Various Extraction and Analytical Techniques for Isolation and Identification of Secondary Metabolites from *Nigella sativa* Seeds

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Abstract: *Nigella sativa* L. (black cumin), commonly known as black seed, is a member of the Ranunculaceae family. This seed is used as a natural remedy in many Middle Eastern and Far Eastern countries. Extracts prepared from *N. sativa* have, for centuries, been used for medical purposes. Thus far, the organic compounds in *N. sativa*, including alkaloids, steroids, carbohydrates, flavonoids, fatty acids, etc. have been fairly well characterized. Herein, we summarize some new extraction techniques, including microwave assisted extraction (MAE) and supercritical extraction techniques (SFE), in addition to the classical method of hydrodistillation (HD), which have been employed for isolation and various analytical techniques used for the identification of secondary metabolites in black seed. We believe that some compounds contained in *N. sativa* remain to be identified, and that high-throughput screening could help to identify new compounds. A study addressing environmentally-friendly techniques that have minimal or no environmental effects is currently underway in our laboratory.

Keywords: Black seeds, environmentally-friendly techniques, high-throughput screening, *Nigella sativa*, secondary metabolites, volatile components.

1. INTRODUCTION

Nigella sativa L. is a widely distributed herbaceous plant belonging to the Ranunculaceae family. The seed of the plant is variously referred to as black seed, black cumin (English), black-caraway seed (U.S.A.), habba-tusawda (Arabic), kalonji (Urdu and Hindi), krishnajirika (Sanskrit), kalajira (Bangali) and shonaiz (Persian) [1]. It has been employed for thousands of years as a spice, a food preservative, and a natural remedy. The earliest known article referring to *Nigella sativa* was published before 1871 [2]. Additionally, according to SCIFinder® statistics in the database of the Chemical Abstracts Service (CAS), more and more investigations of *Nigella sativa* are being conducted, and these studies are providing clear evidence of its profound medicinal properties, which include antioxidant, anti-inflammatory, antihistamine, antimicrobial, antitumor, anti-hepato/nephrotoxic, respiratory, and immunomodulatory effects.

Several previously published review articles have focused on pharmacological and toxicological properties [3], immunomodulatory and therapeutic properties [4], and composition and possible therapeutic rules [1,5]. All of these articles reviewed publications released no later than 2008. Additionally, previous chemical composition studies have generally not been sufficiently comprehensive. Additionally, from 2009 on, new research is being conducted into *Nigella sativa* at a prodigious pace--more than 100 reports are currently being published annually. Moreover, there has been no overview regarding the best methods to extract the active ingredients from black seed and to ensure the quality of the black seed during usage. This review article focuses on the extraction methods for volatile components and analytical methods for active ingredients in the seeds of *Nigella sativa*. The relevant chemical composition studies are also completely reviewed in detail, including the most recently-published articles (Fig. **1**).

2. CHEMICAL COMPOSITION

The seeds of *Nigella sativa* had been and are being globally investigated. Due to the different original sources, different determination methods, and different research scientists, the content levels of each ingredient in black seed have been reported within a broad range. Oil, protein, ash, moisture, fiber, and total carbohydrate content ranged from 24.76-40.35%, 18.6 to 31.2%, 3.7 to 15.0%, 3.8 to 7.4%, 3.7 to 4.7%, and 23.5 to 40.0% in different studies [6-11].

2.1. Essential Oil or Volatile Components

Essential oil is regarded as a bioactive ingredient in black seed. The essential oil content in black seed was measured in a range between 0.08 to 1.7% [12-19]. More than 100 compounds have been identified from the essential oil of *Nigella sativa* seeds *via* GC-MS. These include basic monoterpenoidic constituents (Including hydrocarbons, alcohols, phenols, and ketones), sesquiterpenes, phenol propanoid compounds, and other trace amount constituents [13-15,17,19-

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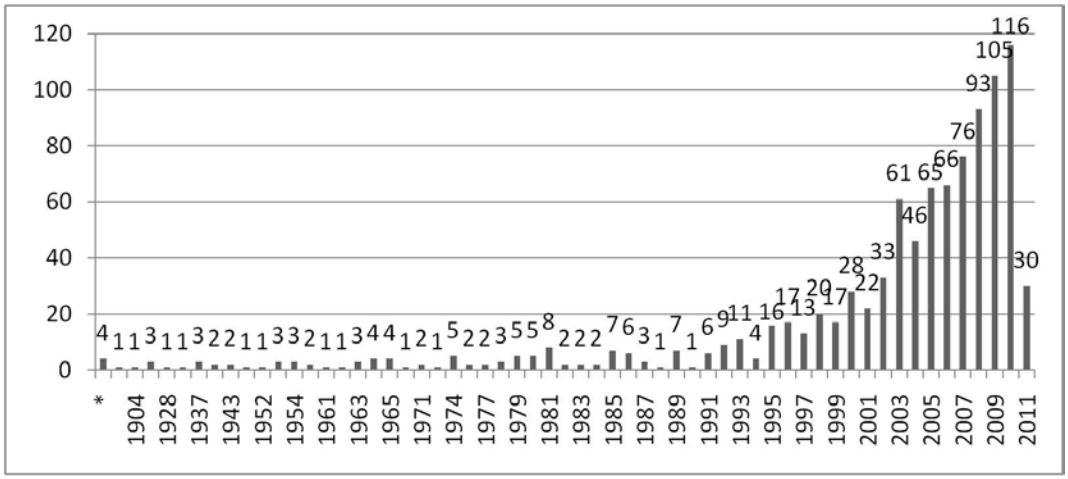

Fig. (1). Publication articles about *Nigella sativa* according to SCIFinder statistics based on Chemical Abstracts Service (CAS) indexing until March 29, 2011 (Totally 960 articles).

*References not containing information for this analysis.

22]. However, the contents of each constituent published by different scientists varied greatly. The major constituents in each of the research articles reviewed herein were published over the past 10 years; the relevant details of the essential oil of *N. sativa* seeds are abstracted and compared in Table **1** and Fig. (**2**).

2.2. Lipids or Fixed Oil

The oil material contents of *Nigella sativa* seeds have been reported in a range of 24.76-40.35%. The so-called fixed oil, stable oil, lipids and crude oil have all been evaluated in previous articles. The principal components of the fixed oil were identified as triacylglycerols (57.5-83.1%), free fatty acids (14.2-16.2%), monoacylglycerols (0.41-5.7%), diacylglycerols (0.65-5.1%), sterols (0.33-5.0%), and other trace materials including phospholipids, fat-soluble vitamins, lipases, etc. [6,11,23,25-28]. Hamrouni-Sellami *et al*. reported very different triacylglycerol and free fatty acid contents and attributed them to the high triacylglycerol lipolytic activity [7]. According to the results published by Ramadan and MÖrsel, *Nigella sativa* seed oil consisted of six triacylglycerol species. The species of C54:3 [Equivalent carbon number (ECN) =48] and C54:6 (ECN=42) were present to an extent of 74% or greater [25].

2.2.1. Fatty Acids

Fatty acid composition was studied after derivatization to fatty methyl esters *via* different approaches. The total unsaturated and saturated fatty acids in *Nigella sativa* oil were in a range of 69.7-77.9% and 22.0-25.5%, respectively. The major fatty acids were identified as linoleic acid ($C_{18:2}$, 49.2-62.1%) and oleic acid ($C_{18:1}$, 19.21-25.0%) as unsaturated fatty acids; however, palmitic acid ($C_{16:0}$, 8.59-18.4%) and stearic acid ($C_{18:0}$, 2.84-3.7%) have been identified as saturated fatty acids in investigations conducted over the past decade. Meanwhile, small or trace amounts of palmitoleic ($C_{16:1}$), margaroleic ($C_{17:1}$), linolenic ($C_{18:3}$), dihomolinoleic ($C_{20:2}$), eicosadienoic ($C_{20:2}$), and erucic ($C_{22:1}$) acid as unsaturated fatty acids, as well as lauric ($C_{12:0}$), myristic ($C_{14:0}$), arachidic ($C_{20:0}$), behenic ($C_{22:0}$), lignoceric ($C_{24:0}$) acid as saturated fatty acids were detected [6,8,11,20,23,25,27].

2.2.2. Sterols

The crude oil of *Nigella sativa* seeds contained significant quantities of sterols (0.33-5.0%). Possibly as the consequence of geographical conditions, climate, and seed maturation, the sterol component contents differed among different studies. In the majority of published articles, β-sitosterol was identified as the major component (44.53-76.8%), followed by stigmasterol (9.5-19.6%). Cholesterol, β-sitostanol, campestrol, Δ^7-avenasterol, Δ^5-avenasterol, Δ^7-stigmasterol were also detected in small quantities [6,7,28]. Nevertheless, Ramadan *et al*. reported that β-sitosterol (32.2–34.1% of total sterol content) represents the main component of the phytosterols, followed by Δ5-avenasterol (27.8–27.9% of total sterol content), and Δ^7-avenasterol (18.5–22.0% of total sterol content) [25,29]; and Δ^7-avenasterol was identified as the dominant sterol of the total sterol pool in black cumin subclasses, followed by β-sitosterol [30]. Moreover, one novel steroid and the other two aliphatic esters were previously isolated from *Nigella sativa* seeds and identified as ergosta-5,24 (28)-dien-2,3-*cis*-diol; pentylundecanoate; and methyloctadeca-14,16-dienoate [31].

2.3. Minerals

Nigella sativa seeds contained significant amounts of important mineral elements. The measurable elements were potassium, calcium, magnesium, phosphorus, sodium, iron, zinc, manganese, and copper, and these constituents were found in various amounts by various researchers (Table **2**). The content level of each element differed hugely, in a range from 10 to more than 10,000 times [8,10,11,32,33].

2.4. Others

Both fat-soluble and water-soluble vitamins (FSV & WSV) have been detected in *Nigella sativa* seeds. FSV and β-carotene together comprise more than 0.2% of the total oil content [26]. The detected FSV included α-tocopherol,

Table 1. Chemical Composition (%) of Major Constituents in the Essential Oil of *Nigella sativa* Seeds of Different Original Places

Original place	Tunisia	Tunisia	Tunisia	India	India	Czech				Czech	Poland	Iran	Iran	Algeria		Algeria
Researcher	Toma *et al.* [14]	Bourgou *et al.* [23]	Ham-rouni-Sellami *et al.* [7]	Venka-tachal-lam *et al.* [12]	Singh *et al.* [17]	Kokoska *et al.* [15]				Hav-lik *et al.* [24]	Wajs *et al.* [13]	Jalali-Heravi *et al.* [16]	Nickavar *et al.* [20]	Benkaci-Ali *et al.* [19]		Benkaci-Ali *et al.* [21]
Published time	2010	2010	2008	2010	2005	2008				2006	2008	2007	2003	2007		2006
Extraction method	HD	HD	DHS	SFE-HD	HD	HD	SD	SE-HD	SFE-HD	HD	HD	HD	SE-HD	HD	MD	MD
Yield	NM	0.39-0.72%	-	1.5% (V/V)	1.2%	0.29%	0.39%	0.34%	0.27%	0.42%	1.7%	2.66%	-	0.08-0.18%	0.11-0.2%	0.57%
α-thujene	ND	9.11-14.88	7.2	ND	10.03	15.1	17.5	4.1	0.3	3.7	7.2	0.11	2.4	0.5-0.7	4.1-6.5	11.91
α-pinene	13.75	1.59-2.60	1.4	ND	3.33	3.3	3.8	0.9	Tr	5.7	2.0	3.98	1.2	ND	ND-2.1	2.29
β-pinene	3.0	2.10-2.73	1.8	0.40	3.78	4	4.2	1.8	Tr	0.1	2.1	6.22	1.3	ND	ND-2.3	1.96
Sabinene	1.66	0.90-1.31	0.7	ND	1.34	2	1.9	0.9	Tr	21.4	0.8	1.14	1.4	0.1-0.4	1.2-5.3	2.79
Limonene	2.55	1.27-1.59	0.1	1.03	1.76	2.9	2.9	2.1	ND	0.7	ND	1.41	4.3	ND	0.4-1.1	1.88
γ- terpinene	1.40	15.20-39.32	1.2	12.87	0.16	1.2	0.7	1	Tr	4.5	12.9	24.40	0.5	0.4-0.6	1.2-2.9	1.43
p- cymene	43.58	31.41-43.65	53.1	ND	36.20	56.2	52.0	42.4	8.6	0.5	60.2	8.33	14.8	7.2-8.9	28.1-32.0	53.83
Terpinolene	9.08	0.26-0.49	0.1	Tr	0.06	ND	ND	ND	ND	0.2	0.6	0.91	ND	ND-0.8	ND-0.1	0.14
Terpinen-4-ol	4.25	0.34-1.58	0.4	ND	2.37	0.9	0.6	0.8	1.4	0.1	0.9	0.58	0.7	0.6-8.9	2.0-3.4	0.38
Thymoquinone	1.65	0.06-0.12	ND	38.41	11.27	0.5	4.3	30.7	76.7	Tr	Tr	ND	0.6	1.6-21.8	10.8-24.6	17.04
Thymol	1.67	0.02-0.08	1.8	16.95	0.13	ND	Tr	ND	Tr	Tr	Tr	ND	ND	0.7-1.5	0.3	0.01
Carvacrol	2.53	0.27-5.30	ND	0.81	2.12	0.8	0.6	1	2.5	ND	3.0	ND	1.6	12.9	3.0-6.0	0.68
2-Undecanone	ND	ND	ND	13.72	0.09	ND	ND	ND	ND	ND	ND	ND	ND	ND-0.1	ND-0.1	0.02
α-Longipinene	0.95	ND	ND	ND	1.54	0.7	0.6	0.6	0.9	0.1	0.1	ND	0.3	0.2-0.7	0.8-2.2	0.36
α-Longifolene	ND	0.13-1.27	ND	0.51	6.32	2.6	2.2	1.8	2.6	0.3	Tr	ND	0.7	Tr-1.8	3.2-6.0	1.21
β-Caryophyllene	ND	ND	ND	4.8	ND	0.1	0.4	ND	0.3	Tr	ND	ND	ND	Tr	Tr	0.02

HD: Hydro distillation; SD: Steam distillation; SE: Solvent Extraction; SFE: Supercritical fluid extraction; DHS: Dynamic headspace; MD: Microwave distillation.
NM: Not mentioned; ND: Not detected; Tr: Trace.

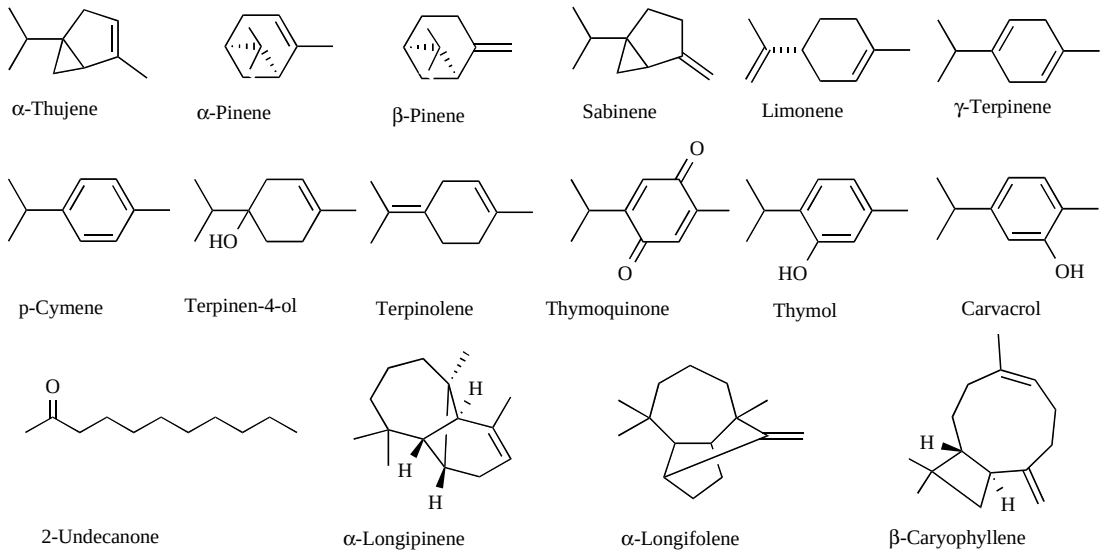

Fig. (2). Chemical structures of the major components in the essential oil of *Nigella sativa* seeds.

Table 2. The Mineral Content of *Nigella sativa* Seeds from Different Original Places and Researchers

Researcher	**Sultan *et al.* [33]**	**Iqbal *et al.* [32]**	**Ashraf *et al.* [8]**	**Cheikh-Rouhou*et al.* [11]**		**Takruri *et al.* [10]**			
Published time	**2009**	**2009**	**2006**	**2007**		**1998**			
Original place	**Pakistan**	**Pakistan**	**Pakistan**	**Tunisia**	**Iran**	**India**	**Jordan**	**Syria**	**Turkey**
Potassium (mg/kg)	8080	8300	6750	783	708	5517	4423	5606	5380
Calcium (mg/kg)	5700	9.13	2809.2	572	564	1932	1867	2005	1544
Magnesium (mg/kg)	2650	10.20	1381.7	235	260	-	-	-	-
Phosphorus (mg/kg)	5430	5700	3781.2	48.9	51.9	5043	5023	5769	5267
Sodium (mg/kg)	176	0.35	4125.0	20.8	18.5	550	419	535	440
Iron (mg/kg)	97	0.26	104.0	8.65	9.42	102	107	93	130
Zinc (mg/kg)	62.3	0.05	44.3	8.04	7.03	62	59	59	56
Manganese (mg/kg)	85.3	0.05	125.3	4.43	3.37	-	-	-	-
Copper (mg/kg)	26	0.03	31.7	1.65	1.48	24	18	17	18

β-tocopherol, γ-tocopherol, δ-tocopherol, vitamin K_1 (2-methyl-3-phytyl-1, 4-naphthochinon), and vitamin A (all-*trans*-retinols). The mean concentrations of selenium, DL-α-tocopherol, DL-γ-tocopherol, and all-*trans*-retinol were 0.177, 9.027, 5.427, and 0.277 mg/kg fresh weight, respectively [34]. Vitamin B1, vitamin B6, niacin, and folic acid as WSV were also reported, at average concentrations of 14.6, 6.6, 56.5, and 614 mg/kg on a dry matter basis, respectively [10].

Several mono triterpene aglycones and saponins have been isolated and identified from the seeds of *Nigella sativa*, and the chemical structures have been elucidated as 3,23-dihydroxy-12-oleanan-28-oic acid [35]; cycloart-23-methyl-7,20,22-triene-3β,30-diol ; cycloart-3-one-7,22-diene-24-ol [36]; α-hederin [37]; 3-O-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-hederagenin; 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-hederagenin; and 3-O-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin [38]; 3-O-[-D-xylopyranosyl-(12)-L-rhamnopyranosyl-(1, 2)-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methyllolean-12-enoate [39]; and 3-O-[β-D-xylopyranosyl-(1→3)-α-D-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl]-11-methoxy-16-hydroxy-17-acetoxy-hederagenin [40].

Three types of alkaloids were found at trace levels in *Nigella sativa* seeds, among which isoquinone alkaloids included nigellimine and nigellimine-N-oxide [41,42]; inda-

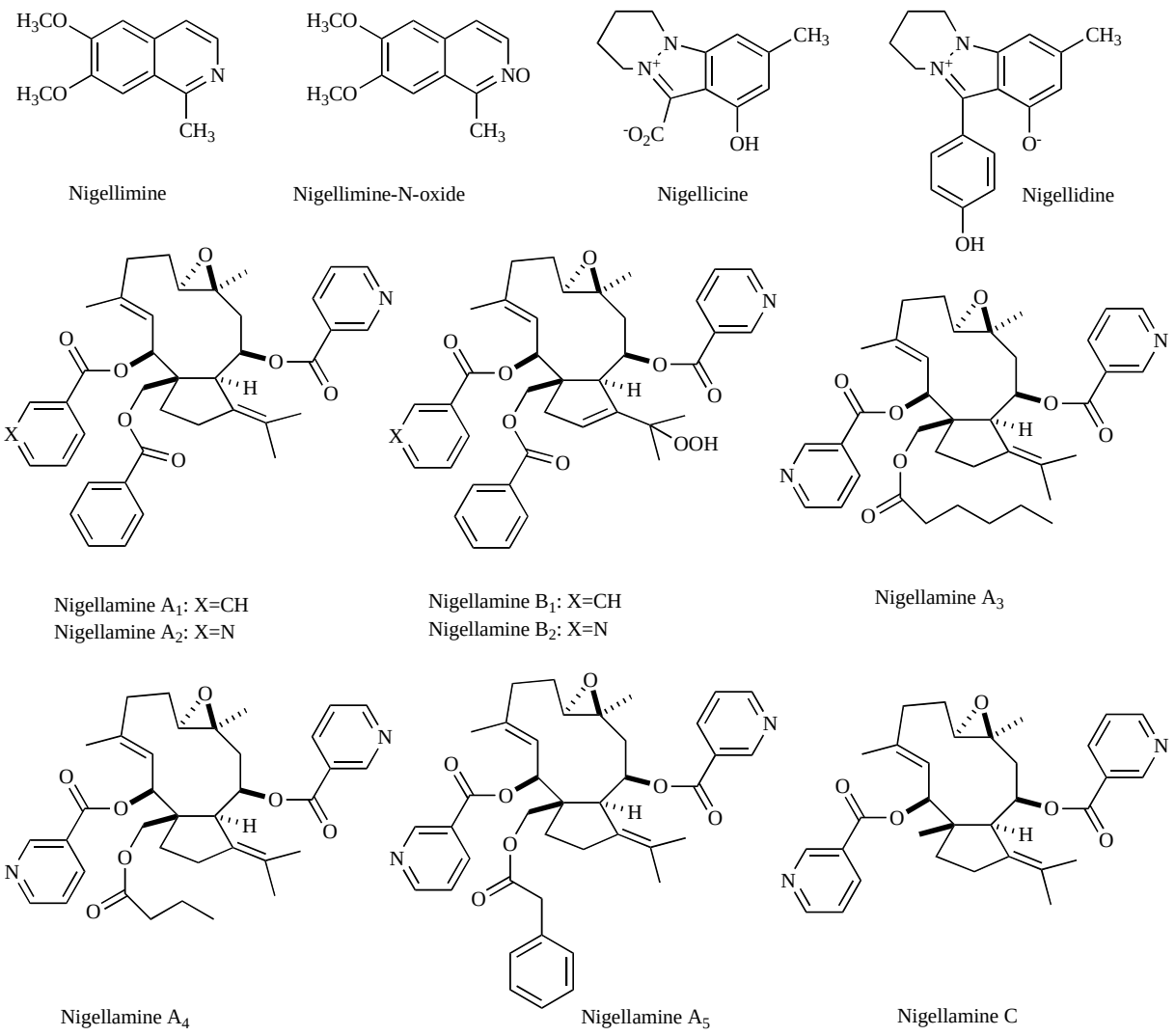

Fig. (3). Chemical structures of alkaloids in *Nigella sativa* seeds.

zole alkaloids included nigellidine, nigellicine, and nigellidine-4-O-sulfite [43,44]; dolabellane-type alkaloids included nigellamines A1, A2, A3, A4, A5, B1, B2, and C [45,46] (Fig. **3**).

Furthermore, a lipid-transporting protein was previously isolated and characterized from the seeds of *Nigella sativa* with a molecular mass of 9602 Da; this protein contains eight cysteine residues that form four disulfide bridges [47]. The component composition of protein-peptide fractions was also evaluated [48].

3. EXTRACTION AND DETECTION METHODS

Technically, a variety of extraction and detection methods should be used for the determination of various component levels in *Nigella sativa* seeds. To overview all of the available published articles, the extraction methods available can be summarized as follows: solvent extraction (SE), supercritical fluid extraction (SFE), hydro distillation extraction (HD), steam distillation extraction (SD), SE-SD, SFE-SD, microwave assisted extraction (MAE), and headspace solid phase micro extraction (HS-SPME). The applied detection methods included gas chromatography equipped with a flame ionization detector (GC-FID), or equipped with a mass spectrometry detector (GC-MSD), gas liquid chromatography equipped with FID (GLC-FID), high performance liquid chromatography (HPLC) coupled with an ultraviolet detector (UVD), HPLC coupled with electrospray ionization (ESI) time of flight mass spectrum detector (TOF-MSD), thin-layer chromatography (TLC), differential pulse polarographic (DPP), and other traditional methods for the determination of dry matter, fat, protein, ash, mineral, and carbohydrate contents.

3.1. Extraction Methods

Hydro distillation (HD) is a traditional method used for the extraction of essential oil or volatile components, along with steam distillation (SD). HD is a popular method, and is almost always employed as a basic method for comparison for newly developed extraction methods in the study of volatile components of *Nigella sativa* seeds [13,14,16,17, 19,20,22,49]. Normally, a Clevenger-type or Likens-Nickerson apparatus is used in HD. When HD or SD is performed, the volatile components from samples are extracted

with hot steam and condensed *via* an adaptor apparatus. Hence, the main factor to be considered is extraction time. HD is an easy extraction method that is simple to perform and inexpensive. However, the long extraction time and comparative constituent loss should be carefully considered before deciding to use the method.

Solvent extraction (SE), including soxhlet extraction, is another traditional extraction method, and perhaps the easiest. Hexane, chloroform, acetone, and methanol are normally used to extract the essential oils and fixed oils from *Nigella sativa* seeds [17,18,23,25,50]. For the extraction of essential oil or volatile compounds, this technique is usually combined with SD [15,20].

However, both HD (SD) and SE had few adjustable parameters by which the selectivity of the extraction processes can be controlled. Consequently, a supercritical fluid extraction (SFE) technique has been developed and studied extensively for the extraction of active compounds from herbs and other materials. SFE is a modified SE technique. The solvation power of supercritical fluids (SFs) can be readily adjusted by altering the extraction pressure and temperature or modifier contents to achieve good selectivity. Normally, the sample preparation method, type of fluid, choice of modifier, extraction pressure, extraction temperature, extraction time, and flow rate should be optimized for successful SFE. Among all the gases and liquids studied, CO_2 remains the most commonly used fluid for SFE applications. SFE with CO_2 can overcome some of the problems associated with conventional techniques, since solvent contamination and hydrolysis reactions can pose problems in SE and HD, respectively. Moreover, CO_2 is nontoxic, cheap, and nonflammable [51].

Many investigations of SFE for *Nigella sativa* seeds have been carried out. The deacidification of *Nigella sativa* seed oil was investigated using supercritical CO_2 at two levels of temperatures, pressures, and polarities [52]. Fullana *et al*. employed neural net computing for statistical and kinetic modeling and supercritical extractor simulation for the extraction of *Nigella sativa* oil with supercritical CO_2, and attempted to obtain higher oil yields [53]. Rao *et al.* considered both oil yield and antioxidant composition yield, and developed two optimized extraction conditions [50]. Not only volatile components, but also nonvolatile components can be extracted out by SFE. Hence, SFE extracts can be continuously extracted *via* HD or SD to obtain volatile components. Kokoska *et al.* previously compared the chemical composition and antibacterial activity of essential oil between SFE-SD and traditional HD and SD methods. The results demonstrated that the extract obtained *via* SFE-SD had a different composition and higher bioactivity [15]. However, according to the data reported by Venkatachallam *et al.*, if it was reliable, the recovery of phenolic compounds extracted by SFE-HD was lower than that achieved *via* direct SFE extraction, although that study contained contradictory descriptions between its abstract and discussion sections [12]. However, as a green technique with properties including relatively lower extraction temperature, higher bioactive extract, and no organic solvent residue, SFE should be paid more attention in studies of *Nigella sativa*.

Microwave assisted extraction (MAE) is a viable alternative to conventional techniques for the extraction of essential oils, aromas, pesticides, phenols, and other organic compounds from various matrices (soils, animal tissues, food and plant materials and so on). In the case of extraction, the principal advantage of microwave heating is the disruption of weak hydrogen bonds promoted by the dipole rotation of the molecules. The higher viscosity of the medium lowers this mechanism by influencing molecular rotation. The effect of microwave energy depends profoundly on the nature of both the solvent and the solid matrix. The matrix itself interacts with microwaves, whereas the surrounding solvent possesses a low dielectric constant and therefore remains cold. This situation presents some obvious advantages in the case of thermosensitive compounds, and has been used successfully for the extraction of essential oils [54].

The MAE technique has been developed rapidly over the past 5 years. Benkaci-Ali *et al.* applied this method to essential oil extraction from *Nigella sativa* seeds and assessed the kinetics of the MAE process over a 10-minute period [21]. Additionally, later they compared the chemical composition of extracted essential oils by MAE distillation using traditional HD. They claimed that MAE distillation offered similar yields, shorter extraction times, and substantial energy savings. Moreover, hydrolytic and oxidative reactions were greatly reduced in the MAE technique because of the short extraction time (10 min) and the small amount of water used (150 g sample, 50 mL water) [19]. Nevertheless, MAE, along with some techniques of dry distillation, solid phase micro-extraction, dry-diffusion and gravity, steam diffusion, and so on, have been developed as excellent techniques for the extraction of essential oil [55-58].

Solid phase microextraction (SPME) was developed in the early 1990s. The extraction is simple and fast, and can be conducted without solvents. The principal parameters that could influence SPME analysis include the choice of fiber, adsorption temperature, and adsorption time. Due to these properties, Wajs *et al.* employed head-space SPME to eliminate the possibility of methyl ether formation during the heat-treatment of *Nigella sativa* essential oil (hydro distillation and fractional distillation), and demonstrated that isomeric methyl ethers are natural components of *Nigella sativa* seeds [13].

Moreover, Tuter *et al.* partially purified the lipase from *Nigella sativa* seeds *via* DEAE-ion exchange chromatography and studied its applications in transesterification and the hydrolytic reaction [59,60]. Akova and Ustun studied a lipase immobization method from *Nigella sativa* seeds *via* adsorption on Celite 535 from phosphate buffer solutions [61].

3.2. Detection Methods

Gas chromatography (GC) has been broadly applied in the identification or determination of the chemical composition of essential oils and lipids in *Nigella sativa* seeds. GC equipped with a Flame ionization detector (FID) and a mass spectrometry detector (MSD) was adopted in nearly all determination studies of essential oil and lipids of *Nigella sativa* seeds. The separation of chemical components was usu-

ally accomplished using a capillary column with a simple program of oven temperature. Using GC-MS combined with iterative and non-iterative resolution methods, the number of volatile components in *Nigella sativa* seeds increased from 39 to 98 [16]. Moreover, 112 compounds were clearly identified from HD- and WAE-extracted essential oils *via* GC-FID and GC-MS techniques [19]. GC-MS was also employed to assess the composition of fatty oil fractionated from silica gel and alumina columns, and 26 compounds were identified [62]. Salih *et al*. studied the differences of chemical composition between ancient and modern *Nigella sativa* seeds by GC-MS. The two seeds were similar in terms of essential oil acids [63].

High performance liquid chromatography (HPLC) is normally used for the determination of chemical compositions with the following properties: high boiling point, instability at high temperatures, or other reasons making it inappropriate for GC. The determination of phenolic components, quinones, saponins, flavonoids, proteins and peptides, vitamins, phospholipids, glycolipids, and amino acids by HPLC from *Nigella sativa* seeds were reported [26,27,30,64-69]. Ultraviolet detectors (UVD) were usually equipped with HPLC for determination studies of *Nigella sativa*. Avula *et al*. used HPLC with electrospray ionization- time of flight mass spectrometry for the confirmation of one alkaloid, one flavonoid glycoside, six saponins, and one quinone in *Nigella sativa* [65].

Thin-layer chromatography (TLC) has also been used for the analysis of sterols and quinones in *Nigella sativa* [6,7,70,71].

4. FUTURE PERSPECTIVES

Nigella sativa is a very attractive plant, which has been employed for thousands of years and is attracting more and more attention from scientists. Focusing on the domain of chemical composition, more and more new extraction and detection techniques are currently being studied. Despite the more than 100 compounds that have been identified from *Nigella sativa* seeds, more unknown compounds will be identified with the development of new and improved techniques. In addition to the published methods of SE, HD, SD, SFE, WAE, and SPME for the extraction of essential oils from *Nigella sativa* seeds, accelerated solvent extraction [72], lipid phase microextraction [73], solvent-free solid injection method [74], purge and trap technique [75], etc. are all appropriate methods for the extraction of volatile components. The optimization of existing methods will also prove an effective approach for new compounds discovered from *Nigella sativa*. On the other hand, new detection techniques such as GC-MS/MS, LC-TOF, and LC-MS/MS should also prove useful for the determination of known or unknown compounds in *Nigella sativa* seeds.

5. CONCLUSIONS

The chemical composition of *Nigella sativa* seeds has been thoroughly investigated in previous studies, and in particular in its essential oil. However, the essential oil from different original sources or studied by various researchers has been shown to contain a variety of different chemical constituents. The worldwide distribution, geographical conditions, climates, extraction methods, and operator skills are possible factors underlying these deviations. With the development of this type of research, better extraction methods, more sensitive detection methods, and more unknown compounds from *Nigella sativa* seeds will certainly be discovered.

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