

**How to Cite:**

Rai, K., Mishra, H., & Alex, A. (2022). In vitro antilithiatic and antioxidant potential of methanolic extract of polyalthia longifolia leaves. *International Journal of Health Sciences*, 6(S6), 2582–2598. <https://doi.org/10.53730/ijhs.v6nS6.10041>

# **In vitro antilithiatic and antioxidant potential of methanolic extract of polyalthia longifolia 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 and antioxidant potential of Polyalthia Longifolia leaves methanolic extract. The extract was 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 assay and lipid peroxidation method. Antilithiatic potential of few secondary metabolites like triterpenoids was confirmed by evaluation calcium oxalate dissolving ability of plant's methanolic extract. The urinary calculi dissolving ability was of extract was assessed in vitro. The overall findings confirmed the antilithiatic and antioxidant potential of methanolic extract of Polyalthia Longifolia Leaves.

**Keywords**---urolithic activity, polyalthia longifolia, kidney stone, antioxidant, peroxidation.

**Introduction**

Kidney stones are becoming more common in Western nations as a result of economic growth over the past five decades. Most urinary calculi are formed by calcium oxalate (CaOx), which accounts for up to 80% of the examined stones. Extracorporeal Shock Wave Lithotripsy (ESWL), which has transformed urology

and has replaced open renal surgery as the gold standard for removing kidney stones, has made open surgery for nephrolithiasis extremely uncommon (Scales et al., 2012). Urolithiasis, despite the use of sophisticated tools, study, and so on, has not been explained. Modern medicine's treatments are both expensive and difficult to buy for the poor. Actually, contemporary medicine does not have a medication that can dissolve the stone, thus doctors must resort to alternate methods of treatment (Khan et al., 2016). Drugs used to dissolve or help pass the stone are associated with additional retention in herbal kidney stone therapy. It is also vital to have a diuretic activity in order to increase the quantity of fluid going through the kidneys and to remove the deposits that have built up (Delfan et al., 2015). To have lithotripsy is to have the prepared stones shatter and disintegrate or dissolve. By lowering salt saturation and preventing crystal formation, certain medications increase urine volume (Delfan et al., 2015). A species of *Polyalthia longifolia* (Sonn.) As an avenue tree in Sri Lanka and India, Thwaites is a tall, beautiful tree with dangling linear leaves that is revered for its religious importance. *Polyalthia longifolia* (Sonn.) Thwaites. 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 *Polyalthia longifolia* (Sonn.) Thwaites 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./2021/04 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. It took seven days to extract the methanol 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 *P. longifolia*'s midrib and lamina area was prepared for microscopic examination. Safranin 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).

$$\text{Stomatal index} = \frac{S}{S + E} \times 100$$

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 *Polyalthia longifolia* (Sonn.) Thwaites. 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 methanolic extract of *Polyalthia longifolia* (Sonn.) (MEPLS) 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<sub>3</sub>PO<sub>4</sub> 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 FeSO<sub>4</sub> (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 dodecyl 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”:

$$\% \text{ inhibition} = (\text{Absorbance of Control} - \text{Absorbance of Test}) / \text{Absorbance of Control}$$

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:

$$A\% = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}}$$

Where  $W_{\text{initial}}$  is weight of the precipitate before the incubation with the saponins fraction (negative control).  $W_{\text{final}}$  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, microscopic and pharmacognostic properties and various evaluated parameters are represented in table 1. Long, straight trunks, thick crowns, and hairless twigs distinguish the 25-meter-tall *P. longifolia* tree. Wavy edges, 11–22 x 2–5 cm ovate-oblong to thin lance-shaped leaves with a 4–8 mm long petiole are the characteristics of the leaves. Figure 1 depicts a leaf's microscopic parameters. The leaf is located on the backside. The epidermis of both the upper and lower layers is one layer thick. The palisade was made up of two separate layers. Calcium oxalate crystals are discovered. Despite its modest size, the mesophyll has a lot of air gaps. The collenchyma has two to three layers. The palisade and lower midrib contain oil glands (Figs. 8.2). A higher number of trichomes were seen on the lower epidermis. Lower epidermis included anomocytic stomata. The guard cells were much bigger than the stomata, which were surrounded by 4–5 smaller subsidiary cells, indicating that the stomata were anomocytic. Sclerenchymatous ring covers a vast vascular bundle. *Plantago longifolia* leaves were green in colour and had a unique aroma when ground into a fine powder. The powder characteristics are

shown in Figure 8.3. Spiral xylem vessels and the multicellular blunt-tip trichomes found in the skin, as well as the mesophyll and epidermis in the epidermis, were shown to be crucial in the powder analysis.

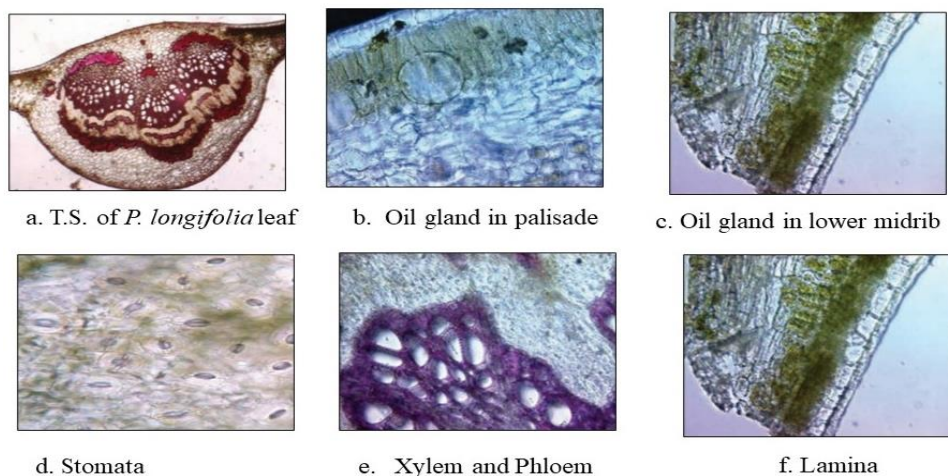


Figure 1. Various microscopic parameters of leaves of *P. longifolia*

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.

Table 1  
Physicochemical characterization of leaves of *P. longifolia*

PARAMETERS	SAMPLE	VALUE
Stomatal Index	Leaves	15.46 %
Loss on drying	crude powder	12 % (w/w)
Total ash	crude powder	5.8 % (w/w)
Acid insoluble ash	crude powder	0.4 % (w/w)
Water soluble ash	crude powder	2.5 % (w/w)
Petroleum ether soluble extractive	crude powder	4.60 % (w/w)
Alcohol soluble extractive	crude powder	20.11% (w/w)
Methanol soluble extractive	crude powder	20.16% (w/w)
Water soluble extractive	crude powder	15.25% (w/w)
pH	methanolic 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. Methanol extract of leaves yielded the good results (20.1 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 methanolic ether extract was  $82.67 \pm 0.04$  mg GAE/g, whereas it was  $114.28 \pm 0.04$  mg GAE/g in the leaves methanolic extract, according to the screening of plant parts. The total phenolic content and total flavonoid content was determined and represented in figure 2 and 3 respectively.

Table 2  
Preliminary photochemical analysis of *P. longifolia*

Name of Tests	Methanolic Extract
Steroids	-ve
Carbohydrate	-ve
Flavonoids	+ve
Triterpenoids	+ve
Glycosides	-ve
Alkaloids	-ve
Saponins	+ve
Tannins"	-ve

(+) = Present; (-) = Absent

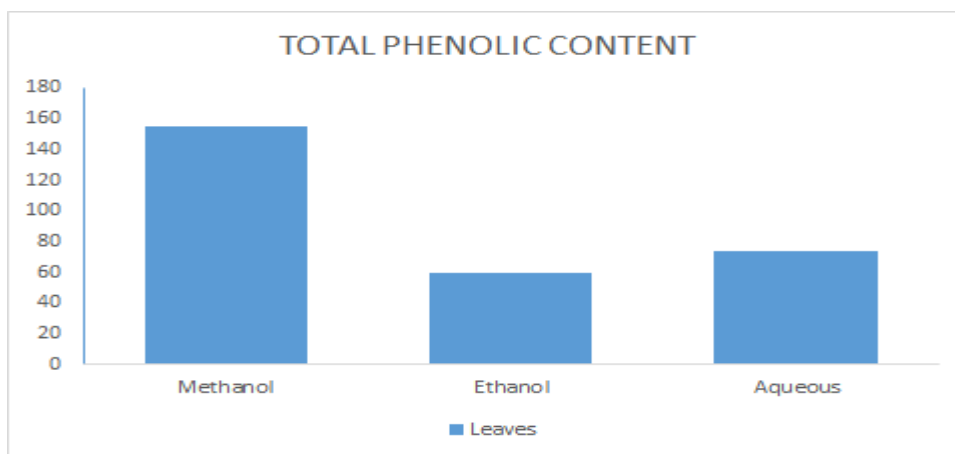


Figure 2. Total phenolic content in various solvents of plant parts of *Polyalthia longifolia* (Sonn.) Thwaites

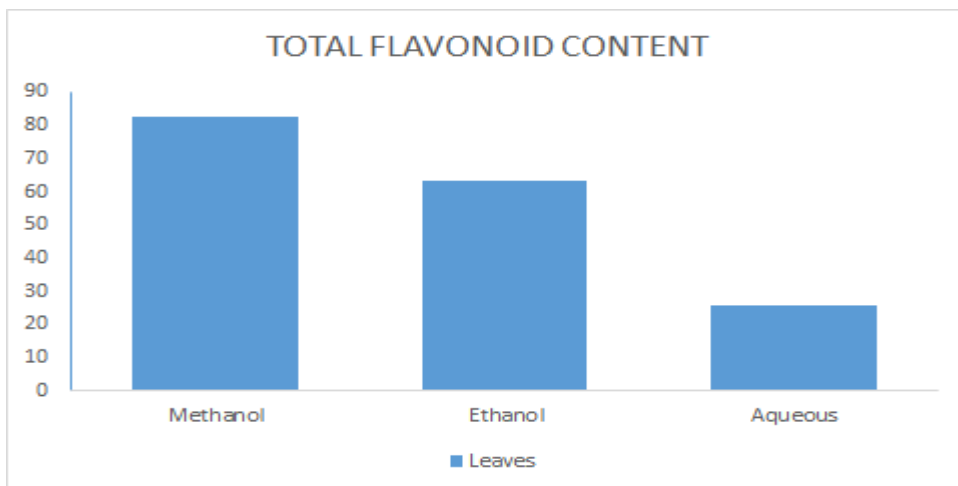


Figure 3. Total flavonoid content in different solvents of plant parts of *Polyalthia longifolia* (Sonn.) Thwaites

The most effective solvent for the TLC of *Polyalthia longifolia* (Sonn.) Thwaites. leaves was found to be Methanol: Glacial Acetic Acid: Formic Acid: Water (3: 0.9: 0.9: 0.5). *Polyalthia longifolia* (Sonn.) Thwaites. leaf care. Iodine vapour is used to identify four compounds with various  $R_f$  values in different colours in methanolic extracts, which indicates the presence of two compounds (Table 3). The developed TLC plate picture with two marked spots is represented in figure 4.

Table 3

TLC of methanolic extract of leaves of *Polyalthia longifolia* (Sonn.) Thwaites

Extract	Solvent System	Number of spots	Colour of Spots	$R_f$ value
Methanolic Extract	Methanol: Glacial Acetic Acid: Formic Acid: Water (3:0.9:0.9:0.5)	02	Dark Yellow	0.57 0.65

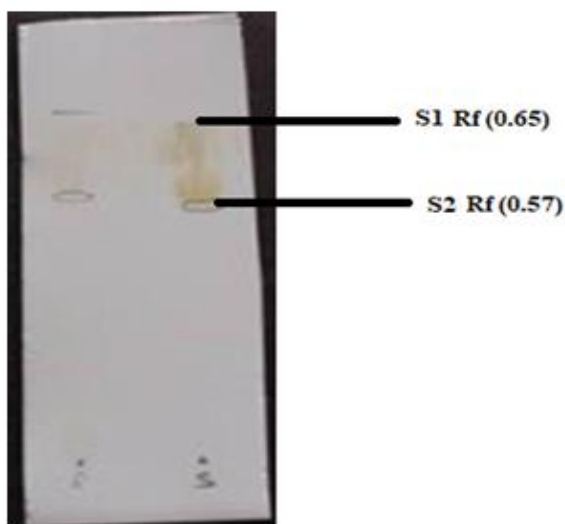


Figure 4. Developed TLC plate of methanolic extract of *Polyalthia longifolia* (Sonn.) Thwaites

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  
Nitric oxide scavenging assay determination

S. no.	Conc. $\mu\text{g/ml}$	% of inhibition		$IC_{50}$ Value ( $\mu\text{g/ml}$ )	
		Ascorbic Acid (n=3)	MEPLS (n=3)	Ascorbic Acid	MEPLS
1	200	83.32 $\pm$ 1.10	19.19 $\pm$ 1.77	94.20	627
2	400	88.27 $\pm$ 1.34	36.63 $\pm$ 1.06		
3	600	87.35 $\pm$ 1.30	43.22 $\pm$ 1.49		
4	800	88.86 $\pm$ 1.54	60.66 $\pm$ 1.08		
5	1000	89.89 $\pm$ 1.27	68.79 $\pm$ 1.38		

"Values are mean  $\pm$  SEM of 3 replicates"

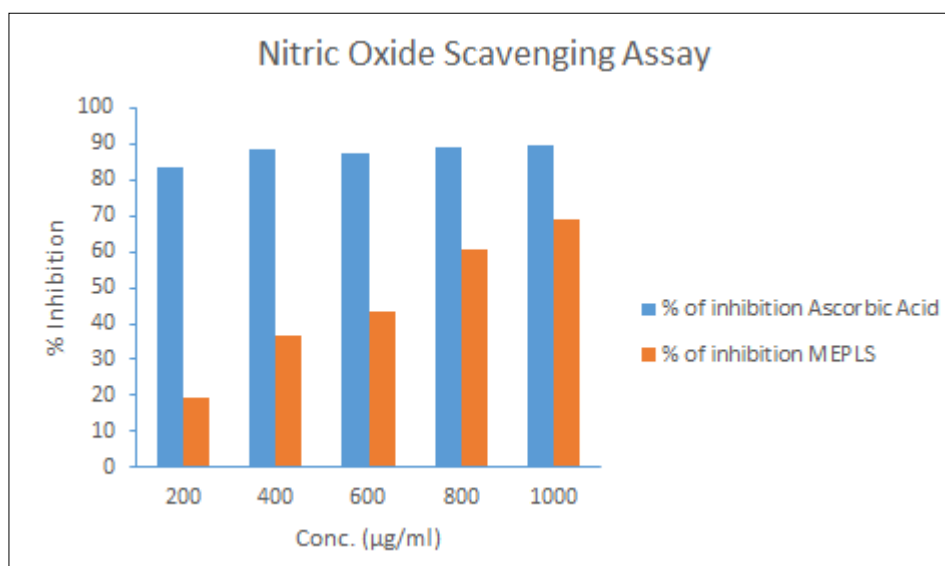


Figure 5. Assay for the determination of nitric oxide scavenging

Lipid peroxidation is the process through which lipid radicals are formed and propagated, ultimately resulting in the destruction of membrane lipids. Assays for lipid peroxidation are the best way to assess cells and tissues for oxidative stress. TBARS formation was employed to test the extract's ability to reduce lipid peroxidation produced by  $\text{Fe}^{2+}$ . In the lipid peroxidation test, the percentage of inhibition and the 50% inhibition concentration ( $\text{IC}_{50}$ ) are determined. Plant extracts have been shown to reduce the  $\text{FeSO}_4$ -induced egg yolk lipid peroxidation, which is a byproduct of ferrous hydroxyl radicals. Scavenging hydroxyl radicals or chelating iron ions, which initiate Fenton's reaction, are two ways to do this. For the lipid peroxidation assay (figure 6), the  $\text{IC}_{50}$  value of Standard Ascorbic Acid was 166.55 micrograms per litre. Lipid peroxidation was effectively inhibited by both extracts, with  $\text{IC}_{50}$  values of methanolic extracts being 721.55 g/ml and 685.43 g/ml, respectively (Table 5).

Table 5  
Lipidic peroxidation test detection and quantification

S. no.	Conc. µg/ml	% of inhibition		$\text{IC}_{50}$ Value (µg/ml)	
		Ascorbic Acid (n=3)	MEPLS (n=3)	Ascorbic Acid	MEPLS
1	200	66.48±1.26	21.96±1.72	166.55	685.43
2	400	75.28±1.56	34.05±1.11		
3	600	77.31±1.16	42.01±0.46		
4	800	82.51±1.39	51.51±1.52		
5	1000	86.72±1.62	56.26±1.18		

"Values are mean ± SEM of 3 replicates"

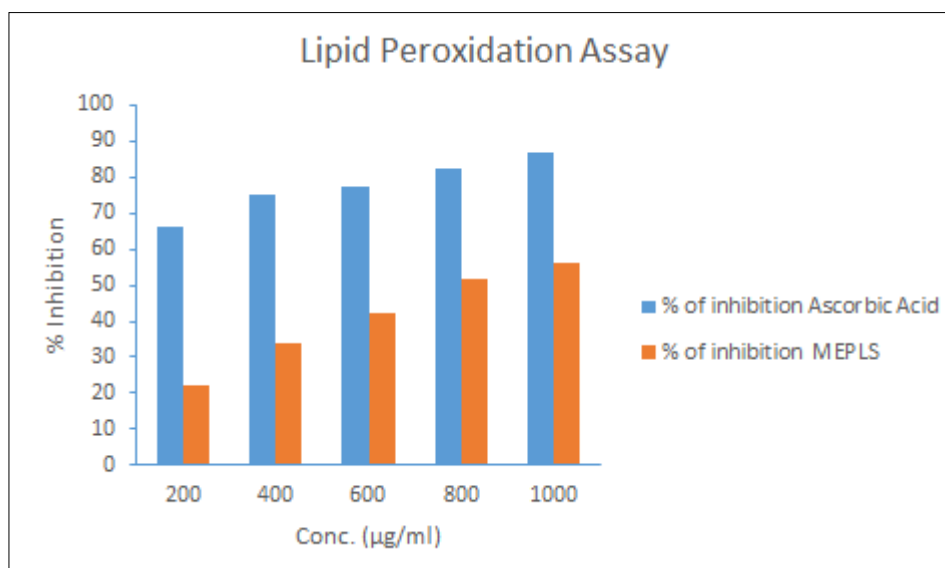


Figure 6. Assay for determining lipid peroxidation

CaOx crystallisation was inhibited to varying degrees by leaf extract at various doses, as shown in Table 8.8. We will take into consideration only the concentrations 10%, 25%, and 50% since these shines which give reliable results. Different quantities of *Polyalthia longifolia* (Sonn.) Thwaites. leaf extract were shown to affect the crystallisation of CaOx in Figure 7. The suppression of nucleation increased as the concentration of the extract increased. At a 50 percent concentration, there is a  $55.23 \pm 1.23\%$  percent inhibitory maximum. After several tests of the concentrations, we are chosen the concentrations illustrated in Table 6. The results indicate the change of dissolving activity as a function of the concentrations of the saponins fraction, as shown in Figure 8.

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)
OD (620 nm)	0.495±0.003	0.377±0.04	0.225±0.004
I (%)	4.05±0.90	22.56±3.20	56.20±1.25
CV (%)	1.95	9.25	6.20

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  $\pm$  standard error of the mean. \*\*\*\*P<0.0001 with F (2.6) = 94.14. Percentage inhibition values are expressed as mean  $\pm$  standard error of the mean. \*\*\*\*P<0.0001 with" F (3.8) = 194.1.

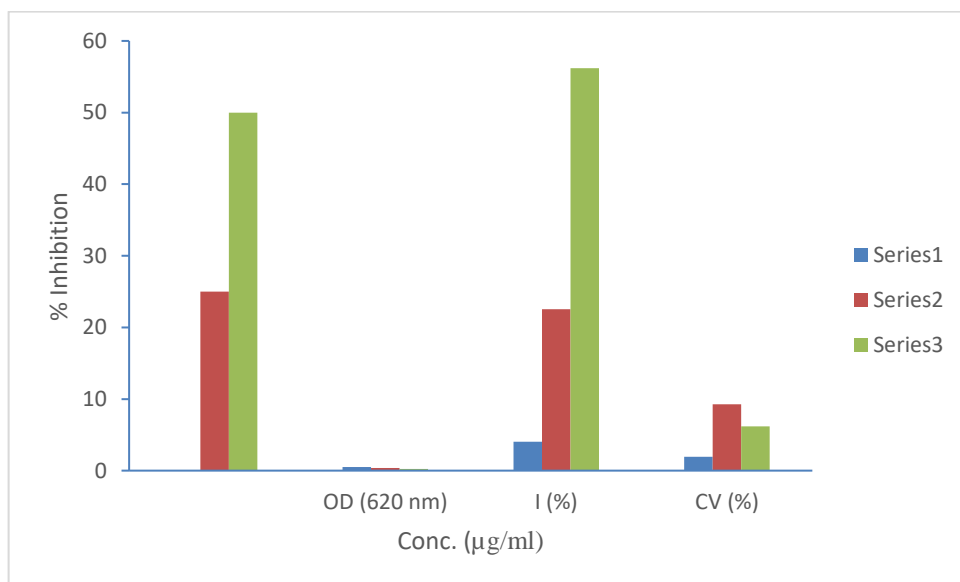


Figure 7. Effect of the extract of the extract leaves of *Polyalthia longifolia* (Sonn.) Thwaites. on the crystallization of calcium oxalate

Table 7

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

C <sub>sf</sub> (%)	10 µg/ml (n=3)	25 µg/ml (n=3)	75 µg/ml (n=3)	100 µg/ml (n=3)
W <sub>CaOX</sub> (mg)	9.85±0.45	9.48±0.20	9.16±0.30	1.26±0.04
A (%)	30.08±0.02	38.02±1.10	50.64±0.43	89.88 ±0.72
CV (%)	7.43	4.25	6.36	7.63

C<sub>sf</sub> (%) concentration of saponins fraction, W<sub>CaOX</sub> (mg) weight of calcium oxalate, C<sub>v</sub> (%) 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

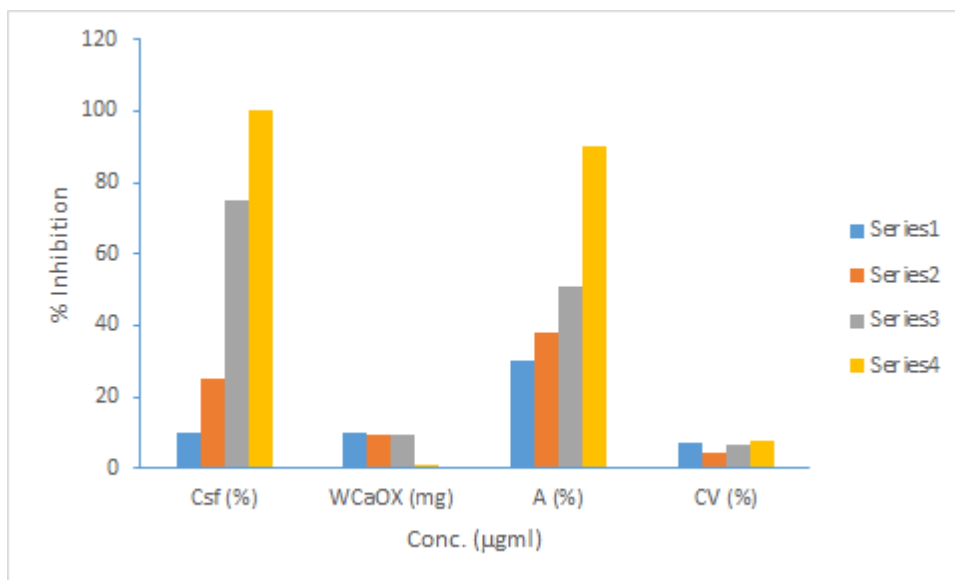


Figure 8. Effect of the different concentration of the extract leaves of *Polyalthia longifolia* (Sonn.) Thwaites. on the dissolving of calcium oxalate

Treatment with antioxidants prevents the buildup of calcium oxalate crystals in the kidneys due to a lack of adequate protection against oxidative stress. Also Cellular harm has been linked to peroxidation of membrane lipids generated by large concentrations of calcium oxalate crystals, according to previous research. 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 8.8. We will take into consideration only the concentrations 10%, 25%, and 50% since these shines which give reliable results. The suppression of nucleation increased as the concentration of the extract increased. Maximum inhibition is  $56.20 \pm 1.25\%$  observed at a concentration of 50%.

## Conclusion

*Polyalthia longifolia* (Sonn.) Thwaites. 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. Methanol was used to extract and then screen the plant's leaves for phytochemicals, which revealed that flavonoids and phenolic compounds were the primary active ingredients. Using iodine vapour as a detection reagent, TLC investigations on an methanolic extract of *Polyalthia longifolia* (Sonn.) Thwaites. leaves indicate two spots of various colours and  $R_f$  values in the solvent system (3: 0.9: 0.9: 0.5). The literature survey reveals that the leaves of *Polyalthia longifolia* (Sonn.) Thwaites. 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 *Polyalthia longifolia* (Sonn.) Thwaites. 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.

### **Acknowledgement**

The authors are thankful to the Director, staff and management of HIMT college of Pharmacy, Greater Noida who gave us opportunity and helped to complete this research work.

### **References**

- Dave, R; Nagani, K; Chanda S. 2010. Pharmacognostic studies and physicochemical properties of the *Polyalthia longifolia* var. *pendula* leaf. *Pharmacognosy Journal*, 2(13), 572-576.
- Delfan, B; Baharvand-Ahmadi, B; Bahmani, et al. 2015. An ethnobotanical study of medicinal plants used in treatment of kidney stones and kidney pain in Lorestan province, Iran. *Journal of Chemical and Pharmaceutical Sciences*, 8(4), 693-699.
- Deyong, Z.H.; Lei, W.A.; Yunting, L.E. 2012. Correlation among SDS sedimentation value, swelling index of glutenin and solvent retention capacity of spring wheat. *Notulae Scientia Biologicae*, 4(2), 132-135.
- Ainsworth, E.A.; Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature protocols*, 2(4), 875-877.
- Atanassova, S; Neykov, K; Gutzow, I. 2000. Solubility and dissolution kinetics of calcium oxalate renal calculi in solutions, containing DL-lysine: in vitro experiments. *Journal of crystal growth*. 212(1-2), 233-238.
- Bensatal, A; Ouahrani, M.R. 2008. Inhibition of crystallization of calcium oxalate by the extraction of *Tamarix gallica* L. *Urological Research*. 36(6), 283-287.
- Chaudhary, A; Singla, S.K.; Tandon C. 2010. In vitro evaluation of *Terminalia arjuna* on calcium phosphate and calcium oxalate crystallization. *Indian journal of pharmaceutical sciences*. 72(3), 340.
- Duh, P.D.; Yen, G.C.; Yen, W.J., et al. 2004. Effects of pu-erh tea on oxidative damage and nitric oxide scavenging. *Journal of Agricultural and Food Chemistry*. 52(26), 8169-8176.
- Kachkoul, R.; Squalli, Housseini, T; Mohim, M; et al. 2019. Chemical compounds as well as antioxidant and litholytic activities of *Arbutus unedo* L. leaves against calcium oxalate stones. *J Integr Med*, 17(6), 430-437.
- Khan, S.R.; Pearle, M.S.; Robertson, W.G.; et al. 2016. Kidney stones. *Nature reviews Disease primers*. 2(1), 1-23.
- L. Yachi, S. Bennis, Z. Aliat, A. Cheikh, et al. 2018. In vitro litholytic activity of some medicinal plants on urinary stones. *African Journal of Urology*, 24(3), 197-201.
- Lin, JY; Tang, CY. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food chemistry*. 101(1),140-147.

- Momin, RK; Kadam, VB. 2011. Determination of ash values of some medicinal plants of genus *Sesbania* of Marathwada region in Maharashtra. *J Phytol.* 3(12), 52-54.
- Moore K, Roberts LJ. 1998. Measurement of lipid peroxidation. *Free radical research.* 28(6), 659-671.
- Nielsen, SS. 2010. Determination of moisture content. In *Food analysis laboratory manual*, pp. 17-27. Springer, Boston, MA.
- Nirmal, SA; Pal, SC; Mandal, SC; Patil AN. 2012. Analgesic and anti-inflammatory activity of  $\beta$ -sitosterol isolated from *Nyctanthes arbortristis* leaves. *Inflammopharmacology*, 20(4), 219-24.
- Roy, H; Chakraborty, A; Bhanja, S; et al. 2010. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. *Journal of Pharmaceutical Science and Technology.* 2(5), 217-221.
- Royer, DL. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO<sub>2</sub> concentration. *Review of Palaeobotany and Palynology.* 14(1-2), 1-28.
- Scales, Jr CD; Smith, AC; Hanley, JM; Saigal CS. 2012. Urologic Diseases in America Project. Prevalence of kidney stones in the United States. *European urology.* 62(1), 160-165.
- Sivaranjan, VV; Balachandran, I. Ayurvedic drugs and their plant sources. *Oxford and IBH publishing*; 1994.
- Sluiter, A; Ruiz, R; Scarlata, C. et al. 2005. Determination of extractives in biomass. Laboratory analytical procedure (LAP).1617, 1-9.
- Xu, DL; Mo Y. 2011. Determination of foreign organic matter in Yemugua tablets by NIR correlation coefficient method. *Chinese Journal of Pharmaceutical Analysis.* 31(7), 1423-4.
- Widana, I.K., Dewi, G.A.O.C., Suryasa, W. (2020). Ergonomics approach to improve student concentration on learning process of professional ethics. *Journal of Advanced Research in Dynamical and Control Systems*, 12(7), 429-445.
- Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, 29(3), 550-561. <https://doi.org/10.1002/cae.22202>
- Santoso, P., Adrianta, K. A., & Wiranatha, I. G. (2021). Phytochemical screening and in vivo test of dewandaru (*Eugenia uniflora* L) fruit extract on mice exposed to cigarette smoke. *International Journal of Health & Medical Sciences*, 4(2), 246-252. <https://doi.org/10.31295/ijhms.v4n2.1722>