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

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Abstract: Objective: In Bryophyllum Pinnatum and Hebiscus Rosa Sinesis study for kidney stone degradation, Bioactive compound extract from soxhlet method, Extract identification, separation of molecule performed by TLC, Column Chromatography, Qualitative, Qualitative test was performed HPLC (High Performance Liquid Chromatography) degradation carried out by egg cell membrane, suger estimate by Sumners 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 teripherenoid, 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. Titation 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 teriferphenoid, 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 Bryophyllum 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-Guddhalal

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 Leaves by Distilled water and dry it, Sun Dry Leaf of Bryophyllum Pinnatum, *Hibiscus rosa-sinensis* For 1 week for powder extract, seperate Crush Dry Leaves 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, Flavonoid, Terphenoid, Deoxysuger, Sterol performed for identification of phytochemicals in *Hibiscus Rosa Sinesis* and *Bryophyllum pinnatum* as following way[7].

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) Flavonoids  
   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 deoxysugger.

7) Sterol  
   3 ml plant extract add 10 ml chloroform filtered it.  
   Take 2 ml filtrate 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 slurry in glass slide, place slide in Hot air oven for 50 °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 (1° dot dry then add 2nd dot continued till 6° 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 overnight 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 °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.
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 (0.45μm)

All samples and standard was further proceed by HPLC.

### Table 1: Parameter

<table>
<thead>
<tr>
<th>S. no</th>
<th>HPLC Parameter</th>
<th>Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Injection Volume</td>
<td>20 ul</td>
</tr>
<tr>
<td>2</td>
<td>Column</td>
<td>RP 18</td>
</tr>
<tr>
<td>3</td>
<td>Solvent System</td>
<td>Methanol+Water+Tetrahydroxy Furfural</td>
</tr>
<tr>
<td>4</td>
<td>Ratio Solvent System</td>
<td>94: 5: 1</td>
</tr>
<tr>
<td>5</td>
<td>pH of Solvent system</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Flow rate of mobile phase</td>
<td>1ml/min</td>
</tr>
<tr>
<td>7</td>
<td>Retention Time</td>
<td>12min</td>
</tr>
<tr>
<td>8</td>
<td>Wavelength</td>
<td>220nm</td>
</tr>
<tr>
<td>9</td>
<td>Temperature</td>
<td>22 °C</td>
</tr>
</tbody>
</table>

### 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 dissolved 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 °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, further 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 °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 KMnO₄ is equivalent to 0.1898 mg of calcium record titrimetry all value recorded by mean [10] .

### Phytochemical Identification

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

### 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

\[ R = \frac{D_{test}}{D_{control}} \]

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 compared with *hibiscus rosa sinesis, bryophyllum pinnatum* which are follows

*Phytophllum Pinnatum*

Solvent: 5.2cm

### Table 4: Spots Calculate in cm

<table>
<thead>
<tr>
<th>Spots</th>
<th><em>H. R. Sinesis</em> (cm)</th>
<th><em>R. Pinnatum</em> (cm)</th>
<th>Ursolic acid (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Rf Values

<table>
<thead>
<tr>
<th>Spots</th>
<th>H. R. Sinesis</th>
<th>B. Pinnatum</th>
<th>Ursolic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.076</td>
<td>0.307</td>
<td>0.423</td>
</tr>
<tr>
<td>2</td>
<td>0.173</td>
<td>0.423</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.307</td>
<td>0.538</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.346</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.384</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.557</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4 shows various spot of *bryophyllum pinnatum* and *hibiscus rosa sinensis* 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 sinensis*. 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 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
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
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 calibration graph subsequently used to calculate mean of sample

<table>
<thead>
<tr>
<th>S. No</th>
<th>STD</th>
<th>Area mAU min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10.6355</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>9.0362</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>121.7506</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>117.1202</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>276.9435</td>
</tr>
</tbody>
</table>

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.

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

Egg placed in sulfuric acid
Semipermeable membrane of egg was removed by needle and scapel washed by distilled water and carefully load kidnet stone crystals and sample also loaded, degradation of kidney stone cheaked by iteration method

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Titeration method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sulfuric Acid</th>
<th>Titeration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinnatum</td>
<td>20 ml</td>
<td>1.8</td>
</tr>
<tr>
<td>Hibiscus</td>
<td>20 ml</td>
<td>1.0</td>
</tr>
<tr>
<td>Blank</td>
<td>20 ml</td>
<td>3.0</td>
</tr>
</tbody>
</table>