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Bryophyllum Pinnatum and *Hebiscus Rosa Sinesis* Evolutionary Study against Kidney Stone Degradation

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Abstract: ***Objective: In Brophyllum Pinnatum and Hebiscus Rosa Sinesis study for kidney stone degradation, Bioactive compond extract from soxhlet method, Extract identification, separation of molecule performed by TLC, Column Chromatography, Quantitative, Qualitative test was performed HPLC (High Performance Liquid Chromatography) degradation carried out by egg cell membrane, suger estimate by Summners Method. Methods: To study Kidney stone degradation properties of Miracle plant B. Pinnatum and H. R. Sinesis for Bioactive compound isolation plant leaf dry powder use for soxhlet extract and various phytochemicals was identified by phytochemical test; plant extract subjected to TLC for Exact identification of Molecule, Ursolic acid which sub compound of penta cyclic teriterphenoid, molecule Purification performed by Column Chromatography. Quantification, qualitative estimation performed by HPLC for exact determination of kidney stone degradation egg cell membrane use and chemically kidney stone and quantified total degradation, spectrophotometer determination also performed by using Standard tablet cystone Result: Hebiscus Rosa Sinesis contains more activity than Bryophyllum pinnatum. Titeration also shows more activity of H. R. Sinesis than B. Pinnatum sample. Conclusion: concluded that B. pinnatum contains less active molecule that H. R. Sinesis, it contains more Pentacyclic Ursolic acid and its shows more Activity than Cystone.***

Keywords: *Hebiscus Rosa Sinesis, Bryophyllum Pinnatum*, Ursolic acid, Pentacyclic teriterphenoid, Cystone

1. Introduction

As per WHO Urinary Tract Infection are widespread Disease in India [1] Medicinal plant constitute major source of active drugs in both traditional and modern medicine [2] *Bryophyllum Pinnatum* Research Originated from my Kindney stone Patients, that take fresh leafs of *Bryophyllym Pinnatum* from my home Garden to diagnosis make fresh juice intake at early morning. Most of chemical compond of *Bryophyllum Pinnatum* that are responsible for therapeutic function are water soluble molecule phenol, glycoside, flavanoid [3]

Despite the immense of scientific technology advancement in modern medicine, many people in the world (approximately 75% of population) still rely on traditional healing practices and medicinal plants for their daily health need [4]. Main background of study to Study effect of *Hebiscus Rosa Sinesis, Bryophyllum Pinnatum* against kidney stone diagnostic *B. pinnatum* has medicinal value, this medicine use in Nigeria (Traditional Medicine of Nigeria), it employed for treatment of earache, lithiasus bburns, earache, burns, ulcer, whitlow, insect bite [5] Invasion of Urinary tract pathogenic bacteria causes inflammatory response in urothelium [6]Main Purpose of study is to study Bioactive compound of *Hebiscus Rosa Sinesis, Bryophyllum Pinnatum* it's miracal role for Kidney stone Degradation.

Figure 1: *Bryophyllum Pinnatum*

Figure 3

Hebiscus Rosa Sinesis
Bryophyllum pinnatum

Taxonomy
Kingdom: Plantae
Vascular plant division: Spermatophyta
Order: Rosales
Family: Crassulaceae-Stonecrop
Genus: Bryophyllum
Species: *Bryophyllum pinnatum {Lam. }*

Vernacular Name

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English: Air Plant
Sanskrit: Parnabeeja, Asthibhaksha
Marathi-Panfuti
Hindi-Zakhmhaiyat, patharchur

Hibiscus rosa-sinensis

Taxonomy
Kingdom: Plantae
Vascular plant division: vascular cryptogams
Order: Malvales
Family: Malvaceae
Genus: Hibiscus
Species: *Hibiscus rosa-sinensis*

Vernacular Name-
English-Hibiscus
Sanskrit-Japa
Marathi-Jaswandh
Hindi-Guddhahal

2. Materials and Methods

Chemicals
Chemicals used in this study including Calsium Oxalate and other all chemical were procured from Sigma Chemicals and High media chemicals, some other chemicals purchase from GeNei Chemical, All chemicals Highest purity grade. Standard Ursolic acid purchase from Sigma Inrich.

Plant Material and Preparation of Extract
The plant of *Bryophyllum pinnatum*, *Hibiscus rosa-sinensis* was collected, identified, and authenticated taxonomically at MGM Institute of Bioscience and Technology, Aurangabad, Maharashtra, for Sample Preparation wash Leafs by Distilled water and dry it, Sun Dry Leaf of Bryophyllum Pinnatum, *Hibiscus rosa-sinensis* For 1 week for powder extract, saperate Crush Dry Leafs of *Bryophyllum pinnatum* and *Hibiscus rosa-sinensis* into each Mortar pistol Fine powder formed, Further use 20 gm of Powder *Hibiscus rosa-sinensis* and 20 gm of Powder *Bryophyllum pinnatum* in two different Filter paper Thimble proceed each Soxhlet it contains 90 ml methanol and 10 ml Distilled water as solvent system 8 cycle performed in 64.7 °C Half of extract dry in pateri plates and half of Extract Store in Reagent bottle for further process.

Phytochemical Identification

Phytochemical test of Tannin, Phlobatanins, Saponin, Flavanoid, Terphenoid, Deoxysuger, Sterol performed for identification of phytochemicals in *Hebiscus Rosa Sinesis* and *Bryophyllum pinnatum* as following way [7].

(Mthanol soxhlet sample use for Phytochemical screening.)

1) **Tannin**
   In 3 ml plant extract add 0.1%ferric chloride appearance of green and blue colour show presence of tannin
2) **Phlobatanins**
   In 3ml plant extract dissolve few drops of 1% hydrochloric acid incubate solution in boiling water bath. Appearance of red precipitates show presence of Phlobatanins.
3) **Saponin**
   In 3 ml plant extract add 7 ml distilled water and agitate it for 10 min, Appearance of foam shows presence of tannin
4) **Flavanoids**
   3 ml extract add in 1% ammonium solution there should form yellow colour then add sulfuric acid then colour less solution indicates positive result.
5) **Terpenoids** Take 3 ml plant extract add some drops of chloroform.
   Red brown colour shows positive result
6) **Deoxysuger**
   In 3 ml plant extract add 0.4 ml acetic acid and few drops of feric chloride solution then add 0.5 ml sulfuric acid in side of test tube. Appearance of blue colour shows presence of deoxysuger.
7) **Sterol**
   3 ml plant extract add 10 ml chloroform filtered it. Take 2 ml filterate add 2 ml acetic unhydrate and concentrated sulfuric acid. Presence of blue green ring shows positive result.

Compound Identification
Bryophyllum Pinnatum, Hibiscus Rosa Sinesis contains various bioactive molecules identified Thin Layer Chromatography; 9gm silica mix in 0.9gm calcium Sulphate by Adding 6.5 ml Distilled water Mix it well in beaker, slurry formed Steam it in micro oven for 60 sec poured slury in glass slide, place slide in Hot air oven for 50 ^{0}C for 50 min further proceed TLC, flamed Capillary in Bunsen Burner for thin spot, samples take in flamed capillary and add 6 drops one by one from one cm above in silics bottom plate (1st dot dry then add 2nd dot continued till 6th dot), ethyl acetate: butenol: acetic acid: water (80: 10: 5: 5) used as a solvent system.

Purification of Molecules
Column Chromatography carried out by using n-Hexane: Acetone (1: 1) as a solvent, 6 gm silica gel overnignt incubated in 60 ml Solvent, edges of column pack my filling glass beads, overning incubated silica poured in column again glass beads place 1 cm above silica, 400ul of soxhlet sample (*Bryophyllum Pinnatum, Hibiscus Rosa Sinesis)* add into column (whole process carried without Dry column/Column always contained solvent, also Air bubble cause error in process) 26 Fractionation collected in clean test tube, eluent collect on the basis of ml.

High performance liquid chromatography
HPLC carried out for Quantification and Qualitative Estimation; Methanol, water, terterahydroxyl furferol (94: 5: 1). Use as Solvent for HPLC, 20 ^{0}C temperature, 0.5ml/min flow rate, 214 nm Absorbance, Injection volume 10 ul, Filter 0.25 um, Column C-18, total time 50 min [8].

Stock: 3mg/ml standard ursolic acid dissolved in HPLC solvent

Working std: 10, 20, 50, 100, 200, 500, 1000ul stock dissolved in per ml of solvent.

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Sample preparation: 3mg/ml soxhlet sample of *B. Pinnatum* and *H R Sinesis* dissolved in HPLC solvent, filtered, replica of sample prepared, all standard and samples

Filtered by sering filter (0.45um)

All samples and standard was further proceed by HPLC.

Table 1: Parameter

S. no	HPLC Parameter	Required
1	Injection Volume	20 ul
2	Column	RP 18
3	Solvent System	Methanol+Water+ Tetrahydroxy Furferol
4	Ratio Solvent System	94: 5: 1
5	pH of Solvent system	5
6	Flow rate of mobile phase	1ml/min
7	Retention Time	12min
8	Wavelength	220nm
9	Temperature	22 ^{0}C

Aggregation Assay

Aggregation assay performed for comparative study of *Bryophyllum Pinnatum*, *Hibiscus Rosa Sinesis* against standard drug cystone.

Preparation of Artificial Kidney Stone

a) Eqvimolar solution of calcium chloride dihydrate dissolve in distilled water
b) Sodium oxalate disolved in 10 ml of 2 N sulfuric acid

Mix A and B in beaker add distilled water drop by drop till precipitate formed precipitate wash with distilled water and dry it completely at 60 ^{0}C for 4 hours [9]

Preparation of Membrane

Egg membrane remove chemically by place eggs in 2 molar HCL solution for overnight next day take inner membrane of egg and wash it by distilled water; hole created by Sharp pointer wash bag by distilled water, furthur it use for kidney stone degradation membrane add crystals of artificial kidney stone (1mg) with our plant extract (10mg) (soxhlet sample) in bag close bag by treat and overnight incubated bag in 0.1M Tris buffer, in one membrane cystone use instead of plant sample it work as standard, 1mg calcium oxalate served as negative control, all beakers placed in Incubator 37 ^{0}C for 24hrs. The contents of SPM pouches were removed into flask this contend further use for titeration.

Titeration Method

In this contend add 2ml of 1N sulfuric acid in each beaker, then ir titerate with 0.9494N $KMnO_4$ is equivalent to 0.1898 mg of calcium record titrimetry all value recorded by mean [10].

3. Results

Sample preparation and extraction

Sample preparation by sun dry for 7 ways for crush leafs and fine powder formed, that used for soxhlet 8 cycles was performed

Phytochemical Identification

Phytochemical test of Tannin, Phlobatanins, Saponin, Flavanoid, Terphenoid, Deoxysuger, Sterol performed by *Hebiscus Rosa Sinesis*, *Bryophyllum Pinnatum* soxhlet samples

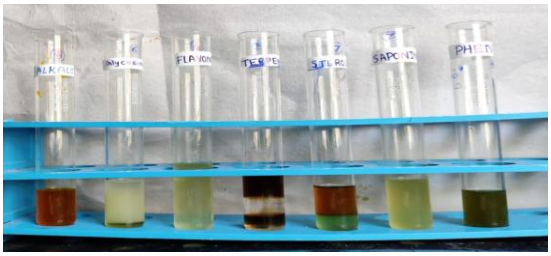

Figure 3: Phytochemical Test Results

Table 2: *Bryophyllum Pinnatum* Result

S. No	Test	Result
1.	Tannin	Positive
2.	Phlobatanins	Negative
3.	Saponin	Negative
4.	Flavonoids	Negative
5.	Terpenoids	Positive
6.	Deoxysuger	Positive
7.	Sterol	Positive

Table 3: *Hibiscus rosa sinesis* Result

Sr. no	Test	Result
1.	Tannin	
2.	Phlobatanins	
3.	Saponin	
4.	Flavonoids	
5.	Terpenoids	
6.	Deoxysuger	
7.	Sterol	

Compound Identification

Various compounds of bryophyllum pinnatum and hibiscus rosa sinesis observed by TLC, Ursolic acid used as Standard; comparatively Retardation factor calculated by following formula

= Distance travelled by solute
Distance travelled by solvent

7 spots observed in *Hibiscus rosa sinesis*

3 spot observed in *Bryophyllum pinnatum*

Standard spots observed in Figure _under UV light; According to standard spots compaired with *hibiscus rosa sinesis*, *bryophyllum pinnatum* which are follows
Bryophyllum Pinnatum
Solvent: 5.2cm

Table 4: Spots Calculate in cm

Spots	*H. R. Sinesis* (cm)	*B. Pinnatum* (cm)	Ursolic acid (cm)
1	0.4	1.6	2.2
2	0.9	2.2	
3	1.6	2.8	
4	1.8		
5	2		
6	2.2		
7	2.9		

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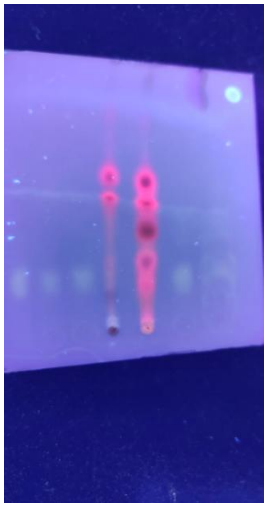
Table 5: Rf Values

Spots	*H. R. Sinesis*	*B. Pinnatum*	Ursolic acid
1	0.076	0.307	0.423
2	0.173	0.423	
3	0.307	0.538	
4	0.346		
5	0.384		
6	0.423		
7	0.557		



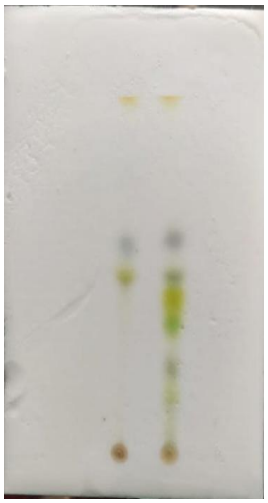


Figure 4: TLC plate in UV & STD **Figure 5:** TLC plate

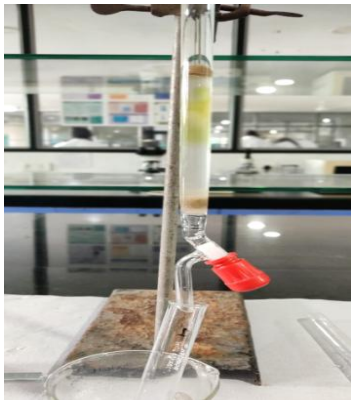


Figure 6: Column Chromatography

Figure 7: Column Fractionations

Figure 4 shows various spot of *bryophyllum pinnatum* and *hibiscus rosa sinesis* shows in 4 and 5 column with standard in 1, 2, 3, 6 column & Figure 5 shows various spot of *bryophyllum pinnatum* and *hibiscus rosa sinesis.* in Table: 4 Spots Calculated in cm and Table: 5 Rf Values calculated.

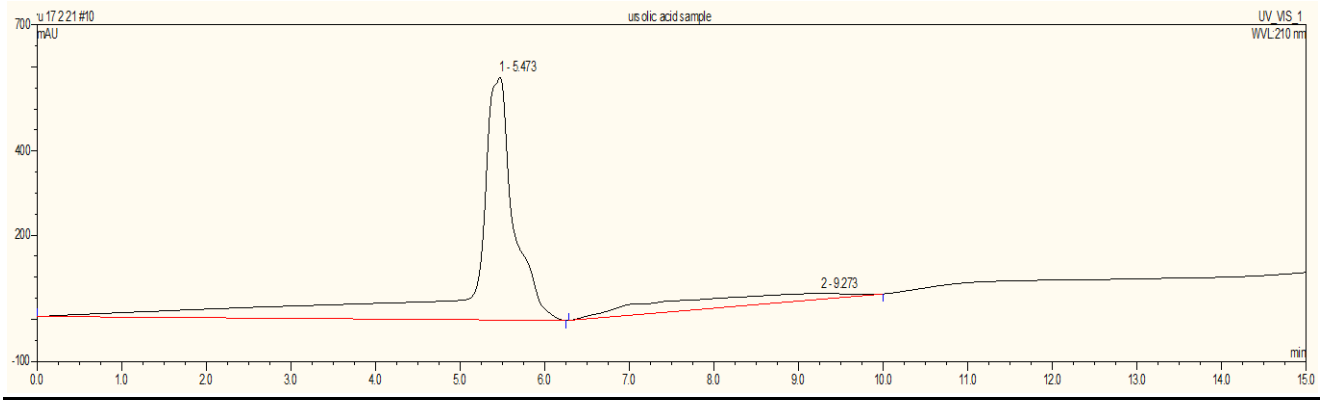
Purification of Molecules

Various Molecules of Bryophyllum Pinnatum and Hibiscus Rosa Sinesis was purified by Column Chromatography, 26 fractionations collected in test tube according to 2 ml in each test tube, according to polarity molecules moved.

Fig 6 shows column chromatography process and Fig 7 shows various fractionation collected in test tube

High performance liquid chromatography

3 sample of *B. Pinnatum* and 3 sample of *H R Sinesis* was proceed for HPLC 6 STD concentration used which explained in procedure.



Sample Graph 2: (3mg/ml *Bryophyylum Pinnatum*)

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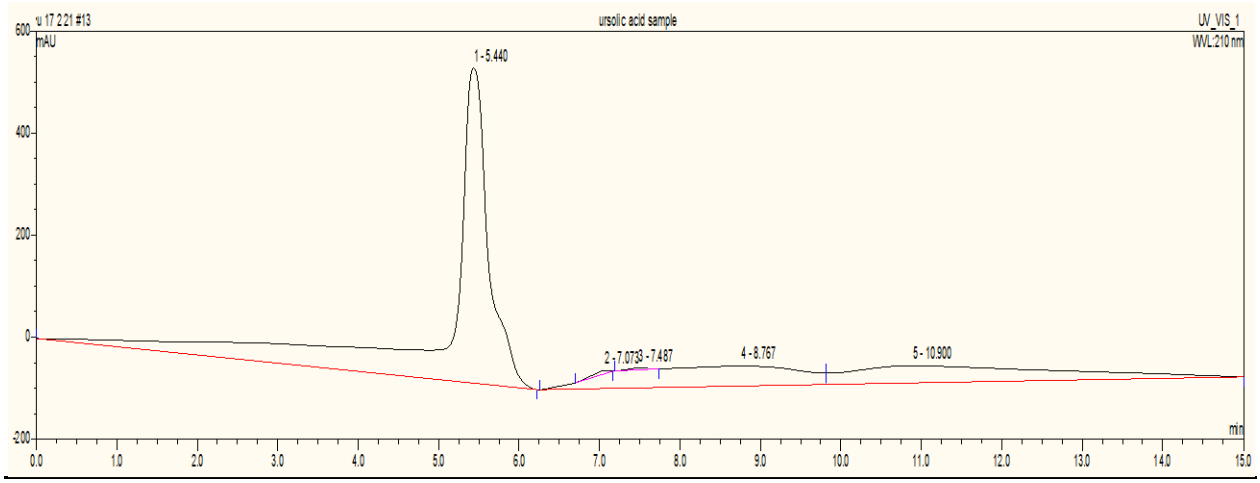
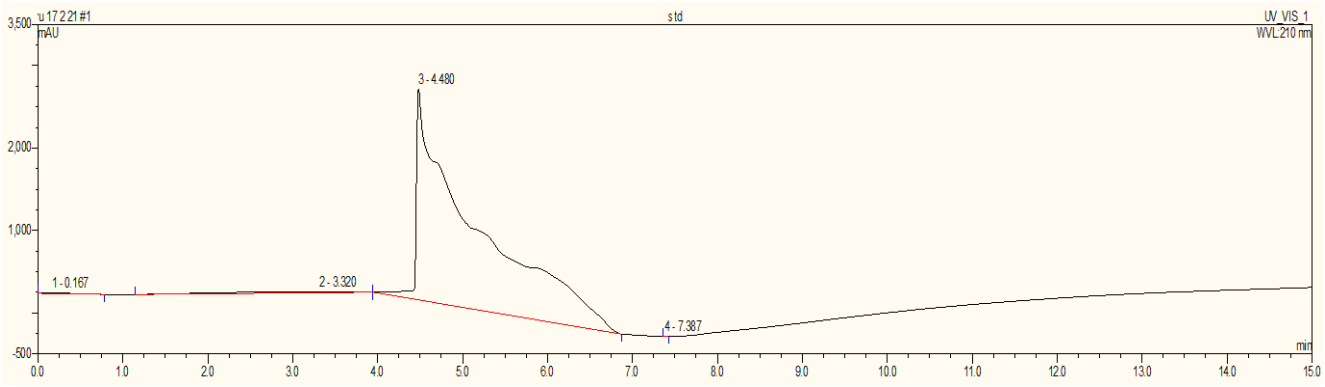
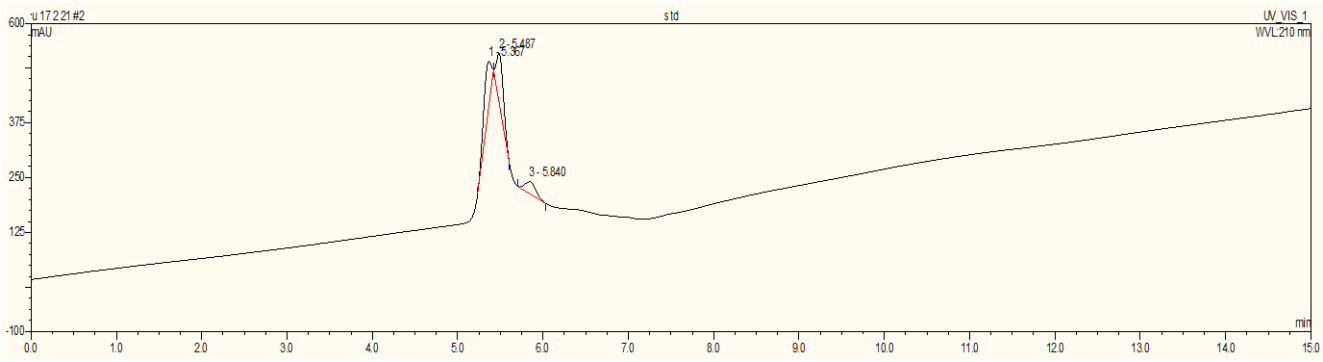


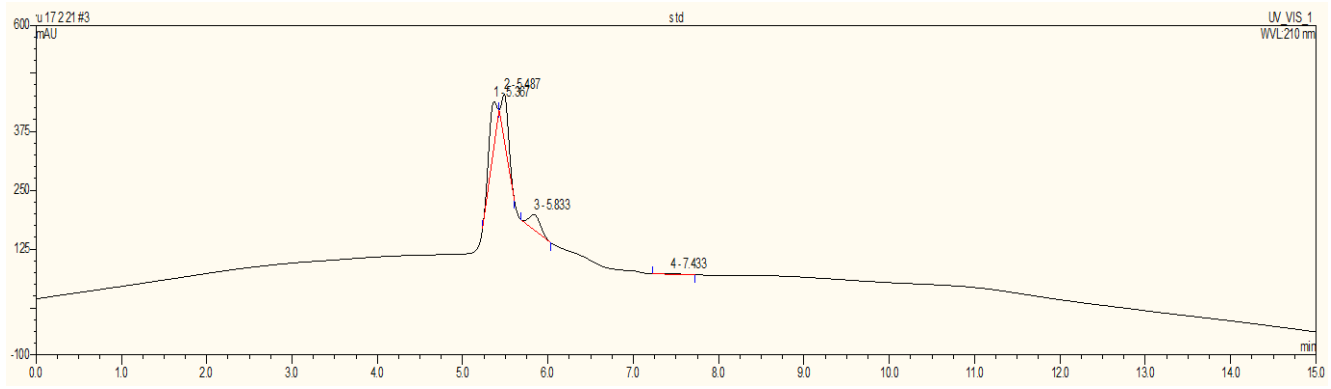
Sample Graph 3: (3mg/ml *H R Sinesis*)



STD Graph 1: (10ul stock/ml solvent)



STD Graph 2: (20ul stock/ml solvent)



STD Graph 3: 50ul stock/ml solvent

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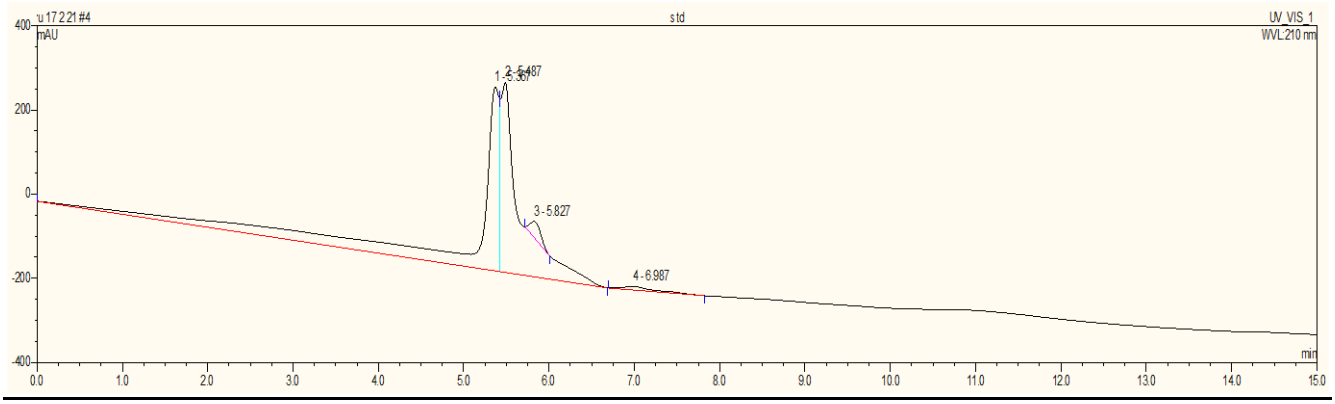
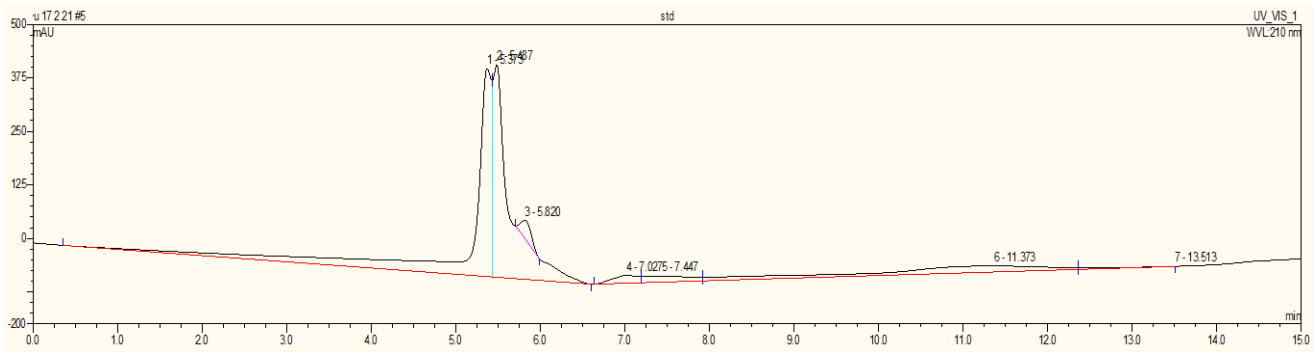
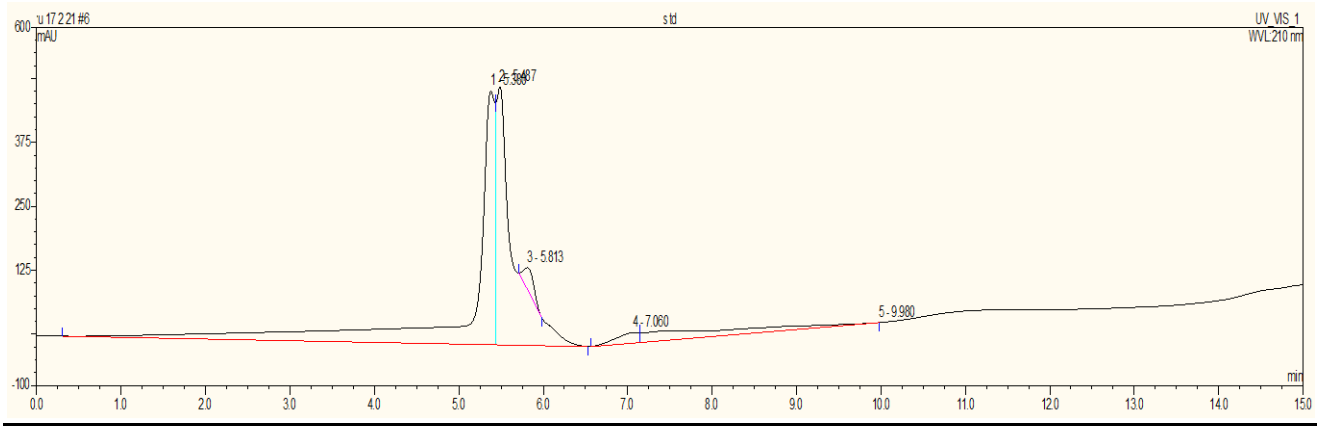


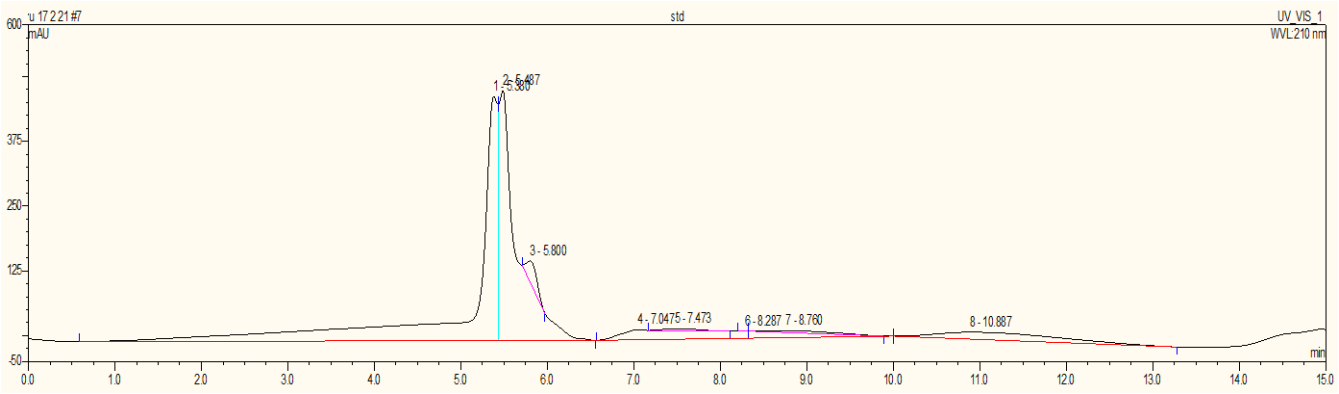
STD Graph 4: 100ul stock/ml solvent



STD Graph 5: 200ul stock/ml solvent



STD Graph 6: 500ul stock/ml solvent



STD Graph 7: 1000ul stock/ml solvent

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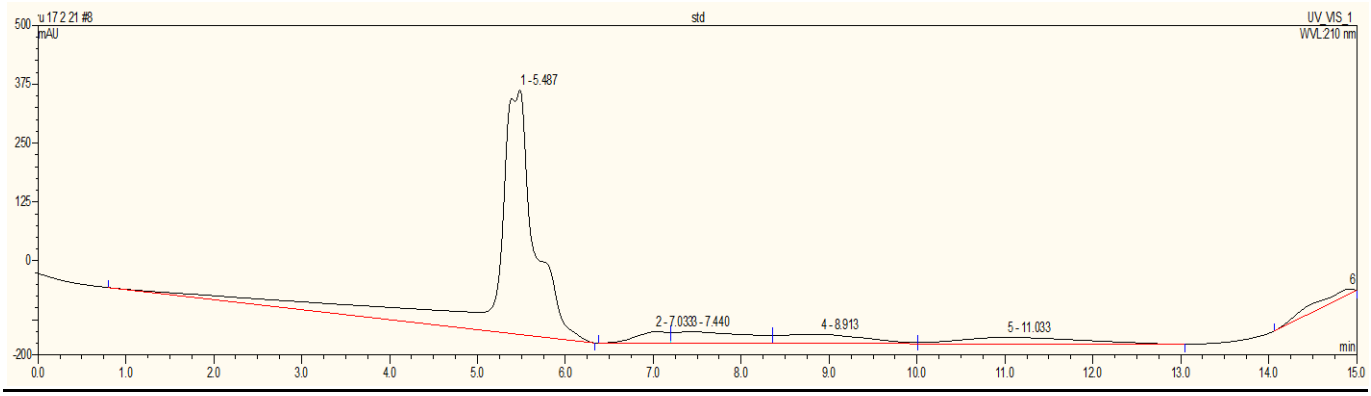
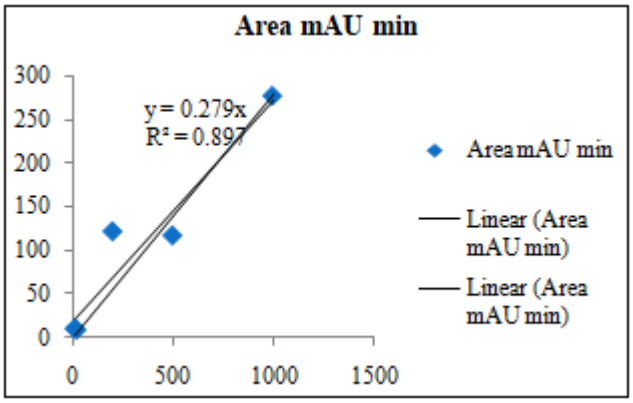


STD Graph 8: Stock solution

As per observation various sample and standard concentration shows Pic in 5.4 in retention time that means as compared to standard in our sample ursolic acid present caliberation graph subsequently used to calculate mean of sample

S. No	STD	Area mAU min
1	10	10.6355
2	20	9.0362
3	200	121.7506
4	500	117.1202
5	1000	276.9435



S. no	Compound/ Samples	Retention time	Area	Height	Quantification in ul	Quantification in ug/ml	Quantification in mg/ml
1	*B. Pinnatum*	5.473	344.659	576.632	x=344.659/0.279 x= 1235.33692	1235.4 ug/ml	1.236 mg/ml
2	*H R Sinesis*	5.44	381.8972	617.090	x=381.8972/0.279 x=13168.80717	1369 ug/ml	1.369 mg/ml

Preparation of Artificial Kidney Stone

Artificial kidney stone prepared by calcium chloride dehydrate and sodium oxalate dihydrate by added 2N sulfuric acid, crystals was formed successfully,

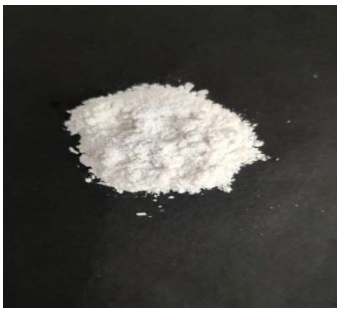


Figure 8: Artificial Kidney Stone

Preparation of semipermiable membrane by egg

Figure 9

Egg placed in sulfuric acid

Semipermeable membrane of egg was removed by needle and scarpel washed by distilled water and carefully load kidnet stone crystals and sample also loaded, degradation of kidney stone cheaked by titeration method

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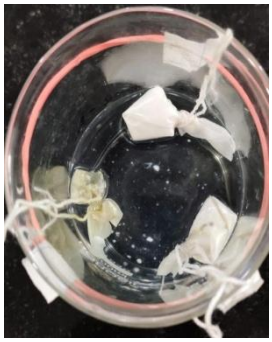

Figure 10: Crystals with Sample placed in Membrane

Titeration method

Figure 11: Titeration Result

Sample	Sulfurc Acid	Titeration
Pinnatum	20 ml	1.8
Hibiscus	20 ml	1
Blank	20 ml	3

Figure 10: Crystals with Sample placed in Membrane

Figure 11: Titeration Result

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