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Assessment Of Antioxidant Potential And Structural Characterization Of Hemicelluloses Extracted From R. Raetam Roots And Stems Via FTIR Spectroscopy

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Abstract

This study evaluates the antioxidant activity of hemicelluloses (HC) extracted from the roots and stems of Retama raetam, a medicinal legume native to the Saharan region. Samples were collected in southern Algeria and subjected to alkaline extraction of polysaccharides. Antioxidant activity was assessed using the DPPH assay, measuring the ability of the extracts to scavenge free radicals. The results showed dose- and time-dependent inhibition, with percentages ranging from 8.97% to 81.22%.

IC_{50} values were determined using the Hill sigmoid model via the curve-fit function (SciPy library, Python 3.10), executed on the Kaggle platform. The obtained values were 6.54 mg/mL for roots, 24 mg/mL for stems, and 0.37 µg/mL for ascorbic acid (used as a reference antioxidant). Root extracts exhibited stronger antioxidant activity, although their action was slower than that of the stem extracts.

Kinetic analysis revealed a biphasic response, characterized by an initial rapid phase followed by a plateau between 72 and 96 hours. At 40 mg/mL, the roots reached a maximum inhibition of 81.22% after 96 hours, compared to 50.88% for the stems at 72 hours. At lower doses, the roots maintained notable effectiveness.

FTIR analysis of the HC in solid state revealed characteristic bands corresponding to OH, C–H, and C=O groups. After solubilization in NaCl (5 mM), attenuation of these bands indicated weakened hydrogen bonding and conformational reorganization of acetylated xylans, promoting greater chain flexibility.

These findings confirm the promising antioxidant potential of R. raetam hemicelluloses, particularly those extracted from the roots.

Keywords: *Antioxidant activity, hemicelluloses, R. raetam, DPPH, IC_{50}, inhibition kinetics, FTIR, acetylated xylans.*

1- INTRODUCTION

The *Retama* genus comprises four main species: *R. monosperma* (L.) Boiss., *R. raetam* (Forssk.) Webb, *R. sphaerocarpa* (L.) Boiss., and *R. dasycarpa* (Cosson). These are perennial shrubby legumes belonging to the subfamily *Papilionoideae* and the tribe *Genisteae*. They are widely distributed across North Africa, the Canary Islands, southern Europe, and western Asia (Zohary, 1982). These shrubs often colonize vast areas, particularly depressions, wadis, sandy regions, and temporary waterways (Fig. 1) (Ozenda, 1991; Quézel & Santa, 1962).

R. raetam is used in traditional medicine in the Béchar region for its anti-inflammatory, sedative, healing, and disinfectant properties. It is also employed against snake and scorpion bites, as well as in the treatment of female infertility. Previous studies have demonstrated significant antioxidant activity in methanolic and aqueous extracts from *R. raetam* leaves and seeds (Conforti et al., 2004), as well as in secondary metabolites of *R. monosperma* (Belmokhtar et al., 2014).

Hemicelluloses, especially xylans, are major structural polysaccharides in the secondary cell walls of plants. They can account for up to 50% of the biomass of herbaceous or woody plants. However, their exploitation remains limited due to their high heterogeneity and variability depending on plant origin (Ebringerová et al., 2005).

In this context, the present study aims to evaluate for the first time the antioxidant activity of hemicelluloses extracted from the roots and stems of *R. raetam*, in order to better understand their bioactive potential and molecular structure.

1- MATERIALS AND METHODS

The biological material used in this study consists of the roots and stems of *Retama raetam*, a wild medicinal plant harvested in the northern Sahara of western Algeria (Béchar region) (Fig. 1). Fresh plant organs were cut into small pieces measuring a few millimeters and weighed to determine their fresh weight. After drying at 50 °C for one week in an oven, the samples were ground into a fine powder using an electric grinder (Kika-Werke M20).

Figure 1. Natural appearance of *R. raetam* stands observed in Ouakda, in the Oued Laâtache region (original photograph).

2.1 Hemicellulose (HC) Extraction

Following the preparation of the cell wall residue (CWR), obtained through selective removal of extracellular compounds such as phenols and lipids following the protocol of Harche et al. (1991), hemicelluloses were extracted using alkaline solutions, according to the method of Chanda et al. (1950). This method enables simultaneous cellulose isolation and targeted solubilization of loosely bound hemicelluloses, while significantly limiting lignin co-extraction (Selvendran & O'Neill, 1987).

2.2 In Vitro Antioxidant Activity Assessment (DPPH Assay)

Antioxidant activity was determined using the DPPH• radical scavenging method, following the protocols described by Blois (1958) and Brand-Williams et al. (1995). Volumes of 0.1 mL of test solutions at concentrations of 10, 20, and 40 mg/mL were mixed with 3.9 mL of freshly prepared DPPH solution (0.004%). After vortexing, the tubes were incubated in the dark at room temperature.

A negative control was prepared with 0.1 mL of NaCl (5 mM) and 3.9 mL of DPPH under the same conditions. This control was freshly prepared and measured separately for each incubation time point (0 min, 30 min, 24 h, 48 h, 72 h, and 96 h) to ensure stability and reliability of long-term measurements. Ascorbic acid, used as a reference antioxidant, served as the positive control. All tests were performed in triplicate.

Absorbance was measured at 517 nm at various incubation times: 0 min, 30 min, 24 h, 48 h, 72 h, and 96 h. No antioxidant activity (Inhibition Percentage, IP%) was observed before 30 minutes. The inhibition percentage was then calculated using the following formula:

$$IP\% = [(Abs\ negative\ control - Abs\ sample) / Abs\ negative\ control] \times 100$$

2.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Infrared spectroscopy is a fast and straightforward physical analysis method that requires minimal sample material. Based on the excitation of molecules by infrared radiation, it can be applied to both crude and purified samples. The absorption of radiation in a wavenumber range of 4000 to 400 cm^{-1} alters the rotational and vibrational states of molecules (Bertrand & Dufour, 2006; Allison et al., 2009; Popper, 2020).

The IR spectral profiles and the relative intensities of absorption bands are similar for polysaccharides with comparable structures. Certain groups or bonds, considered as markers, may indicate the presence of associated molecules (ferulic acids, lignins, proteins) or specific categories of polysaccharides. For example, carboxylic acid functions of galacturonic acids may reveal the presence of pectins (Kacuráková et al., 2000; Popper, 2020).

FTIR analysis was carried out on the *R. raetam* polysaccharide extract both in solid state (powder) and dissolved in NaCl (5 mM). FT-IR spectra were recorded using an Agilent Cary 630 FTIR spectrometer

equipped with a diamond single-bounce ATR (Attenuated Total Reflectance) accessory, which enables the collection of spectra from cell wall biomass samples (solid, liquid, or paste-like) without extensive preparation (Popper, 2020; Agilent Technologies, 2022). This system operates via an evanescent wave, whose penetration depth depends on the infrared frequency—the lower the frequency, the deeper the penetration (up to 7.3 μm at 600 cm^{-1}). This setup is ideal for the rapid, sensitive, and reproducible identification of functional groups in plant polysaccharides (Agilent Technologies, 2022).

2- RESULTS AND DISCUSSION

3.1 Hemicellulose (HC) Extraction

Analysis of the cell wall composition of *R. raetam* organs revealed a clear predominance of cellulose over hemicelluloses (HC), both in the roots and stems.

In the root cell wall residue, cellulose accounted for 84% of the structural components, compared to only 12% for hemicelluloses. This high cellulose content indicates that root cell walls are primarily composed of cellulosic polymers, conferring significant rigidity and mechanical strength, likely related to the root's anchoring and supporting functions underground.

Similarly, in the stems of *R. raetam*, the trend persisted, with cellulose representing 52% and hemicelluloses 15%. Although cellulose remains dominant, its content is lower than in the roots, possibly reflecting a slightly less rigid wall structure associated with the need for aerial support and flexibility in stems.

These findings are consistent with those reported by Bokhari-Taieb Brahimi et al. (2019) on a related species, *Retama monosperma*, in which hemicelluloses made up 16% of the crude cell wall and up to 14% of the delignified walls.

Such values—especially for roots—are particularly high and align with the structural profile of xerophytic plants. Indeed, according to Medina et al. (2009), plant species adapted to arid environments often have cell walls rich in cellulose to ensure rigidity and resistance to desiccation.

Cells in supportive tissues, particularly present in roots, are characterized by high cellulose content, often combined with lignin to ensure stiffness. In contrast, younger and more flexible tissues, such as growing stems, possess primary walls enriched in hemicelluloses, enhancing plasticity (Selvendran & O'Neill, 1987; Mansor et al., 2019). Hemicelluloses are collectively the second most abundant polysaccharides in plant cell walls, accounting for 15–30% of lignocellulosic dry matter (Broda et al., 2022).

The results of Javier-Astete et al. (2021) confirm that the distribution of cellulose and hemicelluloses varies according to the mechanical function of plant tissues. Their study showed that basal stems accumulate more cellulose (up to 48%), while growing apical parts contain higher proportions of hemicelluloses (up to 32%). These observations support our own results for *R. raetam*, where higher cellulose content was found in roots, a structurally demanding organ.

These two polymers exhibit thermal synergy: hemicellulose initiates decomposition, while cellulose sustains it. Their elevated levels in species like *R. raetam* suggest a high energy potential, particularly in arid environments where resources must be both efficient and resilient.

3.2 FTIR Spectral Analysis of Hemicelluloses in Solid State and After Dissolution in 5 mM NaCl

The main application of infrared absorption spectra is the functional analysis and identification of various molecular groups. Even spectra from pure compounds often exhibit more peaks than expected due to fundamental vibration combinations or harmonics (Allison et al., 2009). A typical FTIR spectrum of *R. raetam* hemicelluloses before dissolution in 5 mM NaCl, obtained using the Agilent Cary 630 FTIR spectrometer, is presented below (Fig. 2):

- **Broad band (3196.2–3449 cm^{-1}):** Assigned to O–H stretching vibrations of alcohol groups, indicating hydrogen bonding (Kozarski et al., 2014).
- **Peaks (2298.6–2933.4 cm^{-1}):** Correspond to asymmetric stretching vibrations of CH_3, CH_2, and CH from sugar rings (Gómez-Ordóñez & Rupérez, 2011; Kozarski et al., 2014).
- **Peaks (1718–1733 cm^{-1}):** Attributed to C=O stretching in non-conjugated ketones, carbonyl, and aliphatic xylan groups—primarily associated with acetylated xylans (Chen et al., 2010; Cuello et al., 2020).

• **Peak (1334.4 cm⁻¹):** Corresponds to in-plane bending vibrations of O–H from primary and secondary alcohols—present in both cellulose and hemicellulose.

• **Broad peak (1405.2 cm⁻¹):** Assigned to in-plane bending of CH_3, CH_2, and CH groups (Kozarski et al., 2014).

• **Broad band with peaks (1012.0, 1080, 1150 cm⁻¹):** Strong signatures of glycosidic (C–O–C) bonds and C–O stretching of primary/secondary alcohols and ethers. The wide absorption band in the 1200–1000 cm^{-1} range indicates that *R. raetam* hemicelluloses contain pyranose rings (Chen et al., 2010; Ying et al., 2017; Angelova et al., 2022). This structure also suggests arabinosylated side chains (Bian et al., 2013) and vibrations from acetyl and carboxyl groups of xylans (Cuello et al., 2020). These spectroscopic findings align with our thin-layer chromatography (TLC) results, which identified the hemicelluloses as predominantly arabinoxylans (Kebir et al., 2020).

• **Peak at 1080 cm⁻¹:** Also associated with glucuronic acid (Gómez-Ordóñez & Rupérez, 2011). • **Peak (777.1 cm⁻¹):** Assigned to out-of-plane bending of the O–H group. Small absorptions around 891 cm^{-1} indicate the presence of β-glycosidic linkages between sugar units (Chen et al., 2010; Kozarski et al., 2014).

• **Intense peak (1561.8 cm⁻¹):** Attributed to C=O stretching vibrations resulting from carbonyl groups during carboxymethylation and acetylation, while a band in the 1800–1740 cm^{-1} range is associated with carboxyl groups (Venumadhav & Seshagirirao, 2023).

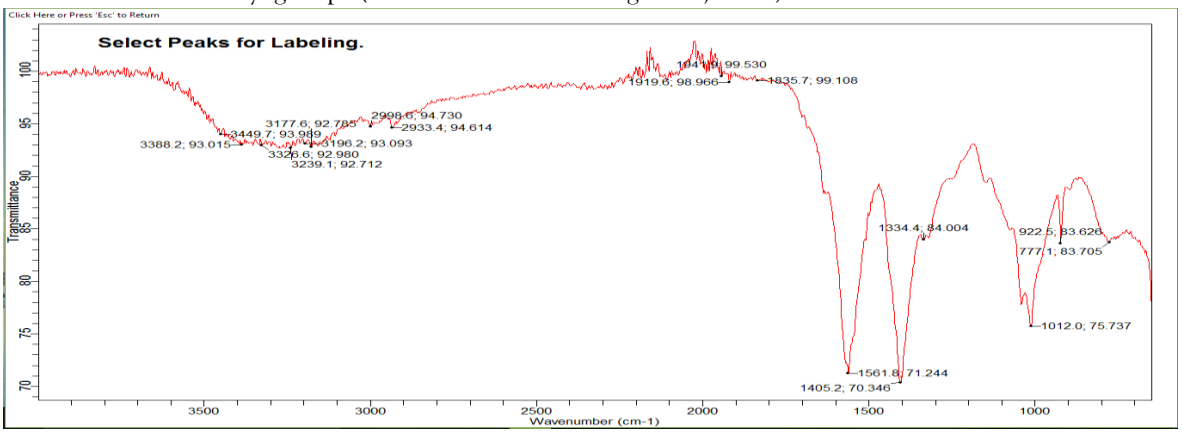

Figure 2: FTIR Spectrum of Hemicellulose in Solid State (Powder).

FTIR Analysis of Hemicelluloses Dissolved in 5 mM NaCl

The infrared (FTIR) analysis of hemicelluloses dissolved in a saline solution highlights the main chemical functions present in their structure (Fig. 3). This spectrum provides valuable insights into molecular interactions, particularly hydrogen bonding and functional groups characteristic of polysaccharides. The observed bands reflect the chemical nature of the bonds involved in the structure of water-soluble hemicelluloses.

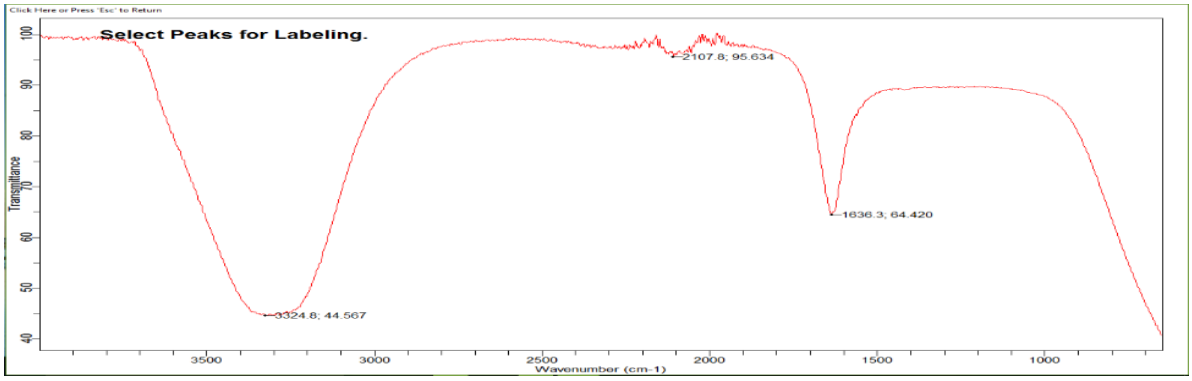

Figure 3: FTIR Spectrum of Hemicellulose Dissolved in NaCl 5 mM.

A broad band at 3324.8 cm^{-1} was observed, attributed to the O–H stretching vibrations of hydroxyl groups from alcohols, also indicating the presence of hydrogen bonding. The peak at 1636.3 cm^{-1} corresponds to primary amide stretching, C=O, and aromatic C=C vibrations (Kozarski et al., 2014).

These spectroscopic observations are consistent with our previous chromatographic analysis (TLC), which revealed a predominance of xylose, accompanied by glucose and traces of arabinose—typical of an arabinoxylan-type structure. This composition was confirmed in our earlier work (Kebir et al., 2020), supporting the hypothesis that the hemicelluloses extracted from *R. raetam* exhibit a branched xylan architecture.

The FTIR spectrum of hemicellulose generally lacks bands characteristic of lignin, such as the aromatic vibration around 1510 cm^{-1} (Rodrigues et al., 1998; Silva et al., 1999; Chen et al., 2010; Cuello et al., 2020). In contrast, bands related to carbonyl (C=O) functions—particularly 1718–1733 cm^{-1} and 1800–1740 cm^{-1}—exhibit stronger intensity. These are attributed to the stretching of free carbonyl groups (Sarkanen et al., 1967; Chen et al., 2010), highlighting the presence of acetylated xylans. This is particularly relevant for understanding the chemical reactivity of hemicelluloses, especially their potential to form complexes with other components.

Although carbonyl groups in cellulose, hemicellulose, and lignin are primarily found in the branched regions of hemicelluloses, the intensity of their bands depends on the holocellulose (cellulose + hemicellulose) to lignin ratio in all wood types (Colom & Carrillo, 2005; Chen et al., 2010).

In solid state, the FTIR spectrum presents a broad band at 3324.8 cm^{-1}, attributed to O–H stretching vibrations within the xylan structure, suggesting the presence of hydrogen bonds, which may influence the solubility and functional properties of the hemicelluloses. Hydrogen bonds play a key role in the stability of polysaccharide structures.

The peaks observed in the 2298.6–2933.4 cm^{-1} region indicate a strong presence of aliphatic groups such as CH_3 and CH_2. This suggests that hemicelluloses contain carbon chains that may affect their interactions with other biomolecules.

When xylan is dissolved in NaCl, the broad band at 3326.8 cm^{-1}, which had an intensity of 92.98 in solid state, becomes less intense in solution. This suggests conformational changes in xylan, promoting more flexible configurations that influence vibrational behavior.

In contrast to the attenuation of certain bands, the 1636.3 cm^{-1} band becomes more pronounced upon dissolution in saline solution. This peak may be attributed to conjugated C=O stretching, amide I bands, or increased water absorption bound within the polysaccharide matrix. This spectral enhancement indicates a modification in the local chemical environment of functional groups, likely due to enhanced solvation. The 1664–1634 cm^{-1} region corresponds to water absorption within the polymer and to N–H bending of primary protein amides (Angelova et al., 2022). Bands above 1600 cm^{-1} are known to be significantly influenced by moisture content (Silva et al., 1999), and peaks in the 1700–1600 cm^{-1} range are often associated with proteins in IR spectra (Ying et al., 2017).

C–H stretching bands (2920–2850 cm^{-1}), corresponding to asymmetric stretching of C–H bonds (Angelova et al., 2022), are attenuated in solution, as methyl/methylene groups become less constrained and interact more freely with the aqueous medium.

In solution, the C–O–C bands (1150–1160 cm^{-1}) and C–O bands (1020–1100 cm^{-1}) are less resolved due to interactions with the solvent.

The peaks at 1334.4 cm^{-1} and 1405.2 cm^{-1}, which correspond to deformation vibrations of hydroxyl and aliphatic groups respectively, reflect the structural diversity of hemicelluloses. The bands around 1012.0, 1080, and 1150 cm^{-1} highlight the complexity of the hemicellulose structure, suggesting the presence of pyranose rings and acetyl and carboxyl functional groups. This structural diversity can influence their mechanical and viscoelastic properties, which are essential for industrial applications.

FTIR spectral analysis of hemicelluloses allows for the identification of the functional groups composing these molecules. In the context of this study, a typical spectrum of *R. raetam* hemicelluloses was obtained both before and after dissolution in a 5 mM NaCl solution. These results underline the structural features

of the hemicelluloses and reveal the complexity of their chemical architecture through the various peaks and bands observed in the FTIR spectrum, as well as their behavior in ionic environments. This provides valuable insights into their chemical composition.

According to Kostryukov et al. (2023), the hemicellulose spectrum displays a broad absorption band in the 1750–1510 cm^{-1} region, corresponding to C=O or COOH groups. Furthermore, the spectra of cellulose and hemicellulose show minimal differences due to their structural similarity.

Our findings are consistent with those of Kostryukov et al. (2023), who assigned the 1750–1510 cm^{-1} region to C=O/COOH vibrations, and with Bazarnova et al. (2002), who reported the presence of acyl, carboxylate, and CH groups within similar ranges. The overall FTIR data confirm the chemical complexity of hemicelluloses and highlight the influence of functional groups (acetyl, hydroxyl, carboxyl) on their behavior in ionic media.

3.3 In Vitro Antioxidant Activity (DPPH Assay)

The DPPH assay revealed that hemicelluloses extracted from roots exhibit notable free radical scavenging capacity, with inhibition percentages ranging from 49.68% to 81.22% (Fig. 4). Although this effectiveness is moderate compared to the reference antioxidant—as the IC_{50} of vitamin C is 0.37 µg/mL—it remains significant in the context of natural biomolecules.

The measured IC_{50} values confirm this observation: roots (IC_{50} = 6.54 mg/mL) demonstrated better antioxidant potential than stems (IC_{50} = 24 mg/mL) *(Fig. ...)*, suggesting a chemical composition more favorable for electron donation or stabilization of DPPH• radicals.

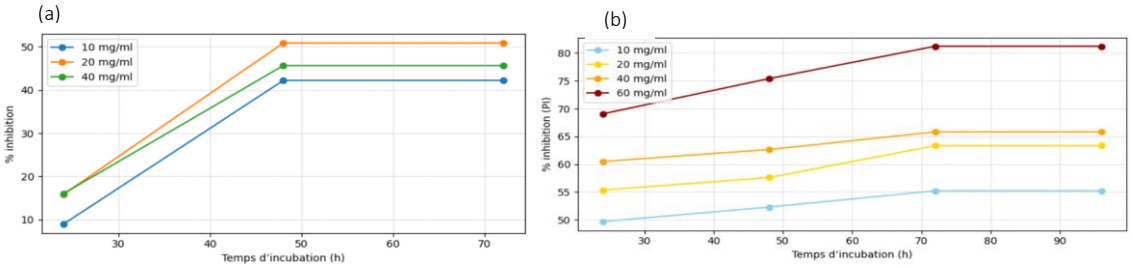

Figure 4. Time-dependent evolution of the antioxidant activity of *R. raetam* extracts: (a) stems, (b) roots.

It is important to note that the poor solubility of hemicelluloses in methanol, the solvent used to prepare the DPPH solution, represents a major experimental limitation. Being hydrophilic polymers, hemicelluloses exhibit very limited dispersion in methanolic media, which may hinder access to DPPH radicals and potentially underestimate their actual antioxidant potential. To mitigate this effect, measurements were taken after prolonged incubation periods (24 h, 48 h, 72 h, 96 h), aiming to improve the slow diffusion of active groups—such as hydroxyl, carbonyl, carboxyl, and acetyl moieties—into the reaction medium.

The difference in antioxidant efficacy between stems and roots could be attributed to variations in the degree of substitution of functional groups on the xylan chains, particularly acetyl, uronic, or phenolic groups, which are known contributors to antioxidant activity. Additionally, the possible presence of bound proteins or residual phenolic compounds in the extracts may enhance or modulate this activity.

The slower activity of hemicelluloses compared to ascorbic acid is typical of water-soluble macromolecules, in which active groups are either less exposed or diffuse more slowly toward the radicals. However, this does not detract from their bioactive potential. In sustained-release formulations or functional food systems, a moderate yet prolonged antioxidant effect can be highly beneficial.

Xylans extracted from the leaves of *Uapaca klainei* exhibit notable antioxidant activity, with an IC_{50} of 3.87 µg/mL in the DPPH assay. This high activity was not observed in four other xylan extracts, despite similar hemicellulose content in plant cell walls. Furthermore, commercial hemicelluloses such as xyloglucan and arabinoxylan showed no radical scavenging activity (Mengome et al., 2014). Previous studies have

demonstrated that phenolic substituents are primarily responsible for the antioxidant activity of xylans (Rao & Muralikrishna, 2006).

Water-soluble polysaccharides extracted from *Plantago asiatica* seeds show antioxidant activity comparable to that of hemicelluloses from *R. raetam* roots, with a DPPH radical inhibition rate of 81.4% at 0.75 mg/mL, compared to 81.22% at 60 mg/mL for the hemicelluloses. However, this similarity in efficacy masks important chemical differences: the *P. asiatica* polysaccharides are more heterogeneous and contain a higher proportion of active reducing groups, such as uronic acids and phenolic derivatives, which contribute to significant antioxidant activity even at low concentrations.

The evaluation of the radical-scavenging ability of a neutral heteropolysaccharide extracted from *Linum usitatissimum* (flaxseed), a complex polymer mainly composed of mannose, glucose, xylose, and arabinose with a mixed α/β anomeric configuration, revealed noteworthy antioxidant activity via the DPPH assay. A maximum inhibition rate of 86.12% was observed at 10 mg/mL, with an IC_{50} of 4.48 mg/mL, indicating moderate but significant effectiveness. The antioxidant activity of the LWSP was found to be dose-dependent, increasing proportionally with concentration. Compared to BHT (a synthetic antioxidant used as a positive control), the polysaccharide showed lower activity but remained promising. This ability to neutralize free radicals may be attributed to the presence of hydroxyl (–OH) and carboxyl (–COOH) functional groups, which are known to donate protons and stabilize radicals through electron or hydrogen atom transfer mechanisms (Trabelsi et al., 2021).

The IC_{50} of *P. asiatica* polysaccharides is 0.32 mg/mL, while that of vitamin C is 0.25 mg/mL (Ye et al., 2011), indicating significant DPPH radical-scavenging activity. Although hemicelluloses display lower antioxidant activity, their ability to gradually release antioxidant functional groups confers interesting potential for applications in controlled-release systems for food and biomaterial technologies.

Polysaccharides extracted from the brown alga *Sargassum pallidum*, rich in fucose and sulfate groups, have been evaluated for their antioxidant activity. Three purified fractions (SP-1, SP-2, and SP-3), isolated using DEAE-52 anion-exchange chromatography, were tested at a concentration of 3.8 mg/mL via the DPPH• assay. At this concentration, their radical scavenging ability was relatively low: SP-1 showed an inhibition rate of 17.8%, SP-2 reached 19.1%, and SP-3 only 10.2% (Ye et al., 2008). Compared to the hemicelluloses studied here (up to 81.22% inhibition), these fractions exhibit limited efficiency. However, it is worth noting that their antioxidant power increases with concentration.

Sulfated polysaccharides extracted from the brown alga *Sargassum graminifolium* (SGP) demonstrated remarkable antioxidant activity, particularly in their ability to neutralize free radicals. The DPPH• assay revealed an IC_{50} value of 0.6 mg/mL (Zhang et al., 2012), significantly more effective than the hemicelluloses extracted from *R. raetam* roots (IC_{50} = 6.54 mg/mL). This highlights the strong bioactive potential of marine sulfated polysaccharides, which surpass their plant-derived counterparts in terms of immediate antioxidant action. However, hemicelluloses offer advantages such as longer-lasting activity and simpler structures that are easier to valorize technologically. This comparison underscores the complementarity of marine and terrestrial resources in the search for new natural antioxidant agents. These findings align with results reported by Stoklosa et al. (2020) on arabinoxylans extracted from sorghum. In their study, fractions from sorghum bran and biomass achieved over 76% inhibition at 1 mg/mL, while sorghum bagasse showed significantly lower activity, reaching only 17% at 5 mg/mL. The higher antioxidant activity of the root extracts (IC_{50} = 6.54 mg/mL) could be attributed to a greater content of acetyl or uronic acid groups, which facilitate charge delocalization. Better exposure of these functional groups on the polymer surface may enhance interactions with DPPH• radicals.

Antioxidant activity is also influenced by the molecular weight of the polysaccharides: higher-molecularweight fractions generally show greater activity (Fan & Liao, 2010).

A dose-dependent relationship between the concentration of purified polysaccharides from *Lycium barbarum* (PLB) and their antioxidant capacity has been reported by Gong et al. (2017). At a concentration of 2 mg/mL, the extracts exhibited strong DPPH radical scavenging comparable to that of vitamin C. The IC_{50} values ranged from 0.018 to 0.027 mg/mL, confirming the high antioxidant potential of PLBs. In

the study by Wang et al. (2010), polysaccharides isolated from *Lycium barbarum* showed variable antioxidant activity depending on their chemical nature. Crude and neutral fractions displayed relatively low activity, with DPPH radical inhibition rates ranging from 38.1% to 58.2%. In contrast, acidic fractions showed significantly higher antioxidant activity, reaching up to 84.9%. This suggests that galacturonic acid content, typical of acidic polysaccharides, plays a major role in free radical scavenging capacity.

The results suggest that *R. raetam* hemicelluloses exhibit genuine antioxidant activity, which may be partially masked under the methanolic conditions used in the DPPH assay. Improved performance could potentially be revealed through alternative tests more suitable for hydrophilic compounds (e.g., ABTS, ORAC, FRAP) or by optimizing solubilization and reaction conditions.

FTIR spectra highlight the presence of hydroxyl (OH), carbonyl (C=O), carboxyl (COOH), and potentially acetyl groups—well-known for their role in radical scavenging due to their ability to donate electrons or stabilize reactive intermediates.

FTIR analysis shows bands associated with hydrogen bonding (~3324 cm^{-1}), suggesting a threedimensional stabilized structure that may be less readily available for rapid interaction with radicals. This could explain the slower antioxidant response of hemicelluloses compared to ascorbic acid, as more time is required to release their antioxidant functional groups.

Overall, the dominant bands in our hemicellulose spectra (OH ~3400 cm^{-1}, aliphatic CH ~2930 cm^{-1}, fingerprint region 1150–1030–920–864–817–777 cm^{-1}) closely match those reported for purified *Lycium barbarum* polysaccharides (PLB) (Gong et al., 2017), indicating a comparable saccharide backbone and βglycosidic pyranosyl linkages. However, the pronounced peaks at 1718–1733 cm^{-1} (acetylated C=O groups) and 1562 cm^{-1} (carboxymethylated carbonyls) in our dry powder indicate a higher degree of acetylation/carboxylation than in PLBP, suggesting additional carbonyl functionalities that may enhance antioxidant activity upon solubilization.

After dissolution in 5 mM NaCl, the FTIR spectrum shows a loss of these intense carbonyl bands and aligns even more closely with the PLBP profile, supporting the observed experimental increase in solubility and DPPH activity (PI ≈ 80–97%). Thus, our FTIR results reinforce the conclusion that the high antioxidant capacity of our polysaccharide, like that of PLBP, primarily depends on the solubilization of polymer chains and the availability of their reactive hydroxyl and carbonyl groups.

In this study, the antioxidant activity analysis of hemicelluloses extracted from *Retama raetam* revealed greater efficacy in root extracts compared to stem-derived fractions. This superiority may be due to a distinct structural composition of the root hemicelluloses or greater reactivity of their functional groups toward free radicals. Conversely, Benahmed et al. (2013), in their study on *Chamaerops humilis L.*, reported higher antioxidant activity in aerial organs, particularly leaflets (IC_{50} = 180.71 µg/mL), compared to roots. This divergence could be attributed to the nature of the bioactive compounds involved: flavonoids in *C. humilis* versus hemicelluloses (xylans) in *R. raetam*.

3- CONCLUSION

The results obtained in this study highlight the antioxidant potential of hemicelluloses extracted from the roots and stems of *Retama raetam*, a Saharan species that remains underexplored**.** Although their activity is lower than that of standard antioxidants such as ascorbic acid, the root-derived hemicelluloses demonstrated a remarkable DPPH• radical scavenging capacity, reaching over 81% inhibition at high concentration. The slow but sustained kinetics of this activity may make them particularly promising for controlled-release applications**.**

FTIR analysis confirmed the rich structural composition of the hemicelluloses, revealing the presence of key functional groups (OH, COOH, C=O, acetyl) that are strongly involved in antioxidant activity. These groups, combined with a conformation that can adapt to the surrounding medium**,** help explain the progressive effectiveness observed.

These findings emphasize the value of utilizing hemicelluloses from Saharan plants like *R. raetam*, both as natural antioxidant agents and as functional materials for use in biomedical, cosmetic, or food-related applications**.**

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