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ORIGINAL ARTICLE

Green and highly extraction of phenolic compounds and antioxidant capacity from kinkeliba (*Combretum micranthum* G. Don) by natural deep eutectic solvents (NADESs) using maceration, ultrasound-assisted extraction and homogenate-assisted extraction

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Abstract Kinkeliba (*C. micranthum*) is a tropical plant widely used for its tremendous phytochemicals and biological activities. In the present study, three green carboxylic acid-based natural deep eutectic solvents (NADESs) were used to assess the extraction of phenolic compounds in terms of total phenolic content (TPC), total flavonoid content (TFC), individual phenolic compounds and antioxidant capacity (DPPH and FRAP assays) from dried *C. micranthum* leaves. For the synthesis of NADESs choline chloride was used as hydrogen bond acceptors (HBA) in combination with lactic acid (ChLa), acetic acid (ChAa) and tartaric acid (ChTa) as hydrogen bond donors (HBDs). The conventional solvents including distilled water, pure methanol and pure ethanol were used for comparison. Three extraction methods including maceration extraction (ME), homogenate-assisted

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extraction (HAE) and ultrasound-assisted extraction (UAE) were tested to determine the best extraction conditions. The solvents combined with the extraction methods were successfully applied for the recovery of phenolic compounds from *C. micranthum* leaves. ChLa exhibited the highest performance giving the TPC (21.12 ± 0.13–23.62 ± 0.58 mg GAE/g, followed by ChAc (15.49 ± 0.13–18.85 ± 0.39 mg GAE/g), water (17.08 ± 0.32–18.13 ± 0.13 mg GAE/g), ChTa (14.49 ± 0.26–17.44 ± 0.19 mg GAE/g), methanol (7.46 ± 0.45–11.64 ± 0.32 mg GAE/g) and ethanol (2.88 ± 0.39–4.60 ± 0.39 mg GAE/g), respectively. For TFC, ChLa (4.38 ± 0.09–5.01 ± 0.09 mg ECE/g) was the most prominent solvent, followed by ChAc (2.84 ± 0.04–5.01 ± 0.36 mg ECE/g), methanol (1.93 ± 053–4.85 ± 0.04 mg ECE/g), ethanol (1.49 ± 0.36–4.16 ± 0.04 mg ECE/g), ChTa (1.09 ± 0.04–3.22 ± 0.13 mg ECE/g) and water (1.15 ± 0.04–1.37 ± 0.44 mg ECE/g), respectively. The acidic NADESs especially ChLa and ChAa exhibited the best efficiencies compared to the conventional solvents. Furthermore, UAE and HAE provided good extraction efficiency in a short extraction time (30 min) in terms of the TPC, TFC, individual phenolic compounds and the antioxidant capacity compared to ME which gave a similar yield with 12 h of extraction time. Principal component analysis (PCA) showed that *C. micranthum* extracts could clearly be discriminated in terms of phytochemical compounds and antioxidant capacity and UAE, HAE or ME combined with ChLa ChAc or ChTa were the best choices to higher extraction efficiency.

1. Introduction

The devasting effects of the COVID-19 pandemic together with other challenges in health, demography and nutrition led to research of preventive and sustainable solutions. For example, foods with high bioactive compounds and biological activities have been suggested to reinforce the immune system during the COVID-19 pandemic (Galanakis, 2020). The phytochemical and biological activity surveys of *Combretum micranthum* G. Don have revealed the presence of many bioactive compounds with tremendous biological activities (Touré et al., 2011; Kpemissi et al., 2019). *C. micranthum* is from the Combretaceae family and is commonly known as kinkeliba. It is a dicotyledonous plant of 4–5 m height widely spread in West African countries. It is popularly employed as a traditional medicine for the prevention and treatment of wounds, sores, fever, malaria, cough, liver ailments, sleep disorder, headache, fatigue and bronchitis. Benoit et al. (1996) have proved the efficacy of *C. micranthum* against malarial, biliary fever, colic and vomiting. It is a rich source of bioactive ingredients and possesses many biological properties such as antioxidant (Touré et al., 2011; Beda et al., 2014), nephroprotective activity (Kpemissi et al., 2019; Kpemissi et al., 2020), anti-inflammatory (Olajide et al., 2003), anti-tyrosinase (Zeitoun et al., 2016), anti-diabetic (Tanko et al., 2017) and antimicrobial (Baba-Moussa et al., 1999; Ayodeji Akeem et al., 2012) activities. Recent clinical studies suggested that *C. micranthum* is a potential plant in preventing and managing hypertension (Seck et al., 2017; Welch et al., 2018; Bourqui et al., 2020) and brain functional damage (Mohammed et al., 2020). These biological activities of *C. micranthum* are closely linked with its richness in bioactive ingredients and particularly in phenolic compounds such as gallic acid, myricetin-3-O-rutinoside, rutin trihydrate, orientin, catechin, vitexin, quercitrin and benzoic acid (Touré et al., 2011; Welch et al., 2018; Kpemissi et al., 2019; Kpemissi et al., 2020; Zeitoun et al., 2020). In Africa, *C. micranthum* is widespread in the fields, streets and forests. It is also sold in the local market as a vegetable, spice or for medicinal purposes.

The efficient extraction of phenolic compounds requires an adequate choice of extraction techniques, solvents and extraction parameters. The phenolic compounds of *C. micranthum* are usually extracted from plants using conventional solvents such as methanol, ethanol, water, acetone and hexane. The use of these conventional solvents requires a long extraction time and a high quantity of solvents (Azmir et al., 2003). They are also lower extraction yields, lower thermal-resistant, lower contents of active constituents in the extracts and higher energy consumption (Azmir et al., 2003; Cui et al., 2018; Zannou et al., 2020). In addition, some of the conventional solvents can be harmful to human beings and environment since they are inflammable, volatile, explosive and toxic (Chemat et al., 2012; Azmir et al., 2003; Bursać Kovačević et al., 2018). Therefore, it is urgent to explore greener ways for the highly efficient extraction of phenolic compounds from *C. micranthum*. Natural deep eutectic solvent (NADES) is a novel class of green and sustainable solvent based on natural components such as carboxylic acids, choline chloride, urea, polyols and sugars. The application of NADES for the extraction of phenolic compounds has emerged as a greener approach, efficient and alternative to organic solvents (Chand Ali et al., 2019; El Kantar et al., 2019; Zannou and Koca, 2020). NADES is generated by mixing two or more natural constituents that are susceptible to self-associated via hydrogen bond interactions by forming a eutectic mixture with a melting point that is hugely below that of the individual constituents (Chand Ali et al., 2019; Alañón et al., 2018). The hydrogen bond interactions take place between the hydrogen bond donor (HBD) and the hydrogen bond acceptor (HBA).

NADESs generated from a wide range of natural constituents have been successfully employed for efficient extraction of phenolic compounds of various plants and derived products (Saha et al., 2019; Buldo et al., 2019; Chakroun et al., 2019; Pal and Jadeja, 2019; Barbieri et al., 2020; Zannou and Koca, 2020; Alsaud et al., 2021). To the best of our knowledge, no study has reported NADESs to extract and enhance the extraction yield of the phenolic compounds from *C. micranthum*. In the present study, NADESs were combined with the extraction techniques such as ultrasound-assisted extraction (UAE), homogenate-assisted extraction (HAE) and maceration (ME) for greener extraction of phenolic compounds from *C. micranthum*. NADES were constituted of choline chloride as HBA and acetic acid, lactic acid, and tartaric acid as HBD. The conventional solvents such as distilled water, ethanol and methanol were compared to the NADESs. The total phenolic content, total flavonoid content, DPPH radical scavenging activity and ferric reducing power, and the individual phenolic compounds of *C. micranthum* were determined.

2. Material and methods

2.1. Plant material

The leaves of kinkeliba (*Combretum micranthum* G. Don) were collected from Abomey-Calavi/Benin Republic. The leaves

sun-dried for seven days and packed in brown bottles with screw caps.

2.2. Chemicals and reagents

Distilled water purified by a Millipore-Q system (Millipore Billerica, Massachusetts, USA). methanol (HPLC grade), ethanol (HPLC grade), acetonitrile (HPLC grade), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, ≥99.0%), Folin-Ciocalteu reagent, Trolox (97%), sodium nitrite (99–100.5%), hydrochloric acid (36.5–38%), sodium carbonate (99.5–100.5%), choline chloride (≥98%), gallic acid (≥99.0) and other standards were bought from Sigma Aldrich Chemical Co. (St Louis, MO). Lactic acid (90%) was purchased from Isolab while aluminum chloride, iron (III) chloride and iron sulfate heptahydrate (≥99.5%) were brought from Merck. Sodium acetate anhydrous (≥99.0%), L(+) tartaric acid (>99%), glacial acetic acid (99.5%), potassium chloride (≥99.0%), and sodium hydroxide (≥97.0%) obtained from Carlo Erba.

2.3. Synthesis of NADESs

Three NADESs were synthesized using choline chloride as the hydrogen bond acceptor (HBA) and lactic acid, tartaric acid and acetic acid as hydrogen bond donors (HBD). The NADESs were obtained according to the preparation procedure described in Chanioti and Tzia (2018) with a slight modification. Briefly, choline chloride was mixed with each hydrogen bond donor at 1:2 M ratio, followed by the addition of 20% of distilled water. Then, the mixture was heated at 80 °C under constant stirring for 2 h. The NADESs obtained from the combination of choline chloride and lactic acid, tartaric acid and acetic acid were encoded as ChLa, ChTa and ChAa, respectively.

2.4. Extraction with NADES and conventional solvents

2.4.1. Ultrasound-assisted extraction (UAE)

A portion of 1 g of the comminuted *C. micranthum* leaves was mixed with 20 g of solvents (NADESs and conventional solvents) in extraction vessels and ultrasonicated in an ultrasonic bath (40 kHz, 296 W, WUC-A03H, daihan scientific Co., Ltd. Seoul, Korea) at room temperature (25 °C) for 30 min.

2.4.2. Homogenate-assisted extraction (HAE)

A portion of 1 g of the comminuted *C. micranthum* leaves was mixed with 20 g of solvents (NADESs and conventional solvents) in extraction tubes and homogenized at 100 rpm in a high-speed homogenizer (Unidrive X1000, CAT Scientific, Inc., Paso Robles, California) at room temperature (25 °C) for 30 min.

2.4.3. Maceration extraction (ME)

The macerationis a traditional extraction method used for the extraction of phenolic compounds from plant materials. The maceration was conducted using the maceration method described in Ćujić et al. (2016) with a slight modification. Briefly, a portion of 1 g of the comminuted *C. micranthum* leaves was mixed with 20 g of solvents (NADESs and conventional solvents) in extraction vessels and roughly shaken for 2 min to homogenize. Afterwards, the mixture was left at room temperature (25 °C) to extract for 12 h.

2.5. Total phenolic content (TPC)

The TPC was determined by the Folin-Ciocalteu method using the method of Singleton and Rossi (1965) with some modifications. Briefly, 150 µL of the appropriately diluted sample was mixed 750 µL of 10% Folin-Ciocalteu reagent and 600 µL of 7.5% (w/v) Na_2CO_3, respectively. The mixture was placed in the dark for 2 h and the absorbance was read at 760 nm using a UV- spectrophotometer (Thermo Spectronic). The TPC was expressed as mg gallic acid equivalent per g (mg GAE/g).

2.6. Total flavonoid content (TFC)

TFC was determined using the described method in Zannou and Koca (2020). Briefly, 1 mL of the appropriately diluted sample mixed with 300 µL of 5% $NaNO_2$ and 500 µL of 5% $AlCl_3$ and 500 µL of 1 M NaOH respectively. Afterwards, the mixture was placed in the dark for 10 min and the absorbance was read at 510 nm. The results were given as mg epicatechin equivalents per g (mg ECE/g).

2.7. DPPH radical scavenging activity assay (DPPH)

The DPPH assay was conducted according to the adopted method of Zannou et al. (2020). The DPPH solution was used as the control and the scavenging ratio was calculated as follows:

$$\text{Inhibition } (\%) = \left(\frac{\text{Absorbance of the control} - \text{Absorbance of extract}}{\text{Absorbance of the control}}\right) \times 100$$

The values of DPPH radical scavenging were determined with a calibration curve as mmol Trolox equivalent per g (mmol TE/g).

2.8. Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was conducted according to the procedure indicated in Benzie and Strain (1996). The values of FRAP was calculated from a calibration curve of $FeSO_4$ and the results were given as mmol $FeSO_4$ equivalents per g (mmol ISE/g).

2.9. Phenolic compounds profile of C. micranthum extracts via HPLC-DAD

The individual phenolic compounds of sumac were determined using the previous method of Bosiljkov et al. (2017) with modifications. The phenolic compounds were identified using a high-pressure liquid chromatography (HPLC) system (Agilent 1260; Agilent Technologies) coupled with a diode array detector (DAD) at 520 nm wavelength for anthocyanins and 280 nm for other phenolic compounds. The phenolic compounds were separated in an Inertsil ODS-4 column (3 µm, 4,6 × 50 mm; GL Sciences Kat No: 5020–0404) at a 1 $mL.min^{-1}$ flow rate. The mobile phases were: (A) 94% 2 mM sodium acetate and

6% acetic acid (v/v); and (B) acetonitrile. The following elution gradient was used, according to solvent B: 0–20 min, 14–23%; 20–40 min, 23–35%; 40–50 min, 40%; 50–60 min, 60%; 60–65 min 95%. The column temperature was set at 30 °C. The individual phenolic compounds were identified by comparing their retention times with their respective standard. The identified phenolic compounds were quantified using a mixture of external standards which were prepared at different concentrations.

2.10. Statistical analyses

The experiments were carried out in triplicate. The results were given as mean ± standard deviation. The one-way analysis of variance (ANOVA) was performed for the statistical analyses using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Duncan's test was applied to determine the significance of the solvents' extractability results ($p < 0.05$). The independent *t*-test was performed to evaluate the significance of the extraction techniques ($p < 0.05$). Pearson's correlation test was used for the evaluation of the correlation between the antioxidant capacities of two independent tests (DPPH and FRAP) and total phenolic content, total flavonoid content and individual phenolic compounds. In addition, the principal component analysis (PCA) was performed (XL Stat software, Addinsoft, New York, NY, USA) to determine the correlations between the variables and properties of the extracts.

3. Results and discussion

3.1. Effects of solvents and extraction methods on phytochemical properties of ***C. Micranthum***

In the present study, the efficiency of the synthesized NADESs to recover phenolic compounds from *C. micranthum* leaves was tested using two representative phytochemical indices such as TPC and TFC. Distilled water, ethanol and methanol were chosen as control solvents since they are the conventional solvents usually used for the extraction of phenolic compounds from plant material. Furthermore, the extractions were carried out using ultrasound-assisted extraction (UAE), homogenate-assisted extraction (HAE) and maceration (ME) techniques to show out the appropriate extraction methods. Extraction is the first and essential step in the isolation and purification of bioactive components from plants. The maceration is a traditional extraction technique applied for the recovery of bioactive compounds such as phenolic compounds. In recent years, new extraction techniques have been experimented for the extraction of bioactive compounds including ultrasound-assisted extraction and homogenate-assisted extraction, (Khoddami et al., 2013; Chanioti and Tzia, 2018). Although the extraction technique is essential in the extraction process, the solvent and other extraction parameters such as time and temperature play a key role in the recovery of bioactive compounds.

The extractability of phenolic compounds with ChLa, ChTa and ChAa, water, methanol and ethanol was shown in Fig. 1. The recovery of phenolic compounds from *C. micranthum* leaves was significantly affected by the type of solvents ($p < 0.05$). ChLa exhibited the highest performance giving the TPC varying between 21.12 ± 0.13 mg GAE/g and 23.62 ± 0.58 mg GAE/g depending on the extraction methods. ChLa was followed by ChAc (15.49 ± 0.13–18.85 ± 0.39 mg GAE/g), water (17.08 ± 0.32–18.13 ± 0.13 mg GAE/g), ChTa (14.49 ± 0.26–17.44 ± 0.19 mg GAE/g), methanol (7.46 ± 0.45–11.64 ± 0.32 mg GAE/g) and ethanol (2.88 ± 0.39–4.60 ± 0.39 mg GAE/g), respectively. For TFC, ChLa (4.38 ± 0.09–5.01 ± 0.09 mg ECE/g) was the most prominent solvent extracting the flavonoid compounds, followed by ChAc (2.84 ± 0.04–5.01 ± 0.36 mg ECE/g), methanol (1.93 ± 053–4.85 ± 0.04 mg ECE/g), ethanol (1.49 ± 0.36–4.16 ± 0.04 mg ECE/g), ChTa (1.09 ± 0.04–3.22 ± 0.13 mg ECE/g) and water (1.15 ± 0.04–1.37 ± 0.44 mg ECE/g), respectively. The NADESs and particularly ChLa and ChAa exhibited the highest extraction efficiency compared to the conventional solvents. This finding is in close accordance with the results reported by others who found that carboxylic-based NADESs have a high ability to extract phenolics from plants (Chanioti and Tzia, 2018; Zannou et al.2020; Zannou and Koca, 2020; Alsaud et al., 2021). The addition of 20% of water to NADESs enhanced their hydrophilicity and decreased their viscosity. Thus, the phenolic compounds of *C. micranthum* leaves were more dissolved in NADESs. Moreover, the interaction between the phenolic compounds and hydrogen bonds of NADESs (OH and/or Cl-) facilitated their removal (Alsaud et al., 2021). Hao et al. (2020) have reported that the higher ability of ChLa and ChAc of extracting the flavonoids is linked with the strong multiple hydrogen-bonding networks that choline chloride and carboxylic acid-based NADESs form with flavonoids. ChLa has shown the best performance compared

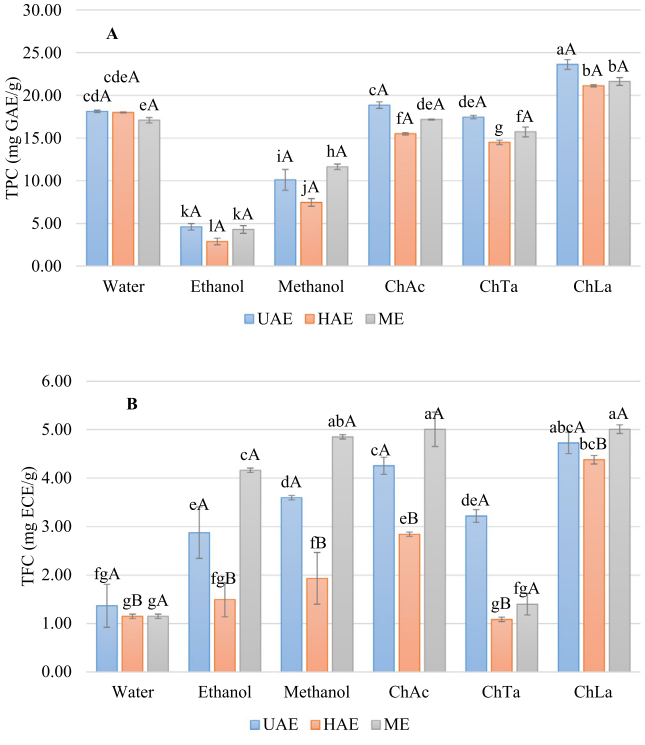

Fig. 1 Recovery of phtychemical content from *C. micranthum*: (A) total phenolic content (TPC) and (B) total flavonoid content (TFC). a-l = Different lowercase letters indicate significant differences between solvents ($P < 0.05$). A-L = Different uppercase letters indicate significant differences between the extraction methods ($P < 0.05$).

to ChAa and ChTa. Therefore, ChLa may have more affinity with the phenolic compounds of *C. micranthum* leaves since the NADES which form high hydrogen bonding can perform better than other NADESs (Dai et al., 2016; Alsaud et al., 2021).

The extraction methods did not have significant effects on the TPC, however, the TFC was significantly affected by the extraction methods ($p < 0.05$). For TPC, UAE was found to be the best method with extraction efficiency higher than HAE and ME. While the ME showed the best performance for the recovery of TFC, followed by UAE and HAE, respectively. It is worth mentioning that the dissolution of the phenolic compounds of *C. micranthum* leaves in the solvents was slow with ME (12 h), whereas UAE and HAE achieved close results with a very short time (30 min). These results are in close agreement with the previous studies where the novel extraction methods such as microwave-assisted extraction and ultrasound-assisted extraction have been reported to reduce significantly the extraction time as compared to maceration (Yılmaz et al., 2020; Tambun et al., 2021; Frohlich et al., 2022). The high yield and short timeframe of UAE and HAE are due to the disruption of the cell walls thanks to the ultrasound and high rotation speed, respectively. Wu et al. (2020) have demonstrated that the NADES-based UAE remarkably showed higher TPC, TFC, DPPH, $ABTS^{+}$, OH^{-} and FRAP values than maceration and stirring extraction. Also, Chanioti and Tzia (2018) have proved that NADES-based HAE was the best method compared to ultrasound-assisted extraction, high hydrostatic pressure-assisted extraction and microwave-assisted extraction for the extraction of phenolic compounds from olive pomace. Although ME requires a long extraction time, UAE, HAE and ME could be good choices to perform the extraction of phenolic compounds with NADES.

3.2. Antioxidant capacity of *C. micranthum*

The antioxidant capacity of *C. micranthum* leaves was measured with DPPH and FRAP assays which are the most common antioxidant capacity assays used in the literature. The DPPH assay displayed the antiradical capacity of the extract, while the FRAP assay measures the ability of the extract to reduce the ferric ions. The results of the antioxidant capacity of *C. micranthum* leaves were given in Table 1. As can be seen, both antiradical activity and FRAP of *C. micranthum* extracts were significantly affected by the types of solvents ($p < 0.05$). The total antiradical activity was ranged from 73.33 ± 19.69 mmol TE/g to 255.22 ± 3.94 mmol TE/g, while the FRAP changed from 43.60 ± 3.73 mmol ISE/g to 160.27 ± 0.39 m mol ISE/g. The extracts obtained from ChLa, ChAa and ChTa provided the highest antioxidant capacity compared to the conventional solvents. Several studies have mentioned the same tendencies, reporting the highest antioxidant capacity from NADES extracts obtained from acidic NADESs when compared to aqueous, methanolic and ethanolic extracts (Bakirtzi et al., 2016; Chanioti and Tzia, 2018; Barbieri et al., 2020; Alsaud et al., 2021). The highest antiradical activity was observed with ChLa, followed by ChAc and ChTa, whereas the best FRAP was achieved with ChTa followed by ethanol and ChAa. Since the NADESs with higher antiradical activity were found different from those giving higher FRAP, it can be concluded that the composition of NADES influences greatly the extraction of the targeted analytes. Many previous

Table 1 Antioxidant characteristics of the extracts obtained from acidic NADESs and conventional solvents.

Solvents		Antioxidant activity					
		DPPH radical scavenging activity			FRAP		
		UAE	HAE	ME	UAE	HAE	ME
Conventional	Water	125.30 ± 17.06fA	116.94 ± 5.25fA	150.35 ± 15.75eA	56.22 ± 0.39ijA	63.43 ± 1.96ghA	67.73 ± efA
	Ethanol	73.33 ± 19.69hA	85.39 ± 7.87ghA	75.18 ± 6.56hA	92.57 ± 1.18dA	71.76 ± 4.71eA	90.49 ± 1.77dA
	Methanol	83.53 ± 0.00ghA	87.25 ± 2.62ghA	103.95 ± 5.25fgA	59.55 ± 3.14hiA	43.60 ± 3.73nA	50.11 ± 1.57lmA
NADES	ChAa	224.59 ± 21.00bcA	200.46 ± 2.62cdA	216.24 ± 6.56bcA	68.43 ± 1.96efA	51.50 ± 0.78klA	52.75 ± jklA
	ChTa	213.45 ± 5.25bcA	181.90 ± 10.50dA	208.82 ± 17.06bcA	154.03 ± 0.59bA	136.55 ± 0.98cA	160.27 ± 0.39aA
	ChLa	255.22 ± 3.94aA	229.23 ± 3.94bA	231.09 ± 17.06bA	65.52 ± 0.98fgA	46.23 ± 0.00mnA	54.56 ± 0.78jkA

a-m = Different lowercase letters indicate significant differences between solvents ($P < 0.05$). a-i = Different uppercase letters indicate significant differences between the extraction methods ($P < 0.05$).

studies have been reported the same behavior of NADESs on the antioxidant capacity of various plant materials (Bakirtzi et al., 2016; Barbieri et al., 2020; Oliveira et al., 2021; Alsaud et al., 2021). Although the UAE proved to be the best method, there was no significant statistical difference was determined between UAE, HAE and ME ($p < 0.05$). Thus, these extraction methods are well-adapted to assist the acidic NADESs for the extraction of antioxidants from plants.

3.3. Phenolic profile of *C. micranthum* leaves

The phenolic compounds extracted from *C. micranthum* using the acidic NADESs and conventional solvents combined with ME, UAE and HAE were shown in Tables 2, 3 and 4. Twelve phenolic compounds including gallic acid, catechin, hydroxybenzoic acid, chlorogenic acid, vanillic acid, epicatechin, syringic, ρ-coumaric acid, ferulic acid, sinapic acid and quercetin-3-glucoside were identified in all the extracts. According to the literature in the *C. micranthum*, gallic acid, catechin, epicatechin, caffeic acid, chlorogenic acid, ρ-coumaric acid, vanillic acid and syringic acid have been reported in various extracts *C. micranthum* (Touré et al., 2011; Kpemissi et al., 2019; Kpemissi et al., 2020; Zeitoun et al., 2020).

3.3.1. Recovery of *C. micranthum* phenolic compounds using acidic NADESs and ME

The extraction yield of *C. micranthum* phenolic compounds obtained from maceration using ChLa, ChAa, ChTa, water, methanol and ethanol was shown in Table 2. Maceration is a traditional and one of the most ancient extraction processes applied for the extraction of bioactive substances such as phenolic compounds. Although maceration is a time-consuming method, it has been reported to be adequate and subsequent for the recovery of antioxidants from various plant materials (Contini et al., 2008; Ćujić et al., 2016). Based on the experimental results, the type of the examined solvents significantly influenced the phenolic compounds of the extracts ($p < 0.05$) suggesting a great variation among the extraction yields (Table 2). The highest amounts of gallic acid, hydroxybenzoic acid and caffeic acid were obtained from the aqueous, ChAa and ChLa extracts, while catechin was highly dissolved in ethanol, water and ChLa. The highest amounts of chlorogenic acid, caffeic acid, vanillic acid and quercetin-3-glucoside were observed with ChAa, followed by ChLa, ChTa and methanol whereas the extracts obtained with ChAa, ChLa, water and methanol exhibited the best recovery of syringic acid, ρ-coumaric acid, ferulic acid and sinapic acid. In addition, ChTa achieved the best performance for the extraction of epicatechin, followed by water, ChAa, ChLa, methanol and ethanol. Considering the sum of the identified phenolic compounds from *C. micranthum*, the general order of the ME efficiency was: ChAa > ChLa > water > methanol > ChTa > ethanol. The synthesized NADESs yielded great amounts of the determined phenolic compounds from *C. micranthum*. It has been revealed that the hydroxyl groups of carboxylic acid (tartaric acid) interact with each other without affecting other protons, indicating a strong hydroxyl attraction from the chlorine anion (Koutsoukos et al., 2019). This kind of attraction between chemical groups leads to the formation of hydrogen bonds (Abbott et al., 2004; Koutsoukos et al., 2019) which increases

Table 2 Phenolic compounds of *C. micranthum* obtained using acidic NADESs and ME (mg/L).

RT (min)	Phenolic compounds	Solvents					
		Water	Ethanol	Methanol	ChAc	ChLa	ChTa
4.87	Gallic acid	1269.54 ± 35.35a	25.09 ± 0.54e	137.97 ± 6.78d	575.46 ± 17.68b	501.02 ± 8.75c	530.46 ± 8.11c
14.29	Catechin	45.73 ± 0.05b	70.36 ± 2.90a	31.31 ± 1.67d	28.54 ± 0.35d	39.73 ± 0.23c	22.65 ± 0.11e
16.34	Hydroxybenzoic acid	65.94 ± 1.10a	0.96 ± 0.06f	23.22 ± 1.39d	54.40 ± 2.02b	40.94 ± 2.73c	3.78 ± 0.36e
17.86	Chlorogenic acid	14.67 ± 0.04e	14.99 ± 0.14e	39.57 ± 0.69d	84.69 ± 1.11a	76.86 ± 2.29b	46.32 ± 0.59c
21.05	Caffeic acid	133.93 ± 3.12a	1.65 ± 0.09f	55.60 ± 0.62d	126.81 ± 0.88b	68.90 ± 1.04c	29.85 ± 0.72e
22.01	Vanillic acid	41.13 ± 0.55e	20.28 ± 0.14f	45.61 ± 1.26d	88.41 ± 0.82a	77.00 ± 0.72b	49.13 ± 0.27c
24.76	Epicatechin	108.72 ± 1.35b	39.18 ± 0.37e	51.34 ± 0.85d	101.41 ± 3.88c	96.89 ± 2.31c	149.56 ± 1.20a
25.90	Syringic acid	48.32 ± 0.14c	10.04 ± 0.07f	36.98 ± 0.71d	69.93 ± 0.14a	49.80 ± 0.41b	28.12 ± 0.47e
33.85	ρ-coumaric acid	807.80 ± 12.05c	132.84 ± 1.75e	588.67 ± 2.94d	1394.24 ± 13.64a	1163.97 ± 18.65b	560.46 ± 11.59d
39.16	Ferulic acid	585.71 ± 6.60c	61.95 ± 2.52f	417.39 ± 5.83d	975.21 ± 5.85a	763.43 ± 6.23b	378.51 ± 3.46e
40.73	Sinapic acid	2321.44 ± 15.44c	330.05 ± 2.54e	1575.60 ± 50.89d	3805.27 ± 60.92a	3165.59 ± 68.15b	1559.95 ± 48.12d
43.11	Quercetin-3-glucoside	5828.66 ± 74.91e	2647.23 ± 51.53f	9578.45 ± 67.35c	16377.36 ± 37.07a	12317.99 ± 35.23b	7104.10 ± 40.83d
Sum		11271.59 ± 144.10	3354.62 ± 62.65	12581.71 ± 146.87	23681.73 ± 84.36	18362.12 ± 146.74	10462.89 ± 115.83

Mean value of three replicates ± standard deviation; a-f = Different lowercase letters indicate significant differences between solvents ($P < 0.05$); Sum = The sum of determined phenolic compounds ± standard deviation.

Table 3 Phenolic compounds of *C. micranthum* obtained using acidic NADESs and UAE (mg/L).

RT (min)	Phenolic compounds	Solvents					
		Water	Ethanol	Methanol	ChAc	ChLa	ChTa
4.87	Gallic acid	1321.52 ± 24.67a	24.03 ± 0.70e	127.61 ± 0.93d	773.76 ± 8.93b	796.63 ± 10.25b	577.94 ± 16.27c
14.29	Catechin	45.03 ± 1.22d	79.18 ± 1.45a	17.47 ± 0.35f	48.25 ± 1.09c	39.81 ± 0.64e	63.81 ± 0.06b
16.34	Hydroxybenzoic acid	69.46 ± 1.41a	1.18 ± 0.28e	16.72 ± 0.36d	47.28 ± 1.60c	59.91 ± 5.13b	18.79 ± 2.19d
17.86	Chlorogenic acid	14.95 ± 0.08e	14.93 ± 0.11e	33.49 ± 0.61d	72.86 ± 0.57b	86.42 ± 3.47a	56.00 ± 0.80c
21.05	Caffeic acid	140.69 ± 1.36a	1.78 ± 0.08e	40.63 ± 0.20d	86.15 ± 1.20b	87.06 ± 1.43b	56.03 ± 4.83c
22.01	Vanillic acid	41.31 ± 0.66d	20.92 ± f	35.55 ± 0.33e	86.72 ± 1.80b	91.37 ± 2.22a	66.30 ± 1.73c
24.76	Epicatechin	109.57 ± 3.24c	38.75 ± 0.02d	40.62 ± 0.34d	113.01 ± 3.97bc	130.13 ± 1.48a	117.76 ± 1.55b
25.90	Syringic acid	50.16 ± 1.22a	10.74 ± 0.04e	21.21 ± 1.67d	45.49 ± 1.23b	44.31 ± 0.70b	37.49 ± 1.30c
33.85	ρ-coumaric acid	800.45 ± 16.65c	133.88 ± 0.11e	418.86 ± 0.14d	1180.75 ± 12.03b	1313.11 ± 9.17a	780.23 ± 53.97c
39.16	Ferulic acid	574.04 ± 8.74c	66.06 ± 0.69e	306.08 ± 0.89d	806.05 ± 13.08b	909.65 ± 31.11a	544.73 ± 31.12c
40.73	Sinapic acid	2315.78 ± 20.46c	333.43 ± 1.88e	1150.56 ± 7.36d	3171.88 ± 72.07b	3518.23 ± 28.02a	2364.94 ± 31.81c
43.11	Quercetin-3-glucoside	5883.32 ± 85.33e	2632.28 ± 6.30f	7174.82 ± 23.67d	13284.00 ± 418.42b	14564.76 ± 394.05a	10729.98 ± 394.05c
Sum		11366.28 ± 165.04	3357.16 ± 32.58	9383.62 ± 36.85	19716.23 ± 522.91	21641.39 ± 487.67	15474.00 ± 538.38

Mean value of three replicates ± standard deviation; a-f = Different lowercase letters indicate significant differences between solvents ($P < 0.05$); Sum = The sum of determined phenolic compounds ± standard deviation.

Table 4 Phenolic compounds of *C. micranthum* obtained using acidic NADESs and HAE (mg/L).

RT (min)	Phenolic compounds	Solvents					
		Water	Ethanol	Methanol	ChAc	ChLa	ChTa
4.87	Gallic acid	976.03 ± 8.72a	28.80 ± 0.64d	105.61 ± 1.67c	771.12 ± 30.06b	779.07 ± 33.72b	741.31 ± 28.26b
14.29	Catechin	45.96 ± 0.68b	38.72 ± 2.16c	12.81 ± 1.02e	41.85 ± 0.54cb	29.93 ± 1.51d	61.64 ± 3.29a
16.34	Hydroxybenzoic acid	63.05 ± 1.70a	0.75 ± 0.01e	11.82 ± 0.78d	32.07 ± 0.80c	35.84 ± 0.58b	12.92 ± 1.67d
17.86	Chlorogenic acid	19.17 ± 0.09e	11.22 ± 0.06f	27.03 ± 0.80d	66.79 ± 1.60b	73.68 ± 1.08a	59.11 ± 0.56c
21.05	Caffeic acid	112.02 ± 8.58a	1.31 ± 0.09e	27.21 ± 0.79d	81.93 ± 1.42b	80.94 ± 3.99b	59.59 ± 2.28c
22.01	Vanillic acid	40.23 ± 0.37c	18.50 ± 0.05e	29.71 ± 0.10d	78.40 ± 1.43a	77.95 ± 0.53a	62.52 ± 1.76b
24.76	Epicatechin	102.07 ± 0.93c	39.22 ± 0.32e	41.83 ± 0.23d	103.45 ± 1.26c	108.26 ± 0.47b	113.03 ± 0.07a
25.90	Syringic acid	40.84 ± 0.37d	8.25 ± 0.19f	20.46 ± 0.42e	45.82 ± 1.70b	49.80 ± 0.12a	43.09 ± 0.19c
33.85	p-coumaric acid	779.97 ± 43.07d	101.51 ± 6.91f	323.80 ± 7.14e	1032.19 ± 23.83b	1168.25 ± 10.84a	847.81 ± 19.32c
39.16	Ferulic acid	505.10 ± 10.63d	44.64 ± 0.67f	226.70 ± 9.31e	735.85 ± 19.44b	789.01 ± 21.87a	561.56 ± 29.06c
40.73	Sinapic acid	1991.51 ± 134.66d	231.05 ± 10.82f	901.56 ± 22.02e	2785.22 ± 95.34b	3160.09 ± 31.09a	2391.19 ± 72.90c
43.11	Quercetin-3-glucoside	5274.02 ± 117.00d	1879.64 ± 28.89e	5688.54 ± 179.41d	14711.80 ± 93.55b	15915.97 ± 158.65a	11383.01 ± 432.67c
Sum		9949.97 ± 326.80	2403.61 ± 50.81	7417.08 ± 223.69	20487.15 ± 178.62	22268.79 ± 264.45	16336.78 ± 592.03

Mean value of three replicates ± standard deviation; a-f = Different lowercase letters indicate significant differences between solvents ($P < 0.05$); Sum = The sum of determined phenolic compounds ± standard deviation.

the extraction yield of bioactive compounds from plants (Dai et al., 2013). Moreover, the carboxylic acid-based NADESs have been reported to be highly polar which therefore facilitate the extraction of polar substances such as phenolic compounds (Bubalo et al., 2016; Chanioti and Tzia, 2018).

3.3.2. Recovery of *C. micranthum* phenolic compounds using acidic NADESs and UAE

The effect of ultrasound-assisted extraction (UAE) on the recovery of *C. micranthum* phenolic compounds with acidic NADESs and conventional solvents was shown in Table 3. The high extraction of phenolic compounds by applying UAE is due to the known acoustic cavitation phenomenon and the bubbles generated on the solid surface, resulting in the disruption of cell walls, penetration of the solvent into the plant material and the release of the phenolic compounds (Chanioti and Tzia, 2017). The efficiency of UAE on the recovery of phenolic compounds from *C. micranthum* was significantly affected by the nature of the solvents ($p < 0.05$). The highest amounts of gallic acid, hydroxybenzoic acid, caffeic acid and syringic acid were extracted with water, followed by ChLa, ChAc, ChTa, methanol and ethanol. Whereas, the highest amounts of chlorogenic acid, vanillic acid, epicatechin, ρ-coumaric acid, ferulic acid, sinapic acid and quercetin-3-glucoside were extracted with ChLa, followed by ChAc, ChTa, methanol, water and ethanol. In addition, catechin was mostly extracted in ethanol, followed by ChTa, ChAa, water, ChLa and methanol. As can be observed, the amount of the target compounds changed greatly depending on the type of solvents. It is well known that solvent polarity, pH and hydrophilicity play a key role in incrementing the solubility of phenolic compounds. Moreover, NADES composition, hydrogen-bunding ability, surface tension and viscosity are important in the extraction of phenolics from plant materials (Dai et al., 2013; Cui et al., 2018). The acidic NADESs combined with UAE provided the best extraction yield of phenolic compounds from *C. micranthum* compared to the conventional solvents. These findings are in the same agreement with the previous studies which reported that the acidic NADESs are prominent for the recovery of phenolic compounds (Radošević et al., 2016; Bosiljkov et al., 2017; Chanioti and Tzia, 2017; Zannou and Koca, 2020). In general, the behavior observed for the sum of the investigated phenolic compounds was: ChLa > ChAa > ChTa > water > methanol > ethanol.

3.3.3. Recovery of *C. micranthum* phenolic compounds using acidic NADESs and HAE

The effect of homogenate-assisted extraction (HAE) combined with the acidic NADESs and conventional solvents on the recovery of *C. micranthum* phenolic compounds was shown in Table 4. The performance of the acidic NADESs combined with HAE on the extraction of phenolic compounds from *C. micranthum* varied significantly depending on the type of the solvents ($p < 0.05$). The highest amounts of gallic acid, hydroxybenzoic acid and caffeic acid were found with water, followed by ChLa, ChAc, ChTa, methanol and ethanol. The best extraction of vanillic acid, ρ-coumaric acid, ferulic acid, sinapic acid and quercetin-3-glucoside were observed with ChLa, followed by ChAc, ChTa, water, methanol, and ethanol. The highest recovery of chlorogenic acid was achieved with ChLa, followed by ChAc, ChTa, methanol, water and ethanol. In addition, the highest amount of catechin was obtained with ChTa, followed by water, ChAc, ethanol, ChLa and methanol, while epicatechin was mostly extracted ChTa, followed by ChLa, ChAc, water, methanol, and ethanol. The HAE is a good dispersion method since it disperses uniformly the sample in the solvent, softens the sample, facilitate penetration of the solvent into the inner part of the matrix and extract the target bioactive compounds from the matrix outer (Duan et al., 2015; Chanioti and Tzia, 2018). The high extraction yield obtained with the application of HAE is related to good disruption of the cell wall due to the stronger mechanical shear and liquid shear exerted by solvent and sample (Duan et al., 2018). The acidic NADESs combined with HAE provided the highest extraction efficiency compared to the water, methanol and ethanol. The general behavior observed for the sum of the investigated phenolic compounds was: ChLa > ChAa > ChTa > water > methanol > ethanol.

3.4. Correlation uisng Pearson's correlation test

3.4.1. Correlation between total phenolic content and antioxidant capacities

The results of correlation between the independent antioxidant capacities (DPPH and FRAP) were given in Table 5. For all the extraction methods, the correlation coefficients were higher and positive ($R = 0.79–0.89$, $p = 0.018–0.064$) between TPC and DPPH, while they were negative ($R = (-0.17)–(-0.04)$, $p = 0.755–0.946$) between TPC and FRAP. Significant correlations between total phenolic content and DPPH provided strong evidence that the predominant source of antiradical activity derives from phenolic compounds in *C. micranthum*. Sariburun et al. (2010) suggested a strong correlation between total phenolic content and DPPH activity. The phenolic compounds are the most important antioxidants of plant materials acting as primary antioxidants or free radical terminators (Sulaiman et al., 2011). The negative correlation between TPC and FRAP confirmed that phenolic compounds are not the only antioxidants of *C. micranthum* extracts. Sulaiman et al. (2011) confirmed that the antioxidant capacity depends on several factors including the extraction solvent, hydrophilicity of compounds, plant material and type of phenolic compounds. The type and concentration of the phenolic compounds and the presence of non-phenolic antioxidants should be accountable of the antioxidant capacity of plant material and extracts (Socha et al., 2009; Sulaiman et al., 2011). Obviously, the non-phenolic antioxidants in *C. micranthum* including amino acids, minerals and vitamins (vitamins C and E) might contribute to the FRAP activity.

3.4.2. Correlation between total flavonoid content and antioxidant capacities

Table 5 showed the Pearson's correlation coefficients among TFC and independent antioxidant capacities (DPPH and FRAP) in *C. micranthum* extracts. The correlation coefficients between TFC and DPPH were positively significant and moderate ranging from 0.60 to 0.68 ($p = 0.136–0.213$) for UAE and HAE, while for the extraction method ME, the correlation coefficient found TFC and DPPH was negative ($R = -0.04$, $p = 0.945$). It can be concluded that although the hydrogen donating abilities of the extracts might depend on TFC, the extraction methods influence this correlation. The correlations

Table 5 Pearson's correlation test showing the relationship between antioxidant activities and TPC, TFC and phenolic compounds.

	Extraction methods					
	UAE		HAE		ME	
Antioxidant activity Factors	DPPH	FRAP	DPPH	FRAP	DPPH	FRAP
TPC	R = 0.88*; p = 0.021	R = −0.08; p = 0.882	R = 0.79; p = 0.064	R = −0.04; p = 0.946	R = 0.89*; p = 0.018	R = −0.17; p = 0.755
TFC	R = 0.60 ; p = 0.213	R = −0.02; p = 0.972	R = 0.68; p = 0.136	R = −0.57; p = 0.242	R = −0.04; p = 0.945	R = −0.65; p = 0.161
Gallic acid	R = 0.43; p = 0.165	R = −0.022; p = 0.947	R = 0.66*; p = 0.019	R = 0.21; p = 0.506	R = 0.41; p = 0.180	R = −0.07; p = 0.834
Catechin	R = 0.10; p = 0.756	R = 0.06; p = 0.856	R = 0.61*; p = 0.036	R = −0.19; p = 0.546	R = −0.72**; p = 0.008	R = 0.34; p = 0.279
Hydroxybenzoic acid	R = 0.35; p = 0.270	R = 0.21; p = 0.516	R = 0.13; p = 0.695	R = p = 0.454	R = 0.28; p = 0.372	R = 0.06; p = 0.850
Chlorogenic acid	R = 0.80**; p = 0.002	R = 0.56; p = 0.059	R = 0.88**; p < 0.0001	R = 0.37; p = 0.235	R = 0.72**; p = 0.008	R = 0.26; p = 0.41
Caffeic acid	R = 0.34; p = 0.279	R = −0.07; p = 0.82	R = 0.47; p = 0.128	R = 0.20; p = 0.528	R = 0.37; p = 0.241	R = −0.15; p = 0.638
Vanillic acid	R = 0.88**; p < 0.0001	R = 0.45; p = 0.141	R = 0.88**; p < 0.0001	R = 0.36; p = 0.254	R = 0.76**; p = 0.004	R = 0.26; p = 0.407
Epicatechin	R = 0.86**; p < 0.0001	R = 0.29; p = 0.368	R = 0.84**; p = 0.001	R = 0.25; p = 0.438	R = 0.87**; p < 0.0001	R = −0.10; p = 0.751
Syringic acid	R = 0.66*; p = 0.020	R = 0.05; p = 0.874	R = 0.80**; p = 0.002	R = 0.31; p = 0.330	R = 0.59*; p = 0.045	R = −0.01; p = 0.971
ρ-coumaric acid	R = 0.78**; p = 0.003	R = 0.41; p = 0.189	R = 0.80**; p = 0.002	R = 0.41; p = 0.182	R = 0.70*; p = 0.012	R = 0.19; 0.555
Ferulic acid	R = 0.77**; p < 0.003	R = 0.38; p < 0.218	R = 0.80**; p = 0.002	R = 0.39; p = 0.214	R = 0.68*; p = 0.015	R = 0.12; p = 0.707
Sinapic acid	R = 0.81**; p = 0.001	R = 0.36; p = 0.256	R = 0.83**; p = 0.001	R = 0.40; p = 0.199	R = 0.70*; p = 0.012	R = 0.18; p = 0.578
Quercetin-3-glucoside	R = 0.80**; p = 0.002	R = 0.49; p = 0.108	R = 0.84**; p = 0.001	R = 0.40; p = 0.196	R = 0.62*; p = 0.032	R = 0.18; p = 0.587

p = p value for a 2-tailed test; * Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

between TFC and FRAP were negative (R = (−0.65)–(−0.02), p = 0.161–0.972) for all the extraction methods (Table 5), suggesting that the TFC might not actively contribute to FRAP activity.

3.4.3. Correlation between phenolic compounds and antioxidant capacities

The results of Pearson's correlation showing the relationship between phenolic compounds of *C. micranthum* and antioxidant capacities were given in Table 5. For all the extraction methods, the phenolic compounds showed a strong or moderate and positive significant linear relationship with DPPH activity, except catechin obtained with ME which showed a negative correlation. These results supported those reported above for TPC and TFC which displayed a positive significant relationship with DPPH. Chlorogenic acid (R = 0.72–0.88, p < 0.008–0.0001), vanillic acid (R = 0.76–0.88, p < 0.004–0.0001), epicatechin (R = 0.84–0.88, p < 0.001–0.0001), ρ-coumaric acid (R = 0.70–0.80, p < 0.002–0.012), ferulic acid (R = 0.68–0.80, p < 0.003–0.015), sinapic acid (R = 0.70–0.81, p < 0.001–0.012), quercetin-3-glucoside (R = 0.62–0.84, p < 0.032–0.001) and syringic acid (R = 0.59–0.80, p < 0.002–0.045) displayed strong positive linear correlation with DPPH. In addition, gallic acid (R = 0.41–0.66, p < 0.019–0.180) displayed a moderate positive linear correlation with DPPH with hydroxybenzoic acid (R = 0.13–0.35, p = 0.270–0.695) exhibited a weak positive linear correlation with DPPH. These findings indicated that these phenolic compounds are the main components responsible for the antioxidant behavior of *C. micranthum* in terms of DPPH activity. This statistically significant correlation was found in agreement with the findings of other authors (Socha et al., 2009; José Jara-Palacios et al., 2018), who also found a strong relationship between DPPH activity and the above mentioned phenolic compounds of *C. micranthum*. In contrast, the phenolic compounds determined in this study displayed negative or linear weak to moderate correlation with FRAP (Table 5). This result corroborates with the results of TPC and TFC which provided a negative or low correlation with FRAP. Thus, it can be assessed that FRAP values of the studied *C. micranthum* extracts were mostly linked to the non-phenolic antioxidant compounds.

3.5. Correlation and properties of differents extracts obtained with acidic NADESs and conventional solvents using ME, UAE and HAE

Principle component analysis (PCA) was performed to display the correlation of TPC, TFC, the antioxidant capacity and the phenolic compounds extracted from *C. micranthum* by using the green acidic NADESs combined with UAE, HAE and ME (Fig. 2). A total of sixteen variables (TPC, TFC, DPPH,

FRAP and 12 individual phenolic compounds) were used for the PCA, contributing to a total of 79.27% of the total variation extraction experiments. The points on the loading plot assigned the contribution of a variable to the score, while the points on the score plot represented an investigated sample. The first principal component factor (PC1) contributed for 64.4% of the total variation of extraction experiments while the second principal component factor (PC2) described 14.83% of the variations. As can be seen in the PCA biplot, most of the investigated variables had positive effects on PC1, except FRAP, catechin and TFC which had a negative effect on PC1. Gallic acid, chlorogenic acid, quercetin-3-glucoside, vanillic acid, DPPH, ferulic acid, sinapic acid, TPC, ρ-coumaric acid and syringic acid positively correlated with each other. Furthermore, there are positive correlations between TPC, syringic acid, epicatechin, hydroxybenzoic acid, caffeic acid and gallic acid. Likewise, there is a positive correlation between FRAP and catechin.

The coded values of the PCA score plot representing the extracts were shown in Table 6. According to the PCA score plot, 4 main groups of extracts were observed: (1) ME4, ME6, UAE4, UAE5, UAE6, HAE4, HAE5 and HAE 6; (2) ME1, UAE1 and HAE1; (3) ME2, UAE2 and HAE2; and (4) ME3, UAE3 and HAE3. The extracts of the group (1) confirmed that the best efficiencies were achieved with UAE, HAE and ME combined with ChLa ChAc and ChTa. These extracts

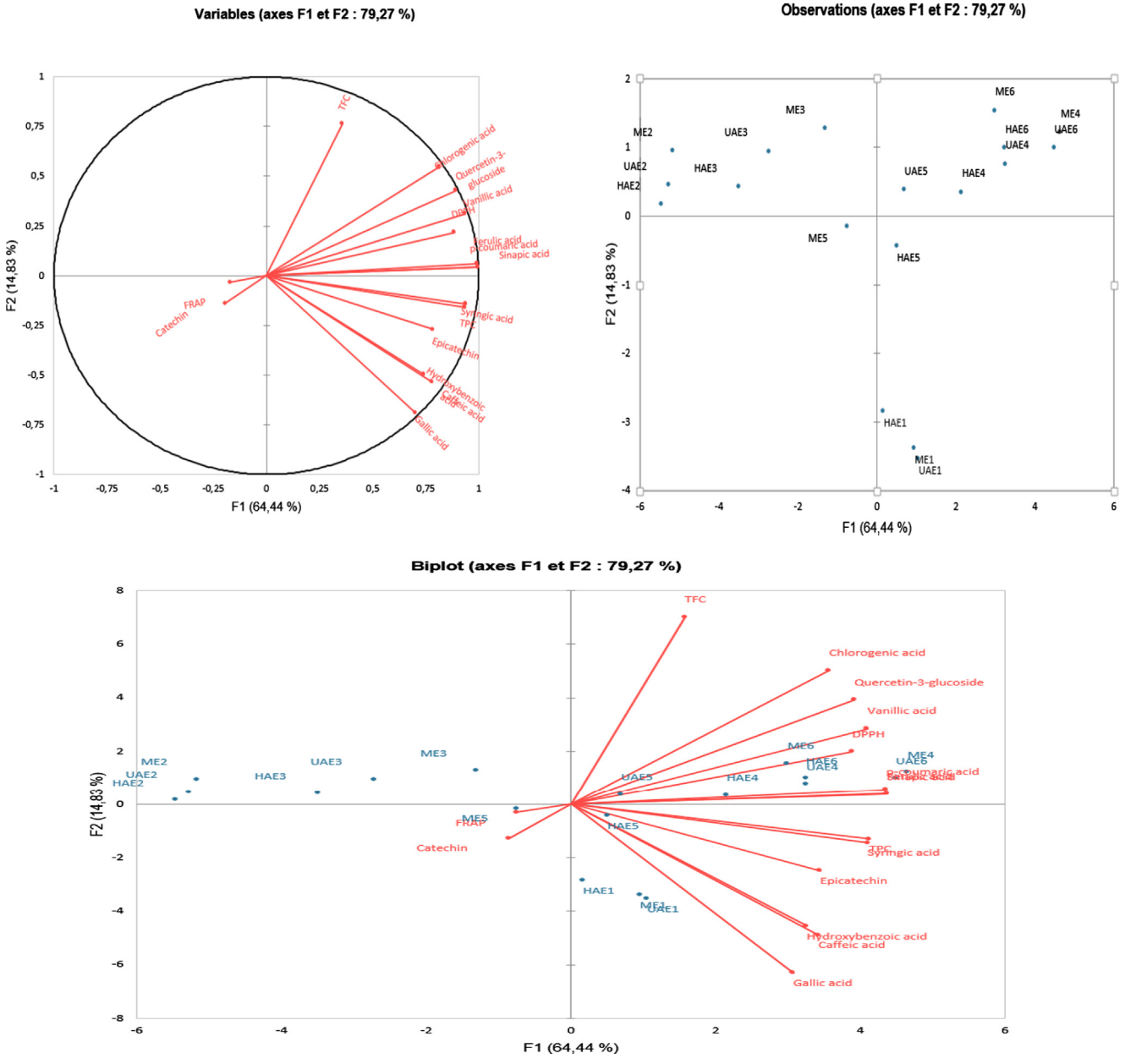

Fig. 2 PCA biplot of the extracts obtained from *C. micranthum* with NADESs and conventional solvents combined with ME, UAE and HAE.

Table 6 Code of solvents and extracts used on principal components analysis (PCA).

Solvents	Extraction methods	Extracts	Code
Water	Maceration extraction	ME-water	ME1
Ethanol		ME-ethanol	ME2
Methanol		ME-methanol	ME3
ChAc		ME-ChAc	ME4
ChTa		ME-ChTa	ME5
ChLa		ME-ChLa	ME6
Water	Ultrasound-assisted extraction	UAE-water	UAE1
Ethanol		UAE-ethanol	UAE2
Methanol		UAE-methanol	UAE3
ChAc		UAE-ChAc	UAE4
ChTa		UAE-ChTa	UAE5
ChLa		UAE-ChLa	UAE6
Water	Homognate-assisted extraction	ME-water	HAE1
Ethanol		ME-ethanol	HAE2
Methanol		ME-methanol	HAE3
ChAc		ME-ChAc	HAE4
ChTa		ME-ChTa	HAE5
ChLa		ME-ChLa	HAE6

conditions were particularly efficient for the recovery of TFC, chlorogenic acid, gallic acid, quercetin-3-glucoside, vanillic acid, DPPH, ferulic acid, sinapic acid, TPC, ρ-coumaric acid and syringic acid. The extraction time (12 h) applied during the maceration extraction was quite long for the solvents to penetrate and break the cell walls of the raw material, allowing the analytes to dissolve efficiently in the solvents. As can be acknowledged, the great performance of HAE at a short time (30 min) could be related to both processes of raw material-pulverizing with mechanical shear force and mixing of solid with solvent (Sun et al., 2017; Duan et al., 2018; Chanioti and Tzia, 2018). Likewise, the highest performances achieved by UAE at a short extraction time (30 min) could be attributed to the ultrasound waves that disrupted the plant cell walls, increased the solvent penetration and improved the mass transfer between the solvents and solute (Hossain et al., 2012; Altemimi et al., 2016).

The values of the group (2) revealed that the extracts obtained with water using ME, UAE and HAE showed similar characteristics of syringic acid, TPC, epicatechin, hydroxybenzoic acid caffeic acid and gallic acid. ME, UAE and HAE were found effective for the extraction of these phenolic compounds. The highest recovery of these phenolic compounds could be attributed to their high hydrophilic properties (Deng et al., 2012; Rodríguez-Roque et al., 2015). The samples of groups (3) and (4) indicated that the extraction of phenolic compounds from *C. micranthum* using ME, UAE and HAE combined with pure methanol and ethanol was less efficient. In addition, the lowest yields were determined with the ethanolic extracts. The previous studies have demonstrated that the extraction of antioxidants such as phenolic compounds with pure methanol or ethanol is less efficient and many authors have suggested the addition of 10–50% of water to pure methanol or ethanol in order to improve their efficiency in extracting the bioactive compounds (Odabaş and Koca, 2016; Kumar and Srinivasa Rao, 2020; Phuong et al., 2020; Saha et al., 2020).

4. Conclusion

In the present study, the extraction efficiency of the phenolic antioxidants of *C. micranthum* was investigated using a combination of three green acidic NADESs (choline chloride-lactic acid (ChLa), choline chloride-acetic acid (ChAa) and choline chloride-tartaric acid (ChTa)) combined with maceration (ME), homogenate-assisted (HAE) and ultrasound-assisted (UAE) extraction methods. The results revealed that the combination of NADESs with MAE, HAE and ultrasounds (UAE) were promising and efficient media for the extraction of phenolic compounds from *C. micranthum*. ChLa exhibited the highest performance giving the TPC (21.12 ± 0.13–23.62 ± 0.58 mg GAE/g, followed by ChAc (15.49 ± 0.13–18.85 ± 0.39 mg GAE/g), water (17.08 ± 0.32–18.13 ± 0.13 mg GAE/g), ChTa (14.49 ± 0.26–17.44 ± 0.19 mg GAE/g), methanol (7.46 ± 0.45–11.64 ± 0.32 mg GAE/g) and ethanol (2.88 ± 0.39–4.60 ± 0.39 mg GAE/g), respectively. For TFC, ChLa (4.38 ± 0.09–5.01 ± 0.09 mg ECE/g) was the most prominent solvent, followed by ChAc (2.84 ± 0.04–5.01 ± 0 .36 mg ECE/g), methanol (1.93 ± 053–4.85 ± 0.04 mg ECE/g), ethanol (1.49 ± 0.36–4.16 ± 0.04 mg ECE/g), ChTa (1.09 ± 0.04–3.22 ± 0.13 mg ECE/g) and water (1.15 ± 0.04–1.37 ± 0.44 mg ECE/g), respectively. The highest yield of TPC was determined with UAE-ChLa, followed by ME-ChLa and HAE-ChLa, while The highest yield of TFC was found with ME-ChLa, followed by ME-ChAa and UAE-ChLa. The total antiradical activity was 73.33 ± 19.69–25 5.22 ± 3.94 mmol TE/g, while the FRAP was found as 43.60 ± 3.7 3–160.27 ± 0.39 mmol ISE/g. The best yield of DPPH radical scavenging activiy was found with UAE-ChLA, followed by ME-ChLa and HAE-ChLa, whereas the best yield of FRAP was achieved with ME-ChTa, followed by UAE-ChTA and HAE-ChTA. The extracts obtained from NADESs were found to be more enriched in phenolic compounds as compared with the conventional solvents such as water, ethanol and methanol. Homogenate-assisted extraction and ultrasound-assisted extraction achieved similar performance to that of maceration very short extraction time. The evaluated acidic NADESs offered sustainability and greener extractability of phenolic compounds from *C. micranthum* leaves. Moreover, the association of these NADESs with homogenate-assisted extraction and ultrasound-assisted extraction are prominent to increase significantly the extraction efficiencies in a very reduced time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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