Free Radicals and Antioxidants. 2019; 9(1):35-42
A Multifaceted Journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.journalonweb.com/fra | www.phcog.net

Original Article

Antioxidant Potential of Cell Wall Polysaccharides Extracted from Various Parts of *Aerva javanica*

Hina Saeed[1], Haq Nawaz[1,*], Muhammad Aslam Shad[1], Dur E Shahwar[1], Hina Andaleeb[1], Saima Muzaffar[2], Raheela Jabeen[3], Tanzila Rehman[4], Anam Waheed[1]

ABSTRACT

Objectives: *Aerva javanica*, an underutilized desert plant, is a rich source of polysaccharide but not investigated properly for the antioxidant potential of its polysaccharides. The antioxidant potential of cell wall polysaccharides in leaves, branches, stem and root of *A. javanica* was determined. **Methods:** In a sequential extraction procedure, the water-soluble fraction (WSF), sodium acetate-EDTA soluble fraction (SASF), sodium carbonate soluble fraction (SCSF), 4% potassium hydroxide soluble fraction (4%KOHSF) and 14% potassium hydroxide soluble fraction (14%KOHSF) were obtained and subjected to antioxidant analysis. **Results:** The addition of salt or alkali in the extraction media resulted in a significant increase in total extractable polysaccharide content. The 14%KOHSF showed higher extract yield, total antioxidant activity, ferrous ion chelating activity, ferric reducing antioxidant power and linoleic acid reduction capacity. The WSF showed higher scavenging capacity against DPPH radical. WSF of branches and root and SCSF of leaf and stem showed highest scavenging capacity against hydroxyl radical. **Conclusion:** The lower extraction yield but high radical scavenging capacity of WSF may be attributed to the presence of some protein linked hydrophilic polysaccharides of pectin nature. The high extractable polysaccharide content with strong antioxidant potential makes *A. javanica* a suitable candidate for the antioxidant polysaccharide based pharmaceutical and industrial applications.

Key words: *Aerva javanica*, Antioxidant potential, Cell wall polysaccharides, desert cotton, Free radical scavenging capacity, Lipid peroxidation.

Hina Saeed[1], Haq Nawaz[1,*], Muhammad Aslam Shad[1], Dur E Shahwar[1], Hina Andaleeb[1], Saima Muzaffar[2], Raheela Jabeen[3], Tanzila Rehman[4], Anam Waheed[1]

[1]Department of Biochemistry, Bahauddin Zakariya University, Multan, PAKISTAN.
[2]Department of Chemistry, University of Education, Vehari, Punjab, PAKISTAN.
[3]Department of Biochemistry and Biotechnology, The Women University, Multan, PAKISTAN.
[4]Department of Chemistry, The Women University, Multan, Pakistan.

Correspondence

Mr. Haq Nawaz

Department of Biochemistry, Bahauddin Zakariya University, Multan-60800, PAKISTAN.

Phone no: +92 300 6373150

E-mail: haqnawaz@bzu.edu.pk

History

- Submission Date: 24-11-2018
- Review completed: 02-12-2018
- Accepted Date: 08-12-2018

DOI : 10.5530/fra.2019.1.7

Article Available online
http://www.antiox.org

INTRODUCTION

The significance of plant originated natural antioxidants in the field of medicine and the pharmaceutical industry is increasing day by day due to their diverse biological activities. The phytochemical antioxidants have been found to be safer than synthetic ones for the human body. These antioxidants have been found to be effective in reducing oxidative stress caused by excess production of free radicals in the human body. The researchers are getting more attention to these natural antioxidant compounds for their use in the treatment of various diseases caused by oxidative stress.[1]

Polysaccharides are a class of carbohydrates which play a vital role in the structure and functioning of plants and animals. Cell wall, the outermost covering of plant cells, is chemically composed of various types of structural polysaccharides including cellulose, hemicellulose, lignin, pectin, glycoprotein and proteoglycan.[2,3] These polysaccharides are synthesized by the condensation polymerization of monosaccharide units. Generally, the structural as well as functional polysaccharides are considered as the non-reducing compounds which are involved in the formation of cellular structures and food storage in plants and as a source of energy for animals. These are used as a food stuff for human and cattle, nutrients for the microbial culture and raw material for various pharmaceutical and industrial applications. However, these compounds have been also reported to show various biological activities including anticoagulant, anti-carcinogenic, immuno-stimulant, nephro-protective, anti-hyperglycemic, antimicrobial, anti-plasmodial and antioxidant activities.[4–11] On account of their antioxidant potential, polysaccharides have scored a great nutritional and medicinal value. These have been found to be effective against neuro-degenerative diseases caused by oxidative stress.[12] Polysaccharides have been reported to be a good source of various prebiotic oligosaccharides used in a number of pharmaceutical formulations.[13]

Aerva javanica, commonly known as desert cotton, is a medicinal herb found in water stressed and desert areas of Pakistan. It is a good source of phytochemicals including carbohydrates, glycosides, tannins, saponins, alkaloids, terpenoids, flavonoids and polyphenols.[14–16]

Cite this article: Saeed H, Nawaz H, Shad MA, Shahwar DE, Andaleeb H, Muzaffar S, *et al*. Antioxidant Potential of Cell Wall Polysaccharides Extracted from Various Parts of *Aerva javanica*. Free Radicals and Antioxidants. 2019;9(1):35-42.

The extracts obtained from different parts of *A. javanica* in solvents of varying polarity have been found to possess good antioxidant potential.[17] The cell wall of *A. javanica* is known to be composed of various types of polysaccharides including cellulose, hemicellulose and pectin which have been reported to possess antioxidant activity.[18] The presence of these polysaccharides and other phytochemicals advocates the medicinal and industrial significance of this plant as a source of bioactive materials.

Previously, studies have been reported which highlighted the mechanism and improvement of biological activities and structure-activity relationships of polysaccharides extracted from animals, plants and bacteria[19–21] but limited data is available on the extraction and antioxidant potential of cell wall polysaccharides of *A. javanica*. Moreover, the extraction of bound polysaccharides from plant cell wall has been also remained a problem for the researcher and still needs to be optimized. Recently, the phytochemical and antioxidant potential of *A. javanica* extracts in various polarity solvents have been reported from our laboratory.[22] In the present study, the antioxidant potential of various fractions of cell wall polysaccharides extracted from different parts of *A. javanica* was evaluated. The study would provide useful information for the researchers and industrialists regarding the extraction of cell wall polysaccharides, antioxidant potential and medicinal importance of *A. javanica*, an underutilized medicinal plant.

MATERIAL AND METHODS

Material

The *Aerva javanica* (desert cotton) plants were collected from roadside water stressed area of Dunyapur, District Lodhran, Punjab, Pakistan. The plant parts including leaves, branches, stem and root were separated manually, washed using distilled water, cut into small pieces with a sharp knife and dried under the shade until a constant weight. The dried material was grinded to form powder using an electric grinder and sieved through a 20µm sieve to obtain fine particle size. The powdered samples were stored in air tight glass containers at standard laboratory conditions (at 25±3°C in dark) throughout the study period.

Isolation of cell wall

The sugar free cell wall material was isolated from selected parts of *A. javanica* by the method described earlier[23] with some modifications. The powdered sample (50 g) was first mixed with 95% ethanol (500 mL) followed by boiling with continuous stirring for 1 h to inactivate the enzymes to release the simple sugars. The mixture was cooled to room temperature and filtered through a fine muslin cloth. The residue was washed thrice successively with methanol, methanol-chloroform mixture (1:1 v/v) and acetone. The simple sugar free residue was used as cell wall material for the extraction of polysaccharide fractions.

Extraction of cell wall polysaccharide

The cell wall polysaccharides (CWP) were fractionated consecutively in five extracting media by previously described method.[24] The fractionation of polysaccharides was done in a sequential manner starting from water followed by sodium acetate, sodium carbonate, 4% potassium hydroxide (KOH) and 14% potassium hydroxide. The sugar free cell wall material (5 g) was first soaked in water (700 mL) for 24 h, filtered through a Whatman filter paper No. 42 using a Buchner funnel. The residue was washed twice with water (100 mL) and the water soluble fraction (WSF) was collected. The residue was extracted consecutively in 0.05M sodium acetate solution (pH 6.5) containing 0.05M ethylenediaminetetracetic acid (EDTA), 0.05M sodium carbonate solution containing 0.01M sodium borohydrate ($NaBH_4$), 4% KOH in 0.1% $NaBH_4$ solution and 14% KOH in 0.1% $NaBH_4$ solution (Figure 1). The residue in each step was washed twice with the respective solution and

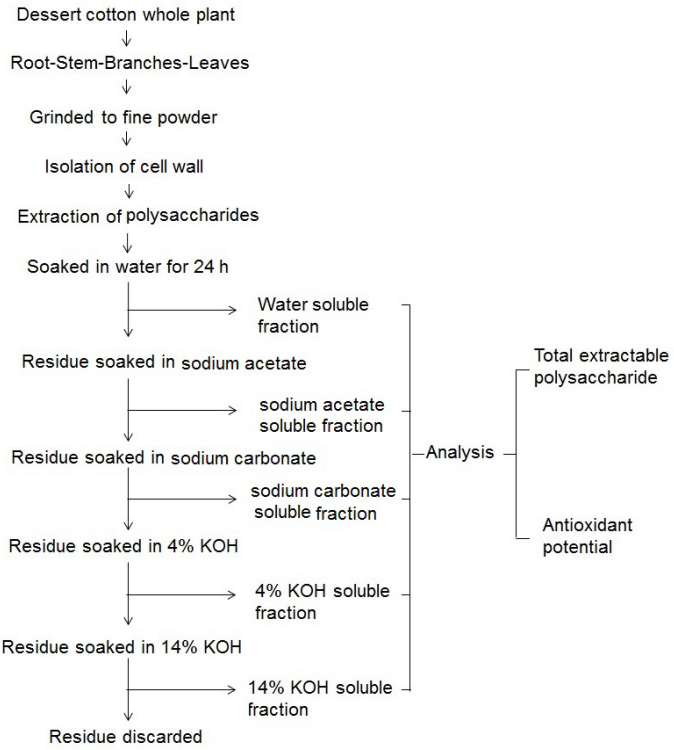


Figure 1: Flow sheet of the experimental plan.

the sodium acetate soluble fraction (SASF), sodium carbonate soluble fraction (SCSF), 4% potassium hydroxide soluble fraction (4% KOHSF) and 14% potassium hydroxide soluble fraction (14% KOHSF) were pooled. The volume of the extracts was reduced to 30 mL using rotary evaporator followed by the precipitation of cell wall components using ethanol (90 mL). The precipitates were centrifuged at 8000 x g at 4°C, washed with distilled water, evaporated to dryness and stored at -20°C till further analysis.

Total extractable polysaccharides

The extractable polysaccharide fractions obtained in each step of the sequential extraction were evaporated to dryness, weighed on an electrical balance and the total extractable polysaccharides (TEP) content was calculated using Eq. 1:

$$TEP(g/(100\ g)\ dw) = (\text{Weight of precipitates})/(\text{Weight of sample}) \times 100 \quad (1)$$

The residue obtained at the end of extraction process was also washed with distilled water, evaporated to dryness and calculated as non-extractable cell wall fraction (NECWF) using Eq. 2.

$$NECWF\ (g/100\ g\ dw) = (\text{Weight of residue})/(\text{Weight of sample}) \times 100 \quad (2)$$

Antioxidant analysis

The residue from each fraction was dissolved in the respective solvent and subjected to their antioxidant analysis. The antioxidant potential of the extracts was determined in terms of their total antioxidant activity, metal chelating activity, metal and lipid reducing capacity and free radical scavenging potential.

Total antioxidant activity

The butylated hydroxytoluene (BHT) equivalent total antioxidant activity (TAOA) of various fractions of cell wall polysaccharides was determined

by two different assays and the results were compared. The TAOA was determined by phosphomolybdenum assay using the previously reported method.[25] The polysaccharide fraction (1 mL) was mixed with the 3 mL of each of the 28mM sodium phosphate solution, 0.6M sulfuric acid and 4mM ammonium molybdate solution 1:1:1 v/v). After incubation at 95°C for 90 min, the reaction mixture was cooled to room temperature (25±5°C) and the absorbance was recorded at 695 nm against a blank (without reagent) using a spectrophotometer (UV-Visible 6405, Jenway, Japan). BHT equivalent total antioxidant activity (g/100 g dw) was calculated from a regression equation (Eq. 3) obtained from the standard curve of BHT (R^2=*0.9811*).

$$\text{TAOA (mg/100 mL)}=(\text{Absorbance of the sample})/0.0539 \quad (3)$$

The BHT equivalent TAOA by phenanthroline assay was determined by previously reported method with some modifications.[26] An aliquot (1 mL) was mixed with reagent solution (a mixture of 0.168 mL 0.1M HCL, 0.218 mL saturated solution of NaCl and 0.15 mL freshly prepared 2mM $FeSO_4$ solution). A reaction mixture without sample was used as a control and that without $FeSO_4$ solution was taken as blank. The reaction mixture was incubated for 5 min followed by the addition of 0.25% w/v 1, 10 phenanthroline (10 µL). The absorbance was recorded at 510 nm. The antioxidant activity was presented in terms of inhibition of phenanthroline-Fe^{2+} complex formation. The BHT equivalent TAOA of extracts was calculated using the following regression equation obtained from the standard curve of BHT (R^2=*0.9811*).

Ferrous ion chelation activity

The ability of CWP to chelate ferrous ions was also determined by the ferozine method reported earlier.[27] An aliquot (2 mL) of extract was added to 2mM $FeCl_3$ solution (0.0 5 mL). The reaction was initiated by mixing with 5mM ferrozine (0.2 mL) and allowed to stand at room temperature for 10 min. The absorbance was recorded at 562 nm and the percent inhibition of ferrozine-Fe^{2+} complex formation was termed as ferrous ion chelating activity (FICA) of extracts and calculated using Eq.4.

$$\text{FICA (\%)}=\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}} \times 100 \quad (4)$$

Reducing properties

The metal and lipid reducing properties of the extracts were determined in terms of ferrous reducing power (FRP) and linoleic acid reducing capacity (LARC) respectively.

Ferric reducing power

The FRP was determined by the previously developed method.[28] The polysaccharide fraction (2.5 mL) was mixed with 2.5 mL of 0.2M solution of phosphate buffer of pH 6.6 followed by the addition of 1% potassium ferricyanide solution (2.5 mL). After a 20 min incubation at 50°C, the reaction mixture was mixed with 10% trichloroacetic acid (2.5 mL). The supernatant (5 ml), obtained after centrifugation of the reaction mixture at 3000 rpm for 10 min, was diluted with distilled water (up to 10 mL). After addition of 1% ferric chloride solution (1 mL), the absorbance was recorded at 700 nm and FRP was expressed in terms of change in absorbance of the reaction mixture as:

$$\text{Absorbance of the reaction mixture at 700 nm} \propto \text{Ferric reducing power}$$

Linoleic acid reduction capacity

The LARC of the extracts was determined by the ferric thiocyanate method described earlier.[29] The polysaccharide fraction (2 mL) was mixed with 2.5% linoleic acid solution in ethanol (2 mL) followed by addition of 0.05M sodium phosphate buffer (2 mL) and distilled water (2 mL). After a 24 h incubation at 40°C in dark, a portion (1 mL) of the reaction mixture was mixed with 75% aqueous methanol (10 mL) followed by the addition of 20mM $FeCl_3$ solution (1 mL). After an addition of 30% ammonium thiocyanate solution (1 mL) to the mixture, the absorbance was recorded at 500 nm against a blank (without $FeCl_3$ solution). The reaction mixture without extract was taken as a control and that containing BHT solution as standard. The LARC was calculated using Eq. 5:

$$\text{LARC (\%)}=\text{Abs}_{\text{control}}\text{-Abs}_{\text{sample}}/\text{Abs}_{\text{control}} \times 100 \quad (5)$$

Free radical scavenging capacity

The scavenging potential of the extracts against nitrogen and oxygen reactive species can be determined using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and hydroxyl radicals respectively. The free radical scavenging capacity of CWP fractions was measured spectrophotometrically in terms of change in color of DPPH radical or hydroxylated salicylate complex after the addition of sample.

DPPH radical scavenging capacity

DPPH radical scavenging capacity (DPPH RSC) of the CWP fractions was determined by the previously described method.[30] The polysaccharide fraction (1 mL) was mixed with 40µM DPPH solution in methanol (3 mL) and allowed to react at room temperature (25±5°C) for 30 min. The absorbance was recorded at 517 nm against methanol as blank. DPPH solution, without extract and that with BHT solution was taken as control and standard respectively. The radical scavenging capacity was calculated using Eq. 6:

$$\text{DPPH RSC (\%)}=\text{Abs}_{\text{control}}\text{-Abs}_{\text{sample}}/\text{Abs}_{\text{control}} \times 100 \quad (6)$$

Hydroxyl radical scavenging capacity

The hydroxyl radical scavenging capacity (HRSC) of the CWP fractions was determined by the previously developed method.[31] An aliquot (1 mL) of the extract was mixed with 1.5mM ferrous sulfate solution (1 mL) followed by the addition of 20mM salicylic acid solution (1 mL) and 6mM hydrogen peroxide solution (1 mL). After a 60 min incubation at 37°C, the absorbance of the reaction mixture was recorded at 562 nm against a blank (without hydrogen peroxide). The reaction mixture without sample was taken as control. The HRSC of the extracts was calculated using Eq. 7:

$$\text{HRSC (\%)}=[1-(\text{Asample-Ablank})/\text{Acontrol}]\times 100 \quad (7)$$

Statistical analysis

The results were expressed as mean± standard deviation of three parallel replicates and the means were separated by one way analysis of variance (ANOVA) at confidence level $p \leq 0.05$ in Tukey's multiple range tests using statistical software SPSS version 19.

RESULTS

Total extractable polysaccharides

The total extractable polysaccharides (TEP) from leaves, branches, stem and root of *A. javanica* in various extracting media were found to be in the range of 6.00±1.02-20.48±1.16, 4.67±1.55-26.94±1.70, 2.50±0.50-23.08±1.61 and 6.17±1.44-19.78±1.26 mg/100 g dw respectively (Table 1). The residue after extraction of TEP in 14% KOH from each of the selected parts, was collected as non-extractable polysaccharide fraction (NEPF) of which ranged from 22.78±3.26-32.56±3.20 mg/100 g dw. A statistically significant difference (p<0.05) was observed in the TEP content of various extracts and NEPF obtained from each part of *A. javanica*. TEP content was found to be comparatively high in 14% KOHSF of branches and the lowest in WSF of the stem. However, the highest NEPF was obtained from leaves followed by stem, root and branches. The results were found

Table 1: Total extractable and non-extractable fractions of cell wall polysaccharides (g/100 g dw) extracted from different parts selected parts of *A. javanica*.

Plant parts	TEP*					NECWF	*p*-value
	WSF	SASF	SCSF	4% KOHSF	14% KOHSF		
Leaves	6.01±1.02^{d**}	12.48±0.45^{c}	10.84±1.20^{c}	20.48±1.16^{b}	18.92±1.12^{b}	32.56±3.20^{a}	0.001
Branches	4.67±1.55^{d}	14.45±0.60^{bc}	12.48±0.58^{c}	24.32±2.04^{a}	26.94±1.70^{a}	15.76±2.73^{b}	0.003
Stem	2.5±0.50^{e}	13.13±0.58^{c}	9.52±0.50^{d}	22.03±1.15^{b}	23.08±1.50^{b}	26.38±2.85^{a}	0.001
Root	6.17±1.44^{d}	17.90±0.76^{b}	14.78±1.50^{c}	17.52±0.90^{b}	19.78±1.26^{ab}	22.78±3.26^{a}	0.023

*TEP: Total extractable polysaccharides, NECWF: Non extractable cell wall fraction

*WSF: Water soluble fraction, SASF: Sodium acetate-EDTA soluble fraction, SCSF: Sodium carbonate soluble fraction, 4% KOHSF: 4% potassium hydroxide soluble fraction, 14% KOHSF: 14% potassium hydroxide soluble fraction

**Values are expressed as mean±SD of three replicates. The means followed by different alphabets in each row are significantly different at confidence level p≤0.05 using Tukey's multiple range test.

Table 2: BHT equivalent TAOA (g/100 g dw) of various extracts of cell wall polysaccharides of selected parts of *A. javanica*.

Parts used	WSF*	SASF	SCSF	4% KOHSF	14% KOHSF	*p*-value
			Phosphomolyebdenum assay			
Leaves	6.60±0.11^{c**}	12.87±2.12^{ab}	16.31±0.65^{a}	10.55±3.20^{b}	16.94±0.96^{a}	0.022
Branches	3.82±0.34^{d}	10.20±1.51^{b}	14.24±0.37^{a}	7.62±1.76^{c}	13.82±0.51^{a}	0.001
Stem	7.55±0.63^{c}	9.49±0.53^{b}	11.68±0.32^{a}	6.43±3.44^{c}	10.36±0.83^{ab}	0.003
Root	3.73±0.24^{d}	5.01±0.25^{c}	8.15±0.81^{b}	5.19±2.30^{c}	12.28±0.48^{a}	0.006
			Phenanthroline assay			
Leaves	11.11±0.82^{a}	5.01±0.36^{c}	8.37±0.58^{b}	7.42±0.62^{b}	12.17±0.24^{a}	0.004
Branches	7.50±0.46^{c}	7.87±0.33^{b}	7.42±0.34^{b}	6.48±0.23^{c}	11.86±0.62^{a}	0.030
Stem	3.92±0.17^{d}	6.83±0.71^{c}	7.92±0.72^{a}	8.18±0.35^{a}	6.13±0.21^{b}	0.008
Root	5.64±0.51^{c}	3.38±0.42^{e}	8.30±0.64^{a}	4.53±0.21^{d}	6.72±0.42^{b}	0.002

*WSF: Water soluble fraction, SASF: Sodium acetate-EDTA soluble fraction, SCSF: Sodium carbonate soluble fraction, 4% KOHSF: 4% potassium hydroxide soluble fraction, 14% KOHSF: 14% potassium hydroxide soluble fraction

**Values are expressed as mean±SD of three replicates. The means followed by different alphabets in each row are significantly different at confidence level p≤0.05 using Tukey's multiple range test.

to be comparable to those reported earlier in similar extracts obtained from *Vaccinium ashei* fruits.[24]

Antioxidant potential

Total antioxidant activity

The results for BHT equivalent total antioxidant activity (TAOA) as determined by phosphomolybdenum assay (PMA) and phenanthroline assay (PA) are presented in Table 2. The TAOA determined by PMA of various fractions of CWP were found to be in the range of 6.60±0.11-16.94±0.96, 3.73±0.24-14.24±0.37, 6.43±3.44-11.68±0.32 and 3.73±1.24-12.28±0.48 g/100g dw respectively. TAOA determined by PA of various fractions of CWP of leaves, branches, stem and root ranged from 5.01±0.36 to 12.17±0.24, 6.48±0.23 to 11.86±0.62, 3.92±0.17 to 8.18±0.35 and 3.38±0.42 to 8.30±0.64 g/100g dw respectively. A statistically significant difference (p<0.05) in TAOA determined by each method was observed among various fractions of CWP of each part. The TAOA determined by PMA and PA was observed to be comparatively high in 14% KOHSF of leaf and low in WSF and SASF of root respectively.

Ferrous ion chelating activity

The ferrous ion chelating activity (FICA) of various fractions of CWP in leaves, branches, stem and root ranged from 5.57±1.15 to 17.43±1.53, 7.59±0.23 to 15.53±0.77, 4.50±1.07 to 16.90±1.35 and 8.70±1.20 to 25.70±2.98% respectively. A statistically significant difference (p<0.05) in FICA was observed among various fractions of CWP of each part. The FICA was observed to be comparatively high in SCSF of root and low in 4% KOHSF of the stem. The FICA of the CWP fraction of each part was found to be significantly lower than that of BHT (35.90±2.23%) taken as standard antioxidant (Figure 2). The results are in agreement with those reported earlier that EDTA associated extraction in sodium acetate increases the FICA of CWP.[32]

Metal and lipid reducing ability

The ferric reducing power (FRP), in terms of absorbance at 700 nm, of CWP fractions of leaves, branches, stems and roots ranged from 0.59±0.06 to 1.54±0.27, 0.84±0.09 to 1.50±0.13, 0.72±0.07 to 1.39±0.12 and 0.48±0.13 to 1.065±0.16 respectively. The linoleic acid reduction capacity (LARC) ranged from 2.23±0.39 to 37.76±6.51, 2.12±0.39 to 7.96±0.53, 2.86±0.39 to 18.32±2.64 and 1.57±0.70 to 16.29±0.76% respectively. A statistically significant difference (p<0.05) in FRP and LARC was observed among various fractions of CWP of each part. The FRP and LARC were found to be comparatively high in 14% KOHSF of leaf and low in SASF and WSF of root respectively. However, the values of both the parameters for *A. javanica* CWP fractions were found to be

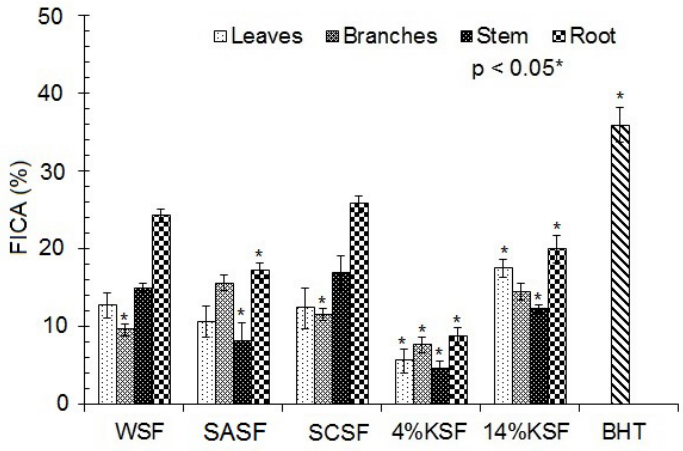


Figure 2: Ferrous ion chelating activity (FICA) of various fractions of cell wall polysaccharides of selected parts of *A. javanica*.
*The bars present the mean±SD of three replicates. The error bars marked with a steric are statistically different from the other fractions of the same part at confidence level p≤0.05 using Tukey's multiple range test.

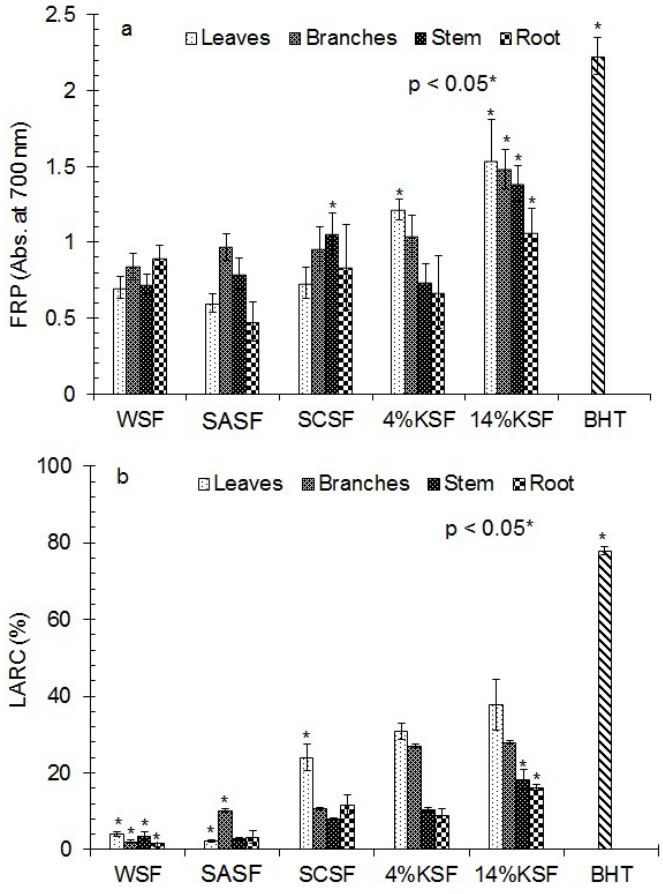


Figure 3: Metal and lipid reducing the ability of various fractions of cell wall polysaccharides of selected parts of *A. javanica*.
a: Ferric reducing power, b: Linoleic acid reduction capacity
*The bars present the mean±SD of three replicates. The error bars marked with a steric are statistically different from the other fractions of the same part at confidence level p≤0.05 using Tukey's multiple range test.

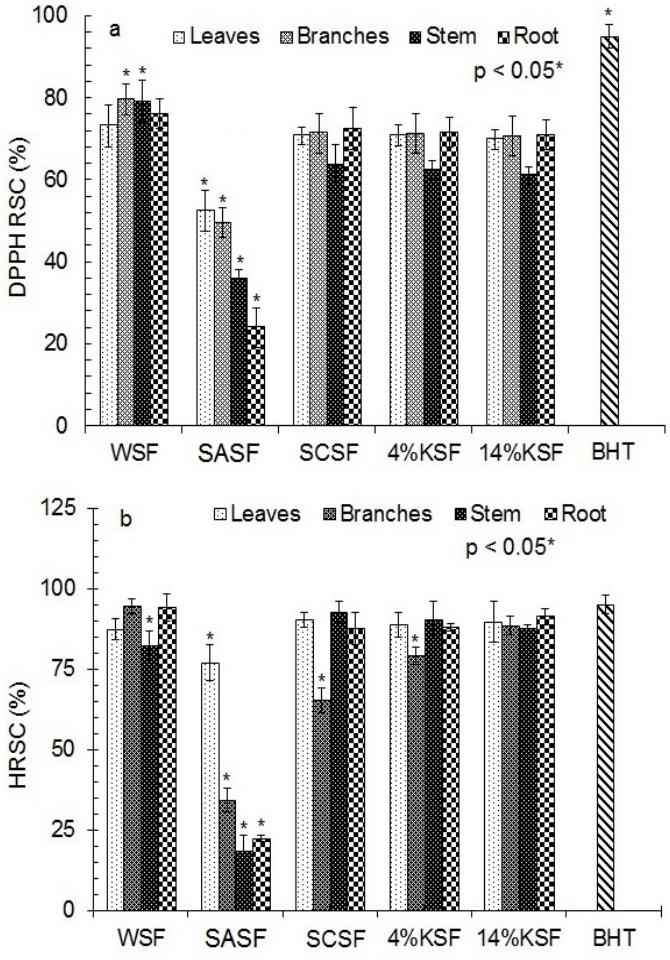


Figure 4: Free radical scavenging capacity of various fractions of cell wall polysaccharides of selected parts of *A. javanica*.
a: DPPH radical scavenging capacity, b: Hydroxyl radical scavenging capacity
*The bars present the mean±SD of three replicates. The error bars marked with a steric are statistically different from the other fractions of the same part at confidence level p≤0.05 using Tukey's multiple range test.

significantly lower than those for BHT (2.23±0.12 and 78±1.2% respectively) (Figure 3a, b).

Free radical scavenging capacities

The free radical scavenging capacity of CWP fractions was determined as DPPH radical scavenging capacity (DPPH RSC) and hydroxyl radical scavenging capacity (HRSC). The DPPH RSC of CWP fractions of leaves, branches, stems and roots ranged from 52.46±4.88 to 73.19±5.10, 49.53±3.73 to 79.64±3.79, 35.72±2.48 to 79.06±5.20 and 23.96±4.81 to 76.14±3.74% respectively. The HRSC ranged from 76.94±5.43 to 90.11±2.26, 34.22±3.68 to 94.57±2.98, 18.17±5.28 to 92.67±3.30, 22.36±2.29 to 93.90±4.24% respectively. A statistically significant difference (p<0.05) in DPPH RSC and HRSC was observed among various fractions of CWP of each part. The DPPH RSC and HRSC were found to be comparatively high in WSF of branches and low in SASF of root and stem respectively. The values of DPPH RSC of CWP fractions were found to be significantly lower than that for BHT (95.00±2.70) while HRSC of CWP fractions was statistically similar to that of BHT (Figure 4a, b). The results support the previously reported data on free radical scavenging potential of CWP extracted from various plants.[5]

DISCUSSION

A. javanica is an underutilized desert plant which needs to be investigated for its bioactive compounds and their activities. The studies on the antioxidant potential of cell wall polysaccharides present in various parts of *A. javanica* would increase its medicinal, pharmaceutical and industrial importance. Antioxidants are the substances known to prevent the oxidative stress caused by over production of endogenous free radicals and other redox reactions. They perform their action by reducing the oxidized metals, inhibiting the lipid peroxidation and scavenging the free radicals. The antioxidant activity of these substances depends on their ability to donate hydrogen to reactive oxygen and nitrogen species.[33] The present study was based on the selection of good extracting medium and determination of the antioxidant potential of leaves, branches, stems and roots of *A. javanica*. Four different extraction media including water, sodium acetate, sodium carbonate and dilute and concentrated sodium hydroxide solution, were used consecutively for extraction of cell wall polysaccharides.

The results showed that the addition of the salt and alkali in the extracting media results in an increase in the extraction yield of CWP. The extract yield of CWP was comparatively low in WSF and high in KOHSF of the studied parts of *A. javanica* plant which suggests that KOH assists the release of bound polysaccharides from plant material. The comparatively highest extraction yield in KOHSF of branches and low in WSF of stem suggests that the CWP can be easily extracted from branches as compared to stem due the structural stiffness.

The antioxidant potential of various fractions of *A. javanica* was determined in terms of total antioxidant content, metal chelating activity, reducing ability and free radical scavenging capacity. BHT equivalent TAOA was also found to be increased with the addition of salts and alkali in the extraction media. The TAOA was found to be comparatively high in SCSF and KOHSF of leaves and low in WSF of stem and root. Although the extract yield was high in various extracts of branches, yet the leaves were found to contain cell wall polysaccharides with strong antioxidant potential. The phosphomolybdenum assay showed comparatively higher values of TAOA as compared to phenanthroline assay.

The FICA of antioxidants is based on their ability to inhibit the formation of ferrozine -Fe^{2+} complex by forming coordination complexes with iron. It has been reported that chelating agent which form σ-bonds with metals are effective as secondary antioxidants because they reduce the redox potential, stabilizing the oxidized form of metal ion.[34] The biological systems contain trivalent iron which has to be converted into a divalent form for its bioavailability. The reduction of ferric (Fe^{3+} to ferrous (Fe^{2+}) form in the human body is usually catalyzed by certain types of reductases such as cytochrome-b. However, the antioxidant substances are also helpful in reducing the ferric stress in the body. The polysaccharides are rich in hydroxyl groups which act as an electron donor and reduce Fe^{3+} to Fe^{2+}. The WSF and SCSF of root showed comparatively high FICA among the extracts indicating the presence of polysaccharides with good chelating ability which would be effective in reducing the ferric ions inside the body. However, the polysaccharides extracted in 14%KOHSF showed good ferric ion reducing capacity. The peroxidation of unsaturated lipids present in the biological membrane is common due to the free radical chain reactions resulting in the formation of a variety of reactive oxygen species including epoxide, peroxide and hydroxyl radicals. The antioxidant compounds prevent the peroxidation of membrane lipids either by reducing the membrane lipids or by scavenging the free radicals.[35,36] The high value of FRP and LARC of CWP extracted in concentrated alkali may be attributed to the alkaline hydrolysis of CWP in to some reducing mono or disaccharides. Comparatively higher values of reducing abilities of KOHSF may also be correlated with the presence of polysaccharides containing relatively high number of hydroxyl groups with good hydrogen donating ability.

Free radicals are the reactive oxygen or nitrogen species which are reduced by antioxidant compounds. Hydroxyl radical is an unstable radical which react with the biomolecules and damage the cellular machinery. The endogenous nitrogen free radicals are also known to cause oxidative damage to the cellular structure. The antioxidants showing high scavenging activity against these radicals may the best tool for the protection of cellular structure from the damage caused by these free radicals. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic compound which produces a stable nitrogen free radical which can be used as a control for determination of antioxidant activity of various molecules against reactive nitrogen species. Hydrogen peroxide is used as a source of hydroxyl radical for the determination of scavenging activity of antioxidants against reactive oxygen species. Although, the exact mechanism still needs to be investigated yet some assumptions have been reported to describe the mechanism of free radical scavenging by polysaccharides. It has been stated that the polysaccharide is rich in hydroxyl groups which act as electron donors for the inhibition of free radical action. In other studies, it has been reported that peptides and proteins associated with polysaccharide structure are responsible for scavenging the reactive oxygen species.[37] The structural and peripheral parts of the plants, which are rich in polysaccharides, have been found to possess higher antioxidant activity as compared to the storage and inner parts.[21] All of the fractions of CWPs obtained from the studied parts of *A. javanica*, except SASF of branches, stem and root, were found to show relatively high scavenging ability against both the nitrogen (DPPH) and oxygen (OH) free radicals. The *A. javanica* plant, due to its high free radical scavenging activity, would be most effective in minimizing the oxidative stress and preventing the cellular damage.

CONCLUSION

In conclusion, the addition of salt or alkali in the extraction media resulted in an increase in polysaccharide yield from leaves, branches, stems and roots of *A. javanica*. The concentrated alkali (14% KOH) was found to be the most effective medium for the extraction of cell wall polysaccharides with the highest yield. The 14% KOHSF obtained from each part was also found to be high in total antioxidant activity, ferrous ion chelating activity, ferric reducing antioxidant power and linoleic acid reduction capacity. The WSF extracted from each part was found to be comparatively most effective against DPPH radical while the polysaccharides extracted in WSF of branches and root and SCSF of leaf and stem showed highest scavenging activity against hydroxyl radical. The lower extraction yield but high radical scavenging activity of water soluble CWP may be attributed to the presence of some protein linked hydrophilic polysaccharides of pectin nature. The high extractable polysaccharide content with strong antioxidant potential makes *A. javanica* a suitable candidate for the antioxidant polysaccharide based pharmaceutical and industrial applications. Moreover, the cell wall polysaccharides of *A. javanica*, being natural and more stable antioxidants, would be preferred over the synthetic antioxidants having potential side effects.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan for providing the facilities for conducting the research work.

CONFLICT OF INTEREST

The authors declare that they do not have a conflict of interest in any capacity including competing or financial.

ABBREVIATIONS

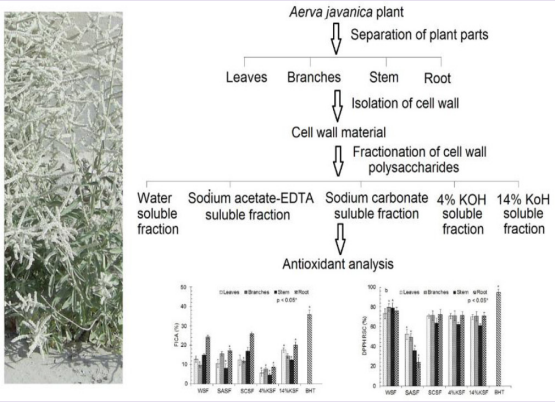
ANOVA: Analysis of Variance; **TAOA:** Total antioxidant activity; **BHT:** Butylated hydroxy toluene; **CWP:** Cell wall polysaccharides; **DPPH RSC:** 2, 2-diphenyl-1-picryl hydrazyl radical scavenging capacity; **DPPH:** 2, 2-diphenyl-1-picryl hydrazyl; **FICA:** Ferrous ion chelation activity; **FRP:** Ferrous reducing power; **HRSC:** Hydroxyl radical scavenging capacity; KOHSF: Potassium hydroxide soluble fraction; **KSF:** Potassium hydroxide soluble fraction; **LARC:** Linoleic acid reducing capacity; **NECWF:** Non extractable cell wall fraction; **NEPF:** Non extractable polysaccharides fraction; **PA:** Phenanthroline assay; **PMA:** Phosphomolybdenum assay; **SASF:** Sodium acetate-EDTA soluble fraction; **SCSF:** Sodium carbonate soluble fraction; **TEP:** Total extractable polysaccharides; **WSF:** Water soluble fraction.

REFERENCES

1. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol. 2001;74(2):113-23.
2. Caffall KH, Mohnen D. The structure, function and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr Res. 2009;344(14):1879-900.
3. Held MA, Jiang N, Basu D, Showalter AM, Faik A. Plant cell wall polysaccharides: structure and biosynthesis. Polysacch Bioactivity Biotechnol. 2015;3-54.
4. Coma V. Polysaccharide-based biomaterials with antimicrobial and antioxidant properties. Polímeros. 2013;23(3):287-97.
5. Francine MK, Louise WA, Pythagore FS, Barbara AT, Gustave S, Boudjeko T. Antioxidant properties of cell wall polysaccharides of *Stevia rebaudiana* leaves. J Coast Life Med. 2014;2(12):962-9.
6. Luo D, Fang B. Structural identification of ginseng polysaccharides and testing of their antioxidant activities. Carbohydr Polym. 2008;72(3):376-81. doi:10.1016/j.carbpol.2007.09.006
7. Movaliya V, Maitreyi Z. Pharmacognostical studies on roots of *Aerva javanica*. Indian J Pharm Sci Rev Res. 2012;16(1):34-7.
8. Mufti FUD, Ullah H, Bangash A, *et al*. Antimicrobial activities of *Aerva javanica* and *Paeonia emodi* plants. Pak J Pharm Sci. 2012;25(3):565-9.
9. Reddy KS, Reddy VM. Antihyperglycaemic activity of ethanol extract of *Aerva javanica* leaves in alloxan-induced diabetic mice. J Pharm Res. 2009;2(7):1259-61.
10. Simonsen HT, Nordskjold JBE, Smitt UW, *et al*. *In vitro* screening of Indian medicinal plants for antiplasmodial activity. J Ethnopharmacol. 2001;74(2):195-204.
11. Srinivas P, Reddy SR. Screening for antibacterial principle and activity of *Aerva javanica* (*Burm. f)* Juss. *ex Schult*. Asian Pac J Trop Biomed. 2012;2(2):S838-45.
12. Li H, Ding F, Xiao L, *et al*. Food-derived antioxidant polysaccharides and their pharmacological potential in neurodegenerative diseases. Nutrients. 2017;9(7):778.
13. Yoo HD, Kim D, Paek SH. Plant cell wall polysaccharides as potential resources for the development of novel prebiotics. Biomol Ther. 2012;20(4):371.
14. Movaliya V, Zaveri M. A review on the Pashanbheda plant *Aerva javanica*. Int J Pharm Sci Rev Res. 2014;25(2):268-75.
15. Mussadiq S, Riaz N, Saleem M, Ashraf M, Ismail T, Jabbar A. New acylated flavonoid glycosides from flowers of *Aerva javanica*. J Asian Nat Prod Res. 2013;15(7):708-16.
16. Nawaz H, Shad MA, Andleeb H, Rehman T. Phytochemical and Antioxidant Composition of Desert Cotton (*Aerva javanica*) Crude Extracts in Solvents with Varying Polarity. Int J Pharm Life Sci. 2015;6(2). http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09767126&AN=101301612&h=38lTOkiQfe%2Fbpl4pSzO7py%2F0QzaSVAzt3KAdXDAjsX%2FjAZV%2BrJV202V8bz9Xl9dbLNyUKoX%2FbfAGQ8%2Br1t5jmQ%3D%3D&crl=c. Accessed February 23, 2016.
17. Nawaz H, Shad MA, Andleeb H, Rehman T. Phytochemical and Antioxidant Composition of Desert Cotton (*Aerva javanica*) Crude Extracts in Solvents with Varying Polarity. Int J Pharm Life Sci. 2015;6(2). http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09767126&AN=101301612&h=38lTOkiQfe%2Fbpl4pSzO7py%2F0QzaSVAzt3KAdXDAjsX%2FjAZV%2BrJV202V8bz9Xl9dbLNyUKoX%2FbfAGQ8%2Br1t5jmQ%3D%3D&crl=c. Accessed December 7, 2015.
18. Movaliya V, Zaveri M. A review on the *Pashanbheda* plant *Aerva javanica*. Int J Pharm Sci Rev Res. 2014;25(2):268-75.
19. He F, Yang Y, Yang G, Yu L. Components and Antioxidant Activity of the Polysaccharide from Streptomyces virginia H03. Z Für Naturforschung C. 2008;63(3-4):181-8.
20. Kardošová A, Machová E. Antioxidant activity of medicinal plant polysaccharides. Fitoterapia. 2006;77(5):367-73.
21. Wang H, Liu YM, Qi ZM, *et al*. An overview on natural polysaccharides with antioxidant properties. Curr Med Chem. 2013;20(23):2899-913.
22. Nawaz H, Shad MA, Andleeb H, Rehman T. Phytochemical and Antioxidant Composition of Desert Cotton (*Aerva javanica*) Crude Extracts in Solvents with Varying Polarity. Int J Pharm Life Sci. 2015;6(2). http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09767126&AN=101301612&h=38lTOkiQfe%2Fbpl4pSzO7py%2F0QzaSVAzt3KAdXDAjsX%2FjAZV%2BrJV202V8bz9Xl9dbLNyUKoX%2FbfAGQ8%2Br1t5jmQ%3D%3D&crl=c. Accessed April 13, 2016.
23. Redgwell RJ, Curti D, Gehin-Delval C. Physicochemical properties of cell wall materials from apple, kiwifruit and tomato. Eur Food Res Technol. 2008;227(2):607-18.
24. Deng J, Shi ZJ, Li XZ, Liu HM. Soluble polysaccharides isolation and characterization from *rabbiteye blueberry* (*Vaccinium ashei*) fruits. BioResources. 2012;8(1):405-19.
25. Prietto P, Pineda M, Aquilar M. Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specification application to the determination of vitamin E. Anal Biochem. 1999;269:337-41.
26. Puntel RL, Nogueira CW, Rocha JB. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. Neurochem Res. 2005;30(2):225-35.
27. Danis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoaminophen, salycilate and 5-amino salycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophy. 1994;315:161-9.
28. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction. Jpn J Nutr. 1986;44(6).
29. Osawa T, Namiki M. A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. Agric Biol Chem. 1981;45(3):735-9.
30. Shad MA, Nawaz H, Hussain M, Yousuf B. Proximate composition and functional properties of *rhizomes* of lotus (*Nelumbo nucifera*) from Punjab, Pakistan. Pak J Bot. 2011;43(2):895-904.
31. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 1989;28(4):1057-60.
32. Yan F, Yang X, Liu C, Huang S, Liao L, Fu C. Extraction optimization of antioxidant polysaccharides from leaves of *Gynura bicolor* (*Roxb. and Willd.*) DC. Food Sci Technol. 2014;34(2):402-7.
33. Podsedek A. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. LWT-Food Sci Technol. 2007;40(1):1-11.
34. Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci Technol. 2007;40(2):344-52.
35. Garrec J, Monari A, Assfeld X, Mir LM, Tarek M. Lipid peroxidation in membranes: the peroxyl radical does not "float." J Phys Chem Lett. 2014;5(10):1653-8.
36. Repetto M, Semprine J, Boveris A. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. In Lipid Peroxidation. 2012.
37. Wang J, Hu S, Nie S, Yu Q, Xie M. Reviews on mechanisms of *in vitro* antioxidant activity of polysaccharides. Oxid Med Cell Longev. 2016;2016.

Cite this article: Saeed H, Nawaz H, Shad MA, Shahwar DE, Andaleeb H, Muzaffar S, *et al*. Antioxidant Potential of Cell Wall Polysaccharides Extracted from Various Parts of *Aerva javanica*. Free Radicals and Antioxidants. 2019;9(1):35-42.

GRAPHICAL ABSTRACT



SUMMARY

- The cell wall of various parts of *Aerva javanica* plant is a rich source of extractable antioxidant polysaccharides.
- The addition of salt or alkali in the extraction media increased the extraction yield of cell wall polysaccharides.
- The KOH soluble fraction of cell wall polysaccharide showed higher extract yield and reducing abilities.
- The water soluble fraction of cell wall showed hydrophilic polysaccharide with higher radical scavenging capacity.

ABOUT AUTHORS

Haq Nawaz, is working as an Assistant Professor and Research Supervisor at Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan. He is working in the field of Biological Sciences with special interest in Food Sciences, Antioxidant Chemistry and Clinical Sciences.