Journal of Pharmaceutical Research International

32(24): 83-92, 2020; Article no.JPRI.61146
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

The Content, Purification Degree, and Molecular Weight of Inulin of Natural Dangshen Roots (*Codonopsis javanica*) in Highland Lam Vien, Vietnam

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i2430813
Editor(s):
(1) Dr. Win Myint Oo, SEGi University, Malaysia.
Reviewers:
(1) Yip Foo Win, Universiti Tunku Abdul Rahman, Malaysia.
(2) Idih Favour Moses, Kogi State University, Nigeria.
(3) Zohra Mohammedi, University of Mascara, Algeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61146

Original Research Article

Received 10 July 2020
Accepted 17 September 2020
Published 15 October 2020

ABSTRACT

Introduction: Inulins are a group of natural active polysaccharides found in ginseng and dangshen and mainly used in pharmaceutical preparations and functional food. The purification condition of inulin from dangshen grown in Vietnam did not occur in previous studies. Hence, the study presented on the content, purification degree, molecular weight, and functional group characteristics of inulin extracted from natural dangshen roots (*Codonopsis javanica*) in other purification conditions.
Methods: Some factors survey impact purification conditions of inulin, for example, the kinds and the concentration of the solvent, the temperature and the times of the precipitation, and the active coal impact. The objects such as the content of inulin, fructan, and crude polysaccharide, purification degree of inulin, and fructan were analyzed. Molecular weight and functional group

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characteristics of purification inulin extracted from dangshen roots (*Codonopsis javanica*) grown in highland Lam Vien, Vietnam, were also analyzed.

Results: The purification degree of inulin purified by using ethanol was higher than that using active coal, corresponding to 97.85a ± 0.84 % and 94.05 %, respectively. Inulin content was the large ratio in crude polysaccharide, exhibited via FTIR and the analysis results. Inulin and fructan were the most precipitated in ethanol, for example, 80 % and 90 % ethanol, respectively, compared to another solvent. The kinds and the concentration of the solvent, the temperature and the precipitation times, and active coal impacted the content and the purification degree of inulin and fructan ($p < 0.05$). The precipitation of inulin and fructan in ethanol solvent was affected by the temperature. The molecular weight of inulin and fructan corresponded to 3,193 Da and 1,112,892 Da, respectively.

Conclusion: Inulin of natural dangshen roots in Lam Vien highland, Vietnam was useful for functional foods and pharmaceutics.

Keywords: Inulin; fructan; dangshen; Codonopsis javanica; purification.

1. INTRODUCTION

Dangshen (*Codonopsis javanica*) is a useful medicinal plant for humans, and naturally distributes in Vietnam with a height of over 800 m. They are classified into the genus *Codonopsis* and mainly contained inulins and fructose oligosaccharides (FOS) that possessed different bioactivities. For example, the improved ability of the immunity system [1,2], anticancer [3-6], probiotic bacteria support [7], prebiotic role [6-8], the treatment of the intestinal disease (infections, colon cancer, inflammatory bowel, the irritable bowel syndrome), Crohn's disease [1], anti-inflammatory [9], anti-hypertensive, reducing triglycerides and glucose in the blood, and cardiovascular disease-preventing [5,6,9], vaccine excipient, increasing calcium absorption and mineralization in bone, limiting osteoporosis [9,10].

Therefore, dangshen was known as a medical resource for the production of functional food and synbiotic [8,11,12]. Inulin and FOS played a role in the absorption improvement of calcium and magnesium but did not change their balance. FOS does not increase the risk for humans, for example, disease, death, or organ toxicity. The fibers did not cause mutagenic, carcinogenic, or teratogenic [8]. Hence, fructose-rich polysaccharides (inulin and FOS) are diverse structures and bioactivities. The complex of inulin (DP 10-70) and FOS (DP 3-10) forms fructan in dangshen [3,11,13-15]. The content and the characterization of inulin and fructan depended on the extracting conditions and initial material [11]. The bioactivities of inulin and fructan were better as the polysaccharides composed of short and long-chain [5]. The content and the purification degree of inulin depended on the solvent (kinds, concentration), the temperature, the time, and the solvent-to-material ratio [16]. However, the impact of the factors in the purification precipitation of inulin and fructan did not exhibit in previous studies.

Hence, the study will focused on the purification degree, and molecular weight of the inulin extracting from dangshen roots (*Codonopsis javanica*) that naturally found highland Lam Vien, Vietnam with some different conditions of the purification process.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Natural dangshen roots in 3 growth years were cleaned, dried at 55°C until the moisture less than 10%. Dried dangshen was removed fatty acids for 6 hours according to the heat reflux method with *n*-hexane [17] and for 15 minutes with absolute ethanol according to 1/9 (w/v) for removing the supernatant (pigments). The residue was extracted for 36 minutes at 71°C according to the ratio of 1/47 (w/v) with the supersonic. The extract selection was then through the Whatman No. 4 paper (20 – 25 µm) and stored at – 52°C for further studies.

2.2 Experience Design

2.2.1 Determining of the precipitation solvent of inulin and fructan

This stage studied the effect of the kinds and the concentration of solvent on inulin and fructan.

Determination of the precipitation solvent kinds of inulin and fructan was as follows: The filtrate was continuously in turn mixed with different polarity solvents such as acetone, ethanol, ethyl acetate,

n-butanol, and *n*-hexane, respectively, for collecting the residue. The residue was dissolved into the water and mixed with 0.1% trichloroacetic acid for the movement of protein. The supernatant was added to absolute ethanol according to the supernatant-to-ethanol ratio of 1/4 and 1/9 (v/v), in turn, for the residue collection [14]. The content of inulin, fructan, and crude polysaccharide was determined and calculated for all different residues (Fig. 1).

Determination of the precipitation solvent concentration of inulin and fructan was following: After supersonic extraction, the supernatant was, in turn, mixed 60, 70, 80, and 90% ethanol, respectively, for 24 hours at the lower temperature of 8 ± 1^{o}C for collecting the residue that was determined the content of inulin, fructan, and crude polysaccharide (Fig. 1).

2.2.2 Determining of the precipitation temperature of inulin and fructan

After supersonic extraction, the extract was, in turn, added to absolute ethanol according to the extract-to-ethanol ratio of 1/4 and 1/9 (v/v), respectively. All mixtures were, in turn, kept at the lower temperature of (8 ± 1)^{o}C, - (11 ± 1)^{o}C, and - (17 ± 1)^{o}C for 12 hours, respectively. The mixture was then centrifugated at -16^{o}C for 15 minutes at 13,000 rpm for the residue and drying the residue at 55^{o}C. The content of inulin, fructan, and crude polysaccharide in all residues was determined and calculated (Fig. 1).

2.2.3 Determining the precipitation times of inulin and fructan

After centrifugation, the residue was continuously dissolved into the distilled water and added to ethanol until 80% ethanol in the mixture that kept for 24 hours at (8 ± 1)^{o}C. The precipitation separation was then via centrifugation and analyzed the content of inulin and fructan in the residue. This cycle repetition was up to 6 times, described in Fig. 1.

2.2.4 Purification of inulin using active coal

After filter using the membrane (PTFE 0.45 µm), the extract was adjusted to pH 10 by using 0.2% $Ca(OH)_2$ and kept for 60 minutes at 25^{o}C for the supernatant collection that continuously adjusted to pH 8 - 8.5 by 0.8 M oxalic acid and hold for 60 minutes for the filter selection that concentrated at 55^{o}C. Then, adding 80% ethanol was into the concentrated filter for keeping at 4-8^{o}C for 24 hours, and centrifugated at 13,000 rpm at 16^{o}C for 15 minutes for selecting purification inulin. The content of inulin and polysaccharide and purification degree of inulin was also determined and calculated (Fig. 1).

2.3 Quantification of Polysaccharide Content

2.3.1 Crude polysaccharide content

Crude polysaccharide content was quantified according to the Dubois method and calculated with glucose standard at the wavelength of 486 nm [18].

2.3.2 The content of inulin and fructan

The content of inulin and fructan was quantified by spectrophotometer at 483 nm according to the method of Pencheva et al. [19] and calculated with the fructose standard.

2.4 Purification Degree and Determination of Inulin and Fructan

Purification degree of inulin and fructan was calculated on crude polysaccharide content and determined on the thin-layer chromatography of Silica gel 60 F_{254} [20] with R_f=a/b. Acetic acid:$CHCl_3$:H_2O according to the ratio (7:6:1, v/v/v) was as the mobile phase. 0.2% resorcinol in 10.0% sulfuric acid was spray solution.

2.5 Determination of Inulin Molecular Weight and Structure Characteristics

The molecular weight of inulin was determined using HP Hewlett Packard Series 1050 HPLC with the column SHODEX OHpak KB – 804 (8.0 x 300.0 mm). The structure characteristics of inulin was measured using the machine Nicolet Impact 410 FTIR spectrometer.

2.6 Data Analysis

Using the Duncan method for the movement of unnormal value and the analysis of statistics and ANOVA was by using MS Excel software. The results were exhibited and expressed as mean ± SD.

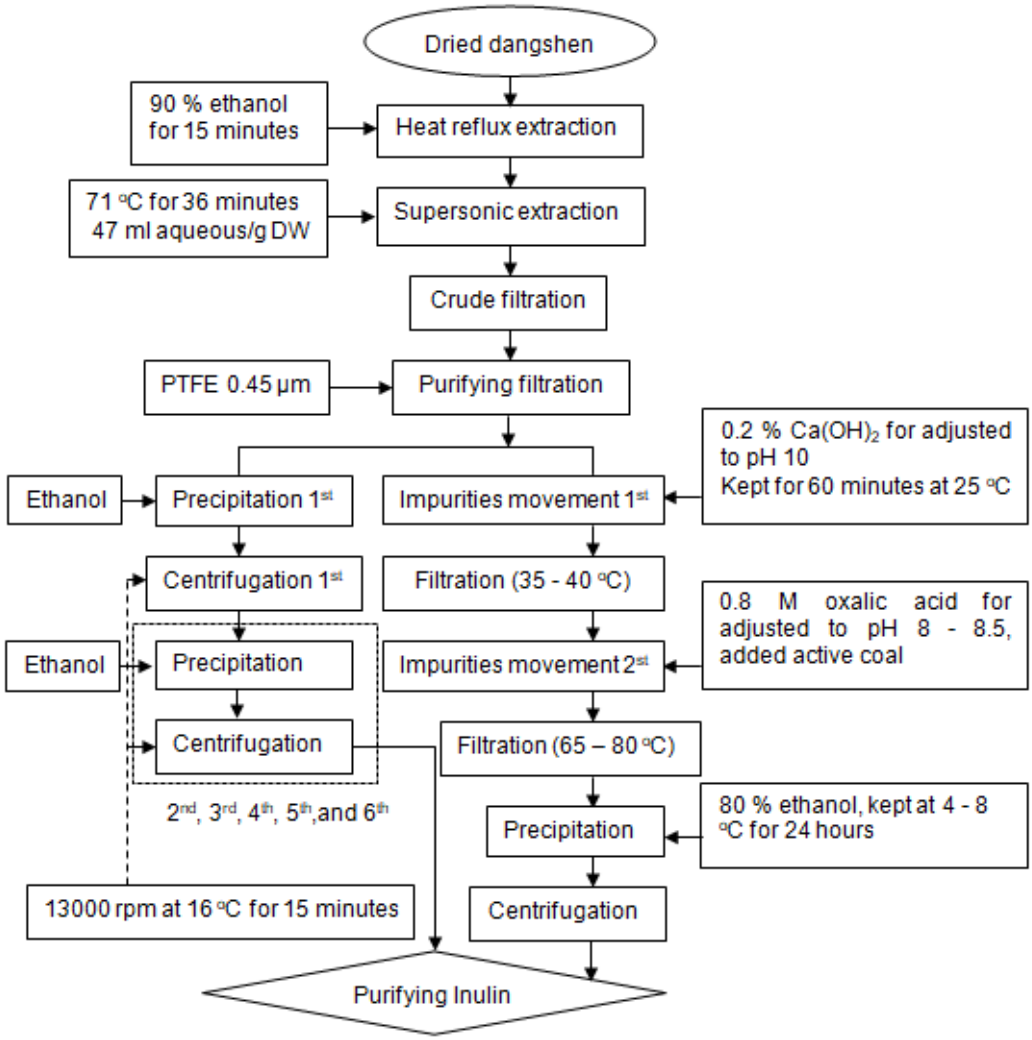

Fig. 1. Diagram of inulin purification from dangshen *Codonopsis javanica*

3. RESULTS AND DISCUSSION

3.1 Effect of the Precipitation Solvent of Inulin and Fructan

The weight of crude polysaccharide precipitating in 90 % ethanol (294.08^{a} ± 6.51) (mg glucose equivalent/g DW) was less than that one in 80% ethanol (233.07^{a} ± 4.97) (mg glucose equivalent/g DW). The precipitating weight of crude polysaccharide depended on the polarity of the solvent and decreased in following order: acetone, ethanol, ethyl acetate, *n*-butanol, and *n*-hexane. The weight of crude polysaccharide precipitating in *n*-hexane was 70.05, 31.12, 7.69, and 7.32 %, compared to n- butanol, ethyl acetate, ethanol, and acetone. The results exhibited inulin and fructan content decreased according to the polarity decrease of the solvent (Table 1). Inulin precipitating by 80% ethanol was 0.77, 0.80, 0.47, 0.61, and 0.51 times of fructan precipitating by 90% ethanol, respectively. After precipitating by acetone and 96% ethanol, the significant difference did not occur ($p > 0.05$) for the precipitation of the crude polysaccharide content continuously by 80% and 90% ethanol.

The solvent polarity caused on the difference in the solid-precipitated content consisting of inulin and fructan. The significant difference in fructan content between the different solvent occurred ($p < 0.05$). The thing was found in the content inulin and crude polysaccharide, except for acetone and ethanol. Inulin was fully precipitated as using

Table 1. The precipitation of the crude polysaccharide, fructan, and inulin in other solvents

Solvent	80% ethanol			90% ethanol		
	Inulin content (mg fructose equivalent/g DW)	Crude polysaccharide (mg glucose equivalent/g DW)	Purification degree (%)	Fructan content (mg fructose equivalent/g DW)	Crude polysaccharide (mg glucose equivalent/g DW)	Purification degree (%)
Acetone	232.92^{a} ± 5.02	244.92^{a} ± 5.28	95.10	301.81^{a} ± 12.30	312.43^{a} ± 12.73	96.60
Ethanol	222.66^{a} ± 5.09	233.07^{a} ± 4.97	95.53	278.90^{b} ± 2.16	294.08^{a} ± 6.51	94.84
Ethyl acetate	46.50^{b} ± 3.87	57.58^{b} ± 6.20	80.76	98.65^{c} ± 5.41	118.08^{b} ± 5.44	83.54
n-butanol	22.54^{c} ± 2.84	25.58^{c} ± 3.79	88.12	36.99^{d} ± 2.39	44.83^{c} ± 2.84	82.51
n-hexane	12.32^{d} ± 1.29	17.92^{d} ± 0.58	68.75	24.25^{e} ± 2.16	33.33^{d} ± 2.92	72.76

Note: Different words in the same column were statistical significance ($p < 0.05$)

80% ethanol because of the low molecular weight of inulin [14]. Ethanol is useful for precipitating polysaccharide in food because of their safety, compared to other solvents (*n*-hexane and acetone) [14,19]. Crude polysaccharide precipitation depended on the solvent concentration ($p < 0.05$), decreased according to the decreasing trend of the solvent concentration and the most precipitated as using 90% ethanol (294.08 mg glucose equivalent/g DW), following 80% ethanol ($232.07^a \pm 4.97$ mg glucose equivalent/g DW). Crude polysaccharide precipitating at 80% ethanol corresponded to 78.91% of total polysaccharide in the extract and 100% at 90%. Methanol, ethanol, and acetone were useful for the sellecting precipitation of the length chain (DP 25-40) [21]. Ethanol concentration was the important factor for the decision of the precipitating yield [21]. The structure characteristics and molecular size also affect the precipitation of naturally polysaccharide in ethanol solvent. Basing on the characteristics, it was easy on the movement of free sugar, pigments, low weight polysaccharide, and amino acid [22].

3.2 Effect of the Precipitation Temperature for Inulin and Fructan

Inulin content precipitating in aqueous extract was a positive ratio to the temperature decrease, got the highest value ($86.23^a \pm 1.66$ mg fructose equivalent/g DW) at (-17°C), following ($36.98^b \pm 2.43$ mg fructose equivalent/g DW) at (-11°C), and the lowest value ($22.33^c \pm 2.04$ mg fructose equivalent/g DW) at 4°C. The precipitation of inulin in aqueous extract containing ethanol was higher than that one containing non-ethanol as keeping at the same temperature and finding a significant difference ($p < 0.05$) (Table 2). The content of inulin and fructan precipitating in 80% and 90 % ethanol at different temperatures was a non-significant difference. The precipitating yield of inulin and fructan was from 95.53 to 96.20% and 94.84 to 95.12%, respectively. Inulin content corresponded to $222.66^a \pm 5.09$ mg fructose equivalent/g DW as using 80% ethanol and keeping at 4°C for precipitating inulin, corresponding to 95.53% of crude polysaccharide. Therefore, inulin was the main composition of crude polysaccharide and dangshen roots. The separation according to the molecular weight usually occurred in the freeze or cold processing, so inulin dissolved easily into hot water [23] and precipitated in cold water [12], and the almost precipitation of inulin was at 4°C [13].

The analysis results showed that inulin and fructan possessed the molecular weight of 3,193 Da and 1,112,892 Da, respectively (Table 3 and Fig. 2). According to Mensink et al. [11] and Shoaib et al. [1], the molecular weight of inulin and fructan was from $5.0x10^2$ to $1.3x10^4$ Da and $1x10^4$ to $1x10^8$ Da, suitable to the current study.

3.3 Effect of the Precipitation Times Using 80% Etoh and Active Coal on the Content and the Purification Degree of Inulin

Inulin of dangshen was white colour that ensured the purification of inulin. The purification degree of inulin got ($75.08^c \pm 0.89$)%, ($84.84^b \pm 4.99$)%, and ($97.85^a \pm 0.05$)%, corresponding to the precipitation times of fourth, fifth, and sixth. The significant difference in inulin content between other precipitations occurred ($p < 0.05$) (Table 4).

The content of inulin and purification polysaccharide corresponded to 179.84 ± 5.61 and 199.67 ± 4.46 mg fructose equivalent/g DW, respectively, as in turn, using 2% $Ca(OH)_2$, 0.8 M oxalic acid, and active coal for aqueous extract, and precipitating inulin and polysaccharide by 80 % ethanol for an aqueous extract that filtered via the membrane PTFE 0.45 µm. Therefore, the purification degree of inulin

Table 2. Effect of temperature and solvents on the precipitation of inulin and fructan

Temperature (°C)	Aqueous	Ethanol			
		80%		90%	
	Inulin content (mg fructose equivalent/g DW)	Inulin content (mg fructose equivalent/g DW)	Purification degree (%)	Fructan content (mg fructose equivalent/g DW)	Purification degree (%)
4-8	$22.33^c \pm 2.04$	$222.66^a \pm 5.09$	95.53	$278.90^a \pm 2.16$	94.84
-(11-12)	$36.98^b \pm 2.43$	$224.19^a \pm 8.71$	96.19	$279.67^a \pm 4.80$	95.10
-(17-18)	$86.23^a \pm 1.66$	$224.21^a \pm 5.38$	96.20	$279.73^a \pm 6.16$	95.12

Note: Different words in the same colume exhibited significant difference ($p < 0.05$)

Table 3. Some parameters of the spectrum of inulin and fructan

Order	Rt (min)	Peak area	Area percent of peak (%)	Molecular weight (Da)
1	4.919	247.161	3.552	1,112,892
2	8.673	6711.200	96.448	3,193

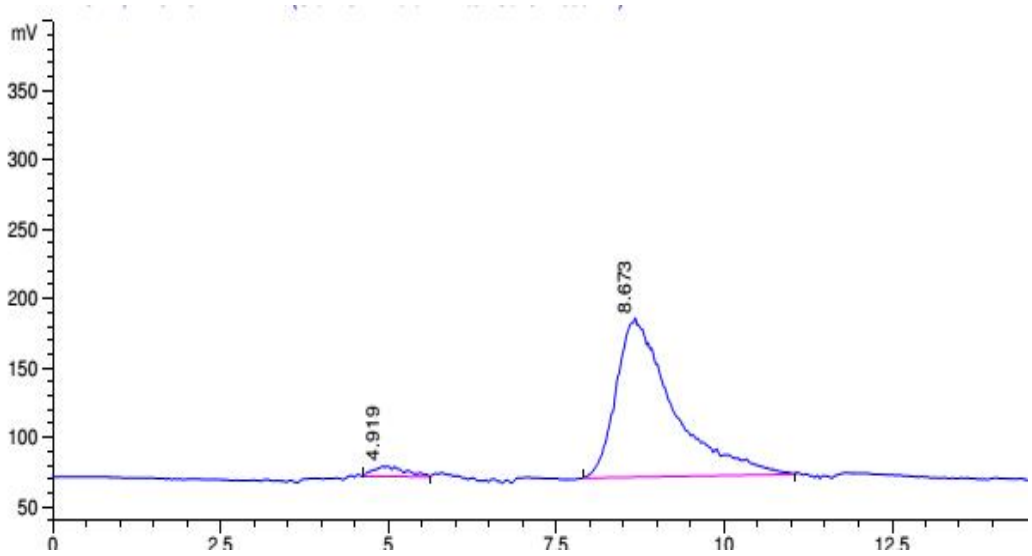

Fig. 2. The chromatogram of purified inulin from dangshen *Codonopsis javanica*

Table 4. The content and the purification degree of inulin after times 4th, 5th, and 6th of precipitation

Times of precipitation	Crude polysaccharide content (mg glucose equivalent/g DW)	Purification using 80% ethanol	
		Purification inulin content (mg fructose equivalent/g DW)	Purification degree (%)
4	$816.67^{a} \pm 28.27$	$613.07^{c} \pm 17.52$	$75.08^{c} \pm 0.89$
5	$783.33^{ab} \pm 28.87$	$663.95^{b} \pm 29.63$	$84.84^{b} \pm 4.99$
6	$746.67^{b} \pm 5.77$	$730.61^{a} \pm 4.02$	$97.85^{a} \pm 0.84$

Note: Different words in the same column were statistical significance ($p < 0.05$)

was from 94 to 98% and 86.35 to 94.05%, corresponding to six precipitation times and using active coal, respectively. The analysis results for Rf of the standard of glucose, fructose, sucrose, and inulin were similar to Rf of inulin and fructan (Rf from 0.411 to 0.415) that purified by ethanol and active coal, respectively.

FTIR spectrum of inulin that purified by using ethanol exhibited the accuracy of bands at 3180 - 3545 cm^{-1}, 2932 cm^{-1}, 2100 cm^{-1}, 1640 cm^{-1}, 1412 cm^{-1}, 1322 cm^{-1}, 1136 cm^{-1}, 1043 cm^{-1}, 927 cm^{-1}, 865 cm^{-1}, and 648 cm^{-1}. The peaks in the range at 3180 - 3545 cm^{-1} exhibited the vibration of the H–bonding and the hydroxyl stretching. The peak 2932 cm^{-1} exhibited the assign of CH and C-H stretching vibration, corresponding to the group of methoxy compounds, similar to the previous study [24]. The peak 2100 cm^{-1} presented to the C–H bonds, described by Aurea et al. [25]. The carbonyl groups stretching (C=O) occurred in the spectrum via the peak 1640 cm^{-1}, described by Rengasamy et al. [26]. The peak 1412 cm^{-1} related to C–C stretch (in-ring) of benzene, found in the current study.. The stretching of C-O groups presented at the peak 1322 cm^{-1} following Luca et al. [27] (Fig. 3). The peak 1043 cm^{-1} belonged to the vibration of hydroxyl groups [27]. The stretching of =C–H and $=CH_2$ and the C–H bend (rocking) of –C≡C–H appeared at the peak 927 cm^{-1} and 648 cm^{-1}, respectively [28]. The peak 865 cm^{-1} and the peak 1136 cm^{-1} were similar to the C-O-C stretching of the fructopyranose rings and the glycosidic linkages of inulin, respectively, described by Amir et al. [29].

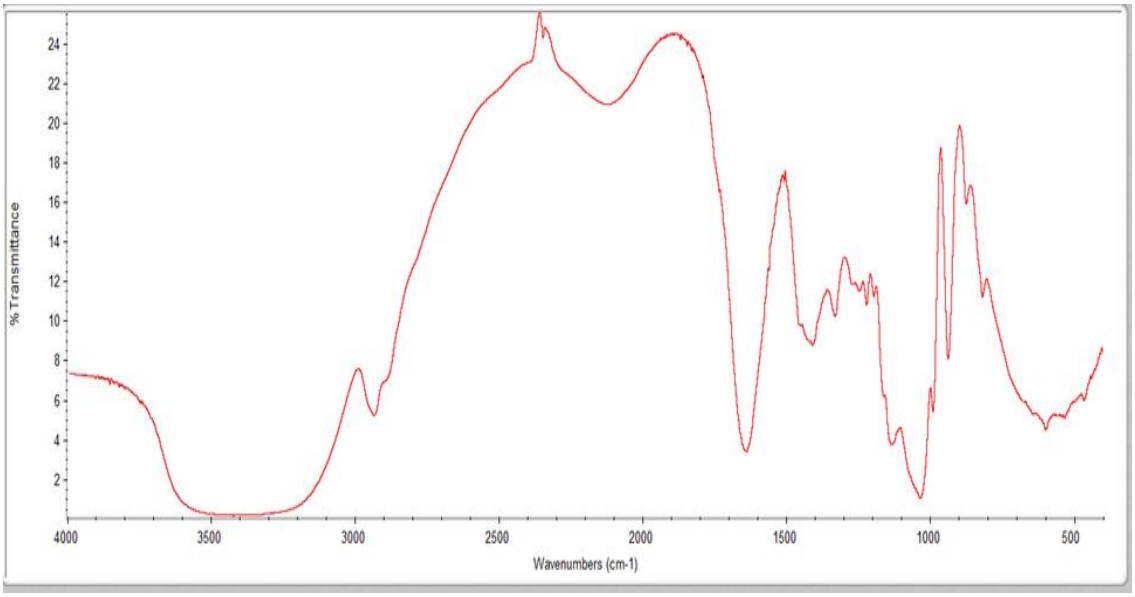

Fig. 3. FTIR spectrum of purified inulin from dangshen *Codonopsis javanica*

4. CONCLUSION

Inulin was the major component in polysaccharide extracted from dangshen naturally grown in highland Lam Vien, Vietnam, and purified by using ethanol and active coal. Purification degree of inulin precipitating by ethanol (97.85^{a} ± 0.84%) was higher than that one by active coal (94.05%). 80% and 90% ethanol was useful for the precipitation of inulin and fructan, respectively. The temperature effect on the precipitation of inulin and fructan in ethanol solvent was a non-significant difference, but a significant difference in an aqueous solvent. The molecular weight of inulin and fructan corresponded to 3,193 Da and 1,112,892 Da, respectively. The results will support to develop the technique of purification for inulin application in functional food and pharmaceuticals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the project - Research on chemical compositions of dangshen (*Codonopsis javanica*) in Lam Dong and apply in functional foods - Code: B2018-DLA-01, granted by Ministry of Education and Training.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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