

# Phytochemical Analysis and Anti-Urolithiatic Potential of *Cyamopsis tetragonoloba* and *Trichosanthes dioica*

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## ABSTRACT

**Objective:** The medicinal and industrial uses of plants have long been acknowledged. Bioactive chemicals, nutritional advantages, and pharmacological characteristics are what make Cluster Bean (*Cyamopsis tetragonoloba*) and Pointed Gourd (*Trichosanthes dioica*) so special. The purpose of this research is to determine whether or not they have anti-urolithiatic properties and to examine their phytochemical makeup.

**Materials and methods:** After collecting and verifying the plant materials, they were extracted using the Soxhlet apparatus and solvents such as petroleum ether and ethyl acetate. To find the most important bioactive chemicals, phytochemical screening was performed. For the purpose of phytochemical profiling, HPTLC and Thin Layer Chromatography (TLC) were employed. The anti-urolithiatic action was assessed in Wistar rats that had hyperoxaluria induced by ethylene glycol. Lactate dehydrogenase (LDH) activity, kidney oxalate levels, and urine parameters were examined.

**Results:** Essential oils, glycosides, tannins, flavonoids, and saponins were all identified through phytochemical research. In terms of extraction efficiency, ethyl acetate extract outperformed petroleum ether. Several bioactive substances were found through chromatographic analysis. Treatment groups showed markedly enhanced renal function and reduced oxalate deposition in in vivo investigations, suggesting substantial anti-urolithiatic activity.

**Conclusion:** The study highlights the potential use of *C. tetragonoloba* and *T. dioica* in nutraceutical and pharmaceutical applications by establishing their pharmacological relevance. To learn more about how effective they are as a treatment, additional studies involving clinical trials and formulation development are needed.

**Keywords:** *Cyamopsis tetragonoloba*, *Trichosanthes dioica*, Phytochemical Screening, Anti-Urolithiatic Activity, Medicinal Plants, Chromatographic Analysis

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**Conflict of interest:** None

## INTRODUCTION

For countless generations, medicinal plants have supplied humans with vital nutrients and healing properties, making them an indispensable component of human society.<sup>1</sup> Cluster beans (*Cyamopsis tetragonoloba*) and pointed gourds (*Trichosanthes dioica*) are two of the most important of these because of the many ways they are used in cooking, medicinal, and manufacturing. The bioactive chemicals and pharmacological potential of these plants have attracted a lot of scientific interest; they are mostly grown in tropical climates.<sup>2</sup>

The drought-resistant leguminous plant *Cyamopsis tetragonoloba* L. is extensively farmed for both its nutritional and industrial use; it goes by several other names, including cluster bean and guar.<sup>3</sup> This resilient crop, which is a member of the Leguminaceae family, does well in a variety of soil types and is especially well-suited to dry and semi-arid climates where irrigation is not an option. While guar pods are eaten raw as a vegetable, the galactomannan content of the dried seeds, which is called guar gum, is what makes them valuable. The emulsifying, thickening, and stabilising qualities of guar gum make it an

indispensable industrial material in many different fields, including the food processing, paper, textile, pharmaceutical, and oil drilling industries. *C. tetragonoloba* produces tiny, ovoid seeds that can be any shade of white, grey, or black (Fig. 1).<sup>4</sup>

In addition to its use in industry, *C. tetragonoloba* has long been acknowledged in traditional medicine as a beneficial plant for the treatment of dyspepsia and anorexia due to its cooling, laxative, appetising, and digestive characteristics. Secondary metabolites have tremendous promise for therapeutic advances, and recent studies have highlighted the importance of improving extraction procedures to increase recovery of these compounds.<sup>5</sup>

A popular vegetable crop in India is *Trichosanthes dioica* Roxb., which goes by many names including pointed gourd and "Parwal" in Hindi. This climbing plant, which is a member of the Cucurbitaceae family, has long been a staple in authentic Indian cooking. In addition to its culinary importance, *T. dioica* has a long history of therapeutic usage in Ayurvedic medicine (Fig. 2).<sup>6</sup>

In addition to its medical usefulness, the plant is a good source of important minerals like vitamins A and C, as well

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Table 1: Extraction of plant material for *C. tetragonoloba* (Seeds) and *T. dioica* (Fruits)

S.No.	Solvent	Weight of drug (g)	% Yield
01	Pt. Ether (60%-80%)	300 ( <i>C. tetragonoloba</i> )	2.340%
02	Ethyl acetate	300 ( <i>C. tetragonoloba</i> )	14.412%
03	Pt. Ether (60%-80%)	300 ( <i>T. dioica</i> )	2.477%
04	Ethyl acetate	300 ( <i>T. dioica</i> )	15.412%

as bioactive chemicals like alkaloids, pentacyclic triterpenes, tannins, and saponins. Some Ayurvedic texts praise *T. dioica* for its possible use in treating diabetes, inflammation, and parasite infections.<sup>7</sup>

There has to be a comprehensive investigation of *T. dioica*'s macroscopy, microscopy, physicochemical characteristics, and phytochemical composition because, despite its extensive medicinal uses, pharmacognostic research on the plant is scarce. Such research is essential for determining its pharmacological importance and developing its possible uses in contemporary medicine.<sup>8</sup>

The purpose of this research is to investigate the pharmacological and phytochemical properties of *T. dioica* and *C. tetragonoloba* so that they can be used effectively in the pharmaceutical, nutraceutical, and food industries.

## METHODOLOGY

### Gathering and Verification of Plant Materials

*Cyamopsis tetragonoloba* seeds and *Trichosanthes dioica* fruits were selected for this investigation after they were gathered and verified for their macroscopic and microscopic features. Different physical and anatomical characteristics were examined to verify the identity (Fig. 3).<sup>9</sup>

### Extraction of Plant Materials

Use of the Soxhlet apparatus was employed for the extraction of *C. tetragonoloba* seeds and *T. dioica* fruit components. For continuous hot extraction, solvents such as petroleum ether (60–80% concentration) and ethyl acetate were utilised. We used 1000 ml of solvent to extract 300 g of dried powder from each plant source. Prior to further investigation, the extracts were concentrated and preserved.<sup>10</sup>

### Preliminary Phytochemical Screening

In order to identify the existence of different bioactive components, the extracts were tested qualitatively using phytochemical methods. As part of the screening process, we looked for things like steroids, glycosides, tannins, flavonoids, saponins, proteins, carbohydrates, and essential oils. We identified the chemical components in the petroleum ether and ethyl acetate extracts by following standard qualitative techniques.<sup>11</sup>

### Chromatographic Analysis

#### Thin Layer Chromatography (TLC)

The phytochemical composition of the extracts was analysed using Thin Layer Chromatography (TLC). A variety of solvent solutions, including n-Hexane, were employed for the separation process: For *C. tetragonoloba*, the ratio of ethyl acetate to methanol is 12:4.5:2.5, while for *T. dioica*, the ratio is 14.5:4:1.5. Following application to silica gel plates, the extracts were allowed to grow in their corresponding solvent solutions. Spots were examined using both visible and ultraviolet light in order to determine the compounds that had been separated.<sup>12</sup>

#### High-Performance Thin Layer Chromatography (HPTLC)

A comprehensive phytochemical fingerprint was obtained by means of HPTLC. Toluene, ethyl acetate, and acetic acid were the optimal solvents for high-performance thin-layer chromatography (HPTLC). For accurate compound identification, the chromatographic spots were seen under 366 nm UV light.<sup>13</sup>

### Evaluation of Anti-Urolithiatic Activity

#### Experimental Animals

Adult Wistar rats weighing 150–250 g and four to five months old were utilised in the research. Under normal laboratory circumstances, the animals were kept at a temperature of  $25 \pm 2^\circ\text{C}$  and subjected to a 12-hour light/dark cycle. Pets were given an unlimited supply of water and a standard pellet feed. With the blessing of the Institutional Animal Ethical Committee (IAEC), the research proceeded in accordance with all applicable ethical standards.<sup>14</sup>

#### Induction of Hyperoxaluria

The first three days of inducing hyperoxaluria in Wistar rats involved giving them water that contained 0.75 percent



Fresh guar green pods



Dried guar pods

Figure 1: *Cyamopsis tetragonoloba* L. (Guar) plant showing different parts

Table 2: Preliminary phytochemical screening for *C. tetragonoloba* (Seeds) and *T. dioica* (Fruits)

S.No.	Test	Pet. Ether Extraction of <i>C. tetragonoloba</i> (PEECT)	Ethyl acetate extraction of <i>C. tetragonoloba</i> (EAECT)	Pet. Ether extraction of <i>T.dioica</i> (PEETD)	Ethyl acetate extraction of <i>T.dioica</i> (EAETD)
01	Alkaloids	-	-	-	-
02	Proteins	-	++	-	-
03	Carbohydrate	-	++	-	+
04	Glycosides	-	+	-	++
05	Tannins and Phenolic comp.	-	++	-	++
06	Flavonoids	-	++	-	++
07	Saponins	-	++	-	++
08	Essential Oils	+	-	+	-
09	Steroids	+	-	+	-

Note: + Present, - Absent

ethylene glycol (EG) and 1 percent ammonium chloride (AC). To encourage stone formation over the rest of the research time, just 0.75 % v/v EG was given.<sup>15</sup>

**Acute Oral Toxicity Study**

Following OECD guideline 423, researchers examined the effects of acute oral toxicity. After 14 days of observation for toxicity symptoms and death, Swiss albino mice were

given a single oral dose of 2000 mg/kg body weight. Regular intervals were used to record the observations.<sup>16</sup>

**Experimental Design**

The research included a total of six groups, with six participants in each. As a control group, Group I got water and a regular diet. Group II served as a control for urolithiatic symptoms; this group received EG and AC but did not get any extract. Cystone (500 mg/kg) was administered as the standard treatment to Group III. Three groups were given different doses of ethyl acetate extracts from different plants: *C. tetragonoloba* (100 mg/kg), *T. dioica* (300 mg/kg), and Group IV (100 mg/kg).<sup>17</sup>

**Biochemical Analysis**

Urine and kidney samples were taken for biochemical analysis after the therapy period. With the use of standard reagent kits, urine samples were tested for several elements including pH, oxalate, calcium, magnesium, phosphorus, creatinine, urea, uric acid, and total protein composition. The levels of lactate dehydrogenase (LDH) were measured in kidney tissue homogenates.<sup>18</sup>

**Statistical Analysis**

A one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to analyse the data.



Figure 2: *Trichosanthes dioica* Roxb. (Parwal) plant

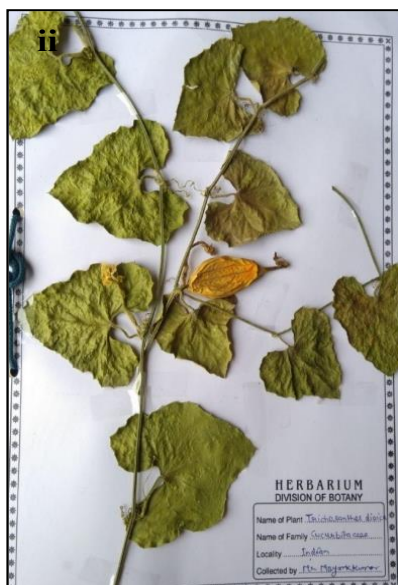


Figure 3: Herbarium sample plants (i) *Cyamopsis tetragonoloba* L. (ii) *Trichosanthes dioica* Roxb. (iii) Certificate obtained with accession number from Central Ayurveda Research Institute (CARI), Jhansi

Table 3: Impact of EAECT on hyperoxaluric rats' LDH, oxalate, and urine pH levels

Parameters	Normal Control	Lithiatic Control <sup>S</sup>	CST 500mg/kg <sup>#</sup>	EAECT 100mg/kg <sup>#</sup>	EAECT 300mg/kg <sup>#</sup>	EAECT 500mg/kg <sup>#</sup>
Ur. Vol. (ml/24 hrs)	18.60±0.72	10.53±0.41***	21.65±0.67***	11.47±0.62 <sup>ns</sup>	12.52±0.62 <sup>ns</sup>	15.09±0.42***
Ur. pH	7.78±0.28	6.24±0.11***	8.16±0.20***	7.91±0.24***	7.48±0.17***	8.07±0.11***
Ur. Ox (mg/dl)	0.33±0.04	4.81±0.41***	0.99±0.14***	3.99±0.14 <sup>ns</sup>	3.21±0.21***	1.95±0.11***
KH. Ox (mg/g)	2.82±0.18	7.32±0.55***	2.72±0.16***	5.83±0.38 <sup>ns</sup>	4.53±0.31***	4.06±0.25***
Sr. LDH (U/I)	845.81±106.21	2387.34±215.50***	1021.24±64.31***	1980.82±133.17 <sup>ns</sup>	1620.12±116.40**	1296.52±88.35***
KH. LDH (U/g)	1.95±0.24	5.02±0.41***	1.88±0.08***	4.05±0.28 <sup>ns</sup>	3.31±0.20**	2.96±0.20***

All values are presented as mean ± SEM (n=6)

Average plus or minus the standard error of the mean (SEM) was used to express the results. If the p-value was less than 0.05, statistical significance was assumed.<sup>19</sup>

## RESULTS

### Macroscopy and Microscopy of *Cyamopsis tetragonoloba* (Seeds) and *Trichosanthes dioica* (Fruits)

*Cyamopsis tetragonoloba*, more often known as guar, is a kind of plant with unusually shaped seeds. The young pods are round, verdant, and packed with 5–12 seeds each. The seeds have a nice aroma and a bitter flavour; they can be either cube- or oval-shaped. Pointed gourd, or *Trichosanthes dioica*, is another plant that looks similar; it produces smooth, green, oblong, or ovoid berries that are encased in a thick rind. In the field of botany, these unique characteristics help with identification and categorisation (Figure 4 and Figure 8).

Under a microscope, the guar pod reveals a protective pericarp covered in parenchymatous cells that form a rectangular shape. Strength is provided mechanically by 6–7 lignified stone cell layers beneath this layer. Figures 5 and 6 show the guar seed's structure, which consists of an outer integument of palisade cells, followed by layers of parenchymatous cells. These layers include oil globules and aleurone grains, which help with germination and nutrient storage. The existence of thicker helical xylem arteries, epidermal cells covered by a thin cuticle, and parenchyma cells are all shown by powder analysis, which indicates that it is fibrous (Figure 7).

Part A. Powder, Parenchyma cell group (B), epidermal cells (C), and stomata (D) magnified 40 times, 10 times, and 40 times, respectively. The Xylem vessels are thickened and twisted. S. Single-fibre and parenchymatous cells, F. Macro sclereides 40x, G. H. One tracheid 40 times

The layers of *Trichosanthes dioica*'s exocarp, mesocarp, and endocarp are shown by its longitudinal and transverse sections. The vascular bundles allow nutrients to be transported, and the stomata on the epicarp allow gaseous exchange. Starch grains found in fruit structure indicate that it stores carbohydrates (Figure 9).

### Extraction and Phytochemical Screening

Soxhlet extraction with petroleum ether (60–80% concentration) and ethyl acetate was used to extract plant components (Fig.10). Table 1 shows that ethyl acetate had

a far better capacity to extract bioactive chemicals than petroleum ether, with an extraction efficiency of 14.412% for *C. tetragonoloba* and 15.412% for *T. dioica* compared to 2.340% and 2.477%, respectively.

Sugars, glycosides, tannins, flavonoids, saponins, steroids, essential oils, and the like were detected in the initial phytochemical screening. Table 2 shows that both plant materials' ethyl acetate extracts had a high concentration of bioactive chemicals, while the petroleum ether extracts mostly included steroids and essential oils. These results highlight the significance of solvent polarity in phytochemical extraction.

### Thin Layer Chromatography (TLC)

Figure 11 shows that different spots identified under visible and UV light were used in TLC analysis to establish the existence of several bioactive components in ethyl acetate extracts. The following solvent systems were employed for separation: For *C. tetragonoloba*, the ratio of ethyl acetate to methanol is 12:4.5:2.5, while for *T. dioica*, the ratio is 14.5:4:1.5. The existence of various beneficial chemicals was further confirmed by HPTLC fingerprinting, which showed unique peaks (Figures 12 and 13), further validating the diversified phytochemical composition.

### High-Performance Thin Layer Chromatography (HPTLC) Fingerprinting

The phytochemical variety of *C. tetragonoloba* (seeds) and *T. dioica* (fruits) was confirmed by the HPTLC fingerprinting results, which showed the presence of numerous compounds with varied retention factor ( $R_f$ ) values. Figure 12 shows the chromatogram of *C. tetragonoloba* (seeds), which contains many bioactive chemicals, showing that there are multiple peaks at 254 nm. The presence of several phytoconstituents was confirmed by peaks observed under UV light at 366 nm in the chromatogram for *T. dioica* (fruits) (Figure 13).

In addition to the results from TLC and phytochemical screening, the complex chemical makeup of the extracts is confirmed by the many peaks identified in both chromatograms. The results of the HPTLC fingerprinting test give a reliable analytical tool for characterising bioactive chemicals in *C. tetragonoloba* and *T. dioica*, which could be useful in pharmaceutical research.

### Anti-Urolithiatic Activity Evaluation



Figure 4: Morphological study of Open guar beans and seeds

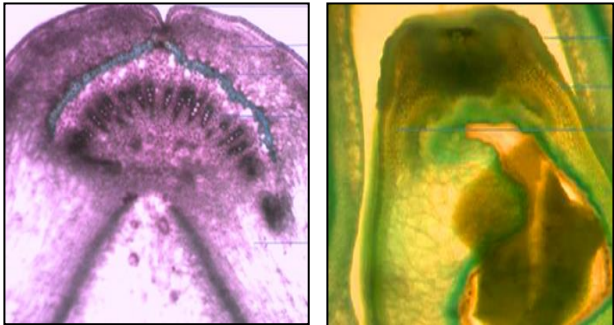


Figure 5: Microscopical study of Guar pod

Figure 6: Microscopical study of Guar seed

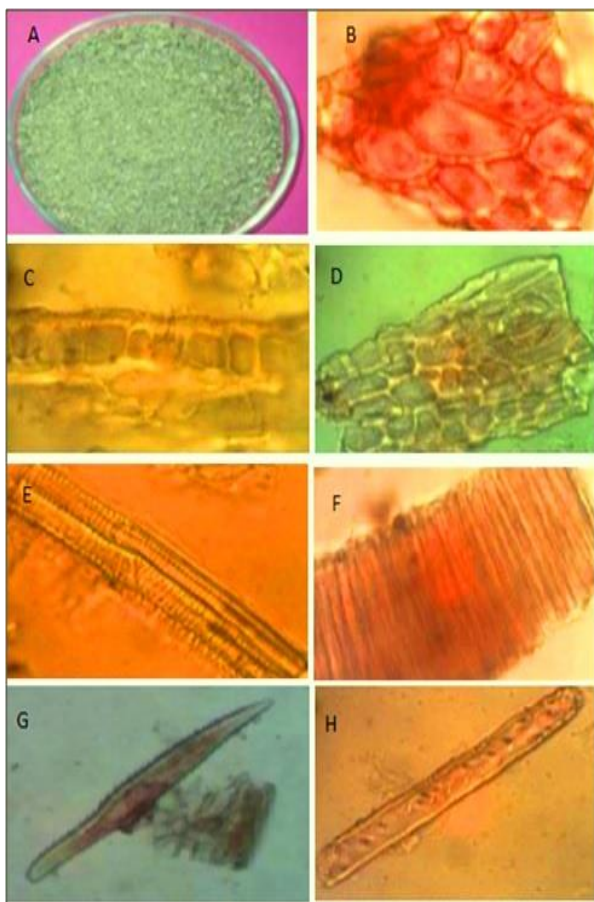


Figure 7: Powder study of Guar seed

One way to induce hyperoxaluria in Wistar rats was to give them water that contained 0.75% ethylene glycol (EG) and 1% ammonium chloride (AC). Urine parameters, kidney



Figure 8: Morphological study of *Trichosanthes dioica* (Fruits)

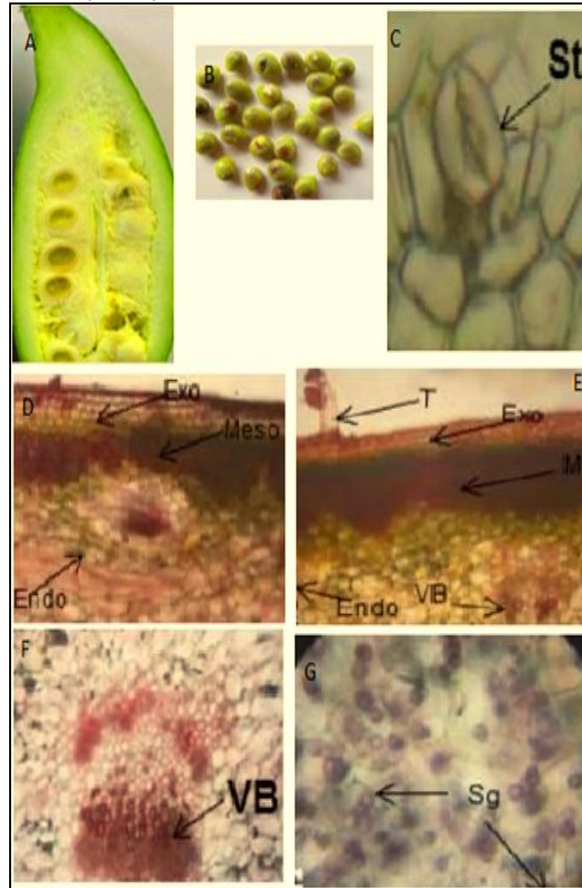


Figure 9: Morphological study of *Trichosanthes dioica* (Fruits)

oxalate levels, and LDH levels were used to evaluate the anti-urolithiatic activity of ethyl acetate extracts.

#### Effect on Urine Output and pH

Reduced urine production and acidity levels were hallmarks of renal impairment in the urolithiatic control group. Cystone and ethyl acetate extracts showed a diuretic and nephroprotective effect in a treatment that greatly improved urine volume and pH (Tables 3 and 4; Figs. 14 and 15).

#### Effect on Oxalate Levels

Extensive stone formation was indicated by dramatically raised oxalate levels in the urine and kidneys of the hyperoxaluric rats. Evidence of prevention of calcium oxalate crystallisation and deposition in renal tissues was

Table 4: Measurements of oxalate, LDH, urine pH, and urine production in rats with hyperoxaluria as a result of EAETD

Parameters	Normal Control	Lithiatic Control <sup>S</sup>	CST 500mg/kg <sup>#</sup>	EAETD 100mg/kg <sup>#</sup>	EAETD 300mg/kg <sup>#</sup>	EAETD 500mg/kg <sup>#</sup>
Ur. Vol. (ml/24 hrs)	18.70±0.72	10.63±0.41***	21.78±0.64***	11.59±0.61 <sup>ns</sup>	12.51±0.62 <sup>ns</sup>	15.12±0.42***
Ur. pH	7.75±0.26	6.24±0.13***	8.16±0.18***	7.97±0.27***	7.48±0.16***	8.03±0.13***
Ur. Ox (mg/dl)	0.33±0.05	4.86±0.43***	0.97±0.13***	3.99±0.12 <sup>ns</sup>	3.20±0.21***	1.97±0.11***
KH. Ox (mg/g)	2.69±0.18	7.35±0.56***	2.72±0.17***	5.72±0.41 <sup>ns</sup>	4.54±0.37***	4.06±0.27***
Sr. LDH (U/I)	847.92±107.21	2398.39±218.50***	1022.25±64.31***	1981.83±135.18 <sup>ns</sup>	1623.15±118.40**	1284.57±88.37***
KH. LDH (U/g)	1.97±0.27	5.03±0.43***	1.91±0.09***	4.05±0.27 <sup>ns</sup>	3.34±0.22**	2.99±0.21***

All values are presented as mean ± SEM (n=6)

suggested by the considerable reduction in oxalate levels observed after treatment with ethyl acetate extracts.

#### Effect on Lactate Dehydrogenase (LDH) Levels

Hyperparathyroidism manifests as an increase in lactate dehydrogenase (LDH) levels in the blood and renal tissues of rats. Nephroprotective potential was indicated by the efficient reduction of LDH levels by cystone and ethyl acetate extracts.

## DISCUSSION

This study set out to examine the bioactive compound composition and anti-urolithiatic potential of Cluster Bean (*Cyamopsis tetragonoloba*) and Pointed Gourd (*Trichosanthes dioica*) via the lens of their phytochemical and pharmacological characteristics. Their nutritional and therapeutic value is enhanced by the presence of essential oils, glycosides, tannins, flavonoids, saponins, and other secondary metabolites that were identified during the phytochemical screening. Solvent polarity plays a significant influence in optimising yield; for example, these bioactive chemicals were better extracted from petroleum ether than from ethyl acetate.

Further validation of the complex chemical content of both plants was provided by chromatographic tests, which included Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC). The extracts' varied chromatographic peaks indicate a wide variety of bioactive components that might be accountable for the pharmacological effects seen. These phytoconstituents found in *C. tetragonoloba* and *T. dioica* suggest that these plants may have medicinal uses in both conventional and alternative medicine.

Results from the study of anti-urolithiatic activity in Wistar rats were encouraging. There were notable changes in urine parameters, kidney oxalate levels, and lactate dehydrogenase (LDH) activity after ethylene glycol and ammonium chloride were used to successfully produce hyperoxaluria. Ethyl acetate extracts of *T. dioica* and *C. tetragonoloba* significantly reduced urinary oxalate levels after treatment, suggesting that these plants may impede the crystallisation of calcium oxalate. The extracts also improved urine pH and decreased LDH levels, two important markers of kidney injury, demonstrating nephroprotective capabilities.

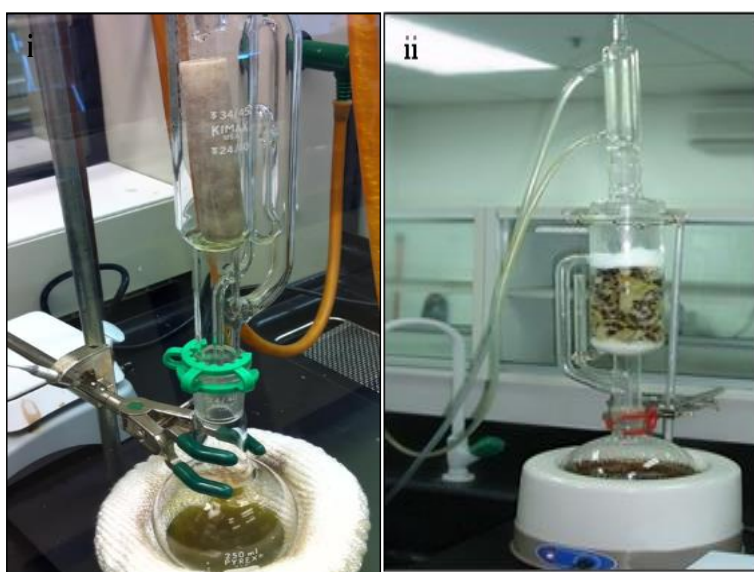


Figure 10: (i) Extraction process for *C. tetragonoloba* (Seeds) and (ii) *T. dioica* (Fruits)

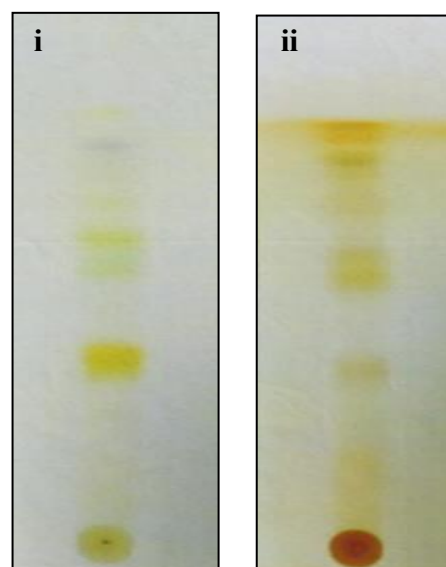


Figure 11: TLC finger printing extract of *C. tetragonoloba* (Seeds) and *T. dioica* (Fruits)

Treatment groups showed a marked decrease in oxalate deposition, which may indicate that the two plants have substantial anti-urolithiatic effects via processes such as antioxidant activity, crystal aggregation inhibition, and diuresis. Traditional use of *C. tetragonoloba* and *T. dioica* in renal health management is further supported by our results, which are in line with prior studies on medicinal plants demonstrating nephroprotective properties.

There are still certain restrictions, though, even with these encouraging results. If we want to know if these plant extracts are safe and effective for humans, we need to do clinical studies, since this study mostly used preclinical animal models. Targeted medication development may be possible if additional study is conducted to discover and extract the particular bioactive chemicals that exhibit anti-urolithiatic action.

This study concludes that *C. tetragonoloba* and *T. dioica* have pharmacological significance and may find use in the

pharmaceutical and nutraceutical sectors. The entire therapeutic potential of these compounds should be investigated in future studies through formulation development, bioavailability studies, and clinical evaluations.

## CONCLUSION

Both Cluster Bean (*Cyamopsis tetragonoloba*) and Pointed Gourd (*Trichosanthes dioica*) were thoroughly investigated for their phytochemical content and pharmacological potential in this investigation. The results validate the existence of key bioactive components that give these plants their therapeutic value, such as tannins, flavonoids, saponins, glycosides, and essential oils. According to the results of the Soxhlet extraction method, bioactive chemicals were better extracted from ethyl acetate than petroleum ether. Using chromatographic methods like TLC

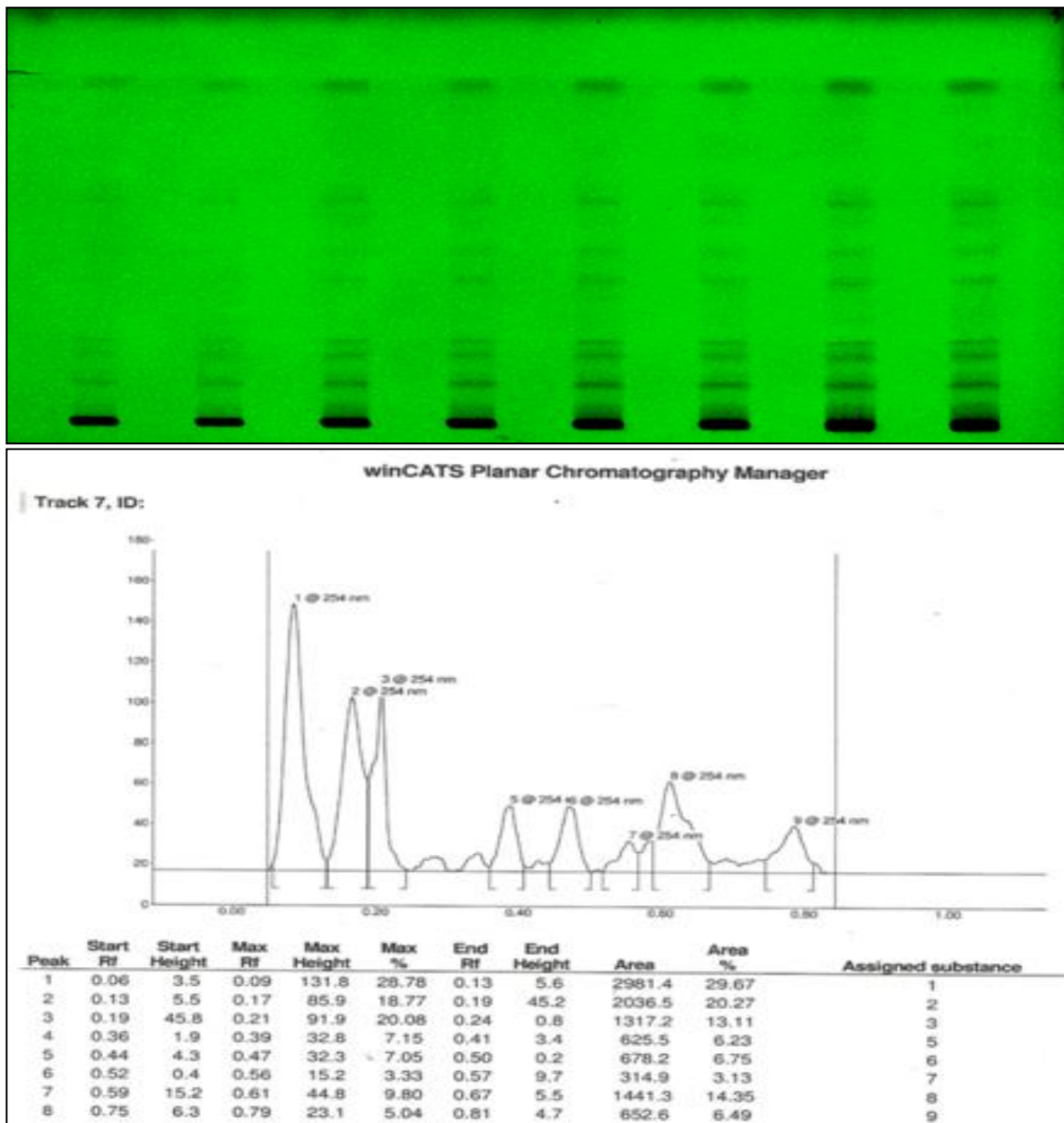


Figure 12: HPTLC finger printing and chromatogram extract of *C. tetragonoloba* (Seeds) under 254 nm

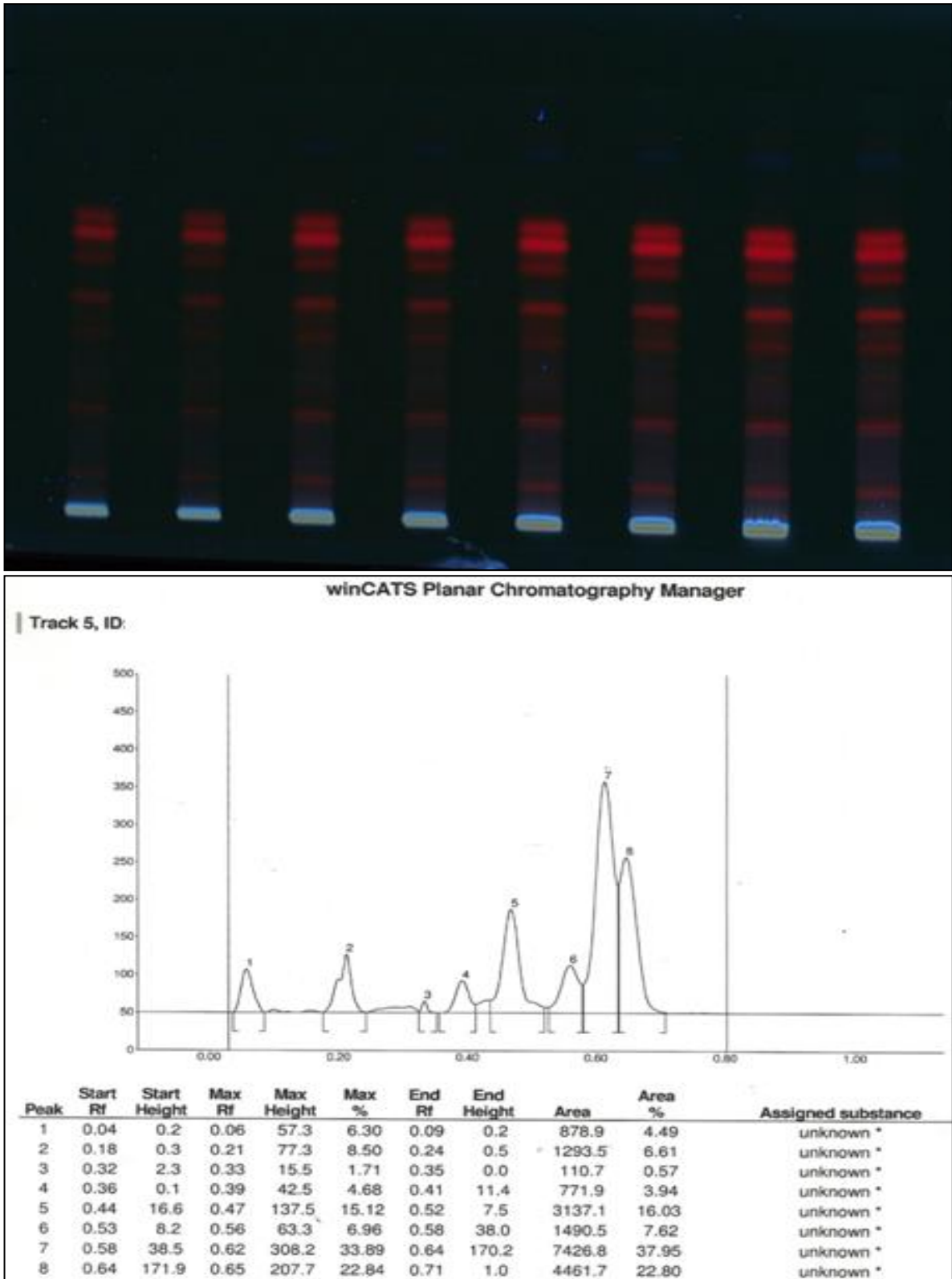


Figure 13: HPTLC finger printing and chromatogram extract of *T. dioica* (Fruits) under 366 nm

and HPTLC, the intricate phytochemical profiles of these plants were further confirmed.

These plant extracts showed promising therapeutic potential in the evaluation of anti-urolithiatic activity in Wistar rats. The protective effect against kidney stone formation was demonstrated by the ethyl acetate extracts of *C. tetragonoloba* and *T. dioica*, which reduced oxalate

deposition, improved urine parameters, and decreased lactate dehydrogenase (LDH) levels. These results indicate that both plants have potential as ingredients in herbal remedies for the treatment of urolithiasis and associated kidney diseases.

In sum, the findings of this study lend credence to the idea that *C. tetragonoloba* and *T. dioica* could find use in the

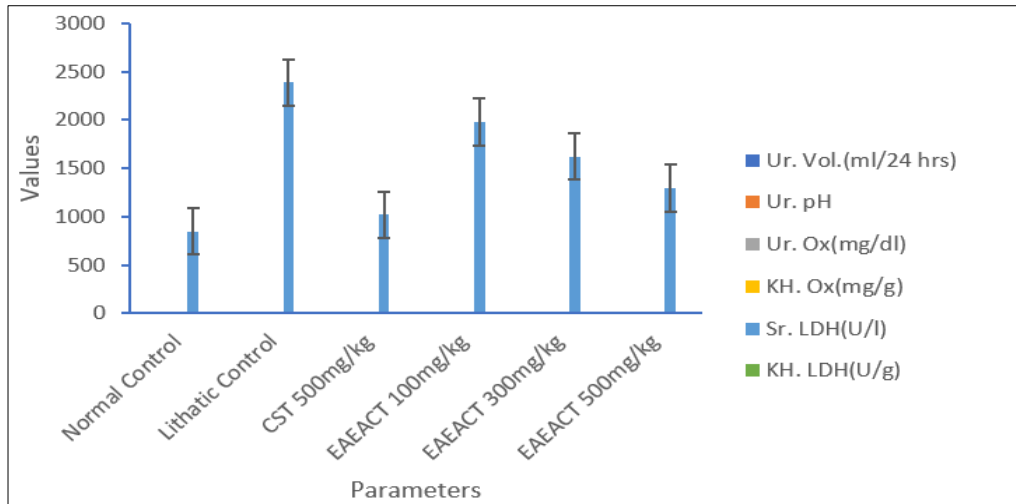


Figure 14: Measurements of oxalate, LDH, urine pH, and urine production in rats with hyperoxaluria as a result of EAETD

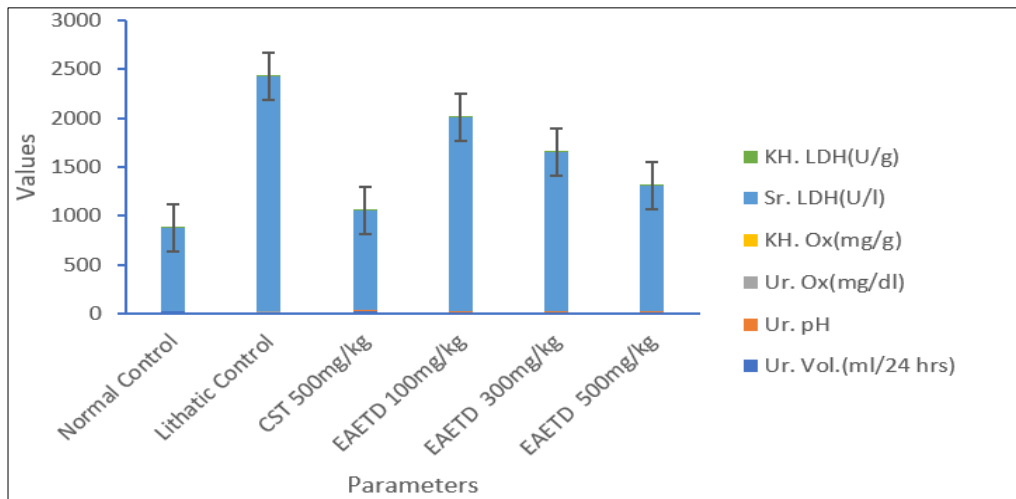


Figure 15: Measurements of oxalate, LDH, urine pH, and urine production in rats with hyperoxaluria as a result of EAETD

food, nutraceutical, and pharmaceutical sectors due to their significant pharmacological properties. Clinical trials and other additional research are necessary to confirm their safety, effectiveness, and the need to standardise dosages for therapeutic purposes.

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