

Valorization of Haloxylon scoparium aqueous extract by chromatographic separation on Sephadex LH-20 column

Sara Haida

Ibn Tofail University

Mohamed Allaoui

Ibn Tofail University

Hayat Elwardani

Ibn Tofail University

Omar Dagdag

Gachon University

Elyor Berdimurodov

elyor170690@gmail.com

National University of Uzbekistan

Abdelaziz Ramadane Kribii

Ibn Tofail University

Abderahim Kribii

Ibn Tofail University

Khasan Berdimuradov

Karshi state technical university

Research Article

Keywords: Medicinal plants, Haloxylon scoparium, Sephadex LH-20, antioxidant activity, alkaloids

Posted Date: November 14th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7977722/v1>

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Additional Declarations: No competing interests reported.

Abstract

Many scientific researches, across various specialties, have been developed to investigate compounds isolated from medicinal plants and agri-food products. In this context, this study focuses on the valorization of *Haloxylon scoparium*, a plant native to southeast Morocco. The objective is to isolate and characterize fractions of plant extracts that exhibit significant antioxidant power using chromatographic separation methods. This work represents a continuation of previously conducted and published research on the same plant. Three separation series were performed on the aqueous extract (AE-HS_{AP}) (IC₅₀ = 0.65 mg/mL) using exclusion chromatography on a Sephadex LH-20 column, resulting in a total 19 of fractions. The antioxidant power of these fractions was assessed by the DPPH test. The results revealed some fractions exhibiting higher antioxidant activity compared to the initial extract. Notably, the fraction (F4") registered an antioxidant activity (IC₅₀ = 0.078 mg/mL) close to that of the reference compound ascorbic acid (IC₅₀ = 0.08 mg/mL). Based on the phytochemical tests conducted in the previous study, alkaloids and polyphenols were identified in the aqueous extract. GC-MS analysis of fraction F4" revealed a prominent presence of three alkaloid classes: tetrahydroisoquinolines (THIQ), phenylethylamines (PHEA), and tryptamines (TRPM). The most abundant compound detected was salsolinol, with a concentration of 252.09 mg per 100 g of dry matter. The high antioxidant activity observed in fraction F4" may be largely attributed to salsolinol. Furthermore, this antioxidant capacity could also result from other polar compounds, such as polyphenols present in the fraction, or from a synergistic effect among these constituents.

1. Introduction

In the present times, the modern medicine depends on the using the bioactive compounds, which are extracted form the plants. Flor example, the various therapeutic compounds were extracted from the plants. Because, these compounds have the versatile biological activities that depends the unique characteristics, such as, the aromatic various and functional groups. Therefore, the plant extracts are key materials in the innovation of modernt pharmacy [1].

The shrub *Haloxylon scoparium* is family member of Chenopodiaceae, it grows in arid and semi-arid regions. It was be adapted to saline soils and sandy regions in North Africa and the Middle East [2, 3]. The *Haloxylon scoparium* contains a variety of chemical constituents, including saponosides and phenolic compounds, which are aromatic rich compounds [4–6]. Additionally, simple tetrahydroisoquinoline alkaloids are frequently present in the Chenopodiaceae family, and have been isolated from different *Haloxylon* species [7–9]. Recent studies have increasingly examined the biochemical and pharmacological properties of plant-derived alkaloids, particularly tetrahydroisoquinoline alkaloids (THIQ), which are known to exert beneficial effects on the human nervous system [10–12]. *Haloxylon scoparium* is mostly used in traditional medicine in the several countries, because, its broad pharmacological activities, including antibacterial, anti-molluscicidal, and larvicidal effects [13–15]. Notably, this plant also demonstrates strong antioxidant potential [13, 16].

Antioxidants play a vital role in preventing or reducing cellular damage caused by free radicals—highly reactive molecules that contribute to oxidative stress and the development of various diseases [17].

Size exclusion chromatography, also referred to as gel permeation, gel exclusion, gel filtration, or molecular sieve chromatography, is a powerful method for separating molecules in extracts based on size. Its effectiveness depends on factors such as the pore size of the stationary material, the choice of mobile phase, and the column length [18]. This separation was widely used for the fractionation of various compounds, including phenolic compounds [19–21], steroids [22, 23], and alkaloids [24, 25], into distinct fractions. In this study, it was employed Sephadex LH-20 to develop a method for separating the aqueous extract of *Haloxylon scoparium*, with the goal of isolating fractions that exhibit enhanced antioxidant activity.

2. Material and methods

2.1. Plant material

Haloxylon scoparium, a member of the Chenopodiaceae family, is native to southeastern Morocco, where it grows at an altitude of 870 m. The plant material was collected during its flowering season at the coordinates 32°10'18"N, 1°13'34"W. The aerial parts were carefully cleaned, dried in a ventilated oven at 40°C for 24 hours, and then ground into fine particles of approximately 300 µm. The powdered material was stored until further use.

2.2. Preparation of the aqueous extract

The aqueous extract (AE-HSAP) of *Haloxylon scoparium* was obtained through maceration followed by liquid–liquid extraction with solvents of increasing polarity [16]. Briefly, 100 g of the powdered aerial parts were mixed with a 30:70 (v/v) water–acetone solution and macerated at room temperature for 2 hours. The mixture was then filtered, and the filtrate was concentrated using a rotary evaporator before being freeze-dried to yield the crude extract (AWE-HSAP). For further fractionation, the extract was dissolved in distilled water and successively partitioned with dichloromethane, ethyl acetate, and butanol. Each solvent fraction was evaporated and lyophilized, resulting in four extracts: dichloromethane (DE-HSPA), ethyl acetate (EAE-HSPA), butanol (BE-HSPA), and aqueous (AE-HSPA). Among these, the aqueous extract was chosen as the primary focus of this study due to its higher yield, and it was subsequently subjected to size-exclusion chromatography.

2.3. Fractionation of the aqueous extract by liquid chromatography on Sephadex LH-20

Phytochemical analyses of the AE-HS_{PA} extract revealed a high concentration of polyphenols and alkaloids [16]. In order to valorize this extract, we carried out fractionation using a Sephadex LH-20 column. The main objective was to investigate its chemical composition, especially in alkaloids, and identify fractions with a higher antioxidant power.

A chromatographic column (1.5 cm x 64 cm) filled with Sephadex LH-20, as stationary phase was prepared. To swell the Sephadex, 15 g of it was soaked in 100 mL of distilled water for 1 hour. Then, 1 g of freeze-dried extract (AE-HS_{AP}) dissolved in 5 mL of distilled water was carefully deposited on the top of the column. Elution was performed by a secession of solvents of decreasing polarity, namely water, acetone and methanol.

Fractions volumes of 12 mL were collected in glass tubes and analyzed using thin-layer chromatography (TLC) to group those with similar properties. The TLC plates were developed under UV light at 365 nm and sprayed with Dragendorff's reagent (an alkaloid developer). The resulting fractions were then dried under reduced pressure, weighed and stored in pillboxes at low temperature.

Three successive separations were carried out by eluting each time with solvent mixtures of decreasing polarity, following the scheme shown in Fig. 1. Fractions F2 and F2' were selected due to their significant quantities:

1. Separation of the extract (AE-HS_{AP}): elution is performed with water, acetone and methanol. Seven fractions are obtained (from F1 to F7).
2. Separation of fraction F2: the elution is carried out with methanol and different methanol/water ratios (90/10, 80/20, 70/30 and 50/50), followed by water. Eight fractions are obtained (from F1' to F8').
3. Separation of fraction F2': the elution is carried out by the mixture of methanol/water (50/50) and water. Four fractions are obtained (from F1" to F4").

Figure 1.

2.4. Evaluation of the antioxidant activity

To determine the antioxidant activity of the different fractions studied, we used the DPPH• (1,1- diphenyl-2-picryl hydrazyl) radical reduction method, as described by Olugbami et al. [26], with some modifications. In this regard, a 76 µM (0.03 mg/mL) solution of DPPH was prepared in ethanol. Then, 2 mL of this solution was added to 0.1 mL of different extracts at varying concentrations (0 to 3 mg/mL). A parallel blank sample was prepared, consisting of only the DPPH solution and ethanol. The mixture was shaken and incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm using a spectrophotometer (UV-2005, Selecta). Ascorbic acid was used as a positive control.

The percent inhibition of the DPPH• radical is calculated using the following relationship:

$$\% \text{ of inhibition} = [(A_b - A_s) / A_b] \times 100.$$

A_b: Absorbance of the blank, A_s: Absorbance of the sample.

To evaluate the antioxidant activity of a sample, the IC₅₀ value is used, which indicates the concentration of sample required to inhibit 50% of the DPPH radical. This value is obtained by using the linear part of the regression curve, which displays the change in the inhibition percentage as a function of the various concentrations for each sample, according to the equation $y = ax + b$.

$$IC_{50} = (50 - b) / a$$

a: Slope of the line, b: Ordinate at the origin.

2.5. GC/MS analysis

Before analysis by GC-MS coupling, the samples were acetylated according to the protocol described by Haida et al. [9]. The analysis was carried out at the University Center of Analysis, Expertise, Technology Transfer and Incubation at the Ibn Tofail University. The equipment used is a Bruker 456-GC, EVOQ TQ system with a Rxi-5Sil MS capillary column (30m × 0.25mm ID × 0.25µm df), operating in electronic impact mode with an ionization energy of 70 eV. The injector was maintained at 280°C and the ion source at 250°C. The furnace was programmed to follow a temperature gradient starting at 60°C for 10 min, then increases at a rate of 10°C/min to 80°C, where it remains stable for 2 min. Then, a second temperature rise of 5°C/min was programmed to reach 300°C, followed by a 300°C isotherm for 9 min. Compound identification and mass spectra were performed using the NIST (National Institute Standard and Technology) mass spectra database.

3. Results and discussion

3.1. Results of antioxidant activity

Previous studies shows that the extracts from *Haloxylon scoparium* by the liquid-liquid separation of the root extracts have good antioxidant activity. Consequently, root-derived extracts typically demonstrate higher activity than those obtained from the aerial parts of the plant [16].

To enhance the antioxidant potential of the aerial parts of the plant, the aqueous extract (AE-HSAP) was selected for this study. This extract was chosen due to its high content (157.53 mg/g DM) and antioxidant capacity (IC₅₀ = 0.65 mg/mL), which is very close to that of the original extract AWE-HSAP (IC₅₀ = 0.66 mg/mL) [16]. Fractionation was performed using a Sephadex LH-20 column with the aim of isolating fractions exhibiting stronger antioxidant activity.

The initial separation of AE-HSAP yielded seven fractions of different quantities. Among them, fraction F2 stood out with a significant yield (143.35 mg/g DM) and the presence of alkaloids. This fraction was subjected to further purification, resulting in eight subfractions. The most abundant of these (82.64 mg/g DM) was then fractionated once more, producing four smaller fractions (Table 1).

Table 1
Quantitative results and antioxidant power of the different fractions
obtained by separating the extract (AE-HS_{AP}) on Sephadex LH-20
column.

Fractions	Masse (mg/g DM)	TLC (Dragendorff)*	Antioxydant activity (IC ₅₀ mg/mL)
Separation of (AE-HS_{AP}) (IC₅₀ = 0.65 mg/mL)			
F1	1.57	-	2.851
F2	143.35	2 oranges spots	1.402
F3	4.72	-	0.173
F4	0.94	-	0.243
F5	5.04	-	1.788
F6	0.78	-	0.615
F7	1.10	-	0.215
Separation of F2 (IC₅₀ = 1.402 mg/mL)			
F1'	8.43	-	0.614
F2'	82.64	1 orange spot	0.636
F3'	3.37	-	1.389
F4'	10.12	-	1.448
F5'	5.06	-	11.911
F6'	6.74	-	17.885
F7'	16.86	-	8.946
F8'	8.76	-	15.126
Separation of F2' (IC₅₀ = 0.636 mg/mL)			
F1"	4.96	-	0.868
F2"	22.04	1 orange spot	1.258
F3"	41.32	1 orange spot	0.940
F4"	9.64	1 orange spot	0.078

The antioxidant activity of the different fractions obtained was evaluated by the DPPH radical reduction method. The results presented in Table 1 indicate that fractionating the aqueous extract (AE-HS_{AP}) using the Sephadex LH-20 column enhanced its antioxidant activity by generating fractions with greater

antioxidant capacities. Examples include fractions F3 (IC₅₀ = 0.173 mg/mL), F4 (IC₅₀ = 0.243 mg/mL) and F7 (IC₅₀ = 0.215 mg/mL). The most interesting result is the F4" fraction which has an antioxidant power (IC₅₀ = 0.078 mg/mL) comparable to that of ascorbic acid (IC₅₀ = 0.08 mg/mL). Analysis of this fraction on a TLC plate by the Dragendorff reagent revealed the presence of alkaloid compounds.

The results of this study demonstrate once again that it is possible to improve the antioxidant power of an extract by separating it into daughter fractions, each with an antioxidant activity that depends on its chemical composition. A phenomenon of synergy between the different compounds of an extract can also explain the results obtained.

The initial separation of the extract (AE-HS_{AP}) resulted in seven fractions of different quantities. The second fraction (F2), which contained alkaloids and had a significant quantity of 143.35 mg/g DM, underwent further fractionation, leading to eight fractions. Among them, the fraction with the highest amount (82.64 mg/g DM) was subjected to additional fractionation, resulting in four fractions (Table 1).

The antioxidant activity of the fractions obtained was evaluated using the reduction capacity of the radical DPPH. As shown in Table 1 and Fig. 2, the fractionation of the aqueous extract (AE-HS_{AP}) on the Sephadex LH-20 column enhanced its antioxidant activity by providing fractions with higher antioxidant capacities. Notably, fractions F3 (IC₅₀ = 0.173 mg/mL), F4 (IC₅₀ = 0.243 mg/mL), and F7 (IC₅₀ = 0.215 mg/mL) exhibited significant antioxidant capacities. The most remarkable result was observed in fraction F4, which demonstrated an antioxidant power (IC₅₀ = 0.078 mg/mL) comparable to that of ascorbic acid (IC₅₀ = 0.08 mg/mL). Analysis of this fraction on a TLC plate using the Dragendorff reagent confirmed the presence of alkaloid compounds.

These findings once again demonstrate that it is possible to enhance the antioxidant power of an extract by separating it into daughter fractions, each of which exhibiting antioxidant activity influenced by its chemical composition. A phenomenon of synergy between the different compounds in an extract can also explain the obtained results.

Figure 2.

Table 1.

3.2. Results of the F4" fraction analysis

The fraction F4", which exhibited an antioxidant power comparable to that of ascorbic acid, was analyzed by GC-MS after an acetylation reaction (Fig. 3). Ten compounds of different structures were identified in this fraction (Table 2). Alkaloids constitute the major compounds (762.99 mg/100g DM), and are categorized into three groups: tetrahydroisoquinolines (THIQ) 4, 8, 9, 10 (404.88 mg/100g DM), phenylethylamines (PHEA) 2, 3, 5, 6 (246 mg/100g DM), and tryptamines (TRPM) 7 (112.11 mg/100gMS). The acetylated derivative of salsolinol 10 (6,7-diacetoxy-2-acetyl-1-methyl-1,2,3,4-tetrahydroisoquinoline) is the most abundant compound (252.09 mg/100g DM).

Table 2. Chemical composition of the F4" fraction.

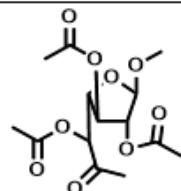
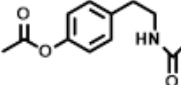
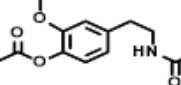
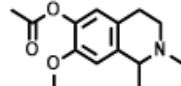
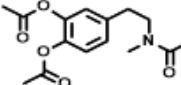
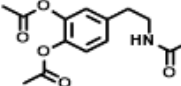
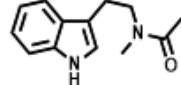
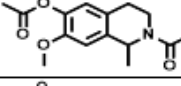
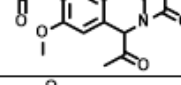
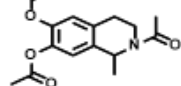
Nº	Chemical compound	RT (min)	Chemical structure	Mass (mg/100gDM)
1	Methyl(methyl-2,3,4-tri-o-acetyl-beta.d-galactofuranosid)uronate	44.36		7.23
2	N,O-diacetyltyramine	44.97		20.44
3	acetamide, N-[2-[4-(acetyloxy)-3-methoxyphenyl]ethyl]-	47.68		5.30
4	O-acetyl-N-methylsalsoline	48.33		13.59
5	4-(2-(N-methylacetamido)ethyl)-1,2-phenylene diacetate	48.80		73.45
6	4-(2-acetamidoethyl)-1,2-phenylene diacetate	50.02		146.81
7	N-methyl-N-acetyltryptamine	50.52		112.11
8	N,O-diacetylsalsoline	50.82		77.02
9	1,2-diacetyl-7-methoxy-1,2,3,4-tetrahydroisoquinolin-6-yl acetate	51.45		62.18
10	6,7-diacetoxy-2-acetyl-1-methyl-1,2,3,4-tetrahydroisoquinoline	52.72		252.09

Figure 3.

Figure 4 represents the mass spectrum of 6,7-diacetoxy-2-acetyl-1-methyl-1,2,3,4-tetrahydroisoquinoline (or acetylated salsolinol). The fragmentation mechanisms of this compound in mass spectrometry are illustrated in Fig. 5. The fragment ion with m/z 290 is formed directly generated from the molecular ion (M^+ 305) through a radical break of the type Subsequently, three hydrogen rearrangements (rH) involving four centers result in the ions with m/z 248, 206 and 164, after elimination of a ketene molecule (CH_2CO).

Figure 4.

Figure 5.

The high antioxidant activity observed in the F4" fraction may be attributed to the presence of salsolinol in significant quantity. This compound possesses a chemical structure that includes two hydroxyl groups attached to the aromatic nucleus (phenolic type), which enables the release of hydrogen atoms for the reduction of free radicals. A study published by Maruyama et al. demonstrated that salsolinol could protect brain neurons against oxidative damage induced by hydrogen peroxide. The researchers suggested that this protective effect might be due to salsolinol's ability of to scavenge free radicals and inhibit the production of reactive oxygen species [27]. Therefore, it would be interesting to investigate the antioxidant power of salsolinol (or even to synthesize it) to test this hypothesis.

146.81mg/100g DM 4-(2-acetamidoethyl)-1,2-phenylene diacetate 6 (or acetylated dopamine) was found, it contribute to the antioxidant activity observed in the F4" fraction. The study by Kanazawa et al. [28] revealed that dopamine exhibits strong antioxidant properties, implying that the antioxidant activity observed in the banana extract may be primarily due to this compound. Furthermore, the antioxidant activity of the F4" fraction could also be linked to other polar constituents within this fraction, which are not detectable by GC-MS, or to a synergistic interaction among these compounds.

Table 2.

Conclusion

This study demonstrated that the aqueous extract of *Haloxylon scoparium* (AE-HSAP) possesses notable antioxidant potential ($IC_{50} = 0.65$ mg/mL), which was further enhanced through sequential fractionation on a Sephadex LH-20 column. From the 19 obtained fractions, several showed stronger antioxidant activities, particularly F3 ($IC_{50} = 0.173$ mg/mL), F4 ($IC_{50} = 0.243$ mg/mL), and F7 ($IC_{50} = 0.215$ mg/mL). The most remarkable result was fraction F4", which exhibited an IC_{50} of 0.078 mg/mL, essentially equivalent to the reference antioxidant ascorbic acid ($IC_{50} = 0.08$ mg/mL).

Chemical characterization of F4" by GC-MS revealed a dominant alkaloid profile, with a total alkaloid content of 762.99 mg/100 g DM, distributed across tetrahydroisoquinolines (404.88 mg/100 g DM), phenylethylamines (246 mg/100 g DM), and tryptamines (112.11 mg/100 g DM). Salsolinol emerged as the most abundant compound at 252.09 mg/100 g DM, followed by acetylated dopamine (146.81 mg/100 g DM) and other alkaloid derivatives.

The strong antioxidant capacity of F4" can be attributed not only to salsolinol and dopamine but also to potential contributions from undetected polar polyphenols and synergistic interactions among constituents. Collectively, these findings underscore *Haloxylon scoparium* as a promising source of bioactive compounds with antioxidant activity comparable to established standards, highlighting its potential for future applications in nutraceuticals and therapeutic formulations.

Declarations

Funding Declaration

No funding

Conflict of Interest

(COI) statement

The authors declare that they have no conflict of interest.

Author Contribution declaration

S.H. designed and performed the experiments, analyzed the data, and drafted the initial manuscript. M.A. supervised the project, contributed to the experimental design, and revised the manuscript. H.E. assisted with plant collection, sample preparation, and biological testing. O.D. contributed to data interpretation, statistical analysis, and manuscript editing. E.B. participated in data analysis, provided critical discussion, and refined the manuscript text. A.R.K. and A.K. assisted with experimental work, literature review, and data verification. All authors read and approved the final manuscript.

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Figures

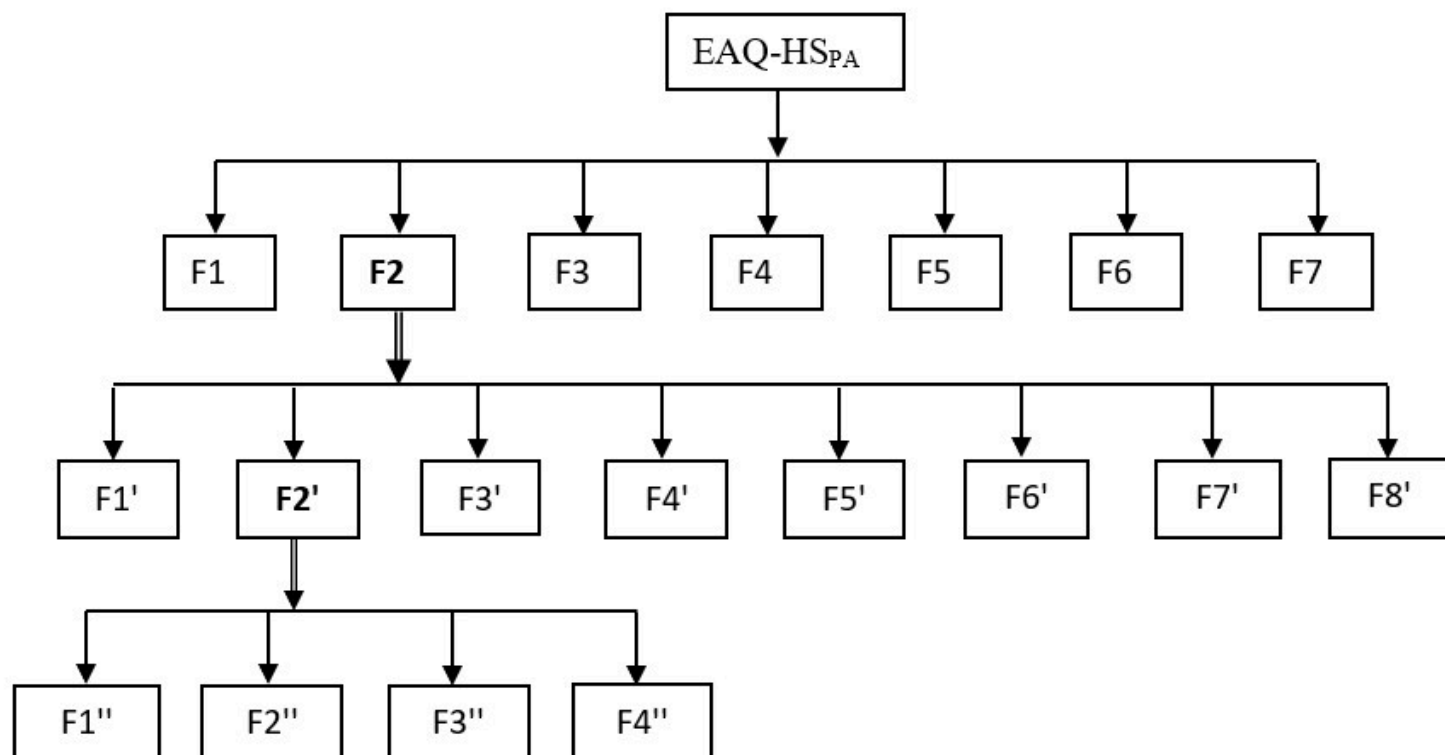


Figure 1

Fractionation scheme of the extract (AE-HS_{AP}) using liquid chromatography on Sephadex LH-20 gel.

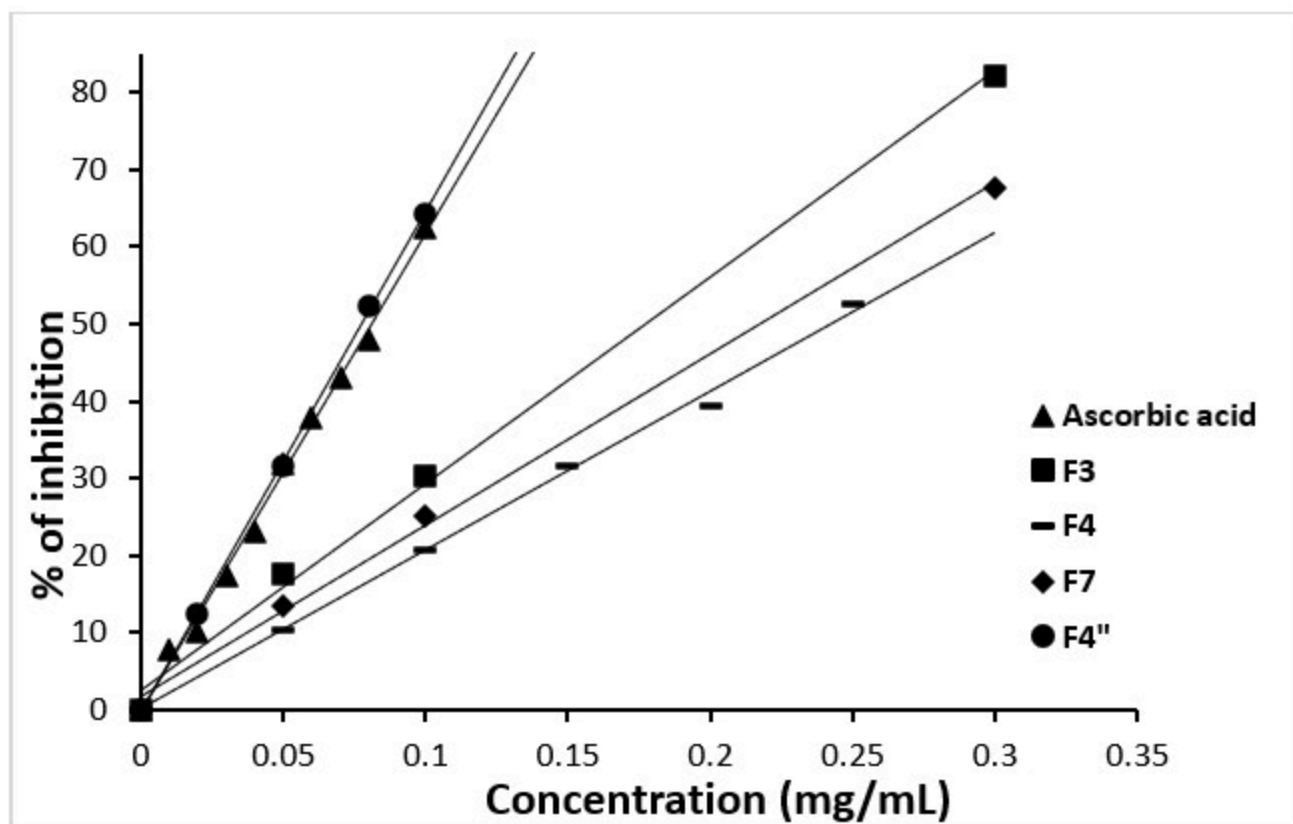


Figure 2

Investigating the variations in DPPH inhibition percentage with changing concentrations of some fractions in comparison to ascorbic acid.

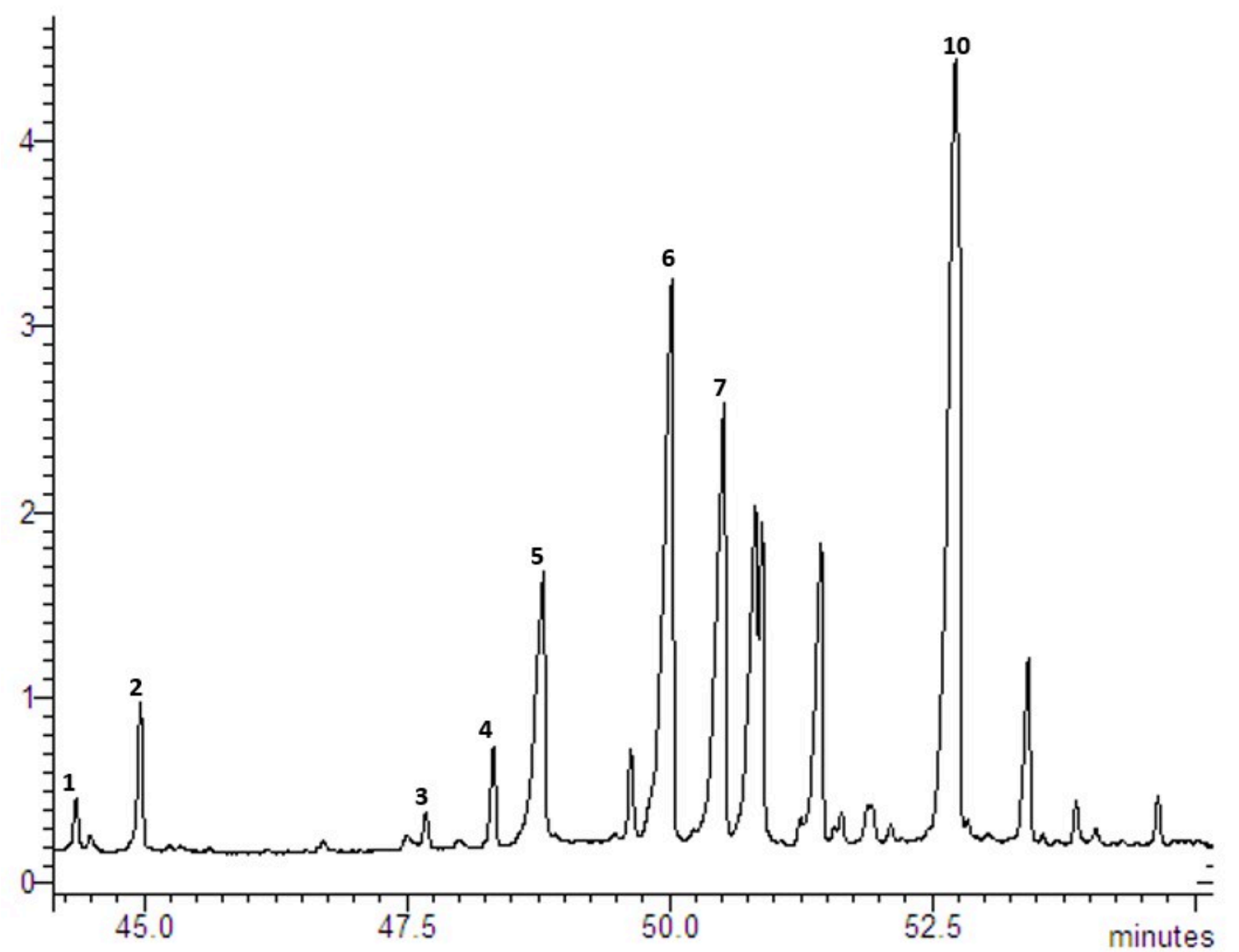


Figure 3
Chromatogram of the F4'' fraction.

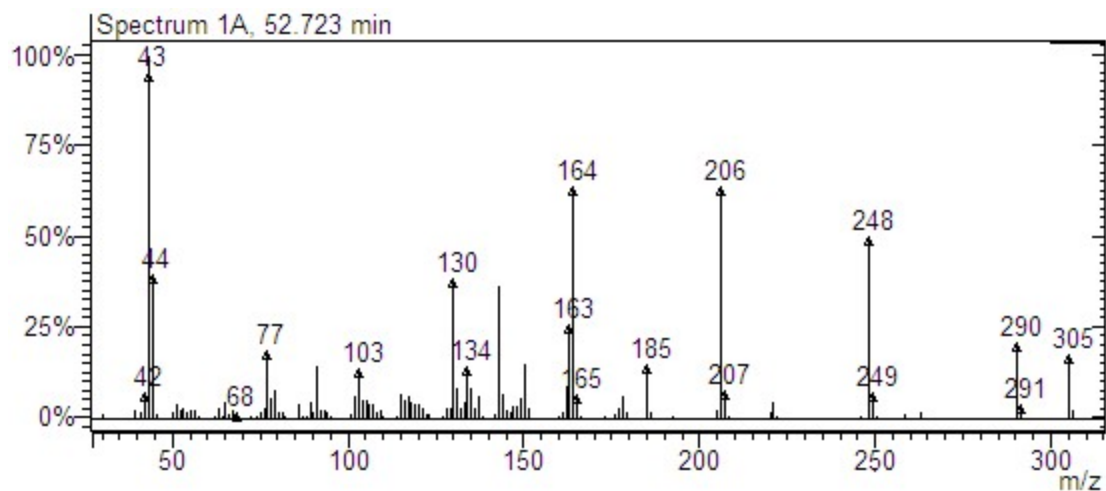


Figure 4

Mass spectrum of 6,7-diacetoxy-2-acetyl-1-methyl-1,2,3,4-tetrahydroisoquinoline.

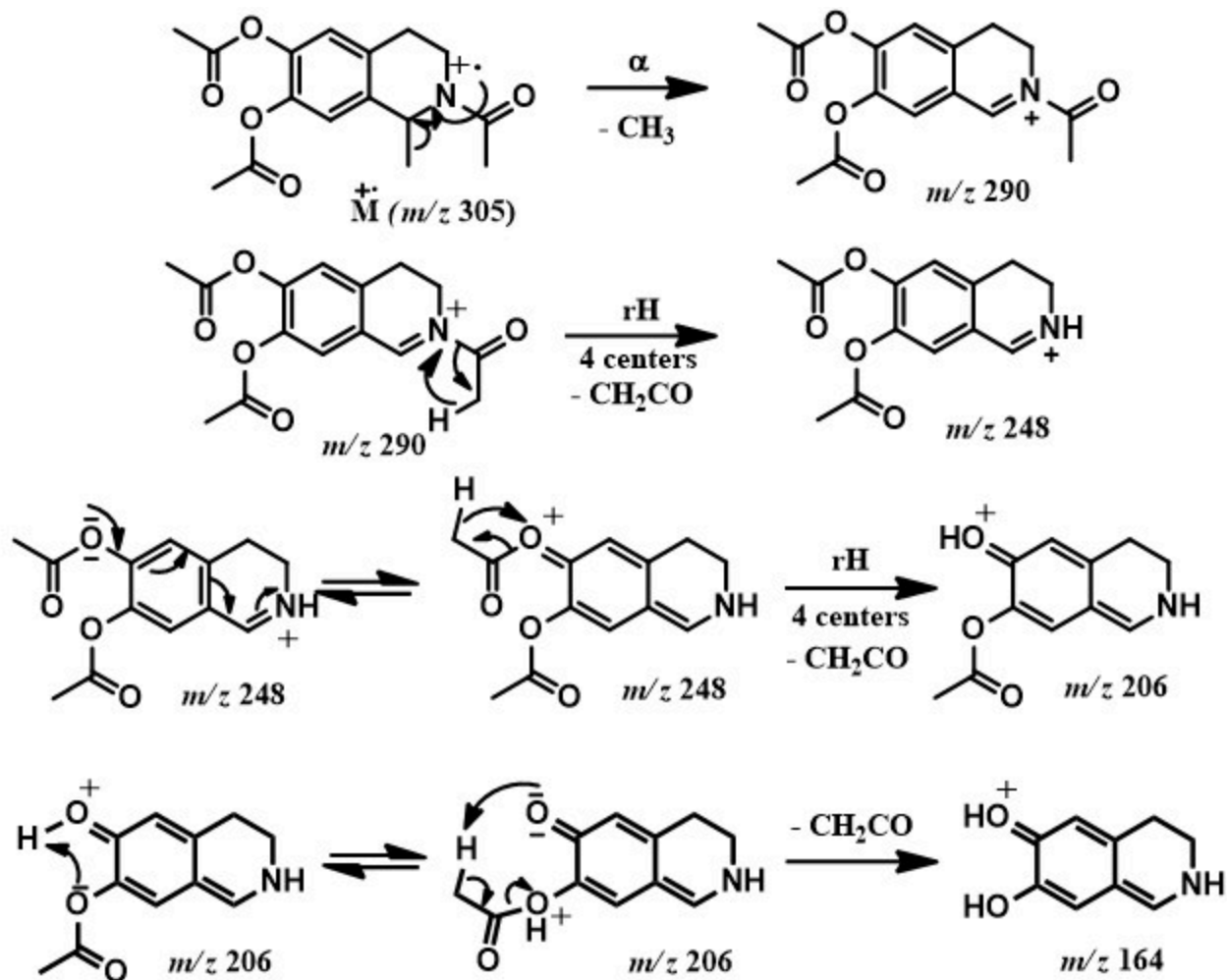


Figure 5

Mass spectrometric fragmentation of 6,7-diacetoxy-2-acetyl-1-methyl-1,2,3,4-tetrahydroisoquinoline.