An Indian Traditional Medicinal Plant For Nutraceutical Properties Of Hydroalcoholic Extract Of Solanum Torvum Fruit Coat

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Abstract

Objective*: To study the effect of the Hydroalcoholic extract(HAE) of Solanum torvum(S.T.) fruit coat by various in-vitro methods for studying its nutraceutical properties to find new, non-toxic, cost-effective and non-resistant drugs.*
Methods: *The fruit coat was extracted in ethanol(organic solvent) and water. This study determined phytochemical constituents, antioxidants properties, anti-diabetic properties, anti-inflammatory properties, anti-bacterial properties, TLC analysis, FTIR analysis and GC-MS analysis.*
Results: *By qualitative and quantitative phytochemical analysis, the HAE of the S.T. fruit coat showed the presence of phenolic compounds, flavonoid and alkaloid compounds majorly, along with, carbohydrates, and saponins compounds minorly. The antioxidant activity ofthe extract possesses the strongest in DPPH; ABTS; FRAPand in Phosphomolybdate assay. The anti-diabetic activity showed the extract can effectively inhibit the α-amylase activity and α-Glucosidases activity. The anti-inflammatory activity by Inhibition of Egg Albumin denaturation and by Inhibition of the BSA denaturation. The antibacterial activity of the extract was tested against Pseudomonas aureus and Bacillus subtilis. TLC chromatogram revealed the presence of Phenolic, Flavanoids and Alkaloids in the extract. FTIR analysis revealed that even though almost similar bands were observed in the spectrum of both solid and liquid medium of HAE of S.Tof fruit coat, the transmittance intensity originating from liquid form HAE of S.T.was lower than the solid form. GC-MS analysis showed the presence of 24 compounds.*
Conclusion: *The study suggested that the hydroalcoholic extract of S.Tfruit coat is promising for the development in the treatment of various human disorders in the future.*

Keywords: *Solanum torvum, hydroalcoholic extract, phytochemical, antioxidants, anti-diabetic, anti-inflammatory, anti-bacterial assay, TLC analysis, FTIR analysis and GC-MS analysis.*

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INTRODUCTION

A spikey shrub 1-3m towering in the Solanaceae family is called *Solanum torvum* and is commonly known as Sundakai. It has wholesome fruits, unpigmented bell-structure flowers, and broadly oval to concise leaves with shallow, enervated borders [1]. It ample-coveringThailand and other tropical regions where it is stumbled[2] and familiarized as turkey berry or Thai eggplant[3].While diverse plant components are commonly employed in ethnomedicine, fruits are used as a vegetable and flavour in cookery [4]. Traditional uses for the whole plant include relaxing, antibiotic, and gastrointestinal properties [5]. Wheezing and expanded liver and spleen are treated using fruit and leaf brewing[6]. Use of

leaves for hemostasia and anti-inflammatory causes[7,8 and 9]. The investigation is done on plant extracts and metabolites, particularly those from fruits and leaves, widely using various extraction techniques and solvents. They contain a range of phytochemical components, notably steroidal alkaloids, phenols, glycosides, flavonoids, saponins, and vitamins C and E [10,11]. Plentiful pharmacological studies have concluded that the phytochemical components of this plant have antioxidant, antiplatelet aggregation, cardiovascular, analgesic, anti-inflammatory, antiviral, anti-microbial, and other properties[12,13]. Subsequently, medicative plants incorporate therapeutic contents, they have been hired to cure human disorders [14].

Due to an increased perception of the persuasiveness, quality, and safety guarantee of ethano- medication, they are now widely used in impoverished nations. Additionally, a great deal of research has been done on the plant's phytochemical components and antibacterial properties as potential sources of therapeutics. Remarkably, the ethanolic extract as a solvent of S.T. showed good antibacterial activity as proved by previous studies[15]. As water and other solvents showed a minimum propensity of compounds to be extracted from solanum fruit coat as proved from earlier data, in our studies, we have used ethanol and water called hydroalcoholic S.T. fruit coat extract in a 1:1 ratio. The kind of extraction solvents employed determines how well natural substances may be anticipated from plant material. Ethanol seems to be the best solvent to extract compounds with different properties by using the Solvent extraction method.

After everything, there is no information till now about the hydroalcoholic extract of *S. torvum* fruit coat regarding its antioxidant, anti-inflammatory, and anti-diabetic properties. Medicinal plants are currently being investigated as options for the treatment of various illnesses due to the defiance issue to solve the problem of drug resistance, fewer side effects and cost-effective treatment of herbal medicines. Determining the phytochemical components of the hydroalcoholic extract of *S. torvum* fruit coat using a gas chromatography-mass spectrometry (GC-MS) test, Thin layer Chromatography test(TLC) and FTIR test. Along with examining the extract's presence of phytochemical constituents by qualitative and quantitative phytoconstituent analysis, antioxidant activity, anti-inflammatory activity, antidiabetic properties and antibacterial effectiveness against two common pathogenic bacteria were the goals of the current investigation.

MATERIALS AND METHODS

Plant materials

S. torvum dried fruits were procured from a Siddha shop in Tambaram, Tamil Nadu, Chennai, India.

Preparation of *Solanum torvum* fruit coat extract

The dried fruit coat of S.T. was coarsely powdered and 50 gm of coarsely powdered sample was extracted by Soxhlet extractor with 100ml ethanol and 100ml distilled water(for 72 h). The extract was filtered using Whatman No. 1 filter paper. The excess solvent was removed by a rotary vacuum evaporator under 60 °C temperature referred to as the hydroalcoholic extract of the *Solanum torvum* fruit coat. 5mg of concentrated dried extract was dissolved in 1 ml of corresponding solvent mixtures of ethanol and D/W(1:1) ratio and mixed. The resultant suspension was centrifuged for 20min at 4°C and 10,000 rpm. The solution was stored at -20°C for further studies[16].

Qualitative phytonutrient analysis of Hydroalcoholic fruit coat extract of *Solanum torvum*

The hydroalcoholic fruit coat extract of *Solanum torvum* berries was subjected to preliminary phytochemical analysis[17]. The presence of alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, flavonoid compounds, terpenoids, and steroids was observed.

Quantitative phytonutrient analysis of Hydroalcoholic fruit coat extract of *Solanum torvum*

The hydroalcoholic fruit coat extract of *Solanum torvum* berries was subjected to determine major organic constituents that is total phenolic compound, total flavonoid and total alkaloid compounds were observed.

Estimation of total phenol content

The entire phenol was predictedspectrophotometrically by applying the Folin-Ciocalteu assay method[18]. Here 20–100 μg/ml of the extract was added to 2.5ml of 10% Folin-Ciocalteu reagent in tubes. It was then swirled for the 30s and approved to stand for 10 min at 25℃. 2 ml of 7.5% anhydrous sodium carbonate was supplemented to the solution and swirled again for another 30s. The tubes were incubated in a water bath at 40℃ for 30min for colour deployment, and absorbance was read at 765nm using a spectrophotometer. The total phenolic content was then deliberated as mg/g gallic acid (GAE/gm) equivalent.

Estimation of total flavonoid content

The entire flavonoid was predicted spectrophotometrically by using the aluminium chloride colourimetric assay[18]. The solution was made up of 0.5ml of the plant extract, 2ml of distilled water in a tube and 0.15 ml of 5% sodium nitrite. The solution was left for 5 min at room temperature then 0.15 ml of 10% aluminium chloride was added and incubated for another 5 min. After incubation, 1 ml of 4% sodium hydroxide was added and the solution was made up to 5 ml with distilled water. It was then swirled and incubated for 15 min to observe a colour change. Absorbance was deliberated at 420nm. The entire flavonoid content was determined as mg/g quercetin proportionate.

Estimation of total alkaloid content

A component of the extract remnant was dissolved in 2N HCL and then processed. 1 ml of this solution was conveyed to a separablefilter and washed with 10 ml chloroform (3 times). The pH of this solution was amended to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were supplemented to this solution. The mixture was shaken and complex excerpted with 1, 2, 3 and 4 ml chloroform by intense shaking, the extract was then possessed in a 10 ml volumetric vial and impoverished with chloroform.Exact measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution were transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution were taken and the concoction was jolted with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then together in a 10 ml volumetric flask diluted to accustom the solution with chloroform. The absorbance of the composite in chloroform was determined at the spectrum of 470 nm in a UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine[19].

In-vitro Anti-oxidant assay

Scavenging Activity of DPPH Radical

The consequences of solvent fractions on DPPH free radicalswere determined bythe method of Brand-Williams et al. [20], andYoo et al. [21]. 4 ml of the fractions were combined to 1 mL DPPH, and the mixture was condensed and left to stand in the dark for 30 min. Absorbance was determined using a spectrophotometer at 520 nm, and ascorbic acid was used as a standard. The scavenging activity of free radicals by the sample was determined as the ratio of multiplication of the absorbance of the control and the absorbance of the extract with the absorbance of control times one hundred.Ascorbic acid was used as a standard. The experiment was completed with 3 times of replication.The results were determined using a calibration curve of ascorbic acid (20–100 μg/ml) and outcomes were forwarded as ascorbic acid equivalents of antioxidant capacity in μM Ascorbic acid per gram of extract.

Scavenging Activity of ABTS Radical

The entire antioxidant activity of the samples was measured using $ABTS^{+}$ radical cation decolourization assay as studied by Re et al. [22]. ABTS was mixed in water to a 7 mM concentration and ABTS radical cations were formed by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was laid to stand at the dark room temperature for 12–16 h before utility. ABTS oxidation was started immediately, but the absorbance was not utmost and balanced until 6 h lapse. The radical cation was balanced in this pattern for extra than 2 days inthe repository in the dark at room

temperature for 16 h to concede the accomplishment of radical generation. It was diluted with ethanol (99.5%) so that its absorbance was adjusted to 0.70 ± 0.02 at 734 nm. To figure out the scavenging activity, 190 µL of ABTS reagent was mixed with 20–100 µg/mlof different extractconcentrations and the absorbance was measured at 734 nm after 30 min of reaction time at room temperature. Ascorbic acid was used as a standard. The experiment was replete 3 times.The results were deliberated using a calibration curve of ascorbic acid (20–100 µg/ml) and results were expressed as ascorbic acid equivalents of antioxidant capacity in µM Ascorbic acid per gram of extract.

Ferric reducing antioxidant power (FRAP) assay

Anabundance of 0.25 g of extract (dry)HAE of *Solanum torvum* fruit coat was dissolved in 2.5 mL of ethanol and 2.5 ml of D/W. The working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ at a (v/v/v) ratio of 10:1:1. A total of 100 µL of each properly diluted extract solution at diverse concentration 20–100 µg/ml was prepared in tubes with 3 mL of working reagent and shaken for 30 s. After 2 h of incubation, the absorbance was read at 593 nm[23,24].

Phosphomolybdenum Method

The approach adopted by Ahmed et al.[25] was used to resolve the total antioxidant capacity with minute changes. 20–100 µg/ml of diverse concentrationsof plant extracts, vitamin C and gallic acid stacked in total concentrations of 0.5 ml were mixed with 3 ml of distilled water and 1 ml of phosphomolybdate reagent in test tubes. The solutions were put in an incubator at 95℃ for 90 min. After incubation, the tubes were normalized to room temperature for about 30min. Absorbance was measured at 695 nm.

In-vitro Antidiabetic assay

In vitro antidiabetic activity was evaluated by α-Amylase inhibition activity and α-Glucosidase inhibition activity.

The α-glucosidase inhibition activity

The inhibitioncapacity of extracts against α-glucosidase was measured to determine in-vitro antidiabetic potential. Diverse concentrations of extracts (20–100 µg/ml) were combined to phosphate buffer (70 mL of 50 mM) trailed by the inclusion of a-glucosidase (one unit/mL). After 10 min incubation at 37°C, 5 mM of ρ-nitrophenol glucopyranoside was combined and absorbance was noted at 405 nm after 30 min. Acarbose was used as a standard reference [26]. % Inhibition = (Ab-As)/Ab*100; Where Ab is the absorbance of blank, As is the absorbance of Sample.

Theα-amylase inhibition activity

The 20–100 µg/ml of individual extractwascombinedwith 0.02M sodium phosphate buffer containing an α-amylase (0.5mg/mL). Thereaction concoction was incubated for 10 min at 25°C. The dinitro salicylic acid was combined with the mixture to stop the reaction. The reaction solutions were then incubated for a time period of 5 min and diluted with distilled water to note absorbance at 540 nm. A control (no extract) was also run and acarbose was used as a standard enzyme inhibitory substance [26]. The % inhibition was calculated by the following relationship. % Inhibition = (Ab-As)/Ab*100; Where Ab is the absorbance of blank, As is the absorbance of Sample.

In-vitro Anti-inflammatory assay by Protein denaturation

In vitro, anti-inflammatory activity is assessed by Inhibition of albumin denaturation and Inhibition of BSA denaturation.

BSA anti-denaturation assay

The method of [27] was used. The reaction mixture was poised of 450 µL of 5% w/v BSA solution and 20–100 µg/ml of HAE of *Solanum torvum* fruit coat or standard drug solutions (aspirin) at different

concentrations (20–100 μg/ml). The sample mixture was conserved for 15 min at 37 °C and then for 5 min at 70 °C. Later cooling the samples, 2.5 mL PBS (pH 6.3) was added to the mixture. The absorbance of the test and control samples was measured at 660 nm. The experiment was run three times and the % inhibition in protein denaturation was calculated using the equation; % Inhibition = (Ab-As)/Ab*100; Where Ab is the absorbance of blank, and As is the absorbance of Sample.

Egg albumin (EA) denaturation inhibition assay

This assay has been done as described previously [28]. The reaction solution was tranquillisedwith 0.2 mL of fresh hen's egg albumin, 2.8 mL phosphate buffer saline (pH 6.4) and 2 mL of various concentrations (20–100 μg/ml) of HAE of *Solanum torvum* fruit coat or standard drugs. All the samples were set aside at 37 °C for 25 min followed by heating for 5 min at 70 °C. The cooled solutions were centrifuged at 3000 rpm for 10 min. Then the absorbance of supernatant solutions was measured at 660 nm. The % of inhibition was calculated as mentioned in the BSA denaturation inhibition assay procedure.

In-vitro Antibacterial assay

Microbial strains

Veritable pious clinical secluded cultures of human virulent bacteria Gram-positive Pseudomonas aureus and Gram-negative bacteria Bacillus subtilis procured from Avigen laboratory, Chromepet(T.N).

Agar well diffusion method

Antibacterial activity ofhydroalcoholic extract of *Solanum torvum*fruit coat assessed by agar well diffusion method on nutrient agar medium[29]. This was verified by the ability of the recognised antibiotic to inhibit bacterial growth as indicated by the inhibited zone.The antiseptic nutrient agar medium (20ml) in Petri dishes was evenlyanointed using a cotton daub with authentic test cultures of Gram-positive Pseudomonas aureus and Gram-negative bacteria Bacillus subtilis. The nutrient agar medium was prepared by dissolving 0.3 beef extract, 0.3 yeast extract, 0.5 peptones, 0.5 NaCl and 1.5% agar in 1 litre of distilled water. The well of 5mm diameter was made using a sterile cork borer in each Petri plate and various extracts of Sundakai fruit coat were added, a blank well loaded without test compound was regarded as the control. For each treatment 3 replicates were made. The plates were incubated at 37°C for 24hrs and the resulting zone of inhibition was measured by comparing the control and the standard antibiotics.

Phytochemical screening by TLC

Thin layer chromatography was doneemployinga 4 × 10 cm TLC plate. The process was carried out with a solvent system of ethanol: distilled water (50:50) as previously described with some modifications[30]. Spot detection was carried out under UV light at 254 and 365 nm. Then calculated R_f (retardation factor) value was, which is the ratio of the solute's distance travelled to the solvent's distance travelled when a given component will always travel the same distance in a given solvent under some conditions.

FTIR analysis – *solanum torvum* solid and liquid phase comparison

The hydroalcoholic extract of the plant was in solid/dried and in liquid form for the analysis of FTIR with an attenuated total reflectance (ATR) accessory was used to record FT-IR transmission spectra from 700 to 4000 cm^{-1}. In the FTIR assay, the extract of the plant'sfruit coat with the concentration of 10 mg was then loaded in the FTIR spectroscope (Shimadzu, IRAffinity-1, Japan) and the resolution was 2 cm^{-1}.

GC-MS analysis

The GC-MS analysis of HAE of *Solanum torvum* fruit coat was performed using Agilent 8890 GC system (Agilent Technologies; Santa Clara, CA, USA) in SAIF (IIT Madras, Chennai, Tamil Nadu). The instrument was equipped with a split-splitless injector. The column used was a DB-5 GC column (30 m × 0.25 mm, 0.25 μm). The name, molecular formula and compound nature of the component in the tested

material were ascertained[32]. The run time was 53.5min and sample overlap is not enabled. Briefly, the injector, detector, and transfer-line temperature were set at 250°C, 160°C, and 230 °C, respectively, with a split ratio of 1:10. The individual extract components, were separated from each other using a linear temperature program. The temperature wasincreased from 75 to 180 °C at an increasing rate of 5 °C min–1. After that, the temperature was held for 5 min at 300 °C at an increasing rate of 5 °C min–1. Using a full scan mode and electron ionization (EI) at 70 eV, the mass detector range was set to scan ions between 40 and 400 m/z. Samples wereanalyzed in duplicate. For each separated component of the extract, the relative percentage of each component (%Area) was calculated by comparing its average peak area to the total area and retention time was calculated. The extract components were then tentatively identified by comparing theircalculated retention indices with those reported in the literature as given in table 9. The mass spectrum of the unknown component was compared with those stored in the Wiley7n.1 library, NIST and ADAMS-2007.

RESULTS

Qualitative phytonutrient analysis of Hydroalcoholic fruit coat extract of *Solanum torvum*

The most abundant phytochemical composition of S.T. fruit coat hydroalcoholic extract arealkaloids, carbohydrates, saponins, phenolic compounds, and flavonoid compounds as shown in Table 1.

Table 1: Major compounds identified in the qualitative estimation of the hydroalcoholic extract of S.torvum exhibits the presence of alkaloids, carbohydrates, saponins, phenolic compounds, and flavonoid compounds.

Bioactive compound	S.T. Hydroalcoholic extract-Presence or Absence	Colour observed
1. Alkaloids	+	Coffee brown ppt
2. Carbohydrate	++	Red ppt
3. Glycosides	-	No pink colour ob.
4. Saponins	+++	Foam formation ob.
5. Proteins	-	No pink col. Ob. in ethanolic layer
6. Amino acid	-	No purple col. Ob.
7. Phenolic compound	+++	Dark green col. ob
8. Flavanoid compound	+++	Yellow col. Ob.
9. Terpenoids	-	No reddish brown layer ob.
10. Steroids	-	No blue green col. Ob.

Quantitative phytonutrient analysis of Hydroalcoholic fruit coat extract of *Solanum torvum*

Our results divested that phenols are the most abundant phytochemical composition of S.T. fruit coat hydroalcoholic extract. The extract also contains good amounts of alkaloids and flavonoidsas shown in Table 2.

Table 2: Data acquired on the quantitative estimation of major organic constituents present in the selected plant drug was tabulated showing the presence of phenols, alkaloids and flavonoids.

Bioactive compound	Quantity(µg/ml)
Total Phenolics	1.448
Total Flavonoids	0.889
Total Alkaloids	0.200

In-vitro Anti-oxidant assay

Scavenging Activity of DPPH Radical

DPPH is a stable nitrogen-based free radical that is violet in colour and turns yellow after hydrogen or electron transfer reduction. The radical scavenging capacity of the HAE of S.T.FC was tested using the stable free radical DPPH. Fig 1. demonstrates the effective concentrations of HAE of S.T.FC required to scavenge DPPH radical and the scavenging values (RSA%) as an inhibition percentage. The antioxidant activity increased with increasing concentration of HAE of S.T.FC in a dose-dependent manner. The order of DPPH radical scavenging activity was as follows: 100 > 80 > 60 > 40 > 20µg/mL.At the highest tested concentration (100µg/mL), HAE of S.T.FC showed 82.2%antioxidant activity, while Vitamin C, the standard antioxidant, showed 96.82% (100 µg/mL) activity depicted in Fig 1.

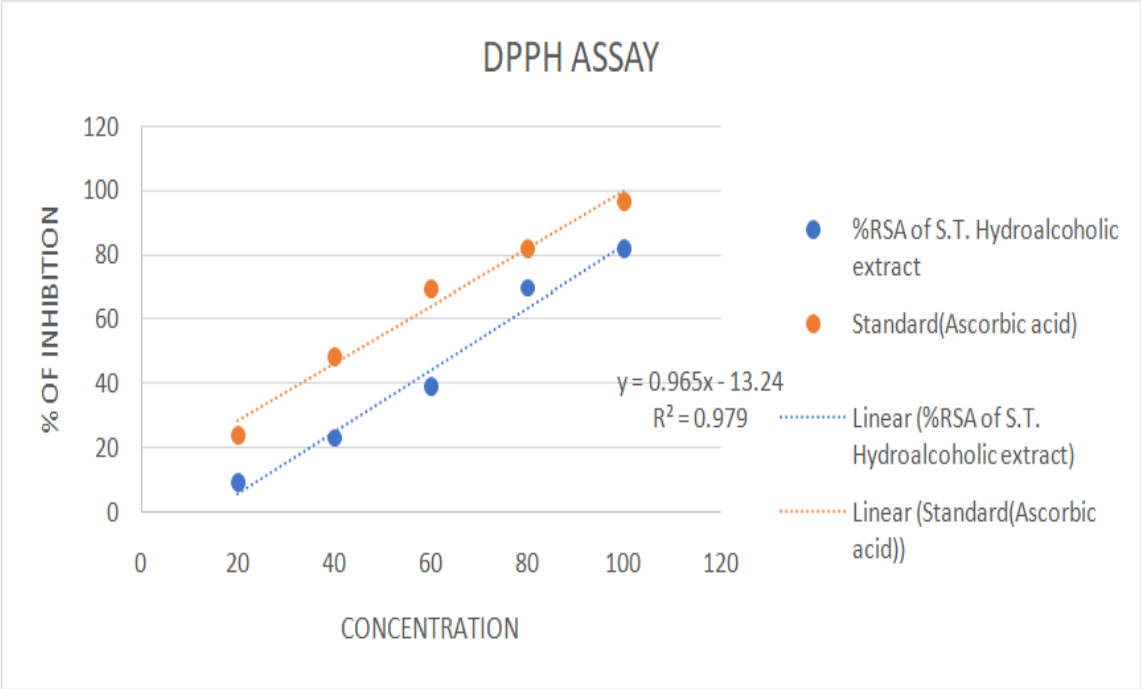


Figure 1: DPPH Antioxidant assayshowed S.T.FC. extract possesses 82.2%(100 µg/mL)of the strongest antioxidant activity, while Vitamin C, the standard antioxidant, showed 96.82% (100 µg/mL).

Scavenging Activity of ABTS Radical

The basis of the ABTS assay is the interaction between an antioxidant and the pre-generated $ABTS^{\bullet+}$ radical cation. $ABTS^{\bullet+}$ scavenging can be easily quantitatively detected due to the bleaching of the absorption spectrum. The radical scavenging capacity of the HAE of S.T.FC was tested using the stable free radical ABTS. Fig 2. demonstrates the effective concentrations of HAE of S.T.FC required to scavenge ABTS radical and the scavenging values (RSA%) as an inhibition percentage. The antioxidant activity increased with increasing concentration of HAE of S.T.FC in a dose-dependent manner. The order of ABTS radical scavenging activity was as follows: 100 > 80 > 60> 40 >20µg/mL.At the highest tested concentration (100µg/mL), HAE of S.T.FC showed 85.8% antioxidant activity, while Vitamin C, the standard antioxidant, showed 92.17% (100 µg/mL) activity as depicted in Fig 2.

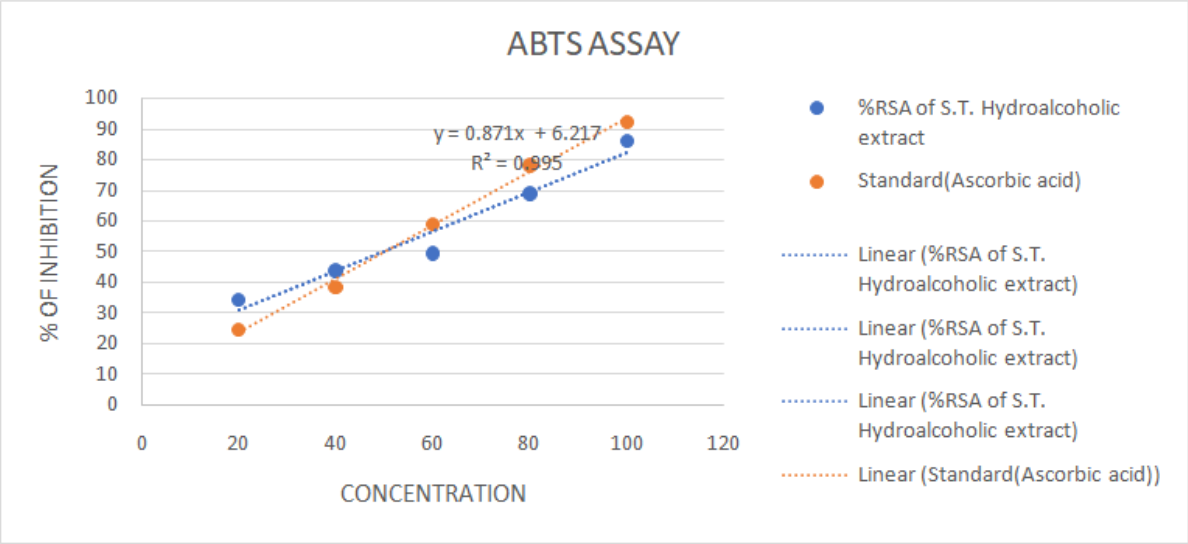


Figure 2: ABTS Antioxidant assayshowed S.T.FC. extract possesses 85.8% (100 µg/mL)of the strongest antioxidant activity, while Vitamin C, the standard antioxidant, showed 92.17% (100 µg/mL).

Ferric reducing antioxidant power (FRAP) assay

The assay was based on the reduction of colourless ferric-tripyridyl triazine to its blue ferrous-coloured form due to electrons donated by antioxidants and the ability of antioxidants to reduce ferric (3+) ions to ferrous(2+) ions. The FRAP was calculated and expressed as ascorbic acid equivalent (AAE) per fresh weight (AAE mg/g). The antioxidant activity of five different concentrations of HAE of S.T.FC was evaluated by measuring the conversion of Fe3+/ferricyanide complex to Fe2+/ferrous form. The results clearly indicate that the activity increases in terms of absorbance with an increase in concentration for all the extracts and the standard. The antioxidant potential of HAE of *Solanum torvum* fruit coat was ascertained from their ferric reducing antioxidant power and the maximum reducing ability was exhibited by the highest concentration of hydroalcoholic extract is 100µg/ml which has the reducing power of 0.89 and the lowest concentration of hydroalcoholic extract is 20µg/which has the reducing power of 0.24 respectively. The standard Ascorbic acid at the same concentration exhibits an absorbance of 0.94 which is depicted in Fig 3.

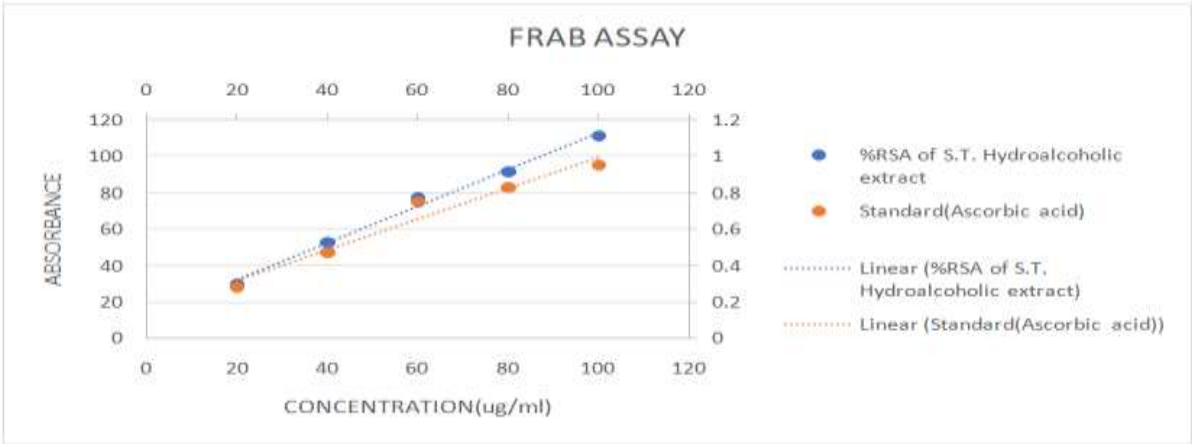


Figure 3: FRAP antioxidant assayshowed S.T.FC. extract possesses the reducing power of 0.89 (100 µg/mL)of the strongest antioxidant activity, while Vitamin C, the standard antioxidant, has 0.94 (100 µg/mL)reducing power.

Phosphomolybdenum Method

The phosphomolybdate assay has been used routinely to evaluate the total antioxidant capacity of plant extracts. In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green-coloured phosphomolybdenum V complex, which shows maximum absorbance at 695 nm. The antioxidant activity of five different concentrations of HAE of S.T.FC was evaluated by measuring the conversion of Fe3+/ferricyanide complex to Fe2+/ferrous form. The results clearly indicate that the activity increases in terms of absorbance with an increase in concentration for all the extracts and the standard. The antioxidant potential of HAE of *Solanum torvum* fruit coat was ascertained from their phosphomolybdate antioxidant power and the maximum reducing ability was exhibited by the highest concentration of hydroalcoholic extract is 100µg/ml which has the reducing power of 0.80 and the lowest concentration of hydroalcoholic extract is 20µg/mL which has the reducing power of 0.20 respectively. The standard Ascorbic acid at the same concentration exhibits an absorbance of 0.96 which is depicted in Fig 4.

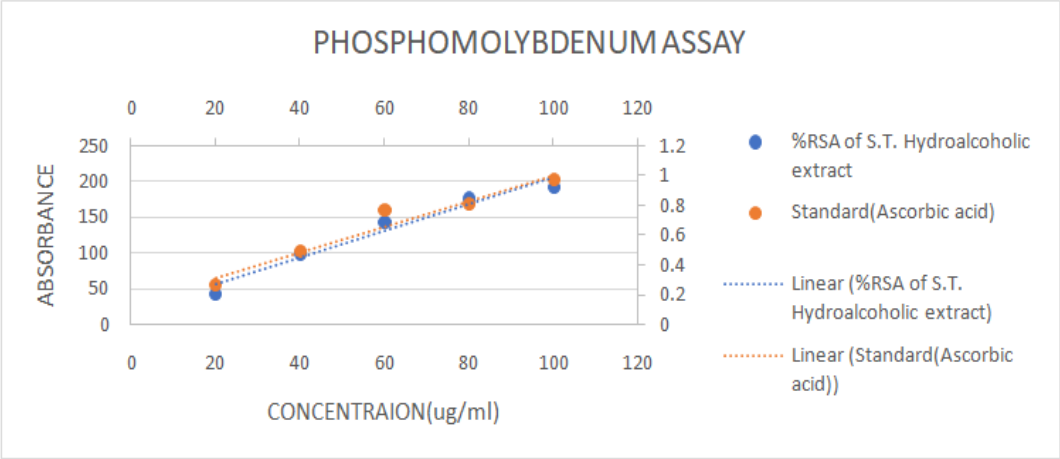


Figure 4: Phosphomolybdate antioxidant assayshowed S.T.FC. extract possesses the reducing power of 0.80 (100 µg/mL)of the strongest antioxidant activity, while Vitamin C, the standard antioxidant, has 0.96 (100 µg/mL)reducing power.

In-vitro Antidiabetic assay

In the present study, HAE of *S.torvum fruit coat* was screened for anti-diabetic activity using inhibiting the α-amylase and α- glucosidases activity. The data of the results obtained were shown in tables 3 and 4.. These results clearly suggest that the extract is capable of effectively inhibiting the α-amylase activity (60.00%) and α-Glucosidases activity (62.00%), while Acarbose, the standard antidiabetic agent, showed 90% (100 µg/mL) activity in α- amylase antidiabetic assay and 79% (100 µg/mL) activity in α-galactosidase antidiabetic assay respectively.

α-amylase antidiabetic assay

Table 3: In-vitro α-amylase antidiabetic assayof the S.T.FC. extract is capable of effectively inhibiting the α-amylase activity at 60%,while, Acarbose, the standard antidiabetic agent, showed 90% (100 µg/mL) activity

Concentration(µg/mL)	Inhibition% of alpha-amylase activity
20ug	29±0.07
40ug	38±0.03
60ug	43±0.05
80ug	56±0.02
100ug	60±0.09

Acarbose(100)	90±0.02

α-galactosidase antidiabetic assay

Table 4: In-vitro α-galactosidase antidiabetic assayof the S.T.FC. extract is capable of effectively inhibiting the α-amylase activity at 62%while, Acarbose, the standard antidiabetic agent, showed 79% (100 µg/mL) activity

Concentration(µg/mL)	Inhibition %alpha-glucosidase activity
20ug	27±0.01
40ug	38±0.04
60ug	45±0.09
80ug	54±0.06
100ug	62±0.03
Acarbose(100)	79±0.05

In-vitro Anti-inflammatory assay

In the present study, 5 different concentrations of the HAE of *S.torvumfruit coat* were evaluated for anti-inflammatory activity employing *in vitro* assays and the data of the results obtained were presented in tables 5 and 6. The HAE of the S.T. fruit coat revealed moderate anti-inflammatory activity 21.87% by Inhibition of Egg Albumin denaturation and 32.25% by the Inhibition of the BSA denaturation at 100µg/ml concentration, which was then compared with that of standard aspirin. While Aspirin, the standard anti-inflammatory agent, showed 75.65% (100 µg/mL) activity in the Inhibition of Egg Albumin denaturation assay and 79% (100 µg/mL) activity in the Inhibition of the BSA denaturation assay respectively.

Inhibition of Egg Albumin denaturation assay

Table 5: In-vitro Egg Albumin Anti-inflammatory assaythe HAE of the S.T. fruit coat revealed moderate anti-inflammatory activity 21.87% by Inhibition of Egg Albumin denaturationat 100µg/ml concentration. While Aspirin, the standard anti-inflammatory agent, showed 75.65% (100 µg/mL) activity.

Concentration(µg/ml)	Inhibition %
20ug	05.49±0.03
40ug	11.71±0.02
60ug	14.64±0.05
80ug	16.52±0.07
100ug	21.87±0.04
Aspirin(100)	75.65±0.09

Inhibition of BSA denaturation assay

Table 6: In-vitro BSA Anti-inflammatory assayof the HAE of the S.T. fruit coat revealed moderate anti-inflammatory activity 32.25% by Inhibition of Egg Albumin denaturationat 100µg/ml concentration. While Aspirin, the standard anti-inflammatory agent, showed 79% (100 µg/mL) activity.

Concentration(µg/ml)	Concentration
20ug	11.37±0.06
40ug	17.48±0.02

60ug	23.57±0.11
80ug	27.84±0.05
100ug	32.25±0.11
Aspirin(100)	66.47±.0.4

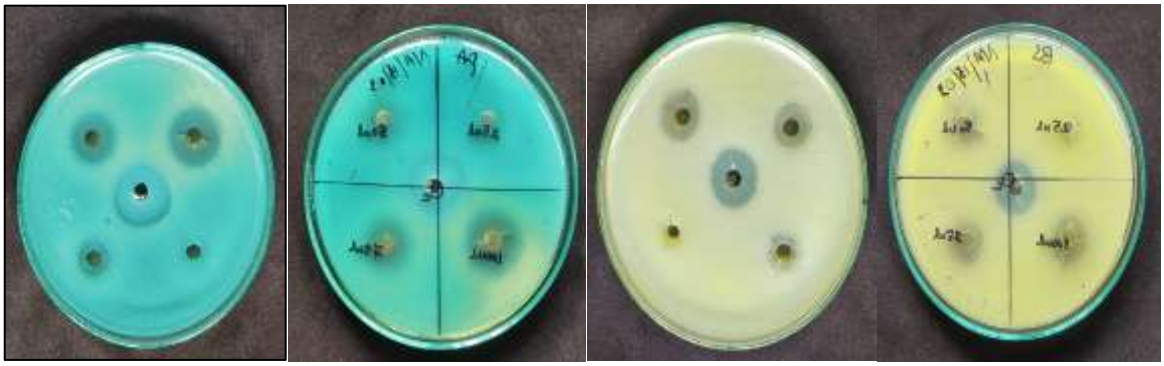
In-vitro Antibacterial assay

As per research, the fruits of *Solanum torvum* are frequently used as potions in ethnomedicine to cure acute pain, cold, and cough symptoms. Researchers found in the current study that the fruit coat of *Solanum torvum* demonstrates substantial antibacterial action against pure isolates of harmful bacteria. Based on the findings, the HAE of ethanol:water(1:1) of the S.T. fruit coat showed significant antibacterial action against gramme-positive bacteria Pseudomonas aureus and gramme-negative bacteria Bacillus subtilis among all different tested concentrations and we have observed that antibacterial activity increases with increasing concentration of HAE of S.T. fruit coat.

Table 7: Antibacterial activity of HAE of S.T. fruit coat in comparison with standard antibiotics

Human virulent bacteria	Zone of Inhibition diameter (mm) of HAE of S.T. fruit coat				Standard antibiotic Gentamycin(20µg/ml)
	25µg/ml	50µg/ml	75µg/ml	100µg/ml	
Gram positive bacteria - Pseudomonas aurues	-	12mm	16mm	24mm	28mm
Gram negative bacteria – Bacillus subtilis	8mm	10mm	14mm	18mm	24mm

The diameter of the zone of inhibition was seen between 12mm at 50µg/ml and 24mm at 100µg/ml concentration showinga clear zone of growth in HAE of *Solanum torvum*fruit coat with Pseudomonas aureus. Along with the diameter of the zone of inhibition was seen between 10mm at 50µg/ml and 18mm at 100µg/ml concentration showinga clear zone of growth in HAE of *Solanum torvum*fruit coat with Bacillus subtilis.The highest zone of inhibition was seen in control Gentamycin as 28mm in Pseudomonas aureus and 24mm in Bacillus subtilis, as depicted in table 7 and figure 5.



a)Pseudomonas aureus **b)Bacillus subtilis**

Figure 5: *Solanum torvum* fruit coat hydroalcoholic extractwith a)Pseudomonas aureus-the diameter of the zone of inhibition was seen between 12mm at 50µg/ml and 24mm at 100µg/ml concentration and b)Bacillus subtilis-the diameter of the zone of inhibition was seen between 10mm at 50µg/ml and 18mm at 100µg/ml concentration.

Phytochemical screening by TLC

The chemical identification of *S. torvum* was done using the TLC technique. In thin-layer chromatography, the retention factor (Rf) is used to compare and help identify compounds. The Rf value of a compound is equal to the distance travelled by the compound divided by the distance travelled by the solvent front (both measured from the origin).TLC chromatogram revealed the presence of Phenolic, Flavanoids and Alkaloidscompounds present in the extract. It was discovered that the level of heavy metal and microbiological contamination was below the quality control requirements, as depicted in table 8andfigure 6.

Table 8: The retention factor (Rf) of the identified compounds of HAE of S.T. fruit coat from TLC chromatogram revealed the presence of Phenolic, Flavanoids and Alkaloidscompounds present in the extract.

Identified compounds	UV_{254} nm color spot	UV_{365} nm color spot	Rf values
Phenol	green	blue	0.73
Flavanoid	green	purple	0.44
Alkaloid	green	pink	0.28

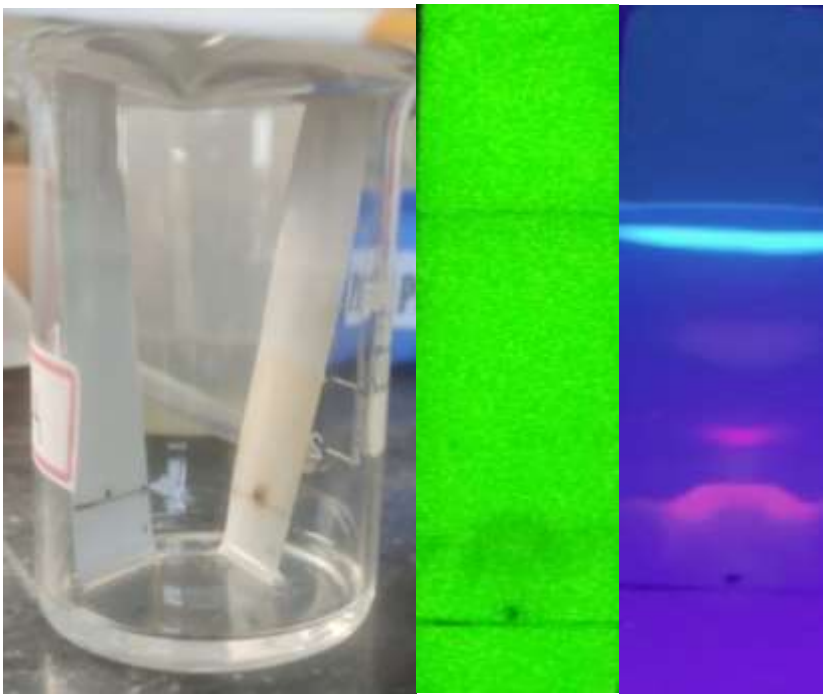


Figure 6: TLC chromatogram of HAE of *S. torvum*fruit coat..
Condition: Stationary phase: Silica Gel GF254, Mobile phase: EtOAc: H_2O = 50:50, Detection: (A) UV 254 nm (B) UV 365 nm

FTIR analysis of HAE *Solanum torvum* fruit coat solid and liquid phase comparison

FTIR interpretation of the HAE of *Solanum torvum* of fruit coat in solid and liquid medium showed the functional groups for the phenols, flavonoids and phenolic compounds. The spectrum of HAE of *Solanum torvum* of fruit coat exhibited bands at 3340 cm-1 and 3296 cm-1, confirming the presence of the O-H stretch. The FTIR spectrum of solid HAE of *Solanum torvum* of fruit coat showing aromatic groups and C-O, C-H, C=C, and C=O at 507 cm-1, 811 cm-1, 764 cm-1, 917 cm-1, 1038cm-1, 1262cm-1, 1595cm-1, 2931cm-1 and 3296cm-1respectively. The FTIR spectrum of liquid HAE of *Solanum torvum* of fruit coat

also shows some similar peaks at 1044 cm-1,1083 cm-1,1637 cm-1 and 2113 cm-1 respectively ascribed to the aromatic groups and C-O, C-H, C=C, and C=O functional groups, respectively. Even though similar bands were observed in the spectrum of both solid and liquid medium of HAE of *Solanum torvum* of fruit coat, the transmittance intensity originating from liquid form HAE of *Solanum torvum* was lower than the solid form of HAE of *Solanum torvum* form. This phenomenon was due to the presence of indeterminate elements present in the prepared liquid (ethanol: water;1:1) of HAE of *Solanum torvum.* The presence of indeterminate elements (ethanol: water;1:1) improves the solubility of the HAE of *Solanum torvum*fruit coat in water. Moreover, the peak obtained at 1083 cm-1 and 1044 cm-1 ascribed to C-H stretching vibration originating from the ethanol substantiates that the ethanol molecules were absorbed with HAE of *Solanum torvum* fruit coat while making it solubilised in it as depicted in figure 7 and 8.

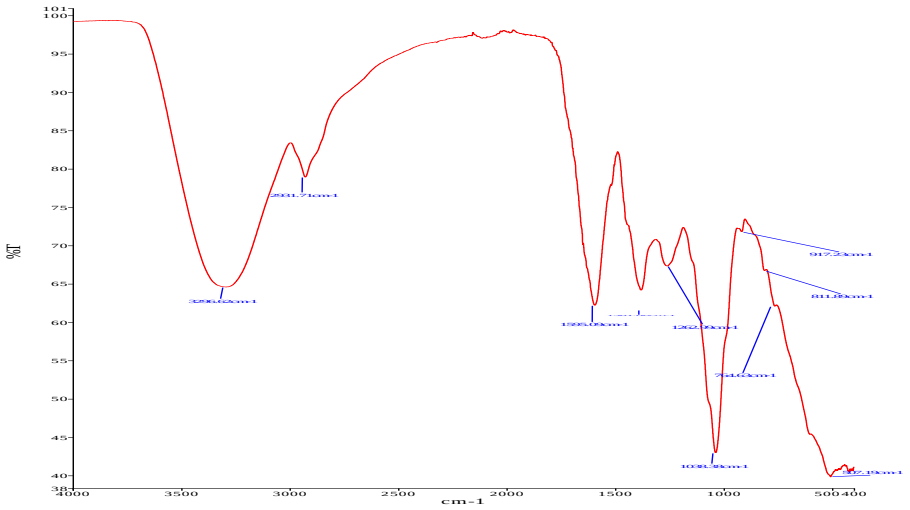


Figure 7: Solid phase FTIR characterization of HAE of S.T. fruit coatshowed the functional groups for the phenols, flavonoids and phenolic compounds

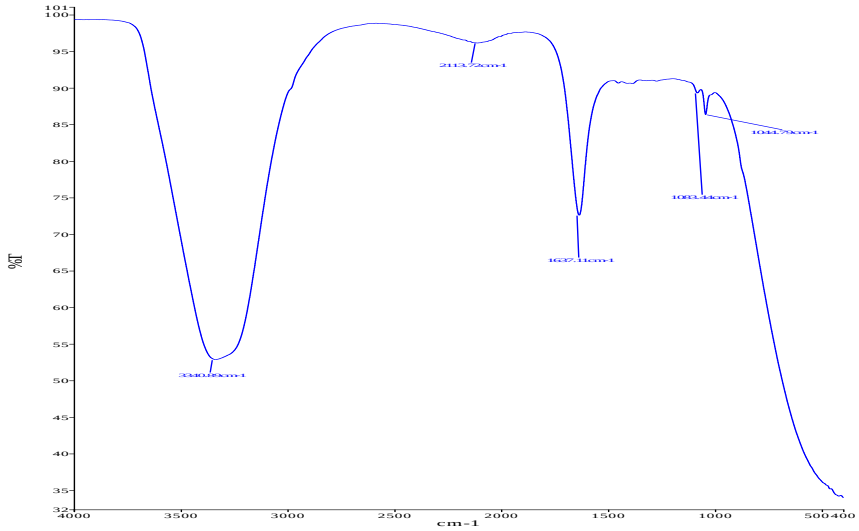


Figure 8: Liquid phase FTIR characterization of HAE of S.T. fruit coatshowed the functional groups for the phenols, flavonoids and phenolic compounds

GC-MS analysis

The GC-MS analysis resulted in the identification of a number of volatile organic compounds from the

GC fractions of the HAE of the *Solanum torvum* fruit coat. In total, 24 compounds were detected and identified by their mass spectra compared with the library scan. The GC-MS spectrum confirmed the presence of various components with different amounts (%Area) and retention times (RT).

The major compounds identified in the *Solanum torvum* fruit coat HAE are shown in Figure 9 and Table 9. They were divided into three major groups—phenolic compounds, terpenoids and fatty acids including their ester and alcohol. This finding corresponded to the study by Nattakarn*et al*.[32] which revealed the presence of phenols, alkaloids, saponin glycosides, cardioactive aglycons, sterol and flavonoids in the ethanolic leaf extract of *Solanum torvum*. However, they performed a different phytochemical assay using the standard methods for alkaloids, saponin, glycosides, flavonoids, phenols, steroids, glycosides-cardio active aglycons, proteins and reducing sugars. The presence of these compounds in the extract probably explains its various uses in traditional medicine.

The 24 compounds were identified in the fruit coat of *Solanum torvum* fruit coat of the plant with hydroalcoholic extraction, such as the Glycerin; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; Heptanediamide, N, N'-di-benzoyl oxy; Catechol; Benzofuran, 2,3-dihydro; 2-Methoxy-4-vinyl phenol; d-Mannose; Ethyl α-d-glucopyranoside; Cyclopentanol, 3,3,4-trimethyl-4-p-tolyl-, (R, R)-(+); 1,8-Diethyl-3,6-diazahomoadamantan-9-ol; Lidocaine; Ethyl (E)-ferulate; Cyclohexanol, 5-methyl-2-(1-methyl-1-phenyl ethyl); Estra-1,3,5(10)-trien-17β-ol; Hexadecanoic acid, ethyl ester; Ethyl trans-caffeate, diacetate; 13-Heptadecyn-1-ol; 9,12-Octadecadienoic acid, ethyl ester ; (E)-9-Octadecenoic acid ethyl ester; Heptadecanoic acid, 15-methyl-, ethyl ester; 5-Benzamido-4-oxo-6-phenyl hexanoic acid; Oxalic acid, mono-(5-[(2-bromophenyl)(2,2-dimethyl propionyl oxy)methyl]-7,8; 5-Methoxy-2-[4-(2-methoxyphenyl)-5-methyl-1H-pyrazol-3-yl]phenol; β-Sitosterol.

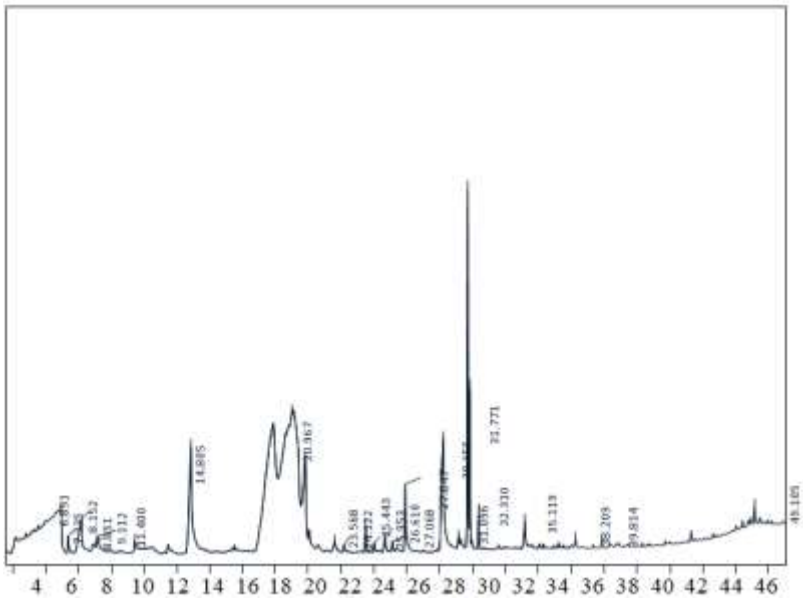


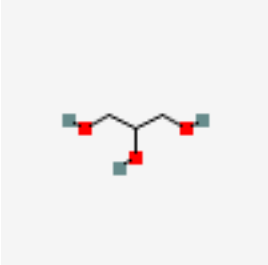
Figure 9: GC-MS analysis of HAE of S.T. fruit coat.A total, of 24 compounds were detected and identified by their mass spectra compared with the library scan.

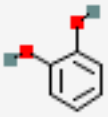
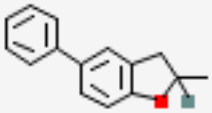
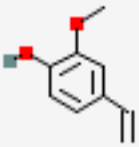
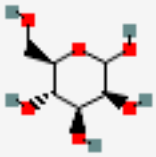
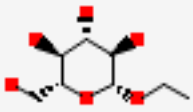
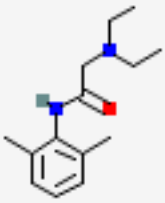
Table 9: GC-MS analysis of HAE of S.T. fruit coat confirmed the presence of various components with different amounts of molecular formula, retention time, % area and molecular weight.

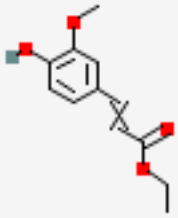
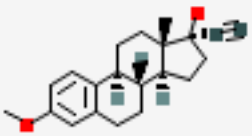
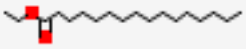
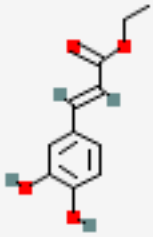
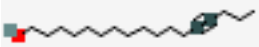
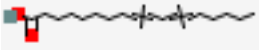
COMPOUND NAME	MOLECULAR FORMULA	RETENTION TIME(min.)	% AREA	MOLECULAR WEIGHT(g/mol)
1. Glycerin	$C_3H_8O_3$	6.894 min	6.44	92.09
2. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	7.338 min	1.09	144.12
3. Heptanediamide, N,N'-di-	$C_{21}H_{22}N_2O_6$	8.157 min	6.31	398.4

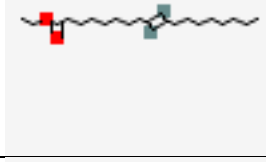
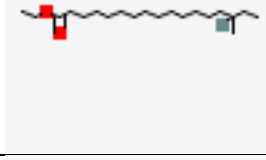
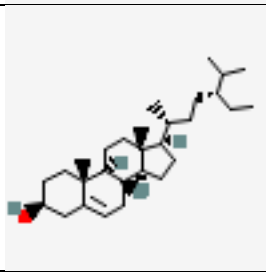
	benzoyloxy				
4.	Catechol	$C_6H_6O_2$	8.851 min	1.23	110.11
5.	Benzofuran, 2,3-dihydro-	C_8H_8O	9.113 min)	2.05	120.15
6.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	11.395 min	0.95	150.17
7.	d-Mannose	$C_6H_{12}O_6$	14.802 min	18.36	180.16
8.	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	20.972 min	2.39	208.21
9.	Cyclopentanol, 3,3,4-trimethyl-4-p-tolyl-, (R,R)-(+)	$C_{15}H_{22}O$	23.566 min	1.31	218.33
10.	1,8-Diethyl-3,6-diazahomoadamantan-9-ol	$C_{13}H_{24}N_2O$	24.122 min	0.56	224.34
11.	Lidocaine	$C_{14}H_{22}N_2O$	25.441 min	1.61	234.34
12.	Ethyl (E)-ferulate	$C_{12}H_{14}O_4$	25.954 min	0.76	222.24
13.	Cyclohexanol, 5-methyl-2-(1-methyl-1-phenylethyl)-	$C_{16}H_{24}O$	26.610 min	1.09	232.36
14.	Estra-1,3,5(10)-trien-17β-ol	Novel	27.066 min	0.72	-
15.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	27.848 min	4.99	284.5
16.	Ethyl trans-caffeate, diacetate	$C_{11}H_{12}O_4$	30.154 min	17.26	208.21
17.	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	31.098 min	0.82	252.4
18.	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	31.655 min	17.04	308.5
19.	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	31.774 min	7.28	310.5
20.	Heptadecanoic acid, 15-methyl-, ethyl ester	$C_{20}H_{40}O_2$	32.311 min	1.70	312.5
21.	5-Benzamido-4-oxo-6-phenylhexanoic acid	$C_{19}H_{19}NO_4$	35.118 min	2.19	325.4
22.	Oxalic acid, mono-(5-[(2-bromophenyl)(2,2-dimethylpropionyloxy)methyl]-7,8-	Novel	38.206 min	0.72	-
23.	5-Methoxy-2-[4-(2-methoxyphenyl)-5-methyl-1H-pyrazol-3-yl]phenol	$C_{18}H_{18}N_2O_3$	39.812 min	0.45	310.3
24.	β-Sitosterol	$C_{29}H_{50}O$	49.102	2.68	414.7

Table 10: GC-MS analysis of HAE of S.T. fruit coat showing molecular structure and the mechanism of action of some of the identified compounds.

Compound Name	Molecular Structure	Mechanism Of Action
Glycerin		Antimicrobial andantiviral properties[33]

Catechol		Antioxidants, anti-inflammatory[34]
Benzofuran, 2,3-dihydro		Anti-tumour, antibacterial, anti-oxidative, and anti-viral activities[35].
2-Methoxy-4-vinylphenol		Antibacterial and antioxidant activity[36]
d-Mannose		Helps in glycosylation of certain proteins,preventing urinary tract infections[37].
Ethyl α-d-glucopyranoside		Increase the proliferation of normal human dermal fibroblasts[38]
Lidocaine		Antimicrobial activity,Anesthetic ,antiarrhythmic agent[39]

Ethyl (E)-ferulate		Anti-inflammatory,antioxidant properties[40]
Estra-1,3,5(10)-trien-17β-ol		Anticancer, has role as an estrogen andhuman metabolite[41]
Hexadecanoic acid, ethyl ester		Antioxidants, hypocholesterolemic, nematicide, and pesticide[42]
Ethyl trans-caffeate, diacetate		Anti-cancer, anti-inflammatory[43]
13-Heptadecyn-1-ol		Anti-inflammatory, anticancer, antimalaria[44]
9,12-Octadecadienoic acid		Anti-inflammatory, ant histaminic, antiandrogenic, antiarthritic[45]

(E)-9-Octadecenoic acid ethyl ester		Anti-inflammatory and Antimicrobial activities[46]
Heptadecanoic acid, 15-methyl-, ethyl ester		Antioxidant[47]
Beta sitosterol		Cardiac disorder, anti-cancer [48]

DISCUSSION

Many study publications backed this plant-based product. Ultimately, there is currently no information about the antioxidant, anti-inflammatory, and anti-diabetic effects of the hydroalcoholic extract of *S. torvum* fruit coat. Secondary metabolites that make up the major organic components were estimated. Preliminary phytochemical analysis of the hydroalcoholic extract of S.torvum exhibits the presence of alkaloids, carbohydrates, saponins, phenolic compounds, and flavonoid compounds. As was shown in earlier research, the water extract of the *Solanum torvum* fruit coat[17], as compared with the hydroalcoholic extract of the S.T. fruit coat contains the highest level of polyphenols, flavonoids, and alkaloids. In contrast to carbohydrates and saponins the HAE of the S.T. fruit coat had a negligible amount of glycosides, proteins, terpenoids, and steroids. In the fruits of *S. torvum*, phenols produced a larger percentage of yield (1.448µg) than flavonoids (0.889µg) and alkaloids(0.200µg). These compounds may be biologically active and have positive effects on human health as antibacterial, anti-inflammatory, antidiabetic, anticholesterolemic, antioxidant, and anti-cancer agents.Polyphenols comprise flavonoids, phenolic acids, tannins, lignans, and coumarins, compounds naturally found in fruits, vegetables, cereals, roots, and leaves among other plant products. In this sense, recent works suggest the potential health benefits of phenolic compounds as antioxidants against oxidative stress diseases. This showed that a greater total phenolic content was linked to a higher flavonoid content. Alkaloids can be categorised into groups based on their structural similarities, such as indoles, quinolines, iso-quinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, terpenoids, and steroids. They have a wide range of physiological effects, including anticancer activity, analgesic, local anaesthetic, hypnotic, antibacterial, antimitotic, and anti-inflammatory properties. From quantitative analysis, our results divested that total phenols are the most abundant phytochemical composition of S.T. fruit coat hydroalcoholic extract. The extract also contains good amounts of total alkaloids and total flavonoids with it.

The DPPH antioxidant assay revealed 82.2%(100 µg/mL) antioxidant activity. In the ABTS assay, the HAE of S.T.FC showed 85.8%(100 µg/mL) antioxidant activity.In the FRAP assay, the HAE of S.T. FC at

100µg/ml has a reducing power or absorbance of 0.89; in the phosphomolybdate assay,the HAE of S.T. FC at 100µg/ml has a reducing power of 0.80 respectively.This study confirms the antioxidant activity of HAE of S.T. fruit coat and its ability to scavenge free radicals. Antioxidants are the compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite[49].An imbalance between antioxidant and reactive oxygen species results in oxidative stress, leading to cellular damage[50].

In the present study, for anti-diabetic activity, the extract is capable of effectively inhibiting the α-amylase activity at 60.0% In α-Glucosidases antidiabetic assay, inhibiting activity is 62.00%,respectively.High blood sugar levels, which can have catastrophic effects on the kidneys, eyes, and cardiovascular system, are a hallmark of diabetes. A significant factor in the regulation of glucose homeostasis is insulin. Protein, fat, and glucose metabolism are all impacted by insulin deficiency[51]. Therefore, minimising blood sugar swings and the ensuing consequences is the major goal of treating diabetes. One of the main anti-diabetic therapy strategies is to reduce the amount of glucose absorbed from the gastrointestinal tract by inhibiting the enzymes α-amylase and α-glucosidase present in the pancreas and intestine. Inhibition of α -amylase leads to a reduction in post-prandial hyperglycemia[52]. The α-amylase inhibitors are currently used for diabetic treatment as oral hypoglycemic agents. Acarbose is a commercially available enzyme inhibitor for type II diabetes. The small intestine's epithelium is home to the membrane-bound enzyme alpha-glucosidase. Disaccharide to glucose conversion is catalysed by this enzyme. Therefore, in order to limit the conversion of glucose from disaccharides, it is necessary to block the enzyme -glucosidase. The plant demonstrated improved in vitro enzyme inhibitory activities that are important in glucose control and absorption.

For anti-inflammatory activity employing *in vitro* assays, the HAE of the S.T. fruit coat revealed anti-inflammatory activity of 21.87% by Inhibition of Egg Albumin denaturation.Also obtained 32.25% (100 µg/mL) inhibition activity in the Inhibition of the BSA denaturation assay. The intricate process of inflammation is frequently accompanied by discomfort. Anti-inflammatory substances can influence different pathophysiological processes by inhibiting the release of performed stored mediators, blocking receptor interactions on target cells, decreasing enzyme expression, lowering substrate levels, and more. These actions lead to a less aggressive immune response to allergen challenges[53]. Inflammatory response to tissue injury involves a complex array of enzyme activation, mediator release, fluids extravasations, cell migration, tissue breakdown and repair[54]. Inflammation has been linked to protein denaturation in several studies. Albumin protein and Bovine serum albumin are employed as a model because it is denatured under inflammatory conditions, and their protection against heat-induced denaturation by plant extract has been assessed [53].

So it may be said that the inhibitory property due to the extract's high polyphenol and flavonoid contents may be the cause of its antibacterial action because of their ability to complex with bacterial cell walls, inhibiting the microbial growth which is having significant activity as compared to the standard. The diameter of the zone of inhibition is 24mm at 100µg/ml concentration in HAE of *Solanum torvum*fruit coat with Pseudomonas aureus. The diameter of the zone of inhibition is 18mm at 100µg/ml concentration in HAE of *Solanum torvum* fruit coat with Bacillus subtilis. TLC chromatogram revealed the presence of high levels of Phenolic compound, Flavanoids and Alkaloids compounds present in the HAE of S.T. fruit coat. It was discovered that the level of heavy metal and microbiological contamination was below the quality control requirements.FTIR interpretation of the HAE of *Solanum torvum* of fruit coat in solid and liquid medium showed the functional groups for the aromatic compounds, phenols, flavonoids, alkaloids, carboxylic acids, esters, alkanes, alkenes, alcohols, alkyl halides, sulfate esters, thiols, amines, phosphoric acids, and nitro compounds.The GC-MS analysis was carried out to discover any anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic or cytotoxic compounds present in the extract of S.T. The GC-MS analysis resulted in the identification of a number of volatile organic compounds from the GC fractions of the HAE of the *Solanum torvum* fruit coat. 24 compounds were detected and identified by their mass spectra compared with the library scan.

CONCLUSION

The hydroalcoholic extract of *Solanum torvum* fruit coat possesses high phenol, flavonoid and alkaloid constituents. The extract has a potential anti-oxidant, anti-diabetic, anti-inflammatory and antimicrobial effect corresponding to its phytochemical constituents, preliminarily confirmed by qualitative and quantitative analysis followed by TLC, FTIR and GC-MS analysis. The study reconfirms the ethnomedicinal property of the *Solanum torvum* plant. Further studies should be carried out to investigate the toxicity and then followed by clinical trials to support its therapeutic usage.

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