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Biochemical Diversity of *Salvia* (Lamiaceae) Species

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Abstract

Fourier transform infrared (FTIR) spectroscopy is one of few analytical methods used successfully in many laboratories worldwide, for huge applications in research and industrial purposes. Chemical composition of 10 *Salvia* species including S. judaica Boiss (S1), S. viridis var. horminum L. (S2), S. *officinalis* L. (S3), S. *palaestina* Bentham (S4), S. *syriaca* L. (S5), S. *pinardi* Boiss (S6), S. *lanigera* Poiret (S7), S. *ceratophylla* L. (S8), S. *dominica* L. (S9) and S. *spinosa* L. (S10) was analyzed using FTIR spectroscopy. FTIR spectra analysis revealed 10, 11, 12, 11, 10, 9, 12, 8, 8 and 12 observed peaks for S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 *Salvia* species, respectively. Overall, FTIR observed peaks belonged to 9 functional groups [(Aromatics, Ethers, Carboxylic acids, Alcohol and hydroxyl, Alkanes, Olefinic (alkene), Common inorganic ions, Ammonium and immonium and Amine and amino compounds)]. These functional groups are known for their biological activity. Performance Salvia analytical analysis to determine the different bioactive components existent in each observed functional groups is required.

Key words: Salvia species, chemical analysis, fourier transform infrared (FTIR) spectroscopy, functional groups

1. Introduction

Salvia is a genus with approximately 1000 species; belongs to Lamiaceae family and is considered as one of the largest plant genera in plant kingdom [1]. In Syria, it has been reported the existence of 28 *Salvia* species in Syrian flora [2].

Salvia species have different properties in medicine *e.g*. as antioxidant, anticholinesterase antimicrobial, anticancer, anti-inflammatory, analgesic, antiseptic and antipyretic agents as well as it is used for herbal teas [3].

Many analytical methods have been employed as main tools for many years for identification and characterization of unknown compounds *e.g.* attenuated total reflectance (ATR)-fourier transform infra-red (ATR-FTIR) and near-infrared (NIR)-fourier-transform raman spectroscopy (NIR-FT-Raman) analyses in 9 genera belongs to Lamiaceae [4], ATR-FTIR spectroscopy [5] and high performance liquid chromatography-photo diode array-electrospray ionization-mass spectrometry (HPLC-PDA-ESI-MS)/ mass spectrometry-liquid chromatography (MS-LC)/ liquid chromatography-high resolution electrospray ionization mass spectrometry (LC-HRESIMS) analysis (HPLC-PDA-ESI-MS/MS-LC/HR-ESI-MS) [6] in *Salvia officinalis*, fourier transform infra-red (FTIR) in Sage Tincture [7], high performance liquid chromatography (HPLC) in Mexican sage *Salvia divinorum* [8], thin layer chromatography (TLC) for 9 *Salvia* species [9] and *Salvia officinalis* [10] phytochemical analysis, fourier transform near infrared (FT-NIR) for biochemical characterization of 5 Syrian wheat varieties [11] and for 5 upland cotton (*Gossypium hirsutum* L.) varieties grown in Syria [12], nuclear magnetic resonance (NMR), ultraviolet (UV), infrared (IR) and mass spectrometry (MS) techniques in wild *S. palaestina* phytochemical analysis [13].

Fourier transform infrared spectroscopy (FTIR) is one of few analytical methods available nowadays; as strong fingerprinting

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technique and popular one in materials molecular structure and composition determination. Its advantages including its rapidity with minimum quantity of materials using non-destructive methods make it as an effective tool for biochemical fingerprints of wide food products [3, 14]. This technique has been employed for chemical composition investigation of different plants species *e.g*. in giant reed (*Arundo donax* L.) and switchgrass (*Panicum virgatum* L.) herbaceous [15]. Recently, this analysis has been employed for juvenile and mature woods in Scots pine (*Pinus sylvestris* L.) [14] and *Acacia mearnsii* [16] chemical composition investigation. No available data regarding the phytochemical *Salvia* analysis in Syria. Thus, the current study aimed for the first time to describe chemical composition of 10 *Salvia* species grown in Syria using FTIR tool. Their phytochemical analysis using FTIR analysis in correlation with their recent published genetic structure using touch-down directed amplification of minisatellite DNA (Td-DAMD) analysis was also examined.

2. Material and methods

2.1. Plant materials

Leaves of 10 *Salvia* species including *S. judaica* Boiss (S1), *S. viridis* var. *horminum* L. (S2), *S. officinalis* L. (S3) species were collected from Lattakia provinces-Syria. Whereas, the remaining *Salvia* species including *S. palaestina* Bentham (S4), *S. syriaca* L. (S5), *S. pinardi* Boiss (S6), *S. lanigera* Poiret (S7), *S. ceratophylla* L. (S8), *S. dominica* L. (S9) and *S. spinosa* L. (S10) were collected from Damascus provinces - Syria (Table 1). Sampling was performed during blooming stage. Plant samples were shade dried for two weeks, milled to a fine powder by special electric mill and stored separately in glass bowls for FTIR test.

Table 1. Description of collection *Salvia* species sites

Species	Code	Collection site	Altitude (m)	Annual rainfall (mm)
S. judaica Boiss	S1	Lattakia	80	750
S. viridis var. *horminum* L.	S2	Lattakia	110	800
S. officinalis L.	S3	Lattakia	118	800
S. palaestina Bentham	S4	Damascus	1200	150
S. syriaca L.	S5	Damascus	1100	300
S. pinardi Boiss	S6	Damascus	975	240
S. lanigera Poiret	S7	Damascus	960	240
S. ceratophylla L.	S8	Damascus	960	240
S. dominica L.	S9	Damascus	970	240
S. spinosa L.	S10	Damascus	950	260

2.2. FTIR assay

The fine powder was used as template for FTIR analysis in the wavenumber range of 3500-500 cm^{-1}. IR measurement has been performed using NXR FTIR (Thermo, USA) instrument for FTIR analysis.

3. Results and Discussion

FTIR spectra analysis revealed 10, 11, 12, 11, 10, 9, 12, 8, 8 and 12 observed peaks for S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 Salvia species, respectively (Figure 1 and Table 2). *Salvia* FTIR spectra revealed four peaks of 811 and 818 cm^{-1} assigned to =C-H oop bend (Aromatics group) as well as 1510 and 1600 cm^{-1} assigned to C=C stretch aromatic (Aromatics group), four peaks of 1007, 1060, 1100 and 1200 cm^{-1} assigned to C–O secondary alcohol stretch C–O stretch (Ethers group), three peaks of 1250, 1300 and 1710 cm^{-1} assigned to C–O stretch (Carboxylic acids group), two peaks of 1322 and 1364 cm^{-1} assigned to phenol or tertiary alcohol, OH bend (Alcohol and hydroxyl group), four peaks of 1410 and 1440 cm^{-1} assigned to C–H bend (Alkanes group) as well as 2864 and 2926 cm^{-1} assigned to C–H stretch (Alkanes group), two peaks of 1630 and 1650 cm^{-1} assigned to alkenyl C=C stretch [Olefinic (alkene) group], one peak of 2024 cm^{-1} assigned to cyanide ion, thiocyanate ion (Common inorganic ions group), one peak of 2350 cm^{-1} assigned to N-H stretching (Ammonium and immonium group) and one peak of 3415 cm^{-1} assigned to N-H Aromatic primary amine (Amine and amino compound group) (Table 2).

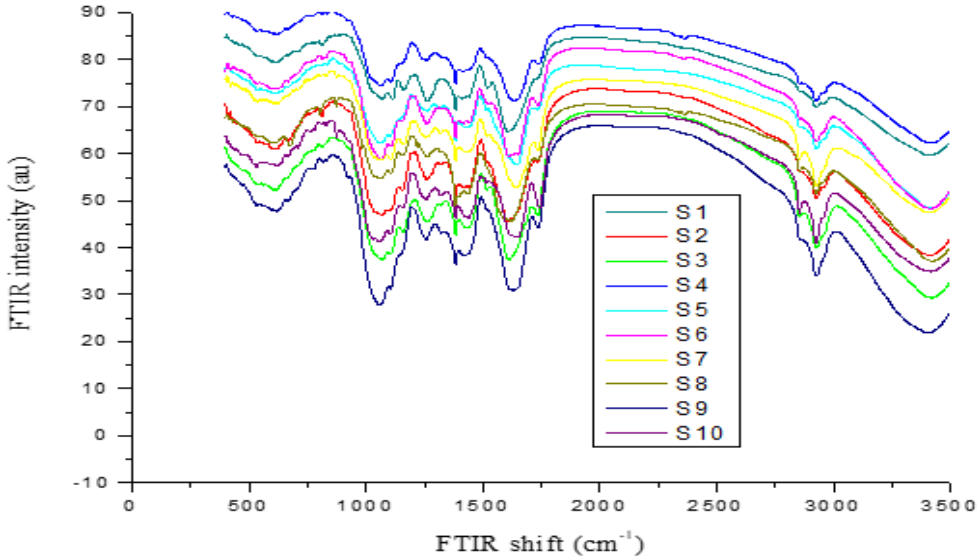

Figure 1. Observed FTIR vibration wavenumbers of the studied *Salvia* species. S1- S. *judaica* Boiss; S2- S. *viridis* var. horminum L.; S3- S. *officinalis* L.; S4- S. *palaestina* Bentham; S5- S. syriaca L.; S6- S. *pinardi* Boiss; S7- S. *lanigera* Poiret; S8- S. ceratophylla L.; S9- S. *dominica* L and S10- S. *spinosa* L

Table 2. FTIR analysis of the studied *Salvia* species

IR (cm^{-1})	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	IR frequency (cm^{-1})	Observed IR (cm^{-1})	Bond	Functional groups
811	+	+	-	-	+	-	+	-	+	+	900-690	811	=C-H oop bend	Aromatics
818	+	+	+	+	+	+	+	-	+	+	900-690	818	=C-H oop bend	Aromatics
1007	-	+	+	+	+	+	+	+	-	+	1200-1000	1007	C–O secondary alcohol stretch C–O stretch	Ethers
1060	+	-	+	-	-	+	+	+	+	-	1200-1000	1060	C–O secondary alcohol stretch C–O stretch	Ethers
1100	+	+	+	+	-	+	+	+	-	+	1200-1000	1100	C–O secondary alcohol stretch C–O stretch	Ethers
1200	+	+	+	+	-	-	-	-	-	-	1200-1000	1200	C–O secondary alcohol stretch C–O stretch	Ethers
1250	-	-	-	-	+	-	-	-	+	+	1300-1200	1250	C–O stretch	Carboxylic acids
1300	+	+	+	+	-	-	-	-	-	-	1300-1200	1300	C–O stretch	Carboxylic acids
1322	-	-	-	-	+	-	-	-	-	+	1410-1310	1322	Phenol or tertiary alcohol, OH bend	Alcohol and hydroxy
1364	-	-	-	-	-	+	+	+	-	+	1410-1310	1364	Phenol or tertiary alcohol, OH bend	Alcohol and hydroxy
1410	+	-	-	+	-	-	-	+	+	-	1475-1365	1410	C–H bend	Alkanes
1440	-	+	+	-	+	+	+	-	-	+	1475-1365	1440	C–H bend	Alkanes
1510	+	+	+	+	-	-	+	-	-	-	1600-1400	1510	C=C stretch aromatic	Aromatics
1600	-	+	+	-	+	-	-	-	-	-	1600-1400	1600	C=C stretch aromatic	Aromatics
1630	-	-	-	-	-	-	+	-	+	-	1680-1620	1630	Alkenyl C=C stretch	Olefinic (alkene)
1650	-	-	-	-	+	-	-	-	-	+	1680-1620	1650	Alkenyl C=C stretch	Olefinic (alkene)
1710	-	-	+	+	+	+	+	-	+	+	1725-1700	1710	C–O stretch	Carboxylic acids
2024	+	-	+	+	-	-	-	-	-	-	2200-2000	2024	Cyanide ion, thiocyanate ion	Common inorganic ions
2350	-	-	-	+	-	-	-	-	-	-	2440-2350	2350	N-H stretching	Ammonium and immonium
2864	-	-	-	-	-	-	+	+	-	-	2970-2850	2864	C–H stretch	Alkanes
2926	-	+	-	-	+	+	+	+	+	+	2970-2850	2926	C–H stretch	Alkanes
3415	+	+	+	+	+	+	+	+	-	+	3415-3380	3415	N-H Aromatic primary amine	Amine and amino compound

Overall, *Salvia* FTIR observed peaks belonged to 9 functional groups [(Aromatics, Ethers, Carboxylic acids, Alcohol and hydroxyl, Alkanes, Olefinic (alkene), Common inorganic ions, Ammonium & immonium and Amine and amino compounds)].

Gruber et al. (1999) [8] reported that salvinorin A content varied between 0.89 - 3.70 mg/g dry weight in the leaves of Mexican sage *S. divinorum* using HPLC analysis. Whereas, Zimmermann et al. (2011) [17] reported polyphenolic composition of aqueous *S. officinalis* extract using rapid ultra-high performance liquid chromatography (UHPLC) with MS/MS and UV analyses. They reported noticeable difference observed in the main either rosmarinic acid or luteolin-7-O-glucoside, phenolic compounds. Moreover, Muttalib and Naqishbandi (2012) [10] reported the presence of flavonoid, saponin, hyrolysable and tannin groups in ethanolic *S. officinalis* leave extracts (with ultrasonic bath for 1 hr at 40 ˚C) (collected from Iraq) using TLC analysis. Whereas, Abu-Dahab et al. (2012) [9] reported phytochemical analysis of 9 *Salvia* species ethanolic extracts (collected from Jordan) using TLC analysis. They reported that flavonoids, coumarins and terpenoids were presented in all *Salvia* species extracts. Whereas, Schulz et al. (2005) [5] reported the occurrence of a-and b-pinene, thymol, carvacrol, 1,8-cineole, p-cymene, g-terpinene and camphor compounds in essential oils of 9 genera (collected from Turkey) belonged to Lamiaceae, using ATR-FTIR and NIR-FT-Raman analyses. Indeed, Al-Qudah et al. (2014) [13] reported phytochemical analysis of the butanol fraction in wild *S. palaestina* using different analytical methods (NMR, UV, IR and MS). They reported the occurrence of new compounds for the first time in nature. These compounds were flavonoid luteolin 7-*O*-(2″-p-hydroxybenzoyl)-β-glucuronide) and the two phenolics salpalaestinin & methyl 3-O-methylrosmarinate compounds besides others that were previously reported. Whereas, Gudi et al. (2015) [5] applied ATR-FTIR spectroscopy for the determination of *S. officinalis* main constituents in its dried leaves. Moreover, Oliveira et al. (2016) [7] reported the presence of 13 peaks in FTIR Sage Tincture spectra.

Whereas, Salimikia et al. (2016) [18] reported two steroids (sitosterol and daucosterol) and three flavonoids (salvigenin, luteolin, and cirsiliol) were identified in *S. chloroleuca* crude areal parts extracts using Nuclear magnetic resonance (1H-NMR and 13C-NMR) analysis. Moreover, ethyl acetate extract has the highest polyphenols compounds compared to n-hexane and methanol once. Indeed, Batista et al. (2017) [19] reported *S. sclareoides* (collected from Portugal) phytochemical analysis. They reported that hexadecanoic acid methyl ester, phytol acetate 2, hexadecanoic acid, linoleic acid ethyl ester and trans-β-caryophyllene oxide, were the main compounds using gas chromatography coupled to mass spectrometry (GC-MS) phytochemical analysis of the *S. sclareoides* volatile oil yielded by supercritical fluid extracts. Moreover, high-performance liquid chromatography (HPLC) with a diode-array detector (DAD). (HPLC-DAD) *S. sclareoides* aqueous extract and its main components identified using liquid chromatography with tandem mass spectrometry (LC-MS/MS) indicated that Rosmarinic acid was the main constituent; whereas, luteolin derivatives [(luteolin diglucuronide and luteolin-7- O-(6´´O-acetylglucoside)] and sagerinic acid, were identified in minor amounts.

Ben Khedher et al. (2017) [20] reported the presence of 49 components including camphor (25.14 %), α-thujone (18.83 %), 1,8-cineole (14.14 %), viridiflorol (7.98 %), β-thujone (4.46 %) and β-caryophyllene (3.30 %) as the major components in leaves *S. officinalis* essential oil (collected from Tunisia), using GC-MS analysis. Whereas, Khiya et al. (2019) [21] reported the presence of polyphenols, saponins and terpenoids, catechics and gallic tannins & flavonoids in Moroccan *S. officinalis.* Moreover, total phenols and flavonoids compounds were highly presented in its methanolic extract. Otherwise, GC–MS analysis of *S. officinalis* leaves essential oils revealed the presence of 105 components, of which trans-thujone (17.74%), 1,8-cineol (12.63%), camphor (12.24%), caryophyllene (9.87%), α-pinene (7.82%), dehydra-aromadendrane (7.29%), and guaiol (7.03%) were the dominant once.

Rosas-Mendoza et al. (2017) [22] reported that olefinic and aliphatic -C-H stretching vibration bands were among spectra analysis observed in FTIR Chia (*Salvia hispanica* L.) seed oil. Whereas, Tulukcu et al. (2019) [3] reported that α-pinene, camphene, β-pinene and eucalyptol were mainly detected in seeds of 6

Salvia species (collected from Turkey) using GC-MS and FTIR analyses. Recently, Jedidi et al. (2020) [6] reported significant variation in total lipids, flavonoids tannins, and polyphenols contents of leaves cultivated *S. officinalis* aqueous extract (cultivated in Tunisia), using HPLC-PDA-ESI-MS/MS-LC/HR-ESI-MS analysis.

The association between phytochemical and genetic variation of plants provided useful and promising information that could help to improve their commercial value. The industrial and economic importance of *Salvia* genus has recently increased and this genus is considered as one of the most attractive natural resource due to its richness in different bioactive compounds [19].

Cluster analysis has been performed using STATISTICA 6 program based on percent disagreement values (PDV). Data presented in Figure 2, FTIR profiling showed that S5 & S10 species were the most closed species (with the lowest PDV of 0.14) among the studied *Salvia* species. These results were in accordance of a recent published data by Saleh 2021 [23] (Figure 3). The later study similarly reported that S5 & S10 species were the most closed species (with the lowest PDV of 0.25) among the studied *Salvia* species based on Td-DAMD analysis. Overall, cluster analysis based on FTIR profiling and recent published data based on Td-DAMD analysis revealed that S5 & S10 species were the most closed species among the studied *Salvia* species.

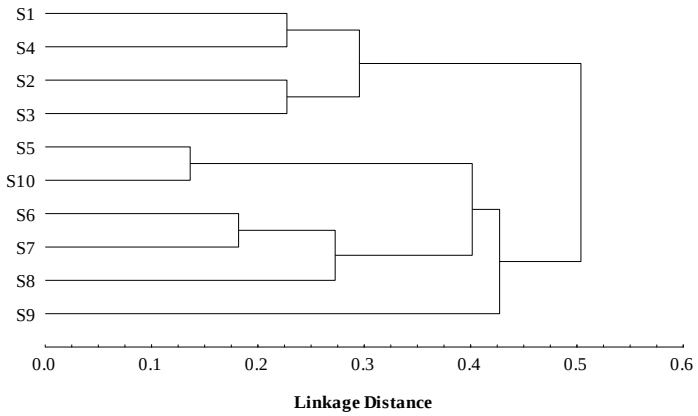

Figure 2. FTIR profiling of the studied *Salvia* species.
S1- *S. judaica* Boiss; S2- *S. viridis* var. *horminum* L.; S3- *S. officinalis* L.; S4- *S. palaestina* Bentham; S5- *S. syriaca* L.; S6- *S. pinardi* Boiss; S7- *S. lanigera* Poiret; S8- *S. ceratophylla* L.; S9- *S. dominica* L and S10- *S. spinosa* L.

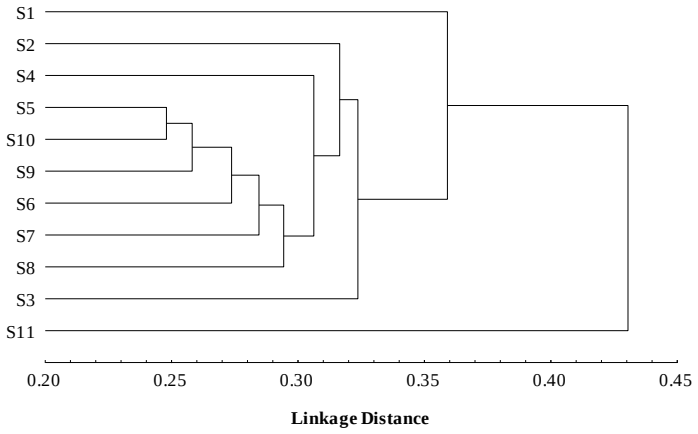

Figure 3. Td-DAMD profiling of the studied *Salvia* species (adopted by Saleh 2021 [23]).
S1- *S. judaica* Boiss; S2- *S. viridis* var. *horminum* L.; S3- *S. officinalis* L.; S4- *S. palaestina* Bentham; S5- *S. syriaca* L.; S6- *S. pinardi* Boiss; S7- *S. lanigera* Poiret; S8- *S. ceratophylla* L.; S9- *S. dominica* L.; S10- *S. spinosa* L. and S11- *Stachys nivea* L. (Lamiaceae).

Skoula *et al.* (1999) [24] suggested that the observed chemical pattern may be based on genetic diversity in S. fruticosa Mill. Species using random amplified polymorphic DNA (RAPD) marker. Similarly, Kremer et al. (2015) [25] reported stronger correlation between the morphological traits and amplified fragment length polymorphism (AFLP) than that those observed between essential oil profile and AFLP in Teucrium arduini; and that the essential oil profile and geographic distribution were strongly correlated compared to that detected between morphological traits and geographic distribution. Whereas, Saleh (2012a) [11] reported that RAPD and AFLP generated profile separated the five Syrian wheat varieties into two separate clusters based on the genome level polidy. Similarly, NIR gave similar pattern. The previous study revealed thereby, correlation between genetic and biochemical diversity.

Moreover, Saleh (2012b) [12] reported that chemical structure using NIR analysis of 5 upland cotton (*Gossypium hirsutum* L.) varieties grown in Syria, was relatively reflected in their genetic variation using AFLP technique.

While, Chahota *et al.* (2017) [26] reported no significant association observed between seeds essential oils analysis based on GC- MS and genetic analysis based on RAPD marker in black cumin (Bunium persicum) populations collected from Himalaya. Similarly, Vieira *et al.* (2001) [27] reported that RAPD profile doesn't reflect necessarily any specific morphological or chemical trait in *Ocimum gratissimum* flavonoids and volatile oils based on Td-DAMD analysis. Further and performance studies required.

4. Conclusions

In conclusion, leaves of 10 Salvia species were phytochemicaly analyzed using FTIR test. Overall, FTIR test revealed different peaks belonged to 9 functional groups [(Aromatics, Ethers, Carboxylic acids, Alcohol and hydroxyl, Alkanes, Olefinic (alkene), Common inorganic ions Ammonium and immonium and Amine and amino compounds)]. These functional groups are known for their biological activity. Performance analytical methods like HPLC, GC-MS, to determine the chemical compounds belonged to each detected functional groups in the current study, are required. Otherwise, cluster analysis revealed that phytochemical diversity of the studied 1o Salvia species is partially correlated with its genetic structure.

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Compliance with Ethics Requirements

Authors declare that he respects the journal's ethics requirements. Authors declare that they have no conflict of interest.

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