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# PHARMACOGNOSTIC STUDIES OF LEAVES OF *Symplocos racemosa* Roxb. AND *Rumex vesicarius* Linn.

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## ABSTRACT

**Objective**: To study pharmacognostic studies of *Symplocos racemosa* Roxb. and *Rumex vesicarius* Linn.

**Method**: The leaves of *Symplocos racemosa* Roxb. and *Rumex vesicarius* Linn. were studied by Microscopic, Physicochemical and Phytochemical Studies.

**Result**: The T.S of the leaf of *Symplocos racemosa* Roxb showed upper epidermis, lower epidermis, lignified xylem & phloem, collenchyma and stomata. The microscopical powder studies of *Symplocos racemosa* Roxb indicate the presence of a lignified vascular bundle (Xylem & Phloem), Trichomes, Cuticle cell, Ca- oxalate crystals and Stomata. The T.S of the leaf of *Rumex vesicarius* Linn showed upper epidermis, lower epidermis, lignified xylem & phloem, collenchyma and anisocytic stomata. The microscopical powder studies of *Rumex vesicarius* Linn indicate the presence of a lignified vascular bundle (Xylem & Phloem), Trichomes, Starch Grain and Stomata.

Physicochemical parameters such as alcohol soluble and hexane soluble extractive value, total ash content, acid insoluble ash value, water soluble ash value and Loss on drying were determined.

The preliminary phytochemical screening result showed the presence of carbohydrate, cardiac glycoside, flavonoids, alkaloids and Tannis in the ethanolic extract of *Symplocos racemosa* Roxb (ESR) And N-hexane extract of *Symplocos racemosa* Roxb (NSR) showed the presence of cardiac glycoside, alkaloids, Tannis. The preliminary phytochemical screening result of ethyl acetate extract of *Rumex vesicarius* Linn (EARV) showed the

presence of cardiac glycoside, flavonoids, alkaloids, tannins, steroids and vitamin C. And an ethanolic extract of *Rumex vesicarius* Linn (ERV) showed the presence of carbohydrate, cardiac glycoside, alkaloids, tannins and vitamin C.

**Conclusion**: The results of the studies can provide standards for the identification of both the plants.

**Keyword: Pharmacognostic study, *Symplocos racemosa* Roxb, *Rumex vesicarius* Linn. and Phytochemical analysis**

## INTRODUCTION

*Symplocos racemosa* Roxb. (Lodhra) belongs to the family Symplocaceae, is a small evergreen tree upto 6 m tall. In traditional system it is mainly used as cardiotonic, antipyretic, antihelmintic and laxative properties. It is beneficial in billow fever, urinary discharge; pharmacologically it is used as antimicrobial, antidiarrhoeal, spasmogenic and heart depressant. The plant mainly contains monomethyl pelargonidin gluocosides, loturidine also contain oxalic acid, phytosterol, ellagic acids and oleanolic acid **[1-3]**.

*Rumex vesicarius* Linn. (Chooka) belongs to perennial herbs to the family Polygonaceae. The plant is an erect usually with a long taproot. Traditionally the plant is used as stomachic, Diuretic, used for the disorders of the lymphatic and glandular system, for bronchitis, asthma, constipation, dyspepsia and the diseases of the liver. Plant leaves are rich in ascorbic acid, citric acid and tartaric acid, it also contains glycoside, alkaloid, flavonoids, tannins and phenolic compounds **[4, 5]**.

The object of present study to evaluate pharmacognostic parameter of *Symplocos racemosa* Roxb. and *Rumex vesicarius* Linn.

## MATERIAL AND METHODS

**Collection and preparation of plant material:** The fresh leaves of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn are used in the study, the plants are collected at the flowering stage (In the month: August -November) from the local area of Satara and Sangli, Maharashtra state, India respectively and authenticated by Botanical Survey of India, Pune, Maharashtra. (BSI/WRC/Iden./2015 dated 4-12-2015)

**Preparation of Extracts**

The leaves of plants were separated from stems and dried under shade at room temperature until it becomes completely dry. After drying leaves were exposed to size reduction. The shade-dried coarsely powdered leaves (500 g) were undergone to Soxhlet extraction. A) *Symplocos racemosa* Roxb. Leaves: (500 g) were subjected to Soxhlet extraction with 95 percent ethanol

and N-hexane to obtain ethanolic and N-hexane extract respectively. B) *Rumex vesicarius* Linn. leaves: (500 g) were subjected to Soxhlet extraction with 95 percent ethanol and ethyl acetate to obtain ethanolic and ethyl acetate extract respectively. The extracts obtained were subjected to the Rotary flash evaporator to remove excess of solvent, and dried extracts were stored in a cool place in airtight pack container for further use.

**Pharmacognostic Studies:** Microscopic character were studied by a preparing a thin section of lamina region and midrib region of the leaf (*Symplocos racemosa* Roxb and *Rumex vesicarius* Linn). This section was stained by phloroglucinol and Concentrated HCL. Leaf powders were observed under a microscope with a different reagent **[6]**.

**Table 1: List of reagents for Microscopic studies**

| Sr. No | Reagent | Observation | Characteristics |
|---|---|---|---|
| 1 | Acetic acid | Insoluble | Calcium oxalate crystals |
| 2 | Iodine Solution | Blue or Black | Starch |
| 3 | Phloroglucinol +Con Hcl | Red | Xylems and Vessels |
| 4 | Sudan Red-III | Red | Cuticle cell |

**Physicochemical studies:**

**1. Determination of alcohol soluble extractive value:** Five gram dried leaf powder of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were macerated separately with 100 ml of alcohol for 1 day, continues shaking required during the first six hours and allowed to stand for eighteen hours. After that filtration, twenty-five ml of the filtrate was evaporated to dryness in a pre-tared dish, dry at 105$^{o}$ and then take weight. The % of ethanol-soluble extractive calculated by the following formula.

$$\textbf{Percentage of ethanol soluble extractive} = \frac{\textbf{Weight of residue}}{\textbf{Weight of the drug}} \times 100$$

**2. Determination of hexane soluble extractive value:** Five gram dried coarsely leaf powder were macerated with 100 ml of hexane for 1 day, continues shaking required during the first six hours and allowed to stand for eighteen hours. After that filtration, twenty-five ml of the filtrate was evaporated to dryness in a pre-tared dish, dry at 105$^{o}$ and weigh. The % of ethanol-soluble extractive calculated by the following formula.

$$\textbf{Percentage of ethanol soluble extractive} = \frac{\textbf{Weight of residue}}{\textbf{Weight of the drug}} \times 100$$

**3. Total Ash content:** 2g of the air-dried leaf powder of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn taken in separate a tared silica dish and incinerate at a high temperature about 450° until it is free from carbon, cool and take a weight. After this process check for carbon ash if ash is not free carbon, then wash with hot water. Then the residue was collected on a filter paper which is free from ash, burn the

residue and filter paper until to get ash white or nearly white. The filtrate was evaporated to dryness and ignite at a high temperature not more than 450°. Total ash value calculated by the following formula.

**Percentage of ash with reference to air dried drug**

$$= \frac{\textbf{Weight of ash (g)}}{\textbf{Weight of the drug (g)}} \times 100$$

**4. Acid-insoluble Ash value:** The ash dissolved in 25 ml of 2 normal Hydrochloric acid and boil for 5 minutes, the residue was collected on filter paper and wash with hot water, burn and cool in a desiccator and then note down weight. The percentage of acid-insoluble ash calculated by the following formula.

**Percentage of acid insoluble ash with reference to air dried drug**

$$= \frac{\textbf{Weight of acid insoluble ash}}{\textbf{Weight of the drug}} \times 100$$

**5. Water-soluble Ash value:** The ash was boil with 25 ml of water for 5 minutes. The residue was collected on an ashless filter paper and wash with hot water, and ignite for fifteen minutes at a temperature of about 450°. The water-soluble ash weight was calculated by subtracting the insoluble matters weight with total ash weight. Calculate the percentage of water-soluble ash by the following formula.

**Percentage of acid soluble ash with reference to air dried drug**

$$= \frac{\textbf{Weight of acid soluble ash}}{\textbf{Weight of the drug}} \times 100$$

**6. Loss on drying:** Dry clean glass-stoppered, bottle for thirty min. Cool to room temp (about 30 mints) in desiccators. Then weight the bottle (W1). Take 1 g of dried leaf powder and transfer to the bottle. And take the weight of the bottle (W2). Distribute the sample as evenly so as to get layer thickness not more than 10 mm. Dry the sample by keeping the bottle in the drying chamber and remove the stopper of the bottle. Dry the sample to get a constant weight of the bottle. After drying, the sample was cool to room temperature in a desiccator before weighing. Then take the weight of the bottle and its contents. (W3) **[7]**.

$$\textbf{Loss on drying} = \frac{W2 - W3}{W2 - W1} \times 100$$

## PHYTOCHEMICAL INVESTIGATION

The following tests were adopted to identify the presence of various chemical constituents in the extracts **[6]**.

**Table 2: Qualitative Chemical Analysis of *Symplocos racemosa* Roxb. and *Rumex vesicarius* Linn.**

| Sr. No | Test | Procedure |
|---|---|---|
| 1 | Test for Carbohydrate<br>✓ Molish's test<br>✓ Benedict's test | Molisch's test: To test solutions add few drops of Molisch's reagent and 2 ml of conc. sulphuric acid added slowly from the sides of the test tube. The purple ring at the junction of two liquids is an observer.<br>Benedict's test: To test solution add Benedict's reagent and boil on a water bath. A test tube shows a reddish-brown precipitate. |
| 2 | Test for protein and Amino acid<br>✓ Biuret test ( General test)<br>✓ Millon's test<br>✓ Ninhydrin test | Biuret test: To test solutions add 40% NaOH and dilute CuSo4 solution. A test tube shows a blue colour.<br>Millon's test: To test solution add Millon's reagent and heated on a water bath, protein show stained yellow on warming.<br>Ninhydrin test: To test solution add ninhydrin reagent gives a blue colour |

| | | |
|---|---|---|
| **3** | **Test for Glycoside**<br>**1. For cardiac Glycoside**<br>✓ **Baljets test**<br>✓ **Legal test**<br>✓ **Keller-killiani test**<br>**2. For Anthraquinone Glycoside**<br>✓ **Borntargers test**<br>✓ **Modified Borntargers**<br>**3. For Saponin Glycoside**<br>✓ **Foam test** | **Baljet's test: To test solutions add sodium picrate. It gives yellow to orange colour.**<br>**Legal test: To aq. or alc. extract add 1 ml of pyridine and 1 ml of sodium nitroprusside gives pink to red colour.**<br>**Keller-killiani test: To 2 ml of extract add glacial acetic acid, 1 drop of 5% Ferric chloride and Conc. sulphuric acid. It gives reddish brown colour at the junction of two layers.**<br>**Borntrager's test: To 3 ml test solution, add dil. $H_2SO_4$. Boil the solution and filter, Cool. To the filtrate, add benzene or chloroform (1:1). Shake well. Collect the organic layer. Then add ammonia. Ammonical layer turns pink or red.**<br>**Modified Borntrager's test for C-glycosides: To 5 ml test solution, add 5 ml 5% ferric chloride and 5 ml dilute. HCl. Heat the solution for 5 min on the water bath. Cool and add any organic solvent like benzene. Shake well. Collect organic layer, add equal volume dilute ammonia. Ammonical layer turns a pinkish red colour.**<br>**Foam test for Saponins Sample shaken with water shows the formation of foam which is stable for 15 mints.** |
| **4** | **Test for Flavonoids**<br>✓ **Sulphuric acid test**<br>✓ **Shinoda test**<br>✓ **Lead acetate solution test** | **Sulphuric acid test: To test solutions add a few drops of the H2SO4. It gives an intense yellow colour.**<br>**Shinoda test: To test solutions add few magnesium ribbons and conc. hydrochloric acid. It shows pink to magenta red colour.**<br>**Lead acetate solution test: To test solution, add a few drops of lead acetate solution (10% w/v). It gives a yellow precipitate.** |
| **5** | **Test for Alkaloids**<br>✓ **Dragendroff's test**<br>✓ **Mayer's test**<br>✓ **Hager's test**<br>✓ **Wagnar's test** | **Dragendroff's test: To acidic test solution, add Dragendroff's reagent which shows a reddish-brown precipitate.**<br>**Mayer's test: To test solution add Mayer's reagent. It gives a cream coloured precipitate.**<br>**Hager's reagent: To acidic test solution, add Hager's reagent. It gives a yellow precipitate.**<br>**Wagner's test: To acidic test solution add Wagner's reagent. It gives a brown precipitate.** |
| **6** | **Test for Tannin and Phenolic compound**<br>✓ **5% $Fecl_3$**<br>✓ **Lead Acetate solution**<br>✓ **Bromine Water**<br>✓ **Potassium dichromate** | **2-3 ml of aqueous or alcoholic extract and add few drops of the following reagent**<br>✓ **5% $Fecl_3$ ( Deep blue-black colour)**<br>✓ **Lead Acetate solution (White ppt)**<br>✓ **Bromine Water (Decolourization)**<br>✓ **Potassium dichromate (Red ppt)** |
| **7** | **Test for Steroids**<br>✓ **Salkowaski test**<br>✓ **Libermann Burchard test** | **Salkowaski test: Add a few drops of concentrated sulphuric acid to test solution. Shaken well then allow to stand. Lower layer turns red indicating the presence of steroids.**<br>**Liebermann Burchard test: To test solution add few drops of acetic anhydride. When conc. H2SO4 is added from the sides of the test tube, it gives a brown ring between the two layers and the upper layer turns to green color.** |
| **8** | **Test for Vitamin C** | **To test solutions added 5 ml of water and 1 drop of 5 percent solution of sodium nitroprusside and 2 ml of Dilute sodium hydroxide. Add 0.6 ml of HCl dropwise and stir, the yellow color turns to blue color.** |

## RESULTS

### Pharmacognostic Studies (Microscopic Studies of Plants)

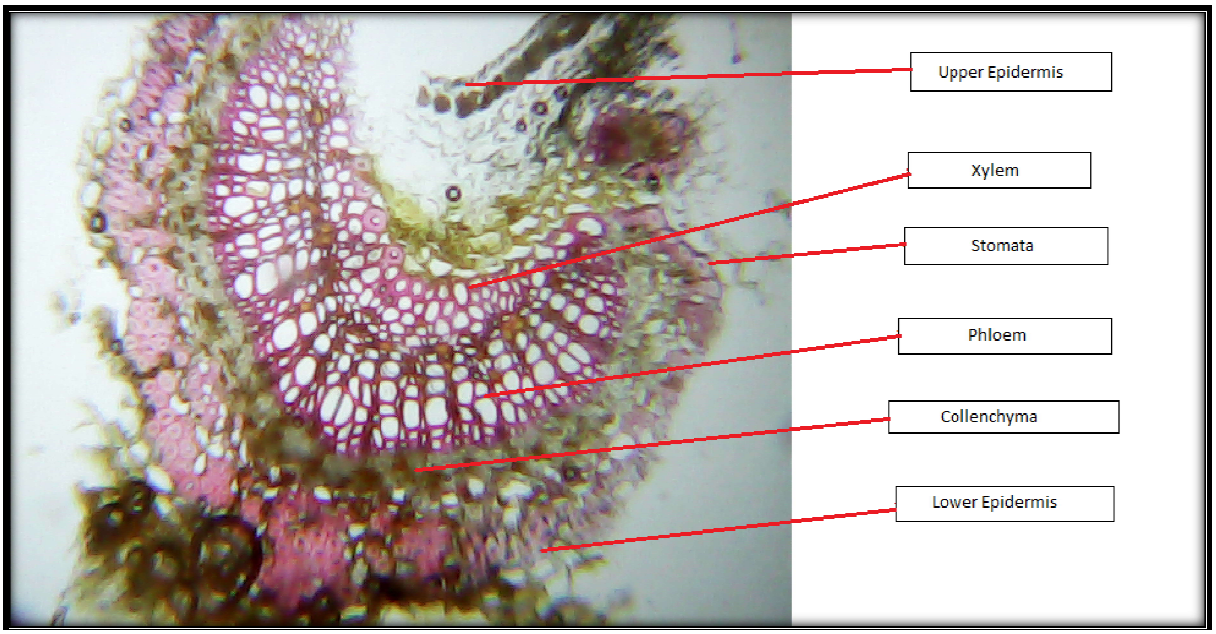


**Figure 1: T.S of *Symlocos Racemosa* Roxb leaf**

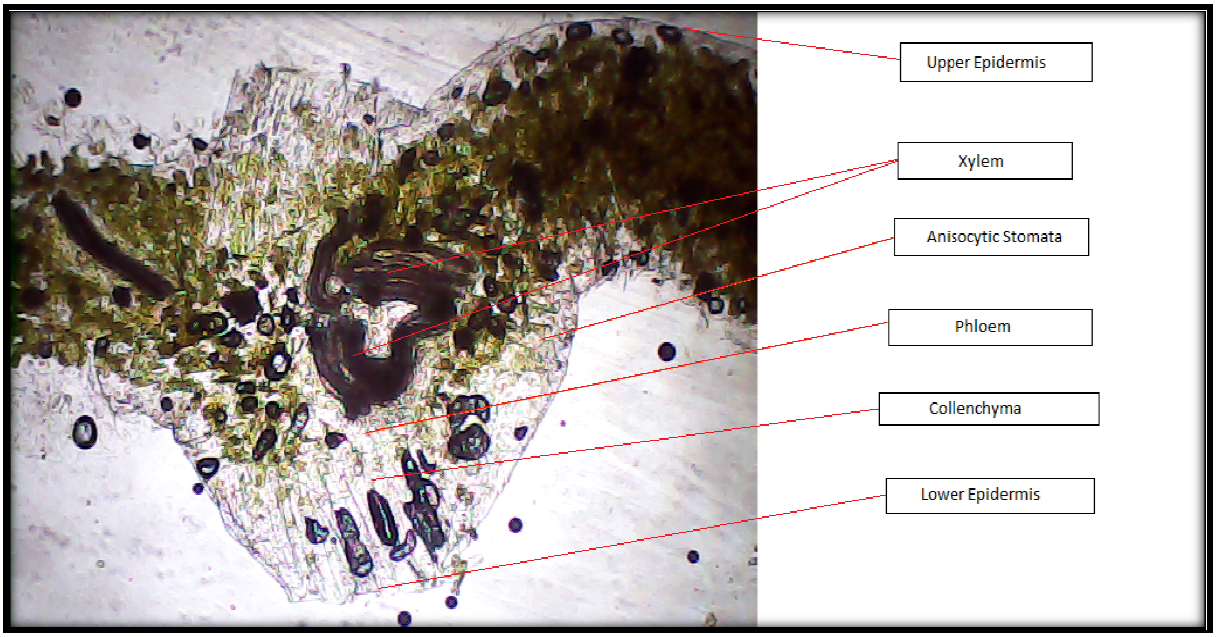


**Figure 2: T.S of *Rumex vesicarius* Linn leaf**

❖ **Powder characteristics of *Symlocos Racemosa* Roxb**

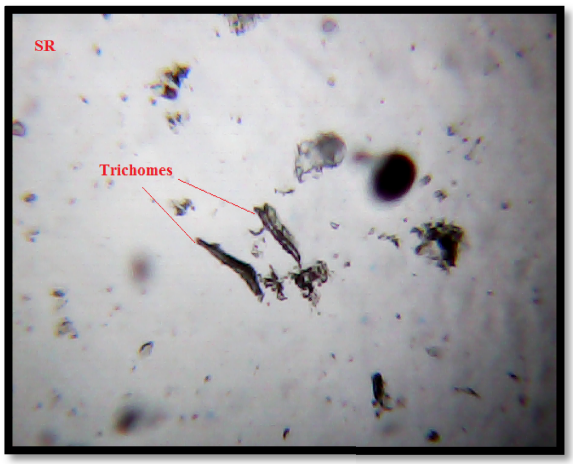


**Figure 3: Trichomes**

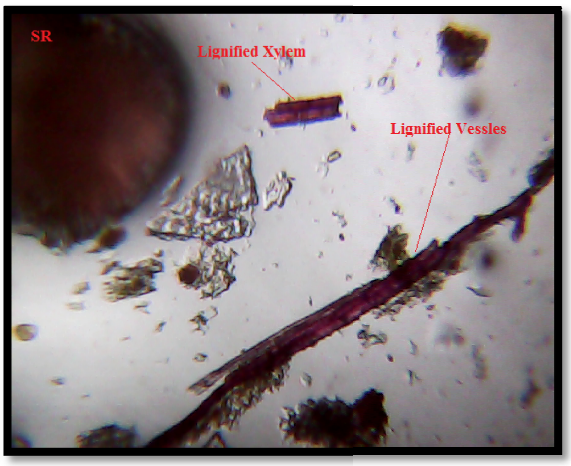


**Figure 4: Xylem and Vessels**

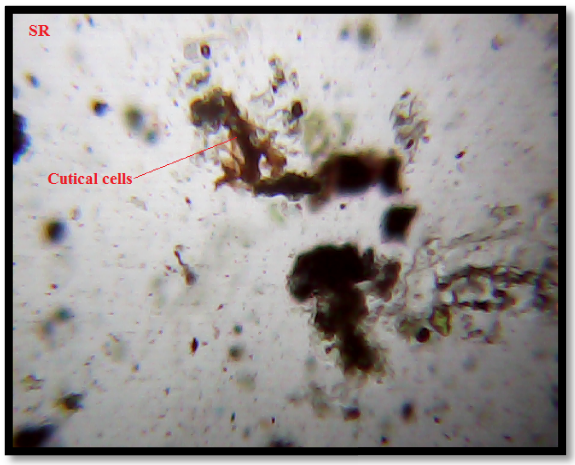


**Figure 5: Cuticle Cells**

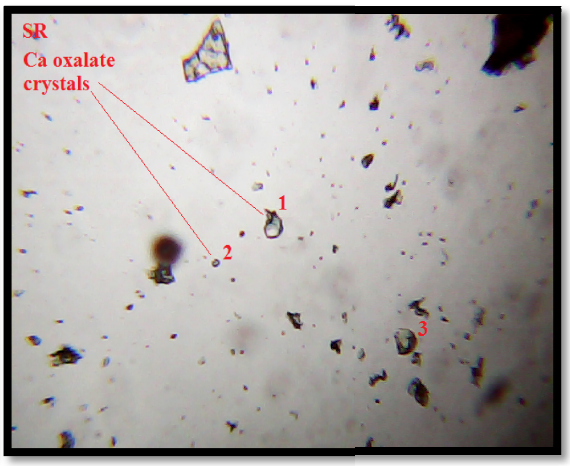


**Figure 6: Ca-oxalate crystals**

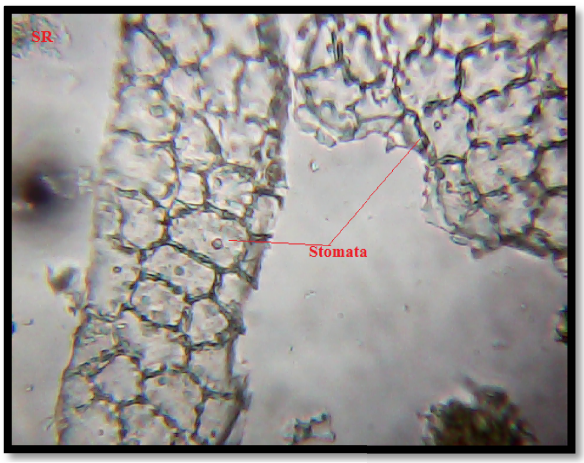


**Figure 7: Stomata**


**Figure 8: Vessels**

❖ **Powder characteristics of *Rumex vesicarius* Linn**

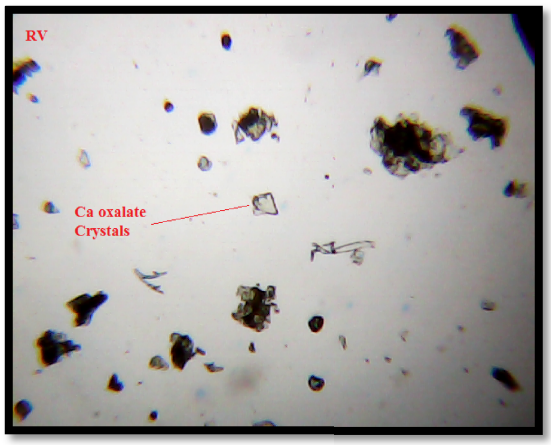


**Figure 9: Ca- oxalate crystals**

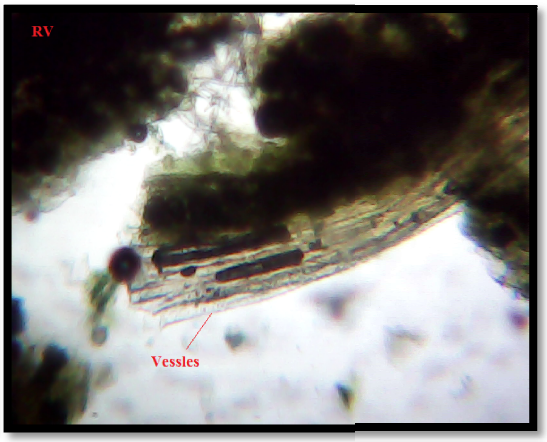


**Figure 10: Vessles**

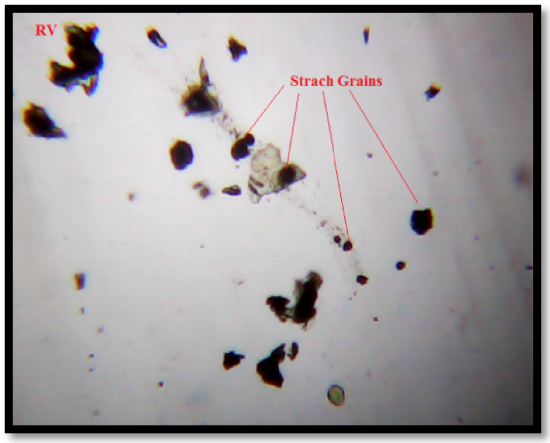


**Figure 11: Starch Grains**

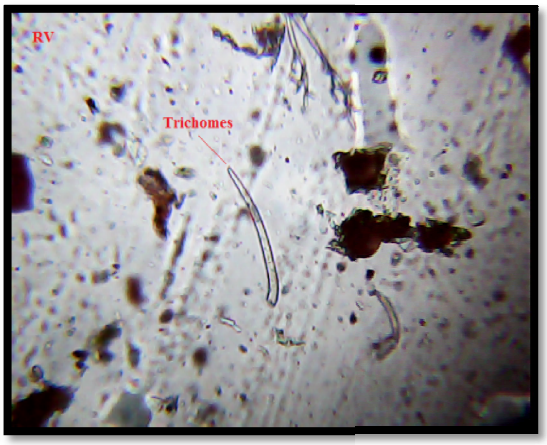


**Figure 12: Trichomes**

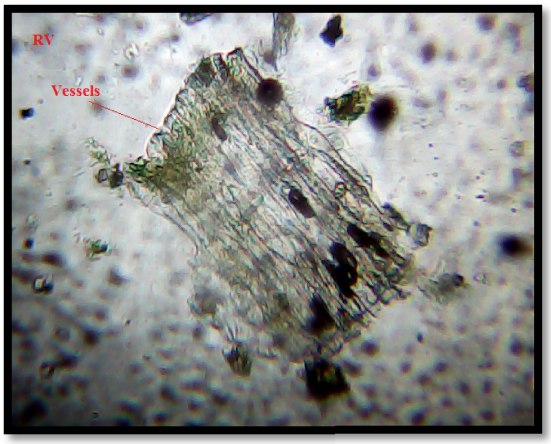


**Figure 13: Vessels**

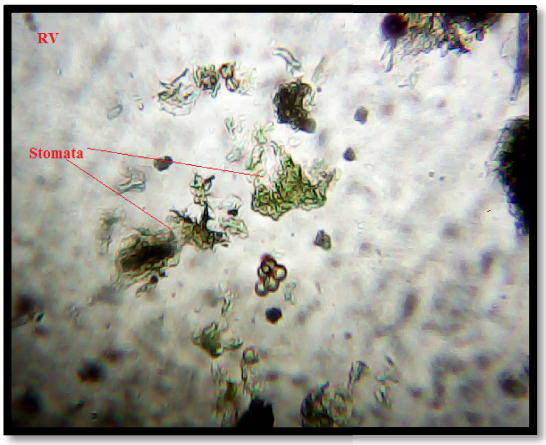


**Figure 14: Stomata**

❖ **Physicochemical studies:**

**Table 3: Physicochemical parameter**

| Sr. No | Physicochemical parameter | Value for *Symplocos racemosa* Roxb | Value for *Rumex vesicarius* Linn |
|---|---|---|---|
| 1 | Alcohol soluble extractive value | 12.37 % w/w | 10.15% w/w |
| 2 | Hexane soluble extractive value | 1.89 % w/w | 2.35 % w/w |
| 3 | Total Ash content | 6.06 % w/w | 7.12 % w/w |
| 4 | Acid insoluble Ash value | 2.78 % w/w | 2.05% w/w |
| 5 | Water soluble Ash value | 3.67 % w/w | 4.69 % w/w |
| 6 | Loss on drying | 0.96 % w/w | 0.79 % w/w |

## Phytochemical Investigation:

**Table 4: Phytochemical Investigation of plants**

| Sr. No | Test | Result | | | |
|---|---|---|---|---|---|
| | | EARV | ERV | ESR | NSR |
| 1 | Test for Carbohydrate | | | | |
| | ✓ Molish's test ( General test) | - | + | + | - |
| | ✓ Benedict's test | - | - | + | - |
| 2 | Test for protein and Amino acid | | | | |
| | ✓ Biuret test ( General test) | - | - | - | - |
| | ✓ Millon's test | - | - | - | - |
| | ✓ Ninhydrin test | - | - | - | - |
| 3 | Test for Glycoside | | | | |
| | 1. For cardiac Glycoside | | | | |
| | ✓ Baljets test | - | - | + | + |
| | ✓ Legal test | + | + | +++ | + |
| | ✓ Keller-Killiani test | ++ | +++ | +++ | ++ |
| | 2. For Anthraquinone Glycoside | - | - | - | - |
| | ✓ Borntargers test | - | - | - | - |
| | ✓ Modified Borntargers | | | | |
| | 3. For Saponin Glycoside | | | | |
| | ✓ Foam test | - | - | - | - |
| 4 | Test for Flavonoid | | | | |
| | ✓ Sulphuric acid test | + | - | +++ | - |
| | ✓ Shinoda test | ++ | - | - | - |
| | ✓ Lead acetate solution test | ++ | + | + | + |
| 5 | Test for Alkaloid | | | | |
| | ✓ Dragendroff's test | + | + | + | + |
| | ✓ Mayer's test | + | + | + | + |
| | ✓ Hager's test | + | ++ | + | + |
| | ✓ Wagnar's test | - | - | + | + |
| 6 | Test for Tannin and Phenolic compound | | | | |
| | ✓ 5% $Fecl_3$ | +++ | - | +++ | - |
| | ✓ Lead Acetate solution | + | - | ++ | +++ |
| | ✓ Bromine Water | ++ | ++ | ++ | - |
| | ✓ Potassium dichromate | + | - | ++ | - |
| 7 | Test for Steroids | | | | |
| | ✓ Salkowaski test | +++ | - | - | + |
| | ✓ Libermann Burchard test | ++ | - | - | + |
| 8 | Test for Vitamin C | ++ | + | - | - |

## DISCUSSION

### Pharmacognostic Studies:

The medicinal plants are used for a variety of disease in a developing country. But these herbal medicines are not used in the developed country due to lack of documentation and poor quality control of herbal medicine. So there is need of

standardization of medicinal plants which is used as medicine. The standardization can be achieved by a pharmacognostic study. Pharmacognostic studies ensure the identity of plants and also adulteration of plant material **[8]**.

The microscopical character of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were studied. The transverse section of the leaf of *Symplocos racemosa* Roxb showed upper epidermis, lower epidermis, lignified xylem & phloem, collenchyma and stomata. The microscopical powder studies of *Symplocos racemosa* Roxb indicate the presence of lignified vascular bundle (Xylem & Phloem), Trichomes, Cuticle cell, Ca- oxalate crystals and Stomata. The T.S of the leaf of *Rumex vesicarius* Linn showed upper epidermis, lower epidermis, lignified xylem & phloem, collenchyma and anisocytic stomata. The microscopical powder studies of *Rumex vesicarius* Linn indicate the presence of lignified vascular bundle (Xylem & Phloem), Trichomes, Starch Grain and Stomata.

The physicochemical character of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were studied and shown in **Table 1**. Ash value of crude drug gives an idea of purity and quality of the crude drug. The acid insoluble ash is due to silica and earthy matter. The water-soluble ash is determined the inorganic compound. Alcohol-soluble extractive value of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were found 12.37 % w/w and 10.15% w/w respectively. Hexane-soluble extractive value of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were found 1.89 % w/w and 2.35 % w/w respectively. Total Ash content of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were found 6.06 % w/w and 7.12 % w/w respectively. Acid-insoluble Ash value of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were found 2.78 % w/w and 2.05% w/w respectively. Water-soluble Ash value of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were found 3.67 % w/w and 4.69 % w/w respectively. Loss on drying *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn were found 0.96 % w/w and 0.79 % w/w respectively.

**Phytochemical Investigation:**

The preliminary phytochemical screening is used to identify phytoconstituents present in the crude extract of plants. Phytochemical analysis of *Symplocos racemosa* Roxb and *Rumex vesicarius* Linn revealed the presence of various metabolites. Medicinal properties of the herb are related to their secondary metabolite such as alkaloids, flavonoids etc. Natural compounds are derived from the living organism. They are produced by the process of metabolism. Metabolism produced primary metabolites and

secondary metabolites. A primary metabolite is responsible for normal Growth, development and reproduction process. Protein, carbohydrate, fat and oil are primary metabolites. Secondary metabolites are not directly involved in growth, development and reproduction process, but they are essential for some an ecological function. The secondary metabolites of plants are present in the stem, leaves, bark or the root of the plant. The secondary metabolites are classified into Phenolic compounds, Nitrogen containing compounds and Terpenes.

Terpenes: They are also called as terpenoids. They are insoluble in water and derived from the union of five carbon atom that has the branched carbon skeleton of isopentane. The structural unit is isoprene. Terpenes are having a function in defense mechanism. At high temperature terpenes are converted into isoprene. Terpenes are toxic to plants. Saponins are the derivatives of terepenes which is steroidal in nature. Carotenoids are also derivative of terepenes.

Phenolic compounds: Plant produces a large number of phenolic or hydroxyl functional group to hydroxyl ring. Plant phenolic compounds are chemically heterogeneous in nature and some are water soluble, some are organic solvent soluble. Some phenolic compounds are activated by light. The anthocyanin, tannins, phenylpropanoid, benzoic acid derivatives, isoflavones, lignin, and flavonoid are the derivative of the phenolic compound. The flavonoids are the largest classes of plant phenols, the basic structure of flavonoids contains 15 carbon arranged in two aromatic rings which are connected by a bridge of three carbon. The flavonoids show different pharmacological activities like antioxidant, anticancer, anti-allergic, anti-inflammatory and anti-microbial.

Nitrogen containing compounds: A maximum secondary metabolites consist of nitrogen in their structure. Examples include alkaloids, cyanogenic glucoside and glucosinate. More than 15000 nitrogen containing metabolites are present in alkaloids. The nitrogen atom is part of the heterocyclic ring. They alkaloids are having a different pharmacological effect on the vertebrate animal. Alkaloids are alkaline in nature, at pH is 7.2. The first medically use alkaloid was morphine **[9]**.

The preliminary phytochemical screening result showed the presence of carbohydrate, cardiac glycoside, flavonoids, alkaloids and Tannis in ethanolic extract of *Symplocos racemosa* Roxb (ESR) And N-hexane extract of *Symplocos racemosa* Roxb (NSR) showed the presence of cardiac glycoside, alkaloids, Tannis.

The preliminary phytochemical screening result of ethyl acetate extract of *Rumex vesicarius* Linn (EARV) showed the

presence of cardiac glycoside, flavonoids, alkaloids, tannis, steroids and vitamin C. And an ethanolic extract of *Rumex vesicarius* Linn (ERV) showed the presence of carbohydrate, cardiac glycoside, alkaloids, tannis and vitamin C.

## CONCLUSION

The pharmacognostic studies of leaves of *Symplocos racemosa* Roxb. and *Rumex vesicarius* Linn. were carried out by different parameter like Microscopic, Physicochemical and Phytochemical. These parameter helpful for identification of both plants.

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