American Journal of Biochemistry and Biotechnology

Research Article

Screening of Bioactive Constituents from the Aerial Parts of *Dictamnus dasycarpus* Turcz. With Antioxidant, Immunoenhancing, and Anticoagulant Potential

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Article history
Received: 05-12-2024
Revised: 22-05-2025
Accepted: 02-06-2025

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Abstract: The cultivation of *Dictamnus dasycarpus* Turcz (DdT) is expensive and difficulty associated with growing, making its root bark the only commonly used medicinal part, while other parts are discarded. Therefore, in order to improve the limited utilization of resources, the new medicinal parts were screened from polysaccharide-rich aqueous extracts. The samples were the purified polysaccharides from the aerial part on ground of *Dictamnus dasycarpus* stems and leaves (PDAP) and dry root bark of DdT (PDBP) by hot water extraction and Diethylaminoethyl Cellulose purification. Then, their physicochemical properties and structural characterization were evaluated. The results of physicochemical properties showed that both had great similarity. However, PDAP contained higher uronic acid content. Furthermore, PDAP exhibited higher antioxidant activity than PDBP in scavenging OH radicals (IC_{50}: 4.73±0.03 mg/mL versus 5.29±0.01 mg/mL), ABTS radicals (IC_{50}: 0.23±0.01 mg/mL versus 5.34±0.01 mg/mL) and DPPH radicals (IC50: 2.08±0.02 mg/mL versus 8.52±0.03 mg/mL). Both showed strong enhanced activity, while PDAP was more prominent than PDBP in phagocytosis activity. Meanwhile, PDAP possessed higher pro-coagulant activity than PDBP, as evidenced by shortening the clotting time of PT and APTT, while PDBP appeared to exert pro-coagulant activity through shortening the clotting time of TT and elevating the content of FIB. PDAP's efficacy surpasses that of conventional PDBP, offering a scientific foundation for utilizing the medicinal benefits of DdT's aerial part.

Keywords: *Dictamnus dasycarpus* Turcz, Polysaccharide, Antioxidant Activity, Immunoenhancement Activity, Coagulation Activity

Introduction

Dictamnus dasycarpus Turcz (DdT), a persistent botanical specimen within Rutaceae, exhibits broad dispersion across numerous Chinese territories. These encompass Heilongjiang, Jilin, Liaoning, plus Inner Mongolia (Gao *et al.*, 2011; Guo *et al.*, 2016). In traditional Chinese medicine, the dry root bark from DdT (DB) is frequently used to address conditions like rheumatism, itching, bleeding, and various skin ailments. Practitioners utilize DB confronting rheumatism, pruritus, hemorrhagic conditions, alongside dermal afflictions (Lv *et al.*, 2015). However, the aerial part on ground of *Dictamnus dasycarpus* stems and leaves (DA) are usually discarded, leading to a waste of resources. Some studies have been conducted to compare the structure and antioxidant activity of the main chemical components in six parts of the DdT, including the aerial part and roots, and found that the highest activity in the leaves and the essential oil of the aerial part show significant inhibitory effect on Candida albicans, suggesting that the aerial part have potential as a medicinal part (Tian *et al.*, 2019; Cao *et al.*, 2022). Hence, uncovering and applying the medicinal benefits of the DA is crucial.

Polysaccharides are considered to be a kind of polymer material with the advantages of low price, few side effects and biodegradability, and have been widely

employed as highly valuable biomaterials in medical and experimental research (Liu *et al.*, 2015; Zhao *et al.*, 2019). Polysaccharides demonstrate intimate correlations with multifarious biological functions encompassing antioxidative, antineoplastic, coagulative, and immunomodulatory functions (Chen *et al.*, 2018a; Wang *et al.*, 2017). Contemporary pharmacological investigations reveal DB contains numerous bioactive entities—limonoids, quinolone alkaloids, sesquiterpenes, coumarins, and steroids—which form the basis of its pharmacological effects: anticancer, anti-inflammatory, antimicrobial, immunoregulatory, antiplatelet aggregation, and radical-scavenging capacities (Chang *et al.*, 2001; Choi *et al.*, 2016, 2019; Han *et al.*, 2015; Kim *et al.*, 2013). Nevertheless, polysaccharides isolated from DB or DA remain inadequately characterized.

Consequently, this investigation procured crude polysaccharides from DA/DB via aqueous extraction-ethanol precipitation methodologies. Subsequent purification employed Diethylaminoethyl Cellulose (DEAE-52), yielding purified DA polysaccharides (PDAP) and DB polysaccharides (PDBP). Structural elucidation entailed UV-visible (UV-vis) spectrophotometry, Fourier Transform Infrared (FT-IR) spectroscopy, Second-Derivative Infrared (SD-IR) spectroscopy, and High-Performance Liquid Chromatography (HPLC). Moreover, the biological activities including antioxidant, immunoenhancement and coagulation activities of PDAP and PDBP were assessed. This paper aims to identify new medicinal parts of DdT to improve the limited utilization of resources.

Materials and Methods

Materials

DA and DB specimens originated from Zhongxin Farmer Specialized Cooperative Society for Chinese Medicinal Materials Cultivation within Liuhe County, Jilin Province, China. Authentication was performed by Xue Jianfei, Jilin University of Chemical Technology associate professor. DEAE-52 procurement occurred through Yuan Ye Biotechnology Co., Ltd. (Shanghai, China).

Fucose (Fuc), Mannose (Man), Glucosamine (GluN), Galactose (Gal), Ribose (Rib), Arabinose (Ara), Glucose (Glu), Glucuronic acid (GluA), Galacturonic acid (GalA), Rhamnose (Rha), and Xylose (Xyl) were sourced from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Beijing, China). RAW264.7 denoted a murine macrophage lineage. Sodium pyruvate, penicillin, and streptomycin derived from Chinese Academy of Sciences culture repositories (Shanghai, China). Standard human plasma (SFDA prospective 2006, 3401635) for coagulant capacity assessment was procured from Dade Behring Marburg Gmbh (Marburg, Hesse, Germany). Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), Prothrombin Time (PT), and Fibrinogen (FIB) quantification kits were furnished by SINNOWA Medical Science & Technology Co., Ltd. (Nanjing, Jiangsu, China).

Extraction and Purification

One kilogram DA or DB desiccated powder commenced aqueous immersion twice employing distilled water, maintaining a 1:12 solid-liquid proportion. Thermal extraction ensued at 100°C across 120 minutes. The resultant slurry underwent filtration followed by pressurized concentration. This concentrate amalgamated with quadruple volumes of 95% ethanol at 4°C, undergoing nocturnal quiescence. Subsequent rotational isolation transpired at 5000 $r \cdot min^{-1}$ for 300 seconds. The sediment underwent cryodesiccation yielding crude polysaccharides. Crude polysaccharide solution (10 mg/mL) received application upon DEAE-52 preconditioned vitreous columns (40 × 5cm). Low-molecular-weight contaminants underwent elution via deionized aqueous passage succeeded by 0.5 M NaCl irrigation, sustaining 1.0 mL/min flux, and each eluate is collected. This was followed by dialysis with flowing water for 24 hours. Ultimately, the primary segments were collected, subjected to dialysis, and freeze-dried to obtain PDAP and PDBP (Li *et al.*, 2020) for further analytical examination.

Physicochemical Property

Total carbohydrate quantification employed phenol-sulfuric acid methodology. Calibration conformed to $Y = 7.09x - 0.0602$ ($r = 0.9998$; Y = absorbance; x = polysaccharide concentration mg/mL; linear span: 0.02–0.1mg/mL) (Dubois *et al.*, 1956). Uronic acid levels underwent meta-hydroxydiphenyl analysis. Standardization yielded $Y = 8.8625x - 0.042$ ($r = 0.9998$; Y = absorbance; x = polysaccharide concentration mg/mL; operational scope: 0.016–0.08 mg/mL) (Blumenkrantz *et al.*, 1973). Protein determination utilized Bradford assay protocol. Reference characterization produced $Y = 7.23x - 0.0022$, ($r = 0.9999$; Y = absorbance; x = protein concentration mg/mL; applicable domain: 0.02–0.1 mg/mL) (Bradford, 1976).

UV-vis, FT-IR and SD-IR

UV-vis and FT-IR assessments were executed as derived from previous investigations (Liu *et al.*, 2021; Nie *et al.*, 2018). SD-IR data underwent computational management per Han *et al.*'s delineated protocol (2024).

Monosaccharide Compositions

After pre-column derivatisation as exhibited in previous research (Hu *et al.*, 2018), monosaccharide composition was analyzed using HPLC.

Molecular Weight

The molecular weight was demonstrated by HPSEC-RID based on the previous research (Hu *et al.*, 2019).

For molecular weight approximation, calibration curves generated via dextran standards (Choi *et al.*, 2016; 2019) were referenced.

Antioxidant Activity

The average value of the 50% free radical inhibition concentration calculated based on vitamin C positive control.

Scavenging capacities toward DPPH radicals (Zhang *et al.*, 2018) and ABTS radicals underwent distinct quantification (Nie *et al.*, 2017). Respective commingling of 0.1 mL specimen with 2.9 mL DPPH methanolic solution and $ABTS^{+}$ solution preceded absorbance recording at 517 nm and 734 nm.

Hydroxyl radical neutralization potency assessment proceeded pursuant to Tang *et al.* (2014). Combination of 0.1 mL specimen with 0.05 mL $FeSO_4$ (9 mmol/L) and 0.1 mL salicylic acid-ethanol solution (9 mmol/L) occurred, succeeded by 0.1 mL H_2O_2 (8.8 mmol/L) addition. The absorbance was measured at 510 nm.

Immunomodulatory Activity

Building upon current methodologies, an MTT assay assessed PDAP and PDBP's influence upon RAW264.7 cells (Hu *et al.*, 2020). Specimens (100 μL) were introduced into cellular suspensions, establishing concentrations spanning 25-800 μg/mL. The entire medium, devoid of any polysaccharide, served as the control for the blank. Absorbance quantification occurred at 490 nm.

Concurrently, phagocytic activity and nitric oxide (NO) generation underwent assessment, precisely documented per Shi *et al.* (2014) and Lv *et al.* (2016). Respective absorbance measurements transpired at 490 nm and 540 nm.

Coagulation Activity

Coagulation activity was tested as described in the reference (Zhai *et al.*, 2021). 100 μL plasma and 100 μL specimen underwent commingling followed by 37°C incubation. Clotting time was recorded separately, and normal saline was used as a blank control. Yunnan Baiyao—a century-renowned Chinese hemostatic agent—served as positive reference within the investigation (Tang *et al.*, 2009).

Statistical Analysis

Three parallel experiments were performed for each sample. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) facilitated analytical procedures, with outcomes rendered as mean ± SD. Intergroup differences underwent evaluation via one-way ANOVA. Probability thresholds beneath 0.05 signified statistical consequence.

Results and Discussion

Extraction and Content Determination

Table 1 delineates polysaccharide, uronic acid, and protein constituents within PDAP and PDBP: 59.82±0.015% contrasted with 64.57±0.012%; 7.51±0.013% versus 6.90±0.011%; and 11.25±0.016% against 9.74±0.017%, respectively. These measurements suggest polysaccharides constitute principal bioactive elements in both polymers, with PDAP demonstrating diminished polysaccharide content relative to PDBP. Furthermore, uronic acid and protein manifested elevated concentrations in PDAP compared to PDBP.

Table 1: Physicochemical properties of PDAP and PDBP

Name	PDAP	PDBP
Polysaccharide (%)	59.82±0.015%	64.57±0.012%
Uronic acid (%)	7.51±0.013%	6.90±0.011%
Protein (%)	11.25±0.016%	9.74±0.017%

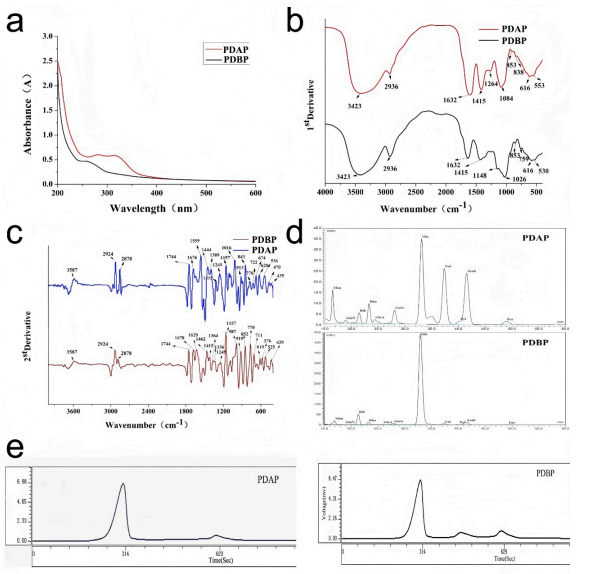

Fig. 1: Structural identification of PDAP and PDBP. (a) UV−vis spectra of PDAP and PDBP. IR spectra (b) second-derivative IR spectra (c) of PDAP and PDBP. (d) Monosaccharide composition of PDAP and PDBP. (e) Molecular Weights of PDAP and PDBP

Analysis of Structural Characterization

As can be seen from Figure 1a, an obvious absorption peak of PDAP was detected around 260 nm to 280 nm, manifesting that the protein content of PDAP was higher than PDBP, which was the same as the determination of protein content by physicochemical property.

In addition, although the FT-IR of PDAP and PDBP were similar and generally consistent, there were some differences as shown in Figure 1b. PDAP exhibited a pronounced absorption peak at 1632 cm^{-1}, conferred upon C=O bond elongation. The 1415 cm^{-1} signal intensity originated from C–H dissymmetrical stretching vibrations (Chen *et al.*, 2018b). An unequivocal spectral feature near 1264 cm^{-1} within PDAP signified S=O tensile vibration absorption (Wang *et al.*, 2018a). Pyranose ring tensile oscillation materialized circa 759 cm^{-1} in PDBP (Liu *et al.*, 2018).

SD-IR spectroscopy is the way that can distinguish the overlapping peaks for more detailed comparison. As

observed in Figure 1c, the peak intensities of PDAP at 1249 and 1016 cm-1 were higher than those of PDBP due to the typical absorption peaks of polysaccharides containing pyranose rings (Zhang *et al.*, 2015). Conversely, PDBP exhibited heightened intensity at 1157 cm^{-1} plus the 760-770 cm^{-1} range versus PDAP. This signifies angular oscillations within C-O bonds and symmetrical C-O-C vibrations, respectively, attributable to the D-glucopyranosyl ring (Liu *et al.*, 2018; Tommonaro *et al.*, 2007).

As shown in Figure 1d and Table 2, the monosaccharide composition (Man, GluN, Rib, Rha, GluA, GalA, Glu, Gal, Xyl, Ara, Fuc) of PDAP and PDBP at a molar ratio was 5.62: 0.72: 2.22: 4.20: 1.96: 5.88: 23.46: 14.60: 1: 20.76: 1.74 and 6.01: 0.44: 12.82: 4.60: 0.95: 5.00: 186.01: 2.40: 1: 8.08: 0.77, respectively. Remarkably, the proportion of Gal and Ara in PDAP was higher than in PDBP, while PDBP possessed a higher proportion of Glu and Rib than PDAP. Figure 1e showed that the molecular weight of PDAP and PDBP was 6.3×10^3 Da and 6.7×10^3 Da, respectively.

Analysis of Biological Activities

First, as shown in Figure 2a to 2d, both PDAP and PDBP exhibited weaker antioxidant activity than VC group, while PDAP possessed stronger capacity than PDBP in scavenging OH radicals (4.73±0.03 mg/mL *versus* 5.29±0.01 mg/mL), ABTS radicals (0.23±0.01 mg/mL *versus* 5.34±0.01 mg/mL) and DPPH radicals (2.08±0.02 mg/mL *versus* 8.52±0.03 mg/mL). The disparity in the impact of PDAP versus PDBP on the elimination of DPPH and ABTS radicals was markedly significant, evidenced by a *p*-value less than 0.001, and similarly, the effect on OH radical scavenging was also noteworthy with a $p<0.01$.

Table 2: Molar ratio of monosaccharide composition of PDAP and PDBP

Monosaccharide	Man	GluN	Rib	Rha	GluA	GalA	Glu	Gal	Xyl	Ara	Fuc
PDAP	5.6159	0.7169	2.2215	4.2032	1.9573	5.8763	23.4601	14.6027	1	20.7572	1.7395
PDBP	6.0064	0.4432	12.8227	4.6044	0.9542	5.001	186.0101	2.3975	1	8.0836	0.7719

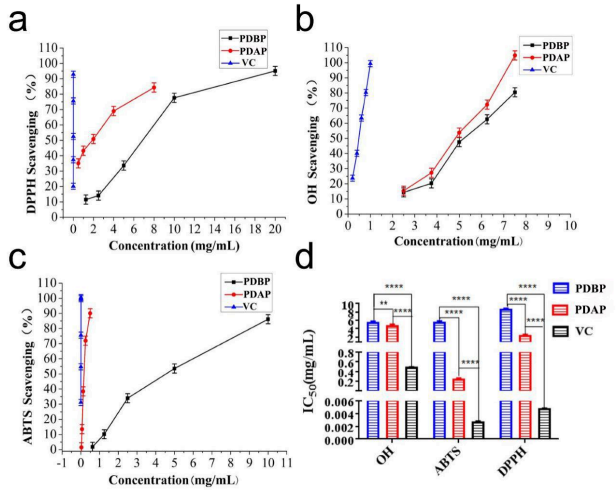

Fig. 2: DPPH radicals (a), OH radicals (b), ABTS radicals (c) and IC 50 value (d) of PDAP and PDBP. (*) expressed significantly different from the control

Next, as can be seen from Figure 3a and 3b, PDAP and PDBP could facilitate the proliferation of RAW264.7 cells within the range of 25-800 μg/mL compared to the blank control. In addition, Figure 3c and 3d showed, in contrast to the blank control, PDAP and PDBP could remarkably enhance phagocytosis activity. However, the concentrations of PDBP between 400 and 800 μg/mL did not remarkably enhance phagocytosis activity, although it showed an upward trend than blank control The ability of both PDAP and PDBP to produce NO secreted by RAW264.7 was clearly demonstrated in Figure 3e and 3f macrophages in the 25-800 μg/mL concentration range in comparison to the blank control. To sum up, PDAP exhibits superior immunomodulatory capabilities over PDBP, potentially due to its elevated uronic acid levels (Georgiev *et al.*, 2017; Wang *et al.*, 2018b)

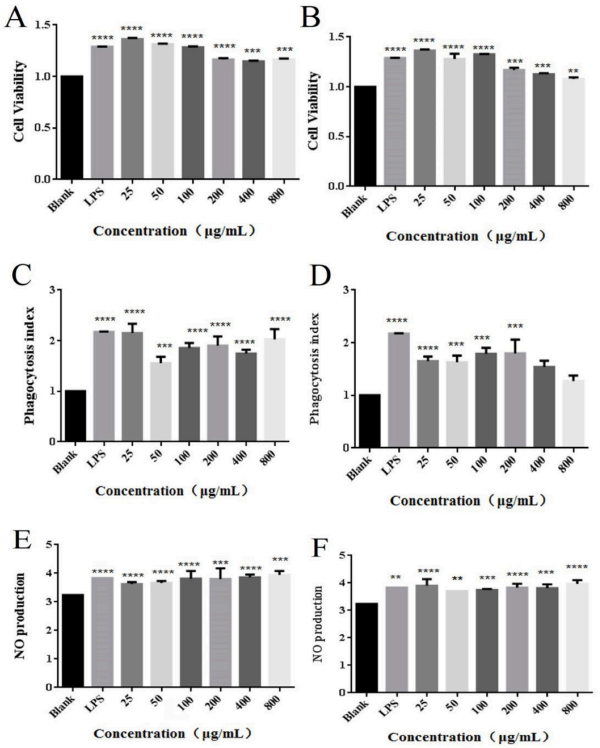

Fig. 3: MTT assay of PDAP (a) and PDBP (b); phagocytosis activity of PDAP (c) and PDBP (d); NO production of PDAP (e) and PDBP (f). (*) expressed significantly different from the blank control

Last, results as demonstrated in Figure 4a and 4b, PDAP possessed higher pro-coagulant activity than PDBP, as evidenced by shortening the clotting time of PT and APTT. However, PDBP appeared to exert pro-coagulant activity through shortening the clotting time of TT and elevating the content of FIB. That is to say, PDAP exerted its pro-coagulant activity via the coagulation pathway of extrinsic and intrinsic (Cao *et al.*, 2019; Sun *et al.*, 2018). while PDBP exerted its pro-coagulant activity via the common coagulant pathways and regulation of fibrinolytic systems (Song *et al.*, 2019; Zhai *et al.*, 2021).

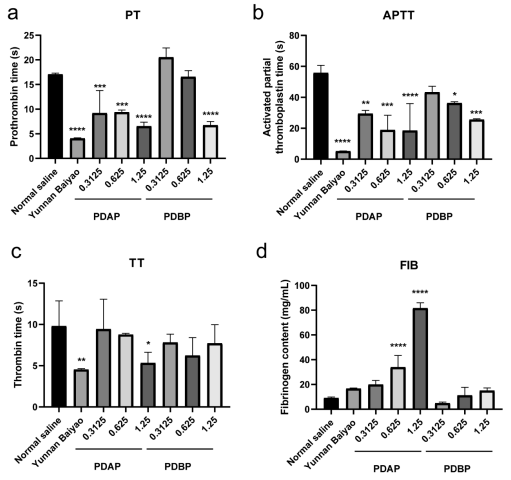

Fig. 4: Coagulant activity was detected by PDAP and PDBP. (*) expressed significantly different from the blank control

Antioxidant activity reduces oxidative damage to damaged tissues by reactive oxygen species (ROS), delays cell necrosis and indirectly enhances immune function (Yu, 1994). Immunomodulation accelerates the removal of necrotic tissue by activating macrophages, inhibits the risk of infection and promotes tissue repair after haemostasis (Li, 2023). PDAP is functionally similar to existing pharmaceutical polysaccharides, such as *Bletilla striata* polysaccharides, which have antioxidant, immunomodulatory and haemostatic effects (Chen *et al.*, 2021), and can be widely used as a kind of plant polysaccharides with rich and significant pharmacological effects in clinical applications. The IC_{50} values of *Bletilla striata* polysaccharides against DPPH, ABTS and OH radicals were 2.601, 3.157 and 6.532 mg/mL, respectively. Compared with the literature, PDAP was more effective in scavenging DPPH, ABTS and OH radicals than *Bletilla striata* polysaccharides. However, its structure is diverse and not fully analyzed, and the mechanism of action has not been clarified, so that its biological activity is still at the surface stage, and further in-depth research is needed.

Conclusion

In this paper, PDAP and PDBP were prepared by hot water extraction method, followed by preliminary structural identification and biological activity analyses. It could be clearly seen that PDAP exhibited antioxidant, immunomodulatory and pro-coagulant and all these activities were superior to those of PDBP. PDAP may serve as become a new and potential resource for pharmaceutical applications, alleviating the over-reliance on root bark. It promotes the broader exploitation and utilization of DdT for future applications such as trauma and post-operative repair, to close wounds through haemostatic function, while reducing oxidative damage to tissues and accelerating healing by utilising antioxidant properties.

Acknowledgment

The equipment is maintained by the Characterisation and Analysis Centre of the Jilin Institute of Chemical Technology to provide testing.

Funding Information

This investigation was supported by the programs of the Science and Technology Department of Jilin Province (Grant No.20190304102YY) and Jilin Medical University (Grant No.HX2025009).

Author's Contributions

Jiaqi Sun and Shiyu Ji: Methodology, Software, Data curation, Writing-original draft.

Dongxue Cao: Formal analysis, Investigation.

Chunyan Wang: Investigation.

Hongli Zhou: Writing-Reviewing and Editing.

Hao Xu: Supervision, Writing-Reviewing and Editing.

Yaoyao Tong: Writing-Review and Editing, Funding acquisition.

Peng Wan: Supervision, Conceptualization, Funding acquisition.

Ethics

This article does not contain any studies with human participants or animals performed by any of the authors. The research complies with ethical guidelines and standards.

Conflict of Interest

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript. The authors state that there are no conflicts of interest.

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