CHAPTER. V
Pharmacognostical Studies
5. PHARMACOGNOSTICAL STUDIES

5.1. Introduction

In many indigenous plant communities, medicinal plants have a long-standing history and continue to provide useful tools for treating various diseases. The development and spread of modern medicine is based on hundreds of year’s practices, belief and observation (Jeya prakash, 2011). The wide spread interest in herbal drugs today stems from the belief that herbal medicines are safe, inexpensive and have no adverse effects (Kaur, 2011). Greater numbers of people seeking remedies and health approach from medicinal plants, these plants are moving from fringe to main stream use (Saha, 2010). For the treatment of various ailments, World’s one fourth population people are dependent on traditional medicines (Jena, 2011).

The promising choice over modern synthetic drugs is “Herbal medicines”, because of their minimum or no side effects and considered to be safe. Herbal medicines formulation generally involves the use of fresh or dries plant parts. However in developed countries, the key obstacle in acceptance of the alternate medicines is the lack of documentation and stringent quality control. With this back drop, making an effort towards standardization of the plant material is to be extremely important. Thus the research work carried out on traditional medicines need to be documented. The very important aspect in preparation, safety and efficacy of herbal products is the correct knowledge of such drug. The most simple and reliable tool to obtain complete information of crude drug is Pharmacognosy (Gokhale, 1979; Mukherjee, 2002;
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Raghunathan, 1982; Trease, 2002). The standardization process can be achieved by stepwise pharmacognostic and phytochemical studies which helps in standardization and identification of the plant material. To ensure reproducible quality of herbal medicine this will contribute to its safety and efficacy. Correct identification and quality assurance of the starting material is essential (Kadam, 2011).

Evaluation of drug is necessary because it means confirmation of identity of drugs. It helps in determination of quality and purity and also determines the nature of adulteration. Thus the three main reasons for evaluation of crude drug are:

1. Biochemical variation in drug.
2. Detoration due to treatment and storage.
3. Substitution and adulteration resulted from carelessness, ignorance or fraud (Mritunjay et al., 2013).

The nature and degree of evaluation of crude drugs has undergone systematic changes over the years. Initially the identification of the crude drugs was done by comparison of drug with the available standard description. But at present due to advancement in the chemical knowledge of crude drugs, evaluation also includes estimation method of active constituents of crude drugs along with morphological and microscopic analysis. And also advent of separation techniques and instrumental analysis made possible to perform physical evaluation of crude drug which could be both qualitative and quantitative in nature (Kokate, 2007; Kokate 2004).
The proper control of starting material is utmost essential to ensure reproducible quality of herbal products. Thus the standardization of medicinal plants of therapeutic potential has been emphasized in recent years. Identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means, despite the modern techniques. According to WHO, the first step towards establishing the identity and purity of medicinal plants, the macroscopic and microscopic description should be carried out before any tests are undertaken (Anonymous, 2002). The qualitative evaluation based on study of morphological and sensory profiles of whole drugs is achieved by organoleptic evaluation (Kokate, 2007). The important characteristics of the plants like the structure of the leaves, the hairy surface of leaves, the typical tongue sensation and the odor which helps to screen the preliminary phytochemical constituents can be obtained by organoleptic studies. And on the bases of the air dried crude drugs the percentage of active chemical constituents can be determined. The test for loss on drying of plant material determines the presence of both water and volatile matter for the materials that absorb moisture easily or deteriorate quickly in presence of water (Kokate, 2007; Anonymous, 2002).

The ash content or ash value is the residue remaining after incineration of plant material, which represents naturally occurring inorganic salts or adhering to it or deliberately added to it as a form of adulteration. The total ash, acid insoluble ash and water soluble ash measure are the three different methods used to determine ash value. The measurement of total amount of material remaining after ignition gives total ash,
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which include both physiological ash and non physiological ash. The physiological ash is derived from the plant tissue itself and non physiological ash is the residue of the extraneous matter adhering to the plant surface. The measurement of amount of silica present in plant material, especially as sand and siliceous earth is the acid-insoluble ash, which is a part of total ash. And water soluble portions of total ash are water soluble ash (Kokate, 2007). The approximate measurement of the chemical constituents in a specific solvent for a specific amount of air dried plant material can be obtained by exhausting the plant material with specific solvents. This parameter is suitable for the plant material which has no suitable chemical or biological assay exists (Anonymous, 1996; Anonymous, 2002).

The preliminary phytochemical screening is useful in finding out the genuinity of drugs. For detecting adulteration in ash values, extractive values are the most reliable aid. These simple but most reliable standards are useful in using drug as a home remedy by a lay person and also can be utilized for identification and selection of raw material for drug production by manufacturer.

**Ash content**

The measurement of total amount of minerals present within a food is the “ash content” and the measurement of the amount of specific inorganic components present within a food such as Ca, Na, N and Cl is the mineral content. Ash and mineral content determination in food is important for a number of reasons:
Nutritional labeling: The type of mineral and concentration present must be stipulated on the label of food package.

Quality: The concentration and type of minerals present, including taste, appearance, texture and stability which determines the quality of foods.

Microbiological stability: The growth of certain microorganisms can be sometime retarded by high content of minerals.

Nutrition: For healthy diet some minerals (e.g., calcium, phosphorous, potassium and sodium) are essential whereas others (e.g., lead, mercury, cadmium and aluminum) can be toxic.

Processing: During food processing it is important to know its mineral content as these effects the physicochemical properties of food.

**Moisture content**

Moisture content is one of the most commonly measured properties by food scientists because of following reasons

- Legal and Labeling Requirements For certain types of food, there are legal limits to the maximum or minimum amount of water that must present in foods.

- Economic. Water is an inexpensive ingredient thus the cost of many food depends on the amount of water they contain. The manufacturers without exceeding some maximum legal requirement often try to incorporate water as much as possible in a food to increase the price.
• Microbial Stability. As many foods are dried below some critical moisture content, the propensity of microorganisms to grow in foods depends on their water content.

• Food Quality. The water content also determines texture, taste, appearance and stability of foods.

• Food Processing Operations. To predict the behavior of foods during processing, e.g. mixing, drying, flow through a pipe or packaging, knowledge of the moisture content is often necessary.

It is therefore important to measure moisture contents. And for this number of analytical techniques have been developed, which vary in their accuracy, cost, speed, sensitivity, specificity, ease of operation, etc. The choice of an analytical procedure for a particular application for food by scientists mainly depends on the nature of the food being analyzed.
5.2. Materials and Methods

5.2.1. Extraction of powdered plant material

The different parts of *Rumex vesicarius* L. (leaf, stem, root and whole plant) powder was subjected to extraction by two methods (Sukhdev et al., 2008).

1. Hot Soxhlet extraction method.
2. Cold Maceration (at room temperature) method.

The procedures for both Hot Soxhlet extraction and Cold Maceration method followed are same as mentioned in Chapter 3 (3.2.3).

5.2.2. Organoleptic studies

The extract obtained with each solvent from both extraction methods was used for the determination of organoleptic characters such as color, nature, taste and the yield. The percentage yields of each extract were calculated by using following formula (Raghunathan, 1976).

\[ \text{Yield} \% = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100 \]

5.2.3. Physical evaluation

The ash values, extractive value and loss on drying were performed according to the officinal methods prescribed in Indian Pharmacopeia, (1996) and the WHO, (1949) guidelines on quality control methods for medicinal plants materials. Fluorescence analyses were carried out according to the method of Kokoshi et al, (1958).
5.2.3.1. Ash values

Weigh and ignite flat, thin, porcelain dish or a tarred silica crucible. Weigh about 2 gm of the powdered drug into the crucible. Support the dish on a pipe clay triangle placed on a ring of retort stand. Heat with a burner, using a flame about 2 cm high and supporting the crucible. About 7 cm above the flame heat till vapors almost cease to be evolved, then lower the dish and heat more strongly until all the carbon is burnt off. Cool in desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug.

Calculation

Weight of the empty dish = X

Weight of the drug taken = Y

Weight of dish + ash (after complete incineration) = Z

Weight of ash = (Z-X) gm.

Y gm of crude drug gives (Z-X) gm of ash.

Therefore 100 gm of the crude drug gives 100/Y (Z-X) gm of the ash.

Total ash value of sample = 100(Z-X) / Y %.

5.2.3.2. Acid insoluble ash value

The ash is obtained by following the steps as mentioned in the procedure for determination of total ash value of crude drug. Further using 25ml of dilute HCl, wash the ash from the dish used for total ash into 100 ml of beaker. Place wire gauze over a Bunsen burner and boil 5 mins. Filter through an ash less filter paper wash the residue.
twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter paper and residue together into the crucible, heat gently until vapors cease to be evolved and the more strongly until all carbon has been removed. Cool in a desiccator. Weigh the residue and calculate acid insoluble ash of the crude drug with reference to the air dried sample of the crude drug.

**Calculation**

Weight of the residue = “X” gm

“Y” gm of the air dried drug gives = “X” gm of acid insoluble ash.

Therefore 100 gm of the air dried drug give = 100 × X/ Y gm of acid insoluble ash.

Acid insoluble ash value of the sample = 100×X / Y %.

**Note:** Acid insoluble ash value of a crude drug is always less than total ash value of the same drug.

5.2.3.3. Water soluble ash

This is determined in a similar way to acid insoluble ash using 25ml of water in place of dilute hydrochloric acid.

5.2.3.4. Extractive value

Extractive values are useful for the evaluation of a crude drug. It gives an idea about the nature of the chemical constituents present in a crude drug. It is also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. In the present study alcohol soluble extractive and water soluble extractive value were carried out.
5.2.3.5. Alcohol soluble extractive value

Weigh about 5 gm of the powder drug in a weighing bottle and transfer it to a dry 250ml, conical flask. Fill a 100 ml graduated flask to the delivery mark with the solvent (90% alcohol). Wash out the weighing bottle and pour the washings together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24hrs shaking frequently (maceration). Filter into a 50 ml cylinder. When sufficient filtrate was collected transfer 25ml of the filtrate to weighed thin porcelain dish and used for the ash value determination. Evaporate to dryness on a water bath and complete the drying in a oven at 100°C. Cool in desiccators and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

25ml of alcoholic extract gives = x gm of residues.

100ml of alcoholic extract gives = 4x gm of residues.

Therefore 5 gm of air dried drug gives = 4x gm of alcohol (90%) soluble residue.

Thus 100 gm of air dried drug gives = 80 x gm of the alcohol (90%) soluble residue.

Alcohol (90%) soluble extractive value of the sample = 80 x %

5.2.3.6. Water soluble extractive value

This is determined in a similar way to alcohol soluble extractive method using chloroform water instead of alcohol.

5.2.4. Moisture content

40gm of the cleaned sample was weighed and dried in an oven at 80°C for 7hrs and the weight was taken after every 2 hrs intervals. The procedure was repeated until a
constant weight was obtained. After each 2 hrs intervals the sample was removed from
the oven and placed in the desiccator for 30 mins to cool. It was then removed and
weighed again. The percentage of moisture content in the seed was calculated by the
following formula.

\[
\text{Moisture} = 100(\text{W1} - \text{W2}) / \text{W2} \%
\]

Where \( \text{W1} \) = original weight of the sample before drying

\( \text{W2} \) = weight of the sample after drying

5.2.5. Florescence analysis

The dried and powdered plant material of \textit{Rumex vesicarius} L. was used for
fluorescent studies. A pinch of powder of drugs such as WP, LF, ST, and RT are taken in
a clean test tube which contain about 10 ml of solvent. Likewise several tubes were made
by adding various solvents like 1N aqueous NaOH, 1N alcoholic NaOH, 50% HNO\textsubscript{3},
50% H\textsubscript{2}SO\textsubscript{4}, 1N HCL, 5% KOH, ethanol, hexane and methanol etc. All the tubes were
shaken well and incubated for about 30 mins. The colors of the drug solutions thus
obtained were observed for their characteristics color reaction under the visible light and
ultra violet light and the colour change is recorded by comparing with a standard color
chart.

5.2.6. Mineral analysis

The total ash content of the plant material obtained by earlier methods as
mentioned in the above text was used for the estimation of few mineral elements present
in the sample (Kumawat and Shimpi 2009).
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The total ash content of the drug was dissolved in 40 ml of 50 % (v/v) HCl in the china dish and covered with a watch glass. This suspension was digested on a water bath for 30 min. the watch glass was removed after cooling and washed with distilled water. This was filtered through a whatman filter paper No. 44 (ash less). 10 ml of filtrate was taken in a 100 ml volumetric flask and made its final volume to 100 ml by adding distilled water and maintained as stock solution.

The stock ash solution was used for the detection and estimation of minerals namely Calcium, Copper, Iron, Magnesium, Potassium, Sodium, and Zinc and Nitrogen in different parts of plant using automated atomic absorption spectrophotometer. The absorbance of elements was recorded. The mineral content is determined by plotting standard graph and calculated using the following formula.

\[
\text{Mineral content (ppm)} = \frac{Z \times S \times A}{Y \times W} \times 100
\]

Where, 
- \(Z\) = mineral content of the standard solution
- \(S\) = Reading of the test sample for a mineral
- \(Y\) = Reading of the standard solution of the mineral
- \(A\) = Dilution factor
- \(W\) = weight of the plant material (g)
- 100 = Volume of the stock ash solution.
5.3. Result

The detailed study of organoleptic characters of extract, ash and extractive values, mineral contents and fluorescent behavior of *Rumex vesicarius* L. whole plant, leaf, stem, and root are identified and further distinguished from their adulterants and substituent by certain standard parameters.

5.3.1. Organoleptic studies of extract (Table 28, 29)

Physical appearance, consistence, color and percentage yield of different extracts of hot and cold extraction were recorded in (Table 28, 29). The physical evaluation of drugs is an important parameter in detecting adulteration or important handling of drug. The results showed greater percentage yield of extract in hot soxhlet extraction when compared to cold maceration extraction method. In case of hot extraction, the aqueous yield is more with 26.0%, 19.8%, 21.7%, 12.1% for WP, LF, ST, RT respectively followed by methanol with 11.9%, 8.13%, 17.3%, 5.2%, for WP, LF, ST, RT respectively. Chloroform with 1.60%, 3%, 1.3%, 2.6% and petroleum ether with 2.35%, 3.4%, 1.66%, and 3% for WP, LF, ST, and RT respectively. In cold maceration process the highest percentage yield is found in case of aqueous extract with 11.21%, 9.80%, 5.5%, 6.21%, for WP, LF, ST, RT respectively followed by methanol with 2.9%, 3.6%, 2.7%, 4.21%, and chloroform with 1.8%, 3.0%, 1.3%, 3.01%, for WP, LF, ST, RT respectively. Petroleum ether has shown lowest extractive value with 0.65%, 1.0%, 1.2%, 2.06%, for WP, LF, ST, and RT respectively. (Table 28 and 29).
<table>
<thead>
<tr>
<th>Extract</th>
<th>Percentage yield</th>
<th>Colour</th>
<th>consistence</th>
<th>Physical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE WP</td>
<td>2.35%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Solid</td>
</tr>
<tr>
<td>PE LF</td>
<td>3.4%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Solid</td>
</tr>
<tr>
<td>PE ST</td>
<td>1.66%</td>
<td>Green</td>
<td>Oily</td>
<td>Solid</td>
</tr>
<tr>
<td>PE RT</td>
<td>3%</td>
<td>Yellowish brown</td>
<td>Amorphous</td>
<td>Solid</td>
</tr>
<tr>
<td>CE WP</td>
<td>1.60%</td>
<td>Greenish yellow</td>
<td>Oily</td>
<td>Solid</td>
</tr>
<tr>
<td>CE LF</td>
<td>3%</td>
<td>Greenish yellow</td>
<td>Oily</td>
<td>Solid</td>
</tr>
<tr>
<td>CE ST</td>
<td>1.3%</td>
<td>Greenish yellow</td>
<td>Oily</td>
<td>Solid</td>
</tr>
<tr>
<td>CE RT</td>
<td>2.6%</td>
<td>Brownish yellow</td>
<td>Amorphous</td>
<td>Powder</td>
</tr>
<tr>
<td>ME WP</td>
<td>11.9%</td>
<td>Reddish Brown</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>ME LF</td>
<td>8.13%</td>
<td>Reddish Brown</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>ME ST</td>
<td>17.3%</td>
<td>Reddish Brown</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>ME RT</td>
<td>5.2%</td>
<td>Reddish Brown</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>AE WP</td>
<td>26.0%</td>
<td>Brown</td>
<td>Waxy</td>
<td>Solid mass</td>
</tr>
<tr>
<td>AE LF</td>
<td>19.8%</td>
<td>Brown</td>
<td>Waxy</td>
<td>Solid mass</td>
</tr>
<tr>
<td>AE ST</td>
<td>21.7%</td>
<td>Brown</td>
<td>Waxy</td>
<td>Solid mass</td>
</tr>
<tr>
<td>AE RT</td>
<td>12.1%</td>
<td>Brown</td>
<td>Waxy</td>
<td>Solid mass</td>
</tr>
</tbody>
</table>
### TABLE 29. NATURE AND % YIELD OF COLD EXTRACT

<table>
<thead>
<tr>
<th>Extract</th>
<th>Percentage yield</th>
<th>Colour</th>
<th>consistence</th>
<th>Physical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP</td>
<td>0.65%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>LF</td>
<td>1.00%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>ST</td>
<td>1.2%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>RT</td>
<td>2.06%</td>
<td>Greenish yellow</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>CE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP</td>
<td>1.8%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>LF</td>
<td>3.00%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>ST</td>
<td>1.3%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>RT</td>
<td>3.01%</td>
<td>Greenish black</td>
<td>Oily</td>
<td>Semi solid mass</td>
</tr>
<tr>
<td>ME</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP</td>
<td>2.9%</td>
<td>Greenish yellow</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>LF</td>
<td>3.6%</td>
<td>Greenish yellow</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>ST</td>
<td>2.7%</td>
<td>Greenish yellow</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>RT</td>
<td>4.21%</td>
<td>Greenish yellow</td>
<td>Gummy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>AE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP</td>
<td>11.21%</td>
<td>Brownish yellow</td>
<td>Waxy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>LF</td>
<td>9.80%</td>
<td>Brownish yellow</td>
<td>Waxy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>ST</td>
<td>5.5%</td>
<td>Brownish yellow</td>
<td>Waxy</td>
<td>Syrupy mass</td>
</tr>
<tr>
<td>RT</td>
<td>6.21%</td>
<td>Brownish yellow</td>
<td>Waxy</td>
<td>Syrupy mass</td>
</tr>
</tbody>
</table>

### TABLE 30. SHOWS PHYSICOCHEMICAL PARAMETERS

<table>
<thead>
<tr>
<th>Sno.</th>
<th>Parameters</th>
<th>Whole Plant</th>
<th>Leaves</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash value</td>
<td>20.81</td>
<td>21.19</td>
<td>22.68</td>
<td>10.57</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble ash</td>
<td>11.15</td>
<td>25.25</td>
<td>22.65</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble ash</td>
<td>10.25</td>
<td>1.9</td>
<td>7.75</td>
<td>4.75</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble extractives</td>
<td>40.84</td>
<td>42.48</td>
<td>28.4</td>
<td>20.5</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol soluble extractives</td>
<td>12</td>
<td>1</td>
<td>12.48</td>
<td>7.2</td>
</tr>
<tr>
<td>6</td>
<td>Moisture content</td>
<td>09</td>
<td>9.5</td>
<td>06</td>
<td>20</td>
</tr>
</tbody>
</table>
The nature of the extract varies from solvent to solvent. In case of hot extraction method the nature of petroleum ether extract is greenish black, oily solid for WP and LF, whereas ST extract is green oily solid and RT extract is Yellowish brown amorphous solid. All the chloroform extract is greenish yellow solid except root extract (RT) which is brownish yellow amorphous powder. The methanol extract is reddish brown gummy syrupy mass aqueous extract is brown waxy solid mass. In case of cold extraction process the petroleum ether and chloroform extract is greenish black oily semisolid mass except root extract (RT) which is greenish yellow oily semi solid for petroleum ether. All the methanol and aqueous extracts are greenish yellow gummy syrupy mass and brownish yellow waxy syrupy mass respectively.

5.3.2 Physical properties of *Rumex vesicarius* L.

5.3.2.1. Total ash, water soluble and acid insoluble values (Table 30)

The ash values, extractive values and moisture content of leaves were determined and the results are shown in (Table 30). Mean of ash value of the plant is obtained as 20.81%, 21.19%, 22.68%, 10.57% for total ash value which is present in whole plant, leaf, stem and root respectively and 10.25%, 1.9%, 7.75% and 4.75% for water soluble ash was present and for acid insoluble the values are 11.15%, 25.25%, 22.65% and 1% in whole plant, leaf, stem, root respectively.

5.3.2.2. Extractive values (Table 30)

The extractive value of different plant parts of *Rumex vesicarius* L. have been analyzed to find out the percentage of extractive values. The water soluble extractive
value of whole plant, leaf, stem, and root was obtained as 40.85%, 42.48%, 28.4%, and 20.5% respectively. The alcohol soluble extractive values of whole plant, leaf, stem, and root were 12%, 1%, 12.48%, 7.2% respectively. The maximum percentage of moisture content was obtained as 20% in roots followed by leaf with 9.5%, whole plant with 9%, and stem with 6% (Table 30).

5.3.2.3. Fluorescence analysis (Table. 31)

Fluorescence analyses are the tool to determine the kind of nature of the drug. The fluorescence analysis of the plant powder was done by treating the plant powder with various chemical reagent and separate observations were made under normal light and UV light, the color changes of different parts of plant powder was recorded and presented in table 28 respectively.

The dried powder of WP, LF, ST, and RT did not show much variation under normal light and UV light but slight variation from grey to green was observed. However when treated with various chemical the plant powder has showed different color characteristics.

The whole plant crude dried powder appears as yellowish green (normal light) and brown (UV light) with aqueous 1N NaOH. And with 1N alcoholic NaOH it appears as pale yellow (normal light), and yellow (UV light). Furthermore, it appears as yellowish brown (normal light), Reddish brown (UV light) with 50% HNO₃, green (normal light), dark green (UV light) with 50% H₂SO₄. In case of 1N HCL and 5 % KOH it appears as pale yellow (normal light), green (UV light) and yellowish green (normal light) brown
## TABLE 31. FLUORESCENCE ANALYSIS OF PLANT POWDER

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whole plant</th>
<th>Leaves</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Light</td>
<td>UV Light</td>
<td>Normal Light</td>
<td>UV Light</td>
</tr>
<tr>
<td>Powder as such</td>
<td>Grey green</td>
<td>Grey</td>
<td>Light green</td>
<td>Green</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Green</td>
</tr>
<tr>
<td>Powder + 1N NaOH (aqueous)</td>
<td>Yellowish green</td>
<td>Brown</td>
<td>Dark green</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td>Yellowish green</td>
<td>Brown</td>
<td>Dark green</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + 1N NaOH (alcoholic)</td>
<td>Pale yellow</td>
<td>Yellow</td>
<td>Light green</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Powder +50%HNO3</td>
<td>Yellowish brown</td>
<td>Reddish brown</td>
<td>Brownish green</td>
<td>Dark green</td>
</tr>
<tr>
<td>Powder +50%H2SO4</td>
<td>Green</td>
<td>Dark green</td>
<td>Light green</td>
<td>Pale green</td>
</tr>
<tr>
<td>Powder +1 N HCL</td>
<td>Pale yellow</td>
<td>Green</td>
<td>Yellow</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Powder +5%KOH</td>
<td>Yellowish green</td>
<td>Brown</td>
<td>Yellow</td>
<td>Orange</td>
</tr>
<tr>
<td>Powder + ethanol</td>
<td>Green</td>
<td>Blackish green</td>
<td>Dark Green</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Powder + Hexane</td>
<td>Yellowish green</td>
<td>Light orange</td>
<td>Light green</td>
<td>Light green</td>
</tr>
<tr>
<td>Powder + methanol</td>
<td>Light yellow</td>
<td>Light blue</td>
<td>Brown</td>
<td>Light brown</td>
</tr>
</tbody>
</table>

|                                | UV Light | UV Light | UV Light | UV Light |
|                                |          |          |          |          |
|                                |          |          |          |          |
|                                |          |          |          |          |
|                                |          |          |          |          |
|                                |          |          |          |          |
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(UV light) respectively. However, with ethanol, hexane and methanol it appears as green, yellowish green, light yellow under normal light and blackish green, light orange, light blue under UV light.

Similarly, Leaf (LF) crude dried powdered with aqueous NaOH, alcoholic NaOH, 50% HNO₃, H₂SO₄, HCl, KOH, ethanol, hexane, methanol appears as dark green, light green, brownish green, light green, yellow, yellow, green, light green, brown under normal light and light green, brown, light green, dark green, pale green, light brown, orange, dark green, dark green, dark brown under UV light respectively.

Similarly with stem, color appears as reddish yellow, light green, yellow, brown, pale yellow, yellow, green, light green, light green under normal light and green, brown, yellow, dark brown, black, black, fluorescent green, yellowish brown, light green, light green under UV light.

Whereas root (RT) appears as green, brick red, red orange, brown, pale yellow, brown, green, pale green, light green under normal light and brown, red, brown, black, lemon green, dark green, lemon green, light green, yellowish green under normal light with aqueous NaOH, alcoholic NaOH, 50% HNO₃, 50% H₂SO₄, 5% KOH, ethanol, hexane, methanol respectively. (Table 30).

5.3.2.4. Mineral analysis (Table 32)

The mineral elements are very essential for the growth and development of plants. The mineral elements namely N, P, K, S, Ca, Mg is expressed in percentage where as Zn, Fe, Mn, Cu as ppm (Table 32).
### TABLE 32. SHOWS MINERAL ANALYSIS

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>N %</th>
<th>P %</th>
<th>K %</th>
<th>S %</th>
<th>Ca %</th>
<th>Mg %</th>
<th>Zn ppm</th>
<th>Fe ppm</th>
<th>Mn ppm</th>
<th>Cu ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHOLE PLANT</td>
<td>4.33</td>
<td>0.40</td>
<td>4.13</td>
<td>0.13</td>
<td>2.44</td>
<td>0.26</td>
<td>34.800</td>
<td>148.100</td>
<td>50.100</td>
<td>29.100</td>
</tr>
<tr>
<td>LEAF</td>
<td>4.74</td>
<td>0.39</td>
<td>2.24</td>
<td>0.13</td>
<td>3.35</td>
<td>0.38</td>
<td>30.100</td>
<td>397.800</td>
<td>62.200</td>
<td>12.000</td>
</tr>
<tr>
<td>STEM</td>
<td>3.24</td>
<td>0.38</td>
<td>2.96</td>
<td>0.10</td>
<td>1.96</td>
<td>0.26</td>
<td>30.600</td>
<td>555.000</td>
<td>24.100</td>
<td>8.600</td>
</tr>
<tr>
<td>ROOT</td>
<td>2.17</td>
<td>0.24</td>
<td>4.43</td>
<td>0.06</td>
<td>1.86</td>
<td>0.13</td>
<td>22.700</td>
<td>153.100</td>
<td>62.800</td>
<td>15.200</td>
</tr>
</tbody>
</table>
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The whole plant consist of highest quantity of N (4.33%), followed by K (4.13%), Ca (2.44%), P (0.40%), Mg (0.26%), S (0.13%), Fe (148.100ppm), Mn (50.100ppm), Zn (34.800ppm), Cu (29.100ppm), further the leaf (LF) consist of highest quantity of N (4.74%) followed by Ca (3.35%), K (2.24%), P (0.39%), Mg (0.38%), S (0.13%), Fe (397.800 ppm), Mn (62.200ppm), Zn (30.100ppm), Cu (12.00 ppm).

In case of stem the highest quantity of minerals present are N (3.24%), followed by K (2.96%), Ca (1.96%), P (0.38%), Mg (0.26%), S (0.10%), Fe (555.00ppm), Zn (30.600ppm), Mn (24.100ppm), Cu (8.600 ppm). However, in case of root the highest quantity of mineral elements present are K (4.43%), N (2.17%), Ca (1.86%), P (0.24%), S (0.06%), Fe (153.100 ppm), Mn (62.80 ppm), Zn (22.700 ppm), Cu (15.200 ppm).
5.4. Discussion

The improvement in the quality control and standardization of herbal drugs has led to the development of effective quality medicines from plants. However, herbal formulations involve use of fresh (or) dried plant parts. Correct knowledge of such crude drug is very important aspect in preparation, safety and efficacy of the herbal products. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (Musa et al., 2006). To ensure reproducible quality of herbal products, proper control of starting material is utmost essential (Venkatesh et al., 2004). Thus, in recent years more emphasis is made on standardization of medicinal plants, and evaluation of plant drugs by pharmacognostical studies has become still more reliable, accurate and inexpensive means.

In the present study, different parts of the plant were evaluated qualitatively by studying various physicochemical parameters. Detailed pharmacognostical studies for different parts of *Rumex vesicarius* L. are undertaken. Further, aerial part of *Rumex vesicarius* L. forms the major ingredient in salad and other preparations, so the present investigation is useful in establishing a monograph detail for the different parts of *Rumex vesicarius* L. The pharmacognostic results on physicochemical characteristics and fluorescence analysis data shows that authentic properties of this crude drug will prevent adulteration and substitution which have a crucial role in standardization of crude drug. The current study on different parts of *Rumex vesicarius* L. powder was evaluated for its ash values, extractive values and loss on drying.
5.4.1. Organoleptic studies of extract

The organoleptic character of *Rumex vesicarius* L. have been revealed in the present investigation. The organoleptic characters of crude extracts can easily be identified owing to its characteristics differential yields, color, taste and nature.

Extraction of various part of plant (WP, LF, ST, and RT) was determined using petroleum ether, chloroform, methanol and water by two different method of extraction i.e. Hot soxhlet extraction and cold maceration method. The percentage yield of extract is higher in hot soxhlet method when compared to cold maceration method.

The yield of the extract depends on the type of extraction method and the solvent system selected. The selection of solvent plays an important role and is taken into consideration which depends on the type of component to be extracted. The specific solvent extracts the specific phytochemical compound. Fats, lipids, Phospholipids, sterols etc have more solubility in petroleum ether, ether, benzene. Amino acids and glycosides are soluble in alcoholic solvent and some alkaloids and glycosides are soluble in water. Ozarkar, (2005) have reported that the extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. Ahirrao et al., (2011) have also carried out the pharmacognostical studies of *Vitex negundo* leaves.

**Total Ash, water soluble and acid insoluble values**

The total ash is particularly important in the evaluation of purity of the drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica.
(Musa et al 2006). In the present study the ash value of whole plant, leaves, stem, and root of *Rumex vesicarius* L. are 20.81%, 21.19%, 22.68%, 10.57% respectively and acid insoluble ash value for whole plant, leaves, stem, and root of *Rumex vesicarius* L. are 11.15, 25.25, 22.65, 1 respectively where as water soluble ash value is found to be 10.25, 1.9, 7.75, 4.75 for whole plant, leaves, stem, and root of *Rumex vesicarius* L. respectively.

Ash content of a drug indicates the presence of various impurities like carbonates, oxalates and silicates present along with the drug. The water soluble ash is used to determine the amount of inorganic compounds present in herbal drugs. Acid insoluble ash gives an idea about the amount of silica present and indicates contamination with earthy material (Ranjeet Singh et al., 2014).

The study of ash value of whole plant, leaves and stem have more acid insoluble ash than the water soluble ash. The ash value is generally the index of purity as well as identity of the drug. Sing et al., (2013) have carried out investigation on pharmacognostical standardization of the roots of *Rumex Hastatus* D. and suggested that the pharmacognostic parameters would serve as a standard reference in terms of quality control for the future studies.

5.4.2. Extractive values

Extractive values are also important parameters for detecting adulteration in drugs. It is the amount of extract that a drug yields to a certain constituents or a group of related constituents that a drug contains. In the present study the water soluble extractives
and alcohol soluble extractives are found to be 40.84, 42.48, 28.4, 205 and 12, 1, 12.48, 7.2 for WP, LF, ST and RT respectively. The amount of extract in a particular substance plays an important role in establishing the index of the purity of drug and any adulteration or substitution may cause change in extractive value (Anonymous, 1992). The water soluble extractive values indicated the presence of sugar, acids and inorganic compounds and the alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids (Mukherjee, 2002; WHO, 1998; Anonymous, 1996). It is evident from the present results that the universal solvent water has higher extractive values than the alcohol soluble extractive. Disha and Sumitra (2014), in their investigation on Phytochemical and pharmacognostic evaluation of leaves of Pongamia pinnata L. (Fabaceae) reported that the extractive value of water is highest followed by methanol.

5.4.3. Moisture content

In herbal drugs, variable limit of water are present. An excess of water in medicinal plant material encourages microbial growth and deterioration following hydrolysis. Estimation of moisture content is important for the material which absorbs moisture easily or deteriorates quickly in the presence of water. The active constituents of drugs are calculated on the basis of moisture free drugs or should be calculated taking consideration the percentage of water therein. Thus the moisture content is a parameter for checking the purity of the drug. Therefore, the parameter must not be neglected. The percentage of moisture content of various part of *Rumex vesicarius* L. in the present study
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was found to be 09, 9.5, 06, 20 % for WP, LF, ST and RT respectively. Vidita et al., (2013) reported that the higher stability of drug is due to Low moisture content present in roots of *Anogeissus latifolia*.

Foreign matter as per standards should not be more than 2% w/w (Soni et al., 2011). Moisture content of drugs could be at minimal level to discourage microbial growth and undesirable enzymatic activity, which accelerates spoilage. The percentage of moisture ranging from 10-20% shows an ideal range for maximum growth of microorganisms.

5.4.4. Fluorescence analysis

Herbal drugs are generally used in the form of powder. It is easy to adulterate them with powders resembling to original drug for making more profit. The fluorescence study of powder under UV light is also helpful in establishing the purity of the drug. It can be checked by observing the change in color of powder under UV light after treating the powder with different chemicals. Many phytocompounds will fluoresce when suitably illuminated. The fluorescence color is specific for each compound. The non fluorescent compound may be fluorescent if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Banoti, 1980). In the present study, the powdered whole plant, leaves, stem, root of *Rumex vesicarius* L. emitted wide range of color under daylight and under UV light. Fluorescence analysis of
powders of different parts of *Rumex vesicarius* L. (whole plant, leaf, stem, and root) gives a clue if powder is in adulteration, thus can be used as a diagnostic tool for testing the adulteration. Presence or absence of certain important compounds in an extract is determined by color reactions of the compounds with specific chemicals which act as dyes. This procedure is pre-requisite before going for detailed phytochemical investigation. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Gupta et al., 2006; Ansari, 2006).

5.4.5. **Mineral analysis**

The mineral analysis in the present study suggests that the *Rumex vesicarius* L. is a good source of nutrient due to the presence of N, P, K, S, Ca and Mg it also shows the presence of Zn, Fe, Mn, Cu. (Table 29). Minerals are required for normal growth, activities of muscles and skeletal development (such as calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium); as well as the regulation of acid-base balance (phosphorus). Iron is useful in prevention of anemia and other related diseases (Oluyemi et al., 2006). Manganese plays a role in energy production and in supporting the immune system. It also works with vitamin K to support blood clotting and with B complex vitamins to control the effects of stress. Zinc is useful for protein synthesis, normal body development and recovery from illness (Muhammad et al., 2011). Deficiency of these nutrients and minerals are known to affect...
the performance and health. In the present study the *Rumex Vesicarius* L. consist of highest quantity of N (4.33%), followed by K (4.13), Ca (2.44%), P (0.40%), Mg (0.26%), S (0.13%), Fe (148.100ppm), Mn (50.100ppm), Zn (34.800ppm), Cu (29.100ppm). Suleiman Idris et al., findings confirmed that the *Rumex acetosa* is rich sources of potassium, magnesium, copper, iron, manganese, and zinc as well as high energy values essential in human and animal nutrition.

Pharmacognostic studies on different plants like *Careya arborea* Roxb stem (Gupta et al., 2012), *Cayratia trifolia* leaf (Kumar et al., 2012), *Cissus quadrangularis* L. stem (Nagani et al., 2011), *Manilkara hexandra* (Roxb.) Dubard leaf (Chanda et al., 2010), *Manilkara zapota* L. leaf (Nagani et al., 2012), *Polyalthia longifolia* var. pendula Leaf (Dave et al., 2010), *Psidium guajava* L. leaf (Kaneria and Chanda, 2011), *Punica granatum* L. leaf (Bapodara et al., 2011) and *Tephrosia purpurea* (Linn.) Pers. Root (Shah et al., 2011), have been reported.