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Geographic variation in water-soluble polysaccharide content and antioxidant activities of *Cyclocarya paliurus* leaves

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ABSTRACT

Cyclocarya paliurus is a multiple function woody plant. To reveal the geographic variation pattern of water-soluble polysaccharide content in the leaves and its antioxidant activities, the leaf samples were collected and analyzed from 29 geographic locations of natural *C. paliurus* populations throughout the distribution areas. Significant differences in the water-soluble polysaccharide content and antioxidant activities were detected among the *C. paliurus* populations. The water-soluble polysaccharide content in the leaves of different populations ranged from 18.72 to 53.59 $mg\ g^{-1}$, and the 29 populations could be classified into four distinct groups. There was a significant correlation between the water-soluble polysaccharide content and scavenging activities for the leaves of *C. paliurus* ($p < 0.01$). Multivariable nonlinear regression analysis showed that no clear geographic variation pattern in water-soluble polysaccharide content was detected for the leaves sampled. However, Pearson correlation analysis indicated that the average annual precipitation was significantly correlated to the leaf polysaccharide content ($r = 0.428$, $p < 0.05$), and its scavenging activities on DPPH radical ($r = 0.386$, $p < 0.05$) and superoxide radical ($r = 0.446$, $p < 0.05$). Based on the water-soluble polysaccharide contents of all populations studied, leaves from the Wufeng, Muchuan and Liping were recommended for food and medical use in the future. Results from this study not only demonstrated that the polysaccharide content in *C. paliurus* leaves could be used either as nutriments, food additives, or antioxidant supplements, but also provided a basis for optimizing cultivation strategies of *C. paliurus* plantations.

1. Introduction

Reactive oxygen species (ROS), including superoxide radical, hydroxyl radical and hydrogen peroxide, can cause oxidative damage to tissues of plants and animals if produced excessively (Chan and Chan, 2015; Gupta et al., 2013; Simon et al., 2000). The oxidative stress induced from them has been reported to be the principal cause of many diseases such as cancer (Poillet et al., 2015), colitis (Van den Ende et al., 2011) and liver injury (Takemoto et al., 2014; Xu et al., 2015). Thus, to protect our human body from excess ROS-induced effects, new natural antioxidants without toxicity and side effect are usually recommended, such as flavone, polyphenol and polysaccharides (Bitanihirwe and Woo, 2010). Of which, polysaccharides from plants are a class of promising anti-oxidants due to their low (or no) toxicity (Cui et al., 2013).

Polysaccharides, known as natural polymers, are widely existed in plants, animals and microorganisms. Recently, polysaccharides from plants have attracted more and more attention due to their extensive biological activities (Chen et al., 2008; Huang and Ning, 2010; Xie et al., 2013a,b; Zhao et al., 2008). It could be learned from some published literatures that many plant polysaccharides had been considered as potential antioxidants because of their evident antioxidant activities such as scavenging activities of DPPH free radical, hydrogen peroxide and hydroxyl radical (Gao et al., 2015; Jiang et al., 2010; Shahidi and Zhong, 2010; Tseng et al., 2008). Hu et al. (2009) reported that polysaccharide from *Potentilla anserine* could protect mice cells against oxidative damage caused by ROS. Moreover, Yue et al. (2014) found that polysaccharide could improve the effect of superoxide dismutase (SOD) to alleviate the oxidative injury.

Cyclocarya paliurus (Batal) Iljinskaja, commonly called "sweet tea tree", belongs to the Juglandaceae family and is widely distributed in mountainous regions of sub-tropical China (Fang et al., 2006; Deng et al., 2015). Leaves of *C. paliurus* are traditionally used in China as a nutraceutical tea because of its unique taste (Birari and Bhutani, 2007;

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Fang et al., 2011a,b). Many studies have indicated that leaves of *C. paliurus* possesses a variety of bioactivities, such as hypoglycemic activity (Kurihara et al., 2003), antihypertensive activity (Xie et al., 2006), anti-HIV-1 (Zhang et al., 2010), anticancer (Xie et al., 2013a,b), and antioxidant activity (Xie et al., 2010). These potential health benefits are attributed to the bioactive compounds in the leaves of *C. paliurus*. Previous investigations indicated that *C. paliurus* leaf contained abundant physiologically active compounds, such as triterpenoids, flavonoids, phenolic acids, and polysaccharides (Xie et al., 2010; Fang et al., 2011a,b; Wu et al., 2017). Thus, most studies on *C. paliurus* were focused on extraction procedures and identification of these substances, as well as their pharmacological effects (Zhang et al., 2010; Xie et al., 2013a,b; Cao et al., 2017).

In recent years, attentions have been cast on polysaccharide from *C. paliurus* leaves due to its biological activities (Xie et al., 2010). For example, the structure and antioxidant activities of polysaccharide as well as sulfated polysaccharides from *C. paliurus* leaves had been investigated (Xie et al., 2010, 2015), while the dynamic variation of polysaccharide accumulation in *C. paliurus* leaves was studied among the plantations of different provenances (Fu et al., 2015). Accumulation of phytochemicals in plants is often affected by various factors, such as genetic, harvesting time and climatic factors during the growing period (Awad et al., 2001; Björkman et al., 2011; Cui et al., 2013). Although some studies have reported variation of phytochemicals in *C. paliurus*, no knowledge of geographic variation in leaf polysaccharide or other compounds (triterpenoids, or flavonoids) is available for natural forests of *C. paliurus*. To our knowledge, this is the first attempt to analyze the geographic variation of *C. paliurus* polysaccharide in natural populations, and to evaluate the phytochemical variation in relation to antioxidant activities. The aims of this study were (1) analyze if there is a clear geographical pattern for the variation of water-soluble polysaccharide content in natural populations of *C. paliurus*, and (2) analyze if the variation of water-soluble polysaccharide content in *C. paliurus* is associated with antioxidant activities and environmental conditions. The information provided by this study could be useful for the selection of natural populations with adequate leaf polysaccharide content, as well as for establishing *C. paliurus* plantations with better food and medical use.

2. Materials and methods

2.1. Plant material

Collections of fully expanded leaves from *C. paliurus* trees took place in 2014 during the same period throughout its distribution areas. At each site or location, sample trees (6–30 trees per site, generally dominant or co-dominant tree in the stand) were selected based on tree age (over 20 years-old), stem form and growth vigor. Number of trees for collecting leaves for each natural population was determined according to stand area and quantity of *C. paliurus* naturally distributed on the area (about 10% of the total), and a total of 287 samples from 29 natural populations were collected. About 400 g fresh fully developed leaves were collected from the middle crown for each tree, and then sealed up with silica gel for transportation. The detailed geographical and climatical information for the 29*C. paliurus* populations was shown in Table 1. Leaves collected in each natural population were mixed together in the lab and then dried (70 °C, 48 h) to constant weight, and ground into fine powder before extraction. All samples were stored at room temperature prior to analysis.

2.2. Extraction and measurement of polysaccharide

Extraction of polysaccharide in *C. paliurus* leaves was carried out as described previously by Fu et al. (2015) with slight modifications. Each sample (0.5 g) of leaves was extracted with 30 ml of 70% ethanol at 70 °C for 60 min to remove most pigments, small molecular sugars and impurities. The insoluble residues were separated, dried and then extracted twice with 20 ml distilled water at 100 °C for 75 min. The extracts were filtered and the filtrate was centrifuged at 5000 × g for 15 min. Finally, the supernatant was combined and the polysaccharide

Table 1
Geographical and climatical information of the 29 populations of *C. paliurus* collected.

Natural populations	Province	County or location	Latitude (N)	Longitude (E)	Annual mean temperature (°C)	Altitude (m)	Annual mean sunshine hours (h)	Annual mean Precipitation (mm)
Jixi (A1)	Anhui	Jixi	30°08′50″	118°53′41″	15.0	680	1926.4	1480.0
Xuancheng (A2)	Anhui	Xuancheng	30°13′34″	118°27′0″	15.5	610	1784.1	1307.6
Qimen (A3)	Anhui	Qimen	30°1′11″	117°31′44″	11.9	400	1800	1762.9
Shucheng (A4)	Anhui	Shucheng	31°01′00″	116°32′30″	15.6	790	1969	1027.7
Pucheng (F1)	Fujian	Pucheng	27°55′43″	118°45′46″	13.2	786	1738.7	1200.0
Mingxi (F2)	Fujian	Mingxi	26°34′7″	116°56′3″	16.5	574	1900	1737.0
Jiumu (Z1)	Zhejiang	Jiumu	30°24′36″	119°38′22″	11.6	774	2009	1476.0
Longwang (Z2)	Zhejiang	Longwang	30°40′00″	119°41′00″	14.0	425	1851	1400.0
Meiwu (Z3)	Zhejiang	Meiwu	27°46′00″	119°17′00″	16.5	678	1862	1600.0
Fenghua (Z4)	Zhejiang	Fenghua	29°45′41″	121°13′4″	16.0	513	1850	1602.0
Lishui (Z5)	Zhejiang	Lishui	27°54′40″	119°11′19″	12.3	1216	1783.2	1415.7
Ningbo (Z6)	Zhejiang	Ningbo	29°48′3″	121°47′33″	16.2	60	1850	1500.0
Wencheng (Z7)	Zhejiang	Wencheng	27°52′30″	119°50′29″	18.5	959	1725	1698.0
Hefeng (H1)	Hubei	Hefeng	29°52′47″	110°25′10″	14.8	1155	1960	1400.0
Wufeng (H2)	Hubei	Wufeng	30°17′00″	110°80′00″	16.7	988	1533	1893.9
Yongshun (H3)	Hunan	Yongshun	28°52′53″	110°19′53″	15.8	730	1266.3	1365.9
Fenyi (J1)	Jiangxi	Fenyi	27°37′37″	114°31′56″	18.0	450	1737.1	1590.0
Xiushui (J2)	Jiangxi	Xiushui	28°9′7″	114°31′8″	16.5	854	1700	1450.0
Nanzhao (H4)	Henan	Nanzhao	33° 28′ 35″	112° 00′ 05″	12.7	880	1897.9	900.0
Lüyang (S1)	Shanxi	Lüyang	33°34′00″	105°84′00″	13.2	1250	1526.2	860.0
Muchuan (S2)	Sichuan	Muchuan	28°58′00″	103°47′00″	12.9	1200	965.3	1533.2
Qingchuan (S3)	Sichuan	Qingchuan	32°45′	104°84′	21.5	825	900	1340.0
Jinzhongshan (G1)	Guangxi	Jinzhongshan	24°36′36″	104°57′00″	17.1	1798	1475	1200.0
Laowangshan (G2)	Guangxi	Laowangshan	24°27′18″	106°20′24″	20.3	1445	1377.7	1115.0
Longsheng (G3)	Guangxi	Longsheng	25°37′12″	109°53′24″	16.4	606	1244	1544.0
Jianhe (G4)	Guizhou	Jianhe	26°22′12″	108°22′48″	15.4	1178	1236.3	1200.0
Liping (G5)	Guizhou	Liping	26°20′24″	109°14′24″	15.6	727	1317.5	1425.9
Shiqian (G6)	Guizhou	Shiqian	27°21′00″	108°06′36″	16.7	1239	1232.9	966.0
Yinjiang (G7)	Guizhou	Yinjiang	27°44′24″	108°30′36″	15.1	1032	1255	1100.0