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Green methods for inulin extraction from common salsify (*Tragopogon porrifolius* L.) roots and its application in metal nanoparticle synthesis

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The aim of the current research is the isolation and chemical characterization of inulin from common salsify (*Tragopogon porrifolius* L.) roots using green extraction technique (microwave- and ultrasound-assisted irradiation) and its further application in the metal nanoparticle synthesis. Functional properties and color characteristics of isolated inulin were also evaluated. The highest yield was obtained for inulin isolated by microwave-assisted extraction (23 %). The degree of polymerization of inulin was 22-23 with average molecular mass of 3.4 kDa. The structure of inulin-type fructan was confirmed by FT-IR and NMR spectroscopy, where the presence of β (2→1) bonds was found. Inulin from common salsify showed better oil-holding capacity than water-holding one, high cohesiveness and good to fair flowability. The possibility of synthesis of gold and nickel nanoparticles was investigated by the reduction reaction of 0.001M $HAuCl_4$ and 0.01M $Ni(NO_3)_2$, respectively, and the effect of temperature on the production of metal nanoparticles was followed. Promising results for synthesis of golden nanoparticles using inulin from common salsify were obtained. Moreover, golden nanoparticles synthesized with common salsify showed potent anti-*Candida* activity and antifungal activity, especially against *Aspergillus*. The obtained results demonstrated the potential application of common salsify inulin in the pharmaceutical industry as a food supplement due to its functional properties and antimicrobial potential of golden nanoparticles synthesized by reduction of $HAuCl_4$ with common salsify inulin solution.

Keywords: *Tragopogon porrifolius*, inulin, nanoparticles, antimicrobial activity.

INTRODUCTION

Inulin and fructooligosaccharides (FOS) are part of the fructans family, widely distributed in various medicinal plants, fruits and vegetables as storage carbohydrates [1]. They are used as dietary fibers or nutritional ingredients in numerous food products. Moreover, the application of inulin in pharmacy as a drug carrier, encapsulating agent, and vaccine adjuvant constantly increases [2-6]. Therefore, the search for new valuable sources of inulin, except traditionally used chicory, dahlia, and Jerusalem artichoke [1-3], gain more and more attention during the last decade. The interest in current research is provoked by one vegetable, common salsify (*Tragopogon porrifolius* L.), as a promising source of inulin with potential use in culinary practice.

Common salsify (*Tragopogon porrifolius* L.) is an annual or biennial plant, used as a root vegetable with excellent nutritional and dietary properties, that belongs to the *Asteraceae* family [7-10]. It has three subspecies, namely: *T. porrifolius* subsp. *australis*, *T. porrifolius* subsp. *cupani* and *T. porrifolius* subsp. *porrifolius* [8]. It can reach 50–110 cm in height and possesses a cylindrical taproot (15–30 cm long and 2.5–3 cm wide, brownish-yellow outer skin and white skin). The root has a very mild and slightly sweet flavor similar to oysters, hence the designation "oyster plant". Its older roots have a milky sap and a slightly distally branched, glabrous stem. Leaves are basal and stalked, alternate, sessile, sheathing lamina linear to linear-lanceolate (grass-like), 20–40 cm long [7-9]. It is rarely cultivated, mainly in the Mediterranean region, but deserves wider attention and use in the human diet. Various parts of the plant are consumed in Southern and Central Europe, North America, and the United Kingdom; it is also used to treat cancer in Lebanese folk medicine. Phytochemical investigations on this plant revealed that it contains carbohydrates, proteins, lipids (mainly monounsaturated fatty acids, essential fatty acids, vitamins, and polyphenol components) [7-11]. Common salsify root is low in calories but rich in protein, small amounts of vitamin A, B1, B2, C, PP,

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B6, fibers, minerals, such as Ca, Fe, Mg, K, P, Fe, and 4-18 % fructans (inulin and/or fructooligosaccharides, dry matter) [8, 11]. However, the most valuable component is inulin – a fructan with prebiotic properties, which has a positive effect on the functions of the human digestive tract [3, 6, 12] The average content of inulin in its roots is 15.17 % (fresh matter). Inulin concentrations are similar in both roots and spring plowing (15.19%) [8-10]. In Portugal and Poland, common salsify (*Tragopogon porrifolius* L.), is alternative inulin source plant, and is also cultivated due to its utilization in culinary practices [8, 9]. From the above mentioned data, it can be concluded that roots of common salsify (*Tragopogon porrifolius* L.) are a promising source of inulin. However, detailed data about inulin characteristics and functional properties are still missing. The aim of the current research is the isolation, chemical characterization and evaluation of the functional properties of inulin from common salsify (*Tragopogon porrifolius* L.) roots using green extraction techniques (microwave and ultrasound-assisted irradiation) and its further application for metal nanoparticles (NPs) synthesis.

MATERIALS AND METHODS

All solvents and reagents were of analytical grade.

Plant material

The plant material used for the analysis was *Tragopogon porrifolius* 'Fiore Blu'. The seeds were planted during March 2021 in vegetable gardens in Kostievo village and Rakovski town (Plovdiv region, Bulgaria). The roots were harvested during the second year of cultivation in July-September.

Isolation of inulin from common salsify (Tragopogon porrifolius L.)

The dried and finely ground roots from common salsify were extracted using deionized water as a solvent (1:10 w/v) by three methods:

1. Conventional extraction under a reflux at 100 °C for 60 min under constant stirring.

2. Ultrasound-assisted extraction in the ultrasonic bath IsoLab (Wertheim, Germany) at a frequency of 40 kHz, 120 W power, at 80 °C for 20 min.

3. Microwave-assisted extraction in a microwave device (Daewoo KOR, with microwave power 700 W and frequency of 2450 MHz) for 5 min [14].

The extraction procedure was performed in triplicate. The water extracts were obtained through a Buchner funnel filtration. The combined extracts were precipitated with the addition of four volumes of acetone, then cooled down to −18 °C, kept for 24 h and filtered. The crude polysaccharide was dried and dissolved in hot water, precipitated, and washed with acetone [14].

Characterisation of inulin from common salsify

The melting point of inulin was measured on a Kofler melting point apparatus. The reducing groups were determined spectrophotometrically by the PAHBAH method at 410 nm, while total fructose content – using resorcinol-thiourea reagent at 480 nm [14]. The purity of the polysaccharide was analyzed by HPLC instrument Elite LaChrome Hitachi (Tokyo, Japan) with a Shodex® Sugar SP0810 (300 × 8.0 mm i.d.) at 85 °C, coupled to a refractive index detector (VWR Hitachi Chromaster, 5450, Tokyo, Japan). Homogeneity and molecular weights were evaluated by high-performance size-exclusion chromatography (HPLC-SEC) performed on ELITE LaChrome (Hitachi, Japan), equipped with column Shodex OH-pack 806 M (i.d. 8 mm) [15]. Polydispersity index (X) of inulin was calculated as the ratio of the two molecular weights (Mw/Mn) [14]. The IR spectra (2 mg) were collected on a Fourier transform infrared (FT-IR) spectrophotometer VERTEX 70v (Bruker, Bremen, Germany) in KBr pellets. The spectra were recorded in the 4000–400 cm^{-1} range at 120 scans and resolution of 2 cm^{-1}. ^{1}H and ^{13}C NMR spectra of polysaccharide samples (20 mg/0.6 mL 99.95 % D_2O) were recorded using a Bruker AVIII 500 MHz spectrometer.

Functional properties

Color measurement of inulin was performed with a portable colorimeter Model WR-10QC D 65 lighting, following the CIELAB (L*, a*, b*) system, as previously described [16]. Functional properties of inulin, such as swelling properties, water- and oil-holding capacity were analysed according to Robertson *et al*. [17]. Angle of repose, densities (true, bulk, and tapped), flowability and wettability were determined as previously described [18].

Synthesis and characterization of metal NPs with inulin from common salfify

Aqueous solution of inulin from common salsify with degree of polymerization (DP) 22, obtained after microwave-assited extraction, with concentrations (0,2 % and 0,5 %), 0.001 M $HAuCl_4$ and 0.01 M $Ni(NO_3)_2$ water solutions (Sigma-Aldrich, Germany) were used for synthesis of metal NPs. In a 2 mL Eppendorf tube 1 mL of inulin solution (0.2 or 0.5 %) and 0.5 mL of chlorauric acid or nickel nitrate solution were mixed. The test tube was shaken and incubated at 85°C (Diterm, Robotics, Velingrad). The synthesized metal NPs (the synthesis time was determined by preliminary

visual and UV-Vis observations) were characterized by transmission electron microscopy (TEM) after placing a drop of the solution onto a standard copper grid coated with amorphous carbon layer and dried for 24 h. Observations were done and photomicrographs were obtained using a JEOL JEM 2100 high-resolution transmission electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 200 kV in conventional mode and high-resolution mode for TEM and HRTEM (high-resolution TEM) images, respectively. Statistical analysis of the nanoparticle size distribution was performed using Image J software. Phase composition identification was performed using the International Center for Diffraction Data (ICDD) PDF-2 Database [19].

Antimicrobial activity of obtained nanoparticles with inulin from common salsify

Eighteen microorganisms (Gram-positive and Gram-negative bacteria, yeasts, and fungi) were used from the collection of the Department of Microbiology (University of Food Technologies, Plovdiv, Bulgaria) for the investigation of antimicrobial activity. The antimicrobial activity of obtained nanoparticles was determined by the conventional agar well diffusion method as described previously by Tumbarski *et al*. [20].

Statistical analysis

All experimental measurements were carried out in triplicate and the values were expressed as average of the three analyses ± standard deviation.

RESULTS AND DISCUSSION

Characterisation of inulin from common salsify

Physicochemical characteristics of inulin from common salsify (*Tragopogon porrifolius* L.) roots are presented in Table 1.

Table 1. Physicochemical characteristics of inulin from common salsify (*Tragopogon porrifolius* L.) roots

Characteristics	*Classical extraction*	*Ultrasound-assisted extraction*	*Microwave-assted extraction*
Yield, %	19	15	23
Purity, %	67	74	64
Melting point, ºC	158-162.5	191-194	170-174.5
Fructose content, %	59	75	74
Reducing groups, %	2.3	3.8	3.3
Molecular weight (Mw), Da	3471	3325 2713	3345
Mn, Da	3310	3183 2602	3192
Polydispersity index (PD)	1.05	1.05	1.05
Degree of polymerization	21	22	22
Degree of polymerization (by NMR)	23	15-16	20-21
Color characteristics			
L	87.41±3.24	85.43±2.14	83.92±1.14
a	3.91±0.30	4.05±0.32	4.16±0.42
b	9.91±1.25	9.92±0.51	9.93±0.53
C	10.65±1.24	10.06±0.72	9.96±0.63
h	68.16±0.99	68.12±1.13	66.10±2.39
ΔE	14.89±0.15	15.08±0.20	19.27±0.61
Functional properties			
Swelling index, g/cm^3	3.70	4.79	5.45
Water holding capacity, g water/ g	1.02	1.34	2.36
Oil holding capacity,g oil/g	6.20	6.52	6.95
Wettability, s	90	112	116
True density (g/ cm^3)	0.73	0.81	0.81
Bulk density (g/ cm^3)	0.20	0.22	0.22
Tapped density (g/ cm^3)	0.20	0.30	0.38
Carr's index	32	34	34
Hausner ratio	1.47	1.49	1.49
Flowability	high	high	high
Cohesiveness	intermediate	intermediate	intermediate

Table 2. Electron diffraction indexing of gold NPs prepared with inulin from common salsify roots

d [Å]	hkl	COD* Entry
2.3925	111	Au cubic S.G. Fm-3m Cell parameter: 4,14500 Å #96-901-3045
2.0720	200	
1.4651	202	
1.2495	311	

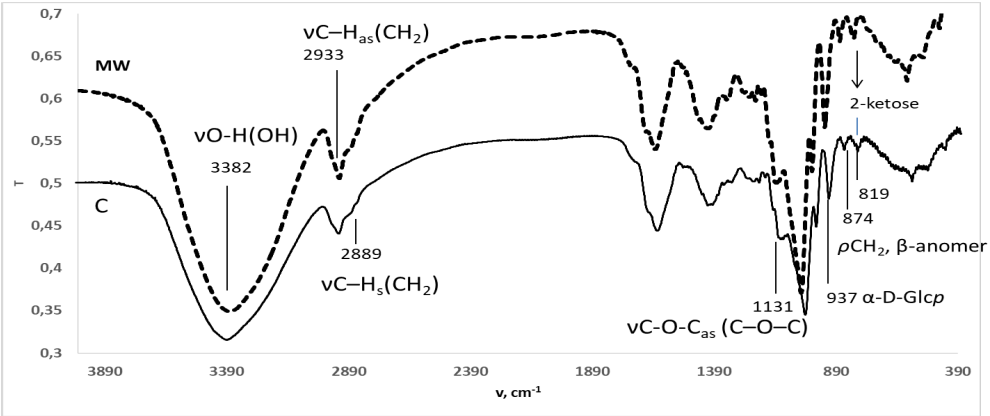

Figure 1. FT-IR spectra of inulin isolated from common salsify (*Tragopogon porrifolius* L.) roots, where C- classical extraction (-), MW – microwave-assisted extraction (--).

The highest inulin yield was observed for microwave-assted extraction – 23 %, together with the highest degree of polymerization 20-22. The highest purity (74 %) and fructose comtent (75 %) were obtained employing ultrasound-assisted extraction (74 %), however the cavitation process reflects on degree of polymerization (15-16) which is lower than the degree of polymerization of inulin extracted using microwave-assisted extraction. There are no significant differences in the color characteristics of isolated inulins by the different extraction methods. Our data for the lightness of inulin were comparable with data for other inulins isolated from different plant sources such as echinacea, chicory, Jerusalem and globe artichoke [16]. The obtained values for water- and oil-holding capacities of common salsify inulin were close to previously reported data for long-chained chicory (1.59 g water/g sample and 3.4 g oil/g sample, respectively) [16], and black salsify (0.5±0.1 water/g sample and 6.1±0.5 oil/g sample, respectively) [15]. However, our results on the oil-holding capacity of inulin were more than five times higher than commercial chicory inulin and globe artichoke inulin (1.37 and 1.38 g oil/g sample, respectively) [15, 21], and Jerusalem artichoke – 1.02 g oil/g sample [22]. The obtained inulin demonstrated high flowability and intermediate cohesiveness, based on Carr's index and Hausner ratio. The highest swelling properties, water and oil-holding capacity were found for inulin from microwave-assited extraction. This could be explained by the highest molecular weight and degree of polymerization. Common salsify (*Tragopogon porrifolius* L.). inulin yield coincided with reported data in the literature: 15 – 20 % [8, 9]. Its content is three times higher than this in roots of meadow salsify (*Tragopogon pratensis* L.) (5-9 % dw) [23]. In general, microwave-asssited extraction significantly reduced the time for extraction to 15 min, while the yield, molecular weight were similar to the inulin extracted by conventional long time (3 hours) extraction.

FT-IR specroscopy

The FT-IR spectra of inulin isolated from common salsify (*Tragopogon porrifolius* L.) roots by classical extraction and microwave-assisted extraction are shown in Figure 1. The spectra contain all typical bands for inulin-type fructans [24], as follows: a broad band at 3300 cm^{-1} due to O–H stretching vibrations assossiated with with inter- and intramolecular hydrogen bonds in the inulin structure. The bands at 2930–2932 cm^{-1} are due to C–H asymmetric stretching vibrations. The bands at 2882 cm^{-1} are characteristics for the symmetric stretching vibrations of C–H. The bands in the region from 1200 to 970 cm^{-1} are mainly due to C–C and C–O stretching in the pyranosyl ring and C–O–C stretching vibrations of glycosidic bonds. The bands at 1120 cm^{-1} were characteristic of C–O–C ring stretching vibrations from glycoside linkage. The bands at 1028–1029 cm^{-1} were assigned to C–O stretching vibrations, together with bands at 987 cm^{-1}. In the fingerprint region typical bands for

inulin and inulin-type fructans are observed. The presence of α-D-glucopyranosyl residue in the polymer chain is observed at 934 cm^{-1}. The band for β-anomer bendings in C1–H was found at 873 cm^{-1} and the occurance of band at 817 cm^{-1} confirmed the presence of 2-ketofuranose or 2-ketopyranose. Similar bands in the FT-IR spectra were reported earlier for inulin type fructan, especially the bands at 935, 873, and 818 cm^{-1}, typical for inulin from different plant sources such as echinacea, dahlia, chicory [14, 16] and black salsify [15].

NMR spectroscopy

This is the first detailed research on the structural characteristics of inulin from common salsify roots. The chemical shifts in ^{1}H NMR and ^{13}C NMR spectra are shown in Figure 2 and Figure 3.

In the ^{1}H NMR spectrum (Figure 2) of common salsify inulin typical chemical shifts for glucose and fructose units were found: ^{1}H NMR (500 MHz, D_2O); δ 5.45, 5.44, 4.30, 4.27, 4.26, 4.21, 4.19, 4.12, 4.11, 4.09, 3.94, 3.92, 3.87, 3.84, 3.78, 3.72, 3.70, and 3.68 ppm. This inulin spectrum contains an isolated resonance for the single anomeric α-glucose proton, observed at 5.45 ppm (Figure 2). In the range from 3.68 to 4.30 ppm all protons for fructose units were found. Anomeric glucose signal H-1 showed low intensity in comparison with the high intensity of fructose units. The integration of the H-1 signal of the glucose unit at δ 5.4 ppm and the H-3 and/or H-4 signals of the fructosyl units between δ 3.6 and 4.30 ppm gave a mean DPn. The DPn distribution of inulin obtained by spectrophotometry analysis ranged from 16 to 23.

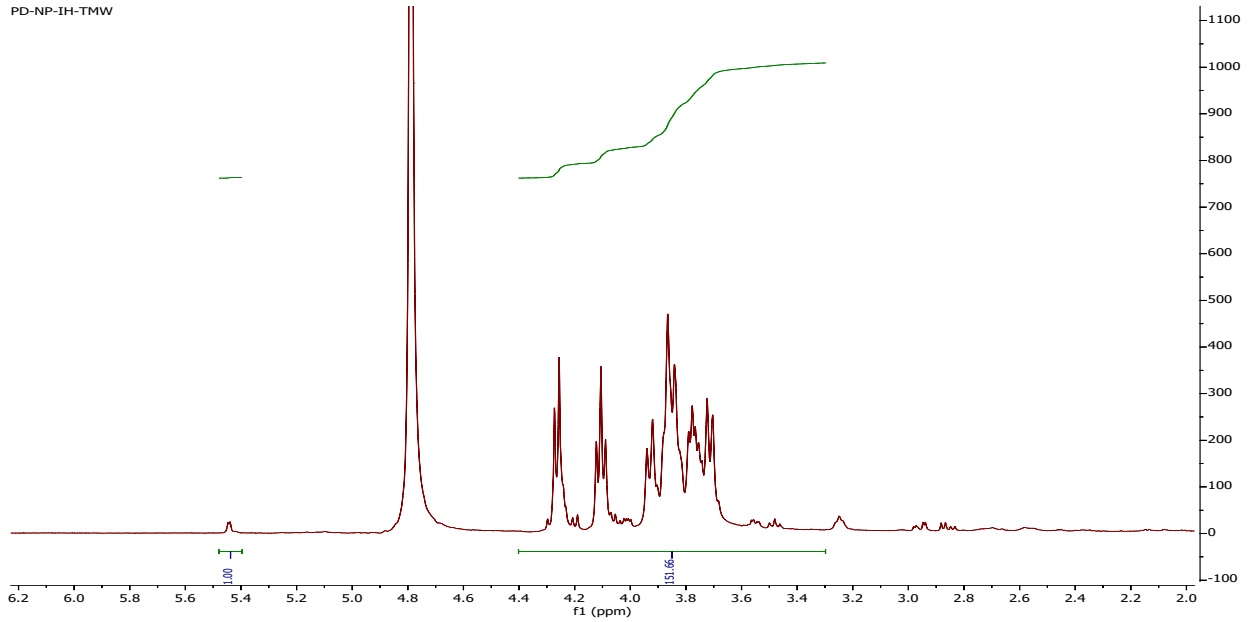

Figure 2. ^{1}H NMR spectrum of inulin isolated from common salsify (*Tragopogon porrifolius* L.) roots (500 MHz, D_2O); δ 5.45, 5.44, 4.30, 4.27, 4.26, 4.21, 4.19, 4.12, 4.11, 4.09, 3.94, 3.92, 3.87, 3.84, 3.78, 3.72, 3.70, 3.68.

In the ^{13}C NMR spectrum of inulin from common salsify roots chemical shifts typical only for fructose units were observed (Figure 3): ^{13}C NMR (126 MHz, D_2O); δ 103.20, 81.03, 76.93, 74.22, 62.09, and 60.85 ppm. The spectrum contains prominent shifts for C1–C6 carbons (C1 60.85 ppm, C2 103.20 ppm, C3 76.93 ppm, C4 74.22 ppm, C5 ~81 ppm, and C6 ~60.85 ppm) of fructosyl residue due to fructose repeated units. Similar shifts in the ^{13}C NMR spectrum were reported for inulin which contained bonds →1)-Fruf-(2→ and Fruf-(2→ [14, 16]. However, ^{13}C shifts due to glucose were not observed (Figure 3). This superposition of glucose shifts was observed in other studies and was reported for inulin from echinacea, dahlia, and stevia [14, 16].

Metal nanoparticle synthesis using inulin from common salsify roots

Inulin coated NPs were used in drug delivery [4, 6]. It was reported that inulin coated plasmonic gold NPs were used as a tumor-selective tool for cancer therapy [4, 5]. Therefore, the synthesis of nanoparticles with inulin is prespective field of application for both inulin from common salsify roots and the sinthesized gold NPs.

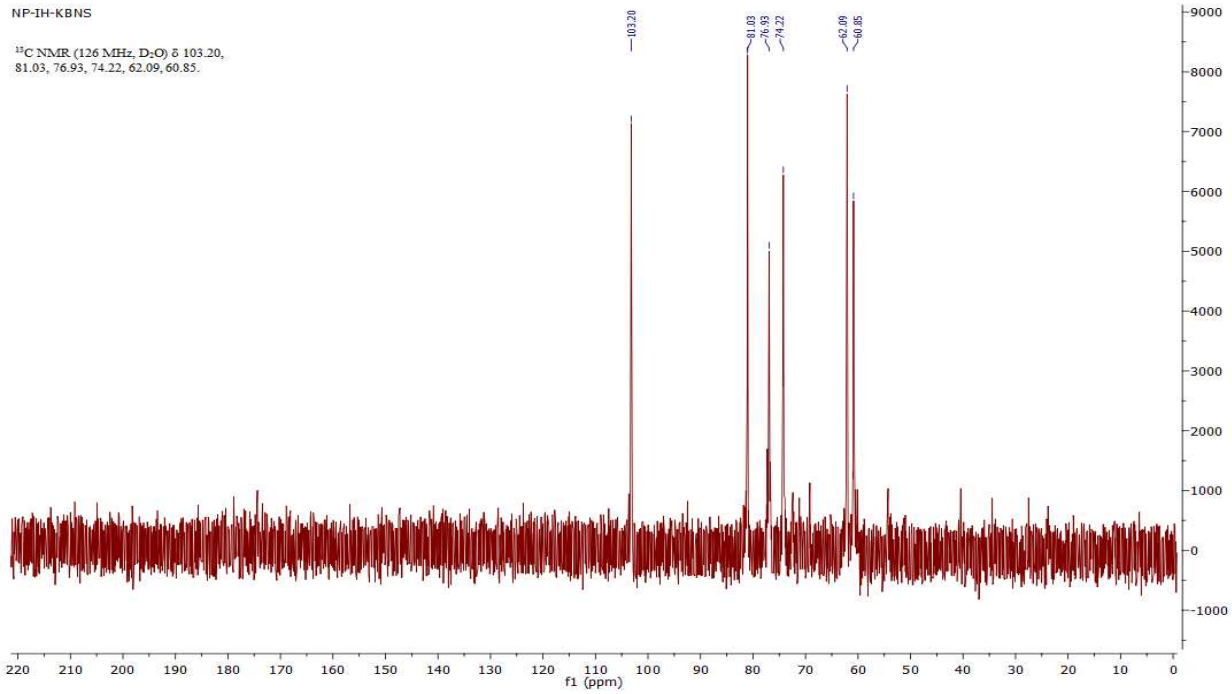

Figure 3. ^{13}C NMR spectrum of inulin from common salsify (*Tragopogon porrifolius* L.) isolated by ultrasound-assisted irradiation 40 kHz (^{13}C NMR (126 MHz, D_2O); δ 103.20, 81.03, 76.93, 74.22, 62.09, 60.85).

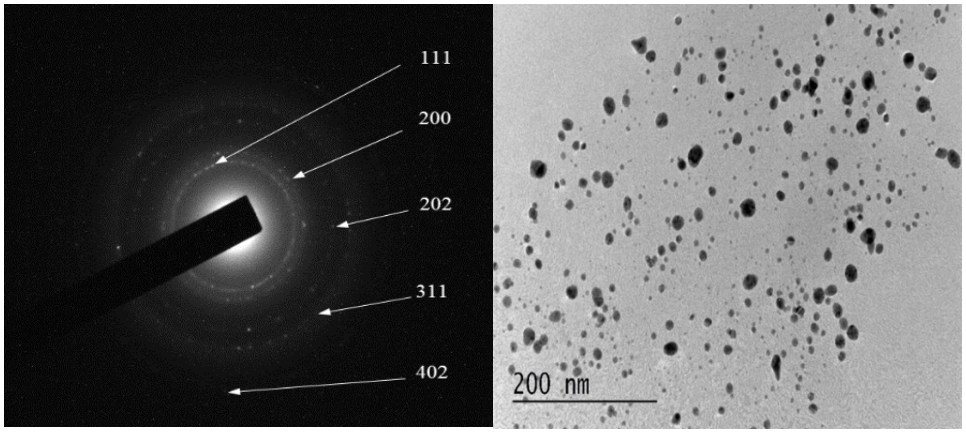

Figure 4. TEM (Transmission electron microscopy) microphotographs and electron diffraction of Au NPs 2:1 with inulin from common salsify roots in concentration 0.5 %

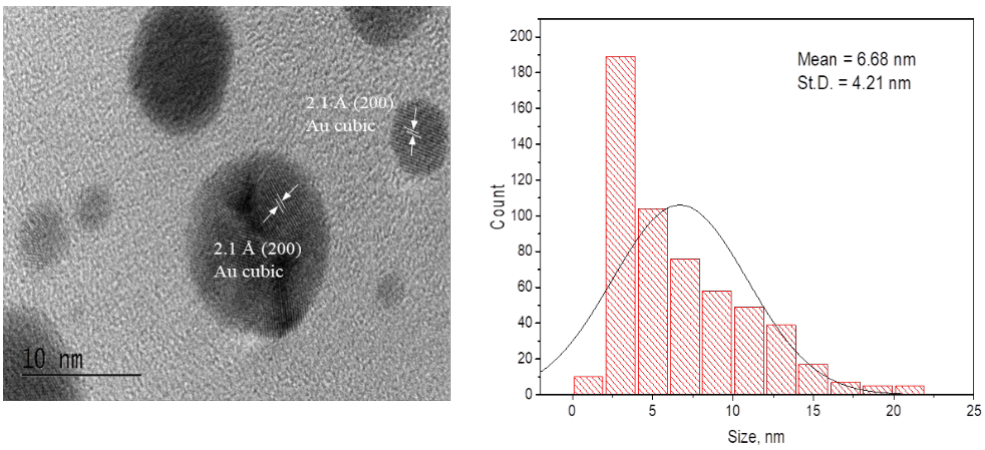

Figure 5. High-resolution TEM microphotograph and particle size distribution (Au NPs 2:1 with inulin from common salsify roots in concentration 0.5 %)

Table 3. Antimicrobial activity of golden and nickel nitrate NPs

Test microorganism	Common salsify inulin Au NPs	Common salsify inulin Ni
Bacillus subtilis ATCC 6633	-	-
Bacillus amyloliquefaciens 4BCL-YT	-	-
Staphylococcus aureus ATCC 25923	-	-
Listeria monocytogenes ATCC 8632	-	-
Enterococcus faecalis ATCC 29212	-	12
Salmonella enteritidis	-	-
Klebsiella sp.	-	-
Escherichia coli ATCC 25922	-	-
Proteus vulgaris ATCC 6380	-	-
Pseudomonas aeruginosa ATCC 9027	-	-
Candida albicans NBIMCC 74	9	-
Saccharomyces cerevisiae	-	-
Aspergillus niger ATCC 1015	9	-
Aspergillus flavus	9	-
Penicillium sp.	-	-
Rhizopus sp.	-	-
*Fusarium moniliforme*ATCC 38932	-	-
Mucor sp.	-	-

The gold NPs were predominantly spherical in shape, but irregularly shaped as could be seen from Figure 4. The size distribution was from 0 to 20 nm, the predominant size being from 2-4 nm (Figure 5). The phase has cubic gold NPs (Table 2) and the information was obtained from the electron diffraction and high resolution imaging. In addition, inulin from common salsify roots successfully supported and coated the gold NPs. Nickel NPs were not observed visually or using TEM and one possible explanation was that inulin did not possess enough reducing power to reduce Ni^{2+} to Ni^{0}. The antimicrobial potential of synthsiezd golden NPs and mixture of inulin and $Ni(NO_3)_2$ were evaluated for the first time. The results are summarized in Table 3.

It was found that gold NPs prepared with inulin from common salsify roots showed moderate antimicrobial activity only against yeasts and fungi, especially *Candida albicans* NBIMCC 74, *Aspergillus niger* ATCC 1015 *Aspergillus flavus.* However, common salsify inulin and $Ni(NO_3)_2$ showed moderate activity only against Gram-negative bacteria *Enterococcus faecalis* ATCC 29212. Both, gold NPs and inulin and $Ni(NO_3)_2$ mixture, were inactive aganst Gram-positive bacteria.

CONCLUSION

To the best of our knowledge this is the first detailed study on the isolation, structural elucidation, and evaluation of the functional properties of inulin from common salsify roots. The study revealed that common salsify contains linear inulin composed of fructose units linked with β-(2→1) bonds and a terminal glucose unit linked α-(1→2), having DP of 20-22 and molecular-weight similar to the chicory inulin. Microwave-assisted extraction was evaluated as a prespective green method for obtaining high-molecular inulin in high yield. The common salsify inulin showed better oil-holding capacities, than water-holding properties, high fowability and intermediate cohesiveness. All these properties revealed the potential of this inulin to be used as a functional ingredient, as taste and structure modifier of formulated food systems. An interesting and promising application of polysaccharides (particularly inulin from common salsify roots) is the synthesis of metal NPs by reduction of their salts. By this way, Au NPs were successfully synthesized and they were characterized by TEM. The antimicrobial potential of golden NPs with common salsify inulin permits its further application in food and pharmacy.

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