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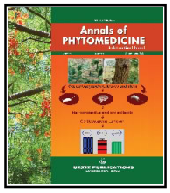

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In vitro and *ex vivo* studies to assess the antiurolithiasis activity of phenolic components of *Ricinus communis* L. and *Euphorbia hirta* L. with simultaneous HPTLC analysis

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Abstract

Traditional Ayurvedic forms containing *Ricinus communis* L. and *Euphorbia hirta* L. have been consumed for decades to cure various diseases. The current study aims to clarify the basic bioactive component in the regulation of calcium oxalate urolithiasis. Significance of chloroform and aqueous components of *R. communis* and *E. hirta* (CFRC) and (AFRC); (CFEH) and (AFEH), in the breakdown of calcium oxalate formation, was investigated for various biochemical parameters, using *in vitro* and *ex vivo* methods by simultaneous HPTLC analysis. Polar components (AFRC) and (AFEH) for the methanolic extract of *R. communis* and *E. hirta* and the marketed drug cystone significantly (p<0.001) inhibit the enlargement of calcium oxalate crystals and inhibition of lipid peroxidation, whereas chloroform components (CFRC) and (CFEH) are unable to disperse calcium oxalate crystals and inhibition of lipid peroxidation. In the HPTLC analysis, 6 peaks were revealed at wavelength 254 and 365 nm with RF values 0.12, 0.23, 0.50, 0.65, 0.75 and 0.84. An RF value of 0.50 was found in the polar components (AFRC) and (AFEH) for the methanol extract of *R. communis* and *E. hirta* compared to that of syringic acid (Phenolic compound). This study revealed the antiurolithic effect of aqueous components (AFRC) and (AFEH) of methanol extract *R. communis* and *E. hirta*. They potentially inhibit the biochemical barriers involved in the development of calcium oxalate, along with its antioxidant effects, which is accompanying its practice in the therapy of kidney disease (KSD).

1. Introduction

Urolithiasis is a form of kidney stone, also known as kidney disease (KSD). It is a progressive disease worldwide with incidence rates and is thought to be related to the enhanced risk of chronic kidney disease (CKD) and end-stage renal disease (ESRD) (Xiao *et al.*, 2020). The cost of healthcare associated with KSD has increased day-by-day (Susmita *et al.*, 2020). Urolithiasis is a complex process of the formation of stones in the kidney, ureter, and urethra. Such processes produce inter-renal multi-crystalline aggregates/fixed structures located in a different area of the kidney system (Kok, 2002). The continued growth of the stone, at a dimension of 4-10 mm in size, which prevents the passage of the ureter or its presence in the kidneys may cause several symptoms such as blood micturition, painful urination, severe pain in the lower extremities (Carlsson *et al.*, 1992).

Most phenolic and flavonoid compounds formed by the degradation of lignin present in the plant cell matrix have conceivable antioxidant, anti-inflammatory, and mast cell alleviating properties, and defend the body's organs from cell-toxic products (Afroz *et al.*, 2019). Euphorbia is the largest genus of the Euphorbiaceae family with about 1600 species with a certain therapeutic value and is characterized by the presence of white milk latex (Kumari and Gupta 2018). *R. communis* in addition to *E. hirta* (Euphorbiaceae) are two renowned herbal medicines containing phenolic and flavonoid composites consumed in different polyherbal formulations in traditional healthcare systems. *R. communis* is a perennial, dense plant (up to 5 m), a weed similar to the wood generally recognized as castor seed oil plant, found in the Sub-Himalayan region and dumping sites in India (Worbs *et al.*, 2011). Castor seeds have been known as traditional medicine since ancient times. Older ayurvedic literature recommends *R. communis* for the medication of liver and urinary disorders. The roots have long been used in various traditional medicine systems to treat various ailments (Elkousy *et al.*, 2021). Roots of *R. communis* have diuretic, anticancer, anti-inflammatory, anthelmintic, hepatoprotective, antibacterial, and antispasmodic activities (Abdul *et al.*, 2018). A combined decoction of *R. communis* root and Gokhuru (*Tribulus terrestris* Linn.) with common salty can reduce kidney stones (Prachi *et al.*, 2009). Roots of *R. communis* contains saponins (lupeol and erandone), steroidal ester (ricinusteryl benzoate), phenolic compound (ricipentatriacontanol), flavonoids

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(rutin, quercetin, isoquercetin, and kaempferol), and fatty acid esters (indole-3-acetic acid, 1-Oleio- 2-palmitoglyceryl phosphate) (Srivastava *et al.*, 2014; Kang *et al.*, 1985).

E. hirta is generally located in India, China, Indonesia, Africa, Myanmar, and Australia. It is usually consumed as a medicinal plant in the medication of various ailments such as stomach ailments, skin pain, scabies, fungal infections, aphthae, tinea, thrush, and respiratory tract diseases such as laryngeal spasms, bronchitis, asthma, cough, and hay fever, as well as kidney and liver diseases (Priya *et al.*, 2013). *E. hirta* is said to contain flavonoids (quercetin and quercitrin), terpenoids (tri-terpenes: α-amyrin, α-amyrin, friedelin, taraxerol), acids (malic and tartaric acid), and phenols (dehydro-ellagi-tannins-euphorbin and terchebinly monomeric tannins-geraniin) (Kausar *et al.*, 2016).

We recently demonstrated the presence of phenolic and flavonoid compounds in methanol extracted from *R. communis* and *E. hirta* by HPTLC methods, which have important antioxidant functions (Singh *et al.*, 2021). It is thought that *R. communis* and *E. hirta* are well known in traditional Indian medicine for their antiurolithic effects, but there are less informative published scientific data available to support the capabilities in the prevention of the development of kidney stones. Therefore, this research was planned to ascertain the antiurolithiasis activity of the phytoconstituents present in polar and the non-polar methanolic extract of *R. communis* and *E. hirta* using *in vitro* and *ex vivo* methods with simultaneous HPTLC analysis.

2. Materials and Methods

2.1 Chemical and instruments

The marker compound syringic acid, sodium oxalate, calcium chloride dihydrate was purchased from a local retailer of Sigma-Aldrich, New Delhi, India. HPLC category solvents, pre-overlay HPTLC plates 60 F_{254}, tris-buffer were procured from Merk Specialties Private Limited, India. Cystone was procured from Himalaya Pharmaceutical Industry, India. All the chemicals solvent for extraction used were analytical grade and the analysis solutions were freshly prepared. The CAMAG HPTLC technique (Muttenz, Switzerland) is furnished with a Linomat 5 applicator, a TLC scanner III used by Win CATS package (Version1.2.0), a Hamilton syringe (100 lL, Reno, NV, USA), and twin-trough development plates.

2.2 Extraction and fractionation

Roots of *R. communis* and aerial segments of *E. hirta* were amassed in September 2018 in the suburbs of Lakhimpur, UP, India and authorized from the NBRI, Lucknow UP. The plant template was also submitted to NBRI with a voucher number (NBRI/CIF/663/2018) for additional recommendation. Dried powdered roots of *R. communis* and aerial parts of *E. hirta* (250 g) extracted in 700 ml of 75% w/v ethanol by the cold process maceration for 72 h. The extract was then clarified *via* a muslin cloth and the filtrate evaporated in boiling bathwater to obtain a constant extract weight of 6.80% and 9.60% w/w. The extracts of both plants (10.0 g) were suspended (100 ml) in double-distilled water and sonicated for 15 min at 45°C. The separation of the components of the prepared water suspension was done using an equal dose of chloroform three times. Both chloroform and water suspension were separated and evaporated to dryness. The yield percentage of chloroform (CFRC) and aqueous fraction (AFRC) in *R. communis* was 1.76% and 4.23% and the yield percentage of chloroform (CFEH) and aqueous extractive (AFEH) in *E. hirta* was 2.65% and 6.74%, respectively. Preliminary phytochemical experiments were performed to assess the presence of chemical elements in all four fragments (Sachan *et al.*, 2011).

2.3 Standard drug

The formulation of cystone polyherbal from Himalayan Drug Company Makali, Bengaluru, India was treated as a model drug in this experiment. Cystone consists of *Tribulus terrestris* 32 mg, *Didymocarpus pedicellate* 130 mg, *Bergenia ligulata* 98 mg, *Rubia cordifolia* 32 mg, *Cyperus scariosus* 32 mg, *Achyranthes aspera* 32 mg, *Onosma bracteatum* 32 mg, and *Vernoni acinerea* 20 g of powdered tablet was extracted in 200 ml of distilled water by keeping on a magnetic stirrer for 6 h and centrifuged at 5000 rpm 15 min, and separates the supernatant and used as soon as needed.

2.4 Animals and plasma sample

The investigational procedure was accepted by the Organized Ethics Agency (IU / Pharm / Ph.D. / CPCSEA / 21/12) ensuing the strategies of the Animal Control and Examination Control Committee (CPCSEA) which complies with international standards of the Indian National Science Academy. Sprague-Dawley rats of either sex weighing 150-200 g (10-12 weeks old) were selected for blood plasma samples. The animals were purchased from the Central Drug Research Institute, Lucknow, and kept under normal laboratory conditions (temperature 22 ± 2°C, relative humidity: 50 ± 15% and darkness 12 h darkness/12 h daylight) in the animal house F/o Pharmacy, I.U., Lucknow. Suitable food pellets and water was given *ad libitum* for seven days. After acquaintance with the rats, the required blood volume is drawn through the Retro-orbital Sinus into each microcentrifuge tube for centrifugation to collect blood plasma.

2.5 *In vitro* analysis of nucleation by microscopy

The chloroform and aqueous fractions of *R. communis* and *E. hirta* (CFRC) and (AFRC); (CFEH) and (AFEH), aqueous extract of cystone, and syringic acid were assayed for inhibition of oxalate aggregation crystallization performed by the procedure designated by Atmani and Khan (2000). The mixture of calcium chloride and sodium oxalate was made at strengths of 26.0 and 28.0 mmol/l, separately, in a buffer including Tris 0.05 mol /l and NaCl 0.15 mol/l at pH 7.4 which is the pH of the kidneys. Calcium chloride (1 ml) solution was assorted with 10 ml (1000 µg/ml) of the tested sample. Crystallization was initiated by adding 1 ml of sodium oxalate solution. In the control group, only buffer solution has been inserted into the calcium chloride solution. The entire reaction mixture was placed at 37°C overnight. The next day, all test tubes are centrifuged for 10 min and discarded the excess water layer (Gupta *et al.*, 2018). The calcium oxalate crystals formed in the test tube were heated to 37 ± 0.2°C on a glass slide until dryness and placed in a compact microscope stage (Leica Microsystem, GmbH; Model-ERG0PLATTE DMI under 45X magnification. The shape and mean size of more than 20 crystals were taken by micrometer (Grohe *et al.*, 2011).

2.6 *Ex vivo* turbidness test

Power to inhibit oxalate crystal embarrassment of *R. communis* and *E. hirta* are made in the plasma of rats to provide a living atmosphere. The rat plasma (950 mmol) was attenuated with saline water to 10

mmol / l of calcium chloride and sodium oxalate, respectively and the resulting mixture was employed for nucleation testing. Plasma sample accomodating sodium oxalate was mixed with 10 ml of 1000 µg/ml CFRC, AFRC, CFEH, AFEH, aqueous extract of cystone, and syringic acid. Crystallization was started by adding plasma containing calcium chloride to the test sample. The mixture was stirred for 1 h at a temperature of 37 ± 2°C and the OD of the resulting suspension was measured at 680 nm. The inhibitory ability of all tested samples was measured in comparison to the controls. Percentage inhibition was estimated by competing for the impairment in the existence of a bioactive component in different fractions than those found in control using the following formula (Sharma *et al.*, 2017).

% Inhibition = (1.66 Sample turbidity/Turbidity control) × 100

2.7 *Ex vivo* kidney stone degradation assay

Surgical kidney stones were found at Varun Arjun Medical College, NH-24, Banthra, Shahjahanpur, UP, India-242307. Experiments were performed on the method of Rao and Bano (2004) through certain slight amendments. The diameter of the kidney stones was evaluated (mm) and the weight was documented (g) and labeled. A 50 ml of 0.052 M Tris-HCl buffer (pH-5.8) having 0.17 M NaCl and (950 mmol) plasma was distributed in several sterile tarson vessels and labeled appropriately. 10 ml 1000 µg/ml (CFRC); (AFRC); (CFEH); (AFEH), an aqueous extract of cystone and syringic acid is added to the labeled tarson vessels. Control was maintained by taking kidney stones into a buffer containing 10 ml of plasma (950 mmol). All the tested sample vessels were vortexed every day to confirm an equal dispersion of the sample in the mixture. The dimension and mass of each kidney stone were calculated every 3 days by drying at 100 ± 5ºC in a hot air oven for 10 min (Sharma *et al.*, 2016). The % weight loss is calculated as

% Dissolution = [(First weight - Last weight)/First weight] × 100

2.8 Entire phenolic substance in different fractions

Entire phenolic content present in chloroform (CFRC) and water fraction (AFRC) in *R. communis* and chloroform (CFEH) and aqueous extractive (AFEH) in *E. hirta* is measured by the reaction of the Folin-Ciocalteu mixture. In various test tubes, the appropriate aliquots for CFRC, AFRC, CFEH, and AFEH are dispersed in clear water, and the volume is composed of 1.0 ml. Thereafter, 0.5 ml of Folin-Ciocalteu mixture and 2.5 ml (20% w/v) solution of sodium carbonate were poured into separate test tubes. The test tubes are centrifuged for 15 min and the afloat was separated. Finally, the absorption of each component was obtained at 700 nm and the total phenolic content was estimated as the equivalent of syringic acid / µg/ml from the extract (Sangeeta *et al.*, 2021).

2.9 Reducing capacity of different fractions

The aggregate reducing capacity of the tested samples CFRC; AFRC; CFEH; AFEH; aqueous extract of cystone and syringic acid were determined. 1000 µg of each sample was dissolved in 0.5 ml of (950 mmol) plasma sample and assorted with 2.6 ml of phosphate buffer (0.2 mM, pH 6.7) and 1% of 2.6 ml of potassium ferricyanide. The combination was gestated at 45 ± 2°C for 40 min. Then, 2.6 ml of trichloroacetic acid (10%) was included in the mixture, which was then centrifugated for 5 min at 2000 pm. The supernatant (1.5 ml) was assorted with 1.5 ml of distilled water and $FeCl_3$ (0.4 ml, 0.2%), and the absorption was taken at 680 nm. High absorption of the reaction mixture showed significant reduction potential (Arif *et al.*, 2011).

2.10 Peroxide radical scavenging action

The peroxide activity of radical scavenging was resolute by the thiocyanate process (Arif *et al.*, 2011). 1000 µg of each sample CFRC; AFRC; CFEH; AFEH; aqueous extract of cystone and syringic acid were dissolved in 0.5 ml of plasma (950 mmol) and assorted with 2.5 ml of 0.02 M linoleic acid emulsion (0.04 M phosphate buffer pH 6.8) and 2 ml phosphate buffer (0.04 M pH 6.8) in a test tube and placed in the dark as 37 ± 2ºC. The amount of peroxide form was determined by studying the improved red absorption of 570 nm by adding 0.1 ml 30% ammonium thiocyanate solution and 0.1 ml 20 Mm ferrous chloride to 3.5% HCl in the reaction mixture. The control was formulated by using buffer and plasma solution.

2.11 HPTLC fingerprint testing

The aqueous fractions of both plants AFRC and AFEH were dissolved in 40 ml methanol, shaken on an orbital shaker separated the supernatant, and evaporated up to 5 ml. Syringic acid (1 mg /ml) is a standard solution made by thawing 25 mg in 25 ml of methanol. The solution system of the elution contains toluene: ethyl acetate: Formic acid in a ratio of 7: 2.5: 0.5. CAMAG Linomat-V automatic applicator was used for applying both the solutions on pre-activated plates as 6-mm bands. Previously saturated for 20 min. A twin-trough glass chamber (CAMAG) was used to develop the plate. The plate was made to track up to 85 mm, and the plate was developed at a wavelength of 254 and 366 nm and scanned with the help of CAMAG TLC scanner III set at reflectance-absorption mode (Baira *et al.*, 2021; Singh *et al.*, 2021).

3. Statistical analysis

All records are presented as a percentage, mean ± SD, and mean ± SEM for samples. One-way variance analysis (ANOVA) and Dunnett tests were performed using Graph Pad Prism V2.01 (GraphPad Software, Inc., San Diego, California, USA).

4. Results

A qualitative chemical test of chloroform fractions of both plants CFRC) and (CFEH) shows the presence of terpenes, triterpenoids, carotenoids, and steroids, whereas aqueous fractions (AFRC) and (AFEH) of both plants have shown the presence of reducing sugars, saponin glycosides, steroids, triterpenoids, alkaloids, flavonoids, and phenolic compounds. These are all major secondary metabolites that are responsible for the activity.

4.1 *In vitro* analysis of nucleation by microscopy

The effect of 10 ml 1000 µg/ml tested sample of CFRC; AFRC; CFEH; AFEH, cystone, and syringic acid on the development of calcium oxalate crystals reticence was determined by measurement with a micrometer. The diameter and shape of calcium oxalate crystals in the different tested sample has been observed by eyepiece and stage micrometer at 45X. The figure and dimension of more than 20 crystals were observed and determined the mean size (Table 1). Aqueous fractions (AFRC and AFEH) of methanol extract of *R. communis* and *E. hirta* and standard drug cystone significantly ($p = {}^{***}p < 0.001$) inhibits the development of calcium oxalate crystals, whereas chloroform fractions (CFRC and CFEH) cannot dissolve calcium oxalate crystals.

Table 1: Effect of *R. communis* and *E. hirta* on calcium oxalate crystallization

Group	Observation	Mean crystal size (µm) ± SEM
Normal control	Large sized (< 85 µm) angular crystals were observed	77.80 ± 0.241
Standard control (cystone)	Very small sized (< 25 µm) and few crystals were observed	19.63 ± 0.442***
Test-1 (CFRC)	Large sized (< 90 µm) bulky crystals were observed	84.72 ± 0.247 ns
Test-2 (AFRC)	Very small sized (< 35 µm) and few crystals were observed	26.34 ± 0.524***
Test-3 (CFEH)	Large sized (< 92 µm) bulky crystals were observed	86.45 ± 0.374 ns
Test-4 (AFEH)	Very small sized (< 37 µm) and few crystals were observed	32.64 ± 0.456***
Test-5 Syringic acid	Small sized (< 55 µm) and few crystals were observed	48.60 ± 0.322**

Values are expressed in Mean ± S.E.M. (n=20) One-way analysis of variance (ANOVA) and Dunnett's were performed p = $^{**}p < 0.01$, $^{***}p < 0.001$ and ^{ns}p (none significant) as compare to control group

Table 2: Percentage dissolution of kidney stones on exposure to chloroform and aqueous fractions of *R. communis* and *E. hirta* CFRC and AFRC; CFEH and AFEH, aqueous extract of cystone, and syringic acid

Group	Initial weight (mg)	Final weight (mg)	Dissolution (%)	Structure	
				Initial	Final
Normal Control	170.84	170.66	0.10		
Standard Control (Cystone)	310.15	20.72	93.31		
Test-1 (CFRC)	187.26	187.10	0.08		
Test-2 (AFRC)	179.15	37.18	79.24		
Test-3 (CFEH)	158.84	158.17	0.42		
Test-4 (AFEH)	278.64	106.12	61.91		
Syringic acid	152.84	24.43	84.01		

4.2 *Ex vivo* turbidity assay

Ex vivo assay, for the antiurolithiasis activity of sample CFRC; AFRC; CFEH; AFEH, cystone, and syringic acid were carried out in rat serum which provides a biological environment. It was found that the aqueous fractions of methanol extracts of both plants AFRC and AFEH, cystone, and syringic acid contribute to preventing the formation of calcium oxalate crystals. Based on the results obtained, the standard drug cystone shows the highest percentage (76%) inhibition of calcium oxalate crystals. The aqueous fractions of *R. communis* and *E. hirta* (AFRC and AFEH) of methanol extract showed 67% and 53% inhibition of calcium oxalate crystals, whereas chloroform fractions CFRC and CFEH showed 9% and 15% inhibition (Figure 1).

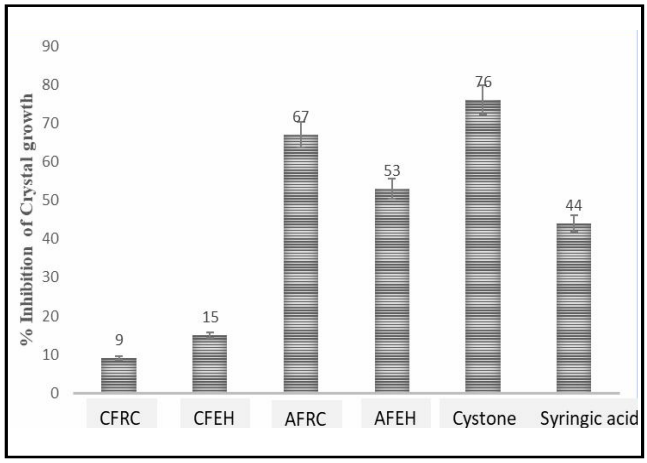

Figure 1: Effects of chloroform and aqueous fractions of *R. communis* and *E. hirta* CFRC and AFRC; CFEH and AFEH, aqueous extract of cystone, and syringic acid on calcium oxalate crystallization. The data represent the percentage of inhibition of crystal growth. Each value represents mean ± SD (n = 3).

4.3 *Ex vivo* kidney stone degradation assay

Surgical human kidney stones were used to analyze the breakdown power of tested samples. Pre-measured kidney stones were gestated with different tested samples accompanied by buffer (pH-5.8) and blood plasma at 37 ± 2 ºC Kidney stones are observed for 21 days at a period of 3 days. The standard drug cystone and syringic acid showed the highest percentage (93.31%) and (84.01%) dissolution of kidney stones on the 21st day. The aqueous fractions of *R. communis* and *E. hirta* (AFRC and AFEH) of methanol extract showed 79.24 % and 61.91% inhibition of kidney stone dissolution whereas chloroform fractions (CFRC and CFEH) showed the least amount (0.08% and 0.42%) kidney stone dissolution (Table 2).

4.4 Entire phenolic substance in different fractions

The entire phenolic substance present in CFRC, AFRC, CFEH, and AFEH were found to have 28, 650, 34, and 720 µg/10 ml of extracts phenolic levels in respect of syringic acid. The aqueous fraction of both plant extract AFRC and AFEH showed the highest concentration of phenolic compounds (Figure 2). The phenolic compounds are polar and can be extracted out in a polar aqueous solvent whereas chloroform is a non-polar solvent that can not solubilize phenolic compounds.

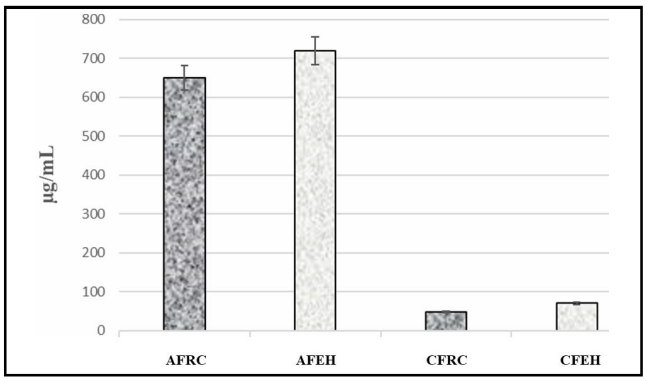

Figure 2: Total phenolic contents of chloroform and aqueous fractions of *R. communis* and *E. hirta* CFRC and AFRC; CFEH and AFEH in respect of syringic acid. Each value represents mean ± SD (n = 3).

4.5 Reducing capacity

Figure 3 exhibits the reductive capacities of the CFRC, AFRC, CFEH, and AFEH compared with cystone and syringic acid. The reducing power of sample AFRC and AFEH was more than CFRC and CFEH, whereas syringic acid showed maximum reductive capabilities.

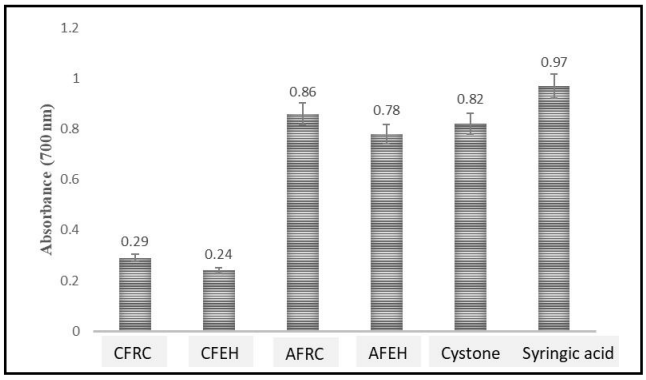

Figure 3: The reductive ability of chloroform and aqueous fractions of *R. communis* and *E. hirta* CFRC and AFRC; CFEH and AFEH, aqueous extract of cystone, and syringic acid. The absorbance (A700) was plotted against the tested sample. Each value represents mean ± SD (n = 3).

4.6 Peroxide radical scavenging activity

The results obtained from ferric thiocyanate tests reveal that AFRC and AFEH have antioxidant ability to prevent chain-breaking lipid peroxidation (Figure 4). The % free radical rummaging actions of AFRC and AFEH were greater (84 and 72%) than CFRC and CFEH, whereas syringic acid showed maximum (93%) inhibitions of peroxide.

4.7 Examination of HPTLC fingerprint

The chromatogram points out the phytoconstituents present in the aqueous fractions of methanolic extract of both plants were separated without any tailing. Seven peaks at a wavelength of UV 254 and 366 nm which have R_f values of 0.12 to 0.84 indicate that at least six separate chemical moieties are present in aqueous fractions of *R. communis* and *E. hirta* (Figure 5). The aqueous fraction contains chemical moieties with an equal R_f value of 0.50 equivalent to the syringic acid (phenolic compound). This technique is very accurate for diagnosing particular constituents of a crude drug.

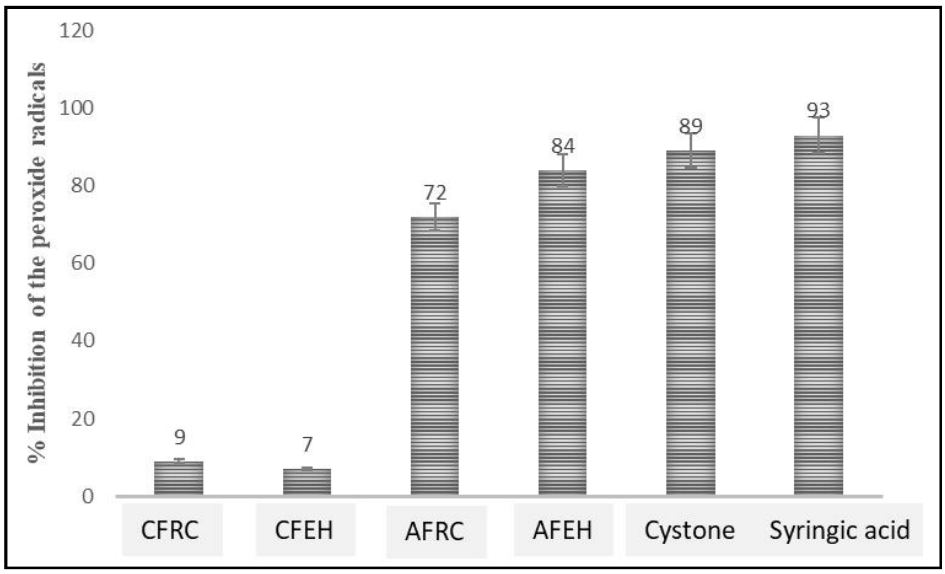

Figure 4: % Inhibition of lipid peroxidation by the chloroform and aqueous fractions of *R. communis* and *E. hirta* CFRC and AFRC; CFEH and AFEH, aqueous extract of cystone, and syringic acid. The data represent the percentage of inhibition of peroxide radicals' formation. Each value represents mean ± SD (n = 3).

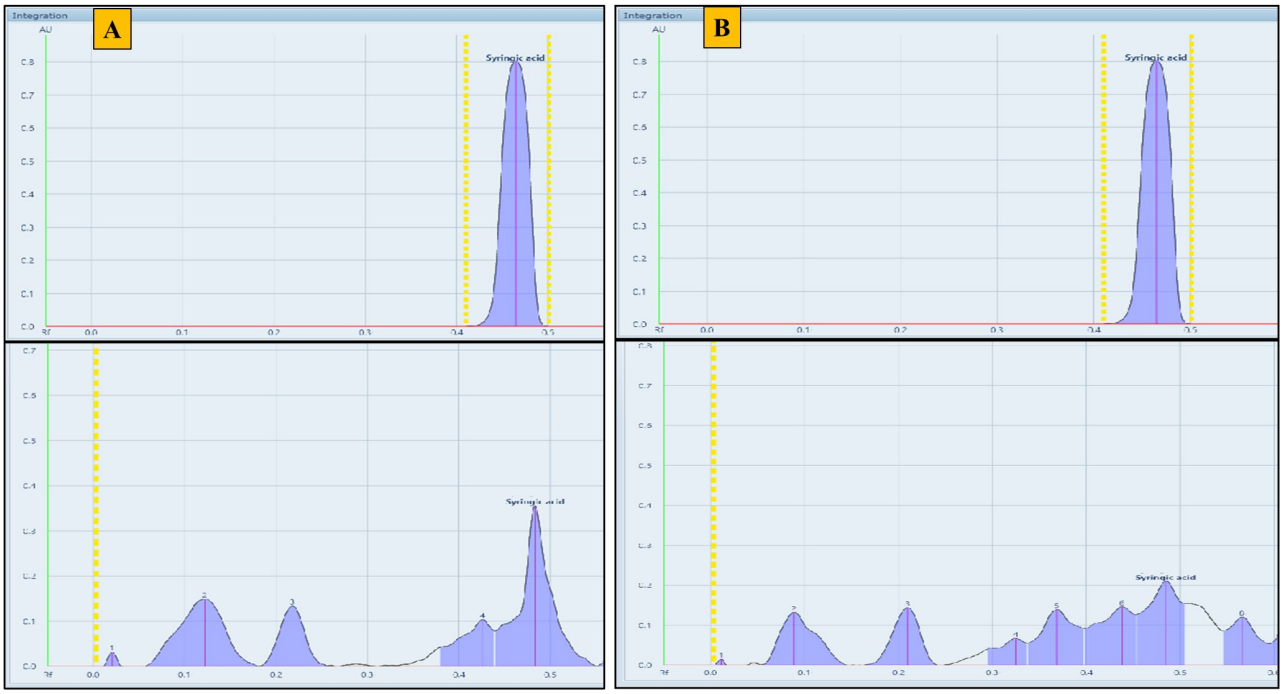

Figure 5: Densitogram of methanolic extract of *R. communis* (A) and *E. hirta* (B) with the standard of syringic acid (R_f 0.50).

5. Discussion

The formation of kidney stones is a complex process that is a consequence due to some physicochemical actions with super-saturation, crystal nucleation, crystal development, aggregation, and conservation within the kidneys. In the current management of kidney stones, surgical procedures and excessive bodily shock waves (Lithotripsy) are often used, harming the kidney soft tissues and leading to cancer. Pretentious advances in the use of phytotherapy in the treatment of nephrolithiasis have been perceived in modern times. The use of many medicinal plants in the treatment of urinary stones has been documented for centuries, even though there is no thought for their use. As a result of the medicinal use of *R. communis* and *E. hirta* aqueous and chloroform fractions of methanol extracts were used to study its antiurolithiasis effects. These fractions were tested to check their ability to prevent nucleation, growth, and

degeneration of the most common kidney stone, calcium oxalate. Inhibition of crystal growth of calcium oxalate by different fractions and model drugs is shown in Table 1. Microscopical studies of crystals in the manifestation of tested samples show that aqueous fractions of methanolic extracts of *R. communis* and *E. hirta* have an important role in preventing the formation of crystals and the growth of crystals. However, in a study on surgically gained kidney stones, it was achieved that AFRC and AFEH exhibited better disintegration of stones and crystals assessed to the chloroform fraction and control group. The antiurolithiasis activity of *R. communis* and *E. hirta* was suggested due to the presence of active phenolic antioxidant compounds in aqueous fractions AFRC and AFEH of the methanol extracts which was assessed by HPTLC and UV-spectroscopy method. Based on preliminary phytochemical analysis and estimation of phenolic content, it has been found that aqueous extractives of both plants are rich in phenolic compounds. Phenolic compounds are works synergistically with the body without disturbing the natural balance. They help clean and purify the organ system without side effects and are triggered by inhibition of glycolate oxidase (GOX) leading to inhibition of oxalate synthesis (Sharma *et al.*, 2017). Oxalate-induced membrane damage mediated the response of lipid peroxidation through the production of oxygen-free radicals. In hyperoxaluria, superoxide-producing enzymes such as glycolic acid oxidase (GAO) and xanthine oxidase (XO) are increased, and OH radical transition metal ions were amassed. Lipid peroxidation consequences such as hydroperoxides and diene conjugates are unreasonably excreted in kidney tissue and plasma of kidney stones. Treatment with antioxidants in urolithic patients activates the cellular antioxidant system, enzymes, and scavengers, as well as disrupts the membrane lipid and protein peroxidation reaction, ATPase inactivation, and its accumulation of calcium. Antioxidant products also reduce oxalate exposure in stone patients (Selvam, 2002). *In vitro* and *ex vivo* analyses will be the cheapest and most scientific way to determine the ability of urolithiasis.

All these observations were suggestive of the antiurolithiasis and antioxidant potency of *R. communis* and *E. hirta* due to the existence of active phenolic antioxidant compounds in aqueous fractions AFRC and AFEH.

6. Conclusion

In summary, the bioactive aqueous fractions AFRC and AFEH in the methanol extract of *R. communis* and *E. hirta* have defensive capabilities to prevent the growth of kidney stones and help dissolve them. Simultaneous measurement of phenolic compounds has resulted in a systematic review of quality-control tests and supports biological activity against urolithiasis. This study will be helpful in further discovery of a phytochemical novel from aqueous components of methanol extracted from *R. communis* and *E. hirta*. Current research will benefit the scientific community around the world by providing a source of effective ingredients in the treatment of kidney stones.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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