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Presented by

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THEME

Phytochemical, antioxidant, and toxicological study of Algerian *Origanum majorana* L: experimental approach in *vitro* and in *vivo*

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Dedication

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Bouthaina

Dedication

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Amira

الملخص

تُعد .*Origanum majorana* L(البردقوش) نباتًا طبيًا ينتمي إلى فصيلة *Lamiaceae*، ويُستخدم تقليديًا لخصائصه المضادة للميكروبات، والمضادة للالتهابات، والمساعدة على الهضم. تهدف هذه الدراسة إلى تقييم القدرة المضادة للأكسدة والتأثير السمي الحاد لمستخلصات إيثانولية، مائية، وهيدروإيثانولية مستخلصة من الأجزاء الهوائية للنبتة، وهذه الأخيرة ماخوذة من منطقة الوادي (جنوب شرق الجزائر). وقد تم تحديد محتوى الفينولات الكلي حيث بلغ أقصاه في المستخلص الهيدروإيثانولي (298.61 ± 5.46 ملغ حمض الغاليك/غ من المستخلص). كما تم قياس محتوى الفلافونويدات ، وسجل أعلى قيمة (61.46 ± 0.29 ملغ كيرسيتين/غ) لنفس المستخلص. تم تقييم النشاط المضاد للأكسدة من خلال اختبار تثبيط جذر DPPH واختبار قدرة الارتباط بأيون الحديد الثنائي. أظهر المستخلص الهيدروإيثانولي فعالية كبيرة في تثبيط الجذور الحرة بقيمةIC_{50} بلغت 0.0241 ملغ/مل، متجاوزًا بذلك مضاد الأكسدة المرجعي BHT (0.0260 ملغ/مل). بينما كانت قدرة هذا المستخلص على ارجاع الحديد متوسطة (IC_{50} = 0.382 ملغ/مل)مقارنة بالـ EDTA (IC_{50} = 0.0034 ملغ/مل).أُجريت دراسة السمية الحادة على فئران بيضاء (سويسرية) ولم تُسجل أية حالات وفاة بجرعات وصلت إلى 5 غ/كغ. غير أن بعض الأعراض الفيزيولوجية مثل النعاس، نقص الشهية، وزرقة الفم لوحظت بجرعات مرتفعة. كما أظهرت النتائج انخفاضًا في أوزان الكبد والكلى بالمقارنة مع مجموعة غير المعالجة. أُرفقت هذه المعطيات بتحليل نسيجي للأعضاء المستهدفة (الكبد، القلب، والكلية)، والذي أظهر عدم وجود أي تغيرات سميّة أو أضرار نسيجية واضحة، مع الحفاظ على البنية العامة للأنسجة، باستثناء احتقان وعائي خفيف في بعض العينات . ختامًا، تُظهر نبتة *Origanum majorana*L قدرة مضادة للأكسدة ملحوظة، خاصة في مستخلصها الهيدروإيثانولي الغني بالبوليفينولات والفلافونويدات، وتُعد آمنة نسبيًا من الناحية السمية وفقًا للظروف التجريبية المعتمدة.

الكلمات المفتاحية:.*Origanum majorana*L، الفينولات، الفلافونويدات، النشاط المضاد للأكسدة، السمية الحادة،الكبد، القلب، والكلية.

Abstract

Origanum majorana L. (marjoram) is a medicinal plant belonging to the Lamiaceae family, traditionally used for its antimicrobial, anti-inflammatory, and digestiveaid properties.This study aimed to evaluate the phytochemical constituent, antioxidant capacity and acute toxic effect of ethanolic, aqueous, and hydroethanolic extracts of the aerial parts of the plant, collected from the El Oued region (southeastern Algeria). The total phenolics content was determined reaching its maximum in the hydroethanolic extract (298.61 ± 5.46 mg gallic acid/g of extract).The flavonoids content with the highest value (61.46 ± 0.29 mg quercetin/g) recorded for the same extract. The antioxidant activity was evaluated by DPPH radical inhibition assay and iron(II) binding capacity assay.The hydroethanolic extract showed important free radical scavenging activity with an IC_{50} value of 0.0241 mg/mL, exceeding the reference antioxidant BHT (0.0260 mg/ml).Meanwhile, the iron chelating capacity of this extract was intermediate (IC_{50} = 0.382 mg/ml) compared to (IC_{50} = 0.0034 mg/ml) of EDTA. An acute toxicity study was performed on Swiss albino mice and no deaths were recorded at doses up to 5g/kg.However, some physiological symptoms such as drowsiness, decreased appetite, and cyanosis of the mouth were observed at higher doses.The results also showed a decrease in liver and kidney weights compared to the control group.These data were accompanied by a histological analysis of the target organs (liver, heart, and kidney), which showed no obvious toxic changes or tissue damage, with the overall tissue structure preserved, with the exception of mild vascular congestion in some samples. In conclusion, *Origanum majorana* L. exhibits a remarkable antioxidant capacity, especially in its hydroethanolic extract, which is rich in polyphenols and flavonoids, and is considered relatively safe in terms of toxicity under the approved experimental conditions.

Keywords:*Origanum majorana*L., polyphenols, flavonoids, antioxidant activity, acute toxicity,liver, heart, and kidney.

Résumé

Origanum majorana L. (marjolaine) est une plante médicinale appartenant à la famille des Lamiacées, traditionnellement utilisée pour ses propriétés antimicrobiennes, anti-inflammatoires et digestives.Cette étude visait à évaluer la composition phytochimique, la capacité antioxydante et l'effet toxique aigu d'extraits éthanolique, aqueux et hydroéthanolique des parties aériennes de la plante, récoltées dans la région d'El Oued (sud-est de l'Algérie). La teneur totale en composés phénoliques a été déterminée, atteignant son maximum dans l'extrait hydroéthanolique (298,61 ± 5,46 mg d'acide gallique/g d'extrait).La teneur en flavonoïdes a été mesurée et la valeur la plus élevée (61,46 ± 0,29 mg de quercétine/g) ayant été enregistrée pour le même extrait. L'activité antioxydante a été évaluée par un test d'inhibition radicalaire du DPPH et un test de capacité de liaison du fer(II).L'extrait hydroéthanolique a montré une activité importante de piégeage des radicaux libres avec une valeur IC_{50} de 0,0241 mg/mL, dépassant l'antioxydant de référence BHT (0,0260 mg/mL).Parallèlement, la capacité de chélation du fer de cet extrait était intermédiaire (IC_{50} = 0,382 mg/mL) par rapport à (IC_{50} = 0,0034 mg/mL) d'EDTA. Une étude de toxicité aiguë a été réalisée sur des souris albinos suisses et aucun décès n'a été enregistré à des doses allant jusqu'à 5g/kg.Cependant, certains symptômes physiologiques tels que somnolence, diminution de l'appétit et cyanose de la bouche ont été observés à des doses plus élevées.Les résultats ont également montré une diminution du poids du foie et des reins par rapport au groupe témoin.Ces données ont été accompagnées d'une analyse histologique des organes cibles (foie, cœur et rein), qui n'a montré aucun changement toxique évident ni lésion tissulaire, la structure tissulaire globale étant préservée, à l'exception d'une légère congestion vasculaire dans certains échantillons. En conclusion, *Origanum majorana* L. présente une capacité antioxydante remarquable, notamment dans son extrait hydroéthanolique, riche en polyphénols et flavonoïdes, et considéré comme relativement sûr en termes de toxicité dans les conditions expérimentales approuvées.

Mots clés :*Origanum majorana*L., polyphénols, flavonoïdes, activité antioxydante, toxicité aiguë, foie, cœur et rein.

List of Abbreviations :

ABTS: 2,2′-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid

$AlCl_3$: Aluminium trichloride

BHT: Butylated hydroxytoluene

CAT: Catalase

DPPH: 2,2′-diphenyl-1-picrylhydrazyl

EDTA: Ethylenediaminetetraacetic acid

EtOH: Ethanol

FRAP: Ferric reducing antioxidant power

GAE: Gallic acid equivalent

GPx: Glutathione peroxidase

IC_{50}: Inhibitory concentration at 50%

LD_{50}: Lethal dose for 50% of population

OM:*Origanum majorana*L.

QE: Quercetin equivalent

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SD: Standard deviation

SEM: Standard error of the mean

SOD: Superoxide dismutase

TFC: Total flavonoid content

TPC: Total phenol content

TEAC: Trolox equivalent antioxidant capacity

OECD: Organization for Economic Cooperation and Development

H&E: Hematoxylin and Eosin

$FeCl_2$: Ferrous chloride

AAE: Ascorbic Acid Equivalent

DWB: Dry Weight Basis

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