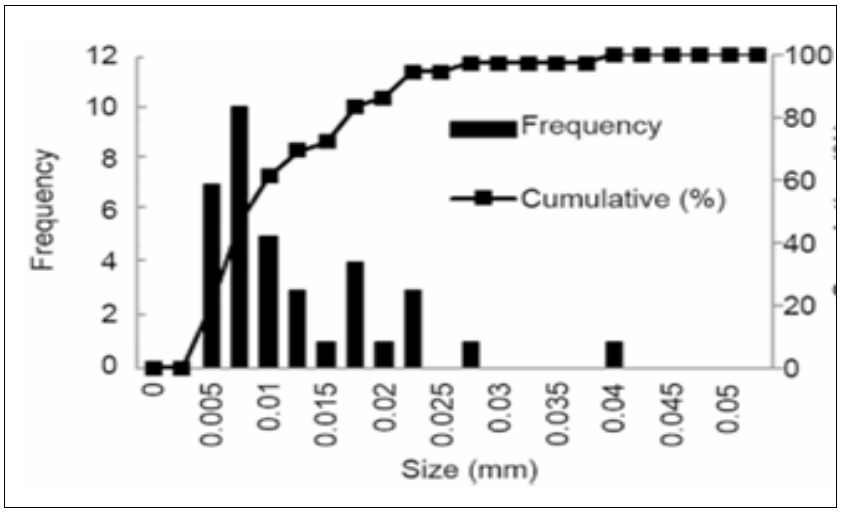

Fig 8:

The mean value of the Feret diameter remains constant at 9.63 ± 0.76 µm, which has a relatively low RSD of 7.84%. The size of the crystals varies by 5.22% or up to 82.69 depending on how one determines it and the level of saturation of the system. The largest difference will occur at supersaturation of 15.0, and the lowest at 12.5.

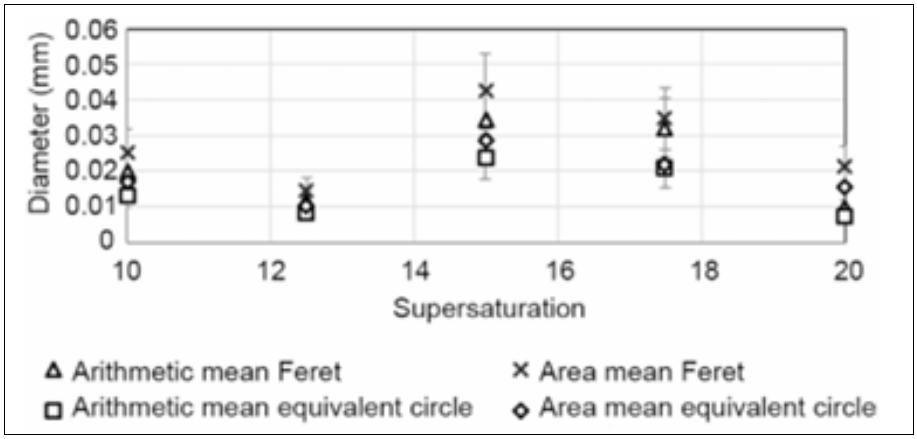

Fig 9:

- **Shape of the Crystal**

The shape gives a lot of information about the type of crystal. The common shapes of the crystals viewed under the microscope are shown in Figure 11. Calcium oxalate monohydrate (COM) crystals consist of elongated rhombohedrons. When viewed along the (010) and (10-1) axes, the COM crystals form a hexagon, as depicted in Figures 11A and 131. The calcium oxalate dihydrate (COD) crystals, on the other hand, form an octahedron and appear as a square or diamond-shaped projection in Figure 11C. Additionally, in the process, a calcium oxalate trihydrate (COT) crystal, a rare type, was identified. The crystal has a short dumbbell-shaped projection, illustrated in Figure 11D.

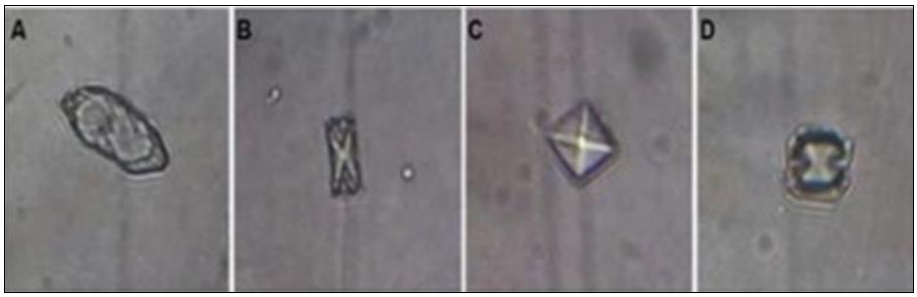

Fig 11:

5. XRD and SEM Techniques

The dried crystals at a higher level of supersaturation of 20 were analyzed for powder X-ray diffraction (PXRD) analysis. The spectra obtained showed strong peaks, indicating that the samples were crystalline in nature. From Figure 12, at a lower concentration of 0.1 mg/mL of B. balsamifera extract, the major peaks were observed to be from calcium oxalate monohydrate (COM) crystals [36]. However, at a higher concentration of 1.0 mg/mL, the peaks associated with COM were greatly reduced. New peaks were observed at 2θ values of 44.5, 64.8, and 77.86, which represent the (200) and (211) planes of a tetragonal crystal structure [37]. This suggests the possibility of the transformation of the crystals from COM to maybe another compound, namely calcium oxalate dihydrate (COD).

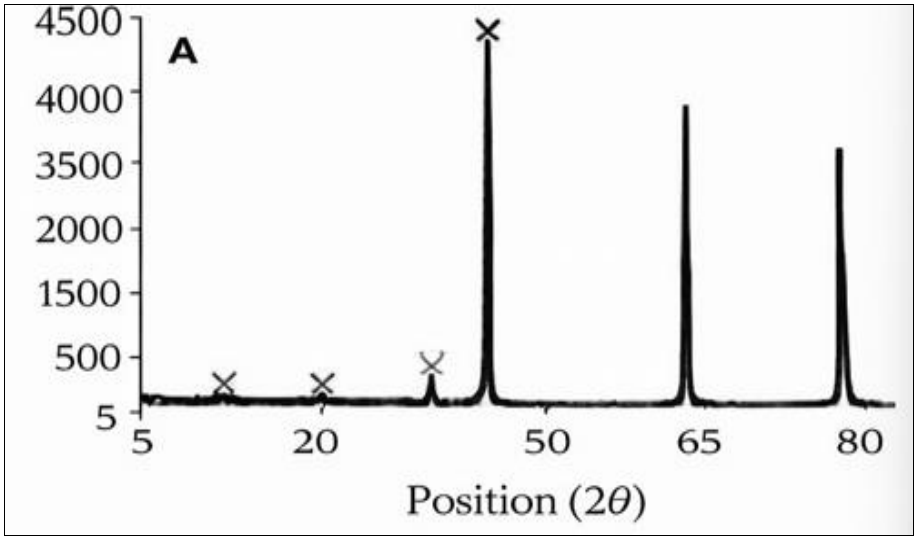

Fig 12:

COD seemed to appear when the extract concentration became higher. Further support can be seen in the SEM images in Figure 13 for crystal appearance at different conditions. In Figure 13A, where no extract was added, the crystals tended to agglomerate and were thus hard to distinguish as individual crystals; this suggests that growth was uneven. In Figure 13B, where there was 0.1 mg/mL extract, the crystals were more distinct and still separated, but less diffused compared with Figure 13A. At the highest concentration, 1.0 mg/mL (Figure 13C), the crystals were smaller, well separated, and exhibited well-defined shapes, with some features characteristic of COM, especially on the (010) and (101) faces.

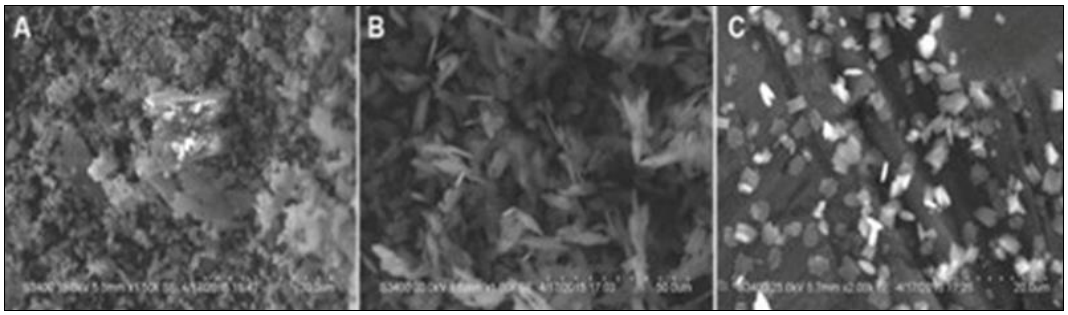

Fig 13:

Taken together, these observations support the previous assertion that B. balsamifera extract causes not only a reduction in crystal aggregation but also affects the size and morphology of crystals. This extract seems to promote the formation of CODs over COMs, probably by affecting the properties of the crystallization medium. A reason for this could be the phytochemicals present in the extract, which, even at small concentrations, can alter the environment of the solution[38]. In other studies, such effects of solutions have been observed; for example, surface free energy reduction by Khella plant extract [34].

6. IC50 Inhibition Assay

The highest inhibition percentage in the aggregation assay was 63.61 ± 0.13% at 10.00 mg/ml of sodium citrate, and the lowest inhibition percentage was 0.00% for the distilled water control sample. The IC50 value of sodium citrate for inhibition in the aggregation assay was 2.68 mg/ml. The highest inhibition percentage in the aggregation assay was 72.19 ± 0.09% at 6 mg/ml of extract, and the lowest inhibition whereas it was 0.00% for the distilled water control sample. For Bluméa balsamifera extract in the aggregation assay, the IC50 value was 2.56 mg/ml. According to the inhibitory effects on calcium oxalate crystallization as evaluated by IC50 values, the IC50 value of 2.68 mg/ml for sodium citrate was higher when compared to that of the extract. IC50 was 2.56 mg/ml [Table]. The explanation for this is because sodium citrate can inhibit the formation of calcium oxalate stones through the formation of salts dissolved with oxalate ions. In the condensation stage of calcium oxalate stones, the effect of condensation inhibition is much lower [39].

This value is higher than that recorded[40], with the extract of Momordica charantia Linn. inhibited 30.00% of crystal aggregation concentration of 0.50 mg/ml, and the findings related [41], which revealed that 100.00% Ocimum gratissimum L. inhibited 62.07% of crystal aggregation. The findings related [42], revealed that Carmona microphylla L. was an inhibitor of calcium oxalate, having an IC50 value of 0.80 mg/ml in an aggregation test. Aggregation is an essential process in stone formation, whereby tiny crystals aggregate to form a group through strong chemical and/or electric forces, which then grow into a larger stone [43]. The Bluméa balsamifera had the highest percentage inhibition because the compound covered the surface of the crystals to avoid aggregation [44]. Saponins and terpenoids may prevent aggregation because they have strong chemical and electric forces, and also as a natural solvent, water has also been able to inhibit aggregation by and mucoprotein interaction, which is the most significant causative factor for the supersaturation of calcium oxalate crystals and hence, the onset of stone formation [30].

However, at concentrations 8.00 mg/ml and 10.00 mg/ml, the percentage inhibition was lower than 6.00 mg/ml because the extracts increase the formation of COD. COD is easily extracted through the renal tubules with no danger from the kidney stone [45]. The IC50 index for the extract (2.56 mg/ml) was lower than that for sodium oxalate (2.68 mg/ml) due to the predominant inhibitory effect being primarily on the growth phase [39]. The findings from testing for inhibition ability to form crystals of calcium oxalate in the three major phases, namely nucleation, growth, and aggregates, yielded IC50 values of 4.25, 2.99, and 2.56 mg/ml respectively. The experimental findings clearly show that a plant extract from a plant called "compassion plant" has the capacity to inhibit the formation of crystals calcium oxalate. It is also in accordance with the study[46] that indicated the capability of the extract of compassion plants to reduce the size of calcium oxalate crystals with an extract dosage of 0.50 mg/mL and 1.00 mg/ml.

The results of research have shown that the extract of compassion plants has potential as a raw material in products used to treat diseases, particularly those caused by kidney stones.

Conclusion

The comparative methodological analysis suggests that Blumea balsamifera has established substantial antiurolithiatic properties by virtue of multi-mechanism modulation in various phases of calcium oxalate stone genesis. The extract displayed the property to prevent nucleation, slow-down crystal growth, and prevent agglomeration, thus minimizing the development of pathologically significant stones. In addition, the morphological alteration from Calcium Oxalate Monohydrate (COM) to the less soluble and easily expellable Calcium Oxalate Di-hydrate (COD) reinforces the inherent advantage for natural urinary expulsion. The

diverse methods of extraction, namely Soxhlet ethanolic and hydro-alcoholic freeze-dried methods, have emerged as prime determinants in terms of phytochemical availability and potency, thus warranting extraction method-standardization for ultimate clinical applications. Conclusively, from the enumerative perquisite, there arises substantial rationale for the suggestion of Blumea balsamifera to act as a promising candidate for phyto-therapeutic management of kidney stones, thus warranting *in-vivo* studies and eventual validation.

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