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Research article

Bioactive component analysis of seed coat hexane extract of Ardahan (Türkiye) walnut

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Abstract

The walnut seed coat (WSC), a significant waste product that is underappreciated compared to other walnut parts, is responsible for the emergence of biological potential, has a favorable and protective effect on health, and includes a wealth of bioactive phytochemicals. Considering this situation, the aim of the study was to clarify the bioactive components in the WSC hexane extract. A hexane WSC extract (H-WSC) was initially produced to conduct the analysis. Four chemical components in the walnut seed coat were identified using the gas chromatography-mass spectrometry (GC-MS) method. Accordingly, the main components in hexane extracts are β-sitosterol (%32.91) and ethyl iso-allocolate (%52.06); other components are 3-(octadecyloxy)propyl (9E)-9-octadecenoate (%8.41) and santa camphor (%4.45).

Keywords: *Gas chromatography-mass spectrometry; phytosterol; steroid; terpenoid; walnut seed coat*

1. Introduction

Since ancient times, people have used plants or items derived from them as supplemental sustenance, as well as for the prevention and treatment of chronic ailments. In terms of global gene diversity, Türkiye is regarded as one of the most significant regions (Karahan et al., 2020). The main causes of this predicament are its unique nature, geographic location, topography, and microclimate (Karakose, 2022). Despite there being about 11707 plant taxa, 3649 of them are endemic, which is a 31.82% percentage (Suzen and Atamov, 2022).

Juglans regia L., a member of the Juglandaceae family, is a type of walnut that is valued highly economically in agriculture. Although it is produced over a range that stretches from Central Asia to the Mediterranean, the majority of it is grown in temperate temperatures (FAO, 2024). In Türkiye, walnut production has spread throughout the Mediterranean, Marmara, Aegean, Anatolian, and Black Sea regions, among other places (Komaki et al., 2019). Walnut seed coat (WSC), which is the woody structure between the seed separated from the outer protective surface, has a very rich chemical content consisting of mostly unsaturated fatty acids (MUFA and PUFA), lipids, proteins, minerals, phytosterols, tocopherols, esters and phenolic compounds (juglone, syringic and ellagic acid) (Martínez et al., 2010; Geng et al., 2021; Yang et al., 2022).

Oxidative stress occurs due to the deterioration of the balance between the antioxidant defense system and free radicals (hydroxyl radicals (OH•), singlet oxygen ($^1O_2$), superoxide anion radicals (O_2•-), and non-free radical species such as hydrogen peroxide (H_2O_2) and causes serious health problems by causing tissue damage (Uguz et al., 2022). Additionally, internal and external factors such as UV and increased glucose levels also contribute to ROS production (Zhu et al., 2024). In addition to showing chelating properties against

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metals that cause oxidative stress, polyphenols have important properties such as forming stable chemical complexes by donating hydrogen and neutralizing free radicals by inhibiting the enzymes involved in the formation of reactive oxygen species (ROS) (Novotny et al., 2017; Demir et al., 2019; Bayrak et al., 2022). It has been supported by many studies that this phytochemical profile, which is used very effectively in traditional medicine and also found in WSC, has antioxidant, antimicrobial, antidiabetic, antitumor (Mates et al., 2023), anti-apoptotic (Askin et al., 2022a), hepato-renal protective effects (Palabiyik et al., 2022; Askin et al., 2022b), anti-inflammatory enzyme inhibition (Palabiyik et al., 2023) and anti-hyperlipidemic (Palabiyik, 2022) properties. Therefore, it has been amply demonstrated that polyphenols control numerous systems that are beneficial to health.

To extract the most phenol from the dried walnut seed coat, the solvent and associated technique were modified for this study. Hexane was employed as the solvent in this case, and the GC-MS method was used to characterize the chemical components.

2. Materials and methods

2.1. Plant materials

The walnuts to be used for the analysis were obtained from the Posof (Türkiye) district of Ardahan in the autumn season between September and October (2022).

2.2. Preparation of walnut seed coat for analysis

After extracting the seed coat samples from the walnuts provided, they were dried in a thin layer at room temperature without being exposed to direct sun. All processes related to the plant were carried out in Atatürk University, Faculty of Science, Genetics Laboratory.

2.3. Hexane extraction of walnut seed coat

The previously dried WSC was weighed and powdered to a weight of 25 g. The solvent 500 ml of hexane (C_6H_{14}) was added, and the mixture was stirred magnetically for 72 hours. Filtering was performed to remove waste materials from the extracted plant sample in a reflux setup (4 hours, 60-80°C). Evaporator was used to remove the solvent remaining in the sample (155 rpm, 50°C). The sample was then dried in the oven and prepared for GC-MS analysis after this procedure, which was carried out three times to increase the yield (Khongthaw et al., 2023; Alkali et al., 2024).

2.4. GC-MS system and chromatographic conditions

GC-MS analyses were performed on an Agilent 7820A gas chromatography system with a 7673 series autosampler chemstation and a 5977 70 series mass selective detector. For separation, an HP-5 MS segment (30 m 0.25 mm I.D., USA) with a 0.25 µm film thickness was used. The input and transfer lines had temperatures of 250 and 300°C, respectively. Injection capability was determined as 1µl indivisible injection mode, carrier gas helium, flow rate 1ml/min, and ionization energy 70 eV (Mawlid et al., 2023; Rutkowska et al., 2023).

Different temperatures were applied while performing the GC-MS procedure. Accordingly, the adjustment was made to increase by 50°C for 1 minute, to increase by 20°C per minute for 1 minute at 100°C, to increase by 10°C per minute for 1 minute at 180°C, and to increase by 5°C per minute for 1 minute. The components of the extract's chromatograms and mass spectra were identified by contrasting them with the reference standard substance.

2.5. Identification of components

In the National Institute of Standards and Technology Library Version (2005), Software, Turbomass 5.2, the range of the obscure segment was compared with the range of the part stored. By comparing the direct Kovats maintenance list and mass spectra with those obtained from the MS library, the pieces could be separated. Utilizing the National Institute of Standards and Technology's database, which has more than 62,000 cases, an understanding of the mass range GC-MS was guided. Every component's relative rate measure was calculated by comparing its typical pinnacle region to the total areas. The test materials' component names, atomic weights, and structures were uncovered.

3. Results

The solvents used during extraction have different polarities, which allows the extracted substance to be separated into different compounds. Plants contain biocomponents that determine bioactivities (Alawode et al., 2021).

Table 1
Chemical composition of compounds identified in walnut seed coat plant.

Peak	Retention Time (min.)	% of total	Compound	Molecular Formula
1	29.52	32.91	β-sitosterol	$C_{29}H_{50}O$
2	33.41	8.41	3-(octadecyl-oxy) propyl (9E)-9-octadecenoate	$C_{39}H_{76}O_3$
3	34.36	52.06	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
4	34.67	4.54	Santa Camphor	$C_{10}H_{16}O$

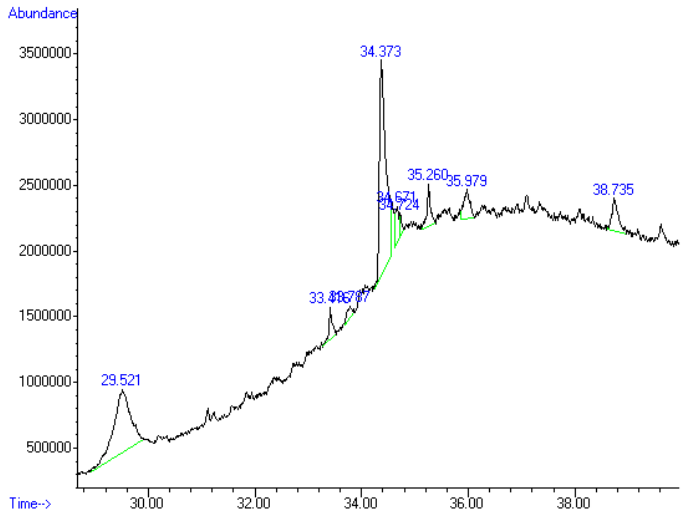

Fig. 1. GC-MS chromatogramı of H-WSC

Accordingly, hexane, a non-polar solvent, was used in our study and four components were detected in the extract of WSC. According to GC-MS analysis, Ethyl iso-alcholate (52.065%),

an ester, and β-sitosterol (32.91%), a steroid, were determined as the main components, while 3-(octadecyloxy) propyl (9E)-9-octadecenoate (8.407%) an unsaturated fatty acid, and the terpenoid Santacamphor (4.455%) were determined as other components. Table 1 provides a detailed summary of the bioactive substances identified in the walnut seed coat. In addition, the GC-MS chromatogram of these substances is presented in Fig. 1 and the three-dimensional view is presented in Fig. 2.

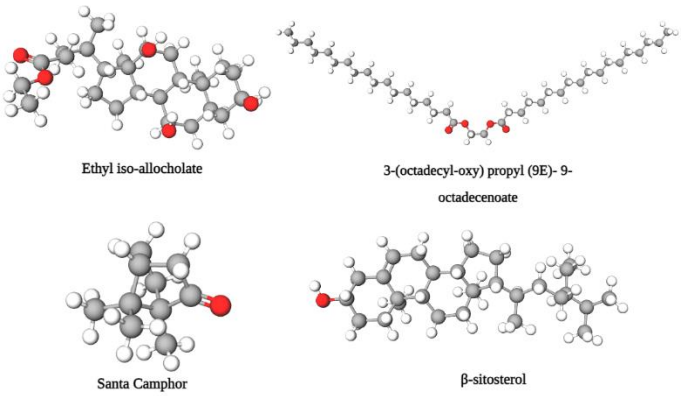

Fig. 2. 3D visualization of H-WSC's bioactive components (KingDraw).

4. Discussion

Considering the available literature, it is possible to say that the most important situation is the existence of therapeutic agents that have little or no side effects and are effective, natural, and easily available.

Table 2
Biological activities of chemical compounds reported in H-WSC.

Chemical constituents	Biological activities	References
β-sitosterol	Anti-inflammatory effect, Anticancer, Hepatoprotective activity, Antioxidant activity, Antidiabetic effect, Cardioprotective, Antimicrobial	von Holtz et al., 1998; Park et al., 2007; Paniagua-Pérez et al., 2008; Sujila et al., 2014; Nasution et al., 2015; Liu et al., 2019; Nweze et al., 2019; Devaraj et al., 2020; Lin et al., 2020
3-(octadecyl-oxy)propyl (9E)-9-octadecenoate	Antifungal	Rech et al., 1998; Diab et al., 2021; El-Naggar et al., 2023
Ethyl iso-allocholate	Anti-inflammatory effect, Antiviral, Antioxidant, Antibebesial potential, Anticancer, Antimicrobial	Okoye et al., 2011; Boligon et al., 2013; Malathi and Ramaiah, 2017; Prakash et al.,2019; Thakur and Ahirwar 2019; Johnson et al., 2020; Poochi et al., 2020; Guz et al., 2021; Shah et al., 2021; Arsana et al., 2022
Santa Camphor	Antifungal, Antibiofilm, Analgesic, Antimicrobial, Antiviral, Antitussive, Anti-inflammatory	Sokolova et al., 2017; Ivanov et al., 2021; Dos Santos et al., 2021

One of the key elements in our study, β-sitosterol (BS), is a steroid that belongs to a significant subclass of phytosterols that are typically found in plants, animals, sea creatures, and fungi. The hydrophobic linear and cyclic molecule BS, which is chemically similar to cholesterol, is considered safe (or non-toxic) because it does not have any negative side effects and works as a supplement. (Choi et al., 2017; Ravi et al., 2023; Mekarunothai et al., 2024). According to studies, BS may offer a wide range of advantages, including anti-inflammatory, anticancer, antibacterial, angiogenic, immunomodulatory, antioxidant properties (Rashed, 2020) and antidiabetic (by inhibition of α-Amylase enzyme) (Ravi et al., 2023), Additionally, studies on animals have revealed that it has no cytotoxic or genotoxic effects (Paniagua-Pérez et al., 2005). In addition, β-Sitosterol's lipophilic properties make it easier for it to be absorbed into the human body and can be considered as a nutritional supplement (Ravi et al., 2023). As a result, it has a significant application in the food and pharmaceutical industries (Rashed, 2020).

The microbial, anti-cancer, anti-fungal and diuretic anti-inflammatory properties of ethyl iso-allocholate, a steroidal molecule obtained in the H-WSC extract, have been reported in other studies (Gaanapriya et al., 2024; Kumar et al., 2024; Okasha et al., 2024). This substance, also known as ethyl cholate, slowed the growth of tumors and caused a decrease in liver metastasis and angiogenesis (Thakur and Ahirwar, 2019). It has demonstrated potent anticancer properties against A549 lung cancer cells *in vitro* and *in vivo* and by showing significant cytotoxicity to HepG2, hepatocellular cancer cells, through upregulation of PCNA, Bax, and caspase 3 activation (Pan et al., 2016; Fachriyah et al., 2019; Sampathkumar et al., 2020). *Bersama engleriana*, which also contains ethyl iso-allocholate, is used in the treatment of various stomach disorders (abdominal pain, colic, diarrhea, cholera, intestinal worms, amebiasis, and dysentery) (Sampathkumar et al., 2020). It was also supported by the study conducted in 2020 that the viral genome has an antiviral character, especially for SARS-CoV, by inhibiting the binding of the viral genome to target protein targets such as angiotensin-converting enzyme 2 (ACE2) and main protease (MPro) (Poochi et al., 2020).

Among other components, 3-(octadecyloxy)propyl (9E)-9-octadecenoate, a bioactive phytochemical compound, plays an important role in the preservation of fresh foods, as well as having an antifungal effect against human and plant fungal pathogens (Abubacker and Devi, 2014; Bhosale et al., 2021). Santa camphor, one of the most well-known bicyclic monoterpenoid compounds obtained naturally and synthetically from turpentine oil, has many remarkable features such as being affordable, having a robust molecular structure, and being synthetically convertible into useful derivatives. These properties of Santa camphor open a new avenue for different applications such as cosmetic products such as creams, lotions, and ointments, as well as fresheners and food products (Dardeer et al., 2022). It is also found in various cell lines (MRC-5, HT-26, HCT116) and has a cytotoxic effect (Nikolić et al., 2015). It is also considered an excellent antifungal by reducing the formation of biofilm and hyphae, that is, the virulence effect of the fungus (Ivanov et al., 2021).

4. Conclusion

It is crucial to find or develop new, powerful medication molecules that can treat diseases including cancer, diabetes,

immune-suppressing illnesses, bacterial and fungal infections, and cholesterol, which is a major factor in the development of many illnesses. Studies done recently with a lot of tenacity have revealed a progression in this direction. The usage of natural products (such as plant sources) since antiquity is also regarded as an amazing factor in the development of curative agents. In our research, the hexane extract of walnut seed coat was found to contain some important chemicals, including β-sitosterol, 3-(octadecyloxy)propyl (9E)-9-octadecenoate, ethyl isoallocholate and Santa campor, according to the results of GC-MS analysis. Accordingly, future studies may reveal that these identified substances are necessary agents for the medical field, and may also help in the evaluation of their pharmacological activities.

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References

Abubacker, M. N., & Devi, P. K. (2014). *In vitro* antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd.(Acanthaceae) inflorescence. *Asian Pacific Journal of Tropical Medicine*, *7*, S190-S193.

Alawode, T. T., Lajide, L., Olaleye, M., & Owolabi, B. (2021). Stigmasterol and β-sitosterol: Antimicrobial compounds in the leaves of *Icacina trichantha* identified by GC-MS. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*, 1-8.

Alkali, K., Dikwa, K. B., Ajibade, G. A., Magaji, Y., & Abdulhamid, M. B. (2024). Qualitative and quantitative phytochemicals screening of aqueous, methanol and hexane leaves extracts of *Senna occidentalis*. *Asian Plant Research Journal*, *12*(3), 27-35.

Arsana, I. N., Juliasih, N. K. A., Ayu Sauca Sunia Widyantari, A. A., Suriani, N. L., & Manto, A. (2022). GC-MS analysis of the active compound in ethanol extracts of white pepper (*Piper nigrum* L.) and pharmacological effects. *Cellular, Molecular and Biomedical Reports*, *2*(3), 151-161.

Askin, S., Askin, H., Dursun, E., Palabiyik, E., Uguz, H., Cakmak, O., & Koc, K. (2022b). The hepato-renal protective potential of walnut seed skin extract against acute renal ischemia/reperfusion damage. *Cytokine*, *153*, 155861.

Askin, H., Askın, S., & Palabiyik, E. (2022a). *Walnut seed coat can attenuate renal ischemia reperfusion injury in distant organ by inhibiting apoptosis*. Health Sciences in International Research (1st edition) (pp 93-104). New York: Eğitim Publishing Group, Inc.

Bayrak, S., Ozturk, C., Demir, Y., Alim, Z., & Kufrevioglu, O. I. (2020). Purification of polyphenol oxidase from potato and investigation of the inhibitory effects of phenolic acids on enzyme activity. *Protein and Peptide Letters*, *27*(3), 187-192.

Bhosale, Y. K., Varghese, S. M., Thivya, P., Hema, V., & Sinija, V. R. (2021). Studies on assessment of safety and nutritional quality of shallot waste fractions. *Journal of Food Processing and Preservation*, *45*(2), e15147.

Boligon, A. A., Schwanz, T. G., Piana, M., Bandeira, R. V., Frohlich, J. K., Brum, T. F. D., ... & Athayde, M. L. (2013). Chemical composition and antioxidant activity of the essential oil of *Tabernaemontana catharinensis* A. DC. leaves. *Natural Product Research*, *27*(1), 68-71.

Choi, N. H., Jang, J. Y., Choi, G. J., Choi, Y. H., Jang, K. S., Min, B. S., ... & Kim, J. C. (2017). Antifungal activity of sterols and *Dipsacus saponins* isolated from *Dipsacus asper* roots against phytopathogenic fungi. *Pesticide Biochemistry and Physiology*, *141*, 103-108.

Dardeer, H. M., Abbas, S. A., El-Sayyad, G. S., & Ali, M. F. (2022). Effect of titanium dioxide nanoparticles and β-cyclodextrin polymer on physicochemical, antimicrobial, and antibiofilm properties of a novel chitosan-camphor polymer. *International Journal of Biological Macromolecules*, *219*, 1062-1079.

Demir, Y., Durmaz, L., Taslimi, P., & Gulcin, I. (2019). Antidiabetic properties of dietary phenolic compounds: Inhibition effects on α-amylase, aldose reductase, and α-glycosidase. *Biotechnology and Applied Biochemistry*, *66*(5), 781-786.

Devaraj, E., Roy, A., Royapuram Veeraragavan, G., Magesh, A., Varikalam Sleeba, A., Arivarasu, L., & Marimuthu Parasuraman, B. (2020). β-Sitosterol attenuates carbon tetrachloride–induced oxidative stress and chronic liver injury in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *393*, 1067-1075.

Diab, T. A., Donia, T., & Saad-Allah, K. M. (2021). Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*(1), 1-13.

Dos Santos, E., Leitão, M. M., Ito, C. N. A., Silva-Filho, S. E., Arena, A. C., de Souza Silva-Comar, F. M., ... & Kassuya, C. A. L. (2021). Analgesic and anti-inflammatory articular effects of essential oil and camphor isolated from *Ocimum kilimandscharicum* Gürke leaves. *Journal of Ethnopharmacology*, *269*, 113697.

El-Naggar, H. M., Shehata, A. M., & Morsi, M. A. A. (2023). Micropropagation and GC-MS analysis of bioactive compounds in bulbs and callus of white squill. *In Vitro Cellular & Developmental Biology-Plant*, *59*(1), 154-166.

Fachriyah, E., Ayu, T., & Kusrini, D. (2019). Identification of phenolic acid from ethanol extract leaves binahong (*Anredera cordifolia* (ten) stennis) and antioxidant activity test. *Journal of Physics: Conference Series*, *1217*(1), 012051.

FAO, (2024). Food and Agriculture Organization of the United Nations, http://www.fao.org/faostat/en/#search/walnut/, Last accessed on August 25, 2024.

Gaanapriya, V., Sivakumar, V. M., & Thirumarimurugan, M. (2024). In-vitro studies of bioactive nanoemulgel from agro-waste and mathematical modeling of drug release. *Indian Journal of Chemical Technology (IJCT)*, *31*(1), 135-142.

Geng, S., Ning, D., Ma, T., Chen, H., Zhang, Y., & Sun, X. (2021). Comprehensive analysis of the components of walnut kernel (*Juglans regia* L.) in China. *Journal of Food Quality*, *2021*, 1-11.

Guz, L., Wawrzykowski, J., & Adaszek, Ł. (2021). Anti-babesial potential and chemical composition of essential oil from yarrow *Achillea millefolium*. *Polish Journal of Veterinary Sciences*, *24*(1), 79-84.

Ivanov, M., Kannan, A., Stojković, D. S., Glamočlija, J., Calhelha, R. C., Ferreira, I. C., ... & Soković, M. (2021). Camphor and eucalyptol—Anticandidal spectrum, antivirulence effect, efflux pumps interference and cytotoxicity. *International Journal of Molecular Sciences*, *22*(2), 483.

Johnson, T. O., Odoh, K. D., Nwonuma, C. O., Akinsanmi, A. O., & Adegboyega, A. E. (2020). Biochemical evaluation and molecular docking assessment of the anti-inflammatory potential of Phyllanthus nivosus leaf against ulcerative colitis. *Heliyon*, *6*(5), 1-10.

Karahan, F., Ozyigit, I. I., Saracoglu, I. A., Yalcin, I. E., Ozyigit, A. H., & Ilcim, A. (2020). Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern Mediterranean region of Turkey. *Biological Trace Element Research*, *197*, 316-329.

Karakose, M. (2022). Vascular plant diversity of Esenli (Giresun) forest planning unit. *Forestist*, *72*(2), 156-164.

Khongthaw, B., Chauhan, P. K., Dulta, K., Kumar, V., & Ighalo, J. O. (2023). A comparison of conventional and novel phytonutrient extraction techniques from various sources and their potential applications. *Journal of Food Measurement and Characterization*, *17*(2), 1317-1342.

Komaki, A., Kordali, S., Bozhuyuk, A. U., & Bostan, H. (2019). Microbial organisms detected on *Juglans regia* in Eastern Anatolia, Turkey. *Fresenius Environmental Bulletin*, 28(9), 6432-6434.

Kumar, R., Ramachaandra, S. K., Kaushik, S., Austin, H., & Rajesh, T. (2024). Bioprospecting of wild botanicals against *Alternaria* leaf blight of radish and their phytochemical profiling by GC-MS. *Research Square*.

Lin, F., Xu, L., Huang, M., Deng, B., Zhang, W., Zeng, Z., & Yinzhi, S. (2020). β-Sitosterol protects against myocardial ischemia/reperfusion injury via targeting PPARγ/NF-κB signalling. *Evidence-Based Complementary and Alternative Medicine*, *2020*(1), 2679409.

Liu, R., Hao, D., Xu, W., Li, J., Li, X., Shen, D., ... & Zhang, Y. (2019). β-Sitosterol modulates macrophage polarization and attenuates rheumatoid inflammation in mice. *Pharmaceutical Biology*, *57*(1), 161-168.

Malathi, K., & Ramaiah, S. (2017). Ethyl iso-allocholate from a medicinal rice Karungkavuni inhibits dihydropteroate synthase in *Escherichia coli*: A molecular docking and dynamics study. *Indian Journal of Pharmaceutical Sciences*, *78*(6), 780-788.

Martínez, M. L., Labuckas, D. O., Lamarque, A. L., & Maestri, D. M. (2010). Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products. *Journal of the Science of Food and Agriculture*, *90*(12), 1959-1967.

Mates, L., Rusu, M. E., & Popa, D. S. (2023). Phytochemicals and biological activities of walnut septum: A systematic review. *Antioxidants*, *12*(3), 604.

Mawlid, O. A., Abdelhady, H. H., & El-Deab, M. S. (2023). Highly active novel K_2CO_3 supported on $MgFe_2O_4$ magnetic nanocatalyst for low-temperature conversion of waste cooking oil to biodiesel: RSM optimization, kinetic, and thermodynamic studies. *Journal of Environmental Chemical Engineering*, *11*(5), 110623.

Mekarunothai, A., Bacher, M., Buathong, R., Intarasam, S., Tayana, N., Kongkiatpaiboon, S., ... & Napiroon, T. (2024). β-sitosterol isolated from the leaves of *Trema orientalis* (Cannabaceae) promotes viability and proliferation of BF-2 cells. *PeerJ*, *12*, e16774.

Nasution, R., Bahi, M., Saidi, N., & Junina, I. (2015). β-sitosterol from bark of *Artocarpus camansi* and its antidiabetic activity. *Proceedings of The Annual International Conference, Syiah Kuala University-Life Sciences & Engineering Chapter*, Banda Aceh, Indonesia. 118-124.

Nikolić, B., Vasilijević, B., Mitić-Ćulafić, D., Vuković-Gačić, B., & Knežević-Vukćević, J. (2015). Comparative study of genotoxic, antigenotoxic and cytotoxic activities of monoterpenes camphor, eucalyptol and thujone in bacteria and mammalian cells. *Chemico-Biological Interactions*, *242*, 263-271.

Novotny, J. A., Chen, T. Y., Terekhov, A. I., Gebauer, S. K., Baer, D. J., Ho, L., ... & Ferruzzi, M. G. (2017). The effect of obesity and repeated exposure on pharmacokinetic response to grape polyphenols in humans. *Molecular Nutrition & Food Research*, *61*(11), 1700043.

Nweze, C., Ibrahim, H., & Ndukwe, G. I. (2019). Beta-sitosterol with antimicrobial property from the stem bark of pomegranate (*Punica granatum* Linn). *Journal of Applied Sciences and Environmental Management*, *23*(6), 1045-1049.

Okasha, N. I., Rahman, M. A., Nafie, M. S., Shama, N. M. A., Mostafa, A., El-Ebeedy, D. A., & Azeiz, A. Z. A. (2024). Identification of potential antiviral compounds from Egyptian sea stars against seasonal influenza A/H1N1 virus. *Journal of Genetic Engineering and Biotechnology*, *22*(1), 100334.

Okoye, F. B. C., Osadebe, P. O., Nworu, C. S., Okoye, N. N., Omeje, E. O., & Esimone, C. O. (2011). Topical anti-inflammatory constituents of lipophilic leaf fractions of *Alchornea floribunda* and *Alchornea cordifolia*. *Natural Product Research*, *25*(20), 1941-1949.

Palabiyik, E. (2022). Triton WR-1339 ile indüklenen hiperlipidemik sıçanlarda ceviz tohum kabuğu ekstresinin hipolipidemik, antiinflamatuar ve antioksidan özelliklerinin değerlendirilmesi, Doktora Tezi, (pp. 1-153). Ataturk University, Erzurum.

Palabiyik, E., Sulumer, A. N., Uguz, H., Avcı, B., Askın, S., Askın, H., & Demir, Y. (2023). Assessment of hypolipidemic and anti-inflammatory properties of walnut (*Juglans regia*) seed coat extract and modulates some metabolic enzymes activity in triton WR-1339-induced hyperlipidemia in rat kidney, liver, and heart. *Journal of Molecular Recognition*, *36*(3), e3004.

Palabiyik, E., Askin, S., & Askin, H. (2022). Effect of walnut seed skin against main organ damage caused by *Hyperlipidemia*. *Avrupa Bilim ve Teknoloji Dergisi*, (34), 652-656.

Pan, Z., Wang, S. K., Cheng, X. L., Tian, X. W., & Wang, J. (2016). Caryophyllene oxide exhibits anti-cancer effects in MG-63 human osteosarcoma cells via the inhibition of cell migration, generation of reactive oxygen species and induction of apoptosis. *Bangladesh Journal of Pharmacology*, *11*(4), 817-823.

Paniagua-Pérez, R., Madrigal-Bujaidar, E., Reyes-Cadena, S., Molina-Jasso, D., Gallaga, J. P., Silva-Miranda, A., ... & Chamorro, G. (2005). Genotoxic and cytotoxic studies of beta-sitosterol and pteropodine in mouse. *Journal of Biomedicine and Biotechnology*, *2005*(3), 242-247.

Paniagua-Pérez, R., Madrigal-Bujaidar, E., Reyes-Cadena, S., Alvarez-González, I., Sánchez-Chapul, L., Pérez-Gallaga, J., ... & Velasco, O. (2008). Cell protection induced by beta-sitosterol: inhibition of genotoxic damage, stimulation of lymphocyte production, and determination of its antioxidant capacity. *Archives of toxicology*, *82*, 615-622.

Park, C., Moon, D. O., Rhu, C. H., Choi, B. T., Lee, W. H., Kim, G. Y., & Choi, Y. H. (2007). β-Sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. *Biological and Pharmaceutical Bulletin*, *30*(7), 1317-1323.

Poochi, S. P., Easwaran, M., Balasubramanian, B., Anbuselvam, M., Meyyazhagan, A., Park, S., ... & Kaul, T. (2020). Employing bioactive compounds derived from Ipomoea obscura (L.) to evaluate potential inhibitor for SARS-CoV-2 main protease and ACE2 protein. *Food frontiers*, *1*(2), 168-179.

Prakash, N. K. U., Sripriya, N. S., Raj, D. D., Deepa, S., & Bhuvaneswari, S. (2019). Antioxidant potency and GC-MS composition of Origanum majorana Linn. *Pakistan Journal of Pharmaceutical Sciences*, *32*(5), 2117-2122.

Rashed, K. (2020). Beta-sitosterol medicinal properties: A review article. *European Journal of Science, Innovation and Technology*, *9*, 208-212.

Ravi, L., Girish, S., D'Souza, S. R., Sreenivas BK, A., Kumari GR, S., O, A., ... & Manjunathan, R. (2023). β-Sitosterol, a phytocompound from *Parthenium hysterophorus*, reveals anti-diabetic properties through α-Amylase inhibition: an *in-silico* and *in-vitro* analysis. *Journal of Biomolecular Structure and Dynamics*, 1-12.

Rech, S. B., Batista, C. V., Schripsema, J., Verpoorte, R., & Henriques, A. T. (1998). Cell cultures of *Rauwolfia sellowii*: growth and alkaloid production. *Plant Cell, Tissue and Organ Culture*, *54*, 61-63.

Rutkowska, E., Wołejko, E., Kaczyński, P., Łuniewski, S., & Łozowicka, B. (2023). High and low temperature processing: Effective tool reducing pesticides in/on apple used in a risk assessment of dietary intake protocol. *Chemosphere*, *313*, 137498.

Sampathkumar, Y., Elumali, S., & Halith, A. M. (2020). GCMS determination of anticancer, anti-inflammatory and anti-bacterial compounds from salt tolerance microalgae (*Lyngbya* sp. *Nostoc* sp. and *Phormidium* sp.) Isolated from Marakkanam Salt Pan, Tamil Nadu, India. *Engineering Sciences*, *11*, 1139-1152.

Shah, S. I. A., Tipu, M. Y., Aslam, A., Khan, A. U., Shafee, M., Khan, S. A., ... & Akbar, A. (2021). Research article elucidating antiviral activity of *Curcuma longa* against H9 N2 influenza virus using embryonated chicken egg model. *Tropical Biomedicine*, *38*(3), 353-359.

Sokolova, A. S., Yarovaya, O. I., Baev, D. S., Shernyukov, A. V., Shtro, A. A., Zarubaev, V. V., & Salakhutdinov, N. F. (2017). Aliphatic and alicyclic camphor imines as effective inhibitors of influenza virus H1N1. *European Journal of Medicinal Chemistry*, *127*, 661-670.

Sujila, S. R., Rajadurai, M., & Shairibha, S. R. (2014). Hepatoprotective effect of β-sitosterol on lipid peroxidation and antioxidant status in ethanol-induced hepatotoxic rats. *International Journal of Integrated Science and Technology*, *3*, 1-11.

Suzen, A., & Atamov, V. (2022). Endemic and rare plant diversity of Ambarlik highland-case study from Çamlihemşin, Türkiye. *Plant & Fungal Research*, 5(1), 29-33.

Thakur, R. S., & Ahirwar, B. (2019). A steroidal derivative from *Trigonella foenum graecum* L. that induces apoptosis *in vitro* and *in vivo*. *Journal of Food and Drug Analysis*, *27*(1), 231-239.

Uguz, H., Palabiyik, E. and Askin, H. (2022). Free radicals, oxidative stress and biological effects of oxidative stress, antioxidant defense system. In: Onay Derin, D. (ed) *International Research in Health Sciences III (1st edition)* (pp 7-24). New York: Eğitim Publishing Group, Inc.

von Holtz, R. L., Fink, C. S., & Awad, A. B. (1998). β-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. *Nutrition and Cancer*, *52*(1), 8-12.

Yang, H., Xiao, X., Li, J., Wang, F., Mi, J., Shi, Y., ... & Wan, X. (2022). Chemical compositions of walnut (*Juglans* spp.) oil: Combined effects of genetic and climatic factors. *Forests*, *13*(6), 962.

Zhu, Y., Wang, K., Jia, X., Fu, C., Yu, H., & Wang, Y. (2024). Antioxidant peptides, the guardian of life from oxidative stress. *Medicinal Research Reviews*, *44*(1), 275-364.

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