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*Research Article*

# Analysis of few crude compounds and separation of leaf pigments in few medicinal plant species

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**Abstract**

**For utilization of a certain biomass it is necessary to know its chemical composition. The present study is to qualitative crude chemical analyses of nutritional value (crude compounds) such as acidity, starch, carohydrates, iron and calcium etc. from stem extracts of *Tinospora cardifolia*, *Centella asiatica, Clerodendrum inerme* and leaf extract of *Ocimum sanctum*, *Lawsonia inermis* and *Piper nigrum* were conducted and also separate the pigments by TLC methods. The results revealed that *Tinospora cardifolia*, biomolecules like starch and carbohydrates were present and elements like iron and calcium were absent. In *Centella asiatica* indicated the absence of biomolecules like starch and carbohydrates and only one molecules i.e., calcium is present and iron is absent. Similarly, in *Clerodendrum inerme,* biomolecules like starch were absent and carbohydrates were present. Both the elements iron and calcium were absent in this plant extracts. In *Ocimum sanctum,* starch is absent carbohydrates are present, iron and calcium are absent. In *Lawsonia inermis,* the results are similar to *Ocimum* sp. i.e., only carbohydrates are present remaining starch, iron and calcium are absent in this leaf extracts. Interestingly, in *Piper nigrum,* all chemical compounds are totally absent. Related to pigments analysis highest distance travelled pigments are carotenoids (4.3 cm) in *O. sanctum* and highest number of pigments was found in *C. inerme* with six pigments and least number of pigments was recorded in *O. sanctum* (four) and *L. inermis* (four). Meanwhile, highest Rf value recorded in *O. sanctum* and *P. nigrum* with 0.97 (chlorophyll a). Among the seven pigments in six species carotenoids, chlorophyll a and xanthophylls are common to all species but chlorophyll b, lutein, anthocyanin and lawsonin are rare to occur in these six species for example Lawsonin occur only in *P. nigrum*.**

**Keywords:** *Chromatography, pigments, extracts, crude compound.*

## INTRODUCTION

In recent years a considerable attention has been given to non-edible plant biomass as a renewable source for production of green energy, bioproducts and biochemicals. The various biomass types like residues of agricultural plants (e.g. stalks, husks, cobs, etc.), forest residues (e.g. sawdust, twigs, shrubs, etc.), waste of wood, textile, pulp, paper and cities, as well as some plant species (e.g. Miscanthus, Switchgrass, Bermuda grass, etc.) (Ioelovich, 2015) were used as a source. In general, the biomass can be used as feedstock for production of liquid biofuel for vehicles and as solid fuel for burning and gasification in order to generate heat, steam, and electricity, and also as feedstock for manufacturing of various bioproducts and biochemicals. To choose the optimal path for utilization of a certain biomass type it is necessary to know its chemical composition. Any type of the biomass consists of three basic plant polymers: cellulose, hemicelluloses and lignin. The other components of the biomass can be mineral substances, organic extractives (waxes, fats, oils), pectin, starch and some other admixtures (Ioelovich, 2015).

Herbal drugs/Medicinal plants are used in traditional medicine practice since prehistoric times. Herbal drugs (herbal medicine raw materials) can be prepared for use in different ways (Keskin, 2018). Plants possess constitutive as well as inducible defense mechanisms (Mithofer and Boland, 2012) against insects, fungi, diseases and herbivorous mammals. Numerous phytochemicals with

established biological activities are identified. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines (Awuchi and Chinaza, 2019). In phytochemical screening the powdered plant parts were subjected to the detection of various plant constituents, characterized for their possible bioactive compounds, which have been separated. Analyses of crude compound contents are very important for estimating chemical composition of plants. Screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses (Sasidharan et al., 2011). Plant derived substances has obtained greater attention in the recent years to prevent and cure human diseases as they are considered to be more bio-friendly. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% medicinal needs of the third world countries (Rajashekaran, 2002). Phytochemical investigations of crude plant extracts shows the presence of active principles in the plant parts like bark, leaves, flowers, roots, fruits, seeds (Tiwari, 2011). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but research works demonstrates that many phytochemicals can protect humans against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances (Edeoga et al., 2005).

*Tinospora cordifolia* (Thunb.) Miers commonly named as "*Guduchi*" in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude (Rana et al., 2012; Parthipan et al., 2011). It is found throughout the India & also in parts of Sri Lanka, Bangladesh and China. The plant is designated as Rasayana in Ayurveda and is very well known for building up the immune system and body's defence against definite infecting microorganisms (Upadhyay et al., 2010).

*Centella asiatica* (L.) Urban has been used as a medicinal herb for thousands of years in India, China, Srilanka, Nepal and Madagascar. It is commonly known as Indian Pennywort, belongs to the family Apiaceae (previously known as Umbelliferae). *Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as a "*Brain food*" in India (Singh et al., 2010).

*Clerodendron inerme* (L.) Gaertn. belonging to family Verbenaceae is very widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. It is commonly called as Wild Jasmine. It is used in the treatment of skin diseases, venereal infections, elephantiasis, asthma, topical burns and for rheumatism (Chethana et al., 2010).

*Ocimum sanctum* L., also known as Tulsi, is an aromatic perennial herb belongs to Lamiaceae family. It is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. *Ocimum sanctum* have been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites, etc (Pattanayak et al., 2010).

*Lawsonia inermis* L. is an evergreen shrub or small tree belongs to Lythraceae family and commonly called as Henna. Henna has been used as antiquity to dye skin, hair and fingernails, as well as fabrics including silk, wool and leather. Henna extracts were investigated for their antibacterial activity against a wide array of different micro-organisms (Habbal et al., 2011).

*Piper nigrum* L. (commonly called as black pepper) is a flowering vine grows in well drained area rich in moist soil and belongs to Piperaceae fmaily. Black pepper has been used in a variety of different remedies. According to Ayurveda, the pungency and heating properties of black pepper work to help metabolize food. Different parts of *Piper nigrum* including secondary metabolites are also used as drug, preservative, insecticidal and larvicidal control agents (Nisar Ahmad et al., 2012).

Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given substance, and determine the purity of a substance. Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. The behavior of an individual compound in TLC is characterized by a quantity Known as Rf and is expressed as a decimal fraction. The Rf is calculated by dividing the distance the compound traveled from the original position by the distance the solvent travelled from the original position (the solvent front).

The present investigation concern with analyses medicinal plant biomass for their chemical constituents help us to known the essential molecules available in such biomass i.e., crude chemical analysis and determination of substance purity. Therefore, for this study decided to select few commonly available medicinal plants, they are, *Tinospora cardifolia, Centella asiatica, Clerodendrum inerme, Lawsonia inermis, Piper nigrum* and *Ocimum sanctum*. A very less work has been conducted on the selected above plant species for the analyses of chemical constituents earlier.

# MATERIALS AND METHOD

## Plant material collection

*Tinospora cardifolia, Centella asiatica, Clerodendrum inerme, Lawsonia inermis, Piper nigrum* and *Ocimum sanctum* fresh plant parts (stem and leaves) were collected from the JSS College of Arts, Commerce and Science campus, JSS Ayurvedic college campus, Mysore District (in and around Mysuru District) and Calicut, Kerala in the month of

January 2020. Carefully placed the collected plant materials in polythene bags and directly brought to laboratory for conducting further analysis.

### Preparation of powder from plant parts

The healthy plant stems were collected and thoroughly washed in distilled water and blotted. The stems were shade dried for one week. The dried stem barks were pulverized in a mixer, sieved with a fine mesh, stored in air tight used for further study.

### Preparation of leaf extracts

From a collected leaves find a suitable leaves of weigh 2 g and grind well by using chloroform and ethyl acetate solution in a mortar and pestle and filter the extract by using Whatsmann filter paper No. 1 and extract the pigments.

### Soxhlet extraction

The stem bark powder was subjected for consecutive extraction in a Soxhlet extractor. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial impacts. The extracts were concentrated to dryness under reduced pressure in desiccators to yield dried extracts.

### Separation of pigments by TLC

Fill the capillary tube by placing the leaf extract and apply the extract at above 2 cm to the lower edge on the TLC plate by quickly spotting on the edge and allow drying. Repeat several times to make a concentrate spot of extract. Carefully place a TLC plate on the coupling jar containing Chloroform and Ethyl acetate in a ratio of 25:25 ml. The TLC plate should sit on the bottom of the coupling jar. Allow TLC plate to develop the pigment separation for approximately 10 mins. Remove the TLC plate from coupling jar with the solvent id approximately 1 cm from the top edge of the TLC plate, with a pencil mark the level of solvent front. Record the Rf value and calculate the percentage of distance travelled by the pigments.

The Rf value can be calculated by using the following formula.

Rf = Distance travelled by the solute/ Distance travelled by the solvent.

Thin-layer chromatography using silica gel G adsorbent and several solvent systems permits rapid separation of small amounts of chlorophylls and related pigments. U.V. radiation is used as a sensitive aid in the detection of very small concentrations of these pigments.

### Qualitative analyses of crude compounds

Test for basic crude chemical compounds like carbohydrates, iron, and calcium, acidic nature from stem extract of all the six species were analyses. Extracts using commonly employed precipitation and colouration procedure to identify the major natural bioactive which are considered during the present study to check their presence or absence in plant extract. Crude chemical analyses carried out by using following standard protocols. Test for acidity:

Take 5ml of the given sample in a test tube and dip a pH paper in it. If pH is less than 7, it is acidic else it is basic.

### Test for Starch:

Take 2 ml of sample in a test tube and add few drops of iodine solution. It turns blue black in colour than the starch is present.

### Test for Carbohydates (Fehling's Test):

Take 2 ml of the given sample and 1 ml of fehling solution A and B and boil it. Red precipitates indicate the presence of producing sugar like maltose, glucose, fructose and Lactose.

### Test for Iron:

Take 2 ml of the given sample add drop of concentrated nitric acid. Boil the solution, cool and add 2-3 drops of potassium thiocyanide solution. Blood red colours show the presence of iron.

### Test for Calcium:

Take 2 ml of the given sample add ammonium chloride and ammonium hydroxide solution. Filter the solution and to the filtrate add 2 ml of Ammonium oxalate solution. White precipitate indicates the presence of calcium.

## RESULT AND DISCUSSION

The collected plant materials Figure 1 from *Tinospora cardifolia, Centella asiatica, Clerodendrum inerme, Ocimum sanctum, Lawsonia inermis* and *Piper nigrum* were subjected to preparation of powder for further qualitative analysis of different chemical compounds and separation of the primary pigments of the plant materials in leaf. The aims of this work to document how different pigments are separated in different samples and determine the Rf value. The tests conducted from aqueous extract of *Tinospora cardifolia* stem powder (Table 1) and the results show that pH is basic in nature, biomolecules like starch and carbohydrates were present and elements like iron and calcium were absent. In *Centella asiatica* stem powder indicated the acidic property with pH 6.44 (Table 1), and indicated the absence of biomolecules like starch and carbohydrates and only one molecules i.e., calcium is present and iron shows negative results in stem powder of *Centella asiatica*. Similarly, the tests conducted on aqueous extracts of *Clerodendrum inerme* showed acidic nature with pH 6.60 (Table 1). Biomolecules like starch were absent and carbohydrates were present. Both the elements iron and calcium were absent in *Clerodendrum inerme* powder extracts. In *Ocimum sanctum* (Table 1), hydrogen ion potential of *O. sanctum* leaf extract is 6.89 and it is slightly basic (neutral). Starch is absent carbohydrates are


**Figure 1:** Stems and powders of T. cardifolia (A and B), C. asiatica (C and D), C. inerme (E and F), O. Sanctum (G and H), Lawsonia inermis (I and J) and P. nigrum (K and L)

**Table 1.** Represents the Analysis of crude chemicals in different plants.

| | *Tinospora cardifolia* | *Centella asiatica* | *Clerodendrum inerme* | *Ocimum sanctum* | *Lawsonia inermis* | *Piper nigrum* |
|---|---|---|---|---|---|---|
| Test for acidity | 7.25 | 6.44 | 6.60 | 6.89 | 5.14 | 5.95 |
| Test for starch | + | _ | _ | _ | _ | _ |
| Test for carbohydtrate | + | _ | + | + | + | _ |
| Test for iron | _ | _ | _ | _ | _ | _ |
| Test for calcium | _ | + | _ | _ | _ | _ |

present, iron and calcium are absent. In *Lawsonia inermis* (Table 1), hydrogen ion potential of the Henna leaf extract is 5.13, it is less acidic. The results are similar to *Ocimum* sp. i.e., only carbohydrates are present remaining starch, iron and calcium are absent in this leaf extracts. Meanwhile, in *Piper nigrum* (Table 1), pH of this plant is 5.95, it indicates that less acidic and all chemical compounds like starch, carbohydrates, iron and calcium are totally absent in this plant extracts.

Chromatograms of pigments in *T. cordifolia, C. asiatica, Clerodendrum inerme Ocimum sanctum*, *Lawsonia inermis* and *Piper nigrum* Figure 2 on thin layer of silica gel sheets are given in detail in Table 2. Complete separation of all major pigment fractions (Distance travelled by the pigments, retention factor and percentage of retention factor) was obtained with the green leaves. In all the pigment separation experiments of the plant species revealed that the major pigments obtained were Carotenoid, Chlorophylls ('a' and 'b'), Xanthophyll. Lutein, Anthocyanin and Lawsonin.

In Table 2 illustrate that distance travelled by the pigments, retention factor and percentage of retention factor of pigments of *T. cordifolia.* The pigment carotenoids travelled highest distance i.e., 3.3 cm from the sample load and it is followed by chlorophyll 'a' with 3.2 cm, xanthophylls with 2.5 cm, chlorophyll 'b' with 1.9 cm and lastely by lutein with 1.2 cm. In case of Rf value of pigments, the higest value 0.93 (93%) was reported in carotenoids, followed by 0.88 (88.33%) of chlorophyll 'a', 0.69 (69.66%) of xanthophylls, 0.53 (53.33%) of chlorophyll 'b' and 0.34 (34.66%) of lutein.

In *C. asiatica* Table 2, the pigment carotenoids travelled highest distance i.e., 2.1 cm from the sample load and it is followed by chlorophyll 'a' with 2.0 cm, anthocyanin with 1.9 cm, xanthophylls with 1.6 cm, chlorophyll 'b' with 1.3 cm and lastely by lutein with 0.8 cm. In case of Rf value of pigments, the higest value 0.89 (89.66%) was reported in carotenoids, followed by 0.85 (85.33%) of chlorophyll 'a', 0.80 (80%) of anthocyanin, 0.69 (69.66%) of xanthophylls, 0.57 (57.33%) of chlorophyll 'b' and 0.35 (35%) of lutein.

Similarly, the distance travelled by the pigments, retention factor and percentage of retention factor of *Clerodendrum inerme* (Table 2). The pigment carotenoids travelled highest distance i.e., 2.8 cm from the sample load and it is followed by chlorophyll 'a' with 2.6 cm, anthocyanin with 2.1 cm,

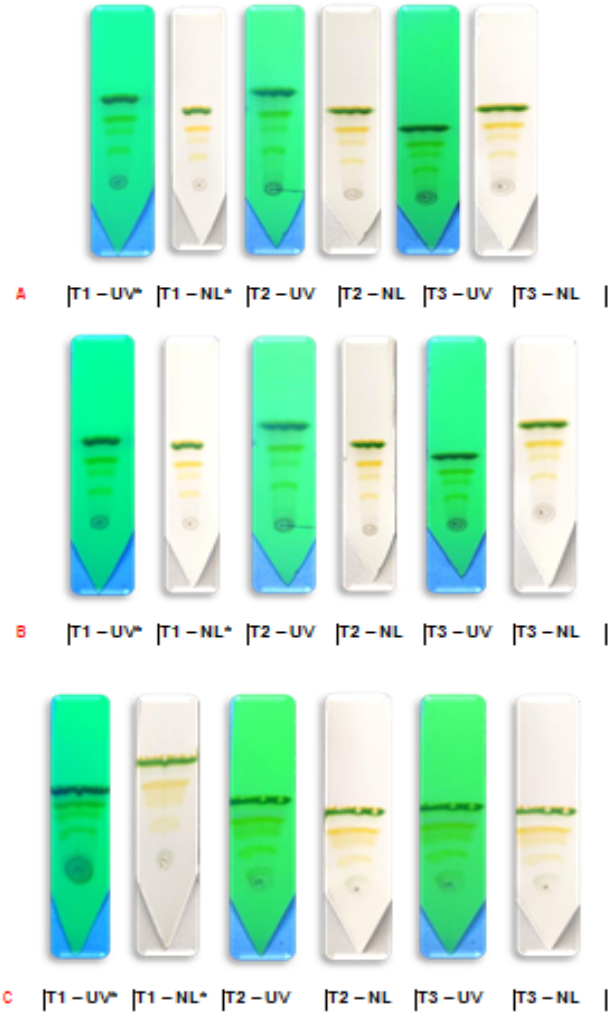


**Figure 2:** Chromatograms of pigments in *T. cordifolia* (A), *C. asiatica* (B) and *C. inerme* (C) on thin layer of silica gel sheets was obtained with the green leaves are shown.

**Note:** * T1, T2 and T3 – Trail Numbers and UV – Ultraviolet light, NL – Normal light

**Table 2.** Complete separation of all major pigment fractions (Distance travelled by the pigments, retention factor and percentage of retention factor) was obtained in the leaf of plants. TC – *T. cardifolia*, CA – *C. asiatica*, CI – *C. inerme*, OS – *O. sanctum*, LI – *L. inermis*, PN – *P. nigrum.*

| Distance travelled by the pigments (cm) | | | | | | | | Rf value of pigments | | | | | | Percentage retention factor | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TC | CA | CI | OS | LI | PN | TC | CA | CI | OS | LI | PN | TC | CA | CI | OS | LI | PN |
| Solvent Front | 3.6 | 2.3 | 3.0 | 4.4 | 3.7 | 4.0 | - | - | - | - | - | - | - | - | - | - | - | - |
| Carotenoids | 3.3 | 2.1 | 2.8 | 4.3 | 3.3 | 3.9 | 0.93 | 0.89 | 0.94 | 0.94 | 0.89 | 0.96 | 93% | 89.66% | 94% | 94% | 90% | 96% |
| Chlorophyll a | 3.2 | 2.0 | 2.6 | 4.1 | 3.5 | 3.9 | 0.88 | 0.85 | 0.86 | 0.97 | 0.95 | 0.97 | 88.33% | 85.33% | 86.33% | 97% | 95% | 97% |
| Xanthophyll | 2.5 | 1.6 | 2.0 | 3.0 | 2.0 | 3.2 | 0.69 | 0.69 | 0.71 | 0.67 | 0.54 | 0.80 | 69.66% | 69.66% | 65.33% | 68% | 55% | 80% |
| Chlorophyll b | 1.9 | 1.3 | 1.5 | 4.2 | - | 3.6 | 0.53 | 0.57 | 0.65 | 0.91 | - | 0.88 | 53.33% | 57.33% | 51.33% | 91% | - | 89% |
| Lutein | 1.2 | 0.8 | 1.1 | - | - | - | 0.34 | 0.35 | 0.51 | - | - | - | 34.66% | 35% | 35.66% | - | - | - |
| Anthocyanin | - | 1.9 | 2.1 | - | - | 2.7 | - | 0.80 | 0.35 | - | - | 0.67 | - | 80% | 71% | - | - | 67% |
| Lawsonin | - | - | - | - | 2.4 | - | - | - | - | - | 0.66 | - | - | - | - | - | 67% | - |

xanthophylls with 2.0 cm, chlorophyll '*b*' with 1.5 cm and lastely by lutein with 1.1 cm. In case of Rf value of pigments, the higest value 0.94 (94%) was reported in carotenoids, followed by 0.86 (86.33%) of chlorophyll 'a', 0.71 (71%) of anthocyanin, 0.65 (65.33%) of xanthophylls, 0.51 (51.33%) of chlorophyll 'b' and 0.35 (35.66%) of lutein.

In *Ocimum sanctum* Table 2, the pigment carotenoids travelled highest distance i.e., 4.4 cm from the sample load and it is followed by chlorophyll 'a' with 4.3 cm, chlorophyll 'b' with 4.2 cm and xanthophylls with 3.0 cm. In case of Rf value of pigments, the higest value 0.97 (97%) was reported in chlorophyll 'a', followed by 0.91 (91%) of chlorophyll 'b',

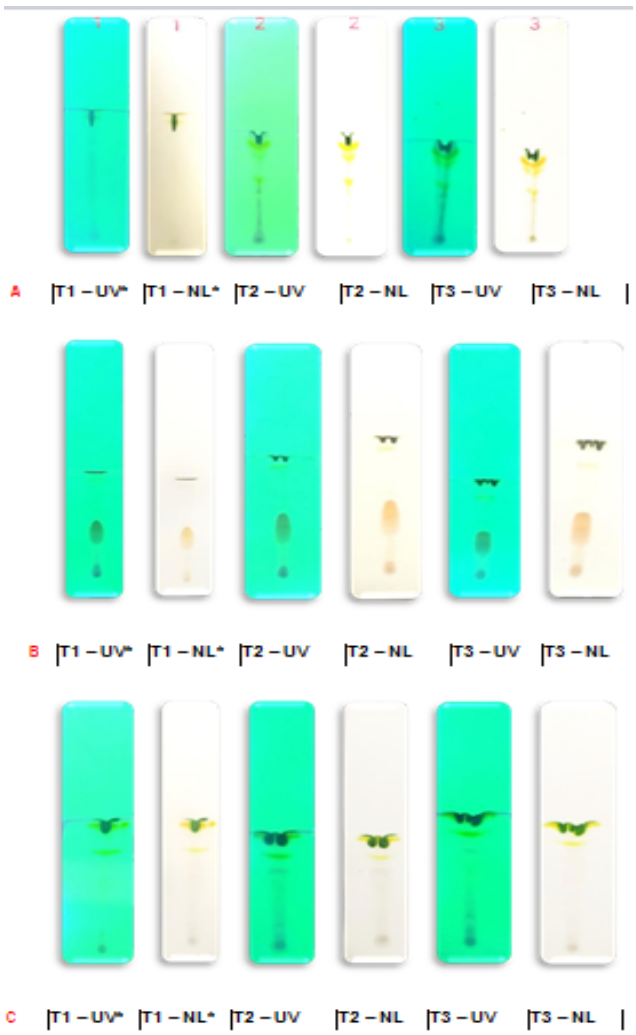


**Figure 3:** Chromatograms of pigments in *Ocimum sanctum* (A), *Lawsonia inermis* (B) and *Piper nigrum* (C) on thin layer of silica gel sheets was obtained with the green leaves are shown.

**Note:** * T1, T2 and T3 – Trail Numbers and UV – Ultraviolet light, NL – Normal light

0.94 (94%) of carotenoids and 0.67 (68%) of xanthophyll. In case of *Lawsonia inermis* Table 2, the pigment chlorophyll 'a' travelled highest distance i.e., 3.5 cm from the sample load and it is followed by carotenoids with 3.3 cm, Lawsonin with 2.4 cm and xanthophylls with 2.0 cm. In case of Rf value of pigments, the highest value 0.95 (95%) was reported in chlorophyll 'a', followed by 0.89 (90%) of carotenoids, 0.66 (67%) of Lawsonin and 0.54 (55%) of xanthophylls. Similarly, Table 2 illustrate that the distance travelled by the pigments, retention factor and percentage of retention factor of *Piper nigrum*. The pigment chlorophyll 'a' travelled highest distance i.e., 3.9 cm from the sample load and it is followed by carotenoids with 3.9 cm, chlorophyll 'b' with 3.6 cm, xanthophylls with 3.2 cm and Anthocyanin with 2.7. In case of Rf value of pigments, the highest value 0.97 (97%) was reported in chlorophyll 'a', followed by 0.96 (96%) of carotenoids, 0.88 (89%) of chlorophyll 'b', 0.80 (80%) of xanthophylls and 0.67 (67%) of Anthocyanin.

Comparatively Table 2, Figures 2 and 3, highest distance travelled pigments are carotenoids (4.3 cm) in *O. sanctum*, followed by chlorophyll b (4.2 cm) in the same plant. The least travelled distance recorded in *C. inerme* of pigment Lutein (1.1 cm) and the same pigment in *T. cardifolia* (1.2 cm) were recorded. Highest number of pigments was found in *C. inerme* with six pigments and least number of pigments was recorded in *O. sanctum* (four) and *L. inermis* (four). Meanwhile, highest Rf value recorded in *O. sanctum* and *P. nigrum* with 0.97 (chlorophyll a), followed by carotenoids in *P. nigrum* with 0.96. Least value recorded in *T. cardifolia* for Lutein with 0.34, it is followed by *C. asiatica* and *C. inerme* with 0.35 of Lutein and Anthocyanin respectively. Similarly, highest percent of retention recorded in Chlorophyll a of *O. sanctum* and *P. nigrum* with 97 % followed by carotenoids in *P. nigrum* with 96 % and least percent recorded in *T. cardifolia* with 34.66 % of Lutein. The same compound in *C. asiatica* and *C. inerme* showed that 35 % and 35.66 % respectively.

Among the seven pigments in six species carotenoids, chlorophyll a and xanthophylls are common to all species but chlorophyll b, lutein, anthocyanin and lawsonin are rare to occur in these six species for example Lawsonin occur only in *P. nigrum*.

Similar kind of work was done on *Phyllanthus acidus*, *P. emblica* and *Citrus limon* revealed that *P. emblica* contain high amount of calcium than the other two varieties. The amount of phosphorous and iron in *C. limon* posses better level than *P. acidus* and *P. emblica* (Suriyavathana and Muthukrishnan, 2011). The dynamics of plant nutrient concentration in leaf petiole sap and carbohydrate accumulation in leaves were studied in walnut tree (*Juglans regia* L.) (Drossopoulos et. al., 1996).

Several procedures have been developed for the separation of the photosynthetic pigments, including column (CC), paper (PC), and thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC) (Hartmut, 1987). The use of thin-Layer chromatography (TLC) for the separation and quantitative determination of natural pigments in various matrices are assembled. The techniques applied for the analysis of individual pigments and pigment classes (anthocyanins, flavonoids, carotenoids, and other pigment classes) are surveyed, critically evaluated (Forgacs and Cserhati, 2002). Simona et al., in 2008 conducts separation of pigments from *Petunia* Juss. petals using TLC. The TLC chromatogram of purple *Petunia*, shown the presence of four spots; in the case of white and violet *Petunia*, the chromatogram shown two spots. The results of the thin-layer chromatography are evaluated and compared with several methods of column and paper chromatography using known compounds. Under the conditions described, as many as 16 compounds can be detected by (Lynn and Schanderl, 1967).

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