Tikrit Journal for Agricultural Sciences (2024) 24 (2): 214-222
DOI: https://doi.org/10.25130/tjas.24.2.15

IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

Phytochemical analysis and biological activity of some local and imported walnut

Shabaz H. Hama[1]*, and Kadhm A. Muhammad[2]

[1] *Dep. of Biotechnology and Crop Science, College of Agricultural Engineering Sciences, University of Sulaimani, Iraq*

[2] *Dep. of Agribusiness and Rural Development (ARD), College of Agricultural Engineering Sciences, University of Sulaimani, Iraq*

*Corresponding author: E-mail: **shabaz.hama@univsul.edu.iq**

KEY WORDS:
Phytochemicals, Biological Activity, Phenolic Acids, Fatty Acids, Antioxidants

Received: 19/09/2022
Revision: 18/10/2022
Proofreading: 20/10/2022
Accepted: 31/10/2022
Available online: 30/06/2024

ABSTRACT

To study the bioactive substances of the walnut kernel of six cultivars which were newly selected from Kurdistan-Iraq and one type import from America, analyzed for their phytochemical contents and antioxidant activities. HPLC (High-performance liquid chromatography) was used for phenolic compound estimation, GC (Gas chromatography) for fatty acid analysis, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging. In terms of fatty acids, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid of all seven types we analyzed, all of them had a significant result. As for antioxidants, the same as before, the antioxidants were significant for all the chosen samples. In terms of phenolic compounds, quinic acid, gallic acid, 1,2,3,6 trigalloyl glucose, vanillic acid, syringic acid, and rutin, all types were significant as well. Finally, our results show that most of them were of high significance. Some regions in the Kurdistan region of Iraq showed high results for important secondary products, while the American counterpart is lower but still better than some of the Kurdistan region walnuts.

التحليل الكيميائي النباتي والنشاط البيولوجي لبعض أشجار الجوز المحلية والمستوردة

شاباز هاشم حمه[١] وكاظم عبدالله محمد[٢]
[١] قسم التكنولوجيا الحيوية وعلوم المحاصيل، كلية علوم الهندسة الزراعية، جامعة السليمانية
[٢] قسم الارشاد الزراعي والتنمية الريفية، كلية علوم الهندسة الزراعية، جامعة السليمانية

الخلاصة
دراسة المواد النشطة بيولوجيا لنواة الجوز لستة أصناف تم اختيارها حديثًا من كردستان العراق ونوع واحد تم استيراده من أمريكا ، وتحليل محتواها الكيميائي النباتي وأنشطتها المضادة للأكسدة. تم استخدام HPLC (High-performance liquid chromatography) لتقدير المركب الفينولي ، GC (Gas chromatography) لتحليل الأحماض الدهنية ، و DPPH (2,2-diphenyl-1-picrylhydrazyl) الكسح الجذري. من حيث الأحماض الدهنية ، وحمض البالمتيك ، وحمض دهني ، وحمض الأوليك ، وحمض اللينوليك ، وحمض اللينولينيك من جميع الأنواع السبعة التي قمنا بتحليلها ، كان لكل منهم نتيجة مهمة. بالنسبة لمضادات الأكسدة، كانت مضادات الأكسدة مهمة لجميع العينات المختارة. من حيث المركبات الفينولية ، وحمض الكينيك ، وحمض الغاليك ، و 1،2،3،6 الجلوكوز الثلاثي ، وحمض الفانيليك ، وحمض السيرينجك ، والروتين ، كانت جميع الأنواع معنوية أيضًا. أخيرًا ، تظهر نتائجنا أن معظمها كان ذا أهمية عالية. أظهرت بعض المناطق في إقليم كردستان العراق نتائج عالية لمنتجات ثانوية مهمة ، في حين أن نظيرتها الأمريكية أقل لكنها مازالت أفضل من بعض حبات الجوز في إقليم كردستان.

الكلمات المفتاحية:
المواد الكيميائية النباتية ، النشاط البيولوجي ، الأحماض الفينولية ، الأحماض الدهنية ، مضادات الأكسدة

INTRODUCTION

World Health Organization (WHO) statistics showed that around 80% of people rely on traditional medicine made from plants. The important medicines we have such as anticancer, antimicrobial, and anti-inflammatory drugs have their origins in nature (Shafaghat, 2011). The walnut tree, or *Juglans regia* L., grows to great heights and lives for a very long time in temperate regions of the world. (Żurek *et al.*, 2022). Consumption of these has been linked to a number of purported health benefits, including decreased change of heart related disease, treatment of type II diabetes, coronary heart diseases, prevention and treatment of specific cancers, and alleviation of symptoms associated with aging and other neurological disorders. Walnuts are good because they contain a great amount of omega-3 and omega-6 fatty acids compared to any tree nut, which is attributable to their high polyunsaturated fatty acid content (Hayes *et al.*, 2016).

Walnuts are good for health because they contain lipids (tocopherol, fatty acid). Approximately 60% of a walnut kernel's total mass is comprised of oil (Macrae *et al.*, 1993). Walnut oil content varies with cultivar, growing region, and watering schedule (Greve *et al.*, 1992; Çağlarırmak *et al.*, 2003). The way that was used to decide the main fatty acids present in heartnuts and walnuts is Gas chromatography. These included linoleic (18:2n-6), alpha-linolenic (18:3n-3), oleic (18:1n-9), palmitic (16:0), and stearic acid (18:0) (LiL 2007). Typically, 100 g of walnuts contain 50 g of multi-unsaturated fatty acids, of which 38.09 g are Omega 6 (linoleic acid) and 9.08 g are Omega 3 fatty acids (linolenic acid). Additionally, phytochemical substances like phenolics are beneficial for human health. By avoiding oxidative stress and biological macromolecule oxidation, they can decrease the risks of degenerative and cardiovascular related diseases (Jahanban-Esfahlan *et al.*, 2019). With the

inclusion of its anti-cancer properties, it has been demonstrated that phenolic compounds have the ability to neutralize free radicals and have metal-chelating properties (Jahanban-Esfahlan *et al.*, 2019). The walnut kernel has previously been assessed in this regard as an antioxidant by Labuckas *et al.*, (2008) and Zhang *et al.*, (2009). Using either the free radical 2, 2-diphenyl-1-picrylhydrazyl assay or the photochemiluminescence method, it was discovered that tocopherols, particularly the gamma-tocopherol, contributed the most to the strong total antioxidant activities of both walnut and heartnut oils. To the best of our knowledge, however, there is no information on the phytochemical components and antioxidant activities of the ehanolic extract from Iraqi walnut kernels.

MATERIAL AND METHODS

Seven different cultivars of walnuts were collected some sample walnuts since October of 2021. The locations of which the samples were gathered from for the research were: Tawella, Byara, Sharbazher, Qaladiza, Sargallu Bargalu, Warte, and walnuts imported from the United States of American origin. At elevations of: 1,688, 1,146, 1113, 882, 972, 1282 respectively (N/A elevation for the US origin walnuts).

Fatty acid composition

The walnuts were grinded inside a blender for a period of 30 seconds to decide their oil content. Through using a Soxhlet apparatus, after grinding (Yerlikaya *et al.*, 2012). The walnuts extracted with hexane in a period of six hours after grinding. Gas chromatography (GC; Varian Chromatograph, Model 1400; Varian Associates, Walnut Creek, CA), outfitted with a flame ionization detector and a column with the measurements of 3.0 m x 0.32 cm, full of LAC-3R-728(20%; Cambridge Ind. Co., Cambridge, UK) on Chromosorb W/AW, has been ascertained for the composition of the fatty acids (80-100 mesh; Merck, Darmstadt, Germany). In accordance with ISO 5508:1990, "Analysis by gas chromatography of methyl esters of fatty acids," nitrogen was used as a carrier gas (flow rate, 24 mL/min) (AOCS, 1993).

Extraction of phenolic compounds

To acquire the crude metabolic extract (CME) including the solid to solvent ratio of 1:10(w/v) for an hour stored at room temperature, 20 grams of dried, ground black cumin seedcake were extracted, containing 80% methanol (3*200ml) by sonication (Hasan Technology, Seoul, Korea). The combination and concentration of CME was taken care of by a rotary evaporator (Bauchi, Flail, Switzerland). The acquired CME (4.4g) was fractionated by using 3 * 100 ml each of hexane, ethyl acetate, and water, using the leftover material to produce the next fraction with each fractionation step. Each extraction procedure started with homogenizing CME and its fractions in the solvent for a period of 15 minutes at a speed of 13,000 rpm, followed by sonication at a constant temperature of 30 °C in the span of an hour. Then, CME was purified using a paper filter including the fractions (hexane fraction HF, ethyl acetate fraction EAF, and water fraction WF). Next up, utilizing a rotary evaporator, the solvents were removed (Shehata & Ibrahima, 2019). Before being stored at -80°C for additional analysis, each extract's yield and its fractions were measured (Mariod *et al.*, 2009).

HPLC-DAD system for phenolic compound analysis:

Chromatographic separations were carried out on a LUNA C-18 column (5 m, 250 x 4.6 mm) and HPLC analysis was carried out using Agilent G1310A pumps (Agilent, Stevens Creek Blvd, Santa Clara, USA) (Phenomena, Torrance, CA, USA). With some tweaks (Chirino and Pedraza-Chaverri, 2009). Described previously, the solvent composition and usage of gradient elution conditions. The solvent (A) water-acetic acid (94:6, v/v, pH 2.27) and the solvent (B) acetonitrile both made up the mobile phase. The gradient of the solvent was 0-15% B in the span of 40 minutes, 15-45% B in the period of 40 minutes, and 45-100% B in the period of 10 minutes as well. 20 ml of sample was injected at a flow rate of 0.5 milliliter per minute. Prior to HPLC injection, samples and mobile phases have been filtered through a 0.22 m Millipore filter, type GV (Millipore, Bedford, MA). Analyses of each fraction were conducted two times. By comparing the data of retention times and UV-Vis spectral data to known, previously injected standards, phenolic compounds were identified and quantified (Chirino & Pedraza-Chaverri, 2009).

Antioxidant

Radical scavenging activity by DPPH:

Started by grinding our walnut samples with a mortar and pestle. The powder material weighing 0.1g was extracted with 1000 ml of 80% (v/v) methanol and incubated for 16 hours at 10°C. Then we started centrifuging our samples for 19 minutes at 10,000 rpm and the supernatant (extract) was retained and used for analysis of the DPPH test. After that, we determined the antioxidant capacity of the extract according to the estimated method, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging as defined by (Fajrina & Tahir, 2019). With some improvements. Standard composition: The standard compound, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Troop), was used to construct the calibration curve using a series of diluted solutions. 11mg of Trolox was mixed with a concentration of 75% ethanol solvent and diluted to obtain some concentrations up to 0.325, 0.625, 1.300, 1.950, 2.600, and 3.25 g/mL.Trolox concentrations were shown to have a linear relationship with absorbance values at 517 nm. The tubes were divided into three groups. In Group 1, 2 ml of DPPH solution (6 x 10-5 M) was mixed with a 10 L sample. In Group 2, 10 L of Methanol and 2 mL of DPPH solution are dissolved in methanol. In the third group, Trolox was mixed with 2 mL of DPPH solution. All tubes (sample extract, control, and Trolox) were incubated for 30 minutes in the dark in order to absorb against a blank that contained just methanol (UV-365, Shimadzu, Japan). It was decided to carry out the triple experiment. Using the formula Trolox Equivalent Trolox per Gram of Dry Matter, the ability of different extracts to fight free radicals was calculated. DPPH antioxidant capacity (g Trolox/g DM) = V/W x C

Where V is the volume of extract (mL), W is the weight of the sample (g), and C is the concentration of Trolox determined from the standard curve.

RESULTS AND DISSCUSSION

Table 1 showed the amounts of some chosen extracted fatty acids, which consists of: Palmtitic, Stearic, Oleic, Linoleic, and Linolenic acids of each type of walnut that were chosen for the analysis.

In this table, the results were laid out for each type of fatty acids per location. In Palmtitic acid, W55 (Qaladiza walnuts) had the highest amount, measured at 12.507 mg/g, meanwhile W155 (Tawella walnuts) had the least amount, measured at 4.184 mg/g. The second one which is Stearic Acid had the highest recorded at W55 (Qaladiza walnuts) measured at 12.540 mg/g, however W99 (Sargallu Bargalu walnuts) had the least amount, measured at 5.665 mg/g. The third one, Oleic Acid had the highest record at W11 (Sharbazher walnuts) measured at 46.502 mg/g, although W33 (Warte walnuts) had the least amount, measured at 10.747 mg/g. Before last, Linoleic Acid had the highest recorded at W133 (American walnuts) measured at 52.070 mg/g, but W155 (Tawella walnuts) had the least amount, measured at 13.936 mg/g. The last one which is Linolenic Acid, had the highest record at W111 (Byara walnuts) measured at 24.199 mg/g, on the other hand, W155 (Tawella) had the least amount, measured at 0.223 mg/g. Overall, all the results for each location were significant. The W133 (American walnuts) had the most amount of interaction with Linoleic Acid, measured at 52.070. Meanwhile, the W155 (Tawella walnuts) which record the least Linolenic Acid among all of them, measured at 0.223.

From previous analysis on Table 1, it was shown the reason behind the differences in characteristics of the walnuts can be mainly geographical features such as: weather (the amount of rain and temperature), soil and its containing minerals, topography of the areas. Another small one could be the amount of helping hand of organizations and higher-ups, helping farmers get enough resources and help to grow the walnuts in terms of quality and quantity. Previously reported results were similar to Bou Abdallah et al. (2016).

Table (1): Fatty acids in *Walnut* sampled at different altitudes (mg/g).

Locations ID	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid
W55	12.507 a	12.540 a	19.043 c	41.183 c	8.257 e
W11	11.156 ab	5.833 c	46.502 a	17.822 d	14.608 d
W111	8.224 c	10.834 ab	13.559 e	42.544 c	24.199 a
W99	9.530 bc	5.665 c	15.881 d	51.882 a	17.042 c
W133	6.167 d	10.407 b	14.440 de	52.070 a	13.537 d
W33	8.495 c	9.510 b	10.747 f	47.712 b	20.693 b
W155	4.184 e	5.773 c	44.175 b	13.936 e	0.223 f

Duncan's Multiple Range Test indicates a 1% significance difference between means that share a letter for all factors and interactions.

Table 2 have the information on the amount of phenolic acids in the samples. The first type was Quinic Acid, which recorded highest at W11 (Sharbazher walnuts) measured at 25.586 mg/g, meanwhile W133 measured the least at 17.287 mg/g. Next up, Gallic Acid was recorded highest at W155 (Tawella walnuts) measured at 18.973 mg/g, although W133 (American walnuts) was the lowest of the bunch measured at 0.952 mg/g. Then, 1,2,3,6 Trigalloyl Glucose was measured the highest at W133 measured at 11.792 mg/g, but W111 (Byara walnuts) was measured the lowest at 1.819 mg/g. After that, in Vanillic Acid the highest record was at W155 (Tawella walnuts) measured at 10.311 mg/g, on the other hand, W33 was measured the lowest at 0.076 mg/g. Before last, Syringic Acid had the highest record at W155 (Tawella walnuts) measured at 13.614 mg/g, however W111 (Byara walnuts) had the least amount measured at 3.736 mg/g. For the last, Rutin was recorded the highest at W11 (Sharbazher walnuts) measured at 11.997 mg/g, Whereas W33 (Warte walnuts) had the least amount measured at 7.882 mg/g. All the results for each sample were significant. Interaction of both W11 (Sharbazher walnuts) and Quinic Acid was the highest between all of them, which was measured at 25.586. But, the interaction between W33 (Warte walnuts) and Vanillic Acid had the least amount among them which measured at 0.076. The contributors in the inconsistency of phenolic acids mostly relate back to table 1 and 2s

Contributors as well, the main ones being common geographical features. Minor contributors such as farmers, as well as the helping hand of organizations and higher-ups. Pre It was found in this study that some walnut varieties had higher concentrations of fatty acids than others. The chemical makeup of walnut oil fluctuates based on factors like cultivar, latitude, weather, and processing. Fertilizers used in the cultivation of walnuts, for instance, change the oil's chemical composition. Walnut oil's chemical make-up is also influenced by factors like the seed's position on the plant and how it's handled after harvest.viously reported results were similar (ERCISLI *et al.*, 2011).

Table (2): Phenolic compounds in Walnut at different altitudes (mg/g)

Locations ID	Quinic Acid	Gallic Acid	1, 2, 3, 6 Trigalloyl Glucose	Vanillic Acid	Syringic Acid	Rutin
W155	24.205 a	18.973 a	6.560 b	10.311 a	13.614 a	10.828 ab
W11	25.586 a	10.171 b	3.573 c	4.063 c	9.675 c	11.997 a
W133	17.287 d	0.952 e	11.792 a	10.076 a	5.979 d	11.515 a
W55	21.076 b	6.643 d	3.238 c	6.886 b	4.666 de	11.148 ab
W99	19.054 c	8.945 bc	3.266 c	4.548 c	11.198 bc	9.341 bc
W111	19.870 bc	10.481 b	1.819 c	3.898 c	3.736 e	10.839 ab
W33	19.775 bc	8.178 cd	3.226 c	0.076 d	12.279 ab	7.882 c

Duncan's Multiple Range Test indicates a 1% significance difference between means that share a letter for all factors and interactions.

This table 3 hands us some great information about the amount of antioxidants in the samples. The highest amount of antioxidants was located in W11 (Sharbazher walnuts), measured at 304.459. But the least amount of antioxidants measured in our testing, was 20.676 in W33 (Warte walnuts). From table 3, it is shown that the differences in the amount of antioxidants are the same ones that we discussed in table 1, the main contributors are: geographical features such as: weather (the amount of rain and temperature), soil and its containing minerals, topography of the areas. Minor contributors can have an effect as well such as farmers using different farming techniques or having different levels of care to the product. As well as small ones such as the helping hand of organizations and higher-ups. Previously reported results were similar (Gharibzahedi *et al.*, 2012).

It was found in this study that some walnut varieties had higher concentrations of fatty acids than others. The chemical makeup of walnut oil fluctuates based on factors like cultivar, latitude, weather, and processing. Fertilizers used in the cultivation of walnuts, for instance, change the oil's chemical composition. Walnut oil's chemical make-up is also influenced by factors like the seed's position on the plant and how it's handled after harvest (Crews *et al.*, 2005). Like fatty acids, the walnut cultivars had more phenolic acid and antioxidant activity than other walnut varieties. Healthy levels of fatty acids, phenolic acid, and antioxidant activity are present in the selected genotypes. This distinction might be the result of genotypes, environmental factors, phenolic compound synthesis and accumulation, or inefficient extraction on our part (Trandafir *et al.*, 2016). This paper's findings corroborated the widespread belief that walnuts are a good source of medicinal, aromatic, cosmetic, and anti-disease agents due to their high nutrient density.

Table (3): Antioxidant in *Walnut* sampled at different altitudes (ug/g)

Locations ID	Antioxidant
W11	304.459 a
W99	240.270 b
W55	146.351 c
W111	113.243 cd
W133	80.811 d
W155	22.027 e
W33	20.676 e

Duncan's Multiple Range Test indicates a 1% significance difference between means that share a letter for all factors and interactions

CONCLUSION

From the results of our analysis that we have done in a lab. The species and samples that we have gathered for the investigation showed that the products had a great amount of Phenolic Acids, Fatty Acids, Antioxidants; they can be used for multiple different things like: Medicinal, Aromatic, Cosmetic, and Anti disease agents. Furthermore, from all the samples of walnuts that we had, we can say that: Phenolic, Fatty Acids, Antioxidants, etc.… but all with

different amounts per sample. For example: generally, W99 (SargalluBargalu walnuts) had the highest amount of Fatty Acids. Meanwhile, W155 (Tawella walnuts) had the least amount of Fatty Acids. It can also be applied for the other secondary products in its own fashion. Lastly, Topography, Environmental, and Experience of farmers all cause the amount of secondary products depending on the region. After all the testing and analysis, we have reached some recommendations from all the different samples that were tested. It can be tried to create a species for walnuts, because walnuts in Kurdistan are planted by seed propagation. It can be tried to grow them by vegetative propagation to obtain walnuts that phytochemically and physically are identical to each other, to also reach higher nutritional values and sellability. To get a successful and accurate enough analysis, we need a great pure set of standard references especially for (GC) and High-performance liquid chromatography (HPLC). From the study and research of walnuts, it can be shown its benefits to medical uses in the economy. The age and method of storage can be contributors for the amount of material in the products, like the previous ones that were explained. Can be impacted. It is shown that it can be done with the help of Biotechnology, the walnuts can be genetically improved for better resilience, more nutritious and the increase in terms of quality and quantity. Because it can be beneficial for the long run as civilization continues to grow.

REFERENCES

Bou Abdallah, I., Baatour, O., Mechrgui, K., Herchi, W., Albouchi, A., Chalghoum, A., and Boukhchina, S. (2016). Essential oil composition of walnut tree (*Juglans regia* L.)' leaves from Tunisia. *Journal of Essential Oil Research*, 28(6), 545–550.

Çağlarırmak, N. (2003). Biochemical and physical properties of some walnut genotypes (*Juglans regia*, L.). *Food/Nahrung*, 47(1), 28-32.

Chirino, Y. I., & Pedraza-Chaverri, J. (2009). Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. *Experimental and Toxicologic Pathology*, 61(3), 223–242.

Crews, C., Hough, P., Godward, J., Brereton, P., Lees, M., Guiet, S., & Winkelmann, W. (2005). Study of the Main Constituents of Some Authentic Hazelnut Oils. *Journal of Agricultural and Food Chemistry*, 53(12), 4843–4852.

ERCISLI, S., KARA, M., OZTURK, I., SAYINCI, B., & KALKAN, F. (2011). Comparison of Some Physico-Mechanical Nut and Kernel Properties of Two Walnut (*Juglans regia* L.) Cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39(2), 227.

Fajrina, N., & Tahir, M. (2019). A critical review in strategies to improve photocatalytic water splitting towards hydrogen production. *International Journal of Hydrogen Energy*, 44(2), 540–577.

Gharibzahedi, S. M. T., Mousavi, S. M., Hamedi, M., & Khodaiyan, F. (2012). Comparative analysis of new Persian walnut cultivars: nut/kernel geometrical, gravimetrical, frictional and mechanical attributes and kernel chemical composition. *Scientia Horticulturae*, 135, 202–209.

Greve, L. C., McGranahan, G., Hasey, J., Snyder, R., Kelly, K., Goldhamer, D., & Labavitch, J. M. (1992). Variation in polyunsaturated fatty acids composition of Persian walnut. *Journal of the American Society for Horticultural Science*, 117(3), 518-522.

Hayes, D., Angove, M. J., Tucci, J., & Dennis, C. (2016). Walnuts (Juglans regia) chemical composition and research in human health. *Critical reviews in food science and nutrition*, 56(8), 1231-1241.

Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar, M., & Amarowicz, R. (2019). A comparative review on the extraction, anتعمtioxidant content and antioxidant potential of different parts of walnut (Juglans regia L.) fruit and tree. *Molecules*, 24(11), 2133.

Labuckas, D. O., Maestri, D. M., Perello, M., Martínez, M. L., & Lamarque, A. L. (2008). Phenolics from walnut (Juglans regia L.) kernels: Antioxidant activity and interactions with proteins. *Food Chemistry*, 107(2), 607-612.

Macrae, R., Robinson, R. K., & Sadler, M. J. (1993). Encyclopaedia of food science, food technology and nutrition.

Mariod, A. A., Ibrahim, R. M., Ismail, M., & Ismail, N. (2009). Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (Nigella sativa) seedcake. *Food Chemistry*, 116(1), 306–312.

Shafaghat, A. (2011). Antioxidant, antimicrobial activities and fatty acid components of flower, leaf, stem and seed of Hypericum scabrum. Natural product communications, 6(11), 1934578X1100601142.

Shehata, T. M., & Ibrahima, M. M. (2019). BÜCHI nano spray dryer B-90: a promising technology for the production of metformin hydrochloride-loaded alginate–gelatin nanoparticles. *Drug Development and Industrial Pharmacy*, 45(12), 1907–1914.

Trandafir, I., Cosmulescu, S., Botu, M., & Nour, V. (2016). Antioxidant activity, and phenolic and mineral contents of the walnut kernel (*Juglans regia* L.) as a function of the pellicle color. Fruits, 71(3), 177–184.

Yerlikaya, C., Yucel, S., Erturk, Ü., & Korukluoğlu, M. (2012). Proximate composition, minerals and fatty acid composition of Juglans regia L. genotypes and cultivars grown in Turkey. *Brazilian Archives of Biology and Technology*, 55, 677-683.

Zhang, Z., Liao, L., Moore, J., Wu, T., & Wang, Z. (2009). Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). *Food Chemistry*, 113(1), 160–165.

Żurek, N., Pawłowska, A., Pycia, K., Grabek-Lejko, D., & Kapusta, I. T. (2022). Phenolic Profile and Antioxidant, Antibacterial, and Antiproliferative Activity of Juglans regia L. Male Flowers. *Molecules*, *27*(9), 2762.