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Purification and characterization of a novel polysaccharide–peptide complex from *Clinacanthus nutans* Lindau leaves

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ABSTRACT

A novel polysaccharide–peptide complex *CNP-1-2* with molecular weight of 9.17×10^4 Da was obtained from *Clinacanthus nutans* Lindau leaves by hot water extraction, ethanol precipitation, and purification with Superdex 200 and DEAE-Sepharose Fast Flow column chromatography. *CNP-1-2* exhibited the highest growth inhibitory effect on human gastric cancer cells SGC-7901 with inhibition ratio of 92.34% and stimulated activation of macrophages with NO secretion level of 47.53 μmol/L among the polysaccharide fractions. *CNP-1-2* comprised approximately 87.25% carbohydrate and 9.37% protein. Monosaccharide analysis suggested that *CNP-1-2* was composed of L-rhamnose, L-arabinose, D-mannose, D-glucose and D-galactose with a molar ratio of 1.30:1.00:2.56:4.95:5.09. Methylation analysis, FT-IR, and ^{1}H NMR spectroscopy analysis revealed that *CNP-1-2* might have a backbone consisting of 1,4-linked Glcp, 1,3-linked Glcp, 1,3-linked Manp, 1,4-linked Galp, 1,2,6-linked Galp and 1,2,6-linked Galp. Its side chain might be composed of 1-linked Araf, 1,6-linked Galp and 1-linked Rhap residues. AFM (atomic force micrograph) analysis revealed that *CNP-1-2* had the molecular aggregation along with branched and entangled structure.

1. Introduction

Recently, polysaccharides obtained from plants and fungi have attracted increasing attentions due to their medicinal values, such as anti-cancer, antioxidant, anti-diabetic and immunobiological activities (Chen et al., 2011; Shen et al., 2013; Sun & Liu, 2009; Xie et al., 2013; Zhou, Yu, Zhang, He & Ma, 2012). Furthermore, most of them are proven to be natural and nontoxic, ideal for producing healthcare foods or medicines (Li, Yuan & Rashid, 2009; Wang et al., 2012).

Clinacanthus nutans (Burm. F.) Lindau, commonly known as "Sabah snake grass" in Malaysia, Indonesia and "Erzuihua" in China, which is a well-known traditional medicinal plant in Southeast Asia, is used as a dermic, febrifuge and diuretic (Chen, Zhang, Zhang, Zhang & Xiao, 2015). It possesses a wide range of pharmacological effects, such as antibacterial, antioxidant, anti-proliferative, anti-inflammatory, and antiviral activities against varicella-zoster virus and herpes simplex virus type-2 (Arullappan, Rajamanickam, Thevar & Kodimani, 2014; Sakdarat, Shuyprom, Pientong, Ekalaksananan & Thongchai, 2009; Wanikiat, Panthong, Sujayanon, Yoosook, Rossi & Reutrakul, 2008; Yong et al., 2013). *C. nutans* contains various chemicals including stigmasterol, lupeol, β-sitosterol, belutin, C-glycosyl flavones, sulfur-containing glycosides, glycoglycerolipids, a mixture of nine cerebrosides and a monoacylmonogalatosyl glycerol, chlorophyll derivatives entadamides and clinamides (Sakdarat et al., 2009; Tu et al., 2014). However, to date, no investigations are available about purification and structural characterization of bioactive polysaccharides from *C. nutans*.

Therefore, the goal of the current study was set to (i) bioassay-guidedly fractionate and purify the bioactive polysaccharides from traditional medicinal plant *C. nutans* by gel-filtration chromatography and ion exchange chromatography, and (ii) elucidate its structural characterization by a combination of chemical and instrumental methods.

2. Materials and methods

2.1. Plant material

C. nutans (Burm. F.) Lindau plant was cultivated by TK MultiHerbs Enterprise located on 3041, Taman Seremban Baru,

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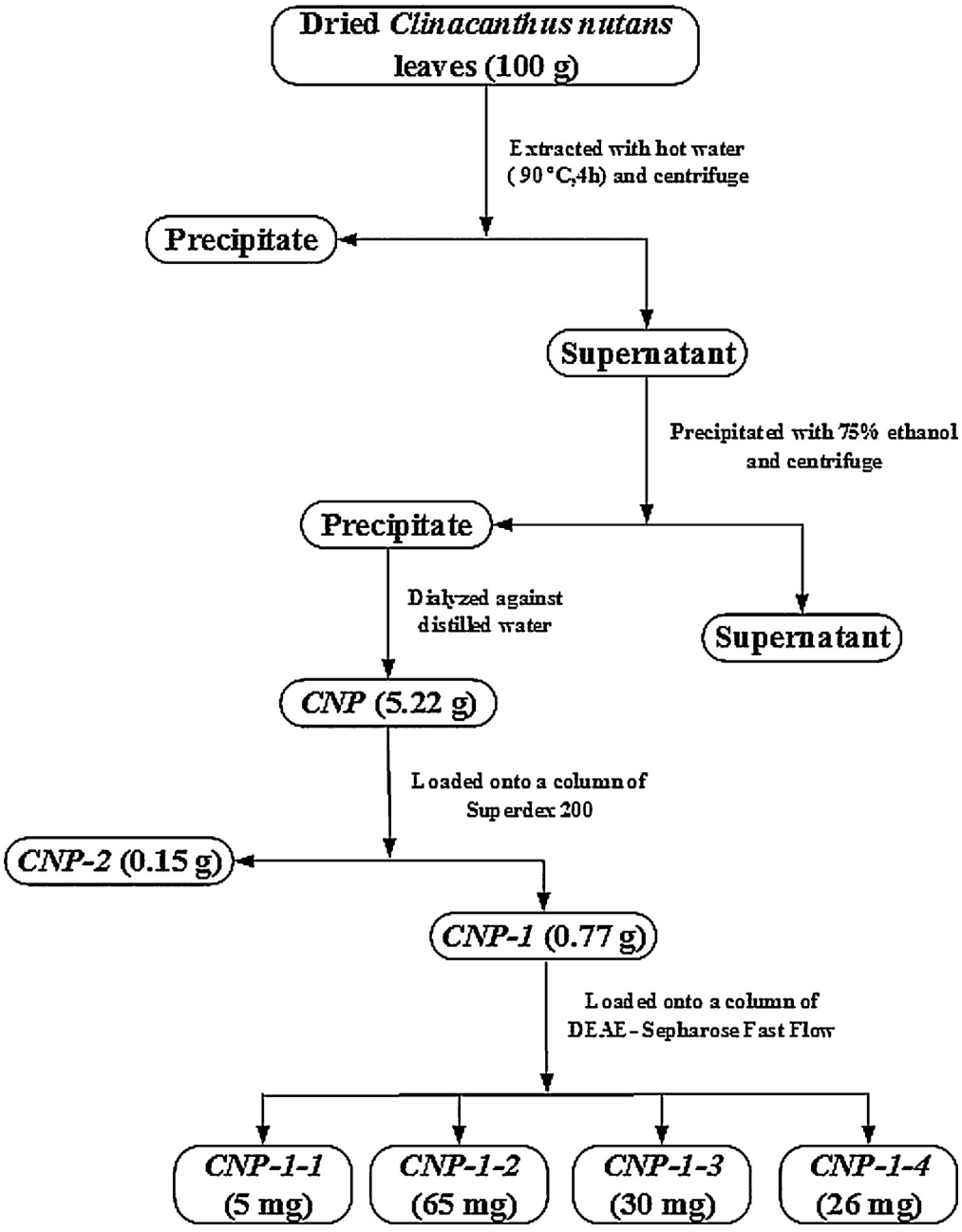

Fig. 1. Summarized extraction scheme of *CNP-1-2* from the leaves of *C. nutans*.

Seremban, N.S.D.K, Malaysia. Plant was identified and authenticated by Prof. Chen Jun with macroscopic and microscopic examinations and kept at the School of Pharmacy, Jiangsu University (Zhenjiang, China).

2.2. Isolation and fractionation of the polysaccharide from C. nutans

The fresh *C. nutans* leaves were oven dried at 60 °C and then ground to fine powder. As shown in Fig. 1, for extracting polysaccharide fraction, the dried leaves were mixed with distilled water (w/v 1:6) at 90 °C for 4 h. After centrifuging at 10,000 × *g* for 20 min, the supernatant was concentrated under reduced pressure and precipitated with a final concentration of 75% ethanol for 12 h at 4 °C. The precipitation was collected by centrifugation (10,000 × *g*, 10 min), followed by dialyzing against distilled water, and lyophilized to yield crude polysaccharides, *CNP*.

CNP (50 mg) were dissolved in distilled water and filtered through a membrane (0.45 μm, Nucleopore), and then the solution was applied to a Superdex 200 (1.6 × 60 cm). The polysaccharide fractions were collected, dialysed against distilled water, and lyophilized. The fraction *CNP-1* was proven to have a much higher antiproliferative activity and a much higher ability to stimulate the NO production by RAW264.7 macrophages than *CNP-2*, so that *CNP-1* was further investigated here.

The fraction *CNP-1* (50 mg) were dissolved in distilled water and filtered through a membrane (0.45 μm, Nucleopore). Then the solution was applied to a DEAE-Sepharose fast flow column (2.5 cm × 20 cm) pre-equilibrated with distilled water (pH 7.0). Fractions were prepared in a stepwise elution with distilled water, 0.1, 0.3, 0.5 and 1.0 M NaCl solutions at pH 7.0 at a flow rate of 2.0 ml/min, and with collection of 8 ml for each tube. The polysaccharide content was detected by phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). In addition, the