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GC-MS and RP-HPLC Analysis Reveals Phytochemical Compositions and Antioxidant Potential in Solanum Schimperia-num, Solanum Cordatum, and Solanum Nigrum Extracts from Saudi Arabia

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Abstract

Folkloric herbal remedies play a significant role in modern medicine, with Solanum plants like S. schimperianum, S. cordatum, and S. nigrum being widely utilized in traditional Saudi Arabian medicine. This study investigates the phytochemical and antioxidant potential of these plants, addressing a gap in existing literature. Two extraction solvents, hydro-methanolic (HME) and hydro-acetonic (HAE), were employed to evaluate total phenolic and flavonoid content, identify polyphenolic compounds using RP-HPLC, and analyze volatilized phytochemicals through GC-MS. Antioxidant activity was assessed using DPPH and ABTS assays, with Pearson correla-tion analysis demonstrating a positive relationship between phenolic content and antioxidant activity. Results indicate that HME extraction was more efficient, with S. nigrum exhibiting the highest phytochemical content. GC-MS analysis identified palmitic acid, alpha-tocopherol ace-tate, phenol, 4,4'-isopropylidenedi-, trimethylgallic acid methyl ester, and 3,9-dodecadiyne as predominant constituents, while RP-HPLC identified twelve phenolic compounds, with chloro-genic acid and rutin being the most abundant. HME extracts displayed superior antioxidant ac-tivity compared to HAE extracts, with S. nigrum demonstrating the highest potency. This study underscores the therapeutic potential of these Solanum species due to their rich phytochemical profiles and robust antioxidant capacity, highlighting their significance as natural sources of phenolic compounds for medicinal applications.

Keywords: Solanum species; RP-HPLC; GC-MS, Phytochemical constituents; antioxidant activity

1. Introduction

The plant genus Solanum, a member of the Solanaceae family, is renowned for its ex-tensive diversity, comprising more than 2000 species [1] Many plants within this genus are recognized for their medicinal properties and have been acknowledged for their ther-apeutic attributes [2, 3]. Currently, a significant area of scientific interest revolves around the exploration of naturally occurring bioactive compounds and their safe and precise uti-lization in the food and pharmaceutical sectors. Solanum species, characterized by their rich and diverse biochemical composition, have emerged as an intriguing subject of study to investigate their potential benefits across multiple domains. The integration of various Solanum species in traditional medicine and as sources of drugs for medical, pharmaco-logical, and therapeutic purposes is firmly established [4].

Extensive pharmacological research has been conducted to validate the traditional therapeutic applications of various plants within the Solanum genus. These investigations have encompassed a wide array of pharmacological properties, including analgesic, an-thelminthic, antibacterial, anticancer, antidepressant, antidiabetic, antifungal, antihyper-tensive, anti-inflammatory, antileishmanial,

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antinociceptive, antipsoriatic, antiplasmodi-al, antiprotozoal, diuretic, hepatoprotective, spasmolytic, and vasorelaxant activities [5]. In Saudi Arabia, approximately 16 species of the Solanum genus are distributed, primarily in the western and southwestern regions of the country [6].

The significance of phytochemicals, particularly antioxidants, in neutralizing harm-ful free radicals and combating severe illnesses such as cancer, stroke, and car-diovascular diseases, is universally acknowledged today. Various classes of phyto-chemicals, including polyphenols (phenolic acids, coumarins, stilbenes, lignans, flavo-noids, and isoflavonoids), are renowned for their potent antioxidant properties and poten-tial health benefits [7,8]. The antioxidant capacity of plant phenolic extracts is influenced by factors such as [7, 8]. The antioxidant capacity of plant phenolic extracts is influenced by factors such as concentration and distribution within plant tissues [9, 10], with envi-ronmental conditions, age, and phenological stage also impacting phenolic levels [11].

Numerous studies have been conducted to investigate the phytochemical composi-tion and antioxidant properties of different Solanum species, including S. betaceum [12], S. erianthum, S. torvum [2], S. xanthocarpum, S. violaceum [13], S. aethiopicum, S. macrocarpon [14], S. indicum, S. surattense [15], S. ferrugineum [16], S. melanocerasum, S. nigrum, S. villosum, and S. retroflexum [17], as well as S. scabrum and S. burbankii [18]. This study primarily aimed to assess the abundance and diversity of phenolic compounds and their antioxidant proper-ties in three Solanum species collected from the southern region of Saudi Arabia, namely S. schimperianum, S. cordatum, and S. nigrum. The study conducted an evaluation of total phenolic content and total flavonoid content, along with GC-MS analysis and high-performance liquid chromatography (HPLC) to detect and analyze phytochemical composition variations. Additionally, antioxidant activity variations among the tested species were investigated using spectrometric methods, including the DPPH and ABTS assays. Pearson correlation analysis was also employed to explore the relationship be-tween total phenolic content and antioxidant activity, offering valuable insights into the role of phenolic compounds in antioxidant properties.

The exclusive sourcing of the three Solanum species from Saudi Arabia provides in-sight into the environmental influences on their phytochemical constituents. By utilizing advanced analytical techniques such as GC-MS and RP-HPLC, the study enhances the precision of phytochemical analysis and correlation with antioxidant activity. This com-prehensive approach addresses a gap in the current literature, as limited studies have ex-plored the phytochemical and antioxidant potential of these species. The investigation of phytochemical constituents and their correlation with antioxidant activity adds a unique and novel perspective to understanding the health-promoting properties of these plants.

2. Materials and Methods

2.1. Plant Material

The complete fruiting aerial components of three Solanum species, specifically S. schimperianum, S. cordatum, and S. nigrum, were harvested in the autumn season from natural populations in the southern region (Abha region) of Saudi Arabia. These botanical specimens underwent authentication by a specialist taxonomist at the Department of Pharmacognosy at King Saud University in Saudi Arabia. A voucher specimen (voucher 15,038, 15,101, and 15,149, respectively) were deposited in the Herbarium of Pharmacy, Department of Pharmacognosy, King Saud University, Riyadh, Saudi Arabia. The collected samples were processed for analysis by meticulous washing with flowing water, subsequent drying in a ventilated oven at 40 °C, and pulverization using a household blender. The resultant desiccated and powdered tissues were then stored in paper pouches at ambient temperature, within a light-free setting, awaiting further scrutiny.

2.2. Chemicals, Reagents and Standards

Analytical-grade reagents and solvents (>99% purity), including methanol, acetonitrile, ethanol, and acetic acid, were employed for the extraction and chromatographic separation procedures. These solvents were procured from VWR International Ltd. (Le Périgares- Bâtiment, France). Standards of polyphenols such as caffeic acid, (+)-catechin, p-coumaric acid, (-)-epicatechin, myricetin, ferulic acid, and chlorogenic acid, were sourced from Tokyo Chemical Industry Co., Ltd (Kita-Ku, Tokyo, Japan). Analytical standards of other phenolics, including gallic acid, quercetin, rosmarinic acid, rutin, apigenin, and kaempferol, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent and ascorbic acid

were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was obtained from the Purelab Flex water purification system (Veolia Ltd., High Wycombe, UK).

2.3. Apparatuses

A rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland) was employed for desiccating extracts, while spectrophotometric analyses were conducted using a UV-1650pc UV-VIS spectrophotometer from Shimadzu Corporation (Nishinokyo Kuwabara-cho, Kyoto, Japan) and a BioTek ELX800 Absorbance Microplate Reader (Winooski, VT, USA). RP-HPLC analysis was performed using an Alliance chromatographic system from Waters Instruments, Inc. (Waters Acquity, Milford, CT, USA), and GC-MS analysis was carried out using the PerkinElmer Clarus GC-MS System from PerkinElmer, Inc. (Waltham, MA, USA). Deionized water was sourced from the Purelab Flex water purification system (Veolia Ltd., High Wycombe, UK) and sample weights were measured using a Mettler digital balance (Greifensee, Zurich, Switzerland).

2.4. Preparation of Extracts

The powdered plant materials from the three species were employed in producing two types of extracts: hydro-methanolic extract (HME) and hydro-acetonic extract (HAE). For the HME, 50 grams of desiccated plant samples were combined with 800 mL of methanol and 200 mL of distilled water. In the case of the HAE, 700 mL of acetone and 300 mL of distilled water were mixed with the powdered plant materials. Prior to filtration, the extracts underwent a maceration process lasting 72 hours. Subsequent to extraction, the samples were filtered using Whatman No. 1 filter paper (Whatman TM 1001-150, Merck KGaA, Darmstadt, Germany). The extracts were then desiccated utilizing a rotary evaporator operating at 45 rpm and 40 °C to yield dry HME and HAE extracts. To shield against light-induced effects, the desiccated extracts were stored in amber glass containers. These dry extracts were then directly utilized for phenolic content analysis and antioxidant capacity assessment. The extraction yield was determined using the formula below, with the extraction yield expressed as a percentage of the weight of the powdered plant material utilized:

Extraction yield (%) = (EQ) / (PQ)×100

Where EQ represent the weight of the extract and PQ represented the weight of the powdered plant.

2.5. Total Phenolic Content

The total phenolic content of HME and HAE was assessed using the Folin-Ciocalteu reagent, following a method outlined by Alqahtani et al. [19] with minor adjustments. To initiate the experiment, each plant sample (0.5 mL containing 1 mg of dry extract) was mixed with 125 µL of Folin-Ciocalteu reagent (1 N) and agitated for 5 minutes. Subsequently, 375 µL of 20% (w/v) anhydrous Na2CO3 solution was added. Following a 30-minute incubation period at room temperature, the absorbance at 765 nm was measured utilizing a UV-VIS spectrophotometer. A gallic acid standard curve (ranging from 50-500 µg/mL in ethanol) was utilized to determine the total phenolic contents, which were then expressed as milligrams of gallic acid equivalent per 10 grams of dry extract (mg GAE/10g DW).

2.6. Total Flavonoid Content

The determination of flavonoid content in the extracts (HME and HAE) from each plant species was conducted using the aluminum trichloride colorimetric assay. In this method, 1 mL of each extract was combined with 5 mL of distilled water, followed by the addition of 0.3 mL of 5% NaNO2. Subsequently, 0.3 mL of 10% AlCl3 was added, and the mixture was allowed to stand at room temperature for 5 minutes. After that, 2 mL of 1 M NaOH was introduced, and the total volume was adjusted to 10 mL with distilled water. The mixture was then left to incubate in a shaded area at ambient temperature for 30 minutes. Following the incubation, the absorbance was measured at a wavelength of 510 nm using a UV-visible spectrophotometer. The flavonoid content was quantified and expressed as milligrams of quercetin equivalents per 10 grams of dry extract (mg QE/10g DW) [20].

2.7. Determination of Polyphenolic Compounds Using RP-HPLC

To analyze individual phenolic compounds, an Alliance chromatographic system from Waters Instruments, Inc. was utilized, featuring a quaternary pump and dual wavelength absorbance detectors. Reverse phase analyses were carried out employing a Pinnacle™ II C18 column (4.6 × 250 mm, 5 µm particle size) with the column

temperature maintained at 24 °C. The mobile phase comprised two solutions: Solution A, consisting of 1% acetic acid in deionized water, and Solution B, a mixture of methanol and acetonitrile in a 75:25 ratio. A gradient flow rate of 0.8 ml/min was achieved by following the prescribed profile detailed in Table 1. The ultraviolet (UV) detection set at a wavelength of 280 nm, a parameter specifically optimized to align with the UV-absorbing characteristics of the polyphenolic compounds.

The HPLC investigation focused on 12 standard polyphenolic compounds: chlorogenic acid, (+)-catechin, caffeic acid, p-coumaric acid, (-)-epicatechin, ferulic acid, rutin, rosmarinic acid, myricetin, quercetin, apigenin, and kaempferol. To establish a calibration curve, a methanol solution containing a standard stock solution (500 µg/mL) was prepared for each standard compound, and calibration concentrations were derived from this stock solution. Prior to HPLC analysis, solutions of each extract (10 mg/mL) were prepared in methanol. To ensure purity, all solutions, including mixed standards and samples, underwent filtration through a 0.20 µm membrane filter from Millipore, with an injection volume of 20 µL for each sample. Data acquisition, peak integration, and calibrations were conducted utilizing Empower 3 software. Compounds were identified by comparing their retention times with those of authentic standards (21).

Table 1. RP-HPLC gradient conditions for polyphenolic compounds analysis.

Time (min)	Solution A (%)	Solution B (%)
0.00	90	10
10.00	65	35
40.00	44	56
51.00	10	90
61.00	10	90
67.00	90	10

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2.8. GC-MS Analysis of Phytochemical

Analysis of the volatilized phytochemicals present in the crude extracts (HME), de-rived from three different Solanum plant species, was conducted using gas chromatog-raphy-mass spectrometry (GC-MS). The experimental setup included a flow rate of 1 ml/min, using helium as the carrier gas. Injection volume was maintained at 1 µl with a split ratio of 20:1. The linear velocity was set at 38.032 cm/sec, and the pressure was regu-lated at 8.1322 psi. A specific temperature program, as detailed in previous literature [22], commenced at 40°C and remained constant for 2 minutes. Subsequently, the temperature gradually increased to 200°C, followed by a further escalation to 300°C. Notably, the rate of oven temperature increase varied among the samples: S. cordatum was subjected to a rate of 10°C/min, whereas the other two samples experienced a rate of 20°C/min. Conse-quently, the total run time for S. cordatum extended to 24 minutes, while it was 16 minutes for the remaining two samples. The identification of the components within the extracts involved comparing the obtained mass spectra with those present in the National Institute of Standards and Technology and WILEY spectral libraries. Furthermore, a comparison was made with compounds in the Adams Library [23] and the Wiley GC/MS Library [24] to aid in the identification process.

2.9. Antioxidant Activity Assay

2.9.1. Determination of DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of each specimen was assessed utilizing a spectrophotometric technique, following a methodology outlined by Chebbac et al. [25] with slight adjustments. Initially, a 0.2 mM solution of diphenyl 1-picrylhydrazyl (DPPH) was prepared in methanol. The baseline absorbance of the DPPH solution in methanol was registered at 515 nm employing a UV-visible spectrophotometer and was consistently maintained throughout the duration of the experiment. Varying concentrations of each botanical sample (100 µL) dissolved in methanol (ranging from 1000 to 20 µg/mL), alongside diverse concentrations of ascorbic

acid (utilized as a positive control), were combined with 100 µL of the DPPH solution for evaluation. Subsequent to a 30-minute incubation period in darkness, the alteration in absorbance at 515 nm was gauged utilizing the Absorbance Microplate Reader. The outcomes are presented as the percentage of inhibition, reflecting the anti-free radical efficacy of the samples, calculated using the provided equation

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

where Abc refers to the absorbance of the DPPH solution in methanol, while Abs represents the combined absorbance of both the DPPH solution and the samples.

2.9.2. ABTS Radical Scavenging Activity.

The assessment of plant extract's ABTS radical scavenging capability was carried out via a spectrophotometric approach, following the methodology outlined by Alamet al. [26]. initially, aqueous solutions of 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) at a concentration of 7 mM and potassium persulfate at 2.45 mM were mixed in equal amounts and incubated for 0.5 hours. The resultant mixture was then frozen for 24 hours and subsequently diluted with ethanol. Subsequently, various quantities of ABTS solution (50 µL) were blended with the plant samples and allowed to incubate in darkness for one hour. The decline in ABTS was quantified at a wavelength of 734 nm (λmax) using the Absorbance Microplate Reader, and the antioxidant potential of the plant extracts was determined utilizing the following equation [27]:

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

2.10. Statistical Analysis

All experiments were conducted in triplicate, unless otherwise specified. The data was presented as the mean ± standard deviation. Statistical comparisons were performed to evaluate the significance of differences between variables, with subsequent Tukey testing. Significance was determined based on a p-value threshold of less than 0.05.

3. Results

3.1. Yield of Extracts

The extraction efficiencies obtained from macerating the aerial parts of S. schimperianum, S. cordatum, and S. nigrum were quantified at 14.56%, 18.47%, and 22.86% for the HME, respectively. Correspondingly, the HAE yielded 21.84%, 15.71%, and 24.38% for each respective species. Variations in extraction yields were observed among species and solvents, influenced by distinct secondary metabolite profiles. The abundance of bioactive compounds and cell wall composition played a significant role. Species possessing robust cell walls potentially requiring harsher extraction conditions for optimal yields [28]. The choice of solvent strongly impacts extraction yields. Methanol and acetone, both polar solvents, differ in polarity, with methanol being more polar. Solvent polarity directly affects the types and amounts of compounds extracted [29, 30]. Furthermore, it is important to note that several factors can impact the extraction yield, such as the duration of maceration and particle size of the material, solvent combination employed, solvent volume to sample mass ratio, temperature of extraction, timing of harvest, drying process, and the specific plant part being used [31-33]. 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. Understanding how these factors interact is vital for refining extraction protocols. Further research could explore the impact of varying extraction conditions on extract yield.

3.2. Quantification of Total Phenolic and Total Flavonoid Content

The quantification of total phenolic content was conducted using the Folin-Ciocalteu reagent and analysis of various concentrations of gallic acid, resulting in the establishment of a regression equation of $y = 12.624x - 1.0824$ ($R2 = 0.9962$). Total flavonoid content was evaluated through the aluminum trichloride colorimetric assay with quercetin concentrations, yielding a regression equation of $y = 6.425x + 0.4125$ ($R2 = 0.9931$). Experimental data on the total phenolic and flavonoid contents of different Solanum species extracts (HME and HAE), are presented in Table 2. A graphical representation of the comparison is illustrated in Figure 1.

Across all three species, HME extracts consistently reigned supreme over their HAE counterparts in both TPC and TFC. This dominance was statistically significant ($p<0.001$), emphasizing the superiority of HME in extracting these valuable compounds. S. nigrum emerged as the phenolic champion, boasting an impressive 79.16 mg GAE/10g DW of total phenolic content, significantly exceeding both S. cordatum (49.35 mg GAE/10g DW) and S. schimperianum (25.07 mg GAE/10g DW). In the evaluation of total flavonoid content, S. nigrum showed the highest concentration at 57.01 mg QE/10g DW, followed by S. cordatum (28.35 mg QE/10g DW) and S. schimperianum (8.86 mg QE/10g DW). Beyond numerical data lies a deeper significance: these results unveil the remarkable abundance of phenolic and flavonoid compounds within the Solanum species native to Saudi Arabia.

Variations in polyphenols and flavonoids among the species emphasize the dynamic nature of these bioactive secondary metabolites in plants, influenced by factors such as plant species, plant part utilized, extraction technique, plant life cycle stage, and solvent selection [34, 35]. The vaeriation in biosynthetic pathways among species explain the observed differences in total flavonoid content. These differences stem from variations in enzymes and metabolic pathways, resulting in diverse flavonoid production across plant species [36].

The results highlight the crucial role of extraction solvents. The clear superiority of HME over HAE emphasizes the importance of choosing the right solvent to maximize the yield of these valuable compounds and unlock the full potential of these plants. An effective solvent should optimize extraction efficiency while preserving the chemical stability of the target compounds [37]. Polyphenols, with varying polarities, are best extracted using polar solvents that facilitate efficient solvation through interactions like hydrogen bonding. Hydro-methanol, a polar solvent, is particularly effective in extracting phenolic compounds and flavonoids due to its superior solvation capacity for polar molecules compared to less polar solvents like hydro-aceton [38]. This may explain the higher total phenolic and flavonoid content in hydro-methanol extracts compared to hydro-aceton, as observed in our study. However, other studies have reported higher polyphenolic compound recovery using hydro-acetonic solvent. Hence, our study aims to compare total phenolic outcomes using both hydro-methanol and hydro-acetonic solvents, considering various factors such as secondary metabolite profiles rather than solely focusing on solvent polarity [39].

Table 2. Total phenolic (TPC) and flavonoid (TFC) contents of hydro-methanolic extract (HME) and hydro-acetonic extract (HAE) of S. schimperianum, S. cordatum, and S. nigrum.

Species	TPC (mg GAE/ 10g DW)		TFC (mg QE/ 10g DW)	
	HME	**HAE**	**HME**	**HAE**
S. schimperianum	25.07 ± 0.52	16.91 ± 0.74	8.86 ± 0.94	9.92 ± 1.83
S. cordatum	49.35 ± 2.26	39.72 ± 0.91	28.35 ± 0.86	11.64 ± 0.37
S. nigrum	79.16 ± 0.61	54.25 ± 1.32	57.01 ± 0.43	46.23 ± 1.52

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

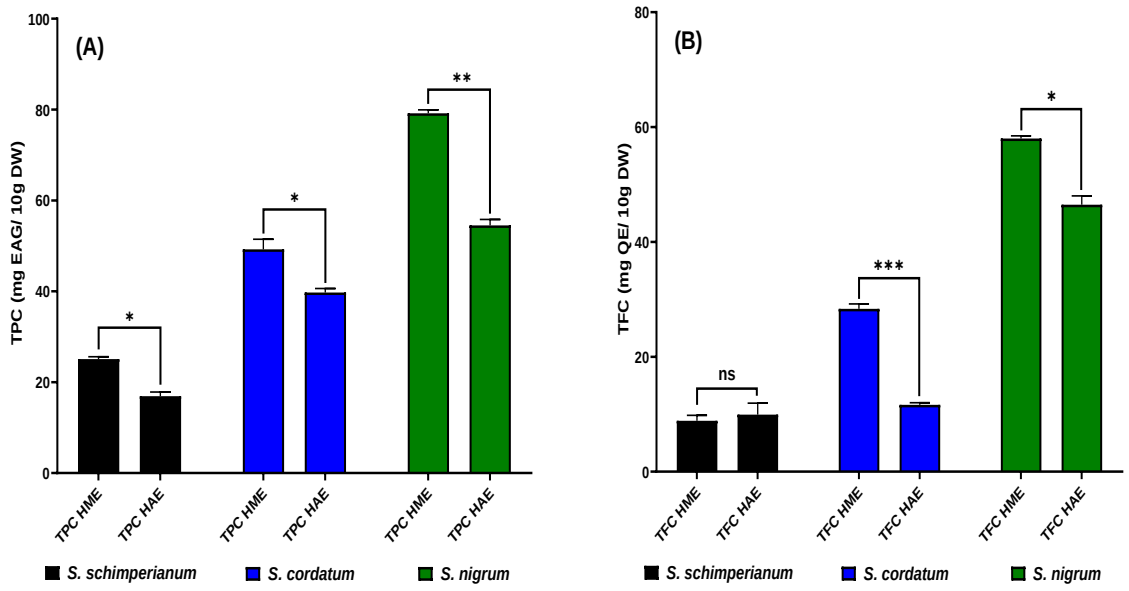


Figure 1. Comparative data of (A) the total Phenolic (TPC) and (B) total flavonoid (TFC) contents obtained from hydro-methanolic (HME) and hydro-acetonic (HAE) extracts of S. schimperianum, S. cordatum, and S. nigrum. Results are expressed as mean ± SD of three parallel measurements (n= 3). The presence of asterisks signifies the application of statistical tests for multiple reciprocal comparisons among the extracts, with significance levels indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; not significant (ns).

3.3. Identification and Quantification of polyphenolic compound using HPLC Analysis

To effectively characterize and quantify the polyphenolic constituents present in the extracts derived from the three solanum species, the utilization of a reversed-phase high-performance liquid chromatography (RP-HPLC) system was deemed appropriate. This selection was based on the diverse polarities exhibited by the individual compounds under investigation. The optimized analytical method effectively separated and identified twelve phenolic compounds, as illustrated in Figure 2. Detection and quantification of these compounds, including chlorogenic acid, (+)-catechin, caffeic acid, p-coumaric acid, (-)-epicatechin, ferulic acid, rutin, rosmarinic acid, myricetin, quercetin, apigenin, and kaempferol, were accomplished through the establishment of external calibration curves. These curves correlated peak areas with prepared concentrations ranging from 0.2 to 100 µg/mL. Importantly, all correlation coefficients from these curves exceeded the threshold of 0.9981.The elution sequence of the compounds from the chromatographic column was dictated by the hydrophobic properties of the stationary phase material and the escalating gradient of methanol and acetonitrile (solvent B) within the mobile phase. The chromatogram depicted in Figure 2, along with the accompanying data table in Table 3, provides a comprehensive insight into the elution sequence of the identified compounds and the polyphenolic profiles derived from the analyzed Solanum species extracts (HME and HAE).

Analysis revealed chlorogenic acid as the most abundant compound in all three species, with S. nigrum exhibiting the highest concentration (63.20 mg/10 g), followed by S. cordatum (38.29 mg/10 g) and S. schimperianum (14.93 mg/10 g). Rutin was the second most prevalent compound in S. nigrum (36.56 mg/10 g) and S. cordatum (14.55 mg/10 g). Myricetin and quercetin were found in all species, with higher levels in S. nigrum. Apigenin was exclusive to S. nigrum (7.03 mg/10 g) and minimally present in S. cordatum (1.98 mg/10 g). Kaempferol was detected in S. nigrum (3.01 mg/10 g) and S. schimperianum (0.35 mg/10 g). Caffeic acid was absent in S. cordatum but present in S. nigrum and S. schimperianum. Rosmarinic acid was found in all species, with higher levels in S. cordatum. Additionally, compounds such as (+)-catechin, p-coumaric acid, (-)-epicatechin, and ferulic acid exhibited varying concentrations among the three species.

Notably, the extraction solvent significantly influenced the phenolic profiles, with HME consistently yielding higher concentrations of chlorogenic acid, rutin, and quercetin compared to HAE counterparts. This observation suggests potential disparities in extractability based on solvent polarity. Beyond the individual constituents, the findings unveiled distinct trends in the overall phenolic content, with species-specific variations noted. S. nigrum exhibited the highest total phenolic content, followed by S. cordatum and S. schimperianum, respectively.

These results provide valuable insights into the phenolic landscape within the Solanum species under scrutiny, underscoring their promise as reservoirs of bioactive compounds. Further exploration into the functional implications of these variations and the impact of environmental factors on their biosynthesis could offer invaluable contributions to future research endeavors and practical applications.

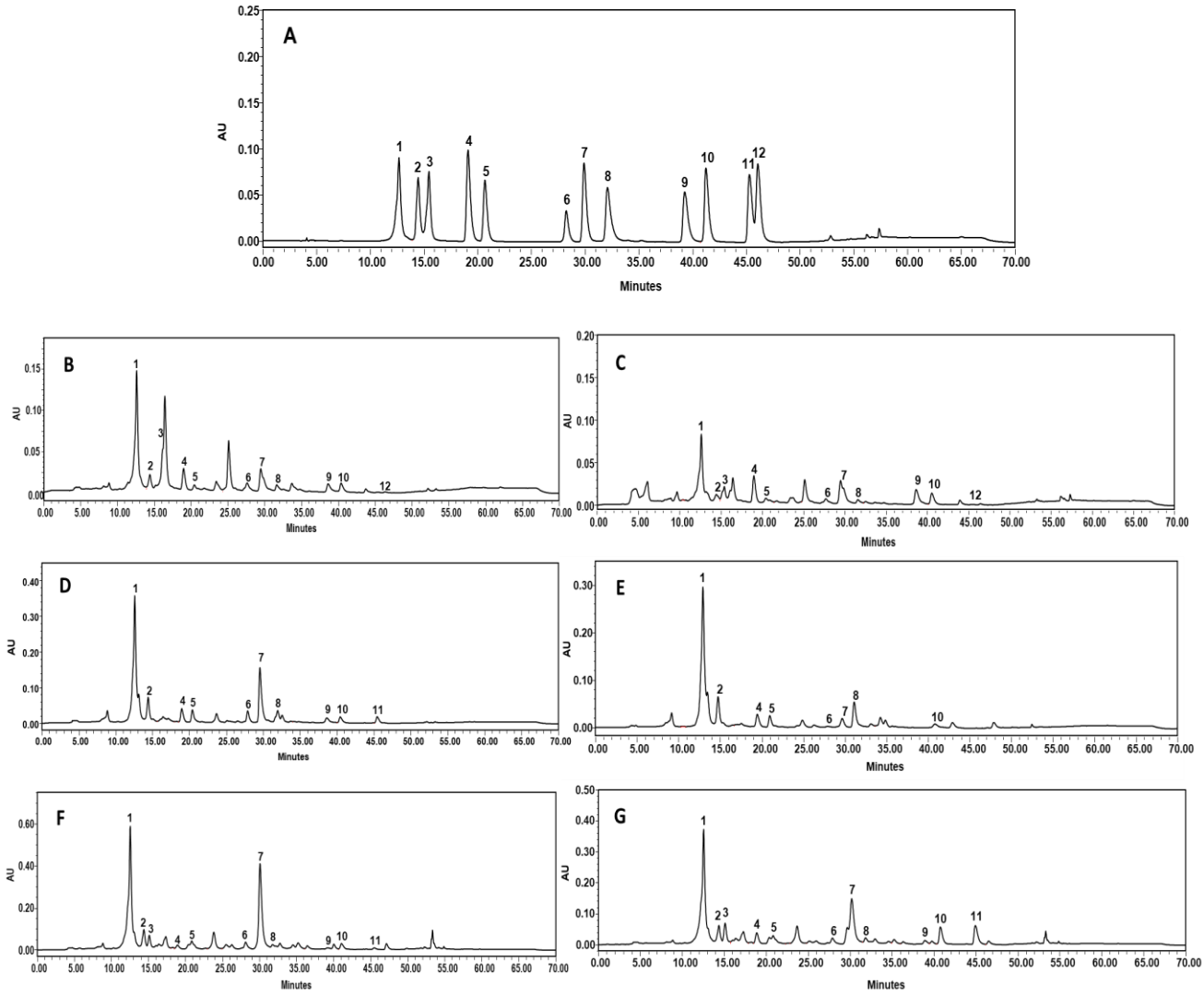


Figure 2. RP-HPLC chromatograms displaying the polyphenolic compound profiles: (A) standard mixture comprising twelve polyphenolic compounds; polyphenolic compound profiles of (B) hydro-methanolic extract (HME) and (C) hydro-acetonic extract (HAE) of S. schimperianum, (D) HME and (E) HAE of S. cordatum, and (F) HME and (G) HAE of S. nigrum. Peak identification numbers are given in Table 3.

Table 3. Detection and quantification data for polyphenolic compounds in *S. schimperianum* (SS), *S. cordatum* (SC), and *S. nigrum* (SN) extracts using RP-HPLC analysis, with values expressed in mg per 10 g of dry weight.

N◦ Peak	Compound	Rt (min)	HME			HAE		
			SS	SC	SN	SS	SC	SN
1	Chlorogenic acid	12.67	14.93 ± 0.20	38.29 ± 0.59	63.20 ± 0.49	9.24 ± 0.62	32.07 ± 0.22	42.08 ± 48
2	(+)- Catechin	14.50	2.05 ± 0.24	5.76 ± 0.63	9.52 ± 0.51	1.86 ± 0.74	5.18 ± 0.49	5.62 ± 0.57
3	Caffeic acid	15.46	4.47 ± 0.17	ND	6.81 ± 0.32	2.58 ± 0.53	ND	4.98 ± 0.30
4	p-coumaric acid	19.11	2.68 ± 0.54	2.87 ± 37	2.06 ± 0.16	3.50 ± 0.19	1.62 ± 0.16	3.68 ± 0.54
5	(-)-Epicatechin	20.68	0.71 ± 0.57	1.91 ± 0.14	3.46 ± 0.18	1.07 ± 0.11	1.91 ± 0.92	2.70 ± 0.27
6	Ferulic acid	28.24	1.19 ± 0.10	3.45 ± 76	3.03 ± 0.65	0.62 ± 0.74	0.59 ± 0.57	1.19 ± 0.44
7	Rutin	30.01	2.87 ± 0.89	14.55 ± 0.45	36.56 ± 0.71	2.99 ± 0.81	1.34 ± 0.18	15.47 ± 0.48
8	Rosmarinic acid	32.08	1.03 ± 0.38	3.11 ± 0.12	1.62 ± 0.52	0.47 ± 0.63	4.21 ± 0.25	0.90 ± 0.39
9	Myricetin	39.27	1.33 ± 0.14	1.45 ± 0.48	2.06 ± 0.24	1.85 ± 0.32	ND	0.85 ± 0.17
10	Quercetin	41.24	1.27 ± 0.32	1.83 ± 0.22	2.60 ± 0.37	1.68 ± 0.58	0.92 ± 0.65	6.13 ± 1.01
11	Apigenin	45.29	ND	1.98 ± 0.09	0.96 ± 0.21	ND	ND	7.03 ± 0.83
12	Kaempferol	46.06	0.35 ± 0.35	ND	ND	0.23 ± 0.18	ND	ND

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

3.4. Phytochemical Analysis by GC/MS

The investigation was conducted to elucidate the chemical composition of three Solanum species through a comprehensive GC-MS comparative analysis. The decision to utilize the HME extract was made based on its established effectiveness in extracting phytochemical constituents. Chemical profiling of the extracts was accomplished using the HP Innowax column, with compound identification relying on various parameters including retention time, molecular formula, molecular weight, and relative peak area percentages. These percentages served as a quantitative measure of the concentration of each compound, with the outcomes detailed in Table 4 outlining the primary compounds present in each species. The chromatograms in Figure 3 provide a detailed insight into the elution sequence of the identified compounds.

The GC-MS evaluation uncovered a distinct chemosphere that characterized the investigated Solanum species. The investigation highlighted significant interspecific variation, manifested in differences in both the number and relative abundance of identified metabolites. S. schimperianum exhibited the highest chemodiversity, containing 13 unique compounds, while S. nigrum had the greatest number with a total of 19 identified metabolites. Notably, the most abundant compound varied significantly across the species. S. schimperianum showed the highest proportion of palmitic acid (30.52%), followed by α-tocopherol acetate (15.02%) and stigmasterol acetate (8.11%).

In contrast, S. cordatum exhibited elevated levels of palmitic acid (16.55%), and trimethylgallic acid methyl ester (13.44%), in addition to significant amounts of phenolic compounds like phenol, 4,4'-isopropylidenedi- which may contribute to its antioxidant properties. On the other hand, S. nigrum showcased a notable presence of palmitic acid (24.11%), along with substantial quantities of 3,9-dodecadiyne (11.37%). An important observation was the consistent presence of palmitic acid, a saturated fatty acid, in all three Solanum species studied, occurring in notable amounts.

The analysis using GC-MS of the chemical composition of these species has revealed a diverse range of bioactive compounds that suggest potential therapeutic properties. Variations in metabolites among the different species underscore the unique complexity of each plant and present opportunities for further investigation. These findings validate the

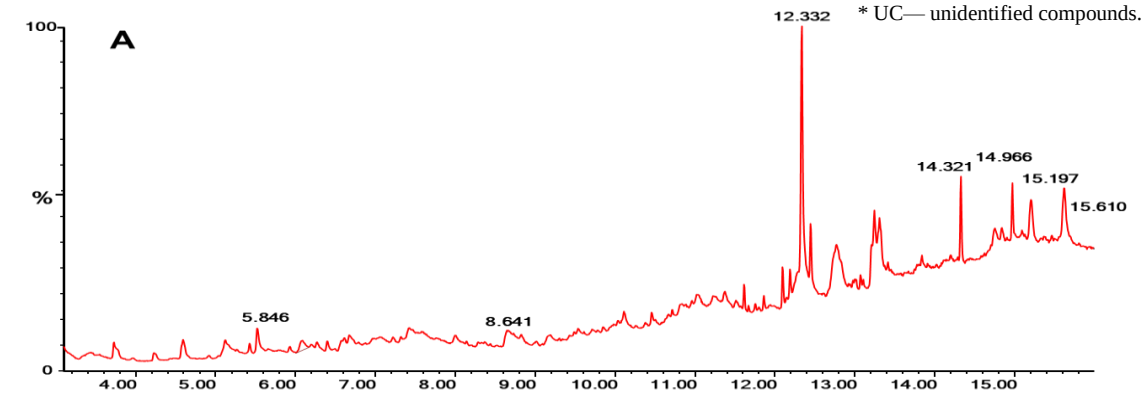
traditional medicinal uses of these plants and offer exciting prospects for targeted research to identify the specific compounds responsible for their therapeutic effects. This sets the stage for a deeper exploration and application of the therapeutic potential of these plants. Further investigation into the potential synergistic effects of the identified compounds and bioassays to assess specific bioactive properties could enhance our understanding of the therapeutic potential of the Solanum species. Correlating the chemical composition with biological activities could lead to the development of novel natural products with enhanced efficacy.

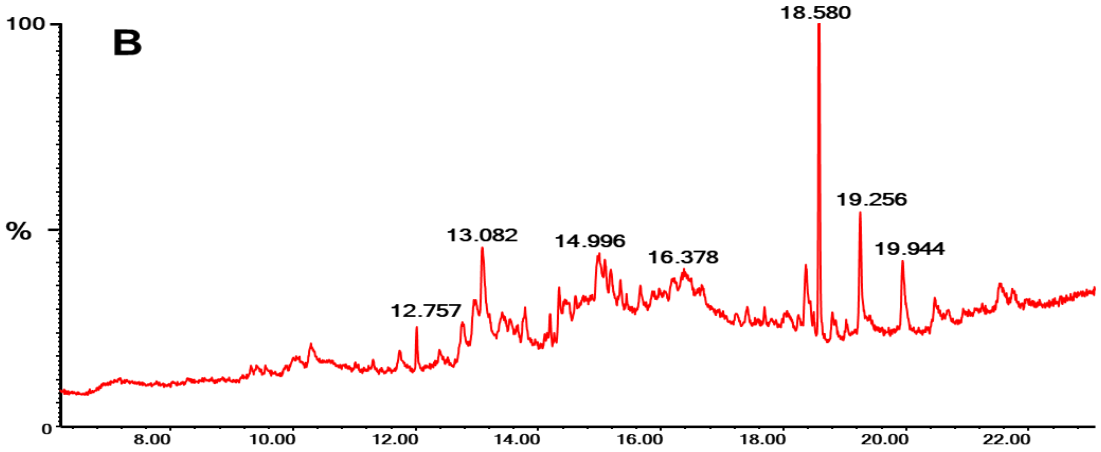
Table 4. GC-MS analysis of phytoconstituents identified in hydro-methanolic extracts (HME) from the three Solanum species.

Solanum Species	Name of Compound	Chemical Formula	Molecular Weight (g/mol)	RT (min)	% Area
S. schimperianum	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143.058	5.514	4.378
	4-Methylproline methyl ester	$C_7H_{13}NO_2$	143.095	8.641	5.846
	1-Pyrrolidinebutyronitrile	$C_8H_{14}N_2$	138.116	9.186	2.931
	2-Octen-1-ol, 7-ethoxy-3,7-dimethyl-, (E)-	$C_{12}H_{24}O_2$	200.178	11.219	2.549
	2-Propenal, 3-(dimethylamino)-3-(1-piperidinyl)-	$C_{10}H_{18}N_2O$	182.142	11.369	2.515
	Methyl isohexadecanoate	$C_{17}H_{34}O_2$	270.256	12.088	3.223
	Palmitic acid	$C_{16}H_{32}O_2$	256.240	12.332	**30.516**
	Ethyl palmitate	$C_{18}H_{36}O_2$	284.477	12.438	4.773
	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264.245	13.239	3.247
	Stearic acid	$C_{18}H_{36}O_2$	284.272	13.302	4.935
	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.308	14.321	6.893
	Adamantane, 1-isothiocyanato-3-methyl-	$C_{12}H_{17}NS$	207.108	14.966	5.096
	Stigmasterol acetate	$C_{31}H_{50}O_2$	454.381	15.197	**8.072**
	Alpha-Tocopherol acetate (Vitamin E acetate)	$C_{31}H_{52}O_3$	472.392	15.61	**15.024**
S. cordatum	Phenol, 4,4'-isopropylidenedi-	$C_{15}H_{16}O_2$	228.286	13.082	**12.786**
	n-Capric acid	$C_{10}H_{20}O_2$	172.264	16.378	7.533
	* UC	$C_{10}H_9N_3O_2S$	235.260	18.368	10.146
	* UC	C21H15N	281.350	18.58	27.958
	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.256	18.799	5.758
	Trimethylgallic acid methyl ester	$C_{11}H_{14}O_5$	226.225	19.256	**13.437**
	Palmitic acid	$C_{16}H_{32}O_2$	256.240	19.944	**16.545**
	Oleic acid chloride	$C_{18}H_{33}ClO$	300.910	20.469	5.837
S. nigrum	* UC	$C_6H_8O_4$	144.042	6.540	10.577
	5-Hydroxymethyl-2-furfural	$C_6H_6O_3$	126.032	7.415	4.272
	p-Vinyl guaicol	$C_9H_{10}O_2$	150.068	7.922	2.486
	Pyrogallol 1,3-dimethyl ether	$C_8H_{10}O_3$	154.063	8.266	2.608
	Naphthalene, 1,2-dihydro-2,5,8-trimethyl-	$C_{13}H_{16}$	172.125	8.723	3.494
	Dehydro-β-ionone	$C_{13}H_{18}O$	190.136	9.336	4.334

2-Acetyl-1,4,5,6-tetrahydropyridine	$C_7H_{11}NO$	125.084	9.599	3.259
* UC	$C_9H_{11}N_3$	161.095	9.830	2.238
3,9-Dodecadiyne	$C_{12}H_{18}$	162.141	10.456	**11.368**
cis-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{24}O$	220.183	11.525	2.978
7-Methyl-1,6-octadiene	C_9H_{16}	124.125	11.607	2.885
Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268.277	11.663	3.081
Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.256	12.088	5.483
Palmitic acid	$C_{16}H_{32}O_2$	256.240	12.363	**24.108**
Stearic acid	$C_{18}H_{36}O_2$	284.272	13.308	2.270
Phthalic acid, mono-(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.152	14.966	5.543

* UC— unidentified compounds.





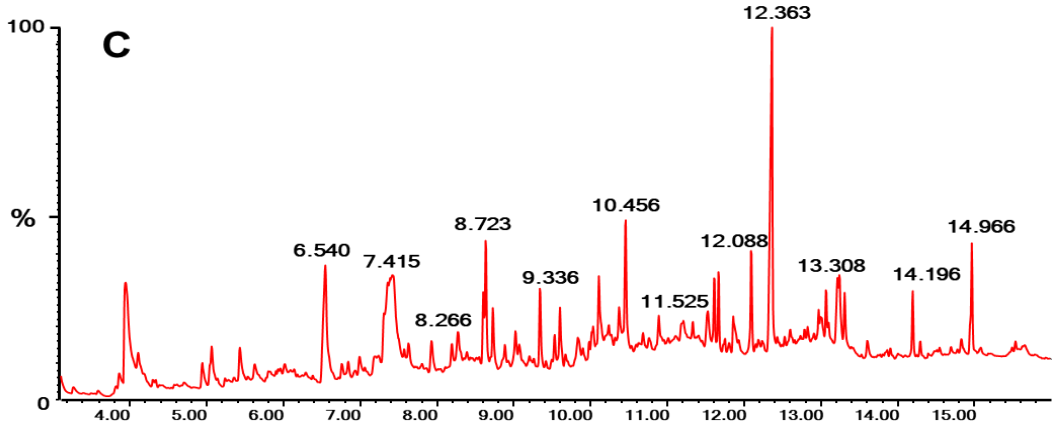


Figure 3. GC-MS chromatograms of hydro-methanolic extracts (HME) from (A) S. schimperianum, (B) S. cordatum, and (C) S. nigrum

3.5. DPPH and ABTS Radical Scavenging Effects

The potential of plant extracts in scavenging free radicals has been investigated through various methodologies [40, 41]. In this investigation, the antioxidant efficacy of HME and HAE derived from three distinct solanum species was evaluated using the DPPH and ABTS radical scavenging assays.

The DPPH assay evaluates the capability of antioxidants present in the samples to reduce the DPPH radical by electron transfer processes, with the absorption at 517 nm being used as a measurement. The ABTS assay measures the ability of antioxidants in the samples to reduce the ABTS radical through electron and/or hydrogen atom transfer, and the measurement at 734 nm was utilized for assessing the absorption. A decreased value for the 50% effective concentration (IC50) generally indicates a more potent radical scavenger [42]. The outcomes derived from these methodologies, as depicted in Figures 4 and 5, clearly demonstrate that the antioxidant potential of the unrefined extracts is contingent on the dosage. Additionally, the findings summarized in Table 5 offer valuable insights into the order of radical scavenging potency among the different extracts.

The evaluation of anti-radical activity using DPPH and ABTS assays revealed a clear trend across the three Solanum species studied. Specifically, S. nigrum demonstrated the most potent anti-radical activity for both extracts (HME and HAE), as evidenced by the lowest IC50 values of 47.53 µg/mL and 36.78 µg/mL for DPPH and ABTS assays, respectively. In comparison, S. cordatum exhibited moderately lower anti-radical activity than S. nigrum, with IC50 values of 80.54 µg/mL and 61.57 µg/mL for DPPH and ABTS assays, respectively. S. schimperianum displayed the weakest anti-radical activity among the three species, with IC50 values of 102.5 µg/mL and 88.13 µg/mL for DPPH and ABTS assays, respectively. These findings are consistent with the higher phenolic content previously reported in S. nigrum in comparison to S. cordatum and S. schimperianum. The fruit extracts have been categorized into three distinct groups based on their DPPH/ IC50 values, delineating those with notable antioxidant efficacy (DPPH/ IC50 $\leq$ 100 µg/mL), those with moderate antioxidant properties (100 µg/mL < DPPH/ IC50 $\leq$ 316 µg/mL), and those with limited antioxidant efficacy (DPPH/ IC50 > 316 µg/mL) [43]. 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 IC50 values of 17.20 ± 0.26 µg/mL.

The observed association between anti-radical activity and phenolic content is consistent with established scientific knowledge. Phenolic compounds, particularly flavonoids and phenolic acids, are well-recognized for their potent antioxidant properties. These compounds possess structural features that enable them to scavenge free radicals, thereby preventing oxidative damage in cells. This relationship has been previously established and

reported by other researchers utilizing similar testing techniques [44-49]. Therefore, the superior anti-radical activity of S. nigrum extracts can be attributed, in part, to its higher abundance of phenolic compounds. This finding further highlights the potential of S. nigrum as a natural source of antioxidants with potential applications in various health-related fields. However, it is important to acknowledge that other factors besides total phenolic content can also influence anti-radical activity. The specific types and synergistic interactions of various phenolic compounds present in the extracts may also play a significant role.

Table 5. IC50 values (µg/mL) of anti-radical activity of hydro-methanolic extract (HME) and hy-dro-acetonic extract (HAE) of S. schimperianum, S. cordatum, and S. nigrum using the DPPH and ABTS methods.

Crude Extract	Scavenging Assay	Species		
		S. schimperianum	*S. cordatum*	*S. nigrum*
HME	DPPH	102.5 ± 1.02	80.54 ± 0.82	47.53 ± 1.18
	ABTS	88.13 ± 0.62	61.57 ± 0.43	36.78 ± 1.40
HAE	DPPH	107.1 ± 0.76	95.06 ± 0.91	75.99 ± 0.96
	ABTS	96.51 ± 0.58	82.76 ± 0.69	68.33 ± 0.91

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

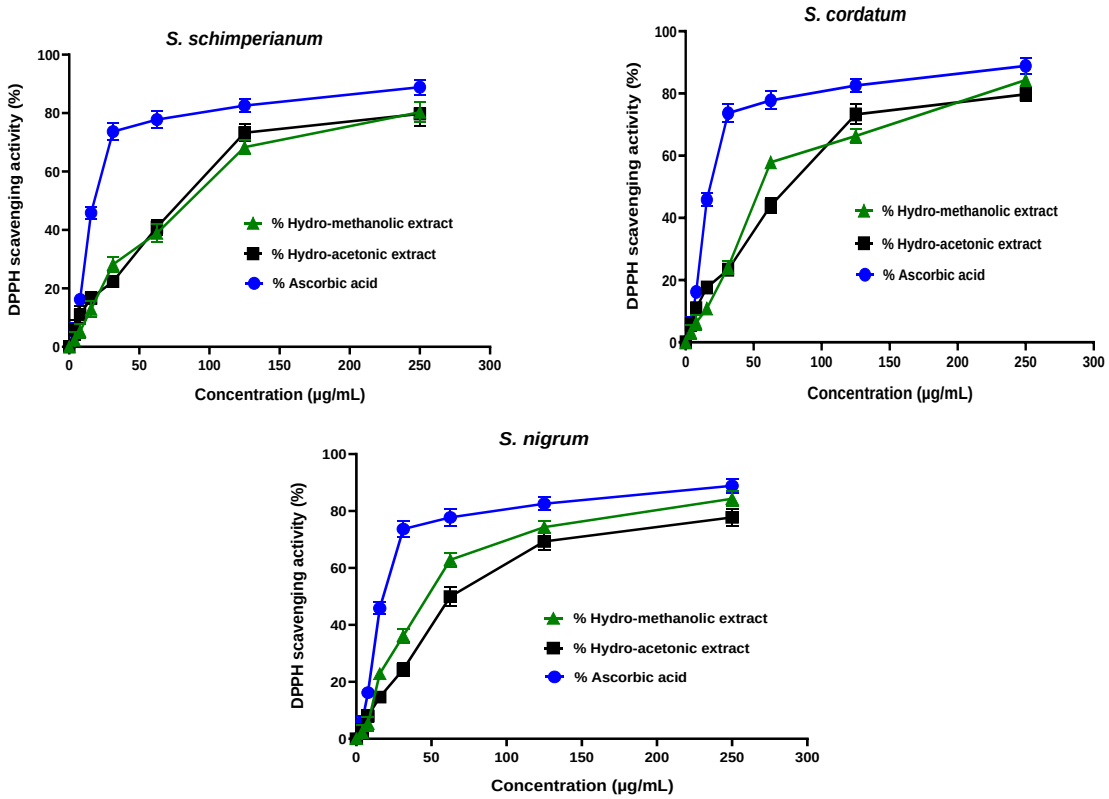


Figure 4. Anti-radical activity of the crude extracts from S. schimperianum, S. cordatum, and S. nigrum and standard ascor-bic acid using the DPPH method.

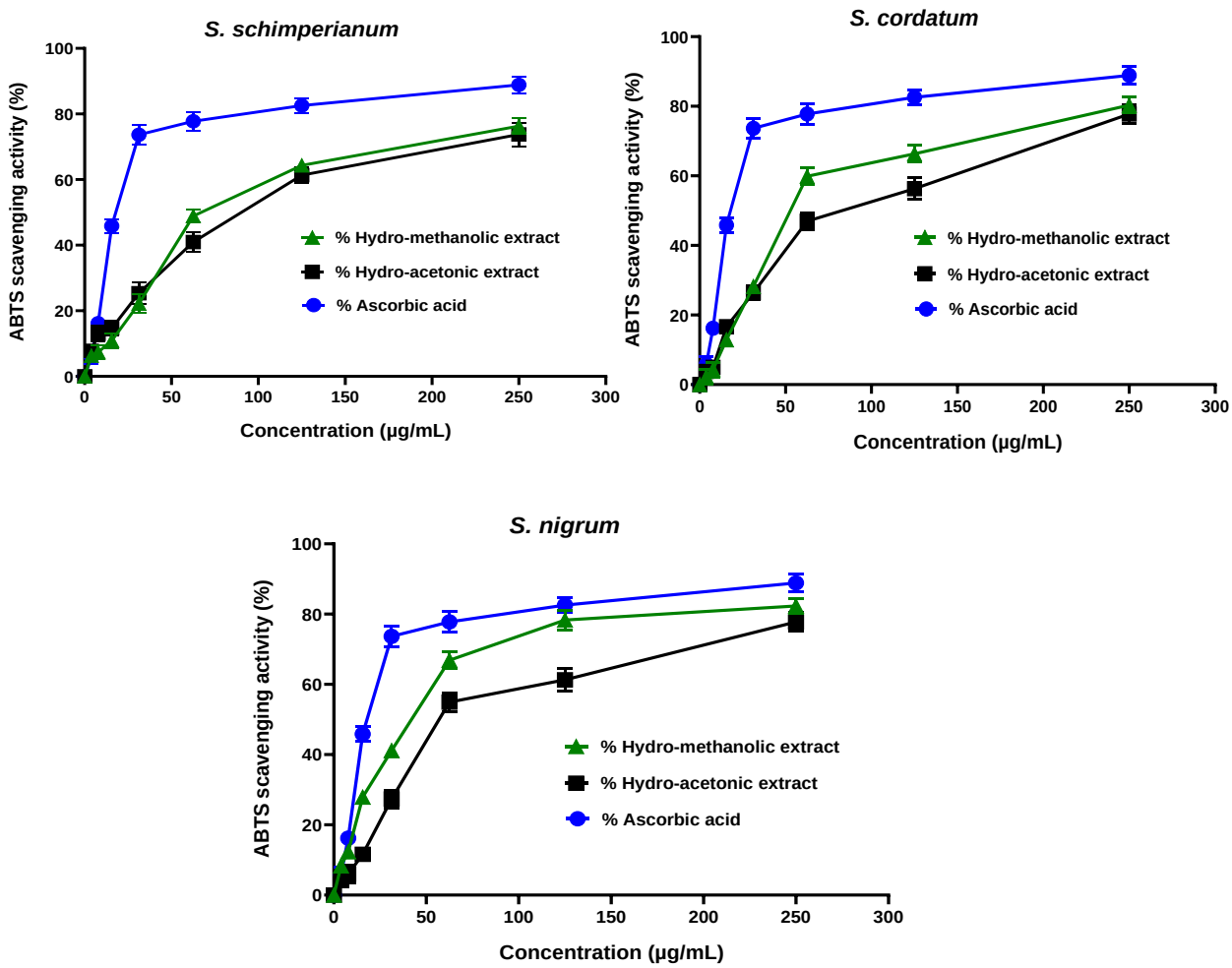


Figure 5. Anti-radical activity of the crude extracts from S. schimperianum, S. cordatum, and S. nigrum and standard ascorbic acid using the ABTS method.

3.6. Correlation between Phenolic Content and Antioxidant Activity

The presence of specific components, notably phenolic compounds, in plant specimens is responsible for the observed antioxidant activity. In this study, we utilized the Pearson correlation coefficient (PCC), also known as Pearson's r, to examine the relationship between total phenolic content (TPC) and total flavonoid content (TFC) with antioxidant radical activity. Figures 6 illustrate the scatter plots showing the PCC association between TPC and TFC with antioxidant radical activity. The correlation analysis carried out in this research revealed a positive correlation between antioxidant activity and the total phenolic compounds content ($r = 0.0.9229–0.0.9345$, $p \leq 0.05$) as well as flavonoids ($r = 0.8503–0.8983$, $p \leq 0.05$).

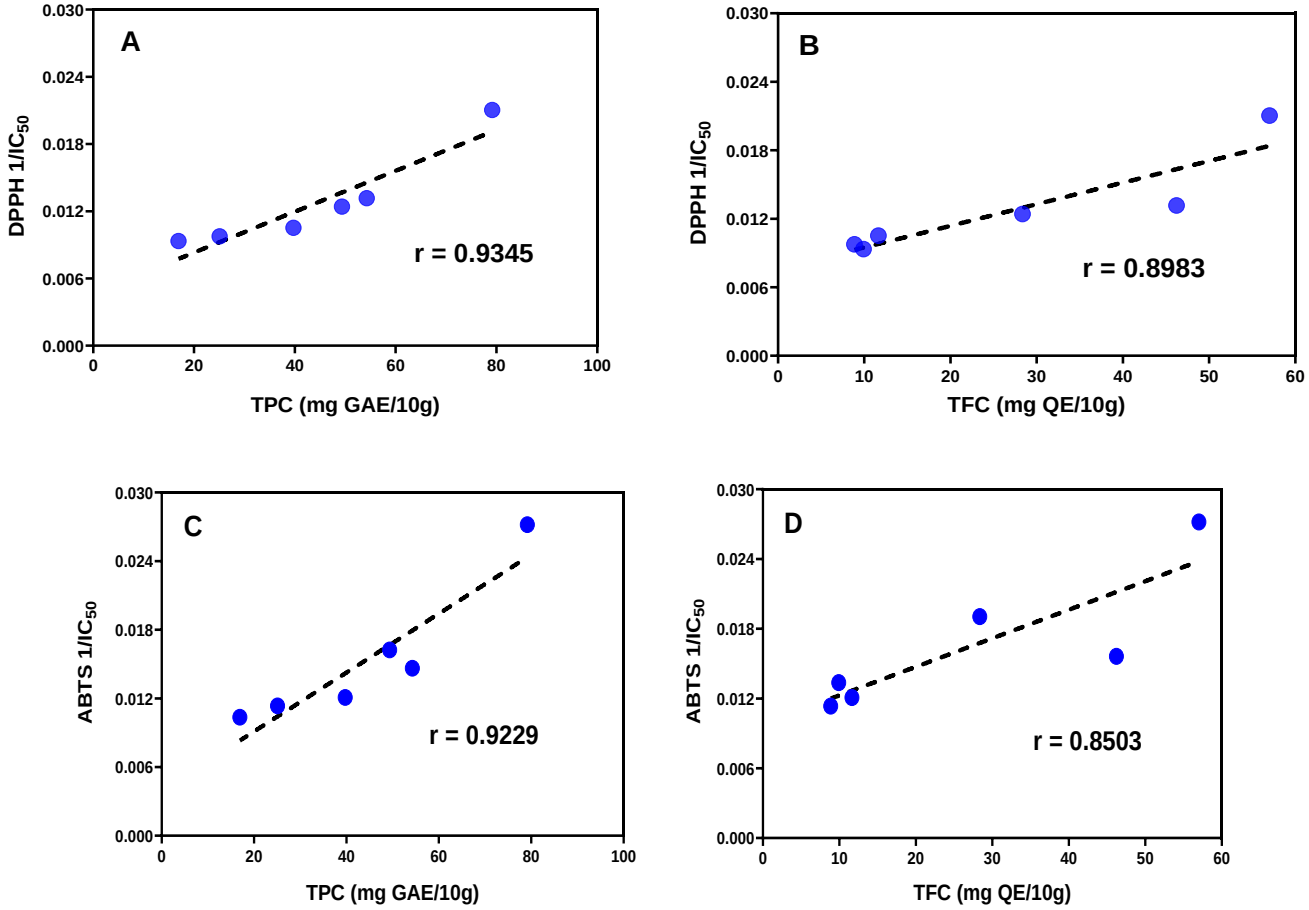


Figure 6. Pearson correlation scatter plot of relationship between (A) total phenolic content (TPC) and DPPH free radical scavenging activity, (B) total flavonoid content (TFC) and DPPH free radical scavenging activity, (C) TPC and ABTS free radical scavenging activity, and (D) TFC and ABTS free radical scavenging activity.

4. Discussion

Plants are crucial sources of diverse bioactive compounds, supporting pharmaceuti-cal development [50]. Extensive research is needed to fully understand medicinal plant phytochemicals and their biological functions [51]. The Solanum genus, with a long history of therapeutic use, stands out in this context [52]. Polyphenols, common in plants, have distinctive properties such as multiple phenolic hydroxy groups and strong interactions with proteins and other molecules [53]. They are widely studied for their diverse benefits, including potent antioxidant, potential anticancer, and notable anti-inflammatory effects [54].

This study explores the phytochemical and antioxidant potential of three species (S. schimperianum, S. cordatum, and S. nigrum), filling a gap in existing literature. Two solvents, hydro-methanolic (HME) and hydro-acetonic (HAE), were used to assess total phenolic and flavonoid content, identify polyphenolic compounds via RP-HPLC, and analyze vo-latilized phytochemicals through GC-MS. The research aimed to gain insights into the potential therapeutic benefits of these botanicals and validate their traditional medicinal uses by examining the disparities in their profiles and activities. This strategic selection of solvents enabled the extraction of a wide range of poly phenolic compounds, facilitating a thorough evaluation and comparison of their composition [39]. Interestingly, our results indicate that the hydro-methanolic extraction method exhibited a remarkably high con-centration of phenolic and flavonoid compounds across the three species investigated. This result is consistent with previous research, particularly the study conducted by Ru-pasinghe et al. [55]. In their study, they demonstrated that solvents with greater polarity, such as hydro-alcoholic mixtures, effectively extracted flavonoid glycosides and higher molecular-weight phenols, leading to higher quantities compared to an equivalent ace-tonic system.

Previous studies assessing the polyphenolic and flavonoid content in S. schimperia-num and S. cordatum have been limited. This study addresses the lack of research in this area for these species. Our results demonstrate notable levels of total polyphenols and flavonoids in both species, with S. cordatum showing higher amounts. These results not only expand our understanding of the phytochemical composition of these plants but also open up opportunities for future research into their potential medicinal and pharmaceu-tical applications. The results of the S. nigrum extract obtained using an 80% (v/v) metha-nol solvent (HME) exhibited the highest total polyphenol and flavonoid content. The total polyphenol content was measured at 79.16 GAE/10g DW, while the total flavonoid con-tent was 57.01 mg GAE/10g DW. These values exceeded those reported in a previous study for seed extracts prepared using methanol (56) and leaf extracts prepared using 50% methanol (57).

Further exploration led to the quantitative assessment of twelve polyphenolic com-pounds in the extracts of the three Solanum species utilizing the RP-HPLC technique. These standards were selected based on their documented occurrence in several investi-gated species, exemplified by S. nigrum and comparable plant species [17, 58, 59], and their direct alignment with our research objectives. The chromatographic profiles depicted clear and distinct peaks corresponding to the 12 polyphenolic compounds, with these peaks appearing at specific retention times in minutes as shown in Table 3, meticulously matched with their respective standards, conclusively confirming the presence of these compounds in the species studied. Notably, chlorogenic acid has consistently been recog-nized as the predominant soluble phenolic compound in a majority of Solanaceous species [3, 60], a trend also observed in this study. Our results validate the prevalence of chloro-genic acid as the primary phenolic compound, with S. nigrum exhibiting a higher concen-tration ranging from 42.08 to 63.20 mg/10 g DW. These values were further compared to a study by Staveckien et al. [17], where chlorogenic acid levels in S. nigrum fruit at varying ripening stages ranged from 361.88 to 539.85 mg/100 g DW.
Furthermore, a novel finding revealed that S. schimperianum and S. cordatum contain high levels of chlorogenic acid, with concentrations of 14.93 and 38.29 mg/10 g DW, re-spectively. The high levels of chlorogenic acid found in the plants align with previous re-search highlighting its antioxidant and anti-inflammatory properties [61], suggesting promising health benefits. This finding underscores the importance of further exploring the therapeutic potential of chlorogenic acid in these plants and its implications for hu-man health.

Rutin, a natural flavone derivative, has been identified as the second most abundant polyphenolic compound in S. cordatum and S. nigrum, with concentrations of 14.55 and 36.56 mg/10 g DW, respectively. Rutin is well-known for its potent antioxidant properties, acknowledged for its ability to reinforce the integrity of erythrocyte membranes [62]. In addition to its antioxidant capabilities, rutin demonstrates a diverse array of beneficial ef-fects, encompassing anti-neoplastic, antibacterial, and antiviral activities [63].
Notable levels of various phenolic acids, such as caffeic acid, p-coumaric acid, ferulic acid, and rosmarinic acid, were observed, with concentrations varying depending on the species and the extracting solvent employed. These compounds play a critical role in in-hibiting the production of reactive oxygen species associated with various diseases [64].

Significant levels of important flavonoids, including myricetin, quercetin, (+)-catechin, and (-)-epicatechin, were found in all three species. In contrast, apigenin and kaempferol were present in relatively low amounts, particularly in S. schimperianum and S. cordatum. Flavonoid compounds exhibit diverse functional properties, acting as antioxi-dants, hydrogen donors, transition metal chelators, and scavengers of reactive oxygen and nitrogen species, while also inhibiting enzymes linked to oxidative stress and regulating the body's natural defense systems. Furthermore, they have been shown to enhance the immune system and help prevent physical disorders associated with cancer, bacteria, and viruses [65]. The presence of a variety of phenolic compounds in the examined Solanum species underscores their potential to offer protection against a range of diseases and sig-nificantly enhance overall health and well-being.

The application of gas chromatography-mass spectrometry (GC-MS) plays a crucial role in the investigation of unidentified phytochemicals present in plants. Given the com-plex composition of plant materials, GC-MS emerges as a highly effective method for their analysis owing to its enhanced sensitivity and specificity. Through the ionization and mass number quantification of compounds, GC-MS aids in the profiling and characteri-zation of these components by providing supplementary and valuable data [66]. The GC-MS examination conducted (as depicted in Figure 6, Table 5) identified a range of compounds with diverse chemical properties. Interestingly, palmitic acid is present in all three species as a predominant component, with the highest levels in S. schimperiatum, followed by S. nigrum, and then S. cordatum. Palmitic acid has been recognized for its bene-ficial properties such as antibacterial, antioxidant, and antifungal effects in previous

scientific studies [67, 68]. S. schimperianum showed high levels of α-tocopherol acetate (15.02%) and stigmasterol acetate (8.11%). Stigmasterol acetate, a sterol known for its health benefits and found in various plants, along with alpha-tocopherol acetate, a form of vitamin E, were identified. Contrastingly, S. cordatum showed elevated levels of trimethyl-gallic acid methyl ester (13.44%), along with substantial quantities of phenolic com-pounds such as phenol, 4,4'-isopropylidenedi- (12.786%), which could enhance its anti-oxidant properties. S. nigrum exhibited significant levels of 3,9-dodecadiyne along with palmitic acid methyl ester (methyl palmitate), and phthalic acid, mono-(2-ethylhexyl) es-ter at 11.368%, 5.543%, and 5.483%, respectively.

In addition to the analysis of phytochemical composition, extensive in vitro experi-ments were carried out to assess the antioxidant activity of HME and HAE from S. schim-perianum, S. cordatum, and S. nigrum. It is crucial to acknowledge that antioxidants exhibit variability in their chemical characteristics and scavenging mechanisms [69]. Therefore, the utilization of multiple methodologies is crucial for accurately evaluating the antioxi-dant potential of plant extracts. In this particular study, two complementary test systems, namely the DPPH radical scavenging assay and the ABTS scavenging assay, were em-ployed to determine the antioxidant capacity of the tested extracts. Radical scaveng-ing-based methods like DPPH and ABTS are commonly used in antioxidant activity stud-ies, especially for assessing herbal extracts. These spectrophotometric assays provide ben-efits such as sensitivity, simplicity, speed, and reproducibility by facilitating direct inter-action between chromogen radicals and antioxidants [70]. The outcomes derived from this study (refer to Table 5 and Figures 4,5) provide valuable insights into the notable antioxi-dant properties of all examined extracts. Furthermore, they elucidate the order of radical scavenging effectiveness among the extracts, which aligns with their polyphenol content. Specifically, the HME extract consistently displayed higher levels of polyphenols and su-perior radical scavenging potential compared to the HAE extract. This pattern is con-sistent with the comparison between S. nigrum (IC50 = 36.78 – 47.53 µg/mL), S. cordatum (IC50 = 61.57 – 80.54 µg/mL), and S. schimperianum (IC50= 88.13 – 102.5 µg/mL). This corre-lation was further supported by the results of the correlation analysis between total phe-nolic and flavonoid content and antioxidant radical activity using Pearson correlation (refer to Figure 6), which clearly demonstrated a positive association between antioxidant activity and the overall content of phenolic and flavonoid compounds (r = 0.8591–0.9345). This finding underscores a clear link between phenolic content and increased antioxidant efficacy, suggesting the potential of the tested extracts as natural antioxidants for diverse applications. Subsequent studies could focus on unraveling the specific mechanisms by which these phenolic compounds combat oxidative stress by interacting with free radi-cals. Moreover, when comparing the antioxidant potential of the three tested species with other Solanum species evaluated using similar bioassays, such as Solanum sessiliflorum, S. torvum, S. nigrum, S. aethiopicum, S. sisymbriifolium, S. melongena, S. muricatum, S. melongena L., and S. Lycopersicum [71-78], it becomes apparent that S. schimperianum, S. cordatum, and S. nigrum exhibited remarkable radical-scavenging activity. Consequently, it can be con-cluded that the species investigated in this study possesses a significant antioxidant ca-pacity that surpasses numerous other species within the Solanum genus.

5. Conclusions

This study investigated the phytochemical profile and antioxidant activity of three Solanum species (S. schimperianum, S. cordatum, and S. nigrum) native to Saudi Arabia. Hy-dro-methanolic and hydro-acetonic extractions were utilized for a comprehensive analy-sis of phytochemical compounds, with RP-HPLC and GC-MS analyses revealing a rich diversity of compounds. Chlorogenic acid was the most abundant, alongside other valua-ble phenolic and flavonoid components. GC-MS analysis identified palmitic acid among a predominant constituent with reported antibacterial, antioxidant, and antifungal proper-ties. The significant antioxidant activity was observed in all extracts through DPPH and ABTS assays, with S. nigrum exhibiting the highest potential, aligns with the diverse phe-nolic profile of the three species. These findings suggest that these Solanum species could serve as promising sources of bioactive compounds with potential health benefits. Further studies are warranted to isolate and characterize the bioactive compounds responsible for the observed antioxidant activity. Additionally, in vitro and in vivo studies are necessary to evaluate the potential health benefits of these Solanum species extracts

6. Author Contributions:

Conceptualization, Ali S. Alqahtani; Data curation, Rashed N. Herqash and Abdelaaty A. Shahat; Formal analysis, Rashed N. Herqash and Syed Ahamad; Funding ac-quisition, Abdelaaty A. Shahat; Investigation, Rashed N. Herqash and Omer I. Fantoukh; Meth-odology, Rashed N. Herqash and Syed Ahamad; Resources, Ali S. Alqahtani; Software, Rashed N. Herqash and Syed Ahamad;

Supervision, Ali S. Alqahtani and Abdelaaty A. Shahat; Visualiza-tion, Ali S. Alqahtani and Omer I. Fantoukh; Writing – review & editing, Rashed N. Herqash, Omer I. Fantoukhand Syed Ahamad.

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Conflicts of Interest: The authors declare no conflict of interest.

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