Research Article

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Exploring the phytochemical profile and antioxidant evaluation: Molecular docking and ADMET analysis of main compounds from three *Solanum* species in Saudi Arabia

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Abstract: This study aimed to comprehensively assess the phytochemical composition, employing gas chromatography–mass spectrometry (GC–MS) and reverse-phase high-performance liquid chromatography (RP-HPLC) analyses, molecular docking, ADMET analysis, and antioxidant activity evaluation, of three *Solanum* species (*Solanum forsskalii* [SF], *Solanum villosum* [SV], and *Solanum incanum* [SI]) from the diverse flora of Saudi Arabia. Two solvents, hydro-methanolic (HME) and hydro-acetonic extract, were utilized for extraction, finding HME more efficient, especially for SV. GC–MS analysis identified diverse compounds, with palmitic acid, linoleic acid, methyl palmitate, *cis*-13-octadecenoic acid, and oleic acid as the main constituents. RP-HPLC quantified 12 phenolic compounds, identifying chlorogenic acid, rutin, and *p*-coumaric acid as abundant. Antioxidant assays showed HME extracts to be more effective in both diphenyl 1-picrylhydrazyl and ABTS assays, with SV exhibiting the strongest antioxidant effect, followed by SF and SI. Pearson correlation analysis indicated a positive correlation between phenolic content and antioxidant activity (r = 0.6067–0.8927). Molecular docking simulations demonstrated robust binding energies between predominant compounds and Cyt-c, underscoring their potential as effective antioxidants. ADMET analysis showcased varied profiles, suggesting promising pharmaceutical prospects. This study explores the phytochemical profiles of these *Solanum* species, emphasizing their strong antioxidant capacity as natural sources of phenolic compounds, advancing our understanding of their promising medicinal applicability.

Keywords: GC, MS, RP-HPLC, *Solanum* species, phytochemicals, antioxidant, molecular docking: ADMET analysis

1 Introduction

Solanum, a plant genus belonging to the Solanaceae family, is known for its vast diversity, encompassing over 2,000 species, many of which are reputed to possess medicinal qualities and have been acknowledged for their therapeutic attributes [1–3]. The investigation of naturally occurring bioactive compounds and their secure and precise implementation in the food and pharmaceutical sectors is a relevant and significant scientific area today. *Solanum* spp., characterized by their abundant and varied biochemical composition, have emerged as an appealing research focus to explore their potential advantages across multiple domains. Traditional medicine incorporates the use of various *Solanum* species, while some species serve as a source of drugs for medicine, pharmacology, and drug therapy [4]. Numerous pharmacological investigations have been conducted to authenticate the traditional therapeutic uses of various plants within this genus. The examined pharmacological properties encompass a wide range of effects, such as analgesic, anthelminthic, antibacterial, anti-cancer, antidepressant, anti-diabetic, anti-fungal, antihypertensive, anti-inflammatory, antileishmanial, antinociceptive, anti-psoriatic, anti-plasmodial, anti-protozoa, diuretic, hepatoprotective, spasmolytic, and vasorelaxant activities [5]. Saudi Arabia is home to

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around 16 species of the *Solanum* genus, which can be found primarily in the western and southwestern parts of the country [6].

The significance of phytochemicals, including antioxidants, in neutralizing harmful free radicals and combating life-threatening illnesses like cancer, stroke, and heart-related ailments, is widely acknowledged today. Polyphenols, including phenolic acids, flavonoids, flavanols, flavones, and isoflavones, are well-known for their significant antioxidant properties and potential health benefits [7,8]. The antioxidant capacity of plant phenolic extracts is influenced by their concentration, as well as their distribution patterns within plant tissues [9,10]. Environmental conditions, age, and phenological stage all have an impact on the concentration of phenolic constituents [11].

Numerous investigations have been conducted to analyze the phenolic composition and antioxidant properties of various *Solanum* species. Among them are *Solanum betaceum* [12], *Solanum erianthum*, *Solanum torvum* [1], *Solanum xanthocarpum*, *Solanum violaceum* [13], *Solanum aethiopicum*, *Solanum macrocarpon* [14], *Solanum indicum*, *Solanum surattense* [15], *Solanum ferrugineum* [16], *Solanum melanocerasum*, *Solanum nigrum*, *Solanum villosum* [SV], and *Solanum retroflexum* [17], as well as *Solanum scabrum* and *Solanum burbankii* [18]. This study aimed to evaluate the abundance and diversity of phenolic compounds and their antioxidant properties in three Solanum species (*Solanum forsskalii* [SF], SV, and *Solanum incanum* [SI]) collected from the southern region of Saudi Arabia. The primary goal was to explore the potential utilization of a specific species from Saudi Arabia as a natural source of antioxidants. To accomplish this, we conducted an evaluation of the total phenolic content (TPC) and total flavonoid content (TFC). Additionally, we employed gas chromatography–mass spectrometry (GC–MS) analysis and high-performance liquid chromatography (HPLC) to detect and analyze the variability in the phytochemical composition. Moreover, we investigated the variations in antioxidant activity among tested species through the implementation of spectrometric methods, including the diphenyl 1-picrylhydrazyl (DPPH) and ABTS assays. Furthermore, we conducted Pearson correlation analysis to examine the relationship between TPC and antioxidant activity, providing valuable insights into the contribution of phenolic compounds to antioxidant properties.

It is worth noting that previous studies assessing the phytochemical composition in SF have been limited. Moreover, the three species analyzed in this study were exclusively sourced from Saudi Arabia, shedding light on the environmental effects on the phenolic constituents and adding a unique aspect to the research.

2 Materials and methods

2.1 Plant material

The fruiting aerial parts of three *Solanum* species were harvested in Al Azizah (end of March 2021), Abha Region (South West Region of Saudi Arabia; harvesting coordinates 18°12′28.8″N, 42°26′24.0″E). During plant collection, sustainable harvesting practices were employed, including timing the harvest during optimal growth periods, utilizing proper techniques to minimize damage, consulting local experts for adaptive management, and adhering to local regulations for documentation and site selection. These specimens underwent authentication by Prof. Abdelaaty A. Shahat of the Department of Pharmacognosy at King Saud University, Saudi Arabia. The process involved macroscopic examination and comparison with authenticated reference specimens [6]. They were subsequently deposited in the departmental herbarium for further reference. The collected samples underwent washing, were then dried in a ventilated oven at 40°C within a controlled environment to prevent contamination or degradation, and closely monitored to avoid overheating. Afterward, they were ground using a domestic blender.

The resulting dry and ground tissues were then preserved in paper bags at room temperature, in a dark environment, until further examination.

2.2 Chemicals, reagents, and standards

Analytical grade reagents and solvents, such as methanol, acetonitrile, ethanol, and acetic acid, were used for extraction and chromatographic separation. These solvents were purchased from VWR International Ltd. (Le Périgares-Bâtiment, France). Standards of polyphenolic compounds, including caffeic acid, myricetin, (+)-catechin, *p*-coumaric acid (PCA), (−)-epicatechin, ferulic acid, and chlorogenic acid (CLA), were obtained from Tokyo Chemical Industry Co., Ltd (Kita-Ku, Tokyo, Japan). Analytical standards of other phenolics (gallic acid, quercetin, rosmarinic acid, rutin (RUT), apigenin, and kaempferol were obtained from Sigma-Aldrich (St. Louis, MO, USA). The Folin–Ciocalteu reagent and ascorbic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA), while deionized water was obtained from the Purelab Flex purification system (Veolia Ltd., High Wycombe, UK).

2.3 Preparation of extracts

The powdered plant materials of the three species were utilized to create two types of extracts: hydro-methanolic

extract (HME) and hydro-acetonic extract (HAE). To obtain the HME, 50 g of dried plant samples were mixed with 800 mL of methanol and 200 mL of distilled water. For the HAE, 700 mL of acetone and 300 mL of distilled water were combined with the powdered plant materials. A consistent extraction ratio of 1:20 (plant material to solvent) was maintained for all samples, informed by prior studies that have validated its efficacy in extracting phenolic, flavonoid, and other phytochemical compounds from plants [1,12]. The extracts were filtered after undergoing a 72-h maceration process. Previous studies indicate that this timeframe is effective for extracting phenolic and other phytochemical compounds from plant materials [19–21]. After the extraction, the samples were filtered through Whatman No. 1 filter paper (Whatman TM 1001-150, Merck KGaA, Darmstadt, Germany). The extracts were then dried using a rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland) set at 45 rpm and 40°C to obtain a dry HME and HAE extracts. To prevent light effects, the dried extracts were stored in amber glass bottles and then kept in a dried and cooled place in the refrigerator. This dried extract was subsequently used directly for the analysis of phytochemical content and evaluation of antioxidant capacity. The percentage extraction yield was calculated based on the weight of the powdered plant material used, as specified in the formula:

$$\text{Extraction yeield (\%)} = \frac{\text{EQ}}{\text{PQ}} \times 100,$$

where EQ represents the weight of the extract and PQ represents the weight of the powdered plant.

2.4 TPC

The TPCs of the extracts (HEE and HAE) were assessed using the Folin–Ciocalteu reagent, following a method described in the study by Alqahtani et al. [22] with slight modifications. For the experiment, each plant sample (0.5 mL containing 1 mg of dry extract) was mixed with 125 µL of 1 N Folin–Ciocalteu reagent and stirred for 5 min. Subsequently, 375 µL of a 20% (w/v) anhydrous Na_2CO_3 solution was added. The mixture was then incubated at room temperature for 30 min, followed by measuring the absorbance at 765 nm using a UV-1650pc UV–VIS spectrophotometer from Shimadzu Corporation (Nishinokyo Kuwabara-cho, Kyoto, Japan). A gallic acid standard curve (5–500 µg/mL in ethanol) was used to calculate the TPC. The regression equation obtained was $y = 12.624x - 1.0824$ ($R^2 = 0.9962$), and the outcomes are presented in milligrams of gallic acid equivalent per 10 g of dry extract (mg GAE/10 g DW).

2.5 TFC

The flavonoid content in the extracts (HME and HAE) from each species was assessed using the aluminum trichloride colorimetric assay. In summary, each extract (1 mL) was diluted with 5 mL of distilled water. To this solution, 0.3 mL of 5% $NaNO_2$ was added, followed by 0.3 mL of 10% $AlCl_3$ after 5 min of incubation at room temperature. Subsequently, 2 mL of 1 M NaOH was added, and the total volume was adjusted to 10 mL with distilled water. After preparation, the mixture underwent incubation in a shaded area at room temperature for a duration of 30 min. Following this incubation period, the absorbance of the solution was measured at a wavelength of 510 nm using a UV–visible spectrophotometer. Flavonoid levels were determined by assessing various quercetin concentrations, resulting in a regression equation of $y = 6.425x + 0.4125$ ($R^2 = 0.9931$). The flavonoid content was expressed as milligrams of quercetin equivalents per 10 g of dry extract (mg QE/10 g DW) [23].

2.6 Determination of polyphenolic compounds using HPLC

To effectively identify and quantify the polyphenolic compounds in the extracts of the three species, it was logical to employ a reverse-phase high-performance liquid chromatography (RP-HPLC) system. This choice was made considering the different polarities of the individual compounds. The method utilized a gradient elution system and UV detection at a wavelength of 280 nm, which was optimized due to the UV-absorbent characteristics of the compounds. For the analysis of individual phenolic compounds, an Alliance chromatographic system from Waters Instruments, Inc. equipped with a quaternary pump and dual wavelength absorbance detectors was used. Reverse phase analyses were conducted using a Pinnacle™ II C18 column (4.6 mm × 250 mm, 5 µm particle size). Throughout the experiment, the column temperature was held at 24°C. The mobile phase consisted of two solutions: solution A, composed of 1% acetic acid in deionized water, and solution B, consisting of a 75:25 ratio of methanol to acetonitrile. A gradient flow rate of 0.8 mL/min was achieved by following the profile set as described in Table 1. The HPLC analysis was based on the following 12 standard polyphenolic compounds: CLA, (+)-catechin, caffeic acid, PCA, (−)-epicatechin, ferulic acid, RUT, rosmarinic acid, myricetin, quercetin, apigenin, and kaempferol. To create a calibration curve, a methanol solution containing a standard stock solution (500 µg/mL) was prepared for each standard compound. The calibration concentrations were then generated using this stock solution. Prior to HPLC analysis,

Table 1: RP-HPLC gradient conditions for polyphenolic compounds analysis of *Solanum* species

Time (min)	Solution A (%)	Solution B (%)
0.00	90	10
9.00	75	25
43.00	49	51
53.00	5	95
61.00	5	95
68.00	90	10

solutions of each extract (10 mg/mL) were prepared in methanol. To ensure purity, all solutions, including mixed standards and samples, were filtered through a 0.20 µm membrane filter from Millipore using an injection volume of 20 µL for each sample. Data acquisition, peak integration, and calibrations were performed using Empower 3 software. The identification of the compounds was achieved by comparing their retention times (RT) to those of authentic reference standards [24].

2.7 Phytochemical analysis using GC–MS

A PerkinElmer Clarus GC System, manufactured by PerkinElmer, Inc. in Waltham, MA, USA, was utilized to perform the GC–MS analysis of HME from the three *Solanum* species. The temperature program followed a specific protocol that had been previously described [25]. The initial program began at 40°C and stayed constant for 2 min. It was then slowly raised to 200°C, increasing by 5°C per minute, and maintained for another 2 min. Afterward, the temperature was incrementally increased from 200 to 300°C, progressing at a rate of 5°C per minute, and then maintained at 300°C for an additional 2 min. The flow rate was set at 1 mL/min, with helium as the carrier gas. The sample injection volume was 1 µL, split ratio at 20:1, linear velocity at 38.032 cm/s, and pressure at 8.1322 psi. To determine the constituents of the HME, the obtained mass spectra were cross-referenced with spectral libraries such as the National Institute of Standards and Technology and WILEY. Additionally, comparisons were made with similar compounds identified in the Adams Library [26] and the Wiley GC/MS Library [27].

2.8 Antioxidant activity assay

2.8.1 Determination of DPPH radical scavenging activity

The DPPH free radical scavenging activity of each sample was assessed using a spectrophotometric method, following the method described by Chebbac et al. [28] with minor modifications. To summarize, a 0.2 mM solution of DPPH was prepared in methanol. The baseline absorbance of the DPPH solution, prepared in methanol, was recorded at 515 nm using a UV–visible spectrophotometer and remained consistent throughout the experiment. Different concentrations of each plant sample (100 µL) prepared with methanol (ranging from 1,000 to 20 µg/mL), as well as various concentrations of ascorbic acid (used as a positive control), were mixed with 100 µL of the DPPH solution for analysis. Following 30 min of incubation in darkness, the variation in absorbance at 515 nm was quantified using the BioTek ELX800 Absorbance Microplate Reader (Winooski, VT, USA). The findings are expressed as the percentage of inhibition, which indicates the anti-free radical effect of the samples using the following equation:

$$\%\ \text{of radical scavenging activity} = \frac{\text{Abc} - \text{Abs}}{\text{Abc}} \times 100,$$

where Abc represents the absorbance of the DPPH solution in methanol and Abs represents the total absorbance of both DPPH and samples.

2.8.2 ABTS radical scavenging activity

The evaluation of the ABTS radical scavenging capacity of plant extracts was conducted employing a spectrophotometric method, following the procedure outlined by Alam et al. [29]. Initially, ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) aqueous solutions (7 mM) and potassium persulfate (2.45 mM) were combined in equal proportions and incubated for 0.5 h. The mixture was then kept in the freezer for 24 h and later diluted with ethanol. Following this, various volumes of the ABTS solution (50 µL each) were combined with the plant samples and allowed to incubate in darkness for 1 h. The decrease in ABTS absorbance was measured at a wavelength of 734 nm (λ_{max}) using BioTek ELX800 Absorbance Microplate Reader, and the antioxidant activity of the plant extracts was determined using the formula below [30]:

$$\%\ \text{of radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100.$$

2.9 Molecular docking

All molecular dockings between the most abundant phytochemicals and Cyt-c were conducted using PyRx software with AutoDock VINA, following the methodology described

by Al-Shabib et al. [31]. The initial step involved downloading the 2D structures of the compounds from PubChem, which were subsequently imported into PyRx. Subsequently, the compounds underwent energy minimization employing the universal forcefield and were converted to pdbqt format with Open Babel. The 3D coordinates of Cyt-c protein (PDB ID: 4A71) were obtained from the RCSB website (www.rcsb.org) and prepared by adding missing hydrogen atoms while removing non-essential foreign and water molecules. The energy of the entire system was minimized using the CHARMM36 force field. The dimensions of the grid box for Cyt-c were set at (52.14 × 48.00 × 62.11) $Å^3$, positioned at (−11.2 × 5.21 × −5.12) $Å^3$ with 0.375 Å spacing. Molecular docking utilized the Lamarckian Genetic Algorithm (LGA) in conjunction with Solis and Wets local search methods [32]. The compounds were given random orientations, starting positions, and torsional angles. Each docking run included up to 2.5 × 106 energy calculations, with each one evaluating the interaction energy between the ligand and the protein. Various factors were considered in the evaluation, including van der Waals forces, electrostatic interactions, and hydrogen bonding. Docking parameters specified a population size of 150, which determined the number of individual ligand conformations generated and assessed. The translational step, set at 0.2 Å, allowed for precise exploration of translational space, enhancing the identification of potential binding sites. Additionally, a torsion step parameter of 5 was employed, broadening the exploration of torsional angles and increasing ligand flexibility. Quaternions were utilized to efficiently describe molecular rotations, with a higher number (5) facilitating a more comprehensive search of ligand orientations during rotation.

The results obtained were analyzed, and final figures were created using Discovery Studio (Accelrys). The dissociation constant of phytochemicals (K_d) for Cyt-c was determined from the docking energy (ΔG) using the equation [32]:

$$\Delta G = -RT \ln K_d,$$

where R represents the universal gas constant and T represents the temperature.

2.10 ADMET analysis

The investigation aimed to explore descriptors characterizing the absorption, distribution, metabolism, excretion, and toxicity profiles of prevalent phytochemicals. This analysis utilized the ADMETlab 3.0 web server [33]. Molecular properties were derived from the chemical structures of the analyzed compounds, represented by SMILES definitions retrieved from the PubChem database [34]. The methodology for interpretation and the definitions of individual descriptors obtained during the analysis are detailed in the "Results" and "Discussion" sections.

2.11 Statistical analysis

All experiments were conducted in triplicate unless otherwise specified. The data is presented as the mean ± standard deviation. An analysis of variance was performed to assess the significance of differences between variables, followed by a Tukey test for multiple comparisons. Statistically significant differences were identified using a p-value threshold of less than 0.05.

3 Results

3.1 Yield of extracts

The extraction yields from maceration of the aerial parts of three *Solanum* species were evaluated. Among the species studied, SF demonstrated the highest yields for both HME at 15.41% and HAE at 13.76%. In contrast, SV and SI exhibited lower yields, with SV showing the lowest overall extraction yields (HME: 10.28%, HAE: 9.15%), while SI had extraction yields of HME: 11.47% and HAE: 12.92%. These results demonstrate that the extraction yields varied among the different species and between the HME and HAE. These variations likely stem from a complex interplay of factors specific to each species, one key factor is the inherent differences in secondary metabolite profiles. Each species possesses a unique set of bioactive compounds, and the abundance of these target molecules can significantly impact the final yield. Additionally, cell wall composition plays a crucial role. Species with more robust cell walls might necessitate harsher extraction conditions or alternative techniques (e.g., sonication) to achieve optimal yields [35]. Beyond these species-specific characteristics, the choice of solvent significantly influences the extraction yields. Both methanol and acetone are polar solvents, with methanol being more polar than acetone. The polarity of the solvent significantly influences the types and quantity of compounds extracted. Studies have shown that highly polar solvents, such as methanol, result in higher extract yields [36,37]. This may explain the observed higher yields with hydroalcoholic mixtures (HME) in most cases. Furthermore, it is important to

Table 2: TPC and TFC of HME and HAE of SF, SV, and SI

Extracts	TPC (mg GAE/10 g DW)			TFC (mg QE/10 g DW)		
	SF	SV	SI	SF	SV	SI
HME	48.11 ± 1.3	86.52 ± 1.9	38.56 ± 0.61	35.95 ± 0.91	55.46 ± 1.4	21.73 ± 0.12
HAE	36.14 ± 0.83	48.21 ± 1.3	16.29 ± 0.53	14.35 ± 0.28	29.87 ± 0.72	10.62 ± 0.23

Values are expressed as mean ± SD of three parallel measurements.

note that several factors can impact the extraction yield, including the duration of maceration, the particle size of the material, the solvent combination employed and its polarity, the solvent volume to sample mass ratio, temperature of extraction, timing of harvest, drying process, and the specific plant part being used [38–40]. Understanding the interplay between these factors is crucial for optimizing extraction protocols. Future research could investigate how different extraction conditions influence the yield of the extracts.

3.2 Quantification of TPC and TFC

Table 2 presents the experimental results obtained for the TPC and TFC of various *Solanum* species extracts using different extraction solvents and a graphic comparison representations are presented in Figure 1. The results clearly indicate that SV has higher phenolic and flavonoid contents compared to the other two species. When extracted using HME, the phenolic contents of SF, SV, and SI were found to be 48.11 ± 1.3, 86.52 ± 1.9, and 38.56 ± 0.61 mg GAE/10 g DW, respectively. However, when extracted using hydro-acetone (HAE), the phenolic contents were lower at 36.14 ± 0.83, 48.21 ± 1.3, and 16.29 ± 0.53 mg GAE/10 g DW, respectively. Flavonoids followed a similar pattern, with higher concentrations in the HME (ranging from 21.73 ± 0.12 to 55.46 ± 1.4 mg QE/10 g DW) compared to the HAE (ranging from 10.62 ± 0.23 to 29.87 ± 0.72 QE/10 g DW). It is worth noting that the HME showed a remarkably high phenolic content, surpassing 60% of the TPC and TFC extracted from both solvents.

The selection of solvents utilized in the extraction process plays a crucial role in determining the TPC and TFC of plant extracts. An effective solvent is characterized by its ability to achieve optimal extraction efficiency while preserving the chemical stability of the target compounds [41]. Polyphenols exhibit a range of polarities, with optimal extraction typically achieved using polar solvents that facilitate efficient solvation through interactions such as hydrogen bonding with the polar sites of these compounds. Polar solvents like hydro-methanol are known for their effectiveness in extracting phenolic compounds, flavonoids, and other bioactive molecules due to their superior solvation capacity for polar molecules than less polar solvents like hydro-acetone, leading to lower TPC and TFC in the resulting extracts [42]. This observation offers a possible

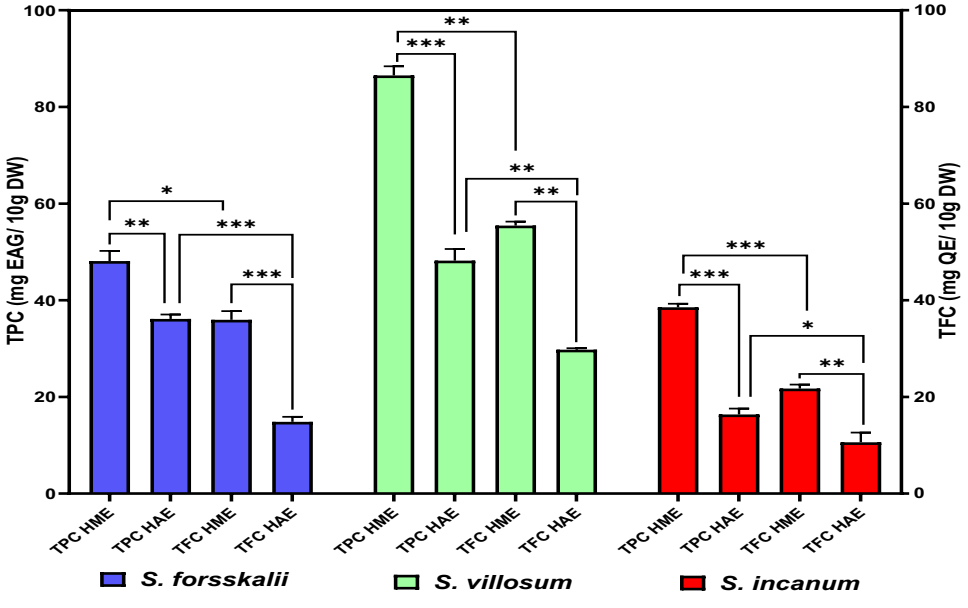

Figure 1: Comparative data of the TPC and TFC obtained from HME and HAE) of SF, SV, and SI. Results are expressed as mean ± SD of three parallel measurements (n = 3). The presence of asterisks signifies the application of statistical tests for multiple reciprocal comparisons among the extracts, with significance levels indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001.

Table 3: Calibration curves, LOD, and LOQ for the determination of polyphenolic compounds by RP-HPLC

Analyte	Rt (min)	Calibration equations	R^2	LOD (µg/mL)	LOQ (µg/mL)	%RSD (n = 5)
CLA	12.01	$y = 4.352x + 1.752$	0.9982	0.42	0.82	0.31
(+)-Catechin	13.83	$y = 9.143x - 6.768$	0.9997	0.11	0.34	0.56
Caffeic acid	14.73	$y = 11.681x - 3.924$	0.9981	0.26	0.65	0.19
PCA	18.19	$y = 2.842x + 5.811$	0.9994	0.15	0.57	0.84
(−)-Epicatechin	19.64	$y = 27.216 + 4.661$	0.9990	0.12	0.38	0.91
Ferulic acid	26.42	$y = y = 8.021x + 0.739$	0.9996	0.10	0.26	0.47
RUT	28.54	$y = 13.543x + 4.022$	0.9983	0.13	0.44	0.21
Rosmarinic acid	30.13	$y = 4.152x - 8.631$	0.9997	0.09	0.26	0.70
Myricetin	37.16	$y = 12.534x - 0.738$	0.9986	0.43	1.35	0.41
Quercetin	38.92	$y = 14.991x - 9.483$	0.9991	0.14	0.39	0.95
Apigenin	43.24	$y = 13.648x + 5.035$	0.9989	0.11	0.35	0.27
Kaempferol	43.89	$y = 9.351x - 8.246$	0.9995	0.08	0.21	0.68

explanation for the higher levels of TPC and TFC in HME compared to HAE, as indicated in our study findings. It is noteworthy that other studies have reported a higher recovery of polyphenolic compounds when utilizing HAE solvent [43]. Therefore, our study was designed to compare the total phenolic outcomes obtained using both hydro-methanol and HAE solvents, considering various factors such as secondary metabolite profiles rather than focusing solely on solvent polarity.

3.3 Identification and quantification of polyphenolic compound using HPLC analysis

Polyphenolic compounds were analyzed and quantified using RP-HPLC in this study. By establishing external calibration curves and plotting peak area against concentrations ranging from 0.2 to 100 µg/mL, we were able to successfully quantify twelve phenolic compounds. These compounds included CLA, (+)-catechin, caffeic acid, PCA, (−)-epicatechin, ferulic acid, RUT, rosmarinic acid, myricetin, quercetin, apigenin, and kaempferol. The correlation coefficients obtained for all cases were higher than 0.9981. Table 3 provides additional information on the determination of these compounds, including detection limits (LOD), quantification limits (LOQ), and RT. The sequence in which substances elute from the chromatographic column is determined by both the hydrophobic characteristics of the packing material and gradual rise in methanol and acetonitrile (solvent B) within the mobile phase. Figure 2 and Table 3 present the chromatogram and data, respectively, illustrating the sequence in which the target compounds were eluted.

Figure 3 displays the chromatographic separations of polyphenols from various *Solanum* species extracts using hydro-methanol as the extraction solvent (HME). On the other hand, Figure 4 illustrates the chromatographic

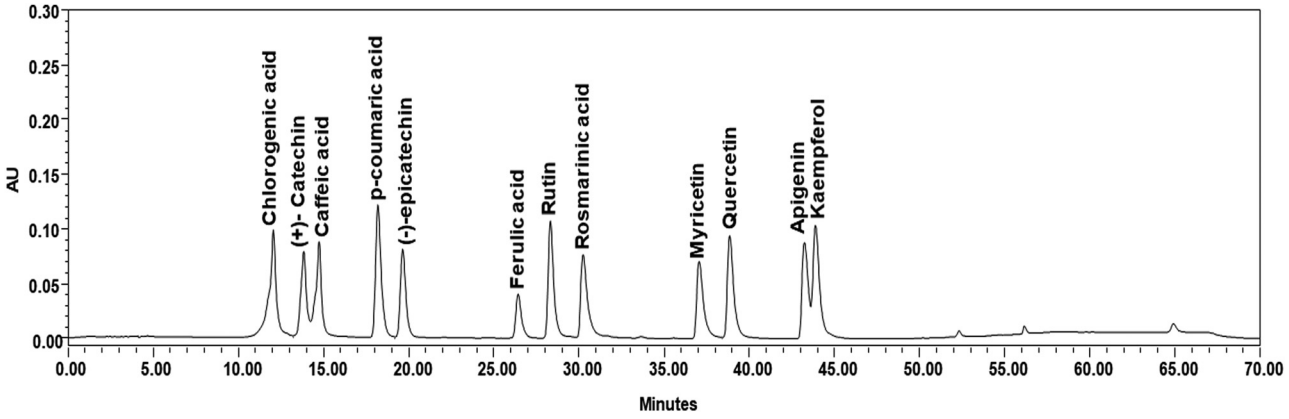

Figure 2: RP-HPLC chromatogram of a mixture of 12 polyphenolic compounds using a Pinnacle™ II C18 column. Mobile phase: (a): 1% (v/v) acetic acid, (b) methanol and acetonitrile (75:25) (v/v) in gradient system. Flow rate: 1.0 mL/min. Absorbance measured at 280 nm, 25°C.

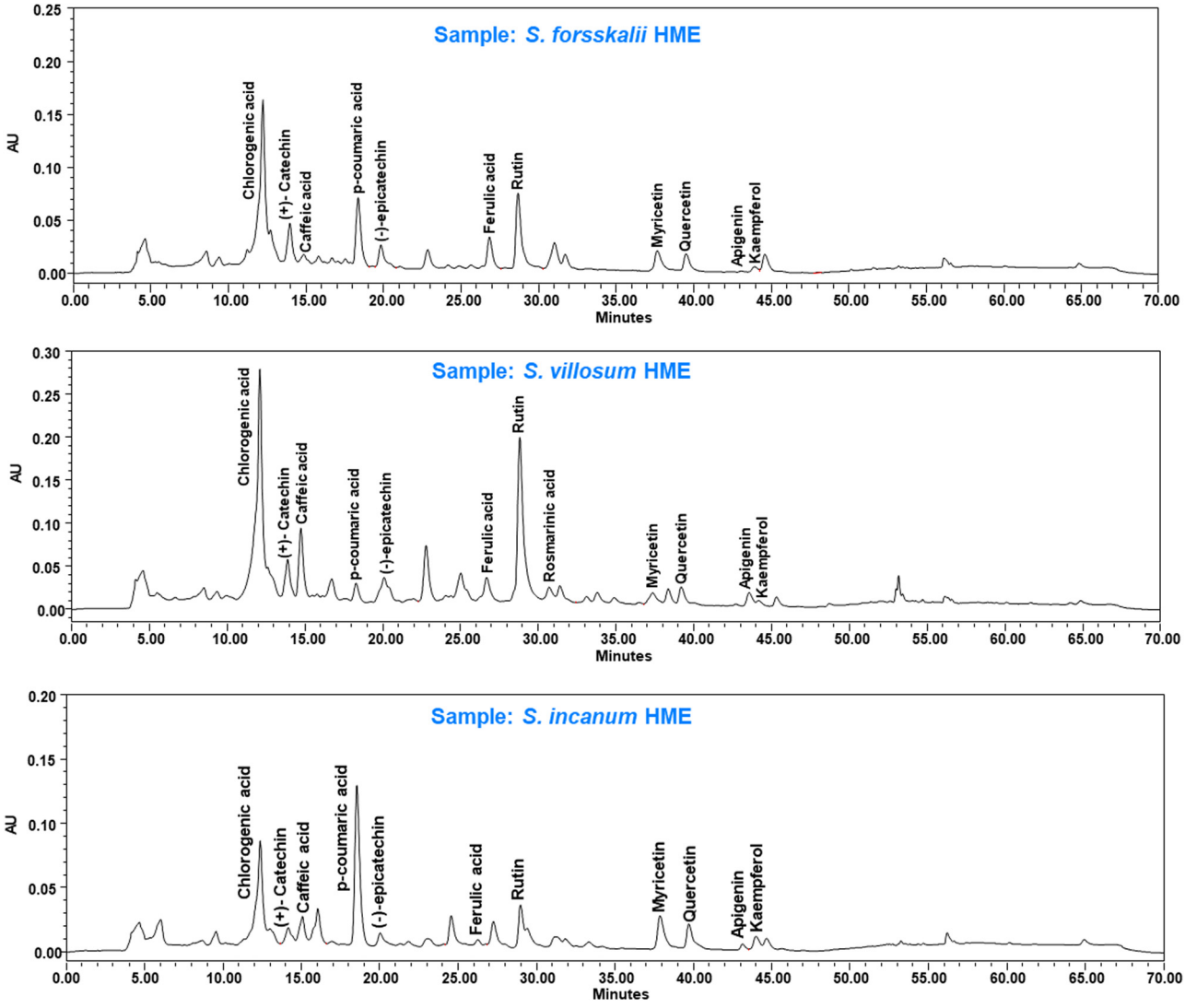

Figure 3: RP-HPLC chromatograms of the polyphenolic compound profiles from three *Solanum* species extracts (SF, SV, and SI) using hydro-methanol as the extraction solvent (HME).

separations using hydro-acetone as the extraction solvent (HAE). The results clearly demonstrate variations in the individual polyphenolic compounds among the three tested species, which are also influenced by the extraction solvent used. Notably, in the case of HME, SV showed the presence of all twelve phenolic compounds, while SF and SI only lacked rosmarinic acid. In the case of HAE, the abundance of phenolic compounds was lower compared to HME in all three species. Specifically, SF lacked rosmarinic acid and apigenin, SI lacked rosmarinic acid, and SV only had kaempferol undetected. Through a comparative analysis of the chromatograms, it was observed that the extracts derived from SV displayed a notably richer content of phenolic acids, specifically CLA, along with a diverse range of individual flavonoid compounds.

The experimental results for quantifying the content of individual polyphenolic compounds from the extracts (HME and HAE) of three *Solanum* species are presented in Table 4. The results clearly show that the levels of most polyphenolic compounds were higher in HME compared to HAE. Additionally, all the extracts contained a significant amount of CLA, ranging from 8.17 mg/10 g in SI to a much higher concentration in SV (42.16 mg/10 g). In the extracts of SF, SV, and SI, RUT was identified as the primary flavonoid, with concentrations of 10.77, 29.13, and 6.85 mg/10 g, respectively. Furthermore, significant levels of PCA were detected, with concentrations of 10.28, 11.49, and 19.73 mg/10 g, respectively. Notably, PCA was identified as the most predominant compound in SI. The content of other phenolic acids, such as ferulic acid, caffeic acid, and rosmarinic acid, showed

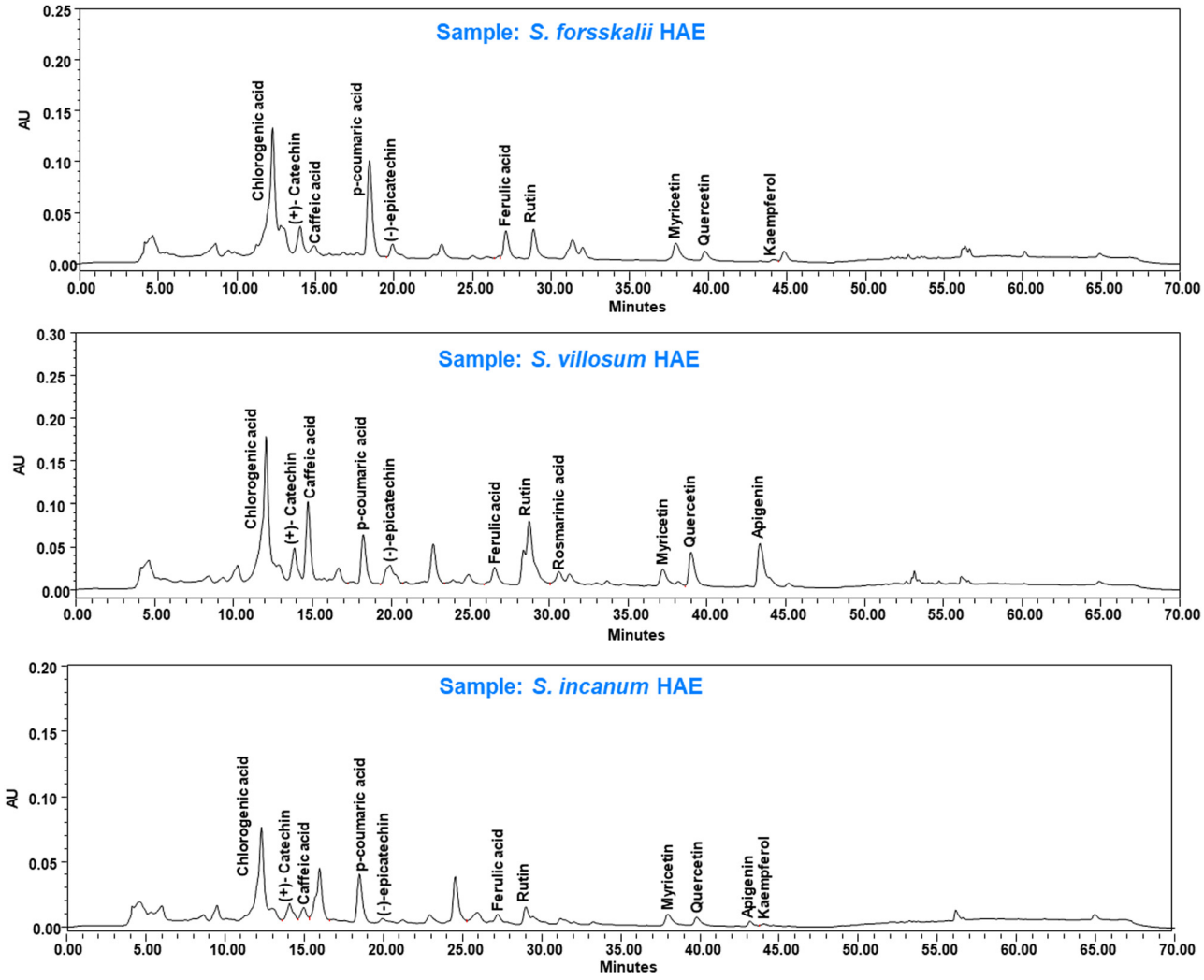

Figure 4: RP-HPLC chromatograms of the polyphenolic compound profiles from three *Solanum* species extracts (SF, SV, and SI) using hydro-acetone as the extraction solvent (HAE).

Table 4: HPLC determination of polyphenolic compounds in SF, SV, and SI extracts in mg/10 g, expressed by dry weigh

Polyphenolic compound	HME			HAE		
	SF	SV	SI	SF	SV	SI
CLA	24.76 ± 0.18	42.16 ± 0.59	10.41 ± 0.37	21.29 ± 0.68	26.82 ± 0.41	8.17 ± 0.33
(+)- Catechin	5.64 ± 0.22	7.33 ± 0.62	3.55 ± 0.53	4.71 ± 0.89	6.91 ± 0.57	2.08 ± 0.92
Caffeic acid	2.19 ± 0.15	10.92 ± 0.21	5.42 ± 0.39	1.86 ± 0.13	2.91 ± 0.25	1.39 ± 0.19
PCA	8.05 ± 0.52	5.27 ± 0.36	19.73 ± 0.14	10.28 ± 0.28	11.49 ± 0.46	4.78 ± 0.30
(−)Epicatechin	2.55 ± 0.09	6.02 ± 0.14	1.31 ± 0.07	1.63 ± 0.12	4.05 ± 0.23	0.77 ± 0.14
Ferulic acid	5.48 ± 0.81	4.73 ± 0.75	0.64 ± 0.56	2.71 ± 0.74	3.86 ± 0.42	0.81 ± 0.37
RUT	10.77 ± 0.34	29.13 ± 0.47	6.85 ± 0.61	4.39 ± 0.19	7.53 ± 0.23	2.19 ± 0.36
Rosmarinic acid	ND	3.65 ± 0.12	ND	ND	2.92 ± 0.17	ND
Myricetin	3.53 ± 0.31	5.77 ± 0.49	4.31 ± 0.23	2.62 ± 0.17	2.46 ± 0.41	1.42 ± 0.29
Quercetin	2.08 ± 0.18	4.11 ± 0.22	3.92 ± 0.37	0.92 ± 0.14	5.81 ± 0.29	1.25 ± 0.13
Apigenin	0.34 ± 0.12	2.32 ± 0.09	0.71 ± 0.16	ND	4.22 ± 0.31	0.94 ± 0.22
Kaempferol	0.87 ± 0.43	0.91 ± 0.36	1.08 ± 0.22	0.29 ± 0.35	ND	0.41 ± 0.26

Values are expressed as mean ± SD (n = 5). *ND – not determined; HME – hydro-methanolic extract; HAE – hydro-acetonic extract.

significant variation depending on the species and extracting solvent. Notably, rosmarinic acid was only detected in SV, with concentrations of 3.65 mg/10 g in HME and 2.92 mg/10 g in HAE. Significant levels of other important flavonoid compounds, including (+)-catechin, (−)-epicatechin, myricetin, and quercetin, were detected among the extracts of the three species. However, apigenin and kaempferol exhibited the lowest contents.

3.4 Phytochemical analysis by GC/MS

To determine the chemical composition of the three *Solanum* species, a comparative analysis was conducted using GC–MS. The HME was selected for its demonstrated efficiency in extracting phytochemical compounds.

The chemical content of the extracts was profiled using the HP Innowax column, and the identified compounds were characterized based on their RT, molecular formula, molecular weight (MW), and peak area percentages. The analysis includes three replicates for each sample, and the results presented are the mean values from these replicates. These percentages were used as an indicator of the relative concentration of each compound. Table 5 and Figure 5 present the data and chromatograms, respectively, showcasing the major compounds found in each species. In the SF extract, the most abundant compounds were 2,4-dimethyl-1-decene (28.54%), palmitic acid (24.65%), and linoleic acid (LNA) (19.71%). For SV, the predominant compounds were (*E*)-cinnamaldehyde (CMA) (19.31%), *cis*-13-octadecenoic acid methyl ester (OAM) (20.37%), methyl palmitate (MEP) (9.81%), and (−)-eburenine (EBU) (12.16%). The major compounds identified in the SI extract were MEP (28.25%), palmitic acid (14.11%), and oleic acid amide (OAA) (8.28%). The presence of diverse bioactive compounds, as revealed by GC–MS analysis, provides support for the traditional medicinal applications of these plants. However, further scientific investigation is required to isolate and study the individual phytoactive compounds in greater detail.

3.5 DPPH and ABTS radical scavenging effects

Plant extracts have been evaluated for their ability to scavenge free radicals using different methods [44,45]. In this study, the antioxidant activity of the HME and HAE from three *Solanum* species was assessed using two methods: the DPPH and ABTS radical scavenging assays (as presented in Table 6). The DPPH assay measures the ability of antioxidants in the samples to reduce the DPPH radical through electron transfers, with the absorption at 517 nm being used as a measurement. Conversely, the ABTS assay assesses the antioxidant capacity to reduce the ABTS radical through electron and/or hydrogen atom transfer, with absorption measured at 734 nm. A lower value for the 50% effective concentration (IC_{50}) typically indicates a more potent radical agent [46]. The results obtained from these methods, illustrated in Figures 6 and 7, clearly demonstrate that the antioxidant activity of the crude extracts is dose-dependent. Furthermore, the results summarized in Table 6 provide valuable insights into the order of radical scavenging potency among the different extracts. It is noteworthy that the HME exhibited superior antioxidant capacity compared to the HAE in all three *Solanum* species. Specifically, the DPPH and ABTS scavenging activities of the HME from SF were measured and the IC_{50} values were 51.24 ± 1.65 and 35.74 ± 0.84 µg/mL, respectively. Similarly, the HME from SV exhibited IC_{50} values of 40.81 ± 1.86 and 44.16 ± 0.61 µg/mL for DPPH and ABTS scavenging activities, respectively. Likewise, the HME from SI demonstrated IC_{50} values of 78.24 ± 2.03 and 58.07 ± 1.57 µg/mL for DPPH and ABTS scavenging activities, respectively. It is important to mention that the fruit extracts have been classified into three groups based on their DPPH/IC_{50} values: those with significant antioxidant properties (DPPH/IC_{50} ≤ 100 µg/mL), those with moderate antioxidant properties (100 µg/mL < DPPH/IC50 ≤ 316 µg/mL), and those with weak antioxidant properties (DPPH/IC_{50} > 316 µg/mL) [47]. Therefore, the results obtained clearly demonstrate the significant antioxidant capacity of the extracts from the three plants, especially when compared to the tested synthetic antioxidant ascorbic acid, which exhibited IC_{50} values of 20.04 ± 0.43 µg/mL. These findings correspond closely with the polyphenol content observed in all the extracts, with the HME consistently displaying higher levels compared to the HAE. Therefore, it can be inferred that these chemical compounds contribute to the observed antioxidant capacity observed in the DPPH and ABTS tests. This relationship has been previously established and reported by other researchers utilizing similar testing techniques [48–53].

3.6 Correlation between phenolic content and antioxidant activity

The antioxidant activity observed in plant samples can be attributed to the presence of certain components, particularly compounds of phenolic nature. To assess the correlation between the TPC and TFC with antioxidant radical activity, we utilized the Pearson correlation coefficient (PCC), also known as Pearson's *r*. Figure 8 displays the

Table 5: GC–MS analysis of phytoconstituents identified in HME from the three *Solanum* species

Solanum species	Name of compound	Chemical formula	MW (g/mol)	RT (min)	% area
SF	*para*-vinylguaiacol	$C_9H_{10}O_2$	150.07	12.050	3.66
	1-Tridecene	$C_{13}H_{26}$	182.20	12.675	2.85
	phenol-2,4-bis (1,1-dimethylethyl)	$C_{17}H_{30}O$	206.17	14.339	2.00
	8-Methyl-6-nonenoic acid	$C_{10}H_{18}O_2$	170.13	14.771	1.57
	2-Tetradecene	$C_{14}H_{28}$	196.22	15.152	2.92
	2,4-Dimethyl-1-decene	$C_{12}H_{24}$	168.19	16.397	28.54
	MEP	$C_{17}H_{34}O_2$	270.45	18.768	2.30
	Palmitic acid	$C_{16}H_{32}O_2$	256.42	19.193	24.65
	LNA, methyl ester	$C_{19}H_{34}O_2$	294.47	20.401	4.34
	LNA	$C_{18}H_{32}O_2$	280.44	20.857	19.71
	Octadecanoic acid	$C_{18}H_{36}O_2$	284.47	21.051	3.28
SV	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.04	6.515	1.82
	Benzaldehyde dimethyl acetal	$C_9H_{12}O_2$	152.08	7.447	4.33
	(***E*)-CMA**	C_9H_8O	132.06	7.597	19.31
	3-Phenyl-2-propyn-1-ol	C_9H_8O	132.06	8.248	0.63
	Copaene	$C_{15}H_{24}$	204.19	8.448	0.96
	Coumarin (2*H*-1-benzopyran-2-one	$C_9H_6O_2$	146.04	9.217	4.50
	γ-Gurjunene	$C_{15}H_{24}$	204.19	9.474	1.28
	2-Propenal, 3-(2-methoxyphenyl)-	$C_{10}H_{10}O_2$	162.07	9.811	5.44
	6-Methoxycoumaran-7-ol-3-one	$C_9H_8O_4$	180.04	10.024	0.94
	o-Cresol	C_7H_8O	108.06	10.456	1.51
	(+/−)-gamma-Muurolene	$C_{15}H_{24}$	204.19	10.55	2.77
	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{18}$	198.14	10.787	1.19
	MEP	$C_{17}H_{34}O_2$	270.26	12.088	9.82
	Palmitic acid	$C_{16}H_{32}O_2$	256.24	12.32	3.16
	***cis*-13-Octadecenoic acid, methyl ester**	$C_{19}H_{36}O_2$	296.27	12.989	20.37
	Cyclohexene, 1-pentyl-4-(4-propylcyclohexyl)-	$C_{20}H_{36}$	276.28	13.208	1.64
	Pyridine, 3-(diphenylmethyl)-	$C_{18}H_{15}N$	245.12	13.308	1.42
	(−)-EBU	$C_{19}H_{24}N_2$	280.19	13.902	12.16
	OAA	$C_{18}H_{35}NO$	281.27	14.259	3.39
SI	Cyclohexanepropanol, 2,2-dimethyl-6-methylene-	$C_{12}H_{22}O$	182.17	10.825	1.05
	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (*E*)-	$C_{10}H_{16}O$	152.12	11.094	1.93
	5,7-Dimethyloctahydrocoumarin	$C_{11}H_{18}O_2$	182.13	11.3	1.07
	Bicyclo[5.2.0]non-1-ene	C_9H_{14}	122.11	11.532	3.20
	MEP	$C_{17}H_{34}O_2$	270.26	12.088	28.25
	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.17	12.182	1.78
	Palmitic acid	$C_{16}H_{32}O_2$	256.24	12.326	14.11
	1,1,10-Trimethyl-trans-decalon	$C_{13}H_{22}O$	194.17	12.432	2.72
	Methyl 14-methylhexadecanoate	$C_{18}H_{36}O_2$	284.27	12.607	1.37
	Caryophyllene oxide	$C_{15}H_{24}O$	220.18	12.776	2.11
	Heneicosane	$C_{21}H_{44}$	296.34	12.951	4.99
	Methyl 16-methyl-heptadecanoate	$C_{19}H_{38}O_2$	298.29	13.095	5.05
	Octadecanoic acid	$C_{18}H_{36}O_2$	284.27	13.296	1.46
	Tricosane	$C_{23}H_{48}$	324.38	13.865	5.08
	2,11-Dodecadiene, 4-chloro-	$C_{12}H_{21}$	200.13	13.934	1.25
	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.32	14.002	1.35
	2-Butyl-3-methyl-5-(2-methylprop-2-enyl) cyclohexanone	$C_{15}H_{26}O$	222.20	14.165	1.13
	OAA	$C_{18}H_{35}NO$	281.27	14.246	8.28
	2-Dodecenylsuccinic anhydride	$C_{16}H_{26}O_3$	266.19	14.421	1.72
	Methyl 20-methyl-heneicosanoate	$C_{23}H_{46}O_2$	354.35	14.828	3.87
	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.28	14.959	1.54
	beta-Phenylethyl butyrate	$C_{12}H_{16}O_2$	192.12	15.497	3.45

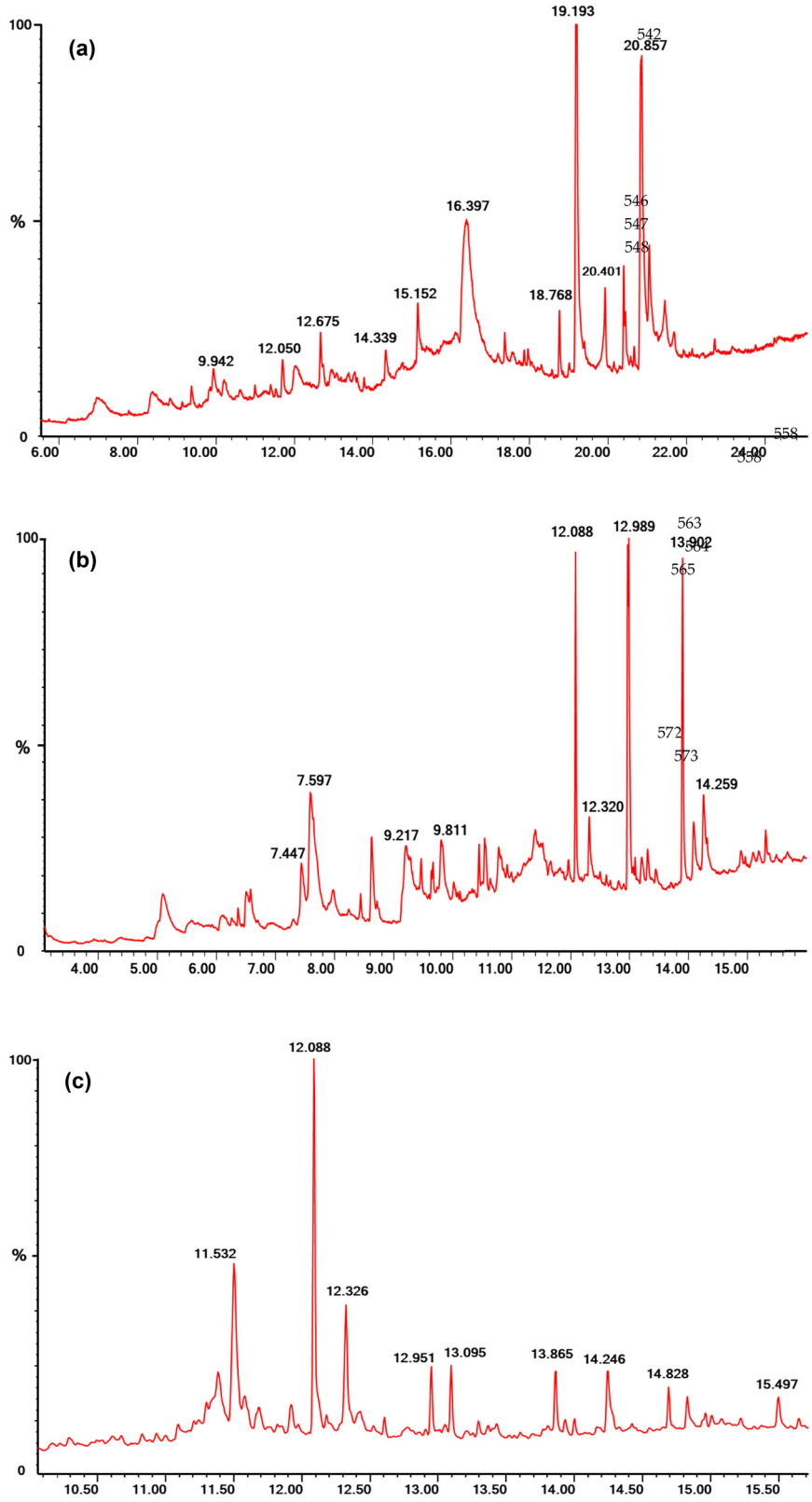

Figure 5: GC–MS chromatograms of crude extracts (HME) from (a) SF, (b) SV, and (c) SI.

Table 6: IC_{50} values (μg/mL) of anti-radical activity of HME and HAE of SF, SV, and SI using the DPPH and ABTS methods

Species	Crude extract	DPPH	ABTS
SF	HME	51.24 ± 1.65	35.74 ± 0.84
	HAE	114.30 ± 2.37	60.93 ± 1.73
SV	HME	40.81 ± 1.86	44.16 ± 0.61
	HAE	68.12 ± 1.43	46.79 ± 1.29
SI	HME	78.24 ± 2.03	58.07 ± 1.57
	HAE	98.89 ± 1.52	84.94 ± 2.16

Values are expressed as mean ± SD of three parallel measurements.

scatter plots illustrating the PCC relationship between TPC and TFC with antioxidant radical activity. The correlation analysis performed in this study revealed a strong positive association between antioxidant activity and both the TPC (r = 0.8904–0.8927, $p \leq 0.05$) and the flavonoid content (r = 0.6067–0.6538, $p \leq 0.05$).

3.7 Analysis of molecular docking

Using the molecular docking approach, the potential antioxidant effect of phytochemicals against Cyt-c was assessed. The results indicated that all the phytochemicals successfully bound to Cyt-c at various sites, as illustrated in Figure 9. The binding energies of all phytochemicals were determined and are summarized in Table 7. These energies spanned from −4.0 to −8.2 kcal/mol, with the most effective compounds having binding energies of ≤−6.0 kcal/mol. Specifically, RUT, CLA, EBU, PCA, and (E)-CMA exhibited docking energies of −8.2, −7.7, −7.4, −6.0, and −6.0 kcal/mol, respectively. For comparison, the positive control, ascorbic acid, had a docking energy of −5.5 kcal/mol. The relative binding position of the phytochemicals is represented in Figure 9.

The Cyt-c and ascorbic acid (control) complex exhibited stability through four conventional hydrogen bonds with ARG48:HH21, ALA147:HN, PRO80:O, and SER81:O along with one carbon hydrogen bond with ARG184:O (Figure 10a, and Table 8). Additionally, the complex involved several van der Waals interactions between ascorbic acid and Cyt-c amino acid residues such as HIS52, ALA83, PRO145, ASP146, SER185, TYR187, and HEME moiety. The docking energy of ascorbic acid towards Cyt-c was −5.5 kcal mol^{-1}, corresponding to the dissociation was 1.08×10^4 M^{-1} (Table 8).

The Cyt-c and PCA complex exhibited stability through four conventional hydrogen bonds with ARG31:HE, TYR42:HN, GLY43:HN, and GLU228:OE1 along with one carbon hydrogen bond with GLY41:CA (Figure 10b, and Table 8). PCA also

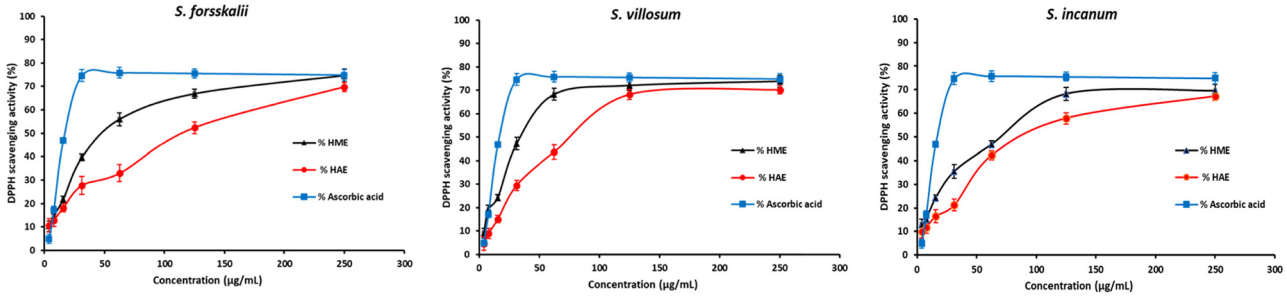

Figure 6: Anti-radical activity of crude extracts from SF, SV, and SI and standard ascorbic acid using the DPPH method. HME = hydro-methanolic extract, HAE = hydro-acetonic extract.

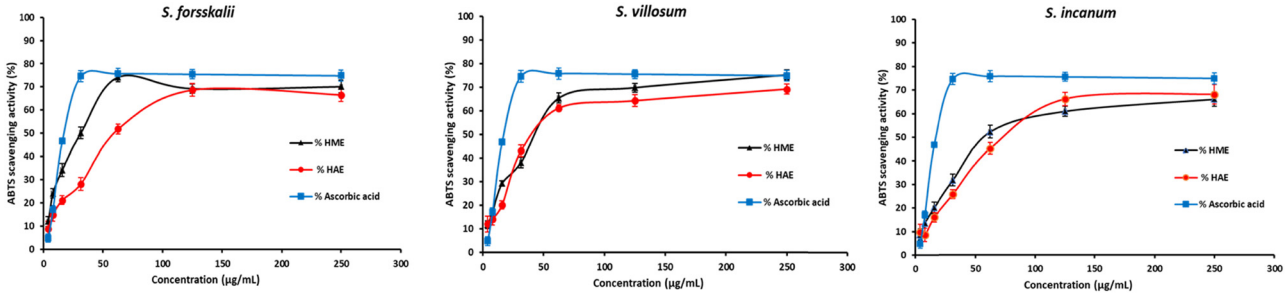

Figure 7: Anti-radical activity of crude extracts from SF, SV, and SI and standard ascorbic acid using the ABTS method. HME = hydro-methanolic extract, HAE = hydro-acetonic extract.

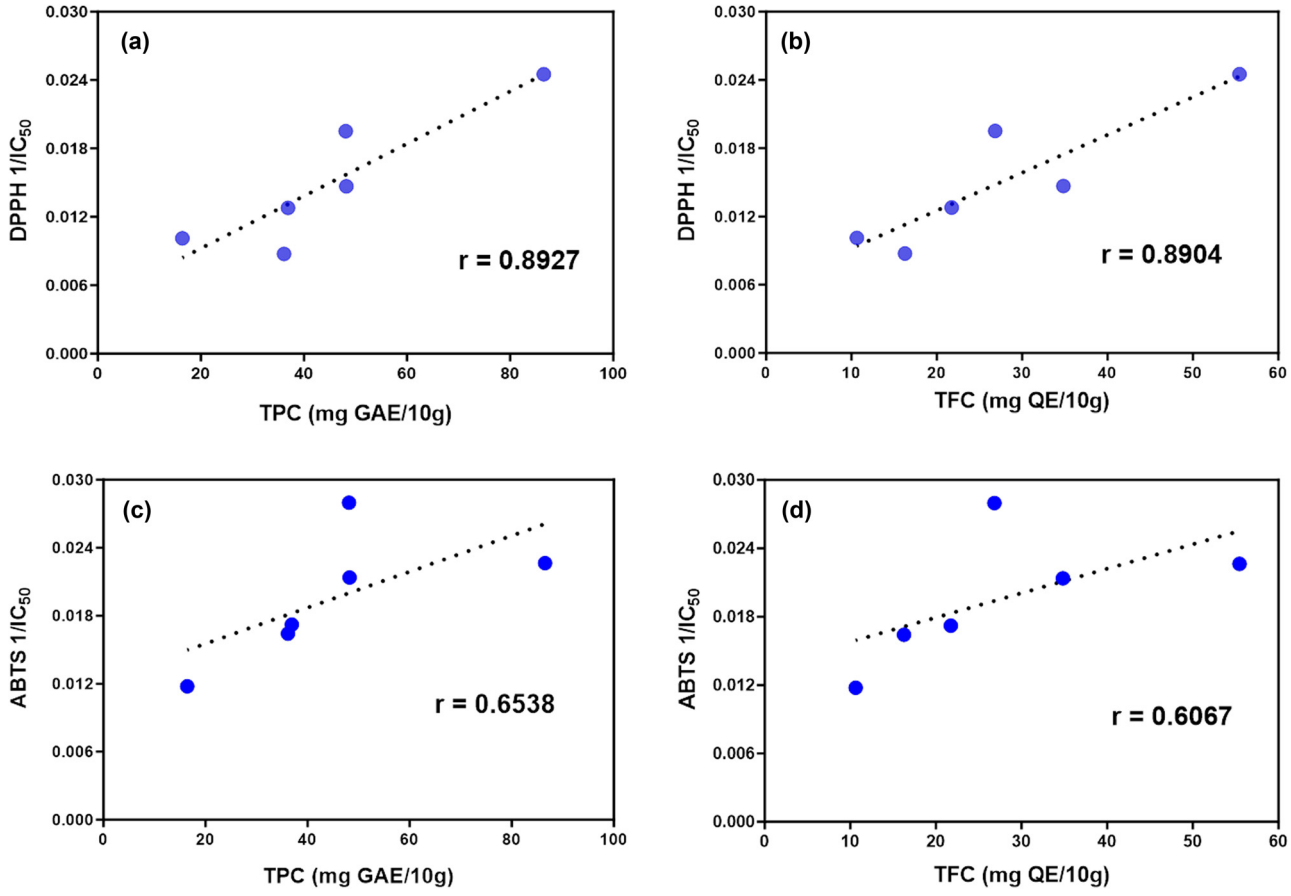

Figure 8: Pearson correlation scatter plot of relationship between (a) TPC and DPPH free radical scavenging activity, (b) TFC and DPPH free radical scavenging activity, (c) TPC and ABTS free radical scavenging activity, and (d) TFC and ABTS free radical scavenging activity.

interacted with Cyt-c through one amide-Pi stacked hydrophobic interaction with LEU30:C,O and ARG31:N, and one Pi-alkyl hydrophobic interaction with ARG31. Additionally, Moreover, it engaged in van der Waals interactions with specific amino acid residues, including ALA27, ILE40, ASN196, and LEU289. The docking energy and binding affinity of PCA towards Cyt-c were estimated to be −6.0 kcal mol^{-1} and 2.52 × 10^{4} M^{-1}, respectively (Table 8).

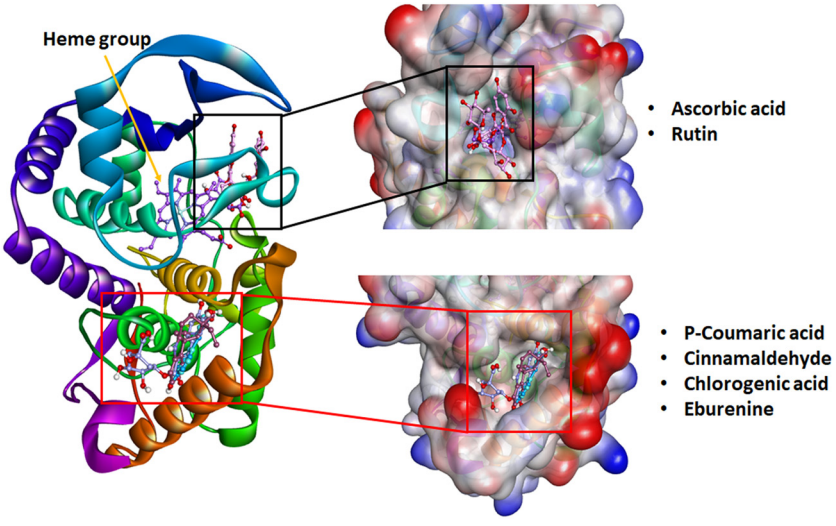

Figure 9: The relative binding position of the phytochemicals bound to different sites on Cyt-c.

Table 7: Molecular docking energy between selected phytochemicals and Cyt-c

S. no.	PubChem ID	Name of compound	Docking energy (kcal mol^{-1})
1	54670067	Ascorbic acid (control)	−5.2
2	521583	4-Dimethyl-1-decene	−4.8
3	1794427	CLA	−7.7
4	637511	CMA	−6.0
5	12541027	OAM	−5.6
6	624448	EBU	−7.4
7	5280450	LNA	−4.2
8	8181	MEP	−4.0
9	5283387	OAA	−4.6
10	985	Palmitic acid	−5.2
11	637542	PCA	−6.0
12	5280805	RUT	−8.2

The stability of Cyt-c and E-CMA complex was maintained due to the formation of two conventional hydrogen bonds with TYR42:HN, and GLY43:HN, along with one carbon hydrogen bond with ILE40:O. Additionally, *E*-cinnamaldehyde interacted with Cyt-c via one amide-Pi stacked hydrophobic interaction with LEU30:C,O and ARG31:N, and one Pi-alkyl hydrophobic interaction with ARG31 (Figure 10c). Furthermore, E-CMA engaged in van der Waals interactions with specific amino acid residues of Cyt-c, including ALA27, GLY41, GLU118, MET119, ASN196, and LEU289. The estimated docking energy and binding affinity of E-CMA towards Cyt-c were $-6.0\,kcal\,mol^{-1}$ and $2.52 \times 10^4\,M^{-1}$, respectively (Table 8).

The estimation of the Cyt-c and EBU interaction revealed that the complex was stabilized through three alkyl hydrophobic interactions with LEU30 (two interactions), and MET119, along with one Pi-alkyl hydrophobic interaction with ARG31 (Figure 10d). Additionally, EBU formed a network of van der Waals interactions amino acid residues like with ALA27, ASP34, ILE40, GLY41, TYR42, GLY43, GLU118, ASN196, and LEU289. The docking energy and dissociation constant of EBU binding to Cyt-c were estimated to be $-7.4\,kcal\,mol^{-1}$ and $2.68 \times 10^5\,M^{-1}$, respectively, as shown in Table 8.

The Cyt-c and CLA complex was stabilized by five conventional hydrogen bonds with ARG31:HE, GLN120:HE22, GLU290:HN, ILE40:O, and GLU118:O. Additionally, CLA interacted with Cyt-c through one amide-Pi stacked hydrophobic interaction with LEU30:C,O and ARG31:N, and two Pi-alkyl hydrophobic interactions with LEU30, and MET119 (Figure 10e). Importantly, van der Waals interactions occurred between CLA and ALA27, ASP31, GLY41, TYR42, GLY43, PRO44, ASN196, THR288, GLU291, and LEU289 of Cyt-c. The binding interaction between CLA and Cyt-c was estimated, revealing an estimated docking energy of $-7.7\,kcal\,mol^{-1}$ and a binding affinity of $4.44 \times 10^5\,M^{-1}$, as detailed in Table 8.

The complex between RUT and Cyt-c was stabilized by four conventional hydrogen bonds with ASN220:HD21, ASN220:OD1, ARG184:O, and SER185:O, along with one carbon hydrogen bond with SER81:OG. Additionally, the RUT and Cyt-c complex was stabilized by one Pi-anion electrostatic interaction with ASP148:OD1, and one Pi-alkyl hydrophobic interaction with HEME group (Figure 10f, and Table 8). Significantly, RUT engaged in van der Waals interactions with a range of Cyt-c amino acid residues. These included ARG48, HIS52, ASP79, PRO80, ALA83, GLY142, PRO145, ASP146, ALA147, GLY186, TYR187, and ALA218. The estimated docking energy and binding affinity of RUT toward Cyt-c were estimated to be $-8.2\,kcal\,mol^{-1}$ and $1.03 \times 10^6\,M^{-1}$, respectively (Table 8).

3.8 ADMET analysis

The crucial ADMET analysis values and molecular properties for the examined phytochemicals are detailed in Table 9 and Figure 11. The absorption potential of the compounds was assessed using several parameters, including human intestinal absorption (HIA), Caco-2 permeability, MDCK permeability, and Pgp factors (Table 9). Most compounds exhibit high HIA, classified as excellent to medium absorption (0–0.3: excellent; 0.3–0.7: medium; 0.7–1.0: poor). However, for orally administered drugs, human oral bioavailability (F (30%)) is vital, indicating the efficiency of drug absorption into the bloodstream. The data in Table 9 suggest a propensity for low oral bioavailability among the compounds studied. In evaluating new drugs, a crucial consideration is assessing their metabolism and the possible effects of their metabolites in the patient's body. As per the prediction data in Table 9, the majority of the compounds exhibit high values of plasma protein binding (PPB) and volume distribution (VD) falling within the optimal range of 0.04–20 L/kg.

Cytochrome P450 (CYP) enzymes play a significant role in regulating drug metabolism in humans. The CYP 1–3 enzyme families, especially CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, are responsible for about 80% of drug oxidative processes and 50% of drug elimination from the body [54]. The ADMET analysis conducted facilitates the prediction of potential pharmacokinetic and toxicological properties for the group of inhibitors being studied. According to the findings in Table 9, compounds like CLA, LNA, palmitic acid, PCA, and RUT, which showed the highest levels through RP-HPLC and GC–MS analyses, demonstrate minimal to zero probability of inhibition. This suggests a very low likelihood of

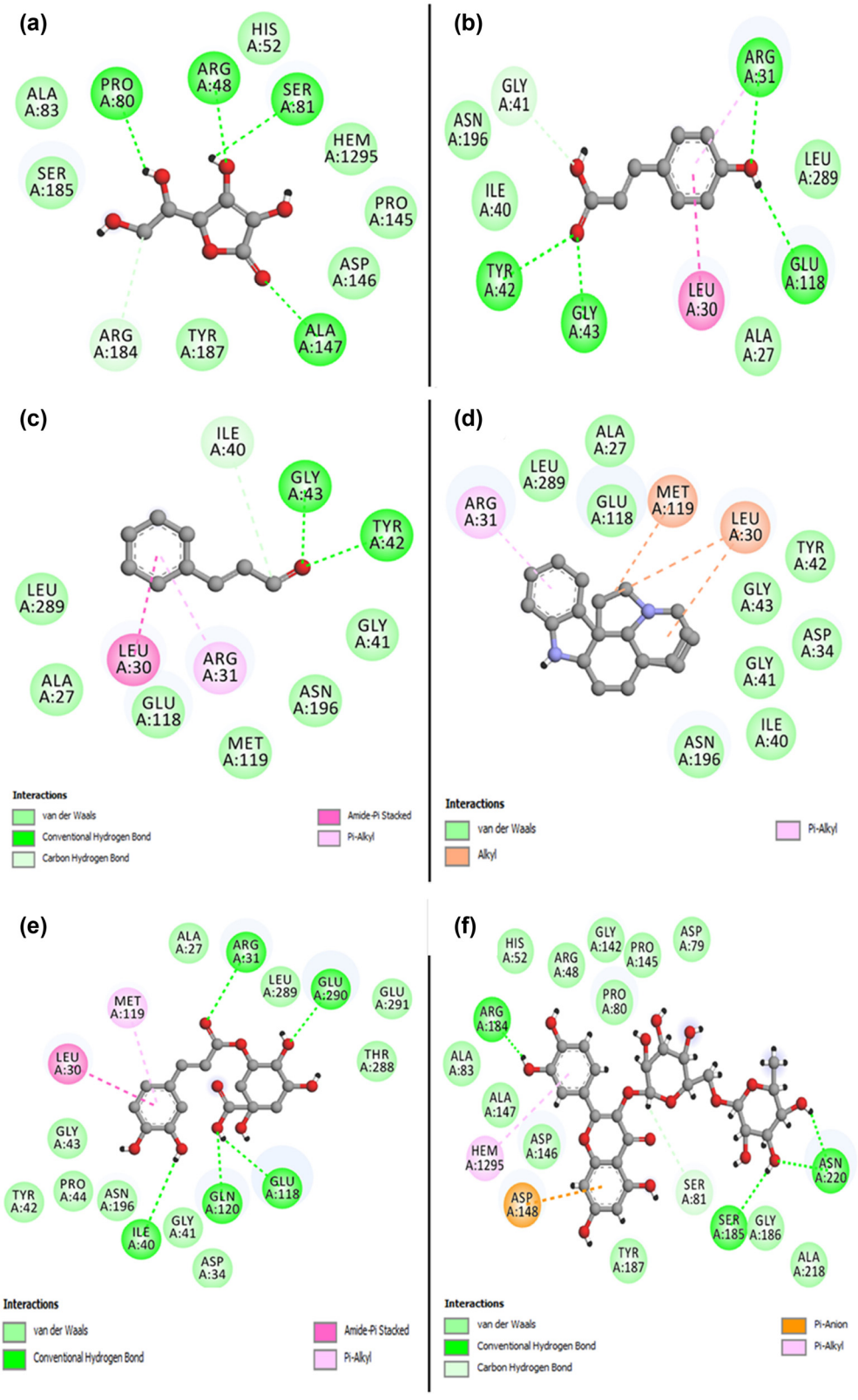

Figure 10: 2D representation of the interaction between Cyt-c and phytochemicals. (a) Ascorbic acid (control), (b) PCA, (c) CMA, (d) EBU, (e) CLA, and (f) RUT.

Table 8: Molecular docking parameters for the interaction of phytochemicals and Cyt-c

Compound	Donor–acceptor pair	Distance (Å)	Type of interaction
Ascorbic acid (control)	ARG48:HH21 – LIG:O	1.97	Conventional hydrogen bond
	ALA147:HN – LIG:O	2.21	Conventional hydrogen bond
	LIG:H – PRO80:O	2.02	Conventional hydrogen bond
	LIG:H – SER81:O	2.50	Conventional hydrogen bond
	LIG:C – ARG184:O	3.50	Carbon hydrogen bond
PCA	ARG31:HE – LIG:O	2.41	Conventional hydrogen bond
	TYR42:HN – LIG:O	2.83	Conventional hydrogen bond
	GLY43:HN – LIG:O	1.98	Conventional hydrogen bond
	LIG:H – GLU118:OE1	2.68	Conventional hydrogen bond
	GLY41:CA – LIG:O	3.63	Carbon hydrogen bond
	LEU30:C,O;ARG31:N – LIG	4.30	Hydrophobic (Amide-Pi Stacked)
	LIG – ARG31	3.97	Hydrophobic (Pi-Alkyl)
CMA	TYR42:HN – LIG:O	2.69	Conventional hydrogen bond
	GLY43:HN – LIG:O	2.13	Conventional hydrogen bond
	LIG:C – ILE40:O	3.75	Carbon hydrogen bond
	LEU30:C,O;ARG31:N – LIG	4.17	Hydrophobic (Amide-Pi Stacked)
	LIG – ARG31	4.01	Hydrophobic (Pi-Alkyl)
EBU	LEU30 – LIG	5.24	Hydrophobic (Alkyl)
	LIG – LEU30	4.53	Hydrophobic (Alkyl)
	LIG – MET119	4.66	Hydrophobic (Alkyl)
	LIG – ARG31	4.20	Hydrophobic (Pi-Alkyl)
CLA	ARG31:HE – LIG:O	2.49	Conventional hydrogen bond
	GLN120:HE22 – LIG:O	2.17	Conventional hydrogen bond
	GLU290:HN – LIG:O	2.51	Conventional hydrogen bond
	LIG:H – ILE40:O	2.73	Conventional hydrogen bond
	LIG:H – GLU118:O	2.41	Conventional hydrogen bond
	LEU30:C,O;ARG31:N – LIG	4.37	Hydrophobic (Amide-Pi Stacked)
	LIG – LEU30	4.42	Hydrophobic (Pi-Alkyl)
	LIG – MET119	5.22	Hydrophobic (Pi-Alkyl)
RUT	ASN220:HD21 – N:UNK1:O	2.25	Conventional hydrogen bond
	LIG:H – ARG184:O	2.34	Conventional hydrogen bond
	LIG:H – ASN220:OD1	2.45	Conventional hydrogen bond
	LIG:H – SER185:O	2.79	Conventional hydrogen bond
	LIG:H15 – SER81:OG	2.60	Carbon hydrogen bond
	ASP148:OD1 – LIG	4.37	Electrostatic (Pi-Anion)
	LIG – HEM1295:CMA	4.49	Hydrophobic (Pi-Alkyl)

interaction with the tested group of enzymes as substrates. Therefore, these tested compounds are not expected to significantly interfere with the metabolic processes of other pharmaceuticals metabolized by the analyzed group of enzymes.

The predicted excretion data, represented by CL plasma penetration (CL) and half-life ($T_{½}$), are vital indicators. A CL value greater than 15 mL/min/kg is considered high clearance, while 5–15 mL/min/kg indicates moderate clearance, and less than 5 mL/min/kg suggests low clearance. According to the data, all compounds exhibit low to moderate clearance, ranging between 2.05 and 9.122 mL/min/kg, and short half-life durations between 0.174 and 3.878 h.

In the exploration of chemical compounds for pharmaceutical purposes, analyzing their toxicity concerning interaction with the human body is crucial. The pharmacological effects of a chemical may entail undesirable side effects. The conducted studies depict a wide range of potential impacts of the tested inhibitors on the human body, summarized in Table 9. Evaluating the potential impact of new drugs involves assessing their effects on the heart, including their ability to inhibit the human ether-a-go-go-related gene (hERG) potassium channel. Inhibition of this channel can disrupt normal cardiac rhythm, potentially leading to adverse effects such as cardiac dysfunction or the development of life-threatening arrhythmias [55]. The collected data suggest a minimal likelihood of adverse effects from the tested compounds in this context, as indicated by the classification of values: 0–0.3 (excellent), 0.3–0.7 (medium), and 0.7–1.0 (poor). Another indicator of drug toxicity is the rat oral acute toxicity (ROA) index, which measures the maximum dose that can cause death in

Table 9: The values of descriptors characterizing ADMET properties (absorption, distribution, metabolism, extraction, and toxicity) of the predominant phytochemicals including 4-dimethyl-1-decene (DMD), CLA, CMA, OAM, EBU, LNA, MEP, OAA, PMA, PCA, and RUT

ADMET parameters	Property	Compound									
		DMD	CLA	CMA	OAM	EBU	LNA	MEP	OAA	PMA	PCA	RUT
Absorption	Caco-2 permeability	−4.374	−6.349	−4.767	−5.023	−4.682	−5.062	−5.028	−5.135	−5.096	−4.985	−6.307
	MDCK permeability	1.5×10^{-5}	−5.029	−4.639	−4.755	−4.889	−4.761	−4.824	−4.723	−4.803	−4.774	−4.996
	Pgp-inhibitor	0.006	0.0	0.945	0.038	0.726	0.001	0.006	0.001	0.0	0.0	0.0
	Pgp-substrate	0.0	0.122	0.029	0.083	0.033	0.007	0.11	0.102	0.014	0.04	0.959
	F 30%	0.695	0.999	0.63	0.894	0.001	0.671	0.959	0.591	0.926	0.768	1.0
	HIA	0.002	0.849	0.031	0.187	0.0	0.208	0.558	0.044	0.85	0.086	0.974
Distribution	PPB	92.87	54.794	94.866	97.941	62.105	96.967	98.45	98.196	98.031	68.241	81.741
	VD	2.85	0.379	−0.415	−0.218	0.575	−0.211	0.837	0.474	0.592	−0.712	−0.151
	BBB	0.972	0.003	0.608	0.025	1.0	0.073	0.013	0.27	0.021	0.003	0.0
	Fu	8.369	44.663	5.263	1.498	36.106	2.766	1.623	1.653	1.672	25.338	19.664
Metabolism	CYP1A2-inh	0.895	0.0	1.0	1.0	0.0	0.566	0.995	0.998	0.073	0.074	0.0
	CYP2C19-inh	0.507	0.0	0.998	0.001	0.0	0.9	0.994	0.991	0.015	0.0	0.0
	CYP2C9-inh	0.375	0.0	0.949	0.992	0.0	0.493	0.931	0.0	0.166	0.0	0.0
	CYP2D6-inh	0.032	0.0	0.225	0.152	0.998	0.005	0.259	0.007	0.147	0.002	0.0
	CYP3A4-inh	0.075	0.0	0.002	0.981	0.668	0.002	0.884	0.108	0.0	0.0	0.001
Excretion	CL	9.122	2.529	11.086	5.431	6.559	3.804	5.278	5.387	3.77	2.609	2.05
	$T_{1/2}$	0.174	3.157	1.395	0.409	1.08	0.598	0.521	0.414	0.932	1.695	3.878
Toxicity	hERG-Blockers	0.023	0.021	0.231	0.394	0.579	0.119	0.352	0.41	0.166	0.046	0.029
	H-HT	0.025	0.431	0.399	0.167	0.718	0.109	0.433	0.282	0.423	0.457	0.291
	ROA	0.012	0.201	0.311	0.057	0.742	0.064	0.148	0.08	0.124	0.258	0.415
	FDAMDD	0.023	0.54	0.324	0.123	0.836	0.063	0.224	0.11	0.178	0.089	0.636
	Carcinogenicity	0.139	0.089	0.399	0.227	0.567	0.1	0.473	0.223	0.27	0.197	0.111

Abbreviations: human oral bioavailability 30% (F 30%); human intestinal absorption (HIA); plasma protein binding (PPB); volume distribution (VD); Blood-brain barrier penetration (BBB); the fraction unbounded in plasma (FU); CL plasma penetration (CL); half-life ($T_{1/2}$); human ether-a-go-go related gene (hERG); human hepatotoxicity (H-HT); rat oral acute toxicity (ROA); FDA Maximum (Recommended) Daily Dose (FDAMDD).

mammals, particularly in rats and mice. This index serves as one of the fundamental indicators of toxicity in the evaluation of potential drugs. The data in Table 9 suggest a low to moderate probability of undesirable effects for each of the compounds, with the highest probability observed for EBU (0.742). Another key aspect of the adverse activity is hepatotoxicity (H-HT), which assesses the risk of drug-induced liver damage, liver injuries, and carcinogenicity. The obtained values clearly indicate a low to moderate probability of these undesirable effects occurring.

4 Discussion

Plants are vital sources of diverse chemical compounds with biological properties for creating effective medications. However, more research is needed to identify phytoconstituents in medicinal plants [56]. The *Solanum* genus is historically significant for the treatment of various illnesses and diseases [57]. This study analyzed the phytochemical composition and antioxidant activity of three S*olanum* species from Saudi Arabia using GC–MS and RP-HPLC. Two polar extraction solvents (HME and HAE) are known for their high efficiency in extracting bioactive compounds, including phenolic compounds and flavonoids, from plants. This selection significantly influenced the levels of extracted polyphenols and overall phytochemical content, shedding light on the solvent's pivotal role in extraction processes. The comparison of these solvents provided valuable insights into their respective capabilities in enhancing the solubility and extraction efficiency of the targeted compounds [58,59].

Interestingly, our findings reveal that the HME exhibited an exceedingly high concentration of phenolic and flavonoid compounds, accounting for nearly 60% of the TPC and TFC extracted from both solvents across the three species investigated. This finding aligns with earlier research, specifically, the study carried out by Rupasinghe et al. [60]. In their study, they found that solvents with higher polarity, like hydro-alcoholic mixtures, were successful in extracting flavonoid glycosides and higher molecular-weight phenols, resulting in greater quantities compared to an equivalent acetonic system. Based on the findings, the SV extract

Figure 11: The charts illustrate the molecular property values of prevalent phytochemicals compared to the recommended lower and upper limits for substances with pharmacological effects. MW, number of rigid bonds (nRig), formal charge (fChar), number of heteroatoms (nHet), number of atoms in the biggest ring (MaxRing), number of rings (nRing), number of rotatable bonds (nRot), Topological Polar Surface Area (TPSA), number of hydrogen bond donors (nHD), number of hydrogen bond acceptors (nHA), logP at physiological pH 7.4 (logD), log of the aqueous solubility (logS), and log of the partition coefficient (logP) are presented.

obtained using an 80% (v/v) methanol solvent (HME) exhibited the highest content of total polyphenols and flavonoids. The total polyphenol content was measured at 86.52 GAE/10 g DW, while the TFC was 55.46 mg GAE/10 g DW. These values are also higher compared to those obtained in a previous study for extracts carried out in solvent 80% methanol [61]. Previous studies assessing the polyphenolic and flavonoid content in SF have been limited. However, our findings reveal that SF contains a significant level of total polyphenols (48.11 mg GAE/10 g DW) and total flavonoids (35.95 mg QE/10 g DW). The results also demonstrate that SI has a lower level of total polyphenols and total flavonoids among the three tested species (38.56 mg GAE/10 g DW and 21.73 mg QE/10 g DW, respectively). These findings are comparable to a previous study conducted by Hlangothi [62], which found that the concentrations of polyphenols and flavonoids in SI extracts varied depending on the extraction solvent. They reported a total phenol content ranging from 0.19 to 3.49 mg GAE/100 mg and a TFC ranging from 0.33 to 1.18 ± 0.93 mg QE/100 mg.

Further investigation led us to a quantitative analysis of twelve polyphenolic compounds in the extracts of the three tested *Solanum* species employing the RP-HPLC method. The chromatograms (Figures 4 and 5) displayed distinct and well-defined peaks corresponding to various polyphenolic compounds. These peaks were observed at specific RT in minutes: CLA (12.01), (+)-catechin (13.83), caffeic acid (14.73), PCA (18.19), (−)-epicatechin (19.64), ferulic acid (26.42), RUT (28.54), rosmarinic acid (30.13), myricetin (37.16), quercetin (38.92), apigenin (43.24), and kaempferol (43.89). The RT were meticulously compared with their respective standards, definitively verifying the presence of these compounds in SF, SV, and SI. CLA has consistently been identified as the main abundant soluble phenolic compound in most *Solanaceous* species [2,63], including the current study. Our findings confirm that CLA is indeed the predominant phenolic compound, and we observed a higher concentration in SV, ranging from 26.82 to 42.16 mg/10 g DW. These values were also compared to a previous study by Staveckien et al. [17], which similarly measured CLA in SV fruit at different ripening stages. In their study, the results ranged from 341 to 415 mg/100 g DW. Additionally, in a novel finding, a high amount of CLA was observed in SF, ranging from 21.29 to 24.76 mg/10 g DW. Similarly, SI was found to have significant levels of CLA, ranging from 8.17 to 10.41 mg/10 g DW, corroborating a previous study [64]. Furthermore, among the phenolic compounds analyzed, PCA exhibited a remarkably higher concentration of 19.73 mg/10 g DW in SI, which was also tentatively identified by Lin et al. [65].

Significant levels of other phenolic acids (such as caffeic acid, PCA, ferulic acid, and rosmarinic acid) were detected and their levels varied depending on the species and extracting solvent used. Specifically, caffeic acid and ferulic acid are noteworthy phenolic compounds known for their cytoprotective and antioxidant properties. These compounds play a crucial role in preventing the production of reactive oxygen species, which are linked to the development of diverse diseases [66]. Additionally, caffeic acid and CLAs have exhibited potential as anti-inflammatory agents and have been explored in the development of novel diabetes drugs to enhance insulin sensitivity and secretion [67]. Furthermore, rosmarinic acid has demonstrated its capacity to diminish reactive oxygen species, thereby reducing oxidative stress. It also possesses antibacterial, immunomodulatory, and antifungal activities [68].

RUT, a natural flavone derivative, has been identified as the second most abundant polyphenol in SF and SV, with concentrations of 10.77 and 29.13 mg/10 g DW, respectively. RUT is highly regarded for its potent antioxidant properties, known for its ability to effectively strengthen the walls of blood cells [69]. In addition to its antioxidant capabilities, RUT exhibits a diverse range of beneficial effects, including anti-tumor, antibacterial, and antiviral activities [70]. Furthermore, RUT has been found to contribute to collagen synthesis and improve the utilization of vitamin C [71]. Significant quantities of the important flavonoids, including myricetin, quercetin, (+)-catechin, and (−)-epicatechin, were detected in all three species, with SV exhibiting the highest concentrations (5.77, 4.11, 7.33, 6.02 mg/10 g DW, respectively). In contrast, apigenin and kaempferol were found in relatively minimal amounts. The functional properties of flavonoid compounds are diverse, as they possess antioxidant capabilities and can act as reducing agents, donors of hydrogen, chelators of transition metals, quenchers of reactive oxygen and nitrogen species, inhibitors of enzymes related to oxidative stress, as well as regulators and protectors of the body's natural defense systems. Additionally, their ability to strengthen the immune system and aid in the prevention of physical disorders caused by cancer, bacteria, and viruses [72]. Given the presence of numerous phenolic compounds in the tested *Solanum* species is clear evidence of their ability to provide protection against a variety of diseases and significantly enhance overall health and well-being.

The utilization of GC–MS is pivotal in the exploration of unidentified plant constituents. Given the complex composition of plant materials, GC–MS is an excellent option for their examination due to its heightened sensitivity and selectivity. By ionizing compounds and quantifying their mass numbers, GC–MS contributes to the characterization

of these profiles by offering additional and valuable information [73]. The GC–MS analysis of (Figure 5 and Table 5) revealed the presence of various compounds belonging to different chemical nature. Notably, palmitic acid, MEP, LNA, and OAA emerged as the major constituents in the tested species. These compounds have been reported in scientific literature for their significant antioxidant, antibacterial, and antifungal properties [74].

Additionally, alongside the analysis of the phenolic content, we extensively conducted *in vitro* experiments to evaluate the antioxidant activity of HME and HAE from SF, SV, and SI. It is important to consider that antioxidants vary in their chemical properties and mechanisms of scavenging [75]. Therefore, it is necessary to employ multiple methods to accurately assess the antioxidant potential of plant extracts. In this particular study, we employed two complementary tests, namely the DPPH radical scavenging assay and the ABTS scavenging assay, to evaluate the antioxidant capacity of the tested extracts. Radical-scavenging properties are crucial for inhibiting lipid oxidation. In studies to determine antioxidant activity, the use of radical scavenging-based methods such as DPPH and ABTS has become standard practice. These spectrophotometric assays are widely used to assess the antioxidant activities of pure antioxidant molecules, particularly herbal extracts or *phenolic* compounds. These assays offer advantages in terms of sensitivity, simplicity, speed, and reproducibility, as they allow for the direct reaction between the chromogen radicals and antioxidant com-pounds [76]. The results obtained from this study (Table 4, Figures 7 and 8) offer valuable insights into the significant antioxidant properties ($IC_{50} \leq 100$ µg/mL) of all the tested extracts. They also shed light on the order of scavenging potency among the extracts, which is consistent with their polyphenol content. Specifically, the HME consistently exhibited higher polyphenolic content and greater radical scavenging potency compared to the HAE extract. This trend is similar to the comparison between SV (IC_{50} = 40.81 µg/mL), SF (IC_{50} = 51.24 µg/mL), and SI (IC_{50} = 78.24 µg/mL). These findings demonstrate a clear relationship between the phenolic contents and the enhancement of antioxidant activity. This suggests that these chemical compounds play a crucial role in the observed antioxidant capacity in the DPPH and ABTS tests.

The study used molecular docking to evaluate the antioxidant capacity of key phytochemicals against Cyt-c. RUT, CLA, EBU, PCA, and (*E*)-CMA displayed strong binding energies, with RUT exhibiting the highest at −8.2 kcal mol^{-1}. Interactions involved hydrogen bonds and van der Waals forces, enhancing stability. These results support previous findings, indicating that phytochemicals in these plants, especially those in high concentrations, possess substantial antioxidant properties. The robust binding energies from the docking analysis suggest a potential for these compounds as effective antioxidants.

ADMET analysis was performed to assess the therapeutic applicability of the main identified phytochemicals, offering valuable insights into their absorption, distribution, metabolism, excretion, and toxicity. Detailed values and molecular properties are presented in Table 9 and Figure 11. The evaluation of these data reveals that most investigated compounds exhibit promising characteristics suitable for therapeutic application, aligning well with fundamental ADMET criteria [77,78]. The high predicted HIA values indicate facilitation of bloodstream entry post-oral administration. However, low oral bioavailability prompts further investigation, possibly due to poor permeability. Permeability assays and *in vivo* studies can illuminate these factors, aiding formulation design for enhancement. Favorable predicted plasma protein binding and volume of distribution suggest sufficient concentration for therapeutic efficacy. Minimal CYP enzyme inhibition minimizes the risk of drug interactions, advantageous in multi-drug treatments. Clearance rates, while generally desirable, may necessitate frequent dosing due to short half-life durations, a challenge potentially addressed by controlled-release formulations. Predicted low to moderate toxicity bodes well for safety, though *in vivo* studies are imperative to confirm these findings and establish safe dosage ranges. Overall, while the ADMET analysis underscores the phytochemicals' medicinal promise, addressing oral bioavailability and optimizing half-life through formulation strategies are pivotal for clinical translation.

Furthermore, when comparing the antioxidant effectiveness of the three tested species with other Solanum species evaluated using the same bioassays, including *Solanum sessiliflorum*, *S. torvum*, *S. nigrum*, *S. aethiopicum*, *Solanum sisymbriifolium*, *Solanum melongena*, *Solanum muricatum*, *Solanum melongena* L., and *Solanum lycopersicum* [79–86], it can be inferred that SF, SV, and SI exhibited remarkable radical-scavenging activity. Hence, it can be deduced that the species under investigation in the present study possesses a notable antioxidant capacity that surpasses numerous other species within the *Solanum* genus. This highlights the therapeutic value of these plant-derived compounds in combating oxidative stress and underscores their potential for future development as natural antioxidants in various applications.

5 Conclusions

This investigation delved into the phytochemical composition, as well as the antioxidant activity, of three *Solanum*

species (SF, SV, SI) from Saudi Arabia. The study employed both HME and HAE for a comprehensive analysis of phytochemical compounds, with RP-HPLC and GC–MS analyses revealing high levels of diverse compounds, particularly in HME. CLA was the most abundant, but other valuable phenolic acids and flavonoids were also detected. GC–MS analysis revealed the presence of various compounds, notably, palmitic acid, LNA, MEP, *cis*-13-octadecenoic acid, and oleic acid emerged as the major constituents. This diverse profile aligns with the significant antioxidant activity observed in all extracts using DPPH and ABTS assays, with SV boasting the strongest potential. The study utilized molecular docking to assess the antioxidant capacity of key phytochemicals against Cyt-c, revealing strong binding energies and interactions, supporting their potential as antioxidants. ADMET analysis revealed promising therapeutic characteristics of the identified phytochemicals, aligning with fundamental criteria. While high predicted HIA values suggest efficient bloodstream entry, addressing issues of low oral bioavailability and optimizing half-life through formulation strategies are crucial for clinical translation. Additionally, favorable plasma protein binding and minimal CYP enzyme inhibition enhance their potential for therapeutic efficacy. Although predicted low to moderate toxicity levels are promising, *in vivo* studies are imperative to confirm safety profiles and establish appropriate dosage ranges. These findings suggest that these *Solanum* species could serve as promising sources of bioactive compounds with potential health benefits. Further research is warranted to explore their specific therapeutic applications and potential contributions to functional foods or natural medicines.

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