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The Antioxidant and Anti-Complementary Activities of Crude Polysaccharides from Trifoliate Orange (*Poncirus trifoliate*) Seeds

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ABSTRACT: In this study, I extracted the crude polysaccharides from trifoliate orange (*Poncirus trifoliate*) seeds, known as TSCP, using water extraction and ethanol precipitation. The monosaccharide composition of TSCP was in the following order: arabinose (28.28 mol%) > galactose (16.76 mol%) > galacturonic acid+glucuronic acid (13.6 mol%) > glucose (12.45 mol%) > rhamnose (4.18 mol%) > mannose (0.57 mol%) > fucose (0.32 mol%). Its total polyphenol contents were 28.66 and 70.96 μg/mL at 1 and 10 mg/mL, respectively ($P<0.01$). Further, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity of 10 mg/mL TSCP (31.67%) was higher than that of 1 mg/mL TSCP (8.07%; $P<0.01$) and also higher than its 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (11.97%) at the same concentration (10 mg/mL; $P<0.01$). The anti-complementary property of TSCP increased in a concentration-dependent manner ($P<0.001$), and at 1,000 μg/mL, it was comparable (61.36%) to the positive control (60%) consisting of polysaccharide-K. In conclusion, TSCP might be a potential immune modulator.

Keywords: anti-complementary property, antioxidant activity, crude polysaccharide, monosaccharide composition, trifoliate orange seed

INTRODUCTION

Recently, polysaccharides from diverse plant sources have been widely researched for various purposes, including medicine, food, animal feed, and cosmetics (Huang et al., 2015). Polysaccharides are natural, easily accessible, inexpensive, and non-toxic (Kaczmarek et al., 2020). Furthermore, they are biologically active with anti-inflammatory, antioxidant, anti-tumor, immuno-modulating, and antimicrobial properties (Wang et al., 2012, 2015).

Citrus fruits are abundant sources of nutrients, such as vitamins A, C, and E, and phytochemicals, including carotenoids, pectins, flavonoids, coumarins, and limonoids (Rafiq et al., 2018). Vitamin E, mainly found in the peel and seeds, protects cell membranes against lipid oxidation due to reactive oxygen species (ROS) (Rafiq et al., 2018). The juice-processing industries generate large amounts of citrus fruit residues, including peels, pulps, and seeds. Citrus fruits represent approximately 50% of the raw fruit processing industry (Anwar et al., 2008). These residues might lead to pollution, disposal, and other environmental problems due to microbial contamination (Fisher and Phillips, 2008). Therefore, several reports have explored the processing of citrus residues to recover the natural value-added components, such as fibers and bioactive components, including flavonoids and additives (Casquete et al., 2014; Sharma et al., 2017).

Citrus by-products, such as peels and seeds, can be potentially used in the food industry owing to their nutrients and bioactive compounds (Casquete et al., 2014; Sharma et al., 2017). Among them, citrus seeds have been reported to contain diverse components, such as polyphenols, phytosterols, tocopherols, and an abundance of unsaturated fatty acids, which can be valuable food additives (Anwar et al., 2008; Adeyeye and Adesina, 2015).

Furthermore, due to the increased demand for safer and more nutritious foods ingredients, researchers have been exploring natural raw materials, such as the natural value-added components from citrus residues (Anwar et al., 2008; Casquete et al., 2014; Adeyeye and Adesina, 2015). Moreover, research interest is steadily rising in uncovering novel biologically active compounds, such as the bioactive substances from citrus residues (Anwar et al., 2008; Casquete et al., 2014; Adeyeye and Adesina, 2015; Sharma et al., 2017; Rafiq et al., 2018).

Seeds are valuable food sources as they can be stored in the dried form for prolonged periods. Recently, edible

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seeds have been used to prepare food items, such as rice balls and rice cakes, in Japan (Kimura et al., 2017). Increased utilization of citrus fruits creates abundant waste, exacerbating environmental pollution. Due to the increasing demand for newer and safer food additives with special health benefits by the food processing industry, their production using by-products, including citrus seeds, has increased worldwide (Sharma et al., 2017).

Trifoliate orange or *Poncirus trifoliate* belongs to the family Rutaceae in the genus *Poncirus*, which is similar to the genus *Citrus*. The hot-water extract of these fruits has been used in traditional Oriental medicine as an anti-inflammatory and anti-allergy agent and to treat stomach disorders (Chun and Sankawa, 1989; Takase et al., 1994). Chemicals, such as limonin and imperatorin, isolated from trifoliate orange seeds, have been shown to have anticancer properties (Rahman et al., 2015). However, studies investigating the bioactive compounds in trifoliate orange seeds are relatively few. Therefore, it has been underutilized as a major residue compared with the *Citrus* genus.

Therefore, I isolated the crude polysaccharides from trifoliate orange seeds and investigated their immuno-potentiating role by evaluating their anti-complementary property and free radical quenching ability.

MATERIALS AND METHODS

Isolation of crude polysaccharides from trifoliate orange seed extraction powder (TSEP)

Trifoliate orange seeds were obtained from the Ceil Food Company. They were processed by washing under tap water and oven drying (Sanyo Electric Co., Ltd.) for 12 h at 80°C. The dried seeds were ground into powder using an electronic grinder (Hanil Electronics Corp.). One hundred of the powder was extracted by l L of distilled water with stirring at a 40°C incubator (Sanyo Electric Co., Ltd.) for 8 h. And then, it was centrifuged at 6,000 *g* for 20 min, and, the supernatant was lyophilized to give the TSEP. Then, 12 g of TSEP was dissolved in 100 mL of distilled water. The sample was precipitated by adding four volumes of 95% ethanol and centrifuging at 6,000 *g* for 30 min. The precipitate was then re-diluted using distilled water. The sample was dialyzed using a 12 kDa molecular weight cut-off filter and then centrifuged at 6,000 *g* for 30 min. The supernatant was lyophilized and used for isolating the trifoliate orange seed crude polysaccharide (TSCP).

Analysis of the sugar and protein content

We determined the content of neutral sugar using the phenol-sulfuric acid method with galactose as the standard (DuBois et al., 1956). The uronic acid content was measured using the *m*-hydroxybiphenyl method using galacturonic acid as the standard (Blumenkrantz and Asboe-Hansen, 1973). The 2-keto-3-deoxy-D-manno-2-octulosonic acid (Kdo) content was measured using the modified thiobarbituric acid method with Kdo as the reference (Karkhanis et al., 1978). The protein content was determined using Bradford's method using bovine serum albumin as the standard (Bradford, 1976). The monosaccharide content was analyzed by modifying the alditol acetate method by Jones and Albersheim (1972) using a gas chromatography (GC) system (6000 series, Young Lin Co.) equipped with an SP-2380 capillary column (0.2 μm ×0.25 mm×30 m; Supelco) and flame ionization detector. The GC temperature program was 60°C for 1 min, 60 → 220°C (30°C/min), 220°C for 12 min, 220 → 250°C (8°C/min), and 250°C for 15 min. The molar ratio of the monosaccharides was calculated using the peak areas and response factors of the slope of the standard curve for each monosaccharide.

Total polyphenol content

The total polyphenol content was measured using the Folin-Ciocalteu method (Sulc, 1984). Briefly, 0.01 mL of the appropriately diluted sample, 0.79 mL of distilled water, and 0.05 mL of Folin-Ciocalteu reagent were mixed into the test tubes. After 1 min, 20% sodium carbonate (0.15 mL) was added, and the mixture was left standing at room temperature for 2 h. The absorbance was measured at 750 nm, and the total polyphenol concentration was calculated using a calibration curve with gallic acid as the reference.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was measured based on the method by Cheung et al. (2003). Briefly, 0.2 mM DPPH ethanolic solution (0.8 mL) was added to 0.2 mL of the diluted sample, mixed vigorously, and left standing for 10 min in the dark. The decrease in absorbance was determined at 520 nm compared to a blank (without sample) using a spectrophotometer. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (1 - As/Ac) \times 100$$

where As and Ac are the absorbance values measured with and without the sample, respectively.

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging activity was measured according to the method by Re et al. (1999). Briefly, the ABTS radical was formed by adding 7 mM ABTS to a 2.45

mM potassium persulfate solution and then leaving the mixture overnight at room temperature in the dark. The ABTS radical solution was then diluted using distilled water to attain an absorbance of 1.4～1.5 at 414 nm. Then, 1 mL of properly diluted ABTS radical solution was added to 50 μL of the sample. The mixture was incubated for 1 h and its absorbance was determined at 414 nm. The ABTS radical scavenging activity was calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = (1 - As/Ac) \times 100$$

where As and Ac are the absorbance values with and without the sample, respectively.

Anti-complementary property

The anti-complementary property was assessed using a complement fixation test based on complement consumption and the degree of red blood cell lysis using the residual complement (Kabat and Mayer, 1964). Briefly, a 50 μL aliquot of the sample was mixed with an equal volume of normal human serum (NHS) and gelatin veronal buffered saline (GVB^2+; pH 7.4), containing 500 mM Mg^{2+} and 150 mM Ca^{2+}. The mixtures were then cultured at 37°C for 30 min, and the residual total hemolytic complement (TCH_{50}) was measured by IgM hemolysin-sensitized sheep erythrocytes (EA cells) at a density of 1×10^8 cells/mL. NHS was cultured with water and GVB^2+ as a negative control. Polysaccharide-K (PSK), a known immune-active polysaccharide from *Coliolus versicolar*, was used as a positive control for the anti-complementary property assay. The anti-complementary property of the samples was calculated as the percentage inhibition of the control TCH_{50}:

$$\text{Inhibition of } TCH_{50}\ (\%) = [TCH_{50}\ (\text{control}) - TCH_{50}\ (\text{treated with sample})] / TCH_{50}\ (\text{control})$$

Statistical analysis

All data were analyzed using IBM SPSS version 20.0 (IBM Corp.). Statistically significant differences among the samples were determined by *t*-test or one-way ANOVA and Duncan's multiple range test. The statistical significance level was set at $P<0.05$, $P<0.01$, and $P<0.001$.

RESULTS AND DISCUSSION

Isolating TSCP and analyzing its sugar and protein content

Polysaccharides are natural polymers in plant cell walls, animal cells, and microorganisms. They comprise diverse monosaccharide units and their derivatives (DelattreI et al., 2011). Mainly, plants contain abundant natural polysaccharides, which structurally consist of homo or hetero monosaccharides and uronic acids linked with glycosidic bonds (Li and Wang, 2015; Zhang and Wang, 2015). Their monosaccharide content varies widely (Zhang and Li, 2018). Naturally occurring polysaccharides have different monosaccharide components, molecular weight, charge properties, and glycosidic bonds, which affect their functional characteristics and enhance their applicability (Yuan et al., 2020).

Table 1. The yield of the TSCP isolated from TSEP

TSEP (g)	TSCP (g)	Yield (%)
12	0.84	7.00

TSCP, trifoliate orange seed crude polysaccharide; TSEP, trifoliate orange seed extraction powder.

Here, I isolated TSCP from the TSEP prepared using water extraction and ethanol precipitation. The final yield of TSCP was 7% (dry weight, Table 1). Table 2 shows the sugar and protein content of the TSCP. The neutral sugar and uronic acid contents were 63.8% and 13.6%, respectively. However, I did not detect any Kdo-like material. The protein content was 22.6%. Table 2 and Fig. 1 show the monosaccharide component of the TSCP that was measured using GC. The monosaccharides with the highest content was arabinose (28.28 mol%), followed by galactose (16.76 mol%) > galacturonic acid+glucuronic acid (13.6 mol%) > glucose (12.45 mol%) > rhamnose (4.18 mol%) > mannose (0.57 mol%) > fucose (0.32 mol%).

Total polyphenol content and antioxidant activities

Recent studies have shown that natural polysaccharides, including microorganisms, plants, and animals, have potential antioxidant properties (Wang et al., 2013; Huang et al., 2017). Especially the polysaccharides of plant ori-

Table 2. The chemical composition of TSCP

Variable	Value
Chemical composition (%)	
Neutral sugars	63.8±8.1
Uronic acid	13.6±1.4
Kdo-like material	0
Protein	22.6±1.5
Monosaccharide component (mol%)	
Arabinose	28.28±2.4
Galactose	16.76±3.8
Rhamnose	4.18±0.5
Xylose	1.24±0.2
Glucose	12.45±0.9
Mannose	0.57±0.1
Fucose	0.32±0.0
Galacturonic acid+glucuronic acid	13.60±1.4
Kdo	0

Values are presented as mean±SD.
TSCP, trifoliate orange seed crude polysaccharide; Kdo, 2-keto-3-deoxy-D-manno-2-octulosonic acid.

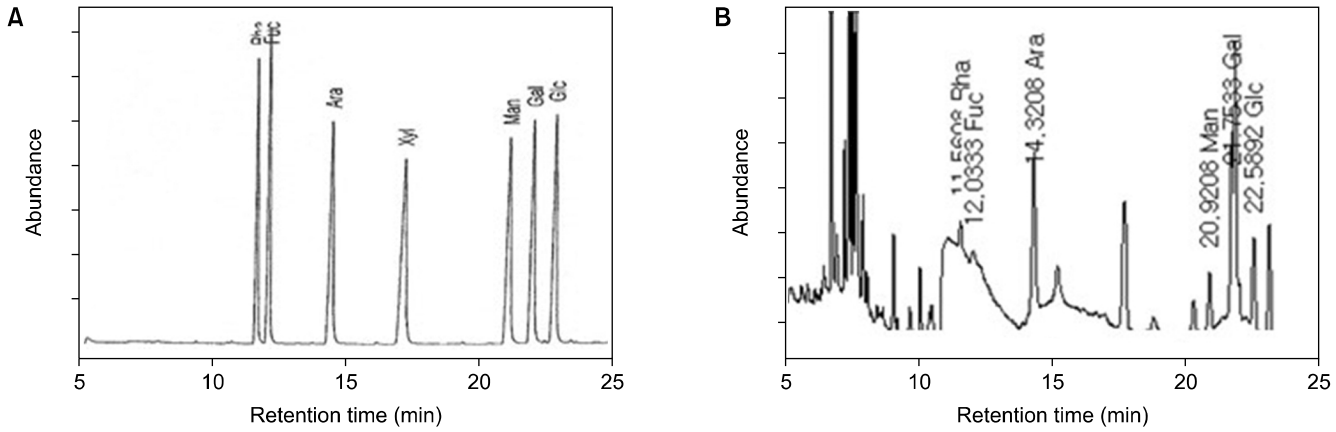

Fig. 1. Gas chromatography chromatogram of the standards (A) and the trifoliate orange seed crude polysaccharide (B) for analyzing the monosaccharide composition.

gin, such as those from leaves, seeds, roots, stems, fruits, and wood, have outstanding antioxidant potential (Krishnaiah et al., 2011). Several polysaccharides have been used to develop safe and effective food additives or medicines due to their potent antioxidant properties (Jiang et al., 2005; Zhang et al., 2011).

Phenolic compounds, such as polyhydroxylated substances, vary in structure, from simple to complex phenolics, and the degree of polymerization. The phenolic compounds with more complex structures and high molecular weights are often known as polyphenols (Balasundram et al., 2006). The total polyphenol content is commonly quantified using the Folin-Ciocalteu method (Singleton et al., 1999), which is also ideal for separating phenolic compounds (Khoddami et al., 2013). Therefore, I used this method to assess the total polyphenol content of TSCP (Fig. 2), which was 28.66 and 70.96 μg/mL for 1 and 10 mg/mL of TSCP, respectively (P<0.01).

Over accumulation of ROS species, such as superoxide ($O_2\cdot^-$) and hydrogen peroxide (H_2O_2) radicals, cause lipid peroxidation and membrane damage (Jaspers and Kangasjärvi, 2010). Antioxidants can scavenge and stabilize unstable free radicals, thus inhibiting ROS-mediated oxidation. The chemical assays used to determine the free radical scavenging property are based on the capability of certain compounds to scavenge synthetic free radicals using diverse free radical generating systems and methods for detecting oxidation endpoints. The DPPH and ABTS radical scavenging assays utilize general spectrophotometric procedures for measuring the antioxidant properties of substances (Güllçin and Daştan, 2007).

Therefore, I determined the antioxidant activities of TSCP using ABTS radical and DPPH radical scavenging assays, as presented in Fig. 3. TSCP showed higher ABTS radical scavenging ability at 10 mg/mL (31.67%) than 1 mg/mL (8.07%; P<0.01). However, there were no significant differences in the DPPH radical scavenging activity between 1 mg/mL (11.72%) and 10 mg/mL (11.97%) of TSCP (P>0.05).

Phenolic compounds are secondary metabolites in plants that have significant biological functions, including anti-

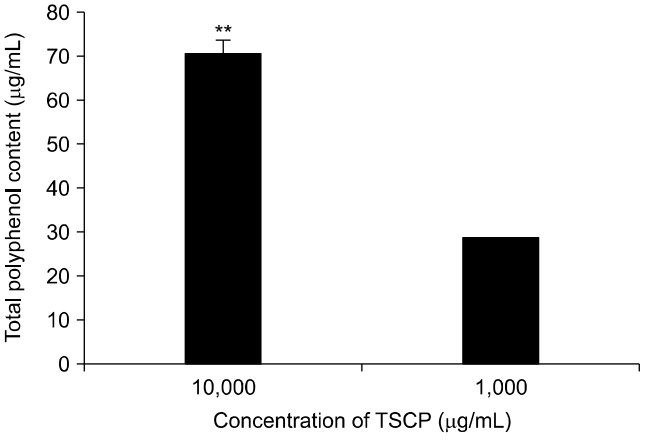

Fig. 2. The total polyphenol content of the trifoliate orange seed crude polysaccharide (TSCP). **Significant difference at P<0.01 based on t-test.

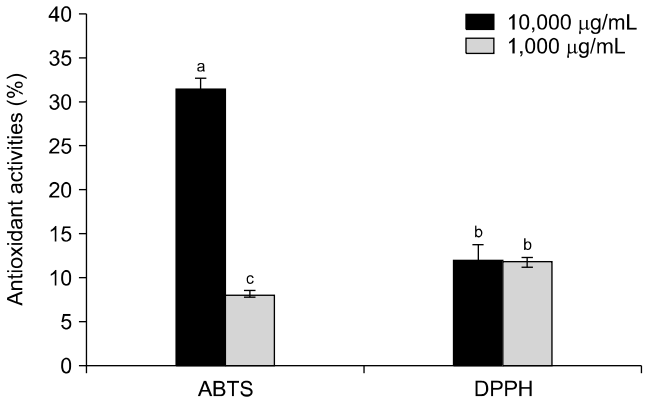

Fig. 3. Antioxidant activities of the trifoliate orange seed crude polysaccharide. ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity. P<0.01 based on ANOVA; different letters (a-c) represent significant differences among samples.

oxidant activities, activation of the endogenous immune systems, and protection against degenerative diseases (Balasundram et al., 2006). Phenolics are potent antioxidants owing to their capacity to scavenge free radicals, such as $O_2\cdot^-$, HO·, HOCl, and H_2O_2 radicals (Aruoma, 1994; 2003). These bioactive phenols are important in food products (Tepe et al., 2006). The antioxidant activity of the TSCP shown here is most likely due to these phenolic compounds. Studies have shown that the association between the total polyphenol content and the ABTS free radical scavenging activity is more robust than that with the DPPH system (Floegel et al., 2011). Here, I found significant differences in the ABTS radical scavenging activity of TSCP with low (30.42 μg/mL) and high total polyphenol contents (86.12 μg/mL). Whereas I did not observe any significant difference in the DPPH radical scavenging activity ($P>0.05$). Based on this, I suggest that TSCP is a potential natural alternative to synthetic antioxidants.

To date, the detailed mechanism underlying the antioxidant systems is poorly understood. Nevertheless, the characteristics of natural polysaccharides, such as monosaccharides content, molecular weight, water solubility, structural conformation, polarity, and intramolecular hydrogen linkages, have been linked to their antioxidant properties (Jia et al., 2013; Zhang et al., 2013). I showed that the TSCP isolated using water extraction had relatively higher monosaccharide content, with arabinose (28.28 mol%) and galactose (16.76 mol%) more abundant than other monosaccharides.

Anti-complementary property

Generally, the complement system is essential for the host's immune system to fight against pathogens, including bacteria and viruses (Yamagishi et al., 2003). This system comprises over 30 plasma- and membrane-bound proteins and is regarded as a nonspecific host immune response, which includes macrophages and lymphocytes (Reid, 1988; Fruchterman, 1998; Gan et al., 2021). Previously, several polysaccharides have been shown to have immuno-modulating properties, such as complement activation, macrophage stimulation, anti-cancer properties, and B-cell proliferation (Khan et al., 2019; Li et al., 2019; Zhao et al., 2020; Gan et al., 2021).

We determined the anti-complementary property of TSCP, as shown in Fig. 4, which showed a significant concentration-dependent increase ($P<0.001$). The values were 61.36, 44.22, and 22.9% at 1,000, 500, and 100 μg/mL of TSCP, respectively. Moreover, 1,000 μg/mL of TSCP revealed a similar anti-complementary property as the positive control (60% PSK).

In summary, I successfully isolated TSCP, a crude polysaccharide, from trifoliate orange seeds. I found that it possesses a significant immuno-modulating effect, based on its anti-complementary property and potent antioxidative potential, as analyzed using *in vitro* systems. Moreover, I also examined the sugar and protein content of TSCP. However, further studies are required using *in vivo* models to reveal the main characteristics underlying its bioactivity, including molecular weight, and glycosidic bond patterns. The details regarding the functional conformations of its sugars also need to be further analyzed.

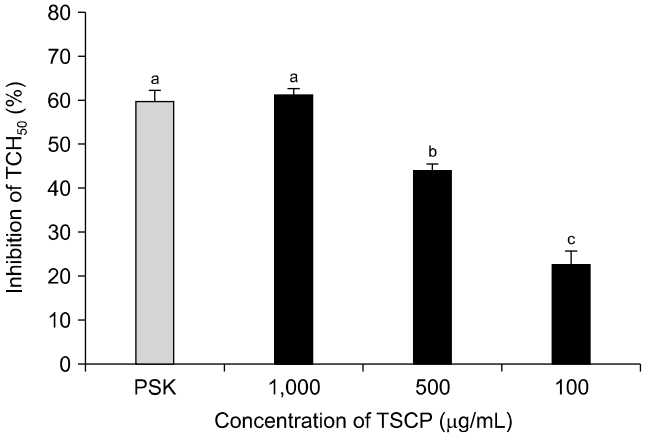

Fig. 4. Anti-complementary activities of the trifoliate orange seed crude polysaccharide (TSCP). Polysaccharide-K (PSK), a known immnoreactive polysaccharide from *Coliolus versicolar*, was used as the positive control. Significant difference at $P<0.001$ based on ANOVA, different letters (a-c) represent significant differences among samples.

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AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

REFERENCES

Adeyeye EI, Adesina AJ. Citrus seeds oils as sources of quality edible oils. Int J Curr Microbiol Appl Sci. 2015. 4:537-554.

Anwar F, Naseer R, Bhanger MI, Ashraf S, Talpur FN, Aladedunye FA. Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. J Am Oil Chem Soc. 2008. 85:321-330.

Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res. 2003. 523-524:9-20.

Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxicol. 1994. 32:671-683.

Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006. 99:191-203.

Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronic acids. Anal Biochem. 1973. 54:484-489.

Bradford MM. A rapid and sensitive method for the quantitation

of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976. 72:248-254.
Casquete R, Castro SM, Villalobos MC, Serradilla MJ, Queirós RR, Saraiva JA, et al. High pressure extraction of phenolic compounds from citrus peels. High Press Res. 2014. 34:447-451.
Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 2003. 81: 249-255.
Chun YT, Sankawa U. Screening of antiallergic effect in traditional medicinal drugs and active constituents of Aurantii Fructus Immaturus. Jpn J Pharmacogn. 1989. 43:314-323.
DelattreI C, Fenoradosoa TA, MichaudI P. Galactans: an overview of their most important sourcing and applications as natural polysaccharides. Braz Arch Biol Technol. 2011. 54:1075-1092.
DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956. 28:350-356.
Fisher K, Phillips C. Potential antimicrobial uses of essential oils in food: is citrus the answer?. Trends Food Sci Technol. 2008. 19:156-164.
Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compos Anal. 2011. 24: 1043-1048.
Fruchterman TM, Spain DA, Wilson MA, Harris PD, Garrison RN. Complement inhibition prevents gut ischemia and endothelial cell dysfunction after hemorrhage/resuscitation. Surgery. 1998. 124:782-791.
Gan T, Feng C, Lan H, Yang R, Zhang J, Li C, et al. Comparison of the structure and immunomodulatory activity of polysaccharides from fresh and dried longan. J Funct Foods. 2021. 76: 104323. https://doi.org/10.1016/j.jff.2020.104323
Güllçin I, Daştan A. Synthesis of dimeric phenol derivatives and determination of *in vitro* antioxidant and radical scavenging activities. J Enzyme Inhib Med Chem. 2007. 22:685-695.
Huang G, Mei X, Hu J. The antioxidant activities of natural polysaccharides. Curr Drug Targets. 2017. 18:1296-1300.
Huang G, Mei X, Xiao F, Chen X, Tang Q, Peng D. Applications of important polysaccharides in drug delivery. Curr Pharm Des. 2015. 21:3692-3696.
Jaspers P, Kangasjärvi J. Reactive oxygen species in abiotic stress signaling. Physiol Plant. 2010. 138:405-413.
Jia S, Li F, Liu Y, Ren H, Gong G, Wang Y, et al. Effects of extraction methods on the antioxidant activities of polysaccharides from *Agaricus blazei* Murrill. Int J Biol Macromol. 2013. 62:66-69.
Jiang YH, Jiang XL, Wang P, Hu XK. *In vitro* antioxidant activities of water-soluble polysaccharides extracted from *Isaria farinosa* B05. J Food Biochem. 2005. 29:323-335.
Jones TM, Albersheim P. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. Plant Physiol. 1972. 49:926-936.
Kabat EA, Mayer MM. Kabat and Mayer's experimental immunochemistry: with chapters on complement and complement fixation and Kjeldahl nitrogen determination. 2nd ed. Charles C. Thomas. 1964. p 133.
Kaczmarek B, Nadolna K, Owczarek A. The physical and chemical properties of hydrogels based on natural polymers. In: Chen Y, editor. Hydrogels Based on Natural Polymers. Elsevier. 2020. p 151-172.
Karkhanis YD, Zeltner JY, Jackson JJ, Carlo DJ. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of gram-negative bacteria. Anal Biochem. 1978. 85:595-601.
Khan T, Date A, Chawda H, Patel K. Polysaccharides as potential anticancer agents-a review of their progress. Carbohydr Polym. 2019. 210:412-428.
Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules. 2013. 18:2328-2375.
Kimura H, Ogawa S, Ishihara T, Maruoka M, Tokuyama-Nakai S, Jisaka M, et al. Antioxidant activities and structural characterization of flavonol O-glycosides from seeds of Japanese horse chestnut (*Aesculus turbinata* BLUME). Food Chem. 2017. 228: 348-355.
Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011. 89:217-233.
Li P, Wang F. Polysaccharides: Candidates of promising vaccine adjuvants. Drug Discov Ther. 2015. 9:88-93.
Li W, Song K, Wang S, Zhang C, Zhuang M, Wang Y, et al. Anti-tumor potential of astragalus polysaccharides on breast cancer cell line mediated by macrophage activation. Mater Sci Eng C Mater Biol Appl. 2019. 98:685-695.
Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Nayik GA. Citrus peel as a source of functional ingredient: A review. J Saudi Soc Agric Sci. 2018. 17:351-358.
Rahman A, Siddiqui SA, Jakhar R, Kang SC. Growth inhibition of various human cancer cell lines by imperatorin and limonin from *Poncirus trifoliata* Rafin. seeds. Anticancer Agents Med Chem. 2015. 15:236-241.
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999. 26:1231-1237.
Reid KBM. The complement system. In: Hames BD, Glover DM, editors. Molecular Immunology. IRL Press. 1988. p 189.
Sharma K, Mahato N, Cho MH, Lee YR. Converting citrus wastes into value-added products: Economic and environmently friendly approaches. Nutrition. 2017. 34:29-46.
Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999. 299: 152-178.
Sulc D. Ernährungsphysiologische und verfahrenstechnische aspekte bei der herstellung von milchsauer vergorenen gemüsesäften. Flussiges Obst. 1984. 51:17-24.
Takase H, Yamamoto K, Hirano H, Saito Y, Yamashita A. Pharmacological profile of gastric mucosal protection by marmin and nobiletin from a traditional herbal medicine, *Aurantii fructus* Immaturus. Jpn J Pharmacol. 1994. 66:139-147.
Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. Food Chem. 2006. 95:200-204.
Wang H, Liu YM, Qi ZM, Wang SY, Liu SX, Li X, et al. An overview on natural polysaccharides with antioxidant properties. Curr Med Chem. 2013. 20:2899-2913.
Wang Y, Li Y, Liu Y, Chen X, Wei X. Extraction, characterization and antioxidant activities of Se-enriched tea polysaccharides. Int J Biol Macromol. 2015. 77:76-84.
Wang Y, Mo Q, Li Z, Lai H, Lou J, Liu S, et al. Effects of degree of carboxymethylation on physicochemical and biological properties of pachyman. Int J Biol Macromol. 2012. 51:1052-1056.
Yamagishi T, Tsuboi T, Kikuchi K. Potent natural immunomodulator, rice water-soluble polysaccharide fractions with anticomplementary activity. Cereal Chem. 2003. 80:5-8.
Yuan Q, Li H, Wei Z, Lv K, Gao C, Liu Y, et al. Isolation, structures and biological activities of polysaccharides from *Chlorella*: A review. Int J Biol Macromol. 2020. 163:2199-2209.
Zhang N, Chen H, Ma L, Zhang Y. Physical modifications of polysaccharide from *Inonotus obliquus* and the antioxidant properties. Int J Biol Macromol. 2013. 54:209-215.
Zhang S, Li X. Hypoglycemic activity *in vitro* of polysaccharides from *Camellia oleifera* Abel. seed cake. Int J Biol Macromol. 2018. 115:811-819.
Zhang Y, Lu X, Fu Z, Wang Z, Zhang J. Sulphated modification

of a polysaccharide obtained from fresh persimmon (*Diospyros kaki* L.) fruit and antioxidant activities of the sulphated derivatives. Food Chem. 2011. 127:1084-1090.
Zhang Y, Wang F. Carbohydrate drugs: current status and development prospect. Drug Discov Ther. 2015. 9:79-87.
Zhao Y, Yan B, Wang Z, Li M, Zhao W. Natural polysaccharides with immunomodulatory activities. Mini Rev Med Chem. 2020. 20:96-106.