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In vitro antilithiatic and antioxidant potential of ethanolic extract of *bauhinia variegata* leaves

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***Abstract*---**Calcium oxalate crystals are responsible for formation of urinary calculi. Traditional therapy based on plants and herbal resources have found beneficial for treatment of kidney stones. The present study aimed to screen antilithiatic activity and antioxidant potential of ethanolic extract of *Bauhinia variegate* leaves. The ethanolic extract of leaves was obtained through continuous heat extraction process and evaluated for the presence of secondary metabolites, total phenolic content and total flavonoid contents. The high phenolic content in leaves is attributable to the presence of phenol, flavonoid, and tannin which all have antioxidant properties, and same was evaluated and confirmed using nitric oxide scavenging method and assessing reducing power of extract. Antilithiatic potential of few secondary metabolites like triterpenoids was confirmed by evaluation calcium oxalate dissolving ability of plant's ethanolic extract. The urinary calculi dissolving ability was of extract was assessed *in vitro.* The overall findings confirmed the antilithiatic and antioxidant potential of ethanolic extract of *Bauhinia variegate* Leaves.

***Keywords*---**Antilithiatic activity, *Bauhinia variegate,* Kidney stone, antioxidant, Calcium Oxalate.

Introduction

For several thousand years, plants were used for food and medicinal purposes. In the world, there are approximately 250 000 higher plant species. The use of

herbal medicines continues to increase in developing countries in particular for the treatment of certain disease categories. The growth in economic affluence in Western countries over the last five decades has contributed to an increase in the occurrence of kidney stones in the population. It's estimated that up to 80% of urinary calculi are made of CaOx, which is a calcium oxalate compound. Surgery for kidney stones is becoming more rare because to ESWL, a procedure that has revolutionised urinary medicine and supplanted open renal surgical procedures as the gold standard for eliminating kidney stones **(Scales *et al.,* 2012)**. Numerous phytoconstituents included in herbal treatments are effective in treating urolithiasis in a variety of ways **(Khan *et al.,* 2016)**. Drugs used to disintegrate or assist in passing the stone have been linked to increased retention in patients undergoing herbal kidney stone treatment. An increase in diuretic activity is also required in order to increase the volume of fluid passing through the kidneys and to flush out the deposits that have accumulated in them **(Delfan *et al.,* 2015)**. In 2022, Kamal *et al.,* studied for the development of new secondary metabolites, the ameliorative activities of B. variegata are of particular interest. This study was carried out since there was a dearth of research on the antiproliferative capabilities of *B. variegata* at the time. In 2020, Sharma *et al.,* reviewed that Kachnar (Bauhinia variegata) is a flowering plant that may be found all throughout India and the rest of the world. Plant leaves were used in this study, and an methanolic extract of these leaves was the goal of the current research assessment of antilithiatic activity (in vitro) model by phytochemical investigation.

Materials and Methods

Plant Material

The leaves of *Bauhinia variegata* Linn. available locally were collected from Knowledge Park, Greater Noida. Leaves of plant was authenticated by Scientist 'E' & In-charge, Botanical Garden of Indian Republic, Noida and voucher specimens no. BSI/BGIR/1/TECH./2021/03 in the herbarium for future research purposes after being submitted.

Extract preparation

To achieve the appropriate particle size, the plant materials were gathered, "dried in the shade at room temperature, and then ground into a fine powder". Stored in air-tight containers after passing through a mesh size of 40. Each of these powdered substances was extracted repeatedly via Soxhlet extraction (Continuous heat extraction). It took seven days to extract the ethanol and water from a total of one kilogram of powdered medicines. To get the dry extract, the filtered extracts were subjected to a rotary evaporator with reduced pressure to evaporate the solvents. Dry extract yield was estimated, desiccators were utilised to store, and following investigations made use of the dry extract yield (Nirmal et al., 2012).

Pharmacognostic studies, physicochemical evaluation and phytochemical screening

The plant's form, size, surface properties, texture, colour, consistency, odour, and

taste were all scrutinised up close and in detail under a stereomicroscope (a powerful microscope) **(Dave *et al.*, 2010).** A thin hand segment of leaves's midrib and lamina area was prepared for microscopic examination. Safranine had discoloured this portion. The dried leaf powder was utilised to examine tiny details **(Dave *et al.*, 2010)**.

Stomatal index

The number of stomata divided by the total number of epidermal cells yields the percentage of epidermal cells that have stomata in the epidermis **(Royer *et al.*, 2001)**.

$$Stomatalindex = \frac{s}{s+E} x100$$

Where, S = Number of stomata per unit area E = Number of epidermal cells in the same unit area. Stomatal count measurements were seen at extreme magnification (45 X). It was necessary to count the epidermal cells as well as the stomata. Stomatal index was determined using the algorithm above based on these variables **(Royer *et al.*, 2001)**.

Determination of foreign organic matter

Spread out a thin layer of air-dried, coarsely ground, and weighed 100g. An unassisted eye or a 6x lens was used to view the sample drug and the foreign organic matter was manually separated and weighed. The weight of the medicine ingested was used to compute the proportion of foreign organic materials **(Xu and Mo, 2010)**.

Determination of moisture content (loss on drying)

In a tarred evaporating plate, 10 grammes of precisely weighed coarsely powdered medicine were inserted. Weighed after drying for five hours at 105°C, the dish was ready to serve. The drying and weighing process was repeated every hour until the difference between the two consecutive weights was less than 0.25 percent. The amount of powder that was taken into account while calculating the drying losses **(Nielsen, 2010)**.

Determination of swelling index

When 1g of plant material is subjected to specific circumstances, the swelling index is the volume in ml that the plant material takes up. In the 25ml glass stoppered measuring cylinder with 25ml water, 1g of the crude powder was weighed and thoroughly shaken every 10 minutes for an hour. For three hours, it was kept at room temperature. In millilitres, the volume filled by the plant material, which includes the sticky mucilage. When calculating the weight, we used the dry weight **(Deyong *et al.*, 2012)**.

Determination of extractive values

During the first six hours of maceration, the air dried coarsely powdered drug was

shaken regularly and left to stand for 18 hours in a closed flask with 100 mL of increasing polarity solvents (petroleum ethers, benzene, chloroforms, ethyl acetates, ethanols, and water). After that, the ethanol was swiftly filtered out to prevent any loss. Dry the filtrate for about 25 minutes at 105°C in a shallow dish with a flat bottom that has been tarred **(Sluiter *et al.*, 2005)**.

Determination of total ash

It was burned at a temperature not exceeding 450 °C to remove carbon before cooling and weighing an accurately weighed 3 g of air-dried coarsely powdered medication. The amount of ash in the medication was determined by comparing it to an air dried sample **(Momin and Kadam, 2011)**.

Determination of acid insoluble ash

Insoluble matter was collected using an ash-free filter paper and then washed with hot water, dried and burned at a temperature not exceeding 450°C, allowed to cool in desiccators, and then weighted after a 5-minute boiling of 2 M hydrochloric acid with the ash was carried out, according to the manufacturer. Based on the air dried medication, the proportion of acid insoluble ash was determined **(Momin and Kadam, 2011)**.

Determination of water soluble ash

Weighed and dried in desiccators, wetted with hot water, dried and re-ignited at a temperature no higher than 450°C for 15 minutes, and then chilled and stored in desiccators. Insoluble components were subtracted from the overall ash weight to arrive at the water-soluble ash content. With the air-dried medication in mind, the proportion of water-soluble ash was determined **(Momin and Kadam, 2011)**.

Phytochemical screening.

Separate methanolic and aqueous extracts of the plant components were produced and tested for their chemical contents. Aqueous and methanol extracts and powdered specimens were subjected to routine chemical testing to determine their contents **(Roy *et al.*, 2010)**. Test for carbohydrates (molish test, fehling test, benedicts test), test for alkaloids (mayer's reagents, dragendorffs reagents), test for flavonoids, test for steroids (salkowaski test, libbbermann burchatd test), test for tannins, test for protein (mellon's reagents, ninhydrin test), test for triterpenoids, test for glycosides (keller-killani test), test for saponins (foam test) were performed using procedures as reported in various literature.

Determination of total phenolic content

Folin-test, Ciocalteu's which uses gallic acid as a standard (1mg/ml), was used to quantify the total phenol level. Extracts of plants were combined with Folin-solution Ciocalteu's (FCR) and sodium carbonate solution (sodium carbonate solution, 7 percent) for 5 minutes before being diluted 1:10 v/v with water. Distilled water was used to fill the tubes to their final capacity, and they were left to stand for 90 minutes at room temperature. A spectrophotometer was used to test the absorbance of the sample against a blank at 750 nm. As a means of

ensuring accuracy, a three-run experiment was conducted and the phenol content (Gallic acid equivalent, GAE) per gramme of dry weight was calculated as the mean and standard deviation **(Ainsworth and Gillespie, 2007)**.

Determination of total flavonoid content

An aluminium chloride technique utilising quercetin as a reference was used to assess the total flavonoid concentration. A volumetric flask was filled with 1 millilitre of the test sample and 4 millilitres of water (10 ml volume). Following the incubation period of five minutes, the addition of 0.3 percent sodium nitrite and 0.3 percent aluminium chloride was performed. After six minutes of incubation at room temperature with sodium hydroxide, it was added to the reaction mixture to complete the reaction (1 M). The final amount was soon raised to 10 ml using just purified water as a source. It was necessary to compare the sample's 510 nm absorbance to that of a control sample. There were three replications of this study for accuracy, and the flavonoid content (Quercetin equivalent, QE) per gramme of dry weight was given as the mean and standard deviation **(Lin and Tang, 2007)**.

Thin layer chromatography

For the purpose of discovering the number of chemicals that support the chemical test, the methanolic extract of powdered leaves of *Bauhinia variegata* Linn. was exposed to thin layer chromatography. Silica Gel G is the adsorbent used in TLC. The coated plates were heated to 110-120°C for one hour in a hot air oven after drying in the air for around 30 minutes to remove water vapour for activation. TLC uses a sample volume of 1 to 5µl, and the concentration of the sample falls within this range. The TLC spot size was not exceed 5 mm at a concentration of 0.01% to 1.00%. The capillary tube was used to spot the sample from the plate's bottom edge at a distance of 1.5 cm. TLC was developed in a chromatographic rectangular chamber via ascending development technique by placing TLC plate at an angle of 45°C along the saturated chamber in order to ensure a good development. Following solvent transfer, the plate is taken from the chamber, dried, and components' positions are determined in a variety of methods. Iodine was used to identify spots on plates after they had been removed from the developing chamber and dried in the air.

***In vitro* antioxidant studies on the extracts**
Nitric Oxide Scavenging

Sreejayan and Rao's modified approach was used to measure the activity. 4 ml of ethanolic extract of *Bauhinia variegata* Linn. at various concentrations (ranging from 100 to 500 g/ml) were incubated for 2 hours at 27 °C with 1 ml of SNP solution before being analysed (5mM). Two millilitres of the incubation solution were withdrawn, and 1.2 millilitres of Griess reagent were added to dilute it (1 percent Sulfanilamide in 5 percent H_3PO_4 and 0.1 percent naphthylethylene diamine dihydrochloride). Chromophore absorbance was measured at 550 nanometers and compared to that of ascorbic acid as a reference standard **(Duh *et al.*, 2004)**.

Lipid Peroxidation Determination:

A 10 percent concentration of egg homogenate in a solution of 1.15 percent potassium chloride was prepared. Test tubes holding the extract/standard and two millilitres of distilled water were combined (100-500 g). The lipid peroxidation process was initiated with the addition of 0.5 ml of FeSO4 (0.07M) to the mixture and allowed to run for 30 minutes after that. After that, 0.5 ml of 0.8 percent TBA (w/v) and 0.5 percent TCA (w/v) were added, vortexed, and then heated for 60 minutes in boiling water with 11 percent sodium dodecyle sulphate was used as a final step. Centrifuged at 3000 rpm for 10 minutes, each tube contained 5.0ml of 1-butanol. The organic top layer's absorbance was measured at 532 nm. In the control experiment, 1.0 mL of deionized water was used in lieu of the extract, and the results were similar **(Moore and Roberts, 1998)**.

***In-vitro* antilithiatic studies on the extracts**
Inhibition of CaOx crystallization by turbidimetric method

Inhibition of CaOx crystallization was studied in a solution containing calcium chloride dehydrate (7.5 mmol/l) and sodium oxalate (2.5 mmol/l), these solutions were prepared using sodium chloride 0.15 mol/l as solvent **(Bensatal and Ouahrani, 2008).**

Study without inhibitor

When 7.5 ml of sodium oxalate solution is introduced to 7.5 ml of calcium chloride solution at 37°C with magnetic stirring, the crystallisation process commences. An ultraviolet–visible spectrophotometer measured the solution's optical density (OD) after 30 minutes at a wavelength of 620 nm (Shimadzu 1240) **(Bensatal and Ouahrani, 2008)**.

Study with inhibitor

0.0425 g of the dry residue is dissolved in 1 percent alcohol and 60 ml of 1 percent ethanol are used to make the inhibitor (100 percent). From this inhibitor, we have prepared numerous diluted inhibitor solutions of 1%, 10%, 25%, 50%, and 75% using solvent sodium chloride (0.15 M). When the sodium oxalate solution was added at 37°C with magnetic stirring, crystallisation began. The room was kept at a constant 37 degrees Celsius. After 30 minutes, the solution's OD was measured at 620 nm. For each experiment, three replicates were taken **(Bensatal and Ouahrani, 2008)**. "The percentage inhibition I (%) produced by the herb extract was calculated as follows":

% inhibition = (Absorbance of Control−Absorbance of Test)/Absorbance of Control

Where, Absorbance test: Absorbance in the presence of inhibitor (extract), absorbance control: Absorbance without inhibitor (control negative)

Dissolution of CaOx by Gravimetric Method
Preparation of a precipitate of CaOx

The preparation of CaOx precipitate based on the references cited by Johannes et al. and Anamarija et al. with a slight modification **(Atanassova *et al.*, 2000)**. A volume of 2 ml of 2.5 mmol/l sodium oxalate pH 7 (at 37°C) and 7.5 mmol/l calcium chloride pH 6 (at 37°C) was mixed in centrifuge tubes. At 37°C, the CaOx was allowed to precipitate for 30 minutes before being dissolved in water. Then, the tubes were centrifuged at 6000 rpm using a centrifuge for 16 min, the supernatant was removed. Then, the precipitates were washed by adding 4 ml of distilled water and centrifuged again as described above. Finally, the supernatant was removed; the tubes were oven-dried at 70°C for 50 min and weighted again to calculate the mass of the precipitates **(Atanassova *et al.*, 2000)**.

Ability of the saponins fraction to dissolve the CaOx precipitate

We evaluated the effectiveness of the saponins fraction in vitro on CaOx dissolution using the method illustrated by Yachi et al. 2018 and Kachkoul et al. 2016, with a slight modification **(Chaudhary *et al.*, 2010)**. A volume of 4 ml of the saponins fraction at different concentrations (1%, 10%, 25%, 75%, and 100%) were added to the CaOx precipitates and the tubes were incubated for 30 min at 37°C. After centrifugation, the tubes and precipitates were cleaned, dried, and weighed as previously mentioned. For each experiment, three replicates were taken. The dissolving activity (A %) was calculated with the following formula:

$$A\% = \frac{Wintial - Wfinal}{Winitial}$$

Where $W_{initial}$ is weight of the precipitate before the incubation with the saponins fraction (negative control). W_{final} is the weight of the precipitate after the incubation with the saponins fraction.

Statistical Analysis

ANOVA and Turkey's multiple comparison test were used to compare the results of three separate experiments (each in triplicate) and the inhibitory activity with and without the inhibitor at varied doses. Significant values were those with a P value less than 0.05. GraphPad Prism 7.00 was used for statistical analysis.

Results and Discussions

The plant leaf was evaluated for its macroscopic (Figure 1), microscopic and pharmacognostic properties and various evaluated parameters are represented in table 1. It shows dorsi-ventral nature; more densely covered upper epidermis with cuticle than lower epidermis and made up of thin walled tangentially elongated rectangular cells. Mesophyll in the lamina shows the presence of 2-3 layers of palisade parenchyma below the upper epidermis and spongy parenchyma above the lower epidermis. Midrib contains 'U'- shape well developed vascular bundle at the centre surrounded by sclerenchyma (pericyclic lignified fibrous tissue in a band). Vascular bundle shows the presence of the xylem at the upper side and

phloem at the lower side and well developed collenchyma below the upper epidermis and above the lower epidermis with scattered prismatic calcium oxalate crystals. TS of the leaf also show the presence of unicellular, 3-5 celled multicellular uniseriate- cuticlerised covering trichomes as well as unicellular sessile and unicellular head with unicellular stalcked glandular trichomes. Trichomes are more prominent on the lower epidermis than upper epidermis.

Total ash content, Water soluble and acid insoluble ash content were determined. The crude powder of P. longifolia leaves included 0.4 percent acid insoluble ash and 2.5 percent water soluble ash. Silica is less prevalent in the leaves of *P. longifolia* because of a low total and acid insoluble ash, as well as a low water-soluble ash. Because the dry powder of *P. longifolia* leaves had just 12 percent moisture, it would hinder the formation of germs and fungus.

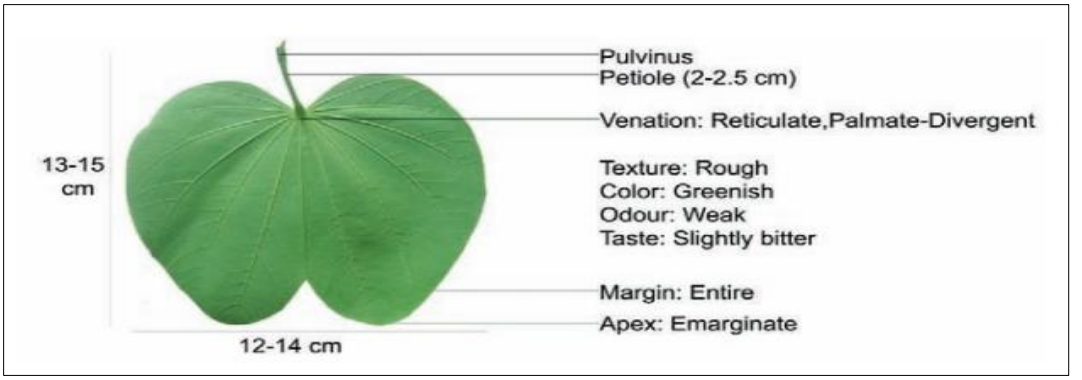

Figure 1. Organoleptic evaluation of Leaf of *Bauhinia variegata* Linn

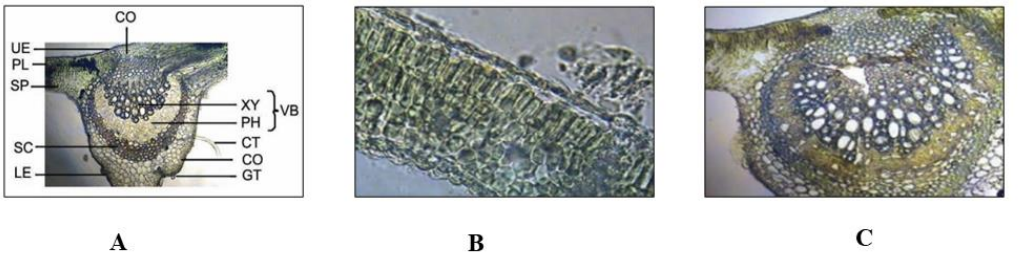

Figure 2. A. TS of Bauhinia variegata Linn. leaf. UE: Upper epidermis; PL Palisade; SP Spongy parenchyma; SC: Sclerenchyma; LE: Lower epidermis; XY: Xylem; PH: Phloem; VB: Vascular bundle; CT: Covering trichome; CO: Collenchyma; GT: Glandular trichome; B. Dorsi-ventral lamina showing 2 layers of palisade & spongy parenchyma (40X); C. Section stained with IKI soin showing starch

Figure 2 depicts the results of powder microscopy, which revealed multicellular uniseriate trichomes measuring 142.8-199.92 m in length, 57.12 m in diameter, prismatic calcium oxalate crystals, lignified annular and spiral xylem arteries, and an area of epidermal cells with anomocytic stomata.

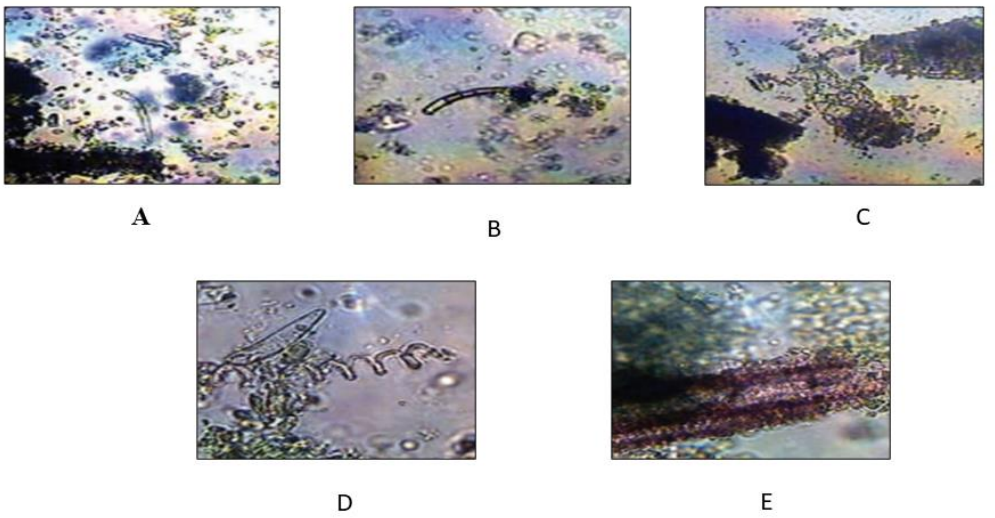

Figure 3. Powder microscopy of leaves of *Bauhinia variegata* Linn
A. Unicellular trichome; B. Multicellular trichome; C. Anomocytic stomata D. Spiral vessel & unicellular trichome; E. Lignified annular xylem vessels

Table 1- Physicochemical characterization of leaves of *Bauhinia variegata* Linn

PARAMETERS	SAMPLE	VALUE
Stomatal Index	Leaves	5.6 %
Palisade ratio	Leaves	4.8
Vein-islet number	Leaves	5.8
Vein termination number	Leaves	3.7
Loss on drying	crude powder	6.27 % (w/w)
Total ash	crude powder	9.42 % (w/w)
Acid insoluble ash	crude powder	5.72 % (w/w)
Water soluble ash	crude powder	3.3 % (w/w)
Alcohol soluble extractive	crude powder	18.23 % (w/w)
pH	ethanolic extract	5.51

The bioactive chemicals usually found in plant components was be determined using phytochemical screening (Table 2). The high phenolic content in leaves is attributable to the presence of phenol, flavonoid, and tannin which all have antioxidant properties. Alkaloids, flavonoids, tannin, saponin and triterpenoids were found in methanol, whereas protein, flavonoids, tannin and triterpenoids were found in ethanol. Ethanol extract of leaves yielded the good results (18.23

percent of yield value). In prepared extracts, carbohydrate (sugar and starch) and protein are present, but lipids are present in very little amounts. The total phenol content in the leaves ethanolic ether extract was 156.64±0.04 mg GAE/g and total flavonoid content was calculated as 88.18±0.25 mg GAE/g according to the screening of plant parts.

Table 2. Preliminary photochemical analysis of *Bauhinia variegata* Linn

Name of Tests	Ethanolic Extract
Steroids	+ve
Carbohydrate	-ve
Flavonoids	+ve
Triterpenoids	+ve
Glycosides	-ve
Alkaloids	+ve
Saponins	-ve
Tannins”	+ve

(+) = Present; (-) = Absent.

The most effective solvent for the TLC of *Bauhinia variegata* Linn. leaves was found to be n-hexane: ethyl acetate: few drops of formic acid (7:3:2-3 drops). TLC plate (Figure 4) showing the 3 spots with different colour with different R_f value in 0.5% vanillin in dil. H_2SO_4 in solvent system (n-hexane: ethyl acetate: few drops of formic acid) in a ratio of 7:3: 2-3 drops of formic acid, which indicates the presence of 3 compounds as detected by iodine vapours (Table 3).

Table 3. TLC of Ethanolic extract of leaves of *Bauhinia variegata* Linn.

Extract	Solvent System	Number of spots	Colour of Spots	R_f value
Ethanolic Extract	n-hexane: ethyl acetate: few drops of formic acid (7:3:2-3 drops)	03	Light yellow	0.51
			Yellowish red	0.44
			Yellowish red	0.36

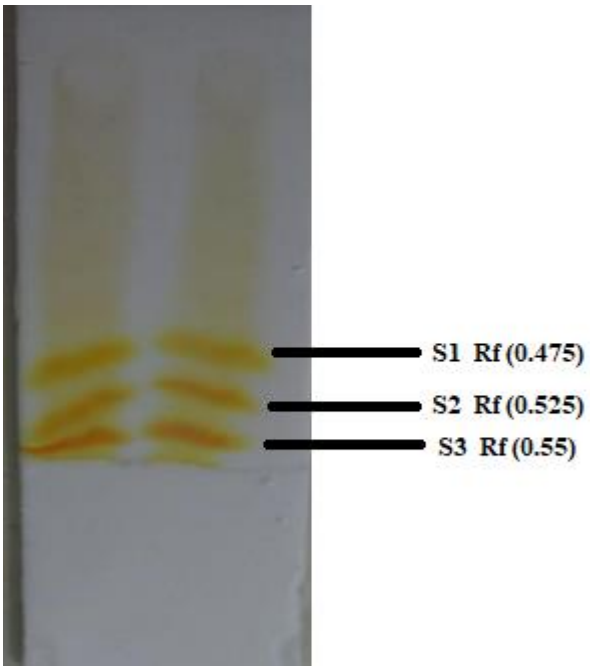

Figure 4. Chromatogram of TLC of ethanolic extract of leaves of *Bauhinia variegata* Linn.

The nitric oxide scavenging assay, and the in vitro lipid peroxidation assay were used to evaluate the antioxidant activity of MEPLS and results are displayed in figure 5. The fact that the aqueous extract of PL had a lower IC_{50} value (187 g/ml) than the methanolic extract (627 g/ml) in a Nitric oxide scavenging experiment suggests that the aqueous extract's antioxidant capacity is stronger than the methanolic extract's. Aqueous extract, on the other hand, has a lesser antioxidant effectiveness (IC_{50}-94.20 g/ml) than normal Ascorbic Acid (Table 4).

Table 4: Assay for the determination of nitric oxide scavenging

S. no.	Conc. µg/ml	% of inhibition		IC_{50} Value (µg/ml)	
		Ascorbic Acid (n=3)	EEBVL (n=3)	Ascorbic Acid	EEBVL
1	200	80.32±1.02	83.32±1.10	95.23	684
2	400	36.27±1.02	88.27±1.34		
3	600	43.35±1.01	87.35±1.30		
4	800	60.86±1.02	88.86±1.54		
5	1000	68.89±1.27	89.89±1.27		

Values are mean ± SEM of 3 replicates

Table 5: Assay for the Determination of Reducing Power

S. no.	Concentration µg/ml	% of inhibition	
		Ascorbic Acid (n=3)	EEBVL (n=3)
1	200	0.14 ± 0.02	1.18 ± 0.03
2	400	0.18 ± 0.02	1.56 ± 0.02
3	600	0.37 ± 0.03	1.63 ± 0.02
4	800	0.60 ± 0.01	1.73 ± 0.01
5	1000	1.02 ± 0.02	1.76 ± 0.03

Values are mean ± SEM of 3 replicates

Inhibition of CaOx Crystallization by Turbidimetric Method

CaOx crystallisation was inhibited to varying degrees by leaf extract at various doses, as shown in Table 8.10. We will take into consideration only the concentrations 10%, 25%, and 50% since these shines which give reliable results. The percent inhibition was calculated using the above mentioned formula. Different quantities of *Bauhinia variegata* Linn. leaf extract were shown to affect the crystallisation of CaOx in Figure 8.8. The suppression of nucleation increased as the concentration of the extract increased. At a 50 percent concentration, there is a 55.23 ± 1.23% percent inhibitory maximum.

Dissolution of CaOx by Gravimetric Method

After several tests of the concentrations, we are chosen the concentrations illustrated in Table 8.11. The results indicate the change of dissolving activity as a function of the concentrations of the saponins fraction, as shown in Figure 8.9.

Table 6: Variation of percentage inhibition, in terms of to the saponins fraction at different concentrations

CI (%)	10 µg/ml (n=3)	25 µg/ml (n=3)	50 µg/ml (n=3)	100 µg/ml (n=3)
OD (620 nm)	0.401±0.02	0.425±0.02	0.476±0.04	0.510±0.02
I (%)	6.05±0.03	22.56±0.02	54.20±0.05	66.48±0.02
CV (%)	2.78	8.56	5.85	10.95

CI (%) concentration of inhibitor, OD optical density at 620 nm, "Cv (%) coefficient of variation of OD, I (%) percentage of inhibition. Density values are expressed as mean ± standard error of the mean. ****P<0.0001 with F (2.6) = 94.14. Percentage inhibition values are expressed as mean ± standard error of the mean. ****P<0.0001 with" F (3.8) = 194.1.

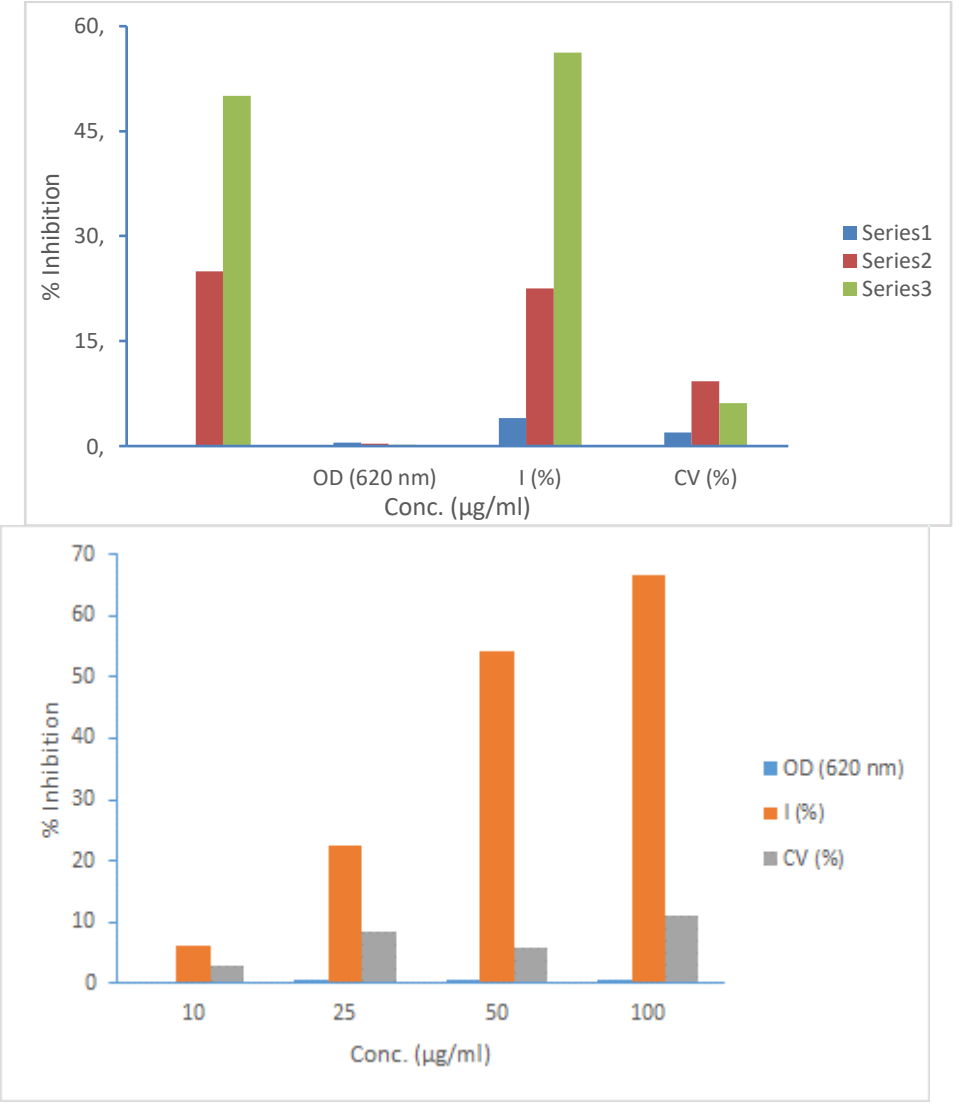

Figure 5: Effect of the extract of the extract leaves of *Bauhinia variegata* Linn. on the crystallization of calcium oxalate

Table 7: Variation of dissolving activity in terms of the saponins fraction at different concentrations

C_{sf} (%)	10 µg/ml (n=3)	25 µg/ml (n=3)	75 µg/ml (n=3)	100 µg/ml (n=3)
W_{CaOX} (mg)	9.76±0.03	9.24±0.02	5.01±0.01	3.26±0.04
A (%)	35.08±0.02	41.02±0.05	51.64±0.03	85.85 ±0.07
CV (%)	6.85	5.34	5.84	7.80

Csf (%) concentration of saponins fraction, WCaOX (mg) weight of calcium oxalate, Cv (%) coefficient of variation of weight, (A %) the dissolving activity. "Values of weigh are expressed as mean±standard error of mean. ****P<0.0001 with F (4.10) = 145, 2. Dissolving activity values are expressed as mean±standard error of mean. **** P<0.0001 with" F (4.10) = 2585

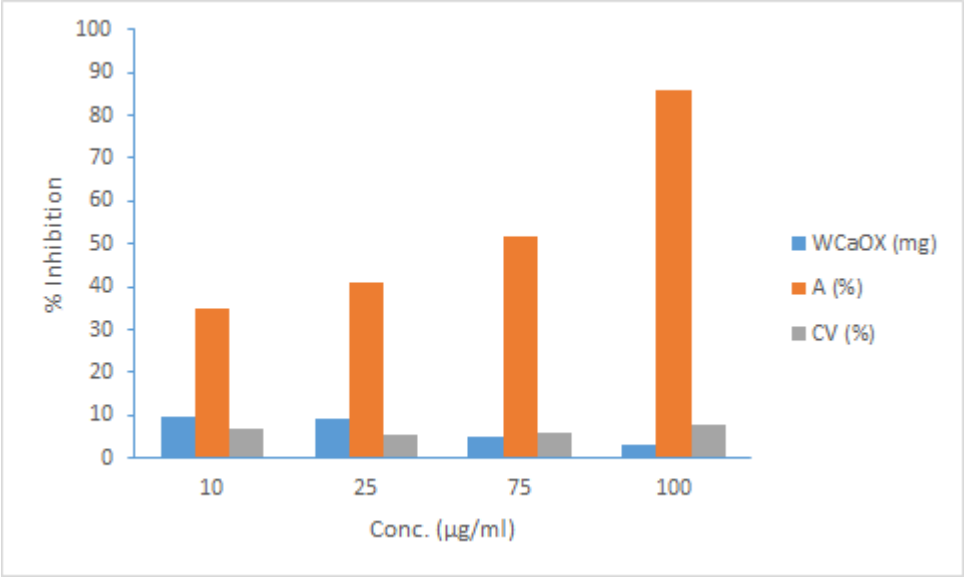

Figure 6: Effect of the different concentration of the extract leaves of *Bauhinia variegata* Linn. on the dissolving of calcium oxalate

All three methods of testing for antioxidant activity in the laboratory were utilised to determine whether EEBVL had any antioxidant activity. These tests included the nitric oxide scavenging test, the reducing power assay, and the in vitro reducing power assay.

The fact that the aqueous extract of PL had a lower IC_{50} value (193 g/ml) than the ethanolic extract (684 g/ml) in a Nitric oxide scavenging experiment suggests that the aqueous extract's antioxidant capacity is stronger than the ethanolic extract's. Aqueous extract, on the other hand, has a lesser antioxidant effectiveness (IC_{50}-95.23 g/ml) than normal ascorbic acid. (See Table 8.8)

A further test is performed to determine the antioxidant activity of the extracts, which is the reducing power test. In this method, a higher absorbance value suggests a greater reductive potential for the chemicals being evaluated, and vice versa. Enhanced reducing power is an essential antioxidant index because substances with high reducing power are electron donors, allowing them to act as primary or secondary antioxidants. In this scenario, the ethanolic extract outperforms the aqueous extract by providing an additional benefit to the patient. It was discovered that the reducing power of the testing substance rose as the concentration of the material grew (Table 8.9).

Treatment with antioxidants prevents the buildup of calcium oxalate crystals in the kidneys due to a lack of adequate protection against oxidative stress. Calcium

oxalate kidney deposits can be prevented if extracts have antioxidant activity. Metal chelating and hydroxyl radical scavenging activities of the extracts' polyphenols and tannins have been documented.

CaOx crystallisation was inhibited to varying degrees by leaf extract at various doses, as shown in Table 8.10. We will take into consideration only the concentrations 10%, 25%, and 50% since these shines which give reliable results. Figure 8.8 depicts the effect of different *Bauhina variegata* Linn. concentrations on the crystallisation of CaOx. (Figure 8.8) Effect of *Bauhina variegata* Linn. concentrations on the crystallisation of CaOx The extract's ability to prevent nucleation increased in proportion to the concentration of the extract. The greatest amount of inhibition achieved at a concentration of 100 percent is 66.48 percent 0.02 percent. After several tests of the concentrations, we are chosen the concentrations illustrated in Table 8.11. The results indicate the change of dissolving activity as a function of the concentrations of the saponins fraction, as shown in Figure 8.9.

Conclusion

Bauhina variegata Linn. Leaves were used in this study, and an ethanolic extract of these leaves was the goal of the current research assessment of antilithiatic activity (in vitro) model by phytochemical investigation. Ethanolic extract was used to extract screen the plant's leaves for presence of various phytochemicals, which revealed that flavonoids and phenolic compounds were the primary active ingredients. Using iodine vapour as a detection reagent, TLC investigations on an ethanolic extract of *Bauhina variegata* Linn. leaves indicate three spots of various colours and R_f values in the solvent system. The Rf values of all three spots were calculated as 0.475, 0.525 and 0.55 respectively. The literature survey reveals that the leaves of *Bauhina variegata* Linn. contain, taraxerol, Quercetin, rutin, lupeol, & kaempferol which are responsible for Antilithiatic activity. Phytochemical screening shows that the presence of triterpenoids which responsible for Antilithiatic activity. According to WHO recommendations for physicochemical and phytochemical analysis, the leaves of *Bauhina variegata* Linn. were assessed in this study for the first time. Having a common monograph for all of the drug's quality control characteristics makes it easier to ensure the purity and validity of various formulations. It may be used to detect and identify the chemical components in the leaf, as well as serve as a foundation for future study.

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