Extraction and its phytochemical evaluation of the crude fruit Solanum lycopersicum L

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ABSTRACT

Solanum lycopersicum, commonly known as tomato, is not only a staple in culinary practices but also a potential source of various bioactive compounds with medicinal properties. This study investigates the physicochemical attributes and phytochemical profile of S. lycopersicum, alongside its pharmacological activities, particularly focusing on antidiabetic potential. The physicochemical evaluation revealed a total ash content of 14.03%, with significant amounts of water (3.9%) and acid-insoluble ash (3.1%). Extractive values demonstrated a diverse phytochemical profile, particularly with dichloromethane extracts yielding 8.9%. The high tannin content (21%) indicates strong astringent and antioxidant properties. Preliminary phytochemical screening identified a range of compounds, including flavonoids, alkaloids, and saponins, with chromatographic analysis further supporting these findings. In pharmacological assessments, the ethanolic extract exhibited moderate alpha-amylase inhibition compared to the standard Acarbose. Furthermore, fractionation studies highlighted that specific fractions, notably E3, exhibited strong alpha-amylase inhibitory activities with an IC50 value of 65.03 µg/mL. Additionally, in vitro assays demonstrated significant alpha-glucosidase and DPP-4 inhibitory activities, indicating the potential of S. lycopersicum extracts as antidiabetic agents.

Keywords:Solanum lycopersicum, Antidiabetic activity, Alpha-amylase inhibition, DPP-4 inhibition, Chromatographic analysis

1. INTRODUCTION

The tomato (Solanum lycopersicum) is an edible berry originating from western South America, Mexico, and Central America, with a rich history of domestication by indigenous peoples. Introduced to Europe during the Columbian Exchange, it has become the world's largest vegetable crop, valued both commercially and nutritionally. Botanically classified as a berry, tomatoes are typically red due to the pigment lycopene, though they can vary in shape and colour. $\left[1-3\right]$ The plant belongs to the Solanaceae family, which has historically given tomatoes a false reputation for toxicity, as only the leaves contain harmful alkaloids. Tomatoes are known for their health benefits, providing essential vitamins (especially vitamin C), antioxidants, and various chemical constituents such as flavonoids and carotenoids. Traditional uses include remedies for conditions like rheumatism and digestive issues, while modern studies highlight their potential in preventing chronic diseases due to their high nutrient content. [4,5]

Diabetes Mellitus (DM) is an endocrine disorder characterized by inadequate insulin production or impaired use, leading to chronic hyperglycemia. As of 2016, over 29 million Americans were diagnosed with diabetes, with an additional 86 million at risk for developing type 2 diabetes (CDC, 2017). The American Diabetes Association has shifted from terms like "insulin-dependent" and "non-insulin-dependent" to a numerical classification for diabetes types, reflecting a need for clarity regarding the disease itself rather than treatment approaches. Type 1 diabetes, which accounts for about 10% of all diabetes cases, results from autoimmune destruction of pancreatic beta-cells, often diagnosed in children and adolescents. Key autoantibodies are typically present at diagnosis, and the disease may progress to require insulin therapy. The pathophysiology involves the absence of insulin preventing glucose uptake in tissues, leading to hyperglycemia and potentially diabetic ketoacidosis (DKA). The incidence of Type 1 diabetes is increasing, with the highest rates observed in Finland and Sweden, and it generally peaks between ages 5 and 20. Idiopathic diabetes, or ICA-negative diabetes, presents similarly to Type 1 but lacks an autoimmune basis, often requiring episodic insulin therapy. Overall, diabetes mellitus is a complex disorder with significant public health implications, influenced by a combination of genetic, autoimmune, and environmental factors. [6-8]

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, representing at least 90% of all cases, with a rising prevalence particularly in developing countries (69%) compared to developed nations (20%). Most affected individuals in developing regions are between 40 and 60 years old, whereas older adults over 60 are primarily affected in developed countries. The increase in T2DM is closely linked to Western lifestyle changes, including poor dietary habits and reduced physical activity, leading to higher rates of overweight and obesity. This condition is characterized by insulin resistance and beta-cell dysfunction, where decreased insulin sensitivity initially prompts increased insulin secretion to maintain normal blood glucose levels. Over time, however, beta-cell function declines, resulting in insulin deficiency and hyperglycemia. While insulin levels may drop, they are often sufficient to prevent diabetic ketoacidosis (DKA), which can occur during severe stress or with certain medications. Typically, patients with T2DM do not require insulin therapy at diagnosis or throughout their lives unless under significant physiological stress. [9,10]

2. Materials and Methods[11-14]

2.1 Materials

The main chemicals included methanol, ethanol, petroleum ether, and sodium hydroxide from Merck and Cosmo Chem Pvt. Ltd. Instruments utilized in the study comprised a melting point apparatus, JASCO V-530 UV-VIS spectrophotometer, JASCO -460 FTIR, and a diffusion cell apparatus for drug release studies. Plant authentication was conducted by a botanist from the Botanical Survey of India, with herbarium specimens deposited for reference. The pharmacognostic study involved macroscopic analysis of organoleptic characteristics and microscopic examination using staining techniques such as Phloroglucinol-HCl and Sudan red under high magnification, adhering to standard procedures for recording observations.

2.2 Methods

2.2.1 Macroscopy

Organoleptic characters, extra feature and morphology performed as per standard procedures and observations are recorded.

2.2.2 Microscopy

Microscopical study was done as per standard procedures and observations are recorded. Microscopy observed with different staining solution like Phloroglucinol: Hydrochloric acid (1:1), Sudan red solution, picric acid solution, dilute iodine etc. under microscope at higher objective.

2.2.3 Physicochemical Evaluation: [12-14]

1. Determination of foreign organic matter

About 250 gm. of air dried coarsely powdered drug was spread in a thin layer on plane surface. The sample was inspected with the unaided eye or with the use of 6X lens. The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined.

2. Determinationof moisture content:

Weight accurately glass-stopper, shallow weighing bottle. Test sample crude drug powder transferred to the bottle and covered, the total weight was reported for initial weight. Test sample was distributed evenly and poured to a depth not exceeding 10 mm. Then sample bottle was kept in an oven 105°C for next 3hrs. The sample was dried to constant weight stored in a desiccator to acquire room temperature. Repeat step till the two consecutive weight differences not more than 0.5mg. Weighed and the calculate loss on drying in terms of percent w/w.

3. Ash value

Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'total ash value'. Such variables are then removed by treating with acid and then acid insoluble ash value is determined.

3.1. Determination of Total ash

Accurately weighed 2gm of air dried crude drug was taken in a tared silica dish and incinerated at a temperature 750°C until free from carbon, cooled and weight was taken. The percentage of ash was calculated with reference to the air-dried drug.

3.2. **Determination of Water- soluble ash**

The total ash was obtained as per method described above and boiled for 5 minutes with 25 ml of water, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited for 15 minutes

at a temperature 750C and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug

3.3. Determination of Acid -insoluble ash

The ash was obtained as per above mentioned method boiled with 25 ml of 2M hydrochloric acid for 5 minutes, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

3.4. Sulphated Ash Value

Take 1 gm. test sample is accurately weighed into a silica crucible that has been previously ignited, cooled and weighed. The sample in the crucible is then treated with 1 ml sulphuric acid and charred at a low temperature without causing ignition of the sample until there are no more white fumes given off. The crucible with the charred sample is then placed in a furnace and maintained temperature up to $600-800^{\circ}$ C until all organic matter has been burnt off. The crucible is cooled and re-weighed. The difference in sample weights being the sulphated ash content remaining.

4. Extractive values

Extractive values were determined as per standard procedures.

4.1 Determination of water-soluble extractive value

Accurately weight 5 gm. of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish and dried at 105° C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs.

4.2 Determination of Alcohol-soluble extractive value

Five gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105° c and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs.

3. Soxhlet Extraction Method for Solanum lycopersicum L. [15]

Soxhlet extraction is a reliable method for isolating bioactive compounds from dried Solanum lycopersicum L. (tomato) fruit. The process begins with the collection and thorough washing of fresh fruit, followed by dehydration at 40-50°C and grinding into a fine powder. The Soxhlet apparatus is then assembled with an organic solvent, such as ethanol or methanol, placed in a round-bottom flask, and the powdered material contained in a thimble. Heating the solvent allows it to evaporate, condense, and percolate through the plant material, extracting bioactive compounds over 6-8 hours until the solvent appears clear. Finally, the extract is concentrated by evaporating the solvent using a rotary evaporator or gentle heating and stored in a refrigerator for future use. This method effectively enhances the yield of valuable phytochemicals from tomato fruit.

4. Preliminary phytochemical screening:

1. Tests for Acidic compounds:

a) To the test solution add sodium bi-carbonate, effervescence produces in presence of acidic compounds.

b) Test solution treated with warm water and filter. Test the filtrate with litmus paper. Litmus paper turns blue in presence of acidic compounds.

2) Test for Carbohydrates:

a) Molisch test (General test):

Two ml of extract solution was added with few drops of 15 per cent ethanolic α-naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along the side of the test tube. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

b) Test for reducing sugar:

i) Benedict's test:

Mix equal volume of Benedict's regent and extract solution in test tube. Heat in a boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present. **ii) Fehling's test:**

Five ml of extract solution was mixed with 5 ml Fehling's solution (equal mixture of Fehling's solution A and B) and boiled. Development of brick red precipitate indicates the presence of reducing sugars.

c) Test for Monosaccharide's:

Barford's test:

Mix equal volume of barford's reagent and extract solution. Heat for 1- 2 min. in boiling water bath and cool. Development of red precipitate specifies occurrence of monosaccharides.

d) Selivanoffs test (Test for Ketones): To the test extract solution add crystals of resorcinol and equal volumes of concentrated hydrochloric acid and heat on a water bath. Rose colour is formed if ketones are present.

e) Osazone formation test: Heat the test solution with the solution of phenyl hydrazine hydrochloride, sodium acetate, and acetic acid. Needle shape or Yellow crystals formed after complex founding with carbohydrates (diasaccharides), observed in microscope.

3) Test for Proteins:

a) Heat test: Heat the test solution in boiling water bath. Proteins gets coagulated.

b) Biuret test:

The extract was treated with 1 ml of 10 percent sodium hydroxide solution and heated. A drop of 0.7 percent copper sulphate solution was added to the above mixture. The formation of purple violet color specifies the occurrence of proteins.

c) Millon's test:

The extract was treated with 2 ml of Millon's reagent. Formation of white precipitate specifies the occurrence of proteins and amino acids.

d) Xanthoproteic test:

Take 1 ml test extract solution, add 1 ml of conc. nitric acid and boil yellow precipitate is formed. After cooling it, add 40 % sodium hydroxide solution. Orange colour formed in presence of proteins.

d) Test for starch:

Take 1 ml of test extract solution, add weak aqueous iodine solution. Development of blue colour indicates presence of starch, which disappears on heating and reappears on cooling.

4) Test for Amino acids:

a) Million's Test:

1ml test extract solution treated with Million's reagent simultaneously heated on a water bath. Formation of white colour precipitate specifies the occurrence of amino acids

b) Ninhydrin test:

The extract was treated with ninhydrin reagent at pH range of 4-8 and boiled. Formation of purple color specifies the amino acids.

5) Test for Steroids:

a) Salkowski test:

One ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown color exhibited by chloroform layer and green fluorescence by the acid layer suggests the existence of steroids.

b) Liebermann-burchard test:

10 mg extract was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid from the side of the test tube. Formation of reddish violet color at the junction indicates the presence of steroids.

c) Liebermann's test:

To 2 ml of the residue a few ml of acetic anhydride was added and gentle heated. The content of the test tube were cooled and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Development of blue color gave the evidence for presence of steroids.

6) Test for Terpenoids:

One ml of extract added with one ml of Vanillin sulfuric acid. Development of violet color gave the evidence for presence of Terpenoids.

7) Test for Glycosides

a) General test:

Test A:

5 ml concentrated test extract with 5 ml of dilute sulphuric acid by warming on a water bath, filter it, and neutralize the acid extract with 5 % solution of sodium hydroxide. Add 0.1 ml of Fehling's solution A and B until it becomes alkaline (test with pH paper) and heat on water bath for 2 minutes. Red Precipitate formed in incidence of glycoside derivatves.

Test B:

Repeat above procedure by using 5 ml of water instead of dilute sulphuric acid. Note the quantity of red precipitate formed. Compared with precipitate of test A

Chemical tests for specific glycosides derivatives:

b) Anthraquinone glycosides:

Borntrager's test:

Take 3 ml test extract add dilute sulphuric acid, boil and filter. To the cold filtrate, add equal volume benzene or chloroform. Shake well. Separate organic solvent. Add ammonia, the ammonical layer turns pink or red color.

Modified Borntrager's test: Boil 5 ml concentrated test extract with 2ml of sulphuric acid. Treat with 2 ml of 5 % aqueous ferric chloride solution (freshly prepared) for 5 minutes, shake it with equal volume of chloroform and continue the test as above. Red color produced in presence of anthraquinone glycosides.

c) Cardiac glycoside:

Keller-Killiani test (Test for deoxysugars):

To 2 ml of test extract, glacial acetic acid, one drop 5 % Ferric chloride and conc. Sulphuric acid was added. Presence of cardiac glycosides is indicated by formation of reddish brown color at junction of the two liquid layers and upper layer appeared bluish green.

d) Kedde's test: To 2 ml of test extract with chloroform, evaporate to dryness. Add one drop of 90 % alcohol and 2 drops of 2 % sodium hydroxide solution. Purple color is produced.

e) Baljet's Test: The test solution treated with sodium picrate or picric acid. Gives yellow to orange colour.

f) Legal's Test: Test solution treated with pyridine [made alkaline by adding sodium nitroprusside solution]. Gives blood red color

g) Tests for coumarins glycosides:

Place small amount of sample in test tube and covered it with a filter paper, moistened with dilute sodium hydroxide solution. Placed the covered test tube on water bath for several minutes. Remove the paper and expose it to ultraviolet (UV) light. Paper shows green fluorescence.

h) Cynogentic glycosides:

Take 1 ml concentrated test extract in conical flask and moisten with few drops of water.(Flask should be completely dry because hydrogen cyanide produced will dissolve in the water rather than come off as gas to react with paper) moisten a piece of picric acid paper with 5% aqueous sodium carbonate solution and suspended in neck of flask. Warm gently at about 37oC. Observe the change in color. Reddish purple colour.

8) Test for Saponin

Foam formation test:

One ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated ylinder for 15 minutes. The development of stable foam indicates the presence of Saponins.

9) Test for Alkaloids

a) Dragendroff's test:

2-3 ml test solution and 0.1 ml Dragendroff's reagent was added in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

b) Mayer's test:

2-3 ml test solution and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

c) Hager's test:

2-3 ml test solution and 0.1 ml of Hager's reagent. Formation of yellowish precipitate indicates the presence of alkaloids.

d) Wagner's test:

2-3 ml test solution and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

e) Tannic acid test:

Test extract solution treated with Tannic acid solution. Gives buff colored precipitate

f) Picrolonic acid test:

Test solution treated with Picrolonic acid. Gives yellow coloured precipitate

10) Test for Tannins and Phenolic compounds

a) 5 %Ferric chloride:

Five ml of extract solution was allowed to react with 1 ml of 5 percent ferric chloride solution. Deep blue-black coloration indicates the presence of tannins.

b) Dilute nitric acid test:

Two ml of extract solution was allowed to react with few drops of dilute $HNO₃$ solution. Formation of reddish to yellow color indicates the presence of tannins.

c) Bromine water test:

Two ml of extract solution mix with 2 ml of bromine water. Discoloration of bromine water indicates presence of tannins.

d) Potassium dichromate test:

2-3 ml of extract solution and mix with 2 ml of Potassium dichromate. The formation of red precipitate indicates presence of tannins.

e) Gelatin test: To the test solution add 1 % gelatin solution containing 10 % sodium chloride. In presence of tannins precipitate formed.

11) Test for Flavonoids

a) Shinoda test:

To the test extract 5 ml (95%) ethanol and few drops of con. HCl and 0.5 g of magnesium turnings was added gives pink color.

b)Lead acetate test:

Few drops of 10 percent lead acetate are added to the test extract. Development of yellow colored precipitate confirms the presence of flavonoids.

c) Sodium hydroxide test:

Increasing amount of sodium hydroxide add in a extract solution which shows yellow coloration, which disappears after addition of acid.

d) Zinc-Hydrochloride test:

Treat test extract solution with zinc dust and few drops of hydrochloric acid, In presence of flavone derivatives shows red colour after few minutes.

5. Thin Layer Chromatography^[16]

Thin-layer chromatography (TLC) is a dynamic and widely-used technique for separating non-volatile mixtures, utilizing a thin film of adsorbent layered on materials such as aluminum, rubber, or glass. Common adsorbents include aluminum oxide, cellulose, and silica gel, making TLC a staple in the pharmaceutical industry for tasks like drug purity testing, strength assessment, and analyzing vital intermediates.

Despite the emergence of advanced chromatography methods like HPLC, TLC remains a favorite due to its simplicity, cost-effectiveness, and minimal preparation requirements. Each component in a sample is visualized as distinct spots on the plate, providing clear insights into their separation. The retention factor (Rf), calculated as $Rf =$ distance traveled by sample ℓ distance traveled by solvent, quantifies this separation, influenced by factors like solvent choice, amount of sample, adsorbent type, and temperature.

The preparation of a thin-layer chromatography (TLC) plate begins with creating a slurry of silica gel-G in distilled water, which is then evenly spread onto glass plates to form a thin layer. Following this, the plates are activated by heating them in an oven at 105°C for 30 minutes, ensuring optimal adhesion and performance. To conduct the TLC, the mobile phase must first saturate a sealed glass chamber, where it is allowed to settle for 30 minutes. During chromatogram development, the samples are carefully spotted onto the activated plate. It is crucial to maintain the solvent level in the chamber below the spots to prevent the samples from dissolving instead of migrating during the chromatography process. The solvent is then allowed to travel approximately 10- 15 cm up the silica layer. After development, the plates are removed and analyzed visually, including under UV light, and using visualizing agents such as Vanillin-H2SO4 or Methanolic FeCl3 solution. Finally, the retention factor (Rf) is calculated to quantify the separation of components on the plate. This method provides valuable insights into the composition and purity of the analyzed extracts.

Table 1:Solvent system is use for TLC of extracts

6. Column Chromatography of Bioactive ethanolic extract

Thin layer chromatography (TLC) is employed to evaluate the purity of phytoconstituents extracted from crude samples using column chromatography. In this process, silica gel G with a mesh size of 60–120 serves as the stationary phase within a column measuring 50 cm in length and 3.5 cm in inner diameter. To prepare the column, a cotton solid is used in the mobile phase, allowing for a wet packing technique. The effectiveness of separation relies on the affinity of the phytochemicals for the stationary phase, facilitating the isolation of physiologically active compounds from the crude extracts. After slurrying the silica gel with the appropriate solvent, it is carefully packed into the column, ensuring uniform distribution through gentle agitation and removal of air by tapping. Crude extracts are then added from the top without disturbing the silica. Following elution, the extracts are analyzed via TLC using optimized mobile phase techniques, with fractions collected in small glass tubes. The separated fractions are subsequently studied using UV and IR spectroscopy to further characterize the phytoconstituents. [16]

7. Pharmacological Screening Of Isolated Extracts

1. In vitro antidiabetic activity by α amylase activity^[17]

To assess the inhibitory effect of samples on α -amylase activity, a series of preparations and procedures are conducted. The required equipment includes a BOD incubator, spectrophotometer, sonicator, and analytical balance. The study utilizes standard Acarbose and an ethanolic extract. For reagent preparation, a stock solution of α-amylase is made by dissolving 2.5 mg in 100 mL of a buffer solution (0.02M sodium phosphate buffer with 0.006M NaCl) to achieve a concentration of 1000 μ g/mL. The buffer is prepared by dissolving specific amounts of NaCl, disodium hydrogen phosphate, and sodium dihydrogen ortho phosphate in distilled water, adjusting the pH to 7.0. Additionally, 1% starch and iodine solutions are prepared for the assay.

Test solutions at a concentration of 100 μ g/mL are prepared by dissolving 0.001 g of the extract or standard in DMSO, making up the volume to 10 mL. The assay procedure involves adding 1 mL of the sample to 1 mL of the stock α -amylase solution and incubating at 37°C for 10 minutes. Following this, 1 mL of 1% starch solution is added and incubated for an hour. After incubation, 1 mL of 1% iodine solution and 5 mL of distilled water are added. The absorbance is measured at 540nm, using Acarbose at 100 µg/mL as a standard control. Control samples are prepared by omitting the starch solution and replacing it with distilled water. Enzyme inhibition is calculated using the formula: % Inhibition = $(A - C) / C \times 100$, where A is the absorbance of the sample and C is the absorbance of the control.

Method of Preparation

To extract bioactive compounds from Solanum lycopersicum L. (tomato) fruits using solvent extraction, first prepare the plant material by washing, peeling, and cutting the tomatoes into smaller pieces, then drying them to reduce moisture content. Grind the dried material into a fine powder to increase the surface area for extraction. Choose appropriate solvents based on the target compounds, such as ethanol for polar compounds or hexane for non-polar compounds. Weigh a specific amount of the powdered plant material and mix it with the solvent in a suitable ratio. Perform the extraction using a Soxhlet extractor for several hours or through batch extraction by allowing the mixture to stand for 24-48 hours with occasional stirring. After extraction, filter the mixture to separate the liquid extract from the solid residue. Remove the solvent from the extract using a rotary evaporator under reduced pressure to concentrate the extract, while keeping the temperature low to avoid degrading heatsensitive compounds. Further concentrate the extract if necessary by drying. Analyze the extract for bioactive compounds using techniques like UV-Vis spectrophotometry or HPLC, and store the final extract in appropriate containers to preserve its stability. This method ensures a systematic and reproducible approach to solvent extraction, optimizing the yield and quality of the extracted bioactive compounds.

2. Alpha-Glucosidase Inhibition Assay[18]

This assay is designed to evaluate the potential of plant extracts or other substances to inhibit the enzyme alphaglucosidase, which breaks down carbohydrates into glucose. By inhibiting this enzyme, it is possible to reduce postprandial (after-meal) glucose levels, which is beneficial in managing diabetes.

For the assessment of α-glucosidase activity, the following materials are required: plant extract, which should be dissolved in a suitable solvent like DMSO; commercially available α-glucosidase enzyme sourced from Saccharomyces cerevisiae; and p-nitrophenyl-α-D-glucopyranoside (pNPG), a synthetic substrate used to measure enzyme activity. A 0.1 M phosphate buffer at pH 6.8 is necessary to maintain optimal conditions for the reaction. Acarbose will serve as a positive control, acting as a known inhibitor of α -glucosidase. To stop the reaction, a 0.1 M sodium carbonate solution is utilized. Finally, a spectrophotometer set to 405 nm, along with test tubes, micropipettes, and other laboratory consumables, will facilitate the experimental procedure.

Method of Preparation

To evaluate the inhibitory effect of plant extracts on α -glucosidase activity, various solutions were prepared. Plant extracts were dissolved in DMSO to create concentrations of 100, 200, 300, 400, and 500 μ g/mL. The α glucosidase enzyme solution was prepared in a 0.1 M phosphate buffer (pH 6.8) at a concentration of 1 U/mL, while a 5 mM solution of p-nitrophenyl-α-D-glucopyranoside (pNPG) was also prepared in the same buffer. For the assay, 50 µL of the enzyme solution was mixed with 50 µL of each plant extract concentration and incubated at 37°C for 10 minutes. Following this, 50 µL of pNPG was added, and the mixture was incubated for an additional 20 minutes to allow substrate hydrolysis. The reaction was stopped by adding 100 µL of 0.1 M sodium carbonate, which also facilitated the development of a yellow color in the resulting p-nitrophenol. Absorbance was measured at 405 nm using a spectrophotometer, with higher absorbance indicating greater enzyme activity. Control experiments included a mixture without plant extract and a positive control with acarbose at equivalent concentrations. The percentage inhibition of α-glucosidase was calculated, and IC50 values were determined by plotting the percentage inhibition against extract concentrations, identifying the concentration required for 50% inhibition.

$$
\% Inhibition = \frac{(Absorbane of Control - Absorbance of Sample)}{(Absorbance of Control)} \times 100
$$

3. DPP-4 Inhibition Assay^[19]

The Dipeptidyl Peptidase-4 (DPP-4) InhibitionAssay is used to evaluate the ability of a compound, such as a plant extract, to inhibit the enzyme DPP-4. DPP-4 plays a role in glucose metabolism by degrading incretin hormones like GLP-1 (Glucagon-like peptide 1), which stimulate insulin secretion in response to meals. Inhibition of DPP-4 results in increased levels of GLP-1, leading to improved insulin secretion and better regulation of blood glucose.

For the assessment of DPP-4 enzyme activity, several key components are utilized, including commercially available DPP-4 enzyme and Gly-Pro-p-nitroanilide (Gly-Pro-pNA) as the substrate. Plant extracts are prepared by dissolving them in DMSO or another suitable solvent. The reaction is buffered with a 0.1 M Tris-HCl buffer at pH 8.0 to maintain optimal enzyme activity. Sitagliptin, a known DPP-4 inhibitor, serves as a positive control in the experiments. A spectrophotometer or microplate reader set to 405 nm is employed to measure absorbance, allowing for the quantification of enzyme activity and the evaluation of the inhibitory effects of the plant extracts. This setup provides a comprehensive approach for investigating potential DPP-4 inhibitors in therapeutic research.

To prepare for the assessment of DPP-4 enzyme activity, the enzyme is dissolved in a 0.1 M Tris-HCl buffer at pH 8.0, typically at a concentration of 0.01–0.1 U/mL, adjustable based on assay optimization. Gly-Pro-pnitroanilide (Gly-Pro-pNA) is prepared in the same buffer at a concentration of 1 mM; upon cleavage by DPP-4, it releases p-nitroaniline, producing a yellow color. Plant extracts are dissolved in DMSO and prepared in serial dilutions ranging from 100 to 500 µg/mL. Additionally, a stock solution of Sitagliptin, a known DPP-4 inhibitor, is prepared at varying concentrations (100–500 µg/mL) to serve as a positive control. This setup allows for effective evaluation of the inhibitory potential of plant extracts against DPP-4 activity.

Method of Preparation

To evaluate the inhibitory effect of plant extracts on DPP-4 enzyme activity, 50 µL of each extract at concentrations ranging from 100 to 500 µg/mL is added to test tubes, followed by 40 µL of a DPP-4 enzyme solution (0.01–0.1 U/mL in Tris-HCl buffer). After a 10-minute incubation at 37°C, 10 µL of Gly-Pro-pNA substrate (1 mM) is introduced to initiate the reaction, which is then allowed to incubate for an additional 30 minutes. Absorbance is measured at 405 nm using a spectrophotometer, reflecting the release of p-nitroaniline and indicating enzyme activity. A negative control (enzyme and substrate without extract) establishes 100% activity, while varying concentrations of Sitagliptin serve as a positive control for comparison. The percentage inhibition of DPP-4 activity by the plant extract is calculated, and the IC50 value—representing the extract concentration required to inhibit 50% of enzyme activity—is determined by plotting the percentage inhibition against extract concentration, allowing for assessment of the extract's potency as a DPP-4 inhibitor.

Spectral Analysis And Structural Confirmation Of The Compound

The Structure elucidation of isolated fractions E3 fractions was carried out by using different spectroscopic analytical methods as Furrier Transfer Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance: H-NMR and Mass spectroscopy (MS) to find out the active component responsible for the pharmacological action.

8. RESULT AND DISCUSSION 8.1 Morphological Study Macroscopy

Figure 1: Solanum lycopersicum linn.

8.2 Microscopy

1. Physicochemical evaluation

The physicochemical evaluation of Solanum lycopersicum reveals significant attributes relevant to its composition and potential applications. With a total ash content of 14.03%, the plant demonstrates a rich inorganic mineral profile, while the water and acid-insoluble ash components (3.9% and 3.1%, respectively) provide insights into its purity. Extractive values indicate substantial yields of soluble compounds, with dichloromethane yielding 8.9%, suggesting a diverse array of phytochemicals. The moderate moisture content (7% loss on drying) is crucial for stability during storage. A foaming index greater than 100 indicates minimal foaming potential, which may affect formulation uses. Notably, the high tannin content of 21% highlights its astringent properties and potential for antioxidant and antimicrobial activities. Collectively, these findings position Solanum lycopersicum as a promising candidate for various industrial and medicinal applications.

Sr. No	Physicochemical properties	Solanum lycopersicum values in % w/w
$\mathbf{1}$	Total ash	14.03 ± 1.40
$\overline{2}$	Water insoluble ash	3.9 ± 1.23
3	Acid insoluble ash	3.1 ± 1.34
$\boldsymbol{4}$	Water extractive	2.0 ± 1.7
5	Methanol extractive	$5.3 + 0.75$
6	Ethanol extractive	5.7 ± 0.87
7	Dichloromethane extractive	8.9 ± 1.18
8	Loss on drying	$7 + 1.20$
$\boldsymbol{9}$	foaming index	>100
10	Tannins contents	21 ± 1.22

Table 4: Physicochemical evaluations of the Solanum lycopersicum

8.2 Soxhlet Extraction

Figure 2: Soxhlet Extraction

Various solvents were used in order of increasing polarity. Air, ethanol, and petroleum ether (60-80). The extraction was carried out in batches.

Bean leaves that have been shade-dried are powdered and treated with a number of solvents. The presentation of the extracts as well as their percent yield are described in the table below.

Sr.	Evaluation	Color	Nature	Percentage Yield
No.	Parameters			$(\% W/W)$
1.	Petroleum ether	Pale yellow	Semisolid	32.47
$\overline{2}$.	Ethanol	Red to dark red	Solid	48.56
3.	Chloroform	Pale yellow	Semisolid	14.56
4.	Ethyl acetate	Yellowish	Semisolid	0.86
5.	Aqueous	Colorless	Semisolid	0.78

Table 5:Yield of various extracts obtained from the Fruits of Solanum lycopersicum

8.3 Preliminary Phytochemical Screening

The extracts included Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate, and Vitamin C. The chemical components of the majority of medications decide their pharmacological and biological activity. A qualitative chemical analysis is used to evaluate a compound's accuracy and purity. To assess the identification, isolation, and purification of active chemical constituents, chemical methods of calculation are used.

Table 6:Preliminary Phytochemical Screening of Various Extracts of Solanum lycopersicum

Extracts	Petroleum ether	Ethanol	Chloroform	Aqueous
Tests for carbohydrates				
Molish Test		$^{+}$		
Fehling Test		$^{+}$		
Benedict Test		$^{+}$		
Test for Monosaccharide				
Barfoed's Test	$^{+}$			
Test for Non-reducing polysaccharides				
Iodine Test				
Test for Proteins				
Biuret test				

+ Indicates presence of phytoconstituents,

- Indicates absence of phytoconstituents

A phytochemical study of Solanum lycopersicum revealed the presence of alkaloids, hormones, flavonoids, tannins, and terpenoids.

8.4 Chromatographic Analysis

1. Thin Layer Chromatography

The TLC studies of ethanol of Solanum lycopersicum extract are as follows

Figure 3: TLC of Steroid ethanol **Figure 4:** TLC of tannins ethanolof Solanum lycopersicum extract of Solanum lycopersicum extract

Table 7: TLC of Extracts

2. Column chromatography of active extract of Solanum lycopersicum

Fractions from the ethanol extract of * Solanum lycopersicum * fruits were collected using column chromatography with a mobile phase of n-Hexane, n-Hexane: Ethyl acetate (5: 5), Ethyl acetate , Ethyl acetate: Methanol(5: 5) and Methanol. These fractions were then further sub-fractionated with a mobile phase of n-Hexane: Ethyl acetate (5: 5) using silica gel with a particle size of 60-120 mesh. The resulting fractions were analyzed using UV, and FTIR study

8.5 Ethanol Extract Column Chromatography Observation Table:

Figure 5: Column chromatography

8.6 Pharmacological Screening Of Isolated Extracts

1. In vitro antidiabetic activity by α amylase activity.[17]

The antidiabetic activity of the ethanolic extract of Solanum lycopersicum fruits was evaluated by measuring its ability to inhibit alpha-amylase, with Standard Acarbose serving as a reference. The results revealed that Standard Acarbose, with an absorbance of 0.3734, achieved an impressive alpha-amylase inhibition of 81.7%. This indicates its strong efficacy as an alpha-amylase inhibitor, underscoring its effectiveness in controlling blood glucose levels. In contrast, the ethanolic extract of the fruit exhibited a lower inhibition of 38.0% with an absorbance of 0.2832. While this demonstrates a moderate antidiabetic potential, it is significantly less effective compared to the standard. This suggests that while the ethanolic extract has some inhibitory activity against alpha-amylase, its potency is not as high as that of Acarbose.

Sr.N 0.	Sample Code	Conc. μ g/ml	Absorbance of sample(A)	% Inhibition of Alpha-amylase
	Control	NA	0.2054	NA
2	Standard Acarbose	100	0.3734	81.7
3	Ethanolic extract	100	0.2832	38.0

Table 11: Antidiabetic activity of Standard and Ethanolic extract Samples

2. Alpha- Amylase Inhibition assay for fraction

Fraction were screened for invitro alpha amylase activity. Five different concentrations (20, 40, 80, 120, 160 µg/mL) of the fractions were used to find out the IC50 value of the fractions. Based on the IC50 value. Acarbose was used as the standard. For alpha amylase inhibition activity, all the fractions showed dose dependent inhibition activity. Among the entire fractions, E3 fraction showed highest activities with lowest IC50 value of

65.03 µg/mL, while other fractions namely C1, C2, C3, E1 and E2 showed IC50 value of 96.45µg/mL, 116.24 µg/mL, 128.07 µg/mL, 89.08 µg/mL and 93.97 µg/mL respectively. The absorbance and percentage of inhibition of the fractions were tabulated as follows.

Figure 6: Alpha- Amylase Inhibition Assay

3. In vitro alpha glucosidase inhibition activity of the extracts[18]

The alpha-glucosidase inhibitory potential of various extracts, including petroleum ether, chloroform, ethanol, and water, was evaluated across sample concentrations ranging from 100 to 500 μg/mL. A dose-dependent increase in inhibitory activity was observed for all extracts. At 500 μg/mL, the inhibition rates were 80.51% for chloroform, 75.27% for petroleum ether, 87.59% for ethanol, and 56.71% for water. Among these, the ethanol extract exhibited the highest inhibition with an IC50 of 197.39 μg/mL, followed by chloroform (223.29 μg/mL), petroleum ether (240.54 μg/mL), and water (399.29 μg/mL). Acarbose, used as the standard, demonstrated 89.54% inhibition at 500 μg/mL, with an IC50 of 130.85 μg/mL.

Figure 7: Alpha-Glucosidase Inhibition

4. In vitro DPP-4 Inhibition activity of the extracts[19]

The DPP-4 inhibition assay is useful for determining the ability of plant extracts to inhibit the DPP-4 enzyme, which is crucial in regulating glucose metabolism. A lower IC50 value signifies a more potent inhibitor, making this data valuable for assessing plant extracts' potential as antidiabetic agents by prolonging the activity of incretin hormones and boosting insulin secretion. The inhibitory potential of petroleum ether, chloroform, ethanol, and water extracts was tested at concentrations ranging from 100 to 500 μg/mL. All extracts showed a dose-dependent increase in inhibition. At 500 μg/mL, inhibition percentages were 66.23% for chloroform, 52.64% for petroleum ether, 72.03% for ethanol, and 50.40% for water. Ethanol extract had the highest inhibition with an IC50 of 333.37 μg/mL, followed by chloroform (431.94 μg/mL), petroleum ether (514.70 μg/mL), and water (573.73 μg/mL). Sitagliptin, used as the reference, showed 83.25% inhibition at 500 μg/mL, with an IC50 of 173.82 μg/mL.

5. Observation Table of DPP-4 Inhibition Assay

	300	0.589	22.30	
	400	0.512	32.45	
	500	0.359	52.64	
Ethanol	100	0.603	20.45	333.37
	200	0.507	33.11	
	300	0.417	44.99	
	400	0.318	58.05	
	500	0.212	72.03	
Water	100	0.656	13.46	573.73
	200	0.624	17.68	
	300	0.599	20.98	
	400	0.532	29.82	
	500	0.376	50.40	
Standard (Sitagliptin)	100	0.465	38.65	173.82
	200	0.329	56.60	
	300	0.267	64.78	
	400	0.198	73.88	
	500	0.127	83.25	
Control		0.758		

Figure 8: Data DPP-4 Inhibition Assay

Structure Elucidation Of Fraction

The Structure elucidation of isolated fractions E3 fractions was carried out. **Fraction E3**

Fraction collected (Fraction E2) was Characterized by IR, NMR and MASS spectra. The molecular formula of the compound is $C_{20}H_{16}O_{10}$ with molecular weight of 416.33. The chemical name of the predicted compound is 2-(4-acetyl-2,3-dihydroxy-5-methylphenyl)-5,6,7-trihydroxy-4-oxo-4H-1-benzopyran-3-yl acetate.

Spectroscopic techniques	Data interpreted
IR (cm ⁻¹) Kbr	3436.53 (HC=CH stretching), 2920.66 (C-H stretching), 1708.62
	(C=O stretching), 1465.63 (CH from CH ₃), 1170.58 (C-0) Ar-
	OH, 1112.73 (Ar C-O-C stretching), 1058.83 (C-C stretch).
	764.63 (C-H bending)
¹ HNMR (DMSO)	1H NMR: δ 2.08-2.20 (6H, 2.13 (s), 2.15 (s)), 2.34 (3H, s), 6.60
	$(1H, s), 7.63$ $(1H, s).$
EIMS (70 ev) : m/z	$417.74[M^+]$
	90.99,126.08,181.21,204.27,209.25,297.55,311.58,325.61,339.62

Table 15. Spectroscopic data of isolated Fraction E3

Figure 9. IR spectra of Fraction E3.

Figure 10. Proton NMR spectrum of Fraction E3

Figure 11. MS spectrum of Fraction E3

On the basis of chromatographic, IR, NMR and MASS spectra data of compound and as per literature elucidated structure is match with spectral data containing steroidal flavonoid.

Figure 12. Structure of elucidated Fraction E3.

9. Summary and Conclusion

The comprehensive evaluation of Solanum lycopersicum reveals its significant potential as a source of bioactive compounds with various industrial and medicinal applications. The physicochemical analysis indicates a robust mineral profile, with a total ash content of 14.03%, suggesting a rich source of inorganic elements that could be beneficial in dietary supplementation. The moderate moisture content (7%) and minimal foaming index further indicate stability, making it suitable for storage and formulation in various products.

The extraction studies demonstrate that different solvents yield varying amounts of phytochemicals, with ethanol and petroleum ether extracts producing the highest yields. This highlights the diverse array of soluble compounds present in Solanum lycopersicum, which may contribute to its bioactivity. The preliminary phytochemical screening confirms the presence of important constituents such as flavonoids, tannins, alkaloids, and terpenoids, all of which are known for their therapeutic properties.

Chromatographic analyses successfully separated key compounds, including carotenoids and sterols, providing a basis for further investigation into their specific roles in health benefits. The pharmacological screenings reveal moderate antidiabetic activity, with ethanolic extracts showing significant inhibition of both alpha-amylase and alpha-glucosidase enzymes, essential for managing blood glucose levels. The identified IC50 values indicate a dose-dependent relationship, reinforcing the potential of Solanum lycopersicum extracts as functional foods or supplements in diabetes management.

In conclusion, Solanum lycopersicum emerges as a promising candidate for further research and application in pharmacology and food industries. Its diverse phytochemical profile and demonstrated bioactivity warrant exploration into its potential uses in developing natural therapeutics and functional foods. Future studies should focus on isolating specific compounds, elucidating their mechanisms of action, and exploring their efficacy in clinical settings, ultimately enhancing our understanding of the health benefits associated with this widely consumed fruit.

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