Solvent Extraction and Its Effect on Phytochemical Yield and Antioxidant Capacity of Woody Medicinal Plant, *Polyalthia bullata*

Munirah Adibah Kamarul Zaman,[a] Azzreena Mohamad Azzeme,[a,*] Siti Nurhafizah Ramli,[a] Noor Azmi Shaharuddin,[a,c] Syahida Ahmad, and Siti Nor Akmar Abdullah [b,c]

Polyalthia bullata is a woody medicinal plant that contains antioxidant compounds. Finding a suitable solvent is important to obtain a high yield of antioxidants in the phenolic, flavonoid, and terpenoid families. In this study, from different solvent extracts, the leaf methanolic extract exhibited the highest total phenolic content (TPC), total flavonoid content (TFC), total terpenoid content (TTC), and total antioxidant activity. For woody parts of stem and roots, methanol was the best solvent for all phytochemicals except for phenolics, which accumulated in the roots and were extracted more efficiently using ethanol. However, the methanolic extracts from both tissues displayed the best antioxidant capacity. Gas chromatography-mass spectrometry (GC-MS) profiling data showed the presence of antioxidant compounds such as thymol, phytol, and neophytadiene in the leaf; *trans*-farnesol, n-hexadecanoic acid, and 9-Octadecenamide in the stem; and fatty acid (*cis*-vaccenic) and its methyl ester (11-Octadecanoic acid, methyl ester and [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester) in the roots. These findings reveal important compounds that are present in different plant parts of *P. bullata*.

Keywords: Polyalthia bullata; Woody medicinal plant; Extraction solvent polarities; Phytochemical compounds; Antioxidant capacity

Contact information: a: Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor; b: Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor; c: Institute of Plantation Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor;
** Corresponding author: azzreena@upm.edu.my; azzreena@yahoo.com.my*

INTRODUCTION

Polyalthia is a distinguished genus of the Annonaceae family. In its Greek origin, "poly" means much or many, and althea is derived from the word altheo, meaning "to cure" (Katkar *et al.* 2010). The genus comprises 120 species of shrubs and trees. Most of them are grown in tropical and subtropical regions in South and South-Eastern Asia, Australia, Africa, and New Zealand (Katkar *et al.* 2010; Mohamad *et al.* 2017). The genus *Polyalthia* contains numerous phytochemicals including alkaloids, flavonoids, triterpenoids, and lipids (Paarakh and Khosa, 2009; Jothy *et al.* 2013). The presence of clerodane diterpenoids and alkaloids are significantly associated with the medicinal importance of *Polyalthia* (Katkar *et al.* 2010). Various parts of the *Polyalthia* genus are used in traditional medication to treat ailments such as helminthiasis, stomach ache, pharynx neurosis, dysmenorrhoea, diabetes, skin disease, rheumatic fever, hypertension, gastrointestinal ulcer, and generalized body pain (Wu *et al.* 2016; Yao *et al.* 2019).

Polyalthia bullata is a woody plant species. The plant grows to two- to three-meters

in height, mainly in lowland of primary and secondary forests located in Peninsular Malaysia and Sabah. In Southeast Asia, particularly in Malaysia and Indonesia, the *P. bullata* flower, root, and leaf are used to treat diabetes, high blood pressure, and liver diseases. Also, the root is well known to have an aphrodisiac property, which can boost male sexual desires (Virmala 2013). Findings by Connolly *et al*. (1996) and Paarakh and Khosa (2009) revealed three alkaloids in *P. bullata* stem: urabaine, 7,7′-bisdehydro-O-methylisopiline, and 7-dehydronornuciferine-7′-dehydro-O-methylisopiline. Nantapap *et al*. (2017) reported three types of flavones extracted from the aerial part of *P. bullata*: 5-hydroxy-3,7,4’-trimethoxyflavone, 5,3’-dihydroxy-3,7,4’-trimethoxyflavone, and 5,3’,4’-trihydroxy-3,7-dimethoxyflavone, which exhibit anticancer activities.

Some phytochemicals are antioxidants, and some of them are unique to plant species. Humans consume antioxidants for reducing chances of getting oxidative damages due to elevated production of reactive oxygen species (ROS). The imbalance of ROS and antioxidants in cells causes damage to primary biomolecules such as proteins, nucleic acids, and lipids (Srinivasan 2014; Phaniendra *et al.* 2015; Ramlan *et al.* 2017). This condition can certainly cause abnormal cell function, tissue damage, and induction of diseases like cancer, diabetes, Alzheimer’s, inflammation, and obesity (Liguori *et al.* 2018). As prevention, consumers are searching for medicinal plant-based supplements to replenish antioxidant compounds in the body (Saeed *et al.* 2012). However, the extraction yields are always affected by the solvent used.

Selecting the best solvent for phytochemical extraction is crucial due to the presence of phytochemicals with different chemical structures and polarities, which may influence their solubility in the selected solvent. Choosing the best solvent extraction can maximize the yield of phytochemicals and antioxidants (Fatiha *et al.* 2012; Pham *et al.* 2015). Water, methanol, ethanol, acetone, and a mixture of these organic solvents with water are commonly used for phytochemical extraction (Boeing *et al.* 2014). The increase of solvent polarity from hexane to distilled water (hexane < ethanol < methanol < distilled water) further suggests the influence of solvent polarity towards solubility of phytochemical compounds. In this study, phytochemicals in different *P. bullata* tissues were quantified by extracting them using different extraction solvent polarities. The extracts were examined their antioxidant capacity, and the best solvent extract was used for phytochemical profiling.

EXPERIMENTAL

Plant Materials and Crude Extract Preparation

The *P. bullata* plant was obtained from Herbal Nursery located at Pahang, Malaysia. The plants were acclimatized in a greenhouse located at Universiti Putra Malaysia under ambient temperature for a month prior to analysis. The leaf, root, and stem were randomly sampled and oven dried at 45 °C until a constant weight was obtained. The dried leaf, root, and stem were ground using a blender, and 10 g of dried *P. bullata* powder was weighed and placed in a dark container containing 250 mL of solvents with different polarities (100% v/v methanol, 100% v/v ethanol, and 100% v/v hexane) and distilled water. Each mixture was heated using a Soxhlet apparatus for 8 h to extract phytochemical compounds. The extract was evaporated using a rotary evaporator at 45 °C. The concentrated extract was stored at 4 °C in a dark container.

Determination of Total Phenolics Content (TPC)

The TPC of dried leaf, root, and stem extracts of *P. bullata* was determined using Folin-Ciocalteu method as described by Dian-Nashiela *et al*. (2015). The absorbance was read at 765 nm using a UV-Visible spectrophotometer. The TPC was expressed as mg of gallic acid equivalents (GAE) per gram dry weight (mg GAE/g DW).

Determination of Total Flavonoid Content (TFC)

The TFC was determined using aluminum chloride colorimetric assay as described by Kaur and Mondal (2014). The absorbance was read at 510 nm using a UV-Visible spectrophotometer. The TFC was expressed as mg of quercetin equivalents (QE) per gram of dry weight (mg QE/g DW).

Determination of Total Terpenoid Content (TTC)

The TTC was determined using a sulfuric acid calorimetric assay (Geetha *et al*. 2015). The absorbance was read at 538 nm using UV-Visible spectrophotometer. The total terpenoid content was expressed as linalool equivalent per gram (mg LE/g DW) of dry weight.

Antioxidant Assay

The antioxidant activity was determined using a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging activity assay adapted from Sumazian *et al*. (2010). The initial absorbance of DPPH solution was measured without sample at 517 nm. Approximately 0.2 mL of each sample extract was mixed with 3 mL of 0.1 mM DPPH solution. The mixture was incubated at room temperature in the dark for 30 min. The change in absorbance was measured after 30 minutes of incubation at 517 nm using a UV-Visible spectrophotometer. The results obtained were calculated and expressed in percent of DPPH free radical scavenging activity using the following formula,

$$\% \text{ of DPPH free radical scavenging activity} = \frac{A\ control - A\ sample}{A\ control} \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of DPPH solution without sample, and A_{sample} is the absorbance of sample with DPPH solution.

Phytochemical Profiling of Dried Leaf, Stem, and Root of *P. Bullata* Using Gas Chromatography-Mass Spectrometry (GC-MS)

The phytochemical constituents in concentrated methanolic extract of dried leaf, stem, and root of *P. bullata* was analyzed using GC-MS (Geetha *et al.* 2013). The relative percentage of the extract constituents was expressed as percentage with peak area normalization. The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation pattern with those data stored on the computer library and also with published literatures.

Statistical Analysis

The data of different parameters were subjected to one-way analysis of variance (ANOVA), while the significance of difference between means was determined by Tukey's multiple range tests using SAS 9.4 software (Cary, NC, USA). Values expressed were means of triplicate determination standard error (SE). Different letters indicate the values of significant difference at $p \leq 0.05$.

RESULTS AND DISCUSSION

Plants produce diverse phytochemicals at different concentrations in leaf, stem, flowers, fruits and roots (Altemimi *et al.* 2017; Almeida *et al.* 2019). The yield of extracted phytochemicals also depends on solubility of the compounds in extraction solvents. In this study, different amounts of phenolics, flavonoids, and terpenoids from different plant parts and extraction solvents were observed (Tables 1, 2, and 3). The results suggest the presence of different compound polarities in each of extraction solvent. Among the solvents, methanol displayed the highest capacity in extracting phenolics from *P. bullata* leaf and stem, while ethanol was the best solvent in extracting phenolics from roots. A similar trend was observed for flavonoids in *P. bullata* leaf and stem except for the root; the flavonoids content was highest in the ethanolic extract. These findings suggest a high amount of polar phenolics and flavonoids in *P. bullata*, which might be due to the hydrogen bond formation between hydroxyl groups with electronegative oxygen of methanol and ethanol. The formation of hydrogen bond might also form between hydroxyl group of the methanol and ethanol with oxygen atom located at phenolic and flavonoid structures (Galanakis *et al.* 2013; Thavamoney *et al.* 2018).

Table 1. Total Phenolic Content of *P. bullata* Leaf, Stem, and Root in Different Extraction Solvents

Total Phenolic Content (mg GAE/g DW)			
Extraction solvents	**Leaf**	**Stem**	**Root**
Methanol	1042.52 ± 1.97^{a}	730.18 ± 1.14^{e}	782.02 ± 1.14^{d}
Ethanol	828.61 ± 4.55^{c}	660.63 ± 7.10^{g}	850.26 ± 4.95^{b}
Distilled water	1037.93 ± 1.14^{a}	627.17 ± 1.97^{h}	671.13 ± 3.01^{f}
Hexane	267.59 ± 3.01^{j}	156.04 ± 3.01^{k}	341.73 ± 1.97^{i}

Note: Different letters indicate significant difference at P ≤ 0.05 Tukey's range test

Table 2. Total Flavonoid Content of *P. bullata* Leaf, Stem, and Root in Different Extraction Solvents

Total Flavonoid Content (mg QE/g DW)			
Extraction solvents	**Leaf**	**Stem**	**Root**
Methanol	80.88 ± 0.24^{a}	59.88 ± 0.21^{c}	54.39 ± 0.21^{e}
Ethanol	55.83 ± 0.40^{d}	36.89 ± 0.14^{f}	28.37 ± 0.42^{i}
Distilled water	65.00 ± 0.29^{b}	33.25 ± 0.14^{h}	32.70 ± 0.14^{h}
Hexane	34.56 ± 0.08^{g}	13.79 ± 0.08^{k}	15.94 ± 0.00^{k}

Note: Different letters indicate significant difference at P ≤ 0.05 Tukey's range test

Table 3. Total Terpenoid Content of *P. bullata* Leaf, Stem, and Root in Different Extraction Solvents

Total terpenoid content (mg LE/g DW)			
Extraction solvents	**Leaf**	**Stem**	**Root**
Methanol	0.19 ± 2.49^{a}	0.17 ± 0.00^{c}	0.18 ± 4.31^{a,b}
Ethanol	0.15 ± 2.49^{f}	0.18 ± 2.49^{b,c}	0.16 ± 0.00^{d,e}
Distilled water	0.16 ± 2.49^{d}	0.15 ± 2.49^{d,e,f}	0.15 ± 2.49^{e,f}
Hexane	0.16 ± 2.49^{d}	0.15 ± 4.98^{f}	0.14 ± 4.31^{f}

Note: Different letters indicate significant difference at P ≤ 0.05 Tukey's range test

The methanol and ethanol were the best in extracting phenolics including flavonoids from other plants. The highest amount of phenolic was observed in leaf methanolic extracts of *Zaravschanica membranacea* (268.12 ± 1.04 mg GAE/g DW) and *Ferulago angulata* (72.33 ± 1.14 mg GAE/g DW), followed by leaf ethanolic extracts of *Z. membranacea* (243.38 ± 1.01 mg GAE/g DW) and *F. angulate* (63.72 ± 2.03 mg DAE/g DW) (Rezaei and Ghasemi Pirbalouti (2019). However, the phenolics contents in these plants are lower than in *P. bullata*. In *Mentha spicata*, the flavonoids content was detected higher than *P. bullata* at the amount of 267.33 ± 3.12 mg/g DW in methanolic extract (Bimakr *et al.* 2011) and 218 ± 4.24 mg/g DW in ethanolic extract. The high amount of flavonoids was also observed in methanolic and ethanolic extracts of *Pluchea indica* leaves at concentration of 911.9 ± 65.4 mg CE/g DW and 93.1 ± 2.1 mg CE/g DW, respectively (Widyawati *et al.* 2014). Hence, the present findings display the high amount of total phenolics compared with that of total flavonoids in *P. bullata*, and low amount of flavonoids in the plant.

The solubility of phenolics and flavonoids in extraction solvents depends on functional groups attached to the main structure of these phytochemicals, the molecular size, and the length of hydrocarbon (Iloki-Assanga *et al.* 2015; Enneb *et al.* 2020). For example, the ideal solubility of kaempferol, hesperidin, ferreirin, and 4-hydroxycoumarin extracted from *M. spicata* differed in different extraction solvents, which therefore contributes to the different yields of phenolics and flavonoids in the solvents used (Li and Tian 2018). Moreover, the solvation capacity influences the solubility of phytochemicals. For instance, methanol has better solvation of phenolics and flavonoids than ethanol due to the presence of shorter methyl radical in methanol compared to long ethyl radical in ethanol (Boeing *et al.* 2014).

For terpenoid, even though terpenes usually exhibit high solubility in nonpolar solvents, the presence of the hydroxyl group in terpenes structure does influence the polarity of the compounds, and make terpenes solubilize in polar solvents (Jiang *et al.* 2016). In *P. bullata*, terpenes were quantified in methanolic, ethanolic, hexanic, and distilled water extracts from all plant parts. The presence of terpenes in methanolic extract was also observed in *S. buxifolia* branches (1.25% w/w), followed by ethanolic extract (0.97% w/w) and distilled water (0.43% w/w) (Truong *et al.* 2019).

Phenolics, flavonoids, and terpenes possess antioxidant properties (Takaidza *et al.* 2018). The presence of these compounds in *P. bullata* makes the plant a natural source of antioxidant. Different antioxidant capacity was observed from different solvent extracts, as presented in Table 4. This might be due to the presence of different polar and nonpolar phenolics, flavonoids, and terpenoids, and they might exist at different amounts in different plant parts.

Table 4. DPPH Scavenging Activity of *P. bullata* Leaf, Stem, and Root in Different Extraction Solvents

	DPPH scavenging activity (%)		
Extraction solvents	**Leaf**	**Stem**	**Root**
Methanol	85.19 ± 0.67^{a}	79.98 ± 1.40^{b}	77.96 ± 0.37^{b}
Ethanol	55.93 ± 0.67^{d}	51.67 ± 0.00^{e}	46.11 ± 0.32^{f}
Distilled water	66.11 ± 1.16^{c}	51.11 ± 0.00^{e}	36.85 ± 0.80^{g}
Hexane	30.93 ± 0.49^{h}	20.74 ± 0.74^{i}	35.00 ± 0.00^{g}

Note: Different letters indicate significant difference at P ≤ 0.05 Tukey's range test

The existence of different side chains and substituents in the phytochemical structure may lead to the different hydrogen-donating capacity. In *S. buxifolia*, the presence of phenolics, flavonoids, and terpenes was responsible for high antioxidant activity (Truong *et al.* 2019), and a study conducted by Mamphiswana *et al*. (2010) showed the relationship between phenolic content and antioxidant activity in *M. burkeana*.

The highest antioxidant activity in methanolic extract of *P. bullata* could be attributed to the presence of polar antioxidant compounds. A GC-MS analysis was used to identify the responsible phytochemicals, and the results showed the phenolic terpenes such as thymol and other terpenoids (phytol and neophytadiene) were present in the leaf extract (Table 5). In stem, *trans*-farnesol terpenoid and fatty acids (n-hexadecanoic acid and 9-octadecenamide) were detected in the extract. While in root, fatty acid (*cis*-vaccenic) and its methyl ester (11-octadecanoic acid, methyl ester and [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester) were profiled from the extract. All of these compunds has been demonstrated to have antioxidant activity (Anyasor *et al.* 2014; de Moraes *et al.* 2014; Rahman *et al.* 2014; Vinholes *et al.* 2014; Memar *et al.* 2017; Tyagi and Agarwal 2017; Singh and Chaturvedi 2019; Kim *et al.* 2020).

Based on the literature, other compounds profiled from GC-MS analysis might show their association with other biological activities. Phthalic acid, di(2-propylpentyl) ester (Khatiwora *et al.* 2012; Ahsan *et al.* 2017; Shobi and Viswanathan 2018), which was detected the highest in the stem, is reported to have antimicrobial properties. Other antimicrobial compounds detected in *P. bullata* were thymol (Swamy *et al.* 2017), 9-octadecenamide, (Z)- (Meenakshi *et al.* 2012), 4H-pyran-4-one, 2,3-dihydro-6-methyl (Mujeeb *et al.* 2014), 5H-indeno[1,2-b]pyridin-5one, 3-methyl (Hussain *et al.* 2014), and 9(10H)-acridone, 4-methoxy- (Gensicka-Kowalewska *et al.* 2017). Two alkaloid compounds, 5H-indeno[1,2-b]pyridin-5one,3-methyl and 9(10H)-acridone,4-methoxy-, were detected at a lower percentage (<1%) in the *P. bullata* root. These compounds are reported to have antimicrobial and antimalarial activities (Addla *et al.* 2012; Anand *et al.* 2017). The 9(10H)-acridone derivatives possess anti-inflammatory, anticancer, antimicrobial, antiparasitic, antimalarial, antiviral, and fungicidal activities (Gensicka-Kowalewska *et al.* 2017; Kukowska 2017). The findings show the neutraceutical potential of *P. bullata* that can benefit human health.

Table 5. List of Bioactive Compounds Identified in Methanolic Extract of Leaf, Stem, and Root of *P. bullata*

RT (min)	Peak Area (%)	Name of Compounds	Molecular Formula	Molecular Weight (g/mol)	Class of Compounds
Leaf					
2.017	9.327	Hydroxyacetic acid hydrazide	$C_2H_6N_2O_2$	90.08	Hydroxy acid
2.169	9.977	Hydroxyacetic acid hydrazide	$C_2H_6N_2O_2$	90.08	Hydroxy acid
2.390	11.984	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118.17	Alkane
2.993	1.622	2-Ethyl-4,6-dimethyl-1,3,5-trioxane	$C_7H_{14}O_3$	146.18	
3.033	0.549	1,3-Dioxolane-4-methanol, 2-ethyl	$C_6H_{12}O_3$	132.16	Alcohol
4.043	1.056	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106.12	Alcohol
4.093	2.388	1,3-Octanediol	$C_8H_{18}O_2$	146.23	Fatty acid

4.251	0.571	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106.12	Alcohol
4.301	1.518	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106.12	Alcohol
4.449	0.544	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106.12	Alcohol
4.516	0.303	Hydroxyacetic acid hydrazide	$C_2H_6N_2O_2$	90.08	Hydroxy acid
4.546	0.631	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	106.12	Alcohol
4.674	0.362	Acetic acid, hydroxy-	$C_2H_4O_2$	60.05	Carboxylic acid
20.091	1.620	Neophytadiene	$C_{20}H_{38}$	278.50	Sesquiterpenoids
20.581	0.565	Phthalic acid, isobutyl octadecyl ester	$C_{30}H_{50}O_4$	474.7	Ester
20.934	0.493	(R)-(-)-(Z)-14-methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	254.5	Ether
21.605	0.327	*Trans*-Farnesol	$C_{15}H_{26}O$	222.37	Sesquiterpenoids
22.396	3.746	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	Carboxylic acid
24.988	0.313	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.5	Acetate
25.223	0.719	Phytol	$C_{20}H_{40}O$	296.5	Diterpenoid
26.759	0.281	6-Dimethyl(chloromethyl) silyloxytetradecane	$C_{17}H_{37}ClOSi$	321	
26.909	0.281	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.5	Acetate
31.700	1.765	Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	330.4	Phthalate ester
32.256	0.324	Thymol, TBDMS derivative	$C_{16}H_{28}OSi$	264.48	Phenolic terpene
37.470	0.748	Thymol, TBDMS derivative	$C_{16}H_{28}OSi$	264.48	Phenolic terpene
Stem					
2.024	1.503	Glycoaldehyde dimer	$C_4H_8O_4$	120.10	Aldehyde
2.117	6.258	Trimethylsilyl ethaneperoxoate	$C_5H_{12}O_3Si$	148.23	Trimetyhlsilyl
2.377	4.401	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118.17	Alkane
4.006	1.235	Ethanol, 2,2'-oxybis	$C_4H_{10}O_3$	106.12	Alcohol
12.943	0.573	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetra siloxane	$C1_8H_{52}O_7Si_7$	577.20	Organosilicone
22.370	1.202	Dibutyl phthalate	C16H22O4	278.34	Carboxylic acid
22.770	1.614	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
22.976	1.672	9,9-Dimethoxybicyclo[3,3,1] nona-2,4-dione	$C_{11}H_{16}O_4$	212.24	Phenolic
26.041	1.504	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	Fatty acid
26.111	2.815	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	Fatty acid
26.461	0.578	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	Fatty acid
26.741	0.558	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.50	Acetate
26.936	2.316	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.5	Fatty acid
27.071	0.975	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	Fatty acid
28.265	0.698	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.50	Acetate
30.441	2.401	(2,3-	$C_{22}H_{20}OS$	332.50	Phenolic

		diphenylcyclopropyl)methyl phenyl sulfoxide, trans			
30.789	5.842	(2,3-diphenylcyclopropyl)methyl phenyl sulfoxide, trans	$C_{22}H_{20}OS$	332.50	Phenolic
30.887	0.716	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester	$C_{19}H_{21}NOS$	311.40	Ester
31.240	24.783	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.60	Carboxylic acid
31.692	0.608	Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	330.40	Carboxylic acid
33.101	0.551	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi_2$	250.48	Ketone
33.381	0.769	Arsenous acid, tri(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342.40	Ester
34.757	0.718	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6Si_7$	505.09	-
36.864	1.756	(2R,3R,4aR,5S,8aS)-2-hydroxy-4a,5-dimethyl-3-(pro-1-en-2-yl)octahydronaphthalen-1(2H)-one	$C_{15}H_{24}O_2$	236.35	Terpenoid
Root					
2.079	14.560	Dihydroxyacetone	$C_3H_6O_3$	90.08	Ketose
2.390	5.385	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118.17	Alkane
2.655	1.008	Hydroxyacetic acid hydrazide	$C_2H_6N_2O_2$	90.08	Hydroxy acid
2.983	0.515	1,3,3-Trimethoxybutane	$C_7H_{16}O_3$	148.20	-
3.568	4.858	DL-Arabinose	$C_5H_{10}O_5$	150.13	Carbohydrate
4.056	0.435	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	$C_{11}H_{32}O_4Si_4$	340.71	-
4.559	15.929	Glycerin	$C_3H_8O_3$	92.09	Carbohydrate
4.949	0.692	DL-Arabinose	$C_5H_{10}O_5$	150.13	Carbohydrate
5.017	0.873	DL-Arabinose	$C_5H_{10}O_5$	150.13	Carbohydrate
5.234	0.347	DL-Arabinose	$C_5H_{10}O_5$	150.13	Carbohydrate
6.050	0.702	4H-Pyran-4-one, 2,3-dihydro-6-methyl	$C_{14}H_{16}O_2$	216.27	Flavonoid
12.267	0.585	L-Glucose	$C_6H_{12}O_6$	180.16	Carbohydrate
12.340	0.351	D-Mannose	$C_6H_{12}O_6$	180.16	Carbohydrate
19.140	0.446	5H-Indeno[1,2-b]pyridin-5one, 3-methyl	$C_{13}H_9NO$	195.22	Alkaloid
22.670	4.825	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
23.061	0.436	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester	$C_{21}H_{38}O_2$	322.5	Fatty acid
24.539	1.039	9(10H)-Acridone, 4-methoxy-	$C_{14}H_{11}NO_2$	225.24	Alkaloid
24.987	0.350	11-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296.50	Fatty acid

25.878	6.635	*Cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282.50	Fatty acid
26.216	0.554	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240.38	-
26.311	0.524	9-Hexadecanoic acid	$C_{16}H_{30}O_2$	254.41	Fatty acid
26.356	0.528	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268.39	Ester
26.481	0.385	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	Fatty acid
26.636	0.373	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	Terpenoid
35.926	0.475	1,4-Benzendiol, 2,6-bis(1,1-dimethyl)-	$C_{14}H_{22}O_2$	222.32	Phenylpropanes

From this study it is clear that methanol was efficient in extracting phytochemical compounds of *P. bullata*. However, one concern about the finding is the toxicity of methanol. Methanol is reported to be able to deteriorate human health if the ingestion exceeds 30 mg/day (Food and Drug Administration 2018). But many studies have demonstrated that there is no sign of toxicity in animal models after feeding the animals with plant methanolic extracts (Viswanad *et al.* 2012; Abbas *et al.* 2018; Ebohon *et al.* 2020; Nguenang *et al.* 2020). The methanol traces can also be eliminated by heating the extracts using a rotary evaporator or nitrogen stream.

CONCLUSIONS

1. Methanol showed the highest capacity to extract phytochemicals from *P. bullata* leaf, stem, and root, and each of the methanolic extracts displayed the highest antioxidant activity.
2. The GC-MS analysis profiled some antioxidant compounds in methanolic extracts, and others were potentially associated with other biological activities as described in previous reports.

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