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High-performance thin-layer chromatography combined with effect-directed assays revealed bioactivity profiles of Salvia aegyptiaca, *S. verbenaca*, and *S. officinalis*

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ABSTRACT

The effect-directed profiling of aqueous ethanol extracts of Tunisian *Salvia aegyptiaca* and *Salvia verbenaca* aerial parts and Tunisian and Hungarian *Salvia officinalis* leaves was achieved by high-performance thin-layer chromatography (HPTLC) coupled with radical scavenging (using DPPH• free radical) and enzyme inhibitory (acetylcholinesterase, AChE) bioactivity assays. The mixture of toluene – ethyl acetate – methanol – formic acid, 11:2:6:1 (*V/V*) was used as a mobile phase for the separation of bioactive zones. The characterization of the compounds present in the active zones was performed by derivatization with aluminum chloride or natural product-polyethylene glycol (NP-PEG) reagent, and HPTLC–heated electrospray ionization-high resolution tandem mass spectrometry (HPTLC–HESI-HRMS/MS). Compounds in the nine antioxidant zones could be characterized by derivatization as phenolic compounds and tentatively identified by MS as caffeic acid derivatives, or flavonoid glycosides or glucuronides. Rosmarinic acid and luteolin 7-*O*-glucuronide were detected in *S. aegyptiaca* and *S. officinalis* extracts, while apigenin 7-*O*-glucuronide and luteolin 7-*O*-glucoside were identified in *S. aegyptiaca* and *S. verbenaca*. The co-presence of an unknown caffeic acid derivative, hispidulin 7-*O*-glucuronide and luteolin 7-*O*-glucoside was proved within a single zone of *S. officinalis* extract. Additionally, an unknown rosmarinic acid derivative and an unknown luteolin derivative were detected in *S. officinalis* and *S. verbenaca*, respectively. These compounds exhibited weak AChE inhibitory activity, with luteolin 7-*O*-glucuronide demonstrating the strongest effect.

1. Introduction

Salvia is one of the most recognizable genera of the Lamiaceae family, comprising over 900 species throughout the world [1,2]. In Tunisia, *S. aegyptiaca* L., *S. verbenaca* L., and *S. officinalis* L. are three of the ten *Salvia* species, which disseminated randomly in arid regions [1]. They have been traditionally used for various medicinal purposes: *S. officinalis* for the remedy of inflammation, tremor, diarrhea, seizure, ulcers, dizziness, paralysis, gout, rheumatism, and hyperglycemia [3], *S. verbenaca* for the treatment of wounds, burns, diabetes, as well as digestive (abdominal colics), respiratory, and genitourinary problems [4], and *S. aegyptiaca* to cure hemorrhoids, eye diseases, diarrhea, and gonorrhoea, as well as for its antiseptic, antispasmodic, and stomachic properties [5]. Most *Salvia* herbs have been employed in traditional medicine due to their biologically active compounds, particularly phenolic acids, flavonoids, anthocyanins, diterpenes, and triterpenes [2,6]. These secondary metabolites are believed to be responsible for the diverse therapeutic effects of *Salvia* plants [7–10]. In fact, the anti-inflammatory, antimutagenic, antidementia, hypoglycemic, anti-cancer, antinociceptive, antioxidant, antimicrobial, and hypolipidemic effects of *S. officinalis* have been confirmed [3]. In addition, the antibacterial, anticancer, antioxidant, antileishmanial, antidiabetic, and immunomodulatory effects of *S. verbenaca* [4], and the antioxidant, anti-Alzheimer, and antidiabetic activities of *S. aegyptiaca* and *S. verbenaca* have been proved [11]. Despite the recognition of the three herbal medicines by their healing activities and abundance of a wide range of

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compounds, there is a lack of comprehensive information on the biological, biochemical, and chemical profiles of their polar or non-polar extracts.

HPTLC is one of the most widely used analytical techniques for the qualitative and quantitative analysis of environmental samples, food and natural products. This technique can be associated with effect-directed assays for the profiling of bioactive natural products in complex matrices [12,13]. This bioanalytical tool offers several advantages, including simple sample preparation, short analysis time, automation during the execution of the analytical steps, simultaneous analysis of multiple samples at the same time under identical conditions, and excluded cross-contamination (new layer is used for every experiment) [14].

Few studies have been formerly conducted on *Salvia* extracts using HPTLC–(bio)assays [15–22]. HPTLC–hyphenations including bioactivity tests, high-resolution mass spectrometry (HRMS) and NMR enabled the identification of acetylcholinesterase (AChE) inhibitory and antioxidant compounds (rosmarinic acid, lithiospermic acid, and salvianolic acid B) in *S. miltiorrhiza* roots [16]. Phenolic acids, tanshinones, and triterpenoids were found as bioactive compounds in ethanol-water and ethyl acetate extracts of Tunisian *S. aegyptiaca* and *S. verbenaca* aerial parts studied by HPTLC combined with antibacterial (against *Aliivibrio fischeri* and *Bacillus subtilis*), antioxidant, AChE, butyrylcholinesterase (BChE), α-glucosidase, β-glucosidase, β-glucuronidase, and tyrosinase assays as well as HPTLC–HRMS [21]. Further investigation of ethyl acetate extracts of Tunisian *S. aegyptiaca* and *S. verbenaca* aerial parts and *S. officinalis* leaves by HPLC–hyphenations revealed the triterpenes corosolic, maslinic, ursolic, and oleanolic acids as antibacterial (against *B. subtilis* and *Rhodococcus fascians*) components with an α-glucosidase inhibitory effect as well [22]. In addition, rosmanol/epirosmanol and methyl carnosate were identified by HPLC-tandem high-resolution MS (LC–HRMS/MS) in the multipotent HPTLC zones of Tunisian *S. officinalis*. Recently, according to HPTLC combined with enzyme inhibitory and antimicrobial effect-directed assays, ingredients of *S. apiana* and *S. officinalis* extracts have been established to exhibit anti-α-amylase (cirsimaritin and a caffeic acid polymer), anti-AChE (16-hydroxyrosmanol, 16-hydroxycarnosic acid, as well as oleanolic and ursolic acids), and antimicrobial (16-hydroxycarnosic acid as well as oleanolic and ursolic acids) effects against *Staphylococcus aureus* [23].

This study aimed to build upon our former studies [21,22] and discover further bioactive compounds in the identical sage samples using an improved separation method. The HPTLC fingerprinting profiles of ethanol-water extracts of Tunisian *S. aegyptiaca* and *S. verbenaca* aerial parts as well as Tunisian and Hungarian *S. officinalis* leaves were compared using chemical reagents as well as antioxidant (using DPPH• free radical) and AChE enzyme inhibitory assays. The major bioactive compounds were tentatively identified by HPTLC–heated electrospray ionization (HESI)-HRMS/MS analysis.

2. Materials and methods

2.1. Materials

Aluminum foil-backed fine particle silica gel 60 F_{254} (HPTLC, 1.05548.0001) layers were provided by Merck (Darmstadt, Germany). Analytical grade-methanol, dimethyl sulfoxide (DMSO), toluene, ethanol, ethyl acetate, formic acid, and aluminum chloride were acquired from Reanal (Budapest, Hungary) or Molar Chemicals (Halásztelek, Hungary). MS-grade methanol (HiPerSolv CHROMANORM®) was delivered by VWR (Debrecen, Hungary). DPPH• (1,1-diphenyl-2-picrylhydrazyl), bovine serum albumin, and acetylcholinesterase (AChE) from *Electrophorus electricus* were purchased from Sigma-Aldrich (Budapest, Hungary). Natural product (NP) (diphenylboryloxyethylamine, or diphenylboric acid β-ethylamino ester) reagent, and polyethylene glycol 400 (PEG 400) were obtained from Carl Roth (Karlsruhe, Germany). 3-Indoxylacetate was purchased from BioSynth (Bratislava, Slovakia).

2.2. Sample preparation

The Tunisian *S. aegyptiaca* (**S1**-**S3** from Bouhedma National Park, Beni Khedache, and Matmata, respectively) and *S. verbenaca* (**S4** from Bouhedma National Park) aerial parts as well as Tunisian *S. officinalis* leaf (**S5** and **S6** from INRGREF and Menzel Elhabib, respectively) samples, identical with those published previously [21,22], were used in this study (Table 1). The voucher specimens of the Tunisian *Salvia* samples were deposited in the herbarium of the University of Gabes. The harvested Tunisian samples (**S1**-**S6**) were dried at room temperature for one month. For comparison, dried leaves of Hungarian *S. officinalis* (three samples, **S7**-**S9**), purchased from the market [22], were also included in this research work (Table 1). A 25% aqueous ethanolic extract of *S. officinalis* leaves (**S10**) obtained from Bioextra Zrt. (Budapest, Hungary), was also employed in this study (Table 1). According to a recently published method [21], each *Salvia* powder (**S1**-**S9**, 1 g) was extracted with 50% aqueous ethanol (10 mL) by vortexing (1 min) and ultrasonication (15 min). After that, the prepared extracts were centrifuged (756 × *g* for 15 min). After a second centrifugation (10,000 × *g* for 5 min), the supernatant was transferred to a vial and kept at 4 °C in refrigerator until further use.

2.3. High-performance thin-layer chromatography

Depending on the (bio)assay or derivatization process used, 1–20 µL/band of the *Salvia* extracts were applied as 5-mm bands with a 7 mm track distance (Automatic TLC Sampler ATS3, CAMAG) on the HPTLC layers at 8 mm height from the bottom edge. HPTLC separation was executed in an unsaturated (20 cm × 10 cm) twin trough chamber (CAMAG, Muttenz, Switzerland) using toluene – ethyl acetate – methanol – formic acid 11:2:6:1 (*V/V*) as a mobile phase to a migration distance of 75 mm from the bottom edge. After development, the plate was dried in a cold air stream for 5 min and then cut with a blade. The individual plate sections were subsequently employed for visualization

Table 1
Collection area and period of the *Salvia* samples from Tunisia and the source of the Hungarian samples, as well as the concentration of the prepared extracts.

ID	*Salvia* species and locations (used part) or sources	Collection period	Extract
S1	*S. aegyptiaca*, Bouhedma National Park, Tunisia (aerial)	between mid-February to mid-March 2019	50% aqueous ethanol 1 g/ 10 mL
S2	*S. aegyptiaca*, Beni Khedache, Tunisia (aerial)		
S3	*S. aegyptiaca*, Matmata-Gabes, Tunisia (aerial)		
S4	*S. verbenaca*, Bouhedma National Park, Tunisia (aerial)		
S5	*S. officinalis*, INRGREF-Gabes, Tunisia (leaf)	December 2020	
S6	*S. officinalis*, Menzel Elhabib-Gabes, Tunisia (leaf)	between December 2020 and February 2021	
S7	*S. officinalis*, Juva Pharma Kft., Budapest, Hungary (leaf)		
S8	*S. officinalis*, Herba Trend, Budapest, Hungary (leaf)		
S9	*S. officinalis*, Herbaria, Budapest, Hungary (leaf)		
S10	*S. officinalis*, Bioextra Zrt., Budapest, Hungary (leaf)		25% aqueous ethanol (extract purchased from Bioextra Zrt.)

and characterization methods. HPTLC chemical fingerprints were obtained by dipping the layer into aluminum chloride (0.1% solution in methanol) or NP (0.5% solution in methanol) / PEG (after drying, 5% ethanolic PEG solution) reagents. Each chromatogram or autogram was documented using a digital camera (Cybershot DSC-HX60, Sony, Neu-Isenburg, Germany) at Vis or under a UV lamp (at 254 or 365 nm, CAMAG).

2.4. HPTLC–bioactivity assays

Effect-directed profiling was achieved using HPTLC–DPPH• and HPTLC–AChE assays. HPTLC–DPPH• was performed based on a reported procedure [24]. Briefly, the developed and dried layer was dipped into the stable free radical DPPH• solution (0.02% in methanol) to reveal bright yellow antioxidant zones on a purple background. In the course of HPTLC–AChE assay, similarly to the published method [25], the developed plate was immersed into the substrate solution of 3-indoxyl acetate (0.5 mg/mL, 4% DMSO in 0.1 M pH 7.5 phosphate buffer), then sprayed with the AChE solution (5 U/mL of AChE and 1 mg/mL of bovine serum albumin in 0.1 M pH 7.5 phosphate buffer). After incubation at 37 °C for 20 min (100% humidity), the autogram was documented at UV 365 nm, and the bioactive zones were detected as dark zones against a blueish-greenish fluorescent background.

2.5. HPTLC–HRMS/MS

HPTLC–HESI-HRMS/MS was performed using a TLC-MS Interface 2 with an oval elution head (4 mm × 2 mm) (CAMAG) installed between the binary pump (Vanquish Flex VF-P10, DionexSoftron, Germering, Germany) and the hybrid quadrupole-orbitrap mass spectrometer with a HESI-II probe (OrbitrapExploris 120, Thermo Fisher Scientific, Bremen, Germany). Compounds from the chromatographic zones were eluted with methanol at a flow rate of 0.2 mL/min. Full scan MS spectra were recorded in negative ionization mode in the range of *m/z* 100–1000 with a resolution of 120.000. Spray voltage was set to −2.0 kV, vaporizer temperature and capillary temperature was maintained at 250 °C and 320 °C, respectively, and nitrogen was used as both sheath and auxiliary gas (10 and 5 arbitrary units, respectively) produced by a Peak Scientific gas generator (Genius XE 35, Glasgow, United Kingdom), a standard automatic gain control target, and a maximum injection time of 100 ms. Tandem mass spectra were acquired in HCD fragmentation mode with a normalized collision energy of 20–40% and a quadrupole isolation window of *m/z* 1 for precursor ion selection. Instrument control, data acquisition and evaluation were realized by Xcalibur 4.7.69 software (Thermo Fisher Scientific).

3. Results and discussion

3.1. Chromatographic fingerprints of bioactive compounds by HPTLC–EDA

Biologically active compounds of ten aqueous ethanol extracts of *Salvia* samples, three *S. aegyptiaca* (**S1**-**S3**), one *S. verbenaca* (**S4**), and two *S. officinalis* from Tunisia (**S5**-**S6**), and four *S. officinalis* from Hungary (**S7**-**S10**), were separated by HPTLC with a mobile phase of toluene – ethyl acetate – methanol – formic acid, 11:2:6:1 (*V/V*) and

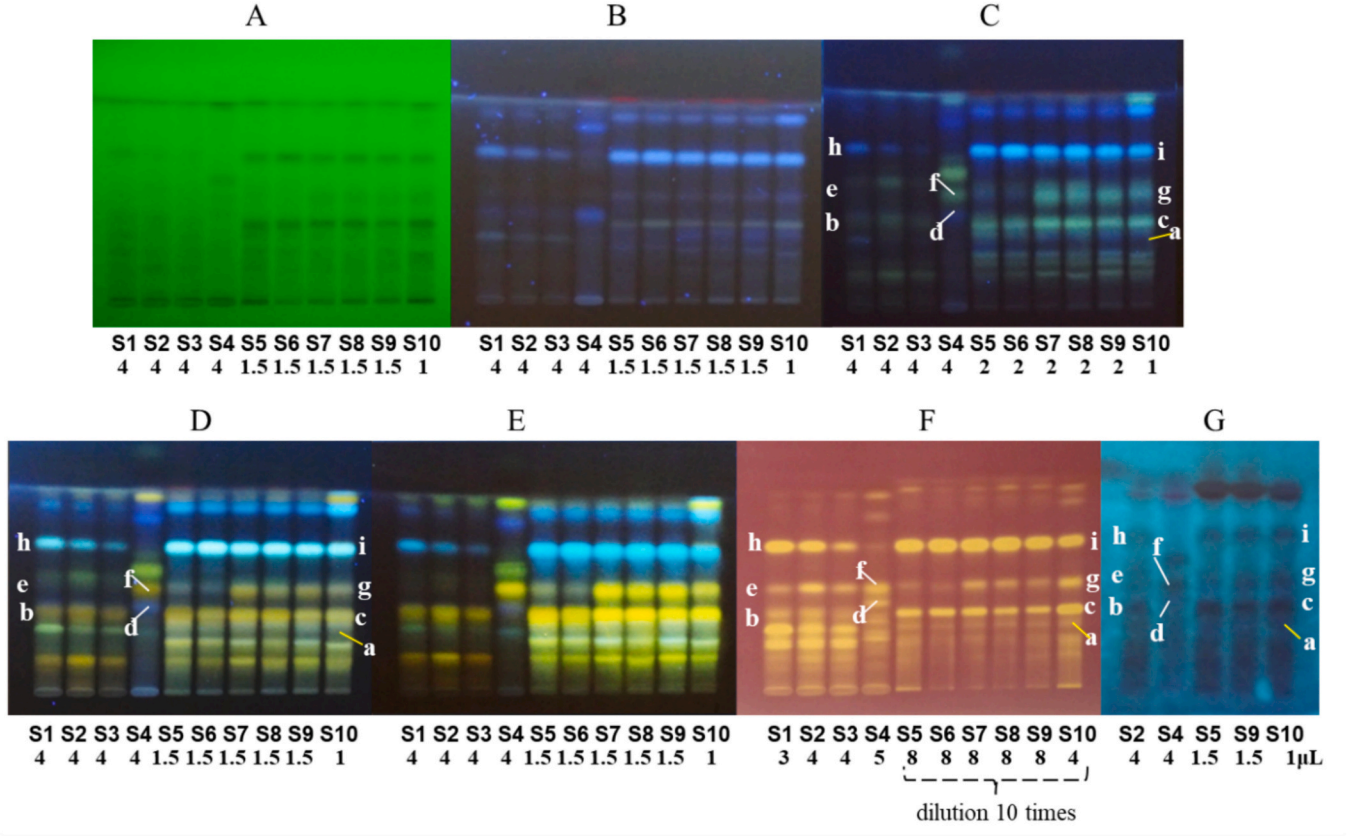

Fig. 1. HPTLC chromatograms and autograms of the Tunisian *Salvia aegyptiaca* (**S1**-**S3**) and *S. verbenaca* (**S4**) aerial parts, and *S. officinalis* (**S5** and **S6**) leaf samples, and four Hungarian *S. officinalis* leaf samples (**S7**-**S10**), developed with toluene – ethyl acetate – methanol – formic acid, 11:2:6:1 (*V/V*), and detected at UV 254 nm (A) and 365 nm (B), after derivatization with aluminum chloride (C, at UV 365 nm), NP reagent (D, at UV 365 nm), NP-PEG reagent (E, at UV 365 nm), and DPPH• assay (F, at white light illumination), and AChE inhibition assay (G, at UV 365 nm). The compounds in the zones **a**-**i** were further characterized by HPTLC–HESI$^-$-HRMS(/MS). The applied sample volumes depicted in the figure are given in µL.

visualized using chemical reagents, and antioxidant and AChE inhibitory assays (Fig. 1). Based on the screening with HPTLC–derivatization and HPTLC–DPPH•, extracts from the same species (**S1**-**S3** and **S5**-**S10**, respectively) provided highly similar profiles. Additionally, compounds in the antioxidant zones exhibited blue, yellow, or orange fluorescence after derivatization with aluminum chloride, or NP, or NP/PEG reagent, indicating the presence of phenolic compounds (Fig. 1C-E). The utilization of aluminum chloride as a derivatization reagent is attributed to the ability of aluminum ions to interact with the hydroxyl and carbonyl groups of flavones and flavonols, leading to the formation of a yellow complex as an indicator of the presence of flavonoids [26]. The use of NP/PEG reagent is associated with the detection of flavonoids, isoflavonoids, and phenolic acids containing phenolic hydroxyl groups, which appear as yellow, orange, blue, and green fluorescent zones [27].

Since the HPTLC profiles were identical for the same species as well as species-specific, five samples were selected (**S2**, **S4**, **S5** from Tunisia, and **S9** and **S10** from Hungary) as representatives of the three different *Salvia* species to evaluate their AChE inhibitory effect (Fig. 1G). Comparing the two biological profiles, three zones of *S. aegyptiaca* (**b**, **e**, and **h**), two zones of *S. verbenaca* (**d** and **f**), and four zones of *S. officinalis* (**a**, **c**, **g**, and **i**) were marked as active in both antioxidant and AChE inhibition assays. However, these zones, except **c**, showed only weak activity in enzyme assays. Furthermore, two antioxidant zones appeared in *S. aegyptiaca* below zone **b**, which provided neither fluorescence after derivatizations, nor a characteristic MS signal. Thus, the compounds responsible for the radical scavenging activity in these zones could not be identified.

3.2. Characterization of phenolic compounds

Further characterization and tentative identification of the compounds in the bioactive zones (**a**-**i**) was carried out by HPTLC–HESI-HRMS/MS. In the investigated zones intense signals were recorded only in the negative ionization mode, which were assigned as deprotonated molecules ($[M–H]^-$). HPTLC–HESI-HRMS/MS data are presented in Table 2 and exemplar spectra are demonstrated in Fig. 2. The recorded spectra were evaluated and compared with literature data regarding their MS/MS fragmentation pattern [28–31]. The results indicated that compounds present in *S. aegyptiaca* zones **b** and **h** were identical to those in *S. officinalis* zones **c** and **i**, and tentatively identified as luteolin 7-*O*-glucuronide ($C_{21}H_{18}O_{12}$, a flavonoid, [28]) and rosmarinic acid ($C_{18}H_{16}O_8$, a phenolic acid, a caffeic acid derivative, [28,29]), respectively. Luteolin 7-*O*-glucoside ($C_{21}H_{20}O_{11}$, a flavonoid, [30,31]) was detected alone in *S. verbenaca* zone **f** and in *S. officinalis* zone **g** along with hispidulin 7-*O*-glucuronide ($C_{22}H_{20}O_{12}$, a flavonoid, [30,31]) and an unknown caffeic acid derivative ($C_{17}H_{28}O_9$, a phenolic acid, [30]). In *S. aegyptiaca* zone **e** apigenin 7-*O*-glucuronide ($C_{21}H_{18}O_{11}$, a flavonoid, [29,30]) was identified. Additionally, compounds in *S. officinalis* zone **a** and *S. verbenaca* zone **d** were assigned as unknown compounds: a rosmarinic acid derivative ($C_{24}H_{22}O_8$, a phenolic acid) and a luteolin derivative ($C_{27}H_{16}O_9$, a flavonoid), respectively. Rosmarinic acid derivative gave the characteristic fragment ions at *m/z* 179, *m/z* 161, and *m/z*135, same as those of rosmarinic acid. Similarly, the MS fragmentation pattern of compound in zone **d** shared fragment ions at *m/z* 285, *m/z* 151, and *m/z* 133 with luteolin 7-*O*-glucuronide and luteolin 7-*O*-glucoside.

Phenolic compounds are well-known for their potential biological activities, including AChE inhibitory [32,33] and antioxidant [34,35] effects. Based on literature data, the flavonoids and phenolic acids identified in the bioactive HPTLC zones have been detected in various *Salvia* extracts. In fact, rosmarinic acid has been found as a recognized phenolic acid in extracts of different *Salvia* species [36–40], including aqueous and methanol extracts of *S. officinalis* aerial part, leaves, and flowers [36,38–40], and methanol extracts of *S. verbenaca* aerial parts [37]. Furthermore, it has been considered as the strongest antioxidant and the main abundant phenolic acid in the ethanol extract of *S. officinalis* [41], and exhibited also AChE inhibitory effect [42–44]. Similarly, luteolin 7-*O*-glucuronide, luteolin 7-*O*-glucoside, and apigenin 7-*O*-glucuronide have been reported from different sages [28,30,36,45–47], also in infusion, decoction, and methanol extracts of *S. officinalis* [30,31,39,40,48–50]. Additionally, the presence of luteolin 7-*O*-glucoside in ethyl acetate extract of *S. aegyptiaca* whole plant [51] has been described. The antioxidant activity and AChE inhibition of luteolin 7-*O*-glucuronide [52–54], luteolin 7-*O*-glucoside [55,56], and apigenin 7-*O*-glucuronide [53,57] have been reported. Hispidulin 7-*O*-glucuronide has been described as an antioxidant [58] compound and constituent of the methanol and aqueous extracts of *S. officinalis* leaves and aerial parts [31,40,48,49]. Also, herbs belonging to Lamiaceae family (especially *S. officinalis*) have been reported to have anti-AChE activity [59]. In fact, the identified hispidulin in *S. officinalis* [60] has been used as one of several flavonoids for the treatment of Alzheimer's disease [61].

Caffeic acid has been detected in different *Salvia* extracts [28,36,62] including aqueous and alcoholic extracts of *S. officinalis* aerial parts [28,31,36,39,40,48,63], and methanolic extract of *S. verbenaca* aerial parts [37]. Moreover, several caffeic acid derivatives have been described as constituents of *Salvia* species [30], known their strong antioxidant [64] and AChE inhibitory [65] activities.

4. Conclusion

HPTLC hyphenations provided a fast and simple tool for obtaining chemical and biological fingerprints of aqueous ethanol extracts of three *Salvia* species: *S. aegyptiaca*, *S. verbenaca*, and *S. officinalis*. Their profiles

Table 2
HPTLC–$HESI^-$-HRMS/MS analysis of compounds in bioactive zones (**a**-**i**) of the aqueous ethanol extracts of the *S. aegyptiaca*, *S. verbenaca*, and *S. officinalis*.

Zone	HPTLC–HRMS (*m/z*) $[M–H]^-$	Molecular formula	HPTLC–HRMS/MS fragment ions (*m/z*)	Tentatively identified compounds	*Salvia* species
a	437.12192	$C_{24}H_{21}O_8^-$	179.04, 161.05, 135.05	Unknown rosmarinic acid derivative	*S. officinalis*
b	461.07245	$C_{21}H_{17}O_{12}^-$	285.04, 151.00, 133.03	Luteolin 7-*O*-glucuronide [28]	*S. aegyptiaca*
c	461.07239	$C_{21}H_{17}O_{12}^-$	285.04, 151.00, 133.03	Luteolin 7-*O*-glucuronide [28]	*S. officinalis*
d	483.06992	$C_{27}H_{15}O_9^-$	285.04, 151.00, 133.03	Unknown luteolin derivative	*S. verbenaca*
e	445.07742	$C_{21}H_{17}O_{11}^-$	269.05	Apigenin 7-*O*-glucuronide [29,30]	*S. aegyptiaca*
f	447.09316	$C_{21}H_{19}O_{11}^-$	285.04, 284.03	Luteolin 7-*O*-glucoside [30,31]	*S. verbenaca*
g	447.09316	$C_{21}H_{19}O_{11}^-$	285.04, 284.03	Luteolin 7-*O*-glucoside [30,31]	*S. officinalis*
	375.16583	$C_{17}H_{27}O_9^-$	179.03, 135.05	Unknown caffeic acid derivative [30]	
	475.08807	$C_{22}H_{19}O_{12}^-$	299.06, 284.03	Hispidulin 7-*O*-glucuronide [30,31]	
h	359.07660	$C_{18}H_{15}O_8^-$	197.05, 179.04, 161.02, 135.05	Rosmarinic acid [28,29]	*S. aegyptiaca*
i	359.07665	$C_{18}H_{15}O_8^-$	197.05, 179.03, 161.02, 135.05	Rosmarinic acid [28,29]	*S. officinalis*

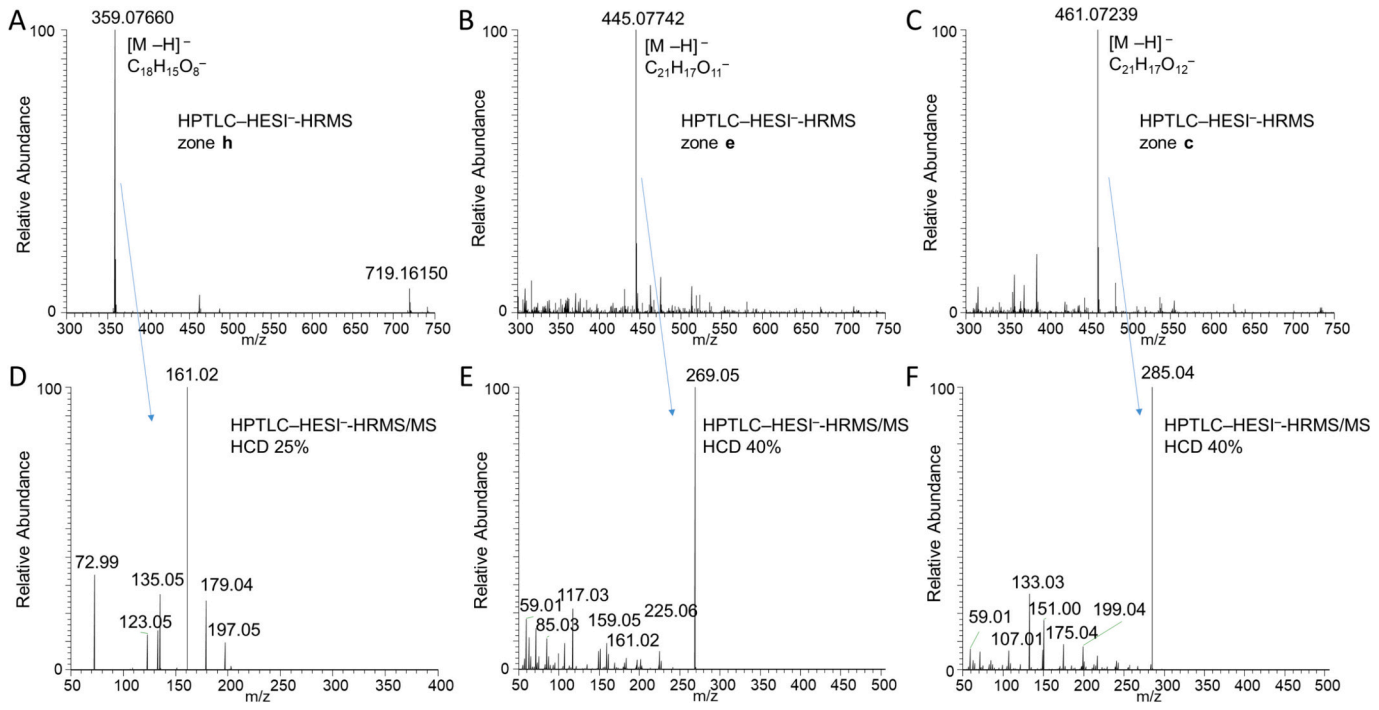

Fig. 2. HPTLC–HESI⁻-HRMS spectra of the compounds in the bioactive zones **h** (A), **e** (B), and **c** (C) and their HPTLC–HESI⁻-HRMS/MS (parent ions: *m/z* 359.08, *m/z* 445.08, and *m/z* 461.07, and normalized HCD collision energies: 25%, 40%, and 40%) spectra (D—F), respectively.

appeared to be species-specific, allowing for their distinction. Compounds in the bioactive zones obtained by HPTLC–DPPH• and HPTLC–AChE assays were tentatively identified by HPTLC–HRMS/MS as phenolic compounds (flavonoids and phenolic acids) that play a key role in the beneficial properties of these sage species.

CRediT authorship contribution statement

Amira Reguigui: Writing – original draft, Investigation, Formal analysis. **Márton Baglyas:** Writing – review & editing, Investigation, Formal analysis. **Anna Cselőtey:** Investigation, Formal analysis. **Mehrez Romdhane:** Supervision, Resources. **Ágnes M. Móricz:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare no conflicts of interest.

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Data availability

Data will be made available on request.

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