LEBDA MEDICAL JOURNAL, Vol. 10 No. 2. 2025. Pages 12-18

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received September 10.2025;
Revised September 19.2025;
Accepted October 13.2025;
Published October 30.2025;

Chemical Composition and Antibacterial Activity of Libyan Propolis

Ali. M Alhalib[a], Alauldin. O Alturshani[a], Adel. M Mlitan[b]

[a] Department of Chemistry, Faculty of Science, University of Alasmarya University, Zliten, Libya
[b] Department of Chemistry, Faculty of Science, University of Misurata, Misrata, Libya
Corresponding author, email: ali.alhalib@asmarya.edu.ly

ABSTRACT

Propolis, a natural resinous substance collected by honeybees, exhibits diverse biological activities due to its rich phytochemical composition. This study aimed to characterize the chemical profile of Libyan propolis and evaluate its antibacterial efficacy. Samples were collected from local beehives, extracted using ethanol and methanol, and analysed via Ultraviolet (UV) spectrophotometric method. The propolis contained carbohydrates (405.97 mg/g), tannins (17.5 mg/g), flavonoids (112.28 mg/g), phenols (50.25 mg/g), alkaloids (24.20 mg/g), saponins (20.50 mg/g), proteins (202.97 mg/g), crude lipids (23.9%), crude fiber (11.7%), and ash (9.43%). The ethanolic extract demonstrated significant antibacterial activity against Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), and Pseudomonas aeruginosa, with inhibition zones comparable to gentamicin. These findings highlight the potential of Libyan propolis as a natural antimicrobial agent.

Keywords: *Propolis, Phytochemicals, Antibacterial activity, Libya, Natural products*

1. Introduction

Throughout history, natural goods have been the main sources of food and medicine, and they have long been essential in the prevention and treatment of human illnesses. Because of their nutritional and medicinal potential, honey and products derived from bees have garnered a lot of scientific interest [1,2]. In addition to honey, several apiculture products have shown various of biological activities that support human health, including pollen, royal jelly, bee venom, wax, and propolis. Also known as "bee glue," propolis is a resinous substance that honeybees gather from plant exudates and then alter with their salivary secretions to give the hive structural and antibacterial protection [1].

Propolis has been used historically by ancient civilizations, such as the Romans for wound healing and the Egyptians for mummification. Avicenna also documented the medical effects of propolis in The Canon of Medicine [3-5]. Propolis' protective function in protecting bee colonies is reflected in its etymology, which comes from the Greek terms "pro" (before) and "polis" (city) [6]. Geographical, climatic, and botanical origins all influence the chemical makeup of propolis, which is a complex matrix of plant-derived resins, waxes, essential oils, and bee-derived enzymes [7-8]. Flavonoids, phenolic acids, terpenes, and aromatic aldehydes are among the more than 300 chemicals that have been found and that contribute to its anti-inflammatory, antibacterial, and antioxidant qualities [3, 9, 10]. Its pharmacological potential has been the subject of much research due to the considerable variance in its phytochemical profile. Propolis has a wide range of biological activity, including antiviral, antifungal, and antibacterial actions against both Gram-positive and Gram-negative bacteria. Its polyphenolic components have also been shown to exhibit antioxidant properties [11, 12]. In light of the increasing prevalence of antimicrobial resistance, a worldwide health issue made worse by the overuse and abuse of traditional antibiotics, these qualities make propolis a promising natural medicinal agent [13]. Little is known about the chemical makeup and bioactivity of Libyan propolis, despite this widespread interest. By describing its main phytochemical components—with special focus on flavonoids, phenolics, alkaloids, and tannins—the current study aims

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

to close this gap. Additionally, the study intends to determine Libyan propolis's antibacterial activity against specific strains of both Gram-positive and Gram-negative bacteria as well as its antioxidant potential utilizing standardized in vitro assays. This work aims to give scientific evidence that may support the medicinal use of Libyan propolis in treating microbial infections and oxidative stress by combining phytochemical profiling with biological evaluation.

2. Materials and Methods

2.1. Preparation of propolis: samples were collected from beehives samples located on western coast of Libya, including the cities of Mis urata, Zliten, Khoms, Meslata, Garabulli, and Tripoli during February–March 2024. Samples were cleaned, fragmented, and stored in glass containers until analysis.

2.2. Extraction

The extract was prepared by cutting the propolis into small pieces and dissolving it in a sufficient amount of ethyl alcohol (95%). Then 30 g of propolis was taken and dissolved in a sufficient amount of ethanol, then transferred to a standard flask and the volume was completed to 100 ml using ethanol to achieve a final concentration of 30%. The extract was then stored in an opaque bottle at room temperature. After ten days of intermittent shaking (daily shaking for a few minutes), and after complete dissolution, the extract was filtered using No. 42 filter paper and stored in the refrigerator at 4°C until use.

-Methanolic extract: Soxhlet extraction with methanol was used for alkaloid quantification.

2.3. Phytochemical Analysis

Quantitative analyses were performed using UV-Vis spectrophotometry**.**

2.3.1. Carbohydrates: phenol-sulfuric acid method, total carbohydrate content = (concentration of glucose from the standard curve × volume of the extract in ml) / (weight of the dry extract in grams). The carbohydrate concentration was expressed as milligram glucose equivalent per gram of extract [14].

2.3.2. Tannins: vanillin-HCl method, total tannin content was determined according to the following equation: total tannin content (concentration of tannic acid for the sample from the standard curve × volume of the extract in ml) / (weight of the dry extract in grams). The tannin concentration was expressed as milligrams tannic acid equivalent per gram of extract [15].

2.3.3 • Flavonoids: aluminium chloride method, the total flavonoid content was determined according to the following equation: total flavonoid content = (concentration of quercetin for the sample from the standard curve × volume of the extract in ml) / (weight of the dry extract in grams). The results were expressed as milligrams of quercetin equivalent (QUE) per gram of extract [16-17].

2.3.4 • Phenols: Folin-Ciocalteu method, the total phenolic content was determined according to the following equation: total phenolic content = (concentration of gallic acid for the sample from the standard curve × volume of the extract in ml) / (weight of the dry extract in grams). The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract [17-18].

2.3.5 • Saponins: vanillin-sulfuric acid method, the total saponin content was determined according to the following equation: total s aponin content = (concentration of aescin for the sample from the standard curve × volume of the extract in ml) / (weight of the dry extract i n grams). The total saponin content was expressed as milligrams equivalents per gram of dry weight (mg AE/g) [19].

2.3.6 • Alkaloids: bromocresol green method, the total alkaloid content was determined according to the following equation: total Alkaloid content = (concentration of atropine for the sample from the standard curve × volume of the extract in ml) / (weight of the dry extract in grams). The total alkaloid content was expressed as milligrams of atropine equivalent per gram [20].

2.3.7 • Proteins: The Bradford method, total protein content = (concentration of albumin for the sample from the standard curve × volume of the extract in ml) / (weight of the dry extract in grams). The total protein content was expressed as milligrams of albumin equivalent per

corresponding author: ali.alhalib@asmarya.edu.ly

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

gram [21].

2.3.8 • Lipids: standard gravimetric methods, the amount of lipid was calculated and expressed as a percentage of the crude lipid content a ccording to the equation: crude Lipid % = (weight of the flask after solvent evaporation - weight of the empty flask) / (weight of the s ample) × 100 [22]. The crucible crude fiber was placed in an oven at 550°C for two hours. It was then taken out of the oven, cooled, and re-weighed to obtain the weight of the ash. Crude fiber content = (weight of the crucible with fibers – weight of the crucible with ash) / (weigh t of the sample) × 100.

2.4. Crude Fiber and Ash: fiber content was determined by boiling propolis with sulfuric acid and then with sodium hydroxide, after which the fibers were collected in a crucible, dried, and weighed.

2.5. Evaluation of Antibacterial Activity: antibacterial activity was evaluated according to the method described by the agar well diffusion method using Mueller-Hinton agar plates. the diameters of the inhibition zones were measured using a ruler and expressed in millimetres [23-25]. The antibiotic Gentamicin was used according to the recommendation of the Clinical and Laboratory Standards Institute.

3. Results & Discussion:

3.1. Phytochemical analysis

As it shown in Table (1) the average carbohydrate estimation was 405.97 mg per gram of extract. This is double the amount of the results of a study for Iraqi propolis, where the carbohydrates content was 200 mg/g, while tannins are similar to our study [26].

Flavonoids were estimated at about 112.28 mg/g which is in the range of the results of Bouchelaghem and his co-workers for Hungarian propolis samples, which ranged between 33.8 and 273.2 mg/g [27], in addition to the results of Touzani and his team for Moroccan propolis, which had a flavonoid content of 98.33 mg/g [28].

Table (1): Phytochemical analysis, calculated crud fat, crude fiber and ash of propolis.

Component	The average value
Carbohydrates	405.97 mg/g
Tannins	17.8 mg/g
Flavonoids	112.28 mg/g
Phenols	50.25 mg/g
Saponins	20.50 mg/g
Alkaloids	24.20 mg/g
Proteins	202.97 mg/g
Lipids	23.9%
Fibers	11.7%
Ash	9.43%,

The phenolic content of the ethanolic extract of propolis was found at 50.52 mg/g, which is consistent with the study of Hungarian propolis samples which ranged between 10.4 and 71.1 mg/g [27], and the study of Brazilian propolis, which ranged between 40.1 and 303.1 mg/g. However, this study's results are higher than those carried out by Al-oklah and his team, who estimated a large group of propolis samples

corresponding author: ali.alhalib@asmarya.edu.ly

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

collected from several different countries, where the phenolic content results for those samples ranged between 2.81 and 30.64 mg/g [30]. This difference is attributed to the geographical location, as all samples were collected from Asian countries. Also, our study's results are lower than those for Moroccan propolis, where the phenolic content reached 141.46 mg/g, which is attributed to the propolis being collected from an area rich in trees with high phenolic content, such as olive and juniper trees.

Saponins as shown in the table were found at 20.50 mg/g which quantitatively agrees with studied of Cameroonian, Iraqi, and Indonesian propolis. [26, 31, 32].

The total alkaloid content in the methanolic extract was found at 24.20 mg/g, which quantitatively agrees with the quantitative detection performed by the Kustiawan group [32] and the Agbor group [33]. However, our study's result differs from what Ramnath's team found in their quantitative estimation of Indian propolis samples, where the alkaloid content ranged between 62-98 mg/g [34].

The average protein content was 202.97 mg/g, as shown in Table (1), which differs from what Devequi-Nunes and co-work study of Brazilian propolis samples, where the protein percentage ranged between 2.12-2.49% [35]. Our study's result is also higher than the results of Shehata and his team for samples from different countries, where the protein percentage ranged between 0.10-2.89% [36]. At the same time, our study's result is close to the results of Indian propolis samples, where the protein percentage ranged between 7.28-9.41% according to Pant's team study [37].

Lipids content in propolis was found with an average of 23.9%, this percentage is higher than what was determined in Brazilian propolis samples, which ranged between 8.19-15.61% [35]. At the same time, our study's result is lower than the results of Indian propolis samples, which ranged between 53.62-68.89% [37], and also lower than what was found in Brunei propolis samples according to Abdullah's team study, which ranged between 45.60-47.86% [38]. This variation in results is attributed to the different sources from which the propolis components were collected due to the different surrounding vegetation.

The fiber content in raw propolis was 11.7%, as shown in Table (1)). This percentage is higher than what was found in Brunei Darussalam propolis samples, where the average fiber percentage was 0.30% [38]. Our study's result also differs from the results of Indian propolis samples, where the fiber percentage ranged between 1.94-3.15% [37]. As for the ash content, the average percentage of ash in our study was 9.43%, as shown in Table (1). This percentage is higher than what was found in Brazilian propolis samples, where the percentage ranged between 1.35-1.44% [35]. It also differs from the ash percentage in Moroccan propolis, which was 4.87% [28]. These differences are attributed to the sources from which the bees collected the propolis components.

3.2. Evaluation of Antibacterial Activity:

Antibacterial activity was evaluated using the agar well diffusion method, where three types of bacteria were used: Staphylococcus aureus (Gram-positive), Pseudomonas aeruginosa (Gram-negative), and the methicillin-resistant MRSA strain. Each type was spread on a Mueller-Hinton plate, and the inhibition of the extract on each type was measured and compared with the inhibition of the antibiotic Gentamicin. The results shown in table (2) indicated that the extract's inhibition on S. aureus bacteria was 26 mm, which was close to the inhibition of the antibiotic used, which was around 28 mm. Also, the inhibitory effect of the extract on the MRSA strain was around 24 mm, which was also close to the antibiotic's inhibition, which gave an inhibition of 26 mm, a difference of 2 mm. At the same time, the extract's inhibition was close to the antibiotic's inhibition when used on Gram-negative bacteria P. aeruginosa, where the inhibition rate was 23 mm for the extract and 24 mm for the antibiotic Gentamicin.

corresponding author: ali.alhalib@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, Vol. 10 No. 2. 2025. Pages 12-18

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Table (2): The extract's inhibition on S. aureus bacteria

Bacterial Type	Propolis Extract Inhibition Diameter (mm)	Gentamicin Antibiotic Inhibition Diameter (mm)
Staphylococcus aureus S	26	28
Strain MRSA	24	26
P.aeruginosa	23	24

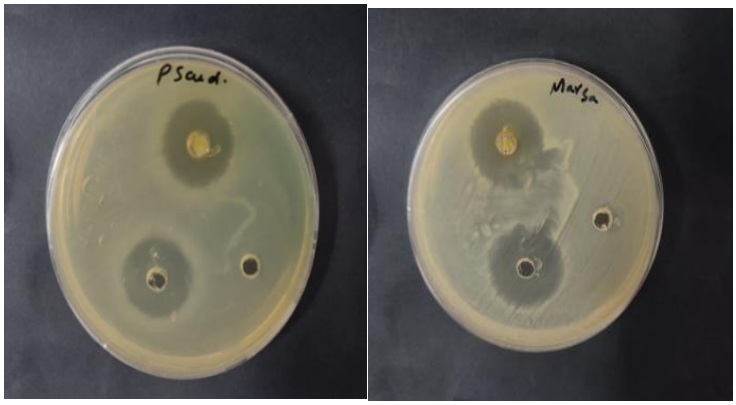

Figure (1): Inhibition of the extract and the antibiotic on the MRSA strain and P. aeruginosa.

Figure (2): Inhibition of the extract and the antibiotic on Staphylococcus aureus.

4. Conclusion

In this research, we studied the basic components of Libyan propolis collected from beehives in the study area, with the aim of estimating these components in its alcoholic extract and assessing its antioxidant and antibacterial activity. The results revealed that the average carbohydrate content of the ethanolic extract was 405.97 mg/g of dry extract, which represented the highest value among the components assessed. The quantitative estimation of phenols and flavonoids revealed that Libyan propolis contained a high amount of these components, with average values of 50.25 and 112.28 mg/g, respectively, which is attributed to the extract's antibacterial activity. The quantitative estimation of tannins is one of the most important additions provided by our study, with a value of 17.5 mg/g, as it is rarely mentioned as a

corresponding author: ali.alhalib@asmarya.edu.ly

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

component of propolis. Estimates indicated that the amount of saponins was 20.50 mg/g in the ethanolic extract, while the amount of alkaloids was 24.20 mg/g in the methanolic extract of propolis. Proteins constituted the second highest value, reaching 202.97 mg/g when raw propolis was treated with the alkali salt extraction method. A study of the lipid content of raw propolis extracted with a mixture of methanol and chloroform revealed that the average lipid content was 23.9% in the propolis from the study area. The study of the antibacterial activity of the extract showed high inhibition against the three bacterial species used, and it was close to the inhibition results of the antibiotic Gentamicin used for comparison.

ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

There is no conflict of interest to declare

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception, design, data collection, analysis, and interpretation of the study.
They jointly participated in drafting, revising, and approving the final version of the manuscript. Each author takes full responsibility for the integrity and accuracy of the work.

References

1- Bankova V. Chemical diversity of propolis and the problem of standardization. Journal of Ethnopharmacology. 2005 Jun 30;100(1–2):114–7. https://doi.org/10.1016/j.jep.2005.05.004.

2- Fernandes FF, Dias ALT, Ramos CL, Ikegaki M, De Siqueira AM, Franco MC. The "in vitro" antifungal activity evaluation of propolis G12 ethanol extract on Cryptococcus neoformans. Revista Do Instituto De Medicina Tropical De São Paulo. 2007 Apr 1;49(2):93–5. https://doi.org/10.1590/s0036-46652007000200005.

3-Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists Society. 1998 Feb 1;75(2):199–212. https://doi.org/10.1007/s11746-998-0032-9.

4- Benzie IFF. Evolution of dietary antioxidants. Comparative Biochemistry and Physiology Part a Molecular & Integrative Physiology. 2003 Jun 30;136(1):113–26. https://doi.org/10.1016/s1095-6433(02)00368-9.

5- Lachance PA, Nakat Z, Jeong WS. Antioxidants: an integrative approach. Nutrition. 2001 Oct 1;17(10):835–8. https://doi.org/10.1016/s0899-9007(01)00636-0.

6- Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Critical Reviews in Food Science and Nutrition. 2004 Jul 1;44(4):275–95. https://doi.org/10.1080/10408690490468489.

7- Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. The Journal of Nutritional Biochemistry. 2007 Mar 24;18(9):567–79. https://doi.org/10.1016/j.jnutbio.2006.10.007.

8- Sen S, Chakraborty R, Sridhar C, Reddy YSR, De B. Fee radicals, antioxidants, diseases and phytomedicines: current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research. 2010 Jan 1;(3):91–100.

9- Baublis A, Decker EA, Clydesdale FM. Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. Food Chemistry. 2000 Jan 1;68(1):1–6. https://doi.org/10.1016/s0308-8146(99)00142-9.

10- Oliboni LS, Dani C, Funchal C, Henriques JA, Salvador M. Hepatoprotective, cardioprotective, and renal-protective effects of organic and conventional grapevine leaf extracts (Vitis labrusca var. Bordo) on Wistar rat tissues. Anais Da Academia Brasileira De Ciências. 2011 Dec 1;83(4):1403–11. https://doi.org/10.1590/s0001-37652011000400027.

11- Pietta PG. Flavonoids as antioxidants. Journal of Natural Products. 2000 May 27;63(7):1035–42. https://doi.org/10.1021/np9904509.

12- Sinnott, M. Carbohydrate chemistry and biochemistry: structure and mechanism. Royal Society of Chemistry; 2007. ISBN, 0854042563, 9780854042562.

13- Hussein N, Zaki WKh, Fahmy GM. Patterns of antimicrobial use and prevalence of antibiotic resistant bacteria study at AIN-SHAMS University Specialized Hospital (ASUSH). Journal of Environmental Science. 2021 Jan 1;50(1):1–22. https://doi.org/10.21608/jes.2021.180068.

14- Masuko T, Minami A, Iwasaki N, Majima T, Nishimura SI, Lee YC. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Analytical Biochemistry. 2004 Dec 30;339(1):69–72. https://doi.org/10.1016/j.ab.2004.12.001.

15- Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 1978 Sep 1;29(9):788–94. https://doi.org/10.1002/jsfa.2740290908.

16- Chang C c., Yang M h., Wen H m., Chern J c. Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis. 2020 Jul 14;10(3). https://doi.org/10.38212/2224-6614.2748.

17- Marinova D, Ribarova F, Atanassova M. Total phenolic and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy. 2005 Jan 1;40(3):255–60. http://dl.uctm.edu/journal/node/j2005-3/Marinova.pdf.

18- Sembiring EN, Elya B, Sauriasari R. Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of Caesalpinia bonduc (L.) Roxb. Pharmacognosy Journal. 2017 Nov 26;10(1):123–7. https://doi.org/10.5530/pj.2018.1.22.

19- Le AV, Parks SE, Nguyen MH, Roach PD. Improving the Vanillin-Sulphuric acid method for quantifying total saponins. Technologies. 2018 Sep 6;6(3):84.

corresponding author: ali.alhalib@asmarya.edu.ly

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

https://doi.org/10.3390/technologies6030084.

20- Shamsa F, Monsef H, Ghamooshi R, Verdian-Rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. The Thai Journal of Pharmaceutical Sciences. 2008 Jan 1;32(1):17–20. https://doi.org/10.56808/3027-7922.2196.

21- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976 May 1;72(1–2):248–54. https://doi.org/10.1016/0003-2697(76)90527-3.

22- Vaishali J. Khilari, Sharma P. P. Determination of total lipids from five underutilized wild edible fruits in Ahmednagar district, Maharashtra (India). International Journal of Advanced Research in Biological Sciences. 2016, 3(7): 14-20. SOI: http://s-o-i.org/1.15/ijarbs-2016-3-7-3.

23- Magaldi S, Mata-Essayag S, De Capriles CH, Perez C, Colella MT, Olaizola C, et al. Well diffusion for antifungal susceptibility testing. International Journal of Infectious Diseases. 2003 Dec 2;8(1):39–45. https://doi.org/10.1016/j.ijid.2003.03.002.

24- Espinel-Ingroff A, Arthington-Skaggs B, Iqbal N, Ellis D, Pfaller MA, Messer S, et al. Multicenter Evaluation of a New Disk Agar Diffusion Method for Susceptibility Testing of Filamentous Fungi with Voriconazole, Posaconazole, Itraconazole, Amphotericin B, and Caspofungin. Journal of Clinical Microbiology . 2007 Apr 12;45(6):1811–20. https://doi.org/10.1128/jcm.00134-07.

25- Espinel-Ingroff A, Canton E, Fothergill A, Ghannoum M, Johnson E, Jones RN, et al. Quality Control Guidelines for Amphotericin B, Itraconazole, Posaconazole, and Voriconazole Disk Diffusion Susceptibility Tests with Nonsupplemented Mueller-Hinton Agar (CLSI M51-A Document) for Nondermatophyte Filamentous Fungi. Journal of Clinical Microbiology. 2011 May 5;49(7):2568–1371. https://doi.org/10.1128/jcm.00393-11.

26- Hindi, N., Al-Charrakh, A., Saheb Naer, H., Abbas, A. Study of chemical analysis of Iraqi propolis and active component of propolis, Iraq. Journal of Science Microbiology. 2015; 15(11) 1095-1103.

27- Bouchelaghem S, Das S, Naorem RS, Czuni L, Papp G, Kocsis M. Evaluation of Total Phenolic and Flavonoid Contents, Antibacterial and Antibiofilm Activities of Hungarian Propolis Ethanolic Extract against Staphylococcus aureus. Molecules. 2022 Jan 17;27(2):574. https://doi.org/10.3390/molecules27020574.

28- Touzani S, Embaslat W, Imtara H, Kmail A, Kadan S, Zaid H, et al. In Vitro Evaluation of the potential use of propolis as a multitarget therapeutic product: physicochemical properties, chemical composition, and immunomodulatory, antibacterial, and anticancer properties. BioMed Research International. 2019 Dec 12;2019:1–11. https://doi.org/10.1155/2019/4836378

29- González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, et al. Evaluation of the Antioxidant Activity of Aqueous and Methanol Extracts of Pleurotus ostreatus in Different Growth Stages. Frontiers in Microbiology. 2016 Jul 11;7. https://doi.org/10.3389/fmicb.2016.01099

30- Al-oklah B, Badr Al-Deen R, Hajali M. In Vitro Antibacterial activity and total phenolic content of propolis alcoholic extracts. Damascus University Journal of Agriculture Sciences. 2021 Jun 30; 36(2);9-24.

31- Talla E, Tamfu AN, Biyanzi P, Sakava P, Asoboe FP, Mbafor JT, et al. Phytochemical screening, antioxidant activity, total polyphenols and flavonoids content of different extracts of propolis from Tekel (Ngaoundal, Adamawa region, Cameroon). The Journal of Phytopharmacology. 2014 Oct 25;3(5):321–9. https://doi.org/10.31254/phyto.2014.3504.

32- Kustiawan PM, Zulfa AF, Batistuta MA, Hanifa DNC, Setiawan IM. Comparative Analysis of Phytochemical, Total Phenolic Content, Antioxidant and Antibacterial Activity of Two Species Stingless Bee Propolis from East Kalimantan. Malaysian Journal of Medicine and Health Sciences. 2022, 18, 50–55.

33- Agbor AM, Tsague R, Kaptue B, Naidoo S, Tembe-Fokunang E, Fokunang CN, et al. Phytochemical Characterisation and Antibacterial Activity of Propolis on Cariogenic Microrganisms. European Journal of Medicinal Plants. 2020; 31 (4):56–66. https://doi.org/10.9734/ejmp/2020/v31i430232.

34- Ramnath S, Venkataramegowda S, Singh C. Chemical Composition of Bee Propolis Collected from Different Regions in India by GCMS Analysis. International Journal of Pharmacognosy and Phytochemistry. 2015 May;30(1):1319–28.

35- Devequi-Nunes D, Machado BAS, De Abreu Barreto G, Silva JR, Da Silva DF, Da Rocha JLC, et al. Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. PLoS ONE. 2018 Dec 4;13(12):e0207676.

36- Shehata MG, Ahmad FT, Badr AN, Masry SH, El-Sohaimy SA. Chemical analysis, antioxidant, cytotoxic and antimicrobial properties of propolis from different geographic regions. Annals of Agricultural Sciences. 2020 Dec 1;65(2):209–17. https://doi.org/10.1016/j.aoas.2020.12.001.

37- Pant K, Thakur M, Chopra HK, Nanda V, Ansari MJ, Pietramellara G, et al. Characterization and discrimination of Indian propolis based on physico-chemical, techno-functional, thermal and textural properties: A multivariate approach. Journal of King Saud University - Science. 2021 Mar 23;33(4):101405. https://doi.org/10.1016/j.jksus.2021.101405.

38- Abdullah NA, Ja'afar F, Yasin HM, Taha H, Petalcorin MIR, Mamit MH, et al. Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee Heterotrigona itama found in Brunei Darussalam. Heliyon. 2019 Sep 1;5(9):e02476. https://doi.org/10.1016/j.heliyon.2019.e02476.

corresponding author: ali.alhalib@asmarya.edu.ly