Carbohydrates are used for the therapeutic purpose as pure substances in pharmacy today. These compounds are widely used, however, in the form of excipients for the manufacture of tablets and capsules, as well as for the purposes of improving flavor and stability of certain liquid preparations. They serve as sources of energy and stores of energy for the metabolic processes of plants and animals [1-4].

Carbohydrates are most abundant organic compounds in nature. They are synthesized and consumed by all plants and animals. They are classified as sugars and non sugars and are first studied by Emil Fischer [5]. They are straight chain polyhydroxy aldehydes or ketones having m.f. C_nH_{2n}O_n; n=3 to 8 and are found as free sugars or linked with glycosides in plants. Carbohydrates are used daily not only as sugars and starches but also in building material wood and glues in paper and in clothing (Cotton, linen and ray on).

Plants contain active polysaccharides which have been found to be branched, water soluble, acidic heteroglycons and have molecular weight ranging from 25,000 to 500,000+. They are tasteless, amorphous and may be classified as homopolysaccharides and heteropolysaccharides. On hydrolysis, the homopolysaccharides yield one kind of monosaccharide units, whereas heteropolysaccharides yield more than one kind of monosaccharide unit. Polysaccharides composed of galactose and mannose or glucose and mannose have been reviewed by various researchers [6-10]. Galactomannan [11-19] is a highly branched polysaccharide in which mannose units form the main chain and the galactose units are present as branches, the former being attached through β (1→4) and branches of single galactose units through α (1→6). The presence of various sugars are analysed by known methods [20-32].

The aqueous extracts of a large number of plants contain polysaccharides used in traditional medicine as “tonics” [33-34]. Screening of extracts and fractions obtained from plants for immunostimulant activity has resulted in the identification of complex polysaccharide as the active constituents of several drugs. Recent reports reveal that
polysaccharides contain plant possesses biological activities like antiviral\textsuperscript{[35]}, hypolipidaemic \textsuperscript{[36]}, antihepatotoxic \textsuperscript{[37]}, anti-HIV \textsuperscript{[38]}, anticomplementary \textsuperscript{[39]}, anticoagulant \textsuperscript{[40]}. Present studies include the analysis and chemical composition of seed polysaccharide and reducing sugar from the seeds of \textit{T. urgentia} and \textit{G. maculata}.

\textbf{Material and Methods}

\textbf{Analysis of polysaccharide}

20 gm each of \textit{Tecom\textit{a urgentia}} seed powder were suspended separately in 200 ml water for 24 hrs and then heated at 100\textdegree C for four hours. The swollen material was blended further with 300 ml water. The viscous solution was filtered and centrifuged to remove suspended seed material. The polysaccharide was precipitated by adding large quantity of ethanol to this solution. The coarse powder was re dissolved in water and saturated barium hydroxide solution was added. Then the polysaccharide was precipitated as the barium complex. This barium complex was made into paste with water and decomposed by drop-wise addition of 2N acetic acid with vigorous stirring, when it was partially dissolved. The insoluble fraction was separated by filtration.

The soluble fraction was recovered by precipitation with ethanol (300 ml). Both the fractions, acetic acid soluble as well as acetic acid insoluble fractions were then successively washed with 90%, 80% and 50% ethanol to remove the acid. The fractions thus obtained did not reduce the Fehling's solution. The polysaccharides were obtained in the yields of 6.23% from the seeds of \textit{Tecom\textit{a urgentia}}.

Polysaccharide (0.1 gm) was hydrolysed with 1N H\textsubscript{2}SO\textsubscript{4} (5 ml) at 100\textdegree C for 17 hours in each case. The resulting solution was neutralized with barium carbonate, filtered and concentrated to a thin syrupy mass. This syrupy solution was used for chromatographic studies.
The ascending and descending [22, 19, 41, 30] paper chromatography techniques were used. The solution was spotted on Whatman No. 1 paper strip and developed in the solvent system, n-butanol:acetic acid:water (4:1:5, upper layer). The chromatogram was sprayed with aniline hydrogen phthalate. On drying two distinct spots of galactose and mannose were revealed in the chromatogram. Rf values of authentic and test samples are given in table II.

Quantitative estimation was done by Harborne’s method[31]. A measured volume of polysaccharide hydrolysate was placed along the starting line of the chromatogram and paper developed in upper layer of n-butanol : acetic acid : water (4:1:5, v/v). Sprayed with aniline hydrogen phthalate and dried in air oven at 100°C for 5 minutes. The coloured spots were cut out and each cut spot was eluted with 3 ml methanol containing 1% stannous chloride. The solutions were subjected to UV analysis and absorbance at maximum wavelength (λmax 420 nm) were recorded on a spectrophotometer. The same treatment was followed with glucose solutions of different molar concentration as references. A graph (Fig. 3.1) was plotted between optical density and molarity of glucose solutions, when a straight line was obtained passing through the origin. From the graph, concentrations of unknown sugars were calculated and tabulated (Table II). Thus the ratio of molar concentrations of galactose and mannose present in Tecoma urgentia is 5:2.

Analysis of Reducing Sugars

Seed powder (10 gm of each) was refluxed separately with a small quantity of calcium carbonate and 100 ml distilled water for one hour. The aqueous extract was separated by decantation and the powder was further refluxed thrice with 50 ml distilled water each time. The aqueous filtrates were combined and a 10% (w/v) solution of lead acetate was added till complete precipitation. It was filtered and H2S gas bubbled through the filtrate to remove excess lead acetate as lead sulphide. It was filtered and the filtrate was neutralised with ammonia. The solution was concentrated and used for chromatography and estimated by Fehling’s method.
Unidimensional ascending paper chromatography[25] was used for the identification of sugars on Whatman No. 1 paper strip (40x12 cm). Spots of authentic sugars as well as test samples were applied on the same paper strip and developed in the solvent system n-butanol : acetic acid : water (4:1:5, upper layer). After developing the chromatogram, it was sprayed with aniline hydrogen phthalate reagent[21]. Finally it was dried in an air oven at 105°C for ten minutes and Rf values are calculated (table III).

The percentage of total reducing sugars was estimated as glucose by titrating the solution obtained with Fehling’s solution (A and B), using methylene blue as indicator. 10 ml of Fehling’s solution A and B (along with indicator) were titrated with the sugar solution. till the blue color of the solution just disappears. The process of drop-wise addition of solution from the burette is repeated till the end point was determined exactly. The titration was also done with standard glucose solution (N/10). The results are given in table III.

Thus the percentage of reducing sugars are glucose present in the seeds was found to be 5.69% in T. urgentia and 6.78% in G. maculata.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration of glucose solution (mole)</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1</td>
<td>0.64</td>
</tr>
<tr>
<td>2.</td>
<td>2</td>
<td>1.30</td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>1.96</td>
</tr>
</tbody>
</table>
REFERENCE GRAPH FOR SUGAR ESTIMATION

FIG 3.1: OPTICAL DENSITY Vs. MOLAR CONCENTRATION OF GLUCOSE
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Plant</th>
<th>Name of the sugar</th>
<th>Rf (reported)</th>
<th>Rf (Obtained)</th>
<th>λ max in UV region (nm)</th>
<th>Optical density</th>
<th>Molarity (from graph)</th>
<th>Cal. Molarity of the sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T. urgentia</td>
<td>1. Acetic acidsoluble fraction</td>
<td>0.16</td>
<td>0.16</td>
<td>367</td>
<td>3.66</td>
<td>5.54</td>
<td>71.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Galactose</td>
<td>0.16</td>
<td>0.16</td>
<td>367</td>
<td>3.66</td>
<td>5.54</td>
<td>71.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Mannose</td>
<td>0.20</td>
<td>0.20</td>
<td>319</td>
<td>1.46</td>
<td>2.22</td>
<td>28.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Acetic acid insoluble fraction</td>
<td>0.16</td>
<td>0.16</td>
<td>367</td>
<td>5.50</td>
<td>4.03</td>
<td>71.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Galactose</td>
<td>0.16</td>
<td>0.16</td>
<td>367</td>
<td>5.50</td>
<td>4.03</td>
<td>71.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Mannose</td>
<td>0.20</td>
<td>0.20</td>
<td>319</td>
<td>2.15</td>
<td>1.57</td>
<td>28.04</td>
</tr>
</tbody>
</table>
### TABLE – III

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the sugar</th>
<th>Rf value of authentic sample</th>
<th>G. maculata</th>
<th>T. urgentia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D-glucose</td>
<td>0.18</td>
<td>0.18</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Lactose</td>
<td>0.09</td>
<td>0.09</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>D-galactose</td>
<td>0.16</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Maltose</td>
<td>0.11</td>
<td>-</td>
<td>0.12</td>
</tr>
<tr>
<td>5</td>
<td>D-fructose</td>
<td>0.23</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td>D-arabinose</td>
<td>0.21</td>
<td>-</td>
<td>0.21</td>
</tr>
<tr>
<td>7</td>
<td>D-rhamnose</td>
<td>0.34</td>
<td>-</td>
<td>0.37</td>
</tr>
<tr>
<td>8</td>
<td>Xylose</td>
<td>0.28</td>
<td>-</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Results and discussion

The reducing sugars of T. urgentia and G. maculata are analysed. T. urgentia is rich in polysaccharides. The results of the analysis are given below.

The reducing sugars present in T. urgentia seeds are D-arabinose, Maltose, D-fructose, D-rhamnose and Xylose and that of G. maculata are D-glucose, lactose, D-galactose and D-fructose. The percentage of these reducing sugar (as glucose) were 5.69% in T. urgentia and 6.78% in G. maculata.

Polysaccharide analysis of T. urgentia revealed that the acetic acid insoluble fraction (2.03%) as well as acetic acid soluble fraction (4.04%) has been found to contain galactose and mannose. The molar ratio of galactose and mannose in Tecoma urgentia is 5:2.

From the above observations it may be concluded that the seeds of G. maculata and T. urgentia are good source of carbohydrates.
References


