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Antioxidant properties of cell wall polysaccharides of *Stevia rebaudiana* leaves

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PEER REVIEW

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Comments

In the present manuscript authors have appropriately described the free radical quenching potential, reducing properties, and chelating ability as well as membrane and DNA protective activities of *S. rebaudiana* leaf polysaccharides. Results have been interpreted suitably with the discussion part. *S. rebaudiana* was found to be a promising antioxidant agent. Hence phytochemicals present in the plant part have potential for developing drug lead compounds against oxidative stress.
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ABSTRACT

Objective: To examine the total phenolic and protein contents, and the antioxidant activities of cell wall polysaccharide fractions of *Stevia rebaudiana* leaves.

Methods: Three different polysaccharide-enriched fractions, namely FPE (extract with 50 mmol/L ethylene diamine tetra acetic acid), FPK (extract with 0.05 mol/L KOH) and FH (extract with 4 mol/L KOH) were extracted from *Stevia rebaudiana* leaves. The antioxidant activity of these fractions was evaluated based on their ability to scavenge DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical, to reduce ferric power, to chelate ferrous ion and to protect human DNA.

Results: The results indicated that protein content was found to be higher in FPK polysaccharide enriched fraction (47.48 μg per mg of FPK). Furthermore, the phenolic compound analysis according to the Folin-Ciocalteu method was higher in FPK (17.71 μg ferulic acid). The DPPH maximal inhibition percentage of the three polysaccharide-enriched fractions at 400 μg/mL was 27.66%, 59.90% and 23.21% respectively for FPE, FPK and FH. All the polysaccharide fractions exhibited a ferric reducing power except the FH one. The three fractions also exhibited lipid peroxidation inhibition, and they completely reverted the DNA damage induced by H_2O_2/$FeCl_2$. FPK showed the strongest scavenging activity against the DPPH radical, the best chelating ability and lipid peroxidation inhibition.

Conclusions: *Stevia* cell wall polysaccharide fractions are potent protective agents against oxidative stress. The analysis revealed major differences in the antioxidant activity in the three polysaccharides fractions. However, the 0.05 mol/L KOH pectin fraction (FPK) showed better antioxidant activity.

KEYWORDS

Stevia rebaudiana, Cell wall polysaccharides, Fractions, Antioxidant properties

1. Introduction

Free radicals are products of cellular metabolism and transition-metal ions, and they seem to play an important role in causing bio-macromolecule damage *in vivo*[1]. Increasing oxidative stress and disorders in energy metabolism may lead to mutations and eventually to many severe diseases. Antioxidants may play an essential role in protecting the body from various oxidative damages that are linked to obesity, diabetes, cancer, cardiovascular diseases and neurodegenerative ones, including Parkinson's and Alzheimer's disease[2,3]. The etiology of these several health disorders implicates free radicals or reactive oxygen species generated by normal physiological processes and various exogenous factors. Reacticve oxygen exogenous can cause oxidative stress and thereby initiate peroxidation of

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membrane lipids, causing damage to a wide range of other biological molecules including DNA, lipids and proteins[4]. The extent of damage caused by free radicals might be mitigated by supplementation with one or more antioxidants, hence the search for new sources of natural antioxidants is currently become the major interest to scientists. Natural antioxidants such as polysaccharides, originating from plants, are safe therapeutic substances providing protection to humans against infections, degenerative and metabolic diseases[5,6].

Non-cellulosic cell wall polysaccharides of higher plants represent a potential source of pharmacologically active polysaccharides. In traditional medicine, extracts of polysaccharide-containing plants are widely used for the treatment of skin problems, epithelial wounds and mucous membrane irritations[6,7]. Moreover, several reports showed that polysaccharides from different plants could be responsible for the effects associated with the healing of wounds. Some of them have an influence on the immune system and are often called immunomodulators when the complement system is involved[8,9]. The wide structural diversity of cell wall polysaccharides reflects the different mechanisms of action that may used to stop, prevent or heal various infections or pathologies. Published data indicates that cell wall polysaccharides can be explored as novel potential antioxidants[9,10].

Stevia rebaudiana Bertoni (*S. rebaudiana*) is a well-known native South American sweet herb. Stevioside, a diterpene glycoside, is an abundant component in the leaf. It has become well-known for its intense sweetness, and is used as a non-caloric sweetener in several countries. In addition, the crude extract and stevioside from leaves have beneficial effects on human health, including antihypertensive[11], anti-hyperglycemic[12], anti-inflammatory, anti-cancer[13], antiviral[14] and antioxidant[15]. However, no reports are available on the antioxidant activity of the cell wall polysaccharides of *S. rebaudiana*. Therefore, the present investigation was undertaken to examine the total phenolic and the protein contents, and the antioxidant activities of pectin and hemicellulose fractions using various established *in vitro* systems. Specifically, we evaluated their possible role in antioxidative defence through their capacity to inhibit lipid peroxidation and to protect human DNA.

2. Materials and methods

2.1. Plant material and preliminary treatment

S. rebaudiana was grown at the Biotechnology Centre-Nkolbisson (Yaounde, Cameroon) and the leaves were collected in February, 2012. The identification of the voucher specimens was confirmed by the National Herbarium of Cameroon. The leaves were chopped into small pieces and dried under shade in the laboratory for further use. The air-dried leaves were then powdered.

2.2. Extraction and isolation of polysaccharide-enriched fractions

The leaf powder (14 g) was suspended in boiling ethanol (85%) for 30 min in order to inactivate any existing enzymes. The residue recovered after centrifugation (4000 r/min) was subjected to a series of extractions to remove the lipids, polyphenols, and other low-molecular weight metabolites as previously described by Boudjeko *et al*[16]. It was then extracted overnight at room temperature with 100 mL of 90% dimethylsulfoxide, 24 h with methanol-chloroform (1:1; v/v), 24 h with methanol-acetone (1:1; v/v), and finally with acetone-water (4:1; v/v). The residue was air-dried at 40 °C and the cell wall material (CWM) obtained was used for the extraction of pectic and hemicellulosic wall polymers. For this, CWM (6.86 g) was subjected to a sequential extraction regime using 50 mmol/L ethylene diamine tetra acetic acid (EDTA), pH 6.5; 0.05 mol/L KOH and 4 mol/L KOH (Figure 1). The pH of alkaline extraction was adjusted to 4.5 with acetic acid. Each fraction was filtrated and dialysed against distilled water, except the EDTA one, which was dialysed against 1 mol/L NaCl to exchange the EDTA anion with the chloride anion before lyophilisation[16].

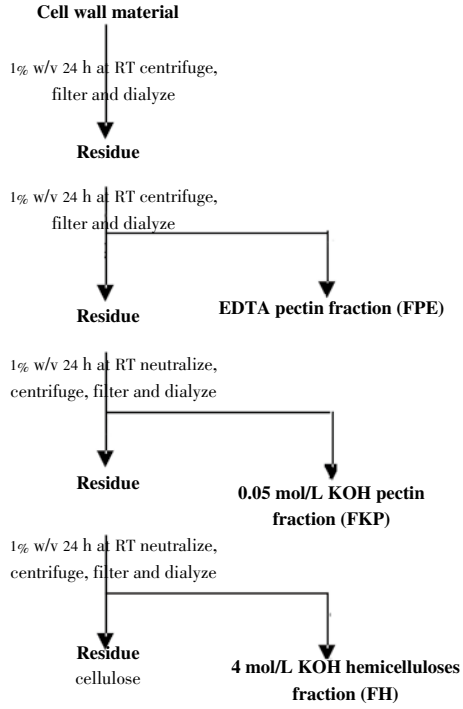

Figure 1. Flow diagram of the cell wall fractionation procedure. All extraction was for 24 h at room temperature.

2.3. Determination of total protein and phenolic content

The protein content of the polysaccharide fractions of *S. rebaudiana* was measured by the Bradford method using bovine serum albumin (BSA) as the standard[17]. The protein content was expressed in μg of BSA equivalent.

The phenolic content of the leaf polysaccharide fractions was determined spectrophotometrically at 760 nm according to the method of Nguimbou *et al*. using the Folin-Ciocalteu reagent[18]. Phenolic contents were expressed in μg equivalent of Ferulic acid per mg of CWM. A volume 75 μL of the polysaccharide fraction at 4 mg/mL was diluted with 675 μL of distilled water. Then 75 μL of Folin-Ciocalteau reagent at 0.2 mol/L was added and mixed thoroughly and incubated for 3 min. This was followed by the addition of 750 μL of 20% sodium carbonate and the mixture was allowed to stand for 1 h at room temperature. The absorbance of the blue colour that developed was measured at 760 nm.

2.4. Antioxidant activities

2.4.1. Determination of DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity

The free radical scavenging activity of the polysaccharide fractions was measured in terms of their hydrogen donating or radical scavenging ability using the DPPH radical[19]. For the assay, 500 μL of the fraction at different concentrations 100-500 μg/mL were introduced into test tubes and 500 μL of the freshly prepared solution of 400 μmol/L DPPH in methanol were then added. The mixture was stirred and left in the dark at 37 °C for 30 min. The absorbance was measured at 517 nm using a UV-1605 Shimadzu spectrophotometer and gallic acid and catechin were used as the positive controls. A low absorbance of the reaction mixture indicated high free radical scavenging activity. The DPPH radical scavenging effect was calculated as "percentage of inhibition" according to the following formula: percentage of inhibition (%)={[(A_o-A_1)/A_o]×100} where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample.

2.4.2. Antioxidant activities by ferric reducing antioxidant power (FRAP) assay

The FRAP reagent containing 1 volume of 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mmol/L HCl, 1 volumes of 20 mmol/L $FeCl_3$ and 10 volume of 0.3 mol/L acetate buffer (pH 3.6) was prepared freshly. Aliquots of 100 μL extract (100 μg/mL) were mixed with 500 μL of FRAP reagent and the absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37 °C for 30 min in the dark using a UV-1605 Shimadzu spectrophotometer[20]. Gallic acid was used as the standard. The final result expressed that the concentration of antioxidant has a ferric reducing ability and was determined by μg of gallic acid equivalent (GAE).

2.4.3. Chelating ability of ferrous ions

The method of Dinis *et al*. was used to determine the ferrous ion chelating ability[21]. Various concentrations 100-500 μg/mL of sample solution (1 mL) were mixed with $FeCl_2$ (2 mmol/L, 100 μL) and left in dark for 1 min at room temperature. The mixture was added to 200 μL of 5 mmol/L ferrozine. After 10 min at room temperature, the absorbance of the mixture was read at 562 nm against a blank (distilled water) using a UV-1605 Shimadzu spectrophotometer. EDTA was used as the standard and the ability of all samples to chelate ferrous ion was calculated using the following equation: Chelating ability (%)={[($Acontrol_{562}$-A $sample_{562}$)/$Acontrol_{562}$]×100}.

2.4.4. Inhibition of lipid peroxidation by thiobarbituric acid-reactive substances method

2.4.4.1. Animal material

Investigations on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines [United States National Institutes for Health Publication No. (85-23) revised in 1985]. The animals were maintained under standard laboratory conditions with a 12 h light and dark cycle, free access to standard laboratory rat food and tap water, temperature of (24±1) °C and humidity of (55±10)%. Prior authorization for the use of laboratory animals in this study was obtained from the Institutional Review Board of the Biotechnology Centre (University of Yaounde I, Cameroon).

Male Wistar rats weighing (200-220) g from the Animal Laboratory of Metabolism of the Medical Institute for the Study of Medicinal Plants, Yaounde, Cameroon, were anaesthetized by ether inhalation and killed by decapitation. The liver was removed, rinsed in normal saline and placed on ice. The organs were weighed, grounded in normal saline and centrifuged at 3000 g for 10 min. The homogenates were prepared at a concentration of 10% the resulting supernatants stored at -20 °C.

2.4.4.2. Lipid peroxidation assay

One millilitre of liver homogenate (10%, w/v) was added to 100 mL of the tested fraction at different concentrations 100-500 μg/mL. Lipid peroxidation was initiated by adding 50 μL of 500 μmol/L $FeCl_2$ and 50 μL of 500 μmol/L H_2O_2. After 1 h of incubation at room temperature, 1 mL of trichloro acetic acid (15%, w/v) and 1 mL of TBA (0.67%, w/v) were added and the mixture was heated in a water bath at 65 °C for 15 min and centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured at 532 nm using a UV-1605 Shimadzu spectrophotometer[22]. The percentage of inhibition was calculated using the formula mentioned above. The concentration of TBARS was determined using the molar extinction coefficient [ε=1.53 10^5 mol/(L×cm)] of MDA. Results were expressed in μmol/L.

2.4.5. Protective effect on human DNA

2.4.5.1. Human material

Human DNA used in this work came from the bank of Laboratory of Biochemistry, Immunology and Biotechnology, Faculty of Medicine and Biomedical Sciences, University of

Yaounde I, Cameroon, with official permission from the faculty board. This DNA was extracted using the Qiagen commercial kit.

2.4.5.2. Assay for protective effect on human DNA

The degree of damage induced on human DNA was evaluated by the difference in migration patterns on agarose gel[23]. The reaction was conducted in a total volume of 30 μL containing 2 μL of tris-buffer 50 mmol/L, pH 7.4; 5 μL of human DNA and 5 μL of polysaccharide fraction prepared at different concentrations (62.5-1000 μg/mL). Then, 12 μL of 30% H_2O_2 and 6 μL of 500 μmol/L $FeCl_2$ were added and incubated at 37 °C for 30 min. The reaction mixture was then homogenised with 6 μL of gel loading dye, loaded into a 1% agarose gel and run at 100 V for 15 min in a submarine gel electrophoretic apparatus (Mupid eXu). The DNA was finally visualized and photographed.

2.5. Statistical analyses

The data was subjected to statistical analysis to verify and evaluate the differences between the antioxidant activities of the three leaf polysaccharide fractions. All data is reported as mean±SEM for three independent samples (n=3). One-way analysis of variance (ANOVA) was used and the least significant difference (LSD) at P<0.05 was calculated using the SPSS 18.0 version for Windows package. The graphical representations were designed in Microsoft Excel 2007.

3. Results

3.1. Total protein and phenolic compounds content

The pre-treatment of leaf powder with organic solvent yielded a CWM from which cell wall polysaccharides were extracted. Three fractions were obtained, namely, pectin EDTA (FPE), pectin 0.05 mol/L KOH (FPK) and hemicellulose (FH). The fractions accounted for 319.24, 77.26 and 517.49 mg per gram of cell wall, respectively for FPE, FPK and FH. The FPK fraction represented the lowest quantity of cell wall polysaccharides in *S. rebaudiana* leaves.

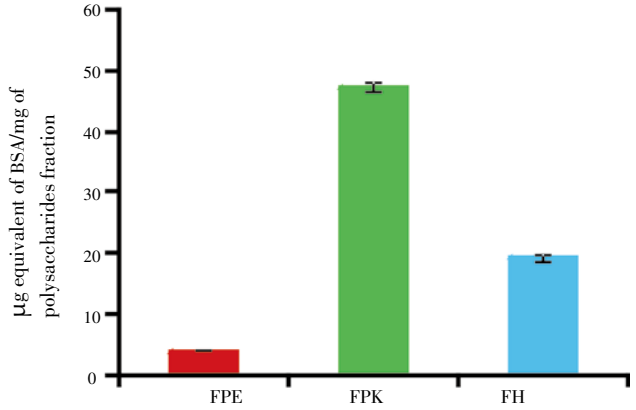

Figure 2. Amount of total proteins in the polysaccharide leaf extracts of *S. rebaudiana* (mean±SEM, n=3).

The partial characterisation of total protein and phenolic content showed the presence of these metabolites in each polysaccharide fraction. The total amounts of protein and phenolic content of the polysaccharide fractions are shown in Figures 2 and 3. The FPK fraction was mostly a complex of proteins (47.48 μg BSA equivalent per milligram of polysaccharide fraction) and phenolic compounds (17.71 μg Ferulic acid equivalent/mg of polysaccharide fraction).

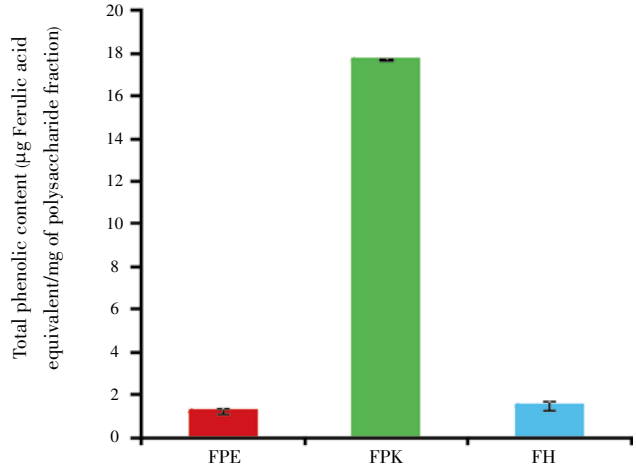

Figure 3. Amount of total phenolic content in the polysaccharide leaf fractions of *S. rebaudiana* (mean±SEM, n=3).

3.2. Inhibition of DPPH radical

The DPPH free radical, a stable radical with purple color and a maximum absorption at 517 nm, has been widely used as a tool to evaluate the free radical scavenging activities of antioxidants[24]. When DPPH encounters antioxidant scavengers, its purple color fades rapidly because it is changed to the non-radical form DPPH-H. On the basis of this principle, the scavenging effects of the three polysaccharide leaf fractions on the DPPH radical were measured and the results are shown in Figure 4.

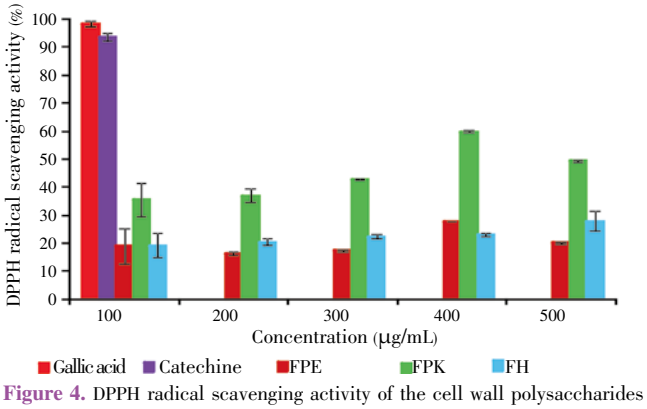

Figure 4. DPPH radical scavenging activity of the cell wall polysaccharides leaf fractions of *S. rebaudiana* (mean±SEM, n=3).

FPK had the strongest scavenging activity on DPPH at every concentration level. The scavenging activities of FPK increased significantly with the increase in sample concentration ranging from 100 μg/mL to 500 μg/mL. Also, FPE and FH had almost the same level of activity. At the

concentration of 400 μg/mL, the scavenging activities were 27.70%, 59.91% and 23.20% for FPE, FPK and FH, respectively. The scavenging activities of the positive controls (gallic acid and catechin) were 98.52% and 93.72 % at the 100 μg/mL concentration. Compared to the polysaccharides, gallic acid and catechin were more effective for scavenging DPPH. The results suggest that all the polysaccharides carry out scavenging activities on DPPH in a concentration-dependent manner, and especially FPK (low alkali fraction) had a much stronger antioxidant activity.

3.3. Antioxidant activities by FRAP assay and ferrous ion chelating ability

3.3.1. Reducing power of polysaccharide fractions

The reducing power of FPE, FPK and FH determined at 700 nm are depicted in Figure 5. As shown in Figure 5, all the polysaccharide fractions had a reducing power. At 100 μg/mL, the reducing powers were (33.79±3.46) μg GAE, (64.11 ±1.28) μg GAE and (36.32±2.75) μg GAE for FPE, FPK and FH, respectively. FPK, with its highest reducing power, showed that there is a direct correlation between antioxidant activities and the reducing capacity of certain plant extracts[24].

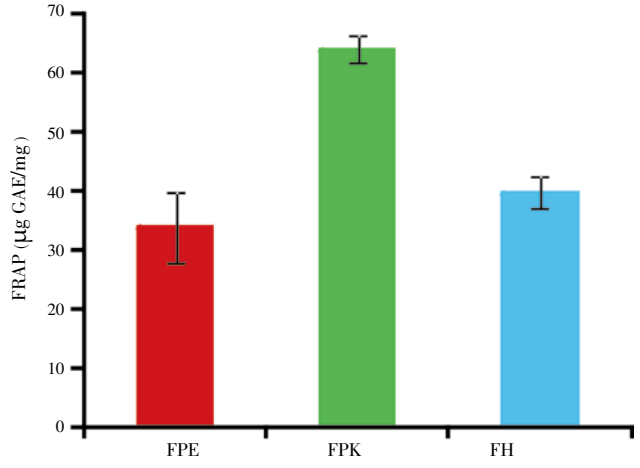

Figure 5. Ferric reducing antioxidant power of the cell wall polysaccharides leaf fractions of *S. rebaudiana* (mean±SEM, *n*=3).

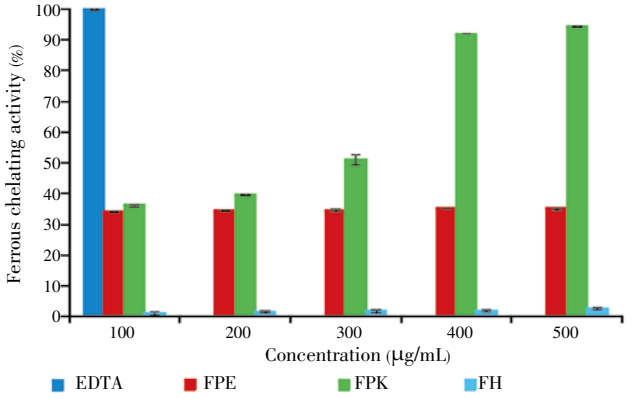

Figure 6. Ferrous ion chelating ability of the cell wall polysaccharides leaf fractions of *S. rebaudiana* (mean±SEM, *n*=3).

3.3.2. Ferrous ion chelating ability

At 100-500 μg/mL, the chelating ability was (34.37±0.13)%-(35.19±0.19)% for FPE, (36.30±0.21)%-(94.51 ±0.09)% for FPK and (1.26±0.36)%-(2.8±0.2)% for FH (Figure 6). Only FPK had a dose dependent activity. When the concentration was higher than 300 μg/mL, the metal chelating activity was the highest for the three different polysaccharide fractions. However, as shown in Figure 6, the chelating ability of all the samples was weaker when compared to that of EDTA [(99.48±0.05)% at 100 μg/mL].

3.3.3. Inhibition of lipid peroxidation

S. rebaudiana leaf polysaccharide fractions inhibited the amount of MDA generated (and thus lipid peroxidation) in liver homogenate (Figure 7). Thus, the decrease in the MDA level in the presence of the leaf polysaccharide fractions indicated the role of the fraction as an antioxidant. The FPK fraction showed the highest inhibition percentage [(35.92±1.8)% and (48.58±0.06)% at 300 and 500 μg/mL, respectively)] compared to the FPE and FH fractions. However, these activities were lower than those of the standard catechin [(47.28±0.17)% and (72.59±0.67)% at 100 and 500 μg/mL respectively].

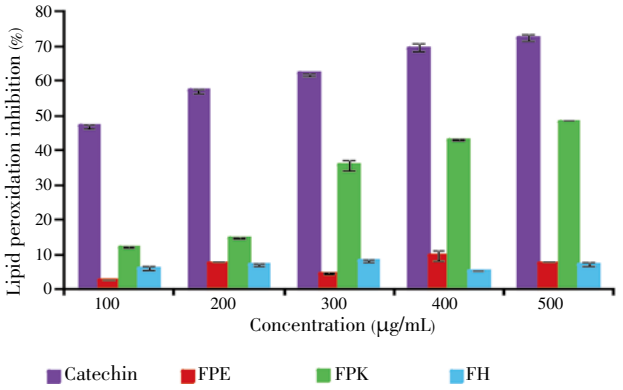

Figure 7. Lipid peroxidation inhibition of the cell wall polysaccharides leaf fractions of *S. rebaudiana* and standard Catechin (mean±SEM, *n*=3).

3.3.4. Protective effect of polysaccharides on DNA damage

The gel pattern of human DNA exposed to the polysaccharide leaf fractions and to radical hydroxide (OH^-) generated *in vitro* by the reaction of $H_2O_2/FeCl_2$ is presented in Figure 8. DNA migration assay is a sensitive biomarker of the DNA damage[25]. From the migration pattern, it can be deduced that OH^--damaged human DNA lead to the shredding and caused the absence of specific band in H_2O_2+$FeCl_2$ treated DNA (Lane 3). The three polysaccharides leaf fractions of *S. rebaudiana* reduced the DNA damage induced by $H_2O_2/FeCl_2$ to human DNA caused the presence of specific band in Lanes 3 to 7. Visually, this protection is not significantly dose dependent. *S. rebaudiana* leaf fractions, by themselves, did not cause any DNA damage at the concentration of 1 mg/mL as evidenced by the intact DNA band (Lane 8). The striking observation that could be made was that the extent of DNA damage induced by $H_2O_2/FeCl_2$ was completely reverted by

the leaf polysaccharides fractions at concentrations of 62.5, 125, 250, 500 and 1000 μg/mL (Lane 3 to 7).

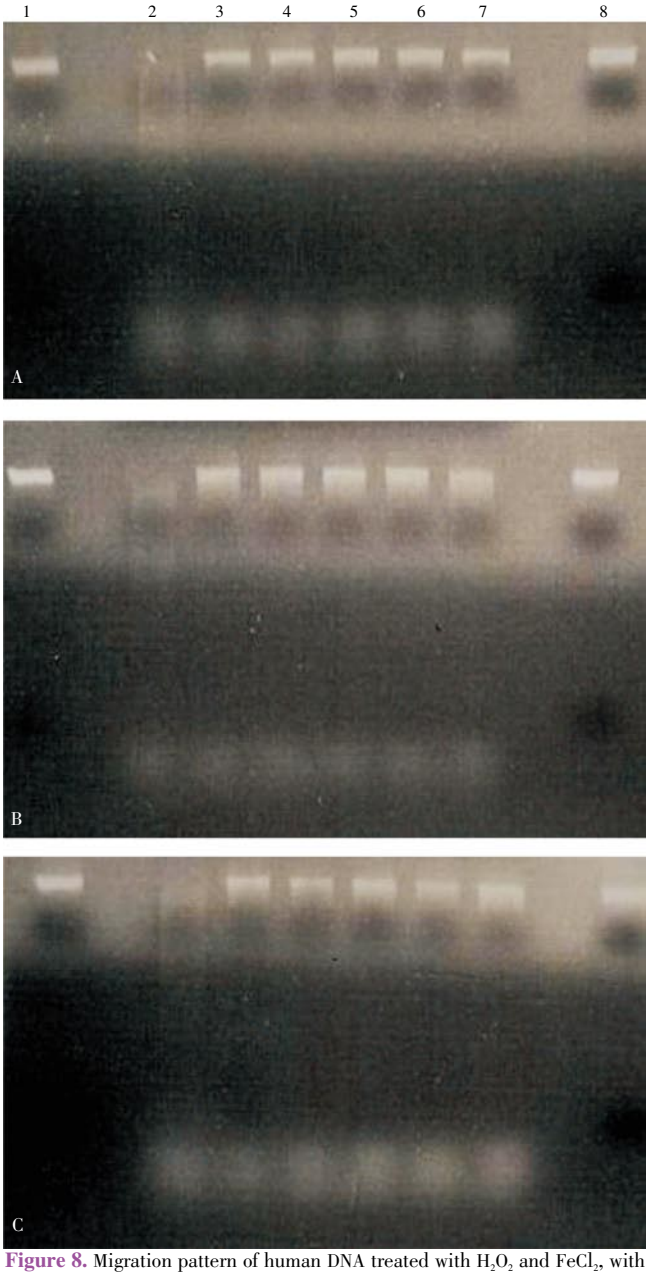

Figure 8. Migration pattern of human DNA treated with H_2O_2 and $FeCl_2$, with and without the polysaccharides leaf extracts.

Agarose gel electrophoretic pattern showing protection of *S. rebaudiana* polysaccharides leaf fractions on H_2O_2 induced strand breaks in human DNA. A: FPE; B: FPK; C: FH. Lane 1: Human DNA control; Lane 2: Human DNA exposed to H_2O_2 and $FeCl_2$; Lane 3 to 7: Human DNA exposed to H_2O_2 and $FeCl_2$ in the presence of polysaccharides fractions at different concentration of 62.5, 125, 250, 500 and 1 000 μg/mL, respectively; Lane 8: Human DNA exposed to polysaccharides fractions at 1 mg/mL.

4. Discussion

Natural polysaccharides are not isolated molecules in plants, animals or microorganisms but are associated with other compounds such as proteins and lipids[10]. Moreover, some plant polysaccharides contain important amounts of polyphenolic compounds with antioxidant activity[2,5]. Phenolic compounds have been reported to have multiple biological effects including antioxidant activity. These polyphenols have both hydrophobic aromatic rings and hydrophilic hydroxyl groups with the ability to bind to polysaccharides and proteins at several sites on the cell wall surface[26].

At 100 μg/mL, the scavenging activity of FPK (the most effective polysaccharide fraction) was 35.68% which was higher than that of the same plant acqueous leaf extract (10.15%) found by Kim *et al*[27]. The concentrations required for IC_{50} of DPPH radicals was 330.12 μg/mL for FPK whereas it was found to be 752.6 μg and 541.3 μg of sample for the water extracts of *Stevia* leaves and callus[28]. However, Schulka *et al*. found that 200 μg/mL of acqueous leaf extract of *Stevia* exhibited 72.37% inhibition and its IC_{50} value was found to be 83.45 μg/mL[29].

It had been previously reported that there was a direct correlation between the antioxidant activities and the reducing power of certain polysaccharide fractions. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity[10]. A measurement of the reducing power may directly reflect the production capacity of the electron donor. Among the three fractions of polysaccharides, FPK showed an obviously higher ferric reducing ability than that of FPE and FH. It suggested that the reductone-associated and hydroxide groups of *Stevia* polysaccharides can act as electron donors and can react with free radicals to convert them into more stable products[30]. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of ferric tripyridyltriazine [Fe(III)-TPTZ] complex to ferrous tripyridyltriazine [Fe(II)-TPTZ] at low pH.

Metal chelating capacity is important since it reduces the concentration of transition metals that may act as catalysts to generate the first few radicals and initiate the radical-mediated oxidative chain reactions in biological systems. Ion-chelating agents may also inhibit the Fenton reaction and hydroperoxide decomposition. Fe^{2+} ion is the most powerful pro-oxidant among various species of metal ions, being able to form complexes with ferrozine. Earlier reports have revealed that Fe^{2+} accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides formed by the Fenton free radical reaction. Therefore, the chelating effect on ferrous ion is a widely used method to evaluate antioxidant activity. Our data on the chelating ability of the fractions revealed that the polysaccharides demonstrated an effective capacity for iron bindings, which suggesting that their action as antioxidants may also be related to this capacity.

Lipids in the membrane are continuously subjected to the action of free oxygen radicals that initiate membrane lipid peroxidation. The oxidant induced subtraction of a hydrogen atom from an unsaturated fatty acyl chain of membrane lipids initiates the process of LPO, which propagates as a chain reaction. Lipid aldehydes (like malonyldialdehyde) generated during the breakdown of lipid superoxides are especially dangerous for the organism. These aldehydes which have a longer half-life (a few minutes) can easily migrate over a considerable distance and can react with

other molecules far away from the site of their origin[31].

DNA contains reactive group in its bases that are highly susceptible to free radical attack. H_2O_2/$FeCl_2$ generates OH^- radicals which cause oxidative damage. OH^- bounded to DNA leads to stranded breakage, deoxysugar fragmentation and base modification. Moreover, H_2O_2 and associated free radical can induce DNA mutations[32]. Many studies have reported the protection against oxidative DNA damage by herbal extracts and formulations[33,34]. For instance, the degraded polysaccharides from *Poria cocos sclerotium* can protect the native SC-DNA against UV irradiation and H_2O_2[33]. Furthermore, water polysaccharides from rhizomes of *Panax japonicas* conferred the protection on H_2O_2 and UV-induced damage of pIC333 plasmid DNA[34]. The results of the present study are also in agreement with the above reports. These findings support the use of the cell wall polysaccharides leaves of *S. rebaudiana* to protect DNA against oxidative damage and indicate a correlation with the antioxidant potential of the polysaccharides.

The researches of natural polysaccharides have attracted a lot of attention because of their abundance in resources and non-toxicity. This article mainly related to the isolation of the antioxidant activities polysaccharides from *S. rebaudiana* leaves. According to the results stated above, it could be concluded that cell wall polysaccharides of *S. rebaudiana* leaves bear a potent antioxidant activity. Their constituents scavenge free radicals and exert a protective effect on cellular macromolecules against oxidative damage. The analysis revealed major differences in the antioxidant activity in the three polysaccharides fractions, the FPK showed better antioxidant activity and it might be attributed to the presence of higher amount of proteins and polyphenolic compounds. They might be equally beneficial to human antioxidant protection system against oxidative damage. What's more, it should be planned for further investigation to know more about the polysaccharides of *S. rebaudiana*, the relationship of structures and various activities.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Free radicals lead to oxidative stress, which has been implicated in many diseases. Antioxidants counteract the action of free radicals by inhibiting their formation, mitigating their activity or scavenging them. Therefore, there is a need of new and potent antioxidants of natural origin. Hence, the manuscript deals with the relevant issues.

Research frontiers

The present work has reported the *in vitro* antioxidant capacity of cell wall polysaccharides of *S. rebaudiana* leaves by measuring their radical scavenging activity, reducing power, metal ion chelating ability and lipoprotective efficacy. The protection of DNA damage has also been shown. In addition, the protein and phenolic contents have also been determined. Screening of natural phytochemicals for bioactivity is required for the development of drug leads.

Related reports

The variety of secondary metabolites is produced in the plant kingdom. Many of them possess medicinal attributes. There are many reports showing effectiveness of herbs in treating various disorders that arise from the oxidative stress. The folklore medicine has been shown to produce antioxidant activity.

Innovations and breakthroughs

The usage of cell wall polysaccharides of *S. rebaudiana* as a source of antioxidants offers a further scope for the use of this plant in addition to its other biological properties. FPK fraction deserves special mention because of its medicinal potential.

Applications

It has been found that antioxidant therapy has been being used in the treatment of many degenerative diseases including cancer, diabetes, cardiovascular diseases and aging. Current research has provided an insight for the utilization of *S. rebaudiana* as potential antioxidant. Further researches are required for characterization of cell wall components as well as the combinations with proteins and polyphenols related with the bioactivity.

Peer review

In the present manuscript, the authors have appropriately described the free radical quenching potential, reducing properties, chelating ability as well as membrane and DNA protective activities of *S. rebaudiana* leaf polysaccharides. Results have been interpreted suitably with the discussion part. *S. rebaudiana* was found to be a promising antioxidant agent. Hence, phytochemicals presented in the plant part have potential for developing drug in lead compounds against oxidative stress.

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