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Chemical Composition of Essential Oils and Supercritical Carbon Dioxide Extracts from Cambodian Spices

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Abstract: Cambodian spices *Amomum kravanh*, *Citrus hystrix* and *Piper nigrum* 'Kampot' have long history of seasoning of food products. In this study, essential oils (EOs) and supercritical CO_2 extracts from all three species have been analyzed using GC-MS with two columns of different polarity, whereas EO and CO_2 extract from *P. nigrum* fruits and CO_2 extract from the peel of *C. hystrix* have been isolated and analyzed for the first time. The results showed that *A. kravanh* EO contained mostly eucalyptol (78.8/ 72.6 %), while CO_2 extract was rich in oleic acid (29.26 %) and tricosane (14.74 %). *C. hystrix* EO was rich in β-pinene (29.95/29.45 %), followed by D-limonene (24.54/23.24 %) and sabinene (9.94/10.23 %). β-pinene (30.2/28.9 %), D-limonene (23.99/23.74 %) and sabinene (13.36/19.55 %) were also predominant in the CO_2 extracts. β-Caryophyllene was the main constituent of both EO and CO_2 extract from *P. nigrum* 'Kampot' with contents ranging from 37.84 to 55.84 %, followed by 3-carene (from 7.18 to 18.72 %). Such findings suggest that supercritical CO_2 can potentially be used for extraction of all three Cambodian spices. Nevertheless, further research determining the most efficient extraction parameters will be needed before its application in the spice processing practice.

Keywords: essential oil; GC-MS analysis; spice; supercritical fluid extraction

1. Introduction

The term spice refers to dried plants or their parts that are used to enhance food flavor, taste, and color [1,2]. Nowadays, more than 400 spices and condiments are used worldwide from which among 275 species have their origin in tropical Southeast Asia. Cardamom, cinnamon, clove, ginger, nutmeg, pepper, and turmeric are examples of commodities of global economic importance. In 2021, the global spice market accounted for 21.3 billion US dollars, and it is forecasted to reach 27.4 billion USD by the end of 2026 [3,4]. Economically, the most important spice in international trade is black pepper, known as "king," of the spices. Pepper or peppercorn refers to dried fruits of *Piper nigrum,* a perennial vine native to Western Ghats in India, which belongs to *Piperaceae* family. Throughout history, black pepper was one of the most traded spices worldwide and was utilized even as a currency all around the commercial routes established between Europe and India [5]. At the present time, the black pepper market is estimated to have reached 4400 million USD in 2022 and is likely to increase to almost 8 million USD by 2032 [6]. Apart from their seasoning properties, spices are used as natural colorants in food industry due to the presence of pigments. Moreover, due to numerous proven beneficial effects attributed to active biochemicals present in spices, they are also utilized in aromatherapy, cosmetics, nutraceuticals, perfumes and pharmaceuticals. Spices can be added to foods in various forms, such as whole, ground or in form of highly concentrated extracts [7].

In most spices, essential oils (EOs) are the main constituents responsible for their taste and olfactory sensations. EOs are usually comprised of large number (up to 400) of individual constituents with one or two dominant compounds, mostly classified as terpenes and their oxygenated derivatives. Other chemicals present in EOs are benzene derivatives which are responsible for the aroma of spices. Significant representatives include phenols or phenolic ethers [8–10]. Furthermore, alkaloids also contribute to the olfactory sensations, especially to pungency, of some spices. [11,12]. The extraction of EOs can be carried out using wide range of techniques while distillation (steam, water or combined) remains the most common method applied in industrial scale. Extracts obtained by solvent extraction are called oleoresins and contain flavor constituents of spices and other compounds soluble in organic solvents [2,4,13]. The most important shortcomings of distillation are high consumption of plant material, loss of thermo-sensitive compounds and long extraction time. The main disadvantages of solvent extraction are environmental and safety hazards associated with accumulation of organic solvents, high energy costs and oxidation of aroma and coloring compounds from spices [14]. To overcome these drawbacks, various green extraction techniques have recently been developed, while supercritical fluid extraction (SFE) belongs to one of them. This method provides multiple advantages associated with utilization of supercritical fluids as solvents, which possess different physicochemical properties such as lower viscosity and higher diffusivity and therefore results in higher extraction rates and overall faster process. Moreover, their density, which is related to the solvent capacity, can be modified by adjusting the extraction pressure and temperature [14,15] Although several solvents can potentially be used during SFE, carbon dioxide (CO_2) remains the most employed supercritical fluid. Its easily obtainable moderate critical pressure and temperature ensure the preservation of labile compounds in the extract. In addition, CO_2 is ubiquitous in the environment, non-toxic to human health, non-flammable and widely available at low cost [16,17]. All above-mentioned characteristics make supercritical CO_2 highly attractive to be used as a “green solvent” and have led to multiple practical applications in different industries. Hop extract, decaffeinated tea and coffee, nicotine-free tobacco, and specialty oils are examples of commercial products obtained by CO_2 extraction at industrial level [18,19]. Extensive research has occurred in the field of possible alternative use of SFE in extraction of bioactive components from spices during last decades and have led to the availability of wide variety of products on the market with CO_2 extract from cinnamon, ginger, black and white pepper as examples [17]. Regarding to differences in chemical composition of EOs and supercritical CO_2 extracts from various spices and aromatic plants, a plethora of studies have been conducted. Most commonly, results indicated significant differences in quantities of individual compounds. EOs obtained by distillation contained higher amounts of low molecular weight components like monoterpenoids and phenylpropanoids, while CO_2 extracts were richer in constituents of higher molecular weight like sesquiterpenoids and diterpenoids [20–23].

Cambodia as well as many Southeast Asian countries has cuisine that is generally considered as healthy and delicious due to the abundant use of fish and incorporation of many vegetables, fruits, herbs, and spices into every meal. Fresh spices and herbs are essential ingredients in Cambodian dishes and approximately 42 g of condiments and spices are consumed daily per person [24,25]. Kampot pepper is one of the most popular spices grown in Cambodia with international recognition. It is a cultivar of *Piper nigrum* L produced in the Kampot province with unique climatic and soil conditions, giving the pepper distinctive flavor and aroma from other kinds of peppercorns. This commodity has been exported to Europe since 1870 during the French Protectorate due to its exceptional organoleptic quality. Currently, four different types of Kampot pepper can be found on the market: green, black, red, and white pepper. Although all Kampot peppers have an excellent reputation regarding to their sensory properties, red peppercorns are especially rare because of the unique process of their production. Red berries are harvested in full maturity, then blanched, sun-dried and manually sorted [26]. Leaves and fruits of numerous *Citrus* species (*Rutaceae*) are widely used to flavor foods and beverages. *Citrus hystrix* DC, known as kaffir lime, can be mentioned as an example of regionally used condiment. Leaves and fruit juice from this citrus are used for various flavoring purposes in Khmer cuisine and EO from the fruit pericarp is utilized in cosmetics and beauty products. Previous research has shown that kaffir lime EO possess various biological activities, namely antimicrobial, antioxidant, repellent and antiviral. Numerous studies analyzing chemical composition of *C. hystrix* EO have been conducted and revealed substantial number of monoterpene hydrocarbons and their oxygenated derivatives, with main compounds α/β-pinene, citronellal, limonene and sabinene. [28–30]. Plants belonging to the *Zingiberaceae* family are also widely used for their unique aroma as spices in Southeast Asia. *Amomum kravanh* Pierre ex Gagnep. (*Zingiberaceae*) is cultivated in Cambodia and other Southeast Asian countries for its fruits and leaves that are used to flavor curries. Fruit EO and SFE extract contain eucalyptol and β-pinene as main components [31–34]. With exception of single report on chemical composition of *C. hystrix* leaf CO_2 extract [35], there is no study dealing with supercritical extraction of fruits of these species of spices that are frequently used in Southeast Asian cuisine. Therefore, the main objective of this study was to determine the chemical composition of EOs and CO_2 extracts obtained from fruits of three traditional Cambodian spices, namely *A. kravanh*, *C. hystrix* and *P. nigrum* 'Kampot'.

2. Results

In this investigation, three EOs and three CO_2 extracts have been isolated from Cambodian spice species with respective yield values ranging from 3.01 to 5.22 % (EOs) and from 0.57 to 8.35 % (CO_2 extracts). In EOs obtained from *P. nigrum* 'Kampot', *C. hystrix* and *A. kravanh*, a total of 35, 38 and 21 individual constituents have been identified using HP-5 column, representing 99.38, 98.68 and 99.88 % of their respective total contents. Using DB-HeavyWAX column, a total of 41, 50 and 24 compounds have been detected constituting 99.15, 98.06 and 99.26 % of the total EOs, respectively. In CO_2 extracts, a total number of 32, 36 and 31 components have been determined amounting to 98.65, 99.36 and 92.69 % of the total extracts. When analyzed with DB-HeavyWAX column, 40, 54 and 40 compounds have been identified which accounted for 96.74, 98.06 and 95.57 % of their total respective contents. Sesquiterpenes, monoterpenes and their oxygenated derivatives were the most predominant chemical groups in almost all tested EOs and CO_2 extracts with exception of *A. kravanh* extract, where higher fatty acids and long-chain alkanes have been identified as the most abundant chemicals.

In *A. kravanh* EO, oxygenated monoterpene eucalyptol has been determined as prevailing ingredient comprising 78.8/ 72.6 % of the total sample. Other compounds occurring in significant amounts were monoterpenes β-pinene (7.68/7.49 %), α-pinene (2.3/ 2.2 %) and oxygenated derivative α-terpineol (4.31/ 4.67 %). In HP-5 analyses, L-terpinene-4-ol amounted to 1.19 % of the sample, however, this constituent has not been detected by DB-HeavyWAX column, where monoterpene D-limonene was identified as the third most abundant component (5.12 %). On the contrary, chemical composition of CO_2 extract differed substantially from the EO. In HP-5 investigation, long-chained alkane tricosane comprised 14.74 % of the total extract followed by monoterpenoid eugenol acetate accounting for 14.02 % of the sample. Oleic acid was the third most prevailing constituent comprising 12.21 % of the extract accompanied by oxygenated monoterpenoid eugenol (7.91 %) and long chained alkane pentacosane (5.19 %). In contrast with these findings, analyses with DB-HeavyWAX column differed considerably. Majority of the sample consisted of oleic and palmitic acids constituting 29.26 and 17.07 % of the total respective content followed by tricosane (5.26 %), eugenol acetate (5.24 %) and linoleic acid (5.17 %). Compared to the hydrodistillation, the yield of CO_2 extract was much lower (0.6 %), and its physical properties were different as the extract had a waxy and semi-solid structure.

Investigation of *C. hystrix* EO revealed monoterpenes as the most prevalent class of chemical compounds. Monoterpenes β-pinene (29.95/29.45 %), D-limonene (24.54/23.24 %) and sabinene (9.94/10.23 %) accompanied by alcohols L-terpinene-4-ol (9.71/9.07 %) and α-terpineol (3.7/3.62 %) were the main constituents of EO. Similarly, β-pinene (30.2/28.9 %), D-limonene (23.99/23.74 %) and sabinene (13.36/19.55 %) followed by aldehyde citronellal (5.21/4.28 %) were predominant components of the CO_2 extract. In HP-5 column analysis, furanocoumarin oxypeucedanin accounted for 2.96 % of the total extract, however, this compound was not detected by DB-HeavyWAX column.

In *P. nigrum* 'Kampot' EO, sesquiterpene β-caryophyllene has been identified as the dominant compound constituting 34.84/39.55 % of the total oil followed by monoterpenes 3-carene (18.72/18.48 %), D-limonene (11.18/10.93 %) and β-pinene (5.42/5.32 %) when detected by HP-5/ DB-HeavyWAX columns, respectively. Similarly, analysis of CO_2 extract has revealed even higher content of β-caryophyllene (54.21/ 55.86 %) accompanied by 3-carene, D-limonene and β-selinene comprising 7.4/ 7.18 %, 6.26/ 6.03 %, and 5.24/ 4.76 % of the total extract. Complete chemical analyses of *A. kravanh*, *C. hystrix* and *P. nigrum* 'Kampot' EOs and CO_2 extracts are provided in Tables 1–3. Chromatograms of EOs and CO_2 extracts can be seen in Figures 1 and 2.

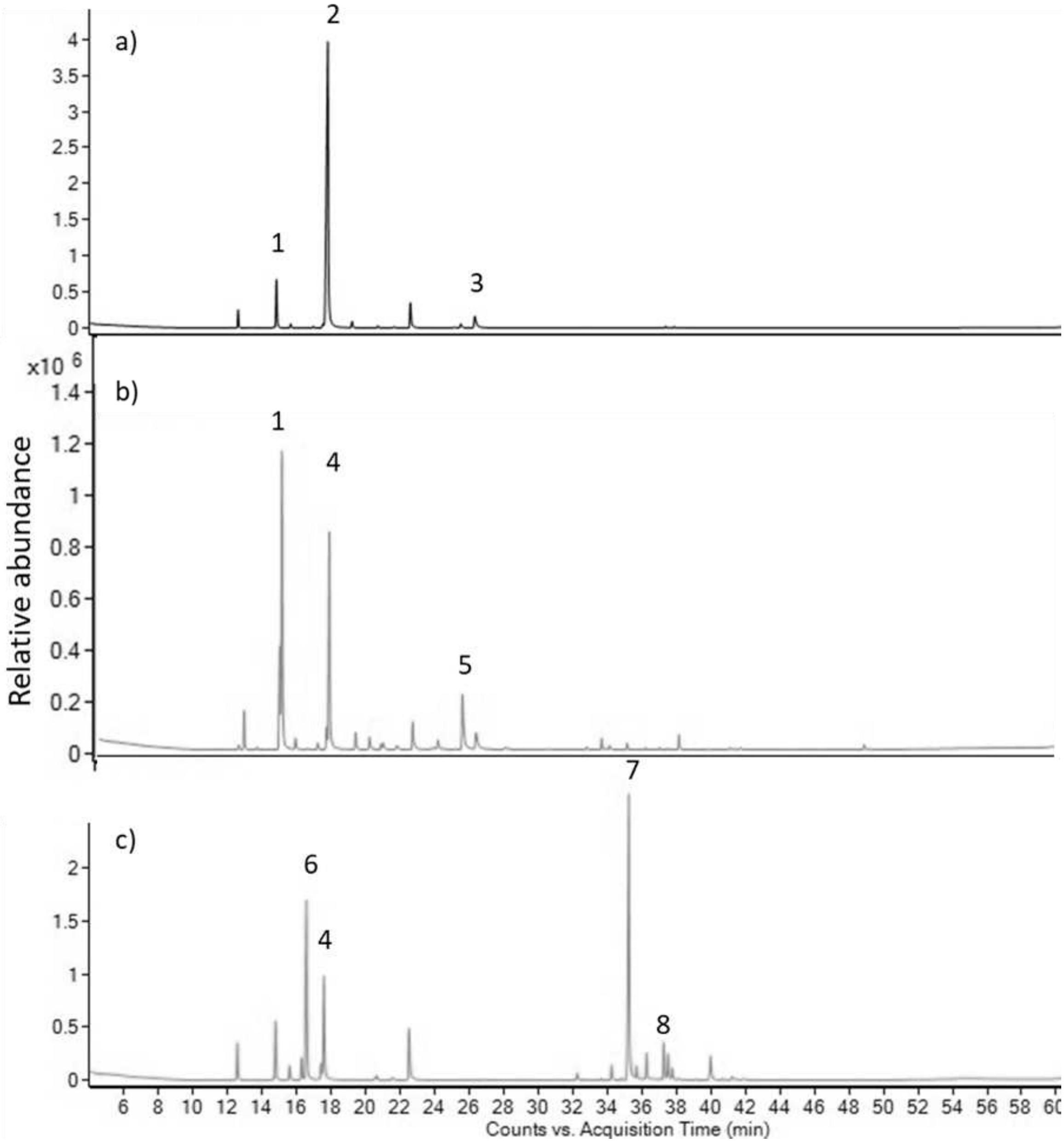

Figure 1. GC-MS chromatograms of EOs of a) *A. kravanh*, b) *C. hystrix* and c) *P. nigrum* 'Kampot' (analyzed with HP-5 column). Peak numbers and constituents' names: 1. β-pinene, 2. eucalyptol, 3. α-terpineol, 4. D-limonene, 5. L-terpinene-4-ol, 6. 3-carene, 7-β-caryophyllene and 8. β-selinene.

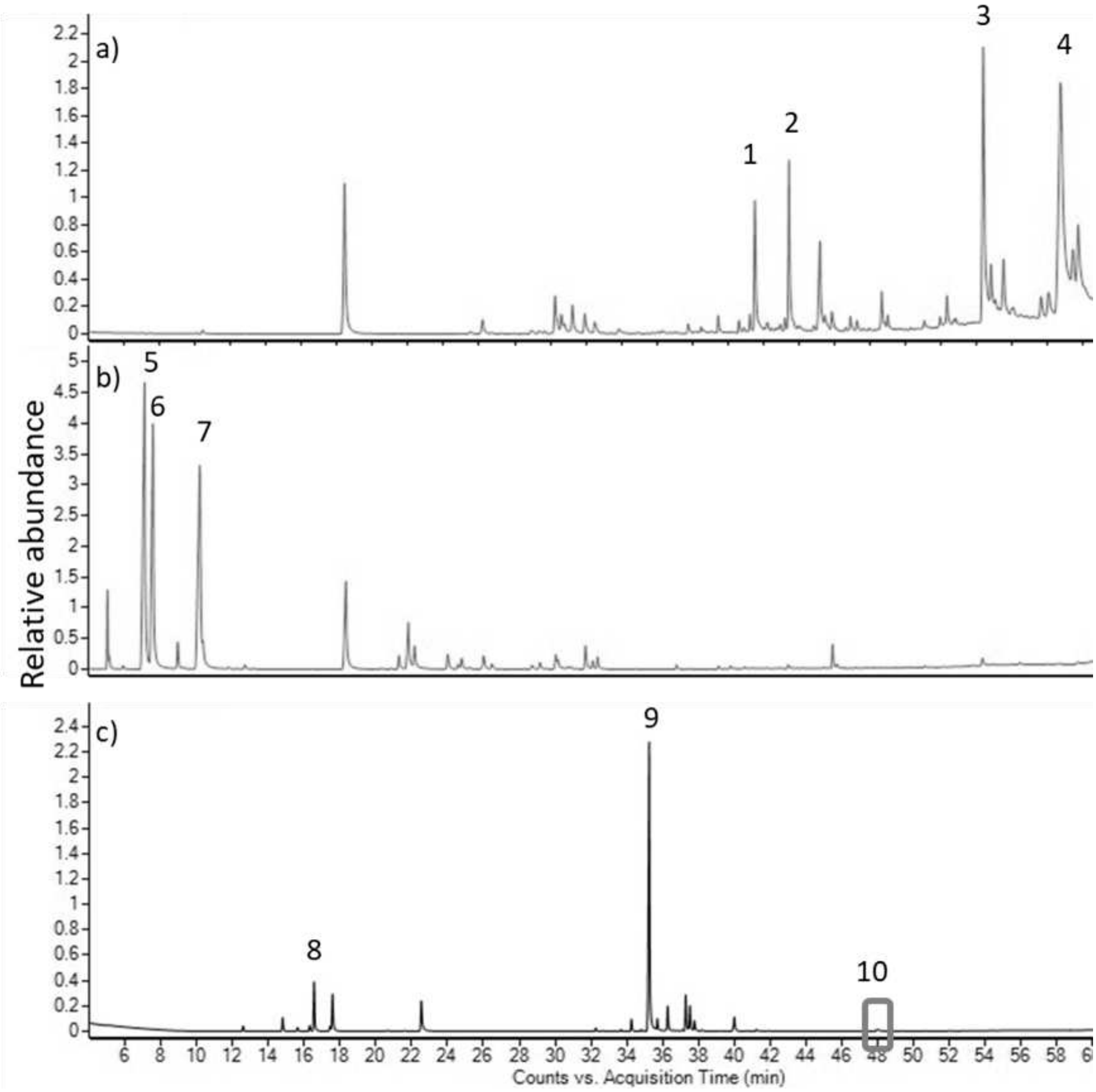

Figure 2. GC-MS chromatograms of CO_2 extracts of a) *A. kravanh*, b) *C. hystrix* (analyzed on DB-HeavyWax column) and c) *P. nigrum* 'Kampot' (analyzed with HP-5 column). Peak number and compound names: 1. eugenol acetate, 2. tricosane, 3. palmitic acidt, 4. oleic acid, 5. β-pinene, 6. sabinene, 7. D-limonene, 8. 3-carene, 9. β-caryophyllene and 10. Pellitorine.

Table 1. Chemical composition of *A. kravanh* EO and CO_2 extract.

RI[a]		Compound[b]	C[c]	Extraction type/Column type/Peak area [%]				Column type/Identification method[d]	
				Essential oil		CO_2 extract			
Obs.	Lit.			HP-5 MS	DB-Wax	HP-5 MS	DB-Wax	HP-5 MS	DB-Wax
923	931	α-Thujene	MH	0.09 ± 0.01	- - -	- - -	- - -	RI, GC-MS	-
929	932	α-Pinene	MH	2.3 ± 0.05	2.20 ± 0.03	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
-	945	α-Fenchene	MH	- - -	- - -	- - -	0.01 ± 0.01	-	GC-MS
944	946	Camphene	MH	0.07 ± 0.01	0.08 ± 0.00	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
970	969	Sabinene	MH	0.2 ± 0.04	0.23 ± 0.00	- - -	- - -	RI, GC-MS	GC-MS
973	974	β-Pinene	MH	7.68 ± 0.08	7.49 ± 0.09	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
989	988	β-Myrcene	MH	0.78 ± 0.03	0.86 ± 0.08	- - -	- - -	RI, GC-MS	GC-MS
1003	1002	α-Phellandrene	MH	0.08 ± 0.01	- - -	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
1015	1009	4-Carene	MH	0.24 ± 0.03	0.17 ± 0.12	- - -	- - -	RI, GC-MS	GC-MS
-	1014	α-Terpinene	MH	- - -	0.22 ± 0.01	- - -	- - -	-	GC-MS
1025	1022	o-Cymene	MH	0.69 ± 0.04	0.87 ± 0.02	- - -	- - -	RI, GC-MS	GC-MS
1031	1026	Eucalyptol	MO	78.89 ± 0.42	72.60 ± 0.89	- - -	0.08 ± 0.01	RI, GC-MS	GC-MS
-	1031	D-Limonene	MH	- - -	5.12 ± 0.09	- - -	0.01 ± 0.01	-	GC-MS
1058	1054	γ-Terpinene	MH	1.05 ± 0.06	1.06 ± 0.02	- - -	- - -	RI, GC-MS	GC-MS
-	1083	Fenchone	MO	- - -	0.18 ± 0.02	- - -	- - -	-	GC-MS
1087	1086	Isoterpinolene	MO	0.43 ± 0.02	- - -	- - -	- - -	GC-MS	-
1105	1095	Linalool	MO	0.45 ± 0.01	0.50 ± 0.01	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
1174	1162	δ-Terpineol	MO	0.39 ± 0.05	0.43 ± 0.01	- - -	- - -	RI, GC-MS	GC-MS
1182	1174	L-terpinen-4-ol	MO	1.19 ± 0.12	1.32 ± 0.02	- - -	- - -	RI, GC-MS	GC-MS
1196	1186	α-Terpineol	MO	4.31 ± 0.17	4.67 ± 0.08	3.68 ± 0.16	1.77 ± 0.15	RI, GC-MS	GC-MS
1350	1346	α-Terpinyl acetate	MO	- - -	- - -	0.17 ± 0.01	- - -	RI, GC-MS	-
1368	1356	Eugenol	MO	- - -	- - -	7.91 ± 0.18	5.06 ± 0.18	RI, GC-MS	GC-MS
-	1416	α-Santalene	SH	- - -	- - -	- -	0.08 ± 0.01	-	GC-MS
1421	1419	β-Caryophyllene	SH	- - -	- - -	1.37 ± 0.25	0.66 ± 0.02	RI, GC-MS, Std	GC-MS, Std
1457	1452	Humulene	SH	- - -	- - -	0.41 ± 0.03	0.12 ± 0.10	RI, GC-MS, Std	GC-MS, Std
1486	1465	(*Z*)-muurola-4(14),5-diene	SH	0.16 ± 0.01	- - -	- - -	- - -	RI, GC-MS	-
-	1478	γ-Muurolene	SH	- - -	0.16 ± 0.02	- - -	- - -	-	GC-MS
1484	1484	Germacrene D	SH	- - -	- - -	1.38 ± 0.02	- - -	RI, GC-MS	-
1490	1489	β-Selinene	SH	0.35 ± 0.03	0.25 ± 0.01	2.06 ± 0.04	0.88 ± 0.16	RI, GC-MS	GC-MS

1497	1496	Valencene	SH	-	-	-	-	-	-	0.69	±	0.10	0.09	±	0.01	RI, GC-MS	GC-MS
1508	1505	β-Bisabolene	SH	0.36	±	0.03	0.23	±	0.01	3.59	±	0.06	1.12	±	0.10	RI, GC-MS	GC-MS
1518	1513	γ-Cadinene	SH	-	-	-	-	-	-	1.34	±	0.09	0.73	±	0.11	RI, GC-MS	GC-MS
-	1514	Cubebol	SO	-	-	-	-	-	-	-	-	-	0.39	±	0.01	-	GC-MS
1525	1521	β-Sesquiphellandrene	SH	0.12	±	0.02	0.15	±	0.00	1.60	±	0.62	0.65	±	0.02	RI, GC-MS	GC-MS
1531	1521	Eugenol acetate	MO	-	-	-	-	-	-	14.02	±	0.74	5.23	±	0.11	RI, GC-MS	GC-MS
1558	1542	(*Z*)-Sesquisabinene hydrate	SO	0.12	±	0.02	0.15	±	0.00	0.51	±	0.11	0.20	±	0.01	RI, GC-MS	GC-MS
1566	1561	(*E*)-Nerolidol	SO	-	-	-	0.12	±	0.00	1.71	±	0.03	0.61	±	0.04	RI, GC-MS	GC-MS
1595	1577	(*E*)-Sesquisabinene hydrate	SO	-	-	-	-	-	-	1.11	±	0.30	0.44	±	0.01	RI, GC-MS	GC-MS
-	1577	Spathulenol	SO	-	-	-	-	-	-	-	-	-	0.15	±	0.00	-	GC-MS
1591	1582	Caryophyllene oxide	SO	-	-	-	-	-	-	0.39	±	0.10	0.25	±	0.02	RI, GC-MS	GC-MS
1675	1674	β-Bisabolol	SO	0.04	±	0.04	0.12	±	0.04	0.21	±	0.00	0.07	±	0.00	RI, GC-MS	GC-MS
1691	1685	α-Bisabolol	SO	-	-	-	-	-	-	0.21	±	0.00	0.07	±	0.00	RI, GC-MS	GC-MS
1714	1715	β-Santalol	SO	-	-	-	-	-	-	0.56	±	0.04	0.90	±	0.01	RI, GC-MS	GC-MS
-	1959	Palmitic acid	FAD	-	-	-	-	-	-	-	-	-	17.07	±	0.23	-	GC-MS
2086	2100	Heneicosane	AH	-	-	-	-	-	-	2.12	±	0.03	0.76	±	0.00	RI, GC-MS	GC-MS
-	2113	Linoleic acid	FAD	-	-	-	-	-	-	-		-	5.17	±	0.36	-	GC-MS
2166	2141	Oleic Acid	FAD	-	-	-	-	-	-	12.21	±	0.25	29.26	±	0.42	RI, GC-MS	GC-MS
-	2172	Stearic acid	FAD	-	-	-	-	-	-	-	-	-	2.16	±	0.09	-	GC-MS
2186	2200	Docosane	AH	-	-	-	-	-	-	1.57	±	0.09	0.51	±	0.10	RI, GC-MS	GC-MS
2286	2300	Tricosane	AH	-	-	-	-	-	-	14.74	±	0.60	5.24	±	0.09	RI, GC-MS	GC-MS
2383	2400	Tetracosane	AH	-	-	-	-	-	-	1.94	±	0.03	0.69	±	0.05	RI, GC-MS	GC-MS
2482	2500	Pentacosane	AH	-	-	-	-	-	-	5.19	±	0.39	1.88	±	0.08	RI, GC-MS	GC-MS
-	2700	Heptacosane	AH	-	-	-	-	-	-	-	-	-	0.46	±	0.02	-	GC-MS
2096	NA	Nonadecan-2-one	K	-	-	-	-	-	-	0.54	±	0.02	-	-	-	GC-MS	-
2261	NA	Tetradec-9-enal	A	-	-	-	-	-	-	0.24	±	0.01	-	-	-	GC-MS	-
2267	NA	Palmitoleic acid	FAD	-	-	-	-	-	-	0.80	±	0.06	1.94	±	0.03	GC-MS	GC-MS
2464	NA	Hexadec-7-enal	A	-	-	-	-	-	-	1.50	±	0.13	0.11	±	0.02	GC-MS	GC-MS
2691	NA	Azelaic acid bis(2-ethylhexyl) ester	E	-	-	-	-	-	-	4.99	±	0.12	1.96	±	0.20	GC-MS	GC-MS
2810	NA	β-Monoolein	E	-	-	-	-	-	-	2.88	±	0.23	-	-	-	GC-MS	-
-	NA	Tricosanol	A	-	-	-	-	-	-	-	-	-	0.38	±	0.01	-	GC-MS
-	NA	Cyclopentadecanone	K	-	-	-	-	-	-	-	-	-	0.46	±	0.01	-	GC-MS
-	NA	Pentacos-1-ene	AH	-	-	-	-	-	-	-	-	-	0.48	±	0.04	-	GC-MS
-	NA	Heptacos-1-ene	AH	-	-	-	-	-	-	-	-	-	1.31	±	0.04	-	GC-MS

-	NA	Hexadec-9-enoic acid	FAD	- - -	- - -	- - -	0.30 ± 0.04	-	GC-MS
-	NA	Octacosanol	A	- - -	- - -	- - -	3.16 ± 0.06	-	GC-MS
-	NA	Squalene	TH	- - -	- - -	- - -	1.17 ± 0.05	-	GC-MS
-	NA	Glyceryl linolenate	E	- - -	- - -	- - -	1.43 ± 0.06	-	GC-MS
-	NA	β-Sitosterol	O	- - -	0.17 ± 0.14	- - -	- - -	-	GC-MS
		Total identified [%]		**99.88 ± 0.05**	**99.29 ± 0.16**	**91.64 ± 0.88**	**95.41 ± 0.46**		

Footnotes 1: [a] RI = retention indices for HP-5 column; Obs = retention indices determined relative to a homologous series of *n*-alkanes (C_8-C_{40}) on a HP-5MS column, Lit = literature RI values (Adams, 2007), NA = RI values were not available in the literature. [b] C = Class; A - Aldehydes, DH - Diteprene hydrocarbons, E - Esters, FAD - Fatty acid and fatty acid derivatives, MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenes, O - Others, SH - Sesquiterpene hydrocarbons, SO - Oxygenated sesquiterpenes, [d]Identification method: GC-MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database; Std = constituent identity confirmed by co-injection of authentic standards. [e] Retention indices were not calculated for compounds calculated only by DB-HeavyWAX column.

Table 2. Chemical composition of *C. hystrix* EO and CO_2 extract.

RI[a]		**Compound[b]**	**C[c]**	**Extraction type/Column type/Peak area [%]**				**Column type/Identification method[d]**	
				Essential oil		**CO_2 extract**			
Obs.	**Lit.**			**HP-5 MS**	**DB-Wax**	**HP-5 MS**	**DB-Wax**	**HP-5 MS**	**DB-Wax**
923	931	α-Thujene	MH	0.31 ± 0.01	0.34 ± 0.02	0.32 ± 0.02	0.31 ± 0	RI, GC-MS	GC-MS
930	937	α-Pinene	MH	2.93 ± 0.16	2.96 ± 0.09	2.54 ± 0.25	2.52 ± 0.09	RI, GC-MS, Std	GC-MS, Std
-	945	Fenchene	MH	- - -	- - -	- - -	0.02 ± 0.02	-	GC-MS
945	946	Camphene	MH	0.16 ± 0.01	0.19 ± 0.01	0.14 ± 0.01	0.15 ± 0	RI, GC-MS, Std	GC-MS, Std
971	976	Sabinene	MH	9.94 ± 0.22	10.2 ± 0.15	19.36 ± 0.97	19.55 ± 0.33	RI, GC-MS	GC-MS
974	980	β-Pinene	MH	29.95 ± 0.54	29.5 ± 0.45	30.2 ± 1.84	28.9 ± 0.55	RI, GC-MS, Std	GC-MS, Std
990	991	β-Myrcene	MH	1.20 ± 0.05	1.38 ± 0.02	1.43 ± 0.06	1.58 ± 0.02	RI, GC-MS	GC-MS
-	1004	Pseudolimonene	MH	- - -	0.97 ± 0.07	- - -	0.97 ± 0.04	-	GC-MS
1003	1005	α-Phellandrene	MH	0.07 ± 0.01	0.06 ± 0	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
-	1008	3-Carene	MH	- - -	0.01 ± 0.02	- - -	- - -	-	GC-MS
1015	1009	4-Carene	MH	- - -	- - -	0.02 ± 0.02	- ± -	RI, GC-MS	-
1015	1009	α-Terpinene	MH	0.51 ± 0.04	0.61 ± 0.01	- - -	- - -	RI, GC-MS	GC-MS
1025	1022	o-Cymene	MH	1.91 ± 0.01	2.34 ± 0.01	0.1 ± 0.01	0.21 ± 0.02	RI, GC-MS	GC-MS
1028	1031	D-Limonene	MH	24.54 ± 0.16	23.2 ± 0.1	23.99 ± 0.45	23.74 ± 0.12	RI, GC-MS	GC-MS
1058	1062	γ-Terpinene	MH	1.67 ± 0.01	1.63 ± 0.02	0.08 ± 0	0.1 ± 0.01	RI, GC-MS	GC-MS
1072	1065	(*Z*)-Sabinene hydrate	MO	- - -	- - -	0.78 ± 0.06	1.06 ± 0.02	RI, GC-MS	GC-MS

-	1071	β-Terpinene	MH	-	-	-	-	-	-	-	-	-	0.03	±	0.01	-	GC-MS
1074	1074	Linalool oxide	MO	1.55	±	0.02	1.57	±	0.03	-	-	-	0.05	±	0.01	RI, GC-MS	GC-MS
1087	1086	Terpinolene	MH	0.54	±	0.03	0.52	±	0.01	0.02	±	0.01	0.05	±	0	RI, GC-MS	GC-MS
1105	1095	Linalool	MO	0.72	±	0.09	0.98	±	0.02	0.52	±	0.27	0.81	±	0.07	RI, GC-MS, Std	GC-MS, Std
-	1098	(*E*)-Sabinene hydrate	MO	-	-	-	-	-	-	-	-	-	0.35	±	0.03	-	GC-MS
-	1114	Fenchol	MO	-	-	-	0.02	±	0.03	-	-	-	-	-	-	-	GC-MS
1145	1137	Sabinol	MO	-	-	-	-	-	-	0.08	±	0.02	-	±	-	RI, GC-MS	-
1150	1145	L-isopulegol	MO	0.22	±	0.04	0.28	±	0.03	0.07	±	0.01	0.11	±	0	RI, GC-MS	GC-MS
1154	1148	Citronellal	MO	1.38	±	0.07	1.05	±	0.04	5.21	±	0.31	4.28	±	0.25	RI, GC-MS	GC-MS
1174	1165	Borneol	MO	0.09	±	0.02	-	-	-	-	-	-	-	-	-	RI, GC-MS	-
1161	1167	dl-Isopulegol	MO	0.12	±	0.02	-	-	-	-	-	-	-	-	-	RI, GC-MS	-
1184	1174	L-terpinen-4-ol	MO	9.71	±	0.16	9.07	±	0.22	0.36	±	0.04	0.42	±	0.02	RI, GC-MS	GC-MS
-	1176	m-Cymen-8-ol	MO	-	-	-	0.09	±	0	-	-	-	-	-	-	-	GC-MS
-	1182	Pinocarveol	MO	-	-	-	0.03	±	0.03	-	-	-	0.06	±	0	-	GC-MS
1199	1189	α-Terpineol	MO	3.70	±	0.09	3.62	±	0.07	0.98	±	0.17	1	±	0.04	RI, GC-MS	GC-MS
-	1194	Myrtenol	MO	-	-	-	0.05	±	0	-	-	-	-	-	-	-	GC-MS
1217	1205	(*E*)-Piperitol	MO	0.02	±	0.04	-	-	-	-	-	-	-	-	-	RI, GC-MS	-
1237	1228	Citronellol	MO	0.75	±	0.15	0.94	±	0.03	0.43	±	0.28	0.92	±	0.04	RI, GC-MS	GC-MS
-	1249	Geraniol	MO	-	-	-	0.1	±	0	-	-	-	-	-	-	-	GC-MS
1291	1273	(*Z*)-Ascaridole glycol	O	0.40	±	0.10	0.69	±	0.02	-	-	-	-	-	-	RI, GC-MS	GC-MS
-	1312	Citronellic acid	MO	-	-	-	0.3	±	0.01	-	-	-	0.25	±	0.01	-	GC-MS
1351	1345	α-Cubebene	SH	-	-	-	-	-	-	0.05	±	0.02	0.07	±	0	RI, GC-MS	GC-MS
1355	1354	Citronellyl acetate	MO	0.27	±	0.03	0.32	±	0.01	0.33	±	0.11	0.44	±	0.03	RI, GC-MS	GC-MS
1384	1365	Neryl acetate	MO	0.24	±	0.04	-	-	-	0.22	±	0.14	0.01	±	0.02	RI, GC-MS	GC-MS
1379	1374	α-Copaene	SH	0.95	±	0.03	0.75	±	0.04	1.43	±	0.13	1.21	±	0.12	RI, GC-MS	GC-MS
-	1379	Geranyl acetate	MO	-	-	-	0.36	±	0.02	-	-	-	0.51	±	0.03	-	GC-MS
1391	1390	β-Cubebene	SH	0.37	±	0.01	-	-	-	1.04	±	0.15	-	±	-	RI, GC-MS	-
1395	1391	β-Elemene	SH	0.05	±	0.00	-	-	-	-	-	-	-	-	-	RI, GC-MS	-
1424	1419	β-Caryophyllene	SH	0.72	±	0.02	0.57	±	0.01	1.12	±	0.09	1.05	±	0.09	RI, GC-MS, Std	GC-MS, Std
-	1430	β-Copaene	SH	-	-	-	0.31	±	0.01	-	-	-	0.96	±	0.09	-	GC-MS
1461	1454	Humulene	SH	0.23	±	0.01	0.2	±	0.01	0.35	±	0.03	0.32	±	0.02	RI, GC-MS, Std	GC-MS, Std
1488	1484	D-Germacrene	SH	0.24	±	0.01	0.07	±	0	0.69	±	0.05	0.49	±	0.06	RI, GC-MS	GC-MS
1502	1495	Bicyclogermacrene	SH	-	-	-	-	-	-	0.14	±	0.07	0.13	±	0.01	RI, GC-MS	GC-MS
1505	1499	α-Muurolene	SH	0.10	±	0.01	0.05	±	0.02	0.1	±	0.05	0.09	±	0.01	RI, GC-MS	GC-MS

-	1514	Cubebol	SO	-	-	-	-	-	-	-	-	-	0.24	±	0.02	-	GC-MS
1528	1524	β-Cadinene	SH	1.49	±	0.04	1.06	±	0.1	1.75	±	0.11	1.57	±	0.07	RI, GC-MS	GC-MS
-	1528	Calamenene	SH	-	-	-	0.01	±	0.01	-	-	-	-	-	-	-	GC-MS
-	1548	Elemol	SO	-	-	-	0.09	±	0.02	-	-	-	0.01	±	0.01	-	GC-MS
1588	1574	Germacrene D-4-ol	SO	-	-	-	-	-	-	0.07	±	0.01	0.17	±	0.01	RI, GC-MS	GC-MS
-	1577	Spathulenol	SO	-	-	-	0.09	±	0	-	-	-	0.09	±	0.01	-	GC-MS
-	1582	Caryophyllene oxide	SO	-	-	-	-	-	-	-	-	-	0.05	±	0	-	GC-MS
-	1608	Humulene epoxide	SO	-	-	-	0.01	±	0.01	-	-	-	-	-	-	-	GC-MS
-	1619	Humulane-16-dien-3-ol	SO	-	-	-	-	-	-	-	-	-	0.04	±	0	-	GC-MS
1641	1627	Epicubenol	SO	0.08	±	0.01	0.08	±	0.02	-	-	-	-	-	-	RI, GC-MS	GC-MS
1647	1631	γ-Eudesmol	SO	0.26	±	0.01	0.41	±	0.03	-	-	-	-	-	-	RI, GC-MS	GC-MS
-	1645	Cubenol	SO	-	-	-	0.07	±	0.02	-	-	-	-	-	-	-	GC-MS
1656	1645	δ-Cadinol	SO	0.10	±	0.00	0	±	0.01	-	-	-	0.02	±	0.02	RI, GC-MS	GC-MS
-	1649	β-Selinenol	SO	-	-	-	0.18	±	0.02	-	-	-	0.04	±	0	-	GC-MS
1671	1652	α-Eudesmol	SO	0.41	±	0.01	0.06	±	0.01	-	-	-	-	-	-	RI, GC-MS	GC-MS
-	1656	Patchouli alcohol	SO	-	-	-	-	-	-	-	-	-	0.04	±	0.01	-	GC-MS
-	1942	Phytol	DO	-	-	-	-	-	-	-	-	-	0.06	±	0	-	GC-MS
-	1984	Palmitic acid	FAD	-	-	-	-	-	-	-	-	-	0.62	±	0.01	-	GC-MS
-	2132	Linoleic acid	FAD	-	-	-	-	-	-	-	-	-	0.28	±	0.03	-	GC-MS
2521	2501	Oxypeucedanin	O	-	-	-	-	-	-	2.96	±	0.64	-	±	-	RI, GC-MS	-
2707	2707	β-Monolinolein	E	-	-	-	-	-	-	0.18	±	0.01	-	±	-	RI, GC-MS	-
1562	NA	Hedycaryol	SO	-	-	-	-	-	-	0.17	±	0.01	0.17	±	0.02	GC-MS	GC-MS
2009	NA	Thunbergol	DO	-	-	-	-	-	-	1.79	±	0.12	1.4	±	0	GC-MS	GC-MS
2010	NA	trans-Geranylgeraniol	DO	0.79	±	0.02	0.64	±	0.01	0.31	±	0.03	0.19	±	0	GC-MS	GC-MS
-	NA	2-p-Menthen-1-ol	MO	-	-	-	0.15	±	0.01	-	-	-	-	-	-	-	GC-MS
-	NA	tau.-Muurolol	SO	-	-	-	0.05	±	0.01	-	-	-	-	-	-	-	GC-MS
-	NA	Tetradecanoic acid	FAD	-	-	-	-	-	-	-	-	-	0.16	±	0.01	-	GC-MS
-	NA	17-Octadecynoic acid	FAD	-	-	-	-	-	-	-	-	-	0.12	±	0.08	-	GC-MS
-	NA	Ricinoleic acid	FAD	-	-	-	-	-	-	-	-	-	0.06	±	0.01	-	GC-MS
		Total identified [%]		**98.69**	±	**0.22**	**98**	±	**0.39**	**99.36**	±	**0.07**	**98.06**	±	**0.32**		

Footnotes 2: [a] RI = retention indices for HP-5 column; Obs = retention indices determined relative to a homologous series of n-alkanes (C8-C40) on a HP-5MS column, Lit = literature RI values (Adams, 2007), NA = RI values were not available in the literature. [b] C = Class; A - Aldehydes, DO -Oxygenated diterpenes, E - Esters, FAD - Fatty acid and fatty acid derivatives, MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenes, O - Others, SH - Sesquiterpene hydrocarbons, SO - Oxygenated sesquiterpenes, [d]Identification method: GC-MS = Mass spectrum was identical to

that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database; Std = constituent identity confirmed by co-injection of authentic standards. e) Retention indices were not calculated for compounds calculated only by DB-HeavyWAX column.

Table 3. Chemical composition of *P. nigrum* 'Kampot' EO and CO_2 extract.

RI[a]		Compound[b]	C[c]	Extraction type/Column type/Peak area [%]				Identification[d]	
				Essential oil		CO_2 extract			
Obs.	Lit.			HP-5 MS	DB-Wax	HP-5 MS	DB-Wax	HP-5 MS	DB-Wax
923	924	α-Thujene	MH	0.059 ± 0	- - -	- - -	- - -	RI, GC-MS	-
929	937	α-Pinene	MH	2.806 ± 0.25	2.568 ± 0.09	0.649 ± 0.02	0.574 ± 0.04	RI, GC-MS, Std	GC-MS, Std
944	946	Camphene	MH	0.04 ± 0.01	0.05 ± 0	- - -	- - -	RI, GC-MS, Std	GC-MS, Std
970	976	Sabinene	MH	0.048 ± 0.03	0.091 ± 0	- - -	- - -	RI, GC-MS	GC-MS
973	980	β-Pinene	MH	5.424 ± 0.45	5.322 ± 0.14	2.039 ± 0.04	1.996 ± 0.14	RI, GC-MS, Std	GC-MS, Std
989	991	β-Myrcene	MH	1.477 ± 0.14	1.682 ± 0.12	0.681 ± 0.05	- - -	RI, GC-MS	GC-MS
-	1001	2-Carene	MH	- - -	- - -	- - -	0.076 ± 0.02	-	GC-MS
1003	1005	α-Phellandrene	MH	1.803 ± 0.14	1.481 ± 0.08	0.762 ± 0.03	0.681 ± 0.03	RI, GC-MS, Std	GC-MS, Std
1008	1008	3-Carene	MH	18.72 ± 1.46	18.49 ± 0.42	7.395 ± 0.17	7.181 ± 0.4	RI, GC-MS	GC-MS
1025	1022	o-Cymene	MH	1.399 ± 0.12	1.495 ± 0.04	0.636 ± 0.01	0.771 ± 0.05	RI, GC-MS	GC-MS
1028	1031	D-Limonene	MH	11.18 ± 0.79	10.93 ± 0.15	6.265 ± 0.11	6.034 ± 0.39	RI, GC-MS	GC-MS
1058	1062	γ-Terpinene	MH	0.056 ± 0.01	0.045 ± 0	- - -	- - -	RI, GC-MS	GC-MS
1084	1086	Isoterpinolene	MH	0.194 ± 0.04	0.4 ± 0.02	0.09 ± 0	- - -	RI, GC-MS	GC-MS
1087	1086	Terpinolene	MH	0.428 ± 0.08	0.169 ± 0	0.156 ± 0.01	0.191 ± 0.01	RI, GC-MS	GC-MS
1104	1095	Linalool	MO	0.354 ± 0.03	0.453 ± 0	0.238 ± 0.05	0.386 ± 0.01	RI, GC-MS, Std	GC-MS, Std
-	1140	Verbenol	MO	- - -	0.185 ± 0.02	- - -	- - -	-	GC-MS
-	1179	p-Cymen-8-ol	MO	- - -	0.05 ± 0.05	- - -	- - -	-	GC-MS
-	1318	2,3-Pinanediol	MO	- - -	0.254 ± 0.01	- - -	- - -	-	GC-MS
-	1329	Piperonal	O	- - -	- - -	- - -	0.04 ± 0.01	-	GC-MS
1339	1339	δ-Elemene	SH	0.559 ± 0.02	0.588 ± 0.01	0.491 ± 0.01	0.516 ± 0.01	RI, GC-MS	GC-MS
-	1340	Piperitenone	MO	- - -	0.063 ± 0.05	- - -	- - -	-	GC-MS
1351	1351	α-Cubebene	SH	0.097 ± 0	0.086 ± 0.02	0.144 ± 0.01	0.117 ± 0.01	RI, GC-MS	GC-MS
-	1357	Octadecanal	A	- - -	0.57 ± 0.1	- - -	- - -	-	GC-MS
1378	1374	α-Copaene	SH	0.194 ± 0.01	0.17 ± 0.01	0.275 ± 0.01	0.247 ± 0.02	RI, GC-MS	GC-MS
1394	1391	β-Elemene	SH	1.483 ± 0.08	- - -	1.887 ± 0.03	1.303 ± 0.03	RI, GC-MS	-
1410	1409	α-Gurjunene	SH	0.164 ± 0.01	0.131 ± 0	0.257 ± 0.01	0.239 ± 0.03	RI, GC-MS	GC-MS

1416	1411	α-Bergamotene	SH	0.093	±	0	0.01	±	0.01	0.154	±	0.01	0.018	±	0	RI, GC-MS	GC-MS
1425	1419	β-Caryophyllene	SH	37.84	±	2.05	39.55	±	1.12	54.21	±	0.85	55.86	±	1.37	RI, GC-MS, Std	GC-MS, Std
-	1434	γ-Elemene	SH	-	-	-	0.057	±	0	-	-	-	0.12	±	0.01	-	GC-MS,
1440	1437	α-Guaiene	SH	0.983	±	0.07	-	-	-	1.363	±	0.02	-	-	-	RI, GC-MS	-
1456	1454	β-Farnesene	SH	0.101	±	0.03	0.058	±	0.05	0.143	±	0	0.167	±	0	RI, GC-MS	GC-MS
1459	1454	Humulene	SH	2.572	±	0.22	2.52	±	0.07	3.7	±	0.02	3.465	±	0.08	RI, GC-MS, Std	GC-MS, Std
-	1475	γ-Gurjunene	SH	-	-	-	0.9	±	0.03	-	-	-	-	-	-	-	GC-MS
1493	1485	β-Selinene	SH	3.653	±	0.33	3.358	±	0.15	5.242	±	0.14	4.757	±	0.11	RI, GC-MS	GC-MS
1486	1492	Valencene	SH	0.136	±	0.01	-	-	-	0.224	±	0.01	-	-	-	RI, GC-MS	-
1501	1494	α-Selinene	SH	2.409	±	0.23	1.972	±	0.29	3.493	±	0.08	3.009	±	0.09	RI, GC-MS	GC-MS
1510	1506	β-Bisabolene	SH	1.131	±	0.1	0.887	±	0.1	1.711	±	0.06	1.284	±	0.05	RI, GC-MS	GC-MS
1524	1520	7-epi-α-Selinene	SH	0.114	±	0.01	-	-	-	0.168	±	0.03	-	-	-	RI, GC-MS	-
-	1528	Calamenene	SH	-	-	-	-	-	-	-	-	-	0.013	±	0	-	GC-MS
1533	1529	γ-Bisabolene	SH	0.069	±	0.01	-	-	-	-	-	-	-	-	-	RI, GC-MS	-
-	1561	Nerolidol	SO	-	-	-	-	-	-	-	-	-	0.076	±	0	-	GC-MS
-	1577	Spathulenol	SO	-	-	-	0.184	±	0	-	-	-	0.118	±	0.09	-	GC-MS
-	1579	Isoaromadendrene epoxide	SO	-	-	-	0.086	±	0	-	-	-	-	-	-	-	GC-MS
1593	1582	Caryophylene oxide	SO	2.941	±	0.24	3.295	±	0.26	2.036	±	1.01	3.013	±	0.2	RI, GC-MS	GC-MS
1621	1608	Humulene epoxide II	SO	0.154	±	0.02	0.158	±	0	0.128	±	0.01	0.139	±	0	RI, GC-MS	GC-MS
1643	1638	Isospathulenol	SO	0.492	±	0.05	0.421	±	0.32	0.463	±	0.02	0.526	±	0.08	RI, GC-MS	GC-MS
1668	1651	Pogostole	SO	0.154	±	0.02	0.215	±	0.1	-	-	-	0.09	±	0	-	GC-MS
-	1658	Neointermedeol	SO	-	-	-	0.054	±	0.01	-	-	-	-	-	-	-	GC-MS
1675	1665	Intermedeol	SO	0.05	±	0.02	0.07	±	0.01	-	-	-	-	-	-	RI, GC-MS	GC-MS
1950	1938	Pellitorine	O	-	-	-	0.006	±	0.01	1.191	±	0.09	1.669	±	0.06	RI, GC-MS	GC-MS
-	1953	Hexadec-9-enoic acid	FAD	-	-	-	-	-	-	-	-	-	0.103	±	0	-	GC-MS
-	1959	Palmitic acid	FAD	-	-	-	-	-	-	-	-	-	0.423	±	0.04	-	GC-MS
-	2141	Oleic Acid	FAD	-	-	-	-	-	-	-	-	-	0.267	±	0.03	-	GC-MS
2707	2707	β-Monolinolein	E	-	-	-	-	-	-	1.84	±	0.98	-	-	-	RI, GC-MS	-
2018	NA	Heptadec-14-enal	A	-	-	-	-	-	-	0.122	±	0.04	-	-	-	RI, GC-MS	-
2815	NA	β-Monoolein	E	-	-	-	-	-	-	0.503	±	0.6	0.52	±	0.06	RI, GC-MS	GC-MS
-	NA	Hexadec-9-en-1-ol	O	-	-	-	-	-	-	-	-	-	0.238	±	0.01	-	GC-MS
-	NA	Octadec-17-ynoic acid	FAD	-	-	-	-	-	-	-	-	-	0.025	±	0.02	-	GC-MS
-	NA	Tetradec-9-enal	A	-	-	-	-	-	-	-	-	-	0.073	±	0.01	-	GC-MS
-	NA	Kalecide	O	-	-	-	-	-	-	-	-	-	0.181	±	0	-	GC-MS

-	NA	Ricinoleic acid	FAD	-	-	-	-	-	-	-	-	-	0.231	±	0.01	-	GC-MS
-	NA	Sabinol	SO	-	-	-	0.072	±	0.01	-	-	-	-	-	-	-	GC-MS
		Total identified [%]		**99.4**	**±**	**0.2**	**99.2**	**±**	**0.1**	**98.7**	**±**	**0.7**	**96.7**	**±**	**0.1**		

Footnotes 3: a) RI = retention indices for HP-5 column; Obs = retention indices determined relative to a homologous series of n-alkanes (C8-C40) on a HP-5MS column, Lit = literature RI values (Adams, 1995), NA = RI values were not available in the literature. b) C = Class; A - Aldehydes, DH - Diteprene hydrocarbons, E - Esters, FAD - Fatty acid and fatty acid derivatives, MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenes, O - Others, SH - Sesquiterpene hydrocarbons, SO - Oxygenated sesquiterpenes, d)Identification method: GC-MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database; Std = constituent identity confirmed by co-injection of authentic standards. e) Retention indices were not calculated for compounds calculated only by DB-HeavyWAX colu.mn.

3. Discussion

As a result of GC-MS analysis, eucalyptol has been detected as the dominant constituent of *A. kravanh* EO. Such finding is in accordance with previously published studies investigating the chemical composition of EO from this plant [31,32], or from other species of *Amomum* genus [39,40]. In correspondence with results of Zhang et al. [33], β-pinene and α-terpineol have been detected as abundant compounds in the analyzed sample of the EO. Contrastingly, Diao et al. [31] reported relatively lower amounts of α-pinene (5.71 %) and β-pinene (2.41 %), while terpinyl acetate (11.2 %) and dipentene (6.1 %) were abundant EO components. These slight differences can be attributed to different geographical origins of the samples. Correspondingly with study of Zhang et al. [33], D-limonene has been identified as third most prevalent compound of the EO when investigated by DB-HeavyWAX column. In the literature, only results for chemical analyses using the DB-HeavyWAX column are available, however, they differ from our results. According to Yothipitak et al. [34], eucalyptol (71. 45 %), β-pinene (8. 64 %) and limonene (4. 77 %) were three dominant constituents of extract obtained by SFE from *A. kravanh*. These variances can be caused by different extraction parameters (33 °C and 175 bars) used during the SFE process and by distinct geographical origin (Thailand) of the plant sample. Different main constituents in HP-5 column and DB-HeavyWAX column analyses could be caused by stronger detection sensitivity and ability of polar DB-HeavyWAX column to separate and quantify fatty acids and their methylesters from the rest of the sample compared to the non-polar HP-5. Furthermore, polar columns based on polyethylene glycol have more accurate results in identification of fatty acid saturation and therefore are commonly employed in analyses of complex fats and oils [41,42].

In *C. hystrix* EO, β-pinene has been determined as the most abundant constituent, which is in accordance with previously published analyses, where percentages of β-pinene ranged from 25.93 to 47.93 % [28,43–45]. Study carried out by Jantan et al. [28] and Tran et al. [44]also revealed limonene as the second most dominant compound, comprising almost 15 and 20 % of the sample, respectively. Sabinene has been detected as the third most abundant constituent and such finding is in agreement with research carried out by the above-mentioned study [44]. However, slight discrepancy can be observed in comparison to investigation conducted by Sato et al. [43], where this monoterpene accounted for more than 20 % of the sample and has been second most dominant compound of the total oil. Moreover, our sample was lower in citronellal in comparison to previously published data. Since the samples from previously published studies were collected in Malaysia [28], Vietnam [46] and Thailand [43], differences in chemical composition can be attributed their different geographical origin. Furthermore, in case of study conducted by Sato et al. [43], steam distillation has been used as extraction method. In addition, maturity of the fruit and processing of the sample before extraction are factors which can affect the chemical composition of the EO [29]. Although CO_2 extraction was previously performed from the leaves of this species in investigation carried out by Norkaew et al. [35], to the best of our knowledge, this is the first report investigating the chemical composition of CO_2 extract isolated from the peel of this species. Due to the existence of large oil sacs or oil glands in the *Citrus* spp. fruit rind, their EOs have traditionally been obtained by cold expression. Cold pressed EOs of citruses comprise of volatile fraction with mono and sesqui-terpenes and their oxygenated derivatives. However, non-volatile fraction represented by coumarins, psoralens and other oxygen heterocyclic compounds, is also present in cold-pressed oils [47,48]. Although investigation of *C. hystrix* cold-pressed EO is currently not available in the literature, several research teams compared cold-pressed and hydrodistilled EOs from more common *Citrus* species. The most common conclusion was higher recovery of terpene hydrocarbons in the cold-pressed oils, which are compounds responsible for the typical aroma of *Citrus* oils [46,49]. Therefore, comparison of cold pressed *C. hystrix* EO with other extraction methods is highly encouraged for future research related to chemical composition or bioactivity assessment.

The main difference between *P. nigrum* 'Kampot' EO and CO_2 extract was presence of pellitorine, belonging to the piperamides, which amounted to more than 1 % of the total CO_2 extract. Such nitrogen containing compound has also been detected in research conducted by Luca et al. [50] in much lower amounts (0.18 %), however, other piperamides like piperine, piperettine and guineesine

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have also been determined in their extracts. This slight dissimilarity can be attributed to the different *P. nigrum* cultivar assessed in our research and different extraction conditions of the SFE process, where higher pressure (up to 300 bars) has been used for selective recovery of piperazines. The predominant compound in EO has been determined as sesquiterpene β-caryophyllene, which is in consonance with numerous previously published studies [51–53] assessing the composition of *P. nigrum* EO. Such sesquiterpene has been present in amounts ranging from 29.9 to 62.3 % of the volatile oil. Other major constituents in our sample were 3-carene and D-limonene. This is corresponding well with research conducted by Li et al. [52], where 3-carene and D-limonene were present in maximal respective amounts of 26.84 and 25.83 % in the various samples of EOs obtained from black and white peppers of Chinese origin. However, slightly different components were discovered in investigation carried out by Andriana et al. [51], where β-thujene and β-selinene accounted for 20.58 and 5.59 % of the sample, respectively. Furthermore, Kapoor et al. and Bagheri et al. [53,54] reported limonene (13.2 %), β-pinene (7.9 %) and sabinene (5.9 %) as predominant compounds of the EO. These slight differences in the main components can be attributed to different cultivar of *P. nigrum* assessed in our study, together with different harvest and post-harvest handling of the peppercorns used to produce red pepper [27]. In CO_2 extract, the amount of β-caryophyllene was even higher than in EO and such finding is in accordance with research executed by Bagheri et al. [54], where the recovery of this sesquiterpene was also higher than in the hydrodistillated EO. Moreover, higher recovery of sesquiterpenes and their oxygenated derivatives for CO_2 extracts opposed to EOs has also previously been reported [50,55]. Following main components were monoterpenes 3-carene and D-limonene and such finding corresponds well with study conducted by Topal et al. [56]. Same compounds amounted to 10.32 and 5.4 % in *P. nigrum* CO_2 extract, respectively. Slight discrepancy can be observed in research executed by Luca et al. [50], where sabinene was present in 8.61 % and limonene comprised 8.21 % of the total *P. nigrum* extract, which can be attributed again to the different cultivar researched in our study. In addition, to the best of our knowledge, the chemical composition of *P. nigrum* 'Kampot' EO and CO_2 extract has been assessed for the first time in our report.

4. Materials and Methods

4.1. Plant material and sample preparation

Fruits of *A. kravanh* and *C. hystrix* were purchased in local markets (Orussey Market, Phnom Penh, KH and Stung Treng Market, KH) and *P. nigrum* 'Kampot' fruits (red peppercorns) were obtained in a pepper farm store (La Plantation, Kampot, KH). *C. hystrix* was peeled and pericarp was used for further analyses. Dried material was homogenized by Grindomix apparatus (GM 100 Retsch, Haan, DE). The residual moisture contents of samples were determined gravimetrically at 130 °C for 1 h by Scaltec SMO 01 analyzer (Scaltec Instruments, Gottingen, DE) in triplicate according to the Official Methods of Analysis of the Association of Official Agricultural Chemists and expressed as arithmetic averages (15.79 %, 22.51 % and 14.39 % for *A. kravanh*, *C. hystrix* and *P. nigrum*, respectively).

4.2. Hydrodistillation of EOs

EOs were extracted by hydrodistillation of 100 g of ground plant materials in one litter of distilled water for 3 h using Clevenger-type apparatus (Merci, Brno, CZ) according to the procedure described in the [37]. Since hydrodistillation belongs to the most utilized methods for commercial production of EOs from *C. hystrix* spp. [29] the properties of samples prepared in our investigation should be alike to those commercially available. All EOs have been stored in 2 ml sealed glass vials at 4 °C until further use.

4.3. Supercritical CO_2 extracts preparation

Supercritical CO_2 extraction was carried out using Spe-ed SFE helix system (Applied Separations, Allentown, PA). Initially, 10 g of ground material have been filled into the 100 ml stainless steel extraction vessel between a glass wool bilayer. Subsequently, the filled vessel was installed into the extraction module and the extraction process was carried out using following parameters: isocratic pressure 200 Ba, temperature 40 °C and flow rate 5 LPM. The extracts have been captured into 60 ml glass collection vials (Applied Separations, Allentown, PA) and stored in 2 ml sealed glass vials at 4 °C until further utilization.

4.4. Gas chromatography-mass spectrometry analysis (GC-MS)

For determination of chemical composition of EOs and supercritical CO_2 extracts, GC-MS analysis has been performed using the dual-column/dual-detector gas chromatograph Agilent GC-7890B. System is equipped with auto sampler Agilent 7,693, two columns, a fused-silica HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm, Agilent 19091s-433) and a DB-HeavyWAX (30 m × 0.25 mm, film thickness 0.25 µm, Agilent 122–7132), and a flame ionization detector (FID) coupled with single quadrupole mass selective detector Agilent MSD-5977B (Agilent Technologies, Santa Clara, CA). Helium has been utilized as a carrier gas at a flow rate 1 ml/min and the injector temperature was set 250 °C for both columns. The oven temperature was raised for both columns after 3 min from 50 to 280 °C. Initially, the heating velocity was 3 °C/min until the system reached temperature 120 °C. Subsequently the velocity increased to 5 °C/min until temperature 250 °C and after 5 min holding time the heating speed reached 15 °C/min until the obtained temperature 280 °C. Heating was followed by 20 min isothermal period. Samples of EOs and supercritical CO_2 extracts were diluted in *n*-hexane for GC–MS (Merck KGaA, Darmstadt, DE) at the concentration 20 µl/ml. 1 µl of the solution was injected in split mode in a split ratio 1:30. The mass detector was set to the following conditions: ionization energy 70 eV, ion source temperature 230 °C, scan time 1 s, mass range 40–600 m/z.

4.5. Identification of constituents, quantification, and statistical analysis

Identification of compounds was based on comparison of their retention indices (RI), retention time (RT) and mass spectra in the National Institute of Standards and Technology Library ver 2.0.f (NIST) as well as in the literature [38]. The certain identified compounds were confirmed by co-injection of authentic standards, namely camphene (97.5 %, CAS: 79-92-5), β-caryophyllene (80 %, CAS: 87-44-5), humulene (96 %, CAS: 6753-98-6), linalool (97 %, CAS: 78-70-6), α-phellandrene (95 %, CAS: 4221-98-1), α-pinene (99 %, CAS: 7785-70-8), β-pinene (99.0 %, CAS: 18172-67-3) and γ-terpinene (97 %, CAS: 99-85-4) (Sigma-Aldrich, Prague, CZ). The RI were calculated for constituents separated by HP-5 column using RT of *n*-alkanes series ranging from C_8 to C_{40} (Sigma-Aldrich, Prague, CZ). For each analyzed EO and CO_2 extract, the final number of individual constituents was computed as the sum of components simultaneously identified using both columns and the remaining compounds detected by individual columns only. Quantitative data are expressed as relative percentage content of constituents determined by FID. Chemical analysis of EO or CO_2 extract was performed in triplicates and relative peak area percentages were expressed as mean average of these three independent measurements ± standard deviation.

5. Conclusions

In summary, this study reports determination of chemical composition of EOs and CO_2 extracts from three Cambodian spices, namely *A. kravanh*, *C. hystrix* and *P. nigrum* 'Kampot', using GC-MS equipped with two columns of different polarity. Differences between chemical composition of EOs and CO_2 extracts have been observed in all species, whereas the most significant difference was detected in *A. kravanh* fruits. Furthermore, the analyses of both columns also differed substantially in the CO_2 extract of this species. In non-polar HP-5 column, long chained alkane tricosane was the main compound, while in DB-HeavyWAX analysis, oleic and palmitic acids were two main constituents of the extract. *C. hystrix* and *P. nigrum* 'Kampot' CO_2 extracts were generally richer in sesquiterpenes and their oxygenated derivatives in comparison to EOs, where monoterpenes were more abundant. Furthermore, fatty acid derivatives and other higher molecular weight constituents were also more prevalent in CO_2 extracts. In addition, to the best of our knowledge, EO and CO_2 extract from *P.*

nigrum 'Kampot' fruits and CO_2 extract from the peel of *C. hystrix* have been isolated and analyzed for the first time in our study. Such finding suggests that supercritical CO_2 can potentially be used for extraction of all three Cambodian spices. Nevertheless, further research determining the most efficient extraction parameters will be needed before its application in the spice processing practice.

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