Original Article

In vitro Pharmacognostical, Phytochemical and Pharmacological evaluation of Tradescantia spathacea: An exploration

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Abstract

Nature has given us a vast array of medicines to treat various chronic and acute illnesses. It is estimated that over 80% of the global population is reliant on traditional medicine for their main health care. Herbal remedies have been used in medicine for thousands of years as a primary treatment in the traditional medical system and have made significant contributions to human health. Since synthetic medicines' toxicity and side effects are now well known, their limits in many therapeutic areas, their relatively expensive cost, and the lack of readily accessible raw pharmaceuticals have all contributed to a surge in worldwide interest in studying and using raw drugs during the last two decades. Because there is a growing interest in herbal medicine throughout the world, the standardization of herbal medicines is the most desired option at this time. Traditional remedies are used by a significant portion of the world's population. As a result, the economic significance of herbal treatments is quickly rising. In addition to a lack of standardization, unethical commercial practices, including adulterating and substituting real herbal drugs, are posing a serious challenge to the general acceptance of tried-and-true herbal-based traditional treatments. The number of pharmaceutical formulations produced by Vaidyas is related to the number of illnesses to be investigated. Tradescantia spathacea (TS) is used to treat a variety of illnesses. TS is an Indian plant that is used as a traditional medicine and belongs to the Commelinaceae family.

Keywords: Traditional medicine, Tradescantia spathacea, Standardisation, Antioxidant activity, Phytochemical & Pharmacognostical study.

INTRODUCTION

Natural products for the treatment and management of severe illnesses may be found in medicinal plants. The use of plant extracts and isolated pure compounds has provided the basis for the production of herbal medicines and phytopharmaceutical compounds (Evans WC, 2005). For a healthcare product to be globally accepted, it must be scientifically validated to ascertain its level of purity, potency, efficacy, and safety (Alam, F., Us Saqib, Q.N., 2015). Standard criteria, such as physicochemical and phytochemical assessment of crude medicines, have been established by the World Health Organization to evaluate herbal plants' quality, safety, and effectiveness (WHO, 1996). Setting these pharmacognostic criteria to create a crude drug monograph entails a number of stages. The evaluation of medicinal plants' quality is critical in order to justify their acceptance in the traditional medical system. The use of standards, which are numerical characteristics by which the quality of herbs can be evaluated, promotes uniformity of quality. Herbal or "botanical" remedies, which have been documented in developing nations with old civilizations such as Egypt and China, offer a vast Pharmacopoeia of items recommended for various illnesses for millennia. Natural ingredients that underpin traditional treatments have lately gained more scientific study & recognition (Han SS et al., 2002).

India has a plethora of plant life. Over 18,000 species of higher plants are thought to exist in various phyto-geological/ecological areas of the nation, with approximately a third of them being medicinally and commercially significant.

Historically, all medical preparations, whether in the form of a single plant component or a more complicated form of crude extract or combination, were produced from plants. The main advantage of utilizing plant-derived medications is that they are believed to be safer than synthetic equivalents while also providing significant therapeutic advantages and lower treatment costs. Plants were responsible for almost a quarter of all medicines given globally. A substantial proportion of synthetic medicines are derived from natural plant precursors, and 11 percent of the 252 pharmaceuticals deemed basic and necessary by the WHO are entire of plant origin (Prabhu et al., 2021). Plants have been utilized as a significant source of therapy for numerous illnesses since ancient times. A variety of plants have been identified as potential candidates with therapeutic properties. Plants have been utilized by all civilizations across the globe from ancient times, with India being one of the most ancient, wealthy, and diverse cultures (Tandon V et al., 2004).

T.S is a vegetative plant that belongs to the Commelinacea family and is first described in the early year of 1788. It is widely cultivated as an ornamental plant in Belize, Guatemala, and southern Mexico (Chiapas, Tabasco, and the Yucatan Peninsula), and grows wild in Florida, Texas, Hawaii, India, and other marine area countries (Maria, I., 2009). This plant has a sturdy subterranean stem and pink-colored waxy spear-shaped leaves that are extensively spread throughout Mexico. This plant has leaflets that are dull to metallic green on top and bright purple on the bottom. This plant's leaves may grow up to 30 cm long and 7.5 cm wide and have very attractive greenery (Prakash, R., and Rajesh, R., 2014).

METHODOLOGY

MATERIALS AND METHODS

Plant material

All the reagents used in our current study of analytical grade. Fresh T.S plant was collected in summer seasons (May–-Jun) from local areas of Hyderabad, Telangana, India. These parts were botanically authenticated by P.V. Prasanna, Scientist ""G" " and Ho O, Botanical Survey of India, Deccan Regional Center, Hyderabad, Telangana, India. A sample voucher (BSI / DRC/2020-21/ Identification / Tech /66) has been provided by the Department of Pharmacognosy, Geethanjali College of Pharmacy, Hyderabad, India. This authentication voucher will be used for the future reference purposes.

Macroscopic evaluation

The plant's macroscopic characteristics were evaluated. Total of five samples have been used to determine the macroscopic characteristics of a plant. (Kokate, C. K et al., 2019). A taxonomic explanation was documented.

Organoleptic evaluation

By utilizing our natural senses, organoleptic assessments of a plant were conducted to define its characteristics, such as size, taste, color, odor, texture, and shape (Deswal. G, and Saini, V et al., 2015).

Microscopic investigations

The freshly collected leaves were promptly fixed for 24 hours in a solution of formalin, acetic acid, and 70% alcohol (5:5:90). A microtome was used to cut the transverse sections of the leaves. Further, the leaves were chopped into tiny pieces and turned into wax blocks. Further, the leaves were chopped into tiny pieces and turned into wax blocks. The slices were stained and examined under the microscope. (Betim et al., 2020).

Transverse sections

Microscopically, the leaf was examined to identify and elaborate the cell patterns. A thick cuticle layer, top epidermis, chlorenchyma, and lower epidermis were all visible in the leaf's transverse slice (Rashid et al., 2018).

Microscopy of powder

The crude drug was pulverised and combined with chloral hydrate to make microscopic inspection easier. The pinch of dried powder was inspected under a microscope using a glass slide (Adekenov.S.M. et al., 2020, TAPI 1999-2017).

Analysis of fluorescence

The leaves powder was exposed to a variety of chemicals and examined in natural light as well as UV light with short (254 nm) and long (366 nm) wavelengths (Ghosh et al., 2017).

Plant material preparation

T.S leaves were cleaned in tap water and dried in the shade for approximately a week before being protected against degradation. With the assistance of a laboratory mixer, the shade dried leaves were ground into powder. These were dried for approximately a week in the shade and kept safe from degradation before being ground and turned into powder. (Gayathri et

al., and D. Kiruba, 2015, Khandelwal and Vrunda K. Sethi, 2015)

Analysis of physicochemical properties

The swelling index, pH value, extraction values , and ash values of plant powder were analyzed by physical method. Examples of physical and chemical parameters are moisture, total ash, acid insoluble ash, water-soluble ash, and water-insoluble ash. The ash content of whole plants was found to be high, followed by acid-insoluble, water-soluble, and sulfuric ash. Similarly, the greatest concentration of methanol-soluble extractive was found, followed by water-soluble extractive. The soluble extract in chloroform and alcohol is almost identical. On the other hand, N-hexane is less valuable. The moisture percentage is high, and the pH is in the base range (Table 6). (Aslam et al., 2019, Kondalkar. A, et al., 2018).

Calculation of extractive values

Extractives are determined using a variety of solvents. The extraction solvent is capable of dissolving a significant amount of the target material. In a closed beaker, about 5 g of coarsely dried air is soaked in 100 ml of alcohol for 24 hours, shaking periodically for the first 6 hours, and then left to stand for 18 hours. Filtered quickly to prevent alcohol loss, the 25 ml filter evaporates dry in a shallow, flat-bottomed dish, dried at 105 ° C, and weighed (Lamari et al., 2018, Bhargava et al., 2013).

Extractives soluble in alcohol

In a closed Erlenmeyer flask, soaked the five grams of coarse powder in 100 ml of 70% ethanol for 24 hours, often stirring for the first six hours, and then letting alone for 18 hours. It is quickly filtered to prevent ethanol loss. In a petri dish, the 25 ml filter is evaporated dry, dried at 1050 C, and weighed. Air-dried materials are used as an indicator to estimate the proportion of alcohol-soluble extract.

Extractives that are soluble in water

In a closed Erlenmeyer flask, soak five grams of dried drug powder in 100 ml of water for 24 hours, regularly shake for the first 6 hours, and then leave for 18 hours. Whatman grade # 100 filter paper was used to filter it. In a petri dish, the 25 ml filter is evaporated dry, dried at 1050 C, and weighed. For air-dried material, the percentage of the water-soluble extract is determined.

Percentage of water-soluble extractive =Weight of the extract × 100 × 100 / 25 × Weight of the sample taken.

Extractives soluble in methanol

In a closed Erlenmeyer flask, sterilize the five grams of coarse powder with 100 ml of methanol for 24 hours, shake regularly for the first 6 hours, and then leave for 18 hours. Methanol is filtered quickly to prevent damage. In a petri dish, the 25 ml filter is evaporated dry, dried at 105 C, and weighed. The percentage of soluble methanol is determined using air-dried drugs as an indication.

Extractives soluble in chloroform

In a closed Erlenmeyer flask, sterilize five grams of coarse powder with 100 ml of chloroform for 24 hours, shake regularly for the first 6 hours, and leave for 18 hours. This was quickly checked to prevent chloroform from leaking out. In a petri dish, the 25 ml filter is evaporated dry and weighed. The percentage of soluble chloroform extract is determined using air-dried using drugs as an indication.

Extractives soluble in hexane

In a closed Erlenmeyer flask, sterilize five grams of coarse powder with 100 ml of hexane for 24 hours, stirring regularly for the first six hours, and then let stand for 18 hours. It is quickly filtered to prevent the hexane from losing anything. In a petri dish, the 25 ml filter is evaporated dry, dried at 1050 C, and weighed. The percentage of soluble hexane extract is determined using air-dried drugs as an indication.

Moisture content and total solid content are determined as follows:

T.S powder (W2, 2g) was weighed and put in a Petri dish (W1). The heated air was put in the oven at 60°C until the Petri dish weight remained steady (W3). After the sample had reached a consistent weight, it was placed in a desiccator and weighed to estimate the total solids and moisture content using the formula (Wei et al., 2015).

Moisture (%) = (W1+W2)-W3/ W2 x100 Where, W1 = Weight of Petri dish, W2 = Weight of sample, W3 = Weight of dried sample + Weight of Petri dish

The following formula was used to calculate the total solid content:

Total solids (%) = 100 – Moisture (%)

Crude fibre content: Eliminate 2 g of powdered material with petroleum ether to remove fat. Boiled the 2 g desiccant in

200 ml sulfuric acid for 30 minutes with the chips heated after extraction with petroleum ether. Make a greasy plate and put the removed residue in it (pre-weighed W1 dish). Dry for 2 hours at 130 degrees Fahrenheit. Weighed W2 after the plate had cooled in the dehydrator. At a temperature of 600 C, ignite for 30 minutes.

Crude fibre content= Loss in weight on ignition (W2-W1) –(W3-W1) / Weight of sample × 100.

Swelling index:

The volume in milliliters taken by 1 g herbal material swelling under certain conditions is explained by the swelling index. Adding water or bulking agent as described in the test method for each specific herbal ingredient is used to determine it (total, cut or ground). Weight about 1gm of a given sample of crude drug; transferred the sample to 25ml measuring cylinder and fill up to 20ml mark with distilled water in it. Close the measuring cylinder with a stopper and leave it undistributed for at least 24 hours after shaking it well. After 24 hours, observe the measuring cylinder and see how much space the swelling has taken up. T.S volume of genuine medication powder must be at least 10ml.

PH determination:

The pH value was determined using a calibrated pH metre. The test was performed three times, with the average reading being recorded. One by one, a ten percent solution of the sample was added, and the reading was taken.

Determination of ash values:

Measuring ash levels can be used to determine the high volume of low-quality goods, expired drugs, and sand or clay materials. All types of ash, total insoluble ash, water-soluble ash, and sulphur ash are used for crude drug detection (Ghasemzadeh et al., 2014, Baidoo et al., 2019).

Total ash:

For crude medicines that have been mixed with different mineral substances such as sand, dirt, calcium oxide, chalk powder or other pharmaceuticals with various inorganic components to improve their appearance, such as T.S, total ash is useful for detecting these mixtures. Higher temperatures might destroy volatile, alkali, and chlorides; therefore, the maximum ash temperature should be limited to 450°C.

Percentage of total ash = Weight of ash / Weight of sample taken × 100

Acid insoluble ash:

Acid insoluble ash is acid ash that is insoluble in dilute hydrochloric acid. Calcium oxalate is found in most raw drugs, and the amount of calcium oxalate varies greatly. As a result, the total grey real samples of the raw drug vary greatly between drug samples. The total ash is ineffective in detecting earthy materials adherent to such medication in this instance. For T.S, acid insoluble ash would be preferred. When the ash is treated with hydrochloric acid, the calcium oxide or carbonate produced by the burned oxalate will be soluble; the remaining ash, known as the acid insoluble ash, is weighed. This allows us to identify the presence of excessive earthy stuff, which is more likely to occur with roots and highly pubescent leaves.

Percentage of Acid-insoluble ash =Weight of the Acid - insoluble residue / Weight of the sample × 100

Water-soluble ash:

For this experiment, we used an empty glass-stopper beaker and added 4 g of water-soluble ash to it. Prior to departing, 100 ml of distilled water was added to the mixture and mixed on a regular basis for 18 hours. The extract is filtered quickly to prevent any solvent damage. The 25 ml filtrate is pre-filtered into a 100 ml flask and evaporated dry in a water bath. A hot air oven at 1050 C was used for six hours before the beaker was quenched in a desiccator before the final weight was calculated. By giving an average value, the experiment was run two times more. The following formula was used to do the computation.

Total Ash weight – water insoluble residue in total ash / Weight of the sample × 100\

Sulphated ash:

The ash is made by adding sulphuric acid to produce sulfate salts, and the percentage of ash is determined using the air-dried medication as a reference. This was done at a temperature of above 600°C.

Extraction

Ultrasound-assisted extraction (UAE)

Ultrasound modifies the material's physical and chemical characteristics and breaks the cell wall, enabling chemicals to leak out and large amounts of solids to be transferred between cells. The ultrasound frequencies range used is from 20 kHz to 2000 kHz. The mechanical impact of ultrasound-induced acoustic cavitation enhances the surface contact of solvents and samples and the permeability of the cell wall. The method is a straightforward and relatively low-cost technology that may be

utilized for phytochemical extraction on a small or big scale. (Lamari et al., 2018, Wei et al., 2015).

Screening for Qualitative Phytochemicals (Ciura et al., 2017, Akhtar et al., 2018, Olivier et al., 2017).

Plant metabolism should be used in systematic and thorough research on raw drugs to encompass primary and secondary metabolic tests. Different qualitative chemical tests must be carried out in order to develop a profile of a particular extract's chemical makeup. The presence of different phytochemicals in the obtained extracts was determined by qualitative phytochemical screening.

Chemical investigation of crude extracts of T.S

Chemical analysis of T.S substances was performed to identify various plant components in the raw materials. The Following chemical tests and thin layer chromatography study were performed for extracts. (Yangui et al., 2021, Seck et al., 2021., The ayurvedic pharmacopoeia, 1996, Manivannan et al., 2021, Dinakaran et al., 2018 and Alam et al., 2015)

1) Test for Alkaloids

The extract is prepared by adding a few cubic centimeters of mild hydrochloric acid to the powder and filtering it. The filterate was analyzed for alkaloids using the following factors:

Dragendorff's test: Add a few drops of Dragendorff's reagent to 2-3 ml of filterate.

Mayer test: 2-3 drops of mayor reagent were added to a few ml filterate along the edges of the test tube.

Wagner's test: 2-3 drops of Wagner's factor were added to a few ml filter along the edges of the test tube.

Hager test: 2-3 drops of Hager's factor were added to a few ml filter along the edges of the test tube.

2) Carbohydrate Test:

Two to three milliliters of an aqueous extract were combined with two drops of an alcohol-naphthalene solution and mixed and added H_2SO_4 through the test tube's edge.

Test for sugars:

Fehling's test: 1 ml Fehling's A and 1 ml Fehling's B solutions were combined, and an equal amount of test solution was added after they had boiled for one minute. 5-10 minutes in a boiling water bath.

Benedict test: In one test tube, Benedict's factor and an equal amount of test solution are mixed. 5 minutes in a hot water bath.

Test for iodine: 3 mL test solution was combined with a few drops of weak Iodine solution.

3) Protein test:

(a) Million tests: 2 drops of 2 ml factor were applied to a 2 ml filter.

(b) Burette test: Two drops of 4 per cent NaOH were added to 3 ml of filtrate and then treated with two drops of 1 per cent $CuSO_4$ solution.

4) Amino Acid Test: Add 3 drops of 5% ninhydrine reagent in 3 ml filter and cook in boiling water bath for 10 minutes.

5) Perform a steroid test: The Liberman-Borchard test used a tiny quantity of the extract dissolved in chloroform. Cold acetic anhydride (1-2 ml) was added and well mixed. Then, around the test tube's walls, 2-3 drops of cooled conc. H_2SO_4 were applied.

Salkowski test: A few drops of concentrated H_2SO_4 were added to the chloroform extract and stirred vigorously before allowing to stand.

6) Determine the presence of tannins and phenolic compounds:

5% ferric chloride test: We used 50 mg of extract in 2 ml of distilled water and stained the solution with a 5 percent neutralised ferric chloride solution after adding 2 drops of that solution.

Lead acetate test: 50 mg of the extract is diluted in 2 ml of distilled water and 3 ml of a 10% lead acetate solution is mixed in.

7) Testing for glycosides:

Anthraquinone glycosides test

Bantrager test: Before filtering, about 50 mg of the extract was hydrolyzed in a water bath with 2 ml of strong hydrochloric acid for 2 h. The chloroform layer was separated and a 10% ammonia solution was added after 3 mL of chloroform was added and mixed into 2 mL of aqueous filter solution.

Saponin glycosides may be detected using the following test:

Test for foam: After dissolving 50 mg of the extract in 2 ml of alcohol, 20 ml of distilled water was added and the mixture was agitated for 15 minutes.

Flavonoid glycosides should be tested:

Shinoda test: 50 mg extract, 5 ml 95% ethanol, 2-3 drops of strong hydrochloric acid, and 0.5 g magnesium nitrate were added. The presence of flavonoids is indicated by the formation of pink color. After dissolving 50 mg of the extract in 2 ml of alcohol, an increased amount of NaOH was added to the extract.

Coumarin glycosides may be detected using the following test:

A) Coumarin glycosides have an odor that is fragrant.

B) When an alcoholic extract is made alkaline, it fluoresces blue or green.

Study of Thin Layer Chromatography (TLC (Baidoo et al., 2019).

A compound's Rf value under certain circumstances is distinctive and may be used to help identify it. As qualitative analysis of extracts of crude medicines are produced and chromatographically compared to any accessible standard reference. TLC profiles of crude drug extracts produced with a specified solvent system and other characteristics may be utilized as fingerprints in qualitative comparisons of herbal medicines. Because of the technique's simplicity and repeatability, this style of herbal assessment is gaining popularity. It may be used to analyze alkaloids, glycosides, isoprenoids, steroids, sugars, and other compounds. Precoated metal plates are used for chromatography. Different solvent systems were used based on the type of the chemical to be examined.

Saturation of Chamber: A sheet of filter paper was placed inside the chamber to cover three sides and was soaked in the solvent solution. To guarantee that the chamber was saturated, it was kept undisturbed for 45 minutes.

Test spots are applied as follows: Prepare a solution of T.S leaf. With the aid of a tiny capillary tube, identical-volume dots were placed 2 cm distant from the plate's bottom border. After each application, the solvent was allowed to evaporate by air drying.

The chromatogram was developed by placing the spotted plates vertically in the room with the lower edge submerged in the rising media. The solvent system was allowed to flow for about 8 cm before the plates were removed and the solvent front was noted.

The resolution of components in all T.S leaf may be examined by finding different locations on the chromatogram. The locations of the dots were first determined visually, then in a UV chamber at 365 nm. Different chemicals glow differently under UV light, so plates were developed in the iodine chamber to detect stains.

1. Steroid detection: Presence of steroids on TLC plates was detected by spraying with antimony trichloride or vanillin - sulphuric acid reagents.

Antimony trichloride reagent: 15-20 ml of a 20% antimony trichloride solution in chloroform was sprayed over the TLC plate and heated for 5-6 minutes at 100°C. The steroidal dots are usually violet or brown in visible light.

Solvent system is Toluene: Ethyl acetate (4: 1).

Spraying with two solutions: 5% ethanolic sulfuric acid (solution I) and 1% ethanolic vanillin (solution II). 10 ml of solution I was sprayed vigorously on the plates, then 5-10 ml of solution II. The plates were heated at 1000 C for 5–10 min before being tested in visible light. The steroid spots gave blue or violet color. The retention factor (Rf) values of the different bands were then calculated using the equation:

Rf = Distance of spot from origin /Distance of solvent front from origin

2.Flavonoids:

The solvent front was allowed to travel until about 1 cm from the top end. The TLC plates were removed, and the solvent front was marked using a soft pencil. They were air-dried and then sprayed with a fine spray of 1% ethanolic aluminium chloride solution, left to dry, and then visualized under UV light at 365 nm. TLC plate was heat for 5-6 minutes at 100°C. The steroidal dots are usually violet or brown in visible light. solvent system is Methanol: Chloroform: hexane (7:2:1, v/v/v). The plates with dried samples were gently lowered into the development tank, closed, and left to develop. The plates were removed from the development chamber when the solvent front had traveled three-quarters of the 'plates' length. The position of the solvent front was immediately marked with a soft pencil. The different bands' retention factor (Rf) values were then calculated.

3. Phenolic compounds:

The solvent front was allowed to travel until about 1 cm from the top end. The TLC plates were removed, and the

solvent front was marked using a soft pencil. They were air-dried and then sprayed with a fine FeCl3 reagent (Green) spray, left to dry, and then visualized under UV light at 365 nm. TLC plate was heated for 5-6 minutes at 100°C. The steroidal dots are usually violet or brown in visible light. the solvent system was Ethyl acetate: Formic acid: Acetic acid: water (100: 11: 11: 26 v/v/v/v). The plates with dried samples were gently lowered into the development tank, closed, and left to develop. The plates were removed from the development chamber when the solvent front had travelled three-quarters of the 'plates' length. The position of the solvent front was immediately marked with a soft pencil. The retention factor (Rf) values of the different bands were then calculated

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Rf = Distance of spot from origin / Distance of solvent front from origin

Quantification of secondary metabolites

Antioxidant properties

Plants contain powerful antioxidants, according to many studies, and they are a significant source of natural antioxidants. The word "antioxidant" refers to a wide range of vitamins, minerals, and phytochemicals' capacity to defend against reactive oxygen species' harmful effects (ROS). ROS are implicated in a variety of physiological processes and illnesses, including ageing, cancer, and atherosclerosis, due to their propensity to react with and harm numerous structures in the body.

Due to their ability to donate electrons, free radical scavenging is the main mechanism of flavonoids' antioxidant activity. Several flavonoids and other phenolic compounds are considered antioxidants not only for their free radical scavenging activity but also for the fact that they chelate metals, increasing antioxidant capacity. Reactive oxygen species (ROS) or antioxidant enzyme deficiency may be induced by various environmental, physical, and chemical stressors on cells. As a result of reactive oxygen species, cells develop abnormalities, including protein depletion, enzyme inactivation, DNA alteration, and lipid peroxidation.

There are many in vitro and in vivo test techniques available in the scientific literature for determining the ability of different compounds to remove free radicals. Antioxidants fall into three classes based on their ability to eliminate free radicals: strong, moderate, and weak antioxidants (Can Ağca et al., 2021, Herrera-Calderon et al., 2016)

In vitro techniques are qualitative and are used to determine whether or not a substance is an antioxidant. To measure the activity, IC50 values (concentrations that produce 50% radical scavenging) or Trolox equivalents (free radical scavenging in terms of Trolox) may be employed.

ANTIOXIDANT ACTIVITY METHODES (Herrera-Calderon et al., 2016 et al., 2016, Al-matani et al., 2015, El Babili et al.,2021).

1) Reducing power by FeCl3

Preparation of standard solution

After diluting 10mg of ascorbic acid in 10ml of deionized water five times, the resulting concentrations are 20, 40, 60, 80, and 100g μg/ml.

Test sample preparation

The test materials were dissolved in a small amount of methanol, and phosphate buffer was used to make a volume of up to 10ml. All samples were diluted in a 10 ml volumetric flask with phosphate buffer to achieve concentrations of (20, 40, 60, 80, and 100 μg/ml). Reagents must be prepared freshly before used.

Phosphate buffer: I.P. produced a 0.2M phosphate buffer with a pH of 6.6.

1% Potassium ferricyanide solution:

2 g of potassium ferricyanide were dissolved in 200 milliliters of distilled water to make a 1% potassium ferricyanide solution. Trichloroacetic acid, 10%: 40 g of Trichloroacetic acid were dissolved in 400 milliliters of distilled water. 0.1 percent ferric chloride solution was prepared by dissolving 0.1 g of ferric chloride in 100 ml of distilled water.

Protocol for Reducing power

2.5ml of 1% percent potassium ferricyanide solution was spiked into 2ml of each sample and reference solution. The mixture should be maintained at 500 C for 20 minutes in a water bath. After cooling, trichloroacetic acid ranging from 2.5 percent to 10 percent was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. It's given for 10 minutes, along with 2.5 ml of distilled water and 1 ml of 0.1 percent ferric chloride. The control was made in the same way as the samples but without the samples. At 700 nm, the Absorbance of the resultant solution was measured. The outcomes are shown in the graph below (Table-9,10 and Fig-15,16)

2) DPPH free radical scavenging activity

Preparation of standard solution: In methanol, the ascorbic acid required to produce concentrations of 20, 40, 60, 80, and 100 µg/ml is dissolved.

Test sample preparation: To make sample stock solutions, 10 mg of dried methanol root and methanol leaf extract were dissolved in 10 ml of methanol, and 1 mg/ml was added. The DPPH solution containing 4.3 mg was dissolved in 3.3 ml methanol, and the test tubes were shielded from light using aluminium foil.

Protocol for estimation of DPPH Scavenging activity (1,1-Diphenyl-2-picryl-hydrazyl assay)

Different levels of test sample volume (20, 40, 60, 80, 100 µg/ml) were examined and 100 liters were produced for each dose level by dilution with methanol. Each test tube is filled with 150 ml of DPPH solution, which is diluted 3 times with methanol. For the control reading, 150 mL of DPPH solution was added to 3 mL of methanol and the absorption at 516 nm was measured immediately. After 15 min, the absorption was measured at 516 nm on a UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) using a methanol blank. IC50 and percentage reduction were determined: Each experiment was repeated three times. The results are depicted in (Table-11, 12, and fig-17, 18). The free radical scavenging activity (FRSA) was calculated using the equation below (ratio of anti-radical activity):

% Antiradical activity =Control absorbance - Sample absorbance/ Control Absorbance X100

3) Iron chelation activity

Antioxidant activity is measured by iron chelation activity. At room temperature, the extract and ascorbic acid solution (2 mL per 5% v / v methanol) were incubated with the O-finantrolin methanol solution (1 mL, 0.05 per cent w / v) and ferric chloride solution (2 mL, 200 m) for 10 min. The absorption of solvents was measured at 510 nm after incubation. The tests were carried out three times.

Principle Fe2+ chelating ability

Through fentanyl chemistry, divalent transition metal ions play an important role as catalysts in the oxidation processes leading to the production of hydroxyl radicals and hydrogen hydroxide decomposition reactions. Iron, a transition metal that may generate free radicals from peroxides, may have a role in human cardiovascular disease. Because Fe2+ produces oxyradicals and promotes lipid peroxidation, lowering its content protects against oxidative damage. In the presence of additional chelating agents, the formation of ferroin complexes is disturbed, resulting in the reddening of the complexes. The absorbance of the Fe2+-ferrozine combination reduced dose-dependently, indicating that activity increased as concentrations rose. The outcomes are shown in the graph below (Table-13,14 and Fig-19, 20).

5) Determination of Hydrogen Peroxide (H2O2) Scavenging Activity of Plant Extract

A modified technique was used to estimate plant extracts' hydrogen peroxide scavenging activity. A 4mM solution of H2O2 was produced (PBS, pH 7.4). H2 O2 concentration is measured using spectrophotometry at 230 nm. In 4 ml of distilled water, 1 mg / mL of plant extract equivalent to 50,100,150,200,250 L of plant extract was added to 0.6 mL of hydrogen peroxide. PBS contains the solution. After ten minutes, the absorption of H2O2 at 230 nm was evaluated in comparison to an empty solution containing the plant extract in PBS without H2O2. After 10 min, the absorption is measured against the empty solution similar to the previous one. The results are shown in (Tables 15,16 and Figures 21, 22).

% H2O2 Scavenging = Absorbance of control – Absorbance of test/ Absorbance of Control × 100

6) Determination of total phenol content (Al-matani et al., 2015, Baba et al., 2015)

The total phenolic component content was determined using the Folin-Ciocaltiu method. For 5 minutes, the extract samples (0.5 ml of different dilutions) were combined with FolinCiocalteu agent (5 ml, diluted with 1:10 distilled water) before being added to aqueous Na2CO3 (4 ml, 1 M). The phenols were detected at 765 nm using a colorimetric method after the mixture had been allowed to stand for 15 minutes. The gallic acid titration curve was used to determine the total phenol content, and the results were reported in gallic acid equivalent (mg gallic acid / g extract). For the reference curve, methanol solutions of gallic acid were used: water at 50, 50, 100, 150, 200, 250, and 300 µg/ml (50:50, v / v). Total phenol levels are specified in gallic acid equivalent units, the most widely used standard. The results are summarized (Table 17,18 and Fig. 23, 24).

7) Total flavonoid content (Al-matani et al., 2015, Baba et al., 2015)

Extraction stock solutions (1 mg/ml) were produced at DMSO. 200 liters of each extract were combined with 100 litres of aluminium chloride (Alcl3) and 100 litres of 1 M potassium acetate (CH3COOK) solution from stock solutions. 4.6 ml of distilled water was used to dilute the resulting formulation. The standard (1 mg/ml) was diluted in the same manner, and serial dilutions were performed (10, 20, 40, 80, 100, and 120 µg/ml). The standard used is quercetin. At 37 degrees Celsius, all of the solutions were incubated for 30 minutes before being observed using a UV-visible spectrophotometer at 415 nm. Using the

quercetin titration curve, the total flavonoid content was calculated in mg quercetin equivalent (per gram of extracted flavonoid content) and reported.

RESULTS

Macroscopic evaluation

Taste, texture, form, size, fracture, odor, and color of fresh and dried T.S. Macroscopic characteristics of Leaves, stem, root, and flower were examined and presented in Table 1, Table 2, Table 3, and Figures 1, 2 and 3.

Microscopy investigations

The fresh stems were promptly fixed for 24 hours in a solution of formalin, acetic acid, and 70% alcohol (5:5:90). The stems are then chopped into tiny pieces and turned into wax blocks. Microtome was used to cut transverse sections of the stems (Schweingruber, Börner, & Schulze, 2011). Under the microscope, the slices were stained and examined (Johansen, 1940).

Transverse sections

Microscopically, the leaf was examined to identify and elaborate the cell patterns. A thick cuticle layer, top epidermis, chlorenchyma, and lower epidermis were all visible in a transverse slice of the leaf (Figures 4, 5).

Microscopy of powders

By analyzing dry microscopy, microscopic research has helped to identify the presence of different types of cells in the dry stem. Pollen, fibrils, exocarp, calcium oxalate crystals, stomata, collenchyma, and parenchyma tissues were detected using powder microscopy. (Table 4 and Figure 6 to Figure 11).

Table 1: Macroscopic characters of Leaves

Leaf	Alternate, Overpopulated, enlarge (Fig-1)
Shape	Pointed, elongated, sword-shaped
Size	Length and width 30-40cm and 4-6cm
Texture	Rough and stiff
Apex	Narrowed or pointed end
Colour	Dark green on the upper surface and rosy purple on the leaf lower side
Taste	Bitter
Margin	Entire
Surface	Both surfaces are smooth
Odour	Characteristics

Fig-1: Leaves of T.S

Table 2: Macroscopic characters of Root and stem

Root		**Stem**	
Root	Branched	Stem	Small (Fig-2)
Shape	Small thin root lets	Shape	Short stout erect
Size	1to 1-5 cm in diameter	Size	20 cm long
Margin	Entire	Margin	Rough
Base	Furrows	Base	Simple
Surface	Dull greay	Surface	Rough
Colour	Balck to brown	Colour	Green
Taste	Bitter	Venation	Similar
Odour	Characteristics	Odour	Characteristics

Fig-2: Roots and Stem of TS

Table 3: Macroscopic characters of Flower

Flower	Auxillary lymph hubs
Colour	White and silver
Odour	Characteristics
Taste	Bitter
Shape	Fan-shaped and oval
Leaflets	3
Stamens	6
Ovary	3-cell, cell 1 ovary

Fig 3: Flower of TS

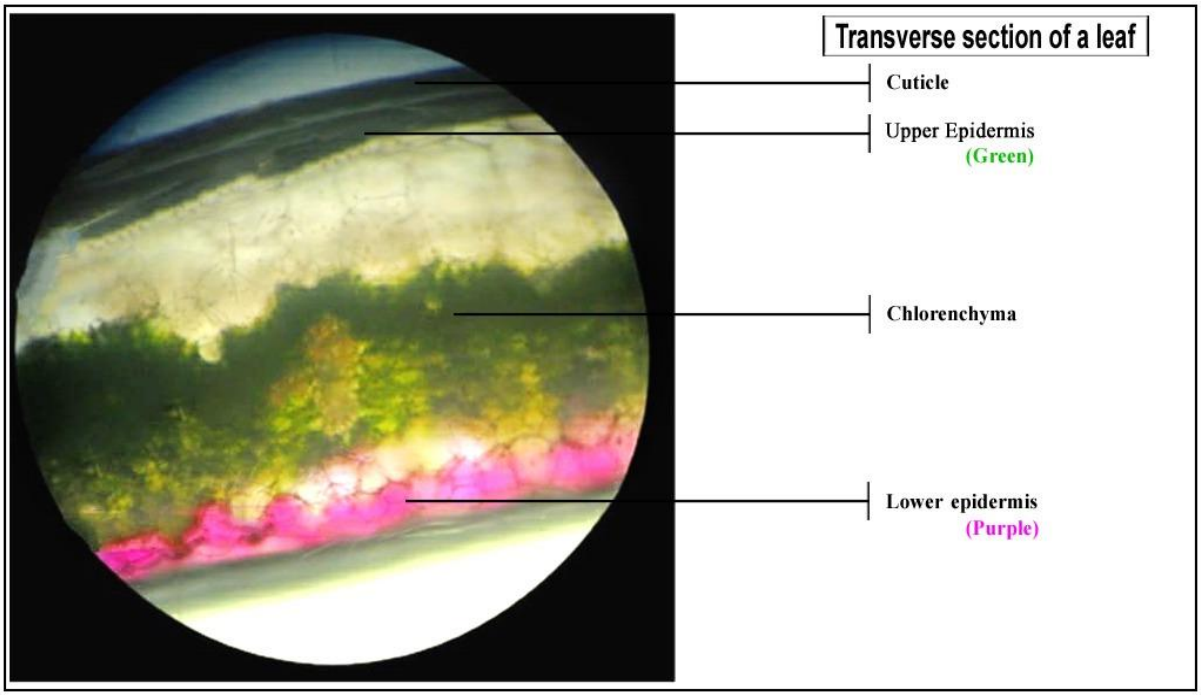


Fig 4: Transverse section of Tradescantia spathacea (TS) Leaf

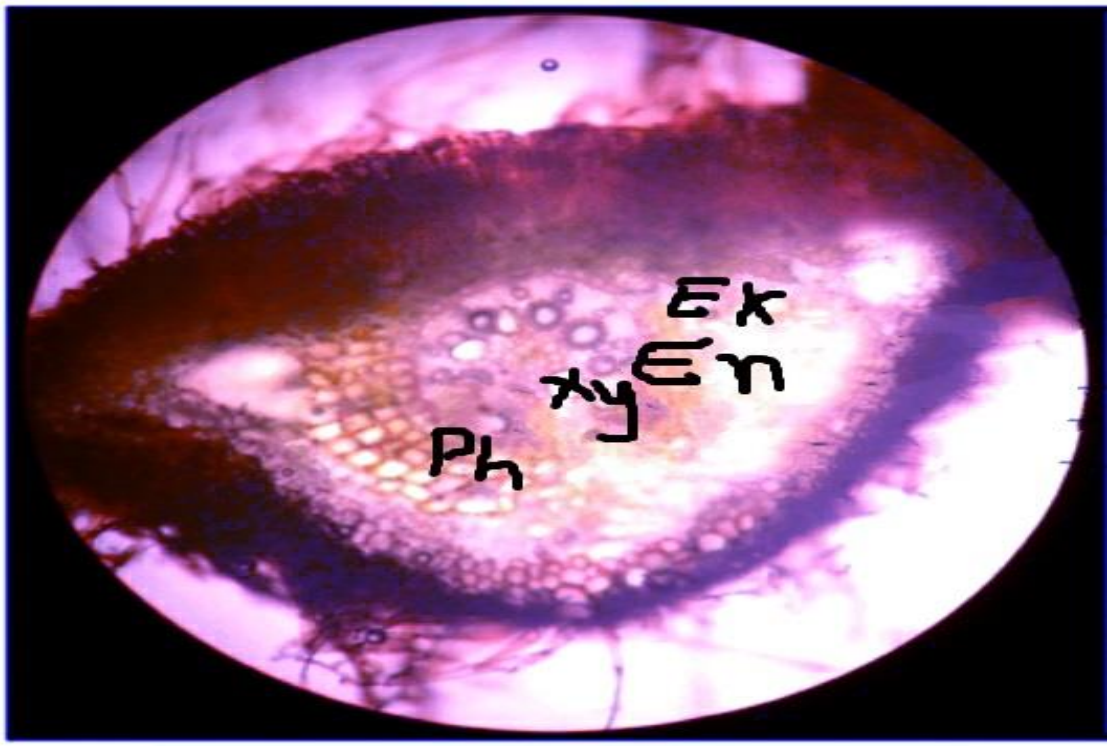


Fig 5: Transverse section of Tradescantia spathacea (T.S) Root. Parts are EX= Exodermis, En= Endodermis, Ph= Phloem, Xy= Xylem.

Table -4: Powder microscopy of Tradescantia spathacea (T.S)

Part of Powder	**Reagent**	**Observation**
Leaf	Iodine solution	Simple and blue starch grains are observed (Fig-6)
	Phloroglucinol	Elongated unicellular 'trichome's are observed. (Fig-7)
	Acetic acid	Prisms and cluster types of Ca-oxalate crystals are observed. (Fig-8)
	Ruthenium red	Red color tissue cells are observed. (Fig-9)
	HCl	Ca –oxalate crystals are not dissolved. (Fig-10)
	H_2SO_4	Ca –oxalate crystals are dissolved. (Fig-11)

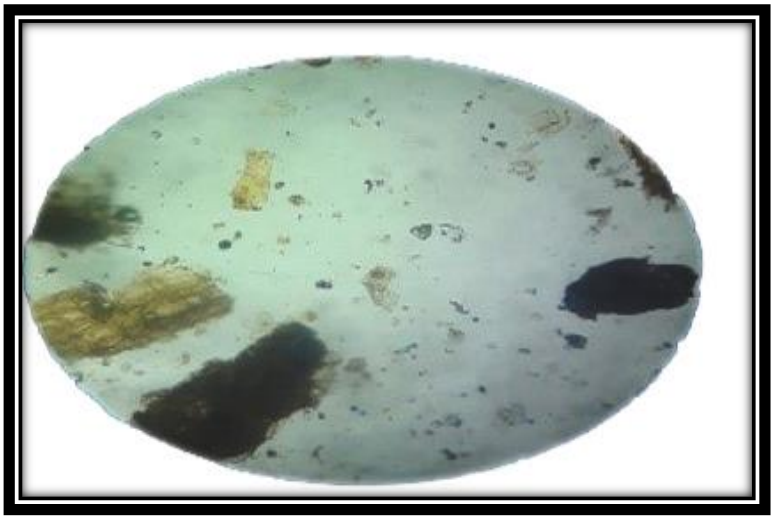


Fig 6: Simple and blue starch grains

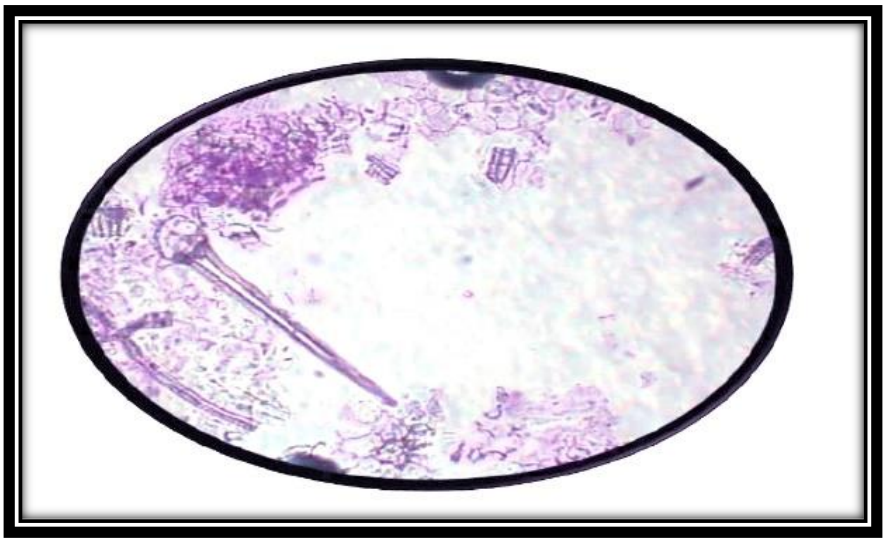


Fig 7: Elongated unicellular trichome's

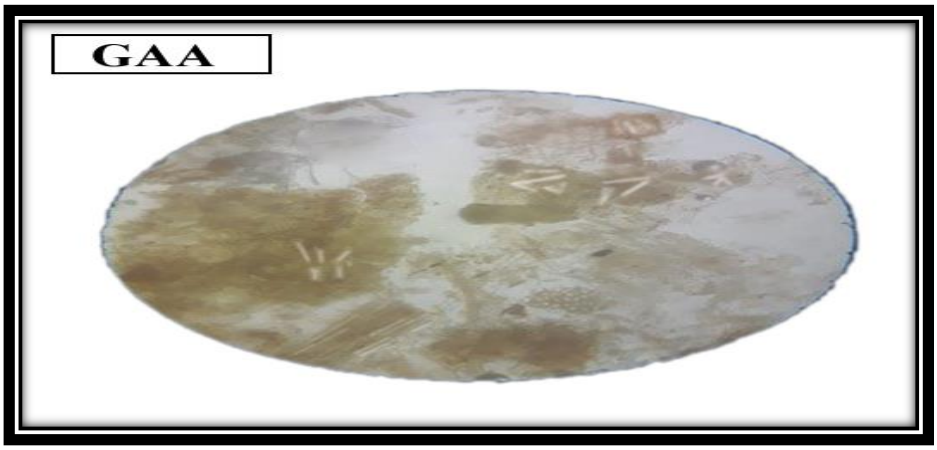


Fig 8: Prisms and cluster type of Ca-oxalate crystals

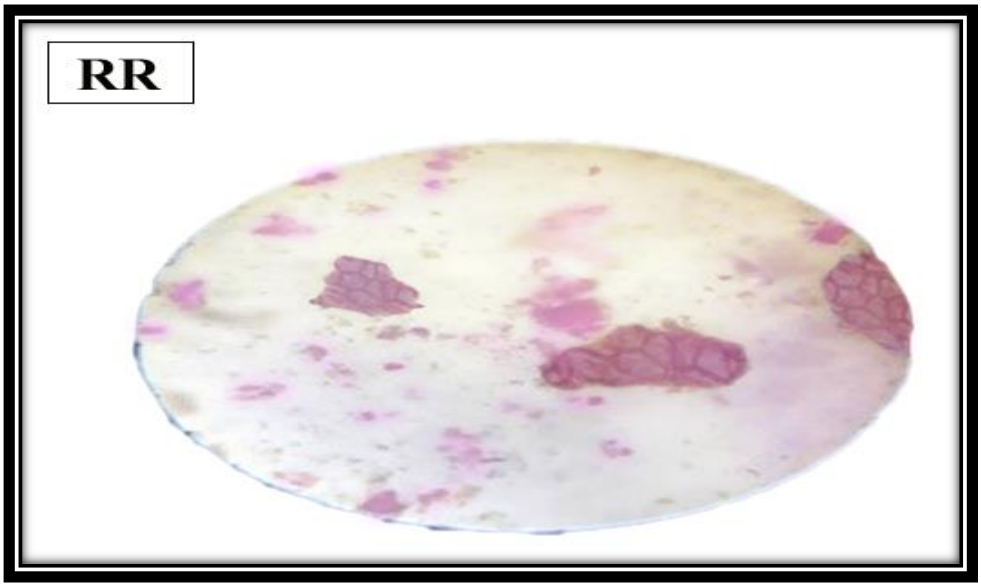


Fig 9: Tissue

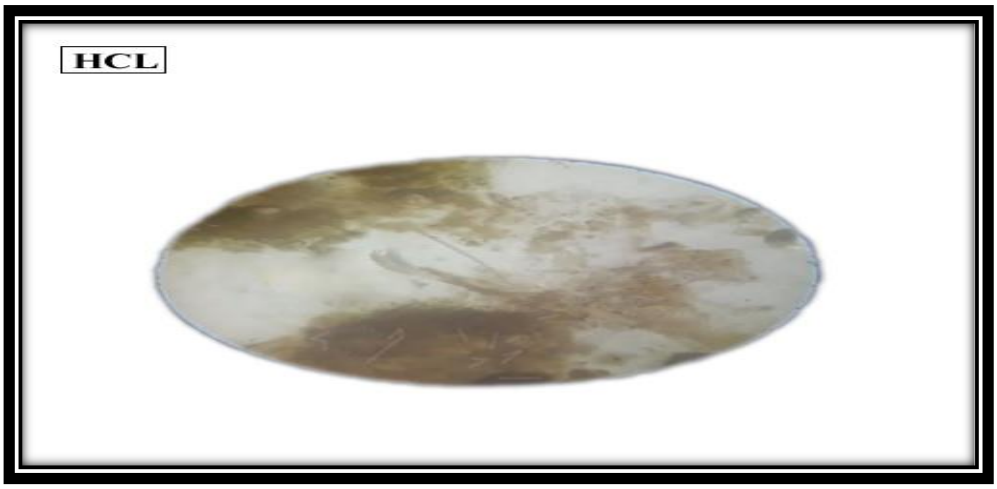


Fig 10: Ca –oxalate crystals not dissolved

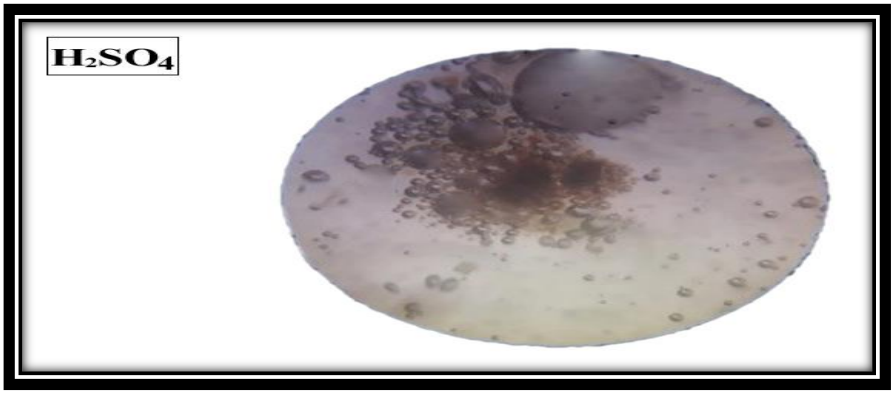


Fig 11: Ca –oxalate crystals are dissolved

Fluorescence analysis

During the day, under short wave-length UV light and long-wavelength UV light, the dried dry plant material was treated with various chemicals and tested for color change. (Table 5).

Table -5: Fluorescence analysis of dried powder of T S Powder

S.NO	**Chemicals**	**Observation**		
		Ordinary light	**Short wavelength 245 nm is**	**Long-wavelength 366 nm**
1	Powder of plant	Greyish green	Light green	Grey
2	Powder + n-Hexane	Whitish grey	Crystal green	White
3	Powder + Chloroform	Light green	Light green	Yellowish green
4	Powder + conc. HCl	Brownish green	Blackish green	Black green
5	Powder + Water	Cream color	Light green	Bluish green
6	Powder + 1 N NaOH in water	Light green	Light green	Grey green

7	Powder + xylene	Golden	Light green	Whitish red
8	Powder + 50% HNO3	Lemon yellow	Whitish green	Light green
9	Powder + con H2SO4	Dark brown	Black	Black
10	Powder + AgNO3	Light green	Light green	Light red
11	Powder + FeCl3	Dark brown	Light green	Light blue
12	Powder + Iodine solution	Brown	Blackish green	Get black
13	Powder + Petroleum ether	Light brown	Light green	Whitish red
14	Powder + Acetone	Light green	Light green	Red
15	Powder + Br2	Yellowish green	Light green	Cream
16	Powder + H2O2	Cream	Whitish green	Whitish grey
17	Powder + aniline	Dark brown	Black	Black
18	Powder + CCl4	Light brown	Green	Cream
19	Powder + Methanol	Light green	Light green	Light red
20	Powder + Ethanol	Light brown	Light green	Whitish red
21	Powder + Glacial acetic acid	Brown	Brownish green	Light red
22	Powder + Ammonia	Dark brown	Green	Whitish green

Physicochemical analysis

The swelling index, pH value, extractive values, and ash values of the plant powder were analyzed physicochemically, and certain techniques were changed. Moisture content, total ash, acid-insoluble ash, water-soluble ash, and water-insoluble ash are examples of physicochemical parameters. The overall ash content of the plant was found to be high, followed by acid-insoluble, water-soluble, and sulfated ash. Similarly, the greatest concentration of methanol-soluble extractive was found, followed by water-soluble extractive. The soluble extract in chloroform and alcohol is almost identical.

On the other hand, N-hexane is less valuable. Humidity percentage is high and within pH base range. (Table 6).

Table 6: Physicochemical profile of dried T.S leaf powder.

Particulars	**Leaf**	**Root**
Alcohol soluble extractive value	5.5	5.3
Water soluble extractive value	3.57	4.12
Methanol-soluble extractive	5.51	5.34
Chloroform-soluble extractive	3.40	3.21

n-Hexane-soluble extractive	2.56	2.3
Total ash	11.65%	9.8%
Acid insoluble ash	7.45	6.5
Water soluble ash	7.75	6.54
Sulphated ash	4.65	5.5
Moisture content	11.23	10.5
Crude fibre content	28.85	26.66
Swelling index	13.00	11.33
pH value	9.30	9.00

Qualitative Phytochemical Screening

Table-7: Phytochemical analysis of the methanolic extract and aqueous extract of T.S

S.NO.	**Phytochemical group**	**Test**	**Methanolic extract**	**Water extract**
1.	Proteins	Millon's test Ninhydrin test Biuret test	Negative Negative Negative	Negative Negative Negative
2.	Carbohydrates	Molich's test Fehling's test Benedict's test	Positive Positive Negative	Positive Positive Negative
3.	Saponins	Foam test Baljet test	Positive Positive	Positive Positive
4.	Lipids	Soap formation test	Negative	Negative
5.	Tannins / Phenolic compounds	Ferric chloride test Gelatin test	Positive Positive	Negative Negative
6.	Terpenoids	Salkowaski test Liebermann's reaction	Positive Positive	Positive Positive
7.	Glycosides	Borntrager's test Keller–Killani test Legal's test	Negative Negative Negative	Negative Negative Negative
8.	Flavonoids	Shinoda test	Positive	Positive

9.	Alkaloids	Dragendorff's test Mayers test Wagners's test Hagers test Tannic acid test	Positive Positive Positive Positive Positive	Positive Positive Positive Positive Positive
10.	Fats and oils	Saponification test	Negative	Negative
11.	Gums and mucilage	Gums and mucilage tests	Negative	Negative
12.	Resins	Resins	Positive	Positive
13.	Coumarins	Coumarins	Positive	Positive
14.	Phytosterols	Phytosterols	Negative	Negative

Thin Layer chromatography of T.S extracts:

TLC analysis helps separate different phytoconstituents in a crude extract. Because herbal extracts include a broad range of compounds with varying physicochemical characteristics, the solvent systems were chosen via trial and error and prior experimental results. The position of different compounds was determined by observation under visible and ultraviolet light before and after spraying with appropriate reagents after TLC plates were developed.

The following table shows the mobile phases and detection systems used in TLC and Rf Values for various phytoconstituents.

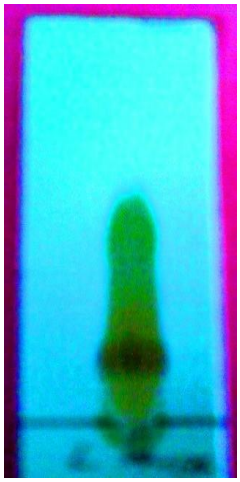
Table 8: Optimisation of solvent system of Detection methods of various phytoconstituents.

Test	**Solvent system**	**Detection**	**Rf Values**
Steroids	Toluene: Ethyl acetate (4:1, v/v)	Antimony trichloride in Chloroform	0.71
Flavonoids	Methanol: Chloroform: hexane (7:2:1, v/v/v)	1% ethanolic aluminum chloride solution Detection under U.V (365 nm)	0.81
Phenolic compounds	Ethyl acetate: Formic acid: Acetic acid: water 100: 11: 11: 26	Detection under U.V (256 nm)	0.80

Detection of various phytoconstituents by TLC in the leaf Extract of T.S

Steroids: **Fig-12**

Flavonoids: **Fig-13**



Detection of phenolic compounds: **Fig: 14**

Antioxidant activity

Table -9: Reduced method Scavenging activity: -

Conc.(µg/ml)	**Ascorbic acid Absorbance**	**Leaf Methanol**	**Root Methanol**
0	0	0	0
20	0.18	0.12	0.1
40	0.34	0.29	0.25
60	0.5	0.39	0.32
80	0.7	0.52	0.44
100	0.85	0.55	0.5

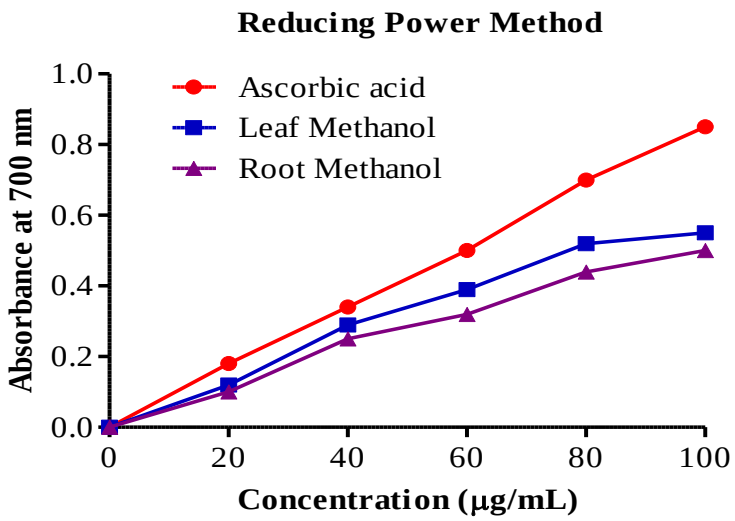


Figure-15: Reducing power method.

Table-10: Reduced method % Inhibition: -

Conc. (µg/ml)	Ascorbic acid	Leaf methanol	Root methanol
0	0	0	0
20	83.33	75	70
40	91.17	89.65	88
60	94	92.30	90.63
80	95.71	94.23	93.18
100	96.47	94.54	94

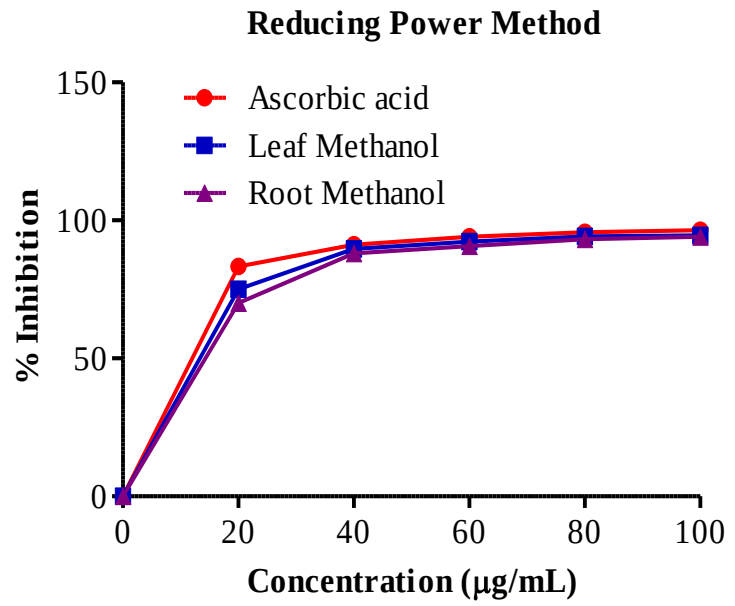


Figure 16: Reduced power method % Inhibition

Table -11: DPPH Method.

Conc. (µg/ml)	Ascorbic acid absorbance	Leaf methanol	Root methanol
0	0	0	0
20	0.1	0.11	0.13
40	0.08	0.12	0.14
60	0.06	0.09	0.12
80	0.05	0.08	0.09
100	0.03	0.05	0.07

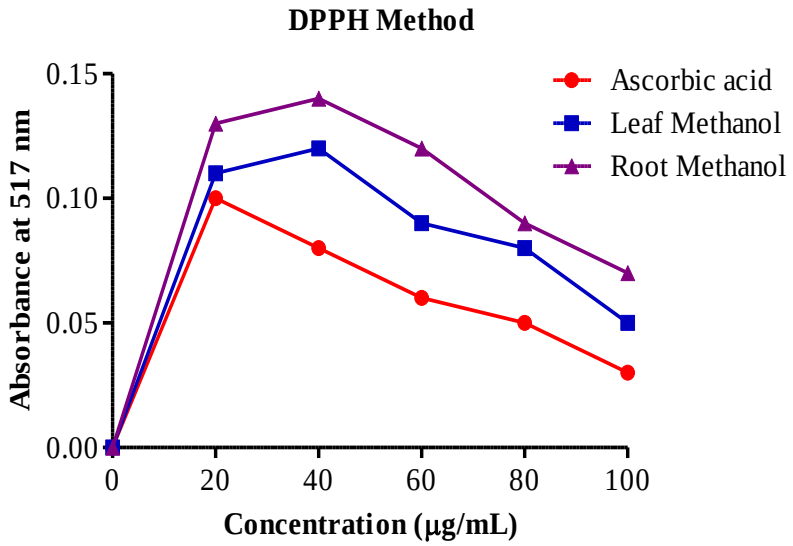


Fig:17 DPPH scavenging activity method

Table -12: DPPH % Inhibition Method

Conc. (µg/ml)	Ascorbic acid	Leaf methanol	Root methanol
0	0	0	0
20	37.5	31.25	18.75
40	50	25	12.5
60	62.5	43.75	25
80	68.75	50	43.75
100	81.25	68.75	56.25

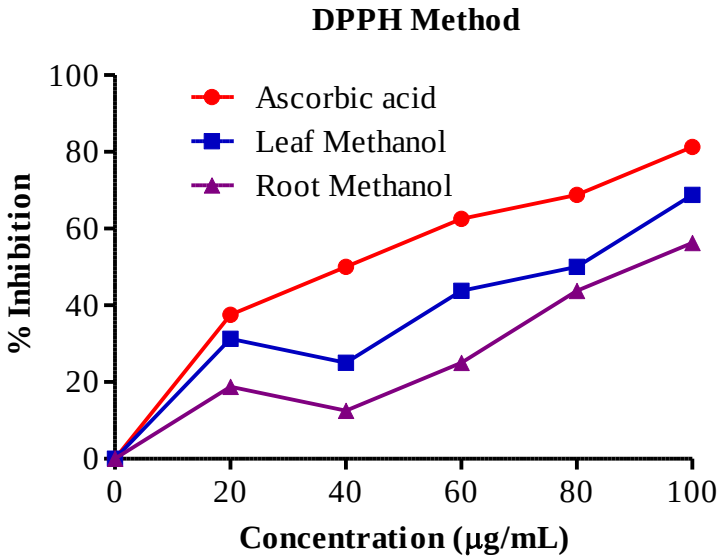


Fig:18 DPPH % Inhibition Method

Table -13: Iron chelation activity

Conc. (µg/ml)	Ascorbic acid	Leaf methanol	Root methanol
0	0	0	0
10	0.07	0.06	0.06
20	0.13	0.12	0.1
30	0.19	0.17	0.14
40	0.25	0.2	0.16
50	0.3	0.24	0.17
100	0.6	0.26	0.18

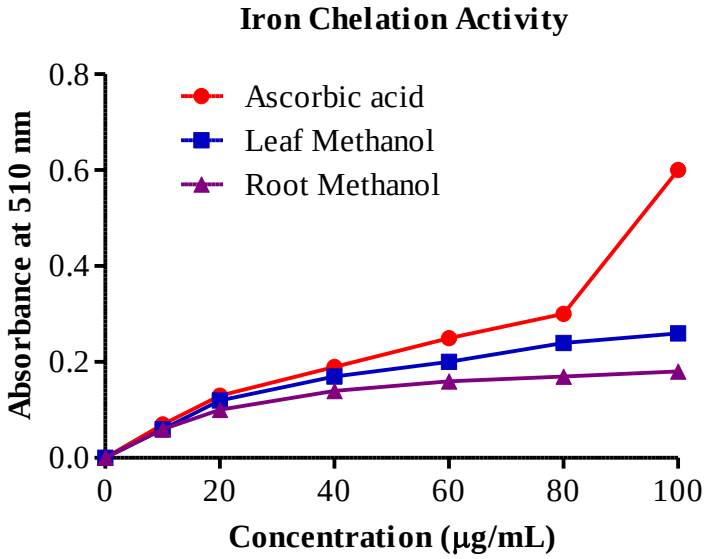


Fig:19 Iron chelation activity

Table -14: Iron chelation Inhibition activity

Conc. (µg/ml)	Ascorbic Acid	Leaf methanol	Root methanol
0	0	0	0
10	14.28	0	0
20	53.84	50	40
30	68.42	64.70	57.14
40	76	70	62.5
50	80	75	64.70
100	90	76.92	66.66

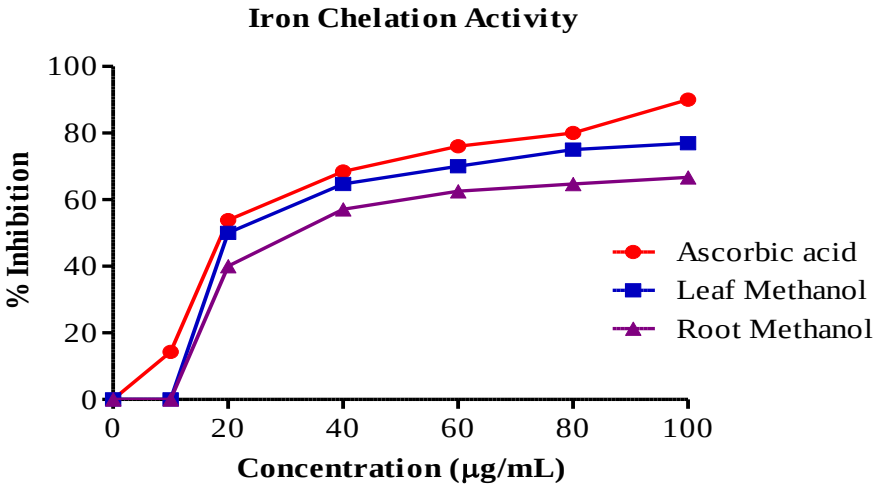


Fig:20 Iron chelation Inhibition activity

Table -15: H2O2 Scavenging activity.

Conc. (µg/ml)	Ascorbic acid absorbance	Leaf methanol	Root methanol
0	0	0	0
50	0.262	0.257	0.248
100	0.389	0.381	0.358
150	0.564	0.486	0.455
200	0.721	0.586	0.556
250	0.943	0.896	0.853

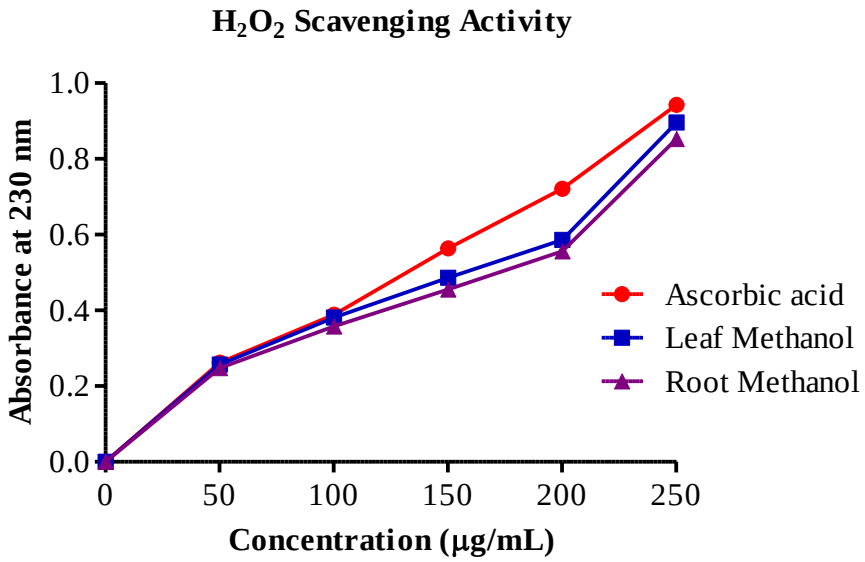


Fig:21 H2O2 Scavenging activity.

Table -16: Hydrogen peroxide % Inhibition: -

Conc. (µg/ml)	Ascorbic acid	Leaf methanol	Root methanol
0	0	0	0
50	27.78	28.68	29.07
100	41.25	42.52	41.96
150	59.80	54.24	53.34
200	76.45	65.40	65.18
250	100	100	100

H2O2 Scavenging Inhibition activity

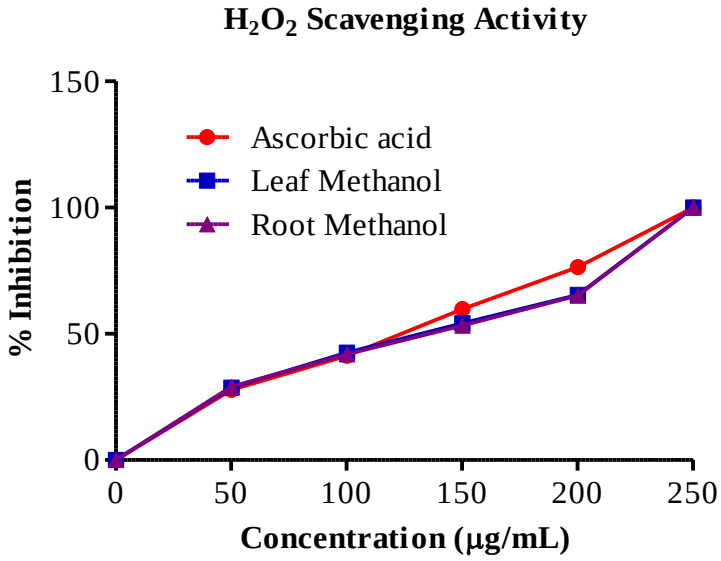


Fig:22 Hydrogen peroxide % Inhibition

Table -16: Total phenolic Activity (TPC)

Conc. (µg/ml)	Gallic acid	Leaf methanol	Root methanol
0	0	0	0
50	0.109	0.08	0.07
100	0.244	0.1	0.08
150	0.337	0.14	0.1
200	0.454	0.16	0.12
250	0.6	0.18	0.14
300	0.721	0.22	0.16

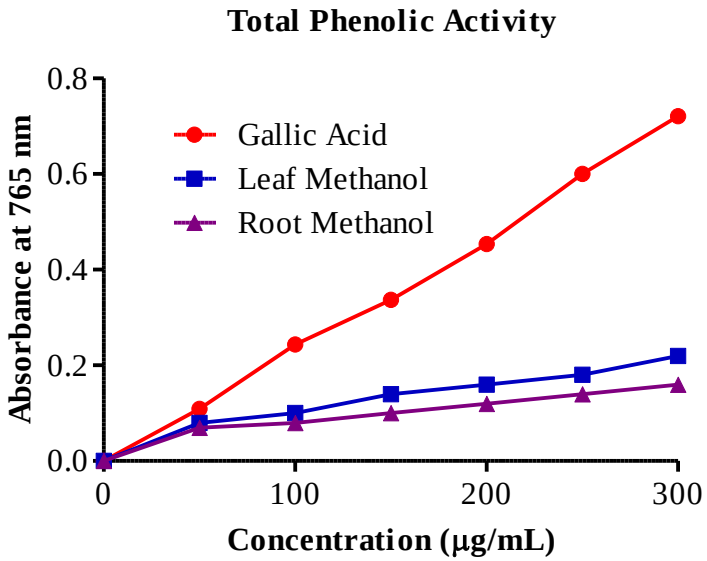


Fig:23 Total phenolic Activity

Table -18: Total phenol % Inhibition (TPC)

Conc. (µg/ml)	Gallic acid	Leaf methanol	Root methanol
0	0	0	0
50	81.65	75	71.42
100	91.80	80	75
150	94.06	85.71	80
200	95.59	87.5	83.33
250	96.66	88.88	85.71
300	97.22	90.90	87.5

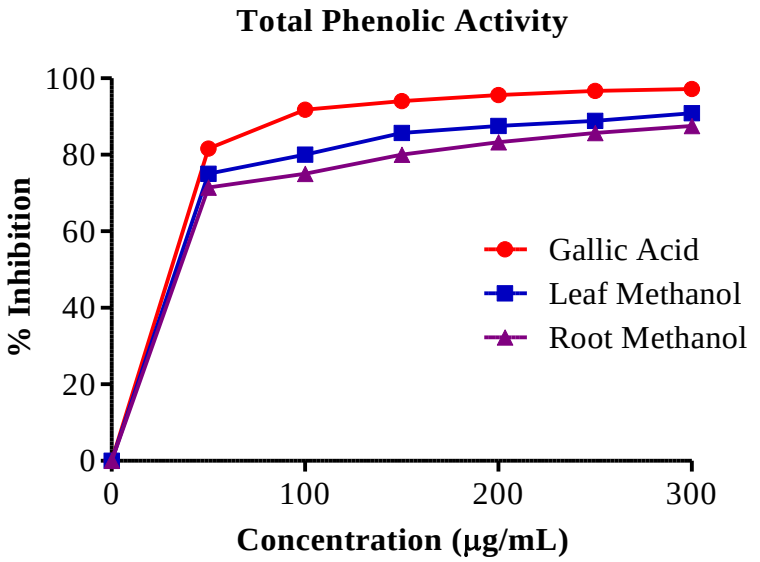


Fig:24 Total phenol % Inhibition

Table -19: Flavonoid Activity

Conc. (µg/ml)	Quercetin	Leaf methanol	Root methanol
0	0	0	0
10	0.05	0.02	0.01
20	0.09	0.08	0.03
40	0.13	0.14	0.09
80	0.18	0.16	0.15
100	0.22	0.21	0.2
120	0.47	0.24	0.25

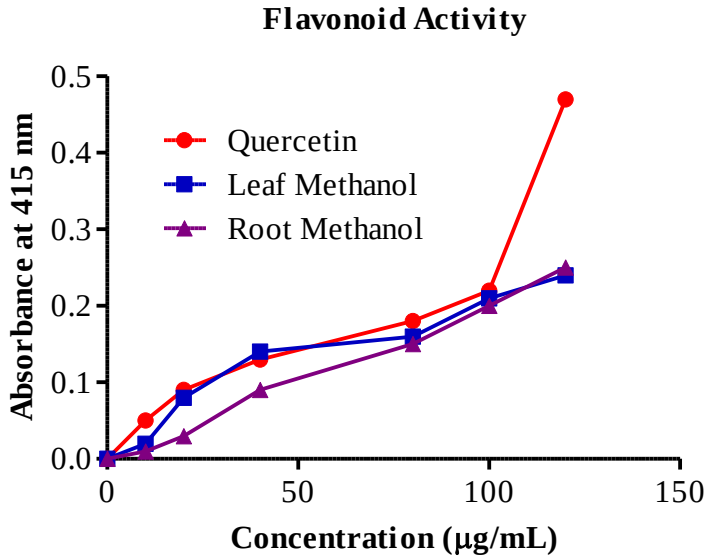


Fig:25 Flavonoid Activity

Table -20: Flavonoid Inhibition Activity

Conc. (µg/ml)	Quercetin	Leaf methanol	Root methanol
0	0	0	0
10	10.63	8.33	4
20	19.14	33.33	12
40	27.65	58.33	36
80	38.29	66.66	60
100	46.80	87.5	80
120	100	100	100

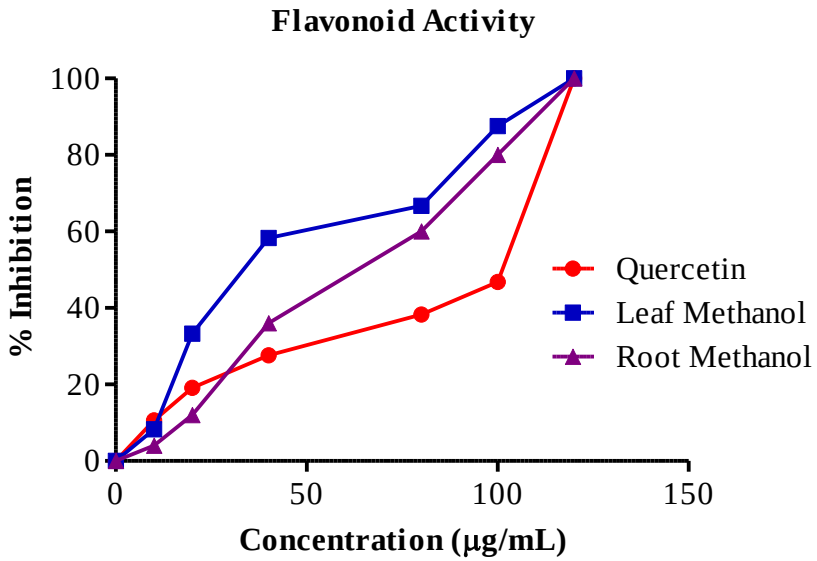


Fig:25 Flavonoid Inhibition Activity

CONCLUSION

Herbal medicines need a high level of standardization since they are made from a variety of sources, which may lead to variances. Variations like this may lead to erroneous findings in pharmacological and phytochemical investigations. In the current study we have performed pharmacognostical, phytochemical, and pharmacological evaluation of Tradescantia Spathacea. This attempt clearly indicates useful information about the T.S The entire plant of T.S has been recognised for a variety of medicinal qualities; therefore, the present research may be useful in supplementing information on its identification, authenticity, and standardisation; no such information exists for the same as of yet. Column chromatography, phytochemical investigations and anti-oxidant activities of T.S plant would give a scientific approach for upcoming researchers.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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