



Faculty of Medical and Health Sciences, University of Poonch Rawalakot

# Journal of Pharma and Biomedics

ISSN: 3007-1984(online), 3007-1976 (Print)

<https://www.jpbsci.com/index.php/jpbs>

## Phytochemical Profiling, In Vitro Antiurolithiatic and Diuretic Activities of Ethanolic Extracts of *Cichorium intybus* L., *Punica granatum* L., and *Terminalia chebula* Retz.

Hira Ahmed<sup>1,3\*</sup>, Muhammad Azam<sup>2</sup>, Muhammad Mohtasheem ul Hasan<sup>3</sup>, Safia Abidi<sup>3</sup><sup>1</sup> Department of Pharmacognosy, Hamdard University, Karachi, Pakistan.<sup>2</sup> Department of Pharmaceutical Chemistry, Hamdard University, Karachi, Pakistan.<sup>3</sup> Department of Pharmacognosy, University of Karachi, Karachi, Pakistan.**Received: August 30, 2025;****Revised: November 15, 2025;****Accepted: December 03, 2025**

### ABSTRACT

The worldwide incidence of urolithiasis continues to rise due to the higher calcium oxalate levels, which are the major cause of most of the urinary stones that form in the body. The current pharmacological treatments for urolithiasis show restricted effectiveness while producing adverse reactions, which drives researchers to explore plant-derived therapeutic options. The present study investigates the phytochemical characterization, *in vitro* antiurolithiatic activity, and *in vivo* diuretic effects of ethanolic extracts obtained from *Cichorium intybus* seeds, *Punica granatum* peels, and *Terminalia chebula* fruits. The extracts contained alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, and phenols, as determined by qualitative phytochemical analysis. The FTIR analysis confirmed that the extracts contained bioactive functional groups, which included hydroxyl, amine, and carbonyl moieties. The *in vitro* antiurolithiatic activity test showed that *C. intybus* extract dissolved 85.47% of calcium oxalate. In comparison, *T. chebula* extract dissolved 66.03%, and *P. granatum* extract dissolved 78.98% compared to the standard drug, which dissolved 90.59%. In diuretic testing, the urinary output matched furosemide levels at 10 mg/kg during *in vivo*. The results of this research confirm the traditional medical applications of these plant extracts for treating renal and urinary diseases; however, additional studies are required to understand their mechanisms of action.

**Keywords:** *Urolithiasis, phytochemicals, Cichorium intybus, Punica granatum, Terminalia chebula, diuretic activity, calcium oxalate.*

**Corresponding Authors:** Hira Ahmed

Email: [hira.maaz@hamdard.edu.pk](mailto:hira.maaz@hamdard.edu.pk)

© 2025 Faculty of Medical and Health Sciences.

### INTRODUCTION

Urolithiasis or urinary stone disease affects 12% of the world population, and with high recurrence rates, it is causing significant global health problems. The main composition of urinary tract calculi consists of calcium oxalate (CaOx) crystals, which form the majority of urolithiasis cases. Development of urolithiasis results from multiple factors, including metabolic disorders, dietary patterns, and environmental exposures. The condition will lead to severe complications such as renal damage,

hypertension, and cardiovascular diseases when left untreated (Alelign and Petros, 2018). The current medical treatments for urolithiasis provide short-term relief, but they remain expensive and require invasive procedures due to recurrence rates. Scientists now focus on phytotherapeutic agents because they present safer, more affordable, and sustainable options for preventing and treating urolithiasis. The majority of urolithiasis cases involve calcium-based stones, which primarily consist of calcium oxalate monohydrate or dihydrate, and calcium phosphate. The

formation of urinary stones depends on the combination of pH levels, ionic concentrations, the interaction between stone-forming substances, like oxalate and calcium, and stone-inhibiting compounds such as citrate and magnesium. The main preventive approach for urolithiasis is to drink plenty of water, reduce sodium intake and animal protein consumption, with calcium levels in normal ranges (Sama et al., 2011) (Samal et al., 2011; Xu et al., 2013).

Traditional medical practices have used herbal remedies for centuries to treat kidney stones. The plant-based preparations used in traditional medicine demonstrate multiple pharmacological effects, which help dissolve stones, stop crystal growth, and reduce the risk of future occurrences (Ahmed et al., 2016; Niharika et al., 2018). This research investigated three plants that have received extensive traditional medical use:

*Cichorium intybus* L. (chicory) serves as a traditional medicine for liver protection and bowel movement regulation and kidney protection (Al-Snafi, 2016).

*Punica granatum* L. (pomegranate) demonstrates strong antioxidant and anti-inflammatory and nephroprotective properties (Jurenka, 2008).

*Terminalia chebula* Retz. (haritaki) serves as a fundamental herb in Ayurvedic medicine because it helps with detoxification and shows antioxidant properties and antiurolithiatic effects (Khan et al., 2015; Rao and Nammi, 2006).

This study aims to investigate the phytochemical profiles in correlation with FTIR spectroscopy, *in vitro* and *in vivo* biological effects of the above-mentioned plants to confirm their traditional medical uses, and evaluate their potential for treating urolithiasis.

## **MATERIALS AND METHODS**

### **Plant Material and Extraction**

The seeds, peels, and fruits of *Cichorium intybus*, *Punica granatum*, and *Terminalia chebula*, respectively, were dried in shade and then ground into powder. The Soxhlet extraction process used 100 g of each plant material with 95% ethanol for eight hours. The extracts were concentrated under a vacuum rotary evaporator before being stored at 4°C for future use.

### **Phytochemical Screening**

#### **Test of Alkaloids**

##### **Wagner's test**

5 ml extract was mixed with Wagner's reagent to observe a reddish brown color precipitate (Sama et al., 2011).

##### **Dragendorff's Test**

2 ml of filtrate was mixed with 1 ml of Dragendorff's reagent. Orange reddish brown precipitate showed a positive test (UC and NAIR, 2013).

### **Test for Anthraquinone glycosides**

#### **Hydroxyanthraquinone Test**

1 ml extract mixed with a few drops of 10% KOH. Red color showed the test +ve (UC and NAIR, 2013).

#### **Test for carbohydrates**

##### **Molisch's test**

Molisch's reagent was added to each extract dissolved in DW, then 1 ml of conc. H<sub>2</sub>SO<sub>4</sub>. The mixture was kept for 2 min and mixed with 5 ml of DW. Red or light violet color showed a positive test (Sama et al., 2011).

##### **Fehling's test**

Take 2 mg extract in 1 ml of DW and treat with 1ml of Fehling's (A+B) solution, heated for 10 min. The brick red precipitate showed the test was positive (UC and NAIR, 2013).

#### **Test for Fats and Fixed Oils**

Take 5 drops of extract, add 1 ml of 1% CuSO<sub>4</sub> solution, and 10% NaOH. The clear blue solution showed a test positive (UC and NAIR, 2013).

#### **Test for flavonoids**

##### **Lead acetate test**

The extract was mixed with 5 drops of lead acetate, and a white or cream precipitate was obtained (Sama et al., 2011).

##### **NaOH test**

Take 1 ml extract mixed with aqueous NaOH and HCl, and observe a yellow-orange color (Sama et al., 2011).

##### **Sulfuric acid test**

The extract was mixed with conc. H<sub>2</sub>SO<sub>4</sub> for orange color formation (Sama et al., 2011).

#### **Test for Glycosides**

##### **Keller-Kiliani test**

Take 0.4 ml of glacial acetic acid and 5% Ferric Chloride, was mix with the extract. Then add 0.5 ml of conc. H<sub>2</sub>SO<sub>4</sub>. The appearance of blue colour confirmed the test (UC and NAIR, 2013).

##### **Salkowski's test**

The extract was treated with 2 ml of CHCl<sub>3</sub>. After which, 2 ml of conc. H<sub>2</sub>SO<sub>4</sub> was added. A reddish-brown color showed steroidal ring (Rawal et al., 2015).

#### **Test for phytosteroids**

##### **Salkowski reaction**

Take 2 mg extract was treated with chloroform, then H<sub>2</sub>SO<sub>4</sub> was added. Red color showed the presence of steroids (Kodangala et al., 2010).

##### **Test with Acetic acid**

Extract mixed with 2ml of CHCl<sub>3</sub>. Then reacted with conc H<sub>2</sub>SO<sub>4</sub> and acetic acid. The appearance of green colour showed the test as positive (Rawal et al., 2015).

#### **Test for phenols: Ferric chloride test**

Each extract mixed with 5% FeCl<sub>3</sub> and showed a dark bluish

black color (Sama et al., 2011).

#### **Test for proteins: Biuret's test**

Take 2 ml extract added with 5 drops of 1% CuSO<sub>4</sub>, add 2 ml of the 10% NaOH and mix well. Appearance of violet color showed protein (UC and NAIR, 2013).

#### **Test for quinone**

1 g of the extract mixed 5 ml of conc. HCl for the yellow color precipitate (Sama et al., 2011).

#### **Test for saponin: Foam test**

Each extract was mixed well with H<sub>2</sub>O for a persistent foam appearance (Sama et al., 2011).

#### **Test for tannins: FeCl<sub>3</sub> test**

The extract was dissolved in water, heated in a water bath at 37 °C for 1 hour, and then the filtrate was treated with FeCl<sub>3</sub>. The appearance of dark green color confirms the presence of tannins (Sama et al., 2011).

#### **Test for terpenoids: Salkowski Test**

The extract was mixed with 1 ml of CHCl<sub>3</sub> and a few drops of conc. H<sub>2</sub>SO<sub>4</sub>. A reddish-brown color appeared at the interface, which showed the test as +ve (UC and NAIR, 2013).

#### **FTIR Spectroscopy**

The FTIR analysis was performed on Nicolet Avatar 330 from Thermo Electron Co., in the range of 500-4000 cm<sup>-1</sup> to show the existence of functional groups containing heteroatoms.

#### **In Vitro Antiuro lithiatic Assay**

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking an equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H<sub>2</sub>SO<sub>4</sub>. Both were allowed to react in sufficient quantities of distilled water in a beaker, and the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water, and dried at 600 °C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed together in a semipermeable membrane. This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. The first group served as a blank containing only 1 mg of calcium oxalate. The second group served as a positive control containing 1 mg of calcium oxalate along with the 10 mg standard drug. The 3rd group, along with 1 mg of calcium oxalate, contains ethanolic extract. The conical flasks of all groups were kept in an incubator preheated to 37 °C for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube, and titrate with

0.9494N KMnO<sub>4</sub> till a light pink colour is obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to determine the total quantity of dissolved calcium oxalate by various solvent extracts (Niharika et al., 2018).

#### **Diuretic Activity**

The study used Wistar rats weighing 150-200 g to evaluate the diuretic effects. The research included five groups with six animals each: a control group received distilled water, three test groups received *C. intybus*, *P. granatum*, and *T. chebula* extracts at 400 mg/kg each, and a standard group received furosemide at 10 mg/kg. The researchers measured urine output for five hours starting from the time of drug administration.

#### **Statistical Analysis**

The research data presented mean values with standard error margins (SEM). The statistical analysis used one-way ANOVA with Dunnett's test to evaluate significance at  $p < 0.05$ .

## **RESULTS**

### **Phytochemical Analysis**

The study analyzed *C. intybus* seeds, *P. granatum* peel, and *T. chebula* fruit extracts through standard methods to determine their phytochemical content, their ability to prevent urolithiasis, and their diuretic effects. The preliminary phytochemical screening test identified all major compound classes, including alkaloids, anthraquinones, carbohydrates, fixed oils, flavonoids, glycosides, phytosterols, phenols, proteins, quinones, saponins, tannins, and terpenoids.

#### **Alkaloids**

The secondary metabolite alkaloids contain nitrogen and produce various pharmacological effects, which include pain relief, muscle relaxation, and antimicrobial properties (Roy, 2017). The Wagner's and Dragendorff's tests produced positive results for all three ethanolic extracts, which confirmed the presence of alkaloids (Figure 1; Table 1). These compounds may contribute to the therapeutic efficacy of the plants.

#### **Anthraquinone Glycosides**

The compound anthraquinones show four different properties because they work as a laxative and display antimicrobial, antioxidant, and anticancer activities (Khan, 2019). The hydroxyanthraquinone test showed that *P. granatum* tested positive, but *C. intybus* and *T. chebula* tested negative (Figure 2), which indicates that *P. granatum* peel contains anthraquinone derivatives.



Figure 1: Phytochemical screening for alkaloids (a) Wagner's test (b) Dragendorff's test.

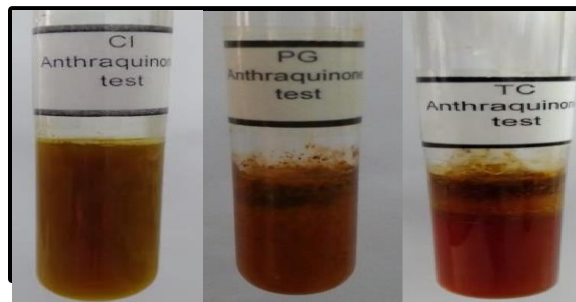


Figure 2: Phytochemical screening for anthraquinone glycosides.

### Carbohydrates

Plants depend on carbohydrates to obtain energy and construct their structural framework (Caffall and Mohnen, 2009). Molisch's test was negative for all extracts, whereas Fehling's test was positive for *P. granatum* and *T. chebula*. At the same time, it appeared negative for *C. intybus* (Figure 3), indicating the presence of reducing sugars in the former

two.

### Fixed Oils and Fats

Fixed oils serve as metabolic agents and pharmaceutical compounds (Rustan and Drevon, 2005). The fixed oils and fats test produced negative results for all three extracts (Figure 4) because the ethanolic extracts contain minimal lipid content.



Figure 3: Phytochemical screening for carbohydrates (a) Molisch's test (b) Fehling's test

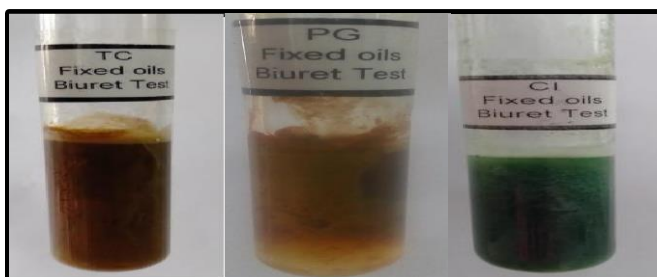


Figure 4: Phytochemical screening for fixed oils.

### Flavonoids

The compound flavonoids show several main effects, which include antioxidant activity, anti-inflammatory, kidney protective, and antiurolithiatic activity, to name a few (Amari et al., 2023). Lead acetate and sulfuric acid tests were positive for all extracts, while the NaOH test was positive for *C. intybus* and *T. chebula* and negative for *P. granatum* (Figure 5). These results confirm the

presence of diverse flavonoid compounds across the three species.

### Glycosides

The medicinal compound glycosides function as drugs because they provide heart benefits and fight cancer (Khan et al., 2019). The Keller–Killiani and Salkowski tests indicated positive results for *P. granatum* and *T. chebula*, while *C. intybus* showed negative results (Figure 6).

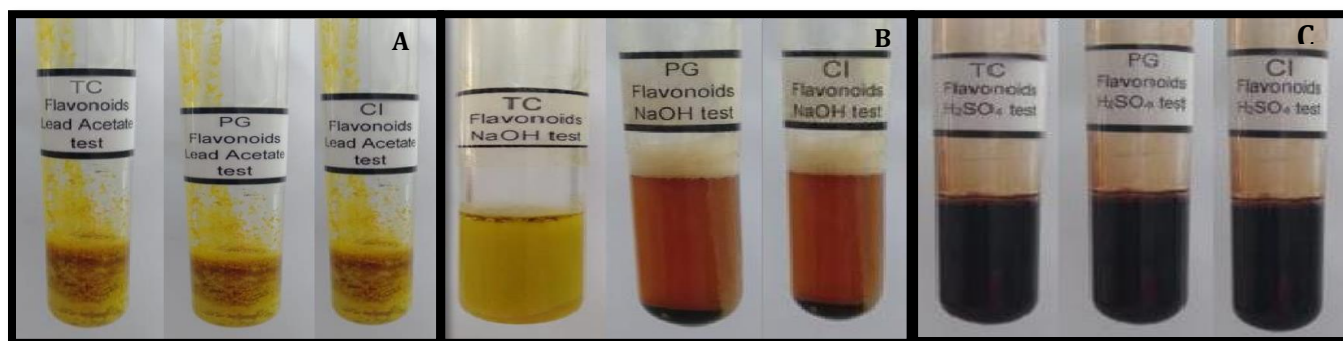


Figure 5: Phytochemical screening for Flavonoids (a) Lead Acetate test (b) NaOH test (c) H<sub>2</sub>SO<sub>4</sub> test.



Figure 6: Phytochemical screening for Glycosides (a) Keller-Kellani test (b) Salkowski test.

### Phytosterols

Phytosterols represent plant sterols, which help decrease blood cholesterol levels and stop the development of atherosclerosis (Rocha et al., 2011). The Salkowski and

acetic acid tests confirmed the presence of phytosterols in all three extracts, except the Salkowski reaction, which was negative for *C. intybus* (Figure 7).

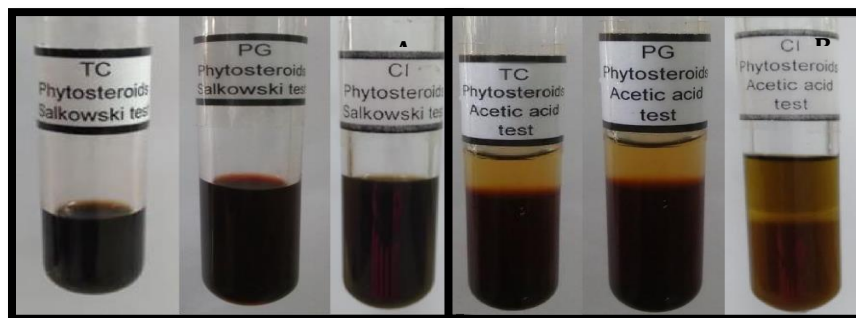


Figure 7: Phytochemical screening for Phytosterols (a) Salkowski test (a) Acetic acid test.

### Phenols

Phenolic compounds serve as strong antioxidants that help halt disease progression (Sinha, 2019). The ferric chloride test yielded positive results for phenols in all three extracts (Figure 8), validating their antioxidant properties.

### Proteins

Proteins are crucial for tissue repair and metabolic processes (Weijs et al., 2014). The Biuret test produced negative results for all three extracts (Figure 9), which showed that there was no substantial protein present in the samples.

### Quinones

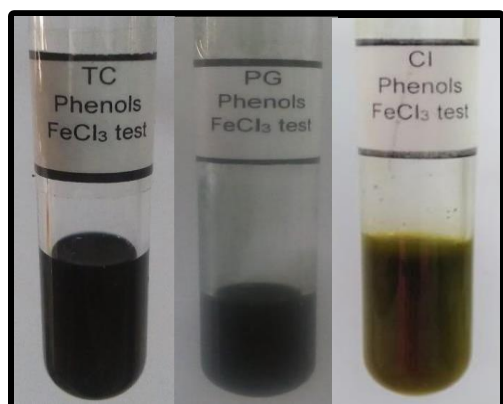


Figure 8: Phytochemical screening for phenols.

Quinones exhibit three primary biological activities through their ability to cause cell death and their potential to fight cancer, and their capacity to delay aging (Madeo et al., 2013). In our study, all extracts showed negative results for quinones (Figure 10).

### Saponins

Saponins demonstrate three main properties which include hypocholesterolemia effects, immunostimulant properties, and antimicrobial activities (Desai et al., 2009). The foam test produced positive results for all three extracts (Figure 11) which indicated their saponin presence.

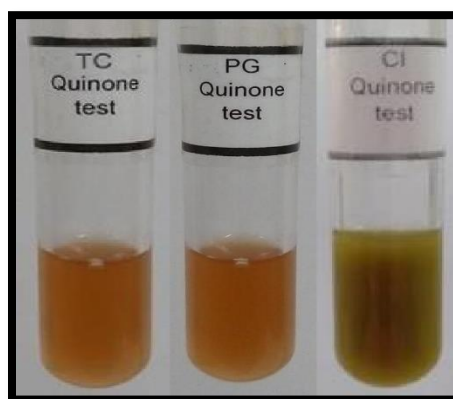


Figure 10: Phytochemical screening for quinone.

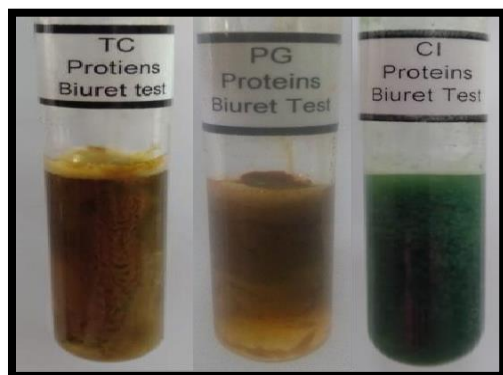


Figure 9: Phytochemical screening for proteins.

### Tannins

Tannins exhibit three main properties which include antioxidant effects and anti-inflammatory properties and astringent properties (Khanbabaee & Teunis, 2001). The ferric chloride test produced positive results for all extracts (Figure 12) which indicates that tannins exist in these extracts to potentially help prevent urolithiasis.

### Terpenoids

The compound terpenoids exist in multiple chemical structures because they show both antimicrobial and wound-healing activities (Narender et al., 2018). The Salkowski test



Figure 11: Phytochemical screening for Saponins.

showed positive results for *P. granatum* and *T. chebula*, and negative results for *C. intybus* (Figure 13).

### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was performed to identify the major functional groups in each plant extract. The spectra were recorded in the infrared region (500–4000  $\text{cm}^{-1}$ ).

### *Cichorium intybus*

The FTIR spectra showed peaks which identified aldehydes,

alkanes, alkenes, amides, carboxylic acids, esters, and ketones through their major absorption at 1095.88, 1158.27,

1460.44, 1539.02, 1635.22, 1744.74, 2854.42, 2924.69, and 3009.31  $\text{cm}^{-1}$  (Table 2; Figure 14).

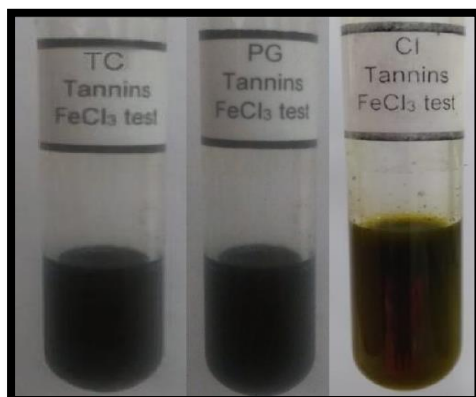


Figure 12: Phytochemical screening for Tannins

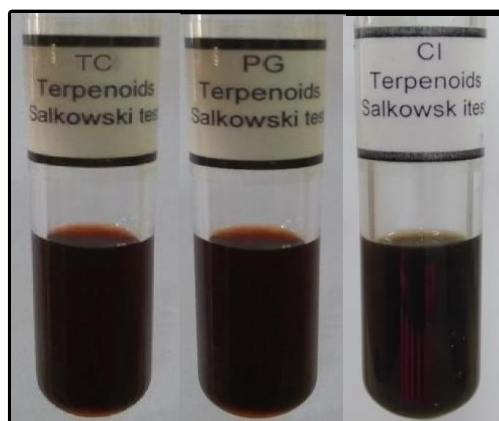


Figure 13: Phytochemical screening for Terpenoids.

Table 1: Phytochemical Screening of Ethanolic Extracts.

Test	<i>T. chebula</i>	<i>P. granatum</i>	<i>C. intybus</i>
Alkaloids (Wagner's & Dragendorff's)	+	+	+
Anthraquinone glycosides	-	+	-
Carbohydrates (Fehling's)	+	+	-
Fixed oils and fats	-	-	-
Flavonoids	+	+	+
Glycosides	+	+	-
Phytosteroids	+	+	-
Phenols	+	+	+
Proteins	-	-	-
Quinones	-	-	-
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	+	+	-

(+ = present; - = absent)

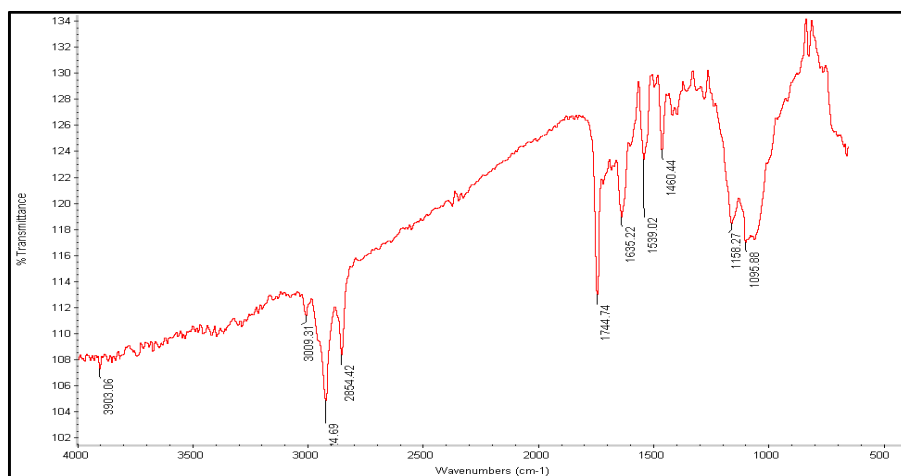


Figure 14: IR spectra of *Cichorium intybus*.

Table 2: FTIR spectral values of *Cichorium intybus*.

Wavenumber cm <sup>-1</sup> test sample	Wavenumber cm <sup>-1</sup> reference range	Functional groups	Compound class
1095.88	1250-1020	C-N, stretching	Amine
1158.27	1250-1020	C-N, stretching	Amine
1460.44	1485-1445	C-H bending	Methylene
1539.02	1550-1500	N-O, stretching	Nitro compound
1635.22	1680-1620	C=C stretching	Alkene
1744.74	1750-1735	C=O, stretching	Esters
2854.42	2865-2845	C-H bending	Methylene
2924.69	2935-2915	C-H bending	Methylene
3009.31	2700-3200	O-H stretching	Alcohol

### *Punica granatum*

The FTIR analysis of *P. granatum* peel extracts revealed alcohols, alkanes, alkenes, esters, ethers, phenols, and nitro

compounds through its absorption peaks at 1045.28, 1162.17, 1620.41, 1715.75, 1745.60, 2363.55, 2856.60, 2927.96, and 3424.88 cm<sup>-1</sup> (Table 3; Figure 15).

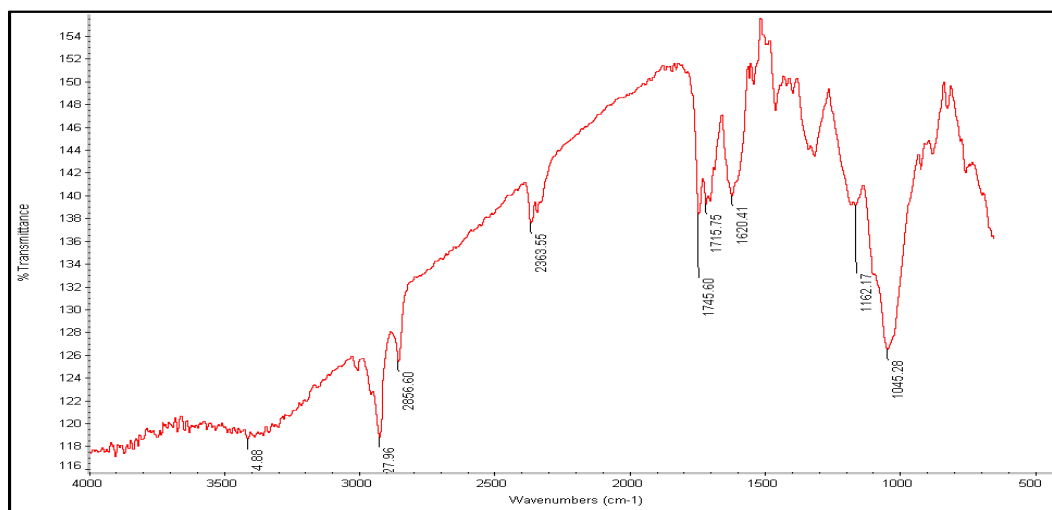


Figure 15: IR spectra of *Punica granatum*.

Table 3: FTIR spectral values of *Punica granatum*.

Wavenumber cm <sup>-1</sup> test sample	Wavenumber cm <sup>-1</sup> reference range	Functional groups	Compound class
1045.28	1075-1020	C-O stretching	Vinyl ether
1162.17	1250-1020	C-N stretching	Amine
1620.41	1650-1600	C=O stretching	Conjugated alkene
1715.75	1725-1700	C=O stretching	Carboxylic acid
1745.60	1750-1725	C=O stretching	Ester
2856.60	2865-2845	C-H bending	Methylene
2927.96	2935-2915	C-H bending	Methylene
3424.88	3400-3500	C-H bending	Primary amine

### *Terminalia chebula*

The FTIR spectra showed evidence of aromatic amines, aldehydes, alkanes, carboxylic acids, and esters through

their distinct peaks at 758.75, 868.53, 1028.35, 1193.87, 1318.50, 1447.21, 1612.19, 1700.11, and 3409.49 cm<sup>-1</sup> (Table 4; Figure 16).

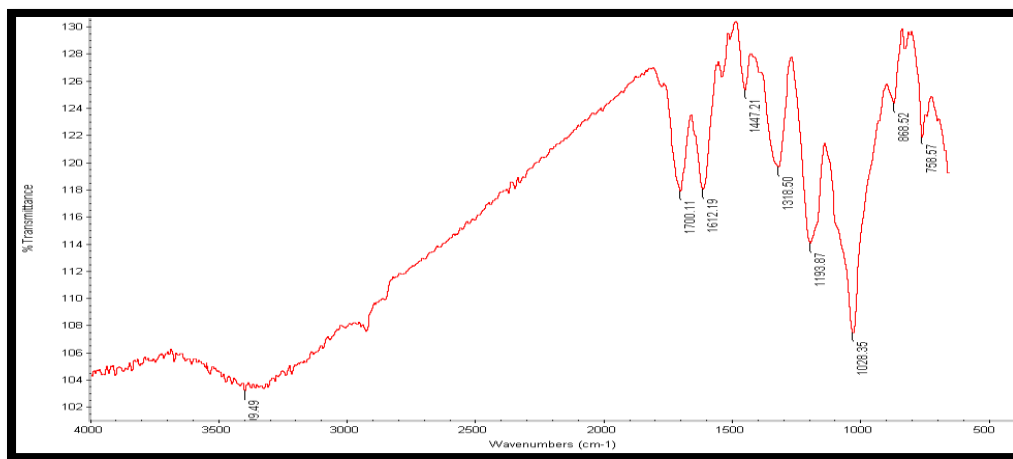


Figure 16: IR spectra of *Terminalia chebula*.

Table 4: FTIR spectral values of *Terminalia chebula*.

Wavenumber cm <sup>-1</sup> test sample	Wavenumber cm <sup>-1</sup> reference range	Functional groups	Compound class
758.57	755 ± 20	C-H bending	1,2 Disubstituted
868.52	880 20	C-H bending	1,3 Disubstituted
1028.35	1075-1020	C-O stretching	Vinyl ether
1193.87	1250-1020	C-N stretching	Amine
1318.50	1342-1266	C-N stretching	Aromatic amine
1447.21	1450-1375	C-H bending	Alkane
1612.19	1650-1600	C=C stretching	Conjugated alkene
1700.11	1680-1710	C=O stretching	Conjugated acid
3409.49	3400-3500	N-H stretching	Primary amine

### In Vitro Antiuro lithiatic Activity

The ethanolic extracts of *C. intybus*, *P. granatum*, and *T. chebula* showed strong ability to dissolve calcium oxalate, which makes them suitable for urolithiasis treatment.

*C. intybus*: 85.47% dissolution

*T. chebula*: 78.98% dissolution

*P. granatum*: 66.04% dissolution

Standard drug (reference): 90.59% dissolution

In light of these results, it has been postulated that phytochemicals such as alkaloids, tannins, terpenoids, and steroids present in plant extracts might be responsible for blocking calcium oxalate crystal growth and expansion,

which ultimately prevents kidney stone development (Patel et al., 2011; Phatak, 2015).

### Diuretic Activity

The diuretic activity of the extracts was evaluated against standard Furosemide (10 mg/kg). All extracts produced

higher urine output than the control group according to Table 6. The observed effect results from flavonoids and saponins and tannins and alkaloids which increase water and electrolyte excretion by the kidneys.

Table 5: Diuretic Activity of Plant Extracts.

Treatment	1 h	2 h	3 h	4 h	5 h
Distilled Water	0.38 ± 0.62	0.66 ± 0.71	0.97 ± 0.92	1.03 ± 0.00	1.47 ± 0.76
Furosemide (10 mg/kg)	1.84 ± 0.86	3.63 ± 1.37	5.04 ± 1.12*	6.47 ± 2.18**	7.04 ± 2.27**
<i>C. intybus</i> (400 mg/kg)	0.33 ± 0.55	1.02 ± 1.58	3.31 ± 2.07	5.01 ± 2.72	6.32 ± 2.02**
<i>P. granatum</i> (400 mg/kg)	0.47 ± 0.64	2.71 ± 1.16	5.63 ± 0.76**	6.81 ± 0.96**	8.10 ± 0.66***
<i>T. chebula</i> (400 mg/kg)	0.43 ± 0.97	2.50 ± 2.71	5.11 ± 2.87*	6.73 ± 2.71**	7.60 ± 2.67**

(\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 vs control)

### DISCUSSION

The current study substantiates the ethnopharmacological importance of *Cichorium intybus*, *Punica granatum*, and *Terminalia chebula* in the therapeutic management of urolithiasis through an integrated approach including phytochemical screening and pharmacodynamic validation. Qualitative screening confirmed the abundant presence of flavonoids, tannins, saponins, phenolics, glycosides, alkaloids, and terpenoids in all three plant extracts (Maletha et al., 2022; Tahir et al., 2018). Fourier-transform infrared (FTIR) spectral analysis revealed hydroxyl, carbonyl, and amine functional groups, confirming the presence of phenolic acids, flavonoid glycosides, and triterpenoid structures (Hedina (Hedina et al., 2016). These molecular classes are major mediators of antioxidative, anti-inflammatory, diuretic, and nephroprotective effects that collectively decrease calcium oxalate (CaOx) crystal formation, aggregation, and growth within renal calculi (Arra et al., 2024; Kachkoul et al., 2020).

Among the evaluated taxa, *Cichorium intybus* showed the highest litholytic activity, achieving 85.46% dissolution of Calcium oxalate crystals. This increased efficacy can be attributed to the synergistic interplay of its major phytoconstituents, particularly quercetin and luteolin, which are potent free-radical scavengers and membrane stabilizers (Radajewska et al., 2023; Tahir et al., 2018). These flavonoids mitigate oxalate-induced reactive oxygen species (ROS) formation, protect lipid membranes from peroxidation, and stabilize renal epithelial cells, thereby impeding crystal adhesion and retention. Saponins such as

chicorin and lactucin derivatives chelate calcium ions, lower urinary supersaturation, and induce fragmentation of preformed CaOx crystals (Maletha et al., 2022). The pronounced diuretic response observed for *C. intybus* is consistent with saponin- and alkaloid-mediated enhancement of glomerular filtration, resulting in increased urinary flow and accelerated clearance of lithogenic solutes (Tahir et al., 2018).

The peel extract of *Punica granatum* exhibited moderate litholytic potential (66.03% dissolution) but pronounced diuretic activity. This pharmacological efficacy correlates with its high content of ellagitannins and phenolic acids, notably ellagic acid, punicalagin, gallic acid, catechin, and kaempferol (Amuthan et al., 2012; Kachkoul et al., 2020). Ellagitannins hydrolyze to release bioactive phenolic acids that enhance cellular redox homeostasis, prevent lipid peroxidation, and preserve tubular membrane integrity (Radajewska et al., 2023). Punicalagin acts as both a calcium chelator and a strong antioxidant, thereby inhibiting CaOx deposition and promoting nephroprotection (Arra et al., 2024). Moreover, the diuretic potential of *P. granatum* appears mechanistically linked to phenolic-mediated regulation of aquaporin expression and renal electrolyte balance, ultimately increasing urinary volume and facilitating the elimination of oxalate and other lithogenic precursors (Kachkoul et al., 2020).

*Terminalia chebula* demonstrated remarkable CaOx dissolution capacity (78.98%) and potent diuretic effects. Its phytochemical matrix, dominated by chebulagic,

chebulinic, and gallic acids, has been associated with calcium-binding, antioxidant, and crystal-inhibitory activities (Anu et al., 2020). FTIR analysis confirmed the presence of triterpenoids such as arjunolic acid and chebulosides, which enhance renal microcirculation and modulate tubular reabsorption mechanisms (Hedina et al., 2016). The combination of hydrolyzable tannins and triterpenoids facilitates calcium ion sequestration, suppresses crystal nucleation, and preserves renal epithelial integrity through oxidative stress mitigation (Anu et al., 2020). This dual mechanism, comprising inhibition of crystal growth and promotion of diuresis, likely underpins the nephroprotective and anti-recurrent efficacy of *T. chebula* (Liu et al., 2024; Tayal et al., 2012).

Collectively, these plant extracts exert antiurolithiatic actions through a tri-mechanistic framework: (i) diuretic activity that reduces urinary supersaturation; (ii) inhibition of CaOx nucleation and aggregation by phenolic and flavonoid antioxidants; and (iii) cytoprotective defense against oxalate-induced oxidative stress in renal epithelial cells (Arra et al., 2024; Kachkoul et al., 2020). While *C. intybus* demonstrated the most potent lithotriptic effect, *P. granatum* and *T. chebula* exhibited superior diuretic efficacy comparable to conventional pharmacological agents (Amuthan et al., 2012; Anu et al., 2020).

The findings of this study align with prior pharmacognostic evidence indicating that polyphenol-enriched botanicals act via dual therapeutic mechanisms, modulating urinary composition while providing direct cellular protection (Hedina et al., 2016; Tahir et al., 2018). Consequently, this research provides robust scientific validation for the traditional therapeutic use of *C. intybus*, *P. granatum*, and *T. chebula* in preventing and treating nephrolithiasis. The synergistic integration of flavonoids, tannins, saponins, and triterpenoids across these botanicals forms a complex phytopharmacological network that confers antioxidant, anti-crystallization, and diuretic properties, positioning them as promising candidates for the development of standardized phytopharmaceutical formulations for renal calculus prevention and dissolution (Jamil et al., 2023; Arra et al., 2024).

## CONCLUSION

The research establishes strong evidence that confirms the effectiveness of *Cichorium intybus*, *Punica granatum*, and *Terminalia chebula* for treating urolithiasis. The phytochemical and FTIR analyses showed that the plant contains bioactive secondary metabolites, which include flavonoids, tannins, saponins,

phenolic acids, alkaloids, and triterpenoids. These compounds have potent antioxidant, anti-inflammatory, nephroprotective, and diuretic properties. The results showed that *C. intybus* produced the most effective crystal dissolution of calcium oxalate while *T. chebula* and *P. granatum* displayed strong diuretic and crystal-inhibitory properties. The biological processes that occur through these activities stem from their ability to block crystal formation, aggregation, their capacity to bind calcium ions, reduce kidney oxidative damage, and enhance urine elimination. These plants can be utilized as promising anti-urolithiasis agents and have the potential to treat, as well as prevent, kidney stone disease.

## REFERENCES

- Ahmed, S., Hasan, M. M., and Mahmood, Z. A. (2016). Antiurolithiatic plants in different countries and cultures. *Journal of Pharmacognosy and Phytochemistry* **5**, 102.
- Al-Snafi, A. E. (2016). Medical importance of *Cichorium intybus*—A review. *IOSR Journal of Pharmacy* **6**, 41-56.
- Alelign, T., and Petros, B. (2018). Kidney stone disease: an update on current concepts. *Advances in urology* **2018**, 3068365.
- Amari, S., Karbab, A., Charef, N., Arrar, L., and Mubarak, M. S. (2023). Anti-urolithiatic, antibacterial, anti-inflammatory and analgesic effects of *Erica arborea* flowers and leaves hydromethanolic extracts: An ethnopharmacological study. *Saudi Journal of Biological Sciences* **30**, 103785.
- Amuthan, A., Chogtu, B., Bairy, K., and Prakash, M. (2012). Evaluation of diuretic activity of *Amaranthus spinosus* Linn. aqueous extract in Wistar rats. *Journal of ethnopharmacology* **140**, 424-427.
- Anu, V., Akhila, S., Kumar, I. A., and Antony, S. (2020). In-vitro antiurolithiatic activity of macerated aqueous extract of *Terminalia chebula* by using titrimetry method. *Int J Pharmacogn* **7**, 144-147.
- Arra, K., Pasupula, R., and Anandam, S. (2024). In Vivo Assessment of *Punica granatum* Leaf Extract: Anti-Urolithiatic and Nephroprotective Effects. *Natural Product Sciences* **30**, 80-92.
- Caffall, K. H., and Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate research* **344**, 1879-1900.
- Desai, S. D., Desai, D. G., and Kaur, H. (2009). Saponins

- and their biological activities. *Pharma times* **41**, 13-16.
- Hedina, A., Kotti, P., Kausar, J., and Anand, V. (2016). Phytopharmacological overview of Terminalia chebula Retz. *Pharmacognosy Journal* **8**.
- Jurenka, J. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): a review. *Alternative medicine review* **13**.
- Kachkoul, R., Houssaini, T. S., Mohim, M., El Habbani, R., and Lahrichi, A. (2020). Chemical Compounds Identification and Antioxidant and Calcium Oxalate Anticrystallization Activities of *Punica granatum* L. *Evidence-Based Complementary and Alternative Medicine* **2020**, 9424510.
- Khan, M., Khalilullah, H., Akhtar, J., and Elhasan, G. O. (2015). Terminalia chebula: an ephemeral glance. *Int J Pharm Pharm Sci* **7**, 40-43.
- Khan, N. T. (2019). Anthraquinones-A naturopathic compound. *Journal of New Developments in Chemistry* **2**, 25-8.
- Kodangala, C., Saha, S., and Kodangala, P. (2010). Phytochemical studies of aerial parts of the plant *Leucas lavandulaefolia*. *Der Pharma Chemica* **2**, 434-437.
- Liu, H., Chen, Z., Liu, M., Li, E., Shen, J., Wang, J., Liu, W., and Jin, X. (2024). The Terminalia chebula Retz extract treats hyperuricemic nephropathy by inhibiting TLR4/MyD88/NF- $\kappa$ B axis. *Journal of Ethnopharmacology* **322**, 117678.
- Madeo, J., Zubair, A., and Marianne, F. (2013). A review on the role of quinones in renal disorders. *Springerplus* **2**, 139.
- Maletha, D., Singh, S. P., and Ramanarayanan, S. (2022). Hepatoprotective and nephroprotective efficacy of *Cichorium intybus* following imidacloprid induced subchronic toxicity in WLH cockerels. *Indian J. Anim. Sci* **92**, 940-945.
- Niharika, M., Suchitha, N., Akhila, S., Himabindhu, J., and Ramanjaneyulu, K. (2018). Evaluation of in vitro antiurolithiatic activity of *Gossypium herbaceum*. *Journal of Pharmaceutical Sciences and Research* **10**, 1236-1237.
- Patel, M. A., Patel, P. K., and Seth, A. K. (2011). I HIBITIO OF CALCIUM OXALATE CRYSTALLIZATIO BY THE FRUIT EXTRACTS OF *Piper longum* L. *growth* **10**, 11.
- Phatak, R. (2015). In-vitro antiurolithiatic activity of *Kalanchoe pinnata* extract.
- Radajewska, A., Szyller, J., Niewiadomska, J., Noszczyk-Nowak, A., and Bil-Lula, I. (2023). *Punica granatum* L. polyphenolic extract as an antioxidant to prevent kidney injury in metabolic syndrome rats. *Oxidative Medicine and Cellular Longevity* **2023**, 6144967.
- Rao, N. K., and Nammi, S. (2006). Antidiabetic and renoprotective effects of the chloroform extract of Terminalia chebula Retz. seeds in streptozotocin-induced diabetic rats. *BMC complementary and alternative medicine* **6**, 17.
- Rawal, G., Yadav, S., and Nagayach, S. (2015). Phytosterols and the health. *Med. Res. Chron* **2**, 441-444.
- Roy, A. (2017). A review on the alkaloids an important therapeutic compound from plants. *IJPB* **3**, 1-9.
- Rustan, A. C., and Drevon, C. A. (2005). Fatty acids: structures and properties. *Encyclopedia of life sciences* **1**, 1-7.
- Sama, K., Xavier, V., and Raja, A. (2011). Preliminary phytochemical screening of root bark of *Delonix regia*. *Int J Pharm Life Sci* **2**, 42-43.
- Samal, L., Pattanaik, A. K., Mishra, C., Maharana, B. R., Sarangi, L. N., and Baithalu, R. K. (2011). Nutritional strategies to prevent Urolithiasis in Animals. *Veterinary World* **4**.
- Sinha, D. (2019). Pharmacological importance of polyphenols: A review. *Inter. Res. Jour. Of Pharmacy* **10**, 13-23.
- Tahir, M., Sadiq, N., Ahmed, S., Ali, A., Rajpoot, N. N., and Riaz, U. (2018). Effects of aqueous and methanolic extracts of *cichorium intybus* seeds on gentamicin induced nephrotoxicity in rats. *Journal of Islamic International Medical College (JIIMC)* **13**, 184-188.
- Tayal, S., Duggal, S., Bandyopadhyay, P., Aggarwal, A., Tandon, S., and Tandon, C. (2012). Cytoprotective role of the aqueous extract of Terminalia chebula on renal epithelial cells. *International braz j urol* **38**, 204-214.
- UC, R., and NAIR, V. M. G. (2013). Phytochemical analysis of successive reextracts of the leaves of *Moringa oleifera* Lam. *International Journal of Pharmacy and pharmaceutical sciences* **5**, 629-634.
- Weijjs, P. J., Cynober, L., DeLegge, M., Kreymann, G., Wernerman, J., and Wolfe, R. R. (2014). Proteins and amino acids are fundamental to optimal nutrition support in critically ill patients. *Critical care* **18**, 591.
- Xu, H., Zisman, A. L., Coe, F. L., and Worcester, E. M. (2013). Kidney stones: an update on current

pharmacological management and future directions. *Expert opinion on pharmacotherapy* **14**, 435-447.