Extraction, Purification, and Isolation of Quercetin from *Bryophyllum Pinnatum (Lam.) Kurz* Leaves Using Analytical Technique and Evaluate its Kidney Stone Dissolving Properties by in-Vitro Means

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Abstract:

Medicinal plants have garnered great interest among researchers in their various fields like pharmacology, biotechnology and many more. Several pharmaceutical industries rely on plant derived compound to develop new chemical entities. *Bryophyllum pinnatum (Lam.) Kurz,* commonly known as the 'Air Plant' hold a promising potential in wound healing, infection, and inflammation. The aim of this research paper is to investigate the active constituents i.e. quercetin present in *Bryophyllum pinnatum (Lam.) Kurz* leaves and evaluate its kidney stone dissolving properties by in vitro experimentation. The study focused on the extraction of these active compounds and their profiling using advanced analytical techniques like HPLC and their effect on calcium oxalate crystals forming kidney stone. This research contributes to a better understanding of the pharmacologically significant compounds within this plant species and their therapeutically effect**.**

Keywords: *Bryophyllum pinnatum(Lam.) Kurz,* GC-MS, HPLC, Calcium oxalate, Quercetin.

1. Introduction

Bryophyllum pinnatum (Lam.) Kurz (BPLK), commonly known as "Patharchur" or "Mother of Thousands," is a succulent plant, native to Madagascar but now widely distributed in tropical and subtropical regions (Nagaratna & Hegde, 2015). Various parts of BPLK are used as a remedy for skin conditions, urolithiasis, respiratory disorders and gastrointestinal diseases(Ahmed et al., 2016). Previous studies had reported anti-urolithiasis activity of BPLK hydro-alcoholic extract(Yadav et al., 2016). Moreover, as a traditional and ethano-medical practice the leaves of BPLK are used to treat urinary insufficiency and kidney stones(Chauhan et al., 2009). Studies have also reported that dried powder of aqueous extract of BPLK when mixed with cider vinegar could completely dissolve calcium oxalate crystals after two weeks(Bashir et al., 2023). A recent study also reported that the ethanolic and hydro-alcoholic extract of BPLK could dissolve calcium oxalate and calcium phosphate crystal, which authors attributed to the presence phytochemicals such as alkaloids, saponin, glycoside, Kaempferol, and flavonoids(Mahesh Kumar, 2019). However, no concrete scientific literature is available to shed light on the phytoconstituents responsible for the anti-urolithiasis activity of BPLK. Urolithiasis represents the clinical condition of stone in urinary tract. It refers to the accumulation of hard, solid and non-metallic minerals in urinary tract (Daudon et al., 2018). Stone formation in the urinary tract has been recognized for thousands of years, but during the last few decades, the pattern and incidence of the disease have changed markedly. Epidemiological data have shown that calcium oxalate (CaOx) is the predominant mineral in the majority of urinary stones(Daudon et al., 1993)**.**The formation of kidney stone begins in the renal tubule and it is a complex process that include total 5 steps- Supersaturation, Nucleation, Aggregation, Growth and Retention of crystals within renal tubules. About 80% of all urinary calculi are calcium stones, the most common type of kidney stone. Calcium stone may contain 50% of pure CaOx, 5% calcium phosphate and 45% brushite (calcium hydrogen phosphate)(Chaudhary et al., 2010). There are many differences in the prevalence of kidney stone across the worldwide as per the report it was 1-5% in Asia, 5-9% Europe, 7-15% in north America, 20% in Saudi Arabia, 4% in China. In India 12% of the total population was affected by kidney stone disease among which 50% population was severely affected that is causing kidney failure(Halinski et al., 2021). In view of the significant research gap in the area; the current study has been designed to perform the phytochemical profiling of the BPLK with efforts to identify potential active constituent responsible for the Anti-urolithiasis potential of BPLK.

2. Chemicals

Methanol, n-hexane, ethyl acetate, acetone, toluene, formic acid, acetic acid, acetonitrile, were procured from FINAR Limited, Sanand, Ahmedabad. All other chemicals used in this study were purchased from Hi-Media Laboratories Pvt. Ltd., Vadhani, Ind. Est., LBS Marg, Mumbai.

2.1 Collection and Preparation of plant extract

BPLK leaves were collected in September 2022 from botanical garden of United Institute of Pharmacy and authenticated by the Department of Horticulture Scientist by Dr. Alok Mukerjee. Leaves were washed properly and shaded dried at room temperature for 4 weeks. The dried leaves were used to make coarse powder by using blender.

Dried BPLK leaves were grounded into coarse powder using blender. The powder was then placed in the thimble of a Soxhlet apparatus. Methanol was added to the flask of the Soxhlet apparatus, and the apparatus was heated at 80^0C. Extract was collected after 72hr and subjected to rotary evaporator to evaporate the solvent at 79^0C for 1hr 100 psi pressure and at the 75 rpm. After complete drying, it was weighed and yield value of extract was calculated by using formula (I). The concentrated extract was stored in an airtight container at a cool and dark place(Akinsulire et al., 2007).

Yield (%) = (Amount of extract / Amount of coarse powder) × 100 Formula (I)

2.2 Qualitative analysis of phytochemicals extract

Initial phytochemical analysis

For methanolic extract of BPLK preliminary phytochemical analysis had been performed for the test of secondary metabolites(Kumari et al., 2017).

2.3 High Performance Liquid Chromatography

Standard solutions of quercetin, rutin, kaempferol, syringic acid, luteolin was prepared by dissolving in 1ml of methanol to achieve final concentration of 1mg/ml. All solutions were filtered through 0.45 µm syringe filter. For sample solution the methanolic extract of BPLK was also filter through 0.45micro syringe and was subjected to HPLC analysis using Quercetin, Rutin, Kaempferol, Digoxin, Luteolin, Syringic acid as a marker.

The HPLC analysis was carried out by using LC 20AP Shimadzu japan model with C18 analytical column with binary pump and Photo Diode Array Detector(Rolim et al., 2005).

a) For Rutin, quercetin, kaempferol Acetonitrile with 0.1% formic acid in water was used. The elution condition was 20minute, the injection volume was 20µl and flow rate was 1ml/min with UV spectral range 220nm, 220nm and 259nm respectively. The column compartment temperature was maintained at 25^0C.(Mortier et al., 2004)

b) For luteolin, syringic acid and digoxin a potassium phosphate buffer with acetonitrile at pH 4.2 maintained with phosphoric acid was used. The elution condition was maintained for 20minutes, the injection volume was 20µl and flow rate was maintained at 1ml/min with UV spectral range 220nm, 347nm, 220nm respectively. The column temperature was maintained 35^0C(Bojarowicz et al., 2011).

2.4 Gas Chromatography Mass Spectrophotometry

GC-MS analysis was performed using a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer. The system was outfitted with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m 250 m, film thickness 0.25 m), and it was interfaced with a 5675C Inert MSD with Triple-Axis detector. As the carrier gas, helium gas was utilized, and the flow rate was regulated to 1.0 millilitre per minute (ml/min). Other settings for the GC-MS include an ion-source temperature of 250^0C, an interface temperature of 300^0C, a pressure of 16.2 pounds per square inch, an out time of 1.8 millisecond, and 1

microliter injector operating in split mode with a split ratio of 1:50 and an injection temperature of 300^0C. The temperature of the column began at 36^0C for five minutes before gradually increasing to 150^0C at a pace of 4^0C per minute. The temperature was brought up to 250^0C at a pace of 20^0C per minute, and it was kept there for five minutes. The duration of the entire elution was 47.5 minute. We were able to determine the relative percentage amount of each component by comparing the average peak area of each component to the total area. The MS solution software that the provider gave was utilized to control the system as well as to obtain the data.

2.5 Proof of concept

Simulated body fluid media (SBF) was prepared by sequentially by adding the required reagents according to the given table S1. The pH of the media was adjusted 1to 7.4 using 1M hydrochloric acid and sodium hydroxide. For performing dissolving properties of kidney stone by fractionated extract containing active constituents and standard chemicals we performed two-set of experiment one's which standard polyphenolic compounds like quercetin. rutin, kaempferol, syringic acid, luteolin and digoxin and others with different extract of BPLK containing specified isolated chemicals leaves containing various active constituent to know which chemicals is responsible for dissolving kidney stone in-vitro. Separate beakers were taken, SBF media was added so that each drug was dissolved separately in the media, beaker was placed on a magnetic stirrer having stirrer speed was set at 300 RPM and temperature was maintained at 25^0C, this stirring condition was maintained for 4hr and 12hr. Record the results after each time interval. The weight variation in kidney stones were calculated by the formula (II) and shown in table for the composition of simulated body fluid media(Baino & Yamaguchi, 2020).

Weight variation = Final weight - Initial weight

3 Results

3.1 Phytochemical screening

Preliminary phytochemical screening was performed for the presence of secondary metabolites in the extract such as carbohydrates, protein, amino acids, alkaloids, flavonoids, glycosides and many more (Yadav et al., 2014) table S2.

3.2 HPLC analysis

HPLC analysis of methanolic extract was performed to confirmed the presence of focus compounds i.e. quercetin, rutin, kaempferol, syringic acid, luteolin and digoxin against their respective markers. The retention time of extract and sample was compared and it was found that both were laying on the approx. similar retention time as shown in figure 1.

Figure 1 Shows compounds that were identified by comparing the retention time of standard and extract of BPLK

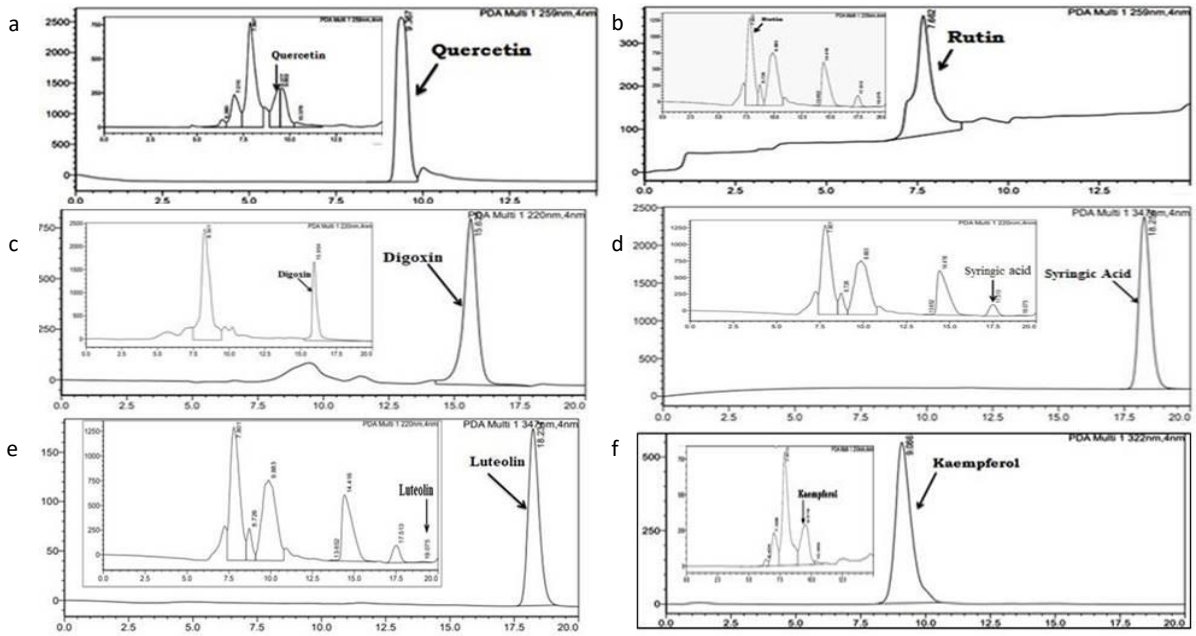
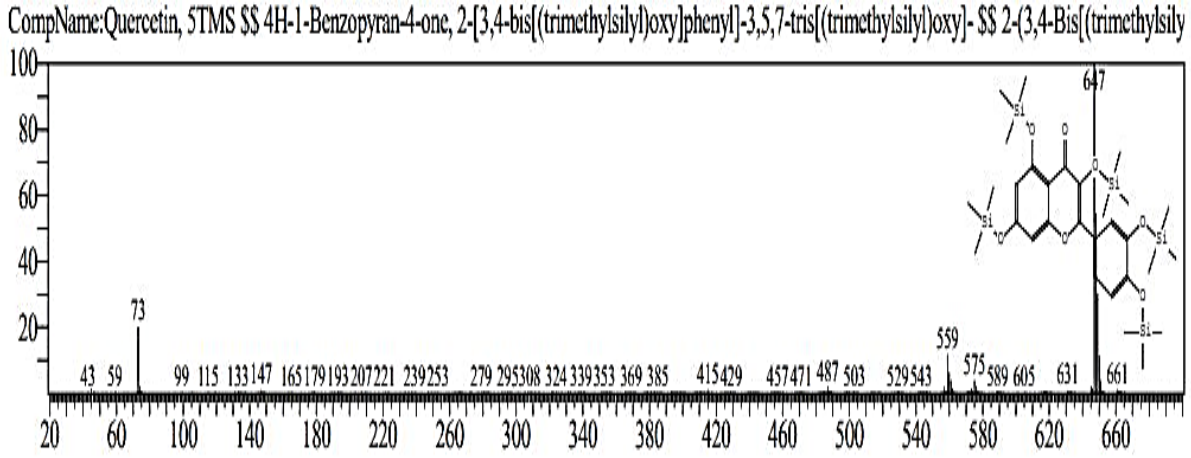


Figure 1 (a) shows the chromatogram of std compound of Quercetin used as marker to confirm the presence of Quercetin in extract, peak appears at the same RT. (b) shows RT of standard Rutin was about 7.662 minutes, while the sample showed a slight shift to 7.801 minutes. (c) Digoxin, the standard RT was 15.625 minutes, with the sample showing a marginal increase to 15.954 minutes. (d) shows the chromatogram of std compound of Syringic acid used as marker to confirm the presence of Syringic acid in extract with slightly lower RT at 17.513 minutes.(e) Luteolin had a standard RT of 18.256 minutes, with the sample displaying a more noticeable shift to 19.075 minutes.(f) shows the chromatogram of std compound of kaempferol used as marker to confirm the presence of kaempferol in extract, peak appears at the same RT

3.3 GC-MS Analysis

GC-MS was performed with the methanolic extract and several peaks were appeared several compounds were resolved as per their molecular mass. Our study specially focused on Quercetin with results presented in figure 2.

Figure 2 GC-MS spectral analysis of ethanolic extract of BPLK. Quercetin, 5TMS, has a molecular formula of $C_{30}H_{50}O_7Si_5$ and molecular weight 663.14 g/mol.



3.4 Proof of Concept

This is performed to confirm that which phytoconstituents shows kidney stone dissolving property. Out of five phytoconstituents, two phytoconstituents Quercetin and Rutin showed good stone dissolving property. Results are summarized in table 1.

Table 1: Shows the effect of different phytoconstituents on kidney stone after 4hr and 12hr.

S. No.	Compound	Initial weight of stone	Final weight of Stone	Weight variation
1	Quercetin (4hr)	0.082gm	0.081gm	-0.001gm
2	Quercetin (12hr)	0.025gm	0.017gm	-0.008gm
3	Rutin (4hr)	0.025	0.023gm	-0.002gm
4	Rutin (12hr)	0.69gm	0.068gm	-0.001gm
5	Kaempferol	0.070gm	0.070gm	00
6	Digoxin	0.028gm	0.028gm	00
7	Syringic acid	0.042gm	0.042gm	00

4. Discussion

Bryophyllum pinnatum(Lam.)Kurz, commonly known as Patharchatta in Indian traditional medicine, is believed to have stone dissolving properties. Tribal and other communities often use the plant's leaves to treat kidney stones. Despite its widespread traditional use, scientific research on its anti-urolithiasis (stone-dissolving) efficacy is limited. This study aimed to verify the ethnomedicinal use of BPLK leaves in the treatment of kidney stones.

The current study reported that methanolic extract of BPLK had a beneficial impact on dissolving kidney stone procured from patients. Preliminary phytochemical screening reveals that the presence of secondary metabolite like reducing sugars, saponins, steroids, tannins, alkaloids, flavonoids like quercetin, kaempferol, rutin and phenols. HPLC method was performed to analyzed quercetin from the mixture using the solvent system n-hexane: ethyl

acetate: acetone (5:4:1) the observed Rf value of quercetin was found to be 0.50. Further GC-MS analysis of methanolic extract the run time is about 60min at which we observed peak at retention time interval of about 50-60min, the peak was further analysis to find hit compounds. Quercetin (3,3',4',5,7-pentahydroxyflavone) is a flavonoid compound that is found in many fruits, vegetables, and herbs, including *Bryophyllum pinnatum*(Lam.)Kurz. It has been shown to possess a wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer properties. In this study, we investigated the methanolic extraction and in vitro profiling of quercetin from BPLK leaves using HPLC and GCMS. We also evaluated the kidney stone dissolving properties of quercetin using a calcium oxalate monohydrate (COM) crystal dissolution assay. Our results showed that the methanolic extract of BP leaves contained a high concentration of quercetin. The GCMS analysis confirmed the identity of quercetin in the extract. The COM crystal dissolution assay showed that quercetin was able to dissolve COM crystals in a time-dependent manner. The highest concentration of quercetin (500 μg/mL) was able to dissolve the COM crystals within 4 and 12 hr. Our findings suggest that quercetin from BP leaves has the potential to be used as a natural remedy for kidney stones. Further research is needed to investigate the efficacy and safety of quercetin in vivo. In addition to the kidney stone dissolving properties of quercetin, it is also important to note its other potential health benefits. Quercetin has been shown to have anti-inflammatory, antioxidant, and anticancer properties. It has also been shown to protect against cardiovascular disease and improve cognitive function. There are few study which showed the co administration of quercetin-rich extracts with other antioxidant have more dissolution capacity (Aghababaei et al., 2023).

Overall, quercetin is a promising natural compound with a wide range of potential health benefits. Further research is needed to fully elucidate the mechanisms of action of quercetin and its potential applications in human medicine.

5. Conclusion

This study showed the potential of quercetin as a natural treatment strategy for kidney stone dissolution, with significant effects demonstrated in vitro. Quercetin's antioxidant, anti-inflammatory, and anticancer properties further enhance its therapeutic value.

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Nil.

Declaration of competing interest

There are no conflicts of interest**.**

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Supplementary

Table S1: Shows the composition of Simulated Body Fluid media.

S. No.	Reagent	Amount (g/L)
1	Sodium chloride	7.996
2	Sodium bicarbonate	0.35
3	Potassium phosphate dibasic tri-hydrate	2.228
4	Magnesium chloride hexa-hydrate	0.305
5	1M HCL	20ml
6	Calcium chloride	0.278
7	Sodium sulphate	0.071
8	Potassium chloride	0.224
9	Tris (hydroxymethyl) amino methane	6.07

Table S 2: Preliminary phytochemical screening of BPLK extracts.

S. No.	Reagents	Methanolic extract
1	Alkaloid test	Positive
2	Terpenoids test	Positive
3	Flavonoids	Positive
4	Tannin	Positive
5	Saponin	Positive
6	Glycosides	Positive
7	Carbohydrate	Positive
8	Protein	Positive
9	Steroids	Positive
10	Carotenoids	Negative
11	Cynogenic Glycosides	Negative
12	Glucosinolates	Negative