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Original Article

CRUDE INULIN DERIVED FROM DAHLIA TUBER AS NANOMATERIAL AND ITS CHARACTERIZATION

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ABSTRACT

Objective: Dahlia tuber (*Dahlia sp.*) is one of the inulin sources that could be planted in Indonesia. Inulin is fructan-based polysaccharide. Therefore, the research aimed to investigate inulin from the extract of dahlia tuber as a drug excipient, especially for nanoparticles based on inulin-cysteamine thiomer.

Methods: Crude inulin was isolated from dahlia tuber using ethanol for maceration. The resulting inulin was characterized using FT-IR and oxidized using sodium periodate ($NaIO_4$) to increase solubility. Afterward, the oxidized crude inulin was further modified by conjugation with cysteamine to produce a cationic thiomer using reductive amination. The thiomer was evaluated regarding the number of thiol groups and solubility. The nanoparticles were prepared using ionic gelation methods. The resulting nanoparticles were evaluated for particle size and zeta potential.

Results: Inulin can be isolated from dahlia tuber with its content of 18.60±4.45% and carbohydrate of 61.75±0.75%. Crude inulin can be conjugated with cysteamine to generate a cationic thiomer using $NaCNBH_3$ as a reductant, which can increase its solubility with free thiol group content of 415.21±40.39 µmol/g. Nanoparticles were generated from crude inulin-cysteamine thiomer with sodium tripolyphosphate (NaTTP), leading to a particle size of 180 nm and zeta potential of-10.8 mV.

Conclusion: As a potential nanoparticulate drug delivery system, a cationic thiomer could be synthesized from inulin derived from dahlia tuber grown in Indonesia.

Keywords: dahlia tuber, inulin, a cationic thiomer, nanoparticles

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INTRODUCTION

Dahlias (*Dahlia* sp.), originating from Mexico, can grow well in Indonesia. Apart from being an ornamental flower, dahlia tubers contain polysaccharides that are beneficial for humans, including inulin. The characteristic of inulin, which cannot be digested in the stomach but will be degraded by the intestine, is an advantage of inulin as a targeted drug delivery system in the intestine [1, 2]. However, inulin in Indonesia still depends on production abroad, resulting in high inulin prices. Thus, dahlia tubers are a solution for inulin sources that can be grown in Indonesia [3, 4].

Development of inulin in pharmaceuticals as a drug delivery system in the intestine has been developing. Modification of inulin into thiomer (polymers conjugated with thiol groups) could increase its solubility in distilled water compared to unmodified inulin [5, 6]. Nanoparticles can increase a drug's solubility permeability to increase drug delivery efficiency [1]. The ionic gelation method is one of the simple methods of nanoparticle preparation. The active compound is dissolved with a positively charged polymer; then, the mixture interacts with a negatively charged crosslinker to nanoparticles [6-8].

Dahlia tubers have the potential to be a source of inulin, which can be used as a raw material for nanoparticles. Thus, inulin isolated from dahlia tubers can be an alternative material for generating inulin-based nanoparticles. Therefore, this study aimed to prepare crude inulin from dahlia tubers (*Dahlia* sp.), evaluate crude inulin and its thiomer, and manufacture crude inulin-cysteamine-based nanoparticles using ionic gelation. Information on the optimal method of isolating inulin from dahlia tubers, the technology for producing crude inulin-cysteamine (thiomer), and its nanoparticles using ionic gelation with its characterization can enrich materials for nanocarriers derived from natural products.

MATERIALS AND METHODS

Material

Dahlia tuber was from Kendal Regency, Central Java, Indonesia, and had a planting age of more than seven months. *Dahlia* sp. was determined at the laboratory of the Biological Research Center, National Research and Innovation Agency, Cibinong, Indonesia, under the reference number B-441/V/DI.05.07/2/2022. Inulin standard, $NaIO_4$, ethylene glycol, $NaCNBH_3$, $NaBH_4$, $KH2PO_4$, Na_2HPO_4, DNTB/5,5′-dithiobis-(2-nitrobenzoic acid), sodium tripolyphosphate, dialyzing membrane 1kDa, MES hydrate, and cysteamine HCl were from Sigma Aldrich, Darmstadt, Germany. HCl and H_2SO_4 were from AR Chemicals, New Delhi, India. D-glucose and phenol were from Mercks, New Jersey, USA.

Equipment

Centrifuge (Kokusan H-103N, Japan), Oven (Memmert, Germany), FT-IR spectrometer (Thermo Fisher Scientific, USA), UV-Vis spectrophotometer (Thermo Fisher Scientific, USA), pH meter (Mettler-Toledo, China).

Preparation and extraction of inulin from dahlia tubers

Dahlia tubers (*Dahlia* sp.) were cut into small pieces and dried at 40 °C. Dried dahlia tubers were added with water in a ratio of 1:2 and ground using a blender. The dahlia tuber juice was boiled at 80-90 °C for 30 min. Afterward, the hot juice was filtered, and the resulting filtrate was collected. The filtrate was mixed with 30% v/v ethanol in a ratio of 5:2 and stored in a freezer at a temperature of-4 °C for 18 h to precipitate inulin, which was then centrifuged at 1500 rpm for 15 min. The resulting inulin was suspended in distilled water and dialyzed using a dialyzing membrane tubing (MWCO), 1 KD [9].

Identification and determination of inulin content

Identification of inulin in crude inulin was evaluated using an FT-IR spectrometer. 500-4000 cm^{-1}. The resulting spectrums were compared with literature and standard inulin [10]. Determination of total carbohydrate content using visible light spectrophotometry (concentrated phenol-sulfuric acid method) [11, 12]. A 10 mg D-glucose was weighed and dissolved in 10 ml distilled to obtain a concentration of 1000 ppm. 2 ml of the solution was withdrawn and put into a 10 ml volumetric flask. Afterward, distilled water was added to the mark. 2 ml of the final solution was withdrawn and put into a test tube with 1 ml of 5% (v/v) phenol and 5 ml of sulfuric acid. Standard series solution was prepared in the same manner as the tested solution in the final concentrations of 30, 45, 60, 75, and 90 ppm. The tested and standard solutions were incubated for 60 min and measured for their absorption at a maximum wavelength of 490 nm.

Determination of inulin level using UV spectrophotometry was done by preparing a 20 mg crude inulin was weighed and put into a 50 ml volumetric flask. 30 ml distilled water was added. Afterward, the final solution was heated (solution A). 2 ml solution A was put in a boiling flask, and 20 ml of 5% (v/v) HCl was added. The flask was refluxed for 2 h, transferred in a 25 ml volumetric flask, and added 5% HCl up to the mark (solution B), which measured its absorption using UV spectrophotometry at a maximum wavelength of 285 nm. Blanks were prepared with 5% HCl [13].

Preparation of crude inulin-cysteamine thiomer

Two grams of crude inulin were dissolved in 100 ml of distilled water in an Erlenmeyer wrapped in aluminum foil. 200 mg of sodium periodate dissolved in 10 ml distilled water was added. The mixture was stirred with a magnetic stirrer until homogeneous for 2 h at room temperature. After 2 h, 0.2 ml of ethylene glycol was added, and the mixture was stirred again for 1 hour at room temperature. The purification process was carried out using a one kDa dialysis membrane in demineralized water for three days with the temperature maintained at 10 °C, then the water was changed every 12 h. The purified polymer was dried using freeze drying and stored at 4 °C.

Further studies were carried out for polymer conjugation with different reducing agents, namely sodium cyanoborohydride ($NaCNBH_3$) and sodium borohydride ($NaBH_4$). 1 g of aldehyde polymer was dissolved in 40 ml of distilled water. One gram of MES buffer and 0.50 g of cysteamine were added. The pH value was adjusted to 5, and distilled water was added to a final volume of 50 ml. The mixture was stirred for 3 h with a magnetic stirrer. Four grams of reducing agent ($NaCNBH_3$ and $NaBH_4$) was added and stirred for three days. Afterward, the mixture was purified using one kDa dialysis membrane for three days in a dark room with demineralized water replaced every 12 h. The purified conjugate was dried using freeze drying and stored at a temperature of 4 °C [6, 8].

Evaluation of crude inulin-cysteamine thiomer

Colour reaction using the Ellman test was done by preparing a 10 mg of thiomer that was dissolved in 5 ml of Ellman buffer. The solution was reacted with 5 ml of Ellman's reagent. Solutions containing thiomer with free thiol groups produced a yellow color.

The solubility test of crude inulin was done by prepare 10 mg tested samples (raw inulin, crude inulin, and crude inulin-cysteamine thiomer (reductant $NaCNBH_3$ and $NaBH_4$) that were weighed and dissolved in 10 ml following solvents: HCl with a pH of 1.2, distilled water with a pH of 7, and 0.1M NaOH with a pH of 14.

Identification of thiomersal by FT-IR was done by generated thiomers and were tested using an FT-IR spectrometer. The spectrum was analyzed by observing the specific functional groups' wavelength numbers. 10 mg of cysteamine-HCl was weighed and dissolved in the Ellman buffer in the total volume of 10 ml to obtain the exact serial concentration for the calibration curve by measuring the absorbance using visible spectrophotometry at a wavelength of 412 nm. The exact concentration of tested samples was treated like the standard series solution to determine the amount of free thiol groups.

Preparation of crude inulin-cysteamine nanoparticles

200 mg crude inulin-cysteamine was weighed and dissolved in 100 ml distilled water with a pH of 5.5. The solution was stirred with a magnetic stirrer at room temperature overnight. Afterward, 0.2% sodium tripolyphosphate was added dropwise and stirred for one hour under permanent stirring using a magnetic stirrer. Transmission electron microscopy evaluated the generating nanoparticles for particle size, zeta potential, and morphology.

RESULTS

Preparation and extraction of inulin from dahlia tubers

The tuber was red dahlia obtained from Kendal Regency, Central Java, Indonesia, and was cultivated and grown for more than 7 mo (fig. 1). The isolated inulin was dark brown; it was suspected that the process of inulin isolation was not optimal (fig. 2). The brown color was caused by the Maillard reaction (browning) in the presence of protein. The solution for browning was to add activated charcoal.

Fig. 1: Dried dahlia tuber

Fig. 2: Crude inulin from dahlia tuber

Identification of inulin using FT-IR spectrometry

Standard inulin and crude inulin detected the presence of hydroxyl groups (-OH) in the range of 3300 cm^{-1}, CH_2 asymmetric stretching in the range of 2930 cm^{-1}(fig. 3). Neither standard inulin nor crude inulin was obtained at 2890 cm^{-1} which shows the CH_3 stretching vibration. This is because the structure of inulin itself does not contain a lot of alkyl CH_3, but most of the polysaccharide chains are CH or CH_2. Hence, it is possible that the 2890 cm^{-1} spectrum has such a small intensity that it cannot be detected (table 1).

Determination of total carbohydrate content

The average result of total carbohydrate content as glucose in crude inulin was 61.75±0.75%. Red dahlia tubers have a carbohydrate content of around 14.77-50.74% [14]. Levels were found to be greater than in the literature. Differences in varieties between

tubers can cause this, and the literature allows them to have different total carbohydrate levels. The presence of fibre in red dahlia tubers affects the number of carbohydrates where the fibre can be hydrolyzed and react with phenol during the reaction.

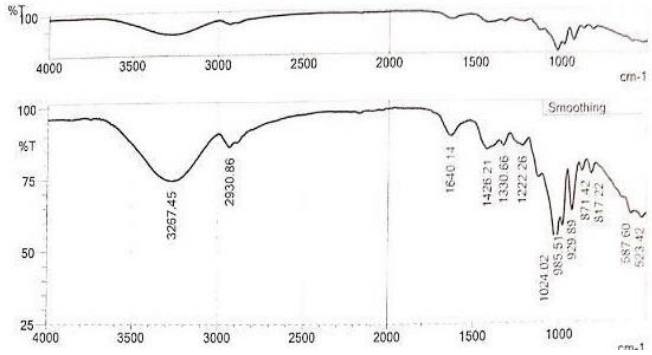

Fig. 3: FTIR spectrum of crude inulin from dahlia tuber

Table 1: FTIR analysis of inulin

S. No.	Specific frequency (cm^{-1})	Functional groups	Standard inulin frequency (cm^{-1})	Crude inulin frequency (cm^{-1})
1	3300	OH stretching, wide pick	3291.70	3267.45
2	2930	stretching asymmetric of CH2	2932.29	2930.86
3	2890	stretching symmetric of CH3	-	-
4	1629	C=C bond	1648.70	1640.14
5	1400	Bending vibrations and internal deformations of CH, CH2, and OH	1454.74	1426.21
6	1330		1334.93	1330.66
7	1130	stretching C-O	1123.85	1222.26
8	1030	stretching C-O-C	1026.87	1024.02
9	800	stretching biomolecule: other carbohydrates	815.79	871.42: 817.22
10	670	pyranose rings	-	-
	650		644.65	-
	590		593.30	587.60

Determination of inulin level using UV spectrophotometry

This study used maximum wavelength optimization to ensure whether the 285 nm wavelength provided maximum absorbance. The maximum wavelength results obtained varied from 274.0 nm to 276.5 nm. The shift in wavelength to a smaller value is called blue shift [15]. This is thought to be due to uncontrolled temperature during the reflux of the solution so that organic compounds such as carbohydrates and proteins are destroyed, and the solution changes color from colorless to yellow. In future research, it is recommended to control the temperature during reflux.

Preparation of crude inulin-cysteamine thiomer

Based on the results, the average free thiol group of thiomer with $NaCNBH_3$ and $NaBH_4$ reducing agents were 415.21±40.39 and 333.06±137.01 µmol/g, respectively. Both thiomers have reached the number of free thiol groups more than 100 µmol/g. The value of the number of thiol groups in the thiomer with the $NaCNBH_3$ as a reducing agent was also proven by the sample solution being yellower than the thiomer with the $NaBH_4$ as a reducing agent [16, 17]. Differences in the reduction selectivity of each reductant caused this difference in results. $NaBH_4$ can also react with aldehydes and ketones (for example, carbohydrates) and disulfide bonds in other compounds in crude inulin, affecting the reduction ability and conjugation of cysteamine to inulin. The crude inulin-cysteamine thiomer has more free thiol groups. Thus, this thiomer was chosen for nanoparticle preparation using the ionic gelation method.

Evaluation of crude inulin-cysteamine thiomer by its colour reaction

The color reaction using the Ellman Test is one way to indicate whether the thiomer is made well and has a free thiol group (-SH). Thiomers with free thiol groups will react with Ellman's reagent to produce a yellow complex compound. The two thiomers with different reducing agents produced a yellow color after being reacted with Ellman's reagent. Therefore, both thiomers contain free thiol groups. Based on the reaction, the crude inulin-cysteamine thiomer with $NaCNBH_3$ as a reductant had a darker yellow color than the thiomer with $NaBH_4$ as a reductant. Regarding this observation, it could be assumed that the thiomer with the reducing agent NaCNBH3 contains more free thiol groups.

Solubility test

Crude inulin-cysteamine thiomer with $NaBH_4$ as a reductant is more soluble at pH 1.2, 7, and 14 than crude inulin before modification. Crude inulin-cysteamine thiomer with $NaBH_4$ as a reductant has increased its solubility. Thiomers with the reducing agent $NaBH_4$ gave better solubility than thiomers with the reducing agent $NaCNBH_3$.

Identification of thiomers by FT-IR

Thiomer identification using FT-IR spectroscopy investigated whether the inulin polymer has been modified into a crude inulin-cysteamine thiomer. Based on the results of the analysis, it was found that both thiomers still show the characteristics of inulin, as indicated by a fingerprint spectrum of 580 $cm^{-1,}$ which indicates the presence of a pyranose ring. Other spectra such as 3300, 2930, 1400, 1330, 1130, and 1030 cm^{-1} also appear, similar to the IR spectrum of crude inulin.

Preparation and evaluation of nanoparticles based on crude inulin-cysteamine

Crude inulin-cysteamine thiomer-based nanoparticles were prepared at pH 5.5 by ionic gelation with NaTTP as a crosslinker. This was considered because inulin-cysteamine thiomer-based nanoparticles formed at pH 5.5. Thiomers positively charged containing free thiol groups will interact with NaTTP, which is negatively charged, resulting in particle encapsulation. The

nanoparticles formed were measured for their particles and zeta potential (fig. 4). In this research, it was found that the formation of crude inulin-cysteamine nanoparticles was not optimal due to the particle sizes being 180.0 nm.

Table 2: Solubility test of crude inulin and its modifications

Solvent	Standard inulin	Crude inulin	Crude inulin-cysteamine (NaCNBH3)	Crude inulin-cysteamine (NaBH4)
HCl pH 1.2	Slightly soluble	Slightly soluble	Soluble	Freely soluble
Distilled water	Slightly soluble	Slightly soluble	Soluble	Freely soluble
NaOH pH 14	Soluble	Soluble	Freely soluble	Freely soluble

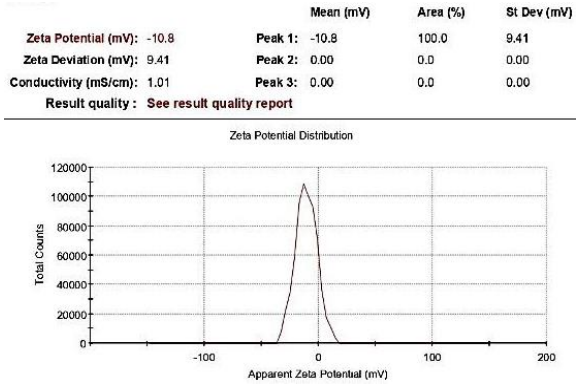

Fig. 4: Zeta potential of crude inulin-cysteamine nanoparticle

DISCUSSION

Activated charcoal will absorb proteins and other compounds, so the yield is expected to be pure inulin. However, the drawback of adding activated charcoal is that the inulin in the solution will also be absorbed into the activated charcoal, resulting in a reduced yield [7]. Regarding this aspect, the researchers did not add activated charcoal during extraction. A cooling process to separate inulin from macerate was considered less efficient. The process at-4 °C for 18 h can freeze the macerate, so it must be thawed before further studies. This thawing period gave rise to the potential for the macerate to be contaminated with bacteria/fungi and degrade the isolate through fermentation. The amount of ethanol in macerate did not guarantee the prevention of contaminants because the amount is small.

Standard inulin and crude inulin detected CH, CH_2, and OH vibrations, C-O and C-O-C stretching at 1400, 1330, 1130, and 1030 cm^{-1}. Crude inulin has two significant spectra in the 800-600 cm-1 range, namely at 871.42 and 817.22 cm^{-1}. The 800-600 cm^{-1} spectrum differentiates commercial inulin from inulin from local dahlia tubers. This spectrum indicates the presence of other carbohydrate substances due to differences in the structure and influence of glucose, sucrose, and mannan on the polymer chain and the purity of the inulin being analyzed [18]. In the fingerprint area, standard inulin only detected two peaks at 644.65 and 593.30 cm^{-1}. Meanwhile, crude inulin was only detected at 587.60 cm^{-1}. The peak area is suspected to be too small, so it cannot be detected, especially in crude inulin, which does not contain pure inulin. Based on this analysis, it was concluded that crude inulin from dahlia tubers possessed inulin.

Determination of total carbohydrate content using visible light spectrophotometry (phenol-sulfuric acid method). In this assay, the operating time was carried out first to determine how long it takes for the color reaction to form at maximum absorption. It takes around 25 min for color formation, with 15 min in a water bath to reduce the temperature of the solution. However, the time the absorbance reaches its maximum is not explained. It was found that the formation of color reactions tended to increase slowly from 0-60 min. At 40 min, the solution was stable and gave an absorbance that was not much different, as evidenced by the sloping graph. So, the operating time is 60 min due to maximum absorbance and an insignificant increase in absorbance.

This study obtained crude inulin-cysteamine thiomer with the reducing agents NaCHBH3 and NaBH4, respectively. Thiomers were prepared from crude inulin with an inulin content of 18.60±4.45 %. Both thiomers have a characteristic odor. Thiomers with the reducing agent $NaCNBH_3$ are powdered into granules with distinct odors. Meanwhile, thiomer with the reducing agent $NaBH_4$ was a fine powder with an odorless characteristic.

The thiomer with the reducing agent $NaCNBH_3$ was yellowish brown, which was pale yellow, different from the thiomer with the reducing agent $NaBH_4$. The brown color comes from crude inulin itself, which is dark brown. However, with the addition of the reducing agent $NaBH_4$, the color of the thiomer changes to pale yellow. This is because $NaBH_4$ was less selective, where $NaBH_4$ can reduce carbonyl groups in aldehydes and ketones [19]. Additionally, using $NaBH_4$ is exothermic and emits heat into the medium. When $NaBH_4$ is dissolved in distilled water, it will emit heat, increasing temperature. Organic compounds such as proteins, fats, and free carbohydrates that remained in the sample were then degraded by the heat. The increase in temperature can be reduced by dissolving NaBH4 in distilled water before the reaction.

One of the properties of thiomers is to increase the polymer's solubility, where the polar thiol (-SH) group will facilitate solubility in water. Solubility tests are carried out at pH 1.2, 7, and 14. pH 1.2 is intended to determine solubility in acidic conditions, which describes the acidic atmosphere of the stomach. pH 7 is taken to determine solubility in neutral water. Meanwhile, pH 14 is intended to determine the solubility of inulin, which is very easily soluble in a pH 14 solution. Solubility in alkaline pH will be applied to determine the number of free thiol groups, and the analysis will be carried out in an alkaline environment. Standard inulin is slightly soluble at pH 1.2 and 7 but soluble at pH 14. Based on the reference, inulin is difficult to dissolve in water and will dissolve in dilute alkali. The solubility properties of crude inulin are worse than those of standard inulin at the examined pH values due to impurities such as protein and ash. Crude inulin-cysteamine thiomer with $NaCNBH_3$ as a reductant is soluble at pH 1.2 and 7, very freely soluble at pH 14 compared with unmodified crude inulin, crude inulin-cysteamine thiomer with $NaCNBH_3$ as a reductant has been shown to increase thiomer solubility. The results of identifying the two thiomers using FT-IR could not show the presence of the crude inulin-cysteamine thiomer because no spectrum of the thiol group (-SH) was found.

However, the color reaction test results using the Ellman test selectively showed the presence of free thiol groups.

Nanoparticle stability occurs when the zeta potential is above ±28 mV. Otherwise, nanoparticles will tend to agglomerate. Agglomeration will result in larger particle sizes, and nanoparticles will not be effective. The resulting nanoparticles had a zeta potential of-10.8 mV. Thereby, the nanoparticles formed were unstable. This result also correlated with the large particle size, which tends to be prone to aggregation. Therefore, the preparation of crude inulin-cysteamine nanoparticles needs to be reviewed, from forming thiomers to formulating crude inulin-cysteamine thiomer nanoparticles.

CONCLUSION

Extraction of inulin from red dahlia tubers obtained an inulin content of 18.60±4.4516% and total carbohydrates of 61.75±0.75%. Crude inulin can be modified into cationic thiomers, which have been identified using FT-IR and have been proven to increase solubility. The largest number of free thiol groups in the crude inulin-cysteamine thiomer was demonstrated with the reducing agent sodium cyanoborohydride ($NaCNBH_3$). Crude inulin-cysteamine thiomer-based nanoparticles with NaTTP as a crosslinker provided a particle size of 180 nm and zeta potential of-10.8 mV.

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AUTHORS CONTRIBUTIONS

Deni Rahmat: conceived and planned the experiments supported the financial research; Vinessa Putri Graciella: carried out the experiments and analyzed the data; Yati Sumiati and Sarah Zaidan: analyzed the data and contributed to the interpretation of the results; Safira Nafisa and Yesi Desmiaty: administration of the research, supervised and verified the result; All authors provided critical feedback and helped shape the research, analysis and manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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