

Vibrational Analysis and Thermal Behavior of *Salvia hispanica*, *Nigella sativa* and *Papaver somniferum* Seeds

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ABSTRACT

Introduction: *Salvia hispanica* L., *Nigella sativa* L. and *Papaver somniferum* L. are involved in opiate-dependent behavior. It is known that the seeds of these three herbs contain high amounts of antioxidants, which are helpful in disease prevention, but further research is needed on some of their other phytochemical components (terpene alkaloids, benzoquinones and others), which are claimed to affect human opioid receptors. **Methods:** Seeds from the three afore mentioned plants have been studied by ATR-FTIR vibrational spectroscopy and thermo analytical techniques (TG/DTG, DTA and DSC). **Results:** The infrared spectrum has confirmed the presence of the ester carbonyl of terpenoid alkaloids (such as nigellamine) and the fully conjugated cyclic dione structure of quinones (e.g., thymoquinone). As regards the thermal stability of these seeds, small differences have been observed in their thermal profiles (endothermic effects at around 333°C for chia, 268°C for black cummin and 319°C for poppy seeds), which can be ascribed to their different content in carbohydrates. **Conclusions:** The functional groups of the main active constituents and the thermal behavior of these three seeds have been elucidated.

Key words: ATR-FTIR, Black cummin, Chia, Poppy, Thermal analysis.

INTRODUCTION

Salvia hispanica L.

Salvia hispanica L., commonly known as *chia*, is a species of flowering plant of the mint family, *Lamiaceae*, native to Central and Southern Mexico and Guatemala.¹ The 16th century Codex Mendoza provides evidence that it was cultivated by the Aztec in pre-Columbian times and economic historians have suggested that it may have been as important as maize as a food crop.² Ground or whole chia seeds are still used in Paraguay, Bolivia, Argentina, Mexico, and Guatemala for nutritious drinks and as a food source.³ Today, chia is grown commercially in its native Mexico, as well as in Bolivia, Argentina, Ecuador, Guatemala and Australia.²

Typically, chia seeds are small ovals with a diameter of approximately 1 mm, mottled-colored with brown, gray, black and white. They are hydrophilic, absorbing up to 12 times their weight in liquid when soaked. While soaking, the seeds develop a mucilaginous coating that gives chia-based beverages a distinctive gel texture.

The composition of chia seeds has been reported by Cordain:⁴ fiber (377 g·kg⁻¹), fat (308 g·kg⁻¹), protein (156 g·kg⁻¹) and total carbohydrates (439 g·kg⁻¹). Calcium, potassium and magnesium were the predominant mineral elements. Saturated fats were 32 g·kg⁻¹ vs. 29 g·kg⁻¹ for monounsaturated fat and 233 g·kg⁻¹ for polyunsaturated fat. Linolenic acid concentra-

tion was three times that of linoleic and nine times that of oleic acid. The amounts of A, B1, B2, B3, B6 and C vitamins found in chia seeds were 37 IU, 8.7 µg·g⁻¹, 1.7 µg·g⁻¹, 58 µg·g⁻¹, 6.9 µg·g⁻¹ and 157 µg·g⁻¹, respectively. Among the water-soluble vitamins determined, pantothenic acid was found at 9.40 µg·g⁻¹. Myricetin was the main flavanol (with a concentration three times higher than that of kaempferol). The antioxidants in chia seeds are polyphenols (namely myricetin, quercetin and kaempferol). Total phenolics have been quantified at 47 mM per 1,000 g of seeds (caffeic acid equivalents).⁵

Nigella sativa L.

Nigella sativa is an annual flowering plant, native to Asia and the Middle East, which grows to 20-30 cm (8-12 in) tall. The flowers of this plant, which appear between June and September, have five petals about 2.5 cm wide, usually white but occasionally tinted with pale blue veins. They yield a seed capsule with five compartments, each of which is topped by a spike, and that open to allow seed dispersion. The seeds are small, matte-black grains with a rough surface and an oily white interior. They are triangulate, 2 mm in length and look similar to onion seeds.

The seeds of *N. sativa* have been traditionally prized for their healing properties.⁶⁻¹¹ While frequently referred to amongst English-speaking cultures as *Roman coriander*, *black sesame*, *black caraway* and *onion seed*, it is known today primarily as *black seed*, which is at the very least an accurate description of its

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physical appearance. The earliest record of its cultivation and use come from ancient Egypt: black seed oil was found in Egyptian pharaoh Tutankhamun's tomb, dating back to approximately 3,300 years ago. Black seed is also mentioned as the curative *black cumin* in the Holy Bible and is described as *melanthion* by Hippocrates and Dioscorides and as *gith* by Pliny. In Arabic cultures, black seed is known as *habbatul barakah*, meaning 'the seed of blessing'.

Nergiz and Ötles¹² reported the following composition for *Nigella* seeds: crude fiber (66 g·kg⁻¹), fat (320 g·kg⁻¹), protein (202 g·kg⁻¹) and total carbohydrates (374 g·kg⁻¹). Potassium, calcium and sodium were again the predominant mineral elements. Oleic, linoleic and palmitic acids were 22%, 61% and 11%, respectively. The amounts of α -, β - and δ -tocopherols found were 40 $\mu\text{g}\cdot\text{g}^{-1}$, 50 $\mu\text{g}\cdot\text{g}^{-1}$ and 250 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. B1, B2, B6 and PP vitamins concentrations were 8.31 $\mu\text{g}\cdot\text{g}^{-1}$, 0.63 $\mu\text{g}\cdot\text{g}^{-1}$, 7.9 $\mu\text{g}\cdot\text{g}^{-1}$, and 63 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. The most important active compounds were thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), *t*-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α -pinene and thymol.¹³

Poppy seeds (*Papaver somniferum* L.)

Poppy plant is a biennial herb of Asia Minor and Eastern Mediterranean origin, belonging to the *Papaveraceae* family and *Papaver* genus. Its scientific name is *Papaver somniferum*. The tiny kidney-shaped seeds from the opium poppy are less than a millimeter in length and have a pitted surface. They have been harvested from dried seed pods by various civilizations for thousands of years. Nutty and pleasant in taste, the seeds are used, whole or ground, as an ingredient in many foods, and they are pressed to obtain poppy seed oil.¹⁴⁻¹⁸ Their primary flavor compound is 2-pentylfuran.¹⁸

In 2006 by Nergiz and Ötles¹⁸ studied the composition of poppy seeds: fat (440 g·kg⁻¹), protein (211 g·kg⁻¹), moisture (50 g·kg⁻¹), ash (63 g·kg⁻¹), crude fiber (62 g·kg⁻¹) and total carbohydrates (236 g·kg⁻¹). Potassium and calcium were the predominant mineral elements. Linoleic acid was the major unsaturated fatty acid, while palmitic acid was the main saturated one. The amounts of α -, β - and δ -tocopherols found in poppy seed were 220 $\mu\text{g}\cdot\text{g}^{-1}$, 40 $\mu\text{g}\cdot\text{g}^{-1}$ and 20 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. Among the water-soluble vitamins determined, pantothenic acid was predominant, followed by niacin and thiamin.

On the interaction of seed components and opiate receptors

It is known that *Papaver* seeds contain minute traces of opiates (alkaloids as opium and codeine occur in poppy seeds, albeit in tiny quantities, as a result of insect damage or through poor harvesting practices), but it is less known that *Salvia* and *Nigella* species seeds contain terpenoids and quinones that interact with opiate receptors. Although *Salvia hispanica* seeds do not share with *Salvia divinorum* seeds the presence of salvinin A diterpenoid (a potent non-nitrogenated agonist at the κ -opioid receptor), it has been claimed that it may alleviate opiate withdrawal symptoms. Likewise, thymoquinone from *Nigella sativa* acts on μ - and κ -opioid receptors. This quinone is a calcium channel blocker, and drugs in this group are frequently used to help opiate addicts overcome their addiction.¹⁹⁻²¹

In the work presented here in, a rapid and accurate method for the fast and simultaneous qualitative and quantitative characterization of natural products and their constituents,²² namely Attenuated Total Reflection-Fourier-Transform Infrared spectroscopy (ATR-FTIR), and three thermo analytical techniques (*viz.*, thermo gravimetry (TG/DTG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC)) have been used to study the chemical properties and thermal behavior of the three aforementioned seeds.

MATERIALS AND METHODS

Samples

Salvia hispanica seeds were obtained from Supreme Pharmaceuticals Mysore Pvt. Ltd. (Mysore, India). *Nigella sativa* seeds were obtained from Natco Foods Ltd. (Buckingham, UK). *Papaver somniferum* seeds were obtained from the Government Opium Factory (Neemuch, Madhya Pradesh, India).

Methodology

Seed moisture content, expressed as percentage by mass, was determined using 5 g of seeds by adapting AOAC method 934.06.²³ A Heraeus T 6030 heating and drying oven (Thermo Scientific, Waltham, MA, USA) regulated at 105°C was used. The difference between the results of two last determinations was 0.1 g of moisture per 100 g of sample. Nutritional composition of the seeds was determined using the recommended methods of the AOAC.²³ Ash content was determined by incinerating 5 g of oil cake at 550°C in a muffle furnace. Crude protein content was calculated from the nitrogen content measured by the Kjeldahl procedure with a Vapodest 20 instrument (C. Gerhardt GmbH & Co., Königswinter, Germany) using a 6.25 factor. An estimation of crude fiber was obtained by difference between dry weight and ash content of the residue.²³ Total fat content was obtained by the Soxhlet extraction method using *n*-hexane as described by IUPAC Method 1.122. Carbohydrate content was obtained by subtracting the sum of protein, fat, ash and moisture from 100. Fatty acid methyl esters were prepared by methylation of the lipids according to IUPAC method 2.301. Gas chromatography (GC) of the methyl esters was conducted on an Agilent Technologies (Santa Clara, CA, USA) 7890A, equipped with a hydrogen flame ionization detector. For the tocopherol analysis, an Agilent Technologies 1200 Series high-performance liquid chromatograph (HPLC) was used.²⁴ Total polyphenols were determined according to Nergiz and Ünal.²⁵

The vibrational spectra of the materials in the 400-4000 cm⁻¹ range were measured using a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 FT-IR Spectrometer, equipped with an in-built diamond attenuated total reflection (ATR) system.

TG/DTG/DTA/DSC analyses were conducted with a Perkin-Elmer (Waltham, MA, USA) STA6000 simultaneous thermal analyser by heating the samples in a slow stream of N₂ (20 mL/min) from room temperature up to 500°C, with a heating rate of 20°C/min. Pyris v.11 software was used for data analysis.

RESULTS AND DISCUSSION

Chemical composition

The analytical results for seed moisture, ash, crude fiber, total fat content, carbohydrate content fatty acids, total tocopherols and total polyphenols for the three vegetal species, summarized in Table 1, were in good agreement with the bibliography.^{4,5,12,18,25}

Vibrational characterization

The ATR-FTIR spectra of the three seeds under study are depicted in Figure 1, and their main bands with their respective assignments are summarized in Table 2. It can be observed that they are closely related, in particular those of *Salvia hispanica* and *Nigella sativa* species. The relationship with the spectra of calafate (*Berberis buxifolia* L.)²⁶ and rosehip (*Rosa rubiginosa* L.)²⁷ seeds, also included in Table 2 for comparison purposes, is also very high.

The highest absorbance for the three spectra occurred at 1022 cm⁻¹. Poppy seeds spectrum showed a main peak at 1313 cm⁻¹ and one at 777 cm⁻¹,

Table 1: Comparison of the compositions of the three seeds under study.

	Chia seeds	Black cumin seeds	Poppy seeds
Moisture (%)	5.9	3.8-6.4	3.5-5.9
Ash (%)	4	4-5	5-6
Crude fiber (gkg ⁻¹)	237-377	84-216	195-272
Fats (gkg ⁻¹)	285-344	320-406	415-440
Saturated (%)	10	15	10
Palmitic (%)	7	11	8.6-10
Stearic (%)	3	3	
Monounsaturated (%)	8	23	5-11
Oleic (%)	7	22	4-11
Polysaturated (%)	82	62	75
Omega 6 – Linoleic (%)	20	61	45
Omega 3 – Linolenic (%)	62	1	27
Protein (gkg ⁻¹)	156-267	202	180-235
Carbohydrate (gkg ⁻¹)	260-439	374	236-281
Tocopherols (μg g ⁻¹)	1000	340	280
α (μg g ⁻¹)	25	40	220
β (μg g ⁻¹)	25	50	40
γ (μg g ⁻¹)	944	250	20
Total polyphenols, including flavonoids (μg g ⁻¹)	920	4120	19377

Table 2: Main bands in the ATR-FTIR spectra of the three seeds under study and their assignments.

Chia seedst	Dry chia seeds	Black cumin seeds	Poppy seeds	Calafate seeds	Rosehip seeds	Assignments
3294	3292	3281	3280	3290		v(OH), v(C-H)
3010	3010	3009	3009	3010	3007	v(C-H), v(=CH)
2925	2925	2923	2924	2925	2920	asym. v(C-H)
2854	2854	2853	2854	2854	2850	symmetric v(C-H) from phytyl chains (tocopherols)
1735	1732	1744	1743	1743	1730	v(CO) ester from terpene alkaloids
1627	1627	1633		1637		v(C=O) quinoid ring / δ(N-H) (amide I),
			1615		1614	v(C=C) quinoid ring
1548	1538	1540	1548	1539		phenolic C-O (tocopherols) /
1519					1521	phenyl rings / δ(N-H) (amide II)
1456/1434	1435	1456	1456	1440	1456	δ(CH ₂)
1417	1417	1416	1416	1412	1412	ωCH ₂ , O-H in plane def.
1373	1373	1378	1379	1379	1371	δ(CH ₂) ₂ quinones
(1313)		(1316)	1313			coupled C-C/C=C vibration
1240	1239	1239	1235	1238	1238	phenolic C-O (tocopherols) / δ(N-H) (amide III)
1154	1151	1143	1140	1140	1161	methyl ester, v(C-O-C)
1098	1099				1115	starch OH, cellulose
				1045	1049	ring resonance
1023	1019	1034	1026	1027	1012	PO ₄ ³⁻ / v(-C=O) / aromatic ring C-H
891	892	871	888	895	825	
		812	778			typical of xanthene and riboflavin
557	671		560			ρ(CH ₂) _n / aromatic ring C-H
533	534	521		521		
452	452	510				
460	510	460				

† Common to all color varieties

which are very weak (or do not appear) in the spectra of the other two seeds. Both peaks can be assigned to polyphenols (flavonoids), in which poppy seeds are very rich.

Phytochemical components of seeds -such as nigellamines, thymoquinone and others- that affect the human opioid receptors, could be identified: the ester carbonyl band of terpene alkaloids appeared at 1735 cm^{-1} and the fully conjugated cyclic dione structure of quinones led to absorbance at 1630 cm^{-1} and 1615 cm^{-1} .

Thermal behavior

The TG curves of chia seeds (Figure 2a) were closely similar for the different varieties (mottled-colored with brown, gray, black and white), so only one curve is shown. A small weight loss ($\sim 4\%$) was recorded up to 150°C (first DTG peak), mainly due to the evaporation of a fraction of free water contained in the seed powder. Upon heating up to ca. 300°C (second DTG peak), approximately an additional 15% of the original weight was lost. In this stage, desorption of all free and bound water took place from the seed molecules. An abrupt pattern of mass loss was observed when seeds were further heated above 300°C , indicating the decomposition of the polysaccharide molecules to low molecular weight volatiles. Seeds lost nearly an additional 60% weight when heated up to 500°C , in two steps associated to two DTG peaks at ca. 350°C and ca. 400°C . The behavior of the other two seeds is analogous, with DTG peaks at ca. 266°C and 425°C for black cumin seeds (Figure 2b) and at 348°C and 410°C for poppy seeds (Figure 2c).

As regards the DTA (Figure 3) and DSC curves, the three of them show several thermal effects similar to those of typical oilseed polysaccharides. A broad endothermic transition is first observed, corresponding to moisture loss (free water) from the seed powder, peaking at around 150°C (142°C in DTA and 158°C in DSC) for chia; 117°C for black cumin (observed in DTA); and 110°C for poppy seeds (both in DTA and DSC). The endothermic peaks at 333°C and 460°C for chia, at 245°C and 268°C (very weak) for black cumin seeds, and at 319°C and 425°C for poppy seeds can be attributed to pyrolysis of glycosyl units. The intermediate exothermic effect which appears at 362°C for chia and the end effect for poppy seeds at 439°C could be related to the oxidation to the volatiles generated in the preceding endothermic process.

CONCLUSIONS

It is known that poppy seeds contain traces of opiates, but it is less known that *Salvia* and *Nigella* species seeds contain terpenoids and quinones that interact with opiate receptors. In this work, the ester carbonyl of terpenoid alkaloids (e.g., nigellamine) and the fully conjugated cyclic di-

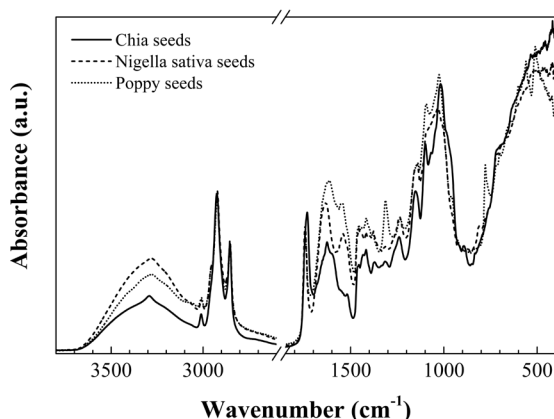


Figure 1: ATR-FTIR spectra of chia (solid line), black cumin (dashed line) and poppy (dotted line) seeds.

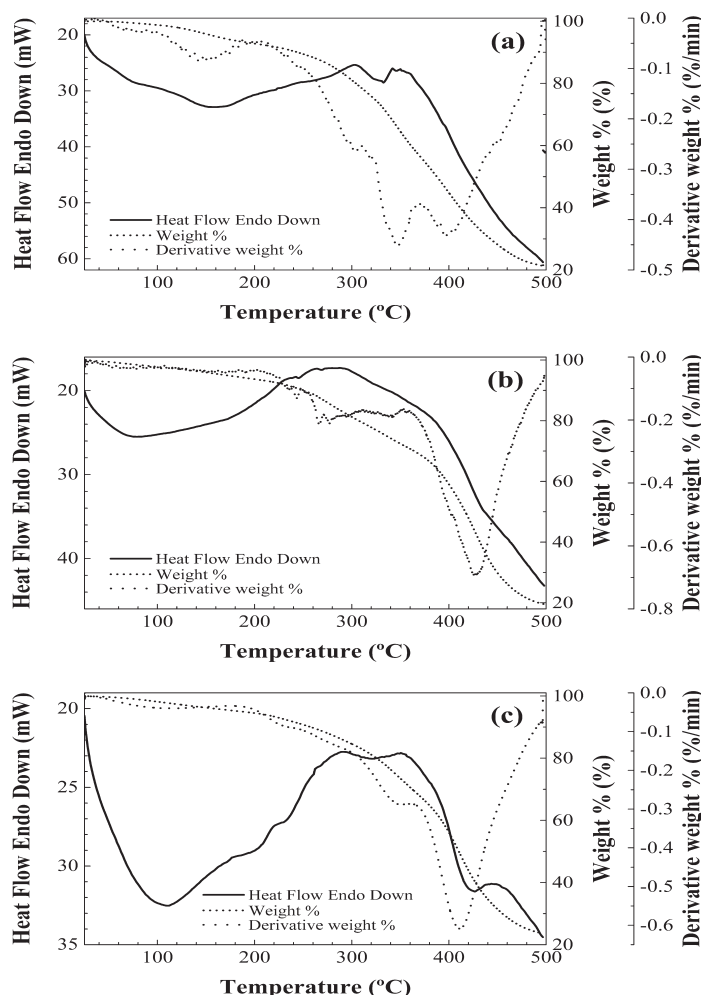


Figure 2: TG/DTG and DSC curves for chia (a), black cumin (b) and poppy (c) seeds.

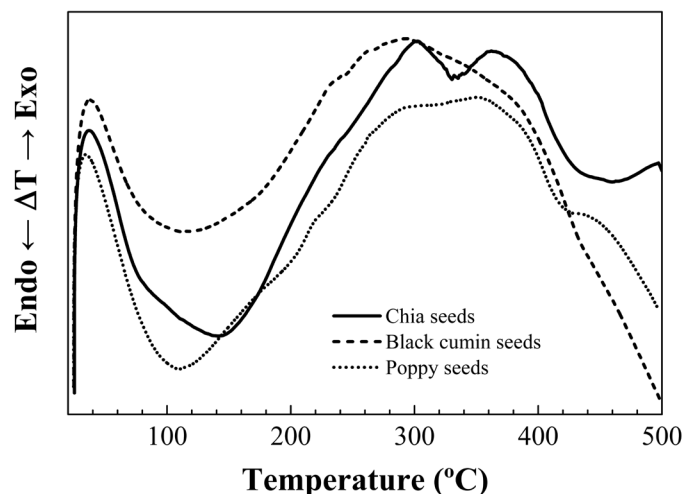


Figure 3: DTA thermograms for chia, black cumin and poppy seeds.

one structure of quinones (e.g., thymoquinone) have been identified by a rapid and precise method (ATR-FTIR spectroscopy). On the other hand, the study of the thermal profiles of the seeds by TG/DTG, DTA and DSC techniques has allowed to observe differences which could be related to the abundance of carbohydrates and fiber: pyrolysis of black cumin seeds

takes place at temperatures 65°C lower than those of chia and poppy seeds, thus evincing a higher thermal stability of the latter two.

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CONFLICT OF INTEREST

None

ABBREVIATION USED

ATR-FTIR: Attenuated Total Reflection–Fourier-Transform Infrared spectroscopy; DSC: differential scanning calorimetry; DTA: differential thermal analysis; DTG: derivative thermogravimetry; TG: thermogravimetry.

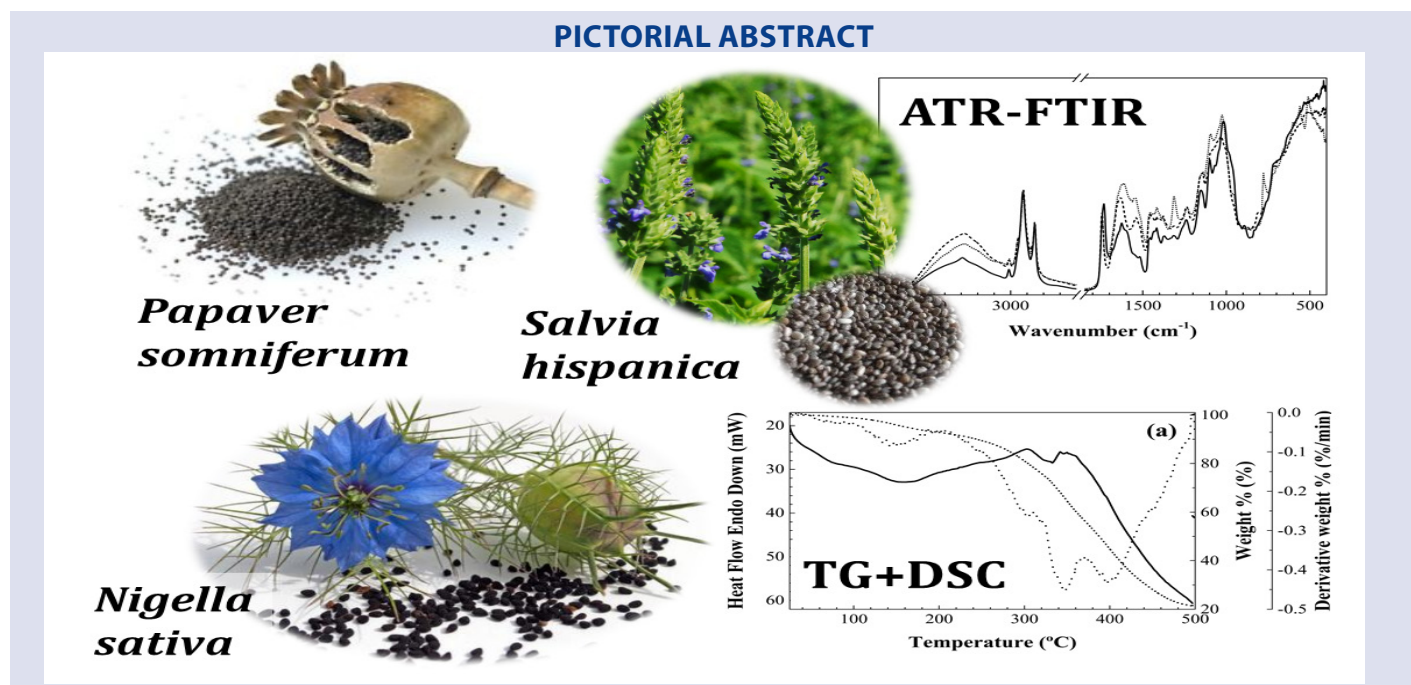
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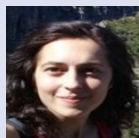
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PICTORIAL ABSTRACT



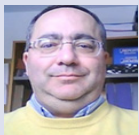
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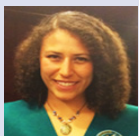
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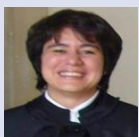
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