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PHYTOCHEMICAL SCREENING AND GC-MS/MS ANALYSIS OF METHANOLIC EXTRACT OF *COLEUS PROSTRATUS*

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ABSTRACT

Coleus prostratus commonly known as “Tangled heart plant or Swedish Ivy” belongs to the family Lamiaceae (Labiatae). This study aims to evaluate the plant by phytochemical studies as well as GC-MS analysis of plant extract. Various types of components and bioactive compounds are found in the herbal preparations and the botanicals which provides opportunities for new drug lead. Integrated approach of the study includes plant identification, extraction followed by phytochemical analysis and GC-MS analysis. Biological or pharmacological activity of plant is mainly depended on the components of plant and the amount bioactive compounds as well as primary and secondary metabolites present in it. Microscopic studies of areal parts of plant were also done and which includes transverse section of leaf and stem, longitudinal section of leaf, powder characteristics. The Soxhlet method were used for extraction and then extract were subjected to Gas Chromatography- Mass Spectrophotometer for the characterization of phytocomponents. Total amount of phenolic compounds, alkaloids and flavonoids were also estimated by spectrophotometric method.

KEYWORDS: Extraction, phytochemicals, Bioactive compounds, GC-MS analysis, characterization, etc.

INTRODUCTION

The family Lamiaceae contains several genera, such as sage (*Salvia*), basil (*Ocimum*) and mint (*Mentha*), with a rich diversity of ethnobotanical uses. Another important genus is *Plectranthus*, a large genus containing about 300 species found in Tropical Africa, Asia and Australia.[1] The potential of phytochemicals to treat human and plant illnesses is substantial. Because of their unmatched chemical diversity, herbal products like plant extracts, whether in their pure form or as standardised extracts, present countless opportunities for the development of new medications. Asia has a

long history of human interaction with nature, which is reflected in the usage of herbal remedies. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[2]

Numerous positive biological benefits have been documented, such as anti-cancer, anti-bacterial, anti-oxidant, anti-diarrheal, analgesics, and wound-healing properties. Most of the people worldwide receive their primary medical treatment from conventional medicine, according to the World Health Organisation (WHO). The WHO also reports that 91 nations, including 12 with notable biodiversity, are home to approximately 20,000 species of medicinal plants. The premier steps to utilize the biologically active compound from plant resources are extraction, isolation and characterization of bioactive compound, pharmacological screening toxicological evaluation and clinical evaluation.[3]

Plants represents the important pool of the drug discovery. Botanicals and plant preparations contain various types of bioactive and pharmacological active compounds and that can be used of medicinal treatment. Phytochemicals isolated from areal parts of plant are safe and broadly effective alternative to the synthetic drug and are with less adverse effects. Characterization of plant extract involves the involve the various analytical methods to identify and quantify the bioactive compound in plant extract.[4] Characterization of a plant extract is a complex procedure which require combination of different analytical methods. Selection of the analytical method is depended upon nature of plant extract as well as the phytochemical or bioactive compound present in it.[5] Characterization is first important step in herbal or plant-based drug discovery and is done by various analytical methods.[6]

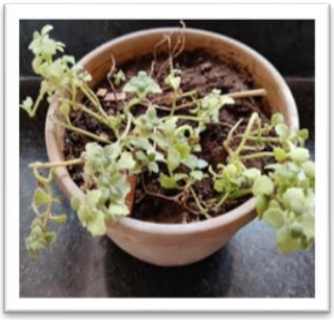

Fig. no. 1: *Tangled heart* Plant.

Plant taxonomy[7]

Common Name: Pillow Plant, Succulent Swedish Ivy

Scientific Name: *Coleus prostratus* (Gürke) A.J. Paton *syn. Plectranthus prostratus*

Kingdom: Plantae - Plants

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida - Dicotyledons

Subclass: Asteridae

Order: Lamiales

Family: Lamiaceae (Labitaeae)

Genus: Plectranthus

Species: *Plectranthus prostratus*/ *Coleus prostratus (Gürke) A.J. Paton*

Preliminary Phytochemical tests

Phytochemicals i.e. in Greek “Phyto” means plant chemicals are the naturally occurring chemicals having positive or negative impact on health. The pharmacological activity of medicinal plant can be determined by the identification and estimation of bioactive phytoconstituents present in plant.[8] Numerous phytochemicals can be found in medicinal plants, which are used to treat a wide range of illnesses and ailments. The phytochemical content of these plants has a significant impact on their medicinal qualities. Numerous phytochemical structures can be found in nature; the main groupings include alkaloids (18%), terpenoids and steroids (27%), and phenolics (45%).[9] Many diseases can now be treated using medications made from the metabolites that plants make to protect themselves from biotic and abiotic stressors. Plants and their phytoconstituents can be characterised and evaluated to validate their medicinal potential in treating a variety of illnesses.[10] Advanced techniques like Gas Chromatography (GC), Liquid Chromatography (LC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC) etc. are very helpful for detection of phytoconstituents both qualitatively as well as quantitatively.[11]

Total Alkaloid content [12,13,14]: The estimation of total alkaloid content by uv-spectrophotometer involves the reaction between alkaloids and bromocresol green (BCG). This method allows for quantitative estimation of total alkaloid content of plant extract providing simple and sensitive approach for assessing the alkaloid level accurately. BCG reacts with the alkaloid having ‘nitrogen(N)’ atom in ring which leads to form the yellow-coloured complex, which is easily extractable by using chloroform at specific pH. The standard calibration curve can be prepared by using atropine solution.

Total Flavonoid content[12,15]: Estimation of total flavonoid content can be done by the aluminium chloride and 2,4-Dinitrophenyl hydrazine colorimetric method. According to the principle, aluminium chloride can form stable acid complexes with flavones and flavanols' C-3 or C-5 hydroxyl groups as well as their C-4 keto group. Furthermore, aluminium chloride forms complexes with the ortho-dihydroxyl group in the flavonoid's A- or B-ring that are sensitive to acid. The principle of, 4-Dinitrophenylhydrazine method is that 2,4-dinitrophenylhydrazine reacts with ketones and aldehydes to form 2,4- dinitrophenylhydrazones.

Total Phenolic content[12,16,17]: The Folin-Ciocalteu reagent (FCR), sometimes called Gallic acid Equivalence (GAE) or **Folin's Phenol reagent** or **Folin-Denis reagent,** is a combination of phosphomolybdate and phosphotungstate. It is frequently employed in the colorimetric analysis of antioxidants that are phenolic and polyphenolic. The reagent functions by determining the quantity of material needed to prevent the reagent from oxidising. It reacts with any reducing agent; therefore, it doesn't precisely quantify just phenolic chemicals. Consequently, the reagent measures a sample's overall reducing capacity instead of just its phenolic concentration**.** This reagent forms part of the Lowry protein assay and will also react with some nitrogen-containing compounds such as hydroxylamine and guanidine.

GC-MS Analysis[18,19]

Gas chromatography and mass spectroscopy are two different analytical techniques in which gas chromatography (GC) is hyphenated with mass spectroscopy (MS). Gas chromatography is the separation science in which separation of various chemical components from mixture and detecting them after, Mass spectroscopy is an analytical technique which measures the mass-to-charge ratio (m/z) of charged particles and can be used to determine the molecular weight and elemental composition, as well as elucidating the chemical structures of molecules.

After being injected into the GC inlet, the sample is evaporated and carried onto a chromatographic column by the helium carrier gas. As the sample passes through the column, the chemicals that make up the mixture of interest are divided according to how they interact with the carrier gas (mobile phase) and the column coating (stationary phase). The column's last section terminates at the entry of the ion source, where substances that elute from the column are transformed into ions, after passing via a heated transfer line. The sample molecules are ionized by an electron beam, forming smaller ions and molecular ions with distinct relative abundances that serve as a molecular structure's "fingerprint." After the ions are separated by the mass analyser, they are detected.

MATERIALS AND METHOD

Plant Materials

Fresh aerial parts of plant were harvested and confirmed the plant material by Head of Department of Botany, "Anekant education society's, Jaysingpur College Jaysingpur. Dist.- Kolhapur, Maharashtra. Harvested fresh aerial parts of *Coleus prostratus* then washed with tap water to remove unwanted material, then that were air dried under shaded condition. Dried materials then powdered to fine particle size.

(Areal parts) **(Powdered material)**

Fig. no.2: Plant materials.

Extraction

Fresh areal parts of plant of *Coleus prostratus* were air dried and powdered it to coarser particle size with mechanical grinder. The samples were then extracted using 150 millilitres of methanol and a Soxhlet device. After passing through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England), the resultant extract was concentrated by letting the solvent evaporate in a fume hood at room temperature. After measuring the extract's yield, the leftovers were kept in the dark for later use.[20]

Solvent for extraction: 150 ml of pure methanol

Temperature of Soxhlet apparatus: 65°C

Weight of powdered material: 15 g

Fig. No. 3: Soxhlet Apparatus.

Preliminary Phytochemical Screening[21]

Table no. 1: Tests for Carbohydrates.

Test	Procedure	Observations
Fehling's test	Add 1 ml of Fehling's solution A + 1 ml of Fehling's solution B and heat the mixture for one minute. Then add 1-2 ml test solution and continue heating for five- ten minutes in boiling water bath.	Firstly, yellow and then red ppt observed.
Benedict's test	In a test tube, mix the test solution and Benedict's reagent in equal amounts. For five minutes, the mixture is heated in a boiling water bath.	Primarily, yellow then brick red ppt is observed.

Table no. 2: Tests for hexose sugar.

Test	Procedure	Observations
Selwinoff's test	In a boiling water bath, heat 1 ml of the test solution and 3 ml of Selwinoff's reagent.	Red colour is formed.
Tollen's phloroglucinol test for galactose	Mix 4 ml of a 0.5% phloroglucinol solution + 2.5 ml of conc. HCl. Heat the mixture after adding 1-2 ml of the test solution.	Yellow to red colour is formed

Table no. 3: Test for saponin glycosides.

Test	Procedure	Observations
Foam test	Shake vigorously the drug extract or dry powder with water.	Foam observed.

Table no. 4: Tests for amino acids.

Test	Procedure	Observations
Ninhydrin test	In a boiling water bath, add 3 ml of the test solution and 3 drops of a 5% Ninhydrin solution. Heat for ten minutes.	Purple/ bluish colour appears.

Table no. 5: Tests for anthraquinone glycosides.

Test	Procedure	Observations
Borntrager's test	3 ml of the extract should be mixed with dil. Sulphuric acid, boiled, and filtered. Add an equivalent amount of benzene or chloroform to the cold filtrate, shake well, and strain off the organic solvent. Ammonia should then be added to the organic layer that has been separated.	Ammoniacal layer shows pink or red colour.
Modified Borntrager's test for C-Glycosides	Add 5 millilitres of 5% FeCl3 and 5 millilitres of diluted HCl to 5 millilitres of the extract. Put the mixture in a boiling water bath and heat it for five minutes. Add benzene or chloroform once it has cooled, shake thoroughly, and then remove the organic layer. The organic layer should next be treated with an equivalent volume of diluted ammonia.	Ammoniacal layer shows pinkish or red colour.

Table no. 6: Tests for alkaloids.

Test	Procedure	Observations
Wagner's test	2-3 ml filtrate of extract + few drops of *Wagner's reagent*	Reddish- brown ppt observed.
Tannic acid test	2-3 ml Test solution + tannic acid solution.	Buff coloured ppt observed.
Mayer's test	2-3 ml filtrate of extract + few drops of May*er's reagent*	Precipitation observed.

Table no. 7: Tests for tannins and phenolic compounds.

Test	Procedure	Observations
Ferric chloride test	2-3 ml of aqueous/ alcoholic extract + few drops of 5% ferric chloride.	Deep blue-black colour.
Dilute nitric acid	2-3 ml of aqueous or alcoholic extract + few drops of dilute nitric acid.	Reddish to yellowish colour.
Iodine solution	2-3 ml of aqueous or alcoholic extract + few drops of dilute iodine solution.	Transient red colour.
Lead acetate solution	2-3 ml of aqueous or alcoholic extract + few drops of lead acetate.	White ppt.

Table no. 8 - Tests for steroids.

Test	Procedure	Observations
Salkowski reaction	2 ml of the extract + 2 ml of $CHCl_3$ + 2 ml of sulphuric acid concentration. Give the mixture a good shake.	Chloroform layer appears red and acidic layer shows greenish yellow fluorescence.
Liebermann – Burchard reaction	2 ml of the extract + chloroform. Next, gently add two drops of concentrated H2SO4 along the test tube's side, followed by 1-2 millilitres of acetic anhydride.	Firstly, red then blue followed green colour appears.

Table no. 9: Tests for flavonoids.

Test	Procedure	Observations
Shinoda test	Plant extract is dissolved in 5ml of alcohol add few Fragments of magnesium + few drops of conc. HCl	Pink to crimson colour
Test with lead acetate	small residue + lead acetate solution.	Yellow coloured ppt is formed.
Sulphuric acid test	66% or 80% of sulphuric acid + test solution, flavones and flavanols dissolves into it.	Gives deep yellow colouration.
Test with NaOH	To test solution, Addition of increasing amount sodium hydroxide, b) Further addition of acid	a) Shows colouration b) Shows decolouration

(NOTE*- All the above observations in the table indicates positive test results.)

Total alkaloid content[12,13,14]

Reagents

Bromocresol green (BCG): Dissolve 69.8 mg of bromocresol green in 3 ml 2N NaOH and 5 ml distilled water. Make up the volume up to 100 ml with distilled water.

Phosphate buffer (pH 4.7): It is prepared by dissolving 71.6 g of sodium phosphate in 1000 ml distilled water to pH 4.7 adjusting with 0.2M citric acid.

Standard atropine solution: Dissolve 1 mg of atropine in 10 ml of distilled water.

Procedure:

1. Fill individual test tubes with 0.4, 0.6, 0.8, 1.0, and 1.2 millilitres of the standard atropine solution.
2. Fill each test tube with 5 ml of BCG solution and 5 ml of phosphate buffer solution (pH 4.7).
3. Give the mixtures a good shake, then use chloroform to remove the yellow-coloured complex.
4. Remove the layer of chloroform and use 10 millilitres of chloroform. Using the blank as a reference, measure each sample's absorbance at 470 nm.

5. Dry the plant material, make a methanolic extract, and dissolve it in 2N HCl. After filtering the mixture, rinse it with chloroform. Use 0.1N NaOH to get the pH down to neutral.
6. To the filtered extract, add 5 ml of BCG solution and 5 ml of phosphate buffer solution (pH 4.7).
7. Give the mixture a good shake, then use chloroform to remove the yellow-colored complex.
8. Measure the absorbance at 470 nm after separating the chloroform layer and adding enough chloroform to reach 10 ml.
9. Utilising the standard atropine calibration curve, determine the concentration of total alkaloids in the plant extract.

Fig. no. 4.

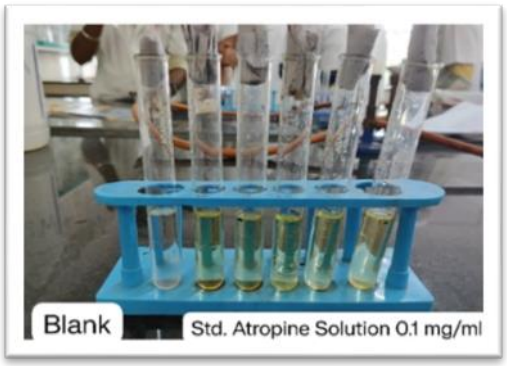

Fig. no. 5.

Total flavonoid content[12,15]

Reagents: Quercetin, aluminium chloride, potassium acetate.

Procedure

1. Create solutions with concentrations ranging from 10 to 100 µg/mL in methanol to create a calibration curve for standard quercetin.
2. Combine 1.5 mL of 95% ethanol, 0.1 mL of 10% aqueous aluminium chloride, 0.1 mL of 1M potassium acetate, 2.8 mL of distilled water, and 0.5 mL of the standard quercetin solution.
3. Let the mixture sit at room temperature for half an hour. Using a UV spectrophotometer, determine the reaction mixture's absorbance at 415 nm following incubation.
4. To make the blank solution, use an equivalent amount of distilled water for the 10% aluminium chloride.
5. In a similar manner, use the calibration method to estimate the flavonoid content after treating 0.5 mL of the plant extract samples with aluminium chloride.

- **Single Beam UV-VIS Spectrophotometer, λ- 415nm**
  - **BioEra –** www.bioeraindia.com

(Fig. no. 6)

Total phenolic content[12,16,17]

Reagents

1. Gallic acid solution (standard) 100 mcg/ml.
2. Folin-Ciocalteu reagent – dilute it with same amount of distilled water.
3. 20% sodium carbonate in water.
4. 1 mg/ml plant extract

Procedure

1. Create solutions with concentrations ranging from 10 to 100 µg/mL in order to create a calibration curve for standard gallic acid.
2. Make plant extract solutions at a concentration of 1 mg/mL. Combine 1.25 mL of a 20% sodium carbonate solution, 0.25 mL of Folin-Ciocalteu's reagent, and 1 mL of each sample.
3. At room temperature, let the mixture react for forty minutes.
4. Mix the materials after the reaction period and compare the absorbance of the blue colour at 725 nm to the standard.
5. Using the calibration curve and the following calculation, determine the total phenolic content as gallic acid equivalent:

Formula:

$$T = \frac{C \times V}{M}$$

[T – Total phenolic content(mg/g), C – Concentration (mg/ml), V – Volume of sample(ml), M – Mass of extract(g)]

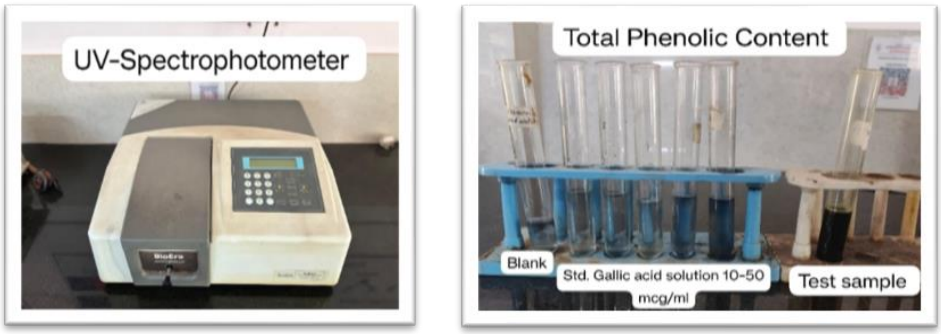

Fig. no. 7.

GC-MS analysis of plant extract

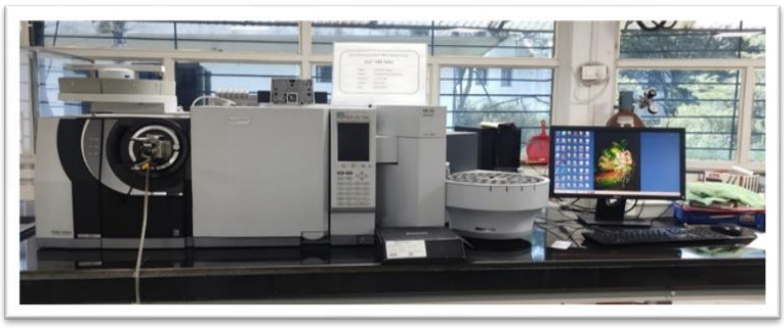

Fig. no. 8: TQ 8050 plus with HS-20 - Shivaji University, Kolhapur.

Equipment Code: **2734616**

Make: **Shimadzu Japan**

Model: **TQ 8050 plus with HS-20**

Institution: **Common facility Centre (CFC)-Sophisticated Analytical Instrument Facilities (SAIF) Kolhapur**

Department: **CFC SAIF DST**

RESULTS AND DISCUSSION

Table no. 10: microscopic characteristics.

Sr. No.	Chemicals	Observations	Characteristics
1	Phloroglucinol + HCl (1:1)	Red/pink	Vascular bundle, lignified fibres
2	Dilute iodine solution	Dark bluish colour	Starch grains
3	Glacial acetic acid	White-pale yellow	Calcium oxalate crystals
4	Sudan red III	Red	Oil globules
5	Alc. Picric acid	Greenish yellow	Mucilaginous cells

Leaf and stem (Microscopic characteristics)[22]

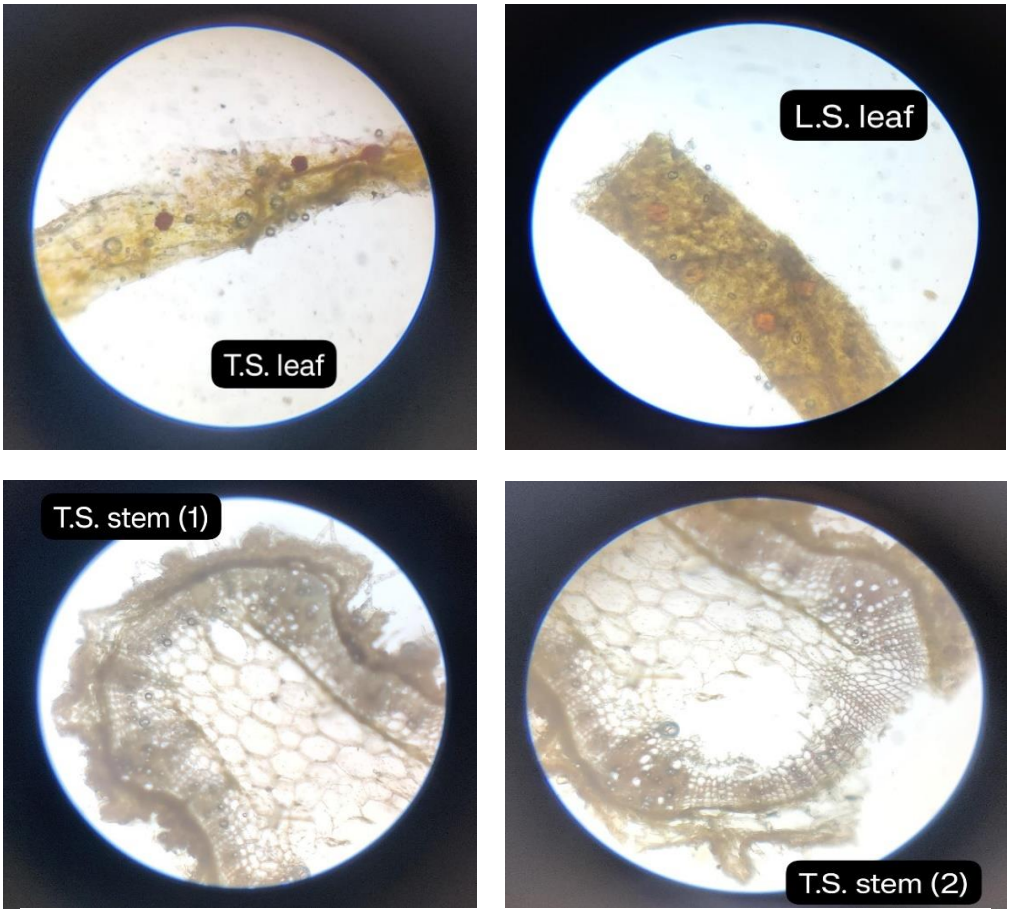

Fig. no. 9: T.S. – Transverse section of leaf & stem, L.S. – Longitudinal section of leaf.

Powder characteristics

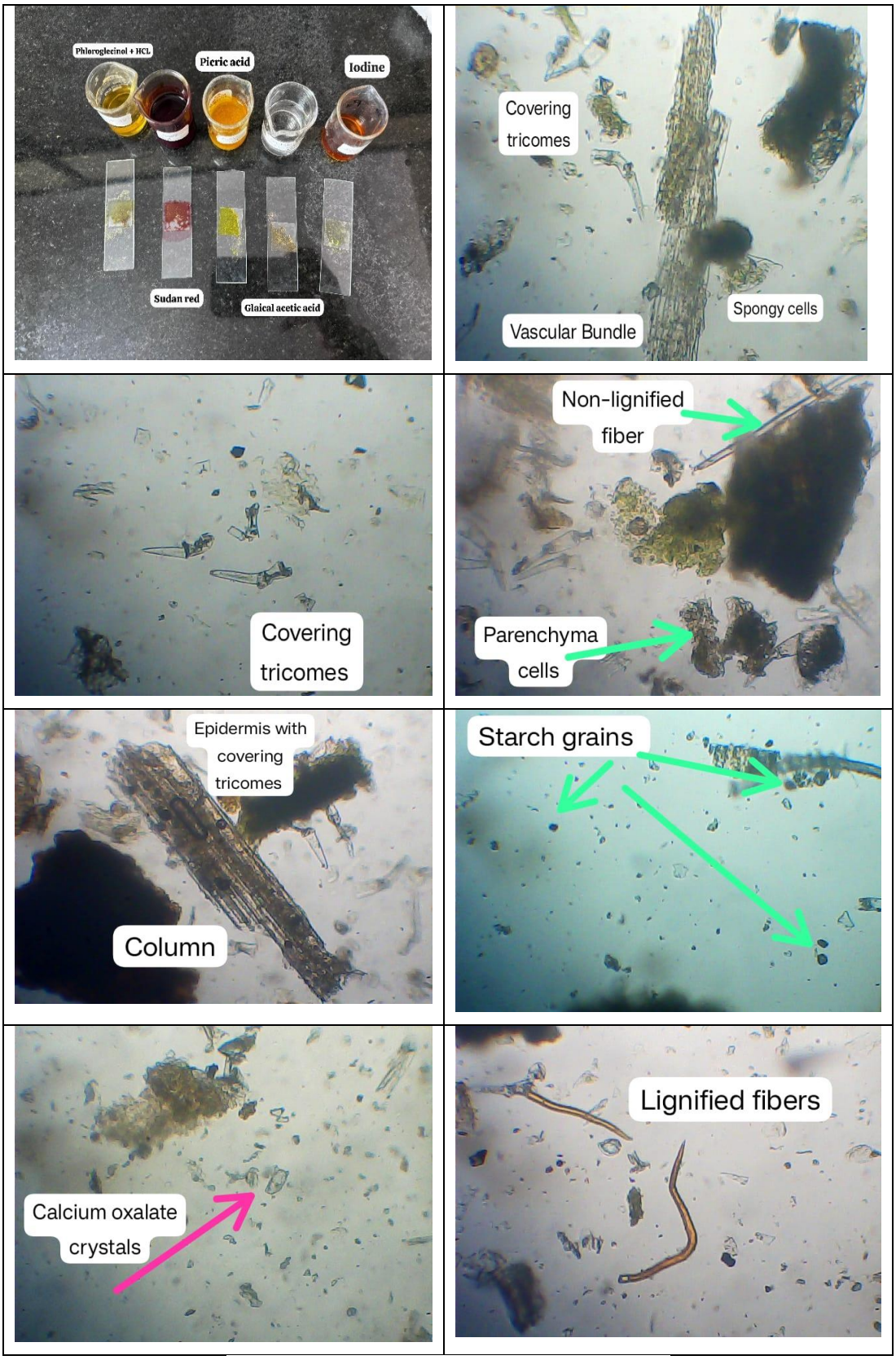

Fig. no. 10: Microscopic powder characteristics.

Table no. 11: Preliminary phytochemical tests.

Sr. No.	Phytochemical	Test	Result
1	**Carbohydrate**	Fehling's test	Positive
		Benedict's test	Positive
2	**Hexose sugar**	Selwinoff's test	Positive
		Tollen's phloroglucinol test	Positive
3	**Saponin glycosides**	Foam test	Positive
4	**Amino acids**	Ninhydrin test	Positive
5	**Anthraquinone glycosides**	Borntrager's test	Positive
		Modified Bontrager's test	Positive
6	**Alkaloid**	Wagner's test	Positive
		Mayer's test	Positive
		Tannic acid	Positive
7	**Tannins and phenolic compounds**	$FeCl_3$ test	Positive
		Dil. HNO_3 acid test	Positive
		Iodine solution test	Positive
		Lead acetate test	Positive
8.	**Steroids**	Salkowski test	Positive
		Liebermann- Burchard Test	Positive
9	**Flavonoid**	Lead acetate test	Positive
		Sulphuric acid test	Positive
		Test with NaOH	Positive

Test for carbohydrates

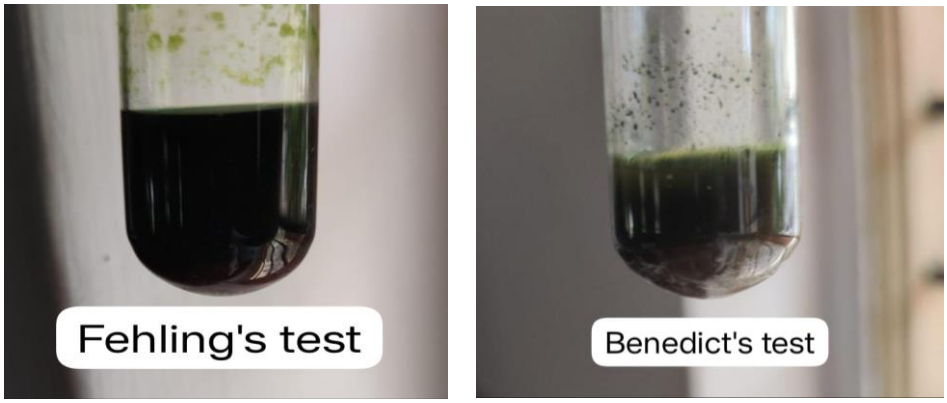

Fig. no. 11.

Test for hexose sugar

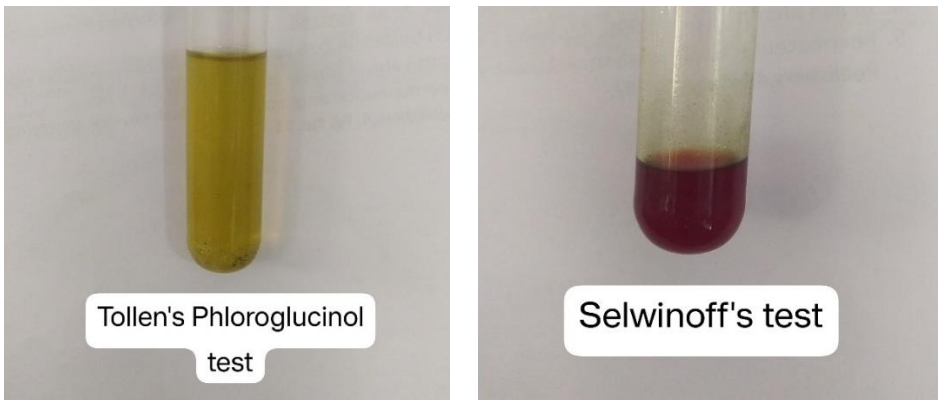

Fig. no. 12.

Test for proteins

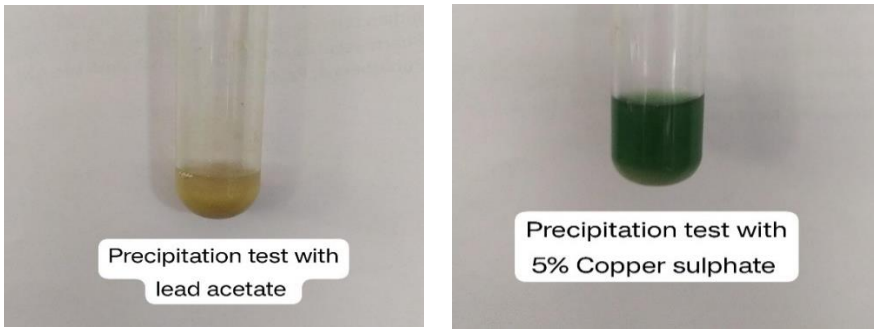

Fig. no. 13.

Test for amino acids: Test for saponin glycosides

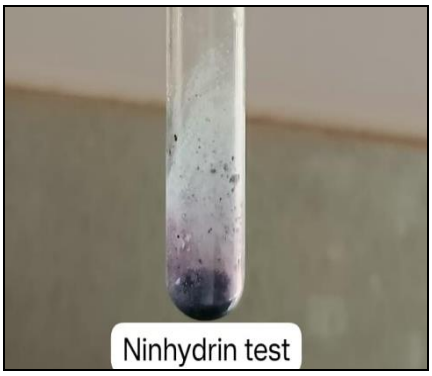

Fig. no. 14.

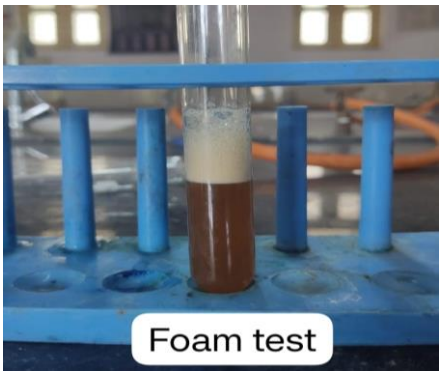

Fig. no. 15.

Test for anthraquinone glycosides

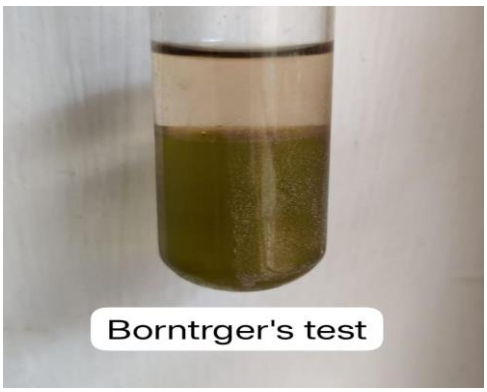

Fig. no. 16.

Test for steroids

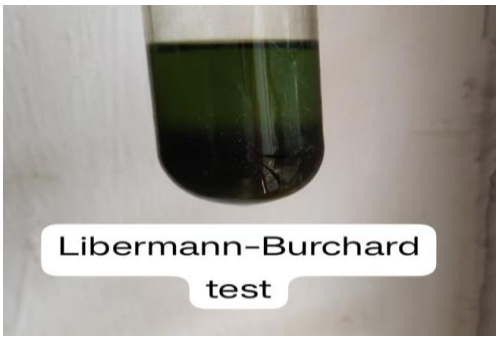

Fig. no. 17.

Test for alkaloids

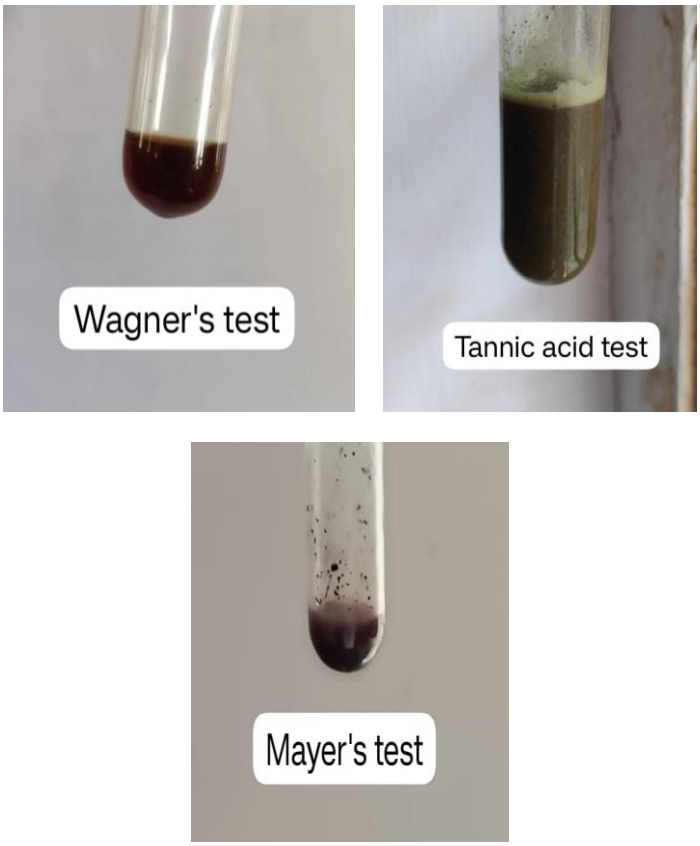

Fig. no. 18.

Test for flavonoids

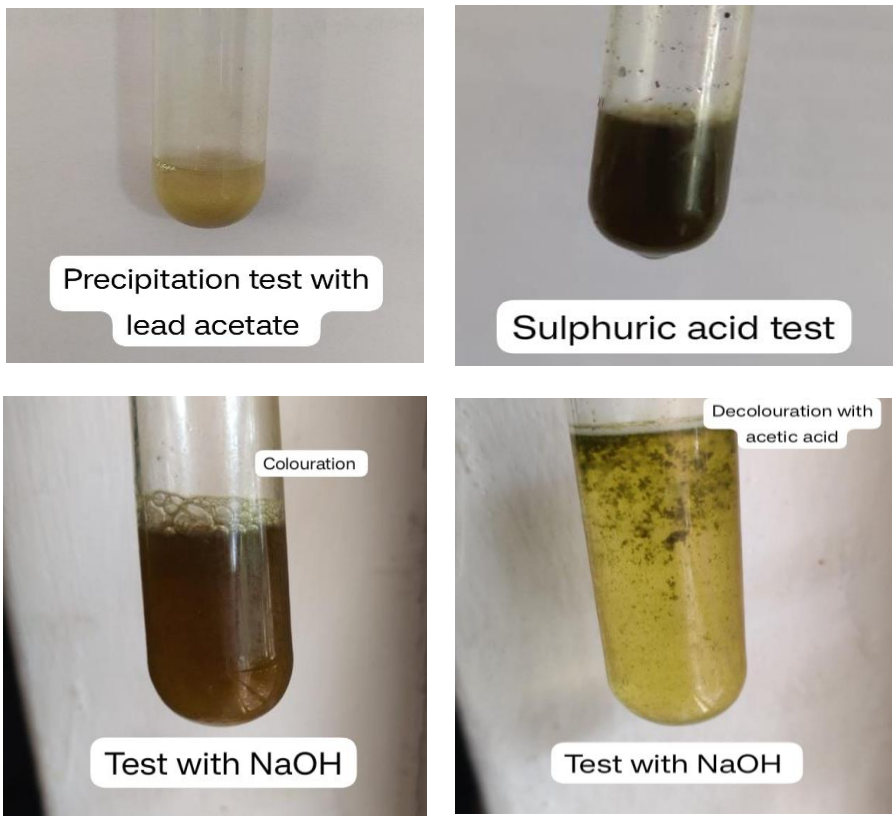

Fig. no. 19.

Test for phenolic compounds

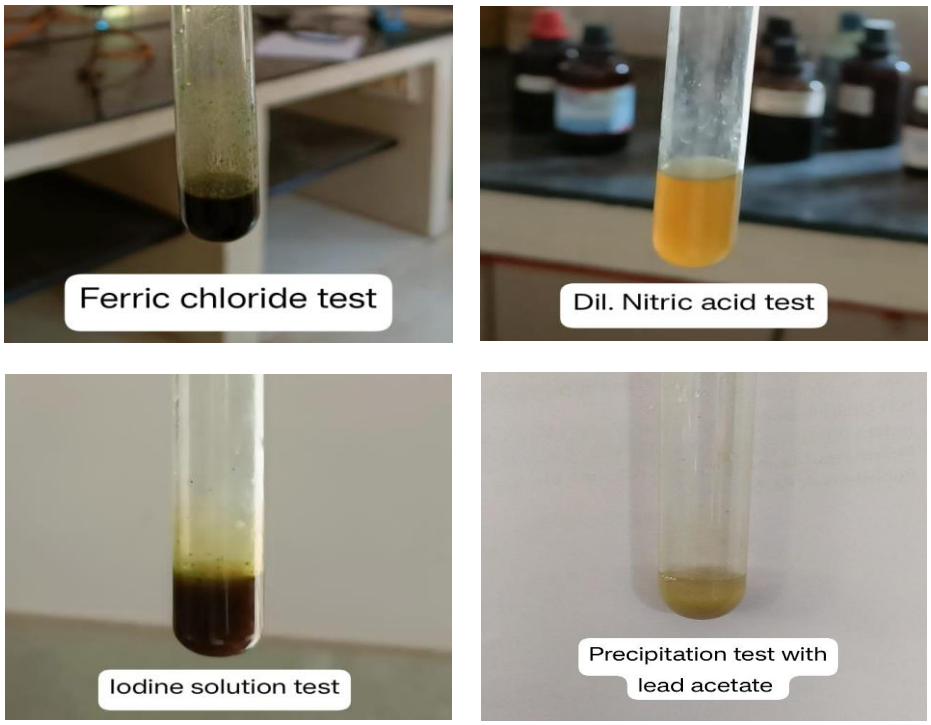

Fig. no. 20.

Estimation of total Alkaloid content

Table No. 12.

Sr. No.	Concentration (ml)	Absorbance			
		1	2	3	mean
1	blank	0	0	0	0
2	0.4	0.113	0.114	0.11	0.112
3	0.6	0.118	0.118	0.111	0.115
4	0.8	0.118	0.11	0.112	0.113
5	1	0.12	0.135	0.122	0.125
6	1.2	0.154	0.158	0.171	0.161
7	test	0.184	0.182	0.182	0.182

Graph

x-axis: conc. of std. atropine solution (ml) y-axis: mean absorbance at 470 nm

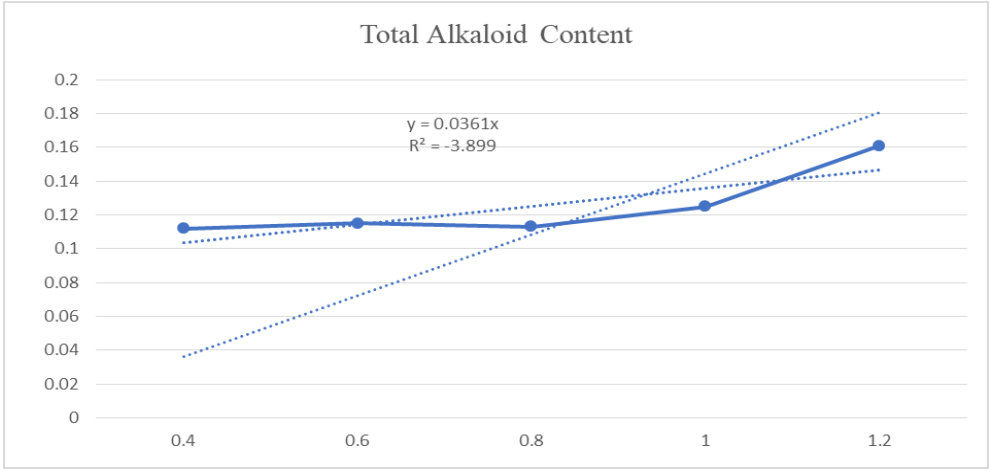

Fig. no. 21/ Graph no. 1.

The **total alkaloid content** from plant extract was found to be **5.04 mg/g.**

Estimation of total flavonoid content

Table No. 13.

Sr. No.	Concentration (mcg/ml)	Absorbance			
		1	2	3	mean
1	blank	0	0	0	0
2	20	0.058	0.059	0.06	0.059
3	40	0.115	0.116	0.116	0.115
4	60	0.182	0.183	0.184	0.183
5	80	0.25	0.251	0.256	0.252
6	test	0.981	0.982	0.981	0.981

Graph

x-axis: conc. of std. quercetin solution (10 mcg/ml) y-axis: mean absorbance at 415 nm

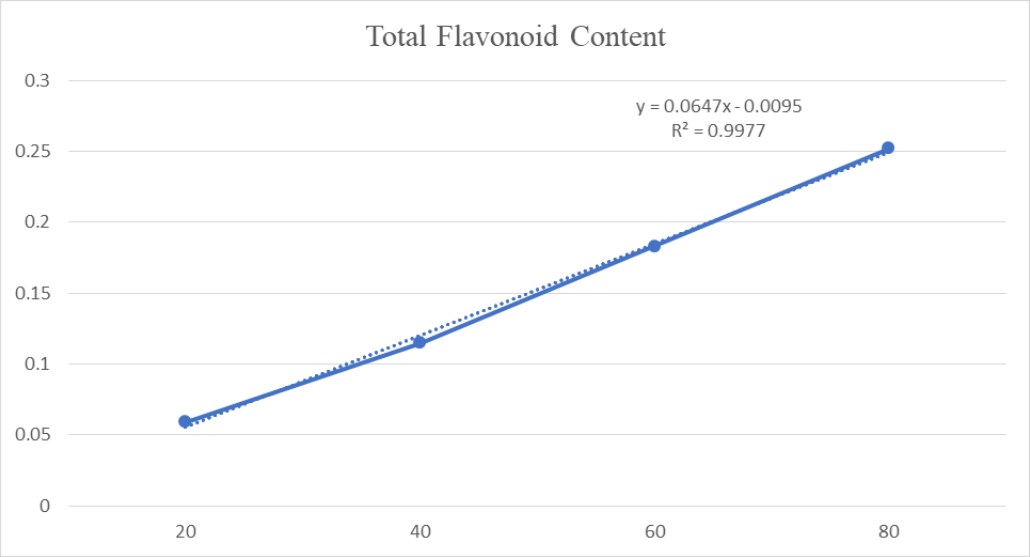

Fig. no. 22/ Graph no. 2.

The **total flavonoid content** from plant extract was found to be **15.30 mcg/g**.

Estimation of Total phenolic content

Table No. 14.

Sr. No.	Concentration mcg/ml	Absorbance			
		1	2	3	mean
1	blank	0	0	0	0
2	10	0.415	0.414	0.414	0.414
3	20	0.416	0.413	0.416	0.415
4	30	0.419	0.417	0.417	0.418
5	40	0.418	0.419	0.419	0.419
6	50	0.423	0.422	0.422	0.422
7	test	0.433	0.43	0.431	0.432

Graph

x-axis: conc. of std. gallic acid solution (100 mcg/ml) y-axis: mean absorbance at 725 nm

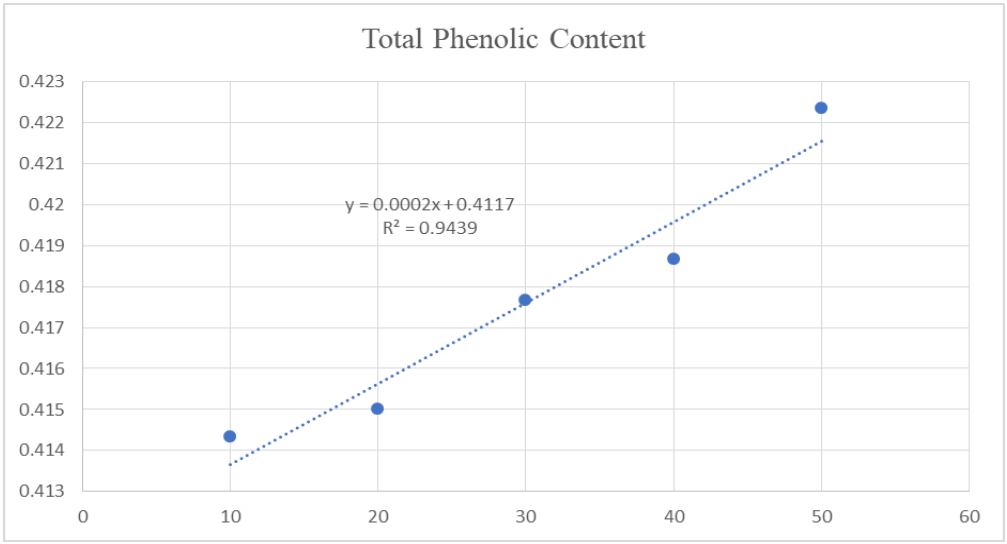

Fig. no. 23/ Graph no. 3.

The **total phenolic content** from plant extract was found to be **6.766 mg/g.**

Chromatogram

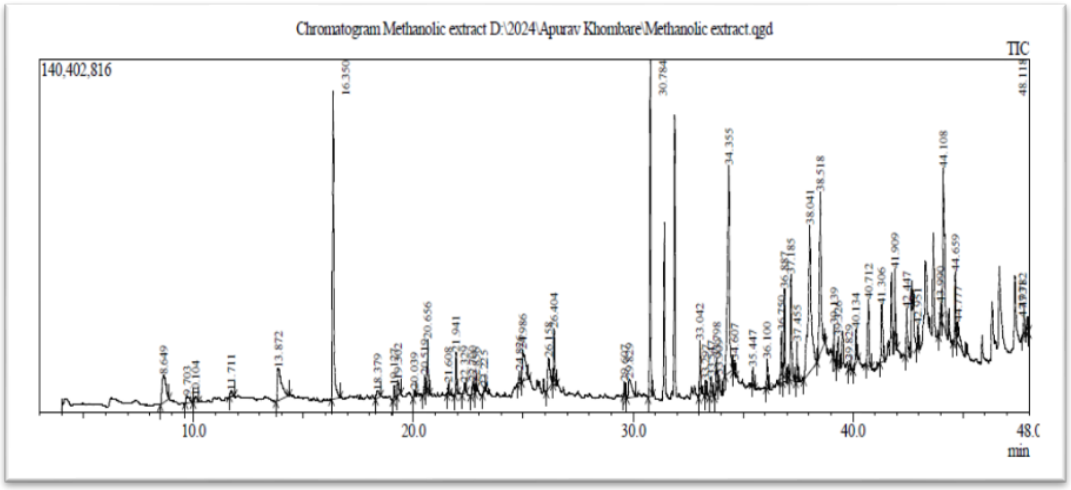

Fig. no. 24/ Graph no. 4.

Table no. 15: GC-MS/MS analysis- Bioactive compounds.

Sr. no.	Retention time	Peak % area	Compound	Molecular formula
1	8.649	1.99	1-Octen-3-ol	C8H16O
2	9.703	0.44	o-Cymene	C10H14
3	16.35	7.89	Thymoquinone	C10H12O2
4	18.379	0.34	4-Hydroxy-2-methylacetophenone	C9H10O2
5	19.302	0.5	Eugenol	C10H12O2
6	20.039	0.13	1-Pentadecene	C15H3O
7	20.519	0.29	(E)-1-(2,3,6-trimethylphenyl) buta-1,3-diene (TPB, 1)	C13H16
8	21.941	0.85	p-Mentha-1,5,8-triene	C10H14
9	24.826	0.32	Megastigmatrienone	C13H18O
10	26.158	1.26	2,5,5,8a-Tetramethyl-3,4,4a,5,6,8a-hexahydro-2H-chromene	C13H22O

11	29.607	0.28	1-Nonadecene	C19H38
12	33.042	1.01	Hexadecenoic acid, methyl ester	C17H34O2
13	33.297	0.26	10,12,14-Nonacosatriynoic acid	C25H42O2
14	33.547	0.56	Isophytol	C20H40O
15	33.798	0.67	Dibutyl phthalate	C16H22O4
16	29.829	1.2	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	C11H16O3
17	35.447	0.28	(7a-Isopropenyl-4,5-dimethyloctahydroinden-Succinic acid	C15H26O
18	37.185	2.65	Phytol	C20H40O
19	37.405	0.7	Methyl stearate	C19H38O2
20	37.760	8.78	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	C18H30O2
21	38.518	5.26	Octadecanoic acid	C18H36O2
22	39.085	0.5	Phytol acetate	C22H42O2
23	39.240	0.63	9,19-Cyclolanost-24-en-3-ol,	C32H52O2
24	39.829	0.16	(3. beta.)-Tributyl acetyl citrate	C20H34O8
25	40.712	1.99	2,3,4,5-tetraethyl-2-Methylhexacosane	C10H22
26	42.951	0.41	geranyl-. alpha. -terpinene	C10H18O
27	43.990	0.29	Hexadecenoic acid, 2-hydroxy-1-(hydroxy meth Phenanthro[3,2-b] furan-7,11-dione	C19H38O4
28	44.659	1.14	1,2,3,4,8,9-Diisooctyl phthalate	C24H38O4
29	44.777	0.80	2,3-diphenyl-Quinoxaline	C20H14N2
30	47.782	0.57	Dotriacontane	C32H66
31	47.931	0.21	Squalene	C30H50
32	48.118	20.20	Lupeol	C30H50O
33	48.608	0.14	2,6,10,14-Hexadecatetraen-1-ol,3,7,11,15-tetramethyl acetate (E, E, E)	C20H34O

DISCUSSION

Coleus prostratus is widely available ornamental plant which contents various phytochemicals and bioactive compounds such as carbohydrates, amino acids, anthraquinone & saponin glycosides, alkaloids, terpenes, flavonoids, tannins, etc. which are extracted using methanol as a solvent. Soxhlet extraction (continuous extraction method) shows high efficiency for extraction of bioactive compounds. The total amount of secondary metabolites i.e. alkaloid content, flavonoid content and phenolic content was found to be 5.04 mg/g, 15.30 mcg/g, 6.766 mg/g respectively. The number of secondary metabolites present in the plant is proportional to potential of showing pharmaco-therapeutic activities. The GC-MS/MS analysis revealed presence of total 58 chemical constituents out of which 33 compounds are found as a natural compound which includes the bioactive compounds such as o-cymene, thymoquinone, lupeol, 1-Octen-3-ol, eugenol, phytol, Isophytol. lupeol acetate, geranyl- alpha- terpinene, etc.

In conclusion, use of an integrated approach which included phytochemical screening, estimation of total amount of secondary metabolites and extraction leads to examine the bioactive potential of plant extracts. Alkaloids, flavonoids, phenols, and terpenoids are among the many phytochemicals noticed in the extracts, indicating their rich chemical composition.

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