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Integrated Phytochemical Profiling and Antioxidant Evaluation of Wild *Thapsia garganica* L. Roots from Western Algeria Using Spectrophotometry, GC–MS and LC–MS/MS

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Abstract

Thapsia garganica L. is a medicinal plant widely used in North African traditional medicine, yet the phytochemical composition and antioxidant potential of its roots remain poorly characterized. This study investigated the phytochemical profile and antioxidant activity of wild *T. garganica* roots collected from western Algeria. Methanolic and *n*-hexane extracts were prepared for phytochemical characterization, while the crude methanolic extract and its flavonoid and alkaloid fractions were evaluated for antioxidant activity. Antioxidant activity of the crude methanolic root extract and the isolated flavonoid and alkaloid fractions were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, complemented by kinetic analysis of radical scavenging. Phytochemical characterization was performed using GC–MS and LC–MS/MS. The crude methanolic extract exhibited the strongest DPPH radical-scavenging response among the tested samples, showing a concentration-dependent increase in radical-scavenging activity and time-dependent DPPH radical-scavenging kinetics, with an initial rapid phase followed by a slower reaction. LC–MS/MS identified ferulic acid (90.2 µg/mg), vanillic acid (9.26 µg/mg), and caffeic acid (2.49 µg/mg) as the major quantified phenolic compounds, while GC–MS revealed a diverse chemical profile comprising fatty acid-related, sterol-related, terpenoid-related, and phenolic-related constituents. Overall, these findings demonstrate promising in vitro DPPH radical-scavenging activity of the crude methanolic root extract and reveal a diverse metabolite profile, providing a basis for further investigation of its phytochemical composition and biological properties.

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Academic Editors: Abuzar Kabir and Victoria Samanidou

Received: 8 September 2026
Revised: 28 October 2026
Accepted: 3 November 2026
Published: 13 November 2026

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Keywords: *Thapsia garganica*; antioxidant activity; DPPH assay; GC–MS; LC–MS/MS; phenolic compounds; medicinal plants

1. Introduction

Medicinal and aromatic plants represent some of the richest natural sources of biologically active compounds and have long played essential roles in traditional healthcare systems worldwide. Due to their diverse phytochemical constituents, medicinal plants have gained much attention for their potential use in the pharmaceutical, cosmetic, food, and nutraceutical industries. Algeria has exceptional botanical diversity because of its varied climatic and ecological conditions, ranging from the Mediterranean coast to the Saharan desert. This rich flora includes many medicinal species that are traditionally used to treat human diseases. Among these, members of the Apiaceae family are especially important because they are widely used as medicinal herbs, spices and sources of valuable secondary metabolites with antimicrobial, anti-inflammatory, antioxidant and anticancer activities [1].

Thapsia garganica L. is a perennial herb belonging to the Apiaceae family, naturally distributed in the Mediterranean Basin (Algeria, Tunisia, Morocco, southern Europe and parts of western Asia). The species has a strongly branched 1–2 m tall stem, yellow–green pinnate leaves, heart-shaped winged fruits and a thick, resinous root, black outside and white inside [2]. The root resin has been known for medicinal properties for centuries and was previously included in the French Pharmacopoeia in the preparation of *Thapsia* plaster [3]. *Thapsia garganica* L. has been widely used in traditional medicine in North Africa and Arabia for the treatment of rheumatic disorders, inflammatory diseases, respiratory diseases, pain and stress-related disorders [4]. Despite the plant's historical association with skin-irritating properties, many of its biological activities have been demonstrated to result from its different phytochemical constituents, not only irritant effects, by modern pharmacological investigations. Over the last two decades, *Thapsia garganica* L. has attracted increasing scientific interest because of its diverse phytochemical composition and reported biological activities. Studies of *T. garganica* and related *Thapsia* species have reported antimicrobial and other biological activities of essential oils, extracts, and isolated constituents [3,5,6].

Sesquiterpene lactones, particularly thapsigargin and related guaianolides, have been a focus of interest in this species due to their strong biological activities and their role as lead molecules for anticancer drugs [3]. *Thapsia garganica* L. contains a diverse spectrum of secondary metabolites, including flavonoids, phenolic acids, coumarins, terpenoids, sterols, and volatile constituents, which may contribute to its reported biological activities [5,6]. The importance of natural antioxidants has increased in the research field because oxidative stress is known to be one of the major factors in aging and many chronic diseases, such as cardiovascular disorders, neurodegenerative diseases, diabetes and cancer. Oxidative stress is the result of an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense mechanisms of biological systems. Excess production of ROS causes lipid peroxidation, protein oxidation, DNA damage and cellular dysfunction. Hence, antioxidants that are able to scavenge free radicals and disrupt oxidative chain reactions are considered important therapeutic agents for preventing oxidative damage [7].

Antioxidants of plant origin predominantly include phenolic compounds such as flavonoids, phenolic acids, tannins and various other specialized metabolites with electron- and/or hydrogen-donating abilities. These components not only help prevent the initiation and propagation of radical-driven processes but also assist in the deactivation of reactive oxygen species (ROS) into more stable products. The medicinal herbs of the Apiaceae family are rich sources of natural antioxidants and display significant free radical scav-

enging potential because of their phenolic and flavonoid contents [1,8]. Therefore, the identification of new antioxidants from medicinal plants is continuously being explored for pharmaceutical and nutraceutical applications.

The phytochemical diversity of medicinal plants depends on many factors, such as geographic location, environmental factors, season of collection, plant part, stage of growth, and methods of extraction and isolation. Thus, it is possible to observe differences in metabolite contents between different populations of the same plant species from distinct geographical areas [9]. For this reason, detailed phytochemical analysis allows not only the identification of major bioactive compounds but also the correlation of chemical composition and biological activity. Currently, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) are sensitive and complementary analytical techniques commonly used for the detection of volatile, semivolatile and nonvolatile metabolites from crude plant extracts. They are valuable tools in the field of natural product research and greatly improve the quality of phytochemical studies.

Although *Thapsia garganica* L. has a history of use in traditional North African medicine, studies combining phytochemical characterization with the evaluation of its antioxidant properties, particularly in Algerian populations, remain relatively limited. Previous studies have investigated the phytochemical composition and antioxidant activity of *T. garganica* roots, while fewer studies have examined these aspects together. In addition, information on the kinetics of DPPH radical reduction by *T. garganica* extracts appears to be limited. Although several studies have evaluated the antioxidant activity of *Thapsia* species, no previous work has simultaneously investigated antioxidant kinetics together with comprehensive GC–MS and LC–MS/MS profiling of wild Algerian roots. We therefore hypothesized that the crude methanolic extract would exhibit greater antioxidant activity than the isolated fractions, consistent with differences in their phytochemical composition.

Hence, this study aimed to assess the antioxidant activity and phytochemical characteristics of the crude methanolic extract and some phytochemical fractions of the roots of the wild *Thapsia garganica* L. from northwestern Algeria. Free radical scavenging DPPH activity was determined by the DPPH assay in the presence of the kinetics evaluation study of DPPH reduction. Furthermore, GC–MS and LC–MS/MS have also been explored in parallel to determine the phytochemical constituents of the crude extract and its derivatives in addition to revealing biocomponents that are likely to have antioxidant activity. These findings provide insight into the phytochemical richness and antioxidant potential of Algerian *Thapsia garganica* L. roots; however, the known toxicity of this species must be considered, and further safety evaluation is required before any application in food, supplement, functional, or therapeutic products can be contemplated.

2. Materials and Methods

2.1. Chemicals and Reagents

Methanol (99.9%), *n*-hexane, ethanol, *n*-butanol, ethyl acetate, chloroform, diethyl ether, hydrochloric acid (HCl), ammonium hydroxide, sodium chloride, anhydrous sodium sulfate, sulfuric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, ascorbic acid, ammonium formate, formic acid, and HPLC-grade water were of analytical or LC–MS grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Helium (99.999%) and nitrogen (99.999%) gases used for GC–MS and LC–MS/MS analyses were obtained from Air Liquide (Paris, France). Whatman No. 1 filter paper was purchased from Cytiva (Marlborough, MA, USA), and TLC silica gel plates were obtained from Merck (Darmstadt, Germany). All reagents and solvents were used as received without further purification.

2.2. Plant Material

The plant material was identified as *Thapsia garganica* L. at the species level; no specific subspecies was designated in the present study. The abbreviation 'L.' refers to Carl Linnaeus, the botanical authority associated with the species name. Roots of *Thapsia garganica* L. were collected on 15 March 2023 from the Mécheria area, Naâma Province, western Algeria. The plant material was collected by Bounoua Nadia and authenticated by Bounoua Nadia, Laboratory of Chemical and Environmental Science (LCSE), 8000, Bechar, Algeria. The Mécheria area is located at approximately 33.54° N latitude and 0.28° W longitude, at an elevation of approximately 1158 m above sea level. The roots were thoroughly rinsed with distilled water to remove attached debris and dirt, air-dried under open and well-ventilated conditions for 14 days, and pulverized to a fine powder using an electric grinder. No voucher specimen was deposited in a herbarium. Plant identification was performed by Bounoua Nadia at the Laboratory of Chemical and Environmental Science (LCSE), Béchar, Algeria.

2.3. Extraction of Thapsia garganica Roots

The dried roots of *Thapsia garganica* L. were powdered to a fine powder. Fifty grams of the powdered material were separately macerated with 500 mL of methanol (99.9%) and 500 mL of *n*-hexane at room temperature with occasional stirring for 72 h for extraction. The mixtures were filtered through Whatman No. 1 filter paper, and the residues were extracted twice under the same conditions to maximize the recovery of phytochemicals. The two filtered fractions were concentrated in a rotary evaporator at 40 °C under reduced pressure affording the crude methanolic and hexane extracts. All the dried crude extracts were accurately weighed, transferred to amber glass vials and kept at 4 °C in refrigerator for subsequent phytochemical studies and antioxidant analysis. The extraction yield was calculated on a dry weight basis via the following Equation (1):

$$\%Yield = \frac{Extract\ Weight}{\text{Dry Plant Weight}} \times 100 \quad (1)$$

where the weight of the dried extract is the weight of the concentrated extract after removing the solvent and the weight of the dried plant is the initial weight of the powdered roots of *Thapsia garganica* L. (50 g) in the extraction procedure.

2.4. Flavonoid Extraction

Ten grams of powdered *Thapsia garganica* L. root was refluxed for 4 h at 90 °C in a 1:1 (v/v) mixture of distilled water and absolute ethanol. After cooling to room temperature, the extract was filtered through a 0.45 µm membrane to remove any remaining plant debris. The resulting filtrate was adjusted to pH 3 with 10% (v/v) HCl to force the flavonoids into the organic phase. The filtrate was acidified and transferred to a separatory funnel and shaken with 200 mL of *n*-butanol. Partitioning of the aqueous extract against *n*-butanol was used to enrich the flavonoid fraction based on differences in polarity and partitioning behavior. The *n*-butanol layer was collected and concentrated to dryness at 40 °C under reduced pressure using a rotary evaporator. The dried residue was re-dissolved in 100 mL of distilled water and subjected to three successive liquid–liquid extractions with 100 mL of ethyl acetate for 1 h each. After allowing the phases to separate for 15 min, the ethyl acetate layer containing the flavonoid-rich fraction was collected and concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. The dried flavonoid fraction was stored in amber vials at 4 °C until phytochemical characterization and antioxidant assays were performed.

2.5. Alkaloid Extraction

The roots of *Thapsia garganica* L. were dried and ground into a very fine powder. A 20 g sample of the powder was macerated with 200 mL of 80% methanol acidified with 1% *v*/*v* hydrochloric acid (HCl) at 25 °C for 24 h on a magnetic stirrer. The filtrate was filtered, and the residue was re-extracted under similar conditions two more times to increase the yield of alkaloids. The combined filtrates were evaporated under reduced pressure at 40 °C to remove the methanol and obtain an aqueous acidic extract.

The aqueous extract was acidified to 2–3 pH with dilute HCl for the alkaloids to reach the protonated (water-soluble) state and then subjected to three extractions with chloroform for the removal of lipophilic impurities. The aqueous phase was then alkalified with ammonium hydroxide to a pH of 9–10 for the alkaloids in free base form, and then this solution was extracted three times with chloroform. The pooled extracts of chloroform were treated with anhydrous sodium sulfate to dryness, filtered and then concentrated under reduced pressure at 40 °C to obtain the crude alkaloid fraction. The dried fraction was then weighed and stored in amber-colored glass vials at 4 °C for further phytochemical analyses and antioxidant investigations [10,11].

2.6. Saponin Extraction

The dried root parts of *Thapsia garganica* L. were washed, air-dried, and then ground into a fine powder. Powder material (20 g) was subjected to continuous shaking for 24 h in 200 mL of 80% methanol solution at room temperature. The residues were extracted twice more under the same conditions and the filtrates were collected. Then, the combined filtrates were vacuum evaporated to a concentrate in a rotary evaporator at 40 °C. The water fraction was re-suspended in distilled water and extracted with diethyl ether to remove lipophilic substances. Then *n*-butanol was added to obtain the *n*-butanol portion. The collected fraction was extracted three times with *n*-butanol and washed with 5% sodium chloride. The pooled *n*-butanol fractions were dried under reduced pressure, and the resultant crude saponin extract was kept as a dry material to constant weight at 4 °C in an amber glass container prior to further analysis [11,12].

2.7. Screening for Phytochemicals

The diethyl ether, methanol, and water extracts were screened for the presence of flavonoids, alkaloids, saponins, sterols, terpenoids, tannins, anthraquinones, anthracenosides, anthocyanosides, coumarins, reducing sugars, fatty acids, and volatile oils using standard phytochemical screening procedures described by Sofowora and Harborne [13,14].

2.8. Preparation of the Methanolic Extract for TLC Screening

The crude methanolic extract obtained as described in Section 2.3 was used for TLC-based antioxidant screening. The dried extract was re-dissolved in methanol prior to TLC analysis.

2.9. Thin-Layer Chromatography (TLC)-Based Antioxidant Screening

The methanolic extract was analyzed by thin-layer chromatography (TLC) using 10:90 (*v*/*v*) methanol: chloroform as the mobile phase. The plate was developed to a migration distance of 70 mm and air-dried for 15 min. It was then sprayed with 0.004% (*w*/*v*) methanolic DPPH solution and dried at 60 °C for 30 min. Yellow or pale-yellow zones against the purple background were considered indicative of radical-scavenging activity [15,16]. To confirm these results, a second TLC plate was prepared under the same conditions and sprayed with 10% sulfuric acid. The presence of positive compounds was indicated by purple staining.

2.10. Preparation of the Methanolic Crude Plant Extract

The crude methanolic extract prepared as described in Section 2.3 was used for the DPPH radical scavenging assay. A 1 mg/mL stock solution of the dried crude methanolic extract was prepared in methanol. The working solutions were serially diluted twofold with methanol, resulting in concentrations ranging from 0.5000, 0.2500, 0.1250, 0.0312, 0.0156 and 0.0078 mg/mL, and these solutions were freshly prepared on the day of analysis. These working solutions were all subjected to a DPPH radical scavenging assay.

2.11. DPPH Radical Scavenging Assay and Kinetic Analysis

A stock solution of DPPH, 2,2-diphenyl-1-picrylhydrazyl, was made by adding accurately 1 mg of DPPH to 25 mL of methanol, resulting in a 0.004% (w/v) solution. This solution was wrapped in aluminum foil to exclude light and was refrigerated at 4 °C. A new DPPH stock solution was made once every 1–4 days to maximize freshness. Reactions were performed in amber tubes. A stock solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (1.0 mM) was prepared by dissolving the appropriate amount of DPPH in methanol. Working standard solutions with concentrations of 10, 20, 30, 40, and 50 µM were prepared by diluting appropriate aliquots of the stock solution with methanol in volumetric flasks. Absorbance readings for each standard solution were taken at 517 nm on a UV–visible spectrophotometer, using methanol as the blank. To ensure that the measured residual DPPH concentrations fell within the linear range of the calibration curve (10–50 µM), aliquots of the reaction mixture were appropriately diluted with methanol, when necessary, before spectrophotometric measurement. The residual DPPH concentration was calculated from the calibration equation, and the resulting concentration was corrected for the dilution factor to obtain the residual DPPH concentration in the original reaction mixture. Linear regression of absorbance against DPPH concentration (µM) gave the equation $y = 0.01311x - 0.00370$ ($R^2 = 0.99967$), where y denotes absorbance and x denotes the DPPH concentration in µM. This calibration equation was used to determine the residual DPPH concentration during the kinetic antioxidant assay.

The antioxidant activity of the crude methanolic root extract and the isolated flavonoid and alkaloid fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay with minor modifications to the method described by Al-Qudah et al. [17]. Briefly, 100 µL of each freshly prepared extract solution at different concentrations was mixed with 1.9 mL of freshly prepared 0.004% (w/v) DPPH solution in methanol (approximately 0.10 mM). The extract concentrations reported for the DPPH assay refer to the concentrations of the prepared extract solutions prior to mixing with the DPPH reagent. Because 100 µL of extract solution was mixed with 1.9 mL of DPPH solution, the resulting extract concentrations in the final 2.0 mL reaction mixture were correspondingly diluted 20-fold. The reaction mixtures were incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm via a UV–visible spectrophotometer. The 30 min incubation period was selected based on the previously reported DPPH procedure [17] and was applied consistently to all samples. Methanol containing DPPH without extract served as the control. For each extract concentration, a corresponding sample blank containing the same volume and concentration of extract in methanol but without DPPH was prepared and measured at 517 nm to account for the intrinsic absorbance of the extract. The absorbance of the sample blank was subtracted from the corresponding sample absorbance before calculation of the radical-scavenging activity. Methanol alone was used as the reagent blank. Ascorbic acid was used as positive control and was analyzed under identical experimental conditions. All measurements were

performed in triplicate, and the results were expressed as mean ± standard deviation (SD). %RSA was calculated using the following Equation (2):

$$\%RSA = \frac{A_{control} - \left(A_{sample} - A_{blank}\right)}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of DPPH without extract, A_{sample} is the absorbance of DPPH in the presence of the extract, and A_{blank} is the absorbance of the corresponding extract without DPPH.

The antioxidant activity was calculated as the percentage of radical scavenging activity (%RSA). The antioxidant activity was expressed as the percentage of radical-scavenging activity (%RSA) at each tested extract concentration. The concentration–response results were evaluated descriptively without estimating IC_{50} values. The calibration equation described above was used to determine the residual DPPH concentration during the kinetic analysis.

The kinetics of DPPH radical reduction were evaluated by monitoring the decrease in absorbance of the DPPH solution over time. For the kinetic experiments, four concentrations of the flavonoids extract were selected and designated C1–C4 as follows: C1 = 0.5000 mg/mL, C2 = 0.0078 mg/mL, C3 = 0.1250 mg/mL, and C4 = 0.0312 mg/mL. These values refer to the concentrations of the prepared extract solutions before mixing with the DPPH reagent. Residual DPPH concentrations were calculated using the calibration equation described above, with correction for any dilution factor applied during spectrophotometric measurement. An aliquot of 1.5 mL of each prepared extract mixture was mixed with 1.5 mL of freshly prepared 0.1 mM DPPH solution in methanol and immediately transferred to a quartz cuvette. The initial absorbance (A_0) was recorded immediately after mixing at 517 nm via a UV–Vis spectrophotometer. The absorbance was subsequently measured at 30 s intervals until a constant value (steady state) was reached. The dilution factor was applied to the concentration obtained from the calibration equation according to the dilution performed for each kinetic sample. Thus, the calibration curve was not used for direct quantification of samples outside its validated linear range. The reduction in DPPH absorbance over time was used to evaluate the radical scavenging kinetics of the extract. All the measurements were performed in triplicate, and the results are expressed as the means ± standard deviations.

2.12. GC–MS Analysis

GC/MS analysis was performed according to a previously described procedure [18,19]. The analysis was performed on a Shimadzu QP2020 GC–MS instrument equipped with a GC–2010 Plus instrument (Shimadzu Corporation, Kyoto, Japan) in split–splitless mode, utilizing a DB–5MS fused silica column (5% phenyl, 95% polydimethylsiloxane, 30 m × 0.25 mm, 25 µm film thickness). For the best component separation, a linear temperature program was used. Briefly, the oven temperature was set to 50 °C for 1 min, and the temperature increased from 50 °C to 280 °C at a heating rate of 7 °C/min and then held at 280 °C for 10 min. The total running time was 44 min. The injector temperature was 260 °C with a split ratio of 20:1, the injection volume was 1 µL, the helium carrier-gas flow rate was 1.50 mL/min, and the flow control mode was as follows: pressure, 88.3 kPa. MS source temperature/detector temperature: 240 °C; interface temperature: 250 °C; ionization energy (EI): 70 eV; scan range: 35–500 amu; scan speed: 1666. The solvent cutoff time was 3 min, and these data were acquired within 4.5 min. These data were collected via Windows-based Lab-Solution GC–MS version 4.45SP1 software. The mass spectra of the detected chromatographic peaks were compared with reference spectra in the ADAMS-2007 and NIST 2017 mass spectral libraries [20]. Compound assignments were further evaluated by comparing the experimentally calculated retention indices (RIs), determined using n-alkanes (C8–C30) under the applied temperature-programmed GC conditions, with literature RI

values reported for comparable stationary phases [21,22]. Compound assignments were considered tentative when they were based on library spectral matching and RI comparison without confirmation using an authentic reference standard. Compounds for which the available spectral and RI evidence was insufficient for a reliable assignment were excluded from definitive compound identification. Accordingly, the GC–MS results are reported as tentative identifications unless otherwise confirmed by an authentic standard. Authentic reference standards were not available for all detected compounds; therefore, definitive structural confirmation was not possible for every GC–MS assignment. The reported identifications should consequently be considered tentative unless independently confirmed by an authentic standard. For sample preparation, an accurately weighed portion (5 mg) of each sample was dissolved in 1 mL of either methanol or *n*-hexane, as appropriate. The solutions were centrifuged and filtered through 0.22 µm nylon membrane filters. An aliquot of 1 µL of each filtrate was injected into the GC–MS system using a split ratio of 20:1.

2.13. LC–MS/MS Analysis

Liquid chromatography–mass spectrometry (LC-MS) analysis of the extract was performed using an Exion LC™ UPLC system coupled with a QTRAP® 4500 LC-MS/MS system equipped with an electrospray ionization (ESI) source (AB Sciex, Framingham, MA, USA). Data acquisition and processing were controlled using Analyst® software (version 1.7). Chromatographic separation was achieved on a Synergi™ C18 reversed-phase column (50 × 3.0 mm i.d., 5 µm particle size, 80 Å pore size; Phenomenex, Torrance, CA, USA) maintained at 30 ± 1 °C [23]. The mobile phase comprises 5 mM ammonium formate in water/methanol (95:5, *v*/*v*) (Mobile Phase A) and methanol containing 1 mM formic acid (Mobile Phase B). A gradient elution was applied at a flow rate of 0.35 mL/min as follows: 5–90% B (0.00–8.00 min), 90% B (8.00–12.00 min), and 90–5% B (12.01–15.00 min). The injection volume was 5 µL. Tandem mass spectrometric detection was performed using multiple-reaction monitoring (MRM) with electrospray ionization (ESI) in the appropriate positive- or negative-ion mode for each analyte. The QTRAP 4500 mass spectrometer was operated at unit resolution for Q1 and Q3, with compound-specific precursor-to-product ion transitions and optimized declustering potential (DP), collision energy (CE), and collision-cell exit potential (CXP). The complete MRM transitions, ionization polarity, dwell times, and compound-specific MS/MS acquisition parameters are provided in the Supplementary Materials (Table S1). The limit of detection (LOD), limit of quantification (LOQ), and linear calibration range for gallic acid, chlorogenic acid, ferulic acid, caffeic acid, rosmarinic acid, caffeic acid phenethyl ester, quercetin, 3-O-methylquercetin, myricetin, apigenin, luteolin, galangin, hesperetin, catechin, rutin, luteolin 7-O-glucoside, apigenin 7-O-glucoside, syringic acid, salvianolic acid A, hesperidin, and ascorbic acid were 100 ng/mL, 300 ng/mL, and 300–8000 ng/mL, respectively. When necessary, samples were appropriately diluted prior to analysis to ensure that the analyte concentrations fell within the validated linear range (300–8000 ng/mL). The corresponding dilution factors were taken into account in the calculation of the final analyte concentrations. For coumarin and 7-hydroxycoumarin, the corresponding values were 300 ng/mL (LOD), 1000 ng/mL (LOQ), and 1000–10,000 ng/mL (linear range). An accurately weighed portion (2 mg) of the dried root extract of *Thapsia garganica* L. was dissolved in 1 mL of methanol to obtain a concentration of 2 mg/mL. The solution was vortex-mixed, centrifuged, and the supernatant was filtered through a 0.22 µm membrane filter prior to LC–MS/MS analysis. The concentrations obtained are calculated and reported as µg/mg of the dried extract.

3. Results and Discussion

The results of this study provide an integrated assessment of the phytochemical composition and antioxidant activity of *Thapsia garganica* L. roots. The findings include the extraction yields of the crude extract and phytochemical fractions, evaluation of antioxidant activity using the DPPH assay, kinetic analysis of DPPH radical reduction, and characterization of the phytochemical constituents by GC–MS and LC–MS/MS. The extraction yields of the crude methanolic extract, flavonoid, alkaloid, and saponin fractions were 4.69%, 0.55%, 0.72%, and 0.44%, respectively (Figure 1). The crude methanolic extract showed the highest extraction yield, whereas the alkaloid fraction showed the highest yield among the isolated phytochemical fractions.

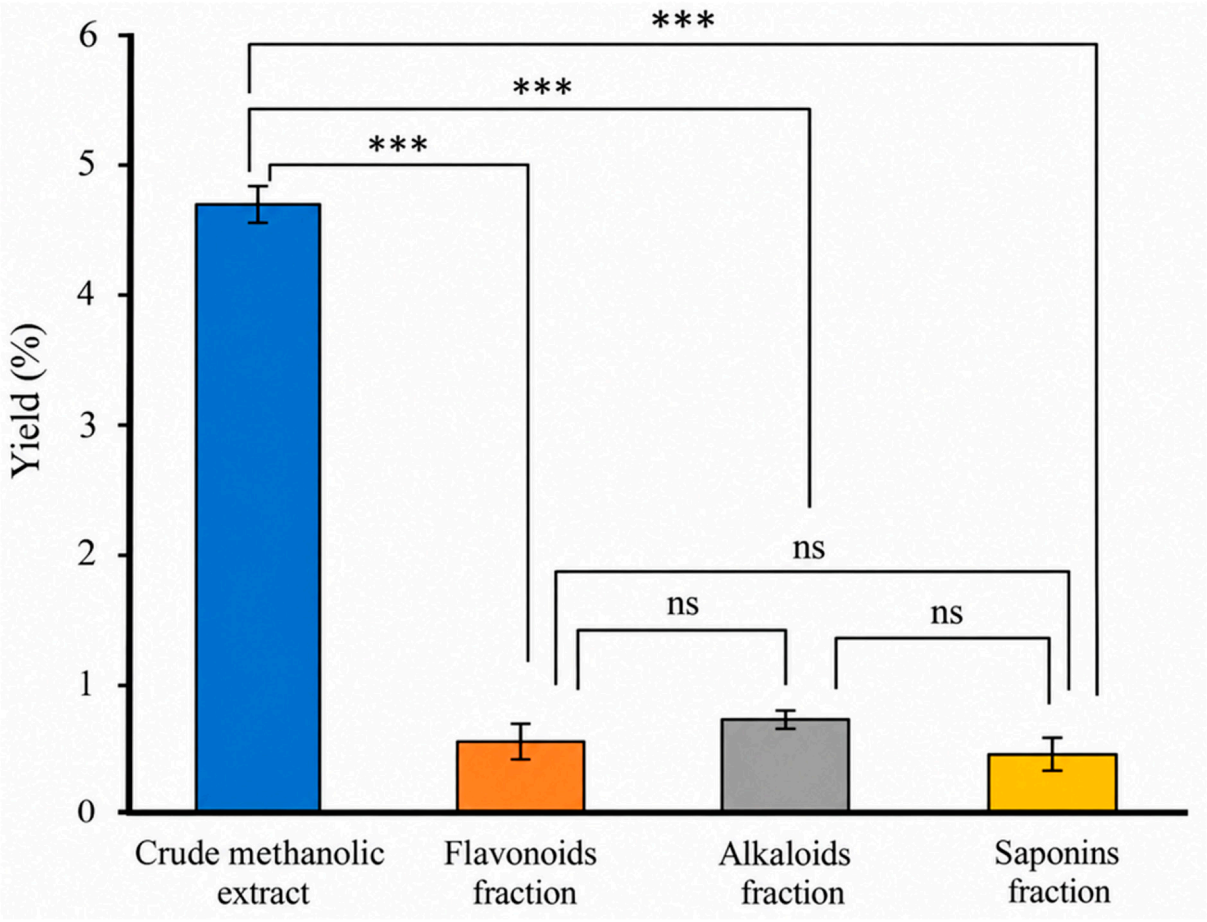

Figure 1. Extraction yields (%) of the crude methanolic extract, flavonoid, alkaloid, and saponin fractions obtained from *Thapsia garganica* L. roots. Values are presented as mean ± SD ($n = 3$). Statistical significance was determined by one-way ANOVA followed by Tukey's HSD test (*** $p < 0.001$; ns, not significant, $p > 0.05$).

The alkaloid fraction showed the highest extraction yield among the isolated phytochemical fractions (0.72%). However, this value represents the yield of the crude alkaloid fraction and does not directly indicate the actual alkaloid content of the roots, as the fraction may contain other co-extracted compounds [5]. Although the acid–base extraction procedure was intended to enrich alkaloids based on their acid–base properties, the actual alkaloid content and purity of the resulting fraction cannot be established from the fraction yield alone. Further quantitative analysis would be required to determine the actual alkaloid content.

The lower yields obtained for flavonoids and saponins, on the other hand, probably reflect differences in polarity, chemical structure, and how readily each class responds to extraction. Methanol, being a strongly polar solvent, tends to pull out a wide range of phytochemicals, which likely explains why the crude methanolic extract itself came out with a comparatively high yield. Extraction yield is influenced not only by solvent properties and chemical composition but also by geographic origin, climate, soil conditions, growth stage, and harvesting season, which can affect the synthesis and accumulation of secondary metabolites in medicinal plants [24].

Interestingly, the crude methanolic extract showed the strongest antioxidant activity overall, despite the alkaloid fraction having the highest yield. That mismatch is telling that antioxidant activity clearly is not determined by any single class of compound. It is more plausible that it arises from the combined action of multiple bioactive groups terpenoids, flavonoids, phenolic acids, and related antioxidant compound the kind of chemical diversity that techniques like GC–MS and LC–MS–MS are well suited to reveal [25]. However, the present experimental design does not permit determination of the individual contribution of these constituents or establish synergistic, additive, or antagonistic interactions among them.

3.1. Antioxidant Activity Based on DPPH Assay

The antioxidant activity of ascorbic acid was investigated through a DPPH radical scavenging assay. This process occurs due to the reduction in a stable, purple-colored DPPH radical (DPPH•) into its uncharged and yellow-colored nonradical form (DPPH-H) through an antioxidant agent. The %RSA was calculated as described in Section 2.13.

As shown in Figure 2, the scavenging activity of ascorbic acid increased sharply with increasing ascorbic acid concentration. The rate of decrease in the concentration of DPPH was very fast even at a low concentration, indicating that ascorbic acid reacts very quickly with free radical species. Nearly 95–97% inhibition occurred above a concentration of 0.05 µg/mL; thereafter, the activity nearly stabilized, and almost all the DPPH radicals were neutralized. The rapid attainment of this steady state demonstrates the high efficiency of ascorbic acid as a radical scavenger.

The potent antioxidant activity of ascorbic acid may be associated with its high reducing potential and electron-donating (or hydrogen-donating) ability, which can react with reactive radical species to form relatively stable products. The high percentage of inhibition observed at very low concentrations indicates that ascorbic acid is a good reference antioxidant and serves as a positive control for comparison with the crude methanolic extract and the purified flavonoid and alkaloid fractions.

TLC analysis of the methanolic root extract showed several separate spots on the developed plate. After spraying with DPPH reagent, yellow/pale-yellow zones were observed against the purple background, indicating the presence of constituents with radical-scavenging activity. The corresponding plate treated with 10% sulfuric acid showed purple-stained zones, providing general visualization of the separated constituents. This staining was used for visualization only and was not interpreted as evidence of a specific phytochemical class [16].

The antioxidant activity of the crude methanolic extract of *Thapsia garganica* L. was evaluated using the DPPH radical-scavenging assay. As shown in Figure 3, the radical-scavenging activity (%RSA) increased from approximately 49% at the lowest tested concentration (0.0078 mg/mL) to approximately 76% at the highest tested concentration (0.5000 mg/mL). These findings demonstrate a concentration-dependent antioxidant response within the investigated concentration range. The observed activity may be associated with phenolic compounds and other secondary metabolites capable of donating hydrogen atoms or electrons to DPPH radicals [1,26].

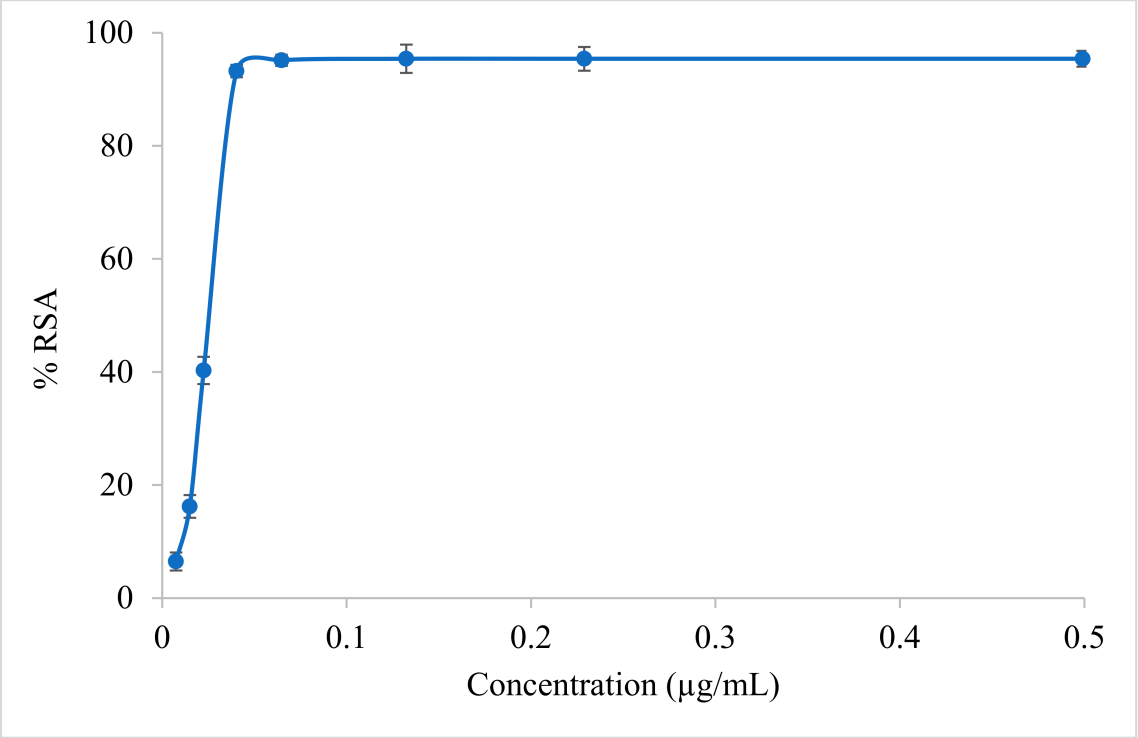

Figure 2. DPPH radical scavenging activity (%RSA) of ascorbic acid at different concentrations. Values are presented as mean ± SD (n = 3).

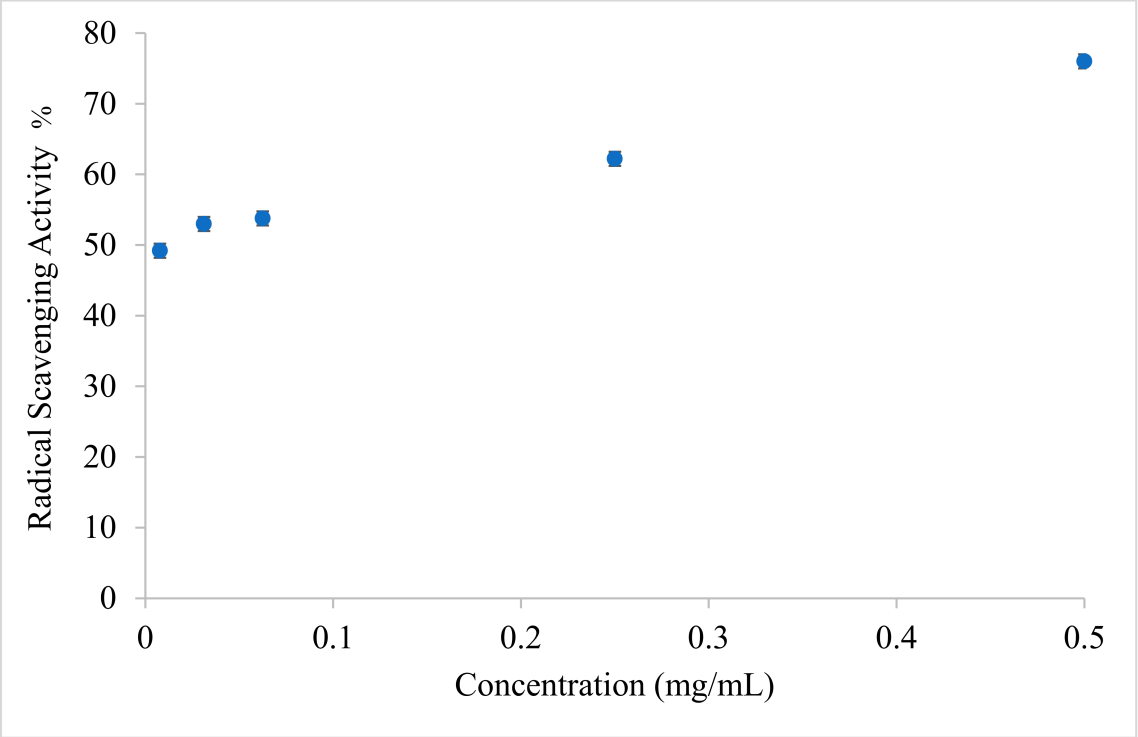

Figure 3. DPPH radical scavenging activity (%RSA) of the crude methanolic root extract of *Thapsia garganica* L. at different concentrations (0.0078–0.5000 mg/mL). Values are presented as mean ± SD (n = 3).

The antioxidant activity of the flavonoid fraction isolated from the roots of *Thapsia garganica* L. was evaluated using the DPPH radical-scavenging assay. As shown in Figure 4, the %RSA in-

creased from approximately 42.3% at the lowest tested concentration (0.0078 mg/mL) to 51.2% at the highest tested concentration (0.5000 mg/mL). These results describe the concentration-dependent radical-scavenging response of the flavonoid fraction under the experimental conditions. No IC_{50} value was estimated from these data.

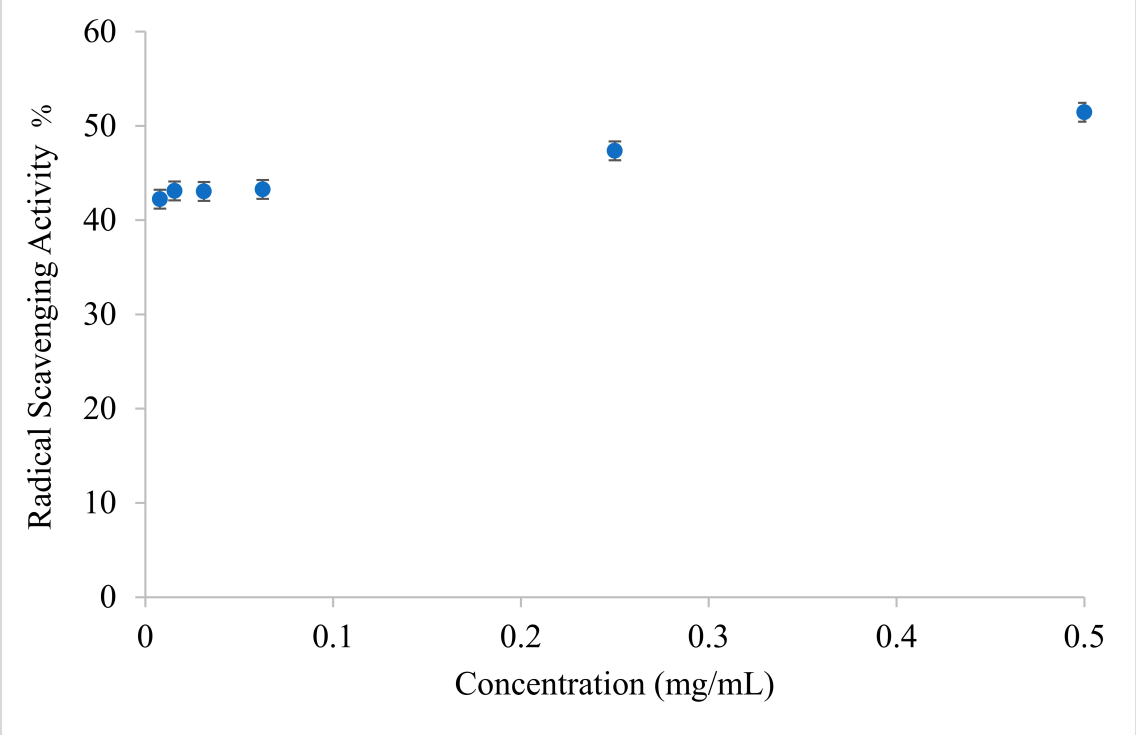

Figure 4. DPPH radical scavenging activity (%RSA) of the flavonoid fraction isolated from *Thapsia garganica* L. roots at concentrations ranging from 0.0078 to 0.5000 mg/mL. The %RSA increased from 42.3% to 51.2%, indicating a concentration-dependent antioxidant effect. Values are presented as mean ± SD (n = 3).

Although the flavonoid fraction showed considerable radical-scavenging activity, its activity was lower than that of the crude methanolic extract under the tested conditions. This difference may be related to differences in the composition and relative abundance of antioxidant constituents between the crude extract and the isolated fraction. The crude extract contains a broader range of phytochemical classes, whereas the flavonoid fraction represents only one fraction of the extracted constituents. However, the present experimental design does not allow synergistic or antagonistic interactions among individual constituents to be established. Flavonoids function as a noteworthy class of antioxidant compounds in *Thapsia garganica* L., as confirmed by their presence in the sample tested.

The antioxidant activity of the alkaloid fraction isolated from *Thapsia garganica* L. roots was evaluated using the DPPH radical-scavenging assay. As shown in Figure 5, radical-scavenging activity increased from approximately 36.1% to 46.0% across the tested concentration range (0.01–1.00 mg/mL). The measured %RSA values remained below 50%, and no IC_{50} value was estimated. The alkaloid fraction showed lower radical-scavenging activity than the crude methanolic extract and flavonoid fraction under the tested conditions. However, the contribution of individual alkaloid constituents to the antioxidant activity of the crude extract cannot be established from the present experimental design.

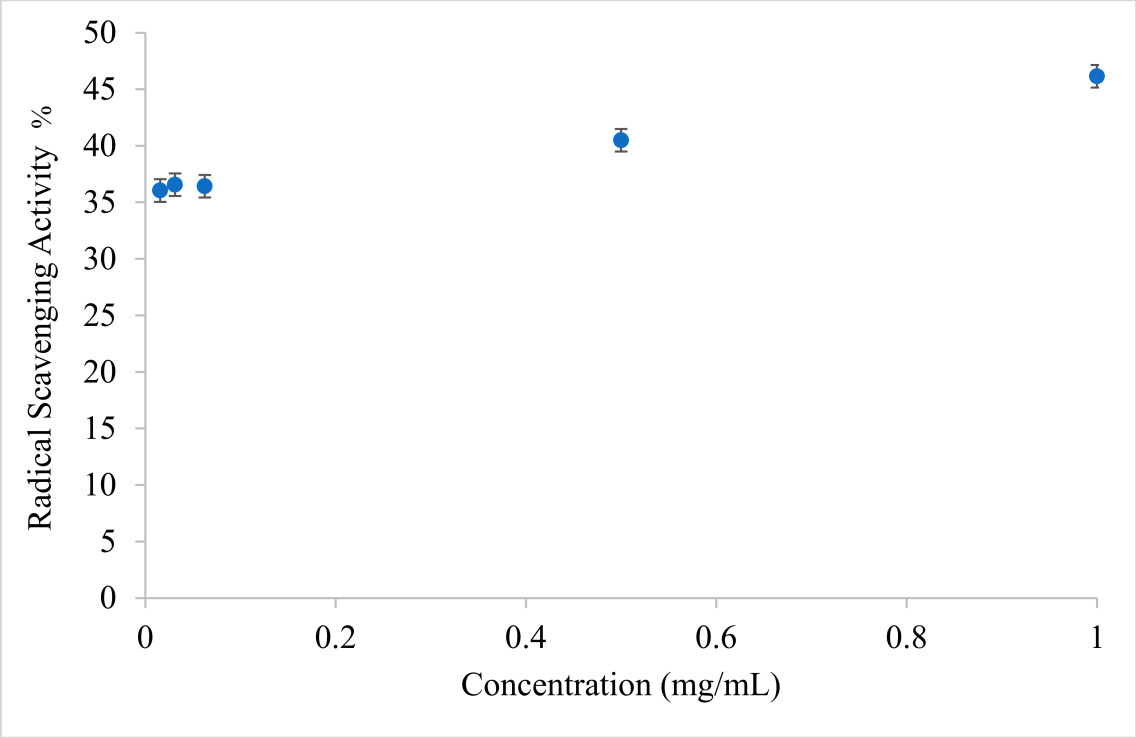

Figure 5. DPPH radical scavenging activity (%RSA) of the alkaloid fraction isolated from *Thapsia garganica* roots at different concentrations (0.01–1.00 mg/mL). The alkaloid extract exhibited concentration-dependent antioxidant activity, with radical scavenging activity increasing from 36.1 to 46.0%. Values are presented as mean ± SD (n = 3).

The alkaloid fraction was evaluated as a crude fraction rather than as a fully characterized collection of individual alkaloid compounds. Therefore, the antioxidant activity observed for this fraction cannot be attributed to specific alkaloid constituents without further chemical characterization. Future work should include detailed characterization and identification of the individual alkaloids present in this fraction using appropriate chromatographic and spectrometric techniques.

This antioxidant activity agrees with previous studies of *Thapsia garganica* L. and other Apiaceae species. Polar extracts tend to have higher free radical scavenging activity than nonpolar extracts due to the presence of phenolic and flavonoid compounds; indeed, the methanolic and ethyl acetate extracts of *Thapsia garganica* L. exhibited significantly higher DPPH radical scavenging activity than the hexane and aqueous extracts, and this was attributed to the increased phenolic content in the polar extracts [27–29]. Likewise, studies have shown that methanolic extracts of *Thapsia garganica* L. are rich in phenolic compounds and display strong antioxidant activity in DPPH, ABTS, and reducing power assays [30].

These results further support the greater antioxidant activity of the crude methanolic root extract identified in this study, probably because a broader range of antioxidant phytochemicals were extracted than those identified in the flavonoid and alkaloid fractions. As antioxidant activity depends on the plant organ studied, geographic origin, environmental conditions, extraction solvent, and experimental protocol, all of which influence the qualitative and quantitative composition of secondary metabolites, we can expect that differences in antioxidant activity will be observed between studies.

In the third manipulation, the antioxidant activity of the alkaloid extract was evaluated. Figure 5 illustrates the results obtained.

To further evaluate the antioxidant performance of the extracts, the percentage of remaining DPPH radicals was determined after 30 min of reaction. As expected, the

percentage of remaining DPPH decreased with increasing extract concentration, confirming concentration-dependent radical scavenging activity [29]. The crude methanolic extract presented the lowest remaining DPPH values, decreasing from 50.80% at 0.0078 mg/mL to 23.98% at 0.50 mg/mL. In comparison, the flavonoid fraction presented DPPH values between 57.78% and 48.55%, whereas the alkaloid fraction presented the highest values (65.99–53.85%), indicating the weakest antioxidant activity.

The lower percentage of remaining DPPH observed for the crude methanolic extract indicates a greater ability to neutralize free radicals than the isolated flavonoid and alkaloid fractions. This higher activity is likely due to the presence of multiple antioxidant compounds, including phenolic acids, flavonoids, terpenoids, coumarins, and other reducing metabolites identified by GC–MS and LC–MS/MS. These compounds may contribute to the overall antioxidant activity through hydrogen atom donation and electron-transfer reactions [30].

The progressive decrease in residual DPPH with increasing extract concentration was consistent with the observed increase in radical-scavenging activity (%RSA). Among the samples studied, the crude methanolic extract exhibited the highest DPPH radical-scavenging response, whereas the flavonoid and alkaloid fractions showed lower responses over their respective tested concentration ranges. These differences may be associated with variations in the phytochemical composition of the extracts. GC–MS and LC–MS/MS analyses revealed several classes of constituents potentially associated with antioxidant activity; however, the contributions of individual compounds to the observed radical-scavenging response were not established in the present study [31].

Figure 6 shows the kinetics of DPPH radical scavenging by the crude flavonoids extract of *Thapsia garganica* L. at four concentrations (C1–C4). C1, C2, C3, and C4 corresponded to 0.5000, 0.0078, 0.1250, and 0.0312 mg/mL, respectively. The percentage of residual DPPH decreased gradually with increasing reaction time, which confirmed the antioxidant activity of all the fractions. The kinetic profiles were fitted using a one-phase exponential decay model described by Equation (3):

$$Y = Y_0 + Ae^{-kX} \quad (3)$$

where Y is the residual DPPH percentage at time X, Y_0 is the equilibrium (plateau) value, A is the amplitude representing the difference between the initial and equilibrium values, and *k* is the apparent first-order rate constant for DPPH radical scavenging. Larger values of *k* indicate faster apparent radical-scavenging kinetics. The initial rapid decline in the curves and the subsequent slower phase approaching the equilibrium state indicate that the majority of the radical scavenging takes place in the early stages of the reaction (Table 1).

Table 1. Kinetic parameters obtained by fitting the DPPH radical reduction curves of flavonoids fraction of *Thapsia garganica* L. roots to a one-phase exponential decay model.

Concentration (mg/mL)	Sample	Y_0	A	k (s^{-1})	R^2
0.5000	C1	23.48	17.91	0.001052	0.986
0.0078	C2	50.97	7.36	0.000548	0.998
0.1250	C3	37.30	10.90	0.000851	0.960
0.0312	C4	49.78	7.25	0.000716	0.999

The flavonoids extract exhibited varying kinetic profiles of DPPH radical reduction across the tested concentrations. After 4000 s, C1 (0.5000 mg/mL) showed the highest radical-scavenging activity, with the lowest percentage of DPPH remaining (approximately 23%), followed by C3 (0.1250 mg/mL), at approximately 38%. In contrast, C2 (0.0078 mg/mL) and C4 (0.0312 mg/mL) showed lower activity, with approximately 51% and 50% DPPH remaining, respectively. These observations indicate that the reduction in DPPH increased with extract

concentration under the tested conditions. The fitted one-phase exponential decay parameters also varied among the samples, with rate constants (k) ranging from 0.000548 to 0.001052 s^{-1}. C1 had the highest rate constant (0.001052 s^{-1}), whereas C2 had the lowest (0.000548 s^{-1}). The goodness of fit varied across the samples, with R^2 values ranging from 0.960 to 0.999 (Table 1). The differences in kinetic behavior may reflect variations in the concentration and composition of antioxidant constituents in the flavonoids extract [30,32].

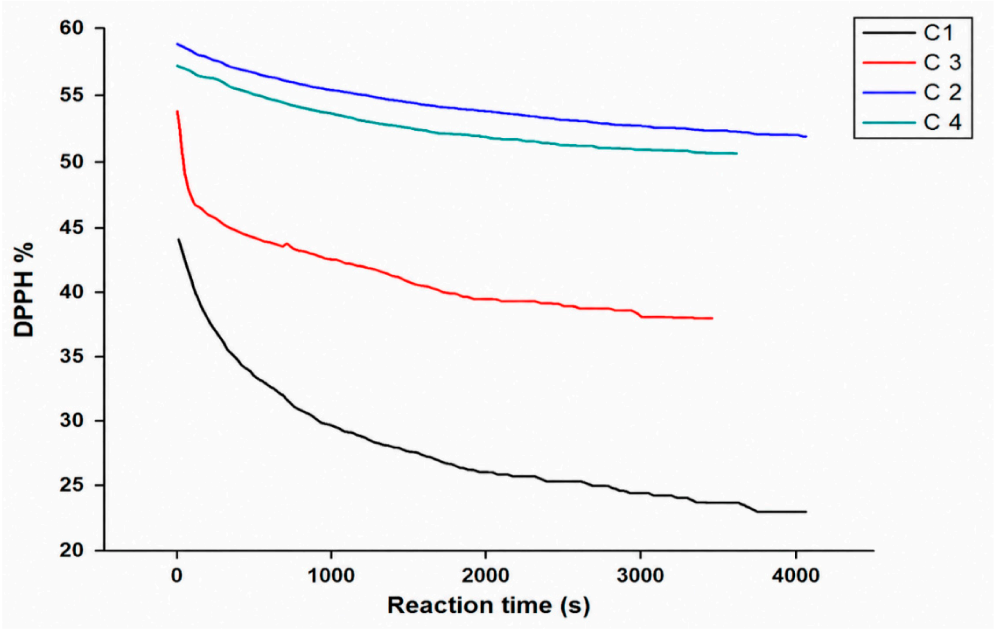

Figure 6. Kinetics of DPPH radical scavenging by the flavonoid fraction of *Thapsia garganica* L. at four concentrations (C1–C4).

Figure 7 shows the kinetics of DPPH radical reduction in the presence of the methanolic crude extract of *Thapsia garganica* L. at four concentrations (C1–C4). The kinetic plot of residual DPPH% versus time indicates the antioxidant properties of an extract or compound in terms of the free radical scavenging capacity of that extract or compound. The rate of DPPH reduction was rapid at first and then slowed, which suggests that the radical is scavenged rapidly at the beginning, while many antioxidant molecules are still able to give away electrons or hydrogen atoms. The rate of reaction then slowed as the percentage of residual DPPH approached a plateau. The concentration dependence of the *Thapsia garganica* L. fraction extract can thus be observed in the results, with the highest concentration resulting in the highest capacity for radical scavenging and the lowest residual DPPH.

However, the lower concentrations had less of a scavenging effect and retained more residual DPPH throughout the duration of the experiment. This confirms that the efficiency of the methanolic crude extract for antioxidants increases with increasing concentration due to the availability of phenolics and flavonoids in greater quantities, which can react with free radicals. The observed good activity of the extract in the DPPH assay could be due to the availability of various bioactive components, such as phenolic compounds, flavonoids, alkaloids and other secondary metabolites derived from methanol.

The time course of DPPH radical reduction by the alkaloid extract of *Thapsia garganica* L. (extract concentrations C1–C4) is shown in Figure 8. In all four samples, the percentage of residual DPPH radical decreased relatively slowly with time, demonstrating that the alkaloid fraction displayed antioxidant activity and was capable of free radical scavenging. The alkaloid fraction showed a somewhat less drastic time course than did the crude and flavonoid extracts, with a less rapid reduction in the DPPH percentage and a slower time

course for antioxidant activity, leading to less radical scavenging. All alkaloid extracts, however, displayed concentration-dependent antioxidant activity, with extracts at higher concentrations capable of scavenging DPPH to a greater extent than those at lower concentrations. However, the most concentrated extract presented the greatest free radical scavenging efficacy and lowest percentage of residual DPPH at the end of the experiment, whereas the least concentrated extract presented a slower rate of reaction.

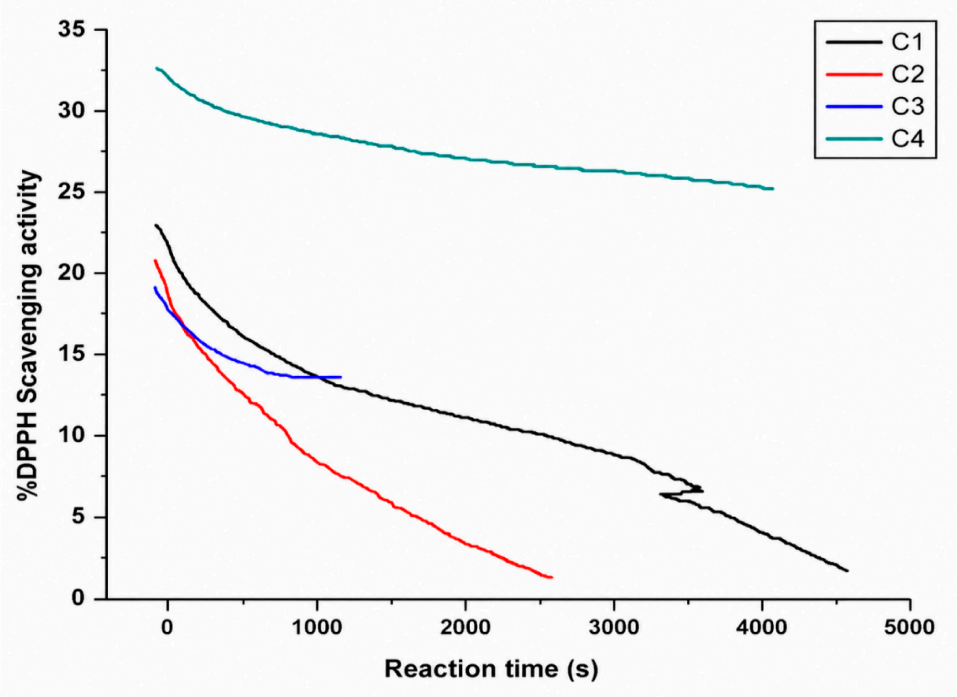

Figure 7. Kinetics of DPPH radical reduction by the methanolic crude extract of *Thapsia garganica* L. at four concentrations (C1–C4). The curves illustrate the decrease in residual DPPH (%) as a function of reaction time, demonstrating concentration-dependent antioxidant activity.

The observed antioxidant activity could be due to the presence of alkaloid compounds that can act as electrons or hydrogen acceptors; thus, the stabilization of DPPH radicals is achieved. The relatively limited reduction observed compared with the other fractions, however, suggests that alkaloids are responsible for only part of the antioxidant potential of *Thapsia garganica* L. These data provide evidence that alkaloids do exhibit antioxidant activity, but other phytochemical groups, namely, flavonoids and phenolics, are likely to be primarily responsible for the observed radical scavenging activity.

Kinetic analysis of DPPH radical reduction has also provided some insight into the antioxidant properties of the test extracts in addition to the endpoint DPPH assay. At the beginning of the experiment, a steep decrease in %DPPH was observed, which indicated an immediate interaction of the antioxidant molecules present in the test extracts with DPPH free radicals. The slope of the decrease in the DPPH free radical content gradually decreased as the reaction continued to a point where no further decrease was observed, which indicated the plateau where the reaction had most likely concluded.

The kinetic profile observed in this work suggests that the antioxidant activity of *Thapsia garganica* L. extracts is limited by the presence of both rapidly and slowly reacting antioxidants. The rapid decrease in the DPPH concentration in the first stage of the reaction is indicative of fast antioxidants that are able to donate hydrogen atoms or electrons via hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms [15,29]. The slower second phase of the reaction is indicative of the less reactive antioxidant compounds. The observed

kinetic pattern is typical of complex plant extracts containing antioxidants with different redox potentials [32]. Thus, kinetic analysis complements the conventional endpoint DPPH assay by evaluating both the antioxidant capacity and the rate of radical scavenging. The rapid initial scavenging followed by a gradual approach to equilibrium is consistent with the presence of chemically diverse antioxidant constituents with different reaction rates. However, the kinetic profiles alone do not establish synergistic interactions among the constituents [25,30].

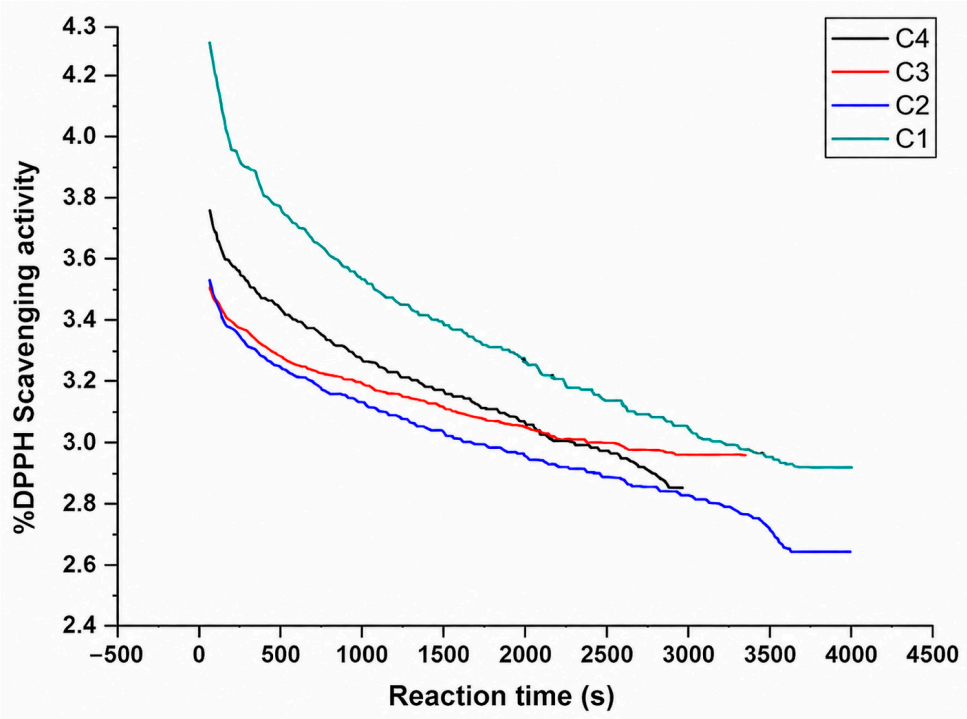

Figure 8. Kinetics of DPPH radical reduction by the alkaloid fraction at four concentrations (C1–C4). The curves show the variation in residual DPPH (%) as a function of reaction time, indicating the concentration-dependent antioxidant activity.

Among all the tested samples, the crude methanolic extract demonstrated the highest rate of reduction in the percentage of the remaining DPPH. These findings indicate that the reaction kinetics of the crude extract are greater than those of the phytochemical fractions. This behavior may be related to the broader range of antioxidant constituents present in the crude extract, which may exhibit different reaction rates toward DPPH radicals. The fractions of flavonoids and alkaloids revealed slower rates of reaction and therefore a reduction in the percentage of the remaining DPPH. The kinetics of this reaction can be explained by the mechanism of the DPPH assay, where antioxidants reduce the stable DPPH radical either through donating a hydrogen atom or an electron and thus forming a nonradical hydrazine, which is associated with decreased absorption at 517 nm. The rapid reduction in the early reaction stage followed by gradual equilibrium is typical for plant extracts containing various antioxidants with different redox potentials.

3.2. LC–MS/MS

LC–MS/MS analysis revealed a diverse profile of phenolic acids, flavonoids, flavonoid glycosides, coumarins, and other phenolic compounds in the *Thapsia garganica* L. root extract. Among the phenolic acids, ferulic acid showed the highest measured concentration (90.2 µg/mg dry extract) [30,33], followed by vanillic acid (9.26 µg/mg), caffeic acid (2.49 µg/mg), syringic acid (0.962 µg/mg), and a trace amount of gallic acid was detected. Chlorogenic acid was not detected under the applied analytical conditions [28] (Table 2).

Table 2. LC-MS/MS data of the phenolic acids, flavonoid, glycoside and other phenolic compounds detected in the *Thapsia garganica* L.

Category	Group	Compound	Molecular Formula	Molecular Mass (g/mol)	LOD (LOQ)	Range	Fragments	Concentration Found
					ng/mL	ng/mL	(*m*/*z*)	(µg/mg in Dried Extract)
Phenolic Acids	Hydroxybenzoic Acids	Vanillic acid	$C_8H_8O_4$	168.2	100 (300)	300–8000	166.828/122.800	9.26
		Gallic acid	$C_7H_6O_5$	170.1	100 (300)	300–8000	168.904/ 124.800	0.0149
		Syringic acid	$C_9H_{10}O_5$	198.17	100 (300)	300–8000	196.912/152.800	0.962
	Hydroxycinnamic Acids	Chlorogenic acid	$C_{16}H_{18}O_9$	354.3	100 (300)	300–8000	252.877/ 96.90	ND
		Ferulic acid	$C_{10}H_{10}O_4$	194.2	100 (300)	300–8000	192.766/ 133.800	90.2
		Caffeic acid	$C_9H_8O_4$	180.2	100 (300)	300–8000	178.888/ 134.900	2.49
		Rosmarinic acid	$C_{18}H_{16}O_8$	360.3	100 (300)	300–8000	358.782/ 160.800	BQ
	Acid Esters	Caffeic acid phenyl ethyl ester	$C_{17}H_{16}O_4$	284.3	100 (300)	300–8000	282.845/ 178.600	ND
Flavonoids	Flavonols (Aglycones)	Quercetin	$C_{15}H_{10}O_7$	302.2	100 (300)	300–8000	300.725/ 150.800	ND
		3-O-Methyl quercetin	$C_{16}H_{12}O_7$	316.3	100 (300)	300–8000	314.404/ 299.600	ND
		Kaempferol	$C_{15}H_{10}O_6$	286.2	100 (300)	300–8000	287.12/ 152.900 [a]	ND
	.	Myricetin	$C_{15}H_{10}O_8$	318.2	100 (300)	300–8000	316.789/ 150.800	0.003
		Galangin	$C_{15}H_{10}O_5$	270.24	100 (300)	300–8000	268.777/168.800	BQ
	Flavones (Aglycones)	Apigenin	$C_{15}H_{10}O_5$	270.2	100 (300)	300–8000	268.810/ 116.900	0.283

Table 2. *Cont.*

Category	Group	Compound	Molecular Formula	Molecular Mass (g/mol)	LOD (LOQ)	Range	Fragments	Concentration Found
					ng/mL	ng/mL	(*m*/*z*)	(µg/mg in Dried Extract)
		Luteolin	$C_{15}H_{10}O_6$	286.2	100 (300)	300–8000	284.726/ 132.800	0.008
		Hesperetin	$C_{16}H_{14}O_6$	302.3	100 (300)	300–8000	300.746/ 163.700	BQ
	Flavan-3-ols	Catechin	$C_{15}H_{14}O_6$	290.3	100 (300)	300–8000	288.837/ 244.600	BQ
Glycosides	Flavonol Glycosides	Rutin	$C_{27}H_{30}O_{16}$	610.5	100 (300)	300–8000	608.799/ 299.700	BQ
	Flavone Glycosides	Luteolin 7-O-glucoside	$C_{21}H_{20}O_{11}$	448.4	100 (300)	300–8000	446.651/ 284.600	ND
		Apigenin 7-O-glucoside	$C_{21}H_{20}O_{10}$	432.4	100 (300)	300–8000	430.757/ 267.700	0.273
	Flavanone Glycosides	Hesperidin	$C_{28}H34O15$	610.6	100 (300)	300–8000	608.785/ 300.700	0.280
Other Phenolics	Coumarins	Coumarin	$C_9H_6O_2$	146.1	300 (1000)	1000–10,000	147.986/ 91.000 [a]	ND
		7-Hydroxy coumarin	$C_9H_6O_3$	162.1	300 (1000)	1000–10,000	162.878/ 107.000 [a]	ND
Organic Acids	Aliphatic Acids/Vitamins	Ascorbic acid	$C_6H_8O_6$	176.1	100 (300)	300–8000	174.854/ 114.700	12.3
	Stilbenoid	Salvianolic acid A	$C_{26}H_{22}O_{10}$	494.2	100 (300)	300–8000	492.732/ 294.70	ND

BQ-below LOQ, [a] = analysis in positive mode, ND = not detected, µg/mg: refers to µg of compound per mg of dry extract (calculated).

Several flavonoids and other phenolic compounds, including myricetin, luteolin, galangin, Hesperetin and catechin were present in trace amounts (below the lower limit of quantification). The calculated concentration of apigenin was 0.283 µg/mg in extract. Quercetin, 3-O-methylquercetin, and kaempferol were not detected. Among the glycosylated flavonoids, apigenin 7-O-glucoside and hesperidin were detected at 0.273 and 0.280 µg/mg, respectively, while rutin and luteolin 7-O-glucoside were below the quantification limit or not detected under the applied conditions.

Ascorbic acid was also detected at 12.3 µg/mg, representing another relatively abundant compound in the analyzed extract. In contrast, coumarin, 7-hydroxycoumarin, and salvianolic acid A were not detected. The LC–MS/MS results indicate that the extract contains a broad range of phenolic constituents, with ferulic acid showing the highest quantified concentration among the phenolic compounds analyzed. The observed diversity of phenolic acids and flavonoids provides a chemical basis for further investigation of the biological properties of the extract; however, the contribution of individual constituents to the observed antioxidant activity cannot be established from the LC–MS/MS data alone.

3.3. GC–MS

GC–MS analysis revealed distinct chemical profiles for the methanolic (Table 3) and *n*-hexane (Table 4) extracts of *Thapsia garganica* L., indicating differences in the relative distribution of volatile and semi-volatile constituents according to the extraction solvent.

Table 3. GC–MS analysis of the methanolic extract of *Thapsia garganica* L. plants.

Peak #	Retention Time (min)	Compound Name	Area (%)	Kovats Index (KI)
1	6.744	Hexanoic acid	0.36	1000
2	11.118	Octanoic acid	3.67	1264
3	11.416	1-Dodecene	0.52	1282
4	15.631	1-Dodecanol	0.97	1537
5	17.85	2,4-Di-tert-butylphenol (tentative identification)	4.84	1672
6	18.598	1,2,3-trimethoxy-5-(2-propenyl)-Benzene	0.79	1717
7	18.894	1,5-dimethyl-Naphthalene	0.25	1735
8	19.415	1-Pentadecene	1.08	1766
9	22.26	n-Hexadecanoic acid	3.00	1938
10	22.413	1-(7-Methoxy-2H-1,3-benzodioxol-5-yl)propan-1-one	1.23	1947
11	22.815	1-Octadecene	0.54	1971
12	23.912	Cis-Lanceol	0.31	2036
13	24.61	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.19	2077
15	24.868	Hexadecanoic acid, methyl ester	0.55	2092
17	25.743	4-Biphenyl methyl carbinol	0.43	2123
18	25.897	1-Hexacosene	0.19	2131
21	27.23	1-Hexadecanol	2.51	2195

Table 3. *Cont.*

Peak #	Retention Time (min)	Compound Name	Area (%)	Kovats Index (KI)
22	27.325	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.71	2200
23	27.417	9-Octadecenoic acid, methyl ester, (E)-	0.33	2204
24	27.857	9,12-Octadecadienoic acid (Z,Z)-	0.74	2225
25	27.935	Oleic Acid	0.46	2229
27	28.259	Pentadecanoic acid	0.32	2245
28	28.555	Hexadecanamide	0.24	2259
38	32.108	Octadecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]	0.23	2432
39	32.799	Unidentified	0.71	2465
41	33.429	Glycidyl palmitate	24.48	2496
45	34.361	9-Tetradecenal, (Z)-	0.25	2541
49	36.275	Unidentified	0.62	2634
53	40.705	3,3-Diethoxy-1-propyne	9.98	2850
57	46.706	Stigmasterol	2.68	3141
58	48.91	γ-Sitosterol	2.22	3248

Table 4. GC–MS analysis of the hexane extract of *Thapsia garganica* L. plants.

Peak #	Retention Time (min)	Compound Name	Area (%)	Kovats Index (KI)
1	6.914	β-Myrcene	0.27	1000
2	11.075	Octanoic acid	0.34	1251
3	18.611	1,2,3-trimethoxy-5-(2-propenyl)-Benzene	0.14	1707
8	25.267	Heneicosane	0.06	2109
11	26.312	Isopropyl palmitate	0.12	2151
50	40.873	Unidentified	21.97	2858
56	45.961	Campesterol	3.65	3105
57	46.865	Stigmasterol	10.30	3149
58	49.029	γ-Sitosterol	5.97	3254
59	49.685	(Unidentified peak)	12.07	3286
60	50.393	Cholest-4-en-3-one	2.65	3320
62	51.450	Ergosta-4,22-dien-3-one	1.98	3372

Although the compositions and relative abundances were very different, both extracts contained a large diversity of bioactive compounds; this pattern is consistent with polar and nonpolar solvents working well together to recover a range of phytochemicals.

Representative GC–MS chromatograms of the methanolic and *n*-hexane extracts are provided in the Supplementary Materials (Figure S1 and Figure S2, respectively). Selected compounds of interest are numbered on the chromatograms and correspond to the compound identifications reported in Tables 3 and 4.

The methanolic extract showed a different distribution of GC–MS-detected constituents, with fatty acids and their derivatives, hydrocarbons, phenolic-related compounds, and

sterol-related compounds represented in the chromatographic profile. Fatty acids are among the common secondary metabolites found in medicinal plants and play vital structural and physiological roles. In addition to playing crucial roles as components of cell membranes, some saturated and unsaturated fatty acids have been reported to have antioxidant, anti-inflammatory, antimicrobial and cytoprotective effects [13,34]. Glycidyl palmitate was the main component found, making up 24. 48% of the entire peak area (KI 2496). Next in line was 3,3-diethoxy-1-propyne (9.98%; KI 2850), followed by 2,4-di-tert-butylphenol (tentative identification) (4.84%; KI 1672), octanoic acid (3.67%; KI 1264), n-hexadecanoic acid (3.00%; KI 1938), stigmasterol (2.68%; KI 3141), 1-hexadecanol (2.51%; KI 2195), and γ-sitosterol (2.22%; KI 3248). Although 2,4-di-tert-butylphenol has been reported as a naturally occurring metabolite in various plants and other organisms [35], its origin in the present extract cannot be confirmed from the GC–MS data alone. Therefore, a possible exogenous or artefactual origin should be considered when interpreting this identification. Other substances that were found included hexanoic acid, 1-dodecene, 1-dodecanol, 1,2,3-trimethoxy-5-(2-propenyl) benzene, 1,5-dimethylnaphthalene, 1-pentadecene, 1-octadecene, cis-lanceol, methyl ester of hexadecanoic acid, 4-biphenyl methyl carbinol, 1-hexacosene, various unsaturated fatty acid methyl esters, oleic acid, pentadecanoic acid, hexadecanamide, and stigmasterol and γ-sitosterol. The detection of n-hexadecanoic acid, its methyl ester, oleic acid, and other related fatty acid compounds show a significant presence of lipid-related substances in the methanolic extract profile.

The *n*-hexane extract contained several prominent constituents distributed among sterol-, fatty acid/ester-, and other chemical groups [36]. The largest chromatographic peak corresponded to an unidentified constituent, accounting for 21.97% of the total peak area. Its identity could not be established confidently from the available mass-spectral and retention-index evidence. Sterol-related constituents were also prominent, particularly stigmasterol (10.30%; KI 3149), γ-sitosterol (5.97%; KI 3254), and campesterol (3.65%; KI 3105). Other reported constituents included ergosta-4,22-dien-3-one (1.98%; KI 3372) and cholest-4-en-3-one (2.65%; KI 3320). An additional unidentified peak represented 12.07% of the total peak area, indicating that some components of the chromatographic profile remained unassigned. Several minor constituents were also detected, including β-myrcene (0.27%), octanoic acid (0.34%), 1,2,3-trimethoxy-5-(2-propenyl) benzene (0.14%), heneicosane (0.06%), and isopropyl palmitate (0.12%). These compounds contributed relatively small proportions of the total chromatographic profile compared with the major constituents.

A comparison of the two extracts revealed differences in their chromatographic profiles. The *n*-hexane extract contained prominent sterol-related peaks and a major unidentified peak representing 21.97% of the total chromatographic area. In contrast, the methanolic extract was characterized by glycidyl palmitate, fatty acid-related constituents, and other chromatographic components. Both extracts contained stigmasterol and γ-sitosterol, although their relative peak areas were higher in the *n*-hexane extract (10.30% and 5.97%, respectively) than in the methanolic extract (2.68% and 2.22%, respectively). These differences may reflect the influence of solvent polarity on the extraction and distribution of GC–MS-detected constituents. However, peak-area percentages represent relative chromatographic abundance and should not be interpreted as absolute concentrations or evidence of biological activity.

The GC–MS analysis showed that the methanolic and *n*-hexane extracts had different chromatographic profiles. The *n*-hexane extract contained prominent sterol-related constituents and a major unidentified peak, whereas the methanolic extract showed substantial peaks associated with glycidyl palmitate, fatty acid-related constituents, and other compounds. These differences indicate that the extraction solvent influenced the relative distribution of the detected constituents. Compound assignments were interpreted cau-

tiously based on the available mass-spectral and retention-index evidence, particularly in the absence of confirmation using authentic reference standards.

The GC–MS profile obtained in this investigation is largely in line with earlier findings about *Thapsia garganica* and other Apiaceae family members. The relative abundances of various chemical classes, however, differ in a few ways. Athmouni & Co. (2015) [28] reported that extracts of *T. garganica* presented greater antioxidant activity and higher concentrations of phenolic compounds than nonpolar extracts did, demonstrating the impact of solvent polarity on phytochemical extraction. Moreover, Jmii et al. (2020) [6] reported that the main components of *T. garganica* were terpenoids, fatty acids, coumarins, and other bioactive metabolites. Other Apiaceae species have also been found to have GC–MS profiles rich in fatty acids and terpenoids, which support their antimicrobial, anti-inflammatory, and antioxidant properties [34]. Variations in geographic origin and environmental conditions may contribute to differences in phytochemical profiles reported among studies [9]. Overall, these findings support the reliability of the present GC–MS analysis and confirm that *Thapsia garganica* L. is a rich source of structurally diverse bioactive compounds.

The GC–MS analysis of *Thapsia garganica* L. extracts showed several types of chemicals that have been noted in *T. garganica* and other plants in the Apiaceae family. The *n*-hexane extract was characterized by prominent sterol-related constituents and a major unidentified chromatographic peak. In contrast, the methanolic extract had a greater amount of glycidyl palmitate, compounds linked to fatty acids, 2,4-di-tert-butylphenol, and other non-aromatic compounds. Previous studies have also found fatty acids, terpenoid compounds, and phytosterols in *T. garganica* and other Apiaceae plants [6,26,36,37]. Differences in the amounts of these compounds may reflect changes due to the type of extraction solvent, the method of analysis, the growth stage of the plant, where it was grown, and the environmental conditions [38]. Overall, the results from the GC–MS show that the roots of *T. garganica* have a wide variety of chemicals, but specific compound identifications need to be made based on the existing mass-spectral and retention-index data.

An important limitation of the present study is the known toxicity of *Thapsia garganica* and its roots. The species contains thapsigargin, a highly bioactive sesquiterpene lactone that is a potent inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and is associated with cytotoxic effects [2,39]. The previous literature has also described the toxic and irritant properties of *T. garganica* and cautioned against considering the plant as a conventional medicinal remedy without appropriate safety assessment [2]. Therefore, the antioxidant activity observed in the present in vitro study should not be interpreted as evidence of the safety or therapeutic suitability of the root extracts. Further investigations are required to determine the levels of thapsigargin and other potentially toxic constituents, establish dose–response relationships, and comprehensively evaluate the safety and toxicity of the extracts before any therapeutic, nutraceutical, or other direct human application can be considered.

4. Conclusions

Using complementary spectrophotometric, GC–MS, and LC–MS/MS methods, this study provides thorough phytochemical and antioxidant characterization of wild *Thapsia garganica* L. roots collected from northwestern Algeria. The crude methanolic extract exhibited greater DPPH radical-scavenging activity than the flavonoid and alkaloid fractions under the experimental conditions. These results indicate that *Thapsia garganica* L. has antioxidant activity. Instead of individual compounds, *Thapsia garganica* L. is associated primarily with the combined action of several phytochemical classes. The presence of both fast-acting and slow-acting antioxidants was indicated by kinetic analysis, which revealed a fast initial scavenging phase followed by a slower reaction phase. LC–MS/MS identified ferulic acid,

ascorbic acid, vanillic acid, caffeic acid, and apigenin as the major quantified metabolites, whereas GC–MS profiling revealed marked chemical diversity, including sterol-related, fatty acid-derived, terpenoid-related, and phenolic constituents with potential biological relevance. Taken together, these findings indicate that *Thapsia garganica* roots have measurable in vitro antioxidant activity. However, due to the known toxicity of the species and the presence of thapsigargin, further safety and toxicity studies are required before any therapeutic, nutraceutical, or functional application can be considered. Future studies should focus on bioassay-guided fractionation and synergy testing, validation of GC–MS identifications using authentic standards, fully validated quantitative LC–MS/MS analysis, comparison of extracts from different organs, seasons, and geographic origins, and complementary antioxidant assays (ABTS, FRAP, and ORAC) and cell-based oxidative-stress models.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/molecules31224101/s1, Figure S1: Representative GC–MS chromatogram of the methanolic extract of Thapsia garganica roots; Figure S2: Representative GC–MS chromatogram of the n-hexane extract of Thapsia garganica roots; Table S1: Multiple reaction monitoring (MRM) transitions, ionization polarity, dwell times, and compound-specific MS/MS acquisition parameters used for the LC–MS/MS analysis of the identified phytochemicals in Thapsia garganica root extracts.

Author Contributions: K.M.A.A. contributed to conceptualization, methodology, investigation, formal analysis, data curation, visualization, writing of the original draft, writing—review and editing, supervision, and project administration. B.N. contributed to investigation, methodology, validation, data curation, and writing—review and editing. S.T.A.-H. contributed to investigation, and writing—review and editing. A.K.S. contributed to formal analysis, methodology, and writing—review and editing. S.A. (Saad Amel) contributed to investigaS1tion, sample collection, and data curation. R.M.N. contributed to investigation, formal analysis, and validation. S.A. (Seghir Abdelhadi) contributed to methodology, resources, and formal analysis. A.A.-S. contributed to resources, and formal analysis. B.L. contributed to formal analysis, and writing—review and editing. Y.A.-M. contributed to investigation and review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The authors declare that ethics committee approval is not needed for this study.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request from the authors.

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

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