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Molecular Weight (Mw) and Monosaccharide Composition (MC): Two Major Factors Affecting the Therapeutic Action of Polysaccharides Extracted from *Cordyceps sinensis*-Mini Review

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***Cordyceps sinensis* (Berk.) Sacc. is one of the well-described fungi that has been extensively used in traditional Chinese medicine for over 700 years. *Cordyceps sinensis* has been cultivated naturally or in artificial media. Fungal mycelia contain some polysaccharides that are responsible for its biological activity. Polysaccharides are the best known and most potent mushroom-derived substances possessing antitumor and immuno modulating properties. However, factors such as molecular weight (M_w) and the type of monosaccharide composition seems to affect the anticancerand immuno modulating properties. This short review focuses on the relationship between molecular weight (M_w) and the composition of extracted polysaccharides for its effectiveness to be used in therapeutic applications of both *in vivo* and *in vitro* conditions. This article also aims to provide some recent information as a benchmark for further researchers to study and exploit it for their future studies.**

Key words: *Cordyceps sinensis*; polysaccharide; antitumor; immuno-modulation; therapeutic mushroom.

Cordyceps is one of the famous medicinal mushroom from the family of *Clavicipitaceae* and the order Hypocreales[1-4]. To date, more than 350 species of *Cordyceps* were being discovered all around the world, which about 120 species have been originated in China[1,5]. *Cordyceps sinensis*(Berk.) Sacc.a well-known genus of *Cordyceps,* as a fungal parasite can be found in the larvae of Lepidoptera. By infecting caterpillar and devouring the host at the end of autumn, the fruiting body like grass protrudes from the head of the lifeless host in the early summer. Regarding to this specific life cycle, the 'worm-grass' or 'winter-worm and summer-grass' were chosen as its label[2]. For thousands of year, *Cordyceps* species have been used widely in China and Japan for their valuable therapeutic activities in making (general tonics and aphrodisiac). However, the slow growth rate and specific altitude condition of this medicinal mushroom are the main concern for inadequate supplies to meet the market demand.

Recently, mycelial fermentation technique and fruiting body as an alternative for steroidgenesis have been used to cultivate *Cordyceps sinensis* artificially. However, the main mechanism of fungal toxicity is not elucidated

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clearly[2]. In addition, it was found that *Cordyceps sinensis* cultivated artificially demonstrates similar bioactivities as its wild or natural type[6]. Furthermore, it is reported that *Cordyceps* and its anamorph contain various bioactive ingredients[7], such as cordycepin, polysaccharides and ergosterol with wide therapeutic effects[8]]

As a traditional Chinese medicine of luxury, compared with other medicinal fungi, *Cordyceps* and its anamorph have various unique pharmacological effects[9], reflected in the biological types and pharmacodynamic studies of *Cordyceps* polysaccharide production from the mycelia[8] and fruiting bodies *of Cordyceps* cultured in the artificial media.

Based on the previous studies, factors such as molecular weight (Mw) and composition seems to have adverse effect in the therapeutic quality and mode of action of the extracted *Cordyceps sinensis* polysaccharide (CP). The main purpose of this study is to summarize some recent available information about polysaccharide extracted from *Cordyceps sinensis,* to show its medical efficiency and relation with morphological properties such as molecular weight (M*w*) and composition to provide some information for further researchers to follow. Literatures were categorized based on the their publish date and some recent *In vitro and In vivo* tests of *Cordyceps sinensis* polysaccharide were also provided as instances.

MATERIALS AND METHODS

The state-of-art bioprocess fermentation techniques have been recently used to cultivate *Cordyceps sinensis* in the enhanced artificial medium and to overcome the issues regarding the specific altitudes and environmental situation of natural *Cordyceps* cultivation.

Molecular weight enhancement and monosaccharide composition analysis

The structure of *Cordyceps sinensis* mycelium was analyzed and elucidated by Smith degradation, methylation, acetolysis, acid hydrolysis and NMR spectroscopy (^{1}H, ^{13}C, ^{13}C-^{1}H 2D-COSY). The $_{D}$-glucan was found as a major content in *Cordyceps sinensis* mycelia. The result illustrated the structure with branched chain of $_{D}$-glucan combined of (1→4) $_{D}$-glucosyl as backbone, which carried a (1→6)-linked $_{D}$-glucosyl residue as side chain. According to IR and NMR spectra, β-$_{D}$-glucosidic linkages were reported in polysaccharide. In addition, short and exterior chains of α-(1→4)-linkage polysaccharides were indicated through a faint blue color with λ_{max}564 nm provided by $_{D}$-glucan with iodine. Finally, the molecular weight of 184 kDa of isolated and fractionated polysaccharide, named as SCP-I, was stated. But, the monosaccharide composition of the extract was not mentioned clearly[10].

In addition, an insoluble polysaccharide (CS-Pp) with main backbone likes glucan and monosaccharide composition of galactose, mannose and glucose in ratio of 1:2:21 was attained from *Cordyceps sinensis* mycelia. The branched chain of (1→3)-β-D-glucan as backbone with (1→6)-side chain was obtained for CS-Pp. As a result, for 80% of particles, less than 5 μm as a particle size (with 1.5 μm as mean diameter) was reported by particle size analysis[11].

One year after, a hetero polysaccharide (PS-A) with monosaccharide composition of D-galactose,D-mannose and D-glucose(molar ratio of 1:1:2), molecular weight of 4.6× 105 Da was water extracted from *Cordyceps sinensis* through activity-guided fractionation. (→3-α-D-Glcp-1→3-β-D-Glcp-1-3-β-D-Galp-1→) was indicated as repeating backbone unit together with the branched residues (α-D-Manp-1→) connected at the O-2 site of residue 3-α-D-Glcp-1[12].

In the same year, an exopolysaccharide (EPS) was also isolated from Cs-HK1, a *Tolypocladium* sp. mushroom extracted from natural (wild) *Cordyceps sinensis*. Later, a new polysaccharide, named as EPS-1A, was fractionated and purified using anion-exchange and gel-filtration chromatography from crude EPS. The molecular weight of 40 kDa was reported for EPS-1A[13]. Characterization of EPS-1A was performed using different methods such as GC, GC–MS, FT-IR, 1H NMR and 13C NMR along with acid hydrolysis, methylation, periodate oxidation and Smith degradation. The outcome of study showed that the monosaccharide composition of galactose, mannose and glucose with the ratio of 1.0:3.6:15.2. Regarding to the structure of polysaccharide, branched chain of (1→6)-β-D-mannose residues (≈23%) and (1→6)-β-D-glucose residues (≈77%) were detected as the backbone

with (1→6)-β-D-glucose residues and (1→6)-β-D-mannose residues as branches linked at O-3 position of (1→6)-β-D-mannose residues and ended with â-D-galactose residues[13].

To investigated on ultrasound as a well-known method, in 2010, A research study was conducted on the physicochemical property modification of high molecular weight exopolysaccharide (EPS) extracted from cultured *Cordyceps sinensis* (Cs-HK) mycelia using high-power ultrasound (20 kHz)[14]. Due to structure-function relationship (e.g. molecular weight and size) and its effects on the quality of polysaccharides immuno biological activities, treatment by ultrasonic was introduced as an appropriate method for controlled bioactive polysaccharides degradation without severe alteration of the main chemical structures. By applying an ultrasound power at 35 W/cm2 or higher, the solubility of EPS in water increased remarkably while a rapid decrease (≈85% during 10 min) in the EPS apparent and intrinsic viscosities were observed. The result showed considerable decrease of highest molecular weight and constant polysaccharide molecular weight distribution around 490 kDa. However, no substantial alteration in the main structure of EPS molecules was reported. In contrast, the EPS intrinsic viscosity was dropped by 20% in the specific condition (in 1.0 M sulfuric acid, at 50 °C for 9 h). Ultrasound was demonstrated as effective and promising methods for enhancing the solubility of bioactive polysaccharides with high molecular weight in slight conditions. Author suggested further investigation to improve the ultrasonic process conditions containing the EPS solution concentration, ultrasound power, and sonication period. Also, bioassay evaluation of sonicated EPS fractions was noted as an imperative issue. At the end, author suggested the investigation on the ultrasonic process feasibility and merits for large-scale application in terms of process efficiency and economy[14].

Also, Another study was investigated on determination of polymeric and free carbohydrates within *Cordyceps* using GC-MS and stepwise-pressurized liquid extraction (PLE) [15].Based on enhanced PLE conditions, hydrolysis and derivatization of acid, *myo*-inositol hexaacetate was used as an internal standard for quantitatively and qualitatively analysis and comparisons between 13 samples of cultured and natural *Cordyceps* containing ten monosaccharides, specifically ribose, arabinose, rhamnose, xylose, mannose, galactose, mannitol, glucose, sorbose and fructose. Interestingly, the results illustrated more than 7.99% of free mannitol and only lesser quantity of glucose in the *Cordyceps sinensis* while the ratio of 1.00:16.61~3.82:1.60~1.28 was reported for the polysaccharide extracted from *Cordyceps sinensis* with monosaccharide composition of mannose, glucose and galactose. However, in cultured *Cordyceps sinensis*, the amount of mannitol was reported lower than 5.83% and regarding free glucose, only few samples were detected with free glucose. As opposed, mannose, galactose and glucose with ratios of 1.00:3.01~1.05~1.09:3.30 and 1.00:3.01~1.05~1.09:3.30 were mainly found as main constituents of polysaccharide extracted from *Cordyceps sinensis.* Authors concluded that cultured and natural *Cordyceps* might be distinguished by hierarchical clustering analysis according to its free carbohydrate contents[15].

In 2011, AIPS and WIPS were introduced as new polysaccharides extracted and fractionated from *Cordyceps sinensis* (strain Cs-HK1) mycelial biomass using hot water and alkaline extraction methods. The main structure of α-D-glucans composed of (1-4)-linked α-D-Glcp (>60%) as backbone was found through the characterization. In addition, Regarding to molecular weight, almost equal molecular weights were recorded for both AIPS and WIPS of 1150 kDa and 1180 kDa, respectively. Furthermore, a linear glucan for AIPS, unique from the branched assemblies of most glucans from therapeutic mushrooms and short branch of (1-6)-linked α-D-Glcp (≈14%) for WIPS were detected. As a result, in aqueous solution of alkaline, WIPS and AIPS demonstrated a random coil structure alongside similar parameters of conformation except considerably distinctive polydispersity indices, 0.37versus 0.19[16].

Furthermore, Methylation analysis and 2D NMR spectroscopy were applied to elucidate the structure of *Cordyceps sinensis* bioactive hydrophilic polysaccharide (CBHP)[17]. Glucose (95.19%), galactose (0.61%) and mannose (0.91%) were revealed as main constituents in the CBHP through the monosaccharide composition analysis. In addition, the average molecular weight of 260 kDa containing 0.09% protein was reported.

Regarding to the molecular structure, the α-(1,4)-linked Glc*p*as main connection type with 65.7% , tracked by t-Glc*p* (20.7%), 1,2,3,6-Glc*p* (4.1%), 1,2,4,6-Glc*p* (3.0%), 1,3,6-Glc*p* (2.0%), 1,4,6-Glc*p* (1.6%), 1,2-Manp (1.9%) and 1,3-Galp (1.0%) were detected. According to 1D and 2D NMR analysis, the initial structure of polysaccharide was proposed as branched chain of Glc*p* linked by (1→4) and (1→3) linkages. Also, the α-terminal-$_D$-Glc*p* as side chain was detected at O-6 or O-2 (branching points) of Glc*p*. Finally, authors presented the proposed structure and they specified that the trace amounts of 1,3-Gal*p*and 1,2-Man*p*bonds are possibly placed accidentally in the side chains[17].

Medium composition effects on M_w and MC of *Cordyceps sinensis* polysaccharide

Exo-biopolymer production from molasses through *Cordyceps sinensis*16 cultivation in submerged culture was investigated thoroughly[18]. The exo-biopolymer attained from submerged culture of *Cordyceps sinensis* 16 composed of main and subunits with molecular weights of 126 and 68 kDa respectively, were reported as the main product of the study. In addition, the molasses with optimized medium composition of 0.3% K_2HPO_4,2% sucrose, 0.4% $CaCl_2$ and 0.9% yeast extract was used to produce the mycelia and exo-biopolymer from the cultures of *Cordyceps sinensis*16. As a result, high exo-biopolymer and mycelial with 28.4 and 54.0 g/l concentrations were attained through the shaking flask culture condition. Authors suggested that high-level extraction of exo-biopolymer and mycelia from *Cordyceps sinensis* 16 can be obtained in submerged culture [18].

Regarding to the optimal production of *Cordyceps sinensis* by submerged culture method, exopolysaccharide productivities was determined by Choi *et al*. (2010) in culture broth containing different sources of carbon, rice bran and citrus peel. High production of EPS with potential immuno stimulating activities were attained through the optimized medium composition of 0.5% molasses, 0.1% KH_2PO_4, 1.5% rice bran, 0.05% $MgSO_4$ and 3% CSL on the optimized condition at 25°C and 5 to 6 days culture time. Enhanced glucosamine content and EPS productivity were reported due to addition of citrus peel to the cultured *Cordyceps sinensis* under optimal conditions[19]. In addition, by comparing the anti-complementary activity between medium with or without the addition of citrus peel, the results indicated higher amount of anti-complementary activity about 58.0 to 80.8% rather than 48.2 to 68.7% for the medium with no addition of citrus peel. Furthermore, the same trend was reported for antioxidant activity (AEAC value)of the culture with citrus peel (about 284.3 to 384.6 mg/100g) that was much higher than the culture with no addition of citrus peel (about 142.8 to 219.5 mg/100g), representing the positive effect of citrus peel on the antioxidant and anti-complementary enhancement of *Cordyceps sinensis*. Also, to optimize the extraction condition and quality of EPS, a novel hetero polysaccharide from rice bran (RBPS2a) with 90 kDa molecular weight, the structure of α-(1→3)-linked $_D$-galactopyranosyl as a main chain and the highest anti-complementary activity *in vitro,* was applied (Wang *et al*. 2008). To sum up, the results showed high efficacy, enhanced antioxidant and anti-complementary activities of submerged cultured *Cordyceps sinensis* with citrus peel[19].

Hypoglycemic activity of polysaccharides with different M_w and MC

Strong hypoglycemic activities of polysaccharide extracted from *Cordyceps sinensis*, namely CSP-1, on streptozotocin (STZ)-diabetic rats and alloxan-diabetic mice was carried out [20]. Ion exchange and sizing chromatography were used to isolate polysaccharide with molecular weight of (M_w) ≈ 210 kDa from the cultured *Cordyceps* mycelia. Analysis showed that obtained polysaccharide contained glucose, galactose and mannose in the ratio of 1:0.75:0.6 respectively. In addition, there was no report on significant change in basal glucose level among normal mice. However, by administration of CSP-1 (at 200 and 400 mg/kg body wt./day for 7 days, p.o.) there was a significant decrease in the level of blood glucose by 12.0 ± 3.2% and 22.5±4.7% in normal mice ($\rho < 0.05$) was observed. Furthermore, the rapid drop in the blood glucose level of both alloxan-induced diabetic mice and STZ-induced diabetic rats after administration of CSP-1 at a dose of greater than 200 mg/kg body wt. daily for 7 days, was recorded. Also, the raise in the level of serum insulin through application of CSP-1 ($\rho < 0.05$) in diabetic rats was reported. Results showed the hypoglycemic property of CSP-1 led to the increase level of circulating insulin in diabetic

Table 1. Polysaccharides, Molecular weight (Mw) and Monosaccharide Compositions (MC)

Polysaccharide	Molecular Weight (Mw) kDa	Monosaccharide Composition (MC)	Application	Reference
CPS-1	210	Glucose, mannose and Galactose	Hypoglycemic and Antioxidation	[20]
SCP-I	184	D-glucan	Antioxidant activity	[10]
Cordyceps sinensis 16	68 to 128	Exobiopolymer from molasses	Antioxidant activity	[18]
EPSF	Not mentioned	Exobiopolymer	Immunocyte activity on H22 tumor bearing mice	[22]
EPS-1	38	β-glucan	Antioxidant activity	[27]
Cs-HK1	5 to 200	β-D-glucan	Moderately antioxidant activities	[28]
Cordysinocan	82	Glucose, mannose and galactose	Induction of the cell proliferation and the secretion of interleukin-2, interleukin-6 and interleukin-8	[23]
Cs-Pp	Not mentioned	Mannose, glucose and galactose	Antioxidant and immuno modulation activity	[11]
PS-A	460	Mannose, glucose and galactose	Antioxidant and immuno modulation activity	[12]
APSF	Not mentioned	Mannose, glucose and galactose	Stimulation of the phagocytosis macrophages	[24]
RBPS2a	90	β-(1→3)-linked D-galactopyranosyl	Antioxidant and anti-complementary activity	[19]
PLE CS PS	Not mentioned	Major: mannose, glucose and galactose Minor: rhamnose, ribose, arabinose, xylose, mannitol, fructose and sorbose	Antioxidant and immuno modulation activity	[15]
CPS-2	43.9	Mannose, glucose and galactose	Protective effects on chronic renal failure	[33]
EPS-1A	40	Mannose, glucose and galactose	Antioxidant activity	[13]
Cs-HK1+	490	β-D-glucan	Antioxidant activity	[14]
WIPS and AIPS	1180, 1150	β-D-glucan (1→4)-linked β-D-Glcp (WIPS: short branch, AIPS: linear)	Antioxidant and immuno modulation activity	[16]
CS-PS	12	Mannose, rhamnose, arabinose, xylose, glucose and galactose	Antioxidant activity	[29]
CBHP	260	Glucose, mannose and galactose	Antioxidant and immuno modulation activity	[17]
AEPS-1	36	Glucopyranose, pyrano glucuronic acid and mannose	Immunomodulatory function	[25]

Sulfated EPS-1A,B,C,D	4.1 to 17.1	Neutral polysaccharide free of than normal EPS monosaccharide, uronic acids, proteins and nucleic acids	Sulfated EPS efficacy more on biological and immuno modulatory activities	[30]
EPS PSP	16	Protein (polysaccharide -protein) dependent on protein content	Strong antioxidant activity	[31]
Poly-N-acetylhexosamine (polyhexNAc)	6	Exobiopolysaccharide	Low molecular weight but notable antioxidant activity	[32]

animals. It suggested that CSP-1 stimulates insulin pancreatic release and/or reduces insulin metabolism. At the end, the assessment on the antioxidation property of polysaccharide demonstrated the protective influential role of CSP-1 in the progress of diabetes[20].

Antitumor activity of polysaccharides with different M_w and MC

Fungus strain G1 isolated form wild (natural) type of *Cordyceps* was analyzed to confirm as an anamorph of *Cordyceps sinensis.* In addition, polysaccharide (PS) from G1 strain was extracted and its biological actions like antitumor were investigated completely by its activity on the H22-bearing mice[21]. For gavage, ICR mice treated by PS for 7 days. Later, H22 cells were subcutaneously added on right oxter of each mouse. The same administration procedure was continued for 9 days. Finally, the tumor weight of each mouse was quantified. Moreover, malondialdehyde (MDA) level and superoxide dismutase (SOD) activity in mouse brain, liver and serum together with glutathione peroxidase (GSH-Px) activity in mouse brain and liver were evaluated. Significant H22 tumor growth inhibition, SOD activity enhancement on brain, liver and serum in addition to GSH-Px activity of brain and liver in tumor–bearing rats were resulted by administrating the PS. Furthermore, reduction in the level of MDA in the brain and liver of tumor-bearing rats was reported as immuno biological activity of PS. In short, polysaccharide from G1 strain, an anamorph of *Cordyceps sinensis* with molecular weight of > 14 kDa was extracted, powdered and introduced as bioactive component with several immuno biological activities. However the composition of obtained PS was not mentioned clearly[21].

Two years later, Immunocyte activities of exopolysaccharide fraction (EPSF) extracted from an anamorph strain of *Cordyceps sinensis* on the H22 tumor bearing mice, was investigated by treating ICR mice with EPSF for 7 days through the intra peritoneal injection of EPSF at 15, 30 and 60 mg/kg as low, mid and high doses and later H22 tumor cells were placed[22]. At the final stage of assessment, the tumor weight of each mouse was measured separately. Neutral red uptakes were applied to test the mouse peritoneal macrophage phagocytosis. In addition, ELISA was used to assess expression of macrophage tumor necrosis factor (TNF)-α. Spleen lymphocytes cytotoxicity and proliferation capability were evaluated by MTT techniques. Also, RT-PCR was applied to detect the mRNA levels of spleen lymphocyte TNF-α and IFN-α mRNA. The results demonstrated significant elevated activity of immunocytes together with considerable H22 tumor progression inhibition by EPSF.In addition, splenic lymphocytes TNF-α and IFN-α mRNA expression was also elevated significantly. However, the exact composition of EPSF was not elucidated, probably because of the complex structure of extracted exopolysaccharide. In addition, authors did not mention the molecular weight or size of the extracted exopolysaccharide. They suggested that EPSF could elevate the immunocytes' activity in H22 tumor bearing mice[22].

Immunostimulating effects of polysaccharides with different M_w and MC

An exopolysaccharide with molecular weight of 82 kDa was isolated from UST 2000, a strain of *Cordyceps sinensis,* using activity-guided purification. The extracted polysaccharide, named as cordysinocan, consisted of galactose, mannose and glucose in a ratio of 1:2:2.4. The secretion of interleukin-8, interleukin-6, interleukin-2 and induction of cell proliferation were described as

cordysinocan activities in the cultured T-lymphocytes. Furthermore, transient induction of extracellular signal-regulated kinases (ERK) phosphorylation by cordysinocan treatment was reported significantly. The result of the study showed increase in the enzymatic and phagocytosis activity of acid phosphatase due to cordysinocan administration in cultured macrophages. The results consequently confirmed the significant role of polysaccharide from *Cordyceps* in activating such immune responses[23].

In 2010, Acid polysaccharide fraction (APSF) modulating effects on the murine macrophage cell line RAW264.7 was evaluated[24]. APSF as a water-soluble polysaccharide was extracted from cultivated *Cordyceps sinensis* mycelia. The immunostimulating ability of APSF on the phagocytosis of macrophages was assessed through the phagocytotic assay by FITC-dextran and neutral red internalization. After APSF treatment NO production was revealed by nitrite level in the culture supernatant quantified using Griess reagent. Also, the promoting effects of APSF on both inducible nitric oxide synthase (iNOS) protein and mRNA expressions were assessed by immuno cytochemistry and RT-PCR analyses. Furthermore, possible-stimulating actions of macrophages through IκB-NF-κB pathway activation was confirmed by the increase in the level of NF-κB in nucleuses demonstrated by Western blotting after treating by APSF. Moreover, the APSF monosaccharide composition was elucidated to be glucose, galactose and mannose at molar ratio of 1:1.5:3.5 using gas chromatography. However, molecular weight of APSF was not indicated it may be perhaps due to the complex structure of APSF[24].

In another study, an exopolysaccharide (EPS), isolated from mycelial culture of therapeutic mushroom *Cordyceps sinensis* (strain Cs-HK1), was fractionated and AEPS-1 as acidic polysaccharide was the result of fractionation [25]. AEPS-1 was characterized using chromatographic and spectral analyses along with periodate oxidation, methylation and Smith degradation derivatization. The monosaccharide composition of AEPS-1 was reported as pyranoglucuronic acid and glucopyranose (Glc*p*) with the molar ratio of 1:8 plus lesser amount of mannose. In addition, 36 kDa as an average polysaccharide molecular weight was stated for AEPS-1. Regarding to the acidic polysaccharide structure, (1→3)-linked α-D-Glc*p* residues as a linear backbone with α-D-Glc*p* and α-D-GlcUp side chains linked to the main chain through (1→6) glycosidic linkages at each seventh α-D-Glc*p* unit, was reported by characterization. Atomic force microscopy exhibited the formation of large networks in water by AEPS-1, which were linked mainly with triple helical strands. Results illustrated that administration of AEPS-1 at proper amounts (from 25 to 250 ìg/ml) in raw 264.7 macrophage cell cultures, expressively

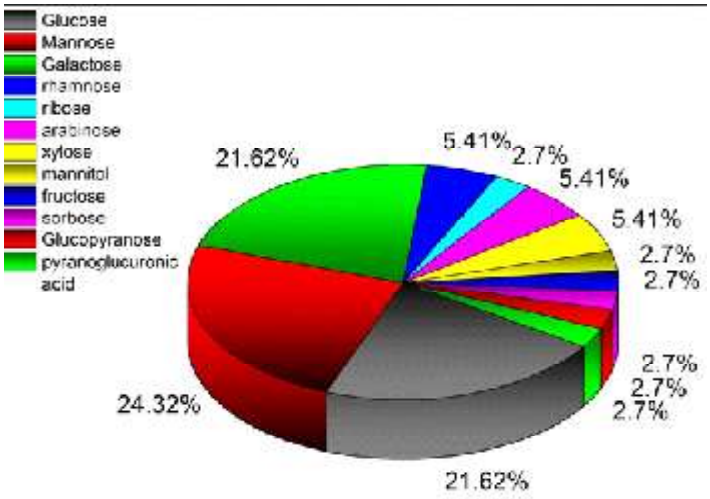

Fig. 1. Monosaccharide composition (MC) of different extracted polysaccharides from natural or cultured *Cordycepssinensis*

stimulated the major cytokine release and exhibiting immunostimulating activities[25].

Based on the previous study by Chen *et al*. (2010), aresearch was conducted to investigate more on immunostimulating activities of APSF; an acidic polysaccharide extracted from an anamorph of *Cordyceps sinensis,* on macrophages. The molecular weight and monosaccharide composition of APSF was determined and the similar values to the previous research published on 2010 were obtained[26]. Research on APSF effects on macrophages phenotypes was started by culturing the cells with culture supernatant of H22 cells to polarize Ana-1 mouse macrophages to M2 phenotype. TNF-α expression, scavenger receptor (SR) and cell surface markers mannose receptor (MR) was measured and checked to determine the M2 phenotype. After 72 h culturing with H22 supernatant, the decrease in the level of TNF-α in Ana-1 cells were observed while both MR and SR expressions were highly regulated. It proposed that the polarization of Ana-1 cells to M2 macrophages occurred correctly. Later, APSF ef6ects on M2 macrophages were explored by determining TNF-α mRNA levels, IL-12, IL-10 and inducible nitric oxide synthase (iNOS). In addition, Nuclear NF-κB was sensed using Western blotting. As a result, increase in TNF-α, IL-12, iNOS expression and decrease in IL-10 of Ana-1 cells expression through the APSF treatment was reported. Moreover, SR and MR expressions were down regulated through the APSF treatment. Also, Western blotting results presented decrease in the level of NF-κB in M2 macrophages and up regulated after treating by APSF. APSF can change M2 macrophages to M1 phenotype by NF-κB pathway activation[26].

Antioxidant activity of polysaccharides with different M_w and MC

EPS-1 as an exopolysaccharide extracted from *Cordyceps sinensis* (Cs-HK1) fungus mycelial culture with an average molecular weight of 38 kDa yielded two main molecular weight fractions of 30 kDa and 3.0 kDa when hydrolyzing with dilute acid solution at 90 °C and pH 1[27]. It is reported that EPS polydispersity (M_w/M_n) dropped gradually while the lower M_w fraction proportion increased in the defined hydrolysis period of 18% and 92% in 0.5 and 10 h, respectively. The only changes recorded were on C-O-H and C-O-C band peaks from EPS-1 through IR spectroscopy of hydrolyzed EPS fractions. Also, degradation of EPS was described by EPS hydrolysis in acidic solution through the glycosidic linkage cleavage, but no modification was found in the main molecular structure. Due to possible complex structure of EPS the exact composition was not elucidated clearly. However,

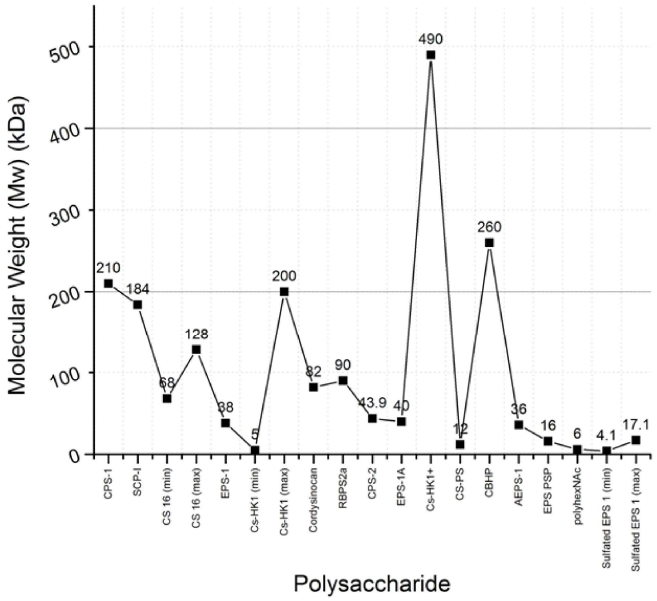

Fig. 2. Molecular weight (M*w*) distribution of different extracted polysaccharides from natural or cultured *Cordycepssinensis*

70% carbohydrates primarily composed of β-glucan and 20-25% protein were stated as isolated EPS broth compositions. The results of the study showed higher antioxidant ability (30-80%) and radical scavenging actions of hydrolyzed low molecular weight EPS fractions due to more hydroxyl groups acting as scavengers with free radical species. Eventually, for better understanding the potential value and health effects of hydrolyzed EPS fractions, more investigation on the structure-activity relationship, degradation mechanism and evaluation of EPS in biological environment like animals and cell cultures were suggested[27].

In the same year, another study on exopolysaccharide production by*Cordyceps sinensis* fungus (Cs-HK1) mycelial liquid culture investigated by Leung *et al*. (2009) at a rate proportional to mycelial growth. The complex polysaccharide-protein composition of EPS was clarified through ethanol precipitation (including 65-70% sugar and 25% protein) of the crude EPS extracted from culture medium. Interestingly, molecular weights of EPS ranging from 5 kDa to more than 200 kDa were resulted. Growth of intrinsic viscosity (index of average EPS molecular weight) during the rapid mycelial progression to 11.0 dL/g after 5 days and its stationary growth period from day 5 to day 8, were reported. Gas chromatography and IR spectrometry of acetylated EPS proposed the structure of α-$_D$-glucan as a backbone of EPS. Also, the moderate ability of plasma ferric reduction of 50-52 ìmol Fe(II)/g and antioxidant actions of EPS with Trolox equivalent antioxidant capacity of 35-40 µmolTrolox/g were informed thoroughly. Because of the complex composition and molecular structure of EPS, it is difficult to conduct chemical properties and their relationship to bioactivity. Thus, researchers suggested more investigations on the biopolymers extraction/ isolation, purification, characterization and their structure-activity relationship[28].

In 2011, Immuno biological activities, chemical composition and molecular weight effects of CS-PS, a polysaccharide from cultured *Cordyceps sinensis* fruiting body, on antioxidation activities of BALB/c mice exposed to 60Co were investigated[29]. Gel-filtration was used to determine the molecular weight of CS-Ps. In addition, gas chromatography-mass spectrophotometer (GC-MS) was applied to test the CS-PS chemical composition. First, CS-PS was administrated on mice with the amounts of 50, 100 and 200 mg/kg body weight, later exposed to 60Co. Also, irradiated and normal control group was used. On the day 4, the activity of macrophage phagocytosis, lymphocyte proliferation, concentration of malondialdehyde (MDA), delayed type hypersensitivity (DTH);cytokine expressions in serum and total-superoxide dismutase (SOD) enzyme activity from the mice were evaluated thoroughly. Extracted polysaccharide (CS-PS) was composed of six monosaccharide, rhamnose, mannose, arabinose, galactose, glucose and xylose with average molecular weight of 12 kDa. Glucose and mannose were reported as main composition monosaccharides of whole carbohydrates. After CS-PS administration, DTH and total-SOD enzyme activity, lymphocyte proliferation and the activity of macrophage phagocytosis in the CS-PS groups were expressively improved in comparison with the irradiated control group. In addition, the level of lipid peroxidation in CS-PS group was considerably dropped in comparison with irradiated control group. Similarly, administrating CS-PS influenced the level of cytokine IL-17, IL-5 and IL-4 in CS-PS group in comparison with the irradiated control group. As a result, the improving effects of CS-PS on the immunity activity in the ionizing radiation treated mice were described through the oxidative injury reduction and cytokine IL-17, IL-5 and IL-4 secretion modulation[29].

Furthermore, to improve the antioxidant activities of exopolysaccharide (EPS-1) extracted from medicinal mushroom *Cordyceps sinensis* (Cs-HK1), a research study on sulfation of EPS-1 was done recently. Sulfation of EPS-1 was performed using chlorosulfonic acid (CSA)-pyridine (Pyr) at various volumes, resulting four sulfated byproducts, SEPS-1A, B, C and D, with distinctive degree of substitution (from 0.25 to 1.38) and molecular weights ranging from 4.1 kDa to 17.1 kDa. Based on the different analyses, EPS-1 was found as a neutral polysaccharide without uronic acids, proteins, nucleic acids and monosaccharides. It was also introduced as appropriately pure bioactive compound for the sulfation trials. Due to the higher reactivity of C-6 hydroxyl groups, the EPS-1 sulfation mostly occurred at that point. Furthermore, formation of single helix was only

reported by sulfated derivatives while normal EPS-1 formed aggregated networks or random coils in aqueous solution. The results showed that increase of DS together with decrease of molecular weight led to significant rise of sulfated EPS-1 derivatives, antioxidant activities for hydroxyl radicals (•OH) and 2,2-azinobis-3-ehtylbenzothiazolin-6-sulfonic acid radicals (ABTS•+) scavenging. Finally, results demonstrated the positive effects of sulfation strategy on bioactivities and physico-chemical properties of mushroom polysaccharides[30].

Recently, gradient precipitations using ethanol with the volume ratio of 1/5, 2/5, 1, 2 and 5 were performed to isolate five different fractions P1/5 , P2/5 , P1 , P2 and P5 from the fermentation broth of therapeutic mushroom *Cordyceps sinensis*[31]. It was reported that P1/5 and P2/5 fractions primarily contained polysaccharides with minor protein content and large particle or molecular size. Also, intrinsic viscosity (η) of 2025 mL/g and hydrodynamic radius (Rh) of 905 nm were stated for above-mentioned fractions. In contrast, higher protein and minor carbohydrate content with lesser molecular size were described for the fraction attained using higher volume ratio of ethanol. Particularly, the structure with average molecular weight of 16 kDa withç = 4.3 mL/g and Rh = 23.5 nm and major composition of protein was reported to be for P5 fraction. Furthermore, an important dependence of EPS antioxidant activity on the protein content was revealed by being slight towards P1/5 and P2/5, low to moderate for P1 and P2 and extremely strong of P5. In addition, EPS key active components for the antioxidant activities were presented as low molecular weight proteins or polysaccharide-protein fractions except protein-free polysaccharide. Gradient precipitation with ethanol was confirmed as a simple and practical way for preliminary polysaccharide and protein fractionation with distinctive molecular sizes and for advance bioactive components identification. At the end, further studies on the EPS purification, polysaccharide and protein molecular structure analysis and structure-function relationships were suggested by the authors[31].

As a most recent research,poly-N-acetylhexosamine (polyhexNAc) was proposed as a novel polysaccharide with the average molecular weight of 6 kDa, which was isolated from crude exopolysaccharide fraction with low molecular weight obtained from *Cordyceps sinensis* (Cs-HK1) through the liquid fermentation[32]. Mass spectroscopy and methylation analysis were applied to determine the linkage and composition of sugar residues of new polysaccharide. Also, NMR was used to confirm chain linkage and anomeric configuration of polyhexNAc. The molecular structure was clarified by analytical methods as are repeating units of [-4-α-$_D$-ManNAc-(1→3)-α-$_D$-GalNAc-(1→] disaccharide in the main chain together with a branch of Gal appearing accidentally at the 3-position of ManNAc. Results exhibited prominent antioxidant actions of polyhexNAc with a plasma ferric reducing ability of 45.7 ìmol Fe(II)/g, a Trolox equivalent antioxidant capacity of 330 ìmol Trolox/g and important cyto-protective actions against injury of PC12 cell induced by H_2O_2. Eventually, therapeutic mushroom liquid fermentation was suggested as a promising method for effective recovery and production of new and bioactive polysaccharides. Authors stated that current study is the first report on the bioactivity and structure of an extracellular amino-polysaccharide from the *Cordyceps* species[32].

Immuno-protective effects of polysaccharides with different M_w and MC

Hot water extraction, anion-exchange and gel permeation chromatography were applied to isolate CPS-2 as a polysaccharide soluble in water[33]. After extractions, PMP pre-column derivation, periodate oxidation, methylation analysis, FT-IR and NMR spectroscopy were used to characterize the structure of isolated polysaccharide. By average, branched chain of α-(1→3)-D-mannose and α-(1→4)-D-glucose along with α-(1→4,6)-D-glucose as a side chain on every twelve residues were found to be the main structure of CPS-2. Regarding to the polysaccharide properties, the monosaccharide composition of galactose, glucose and mannose with ratio of 1:11:4 and molecular weight of 4.39×104 Da were reported for CPS-2. In order to assess the CPS-2 protective effects, specific chronic renal failure model was established using fulgerizing kidney. Finally, the alterations in blood urea nitrogen and serum creatinine demonstrated the substantial renal failure reduction by CPS-2 through the fulgerizing kidney[33].

The polysaccharides, their molecular weights and monosaccharide compositions are listed in Table1 and moreover, common biological and medicinal activities of each extraction are mentioned particularly. Based on the data presented in Table 1, mannose, glucose and galactose are present in the highest level when compared to other extracted monosaccharides. It should be noted that these ranking was made based on the results collected from the recently published works on *Cordyceps sinensis* polysaccharides, which is also being cited in this review. Logically, by focusing on the ratio of three major monosaccharides (mannose, galactose and glucose), we can generalize it to the most other polysaccharide extractions either from fruiting body or mycelia of both natural and cultured *Cordyceps sinensis*. Regarding to exopolysaccharides, mostly composed of the backbone of β-glucan or β-$_D$-glucan, which indicates the basic structure of complex extracted exopolysaccharide from *Cordyceps sinensis*.

Important note that because of complex structure of some extracted polysaccharides and the difficulty of exact structure elucidation, some research studies focused on the quality of action of polysaccharides on different bioassay, *in vitro* and *in vivo* tests rather than clarifications of individual compounds from the complex polysaccharide. Ion exchange, sizing chromatography, methylation, Smith degradation, acetolysis, NMR spectroscopy, acid hydrolysis, FTIR spectra, gas chromatography, mass spectroscopy, gel permeation chromatography, PMP pre-column derivation, periodate oxidation, ultrasonic treatment, gel filtration and atomic force microscopy are the commonly used techniques to analyze and elucidate the structure especially the molecular weight of extracted polysaccharide from *Cordyceps sinensis*.

The monosaccharide composition (MC) of various extracted polysaccharides from either natural or cultured *Cordyceps sinensis*type is shown in (Fig. 1). As mentioned before, mannose (21.43%), glucose (19.05%) and galactose (19.05%) are the highest monosaccharides in the extracted polysaccharides from *Cordyceps sinensis* among those products shown in Table 1.

Based on the data presented in (Fig.2), one can see that the M_w distribution varies from 5 kDa to 490 kDa. It indicates large variety of distribution, which makes it difficult to find an exact trend or principle for it. As mentioned above, minimum molecular weight was recorded at 5 kDa for Cs-HK1 and maximum one was registered for Cs-HK1+ at 490 kDa. Also, the wide range of molecular weight can be seen for different extractions. This condition makes us to refer to the quality of actions of various extractions, which is being stated by different authors as shown in Table 1. As we know that there are not strong statements to denote the stable relationship between the molecular weight distribution and quality of therapeutic actions of different extracted polysaccharides from *Cordyceps sinensis*, thus by referring to the data presented in Table 1, we can see that polyhexNAc (6 kDa) and EPS PSP (16 kDa) are the strong antioxidant while Cs-HK1 (5 to 200 kDa) is mentioned to have moderate antioxidant activity. Also, sulfated EPS 1-A (17.1 kDa) is stated to have more efficient immunomodulatory and biological activities[30] than normal EPS 1-A with molecular weight of 40 kDa as investigated by Yan *et al*., 2010[13].

CONCLUSIONS

Cordyceps sinensis has been used for potential benefits to immunomodulatory, biological activity and high performance therapeutic activity such as antioxidation, which retard the cellular destruction, and demonstrates anti-inflammatory activity. It also helps to protect the liver and kidneys against the damage. The analysis of *Cordyceps sinensis* has revealed the presence of natural substances like *Cordyceps sinensis* polysaccharide (CP), which is effective in repairing immune system, liver protection, hypoglycemic and hypolipidemic effects, and as antitumor agent. The factors like molecular weight (M_w) and monosaccharide composition (MC) shows key relation with *Cordyceps sinensis* polysaccharide quality of action. In this review, various extractions were studied and their M_w and molecular compositions are listed. The overall trend showed that mannose, galactose and glucose are three monosaccharides, which are the most common compounds found during most of the extraction procedures. It indicated that the quality of polysaccharides might be in relationship with molar

ratio of these three monosaccharides. In addition, various molecular weight and therapeutic activities of related polysaccharides clarified no stable relationship between high-level biological activity of the extracted polysaccharides andhigh M_w. Further investigations need to elucidate the relationship of the extracted polysaccharides Mw and their therapeutic action on diseases.

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