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

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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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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.

All the colorimetric tests were performed on Chemito UV–VIS Spectrophotometer UV-2500 at different wavelengths in three most commonly used colorimetric methods. Gas–liquid chromatography of the sugar mixtures was carried out on Thermo Scientific Gas Chromatograph GC-600 fitted with a flame ionization detector (FID). The samples were analyzed in the form of their alditol acetates on a BPX70 capillary column under the following operating conditions: single ramp program with initial temperature 190 °C for 1 min, program rate of 3 °C per min and final temperature 260 °C for 10 min with a Nitrogen flow rate of 1.5–2.0 mL per min.

2.2. Isolation of polysaccharide

The polysaccharide was isolated using standard procedure. The stems (500 g) were pulverized using a laboratory grinder and subjected to vigorous stirring in cold distilled water (3 L) for 6 h at room temperature. The moisture content of the freshly harvested stems was found to be 82.87%. The stirred mixture was filtered and centrifuged to remove water insoluble part. The supernatant part was decanted off and concentrated under reduced pressure to a smaller volume (300 mL). To this concentrated aqueous extract (300 mL), ethanol (900 mL) was added with constant stirring. The cold water soluble polysaccharide (CWSP) was precipitated out and then lyophilized resulting in dried light gray fluffy mass. It was again dissolved in water and re-precipitated with ethanol. The precipitate was successively treated with acetone and dry solvent ether. It was lyophilized and kept in a desiccator. The residue left after filtering the stirred mixture obtained above, was subjected to hot water stirring at 100 °C for 6 h to isolate the hot water soluble polysaccharide (HWSP) using the same procedure as above to give a light gray fluffy polysaccharide. The polysaccharide (17.5 g) so obtained was deionised by passing the aqueous solution successively through the columns of freshly regenerated cation (Dowex-50 W-X8) and anion (Seralite-SRA-400) exchange resins. The columns were washed with distilled water until the washings showed a negative Molisch test for carbohydrates. The combined eluents were concentrated to small volume (1/4th) and subjected to further purification by dialysis, wherein the concentrated polysaccharide solution was transferred to a dialysis membrane (molecular weight 1×10^4–1.5×10^4) and dialyzed for 72 h in distilled water. The dialyzed product was concentrated and re-precipitated with a large volume of ethanol to obtain finally the pure polysaccharide. It was kept overnight, alcohol was decanted off, and the precipitated polysaccharide was treated with solvent ether, acetone and absolute ethanol. It was filtered and lyophilized at −80 °C to obtain finally the pure polysaccharide in the form of a gray and light gray amorphous powder of CWSP (13.80 g) and HWSP (12.68 g) respectively.

2.3. Complete hydrolysis

The complete hydrolysis of CWSP and HWSP of *T. cordifolia* was carried out with sulphuric acid (2 N, 5 mL) for 18 h on a steam bath. The hydrolyzates obtained were cooled and neutralized by drop wise addition of a saturated solution of barium carbonate till the pH of the solution reached at 7. Finally, the solutions were filtered and residues washed three times with water. The combined filtrates were concentrated.

2.4. Alditol acetates

The monosugars obtained after complete hydrolysis of CWSP and HWSP were estimated quantitatively by converting the hydrolyzates to their corresponding alditol acetates and subjecting each to gas–liquid chromatography (Bishop, 1964). Alditol acetates were prepared by the method of Jansson, Kenne, Liedgren, Lindberg, and Lönngren (1976). To hydrolyzate (0.050 g), sodium borohydride (0.020 g) was added and the mixture was kept for 18 h at room temperature. It was neutralized by addition of mixture of acetic acid and methanol (1:49), till the pH reached up to 7. The mixture was concentrated to dryness in the vacuum rotator at 40 °C. Boric acid was removed by codistillations, with methanol (3–5 mL) under reduced pressure. The alditols thus obtained were subjected to acetylation using redistilled acetic anhydride and pyridine, 1:1 (5 mL) and refluxed for 6 h. Toluene (6 mL) was added forming an azeotrope with acetic anhydride, and the mixture was distilled. Toluene (6 mL) was added further and the solution was concentrated to dryness. The final product was dissolved in water (10 mL) and the acetylated sugars were fractionated with dichloromethane (4× 25 mL). Traces of moisture in dichloromethane were removed by adding activated anhydrous sodium sulphate. The alditol acetates were analyzed by GLC (Bishop, 1964).

2.5. Spectrophotometric analysis of uronic acid

The uronic acid present in CWSP and HWSP as indicated by the paper chromatography was qualitatively confirmed by carbazole–sulphuric acid test (Dische, 1950). The quantitative estimation of uronic acid was carried out via spectrophotometry using three colorimetric reagents viz. carbazole, m-hydroxydiphenyl and 3,5-dimethylphenol. In order to ensure the accuracy of results, the polysaccharide samples of CWSP and HWSP were taken in replicates in two dilutions in all three methods.

In first method, i.e. Borate modified carbazole method (Bitter & Muir, 1962; Knutson & Jeanes, 1968), 0.1 mL and 0.2 mL volumes of CWSP and HWSP (100 μg/mL) were added to 6 mL conc. sulphuric acid under ice-cold conditions. The mixture was agitated at vortex and cooled, followed by addition of 0.2 mL of 0.1% alcoholic solution of carbazole. The mixture was then heated at 55 °C for 30 min. The absorbance of the resultant color developed was measured at 530 nm. The content of galacturonic acid was calculated using the absorbance of polysaccharide sample, corresponding to the plotted graph of standard galacturonic acid solutions (0.1–1 mL of 100 μg/mL; absorbance versus concentration).

The estimation of uronic acid using meta-hydroxydiphenyl method was done by Blumenkrantz and Asboe-Hansen method, 1973. In the method, 0.1 mL and 0.2 mL volumes of CWSP and HWSP (100 μg/mL) were added to 1.2 mL borate–sulphuric acid reagent (0.0125 M solution of sodium tetraborate in conc. sulphuric acid) under ice-cold conditions. The mixture was vortexed, cooled and heated on steam bath at 100 °C for 5 min. The mixture was then cooled on ice bath, followed by addition of 20 μL of alkaline solution of m-hydroxydiphenyl [0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) sodium hydroxide]. The absorbance was measured at 520 nm.

In 3,5-dimethylphenol method (Scott, 1979), 0.125 mL of CWSP and HWSP solutions (100 μg/mL) were treated with 2 mL conc. sulphuric acid in the presence of 0.125 mL of 2% NaCl followed by vigorous shaking. The mixture was heated at 70 °C for 10 min, cooled at room temperature, then 0.1 mL of 0.1% of acidic solution of 3,5-dimethylphenol (0.1 g 3,5-dimethylphenol in 100 mL acetic acid) was added and absorbance was instantly measured at 450 nm and 400 nm. In this method, the uronic acid concentration was calculated based upon difference of absorbances taken at two wavelengths.

2.6. Reduction of carboxyl groups of polysaccharide

Both CWSP and HWSP were reduced by the method of Taylor, Shively, and Conard (1976). The pH of polysaccharide (0.06 g) dissolved in distilled water was adjusted to 4.75 by addition of 1 M HCl solution and 1 mmol of solid 1-ethyl-3-(3-dimethylaminopropyl)

carbodimide (EDC) was added. The reaction proceeded for 45 min at pH 4.75 until evolution of hydrogen ion ceases. A 3 M solution of sodium borohydride was added dropwise at 25 °C in the period of one hour. 4 M hydrochloric acid was also added simultaneously to maintain the pH at 7.0. Finally, the remaining sodium borohydride if present was destroyed by adding 1 M HCl and making the reaction mixture slightly acidic. The solution was subjected to exhaustive dialysis against distilled water and lyophilized. The reduced dried polysaccharide (0.052 g) was dissolved in water (5 mL), precipitated with three volumes of ethanol (15 mL) and lyophilized.

The reduced CWSP and HWSP gave a negative carbazole–sulphuric acid test confirming the complete reduction of uronic acid. The alditol acetates of reduced polysaccharides were prepared by method of Jansson et al. (1976) as discussed above and were subjected to gas–liquid chromatography.

3. Results and discussion

Pure polysaccharides, CWSP and HWSP from *T. cordifolia* stems were isolated in 2.99% and 1.99% yield respectively as detailed in experimental. Chemical analysis of pure CWSP and HWSP showed specific rotation, $[\alpha]_D^{28}$ +74.07° (c 0.003%, H_2O) and +214.2° (c 0.001%, H_2O), ash content 1.664% and 0.983% (on a dry basis), and pH 6.46 and 6.37 respectively.

3.1. Monosugar analysis of T. cordifolia

Complete hydrolysis of CWSP and HWSP followed by paper chromatography revealed seven spots indicating the presence of six monosugars and galacturonic acid in both the polysaccharides. The monosugars present in both CWSP and HWSP hydrolyzates were found to be rhamnose, arabinose, xylose, mannose, galactose, glucose along with galacturonic acid. The quantitative investigation of monosugars present was performed by their derivatization to corresponding alditol acetates (Jansson et al., 1976) followed by their GLC analysis (Bishop, 1964). The retention time of six peaks, coincide with the retention time of L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose and D-glucose. In CWSP, the molar ratio of L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose and D-glucose was found to be 0.857, 1.106, 0.727, 0.526, 0.708 and 95.763 while in HWSP, it was 0.697, 0.777, 2.048, 0.777, 0.292 and 95.408. The molar ratios revealed the polysaccharides encompassing a glucose backbone followed by galactose with other monosugars in minor concentrations.

3.2. Comparison of spectrophotometric techniques with GLC methods

The presence of uronic acid was confirmed by qualitative carbazole–sulphuric acid test (Dische, 1950). Its quantitative estimation was carried out by two ways viz. GLC and spectrophotometric analysis. In GLC examination, the carboxyl groups present in both CWSP and HWSP were reduced (Taylor et al., 1976). The reduced polymers were further converted to corresponding alditol acetates, subjected to GLC, the results of which were interpreted to give total molar ratio of monosugars. When this ratio was compared to the molar ratio obtained earlier, the galactose was found to have a greater peak area, signifying the presence of galacturonic acid. The galacturonic acid was found to be 3.06% in CWSP and 5.16% in HWSP.

The other mode of quantifying the uronic acid content present was through spectrophotometry using three colorimetric methods viz. Borate modified carbazole method (Bitter & Muir, 1962; Knutson & Jeanes, 1968), m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), and 3,5-dimethylphenol method (Scott, 1979). The galacturonic acid percentage as calculated from borate modified carbazole method gave anomalous results. It was found to be 23% in CWSP and 63% in HWSP (Fig. 1a), whereas m-hydroxydiphenyl method revealed 0.84% and 1.35% galacturonic acid content in CWSP and HWSP respectively as shown in Fig. 1b. Furthermore, in case of 3,5-dimethylphenol method, it was reported to be 3.425% and 5.304% in CWSP and HWSP, respectively (Fig. 1c). The above spectrophotometric results of galacturonic acid estimation derived from three colorimetric methods when compared to GLC results, it was found that acid content value calculated by 3,5-dimethylphenol method was nearest to the corresponding values derived from GLC.

When such disparity in the results was studied and analyzed, it was found that the three chromogens (carbazole, m-hydroxydiphenyl and 3,5-dimethylphenol) differ in their rate of action with respect to their sensitivity, specificity and selectivity. Carbazole has been used for decades for uronic acid estimation but its specificity declines when neutral sugars are present in substantial amount (Yapo, 2012). Further, individual sugars react differently in terms of speed of development of color and their absorbance (Dische, 1947). Moreover, the method does not distinguish between glucuronic acid and galacturonic acid. Furthermore, the subsequent modifications in the carbazole method allow no possibility of differentiation between uronic acid and hexoses (Blumenkrantz & Asboe-Hansen, 1973). The presence of neutral sugars in excess in both the polysaccharides, thus strongly interfered with uronic acids in *Tinospora* polysaccharides and hence the error occurs. The other colorometric reagent, m-hydroxydiphenyl though claimed to possess greater sensitivity and specificity than former, however, counters few limitations; firstly, it has a short sensitivity range (0.5–15 μg) toward uronic acid content (Blumenkrantz & Asboe-Hansen, 1973). Secondly, the assay is strongly interfered by neutral sugars when present in appreciable amount (Yapo, 2012). Thirdly, quantitative estimation of glucuronic and galacturonic acids cannot be done exclusively for the individual acids. Fourthly, the assay showed very unusual behaviour, i.e. absorbance value was found to decrease as the concentration of polysaccharide sample increased, leading to an underestimation of uronic acid content. When such anomalous behaviour was studied by performing related experiments and analyzing intensively the results, it was found that on addition of sulfuric acid, hexose (glucose) gives 5-hydroxymethyl-2-furancarboxaldehyde, whereas hexuronic acid (galacturonic acid) gives 5-formyl-2-furancarboxylic acid, prime chromogen responsible for color. The absorbance of m-hydroxydiphenyl decreases rapidly at 520 nm with 5-hydroxymethyl-2-furancarboxaldehyde as compared to its relative increase in absorbance due to 5-formyl-2-furancarboxylic acid. The results were also corroborated by the work carried out by Scott (1979). In the present study, both the polysaccharides CWSP and HWSP were comprised mainly of glucose as monosugar, thereby resulting in inaccurate estimation of uronic acid. Interestingly, 3,5-dimethylphenol, has proved to be an appropriate and most selective chromogen, since glucuronic and galacturonic acid can be estimated individually (Scott, 1979). Moreover, interference due to monosugars (glucose in present study) was largely eliminated, thus allowing a pertinent estimation of uronic acid with a far lesser extent of interference of neutral sugars.

On comparison of the molar ratio of two polysaccharides, the study has established an apparent correlation of structure of CWSP and HWSP polysaccharides with their constituent monosugars and uronic acid content, thereby leading to unequivocal structural relationship. The two polysaccharides of the species were however, not yet studied independently and have been reported in the present study, which may prove to be helpful from a prospective of therapeutic applications. Further, based on the spectrophotometric analysis of uronic acids, it has been observed that

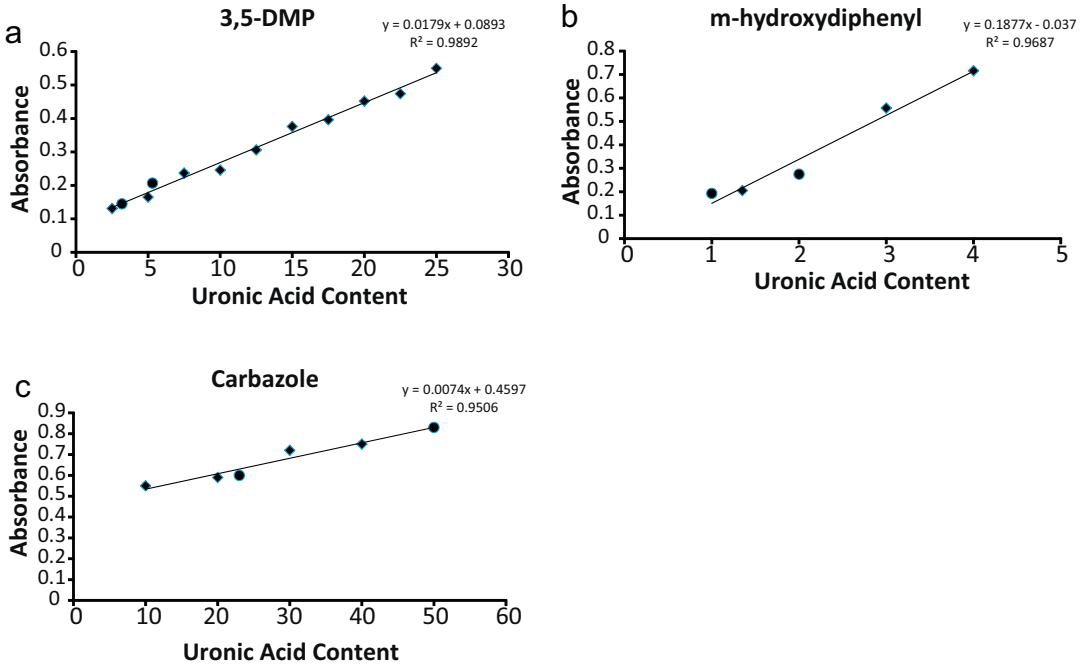

Fig. 1. (a) Determination of uronic acid in CWSP and HWSP with Borate modified carbazole method. ■, pure uronic acid; ●, polysaccharide samples. (b) Determination of uronic acid in CWSP and HWSP with Blumenkrantz and Asboe-Hansen method. (c) Determination of uronic acid in CWSP and HWSP with 3,5-dimethylphenol method.

3,5-dimethylphenol method is the most relevant method for estimation of uronic acid determination using colorimetric methods. The results are expected to reveal the structural moiety of the polysaccharide responsible for its bio-efficacy.

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