GCTOF-MS and HPLC Identification of Phenolic Compounds with Different Fractional Extracts of *Lepironia articulata*

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Abstract: Cyperaceae species have an intrinsic value as a source of active elements with biological activity from the family of monocotyledonous known as sedges. Sedges grow in all types of soils associated with wetlands or poor soils. The aim of this present study is to evaluate the content of phenolic compounds by qualitative and quantitive analysis on *Lepironia articulata*. Dried leaves of *L. articulata* were successfully extracted by using water extraction then separated with different solvent polarities; petroleum ether, ethyl acetate and butanol fractions before being analysed using GCTOF-MS, microplate reader and HPLC. The result from the GCTOF-MS analysis of fractional extracts showed that 48 compounds were found in petroleum ether, ethyl acetate and butanol extracts. From those extracts, only six phenolic compounds were identified in ethyl acetate and butanol extracts which were 2-Methoxy-4-vinylphenol, Phenol, 2,4-bis(1,1-dimethylethyl)-, 4-Hydroxybenzaldehyde, Catechol, Phenol, 2-methoxy- and Vanillin. The total phenolic content was found to be 984.63 ± 5.96 µg GAE/g DW in *L. articulata*. Quantitative analysis of individual phenolic acid by HPLC showed the predominant amount of Vanillic acid (0.48 ± 0.00 µg/g DW) in ethyl acetate while 4-Hydroxybenzoic acid and Caffeic acid, both of which were 0.12 ± 0.00 µg/g DW in butanol extracts. In the present study, the plant extracts demonstrated the highest phenolic compound detected in ethyl acetate and butanol.

Keywords: Phenolic compound, phenolic acid, *Lepironia articulata*, HPLC, GCTOF-MS, total phenolic content.

INTRODUCTION

Aquatic plants consist of an assemblage of diverse taxonomic groups including pteridophytes (fern), bryophytes (mosses, hornworts and liverworts), and angiosperm (flowering plants) that are dominant in wetlands, shallow lakes, pond marshes, streams and lagoons [1, 2]. Emergent macrophytes occur on aerial or submerged soil with the water table 0.5m below the soil. Floating leaved macrophytes are angiosperm that appear on submerged sediment at the water depth of about 0.5 to 3 m. Submerged macrophytes comprise of pteridophytes, mosses and charophytes as well as the angiosperms. Free-floating macrophytes live unattached in water with aerial roots and floating leaves, with well-developed submerged roots, with few roots or without any roots [1, 3-5]. The Cyperaceae family is mostly found in marshy places that comprise 4000 species with 90 genera and are traditionally used as food or medicine [6]. In ecological approaches, wetland plant species have the ability to take up and retain nutrients, particulate matter and other pollutants discharged from greywater [7]. Their function is to form and regulate water quality and oxygen content in natural water bodies [8]. *Lepironia articulata* also known as *rumput kercut* in Malay, derived from the family of Cyperaceae, was found abundant in Terengganu and Putrajaya freshwater lakes [9]. These species become the pioneer producer in a large tropical swamp in West Malaysia [10]. Plants synthesize various phytochemicals such as phenols, flavonoids, alkaloids, tannins, vitamins and carotenoids [11]. As reported by [12], the submerged plant species showed a significantly lower phenolic content compared to emergent or floating species. Phenolic are secondary plant compounds that are important for plant defence against pathogens and herbivores that consists of a hydroxyl group bonded directly to an aromatic hydrocarbon group [13]. Biosynthesis and accumulation of phenolic compounds arise from the highly regulated process via the cell and/or tissue, development and environment-specific controls [14]. Phenol contamination of water bodies has serious environmental implications because of the damaging effects it has on aquatic organisms [15]. Phenolic has been used as a components in dyes, polymer, drugs and other organic substances while their existence in an ecosystem has a relationship with production and

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degradation of numerous pesticides, industrial and municipal sewage [16]. Therefore, this study aimed to identify phenolic compounds profiles from *L. articulata* leaves extract as new sources of dye component in order to be used as natural colorants.

MATERIALS AND METHODS

Sample Preparation

The leaves of the *L. articulata* were removed with a grass cutter and a random 1000 g fresh weight sample was immediately frozen at -20 °C. The leaves samples were freeze-dried for three days, after which the samples were ground into a fine powder and stored at -20 °C until further analysis.

Extraction of Phenolic Compounds

The extraction procedure essentially followed the methods described by [17, 18] with the following modification. For each sample, 10 g of powdered freeze-dried material was mixed with 100 ml of distilled water and then the sample was incubated in an oven at about 60°C for 30 min before being allowed to stand overnight in darkness at room temperature and then the clear supernatants re-extracted with different solvents polarities; petroleum ether, ethyl acetate and butanol.

Separation of Phenolic Compounds

The phenolic compounds were isolated from *L. articulata* water extract according to the method of [18]. The crude extraction (clear supernatant) was extracted using petroleum ether, ethyl acetate and butanol using a funnel separator. For further analysis, the final concentration was resuspended with 5 ml of methanol in tubes then capped and sealed with parafilm to exclude oxygen and immediately stored at -20°C until subsequent analysis. 50 µL of the re-dissolved sample was then transferred in a vial for further analysis by gas chromatography-time of flight mass spectrometry (GCTOF-MS), total phenolic content (TPC) and high-performance liquid chromatography (HPLC).

Determination of GCTOF-MS Analysis

The composition of leaf extracts from *L. articulata* species was qualitatively determined by GCTOF-MS (Agilent 7890 system), equipped with a capillary column (30 m x 0.25 mm, 0.25 µm), based on the method reported by [19]. Split-less injection of a 1.0 µL sample was performed with a purge time of 1.0 min. The solvent delay was set at 4 min. The carrier gas used was helium at the flow rate of 1.0 mL min^{-1}. The column temperature was initially maintained at 80°C for 2 min, then programmed at 5°C min^{-1} to 80 °C min^{-1} and then at 10 °C min^{-1} to 250 °C. The inlet temperature and detector sets were 220 °C and 340 °C, respectively. The time-of-flight mass spectrometer was operated at 1 spectrum/s, acquiring the mass range m/z 50-1000. The identification of the peaks was based on mass spectra of >90 % similarity index with the National Institute of Standards and Technology library (NIST 14) and by comparison with published data.

Determination of Total Phenolic Content (TPC)

TPC was determined by the Folin-Ciocalteau assay as reported by [20]. 90 µL of Folin-Ciocalteu reagent was diluted in deionised water (20% v/v) and had been placed in each well of a flat-bottomed 96-well clear microplate. After that, 1.0 mg/g DW of the sample that was diluted with distilled water (1000 µg/mL) was added and incubated at room temperature for 5 minutes. Next, 90 µL of sodium carbonate in deionised water (7.5% w/v) was mixed and incubated for two hours at room temperature. The absorbance of extracts and standard was then read at λmax = 725 nm against a blank (deionised water without extract or standard) using a TECAN microplate reader.

The total phenolic compound (TPC) was determined using a gallic acid calibration curve whereas the domain for gallic acid (GAE) calibration curves was calculated concerning the dilution factor = 11. Twofold serial dilution (five different concentrations) was performed in 30 mL glass vials using a micropipette, and 1.0 mg/g DW of each concentration (GAE) per gram dry weight sample ± standard error of the mean (SEM). The TPC concentrations were expressed in terms of a microgram GAE per 1.0 g dry weight of freeze-dried matter (µg GAE/g DW).

HPLC Analysis of Individual Phenolic Compounds

The HPLC analysis of phenolic acids was performed on an Agilent 1200 series rapid resolution LC system (Agilent Technologies, Palo Alto, CA, USA) that comprises of a binary pump with autosampler injector, micro vacuum degassers, thermostatted column compartment and a diode array detector (DAD) [21]. The column used was a Zorbax Eclipse XDB-C_{18} end-capped 5 µm, 4.6x150 mm reverse phase column (Agilent Technologies, USA). For the analysis, a linear gradient elution was used, with the two mobile phases consisting of 1% formic acid in water/ acetonitrile 90:10

Table 1: Volatile Compounds Profiles in Different Fractional of Water Extract of *L. articulata* Leaves

Fractional extracts	Volatile compounds	Formula	Exact mass
Petroleum ether	Diallyl carbonate	$C_7H_{10}O_3$	142.06
	2-Decanol	$C_{10}H_{22}O$	158.17
	5-Amino-1-pentanol	$C_5H_{13}NO$	103.10
	1,6-Anhydro-β-D-glucopyranose	$C_6H_{10}O_5$	162.14
	Methoxyphenamine	$C_{11}H_{17}NO$	179.13
	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17
	Pentadecane	$C_{15}H_{32}$	212.25
	(2-Butoxyethoxy)acetic acid	$C_8H_{16}O_4$	176.10
	Hexadecane	$C_{16}H_{34}$	226.44
	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.17
Ethyl acetate	Urea	CH_4N_2O	60.03
	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.15
	4-Hydroxybenzaldehyde	$C_7H_6O_2$	122.12
	1,6-Anhydro-β-D-glucopyranose	$C_6H_{10}O_5$	162.14
	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17
	Catechol	$C_6H_6O_2$	110.11
	Phenol, 2-methoxy-	$C_7H_8O_2$	124.05
	Tryptophol	$C_{10}H_{11}NO$	161.08
	Hexadecane	$C_{16}H_{34}$	226.44
	Phenylethyl alcohol	$C_8H_{10}O$	122.07
	Benzoic acid	$C_7H_6O_2$	122.04
	Hydroquinone	$C_6H_6O_2$	110.04
	Vanillin	$C_8H_8O_3$	152.14
	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
	9,12,15-Octadecatrienal	$C_{18}H_{30}O$	262.37
	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.17
	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	138.07
	4-Ethylcatechol	$C_8H_{10}O_2$	138.07
	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.27
Butanol	Urea	CH_4N_2O	60.03
	Methanamine, N-methoxy-	C_2H_7NO	61.05
	4-Hydroxybenzaldehyde	$C_7H_6O_2$	122.12
	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17
	Acetic acid, (acetyloxy)-	$C_4H_6O_4$	118.03
	Oxalic acid, allyl hexyl ester	$C_{11}H_{18}O_4$	214.12
	1-Nitro-2-propanone	$C_3H_5NO_3$	103.03
	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.17
	Hydroquinone	$C_6H_6O_2$	110.04
	Phenylethyl Alcohol	$C_8H_{10}O$	122.07
	Benzeneacetic acid	$C_8H_8O_2$	136.05

v/v (phase A) and acetonitrile (phase B) using the following gradient: 0-20 min, linear gradient from 0% B to 40% B; 20-25 min, linear gradient from 40% B to 60% B; 25.10-35 min, linear gradient from 100% B to 100% B and 35.10-40 min, isocratic of 0% B. The temperature of the column was set at 25°C. The injection volume was 20 µL, and the flow rate was set at 1.0 mL min^{-1}. Phenolic acid standards of Caffeic acid, Ferulic acid, trans-p Coumaric acid, 2-Coumaric acid, 4-Coumaric acid, Hydroxybenzoic acid and Vanillic acid were purchased from Sigma-Aldrich. The individual phenolic acids were detected at the wavelength of maximum absorption of the phenolic acids in the mobile phase at 280 nm by using photodiode array detection. Individual phenolic acid concentrations were calculated by comparing their relative proportions, as reflected by integrated HPLC peak areas. The individual phenolic acid compound concentrations were expressed in terms of a microgram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

RESULTS AND DISCUSSION

GCTOF-MS Analysis of Volatile Compounds

The GCTOF-MS analysis from the leaves of *L. articulata* (sedge) revealed the presence of 40 compounds belonging to different chemical groups, their formula and exact masses by referring to the National Institute Standard and Technology (NIST) library data. The data is reported in Table **1** with different extracts identified in petroleum ether, ethyl acetate and butanol extracts. The result was based on >90% similarity with NIST library data. The volatile compounds of water extract detected from *L. articulata* were 6 different phenolic compounds. Two phenolic compounds found in the petroleum ether extract were 2-Methoxy-4-vinylphenol and Phenol, 2,4-bis(1,1-dimethylethyl)-. Five phenolic compounds identified in ethyl acetate extract were 4-Hydroxybenzaldehyde, 2-Methoxy-4-vinylphenol, Catechol, Phenol, 2-methoxy-, Vanillin and Phenol, 2,4-bis(1,1-dimethylethyl)- while two phenolic compounds butanol extract were 4-Hydroxybenzaldehyde, 2-Methoxy-4-vinylphenol and Phenol, 2,4-bis(1,1-dimethylethyl)-. There was a report mentioned that phenolic compound is associated with antioxidant activity and play an important role to stabilise lipid peroxidation and positive relationship between total phenolic and antioxidant activity found in many plant species [22].

Total Phenolic Content and HPLC Analysis of Individual Phenolic Compound

The total phenolic content (TPC) of *L. articulata* was determined using the Folin-Ciocalteau method was presented in Table **2**. Based on the value of TPC, *L. articulata* contain 984.63±5.96 µg GAE/g DW. The HPLC chromatogram of the water extract of *L. articulata* was shown in Table **2**. The results indicated that 4-Hydroxybenzoic acid and Caffeic acid were the two major compounds that exist in water extraction of *L. articulata* leaves even though their concentrations were low (<0.20 µg/g DW). No trace of other phenolic acids was found in petroleum ether extract. The highest result for individual phenolic was Vanillic acid

Table 2: Total (µg GAE/g DW) and Individual Phenolic Content (µg/g DW) of *L. articulata* in Water Extraction for Different Fractional Polarities Extracts

Total phenolic (µg GAE/g DW)	Fractional extracts	4-Hydroxybenzoic acid (µg/g DW)	Caffeic acid (µg/g DW)	Vanillic acid (µg/g DW)	*trans-p*-Coumaric acid (µg/g DW)	Ferulic acid (µg/g DW)	3-Coumaric acid (µg/g DW)	2-Coumaric acid (µg/g DW)
984.63±5.96	PE	ND	ND	ND	ND	ND	ND	ND
	EA	0.12± 0.00	0.13± 0.01	0.48± 0.00	0.08± 0.00	0.17± 0.00	ND	ND
	B	0.12± 0.00	0.12± 0.00	ND	ND	ND	ND	ND

Note: PE (Petroleum ether); EA (Ethyl acetate); B (Butanol); ND (Not detected).

(0.48±0.00 µg/g DW) in ethyl acetate extract; meanwhile, the lowest was *trans-p*-Coumaric acid (0.08±0.00 µg/g DW) that was also detected in ethyl acetate extract. The similarity among those fractional extracts in this study for *L. articulata* showed that 2-Coumaric acid and 3-Coumaric acid were not detected in all fractional extracts (Table **2**). Results from the present research showed that among all solvent extracts, ethyl acetate has the highest phenolic content and it decreased with decreasing polarity. A non-polar solvent like ethyl acetate demonstrated to extract phenolic compounds in a higher amount from onions and citrus peel [23]. The polarity of the solvent is an important factor that affects the extraction yield [24]. Besides, the concentration of phenolic acids in the soils depends on the cropping system and the depth of the soil profiles [25]. According to [12], a semi-aquatic plant is the most “expensive” whereby floating and emergent plants invest more energy in chemical defences than submerged plants due to decreased light and carbon availability in water.

CONCLUSION

Phenolic compounds can be extracted from the sedges as seen in this study where 6 different phenolic compounds were extracted from *L. articulata*. Moreover, 4-Hydroxybenzoic acid and Caffeic acid were the two major compounds that exist in water extraction whereas Vanillic acid and trans-p-Coumaric acid were detected in ethyl acetate extract. Hence, it can be ascertained that *L. articulata* can be exploited as a new source of natural colorants that have the potential uses in the dye and food colorant industries.

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