Research Article

Novel Extraction Method for Isolation and Characterization of Bioactive Flavonoid and its Anti-urolithiasis Activity from the Whole Plant Parts of *Fragaria vesca* Linn

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Received: 12-06-2019; **Revised:** 24-07-2019; **Accepted:** 02-08-2019.

ABSTRACT

The objective of the study is to Isolate Plant Extract with Novel Extraction method and Evaluate the abilities of plant extracts used for urolithiasis in animal models can be served as an aid in the evaluation of novel treatments for urolithiasis. A microwave-assisted extraction was used to extract natural Anti urolithiasis from the plant of *Fragaria vesca* Linn. Ethanolic extracts of whole plants was assessed for its protective and curative activity in urolithiasis. Urolithiasis was created in waster albino rats by addition of 0.75% ethylene glycol (EG) to drinking water for 28 days. In preventive treatments the *Fragaria vesca* Linn extracts given from 1st day to 28th day, while in curative regimen, the *Fragaria vesca* Linn extracts was given from 15th day to 28th day. In- vivo tests are in positive manner, Various parameters of renal functional was checked like calcium, phosphate, uric acid, magnesium, urea and oxalate were studied by using urine, serum. Isolate of active constituents like kaempferol from *Fragaria vesca* Linn extract and extract of *Fragaria vesca* Linn were effective on urolithiasis

Keywords: *Fragaria vesca* Linn, Urolithiasis, Isolation, *In-Vivo* study.

INTRODUCTION

Fragaria vesca Linn belongs to family Rosaceae usually known as wild strawberry, woodland strawberry. The root is astringent and used in diarrhea. In ancient system of drugs, the plant is claimed to be possess diuretic and liver tonic property. It is also used as nephroprotective drug. By acting as diuretic drug *Fragaria vesca*, exhibits its urolithiasis action.

Various extraction methods are developed for the extraction of natural plant products, including some innovative technologies such as microwave-assisted extraction (MAE), some conventional methods such as Soxhlet extraction. Among these methods, microwave-assisted extraction is a green and most effective extraction method. Compared with conventional extraction methods, has some advantages, such as lower extraction time and lower temperature, which leads to less degradation of thermally labile compounds.

In the current study, a microwave-assisted extraction method was used to extract natural Anti urolithiasis from the plant of *Fragaria vesca* Linn. In addition Soxhlet extraction was conducted to compare the respective efficacy. Finally, the antiurolithiatic components in the extract were analyzed by the isolation of plant constituents are mainly carried out by one or a combination of several fractionation procedures based on various chromatographic techniques. The most useful chromatographic techniques in phytochemical isolation includes thin layer chromatography (TLC), column chromatography (CC), UV, IR NMR, High performance thin layer chromatography (HPTLC) and gas chromatography-mass spectroscopy (GC- MS). The extracts urolithiasis efficacy was tested by *in-Vivo* tests.

METHODS

Plant material

Whole plants of *Fragaria vesca* Linn collected from the specific area of Mahabaleshwer and at the specific climatic conditions which is suitable for the plant.

Preparation of extract

Conventional extraction

Plant *Fragaria vesca* Linn was dried before extraction under controlled condition or in shade to avoid chemical changes occurring. The powdered material of *Fragarica vesca* whole plant parts (1.5 kg) was used for extraction with various solvent in soxhlet apparatus for 24 hrs selection of solvents were made with increasing polarity in order of petroleum ether (60-80°C), chloroform, ethyl acetate and ethanol, aqueous. All the extracts were concentrated by using rotary evaporator so that solvents were completely recovered in order to get dry extracts, which were preserved in glass desiccators till further use. The quantitative yield was calculated for various extract.[1,2]

Microwave extraction

A microwave oven (scientific microwave synthesis system- Catalyst) used in this study had a total capacity of 850 W. *Fragaria vesca* Linn dry powder weighted 1.5 g were added with the same solvents (20 ml) as in the Soxhlet extraction method in flat bottom, threaded round bottom flask The resulting mixtures were irradiated with

microwaves (250 W power). Then allowed to cool at room temperature. for 20 min based on increasing polarity in order of petroleum ether (60-80°C), chloroform, ethyl acetate and ethanol, aqueous. All the extracts were followed to evaporator and the solvents were completely recovered in order to get dry extracts, which were preserved in glass desiccators till further use. The quantitative yield was calculated for various extract.

Phytochemical Screening

The dried extract of ethanol was subjected to number of qualitative tests (colour reactions) in order to identify the nature of the phytoconstituents present in the different extracts of *Fragaria vesca Linn*.

Isolation and Identification of Phytoconstituents

The isolation of plant constituents are mainly carried out by one or a combination of several fractionation procedures based on various chromatographic techniques. The most useful chromatographic techniques in phytochemical isolation includes thin layer chromatography (TLC), column chromatography (CC), UV, IR NMR further identification was done with High performance thin layer chromatography (HPTLC), gas chromatography- mass spectroscopy (GC- MS).

Thin layer chromatography (TLC)- The required quantity of silica gel-G (eg. 30 g) was used to prepare a suspension. Suspension was prepared by shaking along with 60 ml of distilled water. The slurry was speared over the glass plates set adjacent to each other to form even coated plates which were allowed to dry in air, followed by heating in an oven at 100 –105 ˚C for one hour, cooled and protected from moisture. The plates were kept in dry atmosphere. Whenever essential the plates were activated by heating in hot air oven at 100˚C for 30 mins and used to sample application and separation with the thin layer chromatography. [3,4]

Application of extract for separation of spots

The extracts were dissolved in suitable solvents (approx 1.0 mg in 1.0 ml of solvent) in which the extract shows maximum solubility. The solution of extract was spotted on a TLC plate with the help of a narrow capillary tube 1cm above the bottom of the plate. The spots were equally sized as far as possible and separated equidistant to each other and the edges of plate. The diameter of spots should not exceed 2 mm.

Preparation of chromatographic column

A chromatographic column of 60 cm length and 3 cm width was chosen for the chromatographic separation. The base of the column above the stopper was plugged with cotton wool above which the column packing material (adsorbent) was placed by wet packing technique. The required quantity of adsorbent was mixed with the mobile solvent and poured in to column. The stationary phase settles uniformly in the column and there should not be any entrapped air bubbles. After packing the column, the extract material adsorbed on small amount of silica gel was loaded at the top of the packed column and a piece of cotton wool was placed at the top of the column in order that the packed extract is not disturbed during addition of the mobile phase for the separation.

Sample application

A quantity (10 g) of extract has been used for the isolation of constituents. The extract was dissolved in a minimum volume of ethanol adsorbed on silica gel to form a dry free flowing powder which was loaded over the chromatographic bed and covered with a cotton wool.

UV Spectrum

The fractions obtained by column chromatography were subjected to UV analysis, where 0.1ml fraction was diluted to 10 ml ethanol and UV spectrum was obtained. The UV spectrum was performed by using Jasco UV spectrometer. [5]

Melting point

A capillary tube is taken and an end is sealed by heating it. Fill the capillary tube with the isolated compound. Sealed end of the capillary tube was tapped on the porous plate. Connect the capillary tube to a thermometer with a thread. And observe the melting point.

Refractive index

a few drops of the liquid (dissolve the extracts) to be tested place on the polished surface of the lower refractive prism. Close the upper Incident Prism with hinges and lock it in place with the knob, so that the liquid is distributed evenly on the face of the Refraction Prism [13]

IR Study

The band isolated from Column chromatography was taken; the solvent was evaporated and dried desiccators so as to get the dry powder. The dried compound was observed for IR spectrum. The analysis was carried out by using Jasco-FTIR.

NMR Study

The isolated, dried compound was subjected to NMR spectroscopic analysis. The compound was soluble in DMSO and the spectrum was obtained at 400 MHz. the NMR was performed at Shivaji University, Kolhapur. The instrument Bruker model AV 400 MHz was used for analysis.[6]

GC- MS Study

The compound isolated from column chromatography was subjected to GCMS analysis. From the mass spectrum the molecular formula of the compound was obtained, which has important role in the identification of the compound. Shivaji University Kolhapur the instrument used was GCMS QP 2010- Shimadzu for analysis.[7]

HPTLC Instrumentation and chromatographic conditions

HPTLC was performed on 200 ×100 mm aluminum packed plates coated with silica gel 60 F254 (Merck, Mumbai, India).Standard solution of kaempferol and sample solution were applied to the plates as bands 8.0 mm wide, 30.0 mm apart and 10.0mm from the bottom edge of the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28±20c), with mobile phase in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 minutes. After development. These plates were scanned and visualized under visible light at 525 nm and UV light at 254 nm and 374 nm absorbance/reflection mode using reflection mode by CAMAG Scanner III and Automatic TLC Sampler Camag ATS 4 and deuterium lamp was used to analyze the plates.[8,9]

Evaluation of antiurolithiatic activity in 0.75% Ethylene glycolated water + AC 1% induced urolithiasis in rats[10,11,12]

Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce urolithiasis. One hundred and two Wister Albino rats (180–250 g) were randomly divided into seventeen groups as Group I– V containing six animals in each. Group I served as a vehicle treated normal group and maintained on regular rat food and drinking water ad libitum and All remaining groups received calculi inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water for normal rats ad libitum for 3 days to accelerate lithiasis followed by 0.75% v/v ethylene glycol for 28 days.

Experimental design

- **G**roup – I: Control
- Group – II: Ethylene glycol (0.75%) in drinking water + Vehicle
- Group – III: Ethylene glycol (0.75%) in drinking water + Cystone
- Group – IV: Ethylene glycol (0.75%) in drinking water + extract A – Curative study
- Group – V: Ethylene glycol (0.75%) in drinking water + extract A – Preventive study

(A-Extract of *Fragaria vesca* Linn)

Assessment of antiuroilithiatic activity

Collection and analysis of urine[19,20,21]

Metabolic cages are used to kept all animals and 24-h urine samples were collected on day 28. The animals had free to drinking water during the urine collection period. The urine was analyzed for urea, creatinine, uric acid, magnesium, citrate, calcium, phosphorus, oxalate, PH.

Serum analysis

After the experimental period, blood was collect from the retro-orbital under anesthetic conditions and animals were sacrificed under anesthesia. Collected Serum was separated by using centrifugation at 3000 x g for 10 min and analyzed for creatinine, uric acid, urea, calcium, phosphorus, magnesium and citrate.

Kidney homogenate analysis

Cut the abdomen to remove both kidneys from each animal. The isolated kidneys were cleared of foreign tissue and preserved in 10% formalin for histopathological study. The kidneys were dried at 80 ° C in a hot air oven. A 100 mg sample of dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 minutes and homogenized. The homogenate was centrifuged at 2000 x g for 10 minutes and the supernatant was removed.

RESULTS AND DISCUSSION

Using the microwave assisted extraction technique shows best results which were much faster than the Soxhlet extraction method, and showed higher efficiency in the extraction with time and yield which is shown in table no.1.

Table 1: Comparative account of Conventional extraction and Microwave assisted extraction of *Fragaria vesca Linn* (Rosaceae)

Sr. no.	Solvents	Time (mins)	% yield w/w	Time (mins)	% yield w/w
		Soxlet Extraction		Microwave assisted extraction	
1	Petroleum ether	24hr	8.10±0.20	20	12.10±0.20
2	Chloroform	24hr	7.75±0.12	20	13.75±0.12
3	Ethyl acetate	24hr	8.75±0.13	20	11.75±0.13
4	Ethanol	24hr	10.75±0.15	20	17.75±0.15
5	Aqueous	24hr	7.12±0.34	20	9.12±0.34

Phytochemical Screening

The results of qualitative chemical investigation of *Fragaria vesca* Linn indicated the presence of mainly sterols, flavonoids, phenolic compounds, tannins etc. As shown in table no 2.

Table 2: Chemical Constituents of Ethanolic extracts of *Fragarica vesca*

Chemical Constituents	*Fragarica vesca*
Alkaloid (Dragendroff Test)	+
Glycosides (Borntragers Test)	+
Flavonoid (Shinoda Test)	+
Steroid (Salkovaski Test)	+
Saponins (Foam Test)	+
Carbohydrates (Molisch Test)	++

Isolation of active principle by TLC

TLC of extract Fragaria vesca Linn. extract

The Rf value of the spot was calculated by using formula

Rf (Retention factor): It is a parameter for qualitative analysis

$$Rf = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by solvent front (cm)}}$$

TLC plate was developed in the solvent system Toluene- Acetone- formic acid. And three spot, was observed for Ethanolic extract, at the same one spot observed same with Rf value 0.82 of isolate with the help of iodine vapours which is compare with standard kameferol. As Shown in Table no 3 and 4.

Table 3: TLC of *Fragaria vesca Linn*. extract

		Rf	Colour developed
Spot 1	8.2cm/10cm	0.82	Grayish Yellow
Spot 2	3.4 cm/10cm	0.34	Faint green
Spot 3	5.4 cm/10cm	0.54	Slightly yellow

Table 4: TLC of *Fragaria vesca Linn*. extract

TLC No	Solvent system	Proportion in ml	Spot Observed
1	Ethyl acetate- N Butenol- water	10:5:4	6
2	Ethyl acetate- N Butenol- water- Formic acid	10:10:4:2	5
3	Ethyl acetate- N Butenol- water	10:10:2	3
4	Benzene- Chloroform - Methanol	5:5:5	3
5	Chloroform-Glacial acetic acid-methanol- water	10:10:5	3
6	Chloroform- Methanol	10:5	3
7	Toluene- Acetone- formic acid	7:3: 2drops	3

Column chromatography of extract

The following fractions were collected and were used for isolation purposes As Shown in table no 5.

Table 5: Elution scheme for column chromatography of *Fragaria vesca Linn* Extract

Fraction	Solvent system	Proportion in ml
9	Ethyl acetate- N Butenol- water	70:30
9	ethyl acetate: ethanol	80:20
8	chloroform: methanol (1:1)	50-50
9	Toluene:Ethyl acetetate:Formic acid:Methanol	5:5:1:0.5
6	Toluene- Acetone: formic Acid	70:30:20

Fractions were observed by UV and max fractions were collected. Then dried with evaporation at room temperature. After drying the obtained sample was used further for UV, IR, NMR and GCMS.

Chartarization of active principle from extract of *Fragaria vesca Linn*

a) Melting point of isolated compound was found to be 277-279^{0}c

b) Refractive Index of isolated compound shows 1.42

c) **UV Spectrum**

The UV spectrum of fractions obtained by column chromatography is shown below:

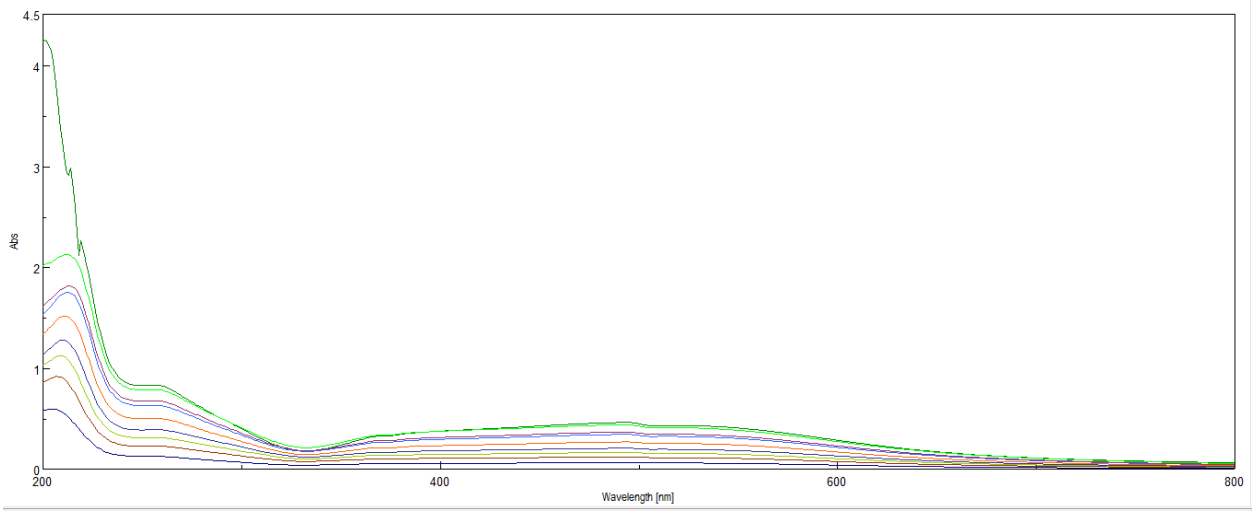


Figure 1: UV spectra of similar fractions

The UV spectra of isolated compound from *Fragaria vesca* Linn was performed using ethanol. The UV spectrum showed characteristic bands at 270 nm. Which is compare with standard drug i.e. kaempferol.

FT-IR Spectrum

The data obtained from FTIR Spectroscopy and possible functional groups present shown in Table 6 and fig no 2. The IR spectral analysis, the Peak at 3322.75a broad band is most probably of O-H stretching vibrations of phenol OH group. The peak 2356.59cm-1 showed C=O aryl ketonic stretch. The peak at 1641.13 indicates the presence of C=O aromatic stretch. The peak at1563.99, 1434.78, 1367.28, 1367.28 showed the presence of C=C aromatic stretch, C=C aromatic stretch, C-O stretch of phenol group respectively. 965.162, 833.098, 707.745 shows because of C-H bending of aromatic hydrocarbon. This is compare with standard kaempferol and gives somewhat similar result.

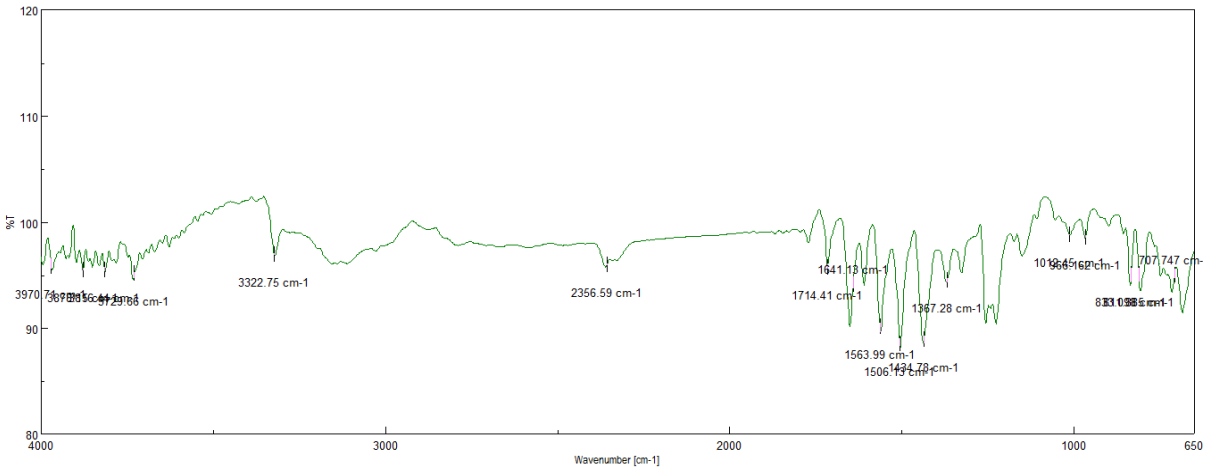
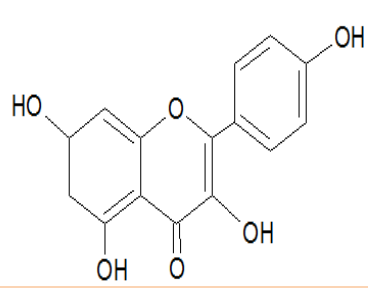


Figure 2: FT-IR spectra of isolated compound from *Fragaria vesca Linn*

Table 6: FT- IR study of isolated compound obtained from *Fragaria vesca Linn*

	Wave number(cm-1)	Functional group
	3322.75	O-H Stretching vibration of phenol
	2356.59	C=O aryl ketonic stretch
	1641.13	C=O aromatic stretch
	1563.99	C=C aromatic stretch
	1434.78	C=C aromatic stretch
	1367.28	C-O stretch of phenol
	965.162, 833.098,707.745	C-H bending of aromatic hydrocarbon
		C-CO-C Stretch and bending in ketone

NMR Data of *Fragaria vesca Linn*

NMR Data- H1 NMR–

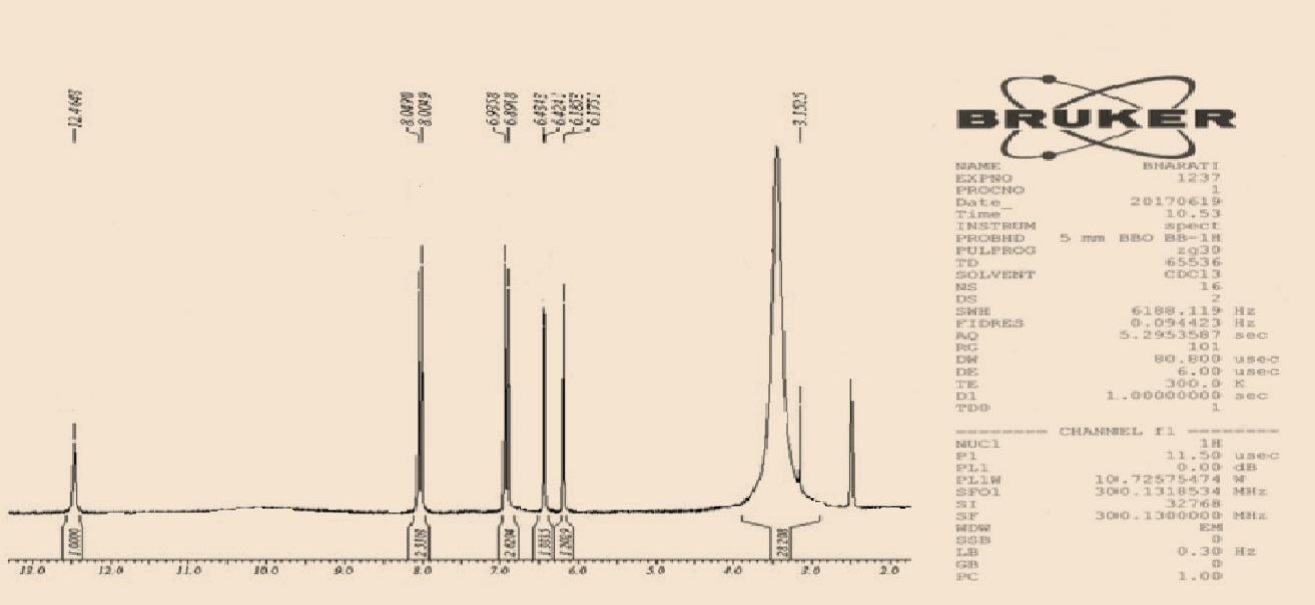


Figure 3: H1-NMR spectra of isolated compound of *Fragaria vesca Linn*

Table 07 shows the data obtained from NMR Spectroscopy. The 1H NMR spectrum of standard kaempferol and Isolated Compound showed two doublets with coupling constant of at δ 6.93, 6.89 and 8.007,8.00 ppm, which were assigned as H-3', H-5 and H-2'and H-6'respectively. 6.17,6.43 gives singlet at H6 and H-8 Position. This is compared with standard kaempferol. So it confirms that isolated compound is kaempferol.

Table 7: H1-NMR study of isolated compound obtained from *Fragaria vesca Linn*

Sr.No	1H Nmr values	Interpretation
1	6.17,6.18	H-6(1H)
2	6.43,6.42	H-8 (1H)
3	6.93, 6.89	H3',H5'(2H)
4	8.007,8.00	H 2', H6'(2H)
5	12.46	1H, Ar-OH

C13-NMR

The 13C-NMR spectrum of isolated exhibited presence of fifteen carbon atoms in the molecule. The 13C chemical shifts of a carbon at δ 177.6 indicated the occurrenceof C-4 carbon group in the molecule. The 13C- chemical shifts of carbon atoms at δ 135.4 (C-3), 147.87 (C-2), indicated that the hydroxyl group are attached at C-3 andC-5 positions. As shown in Fig.no 04 and table no 08.

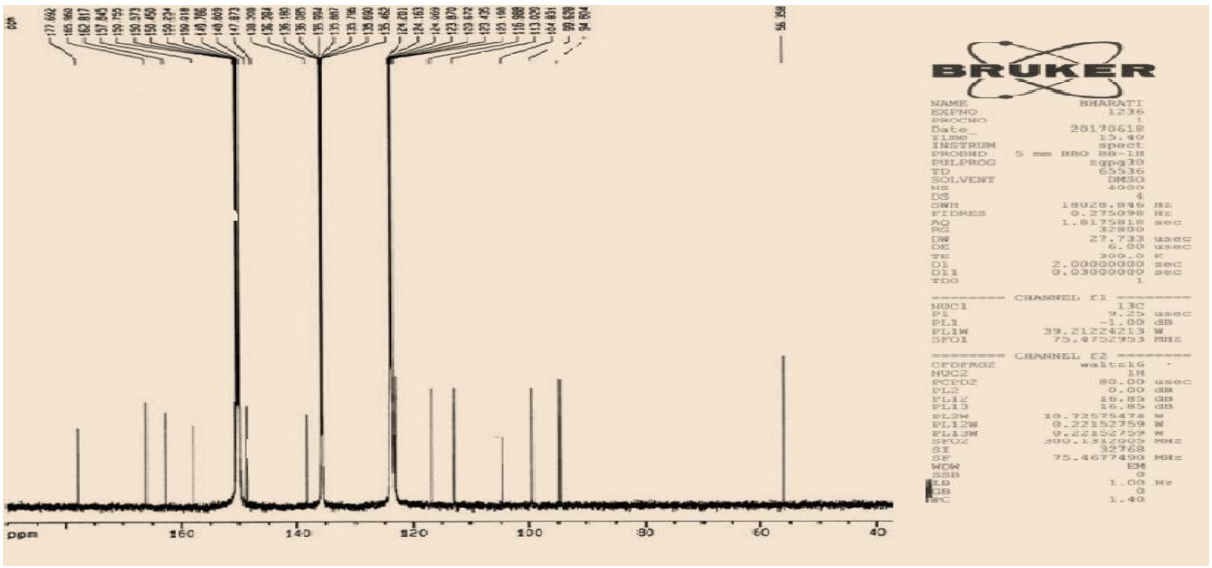


Figure 4: C13-NMR spectra of isolated compound obtained from *Fragaria vesca Linn*

Table 8: C13-NMR study of isolated compound obtained from *Fragaria vesca Linn*

Carbon	Values of C13 NMR
2	147.87
3	135.4
4	177.6
5	162.8
6	99.6
7	165.9
8	94.6
9	157.8
10	104.8
1’	123.1
2’	124.2
3’	116.9
4’	150.8
5’	113.02
6’	130.2

GCMS of isolated compound obtained from *Fragaria vesca* Linn

Analyzed by : ANIL V MOHITE
Analyzed : 8/1/2017 6:36:37 AM
Sample Name : GREEN BOTTLE
Sample ID : GREEN BOTTLE
Data File : D:\Rajaram Babu College\DHOLE\GREEN BOTTLE.QGD
Method File : D:\DI-method.qgm
Tuning File : D:\Tuning 2017\With Rtx-5 60m_24 March 17.qgt

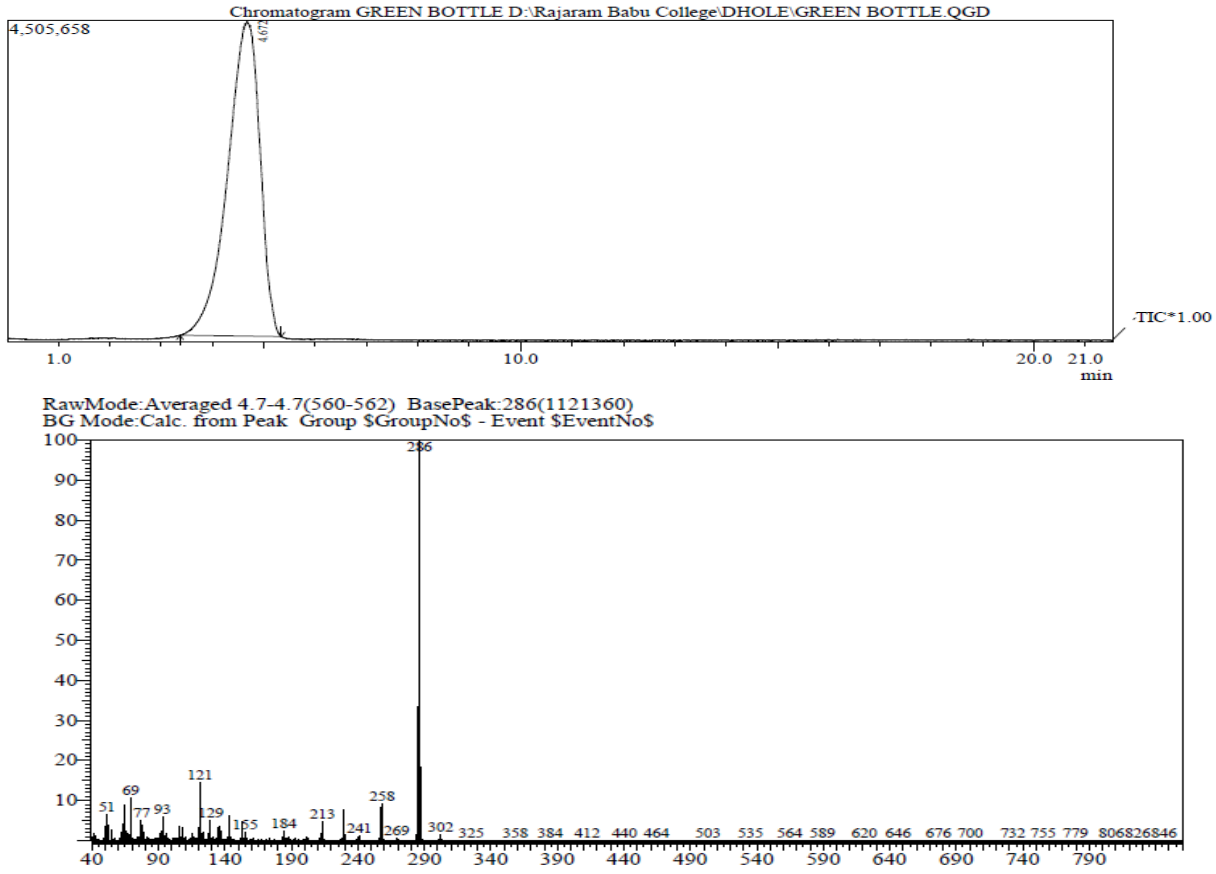
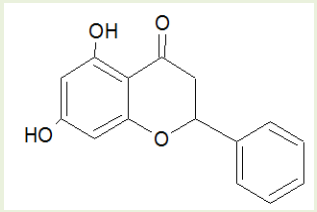
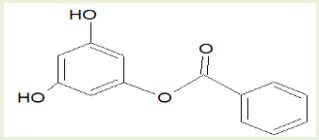
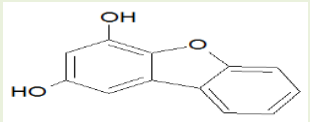
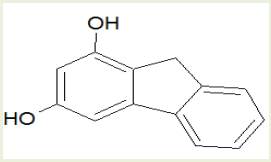
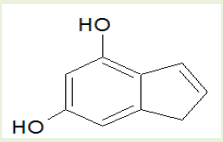
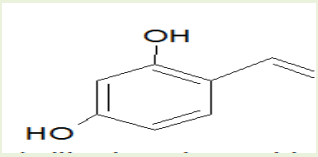
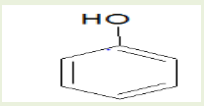
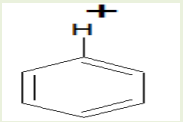


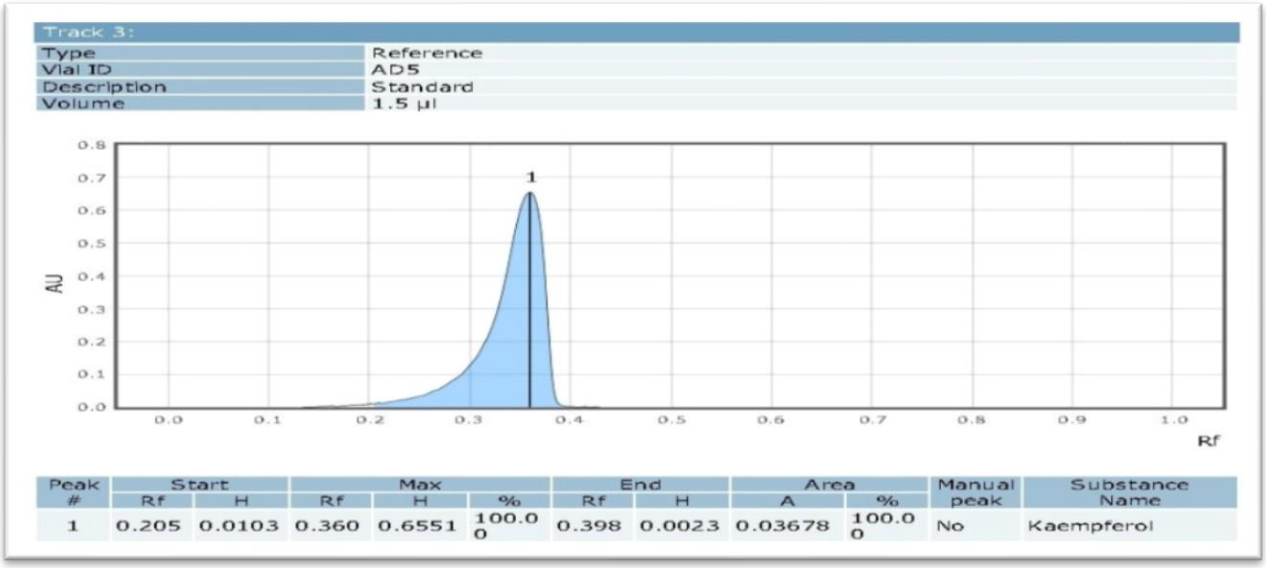
Figure 5: GCMS of isolated compound obtained from *Fragaria vescaLinn*.

Table 9: Fragmentation pattern of isolated compound obtained from *Fragaria vesca Linn*

M/E	Justification for fragment
	269(11) - eliminate OH group
	258(11) ring opining
	241 (17) eliminate oH group
	213 (28) eliminate Co group
	165(48) eliminate closed ring
	129(36) eliminate 3C group
	93(28) eliminate CHO group
	77(16) eliminate O group

HPTLC

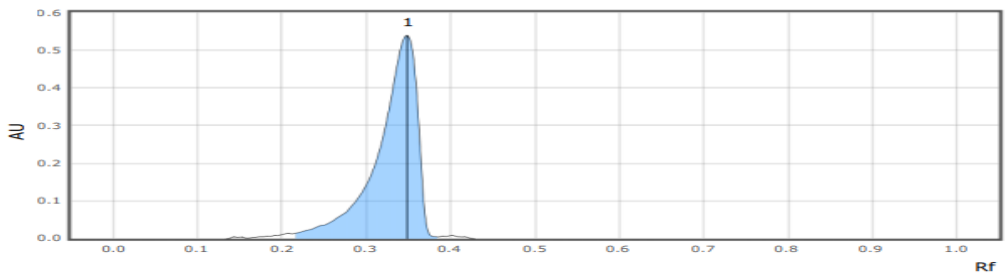
HPTLC chromatogram of Standard kaempferol.



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.205	0.0103	0.360	0.6551	100.00	0.398	0.0023	0.03678	100.00	No	Kaempferol

Figure 6: HPTLC chromatogram of Standard kaempferol

HPTLC chromatogram of isolated compound.



Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual peak	Substance Name
1	0.213	0.0129	0.348	0.5385	100.00	0.384	0.0046	0.02848	100.00	No	Kaempferol

Figure 7: HPTLC chromatogram of isolated compound from *Fragaria vesca Linn*

HPTLC chromatogram of isolated compound at 254 nm

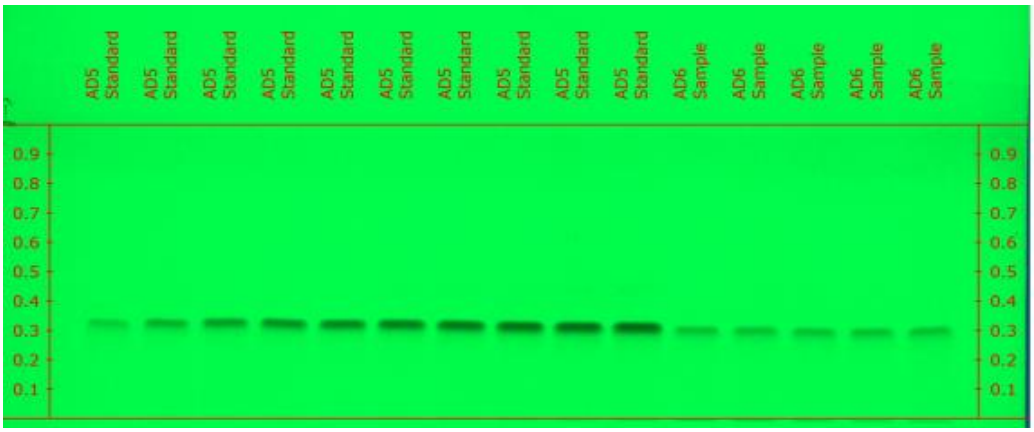


Figure 8: HPTLC of isolated compound at 254 nm obtained from*Fragaria vesca Linn*

HPTLC chromatogram of isolated compound at 366 nm

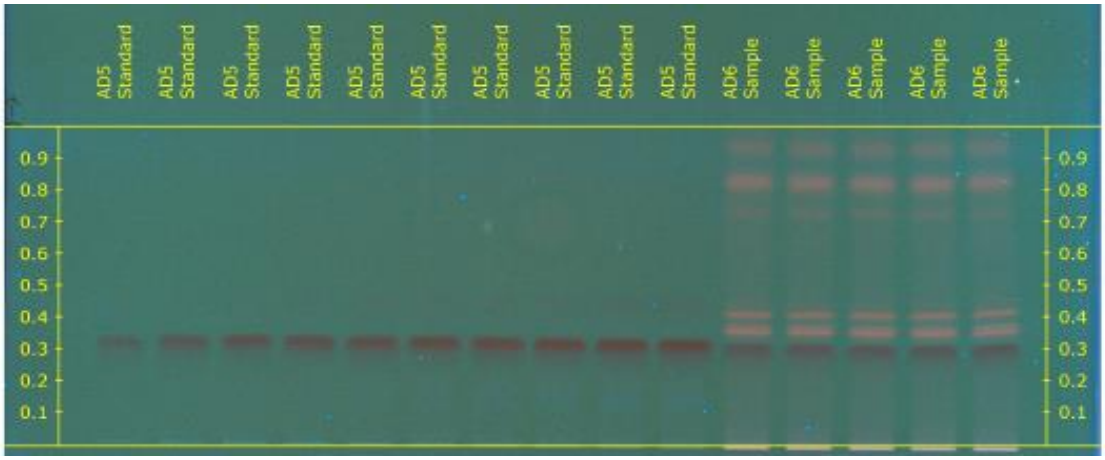


Figure 9: HPTLC of isolated compound at 366 nm obtained from*Fragaria vesca Linn*

HPTLC calibration curve for isolated compound

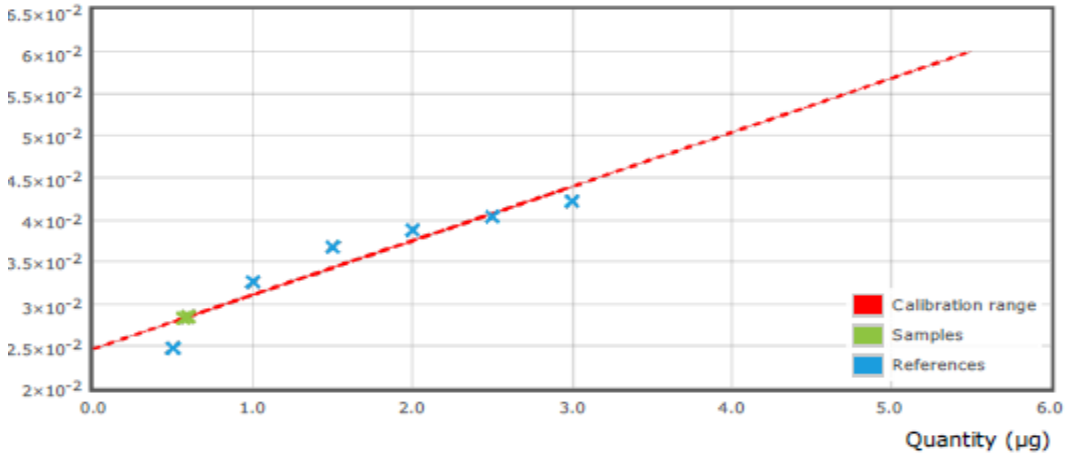


Figure 10: HPTLC calibration curve for isolated compound obtained from *Fragaria vesca Linn*

HPTLC shows clear separation of components present in the ethanol extract of the plant *Fragaria vesca Linn*. HPTLC fingerprint enables a particular plant with its components to be identified i.e Kameferol and distinguished from closely related species. The ethanol extract of *Fragaria vesca Linn* having the potentiality contains flavonoid which was analysed by using HPTLC. (Figure 6,7) The peak table, peak display and peak densitogram were noted. violet colored zones were detected from the chromatogram after derivatization which confirmed the presence of

Flavonoids (Figure 8,9). The extract was run along with the standard kameferol flavonoid compound and it was observed that the extract showed the presence of

presence of kameferol flavonoids and it was confirmed from the chromatogram after derivatization. The Rf value of the compounds present in the extract was found to be 0.384. Calibration curve for isolated compound shown in fig no 10.

Anti- urolithiasis *in vitro* models

Acute toxicity study was carried out according to the OECD/OCDE, OECD Revised draft guidelines 423. Lethal dose of mice was calculated, 1/10th of this lethal dose was taken as an effective dose for subsequent studies. The effective doses therapeutic doses as shown in table no 10.

Table 10: LD50 and the effective doses of *Fragaria vesca Linn*

Sr.No	Extracts	LD50	Effective dose
1	Ethanol extract of *Fragaria vesca* Linn.	4000mg/kg	400mg/kg B.W.

Waster albino rats about 150-200g were used in pharmacological studies. The animals were purchased from the national institute of biosciences, Dhangawadi. Protocol no- RCP/IAEC/16-17/PO5. The animals had free access to standard diet with water supplied ad libitum under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hr prior to experimental protocol to minimize if any of non specific stress. All the protocols and experiments were conducted in strict compliance according to the ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Table 11: Mean standard ± deviation of urinary parameter

Sr. No.	Sample Code	Total Protein (g/dl)	Urine Calcium (mg/dl)	Urine Creatinine (mg/dl)	Urine Oxalate (mg/dl)	urine urea (mg/dl)	Uric acid (mg/dl)
1	Control	10.29 ± 1.56	15.75 ± 1.91	10.31 ± 1.06	20.03 ± 2.84	8.04 ± 0.77	10.73 ± 1.12
2	Normal	3.14 ± 1.17	6.58 ± 1.04	3.98 ± 0.94	4.41 ± 1.46	3.45 ± 0.51	5.83 ± 0.52
3	CYS	5.10 ± 1.49	6.02 ± 1.79	5.32 ± 0.77	5.37 ± 1.45	4.10 ± 0.46	7.54 ± 1.02
4	EFV CR	7.01 ± 1.07	7.52 ± 1.29	7.21 ± 0.47	11.67 ± 1.39	7.90 ± 0.60	7.57 ± 1.88
5	EFV PR	6.45 ± 0.95	6.82 ± 1.48	6.29 ± 0.98	11.80 ± 3.01	0.76 ± 0.15	7.90 ± 1.88

CYS- Cystone, EFV CR -Ethanol extract of *Fragaria vesca* Linn Curative Regimen EFV PR Ethanol extract of *Fragaria vesca* Linn Protective Regimen; * All values are presented as the mean ± standard deviation (n=6)

Table 12: Mean standard ± deviation of Serum parameter

Sr. No.	Sample Code	Serum creatinine (mg/dl)	Serum phosphorus (mg/dl	Serum urea (mg/dl)	Serum uric acid (mg/dl)
1	Control	1.068±0.1329	2.048±0.3603	58.33± 3.409	5.205± 0.5675
2	Normal	0.6262±0.05437	0.6173±0.2698	32.05±5.125	1.852±0.2496
3	CYS	0.6792±0.07774	0.7715±0.1448	34.74±1.990	3.278± 0.2089
4	EFV CR	0.8308±0.1001	0.9158±0.08797	46.15±2.433	3.335± 0.3275
5	EFV PR	0.7542 ± 0.07145	0.7620± 0.1513	29.48± 1.046	3.473± 0.2406

CYS- Cystone, EFV CR -Ethanol extract of *Fragaria vesca* Linn Curative Regimen EFV PR Ethanol extract of *Fragaria vesca* Linn Protective Regimen; * All values are presented as the mean ± standard deviation (n=6)

At the end of urinary biochemical data that were obtained at the end of the experiments in each group. In the present study chronic administration of 0.75%(V/V) ethylene glycol aqueous solution to male waster rats resulted in hyperoxaluria i.e Control there was an in urinary calcium, uric acid, urea, and oxalate in calculi induced animals as in group I, however, supplementation with EFV CR- Ethanol extract of *Fragarica vesca* L Curative Regimen 400mg/k gused gives 7.01 ± 1.07Total Protein (mg/dl), 7.52 ± 1.29 Urine Calcium (mg/dl), 7.21 ± 0.47 Urine Creatinine(mg/dl),11.67 ± 1.39 urine oxalate, 7.90 ± 0.60 urinary urea, 7.57 ± 1.88 uric acid,0.9158±0.08797 serum phosphorus, 46.15±2.433 serum urea, 3.335± 0.3275 Serum uric acid (mg/dl). When it compare with the slandered drug cystone it gives anti urolithiasis activity which is less effective than standard drug cystone. As shown in Table no- 11 Significantly ($P<0.05$) inhibited these changes in urinary calcium, uric acid, urea and oxalate excretion dose dependant in both curative and preventive regimen Renal stone induction caused impairment of renal functions of the untreated rats as evident from the markers of glomerular and tubular damage i.e elevated serum creatinine, uric acid , Serum phosphorus, uric acid and urea. These markers were significantly ($P<0.05$) reduced in the animals which were treated with CYS- Cystone, EFV CR - Ethanol extract of *Fragaria vesca* Linn Curative regimen, EFV PR- Ethanol extract of *Fragaria vesca* Linn, Protective regimen. As shown in Table no -12

SUMMARY AND CONCLUSION

Urolithiasis, or urinary tract stones, is the aggregation of crystals in the urine, calcium oxalate is more common kidney stone. In the case of urolithiasis Unbearable pain is seen that is very severe in intensity than any other disease condition.

Herbal drugs are created more interest among the people by its clinically proven effects like Immunomodulation, adaptogenic and antimutagenic. Number of medicinal plants shows antiurolithiatic activity and plays a vital role in prevention of kidney stone. An attempt has been made to provide potent indigenous herbs for kidney stone. Survey of literature showed that various pharmacological actions of *Fragaria vesca* such as antiulcer, anti-inflammatory, antibacterial, antioxidant activity have been evaluated and documented through scientific publications but a similar scientific evidence for the antiurolithiatic activity in combination gives more prominent effects for curative and preventive action on kidney stone. In the present study the hole plant of, *Fragaria vesca Linn e*xtracts on calcium oxalate Urolithiasis in Rat which is helpful aid in the treatments for urolithiasis.

The Plants were authenticated Botanical survey of India, Pune. The authenticated parts of plant were subjected to organoleptic evaluation. The standardized whole plants of *Fragaria vesca Linn* were subjected to sextet extraction with various solvents like Pet. ether (60-80^{0}C), Chloroform, Ethyl acetate, Ethanol by using soxhlet apparatus. After effective extraction, solvent was distilled off by using hot water bath and rotary evaporator. The concentrated extracts were used for carrying phytochemical investigation. The phytochemical investigation revealed the presence of sterols, triterpenoids, flavonoids and phenolic compounds as a major active chemical constituent. All the extracts were subjected to detail for TLC profile by using Polar, semi polar, nonpolar solvent system and observed under UV and Visible light. The column chromatography was developed by using graded solvent mixtures of Toluene-Acetone: formic Acid for *Fragaria vesca* Linn and Then proceed for UV spectrum of nearby similar isolated fractions were carried out. Further IR, NMR as well as GC-MS study of isolated fractions of Kaempferol *Fragaria vesca* Linn was recorded and gave satisfactory results for confirmation of the structure. The interpretation of IR, NMR and Mass spectra is shown elsewhere in results.

Acute toxicity study was carried out according to the OECD/OCDE, OECD Revised draft guidelines 423. Lethal dose of mice was calculated, 1/10th of this lethal dose was taken as an effective dose for subsequent studies. The glomerular filtration rate decreased in urolithiasis due to obstruction to the outflow of urine by stones in the urinary system and the west product such as urea and uric acid get accumulated. This indicates marked damage of kidney. The uric acid crystals adsorb glutamic acid and other organic compounds and promote calcium oxalate crystal growth. The results showed that a significant increase in uric acid level in serum as well as in urine in the ethylene glycol control group to normal control. The uric acid levels decreased after treatment with extract of *Fragaria vesca* and cystone, therefore hastening the process of dissolving the preformed stone and prevention of new stone formation in urinary system. Renal function was evaluated by measuring serum phosphorus, calcium, urea, and creatinine in Group. The concentration of phosphorus, calcium, urea, and creatinine in the serum was significantly (P< 0.001 vs. Group I) increased in the stone-induced group indicating renal damage.

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Source of Support: Nil, **Conflict of Interest:** None.