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Article

Solvent Effects on the Phenolic Compounds and Antioxidant Activity Associated with *Camellia polyodonta* Flower Extracts

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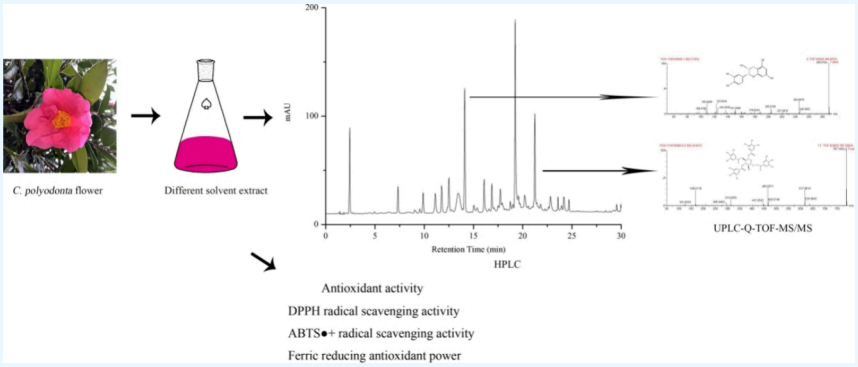

ABSTRACT: *Camellia polyodonta* flowers contain limited information available regarding the composition of their bioactive compounds and activity. The objective of this study was to identify phenolic compounds and investigate the effect of different solvents (ethanol and methanol) on the phenolic content and antioxidant activity in *C. polyodonta* flowers. The analysis using UPLC-Q-TOF-MS/MS revealed the presence of 105 phytochemicals and the most common compounds were flavonols, procyanidins, and ellagitannins. Interestingly, flavonol triglycosides were identified for the first time in these flowers. The study demonstrated that the concentration of the solvent had a significant impact on the total phenolic compound (TPC), total flavonoid compound (TFC), and total proanthocyanidin content (TPAC). The TPC, TFC, and TPAC showed a remarkable increase with the increasing concentration of the solvent, reaching their maximum levels (138.23 mg GAE/g DW, 421.62 mg RE/g DW, 60.77 mg PB2E/g DW) at 70% ethanol. However, the total anthocyanin content reached its maximum at low concentrations (0.49 mg CGE/g DW). Similar trends were observed in the antioxidant activity, as measured by the DPPH· assay (DPPH radical scavenging activity), ABTS·+ assay (ABTS radical cation scavenging activity), and FRAP assay (Ferric reducing antioxidant power). The maximum antioxidant activity was observed at 100% solvents and 70% methanol. Among the 14 individual phenolic compounds, 70% methanol yielded the highest content for 8 (cyanidin-3-*O*-glucoside, procyanidin B2, procyanidin B4, epicatechin, rutin, kaempferol-3-*O*-rutinoside, astragaline and quercitrin) out of the 14 compounds. Additionally, it was found that epicatechin was the most abundant phenolic compound, accounting for approximately 20339.37 μg/g DW. Based on these findings, it can be concluded that 70% methanol is the most effective solvent for extracting polyphenols from *C. polyodonta* flowers. These results provided chemical information and potential antioxidant value for further research in *C. polyodonta* flowers.

1. INTRODUCTION

C. polyodonta is a plant belonging to the Theaceae family. It is primarily found in the high mountains and hilly districts of the subtropical regions in southern China, at elevations ranging from 800 to 1500 m. In China, it is commonly referred to as the "eastern olive oil".[1] The flowers of *C. polyodonta* have a long blooming period, occurring from December to March, and are characterized by their red color. Despite its unique attributes, there have been relatively few studies conducted on *C. polyodonta*. Existing research has primarily focused on the bioactive components and pharmacological properties of *C. oleifera*. Studies on *C. oleifera* have identified triterpenoid saponins,[2] flavonoids,[3] polyphenols,[4] and polysaccharides[5] as major bioactive constituents. According to Chinese Medical Dictionary, various parts of the *C. oleifera* plant possess pharmacological properties due to their bioactive components. For example, the roots of *C. oleifera* have been used to treat common cold, bovillae, and ardent fever.[6] The leaves of *C.*

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oleifera can be utilized to treat itchy skin ulcers and sore carbuncles.[7] Furthermore, the seeds of *C. oleifera* are used to extract oil that is rich in monounsaturated fatty acids, flavonoids, polyphenols, and saponins.[1]

However, research on the bioactive components and potential bioactive properties of *C. polyodonta* flowers remains limited and insufficient. Only one previous study has revealed that *C. oleifera* flowers are a rich source of phenolic compounds, and has isolated quercetin 3-*O*-a-*L*-rhamnopyranoside and kaempferol 3-*O*-a-*L*-rhamnopyranoside from the flower buds of *C. oleifera* Abel.[8] Consequently, further research is needed to explore the appropriate utilization of *C. polyodonta* flowers.

In recent years, there has been increasing interest in the study of phenolic compounds derived from botanical sources, driven by people's improved living standards and their demand for green food options.[9] Plant flowers, rich in bioactive components such as phenolic acids, anthocyanins, and flavonoids, are commonly used as extracts in the form of food additives or functional food ingredients. These phenolic compounds have demonstrated various biological activities, including antioxidant, anticancer, antiobesity, and antidyslipidemia effects.[10,11]

The extraction of phenolic compounds from botanical sources is a crucial initial step in exploring their industrial applications. It is well-established that the composition and functionality of phenolic compounds can be influenced by various parameters, including temperature, time, and solvent choice. Among these factors, the choice of solvent is particularly significant, as the extraction efficiency of phenolic compounds depends on the polarity of the solvent used.[9,12−14] However, to the best of our knowledge, there is a lack of studies investigating the effect of solvents with different polarities on the extraction of phenolic compounds from *C. polyodonta* flowers.

Therefore, the main objectives of this study were to examine the impact of different solvents on the phenolic compounds and antioxidant activity of *C. polyodonta* flowers. The flowers were extracted using solvents of varying types (ethanol and methanol) and concentrations. The quantification and identification of phenolic compounds were performed using HPLC and UPLC-QTOF-MS/MS techniques. Additionally, the antioxidant activity of the extracts was determined. The findings from this research could offer valuable insights into the effective utilization of *C. polyodonta* flowers.

2. MATERIALS AND METHODS

2.1. Materials. The flowers were collected from the Zhougong Mountain (29°N, 103°E, Yaan, China) with altitude of 1721m in December 2021 (Figure 1). The species of flower was identified as *C. polyodonta* by Professor Ding Chunbang, College of Life Sciences, Sichuan Agricultural University. Upon collection, the entire flowers were freeze-dried immediately. Subsequently, they were ground into 60-mesh particles, sealed, and stored at −20 °C for testing purposes.

2.2. Chemicals and Reagents. Folin-Ciocalteu phenol reagent, Na_2CO_3, formic acid, $Al(NO_3)_3$, $NaNO_2$, HCl, methanol, ethanol, and Trolox were obtained from Chengdu Kelong Chemical Reagent Works (Chengdu, China). 1,1-Diphenyl-2- picrylhydrazyl (DPPH, > 99.7%), 2,2′-azino-bis(3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt (ABTS+, > 99.7%), and chromatographic-grade methanol and acetonitrile were purchased from American Sigma (St. Louis, MO, USA). Standard compounds including rutin, afzelin, astragalin, quercitrin, kaempferol-3-*O*-rutinoside, isoquercitrin, (+)-catechin, epicatechin, procyanidin B2, cyanidin-3-*O*-glucoside, and 1,2,3,6-tetragalloylglucose were purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). Standard compounds, including procyanidin B1, procyanidin B4, and procyanidin C1, were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China).

Figure 1. Flower of *Camellia polyodonta*.

2.3. Sample Extraction. In this study, water (containing 1% formic acid), 100%, 70%, 50%, and 30% methanol (1% formic acid), and 100%, 70%, 50%, and 30% ethanol (1% formic acid) were used as solvents.

The samples were extracted using solvents of different types and concentrations. In summary, 1 g of dry sample powder was combined with 15 mL of solvent, which contained 1% formic acid. The mixtures were then subjected to sonication for 30 min at 40 °C, followed by centrifugation at 9000 rpm for 15 min. The supernatant was collected, and the extraction procedure was repeated twice. The resulting extracts were adjusted to a final volume of 50 mL, filtered using a 0.22 μm syringe filter, and stored at 4 °C. Each sample was prepared in triplicate for reliable results.

2.4. Determination of Total Phenolic Content. The total phenolic content (TPC) was determined following the Folin−Ciocalteu colorimetric method.[9] Extracts were diluted to the appropriate ratio. The Folin−Ciocalteu reagent (20 μL) was added to the extracts (20 μL), and the mixture was allowed to stand for 5 min. Subsequently, 5% Na_2CO_3 (160 μL) was added and mixed evenly with the mixture and reacted for 60 min in the dark at room temperature. The absorbance of the mixture was recorded at 765 nm. The standard curve for gallic acid is represented as $y = 0.0071x + 0.0203$ ($R^2 = 0.9996$). The linear range was 2.38−152.48 μg/mL. The results were expressed as mg gallic acid equivalent (GAE)/g dry weight (DW).

2.5. Determination of Total Flavonoid Content. Total flavonoid content (TFC) was determined by conducting the aluminum chloride colorimetric assay.[9] Extracts were diluted to

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Table 1. Characterization of Phenolic Compounds in the Whole Flower of *C. polyodonta* Extract Using UPLC-Q-TOF-MS

Peak no.	R_t (min)	$[M-H]^-$ (m/z)	Formula	Fragment ions MS/MS (m/z)	Identified compounds
Organic acids					
1	0.866	191.0547	$C_7H_{12}O_6$	111,87,85	Citric acid
2	1.024	191.0179	$C_7H_{12}O_6$	111,87,85	Citric acid
Phenolic acids					
3	1.403	169.0119	$C_7H_6O_5$	125	Gallic acid
4	3.603	353.0906	$C_{16}H_{18}O_9$	191,179,135	Caffeoylquinic acid
6	4.307	341.0885	$C_{15}H_{18}O_9$	179,161,133	Caffeoylglucopyranose isomer 1
10	5.099	341.0885	$C_{15}H_{18}O_9$	179,161,133	Caffeoylglucopyranose isomer 2
13	5.528	137.0238	$C_7H_6O_3$	109,93	Hydroxybenzoic acid
15	5.700	343.1111	$C_{15}H_{20}O_9$	181,137,109	3,4-Dimethoxybenzoic acid-hexoside
17	6.011	325.0933	$C_{15}H_{18}O_8$	163,145,117	*p*-coumaric acid-hexoside isomer 1
20	6.646	325.0933	$C_{15}H_{18}O_8$	163,145,117	*p*-coumaric acid-hexoside isomer 2
24	6.988	355.1021	$C_{16}H_{20}O_9$	295,193,175,160,132	Feruloylglucose
28	7.616	163.0409	$C_9H_8O_3$	137	*p*-coumaric acid
29	7.618	137.0238	$C_7H_6O_3$	109,83	Hydroxybenzoic acid
72	10.539	487.1211		323,179,163,161,119	*p*-coumaric acid-dihexoside
Flavonoids					
14	5.563	289.0724	$C_{15}H_{14}O_6$	245,123,109	(+)-Catechin
27	7.563	289.0724	$C_{15}H_{14}O_6$	245,123,109	(−)-Epicatechin
44	9.392	477.0674	$C_{22}H_{22}O_{12}$	315,300,299,271,243	rhamnetin-*O*-pentoside isomer
46	9.568	609.1461	$C_{27}H_{30}O_{16}$	301,300,271,255,243,151	Quercetin-3-*O*-rutinoside
49	9.698	463.0864	$C_{21}H_{20}O_{12}$	301, 300, 271,255, 243, 151	Quercetin-3-O-galactose
52	9.855	463.0864	$C_{21}H_{20}O_{12}$	301,300, 271, 255,151	Quercetin-3-*O*-glucoside
54	9.884	739.2065	$C_{33}H_{40}O_{19}$	285,284,255,227,151	Kaempferol-3-*O*-rhamnosylrutinoside
55	9.931	739.2065	$C_{33}H_{40}O_{19}$	285,284,255,227	Kaempferol-O-dirhamnoside-*O*-glucoside
57	10.106	797.2210	$C_{35}H_{26}O_{22}$	755,301,300,271,255,179,151	Quercetin-3-*O*-rhamnosid-*O*-rhamnosid-*O*-acetylglucoside
58	10.114	433.0761	$C_{20}H_{18}O_{11}$	301,300,271,255,243,151	Quercetin-3-*O*-pentoside isomer
59	10.139	739.2121	$C_{33}H_{40}O_{19}$	285,284,255,227	Kaempferol-*O*-rhamnosylrutinoside isomer
60	10.172	739.2065	$C_{33}H_{40}O_{19}$	285,284,255,227	Kaempferol-*O*-dirhamnoside-*O*-glucoside
61	10.272	433.0761	$C_{20}H_{18}O_{11}$	301,300,271,255,243,151	Quercetin-3-*O*-pentoside isomer
62	10.276	447.0935	$C_{21}H_{20}O_{11}$	285,284,255,227,183	Kaempferol-3-*O*-galactoside
63	10.298	593.1497	$C_{30}H_{26}O_{13}$	285,284,255,227	Kaempferol-3-*O*-rutinoside
64	10.303	781.2222	$C_{33}H_{34}O_{22}$	739,285,284,255,227	Kaempferol-3-*O*-rhamnosid-*O*-rhamnosid-*O*-acetylglucoside
65	10.317	797.2152	$C_{35}H_{26}O_{22}$	755,301,300,271,255,179,151	Quercetin-3-*O*-rhamnosid-*O*-rhamnosid-*O*-acetylglucoside
69	10.429	433.0761	$C_{20}H_{18}O_{11}$	301,300,271,255,243,151	Quercetin-3-*O*-pentoside isomer
70	10.435	477.1077	$C_{22}H_{22}O_{12}$	315,300,299,271,255	rhamnetin-*O*-pentoside isomer
71	10.530	447.0935	$C_{21}H_{20}O_{11}$	285,284,255,227,183	Kaempferol-3-*O*-glucoside
75	10.614	593.1497	$C_{30}H_{26}O_{13}$	447,285,284,255,227	Kaempferol-*O*-rhamnoside-*O*-glucoside
76	10.623	447.0935	$C_{21}H_{20}O_{12}$	301,300,271,255,243,151	Quercetin-3-*O*-rhamnoside
77	10.811	417.0793	$C_{20}H_{18}O_{10}$	285,284,255,227,183	Kaempferol-3-*O*-pentoside isomer
80	10.941	417.0877	$C_{20}H_{18}O_{10}$	285,284,255,227,183	Kaempferol-3-*O*-pentoside isomer
81	10.995	781.2222	$C_{33}H_{34}O_{22}$	739,285,284,255,227	Kaempferol-3-*O*-rhamnosid-*O*-rhamnosid-*O*-acetylglucoside
82	11.111	489.1028	$C_{23}H_{22}O_{12}$	285,284,255,227	Kaempferol-3-*O*-(6″-acetyl)glucoside
83	11.192	417.0793	$C_{20}H_{18}O_{10}$	285,284,255,227,183	Kaempferol-3-*O*-pentoside isomer
84	11.410	431.0968	$C_{20}H_{16}O_{11}$	285,284,255,227	Kaempferol-3-*O*-rhamnoside
86	11.775	823.2294	$C_{35}H_{36}O_{23}$	781,327,285,284,255,227	Kaempferol-3-*O*-rhamnosid-*O*-acetylrhamnosid-*O*-acetylglucoside
88	11.956	823.2294	$C_{35}H_{36}O_{23}$	781,327,285,284,255,227	Kaempferol-3-*O*-rhamnosid-*O*-acetylrhamnosid-*O*-acetylglucoside
89	12.546	623.1398	$C_{28}H_{32}O_{16}$	477,315,300,299,271,255,215,145	Isorhamnetin −3-*O*- (*p*-Coumaryl)glucoside
90	12.547	593.1298	$C_{30}H_{26}O_{13}$	447,285,284,255,227,145	Kaempferol-3-*O*-(*p*-Coumaryl)glucoside
91	12.561	865.2339	$C_{45}H_{38}O_{18}$	823,327,285,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-acetylglucoside
92	12.676	593.1298	$C_{30}H_{26}O_{13}$	447,285,284,255,227,163	Kaempferol-3-*O*-(*p*-Coumaryl)glucoside
93	12.926	623.1398	$C_{28}H_{32}O_{16}$	477,315,300,271,255,243,145	Isorhamnetin −3-*O*- (*p*-Coumaryl)glucoside
94	13.099	865.2339	$C_{45}H_{38}O_{18}$	823,781,635,327,285,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-acetylglucoside
95	13.149	593.1298	$C_{30}H_{26}O_{13}$	285,255,227,145	Kaempferol-*O*-(*p*-Coumaryl)glucoside
96	13.640	865.2339	$C_{45}H_{38}O_{18}$	823,593,327,285,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-acetylglucoside
97	13.695	907.2471	$C_{39}H_{40}O_{25}$	865,823,285,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-(3″,6″-*O*-diacetyl)glucoside

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Table 1. continued

Peak no.	R_t (min)	$[M-H]^-$ (m/z)	Formula	Fragment ions MS/MS (m/z)	Identified compounds
Flavonoids					
98	14.028	907.2532	$C_{39}H_{40}O_{25}$	865,285,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-(3″,6″-*O*-diacetyl)glucoside
99	14.389	907.2471	$C_{39}H_{40}O_{25}$	865,285,327,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-(3″,6″-*O*-diacetyl)glucoside
100	14.539	907.2471	$C_{39}H_{40}O_{25}$	865,823,327,285,284,255,227	Kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-(3″,6″-*O*-diacetyl)glucoside
101	15.112	739.1676	$C_{33}H_{40}O_{19}$	453,307,285,255,227,145	Kaempferol-*O*-(*p*-Coumaryl)rutinoside isomer
102	15.170	739.1620	$C_{33}H_{40}O_{19}$	593,453,285,229,145	Kaempferol-3-*O*-rhamnosid-*O*- (*p*-Coumaroyl)glucoside
103	15.237	739.1676	$C_{33}H_{40}O_{19}$	593,575,453,453,285,255,227,163,145	Kaempferol-*O*-(*p*-Coumaryl)rutinoside isomer
104	15.259	739.1676	$C_{33}H_{40}O_{19}$	593,575,453,285,229,145	Kaempferol-3-*O*-rhamnosid-*O*- (*p*-Coumaroyl)glucoside
Hydrolyzed tannins					
5	3.643	633.0759	$C_{27}H_{22}O_{18}$	463,301,275,169	Galloyl-HHDP-glucose isomer
7	4.593	633.0707	$C_{27}H_{22}O_{18}$	301,275,229	Galloyl-HHDP-glucose isomer
12	5.475	497.0513	$C_{21}H_{22}O_{14}$	331,271,211,169,125	Methylgalloyl−galloyl-glucose
23	6.904	635.0870	$C_{27}H_{24}O_{18}$	575, 483,465,313,169,125	Trigalloyl-glucose isomer
30	7.947	635.0870	$C_{27}H_{24}O_{18}$	465,313,211,169,125	Trigalloyl-glucose isomer
31	8.137	635.0870	$C_{27}H_{24}O_{18}$	483,465,313,211,169,125	Trigalloyl-glucose isomer
33	8.234	635.0870	$C_{27}H_{24}O_{18}$	483,331,313,211,169,125	Trigalloyl-glucose isomer
36	8.424	635.0870	$C_{27}H_{24}O_{18}$	483,465,331,313,211,169,125	Trigalloyl-glucose isomer
37	8.684	785.0847	$C_{34}H_{26}O_{22}$	633,301,275, 229,169	digalloyl-HHDP-glucose
40	8.813	785.0847	$C_{34}H_{26}O_{22}$	633,301,275, 229,169	digalloyl-HHDP-glucose
45	9.507	787.1000	$C_{34}H_{28}O_{22}$	617,465,447,313,169,125	1,2,3,6-Tetragalloylglucose
53	9.875	937.0959	$C_{41}H_{30}O_{26}$	785, 633, 301, 275,169	Trigalloyl-HHDP-glucose isomer
56	10.033	937.0959	$C_{41}H_{30}O_{26}$	785, 633, 483,313,301, 275,169	Trigalloyl-HHDP-glucose isomer
68	10.350	939.1155	$C_{41}H_{32}O_{26}$	787,635,617,465,447,313,2895,169	Pentagalloylglucose isomer
73	10.541	939.1093	$C_{41}H_{32}O_{26}$	787,635,617,465,447,313,2895,169	Pentagalloylglucose isomer
78	10.822	935.0847	$C_{41}H_{28}O_{26}$	765,301,275	Galloyl-bis-HHDP-glucose
Procyanidins					
8	4.739	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B1
9	4.929	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B3
11	5.340	865.1978	$C_{45}H_{38}O_{18}$	739,695,577,407,289,243,161,125	Procyanidin B trimer isomer 1
16	5.942	865.1918	$C_{45}H_{38}O_{18}$	739,695,577,425,407,289,243,161,125	Procyanidin B trimer isomer 2
18	6.128	1153.2623	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 1
19	6.385	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B2
21	6.795	865.1978	$C_{45}H_{38}O_{18}$	739,695,577,425,407,287,161,125	Procyanidin B trimer isomer 3
22	6.862	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B4
25	7.175	865.1978	$C_{45}H_{38}O_{18}$	739,695,577,425,407,287,161,125	Procyanidin B trimer isomer 4
26	7.483	1153.2623	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 2
32	8.224	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B isomer 1
34	8.236	1153.2554	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 3
35	8.380	865.1978	$C_{45}H_{38}O_{18}$	783,695,577,425,407,287,161,125	Procyanidin C1
38	8.697	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B isomer 2
39	8.716	1153.2623	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 4
41	8.957	1153.2554	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 5
42	9.319	1153.2623	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 6
43	9.330	865.1978	$C_{45}H_{38}O_{18}$	695,577,425,407,287,161,125	Procyanidin B trimer isomer 5
47	9.591	1153.2623	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 7
48	9.645	865.1978	$C_{45}H_{38}O_{18}$	739,695,577,425,407,287,161,125	Procyanidin B trimer isomer 6
50	9.742	865.1978	$C_{45}H_{38}O_{18}$	739,695,577,425,407,287,161,125	Procyanidin B trimer isomer 7
51	9.777	577.1349	$C_{30}H_{26}O_{12}$	451,425,407,289,161,125	Procyanidin B isomer 3
67	10.343	1153.2554	$C_{60}H_{50}O_{24}$	983,865,739,577,449,407,289,287,243,125	Procyanidin B tetramer isomer 8
Unknown					
66	10.331	741.2234		269,151	
74	10.554	787.0943	$C_{34}H_{28}O_{22}$	725,617,465,449,285,284,313,169,125	
79	10.842	433.1145		287,269,259,180,152	
85	11.420	285.0407	$C_{15}H_{10}O_6$	255,227,151	
87	11.954	683.2336		323,315,179,161	
105	15.799	949.2590		907,865,327,285,284,255,227	

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the appropriate ratio. A solution of 5% (m/v) $NaNO_2$ (15 μL) was mixed with the extracts (20 μL) evenly and reacted for 6 min at room temperature. Following this, 10 μL of 10% $Al(NO_3)_3$ was added to the mixture and reacted for 5 min. This was followed by the addition of NaOH (1 mol/L; 30 μL) to the mixture before the determination of absorbance at 510 nm. The standard curve was represented as $y = 0.0004x + 0.005$ ($R^2 = 0.9996$), with a linear range of 21.56−1380.00 μg/mL. The results were expressed as mg rutin equivalent (RE)/g dry weight (DW).

2.6. Determination of Total Proanthocyanidin Content. The total proanthocyanidin content (TPAC) was determined by conducting a modified vanillin assay.[15] Briefly, 20 μL of the extract was mixed with 100 μL of a solution of 1% (m/v) vanillin in methanol. Subsequently, 100 μL of 4% (v/v) HCl was mixed with methanol. Then, the mixture was kept at 37 °C for 20 min to complete the reaction before recording the absorbance at 500 nm. The standard curve was represented as $y = 0.7355x + 0.0026$ ($R^2 = 0.999$), with a linear range of 15.62−500.00 μg/mL. The results were expressed as mg procyanidin B2 equivalent (PB2E)/g dry weight(DW).

2.7. Determination of Total Anthocyanin Content. Total anthocyanin content (TAC) was quantified following the method reported by Zhao,[16] using an ultrahigh-performance liquid chromatography system equipped with a photodiode array (PDA) detector coupled to a Waters Xevo G2-XS QTOF micromass spectrometer (Waters, Manchester, UK). The details of the mobile phase and elution gradient maintained are presented in Section 2.8. Absorbance was recorded at 525 nm. Quantification was done following the standard external method using cyanidin-3-glucoside. The results were expressed in mg cyaniding-3-glucoside equivalents (CGE)/g dry weight-(DW).

2.8. Quantification of Phenolic Compounds Using HPLC. The extracts obtained (described in Section 2.3) were analyzed using an Agilent LC-1290 HPLC system (Agilent, Santa Clara, CA, USA) equipped with a DAD. Chromatographic separation was carried out on an Infinity Lab Poroshell 120 PFP column (4.6 mm × 100 mm, particle size: 2.7 μm, Agilent, CA, USA). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile). The gradient program used was based on a previous study:[17] 0−10 min (5−10% B), 10−20 min (10−20% B), 20−35 min (20−40% B), 35−36 min (40−45% B), and 36−37 min (45−95% B). The flow rate was maintained at 0.8 mL/min. The column temperature was set at 30 °C, and the detection wavelengths were 280, 350, and 525 nm. The injection volume was 5 μL. Fourteen phenolic compounds were quantified using corresponding standard compounds. The results were expressed as μg phenolics/g DW.

2.9. Determination of Antioxidant Activity.

2.9.1. DPPH Radical Scavenging Activity. The DPPH radical scavenging activity was determined by following the method described by Ismail.[18] The reported method was modified to conduct the experiments. The extract (100 μL) was diluted to the appropriate ratio and mixed with 100 μL of the prepared 0.2 M DPPH solution (dissolved in methanol). The mixture was kept in the dark for 30 min at room temperature. The absorbance was subsequently recorded at 517 nm. The standard curve was represented as $y = 0.2039x + 0.0142$ ($R^2 = 0.9998$), with a linear range of 0.18−2.98 μg/mL. The results were expressed as mg Trolox equivalent (TE)/g DW of the sample.

2.9.2. $ABTS^{\bullet+}$ Radical Scavenging Activity. The scavenging activity of the $ABTS^{\bullet+}$ radical cation ($ABTS^{\bullet+}$) was determined by following the method reported by Ma.[9] The previously reported method was modified to a small extent to conduct the experiments. A total of 40 μL of the diluted extraction was added to 160 μL of freshly prepared $ABTS^{\bullet+}$ solution, and the mixture was immediately mixed. The reaction mixture was kept in the dark at room temperature for 6 min, after which the absorbance at 734 nm was measured. The standard curve was expressed as $y = 0.0238x + 0.0284$ ($R^2 = 0.9996$), with a linear range of 0.37−23.90 μg/mL. The results were expressed as mg Trolox equivalent (TE)/g DW of the sample.

2.9.3. Ferric Reducing Antioxidant Power (FRAP). The ferric-reducing ability was determined following the method described by Ismail.[18] A total of 30 μL of the diluted extraction was added to 256 μL of freshly prepared FRAP reagent, and the mixture was immediately mixed. The reaction mixture was then kept in the dark at room temperature for 30 min, after which the absorbance at 593 nm was measured. The standard curve was represented by the equation $y = 2.315x - 0.0045$ ($R^2 = 0.9999$), with a linear range of 0.18−23.90 μg/mL. The results were expressed as mg Trolox equivalent (TE)/g DW of the sample.

2.10. Phenolic Compound Identification. The identification of phenolic compounds in the 70% methanol extract of *C. polyodonta* flower was realized using an ultrahigh-performance liquid chromatography system equipped with a photodiode array (PDA) detector coupled to a Waters Xevo G2-XS QTOF micromass spectrometer (Waters, Manchester, UK). The electrospray ionization (ESI) source was operated in the negative mode. The analysis of phenolic compounds was conducted at two wavelengths (280 and 350 nm), using a Waters BEH C18, 1.7 μm (2.1 mm × 100 mm, Waters Corporation; Milford, MA, USA) column. The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile. The following elution gradient was used for analysis: 5−10% B for 5 min, 10−20% B for 3 min, 20−40% B for 6 min, 40−80% B for 6 min, and 80−100% B for 2 min. The injection volume was 1 μL, and the elution was completed within 20 min at a flow rate of 0.3 mL/min. The mass spectrometer (MS) parameters included a capillary voltage of 3.0 kV, a source temperature of 120 °C, a desolvation temperature of 250 °C, a cone gas flow of 50 L/h, and a desolvation gas flow of 600 L/h. The scanning range for mass spectrometry was set from m/z 100 to 1500.

2.11. Statistical Analysis. Experiments were performed in triplicate. The data were evaluated by analysis of variance (ANOVA), and a comparison of means was carried out using Duncan's test. Differences were considered to be significant at $P < 0.05$. Statistical computation and analyses were conducted using SPSS (version 17.0, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Identification of Phenolic Compounds in *C. polyodonta* Flower Extracts. For the identification of phenolic compounds in *C. polyodonta* flower, the 70% methanol extract was selected based on its highest total phenolic compound content (TPC, TFC, TPAC), favorable HPLC results, and antioxidant activity. The MS data obtained from the UPLC-Q-TOF-MS/MS analysis of the 70% methanol extract are summarized in Table 1 (Figure S1). The results revealed that flavonoids, particularly flavonols, and anthocya-

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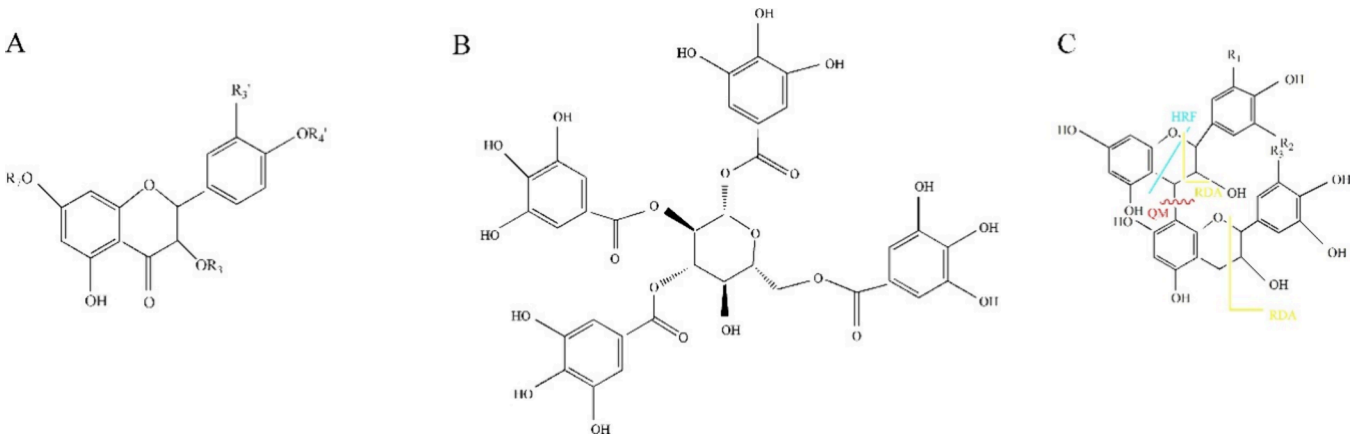

Figure 2. (A) Structure of flavonols. (B) Structure of 1,2,3,6-tetragalloylglucose. (C) Fragmentation patterns for procyanidins dimmers.

nins were the major phenolic compounds present in *C. polyodonta* flower. A total of 99 known phytochemicals were identified, including 2 organic acids, 12 phenolic acids, 44 flavonols, 2 flavanones, 16 hydrolyzed tannins, and 23 procyanidins. Through comparison with our previous study, it was found that the phenolic acids in the *C. polyodonta* flower were similar to those in the *C. oleifera* flower that were harvested from the *C. oleifera* base (Yaan, China).

3.1.1. Flavonoids. Flavonoids, particularly flavonols, are the most abundant compounds found in *C. polyodonta* flower. Among the flavonoids, there were 44 flavonol monoglycosides and 2 flavonol diglycosides, with kaempferol and quercetin as the predominant aglycones. Flavonols are widely distributed in plants and can exist in the form of monoglycosides, diglycosides, and triglycosides, with various substitution patterns in the three rings, particularly at the C-3 and C-7 positions (Figure 2A). Our study identified 24 flavonol monoglycosides, 7 flavonol diglycosides, and 13 flavonol triglycosides. The aglycones of quercetin and kaempferol were connected to glucose, galactose, rhamnose, and rutinose. The mass spectrum characteristics of these aglycones showed fragment ions at m/z 162, 162, 146, and 294, indicating the loss of glycogroups and yielding aglycone fragment ions at m/z 301 and 285. Compounds **46**, **55**, **56**, **59**, **63**, **67**, and **74** (Table 1) were identified as quercetin glycosides, with a characteristic fragment ion at m/z 301, which corresponds to the presence of quercetin after the loss of the specific sugar molecule. Specifically, compounds **46**, **52**, **63**, **71**, **76**, and **84** were identified as quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-glucoside (isoquercitrin), kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside (astragalin), quercetin-3-*O*-rhamnoside (quercitrin), and kaempferol-3-*O*-rhamnoside (afzelin), respectively. The identification was confirmed by comparing these compounds with corresponding standards. These compounds have also been reported in the tissues and pollen of various flowers.[19,20]

To the best of our knowledge, 20 kaempferol glycoside derivatives and 2 isorhamnetin glycoside derivatives were tentatively identified in the *C. polyodonta* flower extract. These compounds have been characterized for the first time in this flower. Compounds **89**, **90**, **92**, **93**, **95**, **101**, **102**, **103**, and **104** (Table 1) were inferred to be glycosides of kaempferol or isorhamnetin based on their fragment ions at m/z 285 or 315, as well as the presence of *p*-Coumaryl indicated by fragment ions at m/z 163 (*p*-Coumaryl) or 145 (*p*-Coumaryl minus H_2O). Specifically, compounds **90** and **92** (Table 1) were tentatively identified as kaempferol-3-*O*-(*p*-Coumaryl) glucoside, as they exhibited an $[M-H]^-$ ion at m/z 593.1298, a fragment ion at m/z 447 ([M-H-146], corresponding to the loss of one *p*-Coumaryl), and a fragment ion at m/z 285 ([M-H-146−162], corresponding to the loss of one glucoside group). Compounds **101** and **103** (Table 1) showed a similar fragment pathway and were tentatively identified as isomers of kaempferol-*O*-(*p*-Coumaryl) rutinoside. They exhibited an $[M-H]^-$ ion at m/z 739.1676, a fragment ion at m/z 593 ([M-H-146], corresponding to the loss of one *p*-Coumaryl), and a fragment ion at m/z 285 ([M-H-453], corresponding to the loss of kaempferol).

In our study, we also detected a significant presence of flavonol triglycosides in the *C. polyodonta* flower extracts. Fang[21] have indicated that the majority of flavonol triglycosides are glycosyl derivatives of quercetin, kaempferol, and myricetin, primarily located at the C-3 position. Flavonol triglycosides have been found to exhibit a wide range of pharmacological effects on the human body. Liu[22] discovered that kaempferol triglycoside extracted from *Camellia* cake demonstrated a therapeutic effect on burn injuries in mice. In our study, compounds **97**, **98**, **99**, and **100** (Table 1) exhibited the same pseudomolecular ion $[M-H]^-$ at m/z 907.2471, along with fragment ions at m/z 865 ([M−H]−42, corresponding to the loss of acetyl) and m/z 865 ([M−H]−42−42, corresponding to the loss of two acetyl groups), and a fragment ion at m/z 285 (corresponding to the loss of kaempferol). These compounds were tentatively identified as kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-(3“, 6″-*O* diacetyl) glucoside. Similarly, compounds **91**, **94**, and **96** with the pseudo-molecular ion $[M-H]^-$ at m/z 865.2339 were tentatively identified as kaempferol-3-*O*-acetylrhamnosid-*O*-acetylrhamnosid-*O*-acetylglucoside. Additionally, Zheng[23] demonstrated that flavonol triglycosides are the most abundant flavonol glycoside group in *Camellia sinensis* (L.) leaves.

3.1.2. Tannins. Tannins are polyphenolic secondary metabolites produced by higher plants, which can be divided into hydrolyzable tannins, condensed tannins, complex tannins, and phlorotannins.[24] Generally, plant extracts contain compounds that exhibit UV spectral characteristics coherent with galloyl and hexahydroxydiphenol (HHDP) derivatives.[25] Specifically, hydrolyzable tannins are divided into ellagitannins and gallotannins. In our research, we identified 16 hydrolyzable tannins from *C. polyodonta* flowers, and these compounds were

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Table 2. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Proanthocyanidins Content (TPAC), and Total Anthocyanin Content (TAC) of Flowers of *C. polyodonta* Extracts Obtained with Different Solvents[a]

Solvent type	TPC (mg GAE/g DW)	TFC (mg RE/g DW)	TPAC (mg PB2E/g DW)	TAC (mg CGE/g DW)
water	76.25 ± 0.80^{d}	214.81 ± 18.08^{e}	41.85 ± 1.83^{d}	0.49 ± 0.02^{a}
30% methanol	127.24 ± 3.57^{c}	340.19 ± 1.44^{bcd}	55.63 ± 0.97^{b}	0.47 ± 0.01^{a}
30% ethanol	129.19 ± 0.48^{bc}	329.08 ± 1.26^{cd}	51.55 ± 1.42^{c}	0.44 ± 0.03^{b}
50% methanol	134.42 ± 1.79^{ab}	348.85 ± 4.14^{bcd}	56.31 ± 0.73^{b}	0.48 ± 0.01^{a}
50% ethanol	137.15 ± 3.60^{a}	344.10 ± 20.28^{bcd}	55.33 ± 0.62^{b}	0.37 ± 0.01^{c}
70% methanol	138.23 ± 3.03^{a}	421.62 ± 4.22^{a}	60.77 ± 0.68^{a}	0.43 ± 0.01^{b}
70% ethanol	132.58 ± 4.28^{abc}	318.91 ± 12.37^{d}	52.07 ± 1.69^{c}	0.26 ± 0.02^{d}
100% methanol	128.22 ± 2.41^{c}	366.99 ± 6.11^{b}	61.03 ± 2.03^{a}	0.37 ± 0.01^{c}
100% ethanol	126.46 ± 3.67^{c}	354.71 ± 30.02^{bc}	60.14 ± 0.65^{a}	0.24 ± 0.01^{d}

[a]Data with different superscript lowercase letters a−e in the same column were significantly different ($P < 0.05$).

associated with different isomers of tri-, tetra-, and pentagalloyl glucopyranose, as well as di- and trigalloyl-hexahydroxydiphenoyl glucopyranose (Table 1). The main characteristic in the mass spectra of these compounds was the pseudomolecule $[M-H]^-$ (m/z 633, 635, 785, 787, 937, and 939), which tended to lose one or more galloyl groups (152 u) and/or gallic acid (170 u).[20] Compounds **51** and **54** were tentatively identified as triallyl-HHDP-glucose isomers based on an $[M-H]^-$ at m/z 937.0959, followed by fragment ions at m/z 785 (loss of a galloyl group, $[M-H-152]^-$), m/z 633 (minus one HHDP group), and m/z 301, which corresponds to one unit of HHDP after the lactonization process to ellagic acid, followed by decarboxylation producing ion m/z 275. Compounds **37** and **40** in *C. polyodonta* flowers with an m/z 785.0847 were identified as isomers of digalloyl-HHDP-glucose, with the fragmentation ions at m/z 633 (loss of galloyl) and m/z 301 (indicating the presence of an HHDP group $[ellagic-H]^-$ in the molecule).

Compounds **23**, **30**, **31**, **33**, and **36** (Table 1) were tentatively identified as trigalloyl-glucose isomers. Compound **45** was identified as 1,2,3,6-tetragalloylglucose using authentic standards (Figure 2B). Compounds **66** and **71** (Table 1) were tentatively identified as pentagalloylglucose isomers. Secondary mass spectrometry analysis revealed that the fragmentation patterns were similar. Taking two pentagalloylglucose compounds as an example, compounds **66** and **71** exhibited an identical $[M-H]^-$ at m/z 939 and shared the same molecular formula $C_{41}H_{32}O_{26}$. The secondary mass spectrometry data indicated similar fragmentation patterns. The possibility of gallic acid attachment at any of the five hydroxyl groups (1, 2, 3, 4, and 6) of the glucose structure and the potential presence of a digallic acyl group at one hydroxyl group make determining the specific structure of the molecule solely through mass spectrometry challenging. These compounds generated a fragment ion at m/z 787 $[M-H-152]^-$ through the loss of a galloyl group, followed by the loss of gallic acid (170 u) to produce m/z 617. Further loss of a galloyl group (152 u) resulted in m/z 465, and finally, the loss of H_2O produced m/z 447. Pentagalloyl glucose has been found in various traditional Chinese medicines, and it has demonstrated strong antioxidant activity,[26] anticancer effects,[27] and potential benefits against Alzheimer's disease.[28]

3.1.3. Procyanidins. Procyanidins are primarily composed of chains of flavan-3-ol units such as (+)-catechin and (−)-epicatechin, connected through C4−C8 or C4−C8 bonds. They are mainly found in flowers, fruits, and seeds of various plants.[29] In our study, *C. polyodonta* flowers were found to contain a variety of procyanidins, totaling 23 compounds, including eight dimers, seven trimers, and eight tetramers (Table 1).

Compounds **8** (Rt = 4.739 min), **9** (Rt = 4.929 min), **19** (Rt = 6.385 min), **22** (Rt = 6.862 min), **32** (Rt = 8.224 min), **38** (Rt = 8.697 min), and **50** (Rt = 9.777 min) (Table 1) exhibited an identical $[M-H]^-$ at m/z 577.1349, as evidenced by characteristic fragment ions at m/z 451, 425, 407, and 289. These compounds corresponded to the typical B-type proanthocyanidin dimer formed by the connection of two proanthocyanidin monomers. The m/z 451 ($[M-H-126]^-$) fragment ion resulted from the loss of one molecule of phloroglucinol through heterocyclic ring fission (HRF, neutral loss of 126 Da). The m/z 425 [M-152] fragment ion was generated through Retro-Diels−Alder (RDA, neutral loss of 152 Da), and the m/z 407 [M-152−18] fragment ion was produced by the subsequent loss of H_2O. The m/z 289 [M-288] fragment ion was derived from the Quinone-Methide (QM) cleavage of the interflavan bond (Figure 2 C). Compounds **8**, **9**, **19**, **22**, and **35** were further confirmed as procyanidin B1, B3, B2, B4, and C1, respectively, using authentic standards. Compounds **11** (Rt = 5.340 min), **16** (Rt = 5.942 min), **21** (Rt = 6.795 min), **25** (Rt = 7.175 min), **43** (Rt = 9.330 min), **48** (Rt = 9.645 min), and **49** (Rt = 9.742 min) (Table 1) exhibited an $[M-H]^-$ ion at m/z 865.1978 and fragment ions at m/z 739, 577, and 289. These compounds were identified as procyanidin B trimer isomers. The fragment ion at m/z 557 was formed due to the loss of a hydroxyl group. Compounds **18** (Rt = 6.128 min), **26** (Rt = 9.319 min), **34** (Rt = 8.236 min), **39** (Rt = 8.716 min), **41** (Rt = 8.957 min), **42** (Rt = 11.244 min), **47** (Rt = 9.591 min), and **65** (Rt = 10.343 min) (Table 1) exhibited an $[M-H]^-$ ion at m/z 1153 and fragment ions at m/z 575, 407, and 289. These compounds were identified as procyanidin B tetramer isomers.

To the best of our knowledge, there was no available information regarding the phenolic composition of *C. polyodonta* flowers. However, our previous study reported the phenolic composition of *C. oleifera* flowers, which contained a total of 85 phenolic compounds.[17] In the *C. oleifera* flower extract, three main groups of polyphenolic compounds were identified, namely flavonoids, ellagitannins, and procyanidins.[17] It is worth noting that the types of polyphenols found in *C. oleifera* flowers were fewer compared to those found in *C. polyodonta* flowers.

3.2. Effect of Solvents on TPC and TFC. The flowers of *C. polyodonta* hold potential economic value for the extraction of bioactive substances, particularly phenolic compounds. To assess the impact of different solvents on the extraction of phenolic compounds from the flower extracts, their TPC and

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TFC were determined. The results are presented in Table 2. The TPC values of the flower extracts exhibited significant variations depending on the solvent used, following the order: 50% ethanol >70% methanol >50% methanol >70% ethanol >30% ethanol >100% methanol >30% methanol >100% ethanol > water. The range of TPC values varied from 76.25 mg GAE/g DW (the lowest value) to 138.23 mg GAE/g DW (the highest value). Previous research studies have demonstrated that the choice of solvent significantly influences the extraction efficiency of polyphenols from different materials.[9,18] In our study, the highest TPC values were obtained from the 50% ethanol extract (138.23 mg GA/g DW) and 70% methanol extract (137.15 mg GA/g DW), which were significantly higher than those obtained from the other solvent extracts. These results indicate that organic solvents with concentrations ranging from 50% to 70% were the most effective in extracting phenolic compounds. Furthermore, while excessively high concentrations (100%) or low concentrations (30%) of organic solvents had minimal impact on enhancing phenolic extraction, their extracts still exhibited higher TPC values than the water extract. The water extract showed the lowest ability to extract phenolics (76.25 mg GAE/g DW) due to their limited solubility in water. Previous studies have also indicated that polyphenols have a higher solubility in solvents less polar than water.[30−32] However, the addition of water to the solvent can contribute to the extraction of phenolic compounds, as observed in our results (Table 2), which aligns with the findings reported by Ye[33], and Escribano-Bailon and Santos-Buelga.[34] These results imply the presence of numerous weak polar and nonpolar phenolic compounds in *C. polyodonta* flowers. In conclusion, based on our findings, 50% ethanol extract and 70% methanol extract are the most suitable solvents for extracting TPC from *C. polyodonta* flowers.

Table 2 presents information on the TFC of *C. polyodonta* flower extracts obtained using different organic solvents. Interestingly, a similar trend to the TPC was observed for TFC. The highest TFC value was recorded in the 70% methanol extract (421.62 mg RE/g DW), while the lowest TFC value was found in the water extract (214.81 mg RE/g DW). In contrast to previous studies, the 100% methanol extract (366.99 mg RE/g DW) and 100% ethanol extract (354.71 mg RE/g DW) exhibited slightly higher TFC values compared to the other solvent extracts.

With the exception of the 70% ethanol extract, the extracts with 70% organic solvents had the highest TFC values, followed by the extracts with 100%, 50%, and 30% solvents. This suggests that TFC increases with decreasing solvent polarity, indicating the presence of numerous weak polar flavonoids in *C. polyodonta* flowers. This observation can be attributed to the lower solubility of flavonoids in water compared to organic solvents. Additionally, less polar solvents are more effective in disrupting cell membranes, allowing for the extraction of flavonoids that are typically located in other organelles.[35]

Furthermore, it was observed that *C. polyodonta* flowers contain higher amounts of flavonoids compared to phenols, with approximately 2−3 times higher concentrations in the solvent extracts. Therefore, it can be concluded that flavonoids are the predominant phenolic group present in *C. polyodonta* flowers.

3.3. Effect of Solvents on TAPC and TAC. The flowers of *C. polyodonta* were found to contain abundant proanthocyanidins, with significant variation ($P < 0.05$) among different organic solvents, as shown in Table 2. It was observed that the TPAC increased with decreasing solvent polarity. The highest TPAC value was observed in the 100% methanol extract (61.03 mg PB2E/g DW), while the water extract exhibited the lowest TPAC content (41.85 mg PB2E/g DW). In this study, the highest TPAC values were obtained in the 100% methanol extract (61.03 mg PB2E/g DW), 70% methanol extract (60.77 mg PB2E/g DW), and 100% ethanol extract (60.14 mg PB2E/g DW), which were significantly higher than the TPAC values of the other solvent extracts. Moreover, the methanol extracts with different concentrations exhibited higher TPAC compared to the ethanol extract. These findings are consistent with those of the study conducted by Pham,[36] which also indicated that methanol was the optimal solvent for proanthocyanidin extraction. It is worth noting that proanthocyanidins are commonly found in flowers, fruits, and seeds of various plants. This study is the first to report a substantial amount of proanthocyanidins (61.03 mg PB2E/g DW) in the flowers of *C. polyodonta*, which is considerably higher than the TPAC of strawberries (97.60 mg/100 g).[37]

The TAC of *C. polyodonta* flower extracted using various organic solvents is presented in Table 2. The range was from 0.24 mg/g DW for 100% ethanol to 0.49 mg/g DW for water. Interestingly, this contradicted the trend observed for TPC, TFC, and TPAC, as the highest TAC values were obtained with water (0.49 mg CGE/g DW), 30% methanol (0.47 mg CGE/g DW), and 50% methanol (0.48 mg CGE/g DW). These values were significantly higher than those of the other solvent extracts. Additionally, it was found that the *C. polyodonta* flower exhibited higher TAC when extracted with methanol at different concentrations compared to ethanol extracts. This finding aligns with the results of Downey, Mazza, and Krstic,[38] who demonstrated that the most effective solvent system for extracting high levels of anthocyanins was 50% aqueous methanol.

3.4. Antioxidant Activity of *C. polyodonta* Flower Extracts. It is widely acknowledged that a comprehensive antioxidant evaluation necessitates the use of multiple assays to account for different mechanisms of antioxidant action.[39] In our study, we employed the DPPH·assay, $ABTS^{\bullet+}$ assay, and FRAP assay to assess the antioxidant properties of the different solvent extracts of *C. polyodonta* flower, as outlined in Table 3. Regarding the DPPH· scavenging activity, the results ranged

Table 3. DPPH, ABTS Radical Scavenging Values, and Ferric Reducing Power (FRAP) of the flowers of *C. polyodonta* Extracts Obtained with Different Solvents[a]

Solvent type	DPPH (mg TE/g DW)	ABTS (mg TE/g DW)	FRAP (mg/g)
water	11.56 ± 0.37^{d}	200.06 ± 4.96^{d}	1.05 ± 0.02^{e}
30% methanol	12.77 ± 0.10^{bc}	328.58 ± 8.26^{bc}	1.52 ± 0.02^{d}
30% ethanol	12.52 ± 0.26$_{c}$	324.71 ± 14.13^{bc}	1.54 ± 0.06^{d}
50% methanol	12.76 ± 0.13^{bc}	356.93 ± 33.26^{a}	1.55 ± 0.02^{d}
50% ethanol	12.44 ± 0.31^{c}	347.87 ± 3.12^{ab}	1.59 ± 0.03^{d}
70% methanol	12.58 ± 0.28^{c}	328.90 ± 12.91^{bc}	2.11 ± 0.04^{a}
70% ethanol	12.52 ± 0.30^{c}	313.48 ± 12.17^{c}	1.80 ± 0.03^{bc}
100% methanol	13.13 ± 0.19^{ab}	359.06 ± 23.28^{a}	1.75 ± 0.12^{c}
100% ethanol	13.28 ± 0.25^{a}	358.69 ± 3.56^{a}	1.89 ± 0.08^{b}

[a]Data with different superscript lowercase letters a−e in the same column were significantly different ($P < 0.05$).

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from 11.56 to 13.28 mg TE/g DW. Ismail[18] suggested that the DPPH· assay demonstrates greater efficacy in scavenging free radicals within a polar solvent system. In our study, the extracts obtained using 100% methanol (13.13 mg TE/g DW) and 100% ethanol (13.28 mg TE/g DW) exhibited the highest values compared to other solvent extracts. This outcome can be attributed to their higher TPAC. Additionally, there was no significant difference ($P > 0.05$) among the remaining organic solvent extracts, all of which demonstrated favorable free radical scavenging activity. The water extract recorded the lowest free radical scavenging activity (11.56 mg TE/g DW). In terms of $ABTS^{\bullet+}$ scavenging activity, the trend mirrored that of the DPPH assay. The extracts obtained using 100% methanol (359.06 mg TE/g DW), 100% ethanol (358.69 mg TE/g DW), 50% methanol (356.93 mg TE/g DW), and 50% ethanol (347.87 mg TE/g DW) exhibited the highest scavenging activity, while the water extract (200.06 mg TE/g DW) exhibited the lowest scavenging activity. The 100% solvent extracts demonstrated the maximum antioxidant activity in the ABTS assay.

In the FRAP assay, the FRAP values of the 70% methanol solvent extracts (2.11 mg TE/g DW) were significantly higher than those of all other solvent extracts. Conversely, the water extract exhibited the lowest FRAP value (1.05 mg TE/g DW), indicating that the water solubility of the active compounds in the samples related to reducing power was very low. This finding aligns with that of the studies conducted by Sepahpour[40] and Meneses.[41] No significant difference was observed between the FRAP values of the 30% methanol, 30% ethanol, 50% methanol, and 50% ethanol solvent extracts. Furthermore, the antioxidant activity assessed by the three methods followed the same trend observed for the total phenolic compounds. There was a positive correlation between antioxidant activity and TPC, TFC, and TPAC, with Pearson's correlation coefficients (R) of 0.574 ($P < 0.01$), 0.829 ($P < 0.01$), and 0.875 ($P < 0.01$), respectively, obtained for the DPPH assay; 0.944 ($P < 0.01$), 0.818 ($P < 0.01$), and 0.867 ($P < 0.01$), respectively, obtained for the ABTS assay; and 0.722 ($P < 0.01$), 0.892 ($P < 0.01$), and 0.854 ($P < 0.01$), respectively, obtained for the FRAP assay. These values confirm the contribution of phenolics, flavonoids, and procyanidins to the antioxidant activity of the *C. polyodonta* flower. Overall, the three antioxidant assays demonstrated the very high antioxidant capacity of *C. polyodonta* flower, with slight variations in values attributed to the different mechanisms employed by the assays.

3.5. Effect of Solvent on Individual Phenolic Compounds. To assess the influence of solvent on individual phenolic compounds in *C. polyodonta* flower extracts, 14 phenolic compounds were quantified, and these are presented in Table 4. The phenolic compounds analyzed in this study encompassed one anthocyanidin (cyanidin-3-*O*-glucoside), two flavanols ((+)-catechin and (−)-epicatechin), six flavonols (rutin, afzelin, astragalin, kaempferol-3-*O*-rutinoside, quercitrin, and isoquercitrin), four procyanidins (procyanidin B1, procyanidin B2, procyanidin B4, and procyanidin C1), and one ellagitannin (1, 2, 3, 6-tetragalloylglucose).

Four procyanidins, six flavonols, and one ellagitannin were reported and quantified for the first time in *C. polyodonta* flowers. The results regarding the individual phenolic compounds indicated that 70% methanol had the highest content for 7 out of the 13 compounds, including cyanidin-3-*O*-glucoside (392.98 μg/g DW), procyanidin B2 (9301.81 μg/

Table 4. Contents of 14 Phenolic Compounds of the Flowers of *C. polyodonta* Extracts with Different Solvents (μg/g DW)[a]

Solvent type	Cyanidin-3-*O*-glucoside	Procyanidin B1	(+) catechin	Procyanidin B2	Procyanidin B4	Epicatechin	Procyanidin C1	Rutin	Isoquercitrin	1,2,3,6-Tetragalloylglucose	Kaempferol-3-*O*-rutinoside	Astragaline	Quercitrin	Afzelin
water	369.21 ± 14.15^{b}	900.81 ± 19.75^{c}	3053.45 ± 174.63^{e}	8949.95 ± 345.78^{ab}	10677.56 ± 533.32^{e}	10106.74 ± 773.92^{f}	6061.80 ± 166.33^{d}	179.28 ± 8.28^{d}	199.49 ± 10.41^{f}	1897.15 ± 8.87^{e}	377.79 ± 13.74^{e}	110.58 ± 5.77^{f}	489.74 ± 27.05^{d}	578.39 ± 38.65^{d}
30% methanol	350.80 ± 12.01^{bcd}	854.30 ± 34.99^{c}	3574.51 ± 196.08^{d}	7930.53 ± 181.55^{e}	10613.77 ± 304.90^{e}	12489.06 ± 67.44^{e}	5760.57 ± 138.35^{e}	169.98 ± 3.20^{e}	198.32 ± 7.16^{f}	4798.96 ± 414.57^{d}	381.57 ± 7.80^{e}	107.56 ± 3.22^{f}	496.79 ± 22.76^{d}	595.14 ± 30.35^{d}
30% ethanol	341.69 ± 13.27^{d}	877.36 ± 37.66^{c}	4365.17 ± 286.56^{c}	8094.68 ± 243.37^{de}	11482.21 ± 605.56^{d}	17199.34 ± 120.97^{d}	5614.50 ± 137.32^{e}	186.58 ± 8.62^{d}	215.87 ± 4.46^{e}	5280.29 ± 28.65^{c}	415.49 ± 17.41^{d}	118.36 ± 6.34^{e}	571.42 ± 29.20^{c}	865.03 ± 39.26^{c}
50% methanol	367.15 ± 6.26^{bc}	958.82 ± 33.63^{b}	4806.53 ± 15.56^{b}	9292.30 ± 61.10^{a}	12496..20 ± 18.90^{c}	19176.35 ± 68.78^{b}	6483.35 ± 65.58^{b}	200.39 ± 1.44^{c}	262.78 ± 2.76^{bc}	6155.05 ± 118.97^{ab}	445.30 ± 3.04^{c}	139.81 ± 0.92^{bc}	668.23 ± 2.36^{b}	864.92 ± 6.34^{b}
50% ethanol	349.70 ± 6.49^{cd}	858.20 ± 17.34^{c}	4965.98 ± 41.55^{b}	8666.59 ± 55.74^{bc}	12829.02 ± 101.76^{bc}	19151.75 ± 213.20^{b}	6296.63 ± 88.87^{bc}	201.47 ± 0.54^{c}	276.05 ± 0.12^{a}	6273.90 ± 164.24^{a}	452.97 ± 3.07^{bc}	136.49 ± 0.10^{c}	672.48 ± 1.96^{b}	908.96 ± 3.34^{a}
70% methanol	392.98 ± 4.39^{a}	968.36 ± 18.41^{ab}	4954.79 ± 17.06^{b}	9301.81 ± 107.71^{a}	13730.30 ± 37.31^{a}	20339.37 ± 421.89^{a}	6914.08 ± 33.62^{a}	235.08 ± 3.07^{a}	249.02 ± 1.87^{d}	6117.51 ± 397.32^{ab}	553.41 ± 3.65^{a}	174.52 ± 3.21^{a}	711.31 ± 12.07^{a}	882.96 ± 5.65^{ab}
70% ethanol	272.28 ± 6.92^{e}	571.47 ± 7.62^{e}	6051.77 ± 86.08^{a}	8237.46 ± 161.52^{de}	12680.08 ± 303.24^{bc}	19249.68 ± 324.57^{b}	6207.61 ± 133.61^{cd}	213.99 ± 3.92^{b}	264.06 ± 1.15^{bc}	5702.12 ± 140.44^{bc}	446.37 ± 5.97^{c}	129.84 ± 2.64^{d}	654.37 ± 6.90^{b}	871.08 ± 17.96^{b}
100% methanol	365.89 ± 11.24^{bc}	682.23 ± 11.94^{d}	6056.23 ± 79.81^{a}	8305.36 ± 134.76^{d}	13189.08 ± 232.73^{ab}	19819.29 ± 192.70^{ab}	7026.06 ± 133.01^{a}	203.12 ± 1.78^{c}	267.89 ± 3.50^{b}	6596.97 ± 9.93^{a}	465.27 ± 10.30^{b}	143.40 ± 1.37^{b}	702.73 ± 8.26^{a}	914.98 ± 9.14^{a}
100% ethanol	244.99 ± 5.93^{f}	1008.40 ± 20.00^{a}	5045.45 ± 34.81^{b}	8343.84 ± 118.98^{cd}	10896.15 ± 146.73^{e}	17992.79 ± 178.04^{c}	6302.64 ± 98.37^{bc}	180.46 ± 0.94^{d}	256.25 ± 0.99^{cd}	6151.40 ± 123.37^{ab}	410.77 ± 3.43^{d}	137.30 ± 0.62^{bc}	706.15 ± 0.18^{a}	910.43 ± 6.24^{a}

[a]Data with different superscript lowercase letters a−f in the same column were significantly different ($P < 0.05$).

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g DW), procyanidin B4 (13730.30 μg/g DW), epicatechin (20339.37 μg/g DW), rutin (235.08 μg/g DW), kaempferol-3-*O*-rutinoside (553.41 μg/g DW), quercitrin (711.31 μg/g DW), as well as astragaline (174.52 μg/g DW). The content of individual phenolic compounds was significantly influenced by the solvent concentrations, which increased with methanol concentrations ranging from 30% to 70%. These findings suggest that 70% methanol is a good solvent for extracting phenolic compounds from *C. polyodonta*. This finding is consistent with other research that utilized a 75% aqueous methanol extract from *Limnophila aromatica*, compared to 50% aqueous methanol and 100% methanol extracts.[42] Additionally, Dai and Mumper[43] mentioned that methanol is excellent for extracting lower molecular weight polyphenols, such as flavonoids, which aligns with our study.

It can be observed that certain phenolic compounds exhibited varying amounts depending on the solvent used for extraction (Table 4). Procyanidin C1 (7026.06 μg/g DW), 1, 2, 3, 6-tetragalloylglucose (6596.97 μg/g DW), (+) catechin (6056.23 μg/g DW), and afzelin (914.98 μg/g DW) had the highest content when extracted with 100% methanol, while procyanidin B1 (1008.40 μg/g DW) had the highest content when extracted with 100% ethanol, and isoquercitrin (276.05 μg/g DW) had the highest content when extracted with 50% ethanol. This variation can be attributed to the different polarities of the solvents used. Each phenolic compound possesses a specific degree of polarity due to the hydroxyl groups present in the aromatic ring. Consequently, when the polarity of the extraction system changes, each compound will be extracted to a lesser or greater extent.[44] Notably, 1, 2, 3, 6-tetragalloylglucose was identified for the first time in *C. polyodonta*, and it exhibited strong antioxidative activity due to its large number of galloyl units. It also demonstrated a significant inhibitory effect on lipid peroxidation and low-density lipoprotein (LDL) oxidation.[45] These findings suggest that *C. polyodonta* flowers possess excellent hypolipidemic capacity.

Furthermore, the most predominant phenolic compounds in *C. polyodonta* flowers were identified as procyanidin B4, procyanidin B2, procyanidin C1, (+)-catechin, 1, 2, 3, 6-tetragalloylglucose, and (−)-epicatechin. Although flavanols are commonly found in edible flowers,[46,47] the content of these compounds in *C. polyodonta* flowers was significantly higher compared to that reported in the study conducted by Morais,[48] which reported the flavanol content in eight other edible flowers.

4. CONCLUSION

This research investigates and identifies the effects of different solvents on TPC, TFC, TPA, TAC, 14 individual phenolic compounds, and antioxidant activity in *C. polyodonta* flowers. The type and concentration of extraction solvents significantly influence the extraction capacity for phenolic and flavonoid contents, as well as antioxidant activity. The TPC, TFC, TPA, and the antioxidant activity of the extracts generally increase with the concentration of solvents, while the TAC is the highest when using low-concentration solvents such as pure water, 30% methanol, and 50% methanol. In terms of antioxidant activity, the extracts obtained with 100% solvent exhibit the highest DPPH and ABTS values, whereas those obtained with 70% methanol show the highest FRAP values. When considering individual phenolic compounds, 70% methanol also yields the highest content for seven major compounds. Furthermore, this research identified several major phenolic compounds, such as procyanidin B1, procyanidin B2, procyanidin B4, and procyanidin C1, in *C. polyodonta* flowers for the first time. In conclusion, 70% methanol can be selected as the most effective solvent for polyphenol extraction in *C. polyodonta* flowers. These results provide insight into the relationship of extraction solvent, phytochemicals, and antioxidant activity for further *in vivo* biological activity research in *C. polyodonta* flowers.

■ ASSOCIATED CONTENT

Data Availability Statement

No data will be made available on request because the patent is filed for these section data and these data was also for another further study.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c01321.

(UPLC-Q-TOF-MS/MS spectra of standard PDF)

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Author Contributions

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Notes

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