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Short communication

Mineral element determination and phenolic compounds profiling of oleoresin extracts using an accurate mass LC-MS-QTOF and ICP-MS

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ABSTRACT

The nutraceutical potential of black and white pepper has drawn the attention of many researchers to unravel their pharmaceutical and nutritional properties. These properties could be attributed to the presence of some essential bioactive compounds and mineral elements. This study, therefore, investigated the phenolic and mineral element composition in their oleoresin extracts, using a set of physicochemical and characterization protocols. The result obtained at optimum condition gave a total of 17 and 20 phenolic compounds in black and white pepper extracts, respectively. Moreover, four essential mineral elements (Na, Mg, K, and Ca) were detected. The presence of hazardous elements such as Ar, Se, Pb, and Cr, below the detection limit (BDL) indicated the edibility of the oleoresin extracts. The total phenolic content (TPC) in both extracts was evaluated using Folin-Ciocalteu assay. The result showed that the white pepper extract exhibited higher total phenol content (63.40 ± 0.096 mg GAE/g d.w) than black pepper extract (51.95 ± 0.025 mg GAE/g d.w). The combined action of the mineral elements, total phenolic content and the phytochemical profiles confirmed the potential of oleoresin extracts as natural antioxidants in food and pharmaceutical industries.

1. Introduction

Black and white peppers (*Piper nigrum*) are tropical fruit from the *Piperaceae* family. These important economic crops are native to India, Thailand, Malaysia and Vietnam (Kamarulzaman et al., 2013). They are traditionally used in ethnomedicine for the treatment of many life-threatening free radical diseases (Capasso et al., 2002; Karsha and Lakshmi, 2010). Their medicinal properties are due to the presence of some phytochemical and mineral elements. The fruit contains numerous medicinal constituents such as polyphenol, flavonoid, alkaloid, terpenoid derivatives, etc (Gupta et al., 2013; Olalere et al., 2017a; Vittal, 1990). These groups of compounds are responsible for the numerous pharmacological characteristics of their oleoresin extracts which include antimicrobial activities (Rani and Saxena, 2013), carminative activities (Madhu et al., 2009; Tiwari, 2008), anti-inflammatory activities (Abdurahman and Olalere, 2016a; Moraes et al., 2013), and bioavailability (Janakiraman and Manavalan, 2008).

In recent times, the use of polyphenols as antioxidant from plant origin has replaced the synthetic ones and this is due to the undesirable effects they portend to human health (Meghwal and Tk, 2012). The polyphenols are the secondary metabolites of plant origin and the most abundant antioxidants with therapeutical effects used for the prevention of oxidative stress-related degenerative diseases (Ilaiyaraja et al., 2015). Due to the thermosensitive nature of these phenolic phytocompounds, the use of traditional extraction methods often leads degradation (Ilaiyaraja et al., 2015; Olalere et al., 2017a). Microwave refluxation provides a rapid, safe, cheap method of extracting phenolic compounds from plant origin without degrading their volatile constituents. It is, therefore, a better extraction technique uniting electromagnetic waves and multi-directional traditional solvent extraction (Abdurahman and Olalere, 2016b; Paunović et al., 2014). It has the merit of shorter extraction time, lower energy absorption, low solvent consumption, higher selectivity and improved extraction rate (Dincutoiu et al., 2006; Xiao et al., 2012).

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The present study, therefore, evaluated the multi-elemental and polyphenolic components of black and white pepper extracts using LCMS-QToF and ICP-MS analysis. The quantitative compositional differences between the two species were adequately evaluated using Folin-Ciocalteu colourimetric assay.

2. Materials and methods

2.1. Preparation of plant extracts

Dried black and white pepper seeds were purchased from Sarawak, Malaysia. The dried samples were powdered using a Grindomix blender (GM-200 model, Germany). The extraction process was carried out using the ETHOS-Milestone extractor (ATC-FO-300, North America). The extraction system is equipped with a temperature control optical fibre coupled with a maximum output power of 1000 W at atmospheric pressure. An assembly of round bottom reactor was attached using a glass rod connected to the condenser. A balance between the microwave power and the solvent boiling temperature was maintained with the aid of an inlet and outlet port. 25 g of the powder sample was mixed with a known quantity of distilled water and thoroughly stirred using a magnetic stirrer to allow for homogeneity. The mixture was then loaded into the microwave cavity and subjected to a rapid microwave heating. The extraction process was conducted at optimum condition viz: 120 min of irradiation time, 350 W of microwave power, 0.105 mm of particle size and 1:12 of a feed-solvent ratio (Olalere et al., 2017a). However, in the extraction of spice oleoresin from white pepper, 90 min of irradiation time, 300 W of microwave power, 0.105 mm of particle size and 1:10 of feed-solvent ratio were used at optimized condition (Olalere et al., 2017b). The extracts were filtered through Whatman No 1 and the filtrates stored at −4 °C prior to analysis.

2.2. Total phenolic content determination

The total phenolic content (TPC) in the extracts were determined based on Folin-Ciocalteu colourimetric method as reported by Saravanan and Parimelazhagan (2014) and Dahmoune et al. (2014) with little modification. 1.58 mL of water was mixed with 20 µl of oleoresin extracts, followed by 100 µl of Folin-Ciocalteu's phenol reagent, 2 N (Sigma Aldrich®, Germany). The mixture was thoroughly stirred and then incubated for 5 min. 300 µl of sodium carbonate (Na_2CO_3) solution was added to the mixture and incubated in the dark for 120 min at room temperature. The absorbance of the plant extracts, standard and blank were then measured at 765 nm using a UV–VIS Spectrophotometer (Hitachi U-1800, Japan). Gallic acid was used as a standard for the calibration curve, to determine the total phenolic concentration of the extracts expressed in mg/L gallic acid equivalents. The total phenolic contents were calculated in triplicates using Eq. (1).

$$TPC = \frac{c(mg_{GATE}/L) * V(L_{solvent})}{m(g_{dw\ dryweight})} \quad (1)$$

where c is the sample concentration from the gallic acid calibration curve (mg/L), V is the volume (ml) of the solvent used in the extraction, and m represents the weight (g) of the dried sample used.

2.3. Untargeted metabolomics based on LC-QToF/MS

The phenolic composition in the extracts was identified using Liquid Chromatography equipped with a Quadrupled time of flight mass spectrometer (Vion Ion Mobility QTOF MS, Waters, USA). The chromatographic separation was carried out with the aid of an ACQUITY UPLCHSS T3 column (2.1 × 100 mm, particle size 1.8 µm). The mobile phase was prepared using the binary solvent manager with solvent A and B. The solvent A was made of 0.1% formic acid (Sigma Aldrich®, Germany) plus water (Milli-Q grade, v/v). However, solvent B comprised of 0.1% formic acid in acetonitrile at a seal wash time of 5 min and maximum pressure limit of 1800 psi. The two solvents were then passed through the vacuum degasser. A known quantity of the oleoresin extracts (3 µl) were injected and phenolic compounds were identified under a negative (−ve) ionization mode with a mass range of 100–1000 m/z, source temperature of 120 °C, desolvation temperature of 550 °C, desolvation rate of 800 l/h, spray voltage of 4 kV and scan time ranging from 0.20 s to 4.0 min.

2.4. Inductively coupled plasma mass spectrometry analysis

The analysis of mineral elements (Na, Mg, K, Ca, Cr, Mn, Fe, Cu, Zn, As, Se, Cd, Pb) in black and white pepper oleoresins was carried out using an Inductively Coupled Plasma/Mass Spectrometer (ICP-MS 7500, Agilent, US) in accordance with the method employed by Wati et al. (2014). An intermediate solution(IS) was prepared using 5 mL of the stock solution into 50 mL volumetric flask to make up a

Table 1
Identified phenolic compounds in black pepper oleoresin extracts via MRE.

S/N	Component name	MF[a]	Observed RT[b] (min)	Observed m/z	Adduct	Total Fragments
1	Forsythoside D	$C_{20}H_{30}O_{13}$	0.31	477.1605	$^{-}$H	25
2	Decaffeoylacteoside	$C_{20}H_{30}O_{12}$	0.38	461.1661	$^{-}$H	10
3	Vanillin	$C_8H_8O_3$	0.44	197.0453	$^{+}$HCOO	2
4	Norbergenin	$C_{13}H_{14}O_9$	0.45	313.0556	$^{-}$H	2
5	N-trans Coumaroyltaramine	$C_{17}H_{17}NO_3$	0.46	328.1191	$^{+}$HCOO	0
6	Moupinamide	$C_{18}H_{19}NO_4$	0.52	312.1239	$^{-}$H	16
7	Tribulusamide A	$C_{36}H_{36}N_2O$	0.65	623.2393	H, $^{+}$HCOO	58
8	Cishinokiresinol	$C_{17}H_{16}O_2$	0.68	297.1136	$^{+}$HCOO	0
9	Moupinamide	$C_{18}H_{19}NO_4$	0.71	312.1239	$^{-}$H	4
10	1,5-Bis(4-hydroxy-3 methoxyphenyl)-1,4 pentadiene-3-one	$C_{19}H_{18}O_5$	0.72	371.1140	$^{+}$HCOO	4
11	N-Dihydro- caffeoyltyramine	$C_{17}H_{19}NO_4$	0.86	300.1249	$^{-}$H	0
12	Moracin C	$C_{19}H_{18}O_4$	0.93	355.1190	$^{+}$HCOO	0
13	2-Octylphenol	$C_{14}H_{22}O$	1.14	251.1655	$^{+}$HCOO	0
14	Octahydrocurcumin	$C_{21}H_{28}O_6$	1.25	421.1871	$^{+}$HCOO	5
15	Kukoamine A	$C_{28}H_{42}N_4O_6$	1.31	529.3042	$^{-}$H	1
16	2,6-Di-tert-butyl-4-hydroxytoluene	$C_{15}H_{24}O$	1.64	265.1806	$^{+}$HCOO	0
17	6-Gingerol	$C_{28}H_{42}N_4O$	3.84	293.1759	$^{-}$H	1

MF[a] – Molecular formula, RT[b] – Retention time.

100 ppm concentration of the stock solution. A multielement standard solution was used in the preparation of calibration solution. The calibration curve was constructed from five concentrations (0–50 ppm) of the standard solution using 2% nitric acid (HNO_3) as blank. The liquid sample was introduced into the ICP-MS nebulizer and spray chamber. The sample was dried, vapourized, atomized and ionized inside the plasma chamber consisting of different heating zones. The elemental composition of the samples was obtained through the transformation of the liquid samples into excited atoms and positively charged ions.

3. Results and discussion

3.1. Total phenolic content (TPC)

The total phenolic content of black and white oleoresin extracts were estimated from gallic acid calibration curve with the regression coefficient (R^2) and an equation of line 0.996 and y = 0.017 + 0.00064×, respectively. The total phenolic content was expressed in gallic acid equivalent per gram of sample dry weight ($mg_{GAE/g\ d.w}$). On the overall, the oleoresin extracted from white

Table 2
Identified phenolic compounds in white pepper oleoresin extracts via MRE.

S/N	Component name	MF[a]	Observed RT[b] (min)	Observed m/z	Adduct	Total Fragments
1	Decaffeoylacteoside	$C_{20}H_{30}O_{12}$	0.31	461.1665	$^{-}$H	19
2	Forsythoside D	$C_{20}H_{30}O_{13}$	0.37	477.1604	$^{-}$H	14
3	Vanillin	$C_8H_8O_3$	0.44	197.0459	$^{-}$H, $^{+}$HCOO	2
4	Norbergenin	$C_{13}H_{14}O_9$	0.45	313.0565	$^{-}$H	7
5	N-trans Coumaroyltaramine	$C_{17}H_{17}NO_3$	0.45	328.1196	$^{+}$HCOO	0
6	Protocatechuic aldehyde	$C_7H_6O_3$	0.45	137.0247	$^{-}$H	0
7	Tribulusamide A	$C_{36}H_{36}N_2O_8$	0.47	669.2453	$^{+}$HCOO	14
8	Moupinamide	$C_{18}H_{19}NO_4$	0.52	312.1244	$^{-}$H	14
9	1,5-Bis(4-hydroxy-3 methoxyphenyl)-1,4 pentadien-3-one	$C_{19}H_{18}O_5$	0.59	325.1070	$^{-}$H	4
10	Moracin H	$C_{20}H_{18}O_5$	0.59	337.1087	$^{-}$H	7
11	Cishinokiresinol	$C_{17}H_{16}O_2$	0.68	297.1118	$^{+}$HCOO	0
12	Moracin O	$C_{19}H_{18}O_5$	0.72	371.1143	$^{+}$HCOO	4
13	Kukoamine A	$C_{28}H_{42}N_4O_6$	0.88	529.3016	$^{-}$H	5
14	Moupinamide	$C_{18}H_{19}NO_4$	0.91	358.1315	$^{+}$HCOO	2
15	6-Gingerol	$C_{17}H_{26}O_4$	0.96	293.1764	$^{-}$H	8
16	Moracin C	$C_{19}H_{18}O_4$	0.97	355.1189	$^{+}$HCOO	8
17	2-Octylphenol	$C_{14}H_{22}O$	1.14	251.1654	$^{+}$HCOO	0
18	Octahydrocurcumin	$C_{21}H_{28}O_6$	1.26	421.1882	$^{+}$HCOO	6
19	2,6-Di-tert-butyl-4-hydroxy toluene	$C_{15}H_{24}O$	1.63	265.1890	$^{+}$HCOO	0
20	6-Gingerol	$C_{17}H_{26}O_4$	3.84	293.1759	$^{-}$H	1

MF[a] – Molecular formula, RT[b] – Retention time.

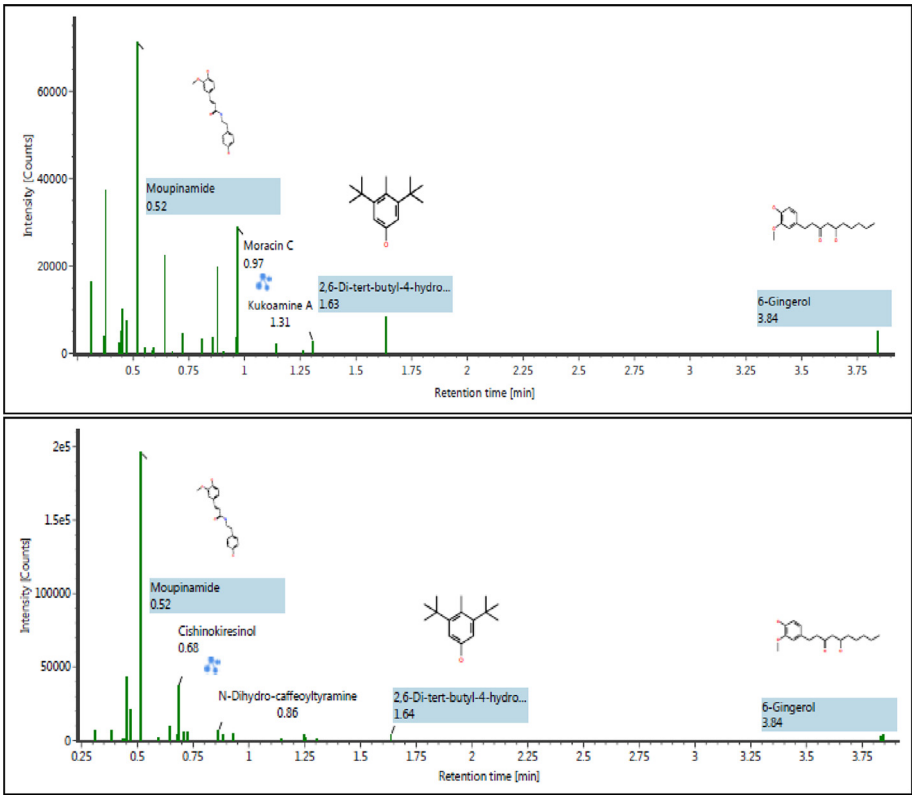

Fig. 1. LC-QToF analysis of optimized oleoresin extract of (a) black pepper extracts (b) white pepper obtained via microwave reflux extraction method.

Table 3
Total concentration of mineral and trace elements in the extracts.

S/N	Mineral elements	Black pepper extracts (mg/L)	White pepper extracts (mg/L)
1	Sodium (Na)	8.1	12.4
2	Magnesium (Mg)	19.4	46.1
3	Potassium (K)	206.2	511.0
4	Calcium (Ca)	2.3	3.0
5	Chromium (Cr)	BDL (<0.5)	BDL (<0.5)
6	Manganese (Mn)	BDL (<0.5)	BDL (<0.5)
7	Iron (Fe)	BDL (<0.1)	BDL (<0.1)
8	Copper (Cu)	BDL (<0.5)	BDL (<0.5)
9	Zinc (Zn)	BDL (<0.5)	BDL (<0.5)
10	Arsenic (As)	BDL (<0.5)	BDL (<0.5)
11	Selenium (Se)	BDL (<0.5)	BDL (<0.5)
12	Cadmium (Cd)	BDL (<0.5)	BDL (<0.5)
13	Lead (Pb)	BDL (<0.5)	BDL (<0.5)

BDL: Below Detection Limit.

pepper has the highest total phenolic content (63.40 ± 0.096) than the black pepper extracts (51.95 ± 0.028). The oleoresin extracts from black pepper have the lowest amount of phenolic when compared with white pepper oleoresin extracts. This further confirmed the higher antioxidant value of white pepper when compared to black pepper as reported by Abd El Mageed et al. (2011) and Olalere et al. (2017b).

3.2. Identification of phenolic phytocompounds in the extracts

The LC-QToF/MS analysis revealed that black and white pepper contains a total of 17 and 20 phenolic phytocompounds, respectively (Tables 1 and 2). The identity of the phenolic profiles was confirmed by the mass fragmentation analysis and mass spectra (Fig. 1). Fig. 1a and 1b show the total ion count chromatographs of the phenolic compounds detected in the two oleoresin extracts from negative electrospray ionization (ESI) mode. The higher numbers of phenolic compounds confirmed the higher medicinal value of white pepper over black pepper. The differences in phenolic constituents in black and white pepper oleoresin extracts is due to the total fragmentation, retention time and observed mass-to-charge ratio as reported by Molina-Calle et al. (2017). The combined action of the phenolic compounds is responsible for their antioxidant activity which indicates their biological activity such as dead cell regeneration, cancerous cell inhibition, cellular metabolism and regulation of cell-cycle (Jeena et al., 2014; Lackova et al., 2017, 2016; Liu et al., 2010; Saha et al., 2013).

3.3. Profiling of mineral elements in black and white oleoresin extracts

The concentration of thirteen mineral elements (Na, Mg, K, Ca, Cr, Mn, Fe, Cu, Zn, As, Se, Cd, Pb) were measured using an inductively coupled plasma/mass spectrometers. On the overall, the mineral and trace elements from both extracts showed a wide variability with the dietary elements such as Na, Mg, K, and Ca classified as essential minerals (Table 3). An increase in the elemental concentration of these mineral elements was noticeable in white pepper extracts. This indicated that in term of the biological activities, white pepper oleoresin extracts have more elemental functionalities in the human body than in black pepper extracts. This could be attributed to their botanical structure occasioned by the method of processing of the two crops as reported by Olalere et al. (2017a). Hence, there is a vast medicinal benefit to explore both in curative and preventive traditional medicine.

4. Conclusion

In this study, the extracts of black and white pepper were characterized using the liquid chromatography QToF –mass spectrometer and inductively coupled mass spectrometer. A total of 17 phenolic compounds were identified in the oleoresins extracted from black pepper via microwave reflux extraction at the optimized condition. However, in white pepper microwave reflux extraction, a total of 20 phenolic compounds were detected. Moreover, the total phenolic content in white pepper oleoresin extract was more that in black pepper. Hence, there is a good correlation between the total phenolic content, phytocompounds, and mineral element profile which indicated the potential of white pepper as a better antioxidant than black pepper. The results, therefore, showed the potential of oleoresin extracts as exploitable natural antioxidants in food and pharmaceutical industries.

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Conflict of interest

The authors declare that there is no conflict of interest whatsoever.

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