الجمهورية الجزائرية الديمقراطية الشعبية

Peoples' Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University Mohamed Khider of Biskra

Faculty of Exact Sciences, Natural and Life Sciences

Department of Natural and Life Sciences

A thesis submitted in conformity with the requirements for the degree of doctorate LMD

Option: Experimental pharmacology

Order N°:……

Serie N°:…….

THEME

Contribution à étudier les propriétés et usage médical des produits de la ruche en Algérie.

Defended by

BAMBRA MOUSSA ABDERRAZAK

On

05 /11/ 2025

Jury's members

Chairperson	**REDOUANESALAH SARA**	**Professor**	**University of Biskra**
Supervisor	**BOUATROUS YAMINA**	**Professor**	**University of Biskra**
Examiner	**TRABSA HAYAT**	**MCA**	**University of Biskra**
Examiner	**BAHLOULI FAYÇEL**	**Professor**	**University of Bordj Bou-Arreridj**
Examiner	**BENMEHAIA RADHOUANE**	**MCA**	**University of Msila**

Academic year: 2024/2025

Remerciement

J’exprime tout d’abord, mon profonds remerciements et louanges à Allah le tout puissant, qui nous guide sur le droit chemin et nous donne le courage et la volonté d’achever et d’entamer ce travail ainsi de nous avoir éclairé sur le chemin de la réussite.

Cet humble travail n'aurait pas eu lieu sans l'aide de mon encadrante, *Pr. Bouatrous Yamina*.

Je tiens à remercier infiniment avec beaucoup d'affection et d’amour mon encadrante, *Pr. Bouatrous Yamina* pour sa patience, ses précieux conseils qu'elle n'a cessé de donner, son aide et sa rigueur scientifique qu'à je illuminer pour l'élaboration de cette thèse. Mme, je ne pouvais pas trouver les mots pour vous exprimer notre profond respect et notre gratitude, vous êtes notre exemple et nous espérons être comme vous un jour.

Je tiens à exprimer ma sincère gratitude au Dr Ramazan Erenler (université d'igdir/turquie) pour son rôle essentiel dans la réalisation de l'analyse moléculaire de notre échantillons. Son expertise et son dévouement ont considérablement enrichi la profondeur scientifique et la qualité de cette recherche. Je tiens également à remercier chaleureusement Mr Ilyes Yildiz (université d'igdir/turquie) pour son aide précieux et son soutien technique au cours de ces analyses. Son engagement et son esprit de collaboration ont largement contribué à la réussite de cet aspect essentiel de mon travail.

Je suis profondément reconnaissant aux membres du jury pour leur temps, leurs efforts et leurs précieuses remarques. Leur évaluation réfléchie et leurs commentaires constructifs ont considérablement enrichi ma thèse et ont contribué à améliorer la qualité de ce travail. J'apprécie énormément leur dévouement à garantir la rigueur et l'intégrité du processus de recherche.

Je tiens à exprimer mon sincères remerciements et mon profond respect avec beaucoup d'affection à tous mes enseignants sans exception durant mes années universitaires.

Dédicace

Toutes les lettres ne sauraient trouver les mots qu'il faut… Tous les mots ne sauraient exprimer la gratitude, l'amour, le respect, la reconnaissance… Aussi, c'est tout simplement que je dédie, Ce modeste travail

❖ *A l'âme des deux très chère « **Oma et Radhia »***

École et les deux premières enseignantes de mon enfance qui n'a jamais cessé de m'encourager et de me soutenir. Rien au monde ne vaut les efforts fournis jour et nuit pour ma réussite, Vos prières et vos conseils m'ont toujours accompagné et ont éclairé mon chemin. Ce travail aujourd'hui est le fruit de tes sacrifices que tu as consentis pour ma formation. Cette œuvre est un gage de fidélité à votre âme.

رحمة الله على روحكما وأسكنكما فسيح جناته

❖ *A ma très chère **MÈRE « Temtaoui Messaouda »***

Tu m'as comblé avec ta tendresse et ton affection tout au long de mon parcours. Tu n'as cessé de me soutenir, de me protéger et de m'encourager durant toutes les années de mes études, En ce jour mémorable, reçoit ce travail en signe de ma vive reconnaissance et ma profonde estime. Puisse le tout puissant te donner santé, bonheur et longue vie afin que je puisse te combler à mon tour.

❖ *A mon très cher **PÈRE « Mohamed saleh »***

Autant de phrases et d'expressions aussi éloquentes soit-elles ne sauraient exprimer ma gratitude et ma reconnaissance. Tes conseils ont toujours guidé mes pas vers la réussite. Ta patience sans fin, ta compréhension et ton encouragement sont pour moi le soutien indispensable que tu as toujours su m'apporter. Je te dois ce que je suis aujourd'hui et ce que je serai demain et je ferai toujours de mon mieux pour rester ta fierté.

❖ *A mon adorable petites sœurs **Hania Ibtissem et son Mari, Radhia** & A mes cher **frères Malek, Badrou, Chamsou et Bahaa***

Mes anges gardien et mes fidèles compagnants dans les moments les plus délicats de cette vie mystérieuse. Pour toute l'ambiance dont vous m'avez entouré. Pour toute la complicité et l'entente qui nous unissent. Je vous dédie ce travail avec tous mes vœux de bonheur, de santé et de réussite.

❖ *A Mes chères Tantes **Naziha, Rachida & Rabia***

Que Dieu vous préserve santé et longue vie.

❖ *A Mes chèrs **fréres et soeurs dans la vie***

❖ A mes adorables amis et collègues

Publications Scientifiques

[1] Bambra, M.A., Bouatrous, Y., Erenler, R., Yildiz, I. (2024). **Estimation of chemical composition by LC-ESI-MS/MS method and the anti-hexokinase activity of Algerian bee pollen**, *Analele Universităţii din Oradea, Fascicula Biologie*, 2, pp. 175-182.

[2] Bambra, M, A ; Bouatrous, Y ; Erenler, R ; Yildiz, I. (2025). **Investigation of Phenolic Profiling Using LC-ESI-MS/MS, Antioxydant and Antihexokinase Activities of Algerian Bee Venom And Algerian Royal Jelly**. *African Journal of Biological Sciences*, 7(1), 503-516.

[3] Bambra, M, A ; Bouatrous, Y ; Erenler, R ; Yildiz, I. (2025). **Preliminary analysis of the chemical characteristics of hydrau-acetonic extracts from algerian bee products, their influence on hexokinase activity, and the antimicrobial properties of honey.** *Analele Universităţii din Oradea, Fascicula Biologie*, 1, 105-116.

[4] Bambra, M, A ; Bouatrous, Y. **ALGERIAN BEE PRODUCTS: INVESTIGATION OF ANTI-HYPERGLYCEMIA EFFECT OF BEE VENOM, BEE POLLEN AND ROYALJELLY IN ALLOXAN-INDUCED DIABETIC MICES « *IN VIVO* STUDY » :** **Le travail est actuellement en phase de soumission.**

Communications Scientifiques

- ***Communication orale***

Abdrrazak Bambra Moussa, Bouatrous Yamina, Ghorbal Imene. **La composition chimique d'un extrait hydro-acétonique du propolis Algérienne : une étude préliminaire,** le 2 ème Séminaire National en ligne sur : Etat des lieux, conservation et possibilités de valorisation des ressources biologiques en Algérie (SNECPVRBA'23), Universitaire Abdelhafid Boussouf Mila, Algeria 12-13 Avril2023.

Abdrrazak Bambra Moussa, Bouatrous Yamina, Ghorbal Imene. **Estimation of chemical composition of Algerian propolis,** The president of the First International Webinar on: Promotion and Exploitation of Plants of Ecological and Economic Interest, University Abbes Laghrour-Khenchela ,Algeria 15 and 16 March 2023.

Abdrrazak Bambra Moussa, Bouatrous Yamina, Ghorbal Imene. **Preliminary analysis of the chemical composition of a hydrau-acetone extract of Algerian propolis,** The first international Seminar on Catalysis, Chemical Engineering & Green Chemistry (CaCEG-2023), 22 – 23 February 2023 University of Echahid Hamma Lakhdar,El Oued, Algeria.

Abdrrazak Bambra Moussa, Bouatrous Yamina. **Ramp Extraction Of Phenolic Compounds: Hydrau-Acetonic Extract Of Algerian Propolis,Chemical Composition By LC-ESI-MS/MS And Antioxidant Activit,** *Séminaire National :* Retombées de l'intensification de l'agriculture sur la sécurité alimentaire, la santé humaine et les bioressources, le 13 novembre 2024, à BISKRA.

- ***Présentations Poster***

BAMBRA Moussa Abderrazak, BOUATROUS Yamina. **Chemical composition of hydro-acetonique extraction « Algerian propolis »,** SNVBNR-2024 : 1er Séminaire National sur la Valorisation de la Biomasse Végétale Naturelle et Résiduelle, *20-21 Avril 2024 Biskra (Algérie).*

Abdrrazak Bambra Moussa, Bouatrous Yamina, Ghorbal Imene, Derardja Imene. **Chemical Composition of Algerian propolis,** 1st International Seminar on Valorization of Bioresources in Environment & Health. 10 – 11 May 2023 University of Echahid Hamma Lakhdar, El Oued, Algeria.

Bambra moussa abderrazak, bouatrous yamina. **Chemical profiling of Algerian propolis characterization of bioactive compound from hydro-acetonic extract,** National conference of phytobiotechnology, 17-18 decembre 2023, university of chadli bendjedid El-Taref.

BAMBRA Moussa Abderrazak, BOUATROUS Yamina. **Analysis Of Chemical Composition Using LC-ESI-MS/MS Technique And The Inhibitory Effect On Hexokinase Of Algerian Bee Pollen**, 2èmes Journées Nationales sur l'Agro-éco-biotechnologie (JNAEB-2), Faculté SNV – Université Blida 01, le 05 Décembre 2024.

BAMBRA Moussa Abderrazak, BOUATROUS Yamina. **Examination Of Chemical Composition Through LC-ESI-MS/MS Technique And The Inhibitory Impact On Hexokinase Of Algerian Bee Pollen**, Séminaire National sur la Valorisation des Bioressources dans les Régions Arides (VBRA-2024), le 16-17 Décembre 2024 au CRSTRA-Biskra.

Résumé

Cette étude porte sur la composition chimique et les effets biologiques des produits de la ruche algériens, notamment :

- ✓ Le venin d'abeille (ABV),
- ✓ La gelée royale (ARJ),
- ✓ Le pollen d'abeille (ABP),
- ✓ La propolis,
- ✓ La cire d'abeille,
- ✓ Le pain d'abeille,
- ✓ Le miel.

Malgré leur diversité en composés bioactifs, ces produits ont fait l'objet de peu d'études portant sur leurs capacités antioxydantes, leur teneur en composés phénoliques et flavonoïdes, ainsi que leurs effets inhibiteurs sur l'enzyme hexokinase, et le potentiel antimicrobien du venin et du miels algériens.

Méthodes d'analyse

- ✓ Identification des composés phénoliques : LC-ESI-MS/MS.
- ✓ Évaluation de l'activité antioxydante : Test du piégeage des radicaux libres DPPH.
- ✓ Analyse de l'effet anti-hexokinase : Mesure de l'inhibition enzymatique.
- ✓ Évaluation de l'activé antimicrobienne du venin et du miel de déférentes régions algériennes.

Principaux résultats

1. Composition phénolique et activité antioxydante
   - ✓ Tous les AHPs contiennent des concentrations élevées de phénols et flavonoïdes.
   - ✓ L'extraction à l'aide d'une solution d'acétone 70 % a permis d'obtenir des rendements d'extraction optimaux.
   - ✓ Les valeurs IC50 pour l'activité antioxydante par DPPH :
     - ABV : 0,431 mg/mL,
     - ABP : 0,19 mg/mL.
   - ✓ L'analyse LC-ESI-MS/MS a révélé :
     - 21 composés phénoliques dans la propolis,
     - 17 composés dans l'ABP,
     - Divers composés bioactifs dans les autres produits de la ruche.
2. Potentiel antimicrobienne du venin d'abeille algérien :
   - *Staphylococcus aureus* (13 ± 1 mm) ;
   - *Escherichia coli* (14,66 ± 1,15 mm) ;
   - *Klebsiella pneumoniae* (10 ± 0 mm) ;
   - *Candida albicans* (13 ± 1,7 mm) ;
   - *Staphylococcus aureus* SA33 (14 ± 1 mm).
3. Potentiel antimicrobienne du miel de déférentes régions algériennes :
   - *Escherichia coli* (33,33 mm) ;
   - *Proteus vulgaris* (24,5 mm) ;
   - *Staphylococcus aureus* (16 mm) ;
   - *Streptococcus* sp. (17,6 mm).
4. Effet anti-hexokinase et potentiel antidiabétique
   - ✓ L'ABP et l'ARJ ont montré une forte inhibition de l'hexokinase :
     - ABP : 97,7 %,
     - ARJ : 99,3 %.
   - ✓ Expériences *in vivo* :
     - Tests sur des souris diabétiques induites par l'alloxane.
     - L'administration d'ABP, ABV et ARJ a permis de réduire significativement l'hyperglycémie et de restaurer les altérations biochimiques et histologiques liées au diabète.

Conclusion et perspectives

Ces résultats soulignent le potentiel thérapeutique des produits apicoles algériens dans :

- La gestion du diabète et ses complications.
- La prévention du stress oxydatif et contre les agents microbiens, grâce à leurs propriétés antioxydantes remarquables.

Des recherches complémentaires sont nécessaires pour approfondir les mécanismes d'action, l'optimisation des extraits, ainsi que leur application clinique potentielle.

Les mots clés : Le venin d'abeille (ABV), La gelée royale (ARJ), Le pollen d'abeille (ABP), La propolis, La cire d'abeille, Le pain d'abeille, Le miel, L'activity antioxydant, L'activity anti-diabétique, LC-ESI-MS/MS.

الملخص:

تتناول هذه الدراسة التركيب الكيميائي والخصائص البيولوجية لمنتجات النحل الجزائرية، وتشمل: سمّ النحل(ABV) ، الغذاء الملكي(ARJ) ، حبوب اللقاح(ABP) ، العكبر (البروبوليس)، شمع النحل، خبز النحل، والعسل. وعلى الرغم من تنوعها بالمركّبات النشطة بيولوجياً، إلا أن الدراسات السابقة حول قدرتها المضادّة للأكسدة، ومحتواها من المركبات الفينولية والفلافونويدية، وتأثيرها المثبّط لإنزيم الهكسوكيناز، والفعالية المضادّة للميكروبات لسمّ النحل والعسل الجزائري، لا تزال محدودة.

منهجية البحث

- ✓ تحديد المركبات الفينولية باستخدام تقنية.LC-ESI-MS/MS
- ✓ تقييم النشاط المضاد للأكسدة بواسطة اختبار DPPH لجذور الأوكسجين الحرّة.
- ✓ دراسة التأثير المثبّط لإنزيم الهكسوكيناز عبر اختبار التحليل الإنزيمي.
- ✓ تقييم النشاط المضاد للميكروبات لسمّ النحل والعسل من مناطق جزائرية مختلفة.

النتائج الرئيسة

1. التركيب الفينولي والنشاط المضاد للأكسدة:

- ✓ أظهرت جميع منتجات النحل مستويات مرتفعة من الفينولات والفلافونويدات.
- ✓ حقّق الاستخلاص بواسطة محلول الأسيتون 70% أفضل مردودية.
- ✓ قيم IC_{50} للنشاط المضاد للأكسدة باختبار DPPH كانت:
  - لسمّ النحل: 0.431 ملغ/مل،
  - لحبوب اللقاح: 0.19 ملغ/مل.
- ✓ كشفت التحاليل بـ LC-ESI-MS/MS عن:
  - 21 مركباً فينولياً في العكبر،
  - 17 مركباً في حبوب اللقاح،
  - وعدداً من المركبات النشطة في المنتجات الأخرى.

2. الفعالية المضادّة للميكروبات لسمّ النحل الجزائري:

- ✓ *Staphylococcus aureus* (13 ± 1 مم)
- ✓ *Escherichia coli (14.66 ± 1.15 مم)*
- ✓ *Klebsiella pneumoniae (10 ± 0 مم)*
- ✓ *Candida albicans (13 ± 1.7 مم)*
- ✓ *Staphylococcus aureus* SA33 (14 ± 1 مم)

3. الفعالية المضادّة للميكروبات لعسل مناطق جزائرية مختلفة:

- ✓ *Escherichia coli (33.33 مم)*
- ✓ *Proteus vulgaris (24.5 مم)*
- ✓ *Staphylococcus aureus (16 مم)*
- ✓ *Streptococcus* sp. (17.6 مم)

4. النشاط المضاد لإنزيم الهكسوكيناز والإمكانات المضادّة للسكري:

- ✓ أظهرت حبوب اللقاح والغذاء الملكي فعالية عالية في تثبيط الإنزيم:
  - حبوب اللقاح: 97.7%؛
  - الغذاء الملكي: 99.3%.
- ✓ كما بينت التجارب على فئران مصابة بالسكري المستحدث بالألوكسان أن حبوب اللقاح وسمّ النحل والغذاء الملكي ساهمت في خفض مستوى السكر في الدم واستعادة التوازن البيوكيميائي والنسيجي.

الاستنتاج والآفاق المستقبلية

تؤكد هذه النتائج الإمكانات العلاجية المتميزة لمنتجات النحل الجزائرية في التحكم بمرض السكري ومضاعفاته، وفي الوقاية من الإجهاد التأكسدي ومقاومة العوامل الممرضة الميكروبية.

وتبرز الحاجة إلى دراسات إضافية لفهم الآليات الجزيئية الفاعلة، وتحسين عمليات الاستخلاص والتنقية، وتقييم الاستخدامات الطبية والصناعية المحتملة لهذه المنتجات كمصادر طبيعية واعدة لمضادات الأكسدة والعوامل المضادّة للسكري.

الكلمات المفتاحية: سمّ النحل، الغذاء الملكي، حبوب اللقاح، العكبر، شمع النحل، خبز النحل، العسل، النشاط المضاد للأكسدة، النشاط المضاد للسكري، LC-ESI-MS/MS.

Abstract

This study investigates the chemical composition and biological properties of Algerian Honeybee Products (AHPs), including:

- ✓ Bee venom (ABV) ;
- ✓ Royal jelly (ARJ) ;
- ✓ Bee pollen (ABP) ;
- ✓ Propolis ;
- ✓ Beeswax ;
- ✓ Bee bread ;
- ✓ And honey.

Despite their diversity in bioactive compounds, limited research has explored their antioxidant capacity, phenolic and flavonoid contents, anti-hexokinase activity, and the antimicrobial potential of Algerian bee venom and honey.

Analytical Methods

- ✓ Identification of phenolic compounds : LC-ESI-MS/MS analysis.
- ✓ Evaluation of antioxidant activity: DPPH free radical scavenging assay.
- ✓ Assessment of anti-hexokinase effect: enzymatic inhibition test.
- ✓ Determination of antimicrobial activity: diffusion assays using honey and venom samples from different Algerian regions.

Main Results

1. Phenolic composition and antioxidant activity :
   - ✓ All AHPs exhibited high phenolic and flavonoid contents;
   - ✓ Extraction using 70% acetone yielded optimal recovery rates ;
   - ✓ DPPH radical scavenging activity (IC_{50}):
   - ABV: 0.431 mg/mL ;
   - ABP: 0.19 mg/mL.
   - ✓ LC-ESI-MS/MS analysis revealed:
   - 21 phenolic compounds in propolis;
   - 17 in bee pollen (ABP);
   - Various bioactive molecules in the remaining bee products.
2. Antimicrobial potential of Algerian bee venom :
   - ✓ *Staphylococcus aureus* (13 ± 1 mm) ;
   - ✓ *Escherichia coli* (14.66 ± 1.15 mm) ;
   - ✓ *Klebsiella pneumoniae* (10 ± 0 mm) ;
   - ✓ *Candida albicans* (13 ± 1.7 mm) ;
   - ✓ *Staphylococcus aureus* SA33 (14 ± 1 mm).
3. Antimicrobial potential of Algerian honey samples :
   - ✓ *Escherichia coli* (33.33 mm) ;
   - ✓ *Proteus vulgaris* (24.5 mm) ;
   - ✓ *Staphylococcus aureus* (16 mm) ;
   - ✓ *Streptococcus* sp. (17.6 mm).
4. Anti-hexokinase and antidiabetic potential
   - ✓ Strong enzymatic inhibition was recorded for:
   - ABP: 97.7% ;
   - ARJ: 99.3%.
   - ✓ *In vivo* experiments on alloxan-induced diabetic mice demonstrated that ABP, ABV, and ARJ significantly reduced hyperglycemia and restored both biochemical and histological parameters.

Conclusion and Perspectives

These findings highlight the therapeutic potential of Algerian honeybee products in the management of diabetes and oxidative stress-related complications, as well as their broad antimicrobial properties.

Further research is required to elucidate the molecular mechanisms of action, optimize the extraction and purification processes, and evaluate their clinical and industrial applications as natural antioxidant and antidiabetic agents.

Keywords: Bee venom (ABV), Royal jelly (ARJ), Bee pollen (ABP), Propolis, Beeswax, Bee bread, Honey, Antioxidant activity, Antidiabetic potential, LC-ESI-MS/MS.

Sommaire

Introduction générale ……..... **1**

Chapitre 01 : Article 01 : Estimation of chemical composition by LC-ESI-MS/MS method and the anti-hexokinase activity of algerian bee pollen…….. **4**

Synthèse d'article 01 ……..…….. **12**

Chapitre 02 : Article 02 : Investigation of phenolic profiling using LC-ESI-MS/MS, antioxydant and antihexokinase activities of algerian bee venom and algerian royal jelly…… **17**

Synthèse d'article 02 …….…….……. **31**

Chapitre 03 : Article 03 : Preliminary analysis of the chemical characteristics of hydrau-acetonic extracts from algerian bee products, their influence on hexokinase activity, and the antimicrobial properties of honey……..….. **36**

Synthèse d'article 03 ……..…….…… **48**

Chapitre 04 : Article 04 : Algerian bee products: investigation of anti-hyperglycemia effect of bee venom, bee pollen and royal jelly in alloxan-induced diabetic mices « *IN VIVO* STUDY » ……..... **56**

Synthèse d'article 04 …….…….…… **67**

Conclusion générale ……...…… **72**

Références bibliographiques ….…….. **74**

LISTE DES TABLEAUX

Tableau 1 : Doses testées pour chaque plante…… **68**

LISTE D'ABREVIATIONS

ABP	Algerian Bee Pollen.
ABV	Algerian Bee venom.
AGE	Acid Gallic Equivalent.
AHPs	Algerian Hive Products.
ARJ	Algerian Royel Jelly.
ATCC	American Type Culture Collection.
D1	Dose 1.
D2	Dose 2.
D3	Dose 3.
F	Femelle.
G6PD	Glucose-6-Phosphate déshydrogénase.
HK	Hexokinase.
IC50	Concentration Inhibitrice de 50% d'une fonction biologique.
LC-ESI-MS/MS	Chromatographie liquide couplée avec deux spectres de masse.
M	Mâle.
MET	Metformine.
MIC	Minimum Inhibitory Concentration.
OMS	Organisation mondial de la santé.
QE	Quercetine Equivalent.
R	Résistance.
Rt	Temps de retention.
S	Sensible.
T	Témoin.
TFC	Total Flavonoïd Content.
TPC	Total Phenolic Content.

INTRODUCTION GENERALE

Introduction Générale

L'abeille est l'un des insectes les plus fascinants et les plus anciens du règne animal. Son importance est telle qu'elle est mentionnée dans la 16 éme sourate du Coran, An-Nahl :

{وَأَوْحى رَبُّكَ إِلَى النَّحْلِ أَنِ اتَّخِذِي مِنَ الْجِبالِ بُيُوتًا وَمِنَ الشَّجَرِ وَمِمَّا يَعْرِشُونَ (68) ثُمَّ كُلِي مِنْ كُلِّ الثَّمَراتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلُلًا يَخْرُجُ مِنْ بُطُونِها شَرابٌ مُخْتَلِفٌ أَلْوانُهُ فِيهِ شِفاء لِلنَّاسِ إِنَّ فِي ذلِكَ لَآيَةً لِقَوْمٍ يَتَفَكَّرُونَ .}(69)

La célèbre citation attribuée à Albert Einstein, « Si les abeilles venaient à disparaître, l'humanité n'aurait plus que quatre années à vivre », illustre leur rôle crucial dans l'équilibre des écosystèmes et la survie des espèces végétales et animales.

Les abeilles appartiennent à l'ordre des hyménoptères et à la superfamille des apoïdes, comprenant des milliers d'espèces à travers le monde (D'Apolito *et al.*, 2010). En Algérie, l'*Apis mellifera intermissa*, une sous-espèce de l'abeille domestique, est la plus couramment élevée (Haddad *et al.*, 2015). Elle produit une grande diversité de substances précieuses, telles que le miel, la cire, la propolis, le pollen, le pain d'abeille, la gelée royale et le venin (Marianna and Franco, 2021). Cependant, l'élevage de cette espèce exige une expertise approfondie, car ces insectes vivent en colonies hautement organisées et communiquent de manière sophistiquée.

L'apiculture, une activité ancestrale, joue un rôle économique et écologique majeur, notamment grâce à la pollinisation. En Algérie, elle a connu une évolution significative, soutenue par des avancées technologiques, et bénéficie d'une demande croissante sur le marché local.

Les produits de la ruche sont depuis longtemps considérés comme de véritables trésors thérapeutiques (Kuropatnicki et *al.*, 2013). L'apithérapie, qui repose sur leur utilisation à des fins médicinales, suscite un intérêt croissant, notamment dans le cadre des médecines douces (Kuropatnicki et *al.*, 2013). De plus en plus de recherches scientifiques s'intéressent aux propriétés antioxydantes et antidiabétiques de ces produits, ouvrant la voie à de nouvelles approches thérapeutiques naturelles.

Les produits de la ruche sont riches en antioxydants naturels, capables d'atténuer le stress oxydatif, un facteur clé dans le développement de nombreuses maladies. L'exploration des composés bioactifs d'origine naturelle devient essentielle pour prévenir et traiter diverses pathologies, notamment le diabète sucré.

Le diabète sucré, véritable problème de santé publique mondiale, ne cesse de croître. Selon l'OMS (2002), on comptait 135 millions de diabétiques dans le monde, un chiffre qui

pourrait atteindre 300 millions d'ici 2025, en particulier dans les pays en développement. Le coût élevé des traitements et l'accès limité aux médicaments conventionnels poussent de nombreuses populations à se tourner vers des alternatives naturelles, encore insuffisamment étudiées dans certaines régions.

Les antibiotiques ont permis de faire considérablement reculer la mortalité associée aux maladies infectieuses au cours du 20^{éme} siècle. Malheureusement, leur utilisation massive et répétée a conduit à l'apparition de bactéries résistantes à ces médicaments. De plus, les animaux d'élevage ingèrent au moins autant d'antibiotiques que les humains, par conséquence la résistance bactérienne est devenue un phénomène global et préoccupant. C'est ce qui a motivé les gens, en particulier les scientifiques, à utiliser des matériaux préventifs et thérapeutiques naturels traditionnels.

Cette recherche s'inscrit dans la quête de nouveaux traitements d'origine naturelle. Son objectif principal est de dresser un inventaire des produits de la ruche algérienne, d'analyser leur composition phytochimique et d'évaluer leurs activités biologiques. Pour ce faire, plusieurs étapes ont été mises en place :

- Collecte des produits de la ruche dans différentes régions d'Algérie ;
- Extraction des composés bioactifs à l'aide d'un solvant acétonique ;
- Analyse qualitative et quantitative des polyphénols et flavonoïdes par LC-ESI-MS/MS ;
- Évaluation *in vitro* du potentiel antioxydant et antidiabétique des extraits obtenus;
- Évaluation le potentiel antimicrobien de venin d'abeille algérien et du miel algérien de déférentes région du pays ;
- Étude *in vivo* de l'activité antidiabétique des extraits sur des souris diabétiques induites par l'alloxane.

Structure du manuscrit

Ce manuscrit est structuré en quatre chapitres principaux :

1. Analyse du profil phénolique du pollen d'abeille algérien, comprenant l'extraction, la détermination des teneurs en phénols et flavonoïdes, ainsi que l'évaluation des propriétés antioxydantes et antidiabétiques *in vitro*, aussi l'évaluation de l'activité antimicrobienne du venin d'abeille algérien.
2. Étude des composés bioactifs de la gelée royale et du venin d'abeille algériens, avec une analyse chimique approfondie et une évaluation de leurs activités biologiques.

3. Évaluation du potentiel thérapeutique du miel, de la cire, de la propolis et du pain d'abeille algériens, en mettant l'accent sur leur composition et leur impact sur le stress oxydatif avec une évaluation du potentiel antimicrobien du miel de déférentes régions algériennes.

4. Étude *in vivo* des effets antidiabétiques des extraits de pollen, de gelée royale et de venin d'abeille chez des souris diabétiques, afin d'explorer leur efficacité potentielle dans la régulation du glucose sanguin.

CHAPITRE 01 :
ARTICLE 01 :

Estimation of chemical composition by LC-ESI-MS/MS method and the anti-hexokinase activity of algerian bee pollen.

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2024, Tom. XXXI, Issue: 2, pp. 175-182

ESTIMATION OF CHEMICAL COMPOSITION BY LC-ESI-MS/MS METHOD AND THE ANTI-HEXOKINASE ACTIVITY OF ALGERIAN BEE POLLEN

Moussa Abderrazak BAMBRA[*], Yamina BOUATROUS[**], Ramazan ERENLER[***], Ilyas YILDIZ[***]

[*] *Laboratory of promotion of agricultural innovation in arid regions (PIARA), Department of Biology, Faculty of Natural Sciences and Life, University Mohamed khider Biskra, Algeria.*
[**] *Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Mohamed khider University, Biskra, Algeria.*
[***] *Research Laboratory Practice and Research Center, Igdir University, 7600, Igdir, Turkiye*
Correspondence author: Yamina Bouatrous, Department of Nature and Life Sciences, Faculty of Exact Sciences a nd Nature and Life Sciences, Mohamed khider University, zip code: 07000, email: y.bouatrous@univ-biskra.dz

Abstract. Few studies aim to evaluate the chemical composition and the effects of algerian bee pollen on antioxidant potential, total phenolics, total flavonoids, and the antihexokinase effect. Also, the algerian bee venom (ABV) possesses potent natural properties with antimicrobial effects. The aim of the study is to investigate the antibacterial effect of the ABV and antidiabetic activity of Algerian bee pollen (ABP). The analyses were performed with ABP extract, which was obtained using aceton 70% as the solvent for the extract. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was used to assess the antioxidant activity. *In vitro* anti-diabetic effects of the isolate of bee pollen from Algeria were evaluated. In this study, phenolic identification, Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC-ESI-MS/MS) were used. Using the method of disk diffusion to evalute the antimicrobial activity of ABV. Our results showed that the highest yield was detected with 70% acetone (72.44%). The phenolic and flavonoid contents of ABP were 54.92 mg GAE/g ± 0.48 and 6.18 mg QE/g ± 1.17, respectively. The half maximal inhibition concentration (IC50) values of ABP for DPPH scavenging activity were found to be 0.19±0.004 mg/mL. The LC-ESI-MS/MS analysis led to the identification of phenolic compounds; seventeen compounds in the extract of ABP were detected. The Algerian bee pollen isolate was assessed at 126 µg/mL concentration for the *in vitro* antidiabetic effect, yielding a 97.7% HK (Hexokinase) inhibition value. The algerian bee pollen has a very important effect as an anti-diabetic agent. The Algerian bee venom has an antimicrobial effect that is very interesting.

Key words: Algerian bee pollen; Algerian bee venom; antimicrobial activity; antioxidant activity; LC-ESI-MS/MS.

INTRODUCTION

Clinicians treat diabetes mellitus by prescribing insulin in addition to synthetic anti-diabetic medications like metformin and glipizide, but these medications have side effects. To eliminate these irritating side effects, scientists are actively studying herbal metabolites, in particular, as potential for new anti-diabetic medications. The abnormal increases in HK2 and the unscheduled glycolysis play an important role in impaired glycolysis control, as suggested by recent research. In this case, the key players in the tissue-specific chronic pathogenesis of hyperglycemia associated with diabetes are abnormal increases in G6P and downstream glycolysis intermediates [43].

The pollen of bees is created from agglutination by the nectar and bee enzymes, i.e., amylases and catalases, which are found in flower pollen that has been collected by bees. The pollen grains are supplied by bees with small amounts of saliva, nectar, and honey. The pollen of bees has been called the only perfectly complete food, as it contains all the essential amino acids that a human organism needs [39]. The bee pollen composition and antioxidative effect are species-specific, dependent very much on the type of plant source coupled with biogeographic regional origin, environmental habitat, season, or entomology [11, 12, 39]. In the case of this natural product, it is a relatively recent evolution, depending mainly on pollen being removed from bee legs as they go into their hives [39]. Proteins (5.6–60%), lipids (4.7–7%), reducing and non-reducing sugars (13–55%), crude fibers (0.3– 20%), vitamins, enzymes, and phenolic compounds, primarily flavonoids, are all present in bee pollen [32].

Polyphenols found in medicinal plants and the human diet, including phenolic acids, flavonoids, and coumarins, are a significant group of secondary metabolites. They play a role in the antioxidant properties of plants and foods [7]. Depending on their chemical structures and the number of hydroxyl groups, phenolic compounds can serve as hydrogen or electron donors, reducing agents, and metal ion chelators [35].

Several studies have examined the natural antioxidant compounds found in medicinal bee products. because, in addition to their use as preservatives in food by replacing synthetic antioxidants, they are involved in the treatment of hyperglycemia. Thus, the study of the extraction of phenolic compounds by aceton 70% aims to reach the most efficient active molecules that have antioxidant and anti-diabtic effects to find a natural source to reduce blood glucose.

Additionally, BV has been shown to exhibit antimicrobial effects against both Gram-negative and Gram-positive bacteria, such as *Escherichia coli* and *Salmonella spp*. [20, 25], as well as *Enterobacter cloacae*, *E. coli*, and *Citrobacter freundii* [8], and *Staphylococcus aureus*, Coagulase-negative *Staphylococcus*, and *E. coli* [38]. The objective of this study was to assess the antimicrobial properties of Algerian bee venom against specific strains of medically significant Gram-positive and Gram- negative bacteria and fungi, to be used for sterilization against microbes as a natural source.

MATERIALS AND METHODS

Collection of the samples

The Algerian bee pollen was collected from a multiflowring plant in Blida (Algeria) in 2022.

The Algerian bee venom was collected from El-Medea (Algeria) in 2023.

Extraction. Acetonic extract of Algerian bee pollen in room temperature

A mixture of 5 g of crude bee pollen and 50 mL of aceton 70% was stirred for a full day (24 h). Then the mixture was filtrated through filter paper. The extraction was repeated three times with the residue.

The solvent was evaporated by rotavapor machine at 40°C and stored in steamroom at 60°C for 6 days. After that, the sample extract was stored at -20 °C.

Determination of the Total Phenol Content

Total polyphenol content in samples was determined by the Folin-Ciocalteau colorimetric method [6]. 100 µL of sample extracts (1 mg/mL of distilled water) were mixed with 500 µL of the folin- ciocalteau (10%) reagent, and 400 µL of Na_2CO_3 (75g/L) were added in a 1.5 mL Eppendorf tube. and the absorbance was measured at 735 nm after 5 min of incubation at 40°C in the dark using a UV-VIS spectro- photometer (JP Selecta UV-2005). Total polyphenol content was expressed as mg/g (gallic acid equivalents) (Fig. 1), (Y= 0.0122x – 0.0052 R^2=0.9995). Triplicate measurements were taken for all samples.

Total flavonoids content

Total flavonoid content in samples was determined by the method of Zhishen *et al.* (1999), with modifications [52]. In a 1.5 mL Eppendorf tube, 200 µL of samples were mixed with 60 µL of $NaNO_2$ (5%). After 5 min, 60 µL of $AlCl_3$ (10%) was added, and the mixture was vigorously mixed. After 6 min, a volume of 400 µL of NaOH 1M was added. The absorbance was read immediately at 510 nm against the control by UV-VIS spectrophotometer. A solution of quercetin has been prepared. For determining the calibration curve, solutions prepared from a stock solution at various concentrations of 0.1 to 1000 g/mL were applied (Fig. 2), (Y= 0.0018x + 0.0357, R^2=0.9991). Triplicate measurements were taken for all samples.

LC-ESI-MS/MS

The phenolics were quantitatively analyzed using an Agilent Technologies 1260 Infinity II, 6460 Triple Quad Mass Spectrometer. There was one Poroshell 120 SB-C18 (3.0 × 100 mm, I.D., 2.7 µm) column used [15]. In a 2 mL Eppendorf tube, 50 mg of the samples were taken and dissolved by adding 1 mL methanol. The resulting extract was extracted with 1 mL of hexane. It was centrifuged at 9000 rpm for 10 minutes. Finally, 100 µL of the methanol phase was taken and diluted with 900 µL as 450 (water)/450 (methanol). In the last step, this mixture was introduced into the instrument after being filtered through a 0.22 µm filter.

5.12 µL was the injection volume, and 0.40 mL/min was the adjusted flow rate. The mobile phase contained ammonium formate (5.0 mM) and formic acid (0.1%) in methanol B and ammonium formate (0.1%) in water A. For the B mobile phase, the gradient program was adjusted to 25% for 1-3 minutes, 50% for 4-12 minutes, 90% for 13-21 minutes, and 3% for 22-25 minutes. The temperature in the column was 40°C. The capillary voltage was 4000 V, the gas temperature was 300°C, the nebulizing gas (N2) flow rate was 11 L/min, and the pressure was 15 psi.

DPPH

DPPH free radical scavenging test: The antioxidant test was performed using the DPPH method [37].

ABP extract was added to 1.0 mL, 0.26 mM of DPPH solution at 20, 40, and 80 µL concentrations for 30 minutes at room temperature. A UV-VIS spectrophotometer measuring at 517 nm was used to measure the absorbance; a lower absorbance of the reaction product indicated a higher activity. Equation (1) was used to calculate the DPPH scavenging activity. We used equation (2) from figure (Fig.3) to calculate the IC50.

$$I\% = ((A1 - A2)/A2) \times 100 \quad (1)$$

$$Y = 0.7079x + 14.604 \quad (2)$$

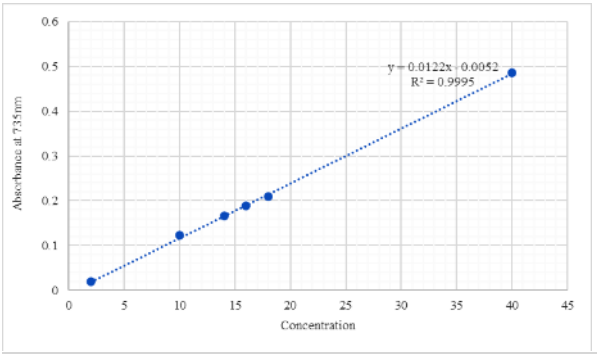

Figure 1. Total polyphenols calibration curve

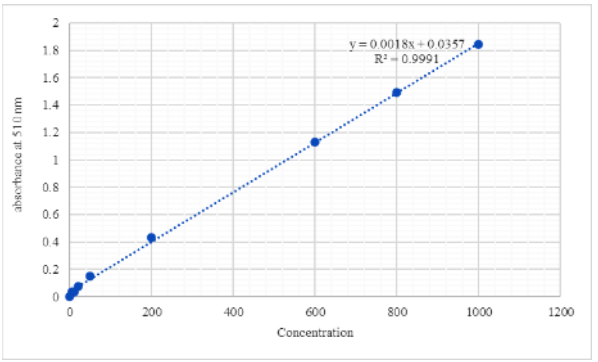

Figure 2. Total flavonoids calibration curve

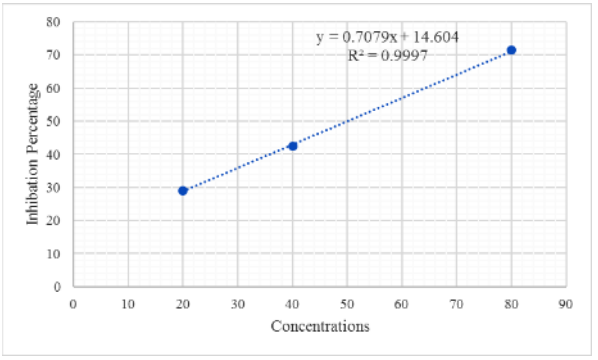

Figure 3. DPPH calibration curve

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2024, Tom. XXXI, Issue: 2, pp. 175-182

Hexokinase (HK) activity

HK activity was assayed spectrophotometrically through a coupled reaction with glucose-6-phosphate dehydrogenase (G6PD), following the NADP reduction at 340 nm [29]. The mix contained: R1 (MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative) and R2 (HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G-6-PDH (*E. coli*): ≥ 300 µkat/L; preservative). The assay was made on yeast HK isoforms (GLUC3, Roche/Hitachi Cobas) as well as on untreated medium. The enzyme was incubated with compounds (126 µg/mL) for 10 min before the assay.

Antimicrobial activity of algerian bee venom

We conducted an agar disk diffusion method with a modification [24]. We assessed the antimicrobial activity of bee venom against microbial and fungic strains using plates containing Mueller-Hinton agar pre-inoculated with a standardized microbial suspension at 10^8 CFU/mL. Whatman paper disks saturated with 15 µL of bee venom were positioned on the agar surface, with each disk having a 6 mm diameter. Following a 24-hour incubation at 37 °C, we interpreted the results by measuring the diameter of inhibition zones in millimeters (mm). All experiments were carried out in triplicate.

Statistical analysis

Tests were executed in triplicate and expressed as mean ± standard error (SE) with $p < 0.05$ as the level of significance by EXCEL 2016. The effect of enzyme inhibitor was analyzed by one-way analysis of variance (ANOVA), by using the SPSS 17.0 software.

RESULTS

Extraction yields

The principal step to evaluate the phytochemical activities of the sample is the extraction method of polyphenols and flavonoids molecules. The extraction with acetone 70% showed a higher yield of bee pollen extract with a value of 72.44%, which indicates the strong concentration and large quantity of extracted polyphenols and flavonoids.

Estimation of concentration of polyphenols and flavonoids molecules

The total phenolic content (TPC) and the total flavonoid content were evaluated for Algerian bee pollen. In this study, 54.92 mg GAE/g ± 0.48 and 6.18 mg QE/g ± 1.17 were calculated.

LC-ESI-MS/MS

The major organic components in algerian bee pollen preparation were identified by the LC-ESI- MS/MS method. 45 phenolic compounds were used as standards (Table 1) (Figure. 4).

Table 1. Esmation of chemical composition of Algerian bee pollen by LC-ESI-MS/MS

Compounds	Analyte	Rt	Phenolics (µg analyte/ g Bee pollen)
0	1	2	3
1	Shikimic acid	1.46	ND
2	Gallic acid	3.23	5.02
3	Protocatechuic acid	5.42	175.16
4	Epigallocatechin	6.82	ND
5	Catechin	6.86	ND
6	Chlorogenic acid	7.38	352.26
7	Hydroxyben zaldeyde	7.63	5.74
8	Vanillic acid	7.73	ND
9	Caffeic Acid	7.81	29.40
10	Syringic acid	8.35	ND
11	Caffein	8.34	1.27
12	Vanillin	8.59	19.55
13	o-coumaric acid	9.39	3.48
14	Salicylic Acid	9.73	20.45
15	Taxifolin	9.68	ND
16	Resveratrol	9.74	ND
17	Polydatine	9.87	ND
18	Trans-ferulic acid	10.05	ND
19	Sinapic acid	10.33	ND
20	Scutellarin	11.16	2.12
21	p-coumaric acid	11.57	ND
22	Coumarin	11.56	1.85
23	Protocatehuic ethyl ester	11.68	ND
24	Hesperidin	11.72	ND
25	Isoquercitrin	11.74	36.05
26	Rutin	12.21	15.91
27	Quarcetin-3-Ksilozid	12.41	ND
28	Kaempferol-3-glucoside	13.07	39.20
29	Fisetin	13.34	2.00
30	Baicalin	13.78	ND
31	Chrysin	14.04	ND
32	Daidzein	14.18	ND
33	Trans-cinnamic acid	14.27	ND
34	Quercetin	14.76	ND
35	Naringenin	14.86	44.54

0	1	2	3
36	Silibinin	15.60	ND
37	Hesperetin	15.83	ND
38	Morin	15.70	ND
39	Kaempferol	16.36	ND
40	Baicalein	17.07	ND
41	Luteolin	17.78	44.57
42	Biochanin A	17.90	ND
43	Capcaicin	18.17	ND
44	Dihydrocapcaicin	18.58	ND
45	Diosgenin	23.53	ND

Rt: Retention time. **ND:** not detected

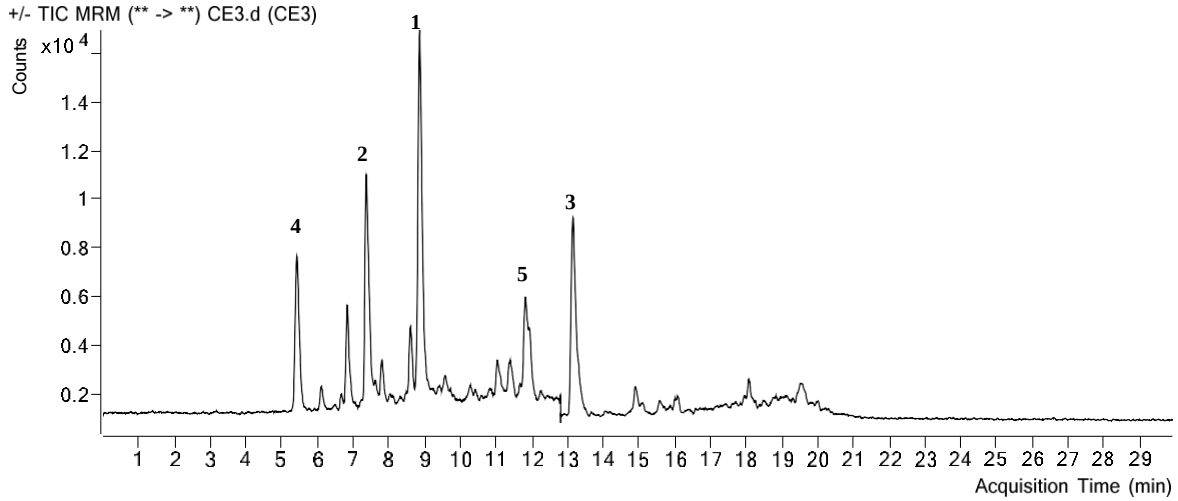

Figure 4. LC-ESI-MS/MS chromatograms of Algerian Bee Pollen; where: **1** - Vanillin, **2** - Chlorogenic acid, **3** - Kaempferol-3-glucoside, **4** - Protocatechuic acid, **5** - Isoquercitrin

Antioxidant activity

The results of the half maximal inhibition concentration (IC50) values of Algerian bee pollen, BHA, BHT, and Trolox measured by the DPPH scavenging activity method are represented in Table 2.

Hexokinase (HK) Inhibitory Assay

In vitro anti-diabetic effects of the isolate of bee pollen from Algeria were evaluated, with a 97.7% HK inhibition value of the isolate at a concentration of 126 µg/mL. The HK inhibitory assay was used to determine the algerian bee pollen isolate's anti-diabetic activity. Consequently, it was discovered that the isolate of algerian bee pollen had high anti-diabetic activity.

Antimicrobial activity of bee venom

The results of the antimicrobial activity (mm) of Algerian bee venom are represented in Table 3 (Fig. 5).

DISCUSSION

Natural sources of nutrients and phytochemicals, specifically polyphenols, can be found in bee pollen. These substances have a variety of biological activities, which make them beneficial to human health. The aim of this study was to investigate some natural products that are traditionally used to treat multiple diseases.

Table 2. The DPPH radical-scavenging activity of Algerian bee pollen.

Antioxidant activity	DPPH scavenging IC50 (µg/mL)	R^2
BHA	4.39	0.04
BHT	10.86	0.06
Trolox	4.95	0.03
Bee pollen	191.64	4.05

Table 3. Results of the antimicrobial activity of Algerian bee venom.

Strain	SAµ *Staphylococcus aureus*	E C *Escherichia coli*	K P *Klebsiella pneumoniae*	CA *Candida albicans*	SA33 *Staphylococcus aureus*
ATCC	29213	25922	70603	10231	33862
Gram	positif	Negatif	negatif	fungi	Positif
Bee venom (100 mg/mL)	13±1	14.66±1.15	10±0	13±1.7	14±1

ATCC: American Type Culture Collection

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2024, Tom. XXXI, Issue: 2, pp. 175-182

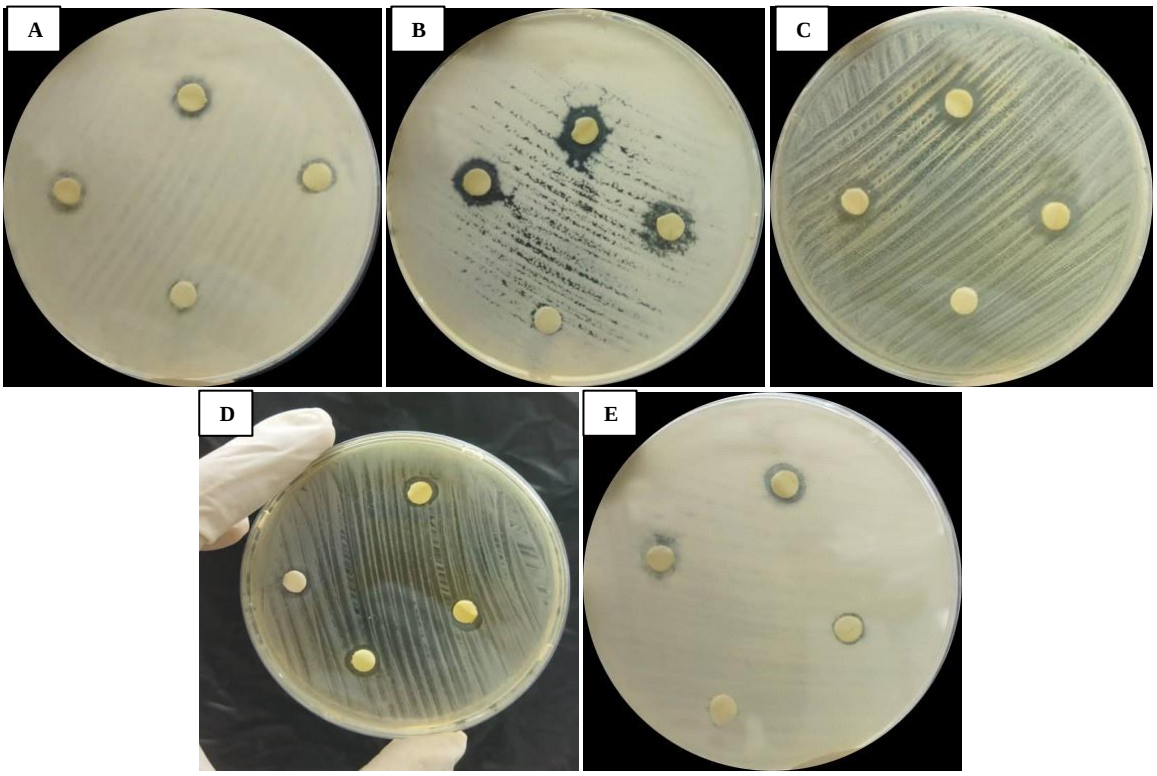

Figure 5. Results of the antimicrobial activity of Algerian bee venom, where: A – *Staphylococcus aureus* SAμ; B – *Escherichia coli* E C; C – *Klebsiella pneumoniae* K P; D – *Candida albicans* C A; E – *Staphylococcus aureus* SA33.

The results demonstrated greater extraction yields, total flavonoid and phenolic content, and antioxidant activity. The most notable characteristic of polyphenols is their highly unequal distribution throughout the various biological materials, which can be attributed to a variety of physiological evolution stages as well as extreme environmental conditions (high salinity, high temperature) [16, 44]. However, when compared to methanol and ethanol in all of the study's analyses, the extraction using 70% acetone produced the best results, with the exception of antioxidant activity, where ethanol performed better [44]. Many researchers proved to the point that acetone extracts antioxidant phenolics better than other organic solvents [18, 19, 22, 31, 34, 42, 44]. Of which the most phenolics have been found in acetone has the lowest polarity [1, 44, 50].

The results about the yield showed that we have a great yield as compared with anouther study [45], which used methanol as a solvent for extraction. These results confirmed that the extraction with aceton gives greater yields than methanol. The extraction yield is dependent on a number of variables, including the extraction method's solvent, temperature, and duration [23, 44, 48].

Significant results were obtained from the quantitative estimation of phenolics and flavonoids in the extracts of the tested algerian bee pollen presented the most important amounts of phenolic compounds (54.92 mg GAE/g ± 0.48) than determined by Rebiai and Lanez (2012) [45], and lower in flavonoids (6.18 mg QE/g ± 1.17) than Rebiai and Lanez (2012) [45], in the three samples of Blida and Boumerdes, but greatest with the other samples. The reason for this is the exceptionally high temperatures and high salinity experienced in Blida in 2010 and 2021.

The research about Algerian bee pollen compounds is very limited. Seventeen phenolic compounds were determinad from algerian bee pollen by the LC-ESI-MS/MS analyses (Table 1). Quantitative analysis (Figure 4) revealed that chlorogenic acid was the major compound with a value of 352.26 (μg/g extract). Moreover, the protocatechuic acid 175.16 (μg/g extract), luteolin 44.57 (μg/g extract), naringenin 44.54 (μg/g extract), kaempferol-3-glucoside 39.20 (μg/g extract), isoquercitrin 36.05 (μg/g extract), caffeic acid 29.40 (μg/g extract), salicylic acid 20.45 (μg/g extract), vanillin 19.55 (μg/g extract), rutin 15.91 (μg/g extract), hydroxyben zaldeyde 5.74 (μg/g extract), gallic acid 5.02 (μg/g extract), o-coumaric acid 3.48 (μg/g extract), Scutellarin 2.12 (μg/g extract), fisetin 2 (μg/g extract), coumarin 1.85 (μg/g extract), caffein 1.27 (μg/g extract) were determined in Algerian bee pollen extract. In another study, thirteen compounds were determinad from Chile bee pollen by HAPLC-DAD [9]. Bee pollen collected in Brazil contains twelve compounds, as shown by the HPLC [46]. In this context, we can say that the time and region of collection of bee pollen (geographic conditions), the extraction method, the solvant of extraction and also the technique used for the esmation of compounds can alter the composition of the samples.

The results of the DPPH test for free radical scavenging were represented as IC50 (mg/mL). According to the test, ABP's DPPH scavenging capacity was lower than that of BHA, BHT, and trolox (Table 2). But it’s higher (0.19±0.004 mg/mL) than

that found in other studies (Table 4) [2, 5, 14, 33, 51]. This finding is consistent with the results of previous studies and could be explained by the nature, quantity, and structure of the synthesized polyphenols [44]. The antioxidant's spatial structure determines the mechanism of the reaction between it and the DPPH radical [27, 44]. In antioxidant activity, the extraction with ethanol was more effective than acetone [44], but in our results, extraction with aceton is more powerful than ethanol as compared with the chine results [51]. This may be explained by the major compound in our sample « Cholorogenic acid » because it has an antioxidant effect [36].

Table 4. IC50 of antioxidant activity of bee pollen for other studies

IC50	Reference
0.39± 0.13 mg/mL	[14]
1.43± 0.00 mg/g	[2]
233.3± 6.1 µg/mL	[5]
2.36 mg/mL	[33]
1.72 mg/mL	[33]
1.28± 0.03 mg/mL	[51]

For the first time bee pollen is tested for anti hexokinase activity. Results of antihexokinase activity showed that our crude acetonic sample has an interesting inhibitory activity of hexokinase. It's possibly due to the absence of Kaempferol in our composition; the polyphenol that increased hexokinase activity [4, 13]. Fisetin (3,7,3',4'-Tetrahydroxyflavone) reduces the activity of glucose-6-phosphate dehydrogenase [13, 41]. In addition, luteolin is an anti- diabetic agent [3, 49]. Also, fisetin has anti-diabetic effects [10]. Furthermore, hexokinase activity is increased by fisetin's anti-diabetic effects on hepatic enzymes, whereas glucose-6-phosphatase (G6Pase) and glucose-6-phosphate dehydrogenase (G6PD) activity are decreased [3]. On the other hand, protocatechuic acid has antihexokinase activity because it has antihyperglycemia activity [36].

The results from Table 3 indicate that bee venom showed effective antibacterial and antifungal properties against the tested microorganisms. The antimicrobial effects of honeybee venom may be due to the presence of various peptides like melittin, apamin, adolapin, mast cell degranulating peptide, enzymes, biologically active amines, and non-peptide components [28, 30]. Fennel et al. [17] noted that the venom contains melittin, which is more effective against Gram-positive bacteria than Gram-negative ones. These findings are consistent with Kondo and Kanai's [26] observations that *mycobacteria* and *staphylococci* were affected by the bee venom fraction (melittin) but not *E. coli*. However, earlier studies [40, 47] have reported a stronger impact on *E. coli* from bee venom. While Hegazi et al. [21] previously showed that bee products had less effect on *E. coli*, the current study provides evidence that bee venom displays antibacterial activity against both Gram-positive and Gram-negative bacteria, with no significant differences between the two groups.

Finally, this study of the crude extracts for the pollen of Algerian bees by aceton 70% According to the LC-ESI-MS/MS analysis, the major components detected in Algerian bee pollen are protocatechuic acid, chlorogenic acid, kaempferol-3-glucoside, naringenin, and luteolin. The algerian bee pollen aceton extract had increased antioxidant activity, phenolic contents, and inhibited hexokinase activity. In diabetes mellitus treatment, clinicians prescribe insulin together with synthetic anti-diabetic helper drugs such as glipizide and metformin, but they have side effects. Scientists are intensively researching herbal metabolites as new anti-diabetic agents to eliminate these irritating side effects. The results suggested that algerian bee pollen isolate could be used as a natural source of compounds with therapeutic potential for treating disorders associated with diabetes. Given the results of the present study, it could be said that algerian bee pollen can play a beneficial role in the treatment of human health. However, further studies are needed to reach a final conclusion. Additionally, the current discoveries enhance the significance of Algerian bee venom in inhibiting microorganisms. These inhibiting properties have potential for broad use as antiseptics and as distinctive inhibitors in biological research. Further research is needed to standardize their precise composition, assess allergy risks, toxicity, and ensure quality.

Acknowledgements. This work was supported by the Ministry of Higher Education and Scientific Research (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, MESRS).

Conflict of interest.: There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Alasalvar, C., Karamać, M., Amarowikz, R., Shahidi, F., (2006): Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana* L.) and hazelnut green leafy cover. Journal of Agricultural and Food Chemistry, 54(13): 4826-4832.

[2] Aličić, D., Flanjak, I., Ačkar, Đ., Jašić, M., Babić, J., Šubarić, D., (2020): Physicochemical properties and antioxidant capacity of bee pollen collected in Tuzla Canton (B&H). Journal of Central European Agriculture, 21: 42–50.

[3] Al-Ishaq, R.K., Abotaleb, M., Kubatka, P., Kajo, K., Büsselberg, D., (2019): Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules, 9(9): 430.

[4] Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K., Liu, D., (2018): The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. Molecules, 23: 2338.

[5] Atsalakis, E., Chinou, I., Makropoulou, M., Karabournioti, S., Graikou, K., (2017): Evaluation of phenolic compounds in *Cistus creticus* bee pollen from Greece. Antioxidant and antimicrobial properties. Natural Product Communications, 12(11): 1934578X1701201141.

[6] Boizot, N., Charpentier, J.P., (2006): Méthode rapide d'évaluation du contenu en composés phénoliques des

organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial, 79-82.

[7] Bota, S.R., Stănășel, O.D., Blidar, C.F., Șerban, G., (2021): Phenolic constituents of *Anethum graveolens* seed extracts: chemical profile and antioxidant effect studies. Journal of Pharmaceutical Research International, 33(54B): 168-179.

[8] Boutrin, M.C., H.A. Foster and V.W. Pentreath, (2008): The effects of bee (*Apis mellifera*) venom phospholipase A2 on *Trypanosoma brucei brucei* and enterobacteria. Experimental Parasitology, 119(2): 246-251.

[9] Bridi, R., Atala, E., Pizarro, P.N., Montenegro, G., (2019): Honeybee pollen load: phenolic composition and antimicrobial activity and antioxidant capacity. Journal of Natural Products, 82(3): 559-565.

[10] Constantin, R.P., Constantin, J., Pagadigorria, C.L., Ishii-Iwamoto, E.L., Bracht, A., Ono Mde, K., Yamamoto, N.S., (2010): The actions of fisetin on glucose metabolism in the rat liver. Cell Biochemistry and Function, 28(2): 149-158.

[11] Denisow, B., Denisow-Pietrzyk, M., (2016): Biological and therapeutic properties of bee pollen: A review. Journal of the Science of Food and Agriculture, 96(13): 4303–4309.

[12] Duarte, A.W.F., Vasconcelos, M.R., Melissa Oda-Souza, M., de Oliveira, F.F., Lòpez, A.M.Q., (2018): Honey and bee pollen produced by Meliponini (Apidae) in Alagoas, Brazil: multivariate analysis of physicochemical and antioxidant profiles. Food Science and Technology, 38: 493–503.

[13] El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C., Lyoussi, B., (2023): Bee pollen as functional food: insights into its composition and therapeutic properties. Antioxidants, 12(3): 557.

[14] El Ghouizi, A., El Menyiy, N., Falcão, S.I., Vilas-Boas, M., Lyoussi, B., (2020): Chemical composition, antioxidant activity, and diuretic effect of Moroccan fresh bee pollen in rats. Vet World, 13: 1251-1261.

[15] Erenler, R., Atalar, M.N., Yildiz, İ., Geçer, E.N., Yildirim, A., Demirtas, İ., Alma, M.H., (2023): Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*. Bütünleyici ve Anadolu Tıbbı Dergisi, 4(3): 3-10.

[16] Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., Abdelly, C., (2008): Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. Comptes Rendus Biologies, 331(5): 372-379.

[17] Fennell, J.F., W.H. Shipman and L.J. Cole, (1968): Antibacterial action of melittin, a polypeptide from bee venom. Proc. Soc. Exp. Biol. Med., 127(3): 707-710.

[18] Gonzàlez-Montelongo, R., Lobo, M.G., Gonzàlez, M., (2010): Antioxidant activity in banana peel extracts: testing extraction conditions and relative bioactive compounds. Food Chemistry, 119(3): 1030-1039.

[19] Gülcin, İ., (2011): Antioxidant activity of eugenol: a stucture-activity relationship study. Journal of Medicinal Food, 14(9): 975-985.

[20] Harwig, S.S., L. Tan, X.D. Qu, Y. Cho, P.B. Eisenhauer and R.I. Lehrer, (1995): Bactericidal properties of murine intestinal phospholipase A2. The Journal of Clinical Investigation, 95(2): 603-610.

[21] Hegazi, A.G., N.Z. Moharram, F.A. Abd-Allah, M.S. Nour and A.M. Khair, (2002): Antibacterial activity of different Egyptian honeys in relation to some bee products. Egypt. J. Vet. Sci., 36: 31-42.

[22] Kajdzanoska, M., Petreska, J.P., Stefova, M., (2011): Comparision of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. Journal of Agricultural and Food Chemistry, 59(10): 5272-5278.

[23] Kavita, S., Eun, Y.K., Awraris, D.A., Soyoung, H.A., Shivraj, H.N., Eul, T.L., Se, W.P., (2014): Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analysis, 23: 243-252.

[24] Klancnik A, Piskernik S, Jersek B, Mozina SS., (2010): Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J Microbiol Methods; 81(2): 121-126.

[25] Koduri, R., J. Gronroos, V. Laine, C. Le Calvez, G. Lambeau, T. Nevalainen and M. Gelb, (2002): Bactericidal properties of human and murine groups I, II, V, X and XII secreted phospholipases A2. The Journal of Biological Chemistry, 277(8): 5849-5857.

[26] Kondo, E. and K. Kanai, (1986): Bactericidal activity of the membrane fraction isolated form phagocytes of mice and its stimulation by melittin. Jpn. J. Med. Sci. Biol., 39(1): 9-20.

[27] Kouri, G., Tsimogiannis, D., Bardouki, H., Oreopoulou, V., (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innovative of Food Science and Emerging Technolgy, 8: 155-168.

[28] Kwon, Y.B., H.J. Lee, H.J. Han, W.C. Mar, S.K. Kang, O.B. Yoon, A.J. Beitz and J.H. Lee, (2002): The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life Sci., 71(2): 191-204.

[29] Lamprecht, W., Trautschold, I., (1974): Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academic press, pp. 1127–1131.

[30] Lariviere, W.R. and R. Melzack, (1996): The bee venom test: a new tonic-pain test. Pain, 66(2-3): 271-277.

[31] Lien, D.T.P., Tram, P.T.B., Toan, H.T., (2015): Effect of extraction process on phenolic content and antioxidant activity of soybean. Journal of Food and Nutrition Sciences, 3(1-2): 33-38.

[32] Martinello, M., Mutinelli, F., (2021): Antioxidant activity in bee products: A review. Antioxidants, 10(1): 71.

[33] Mejías, E., Montenegro, G., (2012): The antioxidant activity of Chilean honey and bee pollen produced in the Llaima Volcano's zones. Journal of Food Quality, 35: 315–322.

[34] Mokrani, A., Madani, K., (2016): Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. Separation and Purification Technology, 162: 68-76.

[35] Negru, P.A., Bota, S.R., Stanasel, O.D., Blidar, C.F., Serban, G., (2021): Molecular docking studies on phenolic constituents of *Anethum graveolens* L. seed extracts. Journal of Pharmaceutical Research International, 33(56B): 119-128.

[36] Nisa, N., Rasmita, B., Arati, C., Uditraj, C., Siddhartha, R., Dinata, R., Gurusubramanian, G., (2023): Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: Small molecule bioinformatics strategies as

amyloid-based therapy. Environmental Science and Pollution Research, 30(17): 51143-51169.

[37] Nusret, G.E.N.Ç., Elmastaş, M., Telci, İ., Erenler, R., (2020): Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects. International Journal of Chemistry and Technology, 4(2): 179-184.

[38] Park, S., B. Park, S. Yun, H. Kang, B. So and S. Yun, (2013): Antimicrobial activities of honey bee venom against pathogens isolated from clinical bovine mastitis in Korea. Planta Med., 79-PL16.

[39] Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., Estevinho, L.M., (2014): Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology, 63: 233–239.

[40] Perumal Samy, R., P. Gopalakrishnakone, M.M. Thwin, T.K. Chow, H. Bow, E.H. Yap and T.W. Thong, (2007): Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J. Appl. Microbiol., 102(3): 650-659.

[41] Prasath, G.S., Pillai, S.I., Subramanian, S.P., (2014): Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. European Journal of Pharmacology, 740: 248–254.

[42] Quy, D.D., Artik, E.A., Phuong, L.T-N., Lien, H.H., Felycia, E.S., Suryadi, I., Yi-H.J., (2013): Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. Journal of Food and Drug Analysis, 22: 296-302.

[43] Rabbani, N., Thornalley, P.J., (2019): Hexokinase-2 glycolytic overload in diabetes and ischemia–reperfusion injury. Trends in Endocrinology & Metabolism, 30(7): 419-431.

[44] Radjah, A., Chorfi, H., Bouatrous, Y., (2019): Stage of development and solvent effects on phytochemistry and antioxidant activity of three algerian plants. Analele Universităţii din Oradea, Fascicula Biologie, 26(2): 91-99.

[45] Rebiai, A., Lanez, T., (2012): Chemical composition and antioxidant activity of *Apis mellifera* bee pollen from northwest Algeria. Journal of Fundamental and Applied Sciences, 4: 155.

[46] Santa Bárbara, M.F., Moreira, M.M., Machado, C.S., Chambó, E.D., Pascoal, A., de Carvalho, C.A.L., da Silva Sodré, G., Delerue-Matos, C., Estevinho, L.M., (2021): Storage methods, phenolic composition, and bioactive properties of *Apis mellifera* and *Trigona spinipes* pollen. Journal of Apicultural Research, 60(1): 99-107.

[47] Stocker, J.F. and J.R. Traynor, (1986): The action of various venoms on *Escherichia coli*. J. Appl. Bacteriol., 61(5): 383-388.

[48] Su, X., Duan, J., Jiang, Y., Shi, J., Kakuda, Y., (2006): Effect of soaking conditions on the antioxidant potenials of oolong tea. Journal of Food Composition and Analysis, 19(4): 348-353.

[49] Tuorkey, M.J., (2016): Molecular targets of luteolin in cancer. European Journal of Cancer Prevention, 25(1): 65-76.

[50] Uma, D.B., Ho, C.W., Wan Aida, W.M., (2010): Optimization of extraction parameters of total phenolic compounds from Henna (*Lawsonia inermis*) leaves. Sains Malaysiana, 39(1): 119-128.

[51] Zhang, H., Wang, X., Wang, K., Li, C., (2015): Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from Monofloral bee pollen. Journal of Apicultural Science, 59(1): 119-128.

[52] Zhishen, J., Mengcheng, T., Jianming, W., (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 64(4): 555-559.

Received: March 30, 2024
Accepted: October 29, 2024
Published Online: November 8, 2024
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House

SYNTHESE D'ARTICLE 01

Introduction

La prise en charge thérapeutique du diabète sucré repose conventionnellement sur l'administration d'insuline et d'agents synthétiques, tels que la metformine et le glipizide, des traitements pouvant induire des effets secondaires indésirables. Cette limite motive l'exploration scientifique de métabolites secondaires d'origine naturelle en tant qu'alternatives thérapeutiques. Parmi les mécanismes physiopathologiques cibles, une régulation anormale de la glycolyse, caractérisée par une surexpression de l'hexokinase 2 (HK2), a été identifiée comme un facteur clé dans l'altération du métabolisme glucidique (Rabbani et Thornalley, 2019).

Le pollen d'abeille, reconnu pour sa composition nutritionnelle complexe incluant protéines, lipides, glucides, fibres, vitamines, enzymes et une fraction notable de composés phénoliques — principalement des flavonoïdes — représente une source prometteuse de ces métabolites. Ses propriétés antioxydantes et antidiabétiques, modulées par l'origine botanique et les conditions environnementales (Pascoal *et al.,* 2014), sont largement attribuées à ces polyphénols, dont le potentiel dans le traitement de l'hyperglycémie fait l'objet d'études approfondies (Bota *et al.*, 2021). Parallèlement, le venin d'abeille (BV) présente une activité antimicrobienne à large spectre, incluant des souches Gram-positives et Gram-négatives telles qu'*Escherichia coli*, *Salmonella* spp. et *Staphylococcus aureus* (Harwig *et al.*, 1995), suggérant son potentiel en tant qu'agent antiseptique naturel.

Objectifs de l'étude

Cette recherche vise à évaluer les caractéristiques bioactives du pollen d'abeille algérien (ABP) et du venin d'abeille (ABV). Plus précisément, elle s'attache à :

- Analyser la composition chimique de l'ABP par la technique LC-ESI-MS/MS ;
- Étudier les propriétés antioxydantes et antidiabétiques de l'ABP ;
- Évaluer les propriétés antimicrobiennes de l'ABV.

Matériels et méthodes

Collecte des échantillons

- L'ABP a été prélevé à Blida, Algérie (2022).
- L'ABV a été obtenu à El-Medea, Algérie (2023).

Extraction et analyse

- L'extraction de l'ABP a été réalisée avec une solution d'acétone à 70 %, obtenant un rendement significatif de 72,44 %.

- La quantification des phénols et flavonoïdes a été effectuée par spectrophotométrie.

- L'identification des composés phénoliques bioactifs a été réalisée par LC-ESI-MS/MS dans laboratoire de recherche, centre de pratique et de recherche à Igdîr, la turquie.

Évaluations biologiques

1. Activité antioxydante : Test du piégeage des radicaux libres DPPH.
2. Activité antidiabétique : Évaluation de l'inhibition de l'enzyme hexokinase (HK).
3. Activité antimicrobienne : Test de diffusion sur disque, permettant d'évaluer l'efficacité de l'ABV contre les bactéries Gram-positives, Gram-négatives et les champignons.

Résultats

Rendement d'extraction

La méthode d'extraction par solvant a été optimisée pour le pollen d'abeille algérien (ABP), révélant que l'acétone aqueuse à 70 % permettait d'atteindre un rendement d'extraction maximal de 72,44 %. Ce rendement, supérieur à ceux obtenus avec d'autres solvants testés, est attribué à la capacité de ce mélange à solvabiliser efficacement une large gamme de composés phénoliques et flavonoidiques, des plus polaires aux plus apolaires. Ces données confirment la pertinence de ce solvant, comme le suggèrent Kajdzanoska *et al.* (2011) et Radjah *et al.* (2019).

Teneur en polyphénols et flavonoïdes

La teneur totale en polyphénols (TPC) et en flavonoïdes (TFC) a été estimée à 54,92 ± 0,48 mg GAE/g et 6,18 ± 1,17 mg QE/g, respectivement. Ces valeurs, indiquent que le pollen d'abeille algérien contient une proportion substantielle de métabolites secondaires responsables de son activité biologique.

Composition chimique

- L'analyse LC-ESI-MS/MS a révélé la présence de 17 composés phénoliques dans l'ABP.
- Les principaux composés identifiés incluent :

✓ Acide chlorogénique (352,26 µg/g),
✓ Acide protocatéchuique,
✓ Lutéoline,
✓ Naringénine,
✓ Kaempférol-3-glucoside.

Ces données révèlent une composition chimique diversifiée et riche, comparable à celles observées dans les pollens du Brésil (Santa Bárbara *et al*., 2021) et du Chili (Bridi *et al*., 2019), tout en présentant des concentrations supérieures en acide chlorogénique, principal composé antioxydant identifié.

Activité antioxydante

L'activité antioxydante, évaluée par le test de piégeage du radical DPPH, a révélé une IC_{50} de 0,19 ± 0,004 mg/mL pour le pollen d'abeille algérien. En comparaison, les standards de référence BHA, BHT et Trolox ont présenté respectivement des IC_{50} de 4,39, 10,86 et 4,95 µg/mL.

Ces résultats suggèrent une forte capacité de neutralisation des radicaux libres, attribuable à la concentration élevée en acide chlorogénique et en flavonoïdes hydroxylés.

Potentiel antidiabétique

L'analyse de l'effet inhibiteur sur l'hexokinase a mis en évidence une activité remarquable de l'extrait acétonique de pollen d'abeille, avec un pourcentage d'inhibition de 97,7 % à une concentration test de 126 µg/mL. Cette inhibition enzymatique prononcée souligne un potentiel antidiabétique substantiel. Cette activité biologique est vraisemblablement attribuable à la synergie de composés phénoliques identifiés dans l'extrait, notamment la lutéoline, la naringénine et l'acide protocatéchuique, dont les effets bénéfiques sur le métabolisme glucidique sont connus (Al-Ishaq *et al.*, 2019 ; Rabbani & Thornalley, 2019).

L'activité antimicrobienne du venin d'abeille algérien

Les essais antimicrobiens réalisés par la méthode de diffusion sur disque ont montré que le venin d'abeille algérien présente une activité inhibitrice significative contre plusieurs souches pathogènes :

- ✓ *Staphylococcus aureus* (13 ± 1 mm),
- ✓ *Escherichia coli* (14,66 ± 1,15 mm),
- ✓ *Klebsiella pneumoniae* (10 ± 0 mm),
- ✓ *Candida albicans* (13 ± 1,7 mm),
- ✓ *Staphylococcus aureus* SA33 (14 ± 1 mm).

Ces résultats confirment l'efficacité du venin contre les bactéries Gram-positives et Gram-négatives, ainsi que contre certaines levures pathogènes.

Discussion

L'optimisation du procédé d'extraction a confirmé la supériorité de l'acétone aqueuse à 70 %, un solvant dont l'efficacité pour l'extraction des polyphénols, en raison de sa polarité intermédiaire, a été préalablement démontrée (González-Montelongo *et al.,* 2010 ; Mokrani et Madani, 2016).

Le rendement significativement plus élevé observé, comparativement à l'utilisation du méthanol (Rebiai et Lanez, 2012), met en exergue l'impact critique des conditions opératoires, telles que la nature du solvant, la température et le temps d'extraction, sur le taux de récupération des métabolites d'intérêt.

La caractérisation phytochimique du pollen d'abeille algérien a mis en évidence un profil distinct. Les teneurs en polyphénols totaux (54,92 ± 0,48 mg EAG/g) se sont avérées plus importantes que celles précédemment documentées (Rebiai et Lanez, 2012). Une variabilité a néanmoins été notée concernant les flavonoïdes totaux (6,18 ± 1,17 mg EQ/g), avec des concentrations inférieures pour les échantillons de Blida et Boumerdes, mais supérieures pour d'autres sites. Cette hétérogénéité inter-régionale est vraisemblablement imputable à des facteurs agroclimatiques, les stress thermique et salin, particulièrement marqués dans la région de Blida, étant susceptibles d'affecter la biosynthèse des métabolites secondaires.

L'identification de 17 composés phénoliques met en évidence la diversité métabolique du pollen d'abeille algérien, notamment l'abondance en acide chlorogénique, protocatéchuique et flavonoïdes. Ces molécules ont été reconnues pour leurs effets antioxydants, anti-inflammatoires et hypoglycémiants (Denisow et Denisow-Pietrzyk, 2016 ; El Ghouizi *et al*., 2023).

Les variations régionales observées dans la composition phénolique du pollen peuvent être attribuées à la flore locale, aux conditions climatiques (salinité et température élevées à Blida) et à la période de récolte (Radjah *et al*., 2019).

L'activité antioxydante du pollen algérien est supérieure à celle rapportée pour les pollens de Bosnie (Alicić *et al*., 2020) et de Grèce (Atsalakis *et al*., 2017). Cette puissance radicalaire s'explique par la présence d'acide chlorogénique et de flavones hydroxylées (luteoline, naringénine), qui agissent comme donneurs d'hydrogène et piégeurs d'espèces réactives de l'oxygène (ROS) (Kouri *et al*., 2007).

Ces composés participent à la protection cellulaire contre les processus oxydatifs et à la réduction du risque de pathologies métaboliques.

L'inhibition enzymatique élevée (97,7 %) observée suggère une forte affinité des composés phénoliques du pollen pour le site actif de l'hexokinase. Ce résultat confirme les

observations de Rabbani et Thornalley (2019), selon lesquelles la surcharge glycolytique et la dérégulation de l'HK2 jouent un rôle central dans la physiopathologie du diabète.

Des flavonoïdes comme la lutéoline (Tuorkey, 2016) et la fisetine (Prasath *et al*., 2014) ont été associés à une réduction de l'activité des enzymes de la gluconéogenèse et à une amélioration de la régulation glycémique.

L'absence de kaempférol libre dans l'extrait, flavonoïde connu pour activer l'hexokinase (Alkhalidy *et al*., 2018), pourrait expliquer la forte inhibition enzymatique observée.

Le venin d'abeille a démontré une activité antimicrobienne efficace contre des bactéries pathogènes comme *S. aureus et E. coli*, corroborant les études de Park *et al*., (2013) et Boutrin *et al*., (2008).

Cette activité est attribuée à la présence de peptides bioactifs tels que la mélittine, l'apamine et l'adolapine, reconnus pour leurs effets bactéricides et antifongiques (Fennell *et al.*, 1968 ; Perumal Samy *et al.*, 2007).

Le venin présente donc un potentiel thérapeutique dans la formulation d'antiseptiques naturels et d'agents antimicrobiens alternatifs, notamment dans un contexte de résistance microbienne accrue.

Conclusion et perspective

Ces résultats démontrent que le pollen et le venin d'abeille algérien constitue une source naturelle prometteuse de composés bioactifs à la fois antioxydants, antidiabétiques pour le pollen et antimicrobiens pour le venin.

L'approche combinée de l'analyse LC-ESI-MS/MS et des essais biologiques *in vitro* permet de relier la composition chimique à la bioactivité des extraits.

Les produits apicoles algériens, en particulier le pollen et le venin, offrent un potentiel d'exploitation biomédicale considérable dans la prévention du stress oxydatif, la modulation du métabolisme glucidique et la lutte contre les infections bactériennes.

Toutefois, des recherches supplémentaires sont nécessaires pour :

- Optimiser les méthodes d'extraction et de purification ;
- Étudier les profils de toxicité et de sécurité ;
- Explorer les applications thérapeutiques potentielles.

CHAPITRE 02 :
ARTICLE 02 :

Investigation of phenolic profiling using LC-ESI-MS/MS, antioxydant and antihexokinase activities of algerian bee venom and algerian royal jelly.

https://doi.org/10.48047/AFJBS.7.1.2025.503-516

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Investigation of Phenolic Profiling Using LC-ESI-MS/MS, Antioxydant and Antihexokinase Activities of Algerian Bee Venom And Algerian Royal Jelly

Moussa Abderrazak Bambra [1*], Yamina Bouatrous [2], Ramazan Erenler [3], Ilyas Yildiz [3]

[1] *Laboratory of promotion of agricultural innovation in arid regions (PIARA), Department of Biology, Faculty of Natural Sciences and Life, University Mohamed khider Biskra, Algeria.*

[2] *Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Mohamed khider University, Biskra, Algeria.*

[3] *Research Laboratory Practice and Research Center, Igdir University, 7600, Igdir, Turkiye*

[*] *Correspondence author: Moussa Abderrazak BAMBRA, Laboratory of promotion of agricultural innovation in arid regions (PIARA), Department of Biology, Faculty of Natural Sciences and Life, University Mohamed khider Biskra, Algeria., zip code: 07000, 0775.66.30.26 email: moussaabdrrazak.bambra@univ-biskra.dz*

.

Volume 7, Issue 1, Jan 2025

Received: 15 Nov 2024

Accepted: 25 Dec 2024

Published: 05 Jan 2025

doi:10.48047/AFJBS.7.1.2025.503-516

Abstract.

Introduction: A limited number of studies investigate the chemical composition and effects of algerian royal jelly (ARJ) and algerian bee venom (ABV) on total flavonoids, total phenolics, and the antihexokinase effect, in addition to their potential as antioxidants. ABV and ARJ samples were used for the analyses. To evaluate the antioxidant activity of ABV, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was employed. Tandem mass spectrometry (LC-ESI-MS/MS), electrospray ionization, and liquid chromatography were utilized for phenolic identification. Both ABV and ARJ had phenolic and flavonoid contents of 23,56 mg GAE/g ± 0.40 and 141.27 mg QE/g ± 25.21, and 08,07 mg GAE/g ± 1,80 and 67,20 mg QE/g ± 12,83, respectively. The half maximal inhibition concentration (IC50) values of ABV for DPPH scavenging activity were found to be 0.431±0.006 mg/mL. The LC-ESI-MS/MS analysis led to the identification of phenolic compounds; two compounds in the extract of ABV were detected, and four in the ARJ sample were detected. The Algerian bee venom and royal jelly isolates were assessed at 126 µg/mL concentration for the in vitro antidiabetic effect, yielding a 07.5% and 99.3% HK inhibition value, respectively. The algerian royal jelly has a very important effect as an anti-diabetic agent.

Key words: Algerian bee venom; Algerian royal jelly; LC-ESI-MS/MS; antioxidant activity; antihexokinase effect; anti-diabetic activity

1. Introduction

Doctors treat diabetes mellitus with insulin prescriptions in addition to synthetic anti-diabetic drugs such as glipizide and metformin, however these drugs may cause adverse effects. In particular, researchers are actively investigating herbal metabolites as possible candidates for novel anti-diabetic drugs in an effort to eradicate these irritating side effects. According to recent research, abnormal increases in HK2 and unscheduled

glycolysis are important factors in impaired glycolysis control. In this context, aberrant elevations in G6P and downstream glycolysis intermediates are important players in the tissue-specific chronic pathophysiology of hyperglycemia linked to diabetes (Rabbani and Thornalley, 2019).

Among complementary medicine, bee products are widely used. Apitherapy, the use of products made from bees in medicine (Sig et al., 2019). More than 20 bioactive compounds that have been found to have anti-inflammatory, anti-arthritic, anti-nociceptive, neuroprotective, anti-tumoral, antimicrobial, anti-diabetic, and anti-rheumatic properties have been isolated and identified from BV to date (Chang and Bliven, 1979 ; Lee et al., 2005; Sig et al., 2019). Also, royal jelly has an antidiabetic effect (Sığ et al., 2019). because RJ's other contents (sulfur, vitamin B3, vitamin H, etc.) and insulin-like peptides can lower blood glucose levels (Pavel et al., 2011 ; Sobral et al., 2016).

Natural antioxidant compounds present in medicinal bee products have been the subject of several studies. because they are used to treat hyperglycemia in addition to serving as food preservatives by substituting natural antioxidants. Thus, the study investigates the phenolic compounds in algerian bee venom and algerian royal jelly by aiming to identify the most efficient active molecules that have antioxidant and anti-diabtic effects.

2. Methods

2.1. Collection of the sample

The Algerian Royal Jelly « ARJ » was collected from Blida in 2022.

The Algerian Bee Venom « ABV » was collected from El-Medea in 2023. Venom is collected on a glass plate and then transferred to bottles for additional processing after bees are exposed to an electric current (fig 1) (Chang and Bliven, 1979).

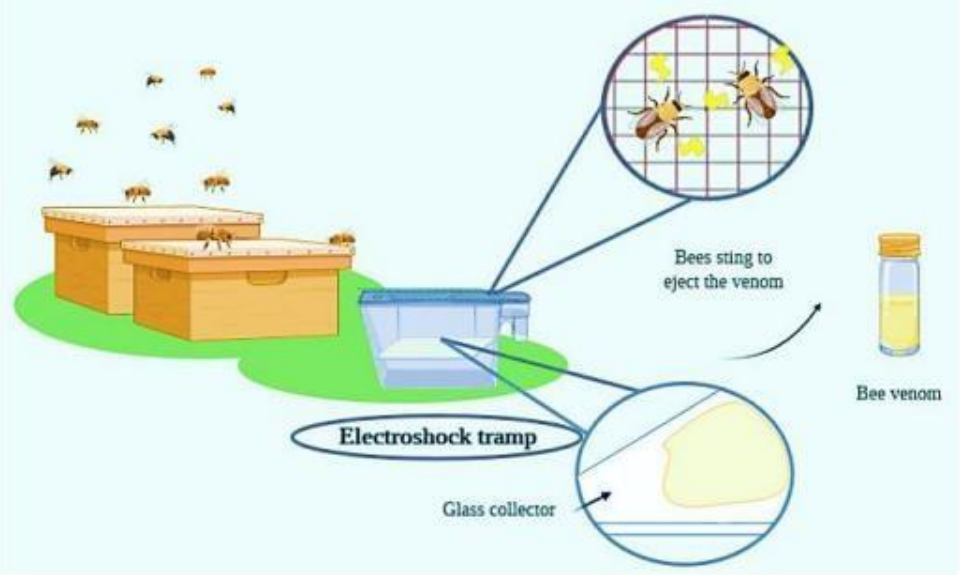

Fig. 1. An electroshock trap is used in the standard procedure flow for collecting bee venom (Carpena et al., 2020).

2.2. Determination of the Total Phenol Content

The Folin-Ciocalteau colorimetric method (Boizot and Charpentier, 2006) was utilized to identify the total polyphenol content present in the samples. 400µl of Na2CO3 (75g/l) was added to a 1.5 mL Eppendorf tube along with 100µl of the sample extract (1 mg/ml of distilled water) and 500µl of the folin-ciocalteau (10%) reagent. and the absorbance was measured at 735 nm after 5 min of incubation at 40°C in the dark. Galllic acid equivalents, or mg/g, were used to express the total polyphenol content.

(R2=0.9995, Y= 0.0122x – 0.0052) Measurements were made in triplicate for every sample.

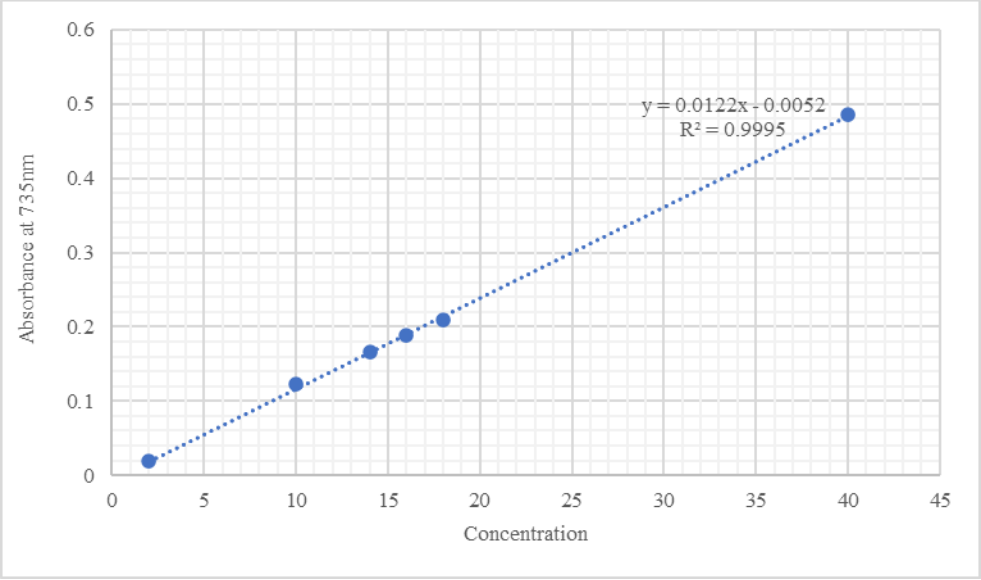

Fig. 2. Total polyphenols calibration curve

2.3. Total flavonoids content

Zhishen et al.'s (1999) method was used to determine the total flavonoid content of the sample. with modifications. 200µl of sample and 60µl of 5% NaNO2 were combined in a 1.5 mL Eppendorf tube. 60 µl of 10% AlCl3 was added after 5 minutes, and the mixture was thoroughly mixed. Following a 6-minute duration, 400µl of 1M NaOH was added. At 510 nm, the absorbance was measured right away in comparison to the control. A quercetin solution has been made. Solutions made from a stock solution at different concentrations ranging from 0.1 to 1000 g/ml were used to calculate the calibration curve. (R2=0.9991, Y=0.0018x + 0.0357). Measurements were made in triplicate for every sample.

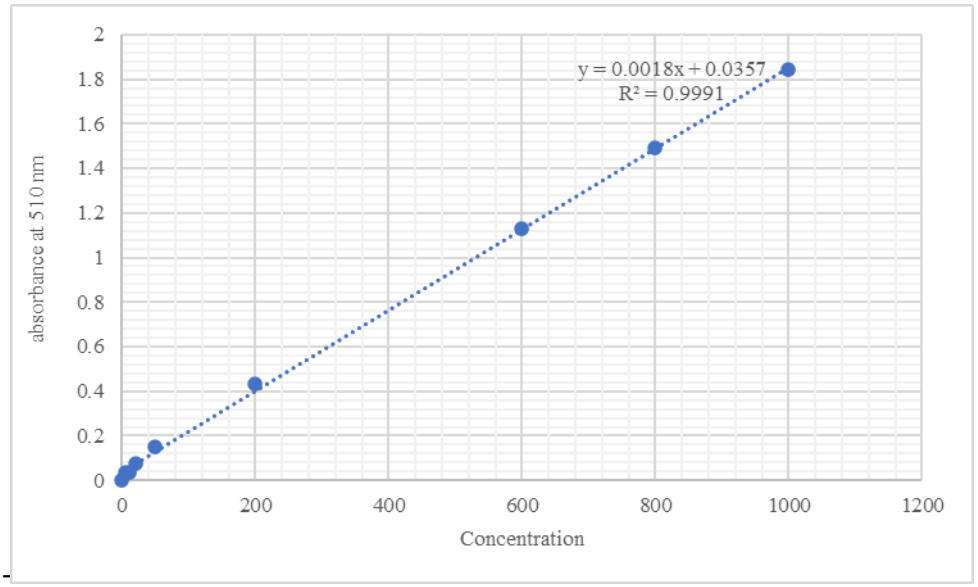

Fig. 3. Total flavonoids calibration curve

2.4. LC-ESI-MS/MS

The phenolics were quantitatively analyzed using an Agilent Technologies 1260 Infinity II, 6460 Triple Quad Mass Spectrometer. Erenler et al. (2023) employed a single Poroshell 120 SB-C18 column (3.0 × 100 mm, I.D., 2.7 µm). A quantity of 50 mg of the samples was dissolved in a 2 mL Eppendorf tube by including 1 ml of methanol. The final product was extracted using one milliliter of hexane. It was spun at 9,000 rpm for a duration of ten minutes. Finally, 100 µl of the methanol layer was isolated and then mixed with 450 µl of water and 450 µl of methanol to make a total volume of 900 µl. The blend passed through a 0.22 µm filter before being introduced into the device as the last stage. The volume injected was 5.12 microliters, with a flow rate of 0.40 milliliters per minute. The mobile phase contained 5.0 mM of ammonium formate, 0.1% formic acid in methanol B, and 0.1% ammonium formate in water A. The gradient program for the B mobile phase was adjusted to 25% for 1-3 minutes, 50% for 4–12 minutes, 90% for 13–21 minutes, and 3% for 22–25 minutes. The temperature of the column reached 40°C. The flow rate of nebulizing gas (N2) was 11 L/min, pressure stood at 15 psi, capillary voltage was set at 4000 V, and gas temperature was maintained at 300 °C.

2.5. DPPH

DPPH free radical scavenging test: The DPPH technique was used to carry out the antioxidant test (Nusret et al., 2020).

ARJ extract was added to 1.0 ml, 0.26 mM of DPPH solution at 20, 40, and 80 µl concentrations for 30 minutes at room temperature. The absorbance was measured using a spectrophotometer set to measure at 517 nm; a lower absorbance of the reaction product signified a higher activity. To determine the DPPH scavenging activity, use equation (1).

$$I\% = [(A1 - A2)/ A2] \times 100 \quad (1)$$

2.6. Hexokinase activity

The activity of HK was measured using spectrophotometry, which involved a linked reaction with glucose-6-phosphate dehydrogenase (G6PD) and measuring NADP reduction at 340 nm (Lamprecht and Trautschold, 1974). The combination included: R1 [MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative] and R2 [HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G 6 PDH (E. coli): ≥ 300 µkat/L; preservative]. The test was performed on yeast HK isoforms (GLUC3, Roche/Hitachi Cobas) and also on medium without treatment. The enzyme was allowed to sit with compounds (126 µg/mL) for 10 minutes prior to the test.

2.7. Statistical analysis

EXCEL 2016 was used to conduct the tests in triplicate and to express the results as mean ± standard error (SE) with $p < 0.05$ serving as the significance level. The impact of the enzyme inhibitor was examined using one-way analysis of variance (ANOVA) with the SPSS 17.0 program.

3. Results

3.1. Estimation of concentration of polyphenols and flavonoids molecules

For Algerian Royal Jelly and Algerian Bee Venom, the total phenolic content (TPC) and total flavonoid content were assessed. The study computed 08,07 mg GAE/g ± 1,80 and 67,20 mg QE/g ± 12,83 for ARJ and 23,56 mg GAE/g ± 0.40 and 141.27 mg QE/g ± 25.21.

3.2. LC-ESI-MS/MS

The major organic components in ABV and AJR preparation were identified by the LC-ESI-MS/MS method. 45 phenolic compounds were used as standards **(Table 1)**.

Moussa Abderrazak Bambra /Afr.J.Bio.Sc. 7(1) (2025) *ISSN: 2663-2187*

Table 1. Esmation of chemical composition of Algerian bee venom and Algerian royal jelly by LC-ESI-MS/MS

Compounds	Analyte	Rt	Phenolics (µg analyte/ g Bee venom)	Phenolics (µg analyte/ g jelly royal)
1	Shikimic acid	1,46	ND	ND
2	Gallic acid	3,23	ND	ND
3	Protocatechuic acid	5,42	ND	ND
4	Epigallocatechin	6,82	ND	ND
5	Catechin	6,86	ND	ND
6	Chlorogenic acid	7,38	ND	3,29
7	Hydroxyben zaldeyde	7,63	ND	ND
8	Vanillic acid	7,73	ND	ND
9	Caffeic Acid	7,81	ND	ND
10	Syringic acid	8,35	ND	ND
11	Caffein	8,34	ND	ND
12	Vanillin	8,59	ND	ND
13	o-coumaric acid	9,39	ND	ND
14	Salicylic Acid	9,73	ND	ND
15	Taxifolin	9,68	ND	ND
16	Resveratrol	9,74	ND	ND
17	Polydatine	9,87	ND	ND
18	Trans-ferulic acid	10,05	ND	ND
19	Sinapic acid	10,33	ND	ND
20	Scutellarin	11,16	ND	ND
21	p-coumaric acid	11,57	ND	ND
22	Coumarin	11,56	ND	ND

23	Protocatehuic ethyl ester	11,68	ND	ND
24	Hesperidin	11,72	3,33	1,93
25	Isoquercitrin	11,74	ND	2,44
26	Rutin	12,21	67,87	ND
27	Quarcetin-3-Ksilozid	12,41	ND	ND
28	Kaempferol-3-glucoside	13,07	ND	1,28
29	Fisetin	13,34	ND	ND
30	Baicalin	13,78	ND	ND
31	Chrysin	14,04	ND	ND
32	Daidzein	14,18	ND	ND
33	Trans-cinnamic acid	14,27	ND	ND
34	Quercetin	14,76	ND	ND
35	Naringenin	14,86	ND	ND
36	Silibinin	15,60	ND	ND
37	Hesperetin	15,83	ND	ND
38	Morin	15,70	ND	ND
39	Kaempferol	16,36	ND	ND
40	Baicalein	17,07	ND	ND
41	Luteolin	17,78	ND	ND
42	Biochanin A	17,90	ND	ND
43	Capcaicin	18,17	ND	ND
44	Dihydrocapcaicin	18,58	ND	ND
45	Diosgenin	23,53	ND	ND

Rt : Retention time. **ND :** not detected

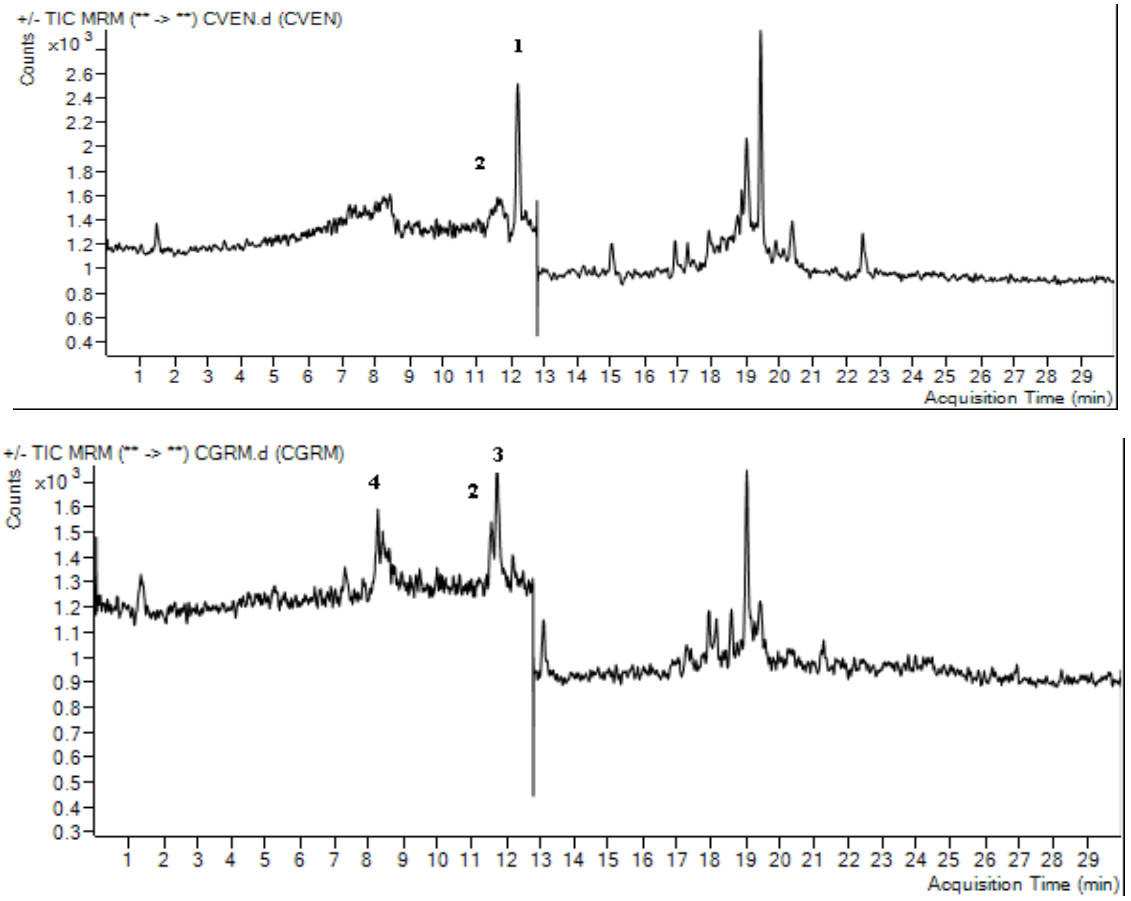

Fig. 3. LC-ESI-MS/MS chromatograms of ABV and AJR respectively; **1**- Rutin **2**-Hesperidin **3**-Isoquercitrin **4**-Chlorogenic acid

3.3. Antioxidant activity

Table 02 shows the half maximal inhibition concentration (IC50) values for ABV, AJR, BHA, BHT, Trolox, and BHA as determined by the DPPH scavenging activity method.

Table 2. The DPPH radical-scavenging activity of Algerian bee venom and Algerian jelly royal

ANTIOXIDANT ACTIVITY	**DPPH SCAVENGING**	
	IC50	**R^2**
BHA	4.39 µg/mL	0,04
BHT	10.86 µg/mL	0,06
TROLOX	4.95 µg/mL	0,03
ALGERIAN BEE VONOM	431.69 µg/mL	6.60
ALGERIAN ROYAL JELLY	73.15 mg/mL	0.02

3.4. Hexokinase (HK) Inhibitory Assay

The isolates of Algerian jelly royal and bee venom were tested for their in vitro anti-diabetic properties; at 126 µg/ml, the isolates' respective HK inhibition values were 99.3% and 7.5%. The anti-diabetic properties of the jelly royal isolate and algerian bee venom were assessed using the HK inhibitory assay. Thus, the high anti-diabetic activity of the Algerian jelly royal isolate was found. However, there was little anti-diabetic effect of the venom of the Algerian bee.

4. Discussion

Bees are more resilient to diseases and pests when they consume royal jelly. It is an essential food for human health as well. There have been a lot pharmacological activities reported for it (Camadan and Akkemik, 2022). Various molecules that could have different effects could be present in BV. These substances may affect several chain reactions that result in different outcomes, rather than just one action [24]. This investigation of several natural treatments that have been used historically to treat a variety of diseases was the goal of the study.

This study is the first to investigate the total phenolic content and total flavonoids content of bee venom 23,56 mg GAE/g ± 0.40 and 141.27 mg QE/g ± 25.21 respectively. The tested Algerian royal jelly extracts found higher amounts of phenolic compounds (08,07 mg GAE/g ± 1,80) than Darwish et al. (2022) and Takruri and Alkhedr (2021), and lower amounts than those found by Pavel et al. (2014) and Ralitsa et al. (2017). These were significant results from the quantitative estimation of phenolics and flavonoids in the extracts of the tested algerian royal jelly, and greatest in flavonoids (67,20 mg QE/g ± 12,83) than Darwish et al (2022).

The estimation of the phenolic compounds of Algerian bee venom and Algerian royal jelly is analyzed for the first time in this study. via LC-ESI-MS/MS analyses, 17 phenolic compounds were identified from venom and royal jelly collected by Algerian bees (Table 1). Rutin was found to be the main compound in bee venom, with a value of 67.87 (µg/g extract), according to quantitative analysis. It was not found in royal jelly. Royal jelly had a value of 3.29 (µg/g extract) for chloroginic acid, whereas bee venom did not contain any of it. Moreover, the hesperidin was determinated in bee venom with a concentration 3.33 (µg/g extract), on the other side was mesured in royal jelly with an amount 1.93 (µg/g extract). Also in royal jelly was detected the Isoquercitrin and kaempferol-3-glucoside with a quantity 2.44 (µg/g extract) and 1.28 (µg/g extract), respectively. Ali et al. (2019) and Frangieh et al. (2019) showed that melittin is one of the chemical compounds of bee venom, Filipa et al., confirmed that melittin is the major compound in bee venom (Sobral et al., 2016).

The DPPH test results for scavenging free radicals are presented as IC50 (mg/mL). The test revealed that ABV had a lower capacity for DPPH scavenging than BHA, BHT, and trolox (Table 2). Additionally, it was

lower (0.431±0.006 mg/mL) than the results of studies conducted by Filipa et al. (2016) in the BV5 sample, Melo da Cunha et al. (2018) and Wu et al. (2022) (Table 3). but higher than four samples in the Filipa et al. study (2016). The nature, amount, and structure of the synthesized polyphenols may account for this finding, which is consistent with the findings of earlier investigations (Radjah et al., 2019). The mechanism of the reaction between the antioxidant and the DPPH radical is determined by its spatial structure (Kouri et al., 2007 ; Radjah et al., 2019). This may be explained by the presence of herpiridine in our sample because it has an antioxidant effect (Al-Ishaq et al., 2019 ; Visnagri et al., 2014). On the other hand, The results of the test showed that ARJ was less capable of scavenging DPPH than BHA, BHT, and trolox (Table 2). The value (73.15 ±0.002 mg/mL) was less than the findings of research carried out by Darwish et al. (2022) (Table 3). But it's higher than that found by Yasemin et al. (2022) (Table 3). The primary component in our sample « AJR », chologenic acid, has an antioxidant effect, which could help to explain this (Nisa et al., 2023).

Table 3. IC50 of antioxidant activity of bee venom for other studies

IC50	Reference
0.178 mg/mL	Wu et al. (2022)
0.033 ± 0.05 mg/g	Melo da Cunha et al. (2018)
0.346 µg/mL	Filipa et al. (2016)
0.487 µg/mL	Filipa et al. (2016)
0.451 µg/mL	Filipa et al. (2016)
0.491 µg/mL	Filipa et al. (2016)
0.512 µg/mL	Filipa et al. (2016)
0.397 µg/mL ±0.1357	Yasemin et al. (2022)
63.10 mg/mL	Darwish et al. (2022)

The anti-hexokinase activity of Algerian royal jelly and Algerian bee venom is tested for the first time. The antihexokinase activity results indicated that our Algerian royal jelly sample exhibited an interesting hexokinase inhibitory activity. It might be because our composition does not have kaempferol, a polyphenol that increased hexokinase activity (Alkhalidy et al., 2018; El Ghouizi et al., 2023). However, the anti- hexokinase activity of Algerian bee venom is significantly lower, Maybe this is because there isn't enough fisetin, which lowers the activity of glucose-6-phosphate dehydrogenase (El Ghouizi et al., 2023; Prasath et al., 2014). Furthermore, luteolin and fisetin have anti-diabetic properties (Al-Ishaq et al., 2018; Constantin et al., 2010 ; Tuorkey, 2016).

5. Conclusion

In the end, this investigation into the venom of Algerian bees and Algerian royal jelly The LC-ESI- MS/MS analysis indicates that rutin and hespiridine are the two components found in the venom of Algerian bees. On the other hand, found that Algerian royal jelly contained four phenolic compounds: "chlorogenic acid, isoquercitrin, hesperidin, and kaempferol-3-glucoside". The amount of phenolic compounds found in royal jelly and bee venom may result from the nutrition of bees. Healthcare providers manage diabetes by administering insulin along with synthetic anti-diabetic medications like metformin and glipizide, however, these drugs come

with potential side effects. Researchers are actively researching herbal metabolites as potential new medications to address troubling side effects of diabetes. Based on the results, algerian royal jelly isolate could be used as a natural source of therapeutic substances for dietary support in diabetes-related conditions.

This research suggests that Algerian royal jelly shows encouraging potential for managing diabetes. More research is required in order to obtain a conclusive answer.

Acknowledgements. This work was supported by the Ministry of Higher Education and Scientific Research (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, MESRS).

Conflict of interest.: There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1]. Al-Ishaq, R.K., Abotaleb, M., Kubatka, P., Kajo, K., Büsselberg, D., (2019): Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules, 9(9): 430. https://doi.org/10.3390/biom9090430

[2]. Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K., Liu, D., (2018): The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. Molecules, 23: 2338. https://doi.org/10.3390%2Fmolecules23092338

[3]. Balkanska, R., Marghitas, L. A., & Pavel, C. I. (2017). Antioxidant activity and total polyphenol content of royal jelly from Bulgaria. *Int. J. Curr. Microbiol. Appl. Sci*, *6*(10), 578-585. https://doi.org/10.20546/ijcmas.2017.610.071

[4]. Boizot, N., Charpentier, J.P., (2006): Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial, 79-82. https://hal.inrae.fr/hal-02669118

[5]. Camadan, Y., & Akkemik, E. (2022). Searching for new natural inhibitors of acetylcholinesterase enzyme. Cumhuriyet Sci. J., 43(1) (2022) 66-71. https://doi.org/10.17776/csj.983869

[6]. Carpena, M., Nuñez-Estevez, B., Soria-Lopez, A., & Simal-Gandara, J. (2020). Bee venom: an updating review of its bioactive molecules and its health applications. *Nutrients*, *12*(11), 3360. https://doi.org/10.3390/nu12113360

[7]. Chang, Y. H., & Bliven, M. L. (1979). Anti-arthritic effect of bee venom. *Agents and actions*, *9*, 205-211. https://doi.org/10.1007/bf02024736

[8]. Constantin, R.P., Constantin, J., Pagadigorria, C.L., Ishii-Iwamoto, E.L., Bracht, A., Ono Mde, K., Yamamoto, N.S., (2010): The actions of fisetin on glucose metabolism in the rat liver. Cell Biochemistry and Function, 28(2): 149-158. https://doi.org/10.1002/cbf.1635

[9]. Darwish, A. M., Abd El-Wahed, A. A., Shehata, M. G., El-Seedi, H. R., Masry, S. H., Khalifa, S. A., ... & El-Sohaimy, S. A. (2022). Chemical profiling and nutritional evaluation of bee pollen, bee bread,

and royal jelly and their role in functional fermented dairy products. *Molecules*, *28*(1), 227. https://doi.org/10.3390/molecules28010227

[10]. El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C., Lyoussi, B., (2023): Bee pollen as functional food: insights into its composition and therapeutic properties. Antioxidants, 12(3): 557. https://doi.org/10.3390%2Fantiox12030557

[11]. Erenler, R., Atalar, M.N., Yildiz, İ., Geçer, E.N., Yildirim, A., Demirtas, İ., Alma, M.H., (2023): Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*. Bütünleyici ve Anadolu Tıbbı Dergisi, 4(3): 3-10. https://doi.org/10.53445/batd.1278048

[12]. Frangieh, J., Salma, Y., Haddad, K., Mattei, C., Legros, C., Fajloun, Z., & El Obeid, D. (2019). First characterization of the venom from *apis mellifera syriaca*, a honeybee from the middle east region. Toxins, 11(4), 191. https://doi.org/10.3390%2Ftoxins11040191

[13]. Kouri, G., Tsimogiannis, D., Bardouki, H., Oreopoulou, V., (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innovative of Food Science and Emerging Technolgy, 8: 155-168. http://dx.doi.org/10.1016/j.ifset.2006.09.003

[14]. Lamprecht, W., Trautschold, I., (1974): Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academic press, pp. 1127–1131. https://doi.org/10.1006/jmcc.2001.1425

[15]. Lee, J. D., Park, H. J., Chae, Y., & Lim, S. (2005). An overview of bee venom acupuncture in the treatment of arthritis. *Evidence-based complementary and alternative medicine*, *2*(1), 79. https://doi.org/10.1093%2Fecam%2Fneh070

[16]. Melo da Cunha, J. D. S., Alfredo, T. M., Dos Santos, J. M., Alves Junior, V. V., Rabelo, L. A., Lima, E. S., ... & de Picoli Souza, K. (2018). Antioxidant, antihyperglycemic, and antidiabetic activity of *Apis mellifera* bee tea. *PloS one*, *13*(6), e0197071. https://doi.org/10.1371/journal.pone.0197071

[17]. Nisa, N., Rasmita, B., Arati, C., Uditraj, C., Siddhartha, R., Dinata, R., Gurusubramanian, G., (2023): Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: Small molecule bioinformatics strategies as amyloid-based therapy. Environmental Science and Pollution Research, 30(17): 51143-51169. http://dx.doi.org/10.1007/s11356- 023-25943-4

[18]. Nusret, G.E.N.Ç., Elmastaş, M., Telci, İ., Erenler, R., (2020): Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects. International Journal of Chemistry and Technology, 4(2): 179-184. http://dx.doi.org/10.32571/ijct.795629

[19]. Pavel, C. I., Mărghitaș, L. A., Bobiș, O., Dezmirean, D. S., Şapcaliu, A., Radoi, I., & Mădaș, M. N. (2011). Biological activities of royal jelly-review. *Scientific Papers Animal Science and Biotechnologies*, *44*(2), 108-108.

[20]. Pavel, C. I., Mărghitaş, L. A., Dezmirean, D. S., Tomoş, L. I., Bonta, V., Şapcaliu, A., & Buttstedt, A. (2014). Comparison between local and commercial royal jelly—Use of antioxidant activity and 10-hydroxy-2-decenoic acid as quality parameter. *Journal of Apicultural Research*, *53*(1), 116-123. http://dx.doi.org/10.3896/IBRA.1.53.1.12

[21]. Prasath, G.S., Pillai, S.I., Subramanian, S.P., (2014): Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. European Journal of Pharmacology, 740: 248–254. https://doi.org/10.1016/j.ejphar.2014.06.065

[22]. Rabbani, N., Thornalley, P.J., (2019): Hexokinase-2 glycolytic overload in diabetes and ischemia–reperfusion injury. Trends in Endocrinology & Metabolism, 30(7): 419-431. https://doi.org/10.1016/j.tem.2019.04.011

[23]. Radjah, A., Chorfi, H., Bouatrous, Y., (2019): Stage of development and solvent effects on phytochemistry and antioxidant activity of three algerian plants. Analele Universităţii din Oradea, Fascicula Biologie, 26(2): 91-99.

[24]. Sig AK, Guney M, Oz Sig O, San H., (2019): Bee venom: A medical perspective. Turk J Clin Lab; 10: 414-421. https://doi.org/10.18663/tjcl.451586

[25]. Sığ AK, Öz-Sığ Ö, Güney M., (2019): Royal jelly: a natural therapeutic? Ortadogu Tıp Derg; 11(3): 333-341. https://doi.org/10.21601/ortadogutipdergisi.500434

[26]. Sobral, F., Sampaio, A., Falcão, S., Queiroz, M. J. R., Calhelha, R. C., Vilas-Boas, M., & Ferreira, I. C. (2016). Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal. *Food and Chemical Toxicology*, *94*, 172-177. https://doi.org/10.1016/j.fct.2016.06.008

[27]. Takruri, H., & Alkhedr, R. (2021). Determination of the Total Phenolic Content, Total Antioxidant Capacity and Radical Scavenging Activity of Jordanian Royal Jelly and Honey Samples. *Jerash Journal for Research & Studies*, *22*(1).

[28]. Tuorkey, M.J., (2016): Molecular targets of luteolin in cancer. European Journal of Cancer Prevention, 25(1): 65-76. https://doi.org/10.1097%2FCEJ.0000000000000128

[29]. Visnagri, A., Kandhare, A. D., Chakravarty, S., Ghosh, P., & Bodhankar, S. L. (2014). Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. *Pharmaceutical biology*, *52*(7), 814-828. https://doi.org/10.3109/13880209.2013.870584

[30]. Wu, Y. H., Zhang, Y., Fang, D. Q., Chen, J., Wang, J. A., Jiang, L., & Lv, Z. F. (2022). Characterization of the composition and biological activity of the venom from vespa bicolor fabricius, a wasp from South China. *Toxins*, *14*(1), 59. https://doi.org/10.3390/toxins14010059

[31]. Zhishen, J., Mengcheng, T., Jianming, W., (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 64(4): 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

SYNTHESE D'ARTICLE 02

Introduction

Les effets indésirables associés aux traitements conventionnels du diabète, tels que l'insuline, le glipizide et la metformine, motivent la recherche de thérapies alternatives basées sur les métabolites secondaires. Des données récentes indiquent que la physiopathologie de l'hyperglycémie diabétique est en partie liée à une augmentation anormale de l'hexokinase 2 (HK2) et à une dérégulation de la glycolyse, caractérisée par une accumulation du glucose-6-phosphate (G6P) (Rabbani et Thornalley, 2019).

Dans ce contexte, les produits de la ruche, relevant du domaine de l'apithérapie, représentent une source prometteuse de principes actifs. Le venin d'abeille, qui renferme plus d'une vingtaine de composés bioactifs, présente diverses propriétés thérapeutiques, dont des effets antidiabétiques (Chang et Bliven, 1979 ; Lee *et al.*, 2005 ; Sığ *et al.*, 2019). La gelée royale démontre également un potentiel hypoglycémiant, attribué à la présence de peptides insulinomimétiques et d'une composition nutritionnelle spécifique (Sığ *et al.*, 2019).

La présente étude vise à caractériser les composés phénoliques du venin d'abeille algérien et de la gelée royale, dans l'objectif d'identifier des molécules efficaces dotées de propriétés antioxydantes et antidiabétiques. L'ambition finale est d'évaluer leur double potentiel d'application en tant qu'agents de conservation alimentaire naturels et comme adjuvants dans le traitement de l'hyperglycémie.

L'objectif principal de cette étude est :

- D'analyser la composition phénolique des extraits de gelée royale (ARJ) et de venin d'abeille algériens (ABV).
- D'évaluer leur activité antioxydante.
- D'étudier leur effet inhibiteur sur l'enzyme hexokinase (HK), un marqueur clé dans la gestion du glucose et du diabète.

Matériels et méthodes

Collecte des échantillons

- La gelée royale (ARJ) a été prélevée à Blida, Algérie.
- Le venin d'abeille (ABV) a été obtenu à El-Medea, Algérie, Le venin est recueilli sur une plaque de verre puis transféré dans des flacons pour un traitement ultérieur après exposition des abeilles à un courant électrique.

Analyses effectuées

1. Détermination des teneurs en phénols et flavonoïdes :

- Méthode Folin-Ciocalteu pour les phénols totaux.
- Approche Zhishen modifiée pour les flavonoïdes.

2. Identification des composés phénoliques :

• Analyse par LC-ESI-MS/MS dans laboratoire de recherche, centre de pratique et de recherche à Igdîr, la turquie.

3. Évaluation de l'activité antioxydante :

• Test du piégeage des radicaux libres DPPH.

• Calcul de la valeur IC50 (concentration inhibant 50 % des radicaux libres).

4. Évaluation de l'activité anti-hexokinase :

• Mesure de l'effet inhibiteur sur l'enzyme hexokinase (HK) impliquée dans le métabolisme du glucose.

Résultat

Teneur en phénols et flavonoïdes totaux

Les teneurs totales en composés phénoliques et flavonoïdiques ont été respectivement de 23,56 ± 0,40 mg GAE/g et 141,27 ± 25,21 mg QE/g pour le venin d'abeille algérien. Pour la gelée royale algérienne, les valeurs obtenues étaient de 8,07 ± 1,80 mg GAE/g pour les polyphénols et 67,20 ± 12,83 mg QE/g pour les flavonoïdes.

Composition chimique

L'analyse chromatographique couplée à la spectrométrie de masse (LC-ESI-MS/MS) a permis d'identifier un ensemble restreint mais caractéristique de composés phénoliques. Chez le venin d'abeille algérien, deux composés principaux ont été détectés, dont la rutine (67,87 µg/g) et l'hespéridine (3,33 µg/g)**.** Dans la gelée royale, quatre molécules ont été identifiées : l'acide chlorogénique (3,29 µg/g)**,** l'hespéridine (1,93 µg/g)**,** l'isoquercitrine (2,44 µg/g) et le kaempférol-3-glucoside (1,28 µg/g)**.** Ces données suggèrent une composition phénolique spécifique à chaque produit, influencée par la nature biologique et la fonction physiologique des sécrétions apicoles.

Activité antioxydante

L'activité antioxydante, évaluée par la méthode de piégeage du radical libre DPPH (Nusret et al., 2020), a révélé des capacités distinctes entre les deux produits apicoles. Le venin d'abeille a démontré une activité modérée à élevée, avec une concentration inhibitrice médiane (IC_{50}) de 0,431 ± 0,006 mg/mL. À l'inverse, la gelée royale a présenté une activité significativement plus faible, comme en témoigne une valeur d'IC_{50} de 73,15 mg/mL, indiquant une capacité de neutralisation des radicaux libres limitée.

La comparaison avec des antioxydants de référence, tels que le BHA (IC_{50} = 4,39 µg/mL), le BHT (IC_{50} = 10,86 µg/mL) et le Trolox (IC_{50} = 4,95 µg/mL), a confirmé la puissance supérieure de ces composés de synthèse. Par conséquent, les activités antioxydantes intrinsèques du venin d'abeille et de la gelée royale se sont avérées inférieures à celles des standards de référence.

Potentiel antidiabétique

Les essais enzymatiques réalisés à une concentration de 126 µg/mL ont permis de quantifier l'inhibition de l'hexokinase (HK), enzyme clé du métabolisme glucidique. Les résultats ont mis en évidence une inhibition de 99,3 % pour la gelée royale algérienne et de 7,5% pour le venin d'abeille, indiquant un effet antidiabétique marqué pour la première et marginal pour la seconde.

Discussion

L'échantillon de la gelée royale algérienne testés ont révélé des quantités plus élevées de composés phénoliques (08,07 mg GAE/g ± 1,80) que celles trouvées par Darwish *et al*., (2022) et Takruri et Alkhedr (2021), et des quantités plus faibles que celles trouvées par Pavel *et al*., (2014) et Ralitsa *et al*., (2017). Il s'agit là de résultats significatifs issus de l'estimation quantitative des composés phénoliques et des flavonoïdes dans la gelée royale algérienne testés, et les plus élevés en flavonoïdes (67,20 mg QE/g ± 12,83) que ceux de Darwish *et al.*, (2022). Ces mesures révèlent une différence marquée entre les deux matrices apicoles, le venin présentant une concentration phénolique significativement plus élevée.

La teneur élevée en polyphénols et flavonoïdes observée dans le venin d'abeille est en accord avec les travaux antérieurs démontrant la présence de nombreuses molécules bioactives à potentiel pharmacologique, telles que la mélittine, l'apamine et divers peptides à activité antioxydante et anti-inflammatoire (Chang et Bliven, 1979 ; Lee *et al.*, 2005 ; Sig *et al.*, 2019). La prédominance de la rutine et de l'hespéridine dans le venin est particulièrement intéressante, car ces composés sont connus pour leur activité antioxydante, vasoprotectrice et antidiabétique (Erenler *et al.*, 2023). Dans la gelée royale, la présence d'acide chlorogénique et d'isoquercitrine, déjà rapportée dans d'autres études (Pavel *et al.*, 2011 ; Sobral *et al.*, 2016), conforte l'hypothèse selon laquelle ces molécules contribuent à la puissant effet antioxydant (Nisa *et al.*, 2023).

La capacité antioxydante observée, bien que modérée par rapport aux standards synthétiques. De plus, il était inférieur aux résultats des études menées par Filipa *et al*., (2016) dans l'échantillon BV5, Melo da Cunha *et al*., (2018) et Wu *et al*., (2022), mais supérieur à celui de quatre échantillons de l'étude de Filipa *et al*., (2016). La nature, la quantité et la structure des polyphénols synthétisés peuvent expliquer ce résultat, qui est conforme aux conclusions d'études antérieures (Radjah *et al.,* 2019). Le mécanisme de la réaction entre l'antioxydant et le radical DPPH est déterminé par sa structure spatiale (Kouri *et al*., 2007 ; Radjah *et al*., 2019). Cela peut s'expliquer par la présence d'hespéridine dans notre échantillon, car elle a un effet antioxydant (Al-Ishaq *et al*., 2019 ; Visnagri *et al*., 2014). Tandis que la gelée royale présentait une IC_{50} plus élevée de 73,15 mg/mL, Cette résultat était inférieure aux résultats des recherches menées par Darwish *et al.,* (2022). Mais elle est supérieure à celle trouvée par Yasemin *et al*., (2022).

La forte concentration en rutine ou la présence d'hespéridine dans l'ABV expliquerait ses propriétés antioxydantes. Quant à la gelée royale, malgré sa moindre activité radicalaire par rapport l'activé des standards utilisés et le venin algérien en peux interpréter son activité par la présence d'acide chlorogénique qu'est le composant principal dans notre échantillon et le connu par leur effet antioxydant (Nisa *et al.,* 2023).

Les différences dans les capacités antioxydantes et anti-hexokinase de l'ARJ et de l'ABV semblent être liées à leurs profils phénoliques respectifs. L'inhibition marquée de l'hexokinase observée avec la gelée royale algérienne (99,3 %) atteste d'un fort potentiel antidiabétique. L'absence du kaempférol dans l'ARJ pourrait être responsable de son puissant effet antidiabétique (Alkhalidy *et al*., 2018 ; El Ghouizi *et al*., 2023). Ce résultat rejoint les conclusions de Sığ *et al.,* (2019), selon lesquelles la gelée royale renferme des peptides analogues à l'insuline, susceptibles d'imiter son effet hypoglycémiant.

En revanche, l'effet du venin d'abeille sur l'hexokinase reste limité (7,5 %), bien que ce produit contienne des peptides bioactifs pouvant agir par d'autres mécanismes, notamment la modulation des voies inflammatoires ou la stimulation de la sécrétion d'insuline (Lee *et al.,* 2005). L'absence du luteolin et la faible concentration du fisetin dans l'ABV pourrait être responsable de son puissant effet diabétique (Al-Ishaq *et al.,* 2019 ; Constantin *et al.,* 2010 ; Tuorkey, 2016).

Ces observations confirment la complémentarité pharmacologique des deux produits : le venin, davantage orienté vers des effets antioxydants et anti-inflammatoires, et la gelée royale, dotée d'un effet antidiabétique direct.

Les différences quantitatives et qualitatives observées entre les produits apicoles algériens pourraient être liées à des facteurs géographiques, floraux et environnementaux influençant leur composition biochimique (Carpena *et al.*, 2020).

Conclusion et Perspectives

L'ensemble de ces résultats s'inscrit dans la continuité des travaux de recherche sur les produits de la ruche comme sources naturelles de molécules thérapeutiques. Ainsi, cette étude met en évidence la composition chimique et les effets biologiques des produits apicoles algériens, aussi apporte une contribution originale à la valorisation des ressources apicoles locales, démontrant leur potentiel d'exploitation en pharmacologie naturelle pour la prévention et la gestion du diabète et des désordres métaboliques associés

- L'ARJ présente une concentration phénolique intéressante et un effet inhibiteur puissant sur l'hexokinase, ce qui la positionne comme un potentiel traitement naturel du diabète.
- L'ABV, bien que moins efficace sur l'hexokinase, se distingue par ses propriétés antioxydantes élevées.
- Des études supplémentaires sont nécessaires pour explorer l'application clinique de ces résultats.
- L'optimisation des méthodes d'extraction et de purification pourrait améliorer leur efficacité thérapeutique.

CHAPITRE 03 :
ARTICLE 03 :

Preliminary analysis of the chemical characteristics of hydrau-acetonic extracts from algerian bee products, their influence on hexokinase activity, and the antimicrobial properties of honey.

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2025, Tom. XXXII, Issue: 1, pp. 105-116

PRELIMINARY ANALYSIS OF THE CHEMICAL CHARACTERISTICS OF HYDRAU-ACETONIC EXTRACTS FROM ALGERIAN BEE PRODUCTS, THEIR INFLUENCE ON HEXOKINASE ACTIVITY, AND THE ANTIMICROBIAL PROPERTIES OF HONEY

Moussa Abderrazak BAMBRA[*], Yamina BOUATROUS[**], Ramazan ERENLER[***], Ilyas YILDIZ[***]

[*] *Laboratory of promotion of agricultural innovation in arid regions (PIARA), Department of Biology, Faculty of Natural Sciences and Life, University Mohamed khider Biskra, Algeria.*
[**]*Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Mohamed khider University, Biskra, Algeria.*
[***]*Research Laboratory Practice and Research Center, Igdir University, 7600, Igdir, Turkiye*
Correspondence author: Yamina Bouatrous, Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Mohamed khider University, zip code: 07000, email: y.bouatrous@univ-biskra.dz

Abstract. A limited number of studies investigate the chemical composition and effects of Algerian bee products specially propolis, beeswax, bee bread. This study investigated the chemical composition and biological activities of Algerian bee products-propolis, beeswax, bee bread, and honey-focusing on total phenolic and flavonoid contents, antioxidant capacity, anti-diabetic potential, and antimicrobial effects. Extracts obtained using 70% acetone showed varying yields, with honey A and propolis exhibiting the highest (78.7% and 24.26%, respectively). All products demonstrated significant levels of phenolic compounds and flavonoids. Antioxidant activity, measured via DPPH radical-scavenging assay, was strongest in Algerian propolis (IC_{50} = 0.0744 ± 0.0007 mg/mL). LC-ESI-MS/MS analysis identified rutin and 21 phenolic compounds in propolis. While all samples were tested at 126 µg/mL for anti-diabetic activity, hexokinase inhibition was minimal. Disk diffusion assays revealed that Algerian bee honey exhibited notable antimicrobial activity. These findings highlight the rich bioactive profile and therapeutic potential of Algerian bee products.

Key words: Algerian bee products; antioxidant activity; antihexokinase effect; anti-diabetic activity; antimicrobial activity; LC-ESI-MS/MS.

INTRODUCTION

Healthcare professionals manage diabetes mellitus by administering insulin alongside synthetic anti-diabetic drugs such as metformin and glipizide, despite the potential side effects of these medications. To mitigate these undesirable side effects, researchers are actively exploring natural metabolites as potential candidates for new anti-diabetic therapies. Recent studies suggest that abnormal increases in HK2 levels and dysregulated glycolysis play a significant role in impaired glucose metabolism. Elevated levels of G6P and downstream glycolysis intermediates have been identified as key contributors to the tissue-specific, chronic progression of hyperglycemia associated with diabetes [47]. Apitherapy involves using bee products for their natural healing qualities and is a part of alternative and folk medicine. Its origins can be traced back to ancient China or present-day Russia. However, the use of bee products for therapeutic purposes can also be found in ancient Egypt and Greece, where they were used for cosmetics and wound healing preparations. It's important to note that while honey or royal jelly cannot cure conditions like diabetes, they do contain anti-diabetic substances which may help improve glycemic profiles when consumed [53].

Numerous research papers have explored the natural antioxidant substances present in medicinal bee products. These compounds are not only used as food preservatives to replace synthetic antioxidants, but they also play a role in managing hyperglycemia. Therefore, the objective of investigating the extraction of phenolic compounds using 70% acetone is to identify the most effective active molecules with antioxidant and anti-diabetic properties. The increasing ineffectiveness of existing antibiotics, coupled with the emergence of more virulent and resistant microorganisms, has effectively compelled the scientific community to explore therapeutic alternatives from natural sources. As a result, plant- and animal-derived products have regained prominence in the search for natural antimicrobials, with honey and its derivatives standing out as a potential solution [21]. Honey is composed of a variety of chemical and mineral substances, vitamins, carbohydrates, proteins, flavonoids, phenolic acids, and additional components, along with physical and chemical characteristics such as acidity, moisture content, viscosity, and crystallinity, which all contribute to its antioxidant properties and its efficacy in combating bacteria and inflammation [3]. Honey demonstrates significant antimicrobial properties. It effectively inhibits a wide variety of bacterial species. Alcohol extracts from honey show a suppressive effect on numerous bacterial types, including both aerobes and anaerobes, as well as Gram-positive and Gram- negative bacteria. Honey exhibits strong antimicrobial action against both harmful and non-harmful microorganisms, such as yeasts and fungi, including those that have developed resistance to various antibiotics. The antimicrobial effects can be either bacteriostatic or bactericidal, depending on the concentration used [3]. The antibacterial and antifungal activities of honey are influenced by its chemical, physical, and biological characteristics. These factors vary considerably among different honey varieties and play a crucial role in determining the specific properties of each type [36]. This study aimed to evaluate the antimicrobial effects of Algerian bee honey against certain strains of clinically relevant Gram-positive and Gram-negative bacteria as well as fungi, to assess its potential use for microbial sterilization as a natural alternative.

MATERIALS AND METHODS

Sample collection

Table 1. Algerian bee products, sampling area and year of collection.

Algerian bee product	Sampling area	Yaer of collection
Algerian bee honey A.	Djelfa.	2022
Algerian propolis.	El-Medea.	2022
Algerian bee honey B.	Sog-Ahras.	2022
Algerian bee-wax.	Sog-Ahras.	2022
Algerian bee-bread.	Tipaza.	2022
Algerian bee honey S1.	Batna.	2024
Algerian bee honey S2.	El-Bayedhe.	2024
Algerian bee honey S3.	Tizi-Ouzou.	2024
Algerian bee honey S4.	Khanchela.	2024
Algerian bee honey S5.	Barika.	2024
Algerian bee honey S6.	Boussaada.	2024
Algerian bee honey S7.	Djelfa.	2022
Algerian bee honey 8 A mixture of seven honey varieties (S8).	/	/

Extraction

Acetonic extract of samples in room temperature

For each sample, 5 grams of bee product were weighed and combined with 50 milliliters of 70% acetone, stirred continuously for 24 hours. The mixture was then filtered through filter paper. The residue was subjected to three separate extractions. The solvent from the two extracts was evaporated using a rotary evaporator at 40°C, and the extracts were stored for six days at 60°C in a steam room. Thereafter, the extract samples were stored at -20°C.

Determination of the Total Phenol Content

The Folin-Ciocalteu colorimetric method was used to determine the total polyphenol content of the samples [18]. In a 1.5 mL Eppendorf tube, 100 µL of sample extract (1 mg/mL in distilled water) was combined with 500 µL of Folin-Ciocalteu reagent (10%) and 400 µL of $Na_2\ CO_3$ (75 mg/mL). After five minutes of incubation at 40°C in the dark, the absorbance was measured at 735 nm. The total polyphenol content was expressed as mg/g of gallic acid equivalents (Fig. 1) using the calibration curve (Y = 0.0122x – 0.0052, R^2 = 0.9995). All measurements were performed in triplicate.

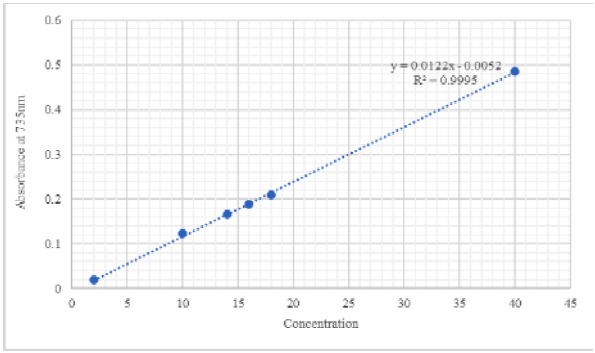

Figure 1. Total polyphenols calibration curve

Total flavonoids content

The method of Zhishen *et al.* (1999), with modifications, was used to determine the total flavonoid content of the samples [60]. In a 1.5 mL Eppendorf tube, 200 µL of sample extract was mixed with 60 µL of 5% $NaNO_2$. After 5 minutes, 60 µL of 10% $AlCl_3$ was added, and the mixture was vigorously mixed. After 6 minutes, 400 µL of 1M NaOH was added. The absorbance was immediately measured at 510 nm against a control. A quercetin solution was prepared, and calibration curves were plotted using standard solutions at concentrations ranging from 0 to 1000 µg/mL (Fig. 2) with the equation (Y = 0.0018x + 0.0357, R^2 = 0.9991). All measurements were performed in triplicate.

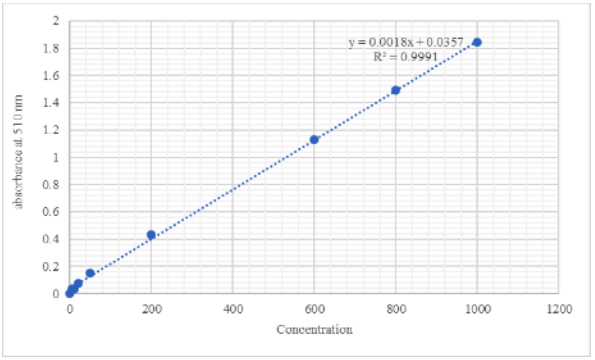

Figure 2. Total flavonoids calibration curve

LC-ESI-MS/MS Analysis

An Agilent Technologies 1260 Infinity II system, coupled with a 6460 Triple Quad Mass Spectrometer, was used for the quantitative analysis of phenolics. A Poroshell 120 SB-C18 column (3.0 × 100 mm, 2.7 µm) was utilized [24]. Samples of 50 mg were dissolved in 1 mL of methanol in a 2 mL Eppendorf tube. One milliliter of hexane was used to extract the end product, followed by centrifugation for 10 minutes at 9,000 rpm. Subsequently, 100 µL of the methanol phase was diluted with 450 µL of water and 450 µL of methanol (900 µL total). After filtering through a 0.22 µm filter, the mixture was injected into the instrument. The injection volume was 5.12 µL, with a flow rate of 0.40 mL/min. The mobile phase consisted of ammonium formate (5.0 mM) and 0.1% formic acid in methanol (B), and 0.1% ammonium formate in water (A). The

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2025, Tom. XXXII, Issue: 1, pp. 105-116

gradient program for the B mobile phase was adjusted as follows: 25% for 1-3 minutes, 50% for 4-12 minutes, 90% for 13-21 minutes, and 3% for 22-25 minutes. The column temperature was maintained at 40°C. The nebulizing gas (N_2) flow rate was 11 L/min, with a pressure of 15 psi, a capillary voltage of 4000 V, and a gas temperature of 300°C.

DPPH Test for Scavenging Free Radicals

The DPPH method was used to assess the antioxidant activity of the extracts [43]. To each methanolic extract solution, 50 µL was added to 1.95 mL of DPPH methanolic solution (0.025 g/L) at varying concentrations (from 0.0125 to 5 mg/mL). Similarly, 50 µL of methanol was mixed with 1.95 mL of the DPPH methanolic solution as a negative control. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 515 nm against a blank prepared for each concentration. Ascorbic acid was used as the positive control, and its absorbance was measured three times for each concentration under the same conditions as the samples. The inhibition of the DPPH radical was calculated using the following equation:

I%= [(Abs contrôle – Abs test)/ Abs contrôle] x 100

Hexokinase activity

Hexokinase (HK) activity was assayed spectrophotometrically using a coupled reaction with glucose-6-phosphate dehydrogenase (G6PD), monitoring the reduction of NADP at 340 nm [33]. The reaction mixture contained: R1 [MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+} : 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative] and R2 [HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+} : 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G6PDH (E. coli): ≥ 300 µkat/L; preservative]. The assay was conducted using yeast HK isoforms (GLUC3, Roche/Hitachi Cobas) as well as untreated medium. The enzyme was incubated with the test compounds (126 µg/mL) for 10 minutes prior to the assay.

Antimicrobial properties of Algerian bee Honey

We utilized a modified agar disk diffusion technique for our study [31]. We evaluated the antimicrobial effect of bee honey on various microbial and fungal strains using Mueller-Hinton agar plates that were pre-inoculated with a standardized microbial suspension at 10^8 CFU/mL. Whatman paper disks that were soaked with 10 µL of bee honey were placed on the surface of the agar, with each disk being 6 mm in diameter. After incubating at 37 °C for 24 hours, we analyzed the results by measuring the inhibition zones' diameters in millimeters (mm). All experiments were performed in triplicate.

To assess the antimicrobial activity of honey samples, five clinically identified bacterial strains were selected, and one clinically identified fungus.

Preparation of honey samples

In a tube, we combined 0.2 mL of each honey sample with 1 mL of dimethyl sulfoxide (C_2H_6OS) (DMSO) solvent [44]. The tubes that were prepared were then vortexed thoroughly.

Minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration that can inhibit the growth of bacteria [56]. This assay is conducted by performing serial dilutions of honey samples at various concentrations on 96-well microplates.

Initially, 100 µL of MH broth was added to every well of the microplates. Introduce 100 microliters of the prepared honey extract into the first well, mix thoroughly, then transfer 100 microliters from the first well to the second well. Continue this process for the remaining wells to create a series of dilutions ranging from a concentration of 10 mg/mL to 0.020 mg/mL. Next, 50 microliters of the bacterial suspension are added to each well of the microplate. The well designated as the negative control contained solely 100 µL of MH broth, while the positive control well contained 100 µL of MH broth with Honey-free Bacterial Suspension. The microplate was then incubated at a temperature of 37°C for 18 hours. Following incubation, each tube is inspected visually for cloudiness [26].

Statistical analysis

EXCEL 2016 was used to conduct the tests in triplicate and to express the results as mean ± standard error (SE) with $p < 0.05$ serving as the significance level. Using SPSS 17.0, a one-way analysis of variance (ANOVA) was used to examine the impact of the enzyme inhibitor.

RESULTS

Extraction yields

The extraction method for evaluating the phytochemical activities of the samples involves the extraction of polyphenols and flavonoids. Extraction with 70% acetone yielded a higher amount of Algerian bee product extract, as shown in Fig. 3.

Estimation of polyphenol and flavonoid concentration

For Algerian bee products, the total flavonoid (TF) and total phenolic content (TPC) were assessed. The highest TPC and TF concentrations were found in Algerian bee propolis, as shown in Fig. 4, with values of 62.65 (1.43) mg EGA/g and 205.16 (18.26) mg EQ/g, respectively. Other products showed lower concentrations: bee bread had 16.49 (0.45) mg EGA/g and 17.57 (4.19) mg EQ/g; bee wax had 6.16 (0.40) mg EGA/g; and bee honey A and 2 had 2.85 (0.31) mg EGA/g and 2.96 (0.54) mg EGA/g, respectively. Bee wax, honey A, and honey B had a TF concentration of 0 mg EQ/g.

LC-ESI-MS/MS

The LC-ESI-MS/MS method was utilized to identify the primary organic components present in Algerian bee products. A total of 45 phenolic compounds were used as standards (Table 2) (Fig. 5).

Bambra, M.A., Bouatrous, Y., Erenler, R., Yildiz, I. - Preliminary analysis of the chemical characteristics of hydrau-acetonic extracts from Algerian bee products, their influence on hexokinase activity, and the antimicrobial properties of honey

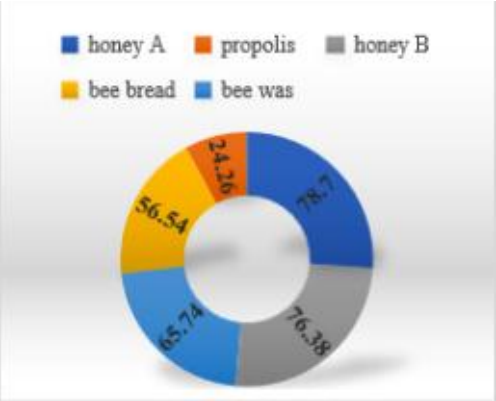

Figure 3. Yields of algerian bee product extracts by aceton 70%

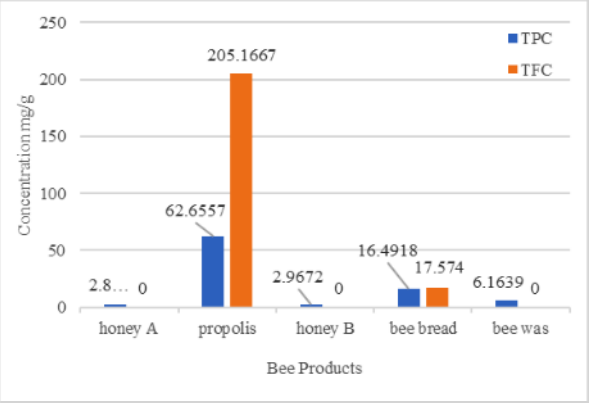

Figure 4. Concentrations of TPC and TFC estimated in acetonic algerian bee product samples.

Table 2. Esmation of chemical composition of Algerian bee products by LC-ESI-MS/MS

Compounds	Analyte	Rt	Phenolics (µg analyte/ g Bee honey A)	Phenolics (µg analyte/ g propolis)	Phenolics (µg analyte/ g Bee honey B)	Phenolics (µg analyte/ g Bee Bread)	Phenolics (µg analyte/ g Bee Wax)
1	Shikimic acid	1.46	ND	ND	ND	ND	ND
2	Gallic acid	3.23	ND	60.79	ND	ND	ND
3	Protocatechuic acid	5.42	ND	50.60	ND	ND	ND
4	Epigallocatechin	6.82	ND	ND	ND	ND	ND
5	Catechin	6.86	ND	ND	ND	ND	ND
6	Chlorogenic acid	7.38	ND	23.01	ND	ND	ND
7	Hydroxyben zaldeyde	7.63	ND	1.93	ND	ND	ND
8	Vanillic acid	7.73	ND	ND	ND	ND	ND
9	Caffeic Acid	7.81	ND	300.08	ND	ND	ND
10	Syringic acid	8.35	ND	ND	ND	ND	ND
11	Caffein	8.34	1.92	3.91	1.11	1.65	1.60
12	Vanillin	8.59	ND	11.75	ND	5.54	4.99
13	o-coumaric acid	9.39	ND	68.45	ND	ND	ND
14	Salicylic Acid	9.73	ND	68.87	ND	ND	ND
15	Taxifolin	9.68	ND	6.00	ND	ND	ND
16	Resveratrol	9.74	ND	ND	ND	ND	ND
17	Polydatine	9.87	ND	ND	ND	ND	ND
18	Trans-ferulic acid	10.05	ND	165.80	ND	ND	ND
19	Sinapic acid	10.33	ND	ND	ND	ND	ND
20	Scutellarin	11.16	ND	2.26	ND	ND	ND
21	p-coumaric acid	11.57	ND	ND	ND	ND	ND
22	Coumarin	11.56	ND	ND	ND	ND	ND
23	Protocatehuic ethyl ester	11.68	ND	ND	ND	ND	ND
24	Hesperidin	11.72	ND	ND	ND	ND	ND
25	Isoquercitrin	11.74	0.69	14.07	ND	ND	ND
26	Rutin	12.21	ND	4489.06	ND	ND	ND
27	Quarcetin-3-Ksilozid	12.41	ND	ND	ND	ND	ND
28	Kaempferol-3-glucoside	13.07	2.21	2.06	ND	ND	ND
29	Fisetin	13.34	ND	ND	ND	ND	ND
30	Baicalin	13.78	ND	ND	ND	ND	ND
31	Chrysin	14.04	ND	ND	ND	ND	ND
32	Daidzein	14.18	ND	ND	ND	ND	ND
33	Trans-cinnamic acid	14.27	ND	23.27	ND	ND	ND
34	Quercetin	14.76	31.18	93.97	ND	ND	ND
35	Naringenin	14.86	9.13	88.82	ND	ND	ND
36	Silibinin	15.60	ND	ND	ND	ND	ND
37	Hesperetin	15.83	ND	12.51	ND	ND	ND
38	Morin	15.70	ND	ND	ND	ND	ND
39	Kaempferol	16.36	268.68	402.56	ND	ND	ND
40	Baicalein	17.07	ND	ND	ND	ND	ND
41	Luteolin	17.78	ND	24.70	ND	ND	ND
42	Biochanin A	17.90	ND	ND	ND	ND	ND
43	Capcaicin	18.17	ND	ND	ND	ND	ND
44	Dihydrocapcaicin	18.58	ND	ND	ND	ND	ND
45	Diosgenin	23.53	ND	ND	ND	ND	ND

Rt: Retention time. ND: not detected

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2025, Tom. XXXII, Issue: 1, pp. 105-116

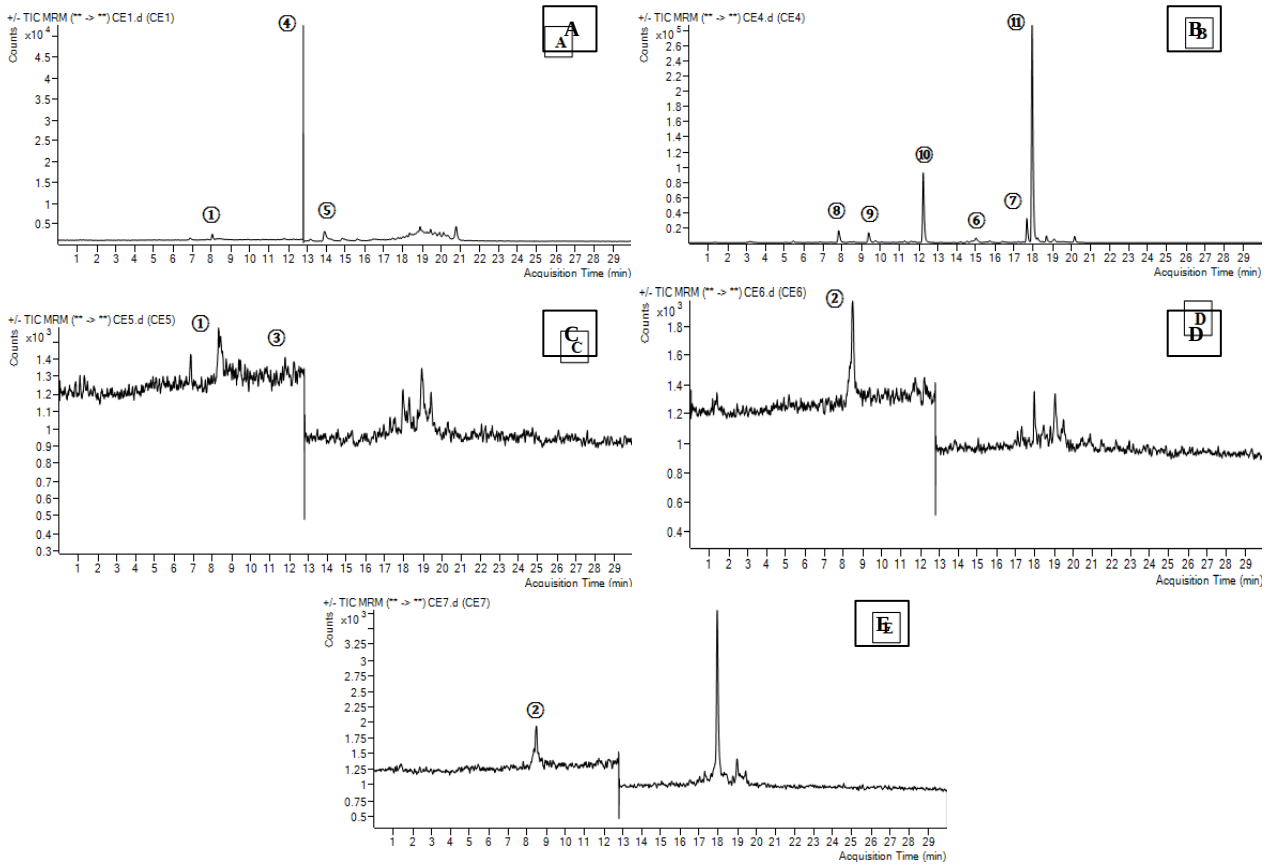

Figure 5. LC-ESI-MS/MS chromatograms of Algerian bee products: A: Honey A; B: Propolis; C: Honey B; D: Bee Bread; E: Bee Wax; ① Caffein; ② Vanillin; ③ Isoquercitrin; ④ Kaempferol-3-glucoside; ⑤ Quercetin; ⑥ Naringenin; ⑦ Kaempferol; ⑧ Caffeic acid; ⑨ Salicylic acid; ⑩ Rutin; ⑪ Luteolin.

Antioxidant activity

The DPPH scavenging activity method was used to determine the half-maximal inhibition concentration (IC50) values for Algerian bee products, BHA, BHT, and Trolox. The results are presented in Table 3.

Table 3. The DPPH radical-scavenging activity of Algerian bee products.

Antioxidant Activity	DPPH Scavenging IC50 µg/mL	R^2
BHA	4.39	0,04
BHT	10.86	0,06
Trolox	4.95	0,03
Algerian Bee Propolis	74.46	0.78
Algerian Bee Bread	198.66	2.63
Algerian Bee Wax	/	/
Algerian Bee Honey A	/	/
Algerian Bee Honey B	/	/

Hexokinase (HK) Inhibitory Assay

The Algerian bee products (honey A, propolis, honey B, bee bread, and bee wax) were tested for their in vitro anti-diabetic properties. At a concentration of 126 µg/mL, the HK inhibition values were 2.9%, 4.7%, 2.2%, 2.2%, and 1.5%, respectively. The anti-diabetic properties of these bee products were assessed using the HK inhibitory assay, indicating a minimal anti-diabetic effect from the Algerian bee products (Fig. 6).

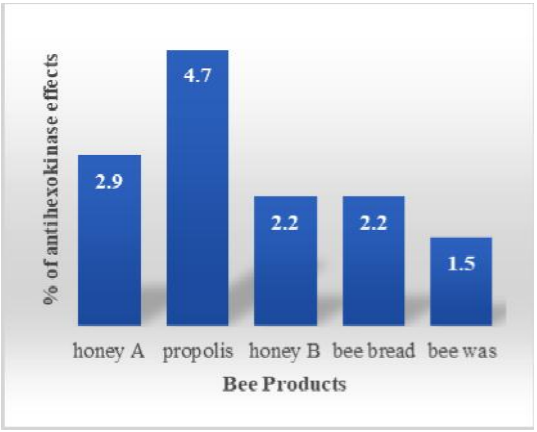

Figure 6. Percentgae of antihexokinase effects of acetonic algerian bee product extracts.

Antimicrobial properties of Algerian bee Honey

Antimicrobial activity results of selected honey samples presented in the table by measuring the inhibition diameter in millimeters for the strains tested (Table 4) (fig. 7-11).

Table 4. Inhibition zone diameters.

Honey samples	*Escherichia coli* Gram -	*Klebsiella pneumoniae* Gram -	*Proteus vulgaris* Gram -	*Staphylococcus aureus* Gram +	*Streptococcus* sp. Gram +	*Candida albicans* Fungi
S1	20.33mm	9.66mm	24.5mm	16mm	-	-
S2	25mm	12.5mm	12.33mm	-	-	-
S3	33.33mm	-	12.66mm	-	-	-
S4	24.66mm	-	17.66mm	-	-	-
S5	22mm	-	15.5mm	-	-	-
S6	20.33mm	-	18mm	-	-	-
S7	22.66mm	-	17.33mm	-	-	-
S8	26.66mm	-	18mm	-	-	-

- : no inhibition zone

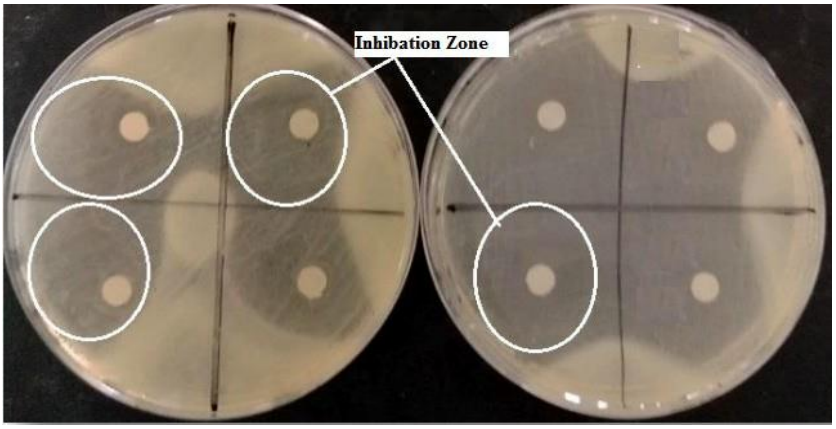

Figure 7. The zone of inhibition in *E.coli* bacteria.

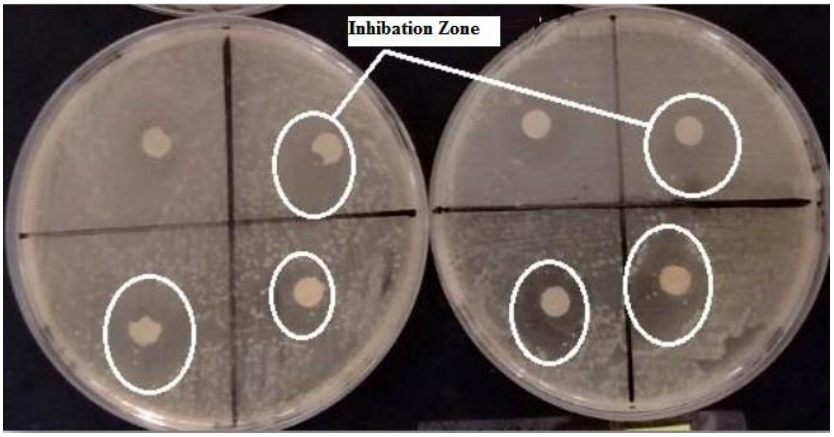

Figure 8. The zone of inhibition in *Proteus vulgaris* bacteria.

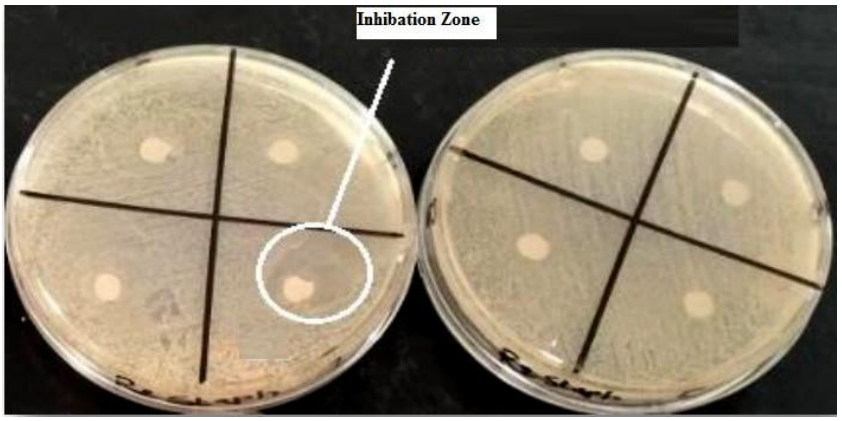

Figure 9. The zone of inhibition in *Staphylococous aureus* bacteria.

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2025, Tom. XXXII, Issue: 1, pp. 105-116

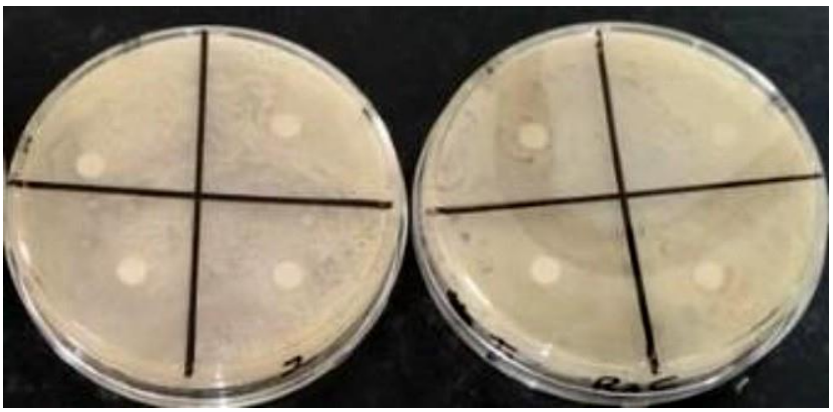

Figure 10. The zone of inhibition in *Streptoccus* sp. bacteria.

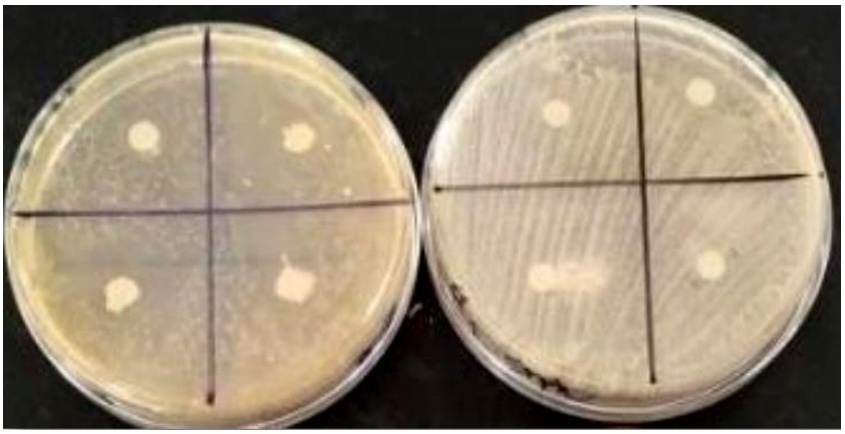

Figure 11. The zone of inhibition in *Candida albicaus* fungi.

Minimum inhibitory concentration (MIC)

The table below displays the minimum inhibitory concentration (MIC) results in mg/mL (Table 5).

Table 5. Result of the minimum inhibitory concentration of honey.

Honey samples	*Escherichia coli* Gram -	*Proteus vulgaris* Gram -	*Klebsiella Pneumoniae* Gram -	*Staphylococcus Aureus* Gram +	*Streptococcus sp.* Gram +	*Candida albicans* Fungi
S1	2.5mg/mL	10mg/mL	5mg/mL	2.5mg/mL	-	-
S2	10mg/mL	10mg/mL	5mg/mL	-	-	-
S3	5mg/mL	10mg/mL	-	-	-	-
S4	10mg/mL	10mg/mL	-	-	-	-
S5	5mg/mL	10mg/mL	-	-	-	-
S6	5mg/mL	10mg/mL	-	-	-	-
S7	5mg/mL	10mg/mL	-	-	-	-
S8	5mg/mL	10mg/mL	-	-	-	-
Cefotaxime Antibiotic	R	R	R	-	S	-

R: Résistance - : no CMI S : sensible

DISCUSSION

Since ancient times, beekeeping products such as honey, bee bread, propolis, and beeswax have been widely used in folk medicine due to their potent medicinal qualities and high concentration of bioactive molecules [38].

In this study, higher extraction yields were observed with 70% acetone compared to methanol and ethanol, except for antioxidant activity, where ethanol performed better [43]. Acetone is known to be more effective at extracting phenolic antioxidants than other organic solvents due to its lower polarity and greater ability to dissolve phenolics [5, 27-29, 31, 40, 46, 48, 54]. The extraction yield is influenced by factors such as the solvent used, temperature, and duration of the extraction process [30, 48, 52].

Bee propolis, bee bread, bee wax, and both varieties of Algerian honey exhibited the highest concentrations of phenolic compounds, with values of 62.65 mg EAG/g, 16.49 mg EAG/g, 6.16 mg EAG/g, 2.96 mg EAG/g, and 2.85 mg EAG/g, respectively. Comparing the Algerian propolis extract to studies by Petar *et al.* (2018) [49] and Nurul Aliah *et al.* (2019) [2], it was

found to have higher concentrations of phenolic compounds and a greater flavonoid content (205.16 mg EQ/g) than reported by Petar *et al.* (2018) [49]. Additionally, the Algerian bee bread extract showed higher levels of phenolics and flavonoids compared to those reported by Carlos *et al.* (2015) [61] and Katarzyna *et al.* (2016) [19]. The Algerian bee wax extract also had higher phenolic content than that reported by Haoan *et al.* (2019) [59]. The Algerian bee honey extracts had higher phenolic levels than those reported by Tania *et al.* (2013) [51], Je-Ruei *et al.* (2013) [35], Ibrahim *et al.* (2018) [8], and Vesna *et al.* (2018) [55]. These variations are likely due to differences in nectar sources and the geographical origin of the samples [6, 11].

There is limited research on the compounds found in Algerian bee products. LC-ESI-MS/MS analyses identified six phenolic compounds in Algerian bee honey sample N°01 (Fig. 5), while only one phenolic compound was identified in sample N°03. In comparison, studies from Cuba identified 16 phenolic compounds using HPLC-DAD-ESI-MS/MS [10], and studies from Brazil identified 20 phenolic compounds by LC-MS and 26 phenolic compounds by HPLC-ESI-MS/MS [15, 16]. Additionally, our analysis determined 21 phenolic compounds in Algerian propolis, while studies from Chile and Portugal reported 29 and 31 phenolic compounds, respectively, using GC-MS and UPLC-DAD-ESI/MS [17, 25]. For Algerian bee bread and bee wax, only two phenolic compounds were identified, whereas Bakour *et al.* (2019) [12] reported 30 phenolic compounds in bee bread. These variations in phenolic compound profiles can be attributed to factors such as the collection time and location of bee pollen (geographic conditions), extraction techniques, extraction solvents, and the methods used to estimate the compounds.

The DPPH test results for scavenging free radicals were expressed as IC50 (mg/mL). The results indicated that the DPPH scavenging capacity of Algerian bee products was lower compared to BHA, BHT, and Trolox (Table 3). However, the bee bread sample exhibited higher antioxidant activity than reported by Bakour *et al.* (2019) [12]. On the other hand, Algerian propolis demonstrated greater antioxidant capacity than other bee product samples and was also superior to the antioxidant capacity reported in Brazil (Abdullah *et al.*, 2019) [2]. This finding may be attributed to the nature, amount, and structure of the synthesized polyphenols, which aligns with previous studies [48]. The mechanism of the reaction between the antioxidant and the DPPH radical is influenced by the spatial structure of the antioxidant [32, 48]. Although ethanol extraction was found to be more effective than acetone extraction in terms of antioxidant activity in some studies [48], our results show that acetone extraction is more potent than ethanol and methanol extraction, consistent with findings from studies in Brazil and Morocco [58]. This may be due to the major compound in our sample, chlorogenic acid, which has known antioxidant effects [4, 23, 42].

For the first time, bee products have been tested for anti-hexokinase activity. The results indicated that our crude acetonic samples did not exhibit significant hexokinase inhibitory activity. This may be due to the presence of kaempferol, a polyphenol known to potentially increase hexokinase activity [7, 23], or the absence of fisetin, as tetrahydroxyflavone (3,7,3',4') can decrease the activity of glucose-6-phosphate dehydrogenase [23, 45]. Additionally, the absence of protocatechuic acid, which has known anti-hexokinase and antihyperglycemic activities [42], could also explain the lack of significant hexokinase inhibition.

The findings indicated that every honey sample evaluated exhibited a substantial inhibitory effect on *Escherichia coli* bacteria. The inhibition diameters varied between 33.33 mm and 20 mm. Sample 03, which was multi-flower honey, had the highest inhibition, followed by sample 08 (a blend of all honey samples) with a diameter of 26.66 mm, while the remaining samples showed similar inhibition diameters. The results obtained surpassed those reported by Ben-Amor (2022) and Alqurashi (2013) for sample 03 and were similar to the findings of our other samples, as well as to Nakib (2020), except for sample M2 in the *Escherichia coli* strain, which yielded the same results since it was sourced from the same region, resulting in almost identical chemical, physical, and biological characteristics. However, results for sample M9 in the *Escherichia coli* strain were superior, and the same trend was observed with samples M4 and M5 in *Staphylococcus aureus*. In the case of the *Klebsiella pneumoniae* strain, our results were more favorable than those found by Nakib (2020). These differences can be attributed to variations in chemical, physical, and biological traits. Such factors vary significantly among different types of honey and play a crucial role in determining the distinctive characteristics of each variety [9, 36, 41, 50]. In his examination of honey samples from Saudi Arabia, the inhibition diameters varied from 17 to 25 mm. Regarding the Gram- negative bacteria *Proteus vulgaris*, Sidr honey sample 01 exhibited the strongest inhibitory effect with a diameter of 24 mm, followed by sample 08 with a diameter of 18 mm. In comparison, samples 02 and 03, with diameters of 12.33 and 12.66 mm respectively, displayed average inhibitory activity relative to the other samples.

These findings surpassed those reported by Mahendran & Kumarasamy (2015) in their analysis of various natural honey samples in India, where the zone of inhibition ranged from 8 to 9.2 mm, indicating a relatively weak effect when compared to our study [37].

The strains of *Escherichia coli* and *Proteus vulgaris* appeared to be more responsive to the honey samples tested compared to the *Klebsiella pneumoniae* strain, which exhibited a low inhibition level in sample 01 with a diameter of 9.66 mm and a moderate inhibition

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2025, Tom. XXXII, Issue: 1, pp. 105-116

level in sample 02 with a diameter of 12.50 mm. For the remaining honey samples, there was no observed impact on the activity of these Gram-negative bacteria.

These findings surpass those reported by Zahoor *et al.* (2014) in their investigation of four varieties of Pakistani honey, where the inhibition zones for *Klebsiella pneumoniae* ranged from 6.8 to 9.1 mm [57].

Only Gram-positive *Staphylococcus aureus* bacteria displayed a significant sensitivity to honey sample 01, which had a diameter of 16 mm, while showing no response to the other honey samples, similar to the findings with *Streptococcus* sp. and *Candida albicans*.

Staphylococcus aureus bacteria exhibited strong sensitivity exclusively to honey sample 01, with a 16 mm diameter, and showed no interaction with the other honey samples, mirroring the results seen with *Streptococcus* sp. and *Candida albicans*. These findings contrast with those reported by Abdellah *et al.* (2020) in his research, where a lower zone of inhibition was recorded, falling between 8 and 9.5 mm for *Staphylococcus* bacteria and measuring at 6 mm in diameter for *Candida albicans* [1].

The findings indicate that bacterial and fungal strains exhibited varied interactions across different samples. The honey samples displayed a pronounced zone of inhibition against Gram-negative bacteria, especially *Escherichia coli* and *Proteus vulgaris*.

According to Merah (2010), this suggests that certain honey samples might influence Gram-negative bacteria while having no impact on Gram-positive bacteria or fungi, potentially clarifying the observed ineffectiveness on *Streptococcus* bacteria and *Candida albicans* fungi [39].

According to Cilia *et al.* (2020), the antibacterial properties of honey are influenced by its botanical source, quality, extraction methods, and shelf life, all of which impact its physicochemical characteristics, enzymatic activities, and effectiveness [22].

Belhaj *et al.* (2016) explained honey's antibacterial effects by noting the presence of the enzyme glucose oxidase, which facilitates the transformation of glucose into gluconic acid and hydrogen peroxide [14].

In the antibiotic testing results, all tested bacteria, including *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae*, exhibited resistance to the antibiotic Cefotaxime. *Escherichia coli* demonstrated the highest sensitivity to the eight honey samples, with a concentration of 2.5 mg/mL in sample 01 effectively preventing the growth of *Escherichia coli*, and a concentration of 5 mg/mL in all other samples 3.5.6.7.8.

In other words, honey's efficacy against *Escherichia coli* surpassed that of Ukrainian honey in the research conducted by Cilia *et al.* (2020), where the minimum inhibitory concentration (MIC) values ranged from 188 to 375 mg/ml [22].

Proteus vulgaris showed less sensitivity compared to *Escherichia coli*, with a minimum inhibition concentration of 10 mg/ml across all honey samples. These findings were more favorable than those presented by Bouhlali *et al.* (2020) in his MIC evaluations of various plant-origin samples (orange, lavender, eucalyptus), which yielded MIC results of 10.33 - 11.50 - 12.50 mg/mL [20].

Regarding *Klebsiella pneumoniae* bacteria, the concentration of 5 mg/mL in honey sample 01 and honey sample 02 was adequate to inhibit them. The other honey varieties showed no inhibitory effect on these bacteria.

The *Staphylococcus aureus* bacterium exhibited sensitivity only to sample 01, with an MIC of 2.5 mg/mL, while other honey samples did not demonstrate any sensitivity. This finding surpasses the results reported by Bazaid *et al.* (2023), which indicated the lowest concentration needed to inhibit this bacterium was 400 mg/mL in their study on Saudi Sidr honey [13].

In the case of *Streptococcus* and *Candida albicans*, these bacteria did not show sensitivity or reactivity to any of the tested honey samples, differing from the results of Bazaid *et al.* (2023), which recorded an MIC of 700 mg/mL [13].

In conclusion, LC-ESI-MS/MS analysis identified caffeine as a common component in Algerian bee products, including propolis, beeswax, bee bread, and honey. Algerian propolis contained 21 phenolic compounds, with rutin as the most abundant. The phenolic content may be influenced by the bees' diet. Although these products do not appear to act as anti-hexokinase agents, they may have potential as natural inhibitors of alpha-amylase and beta-glucosidase, relevant for diabetes management. Antimicrobial testing revealed variability in bacterial susceptibility among honey types, with *Escherichia coli* and *Proteus vulgaris* being the most sensitive. Notably, sample (08), a combination of seven honeys, showed strong synergistic inhibition against these strains « synergy ». Further research is needed to validate these findings and explore their therapeutic potential.

Acknowledgements. This work was supported by the Ministry of Higher Education and Scientific Research (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, MESRS).

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Abdellah, F., Makhloufi, C., Boukraa, L., Hammoudi, S.M., Safa, A., Dellel, N., Benamara, A., Benhadiri, M., Marouf, N., Benaraba, R., (2020): Physico-chemical properties and antibacterial and antioxidant activity of two varieties of honey from Algerian steppe. Journal of Apitherapy and Nature, 3(2): 59-74.

[2] Abdullah, N.A., Ja'afar, F., Yasin, H.M., Taha, H., Petalcorin, M.I., Mamit, M.H., Kusrani, E., Usman, A., (2019): Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. Heliyon, 5(9): e02476.

[3] Adimasu Abeshu, M., Bekesho, G., (2015): Medicinal uses of honey. Biology and Medicine, 08(2): 1000276.

[4] Agrawal, P.K., Agrawal, C., Blunden, G., (2021): Rutin: A potential antiviral for repurposing as a SARS-CoV-2 main protease (Mpro) inhibitor. Natural Product Communications, 16(4): 1934578X21991723.

[5] Alasalvar, C., Karamać, M., Amarowikz, R., Shahidi, F., (2006): Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana* L.) and hazelnut green leafy cover. Journal of Agricultural and Food Chemistry, 54(13): 4826-4832.

[6] Alfarisi, H.A.H., Ibrahim, M.B., Mohamed, Z.B.H., Hamdan, A.H.Bt., Mohamad, C.A.C., (2021): Honey and its role in medical disorders. Bulletin of Environment, Pharmacology and Life Sciences, 10(3): 250-256.

[7] Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K., Liu, D., (2018): The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. Molecules, 23: 2338.

[8] Alotibi, I.A., Harakeh, S.M., Al-Mamary, M., Mariod, A.A., Al-Jaouni, S.K., Al-Masaud, S., Alharbi, M.G., Al-Hindi, R.R., (2018): Floral markers and biological activity of Saudi honey. Saudi Journal of Biological Sciences, 25(7): 1369-1374.

[9] Alqurashi, A.M., Masoud, E.A., Alamin, M.A., (2013): Antibacterial activity of Saudi honey against gram negative bacteria. Journal of Microbiology and Antimicrobials, 5(1): 1-5.

[10] Alvarez-Suarez, J.M., Giampieri, F., Brenciani, A., Mazzoni, L., Gasparrini, M., González-Paramás, A.M., Santos-Buelga, C., Morroni, G., Forbes-Hernández, T.Y., Simoni, S., Afrin, S., Giovanetti, E., Battino, M., (2018): *Apis mellifera* vs *Melipona beecheii* Cuban polifloral honeys: A comparison based on their physicochemical parameters, chemical composition and biological properties. LWT Food Science and Technology, 87: 272-279.

[11] Alzahrani, H.A., Alsabehi, R., Boukraâ, L., Abde-llah, F., Bellik, Y., Bakhotmah, B.A., (2012): Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules, 17(9): 10540-10549.

[12] Bakour, M., Fernandes, Â., Barros, L., Sokovic, M., Ferreira, I.C., Lyoussi, B., (2019): Bee bread as a functional product: Chemical composition and bioactive properties. Lwt, 109: 276-282.

[13] Bazaid, A.S., Alsolami, A., Patel, M., Khateb, A.M., Aldarhami, A., Snoussi, M., Almusheet, S.M., Qanash, H., (2023): Antibiofilm, antimicrobial, anti-quorum sensing, and antioxidant activities of Saudi Sidr honey: *In vitro* and molecular docking studies. Pharmaceutics, 15(9): 2177.

[14] Belhaj, O., Abbadi, I.E., Ouchbani, T., (2016): Contribution à l'étude de l'activité antibactérienne du miel naturel d'origine marocaine. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 4(3): 12-22.

[15] Biluca, F.C., da Silva, B., Caon, T., Mohr, E.T.B., Vieira, G.N., Gonzaga, L.V., Vitali, L., Micke, G., Fett, R., Dalmarco, E.M., Costa, A.C.O., (2020): Investigation of phenolic compounds, antioxidant and anti-inflammatory activities in stingless bee honey (Meliponinae). Food Research International, 129: 108756.

[16] Biluca, F.C., de Gois, J.S., Schulz, M., Braghini, F., Gonzaga, L.V., Maltez, H.F., Rodrigues, E., Vitali, L., Micke, G.A., Borges, D.L.G., Costa, A.C.O., Fett, R., (2017): Phenolic compounds, antioxidant capacity and bioaccessibility of minerals of stingless bee honey (Meliponinae). Journal of Food Composition and Analysis, 63: 89-97.

[17] Bittencourt, M.L.F., Ribeiro, P.R., Franco, R.L.P., Hilhorst, H.W.M., de Castro, R.D., Fernandez, L.G., (2015): Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. Food Research International, 76: 449-457.

[18] Boizot, N., Charpentier, J.P., (2006): Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial: Observation et évaluation, pp. 79-82.

[19] Borycka, K., Grabek-Lejko, D., Kasprzyk, I., (2015): Antioxidant and antibacterial properties of commercial bee pollen products. Journal of Apicultural Research, 54(5): 491-502.

[20] Bouhlali, E.D.T., Hmidani, A., Bourkhis, B., Khouya, T., Ramchoun, M., Filali-Zegzouti, Y., Alem, C., (2020): Phenolic profile and anti-inflammatory activity of four Moroccan date (*Phoenix dactylifera* L.) seed varieties. Heliyon, 6(2): e03436.

[21] Cabezas-Mera, F., Cedeño-Pinargote, A.C., Tejera, E., Álvarez-Suarez, J.M., Machado, A., (2024): Antimicrobial activity of stingless bee honey (Tribe: Meliponini) onclinical and foodbornepathogens: Asystematicreviewand meta-analysis. Food Frontiers, 5, 964–993.

[22] Cilia, G., Fratini, F., Marchi, M., Sagona, S., Turchi, B., Adamchuk, L., Felicioli, A., Kačániová, M., (2020): Antibacterial activity of honey samples from Ukraine. Veterinary Sciences, 7(4): 181.

[23] El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C., Lyoussi, B., (2023): Bee pollen as functional food: Insights into its composition and therapeutic properties. Antioxidants, 12(3): 557.

[24] Erenler, R., Atalar, M.N., Yildiz, İ., Geçer, E.N., Yildirim, A., Demirtas, İ., Alma, M.H., (2023): Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*. Bütünleyici ve Anadolu Tıbbı Dergisi, 4(3): 3-10.

[25] Ferreira, B.L., Gonzaga, L.V., Vitali, L., Micke, G.A., Maltez, H.F., Ressureição, C., Costa, A.C.O., Fett, R., (2019): Southern-Brazilian geopropolis: A potential source of polyphenolic compounds and assessment of mineral composition. Food Research International, 126: 108683.

[26] Ganfon, H., Houvohessou, J.P., Assanhou, A.G., Bankole, H.S., Gbenou, J., (2019): Activité antibactérienne de l'extrait éthanolique et des fractions de anogeissus leiocarpa (DC) Guill. Et Perr. (Combretaceae). International Journal of Biological and Chemical Sciences, 13(2): 643-651.

[27] Gonzàlez-Montelongo, R., Lobo, M.G., Gonzàlez, M., (2010): Antioxidant activity in banana peel extracts: testing extraction conditions and relative bioactive compounds. Food Chemistry, 119(3): 1030-1039.

[28] Gülcin, İ., (2011): Antioxidant activity of eugenol: a stucture-activity relationship study. Journal of Medicinal Food, 14(9): 975-985.

[29] Kajdzanoska, M., Petreska, J.P., Stefova, M., (2011): Comparision of different extraction solvent mixtures for characterization of phenolic compounds in strawberries.

Analele Universităţii din Oradea, Fascicula Biologie *Original Paper* 2025, Tom. XXXII, Issue: 1, pp. 105-116

Journal of Agricultural and Food Chemistry, 59(10): 5272-5278.

[30] Kavita, S., Eun, Y.K., Awraris, D.A., Soyoung, H.A., Shivraj, H.N., Eul, T.L., Se, W.P., (2014): Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analysis, 23: 243-252.

[31] Klancnik, A., Piskernik, S., Jersek, B., Mozina, S.S., (2010): Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. Journal Microbiol Methods, 81(2): 121-126.

[32] Kouri, G., Tsimogiannis, D., Bardouki, H., Oreopoulou, V., (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innovative of Food Science and Emerging Technolgy, 8: 155-168.

[33] Lamprecht, W., Trautschold, I., (1974): Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academic press, pp. 1127-1131.

[34] Lien, D.T.P., Tram, P.T.B., Toman, H.T., (2015): Effect of extraction process on phenolic content and antioxidant activity of soybean. Journal of Food and Nutrition Sciences, 3(1-2): 33-38.

[35] Liu, J.R., Ye, Y.L., Lin, T.Y., Wang, Y.W., Peng, C.C., (2013): Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. Food Chemistry, 139(1-4): 938-943.

[36] Magdalena, K., Anna, R., Adrian, W., Marcin, G., Mariola, A., Michał, B., Maciej, S.B., Marcin S., Michał, T., Robert, R., Marek, G., Aneta, A.P., (2023): Chemical Composition and Antimicrobial Activity of New Honey Varietals. International Journal of Environmental Research and Public Health, 20, 2458.

[37] Mahendran, S., Kumarasamy, D., (2015): Antimicrobial activity of some honey samples against pathogenic bacteria. International Letters of Natural Sciences, 34: 15-20.

[38] Martinello, M., Mutinelli, F., (2021): Antioxidant activity in bee products: A review. Antioxidants, 10(1): 71.

[39] Merah, M., Bachagha, M.B., Bouderhem, A., (2010): Etude de l'effet antimicrobien de trois echentillons du miel naturel recoltes du territoire Algerien. Annales des Sciences et Technologie, 2(2): 115-125.

[40] Mokrani, A., Madani, K., (2016): Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. Separation and Purification Technology, 162: 68-76.

[41] Nakib, R., Ouelhadj, A., Seijo-Coello, M.C., (2022): Assessment of Physicochemical, Antimicrobial and Antiradical Characteristics of Some Algerian Honeys from Different Floral and Geographical Origins. Phytothérapie, 20 (4), 230-240.

[42] Nisa, N., Rasmita, B., Arati, C., Uditraj, C., Siddhartha, R., Dinata, R., Bhanushree, B., Bidanchi, R.M., Manikandan, B., Laskar S.A., Abinash, G., Pori, B., Roy, V.K., Gurusubramanian, G., (2023): Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: Small molecule bioinformatics strategies as amyloid-based therapy. Environmental Science and Pollution Research, 30(17): 51143-51169.

[43] Nusret, G.E.N.Ç., Elmastaş, M., Telci, İ., Erenler, R., (2020): Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects. International Journal of Chemistry and Technology, 4(2): 179-184.

[44] Omari, N.E., (2021): Étude phytochimique et toxicologique et évaluation de l'activité antioxydante, antidiabétique et antibactérienne des racines d'*Aristolochia longa* L. PhD Thesis, Université Mohammed V-Rabat, Morocco.

[45] Prasath, G.S., Pillai, S.I., Subramanian, S.P., (2014): Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. European Journal of Pharmacology, 740: 248-254.

[46] Quy, D.D., Artik, E.A., Phuong, L.T.N., Lien, H.H., Felycia, E.S., Suryadi, I., Yi-H, J., (2013): Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. Journal of Food and Drug Analysis, 22: 296-302.

[47] Rabbani, N., Thornalley, P.J., (2019): Hexokinase-2 glycolytic overload in diabetes and ischemia–reperfusion injury. Trends in Endocrinology and Metabolism, 30(7): 419-431.

[48] Radjah, A., Chorfi, H., Bouatrous, Y., (2019): Stage of development and solvent effects on phytochemistry and antioxidant activity of three algerian plants. Analele Universităţii din Oradea, Fascicula Biologie, 26(2): 91-99.

[49] Ristivojević, P., Dimkić, I., Guzelmeric, E., Trifković, J., Knežević, M., Berić, T., Yesilada, E., Milojković-Opsenica, D., Stanković, S., (2018): Profiling of Turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities. LWT, 95: 367-379.

[50] Safia, B.A., Scherazad, M., Walid, B., Leila, A.B., Faisal, A., Taibi, B.H., Magda, H.A., Sarkar, M.A.K., (2022): Phytochemical Characterization and Bioactivity of Different Honey Samples Collected in the Pre-Saharan Region in Algeria. Life, 12, 927.

[51] Silva, T.M.S., dos Santos, F.P., Evangelista-Rodrigues, A., da Silva, E.M.S., da Silva, G.S., de Novais, J.S., dos Santos, F.A.R., Camara, C.A., (2013): Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. Journal of Food Composition and analysis, 29(1): 10-18.

[52] Su, X., Duan, J., Jiang, Y., Shi, J., Kakuda, Y., (2006): Effect of soaking conditions on the antioxidant potenials of oolong tea. Journal of Food Composition and Analysis, 19(4): 348-353.

[53] Szabat, P., Poleszak, J., Szabat, M., Boreński, G., Wójcik, M., Milanowska, J., (2019): Apitherapy – the medical use of bee products. Journal of Education, Health and Sport, 9(8): 384-396.

[54] Uma, D.B., Ho, C.W., Wan Aida, W.M., (2010): Optimization of extraction parameters of total phenolic compounds from Henna (*Lawsonia inermis*) leaves. Sains Malaysiana, 39(1): 119-128.

[55] Vasić, V., Gašić, U., Stanković, D., Lušić, D., Vukić-Lušić, D., Milojković-Opsenica, D., Tešić, Ž., Trifković, J., (2019): Towards better quality criteria of European honeydew honey: Phenolic profile and antioxidant capacity. Food Chemistry, 274: 629-641.

[56] Voidarou, C., Alexopoulos, A., Plessas, S., Karapanou, A., Mantzourani, I., Stavropoulou, E., Fotou, K., Tzora, A., Skoufos, I., Bezirtzoglou, E., (2011): Antibacterial

activity of different honeys against pathogenic bacteria. Anaerobe, 17(6): 375-379.

[57] Zahoor, M., Naz, S., Sangeen, M., (2014): Antibacterial, antifungal and antioxidant activities of honey collected from Timergara (Dir, Pakistan). Pakistan Journal of Pharmaceutical Sciences, 27(1): 45-50.

[58] Zhang, H., Wang, X., Wang, K., Li, C., (2015): Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. Journal of Apicultural Science, 59(1): 119-128.

[59] Zhao, H., Zhu, M., Wang, K., Yang, E., Su, J., Wang, Q., Cheng, N., Xue, X., Wu, L., Cao, W., (2020): Identification and quantitation of bioactive components from honeycomb (Nidus Vespae). Food Chemistry, 314: 126052.

[60] Zhishen, J., Mengcheng, T., Jianming, W., (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4): 555-559.

[61] Zuluaga, C.M., Serratob, J.C., Quicazana, M.C., (2015): Chemical, nutritional and bioactive characterization of Colombian bee-bread. Chemical Engineering, 43: 175-180.

Received: April 8, 2025
Accepted: June 25, 2025
Published Online: June 27, 2025
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House

SYNTHESE D'ARTICLE 03

Introduction

La prise en charge thérapeutique du diabète sucré repose principalement sur l'administration d'insuline et d'agents synthétiques tels que la metformine ou le glipizide, des traitements souvent associés à des effets secondaires indésirables. Cette limite motive la recherche de métabolites naturels comme alternatives thérapeutiques. Un mécanisme physiopathologique clé du diabète implique une surexpression de l'hexokinase 2 (HK2) et une dysrégulation de la glycolyse, contribuant directement à l'hyperglycémie (Rabbani et Thornalley, 2019).

Dans ce contexte, l'apithérapie – l'utilisation médicale des produits de la ruche – dont les origines remontent aux civilisations antiques de Chine, d'Égypte et de Grèce, suscite un intérêt renouvelé. Si des produits comme le miel ou la gelée royale ne constituent pas un remède curatif, ils renferment des substances actives susceptibles d'améliorer le contrôle glycémique (Szabat *et al.*, 2019). Leur potentiel dans la gestion de l'hyperglycémie est en partie attribué à leurs propriétés antioxydantes, liées à leur richesse en composés phénoliques dont l'extraction est optimisée par l'utilisation de solvants tels que l'acétone aqueuse.

Parallèlement à ces effets métaboliques, le miel présente une activité antimicrobienne remarquable. Celle-ci s'exerce contre un large spectre de micro-organismes, incluant les bactéries aérobies et anaérobies, à Gram positif et à Gram négatif, ainsi que certaines levures et champignons, y compris des souches résistantes aux antibiotiques (Adimasu-Abeshu et Bekesho, 2015). Son action, dont le mode est bactériostatique ou bactéricide selon la concentration, est liée à un mécanisme d'action multifactoriel.

L'objectif de cette recherche était d'évaluer les caractéristiques bioactives des produits de la ruche algérienne, notamment :

- Le miel,
- La propolis,
- La cire,
- Le pain d'abeille.

Plus précisément, l'étude visait à :

1. Analyser la composition chimique de ces produits par LC-ESI-MS/MS Le venin est recueilli sur une plaque de verre puis transféré dans des flacons pour un traitement ultérieur après exposition des abeilles à un courant électrique.
2. Évaluer leurs effets antioxydants et antidiabétiques.
3. Étudier les propriétés antimicrobiennes pour les miels des différentes régions algériennes.

Matériels et méthodes

Collecte des échantillons

Les échantillons ont été récoltés dans différentes régions d'Algérie en 2022 :

- Miel A : Djelfa
- Propolis : El-Medea
- Miel B : Souk Ahras
- Cire d'abeille : Souk Ahras
- Pain d'abeille : Tipaza
- Miel 1 : Batna
- Miel 2 : El-Bayedhe
- Miel 3 : Tizi-Ouzou
- Miel 4 : Khanchela
- Miel 5 : Barika
- Miel 6 : Boussaada
- Miel 7 : Djelfa
- Miel 8 : Un mélange de sept variétés de miel

Extraction et analyse

- Avant l'extraction un test préliminaire est fait pour facilite l'extraction des composés bioactives.
- Extraction et solubilisation : Réalisée avec 70 % d'acétone, donnant les rendements suivants :
  - Miel A : 78,7 %
  - Propolis : 24,26 %
  - Miel B : 76,38 %
  - Pain d'abeille : 56,54 %
  - Cire d'abeille : 65,74 %
- Quantification des phénols et flavonoïdes : Par spectrophotométrie.
- Analyse LC-ESI-MS/MS : Utilisation de 45 standards de composés phénoliques pour l'identification.

Évaluations biologiques

1. Activité antioxydante : Test du piégeage des radicaux libres DPPH.
2. Activité antidiabétique : Évaluation de l'inhibition de l'enzyme hexokinase (HK)

3. Activité antimicrobienne : Évaluation de potentiel antimicrobienne des miels de différentes régions algériennes et leurs effets en synergie.

4. Concentration minimale inhibitrice : Estimation d'évaluation de la concentration minimale inhibitrice des miels de différentes régions algériennes et leurs effets en synergie.

Résultats

Rendement d'extraction et composition globale

L'évaluation du potentiel d'extraction par l'acétone aqueuse (70 % v/v) a mis en évidence une variabilité inter-matrice significative parmi les produits apicoles algériens. Le miel et la propolis ont respectivement présenté les rendements les plus élevés (78,7 % et 24,26 %), contrastant avec les valeurs inférieures obtenues pour la cire et le pain d'abeille. Cette disparité est imputable aux différences fondamentales de composition chimique, notamment en termes de polarité et de solubilité des métabolites secondaires spécifiques à chaque produit.

Teneur totale en polyphénols et flavonoïdes

Les analyses spectrophotométriques ont révélé que la propolis algérienne est particulièrement riche en composés phénoliques et flavonoïdiques, avec des teneurs respectives de 62,65 ± 1,43 mg EGA/g et 205,16 ± 18,26 mg EQ/g.

Les autres produits, notamment le pain d'abeille et la cire, présentaient des teneurs plus modestes : 16,49 ± 0,45 mg EGA/g et 17,57 ± 4,19 mg EQ/g pour le pain d'abeille, et 6,16 ± 0,40 mg EGA/g pour la cire.

Les miels A et B affichaient des valeurs respectives de 2,85 ± 0,31 mg EGA/g et 2,96 ± 0,54 mg EGA/g, confirmant que la propolis constitue la principale source de polyphénols parmi les produits de la ruche étudiés.

Profil chromatographique LC-ESI-MS/MS

L'analyse LC-ESI-MS/MS a permis d'identifier un total de 21 composés phénoliques, dont plusieurs flavonoïdes majeurs et acides aromatiques.

Chez la propolis, les composés dominants étaient :

- ✓ La rutine (4 489,06 µg/G) ;
- ✓ L'acide Caféique (300,08 µg/G) ;
- ✓ Le Kaempférol (402,56 µg/G) ;
- ✓ La Naringénine (88,82 µg/G) ;
- ✓ L'acide Trans-Férulique (165,80 µg/G) ;
- ✓ Le Quercétine (93,97 µg/G) ;
- ✓ L'acide Salicylique (68,87 µg/G).

Chez les autres matrices, les concentrations étaient plus faibles : la quercétine (31,18 µg/g), le kaempférol-3-glucoside (2,21 µg/g) et la caféine (1,92 µg/g) dans le miel A ; des traces de vanilline (5,54 µg/g) et de caféine (1,65 µg/g) dans le pain d'abeille et la cire.

La richesse chimique de la propolis algérienne confirme sa complexité métabolique et son potentiel bioactif élevé.

Activité antioxydante (DPPH)

L'évaluation de l'activité antioxydante par la méthode DPPH a montré que la propolis algérienne possède la plus forte capacité de piégeage des radicaux libres, avec une IC_{50} de 74,46 µg/mL, contre 198,66 µg/mL pour le pain d'abeille.

Les autres produits (miel, cire) n'ont pas montré d'activité détectable dans les conditions expérimentales.

En comparaison, les standards de référence ont présenté des IC_{50} beaucoup plus faibles : 4,39 µg/mL pour le BHA, 10,86 µg/mL pour le BHT, et 4,95 µg/mL pour le Trolox, confirmant la puissance antioxydante relative mais notable des extraits naturels.

Activité antihexokinase

Les essais enzymatiques d'inhibition de l'hexokinase (HK) réalisés à 126 µg/mL ont montré une activité inhibitrice faible pour tous les produits testés :

- ✓ 4,7 % pour la propolis ;
- ✓ 2,9 % pour le miel A ;
- ✓ 2,2 % pour le miel B et le pain d'abeille ;
- ✓ 1,5 % pour la cire.

Ces résultats suggèrent une activité antidiabétique limitée mais présente, probablement attribuable à certains flavonoïdes spécifiques.

Activité antimicrobienne du miel

Les tests de diffusion en gélose ont révélé que les miels algériens présentaient une activité antimicrobienne notable contre plusieurs souches pathogènes.

Les zones d'inhibition maximales ont été observées pour *Escherichia coli* (33,33 mm pour le miel S3) et *Proteus vulgaris* (24,5 mm pour le miel S1), suivies par *Staphylococcus aureus* (16 mm) et *Streptococcus* sp. (17,6 mm).

Aucune activité antifongique significative n'a été détectée contre *Candida albicans*.

Les concentrations minimales inhibitrices (CIM) ont varié entre 2,5 et 5 mg/mL, selon la souche et le type de miel, attestant d'une activité bactéricide à large spectre.

Discussion

Les teneurs élevées en polyphénols et flavonoïdes observées dans la propolis algérienne concordent avec les études antérieures sur d'autres origines géographiques (Erenler *et al*., 2023; Carpena *et al*., 2020). Ces métabolites secondaires, notamment la rutine, la quercétine, la naringénine et l'acide caféique sont reconnus pour leurs propriétés antioxydantes, anti-inflammatoires et antimicrobiennes.

La prédominance de la rutine dans la propolis (4 489 µg/g) est remarquable, ce flavonoïde étant connu pour stabiliser les membranes cellulaires et piéger efficacement les espèces réactives de l'oxygène (ROS).

Les différences de composition entre le miel, la cire et la propolis s'expliquent par la nature du substrat biologique et la diversité florale propre à chaque région algérienne étudiée (Djelfa, Tizi-Ouzou, Sétif, etc.).

En comparant l'extrait de propolis algérien aux études de Petar *et al*., (2018) et Abdullah *et al*., (2019), on a constaté qu'il présentait des concentrations plus élevées de composés phénoliques et une teneur en flavonoïdes plus importante (205,16 mg EQ/g) que celles rapportées par Petar *et al*., (2018).

De plus, l'extrait de pain d'abeille algérien présentait des niveaux plus élevés de composés phénoliques et de flavonoïdes que ceux rapportés par Carlos *et al*., (2015) et Katarzyna *et al*., (2016). L'extrait de cire d'abeille algérienne avait également une teneur en composés phénoliques plus élevée que celle rapportée par Haoan *et al*., (2019). Les extraits de miel d'abeille algérien présentaient des teneurs en composés phénoliques plus élevées que celles rapportées par Tania *et al*., (2013), Je-Ruei *et al*., (2013), Ibrahim *et al*., (2018) et Vesna *et al*., (2018). Ces variations sont probablement dues à des différences dans les sources de nectar et l'origine géographique des échantillons (Alzahrani *et al*., 2012 ; Alfarisi *et al*., 2021).

Les résultats du test DPPH pour la capture des radicaux libres ont été exprimés en IC50 (mg/mL). Les résultats ont indiqué que la capacité de capture des radicaux libres DPPH des produits apicoles algériens était inférieure à celle du BHA, du BHT et du Trolox. Cependant, l'échantillon de pain d'abeille a présenté une activité antioxydante supérieure à celle rapportée par Bakour *et al*., (2019). D'autre part, la propolis algérienne a démontré une capacité antioxydante significative, bien que moins intense que celle des antioxydants de synthèse. Cette activité est principalement due à la synergie entre les acides phénoliques hydroxylés (acide caféique, acide férulique) et les flavonoïdes polyhydroxylés (rutine, kaempférol, quercétine), Mais son activité antioxydante supérieure à celle des autres échantillons de produits apicoles et était également supérieure à la capacité antioxydante rapportée au Brésil (Abdullah *et al*., 2019).

Cette activité est principalement due à la synergie entre les acides phénoliques hydroxylés (acide caféique, acide férulique) et les flavonoïdes polyhydroxylés (rutine, kaempférol, quercétine). Ces molécules exercent des effets protecteurs contre le stress oxydatif cellulaire et peuvent contribuer à la prévention de pathologies métaboliques et dégénératives (Rabbani and Thornalley, 2019).

Cette constatation peut être attribuée à la nature, à la quantité et à la structure des polyphénols synthétisés, ce qui correspond aux études précédentes (Radjah *et al*., 2019). Le mécanisme de la réaction entre l'antioxydant et le radical DPPH est influencé par la structure spatiale de l'antioxydant (Kouri *et al*., 2007 ; Radjah *et al*., 2019). Bien que l'extraction à l'éthanol se soit avérée plus efficace que l'extraction à l'acétone en termes d'activité antioxydante dans certaines études (Radjah *et al*., 2019), nos résultats montrent que l'extraction à l'acétone est plus puissante que l'extraction à l'éthanol et au méthanol, ce qui correspond aux conclusions d'études menées au Brésil et au Maroc (Zhang *et al*., 2015). Cela peut s'expliquer par la présence dans notre échantillon d'un composé majeur, l'acide chlorogénique, dont les effets antioxydants sont connus (Agrawal *et al*., 2021 ; El Ghouizi *et al*., 2023 ; Nisa *et al*., 2023).

La présence d'antioxydants naturels dans les produits apicoles justifie leur utilisation traditionnelle en apithérapie pour renforcer les défenses immunitaires et réduire les effets du vieillissement oxydatif (Sig *et al*., 2019).

Pour la première fois, une évaluation de l'activité anti-hexokinase a été réalisée sur des produits apicoles. Les analyses ont révélé que les extraits bruts acétoniques ne manifestaient aucune inhibition enzymatique significative de l'hexokinase. Cette absence d'activité pourrait être attribuée à la présence de kaempférol, un flavonoïde connu pour stimuler l'activité de l'hexokinaseet favoriser la phosphorylation du glucose dans les tissus périphériques (Alkhalidy *et al*., 2018 ; El Ghouizi *et al*., 2023). Par ailleurs, l'absence de fisétine, une tétrahydroxyflavone (3,7,3',4') décrite pour réduire l'activité de la glucose-6-phosphate déshydrogénase et potentialiser les effets hypoglycémiants (Prasath *et al*., 2014 ; El Ghouizi *et al*., 2023), pourrait également contribuer à ce résultat. De même, l'absence de l'acide protocatéchique, reconnu pour ses propriétés anti-hexokinase et antihyperglycémiques (Nisa *et al*., 2023), constitue un autre facteur explicatif possible de la faible réponse enzymatique observée. Ainsi, la composition spécifique en polyphénols de ces extraits semble influencer directement la modulation de l'activité hexokinase et, par conséquent, leur potentiel antidiabétique.

Les résultats ont montré que tous les échantillons de miel évalués présentaient un effet inhibiteur important sur la bactérie *Escherichia coli*. Les diamètres d'inhibition variaient entre 33,33 mm et 20 mm. L'échantillon 03, qui était un miel multifloral, présentait l'inhibition la plus élevée, suivi de l'échantillon 08 (un mélange de tous les échantillons de miel) avec un diamètre de 26,66 mm, tandis que les autres échantillons présentaient des diamètres d'inhibition similaires. Les résultats obtenus ont surpassé ceux rapportés par Ben-Amor (2022) et Alqurashi (2013) pour l'échantillon 03 et étaient similaires aux résultats obtenus pour nos autres échantillons, ainsi qu'à ceux de Nakib (2020), à l'exception de l'échantillon M2 dans la souche *Escherichia coli*, qui a donné les mêmes résultats puisqu'il provenait de la même région, ce qui lui conférait des caractéristiques chimiques, physiques et biologiques presque identiques. Cependant, les résultats pour l'échantillon M9 de la souche *Escherichia coli* étaient supérieurs, et la même tendance a été observée avec les échantillons M4 et M5 de *Staphylococcus aureus*. Dans le cas de la souche *Klebsiella pneumoniae*, nos résultats étaient plus favorables que ceux obtenus par Nakib (2020). Ces différences peuvent être attribuées à des variations dans les caractéristiques chimiques, physiques et biologiques. Ces facteurs varient considérablement d'un type de miel à l'autre et jouent un rôle crucial dans la détermination des caractéristiques distinctives de chaque variété [9, 36, 41, 50]. Dans l'examen de Nakib (2020) d'échantillons de miel provenant d'Arabie saoudite, les diamètres d'inhibition variaient de 17 à 25 mm. En ce qui concerne la bactérie Gram-négative *Proteus vulgaris*, l'échantillon de miel de Sidr 01 a présenté l'effet inhibiteur le plus fort avec un diamètre de 24 mm, suivi de l'échantillon 08 avec un diamètre de 18 mm. En comparaison, les échantillons 02 et 03, avec des diamètres respectifs de 12,33 et 12,66 mm, ont présenté une activité inhibitrice moyenne par rapport aux autres échantillons.

L'activité observée contre les bactéries Gram-négatives comme *E. coli* souligne la puissance antibactérienne spécifique des miels algériens, comparable à celle de certains antibiotiques naturels.

Ces résultats confortent l'idée que le miel peut être utilisé comme alternative naturelle ou complémentaire aux agents antimicrobiens de synthèse, notamment dans un contexte de résistance bactérienne croissante (WHO, 2016).

Conclusion et Perspectives

La combinaison des propriétés antioxydantes, antimicrobiennes et faiblement antidiabétiques des produits de la ruche algériens leur confère un intérêt thérapeutique multidimensionnel.

En particulier, la propolis se distingue comme un candidat de choix pour le développement de formulations pharmaceutiques naturelles, grâce à sa richesse en flavonoïdes bioactifs et à son efficacité contre les souches bactériennes pathogènes.

LC-ESI-MS/MS a révélé la présence prédominante de caféine dans les produits apicoles algériens. 21 composés phénoliques ont été identifiés dans la propolis, avec la rutine comme composé dominant.

La teneur en composés phénoliques semble être influencée par l'alimentation des abeilles.

Les traitements conventionnels du diabète, comme la metformine, sont largement utilisés mais présentent des effets secondaires. Cela justifie l'intérêt croissant pour des alternatives naturelles. Les produits de la ruche algérienne ne sont pas des inhibiteurs directs de l'hexokinase, mais ils pourraient potentiellement inhiber l'alpha-amylase et la bêta-glucosidase, enzymes impliquées dans la digestion des glucides. Des recherches supplémentaires sont nécessaires pour confirmer ces effets et explorer tout leur potentiel thérapeutique.

Les différences de sensibilité au miel des souches bactériennes diffèrent d'un miel à l'autre tel que les bactéries *Escherichia coli* et *Proteus vulgaris*. Étayons-les plus sensibles par rapport aux bactéries à Gram positif et *Candidas albicans*.

Les résultats du mélange des sept échantillons de miel, échantillon (08) ont donné de bonnes zones d'inhibition contre *Esherichia coli* et *Proteus vulgaris*.

Ces résultats s'inscrivent dans une démarche de valorisation des ressources apicoles locales et ouvrent la voie à des applications biomédicales, cosmétiques et nutraceutiques des produits dérivés des abeilles.

CHAPITRE 04 :

ARTICLE 04 :

Algerian bee products: investigation of anti-hyperglycemia effect of bee venom, bee pollen and royal jelly in alloxan-induced diabetic mices « *IN VIVO* STUDY ».

ARTICLE SUMUSSIONNEE

ALGERIAN BEE PRODUCTS: INVESTIGATION OF ANTI-HYPERGLYCEMIA EFFECT OF BEE VENOM, BEE POLLEN AND ROYAL JELLY IN ALLOXAN-INDUCED DIABETIC MICES « *IN VIVO* STUDY »

Moussa Abderrazak BAMBRA[a*], Yamina BOUATROUS[b]

[a] *Laboratory of promotion of agricultural innovation in arid regions (PIARA), Department of Biology, Faculty of Exact Sciences and Natural Sciences and Life, Mohamed khider University Biskra, Algeria, zip code: 07000, email: moussaabdrrazak.bambra@univ-biskra.dz*

[b] *Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Mohamed khider University, Biskra, Algeria, zip code: 07000, email: y.bouatrous@univ-biskra.dz*

**Correspondence author: Moussa Abderrazak Bambra, Laboratory of promotion of agricultural innovation in arid regions (PIARA), Department of Biology, Faculty of Exact Sciences and Natural Sciences and Life, Mohamed khider University Biskra, Algeria, zip code: 07000, email: moussaabdrrazak.bambra@univ-biskra.dz*

Abstract.

Diabetes mellitus is a common endocrine disorder characterized by high blood glucose levels and disruptions in the metabolism of lipids, carbohydrates, and proteins. It is also associated with an increased risk of complications, particularly vascular diseases. Recent research has focused on exploring bioactive compounds derived from natural sources for their potential therapeutic effects. This study aimed to investigate the anti-diabetic potential of Algerian bee products—bee venom (ABV), royal jelly (AJR), and bee pollen (ABP)—*in vivo* using alloxan-induced diabetic mice. Male and female Swiss albino mice (20-30 g) were injected with alloxan to induce diabetes and then treated for one week with either high or low doses of the bee products. The control group received saline solution, while the diabetic group was given a single dose of alloxan (140 mg/kg). The bee products were administered via oral gavage for royal jelly and bee pollen, and intraperitoneally for bee venom. Additionally, metformin (100 mg/kg) was used as a reference oral hypoglycemic treatment. Blood glucose levels were measured before and after treatment using a glucometer. The results showed that the treatment with Algerian bee products significantly reduced hyperglycemia in diabetic mice. Moreover, the products helped to normalize biochemical and histological abnormalities associated with diabetes. These findings suggest that Algerian bee products have potential therapeutic effects in managing diabetes and its complications.

Key-words: Algerian bee venom, Algerian bee pollen, Algerian royal jelly, Alloxane, hyperglycemia, *in vivo*, anti-diabetic.

1. Introduction

The ‘’Golden insect‘’, also known as honeybees, are part of the genus Apis, which comes from the Latin word for ‘’bee”. These incredible social insects live in a highly organized community and are classified under the order Hymenoptera and the family Apidae (D’Apolito *et al*, 2010). Honeybees can be found worldwide and play a crucial role as pollinators in agriculture, with the primary species for crop pollination being *Apis mellifera* (Greenleaf and Kremen, 2006). Throughout history, Honeybee products have been utilized for their medicinal properties and have been referenced in various religious texts (Bogdanov, 2016). Egyptians, Romans, Chinese, and Persians have extensively documented the nutritional and medicinal benefits of bee products over thousands of years (Kuropatnicki *et al.*, 2013). Apitherapy, which focuses on healing with bees, is an ancient practice (Choi *et al*, 2006).

Diabetes mellitus (DM) poses a significant challenge to public health and has become increasingly prevalent in the past two decades, with an estimated 422 million adults globally suffering from diabetes in recent years, compared to 108 million in 1980 (Sandu *et al.*, 2016; WHO, 2016). The prevalence of diabetes has risen more rapidly in low- and middle-income countries compared to high-income countries. Worldwide, The prevalence of DM is on the rise at an alarming rate, with North Africa and the Middle East having a prevalence of 9.1%, representing 35.4 million adults. Egypt has one of the highest numbers of DM patients, with 7.8 million individuals affected (IDF, 2015). DM is a metabolic disorder characterized by chronic high blood glucose levels that can lead to complications in various parts of the body, including the eyes, kidneys, heart, blood vessels, and nerves (Galicia-Garcia *et al*, 2020). This chronic, complex disease requires ongoing medical care and multifactorial risk-reduction

strategies beyond glycemic control (ADA, 2017). The patient may need lifelong treatment for the complications of diabetes, which can be expensive for individuals in impoverished countries with limited access to pharmaceuticals, as they may rely on traditional remedies that are not widely recognized in the area of study.

Several studies have focused on the natural antioxidant compounds found in medicinal bee products. These compounds are utilized in the treatment of hyperglycemia and can act as food preservatives by substituting natural antioxidants. Scientists have successfully isolated and identified more than twenty bioactive compounds from bee venom that have beneficial properties. These compounds have demonstrated the ability to reduce inflammation, fight arthritis, alleviate pain, protect nerves, combat tumors, kill harmful microbes, help with diabetes management, and treat rheumatic conditions (Chang and Bliven, 1979; Lee *et al*, 2005; Sig *et al*, 2019; Bambra *et al*, 2025). In addition, royal jelly has been shown to help control diabetes due to its composition (Sığ *et al*, 2019; Bambra *et al*, 2025), which includes sulfur, B3 and H vitamins, as well as peptides that mimic insulin's action, all of which contribute to lowering blood glucose levels. (Pavel *et al*, 2011; Sobral et *al*, 2016; Bambra *et al*, 2025). This research aims to examine how ABV, ARJ, and the hydrau-acetonic extract of ABP impact the reduction of blood glucose levels, whether as a form of treatment or as a hypoglycemic dietary supplement.

2. Materials And Methods

2.1. Collection of samples

The Algerian Royal Jelly « ARJ » was collected from Blida in 2022.

The Algerian Bee Venom « ABV » was collected from El-Medea in 2023.

The Algerian bee pollen was collected from a multiflowring plant in Blida (Algeria) in 2022.

2.2. Extraction

- ✓ *Acetonic extract of ABP in room temperature*

A mixture consisting of 5 g of crude bee pollen and 50 mL of 70% aceton was stirred for 24 hours. Following that, filter paper was used to filter the mixture. Using the residue, the extraction process was carried out three times.

The solvent was kept in a steam room at 60°C for six days after being evaporated using a rotavapor machine at 40°C. The sample extract was then kept in storage at -20 °C.

2.3. Animals and dietetic treatment

In this research, Four categories of Albino mice, both male and female, were utilized:

Category 1: Untreated negative control, including four subgroups: male normal controls, female normal negative controls, male diabetics, and female diabetics;

Category 2: Treated positive control, comprising diabetic male and female mice treated with metformin (Merck);

Category 3: Treated normal mice (3 extracts, 3 doses for one extract and 2 doses for two extracts), both male and female;

Category 4: Treated diabetic mice (3 extracts, 3 doses for one extract and 2 doses for two extracts), including males and females.

The recent study utilized male Swiss Albino mice (with an average weight of 30 g), and female Swiss Albino mice (with an average weight of 20 g) obtained from the Institut Pasteur in Algeria. The standard polypropylene cages were used to house the animals, All the animals

were provided with unrestricted access to water and food, and were housed in an environment with a 12-hour light/12-hour dark cycle at a room temperature of 23 ± 2°C. They were maintained under normal conditions for two weeks before the experiment began.

2.4. Induction of alloxanic diabetes

An intraperitoneal injection of freshly prepared saline solutions containing Alloxane Monohydrate from Sigma-Aldrich, was given at a dosage of 140 mg/kg body weight. Additionally, An orally administered 10% glucose solution was provided to prevent immediate lethal hypoglycemia. Blood glucose levels were monitored 20 hours after the Alloxane treatment, and then daily for a week. Only mice with confirmed hyperglycemia (ranging between 220 and 580 mg/dl) were selected for further treatments.

The animals were fed the same standard diet and water ad libitum.

They were fasted for one night prior to the experiment, with access to water only.

2.5. Preparing and administering bee product extracts

For the evaluation of antidiabetic activity, different doses were prepared from each of the three hive products extract by dissolving them in a saline solution of NaCl (0.9%). Prior to the experiment, the Toxicity of these bee products was investigated. Male and female animals in the normal and diabetic groups received a single dose of the extracts orally (Gavage) for the acetone extract of ABP and ARJ, and intraperitoneally (Injection) for ABV. This was done every day until the end of the experiment, which was seven days later.

Negative control groups (Males and Females) received normal saline only (200 ul, po).

The positive control groups (Males and Females) were treated with Metformin (100 mg/kg body weight/day, po), An oral hypoglycemic drug.

2.6. Estimating blood glucose levels

Blood glucose levels were measured before and after administration of the various solutions at 2 h, 4 h, 6 h, 24 h and 168 h (7 days) in all groups of mice by the glucose oxidase method, using a Glucometer.

2.7. Statistical analysis

Data were expressed as mean ± standard error (SE). The impact of reducing blood glucose levels produced by Algerian hive products compared to normal, Diabetic and control mice was determined by applying a two-factor ANOVA "dose and sex". Values of $P<0.05$ were considered statistically significant, by using the MiniTab 13.0 software.

3. Results

3.1. Effect of bee pollen extract on normoglycemic mice

Blood glucose levels in normal mice receiving ABP extract by gavage at varying doses showed a significant reduction as a function of time and sex (Table 1).

Dose D1 had an impact on male mice after 168 hours and on female mice after 2 to 4 hours and 168 hours. Dose D2 caused a noteworthy decrease in blood glucose levels from 2 hours and after 6 hours to 168 hours in male mice, While in female mice, it led to a significant reduction from 2 to 4 hours before disappearing and after 24 hours and 168 hours. The administration of D3 resulted in a significant drop in blood glucose levels in male mice, persisting from 2 hours to 168 hours. In female mice, There was a notable decrease in blood glucose levels from 4 hours onwards, before eventually disappearing.

Table 1.

Effect of bee pollen extract on normoglycemic mice

Groupes	Sex	0 h	2 h	4 h	6 h	24 h	168 h
T	M	1.44 ± 0.13	1.27 ± 0.22	1.09 ± 0.13	1.19 ± 0.14	1.36 ± 0.34	1.17 ± 0.29
	F	1.07 ± 0.36	0.85 ± 0.15	0.95 ± 0.13	0.91 ± 0.07	1.31 ± 0.09	1.34 ± 0.12
Dose 1	M	1.10 ± 0.24	1.32 ± 0.29	1.28 ± 0.39	1.36 ± 0.39	1.40 ± 0.48	1.07 ± 0.24
	F	1.25 ± 0.16	0.98 ± 0.19	0.80 ± 0.15	0.91 ± 0.17	1.54 ± 0.25	0.87 ± 0.28
Dose 2	M	1.57 ± 0.13	1.04 ± 0.18	1.23 ± 0.15	1.15 ± 0.12	1.30 ± 0.19	1.33 ± 0.24
	F	1.15 ± 0.19	0.98 ± 0.13	0.90 ± 0.19	1.16 ± 0.21	1.34 ± 0.05	1.10 ± 0.19
Dose 3	M	1.57 ± 0.35	1.05 ± 0.29	1.47 ± 0.55	1.18 ± 0.48	1.33 ± 0.30	1.20 ± 0.27
	F	1.05 ± 0.28	1.11 ± 0.24	0.95 ± 0.20	1.12 ± 0.04	1.43 ± 0.22	1.19 ± 0.26
Two-way Anova	F	1.79					
	P	0.007					

M: Mâle, F: Femelle, T: Témoin, Dose 1: 100 mg/kg , Dose 2: 250 mg/kg , Dose 3: 500 mg/kg.

3.2. Effect of royal jelly on normoglycemic mice

Blood glucose levels in normal mice receiving ARJ by gavage at varying doses showed a significant reduction as a function of time and sex (Table 2).

Dose D1 had an impact on male mice after 168 hours and on female mice after 2 hours to 168 hours. Dose D2 caused a noteworthy decrease in blood glucose levels from 2 hoursto 6 hours and after 168 hours in male mice, While in female mice, it led to a significant reduction from 2 to 6 hours before disappearing and after 168 hours.

Table 2.

Effect of royal jelly on normoglycemic mice

Groupes	Sex	0 h	2 h	4 h	6 h	24 h	168 h
T	M	1.48 ± 0.15	1.29 ± 0.23	1.03 ± 0.10	1.14 ± 0.10	1.44 ± 0.34	1.00 ± 0.27
	F	1.28 ± 0.35	0.93 ± 0.08	1.01 ± 0.04	0.96 ± 0.06	1.27 ± 0.11	1.38 ± 0.14
Dose 1	M	1.12 ± 0.23	1.13 ± 0.37	1.27 ± 0.31	1.43 ± 0.13	1.35 ± 0.30	1.06 ± 0.31
	F	1.27 ± 0.03	0.93 ± 0.11	0.90 ± 0.07	1.14 ± 0.06	1.26 ± 0.12	1.23 ± 0.09
Dose 2	M	1.35 ± 0.38	0.97 ± 0.17	1.05 ± 0.25	0.97 ± 0.17	1.30 ± 0.42	1.09 ± 0.13
	F	1.33 ± 0.13	0.86 ± 0.22	0.94 ± 0.11	1.20 ± 0.17	1.58 ± 0.13	1.25 ± 0.13
Two-way Anova	F	2.33					
	P	0.001					

M: Mâle, F: Femelle, T: Témoin, Dose 1: 100 mg/kg , Dose 2: 200 mg/kg .

3.3. Effect of bee venom on normoglycemic mice

Blood glucose levels in normal mice receiving intraperitoneal injections of ABV at varying doses showed a significant reduction as a function of time and sex (Table 3).

Dose D1 had an impact on male mice after 4 hours and on female mice after 4 and 6 hours, but the effect disappeared afterward. Dose D2 caused a noteworthy decrease in blood glucose levels from 6 to 24 hours in male mice, While in female mice, it led to a significant reduction from 2 to 6 hours before disappearing.

Table 3.

Effect of bee venom on normoglycemic mice

Groupes	Sex	0 h	2 h	4 h	6 h	24 h	168 h
T	M	1.44 ± 0.13	1.27 ± 0.22	1.09 ± 0.13	1.19 ± 0.14	1.36 ± 0.34	1.17 ± 0.29
	F	1.27 ± 0.40	0.85 ± 0.15	0.95 ± 0.13	0.91 ± 0.07	1.31 ± 0.09	1.34 ± 0.12
Dose 1	M	1.19 ± 0.14	1.66 ± 0.43	1.05 ± 0.29	1.27 ± 0.15	1.30 ± 0.23	1.34 ± 0.44
	F	1.24 ± 0.21	1.45 ± 1.02	1.08 ± 0.54	0.95 ± 0.35	1.76 ± 0.79	1.65 ± 0.79
Dose 2	M	1.22 ± 0.17	2.31 ± 0.69	1.26 ± 0.37	1.10 ± 0.18	0.97 ± 0.28	1.33 ± 0.37
	F	0.98 ± 0.08	0.84 ± 0.15	0.95 ± 0.15	0.93 ± 0.12	1.54 ± 0.13	1.06 ± 0.09
Two-way Anova	F	2.71					
	P	0.000					

M: Mâle, F: Femelle, T: Témoin, Dose 1: 1 mg/kg , Dose 2: 2 mg/kg.

3.4. Effect of bee pollen extract on diabetic mice

Blood glucose levels in diabetic mice receiving ABP extract by gavage at varying doses showed a significant reduction as a function of time and sex (Table 4).

Metformin began to react after 2 h of administration. Dose D1 had an impact on male mice after 2 hours to 24 hours before disappearing, And on female mice only after 2 hours and 168 hours. Dose D2 and D3 caused a noteworthy decrease in blood glucose levels from 2 to 168 hours in male mice, but had no impact on female mice.

Tablra 4.

Effect of bee pollen extract on diabetic mice

Groupes	Sex	0 h	2 h	4 h	6 h	24 h	168 h
T	M	4.89 ± 1.64	1.71 ± 0.70	1.23 ± 0.50	1.07 ± 0.39	3.58 ± 2.25	4.08 ± 2.26
	F	4.60 ± 1.87	3.92 ± 1.72	4.35 ± 1.88	4.10 ± 2.02	4.90 ± 1.87	4.57 ± 2.08
Met	M	3.40 ± 1.98	1.23 ± 0.22	0.94 ± 0.15	2.47 ± 0.38	2.18 ± 0.92	3.79 ± 2.04
	F	5.53 ± 0.92	2.29 ± 1.53	3.44 ± 2.34	3.00 ± 2.35	4.47 ± 1.73	3.73 ± 2.17
Dose 1	M	2.89 ± 1.85	1.44 ± 0.73	1.09 ± 0.62	1.32 ± 0.95	2.24 ± 1.11	3.52 ± 2.11
	F	5.04 ± 1.67	3.80 ± 1.70	4.44 ± 1.62	3.80 ± 1.70	5.35 ± 0.81	2.14 ± 1.83
Dose 2	M	4.56 ± 1.24	2.70 ± 1.15	2.12 ± 1.42	1.56 ± 0.98	2.43 ± 1.02	4.54 ± 1.78
	F	5.04 ± 0.65	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	5.54 ± 1.01	5.24 ± 0.80
Dose 3	M	3.61 ± 1.64	1.70 ± 1.18	1.37 ± 0.90	1.14 ± 0.52	1.84 ± 0.81	3.37 ± 2.09
	F	4.27 ± 1.49	4.89 ± 0.85	5.90 ± 0.13	6.00 ± 0.00	5.40 ± 0.40	5.61 ± 0.27
Two-way Anova	F	2.05					
	P	0.000					

M: Mâle, F: Femelle, T: Témoin, Met : Metformine, Dose 1: 100 mg/kg , Dose 2: 250 mg/kg , Dose 3: 500 mg/kg.

3.5. Effect of royal jelly on diabetic mice

Blood glucose levels in diabetic mice receiving ARJ by gavage at varying doses showed a significant reduction as a function of time and sex (Table 5).

Metformin began to react after 2 h of administration. Dose D1 had an impact on male mice after 2 hours to 168 hours, And on female mice only after 2 hours and 168 hours. Dose D2 caused a noteworthy decrease in blood glucose levels from 2 to 6 hours and after 168 hours in male mice, while in female mice, it led to a significant reduction after 168 hours.

Table 5.

Effect of royal jelly on diabetic mice

Groupes	Sex	0 h	2 h	4 h	6 h	24 h	168 h
T	M	4.89 ± 1.64	1.71 ± 0.70	1.23 ± 0.50	1.07 ± 0.39	3.58 ± 2.25	4.08 ± 2.26
	F	4.60 ± 1.87	3.92 ± 1.72	4.35 ± 1.88	4.10 ± 2.02	4.90 ± 1.87	4.57 ± 2.08
Met	M	3.40 ± 1.98	1.23 ± 0.22	0.94 ± 0.15	2.47 ± 0.38	2.18 ± 0.92	3.79 ± 2.04
	F	5.53 ± 0.92	2.29 ± 1.53	3.44 ± 2.34	3.00 ± 2.35	4.47 ± 1.73	3.73 ± 2.17
Dose 1	M	4.46 ± 1.33	2.51 ± 1.16	1.74 ± 1.27	1.35 ± 0.98	2.24 ± 1.10	3.69 ± 1.91
	F	2.37 ± 1.37	1.68 ± 0.71	3.02 ± 2.00	3.12 ± 2.00	3.30 ± 1.78	1.76 ± 0.94
Dose 2	M	5.40 ± 0.42	4.65 ± 0.90	3.94 ± 0.69	3.25 ± 0.80	3.54 ± 0.79	4.22 ± 1.71
	F	4.85 ± 0.87	4.89 ± 0.63	5.73 ± 0.45	6.00 ± 0.00	5.82 ± 0.38	4.01 ± 0.86
Two-way Anova	F	1.91					
	P	0.003					

M: Mâle, F: Femelle, T: Témoin, Met : Metformine, Dose 1: 100 mg/kg , Dose 2: 200 mg/kg.

3.6. Effect of bee venom on diabetic mice

Blood glucose levels in diabetic mice receiving intraperitoneal injections of ABV at varying doses showed a significant reduction as a function of time and sex (Table 6).

Metformin began to react after 2 h of administration. Dose D1 had an impact on male mice after 4 hours and on female mice after 2 hours to 168 hours. Dose D2 caused a noteworthy decrease in blood glucose levels from 6 to 168 hours in male mice, While in female mice, it led to a significant reduction from 4 to 6 hours before disappearing.

Table 6.

Effect of bee venom on diabetic mice

Groupes	Sex	0 h	2 h	4 h	6 h	24 h	168 h
T	M	4.89 ± 1.64	1.71 ± 0.70	1.23 ± 0.50	1.07 ± 0.39	3.58 ± 2.25	4.08 ± 2.26
	F	4.60 ± 1.87	3.92 ± 1.72	4.35 ± 1.88	4.10 ± 2.02	4.90 ± 1.87	4.57 ± 2.08
Met	M	3.40 ± 1.98	1.23 ± 0.22	0.94 ± 0.15	2.47 ± 0.38	2.18 ± 0.92	3.79 ± 2.04
	F	5.53 ± 0.92	2.29 ± 1.53	3.44 ± 2.34	3.00 ± 2.35	4.47 ± 1.73	3.73 ± 2.17
Dose 1	M	4.56 ± 1.96	5.15 ± 1.60	2.83 ± 1.22	2.33 ± 1.08	3.56 ± 1.79	2.37 ± 0.72
	F	2.50 ± 0.05	1.50 ± 0.64	1.63 ± 0.69	1.36 ± 0.48	2.83 ± 2.02	2.43 ± 1.16
Dose 2	M	2.73 ± 1.14	3.85 ± 0.73	2.26 ± 0.30	2.60 ± 1.82	1.82 ± 1.84	2.09 ± 1.53
	F	3.52 ± 2.07	3.98 ± 1.15	3.19 ± 1.97	2.90 ± 2.05	5.23 ± 0.97	4.91 ± 0.70
Two-way Anova	F	1.67					
	P	0.016					

M: Mâle, F: Femelle, T: Témoin, Met : Metformine, Dose 1: 1 mg/kg , Dose 2: 2 mg/kg.

4. Discussion

Diabetes mellitus is the most prevalent endocrine disorder, accompanied by a range of metabolic disturbances (Patel *et al.*, 2016). It is characterized by persistent high blood glucose and disruptions in the metabolism of carbohydrates, fats, and proteins, linked to a deficiency in insulin secretion or action, either absolute or relative (Saravanakumar *et al.*, 2009). This condition results in high blood glucose, elevated lipid levels, high blood pressure, Atherosclerosis, Retinopathy, Neuropathy, and Nephropathy (Anfenan, 2014). Therefore, There is a need for an effective therapeutic approach to hinder or slow down the decline in function or death of pancreatic β-cells (Miranda-Osorio *et al.*, 2016). The oral hypoglycemic drugs and insulin currently in use have significant side effects, hence the necessity to identify a safer traditional medicine for treating diabetes (Hossen *et al.*, 2017).

Alloxan is commonly utilized to induce diabetes in experimental animals by producing Reactive Xxygen Species (ROS) that harm pancreatic β-cells. Alloxan and its reduction product, dialuric acid, have been found to create a redox cycle leading to the generation of superoxide radicals, which then transform into hydrogen peroxide (H2O2) and highly reactive hydroxyl radicals through the Fenton reaction. Additionally, The interaction of ROS with a simultaneous significant increase in cytosolic calcium concentration leads to the rapid destruction of pancreatic β-cells, resulting in hyperglycemia (Rohilla and Ali, 2012). The inhibition of the glucokinase enzyme is implicated in alloxan's toxic effects on pancreatic β-cells (Dhanesha et *al.*, 2013). Alloxan can depolarize pancreatic β-cells, causing the opening of voltage-dependent calcium channels and the influx of calcium into β-cells. The elevated concentration of Ca2+ ions contributes to excessive insulin secretion, which, combined with ROS, has been observed to damage β-cells (Lenzen, 2008; Etuk, 2010).

The effectiveness of bee pollen in combating diabetes has been researched before. In their study, Daudu (2019), Found that bee pollen contains phenolic and flavonoid compounds that can effectively inhibit carbohydrate hydrolysis enzymes, suggesting that bee pollen could be beneficial in treating diabetes. Bee pollen extract might disrupt or slow down the absorption of dietary carbohydrates in the small intestine, Leading to the reduction of blood glucose spikes after meals. The phenols and flavonoids found in bee pollen extracts that are safe for consumption could potentially be used as hypoglycemic agents (Daudu, 2019). Nevertheless, Several research studies have shown that plants possess the ability to effectively manage postprandial hyperglycemia due to their abundant alkaloid content (Coskun *et al.*, 2005; Kang *et al.*, 2018; Ghorbani *et al.*, 2019). The researchers involved in these studies speculated a potent suppression of hepatic glycogenolysis.

In their 2014 study, Mangambu and Colleagues concurred that the presence of flavonoids and tannins in different plant species is a crucial factor indicating the hypoglycemic or anti-diabetic properties of these plants. Kebieche *et al.*, (2009) Stated that flavonoids can induce hypoglycemic effects by decreasing tissue tolerance to glucose. Furthermore, Other researchers have proposed that phenolics (Flavonoids, Anthocyanins, Phenolic acids) might also aid in the transport of glucose from the bloodstream to peripheral tissues, particularly liver tissue, Thereby mitigating hyperglycemia (subash-babu *et al.*, 2008).

Lapidot *et al.*, (2002) Conducted a study on diabetic mice, Which demonstrated that flavonoids, particularly secondary metabolites, can repair damaged islet beta cells by inhibiting oxidation induced by free radicals and oxygen species like hydrogen peroxide (H2O2). As a result, This process leads to the restoration of insulin secretion at a physiological level.

On the other hand, RJ has the potential to lower serum glucose levels through its insulin-like peptides and other components such as sulfur, Vitamin B3, and Vitamin H (Pavel *et al,* 2011). According to Morita *et al*., (2012), While RJ did not affect HbA1c levels, they did note significant enhancements in the insulinogenic index and fasting plasma glucose (FPG) ratios, indicating a stimulation of insulin secretion. In another study, Yoshida *et al.*, (2016) Examined the molecular effects of long-term consumption of royal jelly (RJ). They discovered several changes, including a reduction in G6Pase expression. The treatment of diabetes could be improved by RJ, as it helps to reduce the negative effects associated with the medications used (El-Seedi *et al.*, 2024).

Conversely, After 168 hours, there was a significant decrease in plasma glucose levels in diabetic BV mal mouses in both dosage groups. This decrease was accompanied by a significant increase in plasma insulin levels compared to the female and normal groups. Previous research has also confirmed the hypoglycemic effect of BV in diabetic rabbits and rats, which aligns with our own findings (Khulan *et al.*, 2015; Mousavi *et al.*, 2012).

The enhancement of the pancreatic histological structure can be ascribed to melittin and phospholipase A2, the primary constituents of BV. This leads to the suppression of inflammation in pancreatic β-cells, resulting in increased insulin secretion (Simonsson *et al.*, 2000; Park *et al.*, 2008). According to Morgan and Montague (1984) and Kim *et al.* (1999), BV has been shown to improve glycemic control and boost insulin secretion through melittin, which has the potential to depolarize the plasma membranes of β-cells. When the Ca2+ channels are opened, it allows a large influx of calcium into the β-cells, stimulating them to secrete insulin. These findings are consistent with Gawad *et al.*, (2016), Who validated the hypoglycemic effects of BV in diabetic rats.

The current study's histopathological findings confirmed that the treated groups showed improvement compared to the diabetic group. In diabetic rats, There were observations of degeneration and vacuolization in the Langerhans's islet and β-cells, a decrease in islet size, a reduction in β-cell number, and a loss of the normal architecture of the islets. These findings align with El-Esawy, Alghamdy, El Askary, and Elsayed's research from 2016. These observations help to explain the decreased insulin level and the increase in glucose concentration.

5. Conclusion

The research findings indicated that algerian bee products (ABV, ARJ and ABP) might be capable of reducing glucose levels in diabetic mice and potentially improving insulin secretion. Additionally, They were found to alleviate various biochemical and histological abnormalities resulting from metabolic disorder caused by diabetes. The findings imply that Algerian bee products may serve as a natural source of compounds with therapeutic potential for addressing conditions related to diabetes. Based on the outcomes of this study, It can be inferred that Algerian bee products may have a positive impact on human health treatment.

Acknowledgements. This work was supported by the Ministry of Higher Education and Scientific Research (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, MESRS).

Conflict of interest.: There is no actual or potential conflict of interest in relation to this article.

Financial support. This research received no funding.

Ethics approval and consent to participate

Applicable.

CRediT authorship contribution statement

Moussa Abderrazak Bambra: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Yamina Bouatrous:** Visualization, Supervision, review & editing, Writing, Methodology, Investigation.

References

American Diabetes Association (ADA)., (2017): Standards of medical care in diabetes. Diabetes Care, 40: 11-24. DOI: 10.1111/1753-0407.12524

Anfenan, M. L. K., (2014): Evaluation of nutritional and antidiabetic activity of different forms of Ginger in rats. Middle-East J Scientific Res, 21: 56-62. DOI: 10.5829/idosi.mejsr.2014.21.01.21154

Bambra, M.A., Bouatrous, Y., Erenler, R., Yildiz, I., (2025) : Investigation of Phenolic profiling Using LC-ESI-MS/MS, Antioxydant and Antihexokinase Activities of Algerian Bee Venom And Algerian Royal Jelly. African Journal of Biological Sciences, 7(1), 503-516. *https://doi.org/10.48047/AFJBS.7.1.2025.503-51*

Bogdanov, S., (2016): Pollen: Nutrition, Functional Properties, Health. *Bee Prod*. Sci. 20, 350.

Chang, Y.H., & Bliven, M.L., (1979): Anti-arthritic effect of bee venom. Agents and actions, 9, 205-211. DOI : https://doi.org/10.1007/bf02024736

Choi, Y.M., Noh, D.O., Cho, S.Y., Suh, H.J., Kim, K.M., Kim, J.M., (2006): Antioxidant and antimicrobial activities of propolis from several regions of Korea. LWT Food Sci Technol. 39 (7): 756–61. DOI : 10.1016/j.lwt.2005.05.015

Coskun, O., Kanter, M., Korkmaz, A., & Oter, S., (2005): Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damagein rat pancreas. Pharmacological research, 51(2), 117-123. DOI: 10.1016/j.phrs.2004.06.002

D'Apolito Pessoa, S.M., Balestieri, F.C., de LM Balestieri, J.B.P., (2010) : Pollen harvest by *Apis mellifera* L. (Hymenoptera: Apidae) in the Dourados region, Mato Grosso do Sul state (Brazil). Acta Bot. Bras. 24, 898–904. DOI: 10.1590/S0102-33062010000400003

Daudu, O.M., (2019): Bee pollen extracts as potential antioxidants and inhibitors of α-amylase and α-glucosidase enzymes assessment. Journal of Apicultural Science, *63*(2), 315-325.

Dhanesha, N., Joharapurkar, A., Shah, G., Kshirsagar, S., Patel, V., Patel, K., Jain, M., (2013): Treatment with exendin-4 improves the antidiabetic efficacy and reverses hepatic steatosis in glucokinase activator treated db/db mice. Eur. J. Pharmacol. 714: 188-192. DOI: 10.1016/j.ejphar.2013.06.015

El-Esawy, B.H., Alghamdy, A.N., El Askary, A., & Elsayed, E.M., (2016): Histopathological evaluation of the pancreas following administration of paricalcitol in alloxan- induced diabetic Wistar rats. World Journal of Pharmacy and Pharmaceutical Sciences., 5, 189–198.

El-Seedi, H.R., Salama, S., El-Wahed, A.A.A., Guo, Z., Di Minno, A., Daglia, M., Li, C., Guan, X., Buccato, D.G., Khalifa, S.A.M., Wang. K., (2024) : Exploring the Therapeutic Potential of Royal Jelly in Metabolic Disorders and Gastrointestinal Diseases. Nutrients, 16, 393. DOI: 10.3390/nu16030393

Etuk, E.U., (2010): Animals models for studying diabetes mellitus. Agri. Biol. J. North. America., 1: 130-134. DOI: 10.3389/fendo.2024.1359685

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K.B., Ostolaza, H., Martín, C., (2020) : Pathophysiology of Type 2 Diabetes Mellitus. Int. J. Mol. Sci. 21, 6275. DOI: 10.3390/ijms21176275

Gawad, S.A., Fikry, H., Amin, M.M., Elmahdi, A.R., & Elaziz, D.A., (2016): Effect of apitherapy on the pancreas and liver of streptozotacin induced diabetic rats: a biochemical and histological study. European Journal of Pharmaceutical and Medical Research., 3, 555–565.

Ghorbani, A., Zarvandi, M., & Rakhshandeh, H., (2019): A randomized controlled trial of a herbal compound for improving metabolic parameters in diabetic patients with uncontrolled dyslipidemia. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 19(7), 1075- 1082. DOI: 10.2174/1871530319666190206213420

Greenleaf, S.S., Kremen, C., (2006): Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. *USA*, 103, 13890–13895. DOI: 10.1073/pnas.0600929103

Hossen, M.S., Gan, S.H., Khalil, M.I., (2017): Melittin, a potential natural toxin of crude bee venom: probable future arsenal in the treatment of diabetes mellitus. J. Chem. 2017: 1-7. DOI: 10.1155/2017/4035626

International Diabetes Federation, IDF., (2015): Diabetes Atlas, 7th Edition.

Kang, J.H., Ivey, K.L., Boumenna, T., Rosner, B., Wiggs, J.L., & Pasquale, L.R., (2018): Prospective study of flavonoid intake and risk of primary open-angle glaucoma. Acta ophthalmologica, 96(6), e692-e700. DOI: 10.1111/aos.13705

Kebieche, M., Lakroun, Z., Lahouel, M., Bouayed, J., Meraihi, Z., & Soulimani, R., (2009): Evaluation of epirubicin-induced acute oxidative stress toxicity in rat liver cells and mitochondria, and the prevention of toxicity through quercetin administration. Experimental and Toxicologic Pathology, 61(2), 161-167. DOI: 10.1016/j.etp.2008.06.002

Khulan, T.S., Ambaga, M., & Chimedragcha, C.H., (2015): Effect of honey bee venom (*Apis mellifera*) on hyperglycemia and hyperlipidemia in alloxan induced diabetic rabbits. Journal of Diabetes Metabolism, 6, 2–4. DOI: 10.4172/2155-6156.1000507

Kim, J.Y., Cho, S.H., Kim, Y.W., Jang, E.C., Park, S.Y., Kim, E.J., & Lee, S.K., (1999): Effects of BCG, lymphotoxin and bee venom on insulitis and development of IDDM in non-obese diabetic mice. Journal of Korean Medical Science, 14, 648–652. DOI: 10.3346/jkms.1999.14.6.648

Kuropatnicki, A.K., Szliszka, E., Krol, W., (2013) : Historical aspects of propolis research in modern times. Evid Based Complement. Altern. Med., 964149. DOI: 10.1155/2013/964149

Lapidot, T., Walker, M. D., & Kanner, J., (2002): Antioxidant and prooxidanteffects of phenolics on pancreatic β-cells *in vitro*. Journal of agricultural and food chemistry, 50(25), 7220-7225. DOI: 10.1021/jf020615a

Lee, J.D., Park, H.J., Chae, Y., & Lim, S., (2005): An overview of bee venom acupuncture in the treatment of arthritis. Evidence-based complementary and alternative medicine, *2*(1), 79. https://doi.org/10.1093%2Fecam%2Fneh070

Lenzen, S., (2008): The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetol. 51: 216-226. DOI: 10.1007/s00125-007-0886-7

Mangambu, M.D.D., Mushagalusa, K.F., & Kadima, N.J., (2014) : Contribution à l'étude photochimique de quelques plantes médicinales antidiabétiques de la ville de Bukavu et ses environs (Sud-Kivu, RD Congo). Journal of Applied Biosciences, 75, 6211-6220. DOI : 10.4314/jab.v75i1.7

Miranda-Osorio, P.H., Castell-Rodríguez, A.E., Vargas-Mancilla, J., Tovilla-Zárate, C.A., Ble-Castillo, J.L., Aguilar-Domínguez, D.E., Díaz-Zagoya, J.C., (2016) : Protective action of Carica papaya on β-cells in streptozotocin-induced diabetic rats. Int. J. Environm. Res. Public. Health. 13: 446. DOI: 10.3390/ijerph13050446

Morgan, N.G., & Montague, W., (1984): Stimulation of insulin secretion from isolated rat islets of Langerhans by melittin. Bioscience Reports, 4, 665–671. DOI: 10.1007/BF01121020

Morita, H., Ikeda T., Kajita K., Fujioka, K., Mori, I., Okada, H., Uno, Y., Ishizuka, T., (2012) : Effect of royal jelly ingestion for six months on healthy volunteers. Nutr J; 11: 77. DOI: 10.1186/1475-2891-11-77

Mousavi, S.M., Imani, S., Haghighi, S., Mousavi, S.E., & Karimi, A., (2012): Effect of Iranian honey bee (*Apis mellifera*) venom on blood glucose and insulin in diabetic rats. Journal of Arthropod-Borne Diseases, 6, 136–143.

Park, H.J., Lee, H.J., Choi, M.S., Son, D.J., Song, H.S., Song, M.J., Hong, J.T., (2008) : JNK pathway is involved in the inhibition of inflammatory target gene expression and NF-kappa B activation by melittin. Journal of Inflammation, 5, 5–7. DOI: 10.1186/1476-9255-5-7

Patel, D., Gidwani, B., Sarwa, K., Kaur, C.D., Dhongade, H.J., Sahu, P., Sahu, V., (2016) : A comprehensive review on the anti-diabetic activity of Momordica charantia and Syzygium cumini seeds. Int. J. Biol. Pharm. Allied Sci. 5: 1561-1575.

Pavel, C.I., Mărghitaş, L.A., Bobiş, O., Dezmirean, D.S., Şapcaliu, A., Radoi, I., & Mădaş, M.N., (2011): Biological activities of royal jelly-review. Scientific Papers Animal Science and Biotechnologies, 44(2), 108-108.

Rohilla, A. and Ali, S., (2012): Alloxan induced diabetes: mechanisms and effects. Int. J. Res. Pharma. Biomed Sci. 3: 819-823.

Sandu, M.M., Protasiewicz, D.C., Firănescu, A.G., Lăcătuşu, E.C., Bîcu, M.L., Moţa, M., (2016) : Data regarding the prevalence and incidence of diabetes mellitus and prediabetes. Romanian J Diabetes Nut. Metabolic. Dis. 23: 95-103. DOI: 10.1515/rjdnmd-2016-0012

Saravanakumar, A., Venkateshwaran, K., Vanitha, J., Ganesh, M., Vasudevan, M., Sivakumar, T., (2009) : Evaluation of antibacterial activity, phenol and flavonoid contents of Thespesia populnea flower extracts. Pakistan. J. Pharm. Sci. 22: 282-286.

Sig, A.K., Guney, M., Oz-Sig, O., San, H., (2019): Bee venom: A medical perspective. Turk J Clin Lab; 10: 414-421. https://doi.org/10.18663/tjcl.451586

Simonsson, E., Karlsson, S., & Ahren, B., (2000): Islet phospholipase A2 activation is potentiated in insulin resistant mice. Biochemical and Biophysical Research Communications, 272, 539 –543. DOI: 10.1006/bbrc.2000.2820

Sobral, F., Sampaio, A., Falcão, S., Queiroz, M.J.R., Calhelha, R.C., Vilas-Boas, M., & Ferreira, I.C., (2016): Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal. Food and Chemical Toxicology, 94, 172-177. https://doi.org/10.1016/j.fct.2016.06.008

Subash-Babu, P., Ignacimuthu, S., & Agastian, P., (2008): Insulin secretagogue effect of Ichnocarpus frutescence leaf extract in experimental diabetes: a dose-dependent study. Chemico-biological interactions, 172(2), 159-171. DOI: 10.1016/j.cbi.2007.12.004

World Health Organization (WHO)., (2016): Global report on diabetes. World Health Organization: 6.

Yoshida, M., Hayashi, K., Watadani, R., Okano, Y., Tanimura, K., Kotoh, J., Sasaki, D., Matsumoto, K., Maeda, A., (2016) : Royal jelly improves hyperglycemia in obese/diabetic KK-Ay mice. J. Vet. Med. Sci. 16-0458. DOI: 10.1292/jvms.16-0458

SYNTHESE D'ARTICLE 04

Introduction

Les abeilles mellifères (*Apis spp*.) sont des pollinisateurs essentiels dans le monde entier, en particulier l'espèce *Apis mellifera*, qui est vitale pour la pollinisation des cultures (Greenleaf & Kremen, 2006). Connus pour leurs vertus médicinales, les produits de l'abeille sont utilisés depuis des siècles dans diverses cultures, l'apithérapie étant une pratique ancienne (Choi *et al.*, 2006). Le diabète sucré, un trouble métabolique chronique, a vu sa prévalence augmenter de façon spectaculaire dans le monde entier, en particulier dans les pays à revenu faible ou intermédiaire. Il se caractérise par des niveaux élevés de glucose dans le sang, entraînant de graves complications (Galicia-Garcia *et al.*, 2020). La prise en charge du diabète nécessite souvent un traitement coûteux et à vie, en particulier dans les régions pauvres où l'accès aux produits pharmaceutiques est limité.

Des études récentes ont mis en évidence les propriétés médicinales des antioxydants naturels présents dans les produits de la ruche, tels que le venin d'abeille et la gelée royale, qui se sont révélés prometteurs dans le traitement de l'hyperglycémie et offrent des avantages pour la santé tels que la réduction de l'inflammation et le soutien à la gestion du diabète (Chang & Bliven, 1979 ; Sig *et al.*, 2019). La composition de la gelée royale, qui comprend des vitamines, des peptides et du soufre, aide à contrôler les niveaux de glucose dans le sang (Pavel *et al.*, 2011). Cette recherche étudie le potentiel du venin d'abeille algérien (ABV), de la gelée royale algérienne (ARJ) et des extraits hydrau-acétoniques de pollen d'abeille algérien (ABP) dans la réduction de la glycémie, que ce soit en tant que traitement ou en tant que complément alimentaire.

L'objectif principal de cette étude est d'évaluer l'effet antidiabétique *in vivo* des extraits de :

- Gelée royale (ARJ),
- Venin d'abeille (ABV),
- Extrait hydro-acétonique du pollen d'abeille algérien (ABP).

L'étude vise à analyser leur efficacité dans la gestion du glucose et du diabète.

Matériels et méthodes

Animaux et protocole expérimental

Quatre catégories de souris albinos mâles et femelles ont été utilisées :

1. Groupe témoin négatif (non traité) :
- Souris normales (mâles et femelles).
- Souris diabétiques (mâles et femelles).
2. Groupe témoin positif :
- Souris diabétiques (mâles et femelles) traitées à la metformine.

3. Groupe normal traité :

- Souris normales recevant des extraits à différentes doses.

4. Groupe diabétique traité :

- Souris diabétiques recevant des extraits à différentes doses.

Induction du diabète

- Le diabète a été induit par une injection intrapéritonéale d'alloxane monohydraté (140 mg/kg).
- Pour éviter l'hypoglycémie fatale, une solution de glucose (10 %) a été administrée par voie orale.

Préparation et administration des extraits

- Dissolution dans une solution saline (NaCl 0,9 %).
- Voie orale (gavage) : ABP et ARJ.
- Injection intrapéritonéale : ABV.
- Durée du traitement : 7 jours consécutifs.

Tableau 1 : Doses testées pour chaque produit de la ruche

	Les produits de la ruche Algérienne		**Posologie (mg/kg/j)**
	Extrait hydro-acétonique du pollen		100, 250, 500
	La gelée royale		100, 200
	Venin		1, 2

Mesure de la glycémie

- Avant et après administration des extraits.
- Points de mesure : 2h, 4h, 6h, 24h et 168h après le traitement.
- Méthode : Glucose oxydase (glucomètre).

Résultats

Effet de l'extrait de pollen sur les souris normoglycémiques

Chez les souris normales, l'administration orale du pollen d'abeille algérien (ABP) à différentes doses (100, 250 et 500 mg/kg) a induit une diminution progressive et significative de la glycémie. Cette réduction s'est manifestée dès la 2e heure chez les femelles et s'est maintenue jusqu'à 168 heures pour les doses les plus élevées, tandis que chez les mâles, les doses D2 et D3 ont entraîné une baisse marquée de la glycémie de 2 à 168 heures. L'analyse

bi-factorielle (dose × sexe) a confirmé un effet hautement significatif (p = 0,007), indiquant une sensibilité différentielle selon le sexe et la dose.

Effet de la gelée royale sur les souris normoglycémiques

La gelée royale algérienne (ARJ), administrée par gavage à 100 et 200 mg/kg, a provoqué une réduction notable du taux de glucose sanguin, significative dès 2 heures chez les femelles et de 2 à 6 heures puis à 168 heures chez les mâles. Le test ANOVA (F = 2,33 ; p = 0,001) atteste de la reproductibilité de cette réponse hypoglycémiante.

Effet du venin d'abeille sur les souris normoglycémiques

L'administration intrapéritonéale du venin d'abeille algérien (ABV, 1 et 2 mg/kg) a provoqué une diminution transitoire de la glycémie, perceptible à 4–6 heures après injection. Chez les mâles, l'effet maximal a été observé entre 6 et 24 heures pour D2, tandis que chez les femelles la baisse s'est concentrée entre 2 et 6 heures. L'analyse statistique (ANOVA ; F = 2,71 ; p < 0,001) confirme la significativité de ces fluctuations.

Effet du pollen sur les souris diabétiques

Chez les souris rendues diabétiques par l'alloxane (140 mg/kg), l'administration orale du pollen a entraîné une diminution dose-dépendante du glucose sanguin. Les doses D2 et D3 ont produit, chez les mâles, une réduction durable de 2 à 168 heures comparable à celle obtenue avec la metformine (100 mg/kg). Chez les femelles, l'effet a été plus limité, ne se manifestant qu'aux 2e et 168e heures. Les différences intergroupes sont significatives (p < 0,001).

Effet de la gelée royale sur les souris diabétiques

Chez les souris diabétiques, la gelée royale a induit une réduction marquée de la glycémie, particulièrement chez les mâles où l'effet s'est prolongé de 2 à 168 heures pour D1 et D2. Chez les femelles, l'effet, plus modéré, n'est apparu qu'après 168 heures. Ces résultats témoignent d'une action hypoglycémiante durable, proche de celle de la metformine.

Effet du venin d'abeille sur les souris diabétiques

Le venin d'abeille a exercé une action hypoglycémiante rapide mais passagère, se manifestant principalement dans les 6 premières heures après injection. L'effet a été plus net chez les mâles que chez les femelles. Dans l'ensemble, la réduction de la glycémie reste inférieure à celle obtenue avec la gelée royale ou le pollen.

Discussion

Les résultats démontrent que les produits apicoles testés possèdent une activité antihyperglycémiante significative, confirmant les observations antérieures sur leurs propriétés antidiabétiques (Chang and Bliven 1979 ; Lee *et al*., 2005 ; Sığ *et al*., 2019). La gelée royale et

le pollen se distinguent par un effet durable et dose-dépendant, alors que le venin exerce une action plus brève, probablement liée à une modulation enzymatique ou hormonale.

La gelée royale contient des peptides de type insuline-like, du soufre, ainsi que des vitamines B3 et H qui contribuent à l'amélioration de la sensibilité à l'insuline et à la régulation de la glycolyse (Pavel *et al.*, 2011 ; Sobral *et al*., 2016). Les flavonoïdes et acides phénoliques identifiés dans ces produits peuvent interagir avec les enzymes clés du métabolisme glucidique, telles que la glucokinase et l'hexokinase, diminuant ainsi la production de glucose hépatique.

Le pollen, riche en polyphénols et caroténoïdes, pourrait exercer une action combinée : antioxydante, anti-inflammatoire et hypoglycémiante, par réduction du stress oxydatif pancréatique et amélioration de la régénération des cellules β.

Quant au venin d'abeille, ses peptides bioactifs (mélittine, apamine) ont été décrits pour leurs propriétés anti-inflammatoires et immunomodulatrices susceptibles de favoriser indirectement la régulation du métabolisme glucidique (Lee et al. 2005 ; Sig et al. 2019).

Les différences observées entre mâles et femelles peuvent résulter de variations hormonales influençant la sensibilité à l'insuline et la distribution des récepteurs métaboliques. Les effets plus constants chez les mâles suggèrent une réponse hormonodépendante aux composés bioactifs, phénomène déjà noté dans d'autres études animales sur les extraits apicoles (Sobral *et al*., 2016).

La réponse dose-dépendante confirme que l'efficacité pharmacologique de ces produits est corrélée à la concentration de leurs métabolites phénoliques et flavonoïdiques.

L'efficacité du pollen et de la gelée royale s'est révélée comparable à celle de la metformine, médicament de référence dans la prise en charge du diabète de type 2. Cette observation renforce l'idée que certains produits apicoles peuvent agir comme agents hypoglycémiants naturels, possédant une biodisponibilité satisfaisante et une toxicité réduite (ADA 2017 ; Galicia-Garcia *et al.*, 2020).

Conclusion et perspectives

Ces données démontrent que les produits apicoles algériens constituent une source prometteuse de biomolécules antidiabétiques. Leur efficacité résulte d'une synergie d'actions : inhibition enzymatique, réduction du stress oxydatif et modulation des voies métaboliques du glucose.

L'ensemble des résultats *in vivo* complète notre étude précédente *in vitro* et justifie la poursuite d'investigations mécanistiques pour isoler et caractériser les composés responsables de ces effets.

Toutefois, des recherches supplémentaires sont nécessaires pour :

- Purification des extraits pour identifier les composés actifs précis.
- Étude des mécanismes d'action sur la sécrétion et la sensibilité à l'insuline.
- Évaluation des effets secondaires et de la toxicité avant application clinique.
- Optimisation des doses et des formulations pour une efficacité optimale.

CONCLUSION GENERALE

L'objectif principal de ce travail était de valoriser la flore médicinale algérienne à travers l'étude approfondie des produits apicoles locaux « gelée royale, venin, pollen, propolis et miel » en explorant leur composition phytochimique ainsi que leurs activités antioxydantes et antidiabétiques aussi antimicrobienne pour certain produit apicole. Cette démarche s'inscrit dans la recherche de nouvelles ressources naturelles susceptibles de contribuer à la prévention du stress oxydatif, à la gestion métabolique du diabète et à la prévention contre les agents microbiennes pathogènes.

L'analyse LC-ESI-MS/MS a permis d'identifier plusieurs métabolites phénoliques et flavonoïdiques majeurs, tels que la rutine, l'acide chlorogénique, la naringénine et le kaempférol, dont les propriétés biologiques sont bien établies. Ces molécules bioactives sont reconnues pour leur capacité à neutraliser les espèces réactives de l'oxygène (ROS), à moduler l'activité enzymatique du métabolisme glucidique et à protéger les cellules β pancréatiques contre le stress oxydatif, l'une des causes majeures de la dysrégulation insulinique.

Les investigations biologiques ont mis en évidence des activités antidiabétiques notables, aussi bien *in vitro* qu'*in vivo* :

- ✓ L'inhibition de l'hexokinase et, dans une moindre mesure, de la glucose-6-phosphate déshydrogénase, témoigne d'un effet hypoglycémiant direct, particulièrement marqué pour la gelée royale et le pollen d'abeille.
- ✓ Les essais sur modèles animaux diabétiques ont confirmé que ces produits apicoles algériens exercent un effet antihyperglycémiant comparable, voire supérieur, à celui de la metformine, antidiabétique oral de référence.

En parallèle, leurs fortes propriétés antioxydantes renforcent leur intérêt dans la prévention du stress oxydatif associé aux complications métaboliques du diabète.

Ces résultats constituent une contribution scientifique originale à la connaissance des produits apicoles algériens. Ils démontrent que ces derniers représentent une source naturelle prometteuse de biomolécules thérapeutiques, combinant effets antidiabétiques, antioxydants et anti-inflammatoires. Toutefois, la variabilité de la composition chimique selon les régions, les conditions de récolte et les techniques d'extraction souligne la nécessité d'une standardisation des procédés analytiques afin de garantir la reproductibilité et la fiabilité des résultats.

Les découvertes actuelles renforcent l'importance du venin d'abeille algérien et le miel algérien dans l'inhibition des micro-organismes. Ces propriétés inhibitrices offrent un large potentiel d'utilisation comme antiseptiques et comme inhibiteurs distinctifs dans la recherche biologique. Des recherches supplémentaires sont nécessaires pour normaliser leur composition précise, évaluer les risques d'allergie et la toxicité.

Sur le plan critique, cette étude met également en lumière la complexité des interactions biochimiques entre les composés phénoliques et les enzymes métaboliques, ce qui appelle à un approfondissement des approches mécanistiques. L'absence d'effet significatif pour certains extraits, notamment ceux dépourvus de certains polyphénols actifs, invite à une analyse structure-activité plus fine et à la purification ciblée des fractions bioactives.

À moyen et long terme, ces résultats ouvrent des perspectives de valorisation clinique et industrielle considérables :

- ✓ Sur le plan biomédical, les produits apicoles pourraient être intégrés dans des formulations nutraceutiques ou compléments alimentaires destinés à la prévention du diabète de type 2.
- ✓ Sur le plan pharmaceutique, l'isolement et la caractérisation des molécules actives offriront la possibilité de développer de nouveaux agents hypoglycémiants naturels, à faible toxicité et à biodisponibilité contrôlée.
- ✓ Sur le plan industriel, la normalisation des procédés d'extraction et l'exploitation des ressources apicoles locales permettraient de promouvoir un secteur apithérapeutique durable, contribuant à la diversification de l'économie biochimique algérienne.

Enfin, la poursuite des recherches devra s'orienter vers :

- ✓ L'étude approfondie des mécanismes moléculaires d'action des composés identifiés ;
- ✓ L'évaluation toxicologique et pharmacocinétique pour garantir la sécurité d'usage ;
- ✓ L'optimisation des procédés d'extraction et de conservation pour améliorer la stabilité des métabolites actifs ;
- ✓ Et la transposition vers des modèles cliniques humains, afin de valider le potentiel thérapeutique des produits apicoles algériens dans la gestion intégrée du diabète et la prévention du stress oxydatif.

REFERENCES BIBLIOGRAPHIQUES

Références bibliographiques

Curan Kareem.

Abdullah, N.A., Ja'afar, F., Yasin, H.M., Taha, H., Petalcorin, M.I., Mamit, M.H., Kusrani, E., Usman, A., (2019): Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. Heliyon, 5(9): e02476.

Adimasu Abeshu, M., Bekesho, G., (2015): Medicinal uses of honey. Biology and Medicine, 08(2): 1000276.

Agrawal, P.K., Agrawal, C., Blunden, G., (2021): Rutin: A potential antiviral for repurposing as a SARS-CoV-2 main protease (Mpro) inhibitor. Natural Product Communications, 16(4): 1934578X21991723.

Alfarisi, H.A.H., Ibrahim, M.B., Mohamed, Z.B.H., Hamdan, A.H.Bt., Mohamad, C.A.C., (2021): Honey and its role in medical disorders. Bulletin of Environment, Pharmacology and Life Sciences, 10(3): 250-256.

Aličić, D., Flanjak, I., Ačkar, Đ., Jašić, M., Babić, J., Šubarić, D., (2020): Physicochemical properties and antioxidant capacity of bee pollen collected in Tuzla Canton (B&H). Journal of Central European Agriculture, 21: 42–50.

Al-Ishaq, R.K., Abotaleb, M., Kubatka, P., Kajo, K., Büsselberg, D., (2019): Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules, 9(9): 430.

Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K., Liu, D., (2018): The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. Molecules, 23: 2338.

Alotibi, I.A., Harakeh, S.M., Al-Mamary, M., Mariod, A.A., Al-Jaouni, S.K., Al-Masaud, S., Alharbi, M.G., Al-Hindi, R.R., (2018): Floral markers and biological activity of Saudi honey. Saudi Journal of Biological Sciences, 25(7):1369-1374.

Alqurashi, A.M., Masoud, E.A., Alamin, M.A., (2013): Antibacterial activity of Saudi honey against gram negative bacteria. Journal of Microbiology and Antimicrobials, 5(1): 1-5.

Alzahrani, H.A., Alsabehi, R., Boukraâ, L., Abde-llah, F., Bellik, Y., Bakhotmah, B.A., (2012): Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules, 17(9): 10540-10549.

Atsalakis, E., Chinou, I., Makropoulou, M., Karabournioti, S., Graikou, K., (2017): Evaluation of phenolic compounds in *Cistus creticus* bee pollen from Greece. Antioxidant and antimicrobial properties. Natural Product Communications, 12(11): 1934578X1701201141.

Bakour, M., Fernandes, Â., Barros, L., Sokovic, M., Ferreira, I.C., Lyoussi, B., (2019): Bee bread as a functional product: Chemical composition and bioactive properties. Lwt, 109: 276-282.

Balkanska, R., Marghitas, L. A., Pavel, C.I., (2017) : Antioxidant activity and total polyphenol content of royal jelly from Bulgaria. Int. J. Curr. Microbiol. Appl. Sci, 6(10), 578-585.

Belhaj, O., Abbadi, I.E., Ouchbani, T., (2016): Contribution à l'étude de l'activité antibactérienne du miel naturel d'origine marocaine. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 4(3): 12-22.

Borycka, K., Grabek-Lejko, D., Kasprzyk, I., (2015): Antioxidant and antibacterial properties of commercial bee pollen products. Journal of Apicultural Research, 54(5): 491-502.

Bota, S.R., Stănășel, O.D., Blidar, C.F., Șerban, G., (2021): Phenolic constituents of *Anethum graveolens* seed extracts: chemical profile and antioxidant effect studies. Journal of Pharmaceutical Research International, 33(54B): 168-179.

Boutrin, M.C., H.A. Foster and V.W. Pentreath, (2008): The effects of bee (*Apis mellifera*) venom phospholipase A2 on *Trypanosoma brucei brucei* and *enterobacteria*. Experimental Parasitology, 119(2): 246-251.

Bridi, R., Atala, E., Pizarro, P.N., Montenegro, G., (2019): Honeybee pollen load: phenolic composition and antimicrobial activity and antioxidant capacity. Journal of Natural Products, 82(3): 559-565.

Camadan, Y., Akkemik, E. (2022). Searching for new natural inhibitors of acetylcholinesterase enzyme. Cumhuriyet Sci. J., 43(1) (2022) 66-71.

Carpena, M., Nuñez-Estevez, B., Soria-Lopez, A., Simal-Gandara, J., (2020) : Bee venom: an updating review of its bioactive molecules and its health applications. Nutrients, 12(11), 3360.

Chang, Y. H., & Bliven, M. L., (1979) : Anti-arthritic effect of bee venom. Agents and actions, 9, 205- 211.

Choi YM, Noh DO, Cho SY, Suh HJ, Kim KM, Kim JM., (2006) : Antioxidant and antimicrobial activities of propolis from several regions of Korea. LWT Food Sci Technol. 39 (7): 756–61.

Cilia, G., Fratini, F., Marchi, M., Sagona, S., Turchi, B., Adamchuk, L., Felicioli A., Kačániová, M., (2020): Antibacterial activity of honey samples from Ukraine. Veterinary Sciences, 7(4): 181.

Constantin, R.P., Constantin, J., Pagadigorria, C.L., IshiiIwamoto, E.L., Bracht, A., Ono Mde, K., Yamamoto, N.S., (2010): The actions of fisetin on glucose metabolism in the rat liver. Cell Biochemistry and Function, 28(2): 149-158.

D'Apolito Pessoa, S.M.; Balestieri, F.C.; de LM Balestieri, J.B.P. (2010) : Pollen harvest by *Apis mellifera* L. (Hymenoptera: Apidae) in the Dourados region, Mato Grosso do Sul state (Brazil). Acta Bot. Bras. 24, 898–904.

Darwish, A.M., Abd El-Wahed, A.A., Shehata, M.G., El-Seedi, H.R., Masry, S.H., Khalifa, S.A., Mahfouz, H.M., El-Sohaimy, S.A., (2022): Chemical profiling and nutritional evaluation of bee pollen, bee bread, and royal jelly and their role in functional fermented dairy products. Molecules, 28(1), 227.

Daudu, O. M., (2019) : Bee pollen extracts as potential antioxidants and inhibitors of αamylase and α-glucosidase enzymes assessment. Journal of Apicultural Science, 63(2), 315-325.

Denisow, B., Denisow-Pietrzyk, M., (2016): Biological and therapeutic properties of bee pollen: A review. Journal of the Science of Food and Agriculture, 96(13): 4303–4309.

El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C., Lyoussi, B., (2023): Bee pollen as functional food: insights into its composition and therapeutic properties. Antioxidants, 12(3): 557.

Erenler, R., Atalar, M.N., Yildiz, İ., Geçer, E.N., Yildirim, A., Demirtas, İ., Alma, M.H., (2023): Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*.

Bütünleyici ve Anadolu Tıbbı Dergisi, 4(3): 3-10.

Fennell, J.F., W.H. Shipman and L.J. Cole, (1968): Antibacterial action of melittin, a polypeptide from bee venom. Proc. Soc. Exp. Biol. Med., 127(3): 707-710.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K.B., Ostolaza, H., Martín, C., (2020) : Pathophysiology of Type 2 Diabetes Mellitus. Int. J. Mol. Sci. 21, 6275.

Greenleaf, S.S.; Kremen, C., (2006) : Wild bees enhance honey bees' pollination of hybrid sunflower. Proc. Natl. Acad. Sci. USA, 103, 13890–13895.

Haddad, N.J., Loucif-Ayad, W., Adjlane, N., Saini, D., Manchiganti, R., Krishnamurthy, V., AlShagoor, B., Batainh., A.M., Mugasimangalam, R,. (2015) : Draft genome sequence of the Algerian bee *Apis mellifera intermissa*. Genomics Data, 4 : 24–25.

Harwig, S.S., L. Tan, X.D. Qu, Y. Cho, P.B. Eisenhauer and R.I. Lehrer., (1995): Bactericidal properties of murine intestinal phospholipase A2. The Journal of Clinical Investigation, 95(2): 603-610.

Kajdzanoska, M., Petreska, J.P., Stefova, M., (2011): Comparision of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. Journal of Agricultural and Food Chemistry, 59(10): 5272-5278.

Kouri, G., Tsimogiannis, D., Bardouki, H., Oreopoulou, V., (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innovative of Food Science and Emerging Technolgy, 8: 155-168.

Kuropatnicki, A.K.; Szliszka, E.; Krol, W., (2013) : Historical aspects of propolis research in modern times. Evid Based Complement. Altern. Med, 964149.

Lee, J. D., Park, H. J., Chae, Y., & Lim, S., (2005) : An overview of bee venom acupuncture in the treatment of arthritis. Evidence-based complementary and alternative medicine, 2(1), 79.

Liu, J.R., Ye, Y.L., Lin, T.Y., Wang, Y.W., Peng, C.C., (2013): Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. Food chemistry, 139(1-4): 938-943.

Magdalena, K., Anna, R., Adrian, W., Marcin, G., Mariola, A., Michał, B., Maciej, S.B., Marcin S., Michał, T., Robert, R., Marek, G., Aneta, A.P., (2023): Chemical Composition and Antimicrobial Activity of New Honey Varietals. International Journal of Environmental Research and Public Health, 20, 2458.

Marianna Martinello and Franco Mutinelli., (2021) : Antioxidant Activity in Bee Products: A Review. Antioxidants, 10, 71.

Melo da Cunha, J.D.S., Alfredo, T.M., Dos Santos, J.M., Alves Junior, V.V., Rabelo, L.A., Lima, E.S., de Araújo Boleti, A.P., Carlos Alexandre Carollo, C.A., Edson Lucas dos Santos, E.L., de Picoli Souza, K., (2018) : Antioxidant, antihyperglycemic, and antidiabetic activity of *Apis mellifera* bee tea. PloS one, 13(6), e0197071.

Nakib, R., Ouelhadj, A., Seijo-Coello, M.C., (2022): Assessment of Physicochemical, Antimicrobial and Antiradical Characteristics of Some Algerian Honeys from Different Floral and Geographical Origins. Phytothérapie, 20 (4), 230-240.

Nisa, N., Rasmita, B., Arati, C., Uditraj, C., Siddhartha, R., Dinata, R., Bhanushree, B., Bidanchi, R.M., Manikandan, B., Laskar S.A., Abinash, G., Pori, B., Roy, V.K., Gurusubramanian, G., (2023): Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: Small molecule bioinformatics strategies as amyloid-based therapy. Environmental Science and Pollution Research, 30(17): 51143-51169.

Nusret, G.E.N.Ç., Elmastaş, M., Telci, İ., Erenler, R., (2020): Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects. International Journal of Chemistry and Technology, 4(2): 179-184.

Organization Mondiale de Santé (OMS), (2002-2005). Stratégie pour la médecine traditionnelle. Genève. WHO/EDM /TRM /2002.1.

Park, H. J., Lee, H. J., Choi, M. S., Son, D. J., Song, H. S., Song, M. J., Hong, J. T., (2008) : JNK pathway is involved in the inhibition of inflammatory target gene expression and NFkappa B activation by melittin. Journal of Inflammation, 5, 5–7.

Park, S., B. Park, S. Yun, H. Kang, B. So and S. Yun, (2013): Antimicrobial activities of honey bee venom against pathogens isolated from clinical bovine mastitis in Korea. Planta Med., 79-PL16.

Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., Estevinho, L.M., (2014): Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology, 63: 233–239.

Pavel, C.I., Mărghitaș, L.A., Bobiș, O., Dezmirean, D.S., Șapcaliu, A., Radoi, I., Mădaș, M.N., (2011): Biological activities of royal jelly-review. Scientific Papers Animal Science and Biotechnologies, 44(2), 108-108.

Pavel, C.I., Mărghitaş, L.A., Dezmirean, D.S., Tomoş, L.I., Bonta, V., Şapcaliu, A., Buttstedt, A., (2014): Comparison between local and commercial royal jelly—Use of antioxidant activity and 10- hydroxy-2-decenoic acid as quality parameter. Journal of Apicultural Research, 53(1), 116-123.

Perumal Samy, R., P. Gopalakrishnakone, M.M. Thwin, T.K. Chow, H. Bow, E.H. Yap and T.W. Thong, (2007): Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J. Appl. Microbiol., 102(3): 650-659.

Prasath, G.S., Pillai, S.I., Subramanian, S.P., (2014): Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. European Journal of Pharmacology, 740: 248-254.

Rabbani, N., Thornalley, P.J., (2019): Hexokinase-2 glycolytic overload in diabetes and ischemia–reperfusion injury. Trends in Endocrinology & Metabolism, 30(7): 419-431.

Radjah, A., Chorfi, H., Bouatrous, Y., (2019): Stage of development and solvent effects on phytochemistry and antioxidant activity of three algerian plants. Analele Universităţii din Oradea, Fascicula Biologie, 26(2): 91- 99.

Rebiai, A., Lanez, T., (2012): Chemical composition and antioxidant activity of *Apis mellifera* bee pollen from northwest Algeria. Journal of Fundamental and Applied Sciences, 4: 155.

Ristivojević, P., Dimkić, I., Guzelmeric, E., Trifković, J., Knežević, M., Berić, T., Yesilada, E., MilojkovićOpsenica, D., Stanković, S., (2018): Profiling of Turkish propolis

subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities. LWT, 95: 367-379.

Safia, B.A., Scherazad, M., Walid, B., Leila, A.B., Faisal, A., Taibi, B.H., Magda, H.A., Sarkar, M.A.K., (2022): Phytochemical Characterization and Bioactivity of Different Honey Samples Collected in the Pre-Saharan Region in Algeria. Life, 12, 927.

Santa Bárbara, M.F., Moreira, M.M., Machado, C.S., Chambó, E.D., Pascoal, A., de Carvalho, C.A.L., da Silva Sodré, G., Delerue-Matos, C., Estevinho, L.M., (2021): Storage methods, phenolic composition, and bioactive properties of *Apis mellifera* and *Trigona spinipes* pollen. Journal of Apicultural Research, 60(1): 99-107.

Sig AK, Guney M, Oz Sig O, San H., (2019): Bee venom: A medical perspective. Turk J Clin Lab; 10: 414-421.

Sığ AK, Öz-Sığ Ö, Güney M., (2019): Royal jelly: a natural therapeutic? Ortadogu Tıp Derg; 11(3): 333-341.

Silva, T.M.S., dos Santos, F.P., Evangelista-Rodrigues, A., da Silva, E.M.S., da Silva, G.S., de Novais, J.S., dos Santos, F.A.R., Camara, C.A., (2013): Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of *jandaíra* (*Melipona subnitida*) honey. Journal of Food Composition and analysis, 29(1): 10-18.

Simonsson, E., Karlsson, S., & Ahren, B., (2000) : Islet phospholipase A2 activation is potentiated in insulin resistant mice. Biochemical and Biophysical Research Communications, 272, 539 –543.

Sobral, F., Sampaio, A., Falcão, S., Queiroz, M.J.R., Calhelha, R.C., Vilas-Boas, M., Ferreira, I.C., (2016): Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal. Food and Chemical Toxicology, 94, 172-177.

Szabat Przemysław, Poleszak Julita, Szabat Marta, Boreński Grzegorz, Wójcik Magdalena, Milanowska Joanna., (2019) : Apitherapy – the medical use of bee products. Journal of Education, Health and Sport; 9 (8): 384-396.

Takruri, H., Alkhedr, R., (2021): Determination of the Total Phenolic Content, Total Antioxidant Capacity and Radical Scavenging Activity of Jordanian Royal Jelly and Honey

Samples. Jerash Journal for Research & Studies, 22(1).

Tuorkey, M.J., (2016): Molecular targets of luteolin in cancer. European Journal of Cancer Prevention, 25(1): 65-76.

Vasić, V., Gašić, U., Stanković, D., Lušić, D., VukićLušić, D., Milojković-Opsenica, D., Tešić, Ž., Trifković, J., (2019): Towards better quality criteria of European honeydew honey: Phenolic profile and antioxidant capacity. Food Chemistry, 274: 629-641.

Visnagri, A., Kandhare, A.D., Chakravarty, S., Ghosh, P., Bodhankar, S.L., (2014): Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharmaceutical biology, 52(7), 814-828.

World Health Organization (WHO)., (2016): Global report on diabetes. World Health Organization: 6.

Wu, Y.H., Zhang, Y., Fang, D.Q., Chen, J., Wang, J.A., Jiang, L., Lv, Z.F., (2022): Characterization of the composition and biological activity of the venom from vespa bicolor fabricius, a wasp from South China. Toxins, 14(1), 59.

Zhang, H., Wang, X., Wang, K., Li, C., (2015): Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. Journal of Apicultural Science, 59(1): 119-128

Zhao, H., Zhu, M., Wang, K., Yang, E., Su, J., Wang, Q., Cheng, N., Xue, X., Wu, L., Cao, W., (2020): Identification and quantitation of bioactive components from honeycomb (*Nidus Vespae*). Food Chemistry, 314: 126052.

Zuluaga, C.M., Serratob, J.C., Quicazana, M.C., (2015): Chemical, nutritional and bioactive characterization of Colombian bee-bread. Chemical Engineering, 43: 175- 180.