

الجمهورية الجزائرية الديمقراطية الشعبية  
وزارة التعليم العالي والبحث العلمي

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Master thesis

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For the fulfillment for the diploma of

Master

Field: Biology

Specialty: Applied Biochemistry

Topic

**Assessment of biological activities of *Marrubium sp*  
hydroethanolic extract**

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2024/2025

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## Acknowledgments

First of all, we would like to express our gratitude to God for giving us strength and patience.

We extend our sincere thanks to Dr. MAMACHE Walid for proposing this research topic to us. We are grateful for your trust, support, availability, and the invaluable guidance you provided us throughout this work. Your assistance and critical insights were tremendously helpful during our research

Next, we would like to express our deepest gratitude to the members of the jury, Pr. Benabdallah Hassiba and Dr. Meziti Hicham, for graciously accepting to examine and evaluate our work.

We would also like to thank Prof. AMIRA Smain (Head of team Effect of Natural Products), for his permission to assess our practical part in the laboratory of Phytotherapy Applied to Chronic Diseases.

# Table of content

Abstract

المخلص

Liste of abbreviations

Liste of figures

List of tables

I-Introduction .....	1
II-Materials and methods .....	4
2-Material and Methods .....	4
2-1-Plant material.....	4
2-2-Laboratory animals .....	4
2-3-Chemicals and reagents.....	4
2-4- Preparation of MHE.....	4
3-Phytochemical assessment.....	5
3-1- Total Polyphenol content assay.....	5
3-2-Total flavonoids content assay .....	6
3-3- Total tannin content assay.....	7
4-Biological activities of MHE.....	8
4-1- The DPPH radical scavenging assay .....	8
4-2- The ABTS radical scavenging assay.....	8
4-3- Ferrous ions chelating activity (ferrozine method).....	9
4-4- Assessment of reducing power (Phenanthroline Method).....	9
5-Acute limited toxicity test.....	10
5-1-Determination of biochemical parameters .....	10
5-2-Reduced glutathione (GSH) content .....	11
5-3-Total protein estimation .....	11
5-4-Assessment of lipid peroxidation (MDA).....	12
5-5-Assessment of catalase activity .....	12
6-Gastric emptying and intestinal transit rate tests .....	13
7-Statistical analysis .....	14
III-Results .....	15
1-Extraction yield .....	15
2-Phytochemical screening .....	15
3-Antioxidant activities.....	16
4-Acute limited toxicity test.....	16

4-1-Effect of on antioxidant parameters .....	18
5-Effects of MHE on Gastric emptying and intestinal transit rate.....	22
5-1-Effects of MHE on Gastric emptying (GE) rate.....	22
5-2-Effects of MHE on intestinal transit (IT) rate.....	23
IV-Discussion .....	25
V-Conclusion and perspectives.....	30
VI-Bibliographic references .....	31

## Abstract

The present study investigated the phytochemical content, antioxidant activity, toxicological effects of *Marrubium* hydroethanolic extract (MHE), and its impact on gastric emptying (GE) and intestinal transit (IT) rates. The extraction yield was 21.66%. Quantitative analysis revealed high levels of bioactive compounds, including total polyphenols ( $95 \pm 1.010$  GAE  $\mu\text{g}/\text{mg}$ ), flavonoids ( $54.43 \pm 11.37$  QE  $\mu\text{g}/\text{mg}$ ), and tannins ( $77.06 \pm 0.47$  TAE  $\mu\text{g}/\text{mg}$ ). MHE exhibited notable antioxidant properties across multiple assays: DPPH radical scavenging ( $\text{IC}_{50} = 98.86 \pm 2.09$   $\mu\text{g}/\text{mL}$ ), ABTS assay ( $\text{IC}_{50} = 24.40 \pm 1.24$   $\mu\text{g}/\text{mL}$ ), ferrozine-based iron chelation ( $\text{IC}_{50} = 210.71 \pm 1.59$   $\mu\text{g}/\text{mL}$ ), and phenanthroline-based reducing power ( $\text{EC}_{50} = 560.18 \pm 20.47$   $\mu\text{g}/\text{mL}$ ).

Acute toxicity studies revealed no mortality or behavioral alterations, even at high doses (up to 5 g/kg). Biochemical assays showed a significant reduction in hepatic enzymes (ALP, AST, ALT), while creatinine levels remained stable, suggesting hepatoprotective activity without nephrotoxicity. At the 5 g/kg dose, oxidative stress biomarkers showed improved antioxidant defense: glutathione (GSH) levels were  $2.10 \pm 0.24$   $\mu\text{mol TNB}/\text{g}$  in kidneys and  $4.06 \pm 0.82$   $\mu\text{mol TNB}/\text{g}$  in liver tissue; malondialdehyde (MDA) levels were 43.6 nmol/g in kidney tissue and 34.87 nmol/g in liver. Catalase (CAT) activity reached  $5.85 \pm 0.33$  mmol/min/mg (kidney) and  $6.86 \pm 4.1$  mmol/min/mg (liver), while total protein content was  $4.56 \pm 0.26$  mg/mL in kidneys and  $6.24 \pm 0.55$  mg/mL in liver.

Gastric emptying and Intestinal transit evaluations showed that MHE at 100 mg/kg significantly inhibited gastric emptying (32.9%), whereas the 50 mg/kg dose showed no significant difference from the control (24.4%). Both doses exhibited similar significant inhibition effects on intestinal transit (13-14%). These findings support the potential therapeutic use of MHE as an antioxidant and hepatoprotective agent with gastrointestinal inhibition effects.

**Key words:** *Marrubium sp.*, antioxidant activity, gastric emptying, intestinal transit and acute toxicity.

## الملخص

هدفت هذه الدراسة إلى تقييم المحتوى الكيميائي النباتي، والنشاط المضاد للأكسدة، والسمية، وتأثير مستخلص الإيثانولي المائي لنبات (MHE) Marrubium hydroethanolic extract على معدلات إفراغ المعدة (GE) وحركة الأمعاء الدقيقة (IT). بلغت نسبة مردود الاستخلاص 21.66%. أظهرت التحاليل الكمية احتواء المستخلص على مستويات مرتفعة من المركبات النشطة حيويًا، شملت: عديدات الفينول ( $1.010 \pm 95$  ميكروغرام مكافئ حمض الغاليك/مليغرام)، الفلافونويدات ( $11.37 \pm 54.43$  ميكروغرام مكافئ كيرسيتين/مليغرام)، والدباغ ( $0.47 \pm 77.06$  ميكروغرام مكافئ حمض التانيك/مليغرام). أظهر مستخلص MHE خصائص مضادة للأكسدة عبر اختبارات متعدد: DPPH ( $2.09 \pm 98.86 = IC_{50}$  ميكروغرام/مل)، ABTS، ( $1.24 \pm 24.40 = IC_{50}$  ميكروغرام/مل)، Ferrozine based ion chelating activity ( $210.71 = IC_{50}$  ميكروغرام/مل)، phenantroline reducing power ( $20.44 \pm 560.18 = EC_{50}$  ميكروغرام/مل). أظهرت دراسة السمية الحادة عدم وجود وفيات أو تغيرات سلوكية حتى عند جرعات مرتفعة (حتى 5 غرام/كيلوغرام). كما أظهرت التحاليل البيوكيميائية انخفاضاً معنوياً في انزيمات الكبد (ALT, AST, ALP) بينما بقيت مستويات الكرياتينين مستقرة، ما يشير إلى تأثير وقائي للكبد دون سمية كلوية. عند جرعة 5 غرام/كيلوغرام، أظهرت مؤشرات الاجهاد التأكسدي تحسناً في النظام المضاد للأكسدة: الجلوتاثيون (GSH):  $0.24 \pm 2.10$  ميكرومول/TNB/غرام (الكلية) و  $0.82 \pm 4.06$  ميكرومول TNB/غرام (الكبد)، المالون (MDA):  $4.19 \pm 43.59$  نانومول/غرام (الكلية) و  $2.41 \pm 34.87$  نانومول/غرام (الكبد). نشاط إنزيم الكاتالاز (CAT):  $0.33 \pm 5.85$  ميليمول/دقيقة/مليغرام (الكلية) و  $4.1 \pm 6.86$  ميليمول/دقيقة/مليغرام (الكبد). محتوى البروتين الكلي:  $0.26 \pm 4.56$  ميلليغرام/مل (الكلية) و  $0.55 \pm 6.24$  ميلليغرام/مل (الكبد). أظهرت تقييمات إفراغ المعدة (Gastric emptying) وحركة الأمعاء الدقيقة (Intestinal transit) ان مستخلص (MHE) بجرعة 100 ملغ/كغ تثبيطاً معنوياً لإفراغ المعدة بنسبة (32.9%)، في حين أن جرعة 50 ملغ/كغ لم تظهر فرقاً معنوياً مقارنة مع المجموعة الشاهدة (24.4%). أما في ما يتعلق حركة الأمعاء الدقيقة، فقد أظهرت كلتا الجرعتين تأثيراً مثبطاً متشابهاً وبنسب متقاربة (13-14%). تدعم هذه النتائج الاستخدام العلاجي المحتمل لمستخلص Marrubium hydroethanolic extract كمضاد أكسدة و كمركب يتمتع بخصائص وقائية للكبد، إضافة إلى تأثيراته المثبطة على وظائف الجهاز الهضمي.

**الكلمات المفتاحية:** مستخلص (MHE) Marrubium hydroethanolic extract، النشاط المضاد للأكسدة، إفراغ المعدة، حركة الأمعاء الدقيقة والسمية.

## Liste of abbreviations

**ABTS:** 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)  
**AlCl<sub>3</sub>:** Aluminum chloride  
**ALP:** Alkaline Phosphatase  
**ALT:** Alanine Aminotransferase  
**AST:** Aspartate Aminotransferase  
**Atr:** Atropine  
**CAT:** Catalase  
**CMC:** Carboxymethylcellulose  
**Creat:** Creatinine  
**DPPH:** 2,2-Diphenyl-1-picrylhydrazyl  
**DTNB:** 5,5'-Dithiobis-(2-nitrobenzoic acid)  
**EC<sub>50</sub>:** Half maximal effective concentration  
**EDTA:** Ethylenediaminetetraacetic acid  
**FCR:** Folin-Ciocalteu reagent  
**GAE:** Gallic acid equivalent  
**GE:** Gastric emptying  
**GSH:** Reduced glutathione  
**H<sub>3</sub>PMo<sub>12</sub>O<sub>40</sub>:** Phosphomolybdic acid  
**H<sub>3</sub>PW<sub>12</sub>O<sub>40</sub>:** Phosphotungstic acid  
**HE:** Hydroethanol  
**HPLC:** High-performance liquid chromatography  
**IC<sub>50</sub>:** Half maximal inhibitory concentration  
**IT:** Intestinal transit  
**LD<sub>50</sub>:** Median lethal dose  
**MDA:** Malondialdehyde  
**MHE:** *Marrubium* hydroethanol extract  
**Na<sub>2</sub>CO<sub>3</sub>:** Sodium bicarbonate  
**NaOH:** Sodium hydroxide  
**NADPH:** Nicotinamide adenine dinucleotide phosphate (reduced form)  
**P:** Weight  
**QE:** Quercetin equivalent  
**rpm:** Revolutions per minute  
**ROS:** Reactive Oxygen Species  
**RNS:** Reactive Nitrogen Species  
**SD:** Standard deviation  
**SEM:** Standard error of the mean  
**TAE:** Tannic acid equivalent  
**TBA:** Thiobarbituric acid  
**TCA:** Trichloroacetic acid

## Liste of figures

<b>Figure 1:</b> Calibration curve of gallic acid for the determination of total polyphenol.....	5
<b>Figure 2:</b> Calibration curve of quercetin for the determination of total flavonoid content .....	6
<b>Figure 3:</b> Calibration curve of tannic acid for the determination of total tannin content. ....	7
<b>Figure 4:</b> 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reaction mechanism (Sadeer et al., 2020) .....	8
<b>Figure 5:</b> The DTNB–GSSG reductase recycling system (Ellman’s reaction) (A. R. Araujo et al., 2008). ..	11
<b>Figure 6:</b> Reaction of malondialdehyde (MDA) with 2 molecules of 2 thiobarbituric acid (TBA) (Jasim et al., 2021).....	13
<b>Figure 7:</b> Effect of Marrubium hydroethanolic extract on biochemical parameters .....	17
<b>Figure 8:</b> Effect of the hydroethanolic extract of Genus <i>Marrubium</i> at 2 and 5 g/kg on GSH levels in the liver and kidneys of rats. ....	19
<b>Figure 9:</b> Effect of <i>Marrubium</i> hydroethanolic extract on MDA levels.....	20
<b>Figure 10:</b> Effects of <i>Marrubium</i> hydroethanolic extract on Catalase levels.....	21
<b>Figure 11:</b> Effects of the hydroethanolic extract of <i>Marrubium</i> on total protein levels. ....	22
<b>Figure 12:</b> Effects of <i>Marrubium</i> hydroethanol extract on gastric emptying in mice .....	23
<b>Figure 13:</b> Effects of <i>Marrubium</i> hydroethanol extract on intestinal transit in mice. ....	24

## List of tables

<b>Table 1:</b> Physical characteristics and extraction yield of MHE .....	15
<b>Table 2:</b> Total polyphenol, flavonoid, and tannin content of the <i>genus Marrubium</i> .....	15
<b>Table 3:</b> IC <sub>50</sub> values for DPPH, ABTS, Ferrozine test and EC <sub>50</sub> value for Phenanthroline test of standard and MHE. ....	16
<b>Table 4:</b> Body weight of female rats untreated and treated with hydroethanolic extract of <i>Marrubium</i> .....	18
<b>Table 5:</b> Relative body weight of female rats' organs untreated and treated with hydroethanolic extract of <i>Marrubium</i> . ....	18

# *Introduction*