

## RESEARCH ARTICLE

# Characterization and Valorization of Cell Wall Polysaccharides of *Ruta chalepensis* L. Taken from Tessala Mountain (North-Western of Algeria)

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## ABSTRACT

As part in the valorization of Mediterranean plant species and in particular the local flora of Tessala Mountain (Sidi Bel Abbes Country, North-Western of Algeria), we conducted a study on the change in composition of cell wall polysaccharides of *Ruta chalepensis* L. content in the stems and leaves taken in natural condition in Tessala mountain. Values obtained from the water content of *Ruta chalepensis* L. shown a capacity of this element in the range of 54.47 % in the stems and 64.02 % in the leaves. Isolation of the wall tissue of *Ruta chalepensis* L. allowed to obtain (35.16±0.11) % of crude cell wall residue for leaves and (29.88±0.09) % for the stems. Moreover, the results of this study reveal a wealth of cellulose from an average of (69.6 ±0.54) % compared to other cell wall fractions; hemicelluloses with an average (12.1±0.6) % and pectins (6.74±0.25) %. The application of the "t" test of Student has to demonstrate a significant difference in the polysaccharide composition between leaves and stems of the species. The study of the saccharide composition of cellulose by thin layer chromatography revealed the presence of glucose in both parts of the species analyzed. For hemicelluloses, it indicates the presence of glucose, xylose, galactose and fructose in the stems and leaves. The existence of these sugars in the constitution of hemicelluloses, encourages us to find purification protocols for uses as a thickener in jams and jellies, or in the field of health that the polysaccharides have a biological activity on diabetes, cancer and viruses.

**Key words:** Tessala Mountain, *Ruta chalepensis* L., valorization, cell wall polysaccharides, TLC.

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## INTRODUCTION

Currently, knowledge, characterization, classification and conservation of different vegetal species are a priority in the scientific assessment and management of plant biodiversity. Important number of species remains unknown at all levels; ecological, biological, biochemical and taxonomic. On the other hand, the biochemistry of different vegetal species is the subject of serious study that allows the conservation of vegetation that knows the value of a species in our daily lives is to preserve it (Bouzidi *et al.*, 2010).

Moreover, the region of Tessala (country of Sidi Bel Abbes), mountain area of western Algeria, with particular characteristics, is known by its rich flora used by the local population (Toumi-Benali *et al.*, 2015). Its

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potential plant resources and the valorization of its species have only been partially studied (Dif *et al.*, 2014; Bouzidi *et al.*, 2009; Attaoui, 2009; Baraka, 2008) and it remains to be evaluated.

In this sense, we wanted to, through a precise taxonomic choice, make this approach to contribute to valorization of the local flora of this region and study for the first time the biochemical composition, in particular the characterization of cell wall polysaccharides of *Ruta chalepensis* L.; *Ruta* genus species commonly known by “fidjel”.

### 1. Methodology:

#### 1.1 Plant material:

Several individuals of *Ruta chalepensis* L. were collected in June 2011 in the Tessala Mountain (Algerian North-West), at a station whose latitudinal coordinates 35°16.125' north, and longitudinal 0°46.283' west located at altitude 797 m. The soil type at this area was loam, deep, with a slightly faster drainage and vegetation characteristic of scrubland.

A specimen was deposited in the herbarium of the faculty of sciences of Djillali Liabes university of Sidi Bel Abbas.

#### 1.2 Polysaccharides extraction:

Preparing the plant powder is made according to standard NF B 51-004 where leaves and stems of *Ruta chalepensis* L. individuals were cut into small pieces and weighed to determine their fresh weight (FW). They were then spread over to metal plates and dried in an oven at a temperature of 60 °C for 72 hrs. Fragments were then re-weighed to determine dry weight (DW); the difference between the two weights gives the water contents (WC) as percentage, and then they were content as powder by a cutter mill type MF 10 basic IKA WERKE with a filter mesh of 0.25 µm (Moine, 2005).

The principle of obtaining the crude cell wall residue was to stir for 14 hrs under a hood and 50 g powder plant with an ethanol-toluene (1V/1V), this operation was repeated twice. It eliminates soluble lipids, tannins and other cytoplasmic constituents (Harche *et al.*, 1991).

Then, a delignification of the cell wall residue was performed; this method allows breaking the lignin-polysaccharides bonds and thus showing the maximum polysaccharide extracts. To do this, 15 g of cell wall residue was mixed with 150 ml of water, 1 ml of acetic acid and 5 g of sodium chlorite. The whole was stirred at 80 °C for 1 hrs under a hood. Then, 5 g of sodium chlorite and 1 ml of acetic acid were added to the mixture, the whole was left straining overnight. After filtration on bolting cloth, the residue was washed with distilled water and ethanol and then dried in an oven at 60 °C overnight (Moine, 2005; Harche *et al.*, 1989).

For the extraction of cellulose and hemicelluloses with Chanda protocol; 5 g of cell wall residue delignified was being stirred for 14 hrs with 100 ml of NaOH at 4 %. This operation was repeated twice for recuperate a maximum of hemicelluloses fraction. After filtration on bolting cloth, the residue was washed with distilled water and acetone and then dried in an oven at 60 °C overnight. The solid part obtained represents the cellulosic fraction. The two filtrates obtained were neutralized by pure acetic acid and then precipitated in ethanol (1V/3V) for 14 hrs. The hemicelluloses fraction was obtained after centrifugation a 3600tr/min for 30minutes (Ray *et al.*, 2004; Habibi, 2004).

Highly methylated pectins (HMP) were extracted with boiling water. About the low methylated pectins (LMP), they were extracted with EDTA (Ethylene-diamine-tetra-acetic-acid) (Harche *et al.*, 1991; Moties, 1980; Fransen *et al.*, 2000).

For accuracy and to have more reliable results, it should be noted that the extraction operations of the different fraction were repeated three times. For the comparison of values obtained from leaves and stems, we used the test “t” of Student (comparison of means two by two) (Dagnelie, 1975).

#### 2.3 Qualitative composition study:

The technique used to determinate the qualitative composition in monosaccharides cell wall was thin layer chromatography (TLC). Before analysis, the cell wall polysaccharides of the leaves and stems have undergone two types of hydrolysis; total acid hydrolysis and partial acid hydrolysis. The method was described by Randerath (Bouzidi *et al.*, 2010).

### 3.Results:

#### 3.1 Determination of the water content:

Plants consist mainly of water; their content of this element varies from 75 % to 95 % of their total weight. 80 % of this water is free (chemically unbound), 18 % to 19 % was bound water and 1 % to 2 % is water content (hydrated molecules) (Camefort, 1972).

Values obtained from the water content of *Ruta chalepensis* L. represented in Table 1 shown a capacity of this element in the range of 54.47 % in the stems and 64.02 % in the leaves. These low levels in this specie can be explained by its adaptive strategy. Indeed, *Ruta chalepensis* L. is a xerophytes plant that grows in the semi-

arid climates and produces its water economy due to its evaporating surface reduced (reduced leaves; stomata sunken in crypts, epidermis thick-walled, deep root system draining the maximum of water) (Camefort, 1972).

**Table 1:** Water content in different part of *Ruta chalepensis* L.

Part of the plant	FW (g)	DW (g)	WC (g)	WC (%)
Stems	534.48	243.35	291.13	54.47
Leaves	254.43	91.55	162.88	64.02

FW: fresh weight, DW: dry weight, WC: water content.

### 3.2 Proportion after different extraction:

Isolation of the wall tissue of *Ruta chalepensis* L. allowed to obtain (35.16±0.11) % of crude cell wall residue for leaves and (29.88±0.09) % for the stems (Table 2). The test “t” of Student has to demonstrate a not significant difference between the cell wall residue continent in leaves and stems.

The results of fractions of the delignified cell wall residue contained in 15 g of crude cell wall presented in Table 2 showed that the stems contains (86.66±0.003) % and leaves (98.40±0.001) %. A significant difference in variation between leaves and stems was due to the volume of lignin contained in the two constituent parts of *Ruta chalepensis* L.

Indeed, the lignin is located in the conducting tissue like the xylem, phloem and in supporting tissues such as sclerenchyma. It has a supporting role by providing mechanical rigidity of cells. Because of its structure rich with phenyl stone and carbon chains, it also makes the cells impervious to water and thus allows the conduction of solutes throughout the plant by conductive tissues. Thus, the quantity and composition of lignin varies according to taxonomic affiliation of the plant species, considered tissue, the cell type (even part of the cell wall in question), the stage of development, environmental conditions and stress factors. The composition and quantity of synthesized lignin also vary depending on time of year and physical constraints which the plant may be submitted such as wind or meet an obstacle. These deposits are so-called reaction wood (Sjöström and Westermark, 1999; Vance *et al.*, 1980).

The amounts of cellulose in the *Ruta chalepensis* L. extracted from 5 g of the delignified cell wall residue represented in Table 2 show that this fraction was (59.2±0.33) % for stems and (80±0.75) % for leaves. For hemicelluloses quantities in 5 g of the delignified cell wall residue, the results indicate that it represent (10.8±0.4)% in the stems and (13.4±0.01) % in the leaves.

The “t” test of Student indicates a significant difference between the two analyzed parts. This can be explained by the fact the leaves were the center of elaboration of various sugars from photosynthesis. So, hemicelluloses were responsible for elasticity of plant cell walls during growth. Thus, reducing in the wall makes it unbreakable (Ray *et al.*, 2004).

In stems, measuring the amount of pectins allowed to demonstrate a capacity of (10.4±0.2) %, while it was (3.08±0.1) % in leaves.

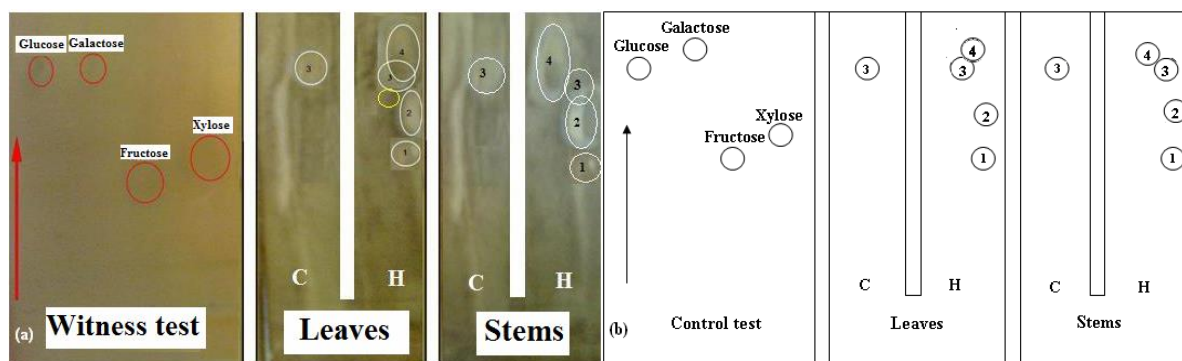
**Table 2:** Fraction of different cell wall polysaccharides proportion after extraction from *Ruta chalepensis* L.

Composition	Plant part	Quantity (g)	Rate (%)
Crude cell wall residue (Quantity / 50 g of plant powder)	Stems	14.94±0.01	29.88±0.09
	Leaves	17.58±0.02	35.16±0.1
Delignified cell wall residue (Quantity / 15 g of crude cell wall residue)	Stems	12.99±0.01	86.66±0.003
	Leaves	14.76±0.06	98.40±0.001
Cellulose (Quantity / 5g of Delignified cell wall residue )	Stems	2.96±0.01	59.2±0.33
	Leaves	4±0.03	80±0.75
Hemicellulose (Quantity / 5g of Delignified cell wall residue )	Stems	0.54±0.02	10.8±0.4
	Leaves	0.67±0.04	13.4±0.8
Pectins (Quantity / 5g of Delignified cell wall residue )	Stems	0.52±0.01	10.4±0.2
	Leaves	0.15±0.005	3.08±0.1

### 3.3 Qualitative analysis by thin layer chromatography:

The thin layer chromatographic result after a total hydrolysis acid represented in Fig. 1, reveal the presence of a single spot for the cellulose corresponds that of glucose in the leaves and stems of *Ruta chalepensis* L. This justifies the saccharide composition of the cellulose which was a tight macromolecule composed solely of glucose monomers bond in  $\beta$  (1→4) (Guignard, 2000).

The results obtained after hydrolysis of hemicelluloses fractions (Fig. 1), showed the existence of four spots correspond to glucose, xylose, galactose and fructose in both parts analyzed plant. It must be said that the hemicelluloses in the stems and leaves of *Ruta chalepensis* L. were xyloglucan type constituted by a linear chain of glucose, as in cellulose, on which are grafted a side chains containing either xylose residues only, whether disaccharide (galactose→xylose) or a trisaccharide (xylose→galactose→fructose). It was this links that give the plant rigidity (Guignard, 2000; Mcneil *et al.*, 1975; Hayashi *et al.*, 1987). Thus, these compounds according to the tissue origin or cell type and stage of maturity according to the tissue generate a large structural diversity (Buchala and Wilkie, 1973; Roland, 1980; Rajnchapel and Vincent, 1986).



C: cellulose, H: hemicelluloses, 1: fructose, 2: xylose, 3: glucose, 4: galactose

**Fig. 1(a, b):** Thin layer chromatography represented a saccharide composition of cellulose and hemicelluloses of *Ruta chalepensis* L. cell walls.

#### Discussion:

The difference in water content between leaves and stems is explained by the mechanism of water conduct in the plant. The roots get water and nutrients they need in the soil in which it enters through a hydrostatic mechanism; it is the leaf transpiration which raises the long stems. Storage of that water is in the cells of the leaves, instead of different metabolic reactions involved in photosynthesis and organic substances which plants need to grow (Hoffmann, 2003).

The rate of the well residue varies from plant to another in some species adapted to extreme condition. For example, it is 65.76 % for *Opuntia ficus-indica* L., 78 % in *Legyum spartum* L., 82 % in *Aristida pungens* L. and 68 % for *Urginea pancration* (Steinh.) Phil. (Bouredja *et al.*, 2015, Bouzidi *et al.*, 2010; Harche *et al.*, 1991; Habibi, 2004). While, our species present a weak yield with an average of 32.52 % for both parties.

Indeed, the lignin is located in the conducting tissue like the xylem, phloem and in supporting tissues such as sclerenchyma. It has a supporting role by providing mechanical rigidity of cells. Because of its structure rich with phenyl stone and carbon chains, it also makes the cells impervious to water and thus allows the conduction of solutes throughout the plant by conductive tissues. Thus, the quantity and composition of lignin varies according to taxonomic affiliation of the plant species, considered tissue, the cell type (even part of the cell wall in question), the stage of development, environmental conditions and stress factors. The composition and quantity of synthesized lignin also vary depending on time of year and physical constraints which the plant may be submitted such as wind or meet an obstacle. These deposits are so-called reaction wood (Sjöström and Westermark, 1999; Vance *et al.*, 1980).

The “t” test of Student indicates a significant difference between the two analyzed parts. This can be explained by the fact the leaves were the center of elaboration of various sugars from photosynthesis. So, hemicelluloses were responsible for elasticity of plant cell walls during growth. Thus, reducing in the wall makes it unbreakable (Ray *et al.*, 2004).

The amount of extracted pectin is relatively weak, this can be explained by the presence of covalent bonds that bind pectins to other cell wall constituents. Pectins extraction encounters many difficulties do to the existence of the structure in “egg box” which makes it difficult for chelation by EDTA (Bouredja and Mehdadi, 2015; Bouzidi *et al.*, 2010; Robert and Roland, 1998). Moreover, the amount of the pectin fraction can also be variable depending on the season (Buchanan *et al.*, 2001).

#### Conclusion:

Quantitative analysis of cell wall fractions of *Ruta chalepensis* L. reveal a wealth of cellulose with an average about (69.6±0.54) %.

Qualitative analysis by thin layer chromatography of cellulose, which was the major constituent in the stems and leaves of *Ruta chalepensis* L., reveals the presence of glucose as unique sugar.

In hemicelluloses, the chromatography in thin layer indicates the presence of glucose, xylose, galactose and fructose in both parts of plant.

The existence of these sugars in the constitution of hemicelluloses, encourages us to find purification protocols for uses as a thickener in jams and jellies, or in the field of health that the polysaccharides have a biological activity on diabetes, cancer and viruses.

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