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Antioxidant Activities of Water Soluble Polysaccharides Extracted From Raw and Sprouted Mung Beans.

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ABSTRACT

The hot water extraction process was used to isolate the water soluble polysaccharides from raw (R-WSP) and sprouted (S-WSP) mung beans (*Vigna radiata*). The chemical characteristics of both the samples were analyzed by FT-IR spectrum. Further, several biochemical assays were carried out to evaluate the antioxidant activity of the samples. The extraction yield of R-WSP and S-WSP were 49.1 mg/g and 10.5 mg/g respectively. The presence of uronic acid was analyzed in both the samples. The raw mung bean polysaccharides (R-WSP) exhibited higher antioxidant ability than the sprouted mung polysaccharides (S-WSP). The results suggested that germination causes the change in polysaccharides content and the water soluble polysaccharides of raw mung beans can be further deduce to identify the principal component for its antioxidant potential.

Keywords Water Soluble Polysaccharides, Mung Beans, Antioxidant Activity.

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INTRODUCTION

In recent years, some naturally occurring bioactive polysaccharides have attracted much attention in the field of biochemistry and pharmacology. As an example, polysaccharides or their glycoconjugates were shown to exhibit multiple biological activities including anticarcinogenic, anticoagulant, immunostimulating, antioxidant etc. The water-soluble polysaccharides have received great attention as free radical scavenger, inhibitors of lipid per oxidation and metal chelators [1,2]. These antioxidant polysaccharides are basically isolated from the plants, fungi, bacteria, animal sources and algae [3]. Legumes are among the three largest families of flowering plants and belong to the orders Gramineae (cereals and grasses) and Leguminosae (legumes or the bean family). Since legumes seeds are rich source of soluble and insoluble non starch polysaccharides (NSP), there has been a worldwide interest in searching for potential utilization of unconventional legumes [4].

Among the NSP, mixed linked β- glucans and pectic polysaccharides are the water soluble polysaccharides. The ubiquitous structural feature of β- glucans polysaccharides is well established. It consists of a linear chain of glucose units joined by both β-(1→3) and β-(1→4) linkages [5]. The term pectic polysaccharides refers to galacturonans or more commonly rhamnogalacturonans in which (1→4)-α-D-galacturonan chains are interrupted at intervals by insertion of (1→2)-α-L-rhamnose residues. Other constituent sugars attached as side chains include D galactose, L- arabinose, D- xylose, and less frequently L - fucose and D - glucuronic acid [6].

Mung bean (*Vigna radiata*) is the most important legume due to its high proteins and carbohydrates and its protein quality is similar to or better than other legumes such as chickpea, black gram, peas, pigeon-pea etc [7,8]. It is one of the most important pulse crops grown in Asia and used in different forms such as whole mung bean, mung bean dhal, sprouted mung bean and dehulled mung bean [9,10]. Mung bean was found to contain 60-65 percent carbohydrate by Paul et al [11] and Agugo et al [12]. Germination of beans enhances the nutritive value of legumes by inducing the formation of enzymes that eliminate or reduce the antinutritional and indigestible factors in legumes [13]. Today there is an increasing interest in Western countries in the sprouting of seeds as consumers demand minimally processed, additive-free, more natural, nutritional and healthy foods. Recently the antioxidant activity of water-soluble polysaccharide (WSP) from mung bean hull were reported to be as potential antioxidants [14] but no investigation has been reported so far on the WSP content extracted from sprouted mung bean. In the present study, the antioxidant potential of water soluble polysaccharides from raw and sprouted mung bean was evaluated.

MATERIALS AND METHODS

Materials

Acetone, Chloroform, Distilled Water, Ethanol (Absolute), n-Butanol, Petroleum Ether, Ascorbic Acid, Ammonium Molybdate, di-amine tetra acetic acid-2Na salt, Ferric Chloride,

Ferrous Chloride, Ferrozine, Hydrogen Peroxide, Potassium Ferricyanide, Sodium Phosphate, Phosphate Buffer, Sulphuric Acid, Tricholoroacetic acid, Extract Samples.

Processing and Sprouting of Mung Beans

Mung beans were first washed by tap water and then twice by distilled water. The beans were air dried for three hours followed by grinding to fine powder and then placed in a sealed glass container for further use [15, 16].

Seeds of mung beans were imbibed for 24 hours at room temperature in distilled water. The seeds were then drained, rinsed twice with distilled water, and kept for germination on moist filter paper in the dark. The sprouted beans were air dried and sun dried respectively, than grinded to fine powder and placed in a sealed glass container.

Extraction of Water Soluble Polysaccharides

A modified method derived from Feng et al [17], Jin Zhe et al [18] and Luo et al [19] was used to extract the water soluble polysaccharides from the raw and sprouted mung beans. 50 g of raw and sprouted mung bean samples was added separately in 200 ml of distilled water. The mixture was heated at 70^{o}C temperature on hot plate for about 2-3 hours, filtered and centrifuged at 5000 rpm for 20 minutes. The supernatant was collected, washed with petroleum ether by continuously stirring for about 5 minutes. 85% ethanol was added to the mixture, stirred for 30 minutes and left for overnight at temperature 4^{o}C. Then, deproteination was carried out by the addition of sevag reagent following centrifugation for 15 minutes at 8000 rpm. The precipitate was obtained and washed with acetone, ethanol and distilled water respectively.

Infrared Spectroscopy

FT-IR spectra were determined by Perkin Elmer Spectrum RX1 at the range of 400- 4000 cm^{-1}. The analysis was carried out at SAIF (Sophisticated Analysis Instrument Facility), Central Drug Research Institute (CDRI), Lucknow.

Antioxidant Activities

Total Antioxidant Activity (TAC) Assay

The method described by Shirwaikar and Somashekar [20] was used. 0.1 ml extract was mixed with 1 ml of TAC reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), incubated at 95^{o}C for 90 min and cooled. The absorbance was measured at 695 nm against blank solution.

Reducing Power Assay

The method of Oyaizu [21] was used with some modifications. 2.5 ml volumes of phosphate buffer (pH 6.6) and 1.5% potassium ferricyanide was mixed with 1 ml sample, incubated at 50^{o}C for 20 min and cooled. Afterwards, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride (0.1%) solution and the absorbance was taken at 700 nm. Vitamin C (ascorbic acid) was used as standard.

Hydrogen Peroxide Radical Scavenging Activity Assay

The method of Ruch et al [22] was adopted. 1 ml of sample was dissolved in 0.6 ml of 40 mM hydrogen peroxide, prepared in phosphate buffer (pH 7.4) and incubated at room temperature for 10 min. The absorbance at 230 nm of the reaction mixture was recorded at 0 min and 10 min later against a blank solution. The percentage of hydrogen peroxide scavenging was calculated as-

$$\% \text{ Scavenged } [H_2O_2] = [(A_o - A_1)/A_o] \times 100$$

where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard.

Metal Chelating Activity Assay

The ability of samples to chelate the ferrous ions was estimated by method of Dinis et al [23]. 1ml sample was mixed with 50 µl of 2 mM ferrous chloride and 0.2 ml of 5 mM ferrozine solution was added. The mixture was vigorously shaken, incubated at room temperature for 10 min and the absorbance was measured at 562 nm. The efficiency of samples was compared with ethylene di-amine tetra acetic acid-2Na salt (Na_2EDTA), as a control. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as-

$$[(A_0 - As)/ As] \times 100$$

where A_0 was the absorbance of the control, and A_s was the absorbance of the extract or control.

Superoxide Anion Scavenging Activity Assay

The superoxide anion scavenging activity was estimated by the spectrophotometric monitoring on the inhibition of pyrogallol autooxidation. Pyrogallol has long been known to autoxidize rapidly which results in pH change and formation of several intermediate products. Thus the solution shows a wide spectrum between 325 - 425 nm [24,25]. An applied modification of this pyrogallol autooxidation is derived enabling the measurement of the antioxidative potential of the water soluble polysaccharides samples. For a proper

measurement of the antioxidative potential, a suitable wavelength was required to obtain. For choosing the suitable wavelength, wavelength scans for 300 to 750 nm were done for measuring and reference cuvette. The scans were run against phosphate buffer. After wavelength scans were done, 0.1 ml α tocopherol was added to both measuring and reference cuvette in order to achieve full reduction of pyrogallol radicals. Then the wavelength scans were repeated. This served to select an adequate wavelength which is influenced only by the pyrogallol radicals (here 400 nm).

The modified method of Marklund and Marklund [24] was adopted for the testing. 0.3 ml sample was mixed with 2.6 ml of 50 mM phosphate buffer (pH 8.2) and 0.1 ml of a 3mM solution of pyrogallol (prepared in 37.5% HCl) was added. The autooxidation reaction rate of pyrogallol was determined at 400 nm by monitoring the absorbance every 30 sec for a total time of 10 min, corresponding to the end of reaction.

The percentage of scavenging by the extracts and a standard compound (ascorbic acid) was calculated as follows:

$$\% \text{ Scavenged} = [(A_o - A_1)/A_o] \times 100$$

Statistical Analysis

Data are reported as mean of three determinations. The results obtained were statistically analyzed with the Student's t-test using a significance level of $p < 0.05$. Microsoft Office Excel 2007 was used for graph plotting.

RESULTS AND DISCUSSION

Polysaccharides modify and control the mobility of water, thus exhibit a profound effect on food properties [26].

Extraction

The resultant crude hot water soluble polysaccharides were brown in colored powder without starch. Similar reports were given by Atkhamova et al [27] and Boual et al [28] for different plants. Siddhanta et al [29] showed that the hot water extraction gave higher yield in comparison to the cold water extraction. Some researchers also used microwave and ultrasonic assisted processes to extract water soluble polysaccharides [30, 31]. The protein content of samples were denatured by sevag reagent [32] and further washed with different solvents.

The extraction yield of R-WSP (Raw Mung bean-water soluble polysaccharide) and S-WSP (Sprouted Mung bean-water soluble polysaccharide) were 49.1 and 10.5 mg/g respectively. Earlier Lai et al [14] reported 6.04% extraction yield of water soluble polysaccharides from mung bean hull with mannose, galactose and rhamnose as its principal components. However it was earlier reported that soaking followed by germination leads to the

activation of some enzymes which reduces the higher polysaccharides [33]. Hooda and Jood [34] also reported that an enzyme β-galactosidase from germinated cereals and pulses attacks galactomannan to yield galactose. The decrease in the polysaccharide content may be attributed to their breakdown and utilization by the growing sprouts.

IR Spectroscopy

The FT-IR spectra of R-WSP and S-WSP were recorded at 4000-450 cm^{-1} to characterize the chemical constituents (figures 1-2). Both the samples showed absorption peak around 3430 cm^{-1}, which was due to the presence of alcohol and hydroxyl group with NH stretch. The absorption near 1400-1220 cm^{-1}, in both the samples, were attributed to simple hydroxyl containing compound with alcohol function [35]. The bands around 2930-2855 cm^{-1} (methylene C-H asymmetric stretch) and 1735 cm^{-1}(esterified carboxylic group) suggested the existence of uronic acid [36, 37]. The presence of uronic acid was also reported in the water soluble polysaccharides extracts by Lai et al [14] and Li et al [32]. Mung beans were observed to contain highly methylated soluble pectins [38].

The absorption band from 1300 cm^{-1} to 800 cm^{-1}, called “finger print” region, was related to conformation and surface structure of molecule. Although these bands are hard to explain [36], it suggested the presence of aromatic ring and secondary amine in both the samples. The spectrum of R-WSP also signifies the existence of cyclic esters/alkyl substituted ether with C-O stretch (1095 cm^{-1}), saturated aliphatic groups (1463 cm^{-1}) and aromatic nitrogenous compound with XO_2 stretch (1513cm^{-1}). The spectrum of S-WSP indicated the presence of cyclohexane ring vibrations (1027 cm^{-1}), transition metal carbonyls (2091 cm^{-1}), alkyne/nitrile (2400 cm^{-1}) and terminal olefinic group (3020 cm^{-1}) [35].

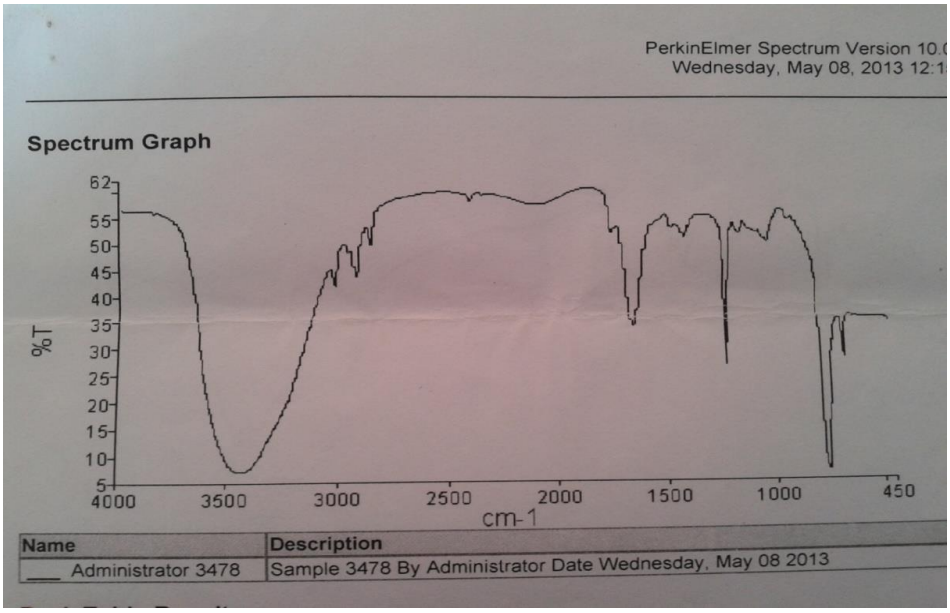

Figure 1: FTIR spectra of Raw-Water Soluble Polysaccharides (R-WSP) of Mung Beans

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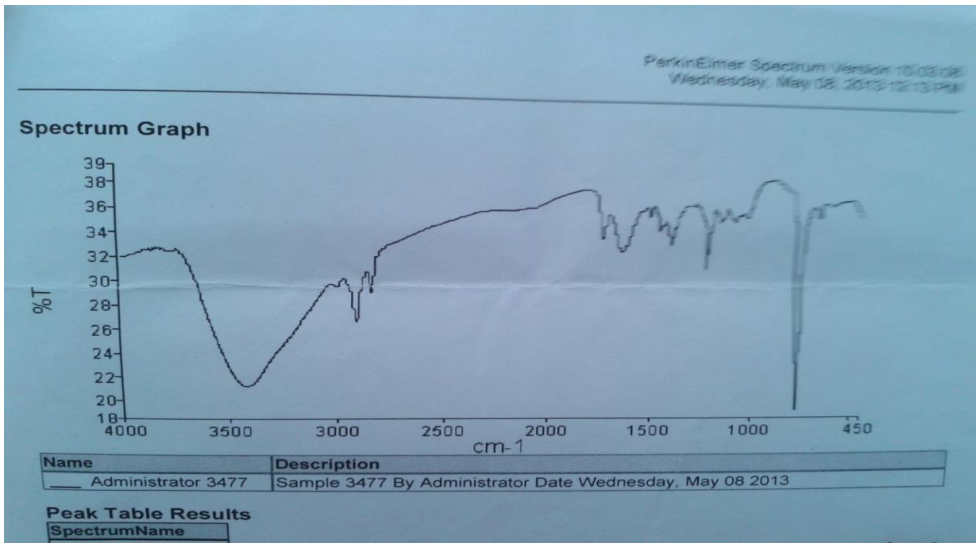

Figure 2: FTIR spectra of Sprouted-Water Soluble Polysaccharides (S-WSP) of Mung Beans

Antioxidant Activity

Total Antioxidant Capacity

The reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phospho-molybdenum complex serves the basis of the total antioxidant assay. The antioxidant activity was expressed as vitamin C equivalent [39]. The water soluble polysaccharides of raw and sprouted mung beans showed 5.5 and 2.7 mg vit C/100 ml of samples respectively. Earlier total antioxidant capacity of mung beans methanolic extracts of sprouts and seeds were reported to be 0.148 and 0.043 µg/mg ascorbic acid equivalent respectively [40].

Reducing Power

It was reported that reducing power and antioxidant activity are directly correlated to each other [41, 42, 43]. Since antioxidants contain reductones which readily donate hydrogen atoms and break the free radical chain [44, 45], thus it can be concluded that the reduction potential of any compound may serve as a significant indicator of its potential antioxidant activity [46]. The reducing power capacity of R-WSP and S-WSP were evaluated and compared with vitamin C. As shown in figure 3, the samples exhibit reducing ability in a concentration dependent manner. The reducing power of R-WSP increased sharply with the increase in concentration whereas slower increase was observed in S-WSP. As compared to standard, R-WSP was found to be more effective. At the concentration of 5mg/ml, R-WSP and S-WSP showed a reducing power of 2.31 and 1.87 respectively, which is higher than the water soluble polysaccharides extracts of mung bean hull [14]. The data suggests that both the samples exhibit strong reducing power but in comparison R-WSP demonstrates higher effect than S-WSP.

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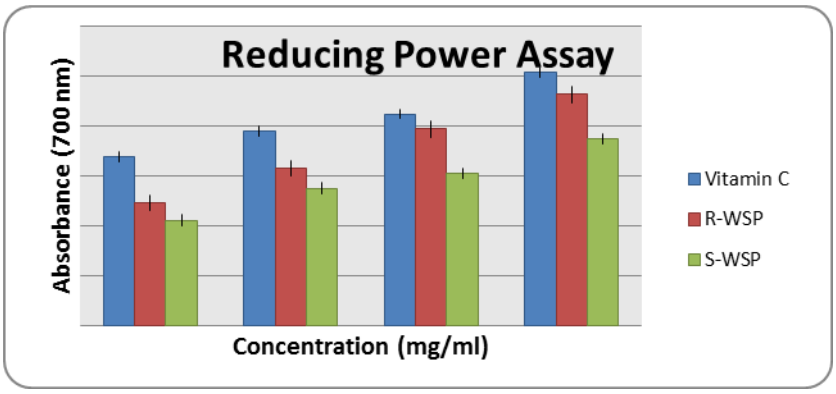

Figure 3: Reducing Power Assay of R-WSP and S-WSP. Vitamin C (Ascorbic Acid) was used as standard. Results are shown as mean ± standard deviation.

Hydrogen Peroxide Radical Scavenging Activity

Hydrogen peroxide can be formed by large number of reactions in our body and supposed to be less reactive. But it becomes harmful when it produces potent species such as hydroxyl radical which interrupts almost each biomacromolecules functioning in the living cell [47, 48, 49]. Figure 4 demonstrates the hydrogen peroxide radical scavenging ability of R-WSP and S-WSP. The samples were capable of scavenging hydrogen peroxide radical in dose dependent manner. At the concentration of 1.25 mg/ml, R-WSP and S-WSP exhibited 52 and 35 percent scavenging respectively. R-WSP showed rapid increase in scavenging percentage upto 3.75mg/ml but slightly slows at 5 mg/ml concentration. S-WSP was found to be less effective than R-WSP at each concentration. In comparison to vitamin C, R-WSP demonstrates significant scavenging effects. These results suggest that water soluble polysaccharides have strong hydrogen peroxide radical scavenging capacity and may be effective in reducing the associated harmful reactive species, for instance hydroxyl radical. Lai et al [14] reported that crude and purified water soluble polysaccharides extracts from mung bean hull efficiently reduce the hydroxyl radicals and also suggested that polysaccharide may have chelating ability. As suggested, metal chelating activity was assessed.

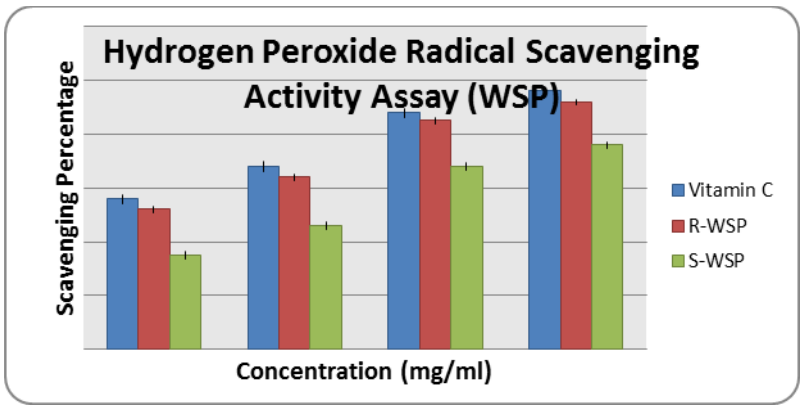

Figure 4: Hydrogen Peroxide Radical Scavenging Assay of R-WSP and S-WSP. Vitamin C (Ascorbic Acid) was used as standard. Results are shown as mean ± standard deviation.

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Metal Chelating Activity

The transition metal ions easily transfer single electrons by virtue of which free radical reactions readily takes place [50]. In this assay, ferrozine quantitatively form complex with Fe^{2+} and the reduction in the complex directly signifies the chelating ability of the samples [51]. Thus the metal chelating activity can be correlated with the antioxidant activity of the test samples. Figure 5 illustrates the metal chelating ability of the polysaccharides. At the concentration of 1.25 mg/ml, R-WSP and S-WSP showed 30 and 25 % chelating ability. With the increase in the concentration, the activity of samples was increased. At each concentration, R-WSP showed better results than S-WSP but was lower than Na_2EDTA (control). A maximum of 62 % chelating capacity was observed by R-WSP at 5 mg/ml whereas 58% activity was exhibited by S-WSP at same concentration. The metal binding capacity of mung beans methanol extract was investigated by Duh et al [41], but no data has been reported for chelating capacity of mung bean polysaccharides. However it is often accepted that polysaccharides with particular spatial structure and functional group like hydroxyl and carboxyl may bind easily to the metal ions [52, 53], which could interrupt the generation of free radicals.

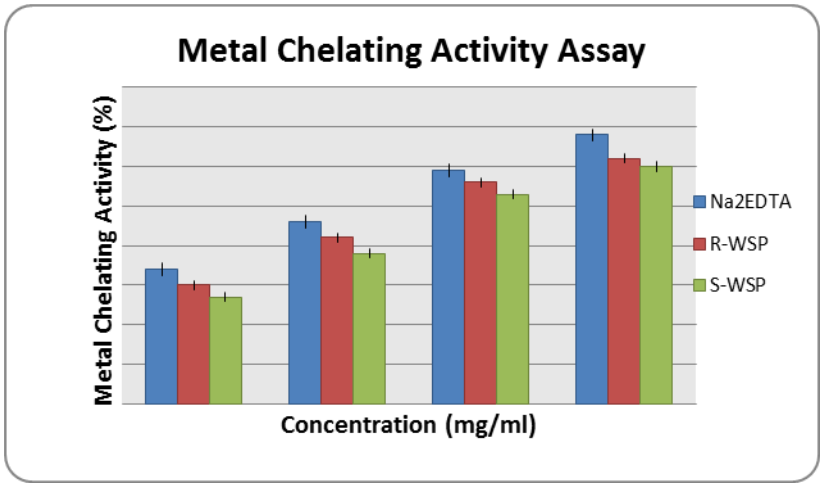

Figure 5: Metal Chelating Assay of R-WSP and S-WSP. Vitamin C (Ascorbic Acid) was used as standard. Results are shown as mean ± standard deviation.

Superoxide Anion Scavenging Activity

The auto-oxidation of pyrogallol (1,2,3 benezenetriol) generates superoxide anions (O_2^-), which is considered as an initial free radical, formed from mitochondrial electron transport systems, to create other cell-damaging free radicals, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems [54]. Earlier studies have reported different wavelengths to measure the pyrogallol auto-oxidation rate [55, 56]. Since the wavelength should be influenced only by the presence of free radicals in the sample [57], the wavelength scanning was carried out. The decrease in absorbance reflects the chemical state of superoxide anions, which was observed at 400 nm (λ_{max}), thus selected.

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The superoxide scavenging percentage of the sample R-WSP and S-WSP is given in figure 6. R-WSP exhibited stronger superoxide radical scavenging ability than S-WSP. At the concentration of 1.25 mg/ml, R-WSP and S-WSP showed 40 and 32% scavenging ability respectively. Gradual increase in the percentage activity with increase in concentration was observed for both the samples. The R-WSP sample illustrated 92% anion scavenging ability at 5 mg/ml concentration which is similar to the report of Lai et al [14]. In comparison to the standard, R-WSP demonstrated slightly lower scavenging activity than the standard (vitamin C). Since superoxide anions produce harmful free radicals, which causes the damage to DNA and membrane of cell [58], its reduction by the samples show evidence of potential antioxidants.

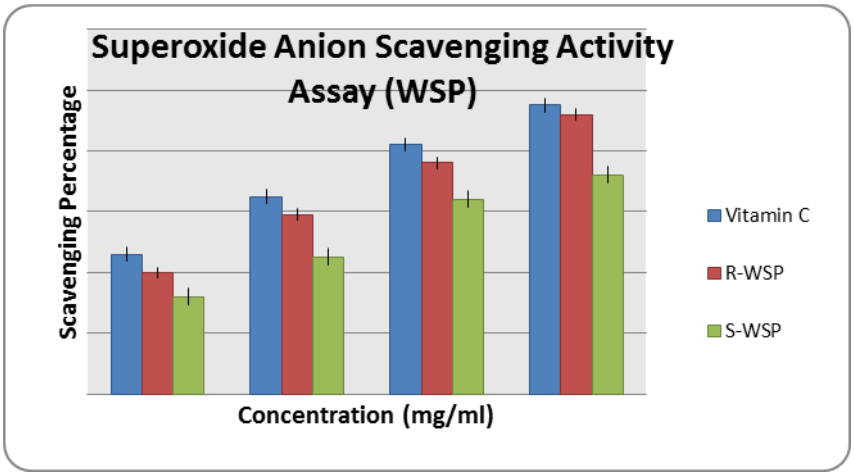

Figure 6: Superoxide Anion Scavenging Activity of R-WSP and S-WSP. Vitamin C (Ascorbic Acid) was used as standard. Results are shown as mean ± standard deviation.

The entire antioxidant assay verified that the polysaccharides of raw mung beans strongly responds as antioxidants while lower activity was observed in the polysaccharides derived from sprouted mung bean. Finally, all the results conclude here that polysaccharides are also able to reduce the free radical reactions and can contribute as a natural source of effective antioxidants. There is a strong need for effective antioxidants from natural sources in order to prevent diseases, maintenance and improvement of health and wellness. Mung beans polysaccharides indicate probable antioxidant compounds, and suggested to be developed as a novel medicine.

REFERENCES

[1] Costa LS, et al. Biomed Pharmacother 2010; 64: 21–28.
[2] Wang ZJ, Luo DH. Carbohydrate Poly 2007; 68: 54–58.
[3] Wang H, Liu YM, Qi ZM, Wang SY, Liu SX, Li X, Wang HJ, Xia XC. Curr Med Chem 2013; 20(23): 2899-2913.
[4] Siddhuraju P, Vijayakumari K, Janardhanan K. Food Chem 1996; 57: 385-391.

[5] Bengtsson S, Aman P, Graham H, Newman CW, Newman RK. J Sci Food Agric 1990; 52: 435- 440.
[6] Choct M. Feed Milling International. 1997; June Issue: 13- 26.
[7] Kataria A, Chauhan BM, Punia D. Food Chem 1989; 32: 9-17.
[8] Jood S, Bishnoi S, Sharma S. Die Nah /Fd 1998; 42: 70-73.
[9] Thirumaran AS, Seralathan MA. "Utilization of Mungbean" in Shanmugasundaram S and McLean BT (eds.) Mungbean: Proceedings of the Second International Symposium, AVRDC, Shanhua, Taiwan, Rep. of China. 1988, 470- 485.
[10] Singh VP, Chhabra A, Kharb RPS."Production and Utilization of Mungbean in India", in Shanmugasundaram S and McLean BT (eds.) Mungbean: Proceedings of the Second International Symposium, AVRDC, Shanhua,Taiwan, Rep. of China. 1988, 486-497.
[11] Paul T, Rubel Mozumder NHM, Sayed MA, Akhtaruzzaman M. Bangladesh Research Publications Journal 2011; 5(3): 207-213.
[12] Agugo UA, Onimawo IA. EJEAFChe 2009; 8(10): 924-930.
[13] Bau HM, Villanme C, Nicolos JP, Mejean L. J Sci Food Agr 1997; 73: 1–9.
[14] Lai F, Wen Q, Li L, Wu H, Li X. Carbohydrate Poly 2010; 81: 323–329.
[15] Rogers CB, Verotta L. (eds). Chemistry and biological properties of the African Combretaceae. In Hostettmann F, Chinyanganya M, Maillard M and Wolfender JL (eds). Chemistry, biological and pharmacological properties of African medicinal plants. University of Zimbabwe Publications, Harare, Zimbabwe, 1996.
[16] Eloff JN. Conservation of plants Genes111, conservation and utilization of African plants. 1998, 71: 209-222.
[17] Feng L, Jia XB, Shi F, Chen Y. Molecules 2010; 15: 5093- 5103.
[18] Jin Zhe H, Qiao MR, Dan Dang D, Sun PL. Molecules 2012; 17: 4373- 4387.
[19] Luo A, Luo A, Huang J, Fan Y. Molecules 2012; 17(7): 8079-8090.
[20] Shirwaikar A, Somashekar AP. Indian J Pharm Sci 2003; 63-68.
[21] Oyaizu M. Japan J Nutr 1986; 7: 307- 315.
[22] Ruch RJ, Cheng SJ, Klaunig JF. Carcinogenesis 1989; 10: 1003–1008.
[23] Dinis TCP, Madeira VMC, Almeida LM. Arch Biochem Biophys 1994; 315: 161–169.
[24] Marklund S, Marklund G. Eur J Biochem 1974; 47: 469- 474.
[25] Xu M, Shangguan X, Wang W, Chen J. Asia Pac J Clin Nutr 2007; 16 (1): 178-182.
[26] Fennema OR. Carbohydrates in Food Chemistry, Third Edition, CRC Press, 1996, pp. 179.
[27] Atkhamova SK, Rakhimov DA, Kristallovieh EL, Karimdzhanov AK, Ismailov AI. Chem Natl Comp 1997; 33: 590- 593.
[28] Boual Z, Kemassi B, Khelil A, Michaud P, Hadj M. International Conference on Biology, Environment and Chemistry 2011; 24: 420- 424.
[29] Siddhanta AK, Goswami AM, Ramavat BK, Mody KH, Mairh OP. Indian J Geo-Marine Sci 2001; 30(3): 166- 172.
[30] Fan F, Fu F, Mazza G, Hu XS, Liao XJ. Trans Chinese Soc Agr Eng 2009; 25(10): 355-360.
[31] Hromadkova Z, Ebringerova A, Valachovic P. Ultrason Sonochem 2002; 9(1): 37- 44.
[32] Li J, Wang Y, Zhang De, Hu X, Zhang Z, Xiang C. J Food Agr Environ 2010; 8 (2): 237-241.
[33] Jood S, Khetarpaul N, Goyal R. J Nutr Food Sci 2012; 2(9): 2- 4.
[34] Hooda S, Jood S. Nahrung 2003; 47: 265-268.

[35] Coates J. Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry. RA Meyers, John Wiley & Sons Ltd, Chichester, 2002; pp 10815–10837.
[36] Gnanasambanda R, Proctor A. Food Chem 2000, 68, 327–332.
[37] Singthong J, Cui SW, Ningsanond S, Goff HD. Carbohydrate Poly 2004; 58: 391- 400.
[38] Goldberg R, Prat R, Morvan C. Carbohydrate Poly 1994; 23: 203-210.
[39] Prieto P, Pineda M, Aguilar M. Annal Biochem 1999: 269: 337-341.
[40] Ramesh CK, Rehman A, Prabhakar BT, Avin VBR, Rao ASJ. J App Pharm Sci2011; 01 (07): 99-103.
[41] Duh PD, Du PC, Yen GC. Food Chem Toxicol 1999; 37: 1055–1061.
[42] Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Food Chem 2004; 84: 551–562.
[43] Qiao DL, Ke CL, Hu B, Luo JG, Ye H, Sun Y, Yan X, Zeng X. Carbohydrate Poly 2009; 78: 199–204.
[44] Shimada K, Fujikawa K, Yahara K, Nakamura T. J Agric Food Chem 1992; 40: 945–948.
[45] Chanda S, Dave R. African J Microbiol Res 2009; 3(13): 981-996.
[46] Kallithraka S, Bakker J, Clifford MN. Food Quality and Preference 2001; 12: 145–152.
[47] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford Science Publications, Oxford University Press, Oxford, 1985, pp. 385-387.
[48] Rollet-Labelle E, Grange MJ, Elbim C, Marquetty C, Gougerot-Pocidalo MA, Pasquier C. Free Rad Biol Med 1998; 24: 563–572.
[49] Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. JAPI 2004; 52: 794-804.
[50] Aboul-Enein AM, El Baz FK, El-Baroty GS, Youssef AM, Abd El-Baky HH. J Med Sci 2003; 3: 87-98.
[51] Geckil H, Ates B, Durmaz G, Erdogan S, Yilmaz I. American J Biochem Biotechnol 2005; 1: 27-31.
[52] Shimada K, Okada H, Matsuo K, Yoshioka S. Biosci Biotechnol Biochem 1996; 60: 125-127.
[53] Yuan YV, Bone DE, Carrington MF. Food Chem 2005; 91: 485- 494.
[54] Bloknina O, Virolainen E, Fagerstedt KV. Annals Botany 2003; 91: 179–194.
[55] Hong WS, Jung HY, Yang SK, Myung SJ, Kim JH, Min YI, Chung MH, Lee HS, Kim HW. Pharmacological Research 2001; 44(4): 293- 297.
[56] Li X, Chen C. International Research Journal of Pure & Applied Chemistry 2012; 2(1): 68-76.
[57] Bondet V, Brand-Williams W, Berset C. Lebensm Wiss Technol 1997; 30: 609–615.
[58] MacDonald J, Galley HF, Webster NR. British J Anaesthesia 2003; 90: 221–232.