European Scholar Journal (ESJ)
Available Online at: https://www.scholarzest.com
Vol. 4 No.08, August 2023
ISSN: 2660-5562

EXTRACTION HYALURONIC ACID FROM SOME PLANT SOURCES AND STUDY ITS ANTIOXIDANT ACTIVITY

[1]Wissam H. Abdel-Hamid; [1]Sawsan A. H. Al-Hilifi
[1]Department of Food Science, College of Agriculture, University of Basrah, Iraq
Corresponding author: e-mail: wesam152008@gmail.com

Article history:

Received: 20th June 2023
Accepted: 20th July 2023
Published: 25th August 2023

Abstract:

Hyaluronic acid, which is one of the many carbohydrates that has unique physical and chemical properties, is one of the important natural polymers, which made it widely used in the fields of pharmaceuticals, biomedical, nutritional and cosmetic supplements. Hyaluronic acid is composed of repeating units of glucuronic acid and N-acetyl glucosamine. Plants are among the important sources for the extraction and production of many effective compounds of nutritional importance and medical, pharmaceutical and cosmetic applications, due to its features, including low production cost, safe in terms of use and rich in natural antioxidants. In this study, light was shed on some plant sources such as sweet potatoes and local potatoes, as well as some plant residues such as lettuce leaves, which are rich in hyaluronic acid, and the modern techniques used in its extraction and displaying its biological properties and antioxidant effectiveness. Acid extracted by FTIR and high performance liquid chromatography (HPLC). The antioxidant activity of extracted hyaluronic acid was also tested. The acid extracted from sweet potato crests had higher antioxidant properties compared to the acid extracted from local potatoes and lettuce leaves

Keywords: Glycosaminoglycans; Hyaluronic acid; antioxidant activity; extraction; Purification

INTRODUCTION

Among the various compounds that are abundant in nature are biopolymers and their derivatives, which are characterized by unique characteristics that have made them the focus of attention of many scientists and researchers in recent times because of the wide applications these compounds have in different aspects of life. Amino acids, sugars and nucleotides are the monounits of biopolymers. Examples of these vital substances are starch, cellulose, proteins, pectin and peptides. (Elnashar, 2010; Abeer etal., 2017)

A great development has been achieved in recent years in the techniques of using natural biodegradable polymeric materials in the inputs of various vital fields, because the polymers have important mechanical, physical and chemical properties in addition to their ability to be enzymatically and hydrolyzed, and conditions must be met in the polymer in order to be allowed to be included in vital applications. It is the bioavailability and that it is non-toxic, and that it is able, through interactions, to form a biological network, build and develop tissues, in addition to the properties of tensile strength, mechanical strength, in addition to other properties in order to be similar to industrial polymers (Walaa et al., 2018; Hassan et al., 2019).). Biodegradable polymers have developed in the applications of various biological compounds. They have entered many fields, including the pharmaceutical industry in the seventies of the last century. The first successful biodegradable polymers are polyesters. It was developed and introduced in the sewing and textile industry. Danhier et al., 2010; Samir et al., 2022). (The biopolymers differ in the structural unit that enters into their structure, and the polymers are found in nature (Widner et al., 2004; Kobayashi et al., 2003).

Hyaluronic acid is a sulfur-free hydrocarbon organic chemical compound that can also be called hyaluronan (Hemshekhar et al., 2016; Zhai et al., 2020). It is a sticky, bio-saccharide substance naturally produced in the body with a high molecular weight. It was discovered by Karl Mayer and his assistant, John Palmer, as cow's eyeballs in 1934. Hyaluronic acid is a natural, biopolymer compound found in body tissues such as cartilage, eyes, cartilage, and skin. It mainly forms the connective tissue of the skin. This substance has the ability to absorb water molecules by 1000 times its weight, as it acts as a moisture absorber (Birajdar et al., 2021)

.It has important biological functions in humans, animals and bacteria alike. It is spread in most of the connective, epithelial and nervous tissues (Necas et al., 2011) and is particularly concentrated in the synovial fluid and the vitreous fluid of the eye. Hyaluronic acid is composed of repeated building blocks of D-glucuronic acid and N-glucuronic acid. -acetyl-D-glucosamine, and they are linked by a β-1→3 glycosidic bond, in addition to molecules of disaccharides, and they are linked by a β-1→4, glycosidic bond. Some studies in recent years have shown that hyaluronic acid has antioxidant effects (Ke et al., 2011). In recent years, there has been a tendency to move away from industrial sources

and replace them with natural sources in the field of food preservation and manufacturing, so the study aimed to extract and diagnose hyaluronic acid and to shed light On the antioxidant properties of haloyurinic acid extracted from sweet potatoes, local potatoes and lettuce leaves.

MATERIALS AND METHODS

Chemicals

Acetic acid, acetone1, chloroform, and acetic acid were obtained from Merck Chemicals Co. (Darmstadt, Germany),1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid. Potassium ferricyanide (K4[Fe(CN)6].3H2O), Butylated hydroxytoluene (BHT), Sodium Chloride (NaCl), Hydrogen Peroxide (H2O2), Ferric chloride (FeCl3) were purchased from Sigma-Aldrich (Merck) Sulphuric acid (H2SO4),.

Raw Materials

Plant samples were obtained from the local markets of the city of Basra. They were washed, cleaned, cut and dried at a temperature of 40 ± 2 °C for a period not exceeding 48 hours, depending on the type of plant. Then they were ground with an electric mill and kept in sealed containers by freezing 18±(- 3) °C until use.

Extraction of hyaluronic acid from animal sources

The method described by Soares. (2017) was followed. With a weight of 500 grams of vegetable samples (sweet potatoes, local potatoes, lettuce), acetone was added in a ratio of 1:1 (weight / volume) at a rate of three times within 24 hours to remove the fat, after which the fat was removed. extraction with acetic acid at a concentration of 0.5 M at a ratio of 1:2 (w/v) for 24 hours, after which the centrifugation process was carried out for 30 minutes at a speed of 7000 revolutions/min. 3000 cycles / minute again, the sediment was collected and dried at a temperature of 40 ° C, and kept in a sealed container until use.

Determination of hyaluronic acid by carbzole method

HA was determined in the extracts using the carbzole method by taking 0.1 mg / ml of each sample and adding 0.025 M of sodium tetraborate solution (in sulfuric acid sp. gr.1.84), and heated in a water bath at 100 ° C for ten minutes and then cooled the tubes by placing them in an ice bath for 10 min after that 0.2 ml of carbzole reagent (in 95% ethanol) was added and boiled for 15 min in a water bath and then left to cool at laboratory temperature The absorbance was measured at a 530 nm.

FTIR spectroscopy

Composite HA samples Fourier-Transform Infrared (FTIR) Spectroscopy were performed by(Perkin Elmer Spectrum One Nicolet 520, Japan, JASCO). The experiment was carried out at laboratory temperature by mixing HA samples with KBr was evaluated at 4000–400 cm^{-1}(Alizadeh-Sani et al., 2018).

High pressure liquid chromatography (HPLC)

Analysis of HA compounds were performed by HPLC Lyophilized HA extracts dissolved in 1 mL of distilled water and filtered before HPLC analysis. The injected sample volume was 10 µL and commercial HA as a standard was used to analyze. HPLC system implemented using a column C_{18} (260 x 4.5mm, 5 µm, Thermo Fisher Scientific, Japan). The solvent flow rate was 10 ml min^{-1} and the gradient was 30 min at 205 nm.

Antioxidant activity of HA

DPPH radicals scavenging activity

A free radical DPPH(f 2, 2-diphienyl-1-picrylhydrazile) was measured according to the method described by Wang et al.(2019) with some modification by mixing 1ml HA (0-125) mg/ml with 1 ml of DPPH(0.01M of ethanol). The mixture was incubated at laboratory temperature for 30 min in the dark, after which the absorption was measured at 517 nm. BHT was used as a comparison sample and at the same concentrations, the control sample was prepared in the same way except for the addition of methanol instead of the sample. for the following equation:

$$\text{DPPH scavenging activity} \quad \frac{A_C - A_s}{A_c} \times 100$$

$A_{Control}$ is the absorbance value of the DPPH, and A_s is the sample's absorbance

Reducing power

The reducing power was estimated according to the method used by Zhang etal.(2022). Briefly, with some modifications, 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 0.1%(w\v) K_3Fe (CN) were added to each sample. After incubating at 50°C for 30 min in the dark, 2. 5 mL of 10% (w/v) trichloroacetic acid solution has been added to the mix. The supernatant 2.5ml was mixed with 2 mL distilled water and 0.1 mL ferric chloride (0.1% w/v), After of 10 minutes, the absorbance measured at 700 nm.

Chelating of ferrous ion assay

The chelating effect of the produced HA on ferrous ions was evaluated by the Kosnett (2013) method and modified by Halfi (2009) and involving the mixing of 0.4 ml of HA(1-5mg\ml) with 0.4 mL 2 mM FeCl2 and with 0.4 ml 8-hydroxy quinolone 5 M (ethanol 98%) Then the reaction mixture was sit at laboratory temperature, for 10 min in a dark. Their absorbance were measured at 522 nm. the control sample was prepared in the same way above except for the addition of the sample. According to the following equation:

Chelating of Ferrous%=[1-A_c \A_s]×100

the absorbance of the control is $A_{control}$, and A_S is the absorbance of the samples.

Hydrogen peroxide (H_2O_2) scavenging activity assay

The ability of HA to scavenge (H_2O_2)was estimated according to the method by (Türkoğlu et al., 2010) , 1 ml of HA with different concentration (1-5mg\ml)was mixed with 0.6 mL of H_2O_2 solution (2 mM) prepared in a phosphate buffer (0.1 M, pH 7.4) and then the absorption was measured at 230 nm after 10 min. Ascorbic acid was used as positive control. The following equation was used to calculate the ability HA to scavenging activity:

Inhibition % =$[A_c$ **- A_s \A_c] ×100**

A_c= Absorbance of the control, A_s = Absorbance of sample

Statistical analysis

Statistical analysis was performed using the SPSS software (version 18.0). The ANOVA was used to analyze the data and results reported as mean ± SD. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Yield hyaluronic acid extraction

The results showed that there were significant differences at the level of probability $p \leq 0.05$ in the percentage of yield of hyaluronic acid extracted from plant sources. In Figure 6, the percentage of yield of hyaluronic acid extracted from sweet potatoes was 3%, while the percentage of yield in local potatoes and lettuce was 2.71%, 2.53%. % respectively, and the reason for the difference in the results may be due to the difference in the content of plant sources in terms of the percentage of carbohydrates and the size of the particles of the sample, as well as the difference in extraction solutions and the duration of extraction (Abdallah et al., 2020).

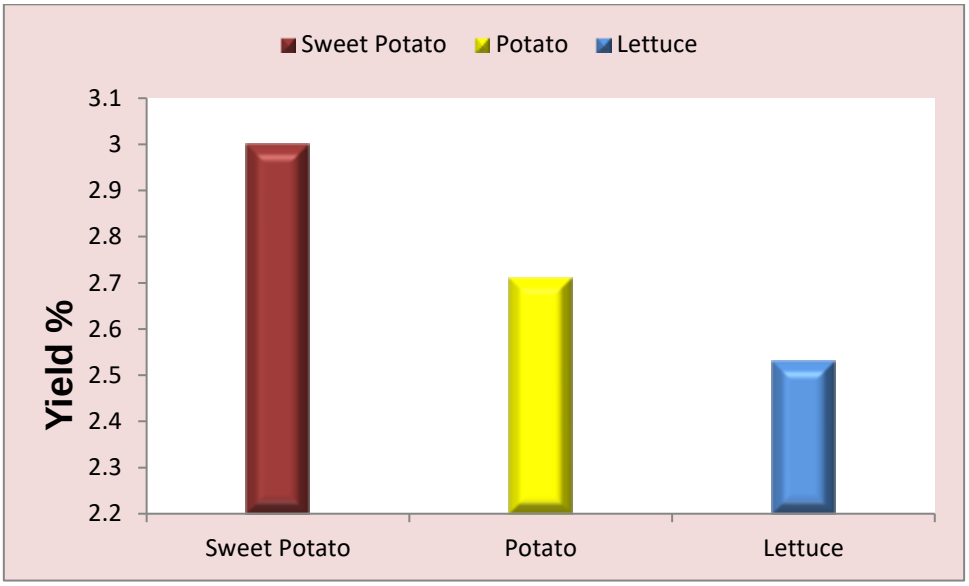

Figure1. The percentage of the yield

FTIR analysis

The active and normal totals of HA extracted Sweet potatoes, potatoes and lettuce leaves were diagnosed by using the infrared spectrum and compared with the spectrum of the standard acid as shown in Figure (2). If the similarity of the peaks of the active groups of the extracted HA with the active groups of the standard HA is observed, this indicates the presence of the active compounds present in the composition of the HA. They belong to the composition of hyaluronic acid, which contains the compound D-glucosamine. Peaks also appeared in the range of 2918-2929 cm-1, which are due to the vibration of the C-H bond and related to the Methylene group, while peaks appeared in the range of 1650-1400 cm-1, which are due to the vibration of the double bond C=O. Which belong to the amid a group of chlorochloronic acid, which is one of the building blocks of hyaluronic acid, and the C-N single bond vibrations of the amine group, as well as peaks appeared in the range of 1064-1040 cm -1, which belong to the C-O single bond vibrations belonging to the alcohol group.

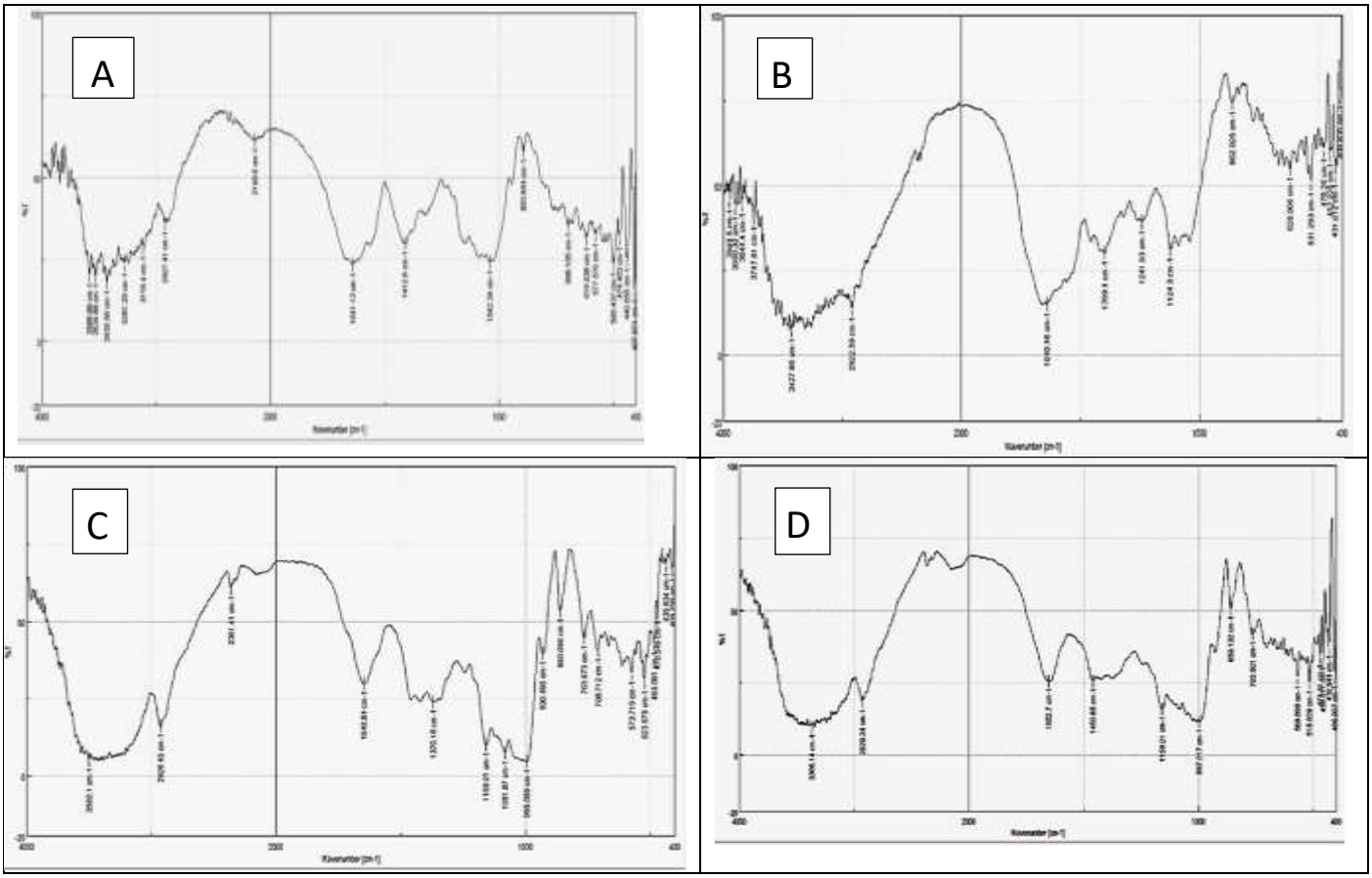

FIGURE 2. FTIR of hyaluronic acid, (A):HA standard, (B): acid extracted from Sweet potatoes, (C): acid extracted from potatoes , (D) : acid extracted from lettuce leaves

High-Pressure liquid chromatography (HPLC)

Figure (3) shows the diagnosis of HA extracted from Sweet potatoes, potatoes and lettuce leaves, compared with standard HA, where one peak appeared for each source with a slight difference in retention time ranging between 1.80-2.052 minutes, where the retention time was for standard HA and the acid extracted from rooster comb and fish eyes And sweet potatoes, local potatoes, and lettuce is 1.945, 1.790, 1.947, 1.978, 1.957, 2.052 minutes, and the appearance of one peak in all sources may indicate the purity of the extracted acid. These results were consistent with what was found by (Liu et al. 2013), where it was found that the retention time 1.85-1.95 minutes (de Oliveira, Nakamura, and Auzély-Velty 2020) found the retention time for standard HA to be 2.4 minutes.

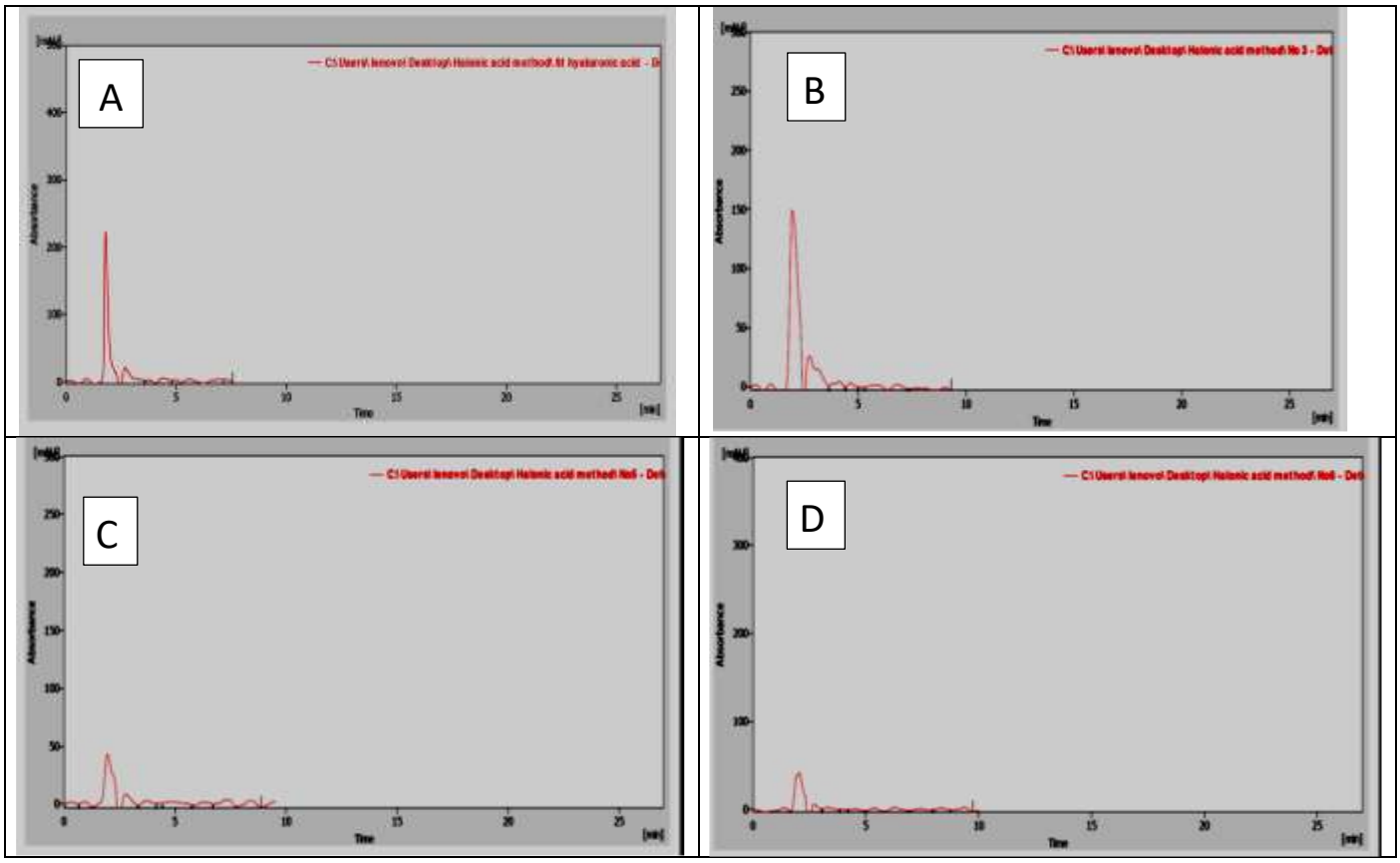

FIGURE 3. HPLC of hyaluronic acid, (A):HA standard, (B): acid extracted from Sweet potatoes, (C): acid extracted from potatoes , (D) : acid extracted from lettuce leaves

Antioxidant activities

DPPH Radical Scavenging Activity

Figure (4) shows the antioxidant effectiveness of HA extracted from plant sources and compared with synthetic antioxidants (BHT) using different concentrations ranging from 25-125 mg ml. It is noted that the extracted HA can scavenging the free radical (DPPH). Where this ability increases with increasing concentration, as the ability to absorb HA extracted from Sweet potatoes reached 27, 30, 45, 53, 59%, respectively, while the ability to absorb HA extracted from potatoes was 21, 29, 38, 44, 51. the ability to absorb HA extracted from lettuce leaves was 17, 26, 31, 40, 46.6 While the industrial antioxidant (BHT) showed the ability to scavenging the free radical (DPPH) higher for all concentrations, if it reached 67, 75, 83, 88.33, 91%, respectively. Antioxidant effect enhanced when the concentration was increase.(Al-Ali et al., 2021; Al-Hilifi et al., 2022).The effectiveness of HA in scavenging free radicals (DPPH) is due to functional groups such as the carboxyl group and the hydroxyl group that bind with free radicals to convert them into more stable compounds and thus end the chain of free radical reactions (Campo et al., 2004; Mohammed and Niamah, 2022)

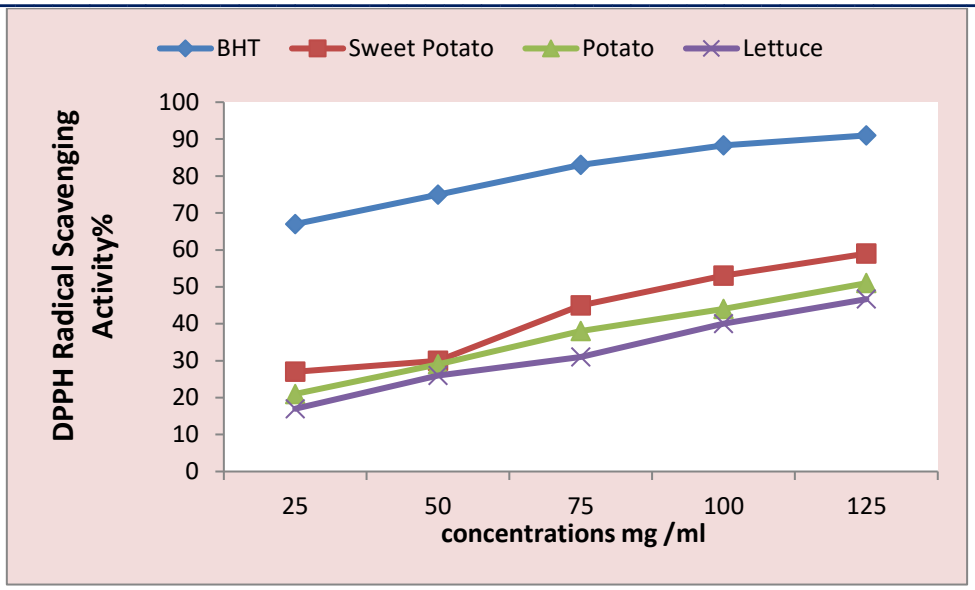

FIGURE. 4. DPPH radical scavenging activity of hyaluronic acid extraction and BHT

Reducing power

The results show in Figure (5) the reductive strength of HA extracted from Sweet potatoes, potatoes and lettuce leaves and compared with industrial antioxidant (ascorbic acid) and using different concentrations ranged between (25-125) mg ml, so it is noted that the extracted HA has a reductive strength this ability increases with increasing concentration, as HA extracted from Sweet potatoes showed the highest reductive power, followed by potatoes and lettuce leaves, and when compared with ascorbic acid, all results were lower than ascorbic acid. For HA extracted from Sweet potatoes, the absorbance was 1.023, 1.288, 1.487, 1.645, 1.896, respectively, while the absorbance of HA extracted from potatoes was 0.957, 1.201, 1.434, 1.59, and 1.721, respectively**.** while the absorbance of HA extracted from lettuce leaves was 0.847, 1.172, 1.298, 1.409, and 1.553, respectively The reducing ferric ion Fe^{+3} increased rapidly as the concentration increase (Zhang et al., 2022).These results are in agreement with what was reported by Sadhasivam et al. (2013) and Al-Hilifi et al. (2022), noting the increase in reductive power with increasing concentration used**.**

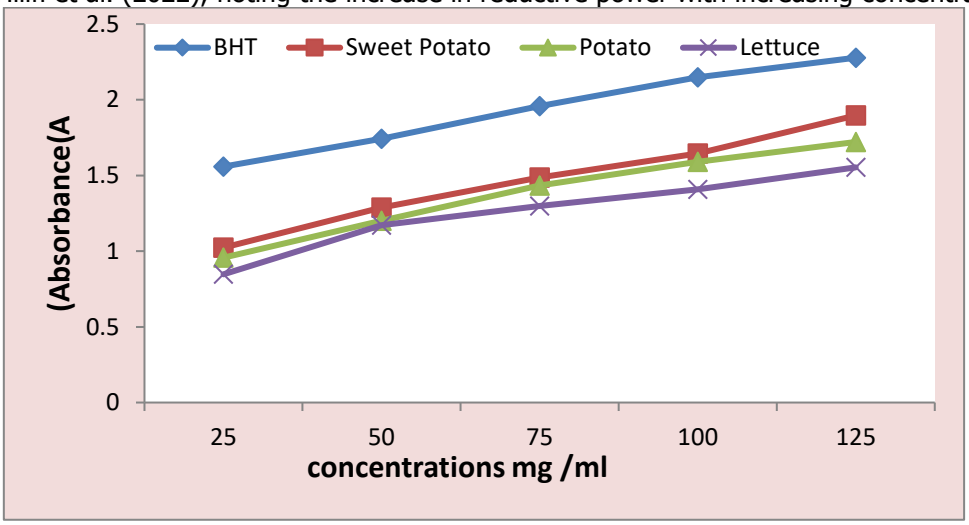

FIGURE.5.. Reducing power of hyaluronic acid extraction and ascorbic acid

Chelating of ferrous ion assay

Figure (6) shows the ability of HA extracted from Sweet potatoes, potatoes and lettuce leaves to bind the ferrous ion and compare it with the industrial antioxidant EDTA-2Na, using different concentrations that ranged between (1-5) mg ml. If it is noted that the extracted HA can bind the ferrous ion. This ability increases with increasing concentration, as the ability to bind the ferrous ion HA extracted from Sweet potatoes was 42, 51, 58, 62, 70.33%, respectively, while the ability to bind the ferrous ion to HA extracted from , potatoes was 41.66, 47, 53, 59.,61%, respectively**.** while the ability to bind the ferrous ion to HA extracted from lettuce leaves was 39, 46, 51, 53,57%, respectively The industrial antioxidant (EDTA-2Na) showed a higher binding capacity for all concentrations, if it reached 33, 42, 58, 78, 96%, respectively, and these results were consistent with what was mentioned by (Mohammed and Niamah 2022), where

was found that with increasing concentration, the ability of HA increases On the binding of iron ions, where the results indicate that the binding ability of HA at a concentration of 50 µg ml was 7.11%, while it reached 73.74% at the highest concentration of 1300 µg ml. The breakdown of peroxides and the decomposition of hydrogen peroxide (H_2O_2) into effective free radicals, and this is done using the Fenton reaction (Pisoschi et al., 2021).

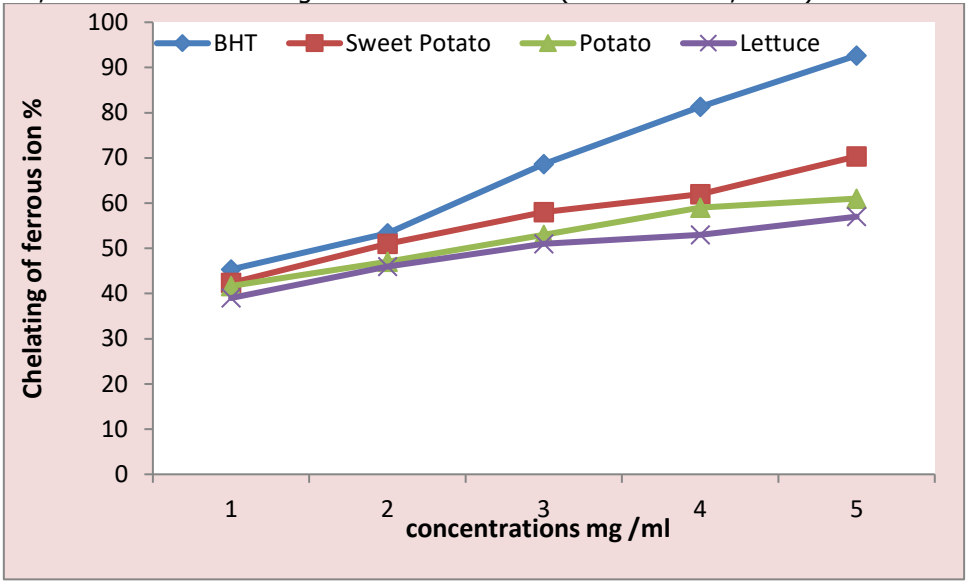

FIGURE. 6. Chelating of ferrous ion of hyaluronic acid extraction and EDTA-2Na

Hydrogen peroxide scavenging activity assay

Figure (7) shows the ability of hyaluronic acid extracted from Sweet potatoes, potatoes and lettuce leaves to radical the hydrogen peroxide radical and compared it with the synthetic antioxidant (BHT) using different concentrations that ranged between (1-5) mg ml. If it is noted that the extracted HA can radical the peroxide radical Hydrogen, as this ability increases with increasing concentration, as the ability to absorb HA extracted from Sweet potatoes reached 23,27, 46,51,58%, respectively**.** While the ability to scavenge HA extracted from potatoes was 9,14, 23, 34, 39%, respectively. While the ability to scavenge HA extracted from lettuce leaves was 8,13, 20, 32, 34%, respectively Hydrogen peroxide (H_2O_2) is a weak oxidizing agent in its natural form, but it is a source for the production of free radicals such as hydroxyl radicals and oxygen radicals, and thus their accumulation and the possibility of their interaction with ions mineral. The ability of HA to absorb hydrogen peroxide may be due to its possession of active groups in its structural composition, such as the carboxyl and the hydroxyl donor of the hydrogen atom, which make the hydroxyl radical (OH) or the free oxygen radical (O) more stable and prevents the start of the reverse reaction (AlMamary and Moussa., 2021; Ofoedu et al., 2021)

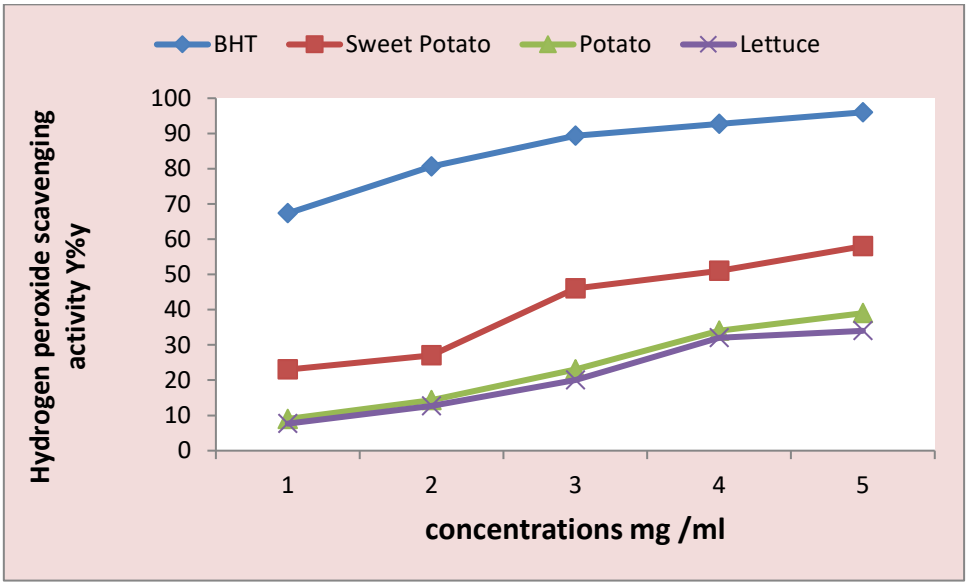

FIGURE. 7. Hydrogen peroxide scavenging activity of hyaluronic acid extraction and BHT

REFERENCES

1. Al-Ali, S.A., Al-Hilifi, and M.M.A. Rashed, (2021). Fabrication, characterization, and anti-free radical performance of edible packaging-chitosan film synthesized from shrimp shell incorporated with ginger essential oil. Journal of Food Measurement and Characterization, 8:9-16. https://doi.org/10.1007/s11694-021-00875-0.
2. Abdallah, M. M., Fernández, N., Matias, A. A., and do Rosário Bronze, M. (2020).
3. Hyaluronic acid and Chondroitin sulfate from marine and terrestrial sources: Extraction and purification methods. Carbohydrate Polymers. (2): 441-449
4. Oliveira, J. R.de Sousa, F. K. Andrade, E. H. Teixeira, M.Â. Cerqueira, A. L.s Coelho da Silva, and B. W. Silva de Souza. 2023. (1999).Extraction and Characterization of Hyaluronic Acid from the Eyeball of Nile Tilapia (Oreochromis Niloticus). International Journal of Biological Macromolecules 226: 172–83.R. T. Wang, "Title of Chapter," in *Classic Physiques*, edited by R. B. Hamil (Publisher Name, Publisher City, pp. 212–213. https://doi.org/10.1016/j.ijbiomac.2022.12.016
5. Al-Hilifi,S.A , R. M. Al-Ali, O. T. Al-Ibresam, N. Kumar, S. Paidari, A. Trajkovska PetkoskaV. , Agarwal(2022). Physicochemical, Morphological, and Functional Characterization of Edible Anthocyanin-Enriched Aloevera Coatings on Fresh Figs (*Ficus carica* L.). *Gels.* 8 (10), 645. **https://doi.org/10.3390/gels8100645**.
6. .Al-Hilifi,S.A R.M.. Al-Ali, A.T. Petkoska(2022). Ginger Essential Oil as an Active Addition to Composite Chitosan Films: Development and Characterization. Gels, 8, 327. https://doi.org/10.3390/gels8060327.
7. Alizadeh-Sani,M, A. Khezerlou,and A. Ehsani. (2018). Fabrication and characterization of the bionanocomposite film based on whey protein biopolymer loaded with TiO2 nanoparticles, cellulose nanofibers and rosemary essential oil. Industrial Crops and Products, vol. 124, pp. 300–315. https://doi.org/10.1016/j.indcrop.2018.08.001.
8. Birajdar, Mallinath S, Haejin Joo, Won-Gun Koh, and Hansoo Park. 2021. "Natural Bio-Based Monomers for Biomedical Applications: A Review." Biomaterials Research 25 (1): 1–14. https://doi.org/10.1016/j.indcrop.2018.08.001.
9. Brigham, Christopher. 2018. "Biopolymers: Biodegradable Alternatives to Traditional Plastics." In Green Chemistry, 753–70. Elsevier.
10. Campo, Giuseppe M, Angela Avenoso, Salvatore Campo, Alida M Ferlazzo, Carmelo Micali, Laura
11. Zanghì, and Alberto Calatroni. 2004. "Hyaluronic Acid and Chondroitin-4-Sulphate Treatment Reduces Damage in Carbon Tetrachloride-Induced Acute Rat Liver Injury." *Life Sciences* 74 (10): 1289–1305. https://doi.org/10.1016/B978-0-12-809270-5.00027-3
12. Elnashar, Magdy. 2010. *Biopolymers*. BoD–Books on Demand.
13. Hassan, Mohamed El Sayed, Jun Bai, and De-Qiang Dou. 2019. "Biopolymers; Definition, Classification and Applications." Egyptian Journal of Chemistry 62 (9): 1725–37. 10.21608/EJCHEM.2019.6967.1580.
14. Hemshekhar, Mahadevappa, Ram M Thushara, Siddaiah Chandranayaka, Larry S Sherman, Kempaiah Kemparaju, and Kesturu S Girish. 2016. "Emerging Roles of Hyaluronic Acid Bioscaffolds in Tissue Engineering and Regenerative Medicine." International Journal of Biological Macromolecules 86: 917–28. 10.1016/j.ijbiomac.2016.02.032
15. Ke, Chunlin, Lanping Sun, Deliang Qiao, Di Wang, and Xiaoxiong Zeng. 2011. "Antioxidant Acitivity of Low Molecular Weight Hyaluronic Acid." Food and Chemical Toxicology 49 (10): 2670–75. 10.1016/j.fct.2011.07.020
16. Ke,C., Sun, L., Qiao, D. , D.i. Wang, X. Zeng (2011). Antioxidant activity of low molecular weight hyaluronic acid, Food Chem. Toxicol. 49 (10): 2670–2675. 10.1016/j.fct.2011.07.020
17. Khabarov, Vladimir N, Petr Ya Boykov, and Mikhail A Selyanin. 2015. Hyaluronic Acid: Production, Properties, Application in Biology and Medicine. John Wiley & Sons.
18. 10.1002/9781118695920
19. Liu, Yanhua, Jin Sun, He Lian, Xin Li, Wen Cao, Liming Bai, Yongjun Wang, and Zhonggui He. 2013. "Determination of Paclitaxel in Hyaluronic Acid Polymeric Micelles in Rat Blood by Protein Precipitation-Micelle Breaking Method: Application to a Pharmacokinetic Study." Journal of Chromatography B 935: 10–15. 10.1016/j.jchromb.2013.07.008
20. Mohammed, Ameer A, and Alaa Kareem Niamah. 2022. "Identification and Antioxidant Activity of Hyaluronic Acid Extracted from Local Isolates of Streptococcus Thermophilus." Materials Today: Proceedings 60: 1523–1529. . https://doi.org/10.1016/j.matpr.2021.12.038.
21. Ofoedu, Chigozie E, Lijun You, Chijioke M Osuji, Jude O Iwouno, Ngozi O Kabuo, Moses Ojukwu, Ijeoma M Agunwah, James S Chacha, Onyinye P Muobike, and Adedoyin O Agunbiade. 2021. "Hydrogen Peroxide Effects on Natural-Sourced Polysacchrides: Free Radical Formation/Production, Degradation Process, and Reaction Mechanism—a Critical Synopsis." Foods 10 (4): 699. https://doi.org/10.3390/foods10040699.
22. Oliveira, Mariana Maciel de, Celso Vataru Nakamura, and Rachel Auzély-Velty. 2020. "Boronate-Ester Crosslinked Hyaluronic Acid Hydrogels for Dihydrocaffeic Acid Delivery and Fibroblasts Protection against UVB Irradiation." Carbohydrate Polymers 247: 116845. https://doi.org/10.1016/j.carbpol.2020.116845.
23. Pisoschi, Aurelia Magdalena, Aneta Pop, Florin Iordache, Loredana Stanca, Gabriel Predoi, and Andreea Iren Serban. 2021. "Oxidative Stress Mitigation by Antioxidants-an Overview on Their Chemistry and Influences on Health Status." European Journal of Medicinal Chemistry 209: 112891. https://doi.org/10.1016/j.ejmech.2020.112891.

24. Rasel, Md, Sojib Raihan, Israt Zerin, Mohammad Tofayel Ahmed, Md Shah Alam, and Habibur Rahman Abir. 2017. "Impact Analysis of Electro Spun Nano Fiber from Biodegradable Polymer for Tissue Engineering-A Review Article." Parameters 39: 40. :
25. 10.5923/j.textile.20170606.03
26. Sadhasivam, Giji, Arumugam Muthuvel, Abirami Pachaiyappan, and Balasubramanian Thangavel. 2013. "Isolation and Characterization of Hyaluronic Acid from the Liver of Marine Stingray Aetobatus Narinari." International Journal of Biological Macromolecules 54: 84–89. https://doi.org/10.1016/j.ijbiomac.2012.11.028.
27. Samir, Aya, Fatma H Ashour, A A Abdel Hakim, and Mohamed Bassyouni. 2022. "Recent Advances in Biodegradable Polymers for Sustainable Applications." Npj Materials Degradation 6 (1): 68. https://doi.org/10.1016/j.biortech.2021.124739.
28. Singh, Akhilesh V. 2011. "Biopolymers in Drug Delivery : A Review." Pharmacologyonline 1: 666–74. 10.3390/polym13162729.
29. Soares, G. C. (2017). Hyaluronic acid and collagen extraction from chicken combs.117.PHD thesis. Universidade Católica.pp 117
30. Song, Richard, Maxwell Murphy, Chenshuang Li, Kang Ting, Chia Soo, and Zhong Zheng. 2018. "Current Development of Biodegradable Polymeric Materials for Biomedical Applications." Drug Design, Development and Therapy, 3117–45. 10.2147/DDDT.S165440.
31. Thakur, Sourbh, Jyoti Chaudhary, Pardeep Singh, Walaa F Alsanie, Sotirios A Grammatikos, and Vijay Kumar Thakur. 2022. "Synthesis of Bio-Based Monomers and Polymers Using Microbes for a Sustainable Bioeconomy." Bioresource Technology 344: 126156. https://doi.org/10.1016/j.biortech.2021.126156.
32. Wang, L.; Guo, H.; Wang, J.; Jiang, G.; Du, F.; Liu, X.(2019).Effects of Herba Lophatheri extract on the physicochemical properties and biological activities of the chitosan film. Int J Biol Macromol, 133, 51-57. https://doi.org/10.3390/coatings11091077.
33. Zhai, Peisong, Xiaoxing Peng, Baoquan Li, Yiping Liu, Hongchen Sun, and Xiangwei Li. 2020. "The Application of Hyaluronic Acid in Bone Regeneration." International Journal of Biological Macromolecules 151: 1224–39. https://doi.org/10.1016/j.ijbiomac.2019.10.169.
34. Zhang Q, Lu L, Zheng Y, Qin C, Chen Y, Zhou Z. (2022). Isolation, Purification, and Antioxidant Activities of Polysaccharides from Choerospondias axillaries Leaves. Molecules.27(24):8881. https://doi.org/10.3390/molecules27248881.