Carbohydrate Polymers 99 (2014) 291–296

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Studies on *Tinospora cordifolia* monosugars and correlation analysis of uronic acids by spectrophotometric methods and GLC

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ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 5 July 2013
Accepted 26 July 2013
Available online 21 August 2013

Keywords:
Tinospora cordifolia
Correlation analysis
Polysaccharide
Spectrophotometry
Colorimetric method
Uronic acid

ABSTRACT

Cold water-soluble (CWSP) and hot water soluble polysaccharides (HWSP) from *Tinospora cordifolia* stems were isolated and purified in 2.99% and 1.99% yield respectively. Complete hydrolysis followed by paper chromatography and GLC analysis indicated the presence of L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose and D-glucose in molar ratio of 0.857, 1.106, 0.727, 0.526, 0.708 and 95.763 in CWSP and 0.697, 0.777, 2.048, 0.777, 0.292 and 95.408 in HWSP. The uronic acid content in the polysaccharide has been studied extensively using assorted approaches. It was quantitatively estimated by GLC analysis and spectrophotometric methods using carbazole, m-hydroxydiphenyl and 3,5-dimethylphenol as colorimetric reagents. GLC analyses indicated galacturonic acid content of 3.06% and 5.16% in CWSP and HWSP respectively. Estimation of uronic acid using 3,5-dimethylphenol corroborated the above analysis. The study resulted in composition of constituent monosugars of CWSP and HWSP and co-relation analysis of uronic acid content, leading to an unambiguous structural analysis.

1. Introduction

Tinospora cordifolia (Willd.) Miers ex. Hook. f. & Thomson, a member of Menispermaceae family is a deciduous climbing shrub with glabrous leaves, succulent stems and aerial roots, found in the tropics of Asia, Africa and Australia ascending to an altitude of 300 m (Anonymous, 1976). The plant, commonly known as Giloy is widely used in Indian folk and Ayurvedic Systems in various 'Rasayanas' for centuries attributing to its wide spectrum of therapeutic activities. All parts of the plant are extensively used in Indian medicinal system for its general tonic, anticancer (Mathew & Kuttan, 1999), antiulcer (Bairy, Roopa, Malini, & Rao, 2002), memory enhancer (Agarwal, Malini, Bairy, & Rao, 2002), antidepressant (Dhingra & Goyal, 2008), antischemic (Rao, Kumar, Viswanath, & Subbaraju, 2005), antifertility (Gupta & Sharma, 2003), chemopreventive (Singh, Banerjee, Kumar, Raveesha, & Rao, 2006), hypolipidemic (Prince, Menon, & Gunasekaran, 1998), neuroprotective (Rawal, Muddeshwar, & Bisis, 2004), blood purifier (Sharma, Gupta, Mishra, & Sharma, 2011); antipyretic (Kumar & Srivastava, 1995); antihepatitis (Prakash & Rai, 1996); cardiotonic, antimicrobial, antileishmanial, antiinflammatory, antiperiodic, anti-spasmodic, antiarthritic, analgesic and diuretic (Sharma, Yelne, & Dennis, 2001) properties. The species is also known to cure obstructive jaundice (Rege, Bapat, Koti, Desai, & Dahanukar, 1993), oxidative stress (Prince & Menon, 2001), throat cancer in humans (Chauhan, 1995) and is being employed in adjuvant therapy in hyperreactive malarious splenomegaly (Singh, 2005). In Ayurveda, the stem is used in treating dyspepsia, debility, bile secretion stimulation, burning sensation, vomiting, vaginal and urethral discharges and urinary diseases. The roots are exploited for antistress (Patil, Patki, Kamath, & Patwardhan, 1997), anti-leprotic and anti-malarial activities (Nayampalli, Ainapure, & Nadkarni, 1982; Zhao, Wang, Rimando, & Che, 1991). The stem and root as constituents of a compound drug taken in amalgamation with other drugs has been recommended as an antidote to snake bite and scorpion sting (Kirtikar & Basu, 2005).

Different extracts of *T. cordifolia* have been reported to exhibit diverse pharmacological activities. Among various extracts, the aqueous extract has been reported to possess immunotherapeutic properties, wherein the active principle is claimed to be a polysaccharide (Chintalwar et al., 2000; De Souza, Yeole, Jha, & Bagchi, 2002). Aqueous fractions of stem parts contain immunologically active arabinogalactan polysaccharide (Chintalwar et al., 1999) together with other major bioactive compounds. The aqueous extract has been investigated for its immunostimulant (Chakraborty & Sengupta, 2012) and immunomodultory properties along with an increase in antibody production in vivo (Ranjith et al., 2008). Hyperlipidemia has been reported to be cured with aqueous extract of *T. cordifolia* stems (Nagaraja, Kammar, & Devi, 2008). The aqueous-methanolic extract of stems was found to show antibacterial and immunomodulatory properties (Mukherjee, De, & Ram, 2010). The aqueous extract of roots has

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0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.07.083

also been reported to show hypoglycemic activity (Stanely, Prince, & Menon, 2000).

There are a number of standard formulations and patents on *T. cordifolia* ascribing its huge spectrum of activities. A novel method of treatment for immunodeficient conditions has been reported using *T. cordifolia* (De Souza et al., 2002). The plant is used as an essential component in anti retroviral herbal formulation (Raja, 2006); health protective herbal soft drink (Pushpangadan et al., 2004); anti-anxiolytic, tranquilizer, and non-narcotic sedative (Managoli, 2007); synergistic antipyretic formulation (Pushpangadan, Rao, Rawat, Srivastava, & Khatoon, 2006); herbal formulation against adenocarcinoma of the prostate (Managoli, 2003) and allergy (Pushpangadan, Rao, Rawat, Ojha, & Reddy, 2006). Herbal compositions containing *T. cordifolia* have been employed in treating AIDS (Ayare, 2005) and cancer (Solanki, 2004). A standardized extract of *T. cordifolia* in the form of an immunoadjuvant pharmaceutical composition has been prepared to treat nephrotic syndrome and chronic recurrent urinary tract infections (Acharya, Mukhopadhyay, & Piramal, 2005). Moreover, there are commercially available products ranging from ayurvedic rasayanas to pharmaceutical products in the Indian market. Adbac and Immumod are commercially available immunostimulants in the form of tablets containing 300 mg and 100/500 mg standardized aqueous extract respectively, while Immumod is also available as syrup (De Souza et al., 2002). Hyponidd and Immu-21 are other known formulations (Khandelwal, Koneri, Balaraman, & Kandhavelu, 2011).

Polysaccharides of different structural characteristics and properties have been isolated and examined for their pharmacological applications. The polysaccharide isolated from *T. cordifolia* was found to be mainly composed of a 1→4 linked glucan, with occasional branch points (Rao & Rao, 1981). A novel (1→4)-α-D-glucan named as RR1 polysaccharide has been isolated from *T. cordifolia* with molecular weight > 550 kDa and structure encompassing 1→4 linked backbone and 1→6 linked glucopyranosyl branches (Nair, Melnick, & Ramachandran, 2006). The polysaccharide has been reported to exhibit immunoprotective potential owing to its water solubility, high molecular mass and other biological characteristics (Nair et al., 2004). In another study, polysaccharide belonging to a class of arabinogalactans, known as G1→4A, extracted from the stem of *T. cordifolia*, has been investigated for its immunomodulatory activity and protection against lipopolysaccharide (LPS) induced mortality (Desai, Ramkrishnan, Chintalwar, & Sainis, 2007; Raghu et al., 2009). The arabinogalactan polysaccharide having a mean Mr (molecular weight) 2.2×10^6 was found to be composed of galactose (32%), arabinose (31%), rhamnose (1.4%) and galacturonic acid (35%) (Chintalwar et al., 1999). Similar polysaccharide isolated by Roja, Bhangale, Juvekar, Eapen, and D'Souza (2005) was found to be composed of glucose, galactose, rhamnose, arabinose, xylose and galacturonic acid wherein galactose, rhamnose and arabinose were present as terminal sugars while galactose, galacturonic acid and arabinose were present as 1→4 linked sugars (Roja et al., 2005). However, the position of glucose and xylose units was not investigated. The arabinogalactan polysaccharide has been reported for its antioxidant (Subramanian, Chintalwar, & Chattopadhyay, 2002) and radioprotective properties (Goel, Prem, & Rana, 2002; Subramanian, Chintalwar, & Chattopadhyay, 2003). Another water soluble polysaccharide isolated from *T. cordifolia* has been reported with constituent monosugars as arabinose 0.5%, rhamnose 0.2%, xylose 0.8%, mannose 0.2%, galactose 0.3% and glucose 98.0% (Jahfar, 2003) that involved glycosyl composition with residues: terminal-glucose, 4-xylose, 4-glucose and linkages such as 4,6-glucose and 2,3,4,6-glucose (Jahfar & Azadi, 2004). Nevertheless, the position and linkage of arabinose, rhamnose, mannose and galactose were not mentioned.

The work carried out above by various researchers reveal that the polysaccharides present in *T. cordifolia* may be a mixture of polysaccharides with high molecular weights. Various polysaccharides isolated by different methods were found to possess a range of active principles attributing to diverse array of activities of the species. Interestingly, despite immense therapeutic significance of the polysaccharide, a closer look reveals that the nature of the backbones reported is entirely different. In addition, the cold and hot water soluble polysaccharides were not studied independently, which may be useful for futuristic pharmacological applications. Interestingly, most of the studies have not even discussed the occurrence of uronic acid in the polysaccharide that prominently influences the biological properties of a polysaccharide (Li, Liy, Fan, Ai, & Shan, 2011).

Keeping in view, the immense biological applications of the polysaccharide as discussed, the present study was undertaken to isolate CWSP and HWSP independently and to determine the monosugar composition of polysaccharides. Further, uronic acid content has been studied extensively using assorted approaches and an unambiguous correlation of the acids present in the polysaccharides has been established based on spectrophotometric methods vis-a-vis gas–liquid chromatography (GLC).

2. Experimental

The stems of *T. cordifolia* were collected from Chandigarh. A voucher specimen was identified by Dr. H.B. Naithani, Consultant, Systematic Botany Discipline, Botany Division, Forest Research Institute Dehradun by comparison with standard specimen from Herbarium. The stems were dried in shade.

2.1. General methods of analysis

Solutions were concentrated at or below 40 °C in a rotary evaporator under reduced pressure. All melting points were uncorrected. All standard and sample solutions were freshly prepared before use. Commercial carbazole, m-hydroxydiphenyl, 3,5-dimethylphenol, D-galacturonic acid and monosugars were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and solvents used were of analytical reagent grade. Optical rotation was determined on Autopol-II, automatic polarimeter (Rudolph Research, Flanders, New Jersey) at 589 nm, D-lines of sodium. Paper chromatography was carried out on Whatmann 1 mm filter paper sheets using the following solvent systems; n-butanol:pyridine:water (6:4:3), n-butanol:formic acid:water (12:1:7) upper layer and ethyl acetate:acetic acid:n-butanol:water (4:3:2:2). Spots were detected with ammoniacal silver nitrate complex (R_1) and aniline phthalate spray (R_2) separately. In the former (R_1), paper was treated with following reagents: (a) to silver nitrate solution (1.25 g in 1 mL water), 100 mL acetone was added with continuous shaking (b) sodium hydroxide (5 g in minimum water) was dissolved in 100 mL of ethanol (c) aqueous ammonia solution. The dried chromatograms were dipped and passed through reagent solution (a) for about 5 min, dried at room temperature and passed through reagent (b); when the dark brown spots were visualized, the paper was dipped in reagent (c) for some time with shaking (5–10 min). Finally, the chromatograms were washed with water and dried in air (Trevelyan, Proctor, & Harrison, 1950). The papers were run in above solvent systems varying from 45 h to 120 h corresponding to the detection of various monosugars and uronic acids from rhamnose to glucuronic acids. Detection with aniline phthalate (0.93 g aniline and 1.66 g of phthalic acid added to 100 mL of butanol saturated with water) reagent (R_2), was done by spraying on paper chromatogram followed by heating at 105 °C for 10 min in oven.