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Waste corn silk for eco-friendly silver nanoparticles: Green synthesis, characterization and determination of enzyme inhibition properties

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Abstract: Due to the increasing population and consumption rate, the sustainable use of resources is very important. Corn is one of the most produced grains in the world. However, many parts of it, such as corn silk, roots and corn husk are disposed of as agricultural waste. Within the sustainability, it is possible to bring waste into the field of technology and develop new products with green synthesis. In this study, the waste corn silk was dried, extracted and used as a precursor in synthesis of silver nanoparticles (CS–AgNPs). The CS–AgNPs were characterized using ultraviolet spectrophotometry, infrared spectrophotometry and scanning transmission electron microscope. Moreover, the inhibition effects of CS–AgNPs on enzymes such as α-amylase, α-glycosidase, urease, acetyl cholinesterase and xanthine oxidase which are important for the treatment of some diseases were determined. The obtained nanoparticles gave the maximum absorbance at 470 nm and the average size of the nanoparticles was found as 65 nm. It was determined that CS–AgNPs showed very good antioxidant activity and inhibitory effects on α-amylase (52.27 %), α-glycosidase (43.51 %), urease (80.33 %), acetyl cholinesterase (66.17 %) and xanthine oxidase (73.67 %). The obtained results show that the nanoparticles synthesized using the green synthesis technique could be used in medicine and pharmaceuticals.

Keywords: eco-friendly; diabetes mellitus; antioxidant activity; inhibition; waste management; sustainability.

INTRODUCTION

In recent years, the whole world is facing various challenges due to the unsustainable use of natural resources and the increasing population. For this reason, European Union countries have planned the European growth strategy for 2020 and set the goal of transitioning from a linear production and consumption model to a recyclable production and consumption model.[1] The amount and content of solid waste produced are affected by different factors, such as the socio-

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economic characteristics of the community or societies, nutritional habits, traditions, geography, professions and climate.[2] It is estimated that the world population, which will be 7.9 billion by 2022, will reach 9.8 billion in 2050. On the other hand, it is known that the food production rate is higher than the human population growth rate and today the food production is sufficient to feed 10 billion people.[3] Agricultural wastes are wastes and residues resulting from the production and processing of plant and animal products. Food waste could be defined as the food lost at every stage of the food supply chain, from farm to table, originating from producers, processors, retailers and consumers.[4] However, every year approximately 30 % of production appears as waste in the food supply chain.[5] The random abandonment and non-use of agricultural, food and domestic waste, which increases day by day, causes air pollution, soil pollution, *etc*. For this reason, the issue of evaluation and management of agricultural wastes has gained great importance in recent years.

Nanotechnology is a popular scientific area that allows to the processing, measurement, design, modelling and editing of materials at sizes of 1–100 nm. It provides technologically advanced or completely new physical, chemical, and biological properties to matter at the atomic and molecule level.[6] Because of their unique properties, nanoparticles could be used in many areas such as food, cosmetic, energy, agricultural and medical industries, which makes the nanoparticles very popular.[6]

Nanoparticles such as gold, nickel, zinc, silver, platinum and copper are synthesized in two ways: top-down or top-up.[6] Different methods such as biological (green), physical and chemical methods are used in the synthesis of nanoparticles. Synthesis with biological resources has attracted more attention than other methods because the synthesis process does not contain toxic chemicals or products, it is fast and provides cheaper synthesis.[7] Various parts of plants are used in synthesis with the biological method.[8] However, especially the use of waste parts of plants is very important in terms of environment and sustainability.[9]

Corn (*Zea mays* L.) is a plant of high commercial importance grown almost all over the world. While world corn production exceeded 1 billion tons, it reached 361 million tons only in America.[10] The corn plant consists of various parts such as stalk, leaves, cobs, husk, silk and roots, each playing a crucial role in its growth and reproduction. But, the majority of the silks found on the cob of produced corn are thrown away as garbage and in general it is not used in industrial processes.[10] Corn silk refers to the fine, thread-like strands that form the outer part of a cob of corn.[10] It’s the long, silky, shiny fibres that are found beneath the green husks and surround the kernels. While they might seem like mere packaging, corn silk actually plays a role in the growth and development of the corn plant. When harvesting corn, the silk is usually removed along with the husk, as it's not typically consumed.[10] The corn silk could be considered a source of antioxidants and may have

potential health benefits.[10] As the waste part of the plant, it contains 70.26 % carbohydrates, 11.06 % ash, 10.10 % oil and 6.26 % protein in average.[10] Additionally, corn silk is rich in valuable biochemical substances such as essential oils, lipids, phenolic compounds and flavonoids.[10] However, the nutritional value of corn silk isn't well-studied compared to other parts of the corn plant and it is destroyed as waste. However, the corn silk has potential in green synthesis of silver nanoparticles with its bioactive compounds.

The silver nanoparticles (AgNPs) are important metallic nanoparticles which are listed in the Organisation for Economic Co-operation and Development (OECD).[11] They have many application areas such as electronic, medical appliances, tableware, clothing, cosmetics, *etc*.[18] Many studies have investigated the microbial activity of AgNPs;[12–14] however, much less is known about the enzyme inhibition properties with nanoparticles. The enzyme inhibition studies are important to develop new drugs for many diseases. For example; the inhibitions of alpha amylase and alpha glycosidase are important for treatment of *Diabetes mellitus*;[15] inhibition of xanthine oxidase is important for the treatment of gout;[16] the inhibition of acetylcholine oxidase is important for Alzheimer's[17] and the inhibition of urease is important for treatment of stomach diseases such as ulcer and gastric.[18]

The synthesizing silver nanoparticles using plant sources is quite common.[6,9,12–14] However, many of the plants used in the synthesis process have nutritional value and are consumed by humans. In this study, the potential of plants that are not preferred to be consumed by humans, but are rich in phytochemicals for the synthesis of silver nanoparticles was determined. This study focused on the synthesis of silver nanoparticles with waste corn silk, and the inhibition properties of synthesized silver nanoparticles on enzymes which has key role in the treatment of diseases such as *Diabetes mellitus*, Alzheimer, gout and ulcer. The antioxidant activity of silver nanoparticles was also determined. As a result of the study, the potential of using nanoparticles as an enzyme inhibitor for the treatment of diseases was identified.

EXPERIMENTAL

Green synthesis of corn silk-based silver nanoparticles

The corn silk silver nanoparticles were synthesized according to the Keskin.[19] For this purpose, corn silk was harvested from a field located in Bilecik, Türkiye (39° and 40°31' north latitude and 29°43' and 30°41' east longitude) in 2023. The corn silk was washed to remove impurities, dried and ground and then the known amount of the corn silk was extracted by distilled water in ratio 1:100, using the maceration technique described previous by Keskin.[19] The prepared extract was filtered and mixed with 5 mM silver nitrate ($AgNO_3$, Sigma–Aldrich) solution in a dark flask at a 1:1 volume ratio for ~2 h at room temperature. The changes of colour and confirmation of nanoparticle synthesis was applied by UV absorption spectroscopy (Hach, DR/4000U) between 250 and 750 nm (Fig. 1). At the end of the synthesis, centrifugation was performed for 15 min at 9000 rpm with a high-speed centrifuge device to precipitate AgNPs

from the aqueous medium. The resulting AgNPs were washed with distilled water to wash off impurities and dried at 75 °C.

Fig. 1. Synthesis of CS–AgNPs.

The obtained silver nanoparticles were characterized using a UV spectrophotometer (Hach, DR/4000U) to detect the colour changes, Fourier transform infrared spectroscopy (FT--IR, Thermo Fisher) to determine the functional groups that were changed (reduced–oxidized), and a scanning electron microscope (SEM, ZEISS/Supra 40 VP) device to determine the sizes of the nanoparticles. The EDX analyses was performed to determine the elemental composition of nanoparticles as combined SEM-EDX.

Determination of optimum conditions for green synthesis of CS–AgNPs

To determine the effect of extract concentration on silver nanoparticle synthesis, 1.0, 2.0 and 3.0 % corn silk extracts were prepared at room conditions, silver nanoparticles synthesized and UV spectrophotometer absorbance values were compared.

To examine the effect of pH on silver nanoparticle synthesis, the corn silk extracts were prepared in different buffer solutions such as acetic acid sodium acetate buffer (pH 5.0 and 6.0) phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 8.0 and 7.0) and the glycine buffer solutions (pH 9.0). The nanoparticle synthesis was performed with each solution separately and the UV spectrophotometer absorbance values were compared.

To determine the temperature effect on silver nanoparticle synthesis, the silver nanoparticles were obtained at 20, 40 and 60 °C and also the UV spectrophotometer absorbance values were compared.[11]

Determination of total phenolic content of CS–AgNPs and corn silk extract

To determine the total phenolic content of both CS–AgNPs and corn silk extract the Folin method was used.[20,21] The phenolic compounds and Folin–Ciocâlteu reagent become a coloured complex and gave a maximum absorbance at 765 nm. Gallic acid (GA) was used as standard. The results were expressed in mg GAE/g dried weight (DW) sample. All the analyses were performed in triplicate.

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

DPPH$^{\bullet}$ is a radical which is used to determine antioxidant activity of various materials.[22] For this purpose, a stock methanol solution (100 μM) of this purchased radical was applied. The sample solutions at different concentrations were prepared by diluting the extracts of the samples with their own solvents. The equal volumes (750 μL) of DPPH$^{\bullet}$ solution and the sample solutions (at different concentrations) were mixed and left at room temperature for 50 min and then the absorbance was recorded at 517 nm.[22] By plotting these absorbance values against the concentrations, the SC_{50} values were calculated and expressed against the Trolox standard.[22] All analyses were performed in triplicate.

Iron reducing capacity (FRAP)

The FRAP method is based on reducing the iron (III) ion in the Fe(III)–TPTZ complex by natural products compounds.[23] Fe(III), which is reduced by the antioxidant substances in the solution, gives absorbance at 593 nm. The results were expressed in μM $FeSO_4 \cdot 7H_2O$ value. All analyses were performed in triplicate.

α-*Amylase enzyme inhibition*

To determine free α-amylase enzyme activity, an equal volume of enzyme solution was incubated with 300 μL of substrate for 30 min at a suitable temperature. At the end of the relevant period, an equal volume of DNS solution was added to the reaction mixture. The reaction mixtures were heated in a boiling water bath for 5–10 min. The colour formation was achieved by boiling. After the tubes cooled in an ice bath to room temperature, the absorbance against the blank was measured at 550 nm. For the inhibition study, increasing concentrations of inhibitor were added to the medium and activity was determined, the results were plotted and the IC_{50} value was calculated.[24] The prosedure was also performed for the corn silk extract. All analyses were performed in triplicate.

α-*Glucosidase enzyme inhibition*

The inhibition properties of *α*-glucosidase was determined according to Gholamhoseinian *et al.*[25] *p*-Nitrophenyl-*α*-D-glucopyranoside was used as substrate and the reaction was performed in 0.1 M pH 6.8 phosphate buffer solution. 5 μL of substrate, enzyme solution, 900 μL of phosphate buffer (50 mM) were mixed and 20 μL of silver nanoparticle solutions was added to the mixture. The mixture was incubated at 37 °C and the calculations were made by recording the absorbance values at 405 nm. The prosedure was also performed for the corn silk extract. All analyses were performed in triplicate.

Acetylcholinesterase inhibition

The acetylcholinesterase (ACT) inhibition was based on the coloration of thiocholine with DTNB.[26] The enzyme solution was prepared in 1% gelatin solution at 2.5 units mL^{-1}. 50 μL of enzyme solution and 50 μL of silver nanoparticle solutions and 3 mL of pH 8 phosphate buffer were mixed and incubated for 5 min at 25 °C. The reaction was started after the addition of 100 μL DTNB and 20 μL ACT. After 10 min, the absorbance was measured at 412 nm and the inhibition values were calculated. The positive control was performed using donepezil hydrochloride. The prosedure was also performed for the corn silk extract. All the analyses were performed in triplicate.

Urease inhibition

Urease is an enzyme that catalyzes the conversion of urea to amonium and carbon dioxide. The formation of urea was determined using the indophenol method.[27] Jack Bean urease (200 μL), 500 μL 0.01 M of phosphate buffer solution with 100 mM urea, 1 mM EDTA and 0.01 M LiCl at pH 8.2 and 100 μL silver nanoparticle solution was incubated for 20 min at room temperature. After the period, 550 μL of phenol solution (1 % phenol and 0.005 % sodium nitroprusside) and 650 μL of alkaline mixture (0.5 % sodium hydroxide and 0.1 vol. % NaOCl) were added to the tubes. The absorbances was recorded at 625 nm after 50 min. IC_{50} values were determined using standards from different concentrations. The prosedure was also performed for the corn silk extract. All the analyses were performed in triplicate.

Xanthine oxidase inhibition

The inhibition properties of xanthine oxidase enzyme were determined according to Baltaş *et al.*[27] The reaction mixture contains 500 µL silver nanoparticles, 770 µL pH 7.8 phosphate buffer and 70 µL xanthine oxidase enzyme. After preincubation for 15 min at 25 °C, 660 µL of substrate was added to the solution and incubated for 15 min at 25 °C. The reaction was stopped by adding 200 µL 0.5 M HCl, the absorbances were determined at 295 nm and IC_{50} values were calculated. The prosedure was also performed for the corn silk extract. All the analyses were performed in triplicate.

RESULTS AND DISCUSSION

The corn silk extracts contain various such as flavonoids, phenolics, terpenoids or enzymes that act as reducing agents.[10] These bioactive compounds interact with silver ions leading to the reduction of Ag^+ to Ag. The reduced silver ions start to aggregate and form nuclei in the solution due to the reducing agents present in the extract. The further reduction and aggregation of these nuclei lead to the growth of silver nanoparticles.[28] The concentration of the extract used in the green synthesis of silver nanoparticles (AgNPs) could significantly influence the synthesis process and the properties of the resulting nanoparticles. The nanoparticle formation and the colour changes to dark brown was monitored using a UV spectrophotometer and the nanoparticles gave a maximum absorbance at 470 nm. In general, because of the free electrons, AgNPs would display a surface plasmon resonance (SPR) band at 450–550,[29] 406,[20] 435,[31], 461.25[32] and 422 nm.[33] The obtained data in this study are similar to the previously reported AgNPs synthesis result.[20–40] The changes of UV-spectrum were recorded in order to determine the optimum extract concentration. The effect of the extract concentration on the synthesis of silver nanoparticle was presented in Fig. 2.

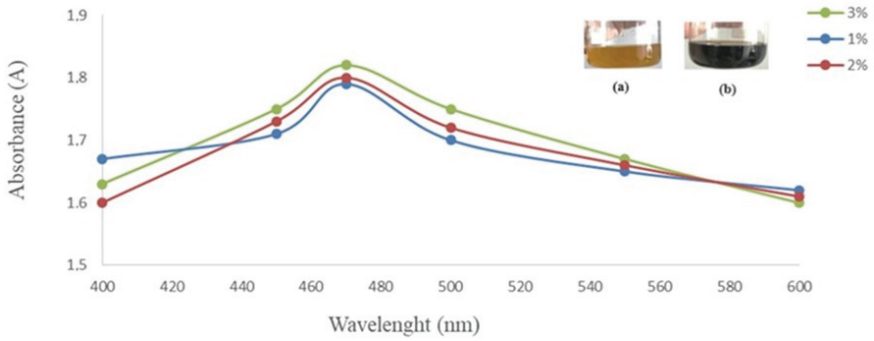

Fig. 2. Effect of extract concentration on synthesis of CS–AgNPs: a) corn silk extract and b) silver nanoparticle solution.

According to Fig. 2, the ratio 3.0 % had the maximum absorbance and it was chosen as optimum extract concentration for the further studies. The presence of large amounts of reductants in the reaction medium, such as the electron-rich

phytomolecules, is believed to cause the rapid reduction of Ag^+. This rapid reduction of Ag ions, in turn, promotes the further growth of nanoparticles. The pH is another important parameter for the green synthesis of silver nanoparticles and it can influence the interaction of the extract or bio-reducing agents with the nanoparticles. When the results of the UV-spectra were compared, it was determined that pH 7.0 is the optimum pH value for the green synthesis of silver nanoparticles (Fig. 3).

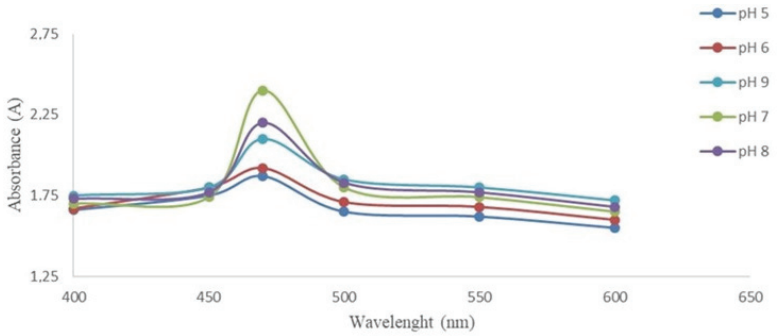

Fig. 3. Effect of pH on synthesis of CS–AgNPs.

Temperature is other crucial parameter for the green synthesis of silver nanoparticles. Elevated temperatures can speed up the reduction process, leading to quicker formation of nanoparticles. 60 °C was found the optimum temperature for the green synthesis of silver nanoparticles (Fig. 4), but synthesis was performed at room temperature to prevent or degradation of the bioactive components in the extract.

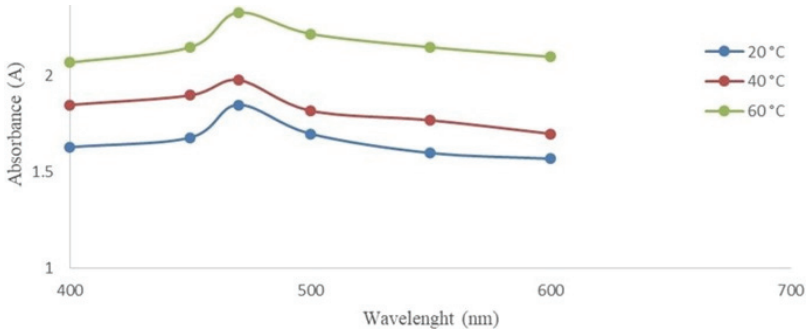

Fig. 4. Effect of temperature on synthesis of CS–AgNPs.

To determine the potential functional groups, FTIR was used. The FTIR peak of corn silk extract and supernatant of AgNPs were presented in Figs. 5 and 6, respectively. The wide peak of nearly 3275–3300 cm^{-1} indicates the presence of an O–H group in the spectrum. Although there are similarities at 2120, 1637.59

and 579 cm^{-1} peaks, there are differences at 2500, 2000–1900, 1300–1200 and 1000 cm^{-1}. These peaks are related with stretching vibrations of C=C, C=O, O–H, C–H, C–N and –COOH groups in alkane, ketone, alkene and nitro compounds.

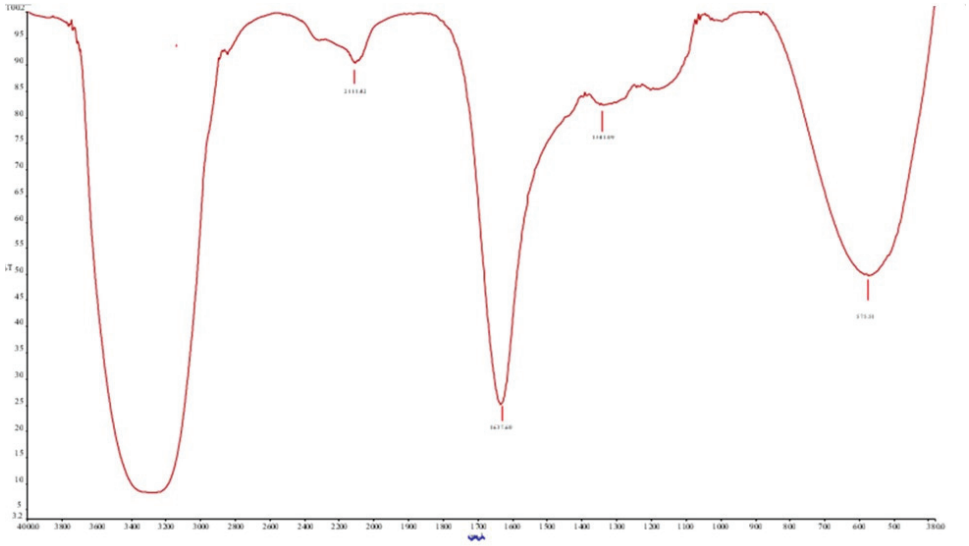

Fig. 5. FTIR spectrum of corn silk extract.

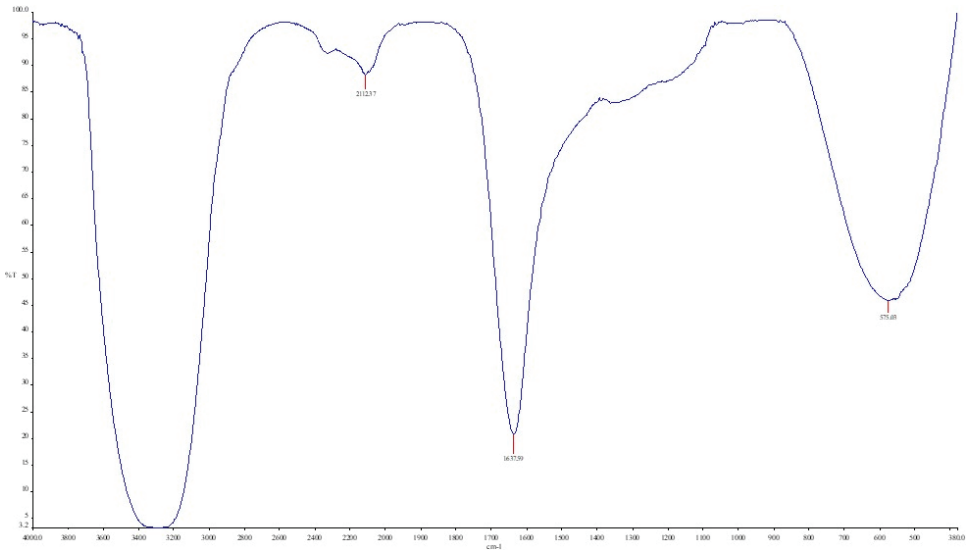

Fig. 6. FTIR spectrum of CS-AgNPs supernatant.

The particle size of the CS–AgNPs was found to be between 63 and 67 nm by the scanning electron microscope (Fig. 7). In literature, the AgNPs were obtained

in different sizes such as 4–10,[20] 65.92,[31] 12.63[32] and 46.26 nm.[33] It was clear that the particle size of AgNPs could change in a wide range.[28–38]

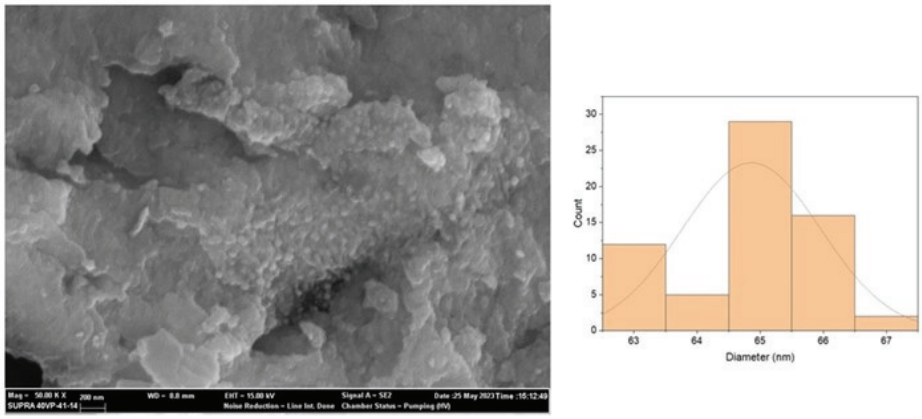

Fig. 7. SEM and histogram of CS–AgNPs.

The synthesized silver nanoparticles had a peak at 2.8 keV in EDX analyses which showed the presence of Ag (Fig. 8). EDX is applied to analyse the principal elemental components of NPs.

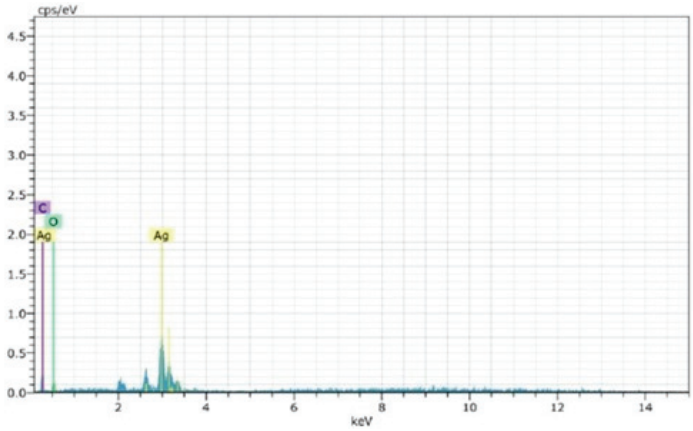

Fig. 8. EDX spectrum of CS–AgNPs.

It was determined that the CS–AgNPs were mainly composed of elemental silver (normalized atomic value 79.13 %), and the C and O contents were relatively lower (Table I).

The antioxidant activity of CS–AgNPs was determined 176.55±1.44 (FRAP) and 82.43±1.22 % μM TE g^{-1} (DPPH). The specific enzymes' inhibitions such as acetylcholinesterase, α-amylase, α-glucosidase urease and xanthine oxidase are

important for the treatment of illness like Alzheimer's disease, *Diabetes mellitus*, stomach infections and gout. The inhibition properties of CS–AgNPs (%) was found to be 52.27±1.09 (*α*-amylase), 43.51±0.98 (*α*-glucosidase), 80.33±1.21 (urease), 66.17±2.08 (acetylcholinesterase) and 73.67±1.70 (xanthine oxidase), respectively (Table II).

TABLE I. EDX results of CS–AgNPs

Element	Series	Unn. C (wt. %)	Norm. C (wt. %)	Atom. C (at. %)	Sigma (wt. %)
Ag	L	23.06	79.13	33.72	0.98
O	K	4.15	14.24	40.91	2.00
C	K	1.93	6.63	25.26	0.85
Total		29.14	100.00	100.00	

TABLE II. Biochemical properties of corn silk extract and CS–AgNPs; different letters represent significant differences at $p < 0.05$ probability level

Parameter	Corn silk extract	CS–AgNPs	Control	Control reagent
Total phenolic content (mg GAE g^{-1} DW sample)	20.16±0.21[a]	24.43±0.19[b]	–	–
FRAP (μM TE g^{-1})	100.18±1.27[a]	176.55±1.44[b]	–	–
DPPH (%)	70.69±1.18[a]	82.43±1.22[b]	–	–
α-Amylase inhibition (%)	48.15±1.11	52.27±1.09	46.84±1.05	Acarbose
α-Glucosidase inhibition (%)	32.21±1.23	43.51±0.98	30.19±1.24	Acarbose
Urease inhibition (%)	50.78±1.15	80.33±1.21	82.66±1.15	Thiourea
Acetylcholinesterase inhibition (%)	47.66±1.98	66.17±2.08	68.41±1.22	Donepezil hydrochloride
Xanthine oxidase inhibition (%)	68.70±1.76	73.67±1.70	76.18±1.18	Allopurinol

The synthesis of metallic nanoparticles using different biological resources has become very popular in recent years.[28,29] By determining the different biological properties of the resulting nanoparticles, their potential for use in areas such as health and environment could be determined. Some biological properties of silver nanoparticles synthesized using different biological sources are presented in Table III.

For example, Amin *et al.*[38] stated that silver nanoparticles based on *Solanum xanthocarpum* L. inhibited the urease enzyme by 64 %. Erenler *et al.*[33] synthesized silver nanoparticles based on *Tagetes erecta* L. in their study. They stated that the antioxidant activities of the nanoparticles they obtained had the IC_{50} values of 23.80 μg mL^{-1} and 2.79 μmol mg^{-1} sample corresponding to the DPPH and FRAP, respectively. Chinnasamy *et al.*[35] stated in their study that the silver nanoparticles they synthesized showed very high antioxidant activity (91±0.5 %, DPPH). Gul *et al.*[37] synthesized silver nanoparticles using different parts of the *Ricinus communis* plant in their study. They stated that the synthesized nanoparticles inhibited xanthine oxidase and urease enzymes by 83.6 and 94.2 %, respectively. Gopal *et al.*[38]

synthesized mushroom-based silver nanoparticles in their study and found that the obtained particles inhibited the α-amylase enzyme by 28 %. Thirumal and Sivakumar[39] synthesized silver nanoparticles based on *Cassia auriculata* and stated that the synthesized nanoparticles inhibited the α-glucosidase enzyme by 80 %. Abdelwahab *et al.*[40] synthesized silver nanoparticles based on *Aspergillus niger* in their study and stated that the nanoparticles they synthesized inhibited the acetylcholine esterase enzyme at a very high rate. The silver nanoparticles could inhibit enzymes by different ways. For example, they could interact with the AChE protein, inhibiting its activity, which demonstrates their affinity for cholinesterase. The lithophilicity of the nanoparticles and the hydrophobic environment of the ChE enzyme molecule facilitate this interaction.[41] As a result of this study, it was determined that the antioxidant properties and the enzyme inhibition properties of the synthesized silver nanoparticles were compatible with the literature.

TABLE III. Synthesis of AgNPs by using different sources

Biological source	UV absorbance, nm	Average size nm	Biomedical application	Reference
Solanum xanthocarpum L.	406	4–18	Antioxidant activity and enzyme inhibition properties	30
Glycosmis mauritiana	435	65.92	Antioxidant activity, antimicrobial activity and enzyme inhibition properties	31
Zea mays L.	461.25	12.63	Antimicrobial activity	32
Tagetes erecta L.	422	46.26	Antioxidant activity	33
Caesalpinia mimosoides	480	20–80	Antibacterial activity	34
Aristolochia bracteolata Lam	430	16.7	Antioxidant and antibacterial activities	35
Mikania cordata	451	26.8–46.0	Antioxidant and antibacterial activities	36
Waste corn silk	470	63–67	Antioxidant activity and enzyme inhibition properties	This study

CONCLUSION

The sustainable use and the protection of natural resources is very important. The natural waste, which increases due to the increasing population, needs to be recycled and used in areas such as technology, health, agriculture and environment. In this study, the environmentally friendly silver nanoparticles were synthesized from the waste corn silk using the green synthesis technique. The potential use of the synthesized nanoparticles in medicine applications was determined and the usability of waste corn silk in the field of health and nanotechnology was determined. As a result of this study, corn silks could be accumulated after the harvest time for recycling and used in the field of nanotechnology for different applications such as drug development, environment, *etc*.

ИЗВОД

ОТПАДНА КУКУРУЗНА СВИЛА ЗА ЕКОЛОШКИ ПРИХВАТЉИВЕ НАНОЧЕСТИЦЕ СРЕБРА: ЗЕЛЕНА СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И ОДРЕЂИВАЊЕ СВОЈСТАВА ИНХИБИЦИЈЕ ЕНЗИМА

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Одрживо коришћење ресурса је веома важно због пораста броја становника и нивоа потрошње. Кукуруз је једна од најпродаванијих житарица на свету. Међутим, многи његови делови, као што су кукурузна свила, корење и кукурузна љуска, одлажу се као пољопривредни отпад. У оквиру одрживости могуће је увести овај отпад у област технологије и развити нове производе зеленом синтезом. У овој студији, отпадна кукурузна свила је сушена, екстрахована и коришћена као прекурсор у синтези сребрних наночестица (CS–AgNPs). CS–AgNPs су окарактерисане коришћењем ултраљубичасте спектрофотометрије, инфрацрвене спектрофотометрије и скенирајућег трансмисионог електронског микроскопа. Утврђени су инхибицијски ефекти CS–AgNPs на ензиме као што су α-амилаза, α-гликозидаза, уреаза, ацетил-холинестераза и ксантин-оксидаза, који су важни за лечење одређених болести. Добијене наночестице су показале максималну апсорбанцију на 470 nm, док је просечна величина наночестица 65 nm. Утврђено је да CS–AgNPs показују веома добру антиоксидативну активност и инхибиторне ефекте на α-амилазу (52,27 %), α-гликозидазу (43,51 %), уреазу (80,33%), ацетил-холинестеразу (66,17 %) и ксантин-оксидазу (73,67 %). Добијени резултати показују да се наночестице синтетизоване техником зелене синтезе могу користити у медицини и фармацији.

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