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Comparative study of extraction yield and antioxidant property of sweet orange peels (*Citrus Sinesis*) essential oil

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ABSTRACT

The research evaluates the extraction yield and antioxidant potentials of essential oil (EO) of sweet orange peels using pressurized liquid extraction (PLE), Soxhlet (Sox) and hydro distillation (HD). The extracts were investigated to find out the antioxidant properties using 2, 2 -diphenyl-1-picryl-hydrazyl (DPPH) and 2, 2 azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical (ABTS•+). PLE and Soxhlet extracted essential oil showed additional polyphenol compounds and tannins using thin layer chromatogram (TLC) and chemical analyses, respectively. Hydrodistillation indicating a pure essential oil without identified tannins and polyphenols with the highest ABTS activity compared to other produced essential oils of PLE and Soxhlet. The major chemical constituents of the pure essential oil were identified by gas chromatography-mass spectrometry (GC-MS) and they include limonene (90.72%), myrcene (2.82%) and octanol acetate (1.24%). PLE had moderate high yield within short extraction time and the highest antioxidant (DPPH) and can be adjusted to individual materials to maximize the extraction yield and antioxidant property.

Introduction

Citrus (*Citrus* spp) is an important fruit and one of the mostly cultivated crops with world production estimated at 115 million tons per year. In 2010, it was reported that the world citrus production is about 82 million tonnes with sweet oranges history of 61% (Alnaimy et al., 2017). Orange fruits have round, rough and green to yellow coloured skin. They are about 20-30 cm in length with a tough peels or skin known as epicarp (or flavedo) that acts as cover which protects the fruit from adverse effects from the environment. An orange peel comprises of epidermis and exocarp with irregular thin-walled cells, which enclose numerous glands or oil sacs (Farhat et al., 2011; Velazquez-Nunez et al., 2013). The oil in these sacs represents the citrus essential oil (EO) that represents secondary metabolites product in the citrus plant (Bousbia et al., 2009a). Citrus fruits have been discovered as excellent sources of essential oils, besides their use as flavouring agents. Citrus essential oil has gained relevance in the food industry due to its antimicrobial effects against both food bacteria and fungi (Rezzoug and Louka, 2009; Velazque-Nunez et al., 2013; Lago et al., 2014). EO is mostly present in peels, when compared to other parts, and it has got a wide application in food industries as additive, nutritious supplement and some other industrial applications (Maria et al., 2012).

The main methods used to extract essential oil from plant material are distillation (hydro, steam and destructive), maceration and expression (Stahl-Biskup and Saez, 2002). However, in order to reduce the limitations associated with the main methods (reduce extraction time, cost of extraction and possibly improve the yield and quality of the extracts) new techniques, such as microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction, and ultrasound-assisted extraction have also been developed (Wang and Weller, 2006).

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PLE is also known as accelerated solvent extraction (ASE). This method is widely used as an extraction technique for sample preparation to discover the presence of minor components in the extract. At higher extraction temperatures, it increases both solubility and mass conveyance rate of the analyte. It also decreases the viscosity and intermolecular forces of solvent, thereby improving extraction rate (Ibanez et al., 2003).

The extraction of essential oil utilizing the ordinary extraction techniques that had been accounted for by Presti et al., (2005). Bousbia et al., (2009b) that the impediment is to be of lower essential oil yield and longer extraction time. Consequently, it is advantageous to enhance these impediments.

The research is focused on extracting essential oil from sweet orange peels using some of the conventional extraction methods (Soxhlet and hydro distillation) with green extraction method (PLE). The antioxidant extract potentials using ABTS and DPPH, the purity (TLC Plate) and chemical constituents using GC-MS for the extracted essential oil were evaluated. The results were then compared to ascertain the best method of extraction in relation to the quality and purity of the extracted essential oil.

Material and methods

Chemicals

Carbon dioxide (CO_2) and nitrogen gases (N_2) used in experiments were 99.5% pure, obtained from White Martins Gases Industrials (Campinas, BR). Ethanol and sodium carbonate were procured from Synth (Diadema, São Paulo, BR), methanol, ethyl-acetate and chloroform from Merck (Darmstadt, GE), gallic acid from Vetec (Rio de Janeiro, BR) and potassium persulfate (Synth, BR), 2,2 -diphenyl-1-picryl-hydrazyl (DPPH), Trolox, and 2,2 azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) were from Sigma (Aldrich, GE).

Raw Material Characterization

Harvested sweet oranges were purchased from fruit and vegetable market centre in Pirassununga, São Paulo, Brazil. Fruits at the same stage of ripeness were used for the research. The ripe fruits were processed at the Laboratory of High Pressure Technology and Natural Products, of the University of Sao Paulo (Pirassununga SP, Brazil). These fruits were sorted and cleaned to remove foreign materials from the epicarp. The fruits were peeled with sterilized knife to remove epicarp or rind (flavedo or shell).

Pressurised Liquid Extraction (PLE)

PLE was performed using an ASE 150 accelerated solvent extraction system (Dionex, Sunnyvale, USA), in which the samples were packed inside a fixed bed and in a vertical position. The stainless-steel extractor with a capacity of 34 mL was filled with approximately 10 g of dried rind samples for each extraction process, with 5 g of diatomaceous earth (Thermo Scientific, Sunnyvale, USA), as adsorbent material, to disperse the vegetal matrix in the extraction cell. The diatomaceous allows a better contact with the solvent and clarifies the extract. Anhydrous ethanol was used as solvent because it is generally recognized as safe (GRAS), (FDA, 2013). A static time of 15 min in each cycle, purge time of 100 seconds, oven heat up time of 10 min, flush volume of 100% and pressure of 10 MPa were the fixed variables. The ethanol extract obtained by PLE was named crude extract, it was evaporated after the extraction and then prepared for analyses. The oven temperature (50-70 ºC) and static extraction cycles time (2-4) were varied in order to ensure that the mechanical and thermal equilibrium is guaranteed in the employed operating conditions.

Classical / Soxhlet Extraction

The soluble content of the essential oil extract was determined in triplicate by Soxhlet extraction using ethanol at 80 ºC for 3 h (12 extraction cycles time), followed by solvent removal at 35 ºC using a rotary evaporator (Yamato, Tokyo, JP). Approximately 10 g of dried orange peels were used for the Soxhlet extraction using methanol as solvent, which was carried out according to a method adopted from AOAC 2000.

Hydrodistillation Process

Dried milled peels 10 g were immersed in 250 mL of water and distilled for 10 h (40 extraction cycles time), using a Clevenger-type apparatus (Ebramhizadeh et al. 2009), which was found to be sufficient for completing the process. The extracted oil was collected and weighed via vial bottle. The extracted essential oils obtained were dried over anhydrous sodium sulfate and stored in a refrigerator prior to analysis (Chegini and Abbasipour, 2017).

Yield Calculation and Purification of Crude Extracts

The extracted weight of essential oil was determined using gravimetrical method. The extractable essential oil yield was determined as the percentage ratio of the extract mass to the mass of orange peels. The crude extracts obtained by PLE and Soxhlet were purified to

eliminate tannins with a high degree of polymerization. The crude extract (100 mg) was diluted in 2.5 mL of methanol and 32.5 ml of chloroform using Lhuiller et al. (2007) standard method. The standard method without any modification was necessary in order not to remove other phenolic compounds in the extract. The diluted extract was stored at 4 ºC for 3h in the dark. The centrifugation of the extract was carried out (Excelsa II Model 26, Fanem, Sao Paulo, BR) at 4,000 rpm and 5 ºC for 10 minutes. The decanted extract was evaporated under nitrogen at room temperature in the dark and named purified methanol extract (Oliveira et al., 2014).

Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The total antioxidant capacity was determined as 2, 2 azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) extracted essential oil according to the method described by Re et al. (1999). ABTS•+ values were determined by reacting ABTS solution (7mM) with $K_2S_2O_8$ (2.45mM, final concentration) in the dark for 16h. The reading was taken between 700 to 734 nm with methanol. Thereafter, 0.2 mL of the essential oil was added to 2.0 mL ABTS•+ solution. The absorbance value was taken at 734 nm after 6 min. Trolox was used as a reference standard, and the results were expressed as mg of trolox equivalent (mg TE) by grams of extract.

Antioxidant by DPPH

The determination of sequestering capacity of the stable free radical 2, 2 -diphenyl-1- picryl-hydrazyl (DPPH) was based on the methodology of Brandi - Williams et al. (1995). Methanol solution of DPPH was prepared with absorbance between 0.700 at 515 nm. Thereafter, 0.4 mL aliquots of each extract diluted in methanol for the control were added to tubes containing 3.6 mL of this DPPH solution and measurements were performed in triplicates. The absorbance reading was taken after 2 h of incubation using a spectrophotometer (Biospectro SP 22, São Paulo, BR). The results were expressed as IC_{50} (µg/mg of extract) which is the amount of antioxidant required to cause 50% reduction of the initial concentration of DPPH (Equation 1). The value was calculated by plotting inhibition percentage against extract concentration (Sokmen et al., 2004).

$$IC_{50} = \left(\frac{A_c - A_t}{A_c}\right) \quad (1)$$

where IC_{50} is the radical scavenging activity (%), A_c is the absorbance of control, and A_t is the absorbance of test sample.

Thin Layer Chromatography

Thin layer chromatography (TLC) was carried out on the extracted oil to determine the authenticity of the oil. Polyphenolic compounds in essential oil extracts were ascertained by thin layer chromatography on TLC plates coated with Silica Gel G. The plates were cleaned and activated by heating at 150 ºC for 60 min to remove moisture. The Silica gel plates 60 F_{254} is the stationary phase which was eluted with commonly used solvent for designation and quantification of phenolic compounds, chloroform and ethyl acetate (70:30, v/v) as the mobile phase. The purified essential oil extract using chromatographic standard (40 - 50 µL) for each essential oil obtained were injected to the plates and eluted with the mobile phase. The plate was placed in 20 mL of mobile phase solution in the developing chamber and allowed to rise by capillary movement until it reached a height of 10 cm from the point of spotting. The plate was dried and heated to visualize the bands that eluted with varied colouration prepared as described by Wagner and Bladt (2009). The image was captured under ultraviolet light (Boitton, model 2909, Porto Alegre, BR), patterns were recorded by camera and all visible spots were outlined with pencil.

Phytoconstituents composition using GC-MS

Gas chromatography coupled with mass spectrometry (GC-MS) is used to evaluate the constituent compounds in the essential oil. Hydrodistillation (HD) essential oil extracts were used for the phyto constituents based on the result from the thin layer chromatogram indicating its essential oil to be pure volatile oil without tanninsor polyphenolic compounds. Phytoconstituent composition of the hydrodistillation (HD) extracts was analysed by gas chromatography coupled with mass spectrometry (GC-MS) (QP 2010 Plus, Shimadzu, Tokyo, Japan) with auto sampler (AOC-5000, SWI, Tokyo, Japan). The compounds were separated on Rtx$^{@}$-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm) (RESTEK, USA) with 5% diphenyl, 95% dimethylpolysiloxane as stationary phase. The injector and detector temperatures were 220 °C, the column temperature was held at 60 °C for 5 min (hold time compound in the column) and then was increased from 60 to 246 °C at 3 °C/min and was finally held at 246 °C for extraction time (taken from method). 1.0 µL of the sample was diluted in methanol (400 mg/L) and then injected by using the split mode (split ratio 1: 20). Helium was used as a carrier gas (extraction

time (taken from method) mL/min). The MSD (EI mode) was operated at 70 eV and the scan range was set to 50 – 500 m/z.

The identification of volatile constituents was based on the comparison of their retention indices (RI), relative to the retention times of a homologous series of *n*-alkanes (C8 – C20), with those reported in the literature and their mass spectra with those of authentic compounds available in our laboratories or those listed in the NIST 08 mass spectral libraries. For accurate and reliable designation of the compounds, kovats retention index (KI) was determined for each compound identified according to Equation 2.

$$KI(x) = 100P_z + 100\left[\frac{logRT(x) - logRT(P_z)}{logRT(P_{z-1}) - logRT(P_z)}\right] \quad (2)$$

where Pz is the number of carbons in the alkane immediately preceding the analyte,
RT(x) is the analyte retention time, and
RT(Pz) is the retention time of the alkane immediately preceding the analyte.

Results and discussion

Extraction Yield

The magnitude of extracted essential oil yield from sweet orange peels for PLE, hydrodistillation and Soxhlet ranged from 5.73 - 53.4 % (Table 1). The highest value 53.4% was recorded by Soxhlet with extraction time of 10 hours (40 cycles) and the lowest value of 5.73% by hydrodistillation with extraction time of 3 hours (12 cycles). Essential oil yield obtained in this study by hydrodistillation is low when compared to solvent extraction (PLE and Soxhlet). Similar trend was obtained by Ahsan et al. (2017) for extracting *Jasminum sambac L* essential oil using hydrodistillation and supercritical fluid extraction. The extract yield of PLE ranged from 21.6 - 49.3 % with mean value of 26.75% and mean extraction time of 45 minutes (3 cycles) (Table 1). The hydrodistillation with extraction time of 3 hours (12 cycles) had essential oil yields of 5.73% with closer value of 5.45% for lime peels (*citrus latifolia Tanaka*) using similar method as reported by Atti-santos et al. (2005).

The yields obtained in this study were higher than those reported in literature. Mercy et al. (2015) reported an improved distillation method for extracting essential oil from peels of *citrus sinesis* and *citrus reticulate* with yield of 4.23% and 5.865% respectively. Franco–Vega et al. (2016) reported orange peels extract yields of 0.92 to 2.73 %. Megha and Mumtaj (2014) reported sweet lime with yields of 1.16% using microwave assisted hydrodistillation. Ahmad et al. (2006) accounted for essential oil yields varying from 0.30 to 1.21 % for four citrus varieties from Pakistan. Also, Kamal et al. (2011) reported that *C. sinensis* had the highest oil value yield of 0.24-1.07 % accompanied by *C. reticulata* with 0.30-0.50 % and the least *C. paradisii* with 0.20-0.40 %. There were significant variations in the yield of essential oils from our study in comparison with those of literature. Such variability could depend on several factors including climatic and environmental conditions, soil variations and season, geographical location, the stage of the vegetative cycle, and the method used to obtain the essential oil (Jing et al., 2014).

In general, extraction yield obtained using the green extraction method and conventional methods in the study were higher than those in literature. However, the yield under Soxhlet was higher than those of PLE, although two methods (PLE and Soxhlet) were extracted with solvent and both showed polyphenolic compounds in addition to essential oil. PLE process is advantageous mainly due to the relatively short duration extraction time and it is more economical than the conventional methods (Soxhlet and hydrodistillation) used in this study.

Table 1. The effects of temperature and static extraction cycle time on the extraction yield, purified extract and antioxidant properties (ABTS and DPPH radical scavenging abilities) of sweet orange peels essential oils from pressurized liquid extraction (PLE), Soxhlet and hydrodistillation (HD)

Test	T (ºC)	Cycles (min)	Yield (%)	Purified extract (g)	Tannins (g)	ABTS (mg TE/g)	DPPH IC_{50} (mg/g)
PLE 1	50	2	21.6	0.016 ± 0.13	0.084±0.02	11.47± 0.13	40.64 ± 0.42
PLE 2	50	4	25.7	0.016 ± 0.02	0.084±0.03	11.45± 0.38	33.44 ± 0.38
PLE 3	60	3	27.06	0.019 ± 0.02	0.081±0.02	11.46± 0.21	25.8 ± 0.28
PLE 4	70	2	40.1	0.018 ± 0.01	0.084±0.02	11.47± 0.23	38.47 ± 0.39
PLE 5	70	4	49.3	0.032 ± 0.03	0.069±0.03	11.56± 0.10	15.27 ± 0.13
HD	70	12	5.73	0.002	0	11.74± 0.13	56.13 ± 0.18
Sox	70	40	53.4	0.935	0.065±0.01	11.44± 0.10	25.78 ± 0.15

Extracts Purification

The value of tannins was calculated gravimetrical and the value varies from 0.065 - 0.084 g. PLE extracts ranged from 0.069 - 0.084 g, Soxhlet had tannins with 0.065 g while in hydrodistillation, tannins were not recorded. The extracts from PLE and Soxhlet were yellowish in colour due to the presence of polyphenols and tannins. The highest value of 0.935 g was recorded by Soxhlet and the lowest value of 0.002 g by hydrodistillation. The hydrodistillation extract is an unadulterated unstable oil which is less dense and colourless which makes it diffuse effectively into the air.
Obviously only volatile oil was extracted under hydrodistillation and the other two methods extracted volatile oil along with other polyphenolic compounds. The tannins identified denote the presence of other compounds with the volatile in the extracts (Table 1).

Antioxidants

Antioxidants carry out their functions in biological system either by preventing the production of free radicals or by negating free radicals produced (Oboh 2006). Due to the chemical complexity of the essential oil, several antioxidant parameters as typified by reducing property of ABTS and DPPH scavenging abilities were measured. Total antioxidant capacity (ABTS) of the essential oil expressed as Trolox equivalent antioxidant capacity (TEAC) had the value ranged from 11.45 to 11.74 mg TE/g of essential oil (Table 1). Hydrodistillation showed the highest activity of 11.74 mg TE/g and the lowest activity of 11.45 mg TE/g was recorded with PLE lowest temperature and lowest static cycles (PLE Test 1).
For the DPPH method, the antioxidant activity of the EO extracts ranged from 15.27 mg/g to 56.13 mg/ g. The lowest temperature and the lowest static cycles (PLE Test 1) showed the highest antioxidant value of IC_{50} = 15.27 mg/g and hydrodistillation showed the lowest antioxidant value of IC_{50} = 56.13 mg/g. Prieto et al. (1999) reported that the smaller the IC_{50} values, the higher antioxidant activity of the plant extracts.

Thin Layer Chromatography

Thin layer chromatography (TLC) is a technique widely used for separating and purifying extracts, due to its simplicity and flexibility. Thin layer chromatography (TLC) was used to identify compounds in extracts as presented in Figure 1, numbered 1 to 5 for PLE, hydrodistillation (HD) and Soxhlet (Sox) on silica plate. The plate exhibited two narrow and intense blue bands for all the PLE and Soxhlet samples whilst the chromatogram in respect of hydro distillation exhibited none. The chromatogram with blue bands implied the presence of additional phenolic compounds with the volatile in the extracts obtained via PLE and Soxhlet. PLE can be used to detect minor compounds in the extract as shown using thin layer chromatogram and chemical analysis to determine polyphenols and tannins.

Identification and quantification of phytoconstituents from orange essential oil

Based on the results from the chemical and thin layer chromatography from this study, it was discovered that hydrodistillation consists of volatile compounds without any additional polyphenols. The essential oil without polyphenol essential oil from hydrodistillation was injected into GC-MS. Essential oils are natural complex mixtures of volatile compounds which had about ten to hundred constituents at different concentrations. The percent composition was computed from the area of the peaks of the gas chromatography (GC) in terms of the components having mass fractions equal to or greater than 0.01. The constituent was identified by GC-MS when there is a quality match of more than 80%.
The individual constituent of the essential oil was identified via mass spectrometry and its identity confirmed in comparison with mass spectra of authentic standard based on the National Institute of standards and technology, (NIST, Gaithersburb, MD, USA) NIST 08 and NIST 08s libraries
The result from the spectrograph showed 62 peaks which were found on the total ion chromatogram and mass spectra from the GC-MS which amounts to 100% of the entire concentration. Figure 2 shows the total ion chromatogram (TIC) of citrus essential oil peel of hydrodistillation from GC-MS.
The result from retention characteristics and GC-MS analysis revealed the identification of 47 constituents in six groups: terpenes (95.13%), aldehydes (1.19%), alcohols (0.68%), esters (1.65%), oxide (0.1%) and ketone 0.05% from Noot ketone representing about 99.8% of the essential oil (Table 2).

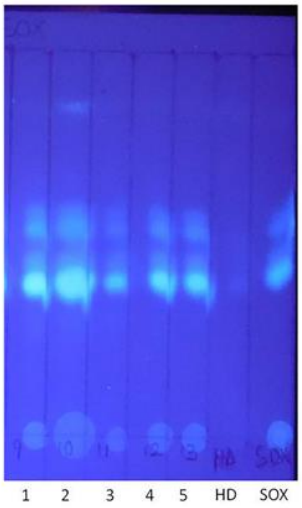

Fig 1. Typical thin layer chromatogram of sweet orange extracts from PLE extracts (1-5), hydrodistillation (HD) and Soxhlet (Sox) on silica gel

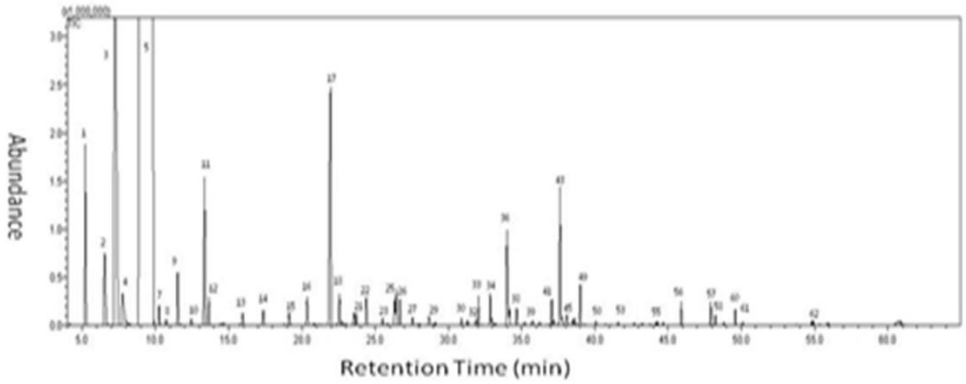

Fig 2. Total ion chromatogram (TIC) of essential oil of sweet orange using Rtx@-5MS capillary column for peak identification

In our study the major constituent groups in the *citrus sinesis* peels essential oil are terpenes (monoterpenes and sesquerpenes), while it also contains aromatic compounds (aldehydes, alcohols, esters, oxide and ketone). The essential oils are characterized by two or more major constituents at fairly high concentrations compared to other constituents present in small quantity. The findings from this research showed that the main constituents in the *citrus sinesis* peels were limonene (90.72%), myrcene (2.82%), octanol acetate (1.24%), nonanal (0.58%), sabinene (0.39%) and elemol (0.14 %). The constituents in essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivatives), and terpenoids (isoprenoids) as reported by Bakkali et al., 2008.

The GCMS analysis revealed that limonene is the most abundant compound in the essential oil. The observed high levels of limonene in this study correlate with the reports of Khaoula et al. (2015), Ademosun et al. (2015) and Yousmel et al. *(*2015). The major constituent from *Citrus sinensis* was limonene of 90.72% using hydrodistillation essential oil extracts and was lower than the one reported by Rodriguez et al., (2011) of same sample with limonene of 97%. The limonene value of our study is higher than the value of Tunisian *Citrus aurantium* of 87.523% as reported by Khaoula et al. (2015). The result from the research showed that *Citrus sinesis* could be used as a source of limonene production. Limonene could be introduced in the nutritional, pharmaceutical and cosmetic fields as reported by Vivian et al., (2016). Moreover, other compounds such as myrcene (2.82%), octanol acetate (1.24%), nonanal (0.58%), sabinene (0.39%) and elemol (0.14%) were present at minimal level.

Table 2. Volatile constituent of sweet orange essential oil from hydrodistillation

Peak	Ret. Time	RI (cal)	KI (lit)	CAS No	Molecular formular	Compounds	Fragmentation ions (m/z)	Identification	Area %
1	5209	937	939	80-56-8	$C_{10}H_{16}$	α-pinene	121, 105,98,79,77	MS, RI	0.6
2	6.529	977	975	3387-41-5	$C_{10}H_{16}$	Sabinene	136,94,93,79,77	MS, RI	0.39
3	7.247	996	990	123-35-3	$C_{10}H_{16}$	Myrcene	93,79,77,69,67	MS, RI	2.82
4	7.751	1008	998	124-13-0	$C_8H_{16}O$	n-Octanal	100,93,85,84,69	MS, RI	0.24
5	9.83	1049	1029	138-86-3	$C_{10}H_{16}$	Limonene	136,121,107,94,93,79,68,53	MS, RI	90.72
6	9.909	1050	1037	3338-55-4	$C_{10}H_{16}$	β-Ocimene	121,105,98,79,77,67	MS, RI	0.01
7	10.26	1056	1050	3779-61-1	$C_{10}H_{16}$	β- Ocimene	121,105,98,80,79	MS, RI	0.01
8	10.736	1064	1059	99-85-4	$C_{10}H_{16}$	γ-Terpinene	136,121,93,77	MS, RI	0.05
9	11.522	1076	1068	111-87-5	$C_8H_{18}O$	n-Octanol	84,70,69,56	MS, RI	0.02
10	12.449	1090	1088	586-62-9	$C_{10}H_{16}$	Terpinolene	136,121,105,93,79	MS, RI	0.18
11	13.35	1102	1096	78-70-6	$C_{10}H_{18}O$	Linalool	121,93,80,71,67,55	MS, RI	0.02
12	13.635	1107	1100	124-19-6	$C_9H_{18}O$	n-Nonanal	98,95,82,70,67,57,55	MS, RI	0.58
13	15.959	1140	1142	4959-35-7	$C_{10}H_{16}O$	Lim.oxide	108,95,94,81,79,67,55,53	MS, RI	0.1
14	17.365	1157	1153	106-23-0	$C_{10}H_{18}O$	Citronellal	121,111,95,69,55	MS, RI	0.05
15	19.124	1178	1177	562-74-3	$C_{10}H_{18}O$	Terpinen-4-ol	111,93,71,69,55	MS, RI	0.06
16	20.358	1191	1188	98-55-5	$C_{10}H_{18}O$	α -Terpineol	136,121,93,81,67,59	MS, RI	0.05
17	21.954	1211	1201	112-31-2	$C_{10}H_{20}O$	n-Decanal	112,95,84,82,70,68,57	MS, RI	0.13
18	22.559	1221	1213	112-14-1	$C_{10}H_{20}O_2$	Octanol acetate	112,83,73,70,61,56	MS, RI	1.24
19	22.758	1224	1216	1197-07-5	$C_{10}H_{16}O$	Carveol	109,91,84,69,55	MS, RI	0.13
20	23.555	1236	1229	106-25-2	$C_{10}H_{18}O$	Nerol	93,84,69,55,52	MS, RI	0.01
21	25.511	1265	1252	106-24-1	$C_{10}H_{18}O$	Geraniol	93,69,67,53	MS, RI	0.11
22	26.338	1276	1271	2111-75-3	$C_{10}H_{14}O$	Perilla aldehyde	135,122,107,93,79,77,68,53	MS, RI	0.02
23	27.551	1292	1295	536-59-4	$C_{10}H_{16}O$	Perilla alcohol	134,119,106,91,79,67,55,53	MS, RI	0.11
24	28.682	1310	1306	112-44-7	$C_{11}H_{22}O$	Undecanal	96,95,82,68,67,57,55	MS, RI	0.02
25	29.106		1316	25152-84-5	$C_{10}H_{16}O$	Decadienal	95,81,79,67,55	MS, RI	0.05
26	30.898	1353	1349	80-26-2	$C_{12}H_{20}O_2$	α -Terpinyl acetate	136,121,107,93,91,79,67	MS, RI	0.01
27	31.303	1360	1352	150-84-5	$C_{12}H_{22}O_2$	Citronelly acetate	123,95,82,81,69,55	MS, RI	0.02
28	31.877	1371	1361	141-12-8	$C_{15}H_{24}$	Neryl acetate	136,121,93,80,69,53	MS, RI	0.02
29	32.07	1374	1376	3856-25-5	$C_{15}H_{24}$	α -Copaene	161,119,105,93,81,55	MS, RI	0.03
30	32.858	1388	1388	13744-15-5	$C_{15}H_{24}$	β-Cubebene	161,119,105,91,81,69	MS, RI	0.1
31	32.972	1390	1390	515-13-9	$C_{12}H_{24}O$	β-Elemene	147,121,107,93,81,79,68,55	MS, RI	0.11
32	34.013	1411	1408	112-54-9	$C_{12}H_{24}O_2$	Dodecanal	96,82,68,67,57,55	MS, RI	0.02
33	34.18	1415	1408	112-17-4	$C_{12}H_{24}O_2$	Decyl acetate	97,83,70,69,61,55	MS, RI	0.36
34	36.221	1461	1456	18794-84-8	$C_{15}H_{24}$	β-Farnesene	133,93,79,69,55	MS, RI	0.01
35	37.054	1479	1479	30021-74-0	$C_{15}H_{24}$	γMuurolene	161,133,119,105,91,81,55	MS, RI	0.01
36	37.625	1491	1496	4630-07-3	$C_{15}H_{24}$	Valencene	161,133,119,107,105,93,79	MS, RI	0.02
37	38.004	1499	1500	31983-22-9		α-Muurolene	161,119,105,93,81	MS, RI	0.02
38	38.102	1501	1509	3691-11-0	$C_{15}H_{24}$	α- Bulnesene	147,119,107,93,81,79,67,53	MS, RI	0.01
39	38.618	1514	1513	39029-41-9	$C_{15}H_{24}$	γ- Cadinene	161,122,107,93,91,81,55	MS, RI	0.02
40	39.004	1524	1523	483-76-1	$C_{15}H_{24}$	δ- Cadinene	204,161,134,119,105,91,81	MS, RI	0.02
41	40.071	1550	1549	639-99-6	$C_{15}H_{26}O$	Elemol	161,149,107,93,91,81,55	MS, RI	0.14
42	40.755	1566	1563	40716-66-3	$C_{15}H_{26}O$	Nerolidol	149,93,69,55	MS, RI	0.01
43	42.698	1613	1612	124-25-4	$C_{14}H_{28}O$	Tetradecanal	96,82,81,71,69,57	MS, RI	0.01
44	43.753	1642	1654	481-34-5	$C_{15}H_{26}O$	α –Cadinol	161,149,107,93,81,67,59	MS, RI	0.01
45	44.661	1666	1671	22451-73-6	$C_{15}H_{26}O$	Bulnesol	161,119,107,93,81,67,59	MS, RI	0.01
46	47.923	1756	1756	17909-77-2	$C_{15}H_{22}O$	α- Sinensal	134,119,107,93,79,55	MS, RI	0.07
47	49.602	1804	1806	4674-50-4	$C_{15}H_{22}O$	Nootkatone	203,175,161,147,133,121,105,91,79,55	MS, RI	0.05

*Retention indices relative to C_8-C_{20} n-alkanes on the BPX5 column, identification based on retention time RT, identification based on retention index RI and identification based on comparison of mass spectra, MF-Molecular Formular. CAS-Chemical Abstracts Service reference number, KI-Kovats index, NI-Not Identified, a Compounds are listed in order of their elution from a DB- 5 FID column, b Kovat Index calculated from retention times, c Linear retention indices from the literature and d Percentages obtained by FID peak-area normalization Source

Conclusion

A comparison of extraction yield results of PLE, Soxhlet and hydrodistillation indicated that PLE process is advantageous mainly due to the relatively short extraction time. PLE extraction time, solvent and extraction temperature can be tailored to individual materials to maximize the extraction yield and antioxidant property. The TLC and chemical purification further showed the presence of minor additional compounds (polyphenol) with essential oil in PLE and Soxhlet. The result from the purified extracts and thin layer chromatogram showed that hydrodistillation extract is pure essential oil without additional polyphenol compounds. Hence, this necessitate further study to determine the constituents of polyphenol compounds in PLE and Soxhlet extracts. The result from GC-MS showed sweet orange essential oils as mixtures of many compounds which include terpenes, aldehydes, alcohols, terpenes oxide, ketone and esters.

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