J Food Sci Technol (February 2022) 59(2):518–531
https://doi.org/10.1007/s13197-021-05036-1

ORIGINAL ARTICLE

Extraction, fatty acid profile, phytochemical composition and antioxidant activities of fixed oils from spices belonging to Apiaceae and Lamiaceae family

Palak Daga[1,2] · Salony Raghunath Vaishnav[1] · Ayushi Dalmia[1,2] · Ajay W. Tumaney[1,2]

Revised: 11 February 2021 / Accepted: 16 February 2021 / Published online: 3 March 2021

Abstract Fixed oil, the non-volatile fraction of oil from spices remains an unexplored entity. The study aimed to extract fixed oils from 13 Indian spices belonging to the Apiaceae and Lamiaceae family. Further fatty acid composition, antioxidant activities and phytochemical profile of the fixed oils was estimated. Among the studied spices, *Ferula assa-foetida* had the highest amount of fixed oil (19.93%). GC–MS profiling of the fixed oils showed palmitic, stearic, oleic, linoleic and alpha-linolenic to be the major fatty acids. The study further identified fixed oils from *Trachyspermum ammi* L., *Petroselinum crispum* L., *Ocimum basilicum* L. and *Rosmarinus officinalis* L. to be major source of protocatecheuic, 4,hydroxybenzoic, *trans*-cinnamic, myristein and *trans*-ferulic acid. *R. officinalis*, *O. basilicum* and *Thymus vulgaris* L. fixed oils also showed highest radical scavenging property. *T. vulgaris*, *Majorana hortensis* Moench. and *O. Basilicum* fixed oils confirmed high amounts of α-, β + γ- and δ-tocopherol respectively. β-sitosterol was found to be the dominating phytosterol in all fixed oils. Principal component analysis revealed existence of variation among spice fixed oils concerning to their fixed oil composition. The study thus identifies spice fixed oils as a rich source of lipid soluble bioactive compounds that are of tremendous industrial and pharmaceutical importance.

Keywords Spices · Non-volatile · Fixed oil · Fatty acid · Apiaceae · Lamiaceae

Introduction

Spices are part of plants used in the form of roots, buds, rhizomes, barks, seeds, leaves, fruits, and flowers (Shahidi and Hossain 2018). They are of immense importance to the medical, pharmaceutical, nutritional, industrial and cosmetic industry as they are reservoirs of bioactive compounds. India owing to its diversified climate and soil conditions is one of the largest producer, consumer, and exporter of spices and its by-products. Spices belonging to the Apiaceae (*Trachyspermum ammi* L., *Pimpinella anisum* L., *Ferula assa-foetida*, *Carum carvi* L., *Coriandrum sativum* L., *Cuminum cyminum* L., *Anethum graveolens* L., *Petroselinum crispum* L.) and Lamiaceae family (*Ocimum basilicum* L., *Majorana hortensis* Moench., *Origanum vulgare* L., *Rosmarinus officinalis* L., and *Thymus vulgaris* L.) were selected for the study as they are annual, medicinal and aromatic plants that are widely distributed in temperate climatic regions. These plants are broadly used across the world as spices, drugs, preservative and flavouring agents owing to the presence of valuable secondary metabolites (Vallverdú-Queralt et al. 2014).

Whole spices, as well as their by-products such as extracts, essential oil and oleoresin, have been immensely studied by researchers stating it's major use as an antioxidative, anti-microbial, anti-inflammatory, anti-mutagenic and analgesic agent (Krishna De and De 2018). However, fixed oils from spices remain an unexplored

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13197-021-05036-1.

✉ Ajay W. Tumaney
ajay@cftri.res.in

[1] Department of Lipid Science, Council of Scientific and Industrial Research-Central Food Technological Research Institute, Mysore 570020, India

[2] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

entity. Technically, fixed oils are the non-volatile oil fraction containing mainly lipophilic components such as fats, resins, and waxes and are generally soluble in organic solvents. Fixed oils are extracted from the aroma free spice matter post essential oil distillation. The understanding of spice fixed oil is still in its infancy as evidenced by a few studies that have been reported on fixed oils. Ashraf et al. (2006) studied the fixed oil content of black cumin and analyzed the fatty acids present in them. Sultan et al. (2009) also highlighted polyunsaturated fatty acids to be the dominating fraction in black cumin. Piras et al. (2012) reported nutmeg to have a fixed oil content of 14.4%. Khalid et al. (2005) studied the fixed oil content of *Carum carvi*, *Anethum graveolens* and *Petroselinum crispum*, belonging to the Umbelliferae family. However for spices of Indian origin, only countable studies have been reported on the fixed oil content of Indian spices where Sowbhagya et al. (2008) found *C. cyminum* to have a fixed oil content of 15% while Ali et al. (2018) reviewed nutmeg fixed oil consisting mainly of myristic, petroselinic and palmitic acid. Manasa et al. (2020) studied the fatty acid profile and nutraceutical composition of fixed oils from *M. fragnans*, *F. vulgare*, *T. Foenum-graecum*, *C. zeylancium* and *A. galanga*. However, for spices belonging to Apiaceae and Lamiaceae family not many studies have been reported on its fixed oil composition and characterization. This lacuna, therefore, allows us to explore the fixed oil content of spices belonging to these families and further report their fatty acid profile for the first time.

Apart from being a potential source of unusual fatty acid spice fixed oils can also be a source of bioactive secondary metabolites such as phenolics, tocopherols, and phytosterols which may also contribute to their antioxidant property. Therefore, the purpose of the present study was to standardize the extraction of fixed oils (non-volatile oil) from 13 Indian spices belonging to two families (Apiaceae and Lamiaceae). Further, the study characterizes the fixed oil by determining the fatty acids present, assessing its antioxidant potential (by three methods namely DPPH, ABTS and FRAP) and additionally understanding the lipophilic compounds (phenolics, tocopherols and sterols) present in the fixed oil attributing to their high antioxidant activity. Lastly, principal component analysis was applied to determine the existence of variation in the fixed oil composition among the spices.

Materials and methods

Plant materials

Thirteen spices belonging to the Apiaceae and Lamiaceae family, present under the purview of Spice Board of India, Cochin, were procured and authenticated from Vriksha Vijnan Private Limited, Bangalore, Karnataka, India. The following are the names of the spices used in the study: *Trachyspermum ammi* L., *Pimpinella anisum* L., *Ferula assa-foetida*, *Ocimum basilicum* L., *Carum carvi* L., *Coriandrum sativum* L., *Cuminum cyminum* L., *Anethum graveolens* L., *Majorana hortensis* Moench., *Origanum vulgare* L., *Petroselinum crispum* L., *Rosmarinus officinalis* L., and *Thymus vulgaris* L.. The images of the spices are shown in Supplementary Fig. S1.

Chemicals and standards

Boron trifluoride (BF3) methanol complex solution (13–15%), heptadecanoic acid, DPPH, TPTZ, ABTS, trolox, ascorbic acid, phenolic standards namely gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, epicatechin, *trans*-cinnamic acid, catechin, caffeic acid, p-coumaric acid, *trans*-ferulic acid, myricetin, quercetin, and kaempferol; tocopherol standards namely α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol; sterol standards namely ergosterol, β-sitosterol, stigmasterol, campesterol and squalene were purchased from Sigma-Aldrich (Saint Louis, MO, USA). HPLC grade solvents were procured from Merck, USA, while other solvents used were obtained from SRL, India. Milli-Q water was used for HPLC analysis. Folin-ciocalteu reagent, sodium carbonate, sodium acetate trihydrate, iron (III) chloride hexahydrate, and potassium persulphate, used in the present study were of analytical grade procured from Himedia, India.

Extraction of fixed oils

The powdered spices were packed in a 2 L capacity steam distillation apparatus. Distillation was carried out to retrieve the essential oil, and the process was continued until the samples were free from volatile compounds, which was confirmed through GC–MS. The time required for each spice to remove volatile components completely was standardized. Once the samples were free from essential oil, they were dried in a hot air oven (Biobee, India) at 60 °C. Further, dried sample was fed into the soxhlet apparatus, and fixed oil extraction was carried out using hexane for about 6 h. The solvent was evaporated under reduced pressure using a rotary evaporator (Heidolph Hei-Vap R-300, Germany). Recovered fixed oils were collected in an amber glass bottle, flushed with a stream of nitrogen gas and stored at −20 °C until use. The fixed oil content of spices were determined on a dry weight basis and expressed as percentage (Khalid et al. 2005).

Fatty acid profiling of fixed oils

The fatty acid composition of fixed oils extracted from Indian spices were determined through GC–MS as described by Prasad et al. (2019). Fatty acid methyl esters (FAMEs) were prepared by esterification of oil with 1 ml of BF_3-methanol complex solution at 60 °C for 30 min. After cooling FAMEs were extracted by adding water:hexane (1:1, v/v). The upper hexane layer containing the FAMEs was transferred into GC vials. 50 µl of heptadecanoic acid (1 mg/ml in hexane) was added as an internal standard to each sample before methylation. The resulting FAMEs were then analyzed using Agilent DB-23 column ((50%-Cyanopropyl)- methylpolysiloxane; 60 m length; 0.25 mm ID; 0.25 µm film thickness) on 7890B GC-5977A MSD system (M/s Agilent Technologies, Singapore). Helium at a flow rate of 1 ml/min was used as a carrier gas; injection volume was 1 µl, and the split ratio was 20:1. The temperature program was as follows: an initial temperature of 40 °C for 1 min, ramped to 130 °C at 70 °C min^{-1} rate with 1 min hold, then increased to 170 °C at 6.5 °C min^{-1} rate and further ramped to 200 °C at 2.75 °C min^{-1} rate with 6 min hold and then increased to 210 °C at a rate of 40 °C min^{-1} with 2 min hold. Finally, the temperature was ramped to 230 °C at 20 °C min^{-1} rate with 7 min hold time. The mass spectrum was recorded under EI (Electron impact ionization) at a fixed electron energy of 70 eV with a mass range of m/z 40–500 da. Fatty acids identified were confirmed by mass spectral library search (NIST version 2.0 g).

Extract preparation

1 g of fixed oil was extracted with 4 ml of 70% methanol containing 0.1% hydrochloric acid by vigorous shaking in a multitube vortexer (Benchmixer XL, Benchmark Scientific, USA) at 2500 rpm for 1 h. The sample suspension was then centrifuged at 12,000 × g for 15 min at 10 °C. The supernatant was then collected and stored in amber tubes at −20 °C. This extract was then further used for determining the phenolic content and antioxidant activity of the fixed oil (Sreeramulu and Raghunath 2011).

Determination of total phenolic content

The total phenolic content present in fixed oils was determined using the Folin-Ciocalteu reagent measured at 725 nm using a plate reader (BMG LABTECH Clariostar Plus, Germany). Briefly, to each well suitable volumes of sample extracts were added. To this 1 ml of 10% Folin-Ciocalteu reagent (v/v) and 0.8 ml of 7.5% Na_2CO_3 (w/v) was added and the final volume was made up to 2 ml with deionized water. The reaction mixture was vortexed thoroughly and incubated in dark at room temperature for 90 min. Reaction without sample or standard was taken as blank. The total phenolic content present in the fixed oils was expressed as mg gallic acid equivalent (GAE) per 100 g oil by using the regression equation obtained from the calibration curve of gallic acid (Sreeramulu and Raghunath 2011).

Antioxidant activity

The antioxidant activity of the fixed oils was estimated by three different methods namely DPPH, FRAP and ABTS.

DPPH Assay

A methanolic solution of 0.1 mM DPPH was prepared freshly in dark conditions. To a series of known concentrations of extracts 1.45 ml of DPPH solution was added. The plate was then vortexed and incubated in dark for 30 min at ambient conditions. The discoloration of DPPH was measured at 517 nm against methanol as blank. The radical scavenging activities of the fixed oils were expressed as mg ascorbic acid equivalent (AAE) per 100 g oil using ascorbic acid as standard (Sreeramulu and Raghunath 2011).

ABTS Assay

The extractives at different concentrations were mixed with 2 ml of the diluted $ABTS^{+\cdot}$ (7 mM) solution and incubated in dark for 6 min at 30 °C, followed by measurement of absorbance at 734 nm. The radical scavenging activity was calculated as mM trolox equivalent (TE) per 100 g oil with reference to the scavenging activity obtained by trolox (Re et al. 1999).

FRAP Assay

The ferric reducing antioxidant power of fixed oils was assessed by employing the method reported by Benzie and Strain (1999). FRAP reagent was prepared freshly by mixing acetate buffer, TPTZ solution and ferric chloride solution in a ratio of 10:1:1. The reagent was then incubated at 37 °C for 30 min in a water bath. Briefly, 1.5 ml of FRAP reagent was added to a known volume of extracts. After incubation in dark for 6 min at room temperature, the absorbance was measured at 593 nm against trolox as standard. FRAP values were expressed as mM trolox equivalent (TE) per 100 g oil.

Quantification of phenolic compounds

Identification of individual phenolic compounds present in the fixed oils was determined by HPLC (Nexera X-2 LC-30A, Shimadzu, Japan). The extracted compounds were separated using a reverse-phase C18 column (Chromasol, 250 × 4.6 mm, 5 µm). The mobile phase consisted of acetic acid in water adjusted to a pH of 2.65 (solvent A) and 20% of solvent A and 80% acetonitrile (solvent B). Gradient elution with a flow rate of 1.2 ml/min was carried out according to Burin et al. 2011. The phenolic compounds present in the sample were detected by comparing their retention time with those of corresponding standards and also by spiking of samples with appropriate standards. A standard mix containing all standards (1 mg/ml in methanol) were analyzed along with samples, and quantification of the identified compounds was carried out using peak area obtained from the standards. The phenolic peaks were detected at 280 nm and 320 nm and annotated using LabSolutions software and expressed as µg per 100 g oil.

Identification of tocopherols

Quantification of tocopherols present in the fixed oils was estimated through HPLC. Weighed amount of oil samples were diluted with hexane and passed through 0.2 µm pore size syringe filters before injection. Separation of tocopherols was done using a reverse-phase C18 column at 30 °C. A mobile phase containing methanol:water (95:5, v/v) was isocratically eluted at a flow rate of 1.5 ml/min. The eluate was detected using a RF 20A fluorescence detector set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The tocopherols present in the fixed oils were expressed as mg per100g oil (Diwakar et al. 2010).

Determination of sterols and squalene

Isolation of sterols and squalene fraction from the fixed oils was carried out by alkali saponification by boiling ethanolic potassium hydroxide under reflux for 2 h (Food Safety and Standard Authority of India 2016). The extracted unsaponifiable matter was dissolved in acetone and separated on a reverse-phase C18 column at 30 °C. A mobile phase consisting of methanol:acetonitrile (98:2, v/v) was isocratically eluted at a flow rate of 1.2 ml/min. Individual sterols and squalene were detected at a wavelength of 205 nm and were expressed as mg per 100 g oil (Sánchez-Machado et al. 2004).

Statistical analysis

All the results were expressed in the form of mean ± standard deviation (SD) of three replicates. The average values within the families were statistically tested using analysis of variance (ANOVA) followed by Tukey test to compare means that showed significant variation ($P \leq 0.05$). The relationship between the phenolic content and antioxidant capacity of the fixed oils were evaluated using Pearson correlation coefficient at $P \leq 0.05$ confidence level. Multivariate Principal Component Analysis (PCA) was performed to determine compositional variability among the spice fixed oils within the families. All the analyses were done using XLSTAT software (XLSTAT statistical and data analysis solution, Boston, USA https://www.xlstat.com).

Results and discussion

Most of the studies on spices have been reported on their volatile oil, their characterization, and their role in combating various metabolic disorders. However, the present study focused on extracting fixed oils from spices, identifying the fatty acids present, and studying their phenolic, tocopherol, sterol and squalene composition and evaluating their antioxidant potential.

Fixed oil content

The fixed oil content of Indian spices, expressed as percentage based on dry weight is presented in Table 1. The time taken for each spice to distill for the extraction of fixed oils is presented in Supplementary Table S1. The fixed oil content was highest in *F. assa-foetida* (19.93%) and *C. cyminum* (14.11%) while lowest in *P. crispum* (0.96%) in the Apiaceae family. Our results were in agreement with Sowbhagya et al. (2008) where 15% of fixed oil was obtained from *C. cyminum*. While amongst the Lamiaceae family *R. officinalis* had the highest fixed oil content (4.57%). Manasa et al. (2020) also reported the fixed oil content of Indian spices where *M. fragnans* (26.43%) had the highest amount of fixed oil.

Fatty acid composition of fixed oils

GC–MS analysis was performed to study the fatty acid profile of fixed oils, which is presented in Table 2. A wide spectrum of 23 fatty acids was confirmed, ranging from C12:0 to C28:0 in *F. assa-foetida* and *P. crispum* fixed oils, respectively. Palmitic, oleic, and linoleic were the predominant fatty acids present in the spice fixed oils belonging to the Apiaceae family (Table 2). However, for

Table 1 Fixed oil content (%) of Indian spices

Family	Spices	Common name	Part used	Fixed oil
Apiaceae	*T. ammi*	Ajowan	Fruit	6.77 ± 0.37[a]
	P. anisum	Aniseed	Fruit	7.03 ± 0.20[a]
	F. assa-foetida	Asafoetida	Gum oleoresin	19.93 ± 0.66[b]
	C. carvi	Caraway	Fruit	4.88 ± 0.16[c]
	C. sativum	Coriander	Fruit	12.52 ± 0.95[d]
	C. cyminum	Cumin	Fruit	14.11 ± 0.53[e]
	A. graveolens	Dill	Fruit	9.81 ± 0.29[f]
	P. crispum	Parsley	Leaf	0.96 ± 0.06 [g]
Lamiaceae	*O. basilicum*	Basil	Leaf	2.52 ± 0.06[ac]
	M. hortensis	Marjoram	Leaf	3.51 ± 0.01[b]
	O. vulgare	Oregano	Leaf	2.27 ± 0.31[c]
	R. officinalis	Rosemary	Leaf	4.57 ± 0.18[d]
	T. vulgaris	Thyme	Leaf	2.97 ± 0.07[a]

Results are expressed as Mean ± SD, n=3; Different superscript letters across the column within families indicate significant difference between values at $P < 0.05$

spices belonging to the Lamiaceae family (Table 3) palmitic and alpha-linolenic were the major fatty acids. It is a well-documented fact that petroselinic acid is the dominating fatty acid in *C. cyminum*, *C. sativum* and *C. carvi* seed oil (Rebey et al. 2012; Laribi et al. 2009; Ramadan 2013) however, in the fixed oil obtained from these seeds oleic acid was found to be the major fatty acid. This further stirs in more interest in exploiting the less discussed facet of fixed oils from Indian spices. Furthermore, the presence of alpha-linolenic acid majorly in *O. vulgare*, *M. hortensis*, *R. officinalis*, *T. vulgaris*, and *O. basilicum* fixed oils generates potential application of these fixed oils for various pharmaceutical and industrial applications. Similar findings were also reported by Matthaus et al. (2018) where linolenic was the prime fatty acid in several *Origanum* seeds. Some rare fatty acids such as C15:0 in *P. crispum* and C20:2 (*cis* 11,14) and C24:1(*cis* 15) in *F. assa-foetida* fixed oils were also observed. Mustard is a well-known source of erucic acid (C22:1, *cis* 13) (Al-Jasass et al. 2012). However, the present study identified erucic acid to be the dominant fatty acid in *F. assa-foetida* fixed oil thereby making it an alternative source of erucic acid which is widely used as lubricants and act as a precursor for biodiesel production. Thus, the study lays importance in identifying spice fixed oils as a source of fatty acids that are of immense commercial importance.

Figure 1 represents the distribution of saturation and unsaturation of fatty acids present in fixed oils. It was observed that the degree of monounsaturated fatty acids was major in spice fixed oils belonging to the Apiaceae family. Our results were in concordance with other studies where *C. sativum* (Kiralan et al. 2009), *T. ammi*, *P. anisum* and *C. carvi* (Laribi et al. 2010, 2013) and fennel showed the highest amount of monounsaturated fatty acid. While on the other hand fixed oils from Lamiaceae family had significantly higher amounts of polyunsaturated fatty acids. Similar findings were also observed by Chernenko and Glushenkova (2001) where *M. hortensis*, *O. vulgare*, and *T. vulgaris* were a rich source of polyunsaturated fatty acid.

Total phenolic content of fixed oils

The total phenolic content of the studied spice fixed oils estimated through Folin-Ciocalteu assay, showed significant variation ranging from 7.32 to 1264.32 mg GAE/100 g oil (Table 4). *R. officinalis* fixed oil (1264.32 mg GAE/100 g) had the highest phenolic content followed by *O. basilicum* (272.79 mg GAE/100 g oil) and *T. vulgaris* fixed oil (212.31 mg GAE/100 g oil). However, on the contrary *F. assa-foetida* and *A. graveolens* fixed oils showed the least amount of phenolics. Rebey et al. (2012) also investigated the total phenolic content of *C. cyminum* seed extract. Manasa et al. (2020) when explored the phenolic content of fixed oils reported the highest phenolic content in *C. zeylancium* and *M. Fragnans*.

Antioxidant activity of fixed oils

Since no single method is sufficient to conclude the antioxidant capacity of the plant extracts so we tested the antioxidant potential of the spice fixed oils through DPPH, ABTS and FRAP assay (Table 4). DPPH radical scavenging assay is one of the most widely used assay because of its simplicity and sensitivity. It was observed that *R. officinalis* fixed oil had the highest radical scavenging activity (756.88 mg GAE/100 g oil) whereas *F. assa-foetida* (7.99 mg GAE/100 g oil) and *P. anisum* (5.21 mg GAE/100 g oil) fixed oil exhibited the least radical

Table 2 Fatty acid composition (μmol %) of fixed oils belonging to Apiaceae family

Fatty acids	*T. ammi*	*P. anisum*	*F. assa-foetida*	*C. carvi*	*C. sativum*	*C. cyminum*	*A. graveolens*	*P. crispum*
C12:0	ND	ND	0.34 ± 0.07^{a}	ND	ND	ND	ND	1.30 ± 0.06^{a}
C14:0	0.52 ± 0.15^{a}	0.72 ± 0.01^{a}	0.47 ± 0.05^{a}	ND	1.62 ± 0.07^{b}	ND	ND	1.81 ± 0.04^{b}
C15:0	ND	ND	ND	ND	ND	ND	ND	0.82 ± 0.05
C16:0	9.53 ± 0.34^{ab}	10.86 ± 0.34^{a}	9.58 ± 0.72^{ab}	10.37 ± 2.91^{ab}	9.50 ± 0.28^{ab}	5.92 ± 1.46^{b}	9.98 ± 0.53^{ab}	38.80 ± 0.88^{c}
C16:1 (*cis* 7)	ND	0.30 ± 0.05^{a}	0.16 ± 0.04^{a}	ND	ND	ND	ND	ND
C16:1 (*cis* 9)	ND	0.40 ± 0.06^{a}	0.30 ± 0.00^{a}	ND	ND	0.33 ± 0.02^{a}	ND	2.93 ± 0.07^{b}
C16:3 (*cis* 7,10,13)	ND	ND	ND	ND	ND	ND	ND	2.98 ± 0.01
C18:0	1.60 ± 0.32^{ab}	2.22 ± 0.68^{ab}	2.77 ± 0.22^{a}	1.83 ± 0.24^{ab}	1.63 ± 0.02^{ab}	1.25 ± 0.68^{b}	2.11 ± 0.11^{ab}	4.67 ± 0.32^{c}
C18:1 (*cis* 9)	61.59 ± 1.06^{ac}	65.48 ± 1.33^{a}	11.72 ± 0.28^{b}	51.47 ± 8.58^{c}	66.86 ± 0.48^{ad}	59.89 ± 1.83^{ac}	76.14 ± 1.07^{d}	3.68 ± 0.22^{b}
C18:1 (*cis* 11)	0.97 ± 0.12^{abc}	0.62 ± 0.09^{bc}	1.30 ± 0.06^{ab}	1.36 ± 0.46^{a}	0.96 ± 0.04^{abc}	1.45 ± 0.03^{a}	ND	0.47 ± 0.02^{c}
C18:2 (*cis* 9,12)	23.98 ± 1.12^{ad}	17.50 ± 0.25^{a}	24.64 ± 1.77^{bd}	33.32 ± 4.49^{c}	18.26 ± 0.61^{ad}	30.59 ± 0.71^{bc}	10.73 ± 0.36^{e}	21.06 ± 0.52^{ad}
C18:3 (*cis* 9,12,15)	1.53 ± 0.18^{a}	1.55 ± 0.09^{a}	10.78 ± 0.27^{b}	1.65 ± 0.49^{a}	1.15 ± 0.23^{a}	0.58 ± 0.35^{a}	ND	13.35 ± 0.44^{c}
C20:0	ND	ND	ND	ND	ND	ND	1.04 ± 0.09^{a}	2.48 ± 0.24^{b}
C20:1 (*cis* 11)	ND	ND	4.20 ± 0.30	ND	ND	ND	ND	ND
C20:2 (*cis* 11,14)	ND	ND	0.62 ± 0.07	ND	ND	ND	ND	ND
C22:0	0.28 ± 0.02^{a}	0.36 ± 0.06^{a}	0.87 ± 0.04^{b}	ND	ND	ND	ND	ND
C22:1 (*cis* 13)	ND	ND	29.99 ± 2.35	ND	ND	ND	ND	ND
C22:2 (*cis* 13,16)	ND	ND	0.91 ± 0.04	ND	ND	ND	ND	ND
C23:0	ND	ND	ND	ND	ND	ND	ND	0.65 ± 0.10
C24:0	ND	ND	ND	ND	ND	ND	ND	2.24 ± 0.06
C24:1 (*cis* 15)	ND	ND	1.35 ± 0.03	ND	ND	ND	ND	ND
C26:0	ND	ND	ND	ND	ND	ND	ND	1.14 ± 0.16
C28:0	ND	ND	ND	ND	ND	ND	ND	1.62 ± 0.01

Results are expressed as Mean ± SD n=3; ND—Not Detected; C12:0—Lauric; C14:0—Myristic; C15:0—Pentadecylic; C16:0—Palmitic; C16:1 (*cis* 7)—Palmitoleic; C16:1 (*cis* 9)—Palmitelaidic; C16:3 (*cis* 7,10,13)—Hexadecatrienoic; C18:0—Stearic; C18:1 (*cis* 9)—Oleic; C18:1 (*cis* 11)—Vaccenic; C18:2 (*cis* 9,12)—Linoleic; C18:3 (*cis* 9,12,15)—Alpha-linolenic; C20:0—Arachidic; C20:1 (*cis* 11)—Eicosenoic; C20:2 (*cis* 11,14)—Eicosadienoic; C22:0—Behenic; C22:1 (*cis* 13)—Erucic; C22:2 (*cis* 13,16)—Docosadienoic; C23:0—Tricosylic; C24:0—Lignoceric; C24:1 (*cis* 15)—Nervonic; C26:0—Cerotic; C28:0—Montanic acid; Different superscript Letters across the row indicate significant difference between values at $P < 0.05$

Table 3 Fatty acid composition (μmol %) of fixed oils belonging to Lamiaceae family

Fatty acids	*O. basilicum*	*M. hortensis*	*O. vulgare*	*R. officinalis*	*T. vulgaris*
C14:0	ND	0.70 ± 0.03^{a}	1.34 ± 0.02^{a}	1.44 ± 0.00^{a}	2.03 ± 1.21^{a}
C16:0	36.45 ± 0.06^{a}	19.35 ± 0.57^{b}	29.22 ± 0.40^{c}	35.53 ± 0.25^{a}	31.81 ± 0.55^{d}
C16:1 (*cis* 9)	ND	0.98 ± 0.14	ND	ND	ND
C18:0	8.02 ± 0.21^{a}	5.28 ± 0.39^{b}	4.14 ± 0.07^{c}	4.34 ± 0.09^{c}	4.84 ± 0.06^{bc}
C18:1 (*cis* 9)	4.17 ± 0.07^{a}	10.06 ± 0.27^{b}	8.11 ± 0.23^{c}	8.18 ± 0.05^{c}	8.64 ± 0.36^{c}
C18:1 (*cis* 11)	0.91 ± 0.01^{ac}	1.06 ± 0.06 ^{ab}	ND	1.14 ± 0.06^{b}	0.85 ± 0.04^{c}
C18:2 (*cis* 9,12)	15.55 ± 0.28^{a}	21.50 ± 0.24^{b}	12.26 ± 0.45^{c}	12.04 ± 0.17^{c}	14.77 ± 0.28^{a}
C18:3 (*cis* 9,12,15)	34.13 ± 0.39^{a}	39.01 ± 1.49^{b}	43.95 ± 0.20^{c}	35.72 ± 0.06^{ab}	35.32 ± 1.19^{a}
C20:0	ND	1.04 ± 0.13	ND	ND	ND
C22:0	0.78 ± 0.11^{a}	ND	1.00 ± 0.03^{ab}	1.62 ± 0.06^{b}	1.74 ± 0.35^{b}
C24:0	ND	0.60 ± 0.06	ND	ND	ND

Results are expressed as Mean ± SD n=3; ND—Not Detected; C14:0—Myristic; C16:0—Palmitic; C16:1 (*cis* 9)—Palmitelaidic; C18:0—Stearic; C18:1 (*cis* 9)—Oleic; C18:1 (*cis* 11)—Vaccenic; C18:2 (*cis* 9,12)—Linoleic; C18:3 (*cis* 9,12,15)—Alpha-linolenic; C20:0—Arachidic; C22:0—Behenic; C24:0—Lignoceric; Different superscript letters across the row indicate significant difference Between values at $P < 0.05$

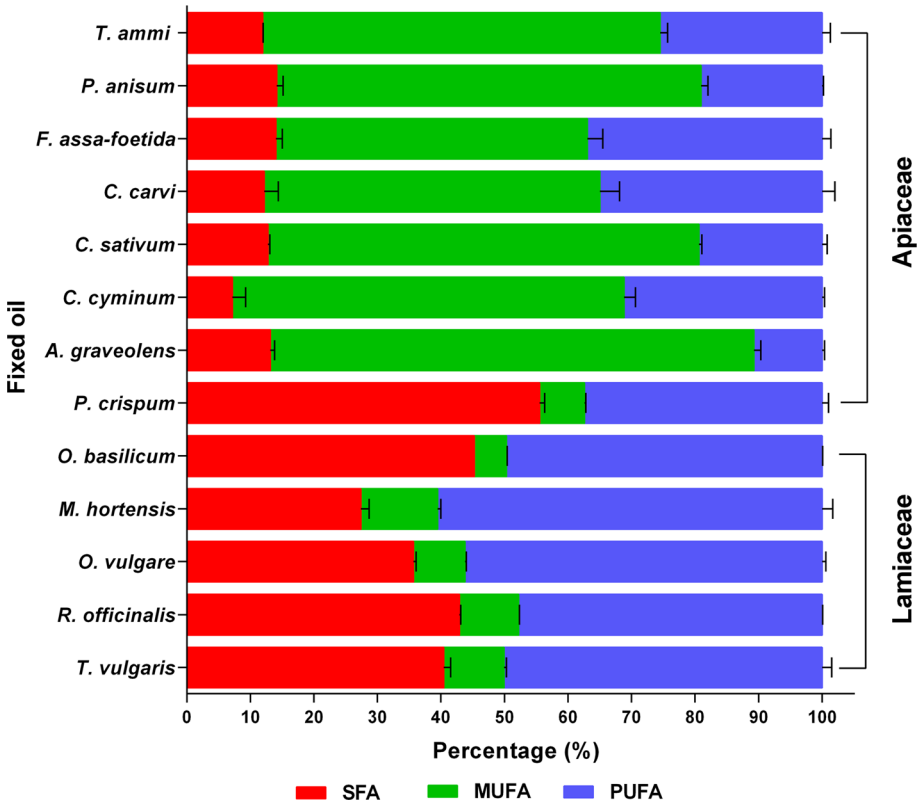

Fig. 1 Levels of SFA, MUFA and PUFA in fixed oils: Distribution of saturation of fatty acids present in spice fixed oils. Values are expressed as percentage. SFA - saturated fatty acid, MUFA - monounsaturated fatty acid, PUFA - polyunsaturated fatty acid

scavenging activity. Spice fixed oils identified with high antioxidant activity can also be explored as a potential candidate for blending with conventional vegetable oils. ABTS radical scavenging activity assay also showed results similar to the DPPH assay results where *R. officinalis* fixed oil showed the highest radical scavenging activity (101,096.19 mM TE/100 g oil) followed by *O. basilicum* (13,703.3 mM TE/100 g oil) fixed oil. While, *A. graveolens* fixed oil (85.03 mM TE/100 g oil) showed the least radical scavenging activity. Our results were in concordance with Vallverdú-Queralt et al. (2014) where ethanolic extract of *R. officinalis* showed the highest radical scavenging property. However, it should also be noted that the aqueous extract from spices has been thoroughly

Table 4 Total phenolic content and antioxidant profile of fixed oils

Family	Spices	Total Phenolic Content (F–C assay) (mg GAE/100 g oil)	DPPH (mg AAE/100 g oil)	ABTS (mM TE/100 g oil)	FRAP (mM TE/100 g oil)
Apiaceae	*T. ammi*	13.62 ± 0.23^{a}	8.25 ± 0.86^{ab}	253.81 ± 12.21^{a}	497.88 ± 33.67^{a}
	P. anisum	10.89 ± 0.19^{a}	5.21 ± 1.77^{b}	798.84 ± 35.13^{b}	654.77 ± 96.44^{a}
	F. assa-foetida	8.76 ± 1.92^{a}	7.99 ± 1.32^{ab}	202.12 ± 16.07^{ac}	598.31 ± 32.02^{a}
	C. carvi	28.58 ± 2.96^{b}	7.72 ± 0.69^{ab}	455.96 ± 70.25^{d}	899.84 ± 25.40^{b}
	C. sativum	28.48 ± 2.88^{b}	8.15 ± 2.18^{ab}	599.22 ± 3.58^{e}	956.50 ± 110.37^{b}
	C. cyminum	23.63 ± 6.57^{b}	7.99 ± 0.8^{ab}	219.58 ± 29.03^{a}	575.98 ± 25.70^{a}
	A. graveolens	7.32 ± 1.59^{a}	10.46 ± 2.75^{ac}	85.03 ± 4.41^{c}	472.27 ± 36.91^{a}
	P. crispum	40.81 ± 0.7^{c}	13.31 ± 1.29^{c}	788.39 ± 42.61^{b}	2104.38 ± 109.64^{c}
Lamiaceae	*O. basilicum*	272.79 ± 12.07^{a}	202.52 ± 36.86^{a}	13,703.3 ± 869.53^{a}	13,098.74 ± 2311.16^{a}
	M. hortensis	26.73 ± 0.61^{b}	11.14 ± 1.23^{b}	427.91 ± 23.56^{b}	1711.17 ± 273.66^{b}
	O. vulgare	19.24 ± 0.55^{b}	16.89 ± 1.99^{b}	455.79 ± 29.55^{b}	793.96 ± 157.76^{b}
	R. officinalis	1264.32 ± 144.97^{c}	756.88 ± 24.16^{c}	101,096.19 ± 1547.4^{c}	15,427.89 ± 651.28^{a}
	T. vulgaris	212.31 ± 6.87^{a}	155.62 ± 18.32^{a}	6674.5 ± 43.91^{d}	7584.63 ± 618.10^{c}

Results are expressed as Mean ± SD, n=3; F–C- Folin Ciocalteu; GAE: gallic acid equivalent; AAE: ascorbic acid equivalent; TE: trolox equivalent;

Different superscript letters across the column within families indicate significant difference between values at $P < 0.05$

exploited by most of the researchers but the lipophilic extracts have not been much explored thereby allowing us to study the less studied aspect of the spices. Ferric reducing antioxidant power of the fixed oils was also estimated where similar results were obtained as that of DPPH and ABTS assay. The highest reducing power was exhibited by *R. officinalis* (15,427.89 mM TE/100 g oil), *O. basilicum* (13,098.74 mM TE/100 g oil) and *T. vulgaris* (7584.63 mM TE/100 g oil) fixed oil. Zhang et al. (2014) studied the ferric reducing power of cold-pressed *O. vulgare* oil however our results were in slight divergence with them due to differences in the extraction method used.

Pearson's correlation coefficient was performed to examine the correlation among the antioxidant assays and the Folin-Ciocalteu assay as shown in Table 5. A strong positive correlation was observed between total phenolic content and DPPH (R = 0.998); total phenolic content and TEAC (R = 0.994) and DPPH and TEAC (R = 0.985). Correlation between DPPH and FRAP was R = 0.877 while between total phenolic content and FRAP it was R = 0.846. The lowest correlation was observed between FRAP and TEAC (R = 0.788). Thus the results obtained were quite conclusive of the fact that the high antioxidant capacity of the fixed oils is attributed to their high phenolic content as observed from the correlation coefficient. Overall the antioxidant potential of the studied fixed oils in decreasing order is as *R. officinalis* > *O. basilicum* > *T. vulgaris* > *P. crispum* > *C. carvi* > *C. sativum* > *M. hortensis* > *C. cyminum* > *O. vulgare* > *T. ammi* > *P. anisum* > *F. assa-foetida* > *A. graveolens*.

Table 5 Correlation coefficient (R) between assays

	Folin-Ciocalteu	DPPH	ABTS	FRAP
DPPH	0.998	1		
ABTS	0.994	0.985	1	
FRAP	0.846	0.877	0.788	1

Phenolic profile of fixed oils

The high antioxidant activity of spices has always been associated with the phenolic compounds present in them as shown in Table 6. We observed that the fixed oils from *T. ammi*, *P. crispum*, *O. basilicum* and *R. officinalis* were the major source of phenolic compounds. *P. crispum* (417.53 µg/100 g oil) and *O. basilicum* (493.47 µg/100 g oil) fixed oil were found to have high levels of protocatecheuic acid. *trans*-Cinnamic acid was present in abundance in *P. crispum* (1438.14 µg/100 g oil) and *M. hortensis* (849.46 µg/100 g oil) fixed oils. High amounts of hydroxybenzoic acid was detected in *O. basilicum* fixed oil (1419.56 µg/100 g oil). It was noted that moderate amounts of vanillic acid, syringic acid, epicatechin and quercetin were significantly present in spices belonging to

Table 6 Phenolics, tocopherols and sterols present in fixed oils belonging to Apiaceae family

	T. ammi	*P. anisum*	*F. assa-foetida*	*C. carvi*	*C. sativum*	*C. cyminum*	*A. graveolens*	*P. crispum*
Phenolics (μg/100 g oil)								
Gallic acid	ND	997.08 ± 11.02^{a}	6.03 ± 0.00^{b}	189.37 ± 0.00^{c}	ND	ND	ND	ND
Protocatecheuic acid	ND	ND	ND	ND	ND	ND	ND	417.53 ± 21.87
4,Hydroxybenzoic acid	ND	ND	ND	239.49 ± 7.88^{a}	470.29 ± 32.18^{b}	86.66 ± 8.75^{c}	ND	ND
Chlorogenic acid	ND	ND	ND	ND	ND	ND	ND	ND
Vanillic acid	211.77 ± 6.11^{a}	ND	54.30 ± 0.00^{a}	ND	ND	ND	100.47 ± 0.66^{a}	850.52 ± 153.08^{b}
Syringic acid	51.86 ± 0.00^{a}	ND	ND	ND	ND	15.48 ± 4.38^{b}	ND	ND
Epicatechin	73.47 ± 6.11^{a}	ND	ND	33.42 ± 0.00^{b}	ND	ND	ND	ND
trans-Cinnamic acid	43.21 ± 0.00^{a}	93.47 ± 0.00^{a}	24.13 ± 0.00^{a}	38.98 ± 7.87^{a}	348.93 ± 10.73^{b}	ND	ND	1438.14 ± 109.35^{c}
Catechin	ND	50.63 ± 5.5^{a}	ND	122.53 ± 0.00^{b}	ND	ND	ND	355.67 ± 21.87^{c}
Caffeic acid	17.24 ± 0.06	ND	ND	ND	ND	ND	ND	ND
p-Coumaric acid	ND	ND	ND	ND	227.56 ± 10.73^{a}	ND	11.99 ± 3.39^{b}	ND
trans-Ferulic acid	21.61 ± 6.11^{a}	ND	12.06 ± 0.00^{a}	22.27 ± 0.00^{a}	ND	ND	ND	402.06 ± 0.00^{b}
Myricetin	30.25 ± 6.11^{ab}	38.94 ± 0.00^{a}	24.06 ± 0.09^{b}	ND	ND	ND	23.98 ± 0.00^{b}	ND
Quercetin	17.28 ± 0.10^{a}	ND	ND	ND	ND	ND	86.34 ± 0.11^{b}	ND
Kaempferol	51.86 ± 0.00^{ab}	ND	75.43 ± 4.26^{a}	ND	91.02 ± 0.00^{a}	ND	ND	2474.23 ± 43.74^{b}
Tocopherols (mg/100 g oil)								
α-Tocopherol	84.33 ± 18.48^{a}	10.89 ± 0.00^{bc}	6.67 ± 0.30^{bc}	32.46 ± 8.79^{b}	4.02 ± 0.17^{bc}	9.24 ± 0.45^{bc}	3.81 ± 0.12^{c}	ND
(β + γ)–Tocopherol	3.26 ± 0.09^{a}	0.86 ± 0.33^{b}	19.95 ± 0.25^{c}	0.79 ± 0.00^{b}	0.83 ± 0.07^{b}	0.10 ± 0.00^{b}	26.32 ± 0.35^{d}	1.99 ± 0.26^{e}
δ–Tocopherol	ND	ND	0.98 ± 0.18^{a}	ND	3.99 ± 0.06^{b}	ND	0.36 ± 0.13^{c}	ND
Sterols (mg/100 g oil)								
Ergosterol	170.32 ± 1.28^{a}	11.98 ± 0.14^{b}	ND	ND	ND	ND	6.43 ± 0.32^{c}	ND
Stigmasterol + Campesterol	3411.80 ± 1.73^{a}	270.61 ± 1.28^{b}	250.99 ± 1.51^{c}	212.48 ± 2.09^{d}	214.65 ± 0.67^{d}	141.53 ± 1.57^{e}	85.55 ± 4.33^{f}	1825.28 ± 8.68 ^{g}
β–Sitosterol	3307.64 ± 17.35^{a}	31.85 ± 1.19^{b}	520.63 ± 0.87^{c}	461.28 ± 2.57^{d}	61.16 ± 1.24^{e}	173.57 ± 3.39^{f}	99.01 ± 6.46 ^{g}	1287.84 ± 0.29 ^{h}
Squalene	190.26 ± 11.24^{a}	2.96 ± 0.13^{b}	2.69 ± 0.14^{b}	10.69 ± 0.47^{b}	14.51 ± 0.21^{b}	2.68 ± 0.13^{b}	2.72 ± 0.52^{b}	1.52 ± 0.11^{b}

Results are expressed as Mean ± SD, n=3; ND—Not Detected; Different superscript letters across the row indicate significant difference between values at $P < 0.05$

Apiaceae family (Table 6) though they were absent in Lamiaceae family (Table 7). However, levels of myristein (88.24–526.88 µg/100 g oil) were majorly high in spice fixed oils belonging to Lamiaceae family. *P. crispum* fixed oil showed high levels of kaempferol (2474.23 µg/100 g oil). The phenolic compounds identified in the studied spice fixed oils were similar to those reported by Vallverdú-Queralt et al. (2014). Notably, the current work confirms the presence of well-studied bioactive compounds even from the lipophilic fraction of spices that could be of immense industrial importance.

Tocopherol composition of fixed oils

The tocopherol composition of the fixed oils is described in Table 6. The fixed oils were screened for α-, β-, γ- and δ-tocopherol. A wide range in tocopherol level was seen, where *T. vulgaris* fixed oil showed the highest amount of α-tocopherol (551.49 mg/100 g oil) while the least was found in *A. graveolens* fixed oil (3.81 mg/100 g oil) (Table 6). Overall the levels of α- tocopherol was higher in lamiaceae family (Table 7). β + γ- tocopherol were found to be highest in *M. hortensis* (26.97 mg/100 g oil) and *A. graveolens* (26.32 mg/100 g oil) fixed oils while *C. cyminum* fixed oil (0.10 mg/100 g oil) showed the least amounts. Remarkably, it was observed that the fixed oils from spices belonging to the *Apiaceae* family such as *T. ammi*, *P. anisum*, *C. carvi*, and *C. cyminum* showed no presence of δ- tocopherol. Our findings were in agreement with that of Matthäus et al. (2015), where *P. anisum* and *C. cyminum* oil also showed the absence of δ- tocopherol. Among all the spices, *O. basilicum* fixed oil had the highest level of δ- tocopherol (9.07 mg/100 g oil). Spice fixed oils such as *R. officinalis*, *T. vulgaris*, *O. vulgare* and *T. ammi* showed remarkably high levels of tocopherols when compared with conventional vegetable oils such as corn, peanut, sunflower, soybean, olive (Gliszczyńska-Świgło and Sikorska 2004) and garden cress seed oil (Diwakar et al.

Table 7 Phenolics, tocopherols and sterols present in fixed oils belonging to Lamiaceae family

	O. basilicum	*M. hortensis*	*O. vulgare*	*R. officinalis*	*T. vulgaris*
Phenolics (µg/100 g oil)					
Gallic acid	ND	ND	ND	ND	99.50 ± 7.97
Protocatecheuic acid	493.47 ± 9.56	ND	ND	ND	ND
4,Hydroxybenzoic acid	1419.56 ± 0.00^{a}	225.80 ± 45.62^{b}	ND	ND	264.71 ± 15.93^{b}
Chlorogenic acid	662.46 ± 0.00	ND	ND	ND	ND
Vanillic acid	ND	ND	ND	ND	ND
Syringic acid	ND	ND	ND	ND	ND
Epicatechin	ND	ND	ND	ND	ND
trans-Cinnamic acid	ND	849.46 ± 15.20^{a}	ND	131.69 ± 3.61^{b}	ND
Catechin	ND	ND	ND	28.24 ± 11.84	ND
Caffeic acid	ND	ND	ND	11.07 ± 1.61	ND
p-coumaric acid	270.39 ± 0.00^{a}	ND	ND	246.20 ± 11.02^{a}	ND
trans-Ferulic acid	ND	43.01 ± 0.00	ND	ND	ND
Myricetin	ND	526.88 ± 15.20^{a}	125.73 ± 9.36^{b}	230.17 ± 2.41^{c}	88.24 ± 5.31^{b}
Quercetin	ND	ND	ND	ND	ND
Kaempferol	ND	ND	26.47 ± 0.00	ND	ND
Tocopherols (mg/100 g oil)					
α-Tocopherol	ND	36.15 ± 3.83^{a}	134.61 ± 0.42^{b}	382.39 ± 1.40^{c}	551.49 ± 0.87^{d}
(β + γ)–Tocopherol	19.05 ± 0.42^{a}	26.97 ± 4.41^{a}	2.84 ± 0.00^{b}	8.24 ± 0.00^{b}	3.00 ± 0.46^{b}
δ–Tocopherol	9.07 ± 0.58^{a}	5.25 ± 0.99^{b}	0.11 ± 0.01^{c}	0.13 ± 0.07^{c}	0.51 ± 0.04^{c}
Sterols (mg/100 g oil)					
Ergosterol	15.48 ± 0.60	ND	ND	ND	ND
Stigmasterol + Campesterol	1126.34 ± 0.15^{a}	127.38 ± 1.21^{b}	ND	371.22 ± 3.02^{c}	141.55 ± 0.05^{d}
β–Sitosterol	1720.65 ± 87.46^{a}	1392.89 ± 8.97^{b}	1089.22 ± 32.06^{c}	1287.47 ± 1.10^{b}	824.27 ± 1.69^{d}
Squalene	333.09 ± 3.31^{a}	55.03 ± 0.25^{b}	112.82 ± 1.37^{c}	65.71 ± 0.11^{b}	36.17 ± 0.22^{d}

Results are expressed as Mean ± SD, *n*=3; ND—Not Detected; Different superscript letters across the row indicate Significant difference between values at $P < 0.05$ level

2010), thus giving an opportunity to investigate spice fixed oils as a rich source of tocopherols.

Sterol and squalene composition of fixed oils

Identifying novel sources of sterols and squalene is of immense importance because of their anti-oxidative, anti-inflammatory, anti-carcinogenic and lipid-lowering effect (Ryan et al. 2007). Table 6 describes the various sterols and squalene present in spice fixed oils. Amongst the four phytosterols (ergosterol, stigmasterol, campesterol, β-sitosterol) identified, β-sitosterol was found to be in abundance as observed in most vegetable oils. *T. ammi* (3307.64 mg/100 g oil) (Table 6) fixed oil showed the highest levels of β-sitosterol while the least was observed by *P. anisum* (31.85 mg/100 g oil) fixed oil. Stigmasterol and campesterol were the next major sterols found in all spice fixed oils except for *O. vulgare* (Table 7). These sterols were recorded to be majorly present in *T. ammi* (3411.80 mg/100 g oil) fixed oil while *A. graveolens* (85.55 mg/100 g oil) fixed oil showed the least amounts. The presence of ergosterol was confirmed in 4 spice fixed

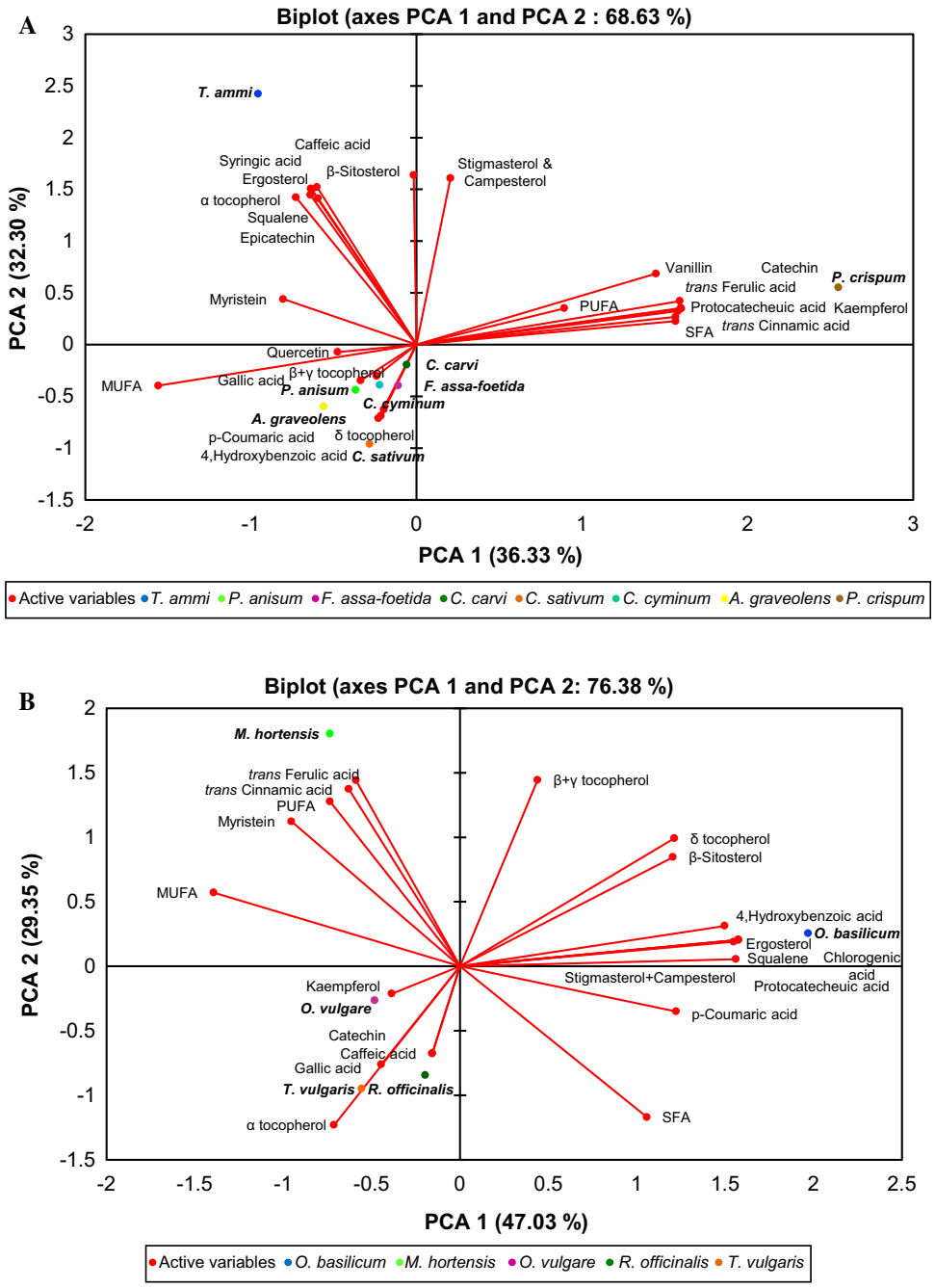

Fig. 2 PCA plot of spice fixed oils belonging to **a** Apiaceae family **b** Lamiaceae family

oils of which *T. ammi* (170.32 mg/100 g oil) fixed oil contained high levels of it. Ramadan and Morsel (2002) reported 29.8% of stigmasterol and 28.2% of β-sitosterol present in *C. sativum* seed oil. High levels of β-sitosterol (960 mg/100 g oil) was also present in nigella seed oil (Atta 2003). Besides, it was interesting to note spice fixed oils as an alternative source of squalene, where *O. basilicum* (333.09 mg/100 g oil) fixed oil along with *T. ammi* (190.26 mg/100 g oil) fixed oil exhibited remarkably high amounts of squalene. Identifying spice fixed oils as a source of sterols would be of great interest to the oil processing and food industries because of the richness of phytochemicals present in the unsaponifiable fraction.

Principal component analysis

This statistical analysis was implemented in our study to identify variation among the fatty acids and bioactive compounds present in spice fixed oils belonging to both the families. The biplot graphs representing the PCA is shown in Fig. 2. In PCA of Apiaceae family (Fig. 2a), the first two principal components accounted for 68.63% of the total variation (PC1 = 36.33% and PC2 = 32.30%, respectively) while in PCA of Lamiaceae family (Fig. 2b) 76.38% of the total variation was observed (PC1 = 47.03% and PC2 = 29.35%, respectively). In the Apiaceae family, SFA (%), protocatechuic acid, *trans*-cinnamic acid, catechin, *trans*-ferulic acid, kaempferol and MUFA (%) contributed majorly to PC1 while ergosterol, stigmasterol and campesterol, β-sitosterol, squalene, α- tocopherol, syringic acid and epicatechin were responsible for variation in PC2. In the Lamiaceae family, variability in PC1 was positively contributed by ergosterol, stigmasterol and campesterol, β-sitosterol, squalene, protocatechuic acid, 4, hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, δ- tocopherol and SFA (%) while β + γ- tocopherol, *trans*-ferulic acid, *trans*-cinnamic acid myristein, PUFA (%) and α- tocopherol were responsible for variation in PC2.

Conclusion

Numerous studies have been reported on spice extracts, essential oils and oleoresins, however not much interest has been shown in studying the fixed oil from the spices. Therefore, the present study was proposed to extract fixed oils from Indian spices and extensively explore its fatty acid profile and bioactive composition. As we studied a spectrum of spices belonging to Apiaceae and Lamiaceae family, *F. assa-foetida* had the highest amount of fixed oil while *P. crispum* had the least. A high degree of unsaturation was observed which thereby paves a pathway for use of fixed oils in food, pharmaceutical and cosmetic industry. The presence of some unusual fatty acids also stirs in a lot of excitement. Interestingly, the study also identifies spices to be a storehouse of lipid-soluble bioactive compounds wherein fixed oils from *T. ammi*, *P. crispum*, *O. basilicum* and *R. officinalis* were the major source of phenolic compounds. A strong positive correlation was observed between Folin-Ciocalteu, DPPH and TEAC. *T. vulgaris*, *M. hortensis* and *O. basilicum* fixed oils confirmed high amounts of α-, β + γ- and δ- tocopherol respectively. β-sitosterol was found to be the dominating phytosterol in all fixed oils with *T. ammi* fixed oil recording at the highest levels. PCA confirmed the difference of *T. ammi*, *P. crispum*, *M. hortensis* and *O. basilicum* fixed oils from other spice fixed oils. The data obtained from the study can also be used by the regulatory bodies to assess the quality and authenticity of Indian spices and set standards for national and international trade. The antioxidant richness of fixed oils also paves a pathway for its use as potential adducts in vegetable oil, and in various cosmetic, industrial, and pharmaceutical applications. Therefore, the study considerably enhances our understanding of lipids and secondary metabolites from the non-volatile fraction of spices and also gives us lucrative leads for various industrial applications.

Acknowledgements The author PD is thankful to University Grants Commission, New Delhi for the award of Senior Research Fellowship. We greatly acknowledge the Director, CSIR-CFTRI for his kind support. The authors would also like to thank B Eswaran M and Asha M, Senior Technical Officer, CSIR-CFTRI, Mysore, for their support in GC-MS and HPLC analysis.

Author contributions The authors PD, SRV and AD planned, designed, performed the experiments and interpreted the research data. PD performed data analysis, statistical analysis and drafted the manuscript sections. AWT fully supervised the study plan, methodology, data execution, interpretation and manuscript corrections. All the authors read and approved the final manuscript.

Funding This research was funded by Science and Engineering Research Board (SERB)—Department of Science and Technology, Government of India, New Delhi (ECR/2016/001548).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Availability of data and material The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not Applicable.

Ethical approval Not Applicable.

Consent to participate Not Applicable.

Consent for publication Not Applicable.

References

Ali MA, Hamiduddin ZM, Ikram M (2018) Phyto-pharmacological potential of Jaiphal (*Myristica fragrans* Houtt): A spice of medicinal importance and its utilization in Unani Medicine. Int J Green Pharm 12(1):S26–S36

Al-Jasass FM, Al-Jasser MS (2012) Chemical composition and fatty acid content of some spices and herbs under Saudi Arabia conditions. Sci World J. https://doi.org/10.1100/2012/859892

Ashraf M, Ali Q, Iqbal Z (2006) Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. J Sci Food Agric 86:871–876. https://doi.org/10.1002/jsfa

Atta MB (2003) Some characteristics of nigella (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile. Food Chem 83:63–68. https://doi.org/10.1016/S0308-8146(03)00038-4

Benzie IFF, Strain JJ (1999) Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 299(1995):15–27. https://doi.org/10.1016/S0076-6879(99)99005-5

BurinV AS, Costa L, Luiz M (2011) Determination of some phenolic compounds in red wine by RP-HPLC: Method development and validation. J Chromatogr Sci 49(8):647–651

Chernenko TV, Glushenkova AI (2001) Lipids of *Acorus calamus*. Chem Nat Compd 37(4):304–306

Diwakar BT, Dutta PK, Lokesh BR, Naidu KA (2010) Physicochemical properties of garden cress (*Lepidium sativum* L.) seed oil. JAOCS 87(5):539–548. https://doi.org/10.1007/s11746-009-1523-z

Food Safety and Standard Authority of India (2016) Manual of methods of analysis of foods food safety and standards authority of India Ministry of Health and Family Welfare Government of India New Delhi. Ministry of Health and Family Welfare 1–86. Retrieved from https://old.fssai.gov.in/Portals/0/Pdf/Manual_Metals_25_05_2016.pdf

Gliszczyńska-Świgło A, Sikorska E (2004) Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. J Chromatogr A 1048(2):195–198. https://doi.org/10.1016/j.chroma.2004.07.051

Krishna De A, De M (2018) Functional and therapeutic applications of some important spices. The Role of Functional Food Security in Global Health. Elsevier Inc. https://doi.org/10.1016/B978-0-12-813148-0.00029-3

Khalid B, Hamid S, Ashraf CM (2005) Chemical constituents of seed oils/meals of Pakistani Umbelliferae spices as potential industrial raw material. Journal of the Chemical Society of Pakistan 27(4) Retrieved from http://jcsp.org.pk/ArticleUpload/870-3707-1-RV.pdf

Kiralan M, Calikoglu E, Ipek A, Bayrak A, Gurbuz B (2009) Fatty acid and volatile oil composition of different coriander (*Coriandrum sativum*) registered varieties cultivated in Turkey. Chem Nat Compd 45(1):89–90

Laribi B, Bettaieb I, Kouki K, Sahli A, Mougou A, Marzouk B (2009) Water deficit effects on caraway (*Carum carvi* L.) growth, essential oil and fatty acid composition. Ind Crops Prod 30(3):372–379. https://doi.org/10.1016/j.indcrop.2009.07.005

Laribi B, Kouki K, Mougou A, Marzouk B (2010) Fatty acid and essential oil composition of three Tunisian caraway (*Carum carvi* L.) seed ecotypes. J Sci Food Agric 90(3):391–396. https://doi.org/10.1002/jsfa.3827

Laribi B, Kouki K, Bettaieb T, Mougou A, Marzouk B (2013) Essential oils and fatty acids composition of Tunisian, German and Egyptian caraway (*Carum carvi* L.) seed ecotypes: A comparative study. Ind Crops Prod 41(1):312–318. https://doi.org/10.1016/j.indcrop.2012.04.060

Manasa V, Vaishnav SR, Tumaney AW (2020) Physicochemical characterization and nutraceutical compounds of the selected spice fixed oils. J Food Sci Technol. https://doi.org/10.1007/s13197-020-04813-8

Matthäus B, Özcan MM, Al Juhaimi F (2015) Variations in oil, fatty acid and tocopherol contents of some *Labiateae* and *Umbelliferae* seed oils. Qual Assur Saf Crops Foods 7(2):103–107. https://doi.org/10.3920/QAS2013.0296

Matthäus B, Özcan MM, Dogu S (2018) Fatty acid composition and sterol contents of some *Origanum* seed oils. Eur J Lipid Sci Technol 120(7):1800094. https://doi.org/10.1002/ejlt.201800094

Piras A, Rosa A, Marongiu B, Atzeri A, Dess MA, Falconieri D, Porcedda S (2012) Extraction and separation of volatile and fixed oils from seeds of *Myristica fragrans* by Supercritical CO 2: Chemical composition and cytotoxic activity on Caco-2 cancer cells. J Food Sci 77(4):448–453. https://doi.org/10.1111/j.1750-3841.2012.02618.x

Prasad P, Savyasachi S, Prasanna Anjaneya Reddy L, Sreedhar RV (2019) Physico-chemical characterization, profiling of total lipids and triacylglycerol molecular species of omega-3 fatty acid rich *B*. arvensis seed oil from India. J Oleo Sci 68(3):209–223. https://doi.org/10.5650/jos.ess18219

Ramadan MF (2013) Healthy blends of high linoleic sunflower oil with selected cold pressed oils: Functionality, stability and antioxidative characteristics. Ind Crops Prod 43(1):65–72. https://doi.org/10.1016/j.indcrop.2012.07.013

Ramadan MF, Morsel J (2002) Oil composition of coriander (*Coriandrum sativum* L.) fruit-seeds. Eur Food Res Technol 215(3):204–209. https://doi.org/10.1007/s00217-002-0537-7

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 26(9–10):1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rebey I, Jabri-Karoui I, Hamrouni-Sellami I, Bourgou S, Limam F, Marzouk B (2012) Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Ind Crops Prod 36(1):238–245. https://doi.org/10.1016/j.indcrop.2011.09.013

Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM (2007) Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum Nutr 62(3):85–91. https://doi.org/10.1007/s11130-007-0046-8

Sánchez-Machado DI, López-Hernández J, Paseiro-Losada P, López-Cervantes J (2004) An HPLC method for the quantification of sterols in edible seaweeds. Biomed Chromatogr 18(3):183–190. https://doi.org/10.1002/bmc.316

Shahidi F, Hossain A (2018) Bioactives in spices, and spice oleoresins: Phytochemicals and their beneficial effects in food preservation and health promotion. J Food Bioact 3:8–75

Sowbhagya HB, Sathyendra Rao BV, Krishnamurthy N (2008) Evaluation of size reduction and expansion on yield and quality of cumin (*Cuminum cyminum*) seed oil. J Food Eng 84(4):595–600. https://doi.org/10.1016/j.jfoodeng.2007.07.001

Sreeramulu D, Raghunath M (2011) Antioxidant and phenolic content of nuts, oil seeds, milk and milk products commonly consumed in India. Food Nutr Sci 02(05):422–427. https://doi.org/10.4236/fns.2011.25059

Sultan MT, Butt MS, Anjum FM, Jamil A, Akhtar S, Nasir M (2009) Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. Pak J Bot 41(3):1321–1330

Vallverdú-Queralt A, Regueiro J, Martínez-Huélamo M, Rinaldi Alvarenga JF, Leal LN, Lamuela-Raventos RM (2014) A

comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay. Food Chem 154:299–307. https://doi.org/10.1016/j.foodchem.2013.12.106

Zhang XL, Guo YS, Wang CH, Li GQ, Xu JJ, Chung HY, Ye WC, Li YL, Wang GC (2014) Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities. Food Chem 152:300–306. https://doi.org/10.1016/j.foodchem.2013.11.153