

Multifunctional properties of polysaccharides from *Dalbergia sissoo*, *Tectona grandis* and *Mimosa diplotricha*



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ABSTRACT

Three water-soluble polysaccharides were isolated and purified from the leaves of *Dalbergia sissoo* Roxb. (DSL), bark of *Tectona grandis* L. f. (TGBP) and seeds of *Mimosa diplotricha* var. *diplotricha* Sauvalle (MDSP). Antioxidant and moisture preserving activities of these three polysaccharides were investigated using in vitro methods. The antioxidant activities studied include superoxide ($O_2^{\bullet-}$), 1,1-diphenyl-2-picrylhydrazyl (DPPH $^{\bullet}$), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS $^{\bullet+}$), hydroxyl ($OH^{\bullet}$), nitric oxide ($NO^{\bullet}$), N,N-dimethyl-p-phenylenediamine (DMPD $^{\bullet+}$) radical scavenging activities, ferric ion (Fe^{3+}) reducing ability, ferrous ion (Fe^{2+}) chelating and lipid peroxidation activities. The study revealed higher activity of TGBP in all antioxidant assays than DSL and MDSP. Further, the three polysaccharides showed effective moisture retention properties in comparison with hyaluronic acid and glycerol.

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1. Introduction

Antioxidants protect living cells against the damaging effects of reactive oxygen species (ROS) like singlet oxygen, and other free radicals such as superoxide, hydroxyl, peroxy, and peroxy-nitrite. ROS are generated during normal or aberrant consumption of molecular oxygen, which are associated with neurodegenerative disorders (Good, Werner, Hsu, Olanow, & Perl, 1996), coronary heart disease, carcinogenesis, and early aging, etc. (Patel, Cornwell, & Darley-Usmar, 1999). Oxidation during catabolism of consumed foodstuffs is indeed essential for sustenance of essential biological processes. Generally free radicals and ROS are developed during the processes of signal transduction, energy production, synthesis of biologically essential compounds, phagocytosis, and critical processes of the immune system, etc. (Wang, Zhang & Cheng, 2011). Human diseases like rheumatoid arthritis, and atherosclerosis, etc., are also developed due to the causative effects of uncontrolled production of oxygen derived free radicals (Blander, de Oliveira,

Conboy, Haigis, & Guarente, 2003; Liu, Ooi, & Chang, 1997; Mau, Lin, & Song, 2002). The adverse effects of free radicals can be reduced by simply adopting several defense mechanisms. One of the important defense mechanism against these adverse effects is the detoxification using antioxidants (Aruoma, 1996). Keeping this in view synthetic antioxidants e.g., butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), etc. have been used. However, the synthetic counterparts are responsible for liver damage and carcinogenesis (Grice, 1988; Qi et al., 2005). Hence, development of potential antioxidants based on natural sources is essential to replace synthetic analogs with no side effects. In recent years efforts have been focused on the development of antioxidants based on polysaccharides derived from abundantly available plant biomass, microbes, etc. due to their non-toxicity even at higher concentration, higher efficacy and potential therapeutic applications (Forabosco et al., 2006; Xiong & Jin, 2007). The antioxidant activity of polysaccharides depends on several structural parameters such as monosugar composition, type of glycosidic linkages, molecular weight, and degree of substitution (Lu, Wang, Hu, Huang, & Wang, 2008; Wang et al., 2010).

Among the selected plant materials for our study, *Tectona grandis*, also known as Sagwan in Hindi language, is native to south and southeast Asia, mainly India, Indonesia, Malaysia, Thailand and Burma, but is naturalized and cultivated in many countries in Africa and the Caribbean. *T. grandis* is widely distributed forest tree species throughout India and used for timber production. The species also shows a number of medicinal properties like antimicrobial,

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antioxidant (Jagetia & Baliga, 2004) and wound healing activity (Krishna & Nair, 2010; Majumdar, Nayeem, Kamath, & Asad, 2007; Varma & Jaybhaye, 2010). In traditional system of medicine, the bark is used as a cure for bronchitis, constipation, anthelmintic, depurative, hyperacidity, dysentery, verminosis, burning sensation, diabetes, leprosy, skin diseases, leucoderma, headache, difficult labor and piles, etc. (Aradhana, Rao, Banji, & Chaithanya, 2010). The bark is also effective against free radical mediated diseases (Ghaisas, Navghare, Takawale, Zope, & Deshpande, 2008). Ethanolic extract of bark showed potent in vitro antioxidant potential and marked hyperglycemic activity (Mazumder, Rathinavelusamy, & Sasmal, 2012).

Dalbergia sissoo or Indian Rosewood is a deciduous tree and native to the Indian Subcontinent and Southern Iran. The species, commonly known as Sheesham, in India is naturally distributed in the foothills of the Himalayas from eastern Afghanistan through Pakistan and India to Nepal (Rana, Kumar, & Soni, 2012). Dried leaves of *D. sissoo* show antibacterial, anti protozoal and anti inflammatory activities (Hajare, Chandra, Sharma, & Tandon, 2001; Niranjana, Singh, Prajapati, & Jain, 2010). Leaf juice of *sissoo* is good for eye ailments. Leaf extract is used in sore throat, heart problem, dysentery, and syphilis. (Al-Qura'n, 2008; Duke, 1981; Shah, Mukhtar, & Khan, 2010). *Sissoo* leaf extract possess strong antioxidant activity (Shah et al., 2010) and is also effective in the treatment of colorectal cancer. Leaf juice is also effective in scabies, burning sensation of the body, scalding urine, syphilis, and digestive disorders (Ishtiaq, Khan, & Hanif, 2006). Aqueous leaf extract is used as a cure for dandruff (Sultana, Khan, Ahmad, & Zafar, 2006). The mucilaginous polysaccharide of the *sissoo* leaves is responsible for a wide spectrum of medicinal properties, leading to its use in treating several ailments like eye diseases, acute stage of diarrhea, dyspepsia, hemorrhoids and burning sensation, skin excoriations and acute stage of gonorrhea (Rana et al., 2012). Despite strong medicinal activity of the *D. sissoo* leaves, no attempt has been made to study the antioxidant activity of the leaf polysaccharide.

The third plant, *Mimosa diplotricha* is a fast growing, annual (short-lived) or perennial shrubby leguminous vine native to the Americas. The leguminous vine has invaded large parts of Asia (India, Sri Lanka, Indonesia, Thailand, Vietnam, Malaysia, the Philippines, China, Cambodia and Taiwan), Africa (Nigeria, Mauritius and Reunion) and several Pacific Islands including Australia and Papua New Guinea (PNG) forming dense tangled, thorny clumps that smother other vegetation (Ekhtor, Uyi, Ikuenobe, & Okeke, 2013). In India, it currently occurs throughout Kerala state and in northeast India, especially in the state of Assam, where it is abundantly available and has been listed as an invasive weed of normal grasslands (Sankaran, 2012). However, there is no utilization of the species, despite its availability in abundance. The plant is still unexplored for its medicinal uses. Only a 5-deoxyflavones has been isolated from the plant which showed potent antiproliferative activity (Lin, Chiou, Cheng, 2011).

Interestingly, the bark of *T. grandis* and leaves of *D. sissoo* tree are potentially unutilized resource despite their vast abundance due to huge plantations under social forestry program for timber requirement. Further, *M. diplotricha* is an invasive weed and tends to overrun forestry plantations. The utilization based management of the species may lead to interesting products for the welfare of mankind.

The present study aims to evaluate antioxidant activity of water-extracted polysaccharides from the leaves of *D. sissoo*, bark of *T. grandis* and seeds of *M. diplotricha*. The isolated polysaccharides were examined for scavenging activities using reactive oxygen species (ROS) including superoxide, nitric oxide, hydrogen peroxide, diphenylpicrylhydrazyl, dimethylphenylenediamine dihydrochloride. The reducing ability, iron-chelating capacity, total antioxidant and lipid peroxidation activities of the polysaccharides

were also evaluated. In addition, moisture-retaining activities of these polysaccharides were evaluated and compared to glycerol (Gly) and hyaluronic acid (hyal).

2. Experimental

2.1. Materials

2.1.1. Materials

The stem bark of *T. grandis*, and seeds of *M. diplotricha* were collected from Rain Forest Research Institute campus, Jorhat (Assam). Leaves of *D. sissoo* were selected from village Sotai, A.T. Road Jorhat (Assam). Voucher specimens of the three species were identified by Dr. P.K. Verma, Plant Taxonomist, Rain Forest Research Institute Jorhat.

2.1.2. Chemicals

All chemicals and solvents were of analytical grade. Nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide reduced (NADH), phenazine methosulphate (PMS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonate) (ABTS), N,N-dimethyl-p-phenylenediamine dihydrochloride (DMPD), 3-(2-pyridil)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) and ethylene diamine tetra acetic acid (EDTA) were purchased from Himedia Biosciences, Mumbai, India. Hyaluronic acid was purchased from Sigma-Aldrich, Milwaukee, US. Ascorbic acid (Vc), monosodium hydrogen phosphate, disodium hydrogen phosphate, potassium chloride, potassium hydrogen phosphate, sodium chloride, potassium ferricyanide, ferric chloride, trichloroacetic acid, hydrochloric acid and sodium hydroxide were purchased from Merck (India) Limited, Bombay, India. All spectrophotometric studies were carried out using Thermo Scientific Spectrascan UV 2700 spectrophotometer.

2.2. Analytical methods

The fresh and mature seeds of *M. diplotricha* were collected in the month of January and dried in shade. These were kept in an airtight container until utilized for experiment. The fresh leaves of *D. sissoo* and stem bark of *T. grandis* were collected in the month of June. The leaves and stem barks were initially washed with tap water followed by double distilled water and dried in shade at room temperature. The air-dried materials of *D. sissoo* leaves and *T. grandis* stem barks were pulverized using laboratory grinder and kept in laboratory seal bags separately. All plant materials were stored under cold condition at 0–5 °C.

The neutral sugar content of polysaccharide samples was determined by phenol-sulfuric acid colorimetric method using D-glucose as standard at 490 nm (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956; Huang, Ding, & Fan, 2012). The quantitative estimation of uronic acid in polysaccharide samples was carried out using carbazole-sulfuric acid reaction with D-glucuronic acid as standard (Dishe, 1947, 1950). The protein contents of polysaccharide samples were determined according to the Bradford's method with bovine serum albumin as standard (Bradford, 1976). In all assays performed for evaluation of antioxidant activity, water was taken as control and ascorbic acid (Vc) as standard or positive control except in methods in Sections 2.4.4–2.4.6.

2.3. Isolation of polysaccharides

The polysaccharides, each from the leaves of *D. sissoo* and bark of *T. grandis* were isolated by the method of Rana et al. (2009, 2012). For isolating *M. diplotricha* seed polysaccharide, 100 g of dried seeds were treated with boiling water for 5 min and endosperm of seeds were separated mechanically. Separated endosperms were

extracted with water and concentrated followed by precipitation with five fold volume of ethanol. The polysaccharide thus obtained was dried in a vacuum desiccator at room temperature. The crude polysaccharide sample of *M. diplotricha* was refluxed three times with chloroform: methanol (2:1, v/v) and filtered to remove lipids. The residue obtained was air dried and refluxed with 80% ethanol. After filtration, the residue was dissolved (three times) in water and then centrifuged. The combined supernatant was taken and precipitated using threefold volume of ethanol and further subjected to centrifugation. After centrifugation, the precipitate was collected and vacuum dried at room temperature. All three polysaccharide samples were purified by ion exchange chromatography followed by dialysis (Rana, Kumar, & Soni, 2009) to obtain finally the pure polysaccharide in the form of a light brown amorphous powder (yield ~13%), dark brown amorphous powder (yield ~9.5%) and white fluffy mass (yield ~18.6%) from *D. sissoo* leaves, *T. grandis* bark and *M. diplotricha* seeds respectively. Pure and vacuum dried polysaccharides were further pulverized with an impact grinder to give fine powder and stored at 0–5 °C for further analysis.

2.4. Assays of antioxidant activity of polysaccharide fractions

2.4.1. Superoxide radical scavenging assay

The superoxide radical scavenging abilities of DSLP, MDSP and TGBP were studied by the generation of superoxide radicals in PMS-NADH system and assayed by reduction of NBT (Li, Ma, & Liu, 2007). 1 ml NBT solution (156 µM NBT solution in 0.1 M Phosphate buffer pH: 7.4), and 1 ml NADH solution (468 µM NADH solution in 0.1 M Phosphate buffer pH: 7.4) was mixed with 1 ml polysaccharide sample solution of increasing concentration (0.2–2.0 mg/ml in double distilled water) were added in a test tube and vortexed using cyclomixer. The reaction was initiated by adding 1 ml of PMS solution (60 µM solution of PMS in 0.1 M phosphate buffer pH: 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and absorbance was measured at 560 nm (Lan, Guo, Zhao, & Yuan, 2012). The scavenging capability of superoxide anion radicals was calculated using formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{S-560}}{A_{C-560}} \right] \times 100$$

where A_{S-560} is the absorbance of the samples at different concentrations and A_{C-560} is the absorbance of control.

2.4.2. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging abilities of three polysaccharides were determined by the method of Li et al. (2007). 1 ml sample solution of polysaccharide of increasing concentration (0.2–2.0 mg/ml in double distilled water) mixed with 2.4 ml phosphate buffer (0.1 M, pH: 7.4) and 0.6 ml H_2O_2 solution (0.4 M). The reaction mixture was vortexed and incubated at room temperature for 10 min. The absorbance of resultant solution was measured at 230 nm. The scavenging ability for hydroxyl radicals was calculated using the formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{S-230}}{A_{C-230}} \right] \times 100$$

where A_{S-230} is the absorbance of samples at different concentration and A_{C-230} is the absorbance of control.

2.4.3. 1,1-Diphenyl-2-picryl-hydrazyl radicals (DPPH•) scavenging assay

For DPPH radical scavenging assay, 1 ml DPPH solution (0.1 M in 50% ethanol) was added to 1 ml sample solutions of polysaccharide of increasing concentration (0.2–2.0 mg/ml in double distilled water). The reaction mixture was vortexed and incubated at room temperature in dark conditions for 20 min. The absorbance of the

resulting solution was measured at 517 nm (Yamaguchi, Takamura, Matoba, & Terao, 1998). Scavenging activity was calculated using the formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{S-517}}{A_{C-517}} \right] \times 100$$

where A_{S-517} is the absorbance of the samples at different concentration and A_{C-517} is the absorbance of control.

2.4.4. Reducing power assay

In order to determine the reduction potential of polysaccharide samples, the reaction mixture of 1 ml phosphate buffer (0.2 M, pH: 6.6), 0.5 ml potassium ferricyanide (1%, w/v) and 1 ml of polysaccharide sample of different concentration (0.2–2.0 mg/ml) was incubated at 50 °C in water bath for 20 min. It was cooled followed by addition of 1 ml trichloroacetic acid (10%, w/v) and 0.2 ml freshly prepared ferric chloride ($FeCl_3$ 0.1%, w/v). The resulting solution was shaken on vortex mixer. The absorbance was measured at 700 nm (Lin, Wang, Chang, Inbaraj, & Chen, 2009). The reducing power was calculated using the formula:

$$\text{Reducing power} = [A_{S1-700} - A_{S2-700}]$$

where A_{S1-700} is the absorbance of the sample and A_{S2-700} is the absorbance of control wherein $FeCl_3$ solution has been replaced by water.

2.4.5. Nitric oxide radical scavenging assay

To study nitric oxide radical scavenging assay, 1 ml of sodium nitroprusside (5 mM) solution in standard phosphate buffer saline (pH: 7.4) was added to 2 ml of polysaccharide solution having different concentrations (0.2–2.0 mg/ml) prepared in phosphate buffer saline (pH: 7.4). The mixture was incubated at 25 °C for 5 h. Nitric oxide generated from sodium nitroprusside was measured by Griess' reaction in which 0.5 ml of above mixture was treated with 0.5 ml of Griess reagent (1% sulphanilamide, 2% *o*-phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride in equal volumes). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylenediamine was measured at 546 nm. Control experiment with an equivalent amount of buffer was performed. The scavenging ability of nitric oxide radical was calculated using the formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{S-546}}{A_{C-546}} \right] \times 100$$

where A_{S-546} is the absorbance of sample and A_{C-546} is the absorbance of the buffer (Green et al., 1982 and Marcocci, Maguire, Droylefaix, & Packer, 1994).

2.4.6. Determination of Fe^{2+} ion chelating ability

Chelating ability of all polysaccharide fractions with Fe^{2+} was investigated by taking 1 ml polysaccharide sample solutions at increasing concentrations (0.2–2.0 mg/ml) followed the addition of 2.7 ml water. The solution was treated with 0.1 ml of 2 mM ferrous chloride ($FeCl_2$) solution. The reaction was initiated by the addition of 0.2 ml ferrozine solution followed by vigorous shaking. The resulting mixture was incubated at room temperature for 10 minutes and the absorbance was measured at 562 nm. EDTA at different concentrations (0.2–2.0 mg/ml) was taken as positive control (Dinis, Madeira, & Almeida, 1994). The chelating rate of the polysaccharide samples was calculated using the formula:

$$\text{Chelating rate (\%)} = \left[1 - \frac{A_{S-562}}{A_{C-562}} \right] \times 100$$

where A_{S-562} is the absorbance of test sample and A_{C-562} is the absorbance of control.

2.4.7. Total antioxidant activity

To examine total antioxidant activity, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cations (ABTS^{•+}) were generated by taking equal volumes of aqueous ABTS (7 mM) and potassium persulfate (2.45 mM) solutions. The reaction mixture was kept under dark conditions at room temperature for 16 h. To 0.4 ml of polysaccharide sample at different concentrations (0.2–2.0 mg/ml), 3.6 ml ABTS solution was added. The resulting mixture was incubated at room temperature for 20 min. Absorbance of the above solutions was measured at 734 nm (Jin et al., 2012). The scavenging ability of polysaccharides was calculated using the formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{S-734}}{A_{C-734}} \right] \times 100$$

where A_{C-734} and A_{S-734} are the absorbance of control and sample respectively.

2.4.8. Determination of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride radical (DMPD^{•+}) scavenging ability

In DMPD^{•+} radical scavenging assay, 1 ml DMPD solution (100 mM prepared in deionized water) was mixed with 100 ml of acetate buffer (0.1 M, pH 5.3) to prepare a stock solution. 0.5 ml FeCl₃ solution (0.05 M) was added to the above solution to obtain freshly prepared working solution of DMPD^{•+} that is stable for 12 h at ambient temperature. 0.5 ml of polysaccharide samples were prepared, to which 1 ml of DMPD^{•+} solution was added. The reaction mixture thus obtained was vortexed and incubated in the dark for 15 minutes. The absorbance was measured at 505 nm (Koksal, Bursal, Dikici, Tozoglu, & Gulcin 2011 and Peksel, Altas-Kiyimaz, & Imamoglu, 2012). The scavenging ability for DMPD^{•+} was calculated using following formula:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{S-505}}{A_{C-505}} \right] \times 100$$

where A_{C-505} and A_{S-505} are absorbance of control and test sample respectively.

2.4.9. Determination of inhibition effect of polysaccharides on lipid peroxidation

The effect of FeCl₂-ascorbic acid induced lipid peroxidation on egg yolk as substrate was determined as follows: the yolk taken out of egg was added to phosphate buffer saline (0.1 M, pH 7.45) in the ratio of 1:25 ($V_{\text{egg yolk}}:V_{\text{PBS}} = 1:25$) and suspension was prepared by vigorous stirring for 30 min using magnetic stirrer. From the above suspension, 0.8 ml was added to 0.5 ml of polysaccharide samples at different concentrations (0.2–2.0 mg/ml) followed by the addition of 0.4 ml FeCl₂ (25 mM) to initiate lipid peroxidation. The reaction mixture was incubated at 37 °C for 1 h. After incubation, the reaction was quenched by the addition of 1 ml trichloroacetic acid (20%, w/v) and 1 ml thiobarbituric acid (0.8%, w/v). The reaction mixture was heated at 100 °C for 15 min, cooled and centrifuged at 2400 × g for 10 min. The absorbance of the upper layer was measured at 532 nm. The inhibition effect of lipid peroxidation was calculated using the formula:

$$\text{Inhibition effect (\%)} = \left[1 - \frac{A_{S-532}}{A_{C-532}} \right] \times 100$$

where A_{C-532} is the absorbance of control and A_{S-532} is absorbance of the test sample (Kimura, Kubo, Tani, Arichi, & Okuda, 1981).

2.5. Evaluation of moisture preserving properties

Moisture preserving properties of isolated polysaccharides was evaluated as per the reported protocol using glycerol and

Table 1a

Preliminary analysis of the samples (% (w/w) of dry weight).

Fraction	Yield (%)	Total sugar (%)	Uronic acids (%)	Protein content (%)
DSLIP	13.0	86.56	15.7	1.26
MDSP	18.6	91.04	–	2.43
TGBP	9.5	78.60	29.74	4.75

hyaluronic acid as positive control (Zhang, Wang, Han, Zhao, & Lin, 2012) The method in brief is as follows:

2.5.1. Moisture absorption activity

The polysaccharide samples were pulverized to fine powder and dried in hot air oven at 100 °C for 4 h. Oven dried samples (0.1 g) were kept at 25 °C for specified time intervals in a humidity chamber. The relative humidity (RH) of 43% was maintained by saturated K₂CO₃, and 81% by saturated (NH₄)₂SO₄. The samples were weighed successively after 0, 6, 12, 24, 48, 72, 96, 120 and 144 h. The moisture absorption ability (R_a) was evaluated by the gain in weight of the resulting polysaccharide samples and the standards after moisture absorption.

$$\text{Moisture absorption rate, } R_a (\%) = \left[\left(\frac{W_t}{W_0} \right) - 1 \right] \times 100$$

where W_0 is the weight of the oven dried sample and W_t is the weight of the sample after moisture absorption at definite time intervals in humidity chamber.

2.5.2. Moisture retention activity

The polysaccharide samples were finely ground into powdered form and dried in hot air oven at 100 °C for 4 h. Distilled water (0.1 ml) was added to the sample (0.1 g) and kept for dehydration at 25 °C using dried silica gel in humidification chambers containing K₂CO₃ (relative humidity 43%) and (NH₄)₂SO₄ (relative humidity 81%) for precise time intervals. Moisture retention rate (R_r %) of all the polysaccharide samples was evaluated by the weight loss after 0, 6, 12, 24, 48, 72, 96, 120 and 144 h as follows:

$$\text{Moisture retention rate, } R_r (\%) = \left(\frac{W_t}{W_0} \right) \times 100$$

where W_0 and W_t are the weights of added distilled water in the sample before and after the sample were put into the humidification chamber, respectively.

2.6. Statistical analysis

The results for all the tests were expressed as the mean ± standard deviation of triplicate analysis. Statistical comparisons were performed using Students' *t*-test. The difference was considered significant at $p < 0.05$.

3. Results and discussion

All the polysaccharides were isolated, purified and examined for total sugar (%), uronic acids (%) and protein content (%). Among three polysaccharide samples, the uronic acid content was found higher in TGBP. The preliminary analysis of DSLIP, MDSP and TGBP is shown in Table 1a.

3.1. Scavenging effect of polysaccharides on superoxide radicals (O₂^{•−})

Among different reactive oxygen species, superoxide anion is generated first. The superoxide radicals are highly toxic species which possess greater oxidative and oleophilic ability than the precursor to initiate peroxidation of lipids in a longer time (Chen, Ju,

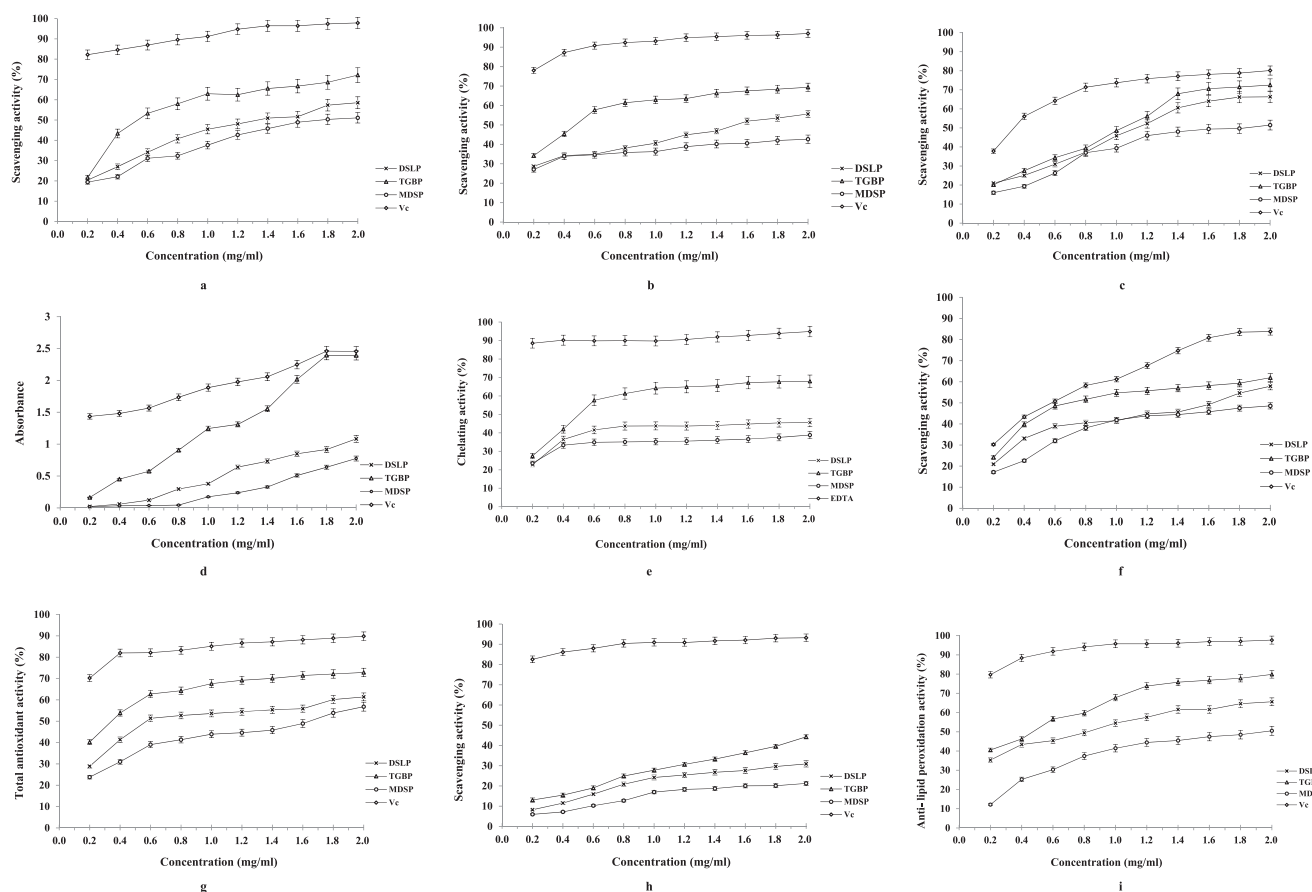


Fig. 1. (a) Superoxide radical scavenging activity of DSLP, TGBP and MDSP. (b) DPPH radical scavenging activity of DSLP, TGBP and MDSP. (c) Hydrogen peroxide scavenging activity of DSLP, TGBP and MDSP. (d) Ferric reducing power of DSLP, TGBP and MDSP. (e) Fe^{2+} ion chelating activity of DSLP, TGBP and MDSP. (f) Nitric oxide scavenging activity of DSLP, TGBP and MDSP. (g) Total antioxidant activity of DSLP, TGBP and MDSP. (h) $\text{DMPD}^{\bullet+}$ radical scavenging activity of DSLP, TGBP and MDSP. (i) Anti lipid peroxidation of DSLP, TGBP and MDSP.

Table 1b

Activity (%)^a of different polysaccharides at the concentration of 1.0 mg/ml for different antioxidant assays.

Activity/assays (%)	Sample			
	DSLP	TGBP	MDSP	Vc
Superoxide radical scavenging	45.49 ± 1.20	62.96 ± 1.44	37.66 ± 1.28	91.23 ± 0.54
Hydroxyl radical scavenging	45.85 ± 1.04	48.53 ± 1.10	39.29 ± 1.02	73.69 ± 1.02
DPPH radical scavenging	40.6 ± 1.08	62.93 ± 1.07	36.27 ± 1.08	93.12 ± 1.02
Reducing power assay	0.38 ± 0.03	1.245 ± 0.05	0.174 ± 0.06	1.887 ± 0.03
Nitric oxide radical scavenging	41.55 ± 1.12	54.74 ± 1.28	41.86 ± 1.18	61.17 ± 1.06
Fe^{2+} ion chelating	43.77 ± 1.04	64.21 ± 1.05	35.25 ± 1.05	89.75 ± 1.10
Total antioxidant activity (ABTS ion assay)	53.64 ± 0.77	67.62 ± 0.70	43.90 ± 0.95	85.13 ± 1.08
$\text{DMPD}^{\bullet+}$ radical scavenging	24.19 ± 1.00	27.79 ± 1.11	16.96 ± 0.83	90.95 ± 0.82
Lipid peroxidation inhibition	54.56 ± 2.96	67.79 ± 4.62	41.41 ± 1.73	95.75 ± 0.52

^a Values are expressed mean ± standard deviation.

Table 1c

IC_{50} value^a of DSLP, MDSP and TGBP for various antioxidant assays.

Scavenging activity	IC_{50} (mg/ml)		
	DSLP	TGBP	MDSP
Superoxide radical scavenging	1.420 ± 0.060	0.755 ± 0.065	1.730 ± 0.075
DPPH radical scavenging	1.570 ± 0.075	0.510 ± 0.070	2.850 ± 1.350
Hydroxyl radical scavenging	1.210 ± 0.035	1.070 ± 0.040	1.665 ± 0.045
Fe^{2+} ion chelating	2.120 ± 0.120	0.630 ± 0.060	3.920 ± 0.170
Nitric oxide radical scavenging	1.535 ± 0.070	1.030 ± 0.072	1.810 ± 0.070
Total antioxidant activity (ABTS ion assay)	0.990 ± 0.017	0.110 ± 0.050	1.645 ± 0.037
$\text{DMPD}^{\bullet+}$ radical scavenging	3.360 ± 0.080	2.360 ± 0.070	4.980 ± 0.100
Lipid peroxidation inhibition	0.851 ± 0.153	0.400 ± 0.121	1.720 ± 0.220

^a Values are expressed mean ± standard deviation.

Li, & Yu, 2012; Li, Liu, Fan, Ai, & Shan, 2011). Superoxide is also responsible to induce lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Dahl & Richardson, 1978). The three polysaccharide samples were to scavenge superoxide radical at a concentration level range between 0.2 and 2.0 mg/ml. Scavenging activities of DSLP, MDSP and TGBP on superoxide anion are presented in Fig. 1a. It was observed that the scavenging ability of DSLP, MDSP and TGBP on superoxide radicals increased significantly ($p < 0.05$) with an increase in polysaccharide concentration. The scavenging effect of three polysaccharides on superoxide anion increased in the order TGBP > DSLP > MDSP with values $62.96 \pm 1.44\%$, $45.49 \pm 1.20\%$ and $37.66 \pm 1.28\%$ at concentration level of 1.0 mg/ml respectively (Table 1b). Among the three polysaccharide fractions, TGBP showed the strongest scavenging ability which may be due to higher uronic acid content in polysaccharide concomitant with protein content. These results are also corroborated by the findings of previous workers (Li et al., 2011; Liu et al., 1997). The IC_{50} values for three polysaccharides to scavenge superoxide anion were also calculated. The validated IC_{50} values (Table 1c) of DSLP, MDSP and TGBP for superoxide radical were 1.420 ± 0.060 mg/ml (R^2 : 0.945), $1.730 \pm 0.075.5$ mg/ml (R^2 : 0.959), and 0.75 ± 0.065 mg/ml (R^2 : 0.769) respectively. Experimental data on the scavenging ability of superoxide indicated that TGBP has the best activity.

3.2. Scavenging effect of polysaccharides on DPPH• radicals

The DPPH is a stable organic free radical with a maximum absorption band around 515–528 nm and thus, it is widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu, Lu, Huang, & Ming, 2004; Li et al., 2011; Wang, Yang, & Wei, 2012). When an electron or hydrogen atom donating antioxidant is added to DPPH system, a number of DPPH radicals get reduced equal to their number of available hydroxyl groups (Singh & Rajini, 2004; Wang et al., 2012). At this stage of reaction, the stable yellow colored diphenylpicrylhydrazine (DPPH-H) is formed and the extent of reaction depends on the hydrogen donating ability of antioxidants (Brand-Williams, Cuvelier, & Berset, 1995). The hydrogen donating activity of three polysaccharides, measured using DPPH revealed that these polysaccharide fractions possessed hydrogen donating capabilities and would act as antioxidants. The results of DPPH free radical scavenging activity of the polysaccharides (DSLP, MDSP and TGBP) are shown in Fig. 1b and compared to Vc as standard. The scavenging effect of various polysaccharides increased significantly ($p < 0.05$) with increasing concentration in a dose range of 0.2–2.0 mg/ml. The DPPH scavenging ability was found to decrease in the order TGBP > DSLP > MDSP. The IC_{50} values of DSLP, MDSP and TGBP (Table 1c) were 1.570 ± 0.075 mg/ml (R^2 : 0.989), 2.850 ± 0.135 mg/ml (R^2 : 0.895), and 0.510 ± 0.070 mg/ml (R^2 : 0.766) respectively. The DPPH radical scavenging activity of TGBP is distinctly greater than other two polysaccharides (DSLP and MDSP). The activities of DSLP and MDSP are almost similar at a concentration level 0.2–0.8 mg/ml. However, at concentration level 1.0–2.0 mg/ml, DSLP showed higher activity than MDSP. At a concentration of 1.0 mg/ml the DPPH radical scavenging activity of DSLP, MDSP and TGBP were found to be $40.6 \pm 1.08\%$, $36.27 \pm 1.08\%$, and $62.93 \pm 1.07\%$ respectively (Table 1b).

3.3. Scavenging effect of polysaccharides on hydroxyl radicals

Hydrogen peroxide is not a very active species but is a precursor to potential radical species such as peroxy radical, hydroxyl radical and superoxides. Hydroxyl radical, an extremely potent oxidant, has an ability to react with most biomacromolecules of living cells, and may cause severe damage to the adjacent biomolecules (Huang et al., 2012). Hydrogen peroxide and superoxide molecules

can lead to oxidative injury in the biomolecules indirectly by producing hydroxyl radical via the Fenton reaction and/or iron catalyzed Haber-Weiss reaction, which can be prevented or inhibited by antioxidants (Erel, 2004). Fig. 1c indicates H_2O_2 scavenging activity of DSLP, TGBP and MDSP. For all samples, H_2O_2 scavenging effect was found to be concentration dependent. At lower concentration (0.2 mg/ml), the activities of TGBP and DSLP were almost equivalent but higher than MDSP. At the concentration of 0.8 mg/ml, all the polysaccharide samples showed almost similar scavenging activity against H_2O_2 . The scavenging effect of DSLP, MDSP and TGBP increased significantly ($p < 0.05$) with sample concentration ranging from 0.2 mg/ml to 1.4 mg/ml after which, it was found to increase slowly with increasing sample concentration. The hydroxyl radical scavenging ability decreases in the order TGBP > DSLP > MDSP. The IC_{50} values of DSLP, MDSP and TGBP (Table 1c) were 1.210 ± 0.035 mg/ml (R^2 : 0.967), 1.665 ± 0.045 mg/ml (R^2 : 0.903), and 1.070 ± 0.040 mg/ml (R^2 : 0.960) respectively. At a concentration of 1.0 mg/ml, the scavenging activity of DSLP, MDSP and TGBP was $45.85 \pm 1.04\%$, $39.29 \pm 1.02\%$, $48.53 \pm 1.1\%$, respectively (Table 1b). The results established that the scavenging activity of TGBP was strongest followed by DSLP and MDSP. In the present study, MDSP that contains no uronic acid exhibited lowest activity amongst all polysaccharides. These results confirmed that uronic acid content is an important factor which may reduce the formation of hydroxyl radicals. It was in conformity with earlier reported results of Li et al. (2011) wherein it was observed that polysaccharide with higher uronic acid content exhibited stronger hydroxyl radical scavenging activity.

3.4. Ferric (Fe^{3+}) ion reducing power

The antioxidant capacity of a molecule can also be evaluated by its reducing power assay, wherein the presence of reductant in the test samples would reduce Fe^{3+} (Ferricyanide complex) to the Fe^{2+} , with change in color of the mixture from yellow to various shades of blue or green depending upon the reducing power of a polysaccharide. The reducing capacity of a compound is a significant indicator of its antioxidant potential (Yang, Liu, Wu, Chen, & Wang, 2011). A reductant may also prevent peroxide formation by carrying out reaction with certain precursors of peroxides. In reducing power assay determination of a compound, higher value of absorbance at 700 nm indicates higher reducing power or stronger antioxidant activity. The reducing power of various polysaccharides and Vc is shown in Fig. 1d. The reducing power (absorbance at 700 nm) of various polysaccharides was found to increase significantly ($p < 0.05$) with increasing doses. Among the fractions tested, TGBP exhibited greater reducing power than DSLP, and MDSP and is comparable with the reducing ability of Vc at higher concentrations (1.8 mg/ml and 2.0 mg/ml). At a concentration of 1.0 mg/ml, the reducing power of DSLP, MDSP and TGBP was 0.38 ± 0.03 (R^2 : 0.966), 0.174 ± 0.06 (R^2 : 0.872), and 1.245 ± 0.05 (R^2 : 0.984), respectively (Table 1b). The order of reducing power of three tested polysaccharides was found to be TGBP > DSLP > MDSP. These results revealed that the reducing property is associated with breaking of free radical chain by donating hydrogen atoms. The polysaccharide fractions act as an electron donor and react with free radicals to convert them into more stable products with the termination of radical chain reactions.

3.5. Chelation with Fe^{2+} ion

Transition metals such as Fe^{2+} , Cu^+ , and Co^{2+} would trigger the progression of free radical reactions to amplify the cellular damage (Huang et al., 2012). The activity to chelate these metal ions is also claimed as one of the antioxidant mechanisms, since it reduces the concentration of transition metals catalyzing lipid

peroxidation (Benzie & Strain, 1996). Ferrozine reacts with Fe^{2+} to form red colored ferrozine- Fe^{2+} complex, which has a maximal absorbance value at 562 nm. In the presence of other chelating agents, like EDTA or polysaccharide, the ferrozine- Fe^{2+} complex formation is disrupted resulting in a decrease in intensity of red color and hence absorbance at 562 nm. This reaction is often used for the quantitative determination of Fe^{2+} (Huang et al., 2012; Wang et al., 2012). Dose dependent Fe^{2+} chelating effects of DSLP, MDSP, and TGBP are shown in Fig. 1e. The chelating effect of TGBP with Fe^{2+} was highest among all polysaccharide fractions, though it was lower than that of EDTA at given concentration range. The Fe^{2+} chelating capacity of polysaccharide fractions was found to decrease in order TGBP > DSLP > MDSP. The IC_{50} values of DSLP, MDSP and TGBP, were found to be 2.120 ± 0.120 mg/ml (R^2 : 0.561), 3.920 ± 0.170 mg/ml (R^2 : 0.613), and 0.630 ± 0.060 mg/ml (R^2 : 0.687) respectively (Table 1c). At a concentration of 1.0 mg/ml, the scavenging activity of DSLP, MDSP and TGBP was $43.77 \pm 1.04\%$, $35.25 \pm 1.05\%$, $64.21 \pm 1.05\%$, respectively (Table 1b). The chelating ability of Fe^{2+} was observed to be a function of hexuronic acid content, thereby resulting in higher chelating effect of TGBP and lower chelating effect of MDSP. The results are in agreement with previously reported findings (Jin et al., 2012; Wang et al., 2012) which indicate that the greater uronic acid content is responsible for higher chelating activity.

3.6. Nitric oxide radical ($\text{NO}^\bullet$) scavenging activity

Nitric oxide is a gaseous free radical that play an important role in various inflammatory processes. The production of this radical is toxic to tissues and attributes to the vascular collapse. Chronic exposure of nitric oxide radical results in various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tyler et al., 1997). The radical is more dangerous when it reacts with superoxide radical forming highly reactive peroxynitrite anion (ONOO^-) (Hazra, Biswas, & Mandal, 2008). The nitric oxide produces nitrite after reaction with oxygen, but in the presence of an antioxidant, nitrite formation is inhibited by the competitive reaction with oxygen. Fig. 1f shows the NO scavenging activity of DSLP, MDSP, TGBP and Vc. For all the samples, the effects against NO were concentration dependent. At a concentration level of 1.0 mg/ml, DSLP and MDSP showed similar results while at the concentration of 1.2 mg/ml and 1.4 mg/ml, DSLP and MDSP showed comparable activity. The scavenging effect of DSLP, MDSP and TGBP increased significantly ($p < 0.05$) with given sample concentration. NO scavenging activity of the three polysaccharides decreased in the order of TGBP > DSLP > MDSP. The IC_{50} values of DSLP, MDSP and TGBP for NO scavenging activity, were 1.535 ± 0.070 mg/ml (R^2 : 0.913), 1.810 ± 0.070 mg/ml (R^2 : 0.854), and 1.030 ± 0.072 mg/ml (R^2 : 0.764) respectively (Table 1c). At a concentration of 1.0 mg/ml, the scavenging activity of DSLP, MDSP, and TGBP was $41.55 \pm 1.12\%$, $41.86 \pm 1.18\%$, and $54.74 \pm 1.28\%$ respectively (Table 1b). The results showed that the scavenging activity of TGBP was the strongest followed by DSLP and MDSP. In the present study, MDSP which contain no uronic acid exhibited lowest activity amongst all polysaccharides. The results demonstrated that the uronic acid content is an important factor which may reduce the generation of NO radicals. This was in accordance with the results of Yuan, Zhang, Fan, and Yang (2011) who reported that polysaccharides with higher uronic acid content exhibited stronger activity.

3.7. Total antioxidant activity

The ABTS radical cation ($\text{ABTS}^{\bullet+}$) decoloration assay, which employs a specific absorbance at a wavelength 734 nm, well

separated from the visible-light range and requires only a short reaction time. The reaction has been widely applied to evaluate the total antioxidant activity in both lipophilic and hydrophilic samples (Wu et al., 2006). Total antioxidant power of single compound can be evaluated by the ABTS assay (Huang et al., 2008; Katalinic, Milos, Kulisic, & Jukic, 2006). In case of polysaccharide samples, specific absorbance at 734 nm is used in both organic and aqueous solvents as an indicator reflecting the antioxidant ability (Han, Weng, & Bi, 2008; Wu et al., 2006). The scavenging ability of DSLP, MDSP and TGBP on ABTS free radical is shown in Fig. 1g. The scavenging powers of DSLP, MDSP and TGBP correlated well with increasing concentration ($p < 0.05$). The results indicated that TGBP have stronger scavenging power for ABTS radical than DSLP and MDSP and may be explored as a potential antioxidant. ABTS free radical scavenging activity of the three polysaccharides was found to decrease in the order of TGBP > DSLP > MDSP. The IC_{50} value of DSLP, MDSP and TGBP, for ABTS free radical scavenging, were 0.990 ± 0.017 mg/ml (R^2 : 0.752), 1.645 ± 0.037 mg/ml (R^2 : 0.927), and 0.110 ± 0.050 mg/ml (R^2 : 0.751) respectively (Table 1c). At a concentration of 1.0 mg/ml, the scavenging activity of DSLP, MDSP and TGBP was $53.64 \pm 0.77\%$, $43.90 \pm 0.95\%$, and $67.62 \pm 0.70\%$, respectively (Table 1b).

For all samples, the effects of ABTS radical were concentration dependent. The scavenging effect of DSLP, MDSP and TGBP increased significantly ($p < 0.05$) with increasing sample concentration ranging from 0.2 to 2.0 mg/ml. The results showed that the scavenging activity of TGBP was the strongest followed by DSLP and MDSP. In the present study, MDSP, which contained no uronic acid, exhibited lowest activity. The results demonstrated that the uronic acid content is an important factor, responsible for the total antioxidant activity. It is in accordance with the previously reported findings (Mateos-Aparicio, Mateos-Peinado, Jiménez-Escrig, & Rupérez, 2010) illustrating a positive correlation of high uronic acid content with strong antioxidant activity.

3.8. $\text{DMPD}^{\bullet+}$ radical scavenging activity

DMPD forms a stable and colored radical cation ($\text{DMPD}^{\bullet+}$) in acidic medium containing suitable oxidant solution. The UV-vis spectrum of $\text{DMPD}^{\bullet+}$ shows absorbance maxima at 505 nm. Compounds containing transferable hydrogen atom react with $\text{DMPD}^{\bullet+}$ and quench the compound responsible for color leading to colorless solution. The terminus of this rapid reaction is stable and taken as a measure of antioxidant efficacy. Therefore, DMPD assay is a measure of efficiency of hydrogen donating antioxidants to scavenge the single electron from $\text{DMPD}^{\bullet+}$ (Gülçin, Bursal, Şehitoğlu, Bilsel, & Gören, 2010). The assay is particularly suitable for polysaccharides, which are hydrophilic in nature, but is less sensitive to hydrophobic bioactive compounds. When compared to ABTS procedure, the $\text{DMPD}^{\bullet+}$ method assures a very stable endpoint and also pertinent when extensive screening is required. Standards such as α -tocopherol or butylated hydroxyl toluene (BHT) do not give true and reproducible results due to their hydrophobic nature. The scavenging ability of DSLP, MDSP, and TGBP on $\text{DMPD}^{\bullet+}$ free radical is shown in Fig. 1h. The scavenging powers of DSLP, MDSP, and TGBP were found to be correlate well with increasing concentration ($p < 0.05$). The results indicated that TGBP had stronger scavenging power for $\text{DMPD}^{\bullet+}$ radical. $\text{DMPD}^{\bullet+}$ scavenging activity of the three polysaccharides decreased in the order TGBP > DSLP > MDSP. The IC_{50} values of DSLP, MDSP and TGBP, for $\text{DMPD}^{\bullet+}$ free radical scavenging, were 3.360 ± 0.080 mg/ml (R^2 : 0.925), 4.980 ± 0.100 mg/ml (R^2 : 0.919), and 2.360 ± 0.070 mg/ml (R^2 : 0.992) respectively (Table 1c). At a concentration of 1.0 mg/ml, the scavenging activity of DSLP, MDSP and TGBP was $24.19 \pm 1.00\%$, $16.97 \pm 0.83\%$, and $27.79 \pm 1.11\%$, respectively (Table 1b). It can be interpreted from the results of above assay that the $\text{DMPD}^{\bullet+}$

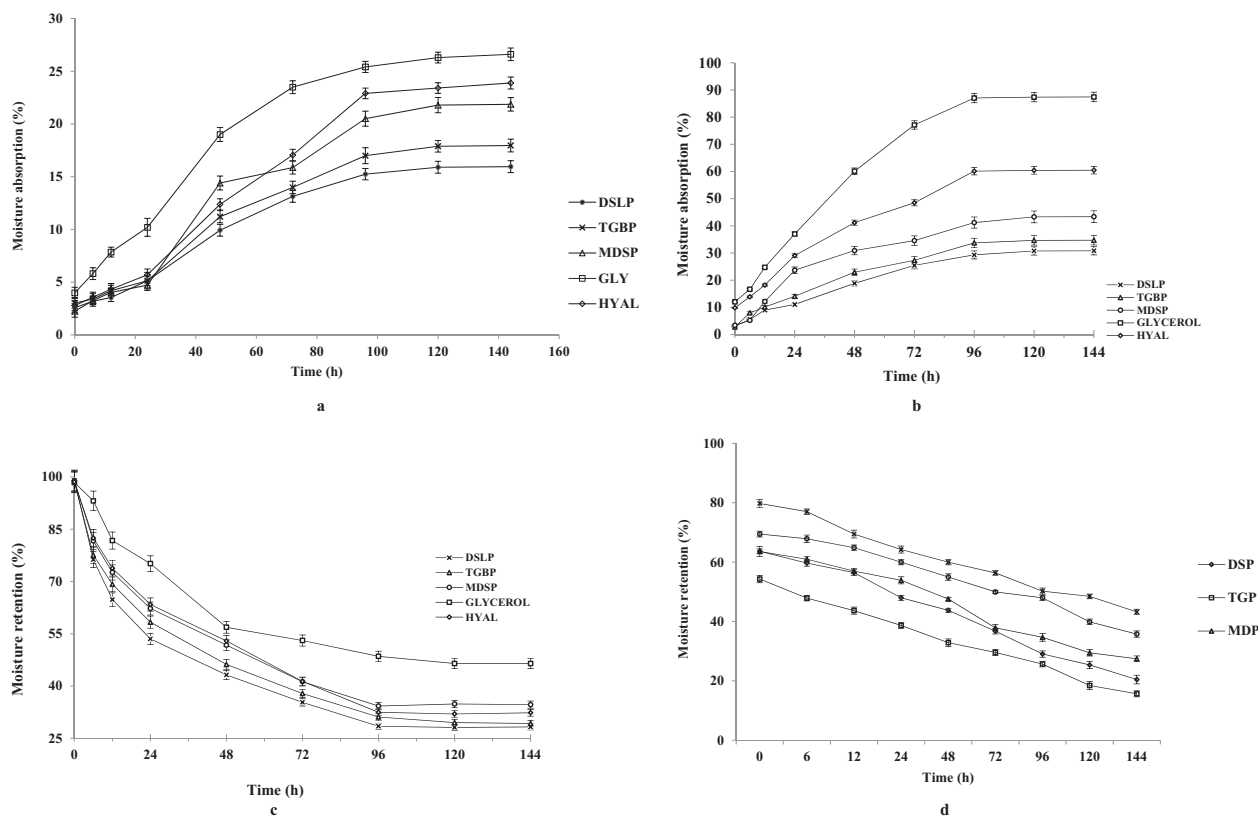


Fig. 2. (a) Moisture absorption by three polysaccharide sample (DSLP, MDSP, TGBP) at RH = 43%. (b) Moisture absorption by three polysaccharide sample (DSLP, MDSP, TGBP) at RH = 81%. (c) Moisture retention by three polysaccharide sample (DSLP, MDSP, TGBP) at RH = 43%. (d) Moisture retention by three polysaccharide sample (DSLP, MDSP, TGBP) at RH = 81%.

radical scavenging activity for all the three polysaccharides was not as strong as in the case of other assays.

3.9. Lipid peroxidation assay

Lipid peroxidation is a complex process which consists of a series of free radical mediated chain reactions and responsible for several types of biological damage like destruction of membrane lipids with the production of breakdown product such as malondialdehyde (MDA). MDA is an active participant in cross-linking reaction with DNA and proteins and acts as a catalyst in the formation of N-nitrosamines in foods containing secondary amines and nitrites. The scavenging ability of DSLP, MDSP, and TGBP on lipid peroxidation assay is shown in Fig. 1i. The scavenging powers of DSLP, MDSP, and TGBP were found to increase with increasing concentration ($p < 0.05$). The results indicated that TGBP have higher anti lipid peroxidation activity than DSLP and MDSP at all concentrations. Antilipid peroxidation activity of the three polysaccharides was found to decrease in order of TGBP > DSLP > MDSP. The IC_{50} values of DSLP, MDSP and TGBP were 0.851 ± 0.153 mg/ml (R^2 : 0.943), 1.720 ± 0.220 mg/ml (R^2 : 0.975), 0.400 ± 0.121 mg/ml (R^2 : 0.902) respectively (Table 1c). At a concentration of 1.0 mg/ml, the scavenging activity of DSLP, MDSP and TGBP was $54.56 \pm 2.96\%$, $41.41 \pm 1.73\%$, $67.79 \pm 4.62\%$, respectively (Table 1b).

3.10. Moisture absorption and retention properties

The in vitro moisture absorption and retention properties of three polysaccharides were examined gravimetrically and compared to humectant standards, hyaluronic acid and glycerol. The moisture absorption properties of three polysaccharides are shown in Fig. 2a and b. At 43% RH, the moisture absorption rate (R_a) of

three polysaccharide samples and hyaluronic acid were in range but lower than glycerol up to 24 h. From 24 to 96 h of the experiment, the R_a values for all samples and standards increased gradually and attained a constant value at 144 h. At RH 81%, the R_a (%) increased gradually up to 96 h and became constant at 120 h. In the initial hours of the experiment, the R_a (%) of MDSP was higher than hyaluronic acid. At 144 h, the values of R_a for DSLP were 15.95% and 30.82%, and that of TGBP was 17.96% and 34.72% at 43% and 81% RH respectively, while the values for MDSP were 21.86% and 43.88%. Among the three tested samples, value of R_a was found in the order MDSP > TGBP > DSLP. At 144 h, the R_a (%) of MDSP (21.86%) was found to be very close to the standard hyaluronic acid (23.88%).

Moisture retention properties of three polysaccharide samples were also examined at RH 43% and 81% (Fig. 2c and d). At RH 43%, all the samples showed continuous decrease in moisture retention ability with time up to 96 h and then attained a stabilized value. The moisture retention (%) rate of three polysaccharides was in order MDSP > TGBP > DSLP as 34.28%, 29.26% and 28.26% respectively. The results also indicated that moisture retention property of MDSP was found to be comparable with standard hyaluronic acid. At 81% RH, DSLP showed even higher moisture retention (%) than TGBP. At 144 h, the moisture retention rate (%) was found in order MDSP > DSLP > TGBP as 27.45%, 20.45% and 15.65% respectively.

In this study, in vitro antioxidant activities and moisture preserving properties of three polysaccharides DSLP, MDSP and TGBP were evaluated by different assays. Results showed that all samples possessed antioxidant activities in a concentration-dependent manner. In all antioxidant assays, TGBP showed higher activities than DSLP and MDSP, which may be due to the higher content of uronic acid in the former.

The mechanism of these polysaccharides for different assays of antioxidant activity is not clear yet. However, hydrogen

atom-donating ability of a molecule to a radical, which result in terminating radical chain reactions and converting free radicals to non harmful products, may be the expected mechanism of action (Hu, Zhang, & Kitts, 2000). Presence of electron-withdrawing groups such as carboxyl or carbonyl group in polysaccharide could lower the dissociation energy of O–H bond leading to release of hydrogen atom. Further, compounds containing two or more of the following functional groups: –OH, –SH, –COOH, –PO₃H₂, –CO, –NR₂, –S– and –O– in a favorable structure–function configuration could benefit metal chelating activity (Chen et al., 2012). Monosaccharide composition and molecular weight distribution are other additional factors, which may affect the potency against oxidation.

It has been established that uronic acid residues prominently influence the physiological, biological and pharmacological properties of polysaccharides. This is due to alteration in the basic structure of biopolymers, thereby, modifying their physical and chemical properties, resulting in characteristic biological activities and solubility behavior (Chen, Zhang, & Xie, 2004; Li et al., 2011). Interestingly, the binding abilities of polysaccharides are strongly influenced by uronic acid content. Further, it has also been established that with gradual increase in uronic acid monomers in polysaccharides, the free radical scavenging activity increases progressively with increasing uronic acid proportion (Chen, Qu, Fu, Dong, & Zhang, 2009). It is reported that the polysaccharides with a greater proportion of uronic acid are negatively charged leading to lesser steric hindrance and the reaction with free radicals proceeds in a facile way. Thus higher content of uronic acid in polysaccharides have better radical scavenging activities (Ye, Liu, Wang, Wang, & Zhang, 2012).

Some recently studied polysaccharides from *Pinus koraiensis*, *Pinus sylvestris* (Xu et al., 2012), *Inonotus obliquus* (Mu, Zhang, Zhang, Cui, Wang, & Duan, 2011), *Houttuynia cordata* (Tiana, Zhaob, Guoa, & Yang, 2011), *Sargassum fusiforme* (Zhou, Hu, Wu, Pan, & Sun, 2008), *Ampelopsis grossedentata* (Wang, Bian, et al., 2011), *Auricularia polytricha* (Suna, Liua, & Kennedy, 2010), *Zizyphus Jujuba* (Li et al., 2011), *Ophiopogon japonicus* (Xiong, Li, Huang, Lu, & Hou, 2011), *Sarcandra glabra* (Jin et al., 2012), marine *Pseudomonas* (Ye et al., 2012), *Grifola umbellata* (Bia, Miaob, Hand, Xue, & Wang, 2013), and *Lactobacillus planterum* (Li, Jin, Meng, Gao, & Lu, 2013), were found to exhibit antioxidant activities. The studies also indicate relationship between the uronic acid contents and the antioxidant properties.

The results of moisture related properties also reveal that MDSP, DSLP and TGBP have strong in vitro moisture absorption and retention capacities and may be used as an ingredient in skin care, cosmetics or other moistening products. In skin care cosmetics, moisturizing properties are of great interest for cosmetologists and dermatologists as the moisture content is very important to skin health. In polysaccharide structure, the hydroxyl, carboxyl and other polar functional groups are involved in the formation of hydrogen bonds with water to coalesce substantial moisture content, and simultaneously, polysaccharide chains have ability to mutually interweave in the lattice, thereby ensuring the moisture content. Based on this mechanism, the DSLP, MDSP TGBP could prove to be useful as moisturizing agents.

Structure of one polysaccharide (DSLP) is previously described (Rana et al., 2009, 2012) and detailed structural characterization of other two polysaccharides (MDSP and TGBP) is in progress in our laboratory, which may provide a good opportunity to elucidate the structure–function relationship and to explore highly potent antioxidant agents.

4. Conclusions

The study revealed antioxidant activity of TGBP, DSLP and MDSP in all assays with highest activity of TGBP. Further R & D efforts are

required to develop these polysaccharides as commercial antioxidants based on structure–activity relationship and in vivo studies. In addition, the studied polysaccharides demonstrated effective moisture absorption and retention abilities and are futuristic ingredients in skin care, cosmetics or other moistening products after detailed analysis.

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