جامعة الشرق الأوسط
MIDDLE EAST UNIVERSITY
Amman - Jordan

Potential Effects of *Moringa Peregrina* Extract on International Normalization Ratio and Prothrombin Time in Normal and Warfarin Treated Animal Models

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جـــامـعـــة الـــشـرق الأوسـط
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التأثيرات المحتملة لمستخلص نبات مورينجا على عوامل تخثر الدم والميوعة في النموذج الحيواني العادي والمعالج بالوارفارين

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This Thesis, titled **"Potential effects of *Moringa peregrina* extract on International Normalization Ratio and Prothrombin Time in normal and warfarin treated animal models"** By Researcher **Omar Mohammed Majeed** Was Successfully Defended and Approved On 24-12-2025

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Acknowledgments

Writing this acknowledgment was more than an academic formality — it was a moment of honest reflection and deep gratitude.

To the one whose name has always stood beside mine, who walks ahead of my steps to clear the road… **my beloved father**.

And to the light of my life, the one whose prayers carried me forward, who gave her life so mine could grow… **my dear mother**.

To my siblings — my constant support and endless sources of strength and kindness — you hold my deepest love and appreciation.

To my friend, who lifted me in exhaustion, steadied me in falling, and shared every dream in success… thank you for always being there.

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My gratitude also extends to the discussion committee members for accepting to review my modest academic work.

Praise be to Allah, at the beginning and the end.

I pray that this work is beneficial, accepted, and written purely for His sake, contributing to academic and practical knowledge.

Omar Majeed

Dedication

To the absent one, who was never truly absent,

To my dear friend — Dr. Pharmacist Mohammed Khudair Abbas…

You have left this world, yet your place in our hearts remains untouched, unshaken, irreplaceable. Your departure carved a quiet pain deep in the soul — a pain that mirrors your presence — and gifted us a warm memory that time shall never cool nor erase.

To you, who taught us the language of kindness without uttering a word, whose mere existence was life itself, and whose leaving became our daily lesson in patience, yearning, and the heavy beauty of missing someone sincerely loved.

May Allah grant you mercy equal to the longing you left behind, equal to the prayers that still rise for you every day, and equal to the love that continues to bloom in our hearts because of you.

I write this for your blessed soul — hoping it reaches you there, in a place where nothing arrives except mercy. Let these words stand as a pulse of your memory, an echo of your name, and a reflection of a heart that still carries you gently within it.

May your soul rest in eternal peace.

Omar Majeed

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List of Abbreviations

Abbreviation	Full Term
aPTT	Activated Partial Thromboplastin Time
ASU	Applied Science Private University
B	Solvent B (Acetonitrile)
Bw	Body weight
C_1, C_2	Initial and final concentrations in dilution formula
COX	Cyclooxygenase
CYP	Cytochrome P450
CYP1A2	Cytochrome P450 isoenzyme 1A2
CYP2C9	Cytochrome P450 isoenzyme 2C9
CYP3A4	Cytochrome P450 isoenzyme 3A4
Ct	Healthy control: received no treatment
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EIC	Extracted Ion Chromatogram
ESI	Electrospray Ionization
FC	Folin–Ciocalteu
GA	Gallic Acid
GAE	Gallic Acid Equivalent(s)
HC	Healthy Control group
HPLC	High-Performance Liquid Chromatography
INR	International Normalized Ratio
LC/MS	Liquid Chromatography / Mass Spectrometry
MAE	Microwave-Assisted Extraction (also: Mean Absolute Error – context-dependent)
MEU	Middle East University
MoPI	Moringa oleifera Papain Inhibitor
m/z	Mass-to-Charge Ratio
MS/MS	Tandem Mass Spectrometry
Na_2CO_3	Sodium Carbonate

Abbreviation	Full Term
NMR	Nuclear Magnetic Resonance
OECD	Organization for Economic Co-operation and Development
ppm	Parts Per Million
PT	Prothrombin Time
QTOF	Quadrupole Time-of-Flight
Rpm	Revolutions Per Minute
RT	Retention Time
SD	Standard Deviation
TEAC	Trolox Equivalent Antioxidant Capacity
TM	Thrombomodulin
TOF	Time-of-Flight
TPC	Total Phenolic Content
UV–Vis	Ultraviolet–Visible (Spectrophotometry)
V_1, V_2	Initial and final volumes in dilution formula
W	Warfarin-only group
WHO	World Health Organization
UHPLC	Ultra-High Performance Liquid Chromatography
UHPLC–QTOF–MS/MS	Ultra-High-Performance Liquid Chromatography with Quadrupole Time-of-Flight Tandem Mass Spectrometry.

Potential effects of *Moringa pregrina* extract on International Normalization Ratio and Prothrombin Time in normal and warfarin treated animal models

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Abstract

Background: Given the increasing regional use of *Moringa*-based supplements in Jordan and the Middle East, the potential herb–drug interactions between *Moringa peregrina* and chronic medications including warfarin, is not fully investigated yet.

Aims: The present study aimed to investigate the phytochemical profile and anticoagulant potential of *M. peregrina* ethanolic leaf extract, with a focus on its effects on prothrombin time (PT), international normalized ratio (INR), and thrombomodulin (TM) in normal and warfarin-treated Wistar rats.

Methods: *M. peregrina* leaves ethanolic extract were prepared using the Soxhlet method. Phytochemical composition of the extract was investigated UHPLC–ESI–QTOF–MS/MS analysis, focusing on phenols and flavonoids. Antioxidant and total phenol content were measured using the DPPH and Folin-Cicalo methods. Serum levels of PT, INR and TM were evaluated in Wistar rat's prior and post treatment with warfarin. Kidney functions tests including alanine aminotransferase (ALT), creatinine, (Cr) and urea levels **(**U) were also evaluated.

Results: The total phenolic content was measured as gallic acid equivalent (8.96 mg/ g plant extract) with maximum DPPH radical scavenging assays of 77.95% at concentration of (0.5 mg/mL). UHPLC–ESI–QTOF–MS/MS analysis revealed the presence of rutin (32.86 ppm), chlorogenic acid (5.88 ppm), and varied fatty acids.

In vivo evaluation demonstrated that pre-administration of *M. peregrina* extract (500, 1000, and 1500 mg/kg Bw) for 10 days resulted in dose-dependent modulation of coagulation parameters. Significant lower effects in the mean INR values between study groups before ($F = 4.901$, $P = 0.011$) and after ($F = 20.05$, $P < 0.001$) warfarin administration were observed. Where a non-significant difference in the mean PT values between study groups before ($F = 2.36$, $P = 0.123$), but significantly lower ($F = 27.14$, $P = <0.001$) after warfarin administration was measured. There was a non-significant difference in the mean TM values between study groups before ($F= 1.14$, $P = 0.36$), but significantly increased after ($F= 3.47$, $P = 0.035$) warfarin administration. Furthermore, TM levels showed a significant positive correlation with PT values ($r = 0.9041$, $T= 4.23$, $P = 0.0134$) after warfarin treatment.

Biochemical analyses indicated that the extract exert hepatoprotective and nephroprotective effects after treatment with warfarin, as evidenced by non-significant changes between the study groups for levels of Cr ($F=2.292$, $P = 0.109$), but significant decrease in U levels ($F = 5.116$, $P = 0.0087$). Similarly, the ALT levels were significantly lowered ($F= 10.24$, $P= 0.0003$) compared to the control group.

Conclusion: The study underscores the clinical importance of monitoring patients using *Moringa*-based herbal products in combination with anticoagulant therapy. This will provide a scientific foundation for future pharmacokinetic and toxicological investigations.

Keywords: *Moringa peregrina*, phenols, warfarin, prothrombin time, international normalized ratio, thrombomodulin, antioxidant activity, herb–drug interaction, rutin, chlorogenic acid.

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الملخص

الخلفية: نظرًا للاستخدام الإقليمي المتزايد للمكملات الغذائية المستخلصة من المورينجا في الأردن والشرق الأوسط، لم تُدرس بعدُ التفاعلات العشبية الدوائية المحتملة بين المورينجا بيريجرينا والأدوية المزمنة، بما في ذلك الوارفارين.

الأهداف: هدفت هذه الدراسة إلى دراسة التركيب الكيميائي النباتي وإمكانية استخدام مستخلص أوراق المورينجا بيريجرينا الإيثانولي كمضاد للتخثر، مع التركيز على آثاره على زمن البروثرومبين (PT)، والنسبة المعيارية الدولية (INR)، والثرومبوموودولين (TM) في التجارب فئران ويستار السليمة والمعالجة بالوارفارين.

الطريقة: تم تحضير مستخلص أوراق المورينجا بيريجرينا الإيثانولي باستخدام طريقة سوكسليت. ودُرست التركيبة الكيميائية النباتية للمستخلص باستخدام تحليل UHPLC–ESI–QTOF–MS/MS، مع التركيز على الفينولات والفلافونويدات. كما تم قياس محتوى مضادات الأكسدة والفينول الكلي باستخدام طريقتي DPPH وFolin–Cicalo. تم تقييم مستويات PT وINR وTM في مصل فئران ويستار قبل وبعد العلاج بالوارفارين. كما تم تقييم اختبارات وظائف الكلى، بما في ذلك مستويات ألانين أمينوترانسفيراز (ALT) والكرياتينين (Cr) واليوريا (U).

النتائج: تم قياس إجمالي محتوى الفينول كمكافئ لحمض الغاليك (8.96 ملغم/غ من المستخلص النباتي) مع أقصى نسبة إزالة جذرية DPPH بلغت 77.95% عند تركيز (0.5 ملغم/مل). كشف تحليل UHPLC–ESI–QTOF–MS/MS عن وجود روتين (32.86 جزء في المليون) وحمض الكلوروجينيك (5.88 جزء في المليون) وأحماض دهنية متنوعة.

أظهر التقييم الحيوي أن الإعطاء المسبق لمستخلص *M. peregrina* (500،1000 ، و1500 ملغم/كغم من وزن الجسم) لمدة 10 أيام أدى إلى تعديل يعتمد على الجرعة لمعايير التخثر. لوحظ انخفاض ملحوظ في متوسط قيم INR بين مجموعات الدراسة قبل (F = 4.901، P = 0.011) وبعد (F = 20.05، P <0.001) من إعطاء الوارفارين. كما تم قياس فرق غير ملحوظ في متوسط قيم PT بين مجموعات الدراسة قبل (F = 2.36، P = 0.123)، ولكنه أقل بشكل ملحوظ (F = 27.14، P = <0.001) من إعطاء الوارفارين. كما كان هناك فرق غير ملحوظ في متوسط قيم TM بين مجموعات الدراسة قبل (F = 1.14، P = 0.36)، ولكنه زاد بشكل ملحوظ بعد (F = 3.47، P = 0.035) من إعطاء الوارفارين. علاوة على ذلك، أظهرت مستويات TM ارتباطًا إيجابيًا ملحوظًا مع قيم (r = 0.9041 PT، T = 4.23، P = 0.0134) بعد العلاج بالوارفارين. أشارت التحاليل الكيميائية الحيوية إلى أن المستخلص يُظهر تأثيرات وقائية للكبد والكلى بعد العلاج بالوارفارين، كما يتضح من التغيرات غير المعنوية بين مجموعات الدراسة في مستويات الكروم (F = 2.292، P = 0.109)، مع انخفاض ملحوظ في مستويات اليوريا (U = 5.116، P = 0.0087). وبالمثل، انخفضت مستويات إنزيم ألانين أمينو ترانسفيراز (ALT) بشكل ملحوظ (F = 10.24، P = 0.0003) مقارنةً بالمجموعة الضابطة.

الخلاصة: تُؤكد الدراسة على الأهمية السريرية لمراقبة المرضى الذين يستخدمون منتجات عشبية قائمة على المورينجا مع العلاج بمضادات التخثر. سيوفر هذا أساسًا علميًا للدراسات الدوائية والسمية المستقبلية.

الكلمات المفتاحية: المورينجا بيريجرينا، الفينولات، الوارفارين، زمن البروثرومبين، النسبة الدولية المُوَحَّدة، الثرومبوموودولين، النشاط المضاد للأكسدة، التفاعل بين الأعشاب والأدوية، الروتين، حمض الكلوروجينيك.

Chapter One
Background and Problem Statement

1. Introduction

1.1 Background of the Study

Warfarin is widely used as anticoagulant agent (Greenblatt & von Moltke, 2001). However, the safe use of warfarin requires extreme caution due to its interaction with multiple types of foods, drugs, and herbs, in addition to its narrow therapeutic range (Divya & Abiram, 2025).

The effect of warfarin is monitored via the prothrombin time (PT) and international normalized ratio (INR) test, which are a standardizes laboratory tests (Souza, 2024). Departure from the therapeutic range of INR leads to real risks such as bleeding or the formation of blood clots(Souza, 2024).

Thrombomodulin (TM) analysis involves measuring TM levels in the body, which is a type-I transmembrane protein that is mainly expressed on endothelial cells and plays important roles in many biological processes. Measurement of the levels of circulating TM has great potential as a biomarker for diagnosis and tracking of different diseases. Studies found thar TM levels were elevated in several pathological conditions associated with endothelial dysfunction such as cardiovascular, inflammatory, infection, and metabolic diseases (Giri et al., 2024).

1.2 Herbal Medicine and Drug Interactions

Herbal medicine is expanding significantly around the world, with the World Health Organization reporting that between 70% and 80% of the population among developing countries rely on traditional medicine for health care (WHO, 2004). Herbs are often combined with prescription medications, especially anticoagulants, raising concerns about interactions that may affect the absorption, distribution, and metabolism of the drugs (Tan & Lee, 2021).

Many Herbal treatments proved to interact with warfarin pharmacokinetically or pharmacodynamically via coagulation cascade or platelet function (Abebe, 2019), or interact with warfarin metabolism (Piersma et al., 2020), such as garlic, ginkgo, ginger,

and turmeric. These interactions may result in significant changes in INR values (Greenblatt & von Moltke, 2001; aszczych et al., 2024).

1.5 *Moringa oleifera* and Hemostatic Activity

Multiple studies have documented the effects of *M. oleifera* as an antioxidant and anti-inflammatory, along with its lipid-lowering and anti-bleeding properties (Pareek et al., 2023; Xu et al., 2019; Zheng et al., 2023). Moreover, leaf extract showed the ability to inhibit platelet aggregation, due to the presence of enzymes like thrombin and plasmin in the leaves extract, which may affect the clotting process (Pareek et al., 2023; Saputri et al., 2022).

Despite these results, the evidence is still mixed; some studies have reported anticoagulant properties (Ahmed et al., 2012), indicate that high concentrations may shorten clotting time. While other studies indicate the possibility of a procoagulant effect (Tan & Lee, 2021; Ebhohon & Miller, 2022).

1.6 *Moringa* and Warfarin Interaction Potential

Reports by (Łaszczych et al., 2024) showed that *Moringa* extracts processes an inhibitory effect on the cytochrome's enzymes mainly CYP2C9 and CYP3A4, which may reduce the elimination of warfarin. Moreover, Tan & Lee, (2021) reveeled that some herbs, such as *Moringa*, may bind to serum albumin, leading to increasing the bioavailability of warfarin and hence increasing its therapeutic effect.

These effects may result in an unpredictable increase in INR values, increasing the risk of bleeding. For this reason, WHO (2004) recommend that herbal supplements should be investigated for safety and risk of drug interactions.

1.7 *Moringa peregrina*: An Underexplored Medicinal Tree

M. Peregrina, which is grown in Jordan, Saudi Arabia, and East Africa, is widespread among medicinal plants that share phytochemical properties with *M. oleifera* (Hamada et al., 2024; Hassan et al., 2025). This plant is widely used traditionally as anti-diabetes, anti-inflammation, and liver protective agent (Senthilkumar et al., 2018). However, its effect on blood clotting processes or its potential interaction with warfarin have not been evaluated.

1.8 Justification and Significance of the Study

With the popular use of *M. peregrina* as an herbal drink and nutritional supplement

in Jordan and the Middle East, the potential for *M. peregrina* to affect coagulation parameters whether used independently or in conjunction with warfarin represents an incomplete but critically and clinically important area of research. Since it has a similarity in pharmacological properties to *M. oleifera*, it is likely to possess anticoagulant or procoagulant effects, However, the scientific evidence available so far is not sufficient to confirm or deny this hypothesis.

This study is justified by:

- Previous studies have not been conducted to determine the effect of *M. peregrina* on TM, INR and PT coagulation indices.
- This plant is distinguished by its ethnopharmacological importance and the widespread use of its traditional use locally.
- Knowledge of drug interactions between herbs and anticoagulants is crucial to ensuring therapeutic safety and minimizing risks.

1.9 Scope and Limitations

1.9.1 Scope

- The extract is prepared using ethanolic Soxhlet extraction method.
- Study procedures include phytochemical analysis of phenolic compounds, experiments on animal models, and statistical analysis of coagulation indices.
- The study investigates the effect of *M. peregrina* leaf extract on coagulation factors (TM, INR and PT) values in normal and warfarin-treated Wistar rats.
- The study investigates the effect of *M. peregrina* leaf extract on Kidney and liver functions (ALT, Cr and U) values in normal and warfarin-treated Wistar rats.

1.9.2 Limitations

- Measured parameters include only TM, INR and PT, to the exclusion of other important coagulation indicators such as aPTT, D-dimer.
- Neither the difference between acute administration and chronic administration of the extract, nor the influence of seasonal variations on the chemical composition of the plant, are addressed in this study.

1.10 Operational Definitions

- *M. peregrina*: A drought-tolerant medicinal tree found in the south of Jordan, used for medicinal purposes, this study investigated its leaf extracts.
- Phyto Chemical analysis of plants: laboratory tests to identify active compounds such as flavonoids and phenols.
- Warfarin: An anticoagulant by inhibiting vitamin K, and its effect is monitored using INR and PT.
- TM: Indicate the health of endothelial cells and used to study its roles in anticoagulation, inflammation, and disease.
- INR: Standardized test to measure blood clotting time to adjust warfarin dose.
- PT: A test that measures the activity of the extrinsic pathway of blood clotting by prothrombin time.
- Anticoagulant effects: Increased INR or PT time means a decreased ability of the blood to clot.
- Thrombogenic effects: Decreased INR or PT time indicates an increased risk of thrombosis.
- Interactions between herbs and medications: An interaction that can affect the effectiveness of a medication when used with herbs.
- Biological studies: Research conducted on living organisms, often animals, to study drug effects such as liver and kidney functions.

Chapter Two

Theoretical Literature and Previous Studies

2. Literature Review

2.1 Introduction

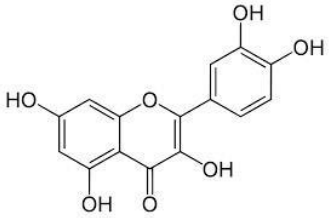
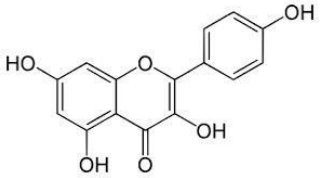
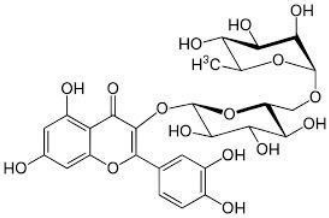
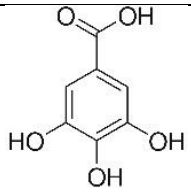
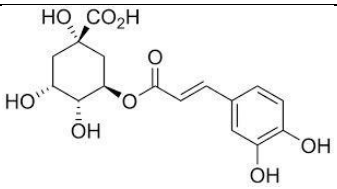
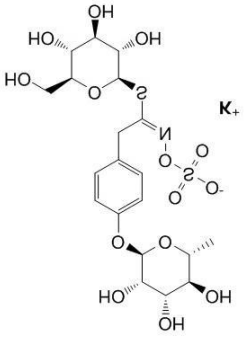
Herbal medicine forms the cornerstone of many traditional and integrative healthcare systems around the world, as synthetic drugs such as warfarin continue to be used to treat blood clotting disorders. Therefore, potential interactions between *M. peregrina* extract and warfarin have become a vital topic in pharmacological research.

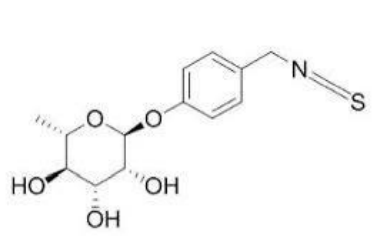
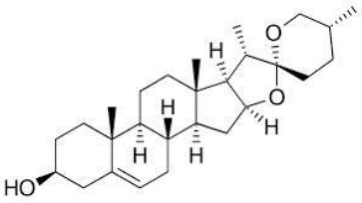
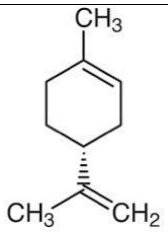
2.2. Phytochemical Profile of *Moringa oleifera*

Phytochemical investigations of *M. oleifera* have revealed a wide spectrum of biologically active compounds. Reviews by (Krishnaiah et al., 2011a) and (Kou et al., 2018a) emphasize that compounds such as **isothiocyanates** (**moringin**), **quercetin**, **kaempferol**, **rutin**, and **chlorogenic acid** contribute significantly to the plant's pharmacological profile, particularly its **antioxidant**, **anti-inflammatory**, **blood-modulating** properties and anticarcinogenic potential (Fahey et al., 2001).

Table (2-1) presents selected compounds identified previously in *M. oleifera* extracts, including their chemical class, structure, detection method, and supporting literature.

Table (2-1): Major Phytochemical Compounds in *Moringa oleifera*

Compound	Class	Chemical Structure	Detection Method	Reference
Quercetin	Flavonol		HPLC, LC–MS, UV–Vis	Anwar et al., 2007; Taher et al., 2012
Kaempferol	Flavonol		HPLC, LC–MS	Sreelatha & Padma, 2009
Rutin	Flavonoid glycoside		UHPLC–QTOF–MS/MS	Coppin et al., 2013
Gallic Acid	Phenolic acid		UV–Vis (Folin–Ciocalteu), HPLC	Krishnaiah et al., 2011
Chlorogenic Acid	Phenolic acid		HPLC, MS	Verma et al., 2009
Glucomoringin	Glucosinolate		LC–MS/MS	Fahey et al., 2001

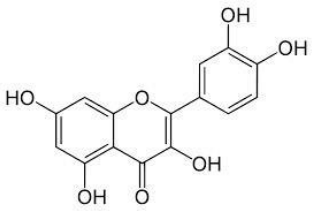
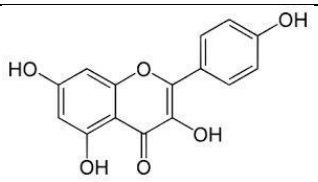
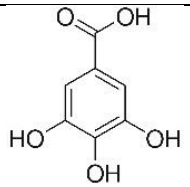
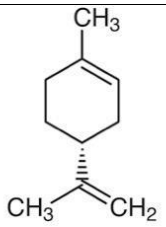
Compound	Class	Chemical Structure	Detection Method	Reference
Moringin	Isothiocyanate		MS, NMR	Fahey et al., 2001
Diosgenin	Steroidal saponin		GC–MS	Taher et al., 2012
Limonene	Monoterpenoid		GC–MS	Krishnaiah et al., 2011

2.3 Phytochemical and Medicinal Profile of *Moringa peregrina*

Although *M. oleifera* has been extensively studied for its phytochemical constituents and pharmacological activities, its least-known relative, *M. peregrina*, remains under-investigated—particularly with respect to its potential impact on hemostasis and anticoagulation.

Ethnobotanically studies and reviews focus on the use of *M. peregrina* traditionally as medicine for treating fever, inflammation, and microbial infections (Al-Dabbas, 2017; Fitriana et al., 2018). In addition, Hussein et al., (2022) have studied the extracts of leaves for their nephroprotective effects in mice model. Similarly, Lali et al. (2023) have studied the extract protective effect on reproductive toxicity caused by the drug gentamicin. These studies linked these effects to the extract phytochemical content. Table (2-2) presents selected compounds identified in *M .peregrina*, including their chemical class, structure, detection method, and supporting literature.

Table (2-2): Major Phytochemical Compounds in *M. peregrina*

Compound	Class	Chemical Structure	Detection Method	Reference
Quercetin	Flavonol		HPLC, LC–MS, UV–Vis	(Anwar et al., 2007)
Kaempferol	Flavonol		HPLC, LC–MS	(Sreelatha & Padma, 2009)
Gallic Acid	Phenolic acid		UV–Vis (Folin–Ciocalteu), HPLC	(Krishnaiah et al., 2011b)
Limonene	Monoterpenoid		GC–MS	(Krishnaiah et al., 2011b)

2.4 Pharmacological Actions of Moringa species

2.4.1 Antiplatelet and Hemostatic Modulation

M. oleifera has proved a great ability to inhibit platelet aggregation, as reported by (Arabshahi-Delouee et al., 2009) for its aqueous leaf extract. Findings showed reduce platelet aggregation in a dose-dependent effects via **inhibiting the cyclooxygenase (COX) pathway**. (Pareek et al., 2023; Sharifi-Rad et al., 2022; Tan et al., 2015) also observed **thrombin and plasmin-like enzymatic activity** for the extract, suggesting a mechanism for its effect in delaying clot formation via enzymatic reaction.

Previously experimental animal models reported similar findings, recording either prolongation of blood clotting time (Singnap et al., 2019), or significant hematology (Nurhayati et al., 2023), or prolonged activated partial thromboplastin time (aPTT) (Cotabarren et al., 2021) in Wistar rats treated with ethanolic *Moringa* seed and leaf extracts.

2.4.2 Coagulant or Procoagulant Effects

Reviews of herb–warfarin interactions consistently highlight enhanced anticoagulant responses when warfarin is co-administered with polyphenol-rich plant extracts (Łaszczych et al., 2024). Thes effects were proposed as follows:

- **Pharmacokinetic inhibition of warfarin metabolism**: Polyphenolic compounds, notably rutin or chlorogenic acid, are known to interfere with hepatic cytochrome P450 isoenzymes responsible for warfarin clearance. Rutin or chlorogenic acid, have demonstrated inhibitory effects on CYP2C9, CYP3A4 and CYP1A2 (Sousa et al., 2021), leading to slower warfarin metabolism and higher plasma concentrations **(**Greenblatt & von Moltke, 2005**).**
- **Pharmacodynamic synergy**: Rutin or chlorogenic acid were found to inhibit platelet aggregation by blocking thromboxane (aseu et al., 2004) or reduces thrombin activity and suppresses fibrin polymerization, contributing to anticoagulant effects (Huang et al., 2023; L. Wang et al., 2022).

Mechanistically, several other explanations can be proposed:

1. **Vitamin K-like or coagulation-supportive phytochemicals:**

Some phytoconstituents in *Moringa* species (notably phylloquinone derivatives and certain flavonoid conjugates) can mimic vitamin K_1 or modulate its reductase cycle, potentially offsetting warfarin-induced inhibition of vitamin K epoxide reductase (VKOR) (Cotabarren et al., 2021). This action would reduce INR, counteracting excessive anticoagulation. Although *M. peregrina* is less studied in this regard, the structural similarity of its flavonoids (rutin, kaempferol glycosides) suggests possible interference with the VKOR pathway.

2. Antioxidant preservation of hepatic function:

Rutin or chlorogenic acid have been shown to protect hepatocytes against oxidative stress and preserve vitamin K metabolism enzymes, indirectly maintaining prothrombin synthesis (Tian et al., 2025). Improved hepatic redox balance could enhance clotting factor production, leading to relatively lower INR values.

3. Homeostatic (hemo) endothelial modulation:

While these compounds can exhibit anticoagulant tendencies at high concentrations, low to moderate doses may enhance endothelial nitric oxide balance and reduce abnormal thrombin activation without causing sustained hypocoagulation (Wang et al., 2021). Thus, a mild INR reduction may represent physiological normalization rather than pharmacologic antagonism.

2.5 Herb-Drug Interactions: Warfarin and Moringa

2.5.1 Mechanistic Pathways of Interaction

The study performed by Łaszczych et al. (2024) showed that an ethanolic extract of *M. oleifera* leaves inhibit the enzymes CYP3A4, CYP1A2, and CYP2C9, which play an important role in the metabolism of warfarin, leading to increased blood concentrations and possible bleeding.

Previous studies such as those by (Greenblatt & von Moltke, 2005) have confirmed the effect of herbal inhibition of enzymes and competition for serum protein binding, which raise levels of active warfarin.

Moringa has shown a complex effect on thrombomodulin levels (TM), with some studies (Ahmed et al., 2012) showing it can dissolve existing blood clots by releasing a fibrinolytic protease, while other research (Sander et al., 2020) suggests certain extracts may accelerate blood clotting, and a case report links long-term use to pulmonary embolism (Ebhohon & Miller, 2022).

The specific effects depend on the part of the plant used and the concentration, with some compounds potentially having an anticoagulant effect and others a procoagulant effect (Kunwar et al., 2023). Therefore, the effect of *Moringa* on TM levels is not straightforward and requires further investigation.

2.6 Research Gaps Identified

Despite the significant research on *M. oleifera*, key gaps remain:

- To date; in vivo studies targeting the effect of *M. peregrina* on TM, INR or PT have not been conducted.
- Published studies discussing the interaction between *M. peregrina* and warfarin are not available.
- The mechanistic pathways of any anticoagulant or procoagulant effects via *M. peregrina* remain undiscovered.

These gaps form the basis for the current study.

Chapter Three

Methodology (Methods and Procedures)

3. Methodology

3.1 Materials and Equipment Used

A list of materials, reagents, and instruments used in the current study is provided in **Table (3-1).**

Table (3-1): List of Materials and Equipment Used

Item	Manufacturer / Source
Moringa peregrina dried leaves	AlRayhan, Amman, Jordan
Soxhlet extractor	Standard laboratory glassware (Generic)
Ethanol (99%)	Sigma-Aldrich or equivalent
Rotary evaporator	Heidolph or equivalent , china
UV–Vis Spectrophotometer (Model: U-1800)	Hitachi, UK
UHPLC–MS/MS System (Impact II, Elute UHPLC)	Bruker Daltonik GmbH, Bremen, Germany
Bruker Solo 2.0 C-18 UHPLC Column	Bruker Daltonik GmbH
CoaguChek® XS PT Test System	Roche Diagnostics, Germany
Microplate Reader	Thermo Fisher Scientific or equivalent
Analytical Balance	Mettler Toledo or equivalent
Centrifuge	Hettich Universal 320
Vortex Mixer	IKA or equivalent
Freeze Dryer / Lyophilizer	Martin Christ or equivalent
Isoflurane (anesthetic agent)	Bayer or equivalent
Eppendorf tubes (heparinized)	Eppendorf AG, Germany
Trolox (standard antioxidant)	Sigma-Aldrich
DPPH (radical reagent)	Sigma-Aldrich
Gallic Acid (standard phenolic)	Sigma-Aldrich
Quercetin (standard flavonoid)	Sigma-Aldrich
Aluminum chloride ($AlCl_3$)	Sigma-Aldrich
Sodium carbonate (Na_2CO_3)	Sigma-Aldrich
Formic acid (LC/MS grade)	Sigma-Aldrich
Methanol, Acetonitrile, Deionized Water (LC/MS)	Sigma-Aldrich
Dimethyl sulfoxide (DMSO, analytical grade)	Sigma-Aldrich
Wistar albino rats	Animal House, Faculty of Pharmacy, ASU

3.2 Research Design

This study was performed on a randomized controlled animal model of Wistar albino rats. *M. peregrina* leaf extracts are administered in multiple serial doses, either alone or combined with warfarin, in attempt to evaluate extract treatment effects on coagulation

indices including TM, PT and INR values.

3.3 Study Site and Duration

The experimental work was conducted as follows:

•Extraction and phytochemical analysis:
Middle East University (MEU), February–June 2025.
•Animal study and sample collection:
Applied Science Private University (ASU), June–July 2025.
•Statistical analysis and thesis writing:
MEU, June–August 2025.

3.4 Ethical Considerations

In this study, all experiments performed on the animal model were proceeded according to approved ethical standards by the Animal Ethics Committee at ASU-Pharmacy (Approval Number [2025-PHA-31]). The procedures also adhered to the 2001 OECD guidelines and the ARRIVE principles, which aim to ensure ethical treatment of laboratory animals and reduce pain and stress during the experiment.

3.5 Collection and Preparation of *Moringa peregrina* Plant Materials

3.5.1 Collection of Plant Material

The *M. peregrina* leaves were purchased from a local herbal distributor in the capital Amman, Jordan, during the first quarter of the year 2025.The plant was identified by Dr. Reem Issa, (Professor of phytochemistry, faculty of pharmacy, Middle East University, Amman, Jordan), as *Moringa peregrina* (Forssk.) Fiori, based on its distinctive vegetative leaves morphology as described by (Hamada et al., 2024).

Figure X illustrates the leaflets are imparipinnate leaf structure, elliptic to obovate leaflet shape, entire margins, and thick, coriaceous texture, measuring approximately 2–3 cm in length, with entire margins, a rounded apex, and a cuneate base. They exhibit a thick, coriaceous texture and a glabrous, dark green surface, consistent with xerophytic adaptation.

A voucher specimen of *M. peregrina* was prepared following standard herbarium procedures and deposited in the Herbarium at the faculty of pharmacy, Middle East

University, Amman, Jordan, for future reference.

Leaves were ground into a fine powder using a mechanical blinder, then were stored in sealed containers and stored at room temperature, until used for extraction.

Figure No (3.1). Morphological characteristics of *Moringa peregrina* leaves and branching habit

Identification based on morphological characteristics of *M. peregrina*. The figure shows the aerial parts and branching habit (left and middle panels) and individual leaflets (right panel) illustrating the imparipinnate leaf structure, sparse leaflet arrangement, elliptic to obovate leaflet shape, entire margins, and thick, coriaceous texture. Scale bars are shown in centimeters. (Hamada et al., 2024).

3.5.2 Extraction method

For the preparation of the ethanolic extract of *M. peregrina* leaves, Soxhlet extraction technique was used. Briefly, a proportion equivalent 100 grams of dried and ground leaves were placed inside a thimble loaded in a Soxhlet device, and 500 ml of 99% pure ethanol was used as extraction solvent. Using a mantle heater, the mixture was heated at 60 °C with continuous reflux of ethanol over the plant material for a duration of 6 to 8 hours.

After the extraction was completed, the solution was filtered to eliminate impurities, and then the extract was concentrated using a rotary evaporator operating at 90 rpm at 60 °C and under reduced pressure to evaporate the solvent. The final thick, semi-solid extract was weighed to calculate the percent yield and kept in sterile opaque containers at 4 °C for later use in further animal model experiments and phytochemical analysis.

3.6 Phytochemical Screening

3.6.1 Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the *M. peregrina* ethanolic leaf extract was determined using the Folin–Ciocalteu (FC) colorimetric assay, following the procedure described by Sadeghi et al. (2015), with slight modifications. The assay relies on the reduction of the FC reagent by phenolic compounds under basic conditions, leading to the formation of a blue chromophore measurable at 765 nm.

3.6.1.1 Preparation of Reagents and Standards

A stock solution of *M. peregrina* ethanol extract was prepared at a concentration of 10 mg/mL in 1% ethanol. Serial dilutions were then prepared for analysis at serial concentrations of (0.25, 0.5, and 1.0 mg/mL).

Gallic acid (GA) was used as the standard phenolic compound for constructing the calibration curve. A series of GA standards were prepared in the concentration range of (1–100 µg/mL).

3.6.1.2 Assay Procedure

For sample or standard solution, 1.0 mL of extract or GA solution was pipetted into a clean test tube. Then, 5.0 mL of distilled water and 1.0 mL of freshly prepared Folin–Ciocalteu reagent were added. The mixture was gently mixed and allowed to stand in the dark for 5 minutes. 1.0 mL of 7.5% sodium carbonate (Na_2CO_3) solution was then added to initiate the color development. The tubes were covered with aluminum foil to protect from light and incubated at room temperature (~25°C) for 30–60 minutes.

The absorbance of each reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer (Hitachi U-1800, UK). TPC was calculated by substituting the absorbance values into the linear equation to obtain gallic acid equivalents (GAE, µg/mL).

3.6.2 Determination of Antioxidant Activity (Using DPPH assay)

The antioxidant capacity of *M. peregrina* ethanolic extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

The DPPH assay relies on the reduction of the stable violet-colored DPPH radical to a yellow-colored non-radical form (DPPH-H) in the presence of an antioxidant. The decrease in absorbance at 517 nm reflects the radical scavenging ability of the extract. The assay is standardized against Trolox, a water-soluble analog of vitamin E, and results are expressed in % inhibition and **µM Trolox equivalents (TEAC)** (Brand-Williams et al., 1995; Thaipong et al., 2006).

3.6.2.1 Preparation of Trolox Standards

Trolox stock solution was serially diluted in methanol to obtain working standards of: 0.005, 0.01, 0.05, 0.1, 1.0, 2.0, 3.0, and 5.0 µg/mL. All solutions were freshly prepared and stored in the dark.

3.6.2.2 Preparation of Plant Extract Samples

The *M. peregrina* ethanolic extract was prepared at an initial concentration of 10 mg/mL, and serial dilutions were made (1, 0.5, 0.1 µg/mL) to cover a range of antioxidant activities.

3.6.2.3 DPPH Assay Procedure

1. 1.0 mL of either extract dilution or Trolox standard was added to 5.0 mL of 0.1 mM DPPH solution in methanol in a test tube.
2. The mixture was vortexed and incubated at room temperature (25 °C) in the dark for 30 minutes.
3. Absorbance was recorded at 517 nm using a UV–Vis spectrophotometer.
4. A control (DPPH + methanol only) and blank (methanol only) were used for calibration.

3.6.2.4 Calculation of Antioxidant Activity

- **% Radical Scavenging Activity**

For each sample:

$$\%\text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Where:

- $A_{control}$ is the absorbance of the DPPH solution without sample
- A_{sample} is the absorbance after addition of extract or Trolox

3.7 Identification of Plant Constituents Using UHPLC–ESI–QTOF–MS/MS

The phytochemical constituents of *M. peregrina* extract were analyzed using ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC–ESI–QTOF–MS/MS). The goal was to identify phenolic and flavonoid compounds based on their mass-to-charge ratios (m/z) and retention times, based on a library of authenticated standards.

3.7.1 Chemicals and Reagents

- Dimethyl sulfoxide (DMSO), acetonitrile, methanol, water, and formic acid were all of LC/MS grade (Sigma-Aldrich, Germany).
- Reference standards of selected phytochemicals were used for the accurate identification of compounds by comparison of their retention times and exact m/z values.

3.7.2 Sample Preparation

- A 20 mg of the dried ethanolic extract was first dissolved in 200 µL of DMSO, followed by 1800 µL of methanol, and vortexed until fully solubilized.
- From this solution, 250 µL was transferred and diluted with 1.0 mL of methanol.
- The mixture was centrifuged at 4000 rpm for 2 minutes to remove any particulate matter.
- 1.0 mL of the clear supernatant was transferred into an autosampler vial.
- 3.0 µL of the prepared sample was injected into the UHPLC system for analysis.

3.7.3 Instrumentation and Analytical Conditions

The chromatographic analysis was performed on a Bruker Daltonik Impact II ESI-QTOF mass spectrometer coupled with a Bruker Elute UHPLC system (Bruker Daltonik GmbH, Bremen, Germany).

Chromatographic Conditions:

- **Column:** Bruker Solo 2.0 C-18 UHPLC column (100 × 2.1 mm, 2.0 µm)
- **Flow rate:** 0.51 mL/min
- **Column temperature:** 40°C
- **Injection volume:** 3.0 µL
- **Mobile phases:**
- **Solvent A:** Water with 0.01% formic acid
- **Solvent B:** Acetonitrile
- **Gradient profile:** 80% to 95% B over 35 minutes
- **Run time:** 35 minutes in positive mode and 35 minutes in negative mode

Mass Spectrometry Settings:

- **Ionization source:** Apollo II ion funnel electrospray ionization (ESI)
- **Capillary voltage:** 2500 V
- **Nebulizer pressure:** 2.0 bar
- **Drying gas (nitrogen):** 8 L/min
- **Drying temperature:** 200°C
- **Mass accuracy:** < 1 ppm
- **Resolution:** 50,000 FSR (Full Sensitivity Resolution)
- **TOF repetition rate:** up to 20 kHz

3.7.4 Compound Identification

Each analyte was identified based on:

- Accurate mass (m/z) from high-resolution TOF-MS.
- Retention time after chromatographic separation.
- Comparison with spectral libraries provided by Bruker Daltonik.

Both positive and negative ionization modes were employed to maximize compound detection, particularly for phenolic acids and flavonoids, which may ionize differently under varying conditions.

3.7.5 Quantitative analysis

3.7.5.1 Quantitative analysis for Rutin

The quantification of rutin in the plant extract was carried out using a standard calibration curve method, at two concentrations of the plant extract.

Stock Solution Preparation: 20 mg of extract was dissolved in 200 µL of dimethyl sulfoxide (DMSO) and 1800 µL of methanol. The procedure involved a two-step dilution protocol to prepare the sample for HPLC analysis. This resulted in a total dilution factor of 10 for the final sample analyzed. Ultra High-performance liquid chromatography (UHPLC–ESI–QTOF–MS/MS UHPLC–ESI–QTOF–MS/MS) was used to determine the rutin content in the plant extract based on rutin standard calibration curve.

3.7.5.2 Quantitative analysis for chlorogenic acid

The quantification of chlorogenic acid in the plant extract was carried out using a standard calibration curve method. The procedure involved a dilution protocol to prepare the sample for HPLC analysis.

Standard chlorogenic acid Calibration curve was performed using three serial concentrations solutions: 0.5 ppm, 1.0 ppm, and 5.0 ppm in methanol with DMSO.

3.8 Experimental Animals

3.8.1 Animal Model Selection

The study used Wistar albino rats known for their genetic uniformity and ease of handling, as well as their consistent physiological response in pharmacological and hematological studies (Krubaa & Yogitha, 2024). These mice are suitable for assessing anticoagulant activity, especially through PT and INR tests, to study the effects of interactions between herbs and drugs (OECD, 2001).

The sample included 32 male Wistar rats, approximately 60 days of age, body weighing (Bw) between 180 and 200 grams, obtained from the Animal House at the Faculty of Pharmacy, Applied Science Private University (ASU), Amman, Jordan.

Rats Bw for each animal was recorded at baseline and at the end of the experiments**.** Before the experiments began, the rats were housed in metabolic cages with a temperature controlled between 21 and 22 °C and a 12-hour light/dark cycle for one week to allow them to acclimate.

3.8.2 Housing and Acclimatization

All rats were housed under standard laboratory conditions in clean, ventilated cages:

- Temperature: 21–23°C
- Humidity: 50–60%
- Photoperiod: 12-hour light/dark cycle
- Diet: Standard rat chow
- Water: Provided ad libitum

3.9 Experimental Groups and Treatment Protocol

3.9.1 Group Assignment

The animals were randomly assigned into four experimental groups (n = 8 per group), as shown in Table (3-2). Group assignments were randomized using a computer-generated list to avoid selection bias.

Table (3-2): Experimental Group Design

Group (n=8)	Treatment
Ct	Healthy control: received no treatment
Dx	500 mg/kg extract orally for 9 days, + warfarin 2 mg/kg on Day 10
D2x	1000 mg/kg extract orally for 9 days, + warfarin 2 mg/kg on Day 10
D3x	1500 mg/kg extract orally for 9 days, + warfarin 2 mg/kg on Day 10

3.9.2 Administration Method

Extract was freshly reconstituted in distilled water prior to administration. Warfarin (2 mg/kg) was administered 16 hours before the final blood collection on Day 10, based on protocols described (Zhang et al., 2023).

M. peregrina extract and warfarin were administered by oral gavage using a sterilized gastric tube to ensure accurate dosing. Administration volume was calculated based on animal body weight of (1 mL/100 g BW). Body weight (BW) and water intake were monitored at baseline and the end of the experiments.

3.10 Blood Collection and Coagulation Assays

3.10.1 Sampling Timepoints

Blood samples were collected at three timepoints:

- T0 (Baseline): Prior to any treatment on Day 0
- T1: After the 9-day extract administration period
- T2: 16 hours post-warfarin administration (Day 10)

3.10.2 Sample Collection Technique

Blood sample of (0.2 mL) was drawn from the retro-orbital plexus using microcapillary tubes under light isoflurane anesthesia based on the method described by(van Herck et al., 2001). Samples were placed in heparinized Eppendorf tubes and immediately analyzed.

3.10.3 PT and INR Measurement

PT (in seconds) and INR scores were automatically recorded using the CoaguChek® XS PT Test (Roche Diagnostics, Germany), which has been validated for rodent blood testing (Y. Sander et al., 2021).

3.10.4 Thrombomodulin (TM) Measurement

Serum TM in (mg/dL) were measured using ELISA kits (Cat No. MBS265115), San Diego, USA) according to the method described by (Lin et al., 2017), blood samples had taken at the beginning and end of the experiments to assess serum TM levels.

3.10.5 Alanine aminotransferase (ALT), creatinine, (Cr) and urea levels (U) Measurements

At the end of the study, serum biochemical parameters were measured as described by (Abdullah et al., 2024)including levels of ALT, Cr, and U were determined using commercial reagent kits: ALT liquicolor, auto-CREATININE liquicolor, UREA liquiUV, (Multipurpose reagents).

Human Fully Automatic Huma Star 200 Biochemistry analyzers (serial number 21190008001, Germany) were used.

3.11 Variables of the Study

Independent Variables

- Dose of *M. peregrina* extract: 500, 1000, and 1500 mg/kg
- Warfarin administration: present or absent

Dependent Variables

- Prothrombin Time (PT) in seconds
- International Normalization Ratio (INR)
- Thrombomodulin (TM) serum level (mg/dL)

These values were selected as primary indicators of coagulation status and anticoagulant effect, as recommended by (Shikdar et al., 2025).

3.12 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics, version 27.0:

- Descriptive statistics were reported as mean ± standard deviation (SD).
- Independent-samples t-tests were conducted to compare control versus treated groups (HC vs. W).
- One-way ANOVA was used to compare the mean values among three the group.
- Dunnett's post-hoc test was applied to determine differences between experimental groups and the warfarin control group (W).
- A p-value < 0.05 was considered statistically significant.

Chapter Four

Study Results

4. Results

4.1 Extraction Yield

The extraction yield of *M. peregrina* ethanolic extract was calculated as follows:

- Extract weight (W_e) = 8.089 g
- Plant material used (W_p) = 100 g

Extraction Yield (%) = 8.089%

This indicates acceptable extraction efficiency using ethanol Soxhlet extraction method.

4.2 Phytochemical Screening

4.2.1 Determination of Total Phenolic Content (TPC)

4.2.1.1 Development of GA Calibration curve

A calibration curve was constructed from the absorbance values of gallic acid standards.

Table (4-1) displays the absorbance values, and **Figure (4-1)** shows the resulting curve.

Table (4-1): Gallic acid standard curve data

Concentration (µg/ml)	Absorbance
1	0.02
5	0.056
25	0.244
50	0.472
75	0.71
100	0.937

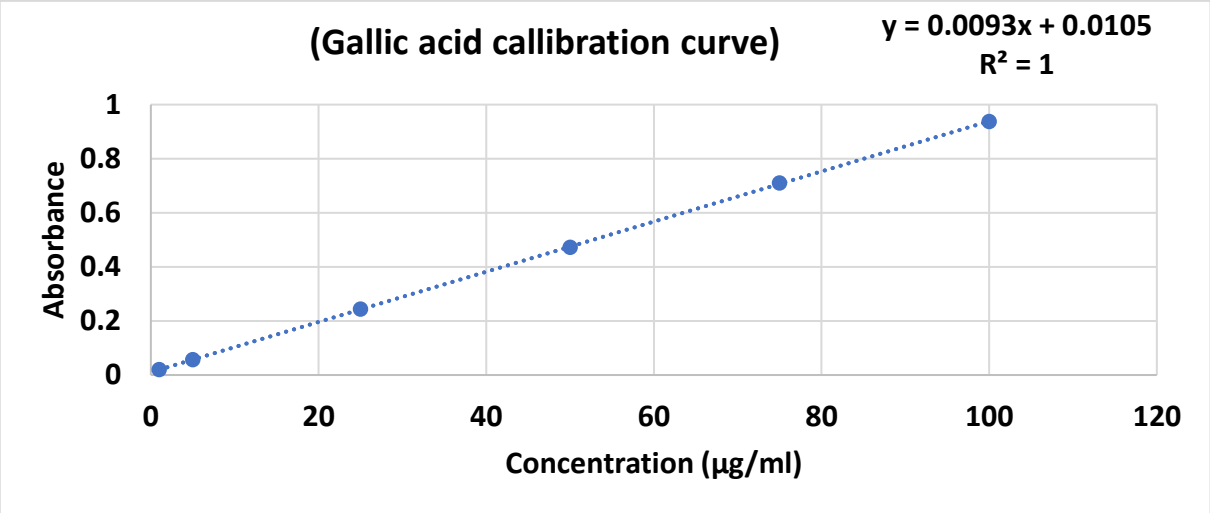


Figure (4-1): Gallic acid calibration curve

Figure (4-1): Gallic acid calibration curve ($R^2 = 1$).

The linear regression equation was:

y = 0.0093x + 0.0105, where:

- y = absorbance at 765 nm
- x = gallic acid concentration (µg/mL)

4.2.1.2 Determination of Total phenols content (TPC)

Using Folin-Cicalo spectroscopic methods, the absorbance values was recorded at 765 nm for different plant extract concentrations, measured in triplicate. Mean values and standard deviations are reported in **Table (4-2).**

Table (4-2): Absorbance (Mean ± SD) of TPC in *M. peregrina* extracts (n=3)

Concentration (mg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	Mean ± SD
0.10	0.046	0.046	0.046	0.046 ± 0.000
0.25	0.133	0.132	0.132	0.132 ± 0.001
0.50	0.271	0.271	0.272	0.271 ± 0.001
10.00	0.851	0.851	0.855	0.852 ± 0.002

Using the regression equation (y = 0.0093x + 0.0105), absorbance values were converted into GAE concentrations. Total phenolic content was then calculated and expressed as mg GAE/ g of extract.

The total phenolic content was calculated as 8.96 mg GAE / g plant extract, indicating a relatively high content of polyphenolic compounds in the *M. peregrina* leaves.

4.2.2 Determination of Antioxidant Activity (DPPH Assay)

Results are presented as mean absorbance, % inhibition, and calculated Trolox equivalent antioxidant capacity (TEAC).

4.2.2.1 Development of Trolox Calibration curve

Findings of Trolox DPPH assay are shown in **Table 4-3**. The data show a consistent decline in absorbance as Trolox concentration increases, indicating a dose-dependent antioxidant activity.

Table (4-3): Absorbance values of Trolox at increasing concentrations used for TEAC calibration in the DPPH assay.

Trolox concentration (µg/ml)	Absorbance
0.005	0.261
0.01	0.268
0.05	0.26
0.1	0.258
1	0.213
2	0.162
3	0.111
5	0.01

A standard calibration curve was constructed based on the absorbance reduction of Trolox solutions at known concentrations, as illustrated in **Figure (4-2).**

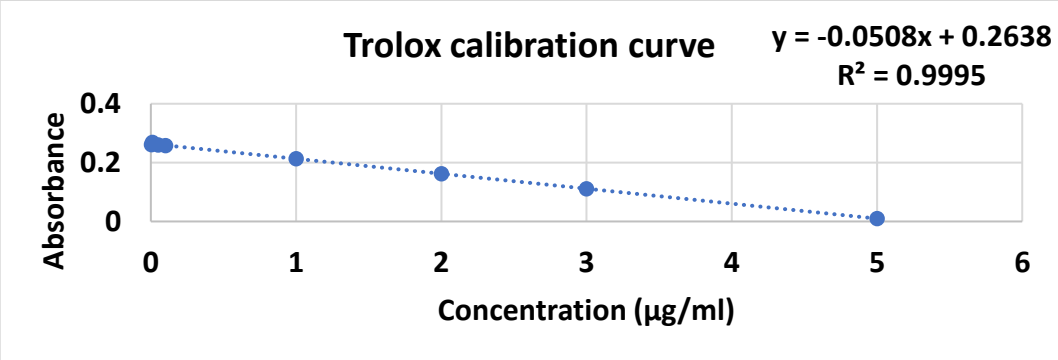


Figure (4-2): Calibration curve of Trolox (R^2 = 0.9995).

A typical linear regression from Trolox standards yielded the equation:

$y = –0.0508x + 0.2638$ $(R^2 = 0.9995)$

Where: y = absorbance at 517 nm, and x = Trolox concentration in µg/ml

This equation was used to calculate antioxidant capacity of the extract as equivalent to Trolox (TEAC), in triplicate and reported as (mean ± SD).

4.2.2.2 Determination of plant extract antioxidant activity

Using the DPPH assay method, the absorbance readings for various plant extract concentrations and the control sample are summarized in **Tables (4-4 and 4-5).**

Table (4-4): Absorbance values of *M. peregrina* extract at different concentrations

Extract Concentration (µg/mL)	Absorbance T1	Absorbance T2	Absorbance T3	Mean ± SD
0.10	0.373	0.366	0.364	0.368 ± 0.005
0.50	0.057	0.058	0.058	**0.058 ± 0.001**
1.00	0.105	0.106	0.106	0.106 ± 0.001
10.00	0.232	0.229	0.238	0.233 ± 0.005

Table (4-5): Control absorbance (n = 3)

Control			
Absorbance T1	**Absorbance T2**	**Absorbance T3**	**Average**
0.267	0.267	0.267	**0.267**

The % inhibition corresponding as Trolox equivalents were calculated and summarized in **Table (4-6).**

Table (4-6): Antioxidant activity of *M. peregrina* extract using DPPH assay method.

Extract Concentration (mg/mL)	% Inhibition
0.10	–39.92%
0.50	77.95%
1.00	59.70%
10.00	11.41%

Results showed that *M. peregrina* extract exhibited dose-dependent antioxidant activity, with maximum activity observed at 0.5 mg/ mL. Anomalies observed at the lowest dilution (0.10 mg/ mL) may be due to color interference or pipetting errors and warrant re-evaluation.

4.3 UHPLC–MS/MS Identification

4.3.1 Compounds detected using Positive Ion Mode

Findings revealed the presence of eight compounds detected in the positive ion mode based on their peak intensity. These compounds are listed in **Table (4-7).**

Table (4-7): Major Compounds Detected in Positive Ion Mode

RT (min)	m/z	Ion Type	Compound Name	Formula	Intensity
0.58	127.0391	[M+H]+	5-Hydroxy methylfurfural	$C_6H_6O_3$	11,474
0.61	160.0369	[M+Na]+, [M+K]+	Trigonelline	$C_7H_7NO_2$	40,728
0.72	118.0863	[M+H]+	L-Valine	$C_5H_{11}NO_2$	21,374
0.77	132.1020	[M+H]+	L-Leucine	$C_6H_{13}NO_2$	18,956
1.23	166.0863	[M+H]+, [M+Na]+	L-Phenylalanine	$C_9H_{11}NO_2$	13,896
9.58	611.1600	[M+H]+, [M+Na]+	**Rutin**	$C_{27}H_{30}O_{16}$	29,214
26.3	279.2313	[M+H]+	α-Linolenic acid	$C_{18}H_{30}O_2$	24,254
29.1	263.2365	[M+H-H_2O]+, [M+H]+	Pinolenic acid	$C_{18}H_{32}O_2$	16,818

4.3.2 Compounds detected using Negative Ion Mode

In negative ionization mode, the extract was particularly rich in **polyphenolic acids** and **unsaturated fatty acids**, as shown in **Table (4-8)**.

Table (4-8): Major Compounds Detected in Negative Ion Mode

RT (min)	m/z	Ion Type	Compound Name	Formula	Intensity
27.45	277.2170	$[M-H]^-$	γ-Linolenic acid	$C_{18}H_{30}O_2$	327,554
28.65	279.2323	$[M-H]^-$	9Z,12Z-Linoleic acid	$C_{18}H_{32}O_2$	183,382
29.67	255.2327	$[M-H]^-$	Palmitic acid	$C_{16}H_{32}O_2$	90,594
1.49	337.0927	$[M-H]^-$	4-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	38,886
1.31	266.0898	$[M-H]^-$	Adenosine	$C_{10}H_{13}N_5O_4$	1,512
1.10	353.0875	$[M-H]^-$	**Chlorogenic acid**	$C_{16}H_{18}O_9$	34,032
3.70	137.0246	$[M-H]^-$	Protocatechualdehyde	$C_7H_6O_3$	7,442
0.51	191.0561	$[M-H]^-$	Quinic acid	$C_7H_{12}O_6$	33,922
28.24	253.2170	$[M-H]^-$	Palmitoleic acid	$C_{16}H_{30}O_2$	17,438

4.3.3 Extracted Ion Chromatograms (EICs)

The flavonoid compounds rutin or chlorogenic acid are compounds widely recognized for their antioxidant and anticoagulant properties, which have been previously identified in *M. peregrina* extract (Abdullah et al., 2024). Therefore, further investigations into these two compounds were performed as shown in Figures 4-3 and 4-4, presenting EICs and Mass spectrum for rutin or chlorogenic acid, respectively

In the EIC, the peak appears at ~9.58 minutes corresponds to m/z=611.16 $[M+H]^+$, confirming the presence of rutin in *M. peregrina* extract. For mass spectrum in the negative ion mode, showing a peak at ~1.10 min corresponds to m/z= 353.10 $[M-H]^-$, confirms the presence of chlorogenic acid in *M. peregrina* extract.

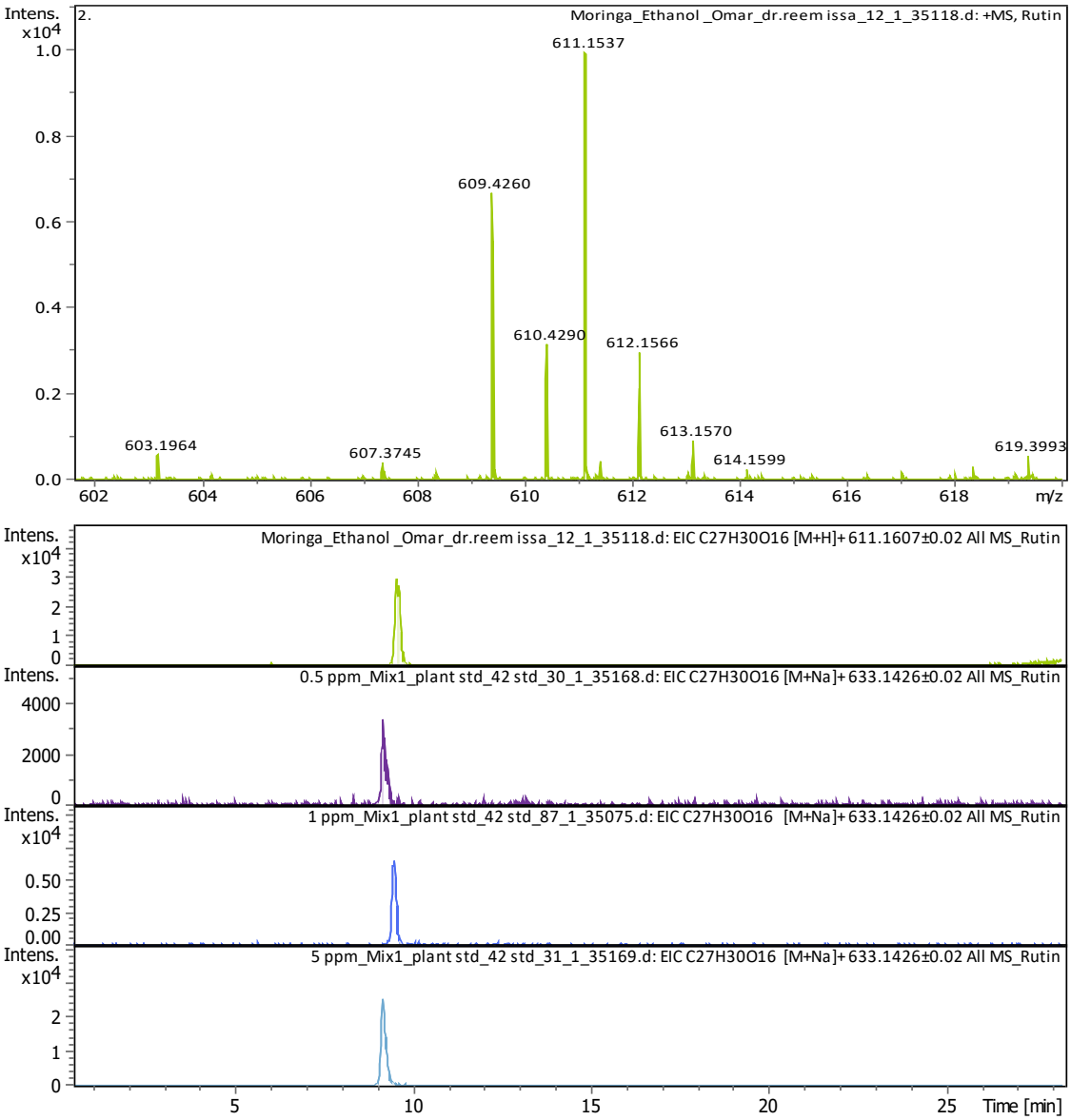


Figure (4-3): Extracted Ion Chromatogram for Rutin in positive ion mode

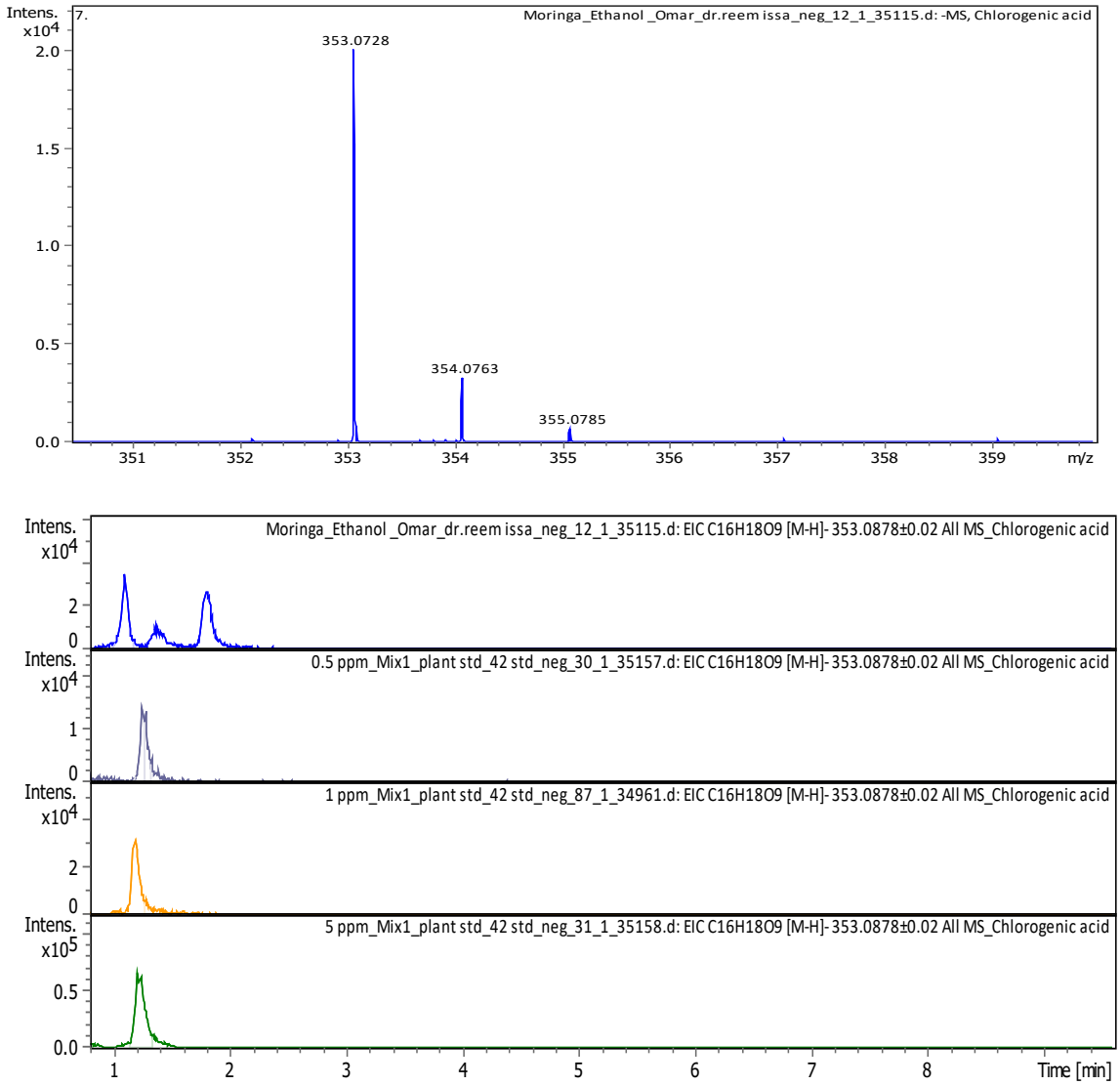


Figure (4-4): Extracted Ion Chromatogram for Chlorogenic acid in negative ion mode

4.3.4 Quantification of Rutin

Results including the standard calibration curve, regression equation, and quantitative data, are presented in **Table (4-9)** and **Figure (4-5).**

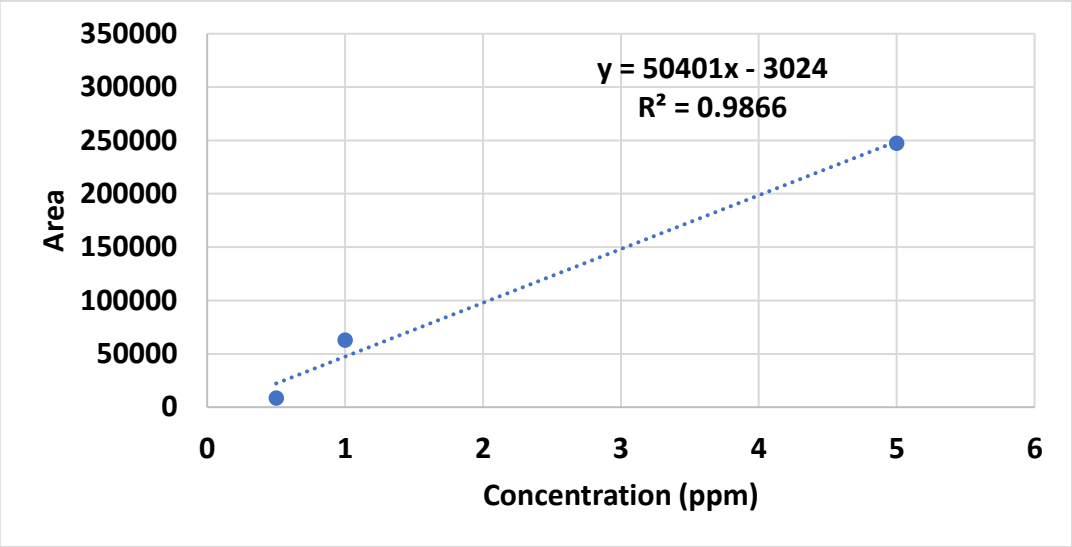


Figure (4-5): Calibration curve for Rutin Standard

Table (4-9): Concentration and peak area values of rutin standards and in *M. peregrina* extract

Sample	Concentration (ppm)	Area
Rutin Standard	5	247,282
Rutin Standard	1	62,658
Rutin Standard	0.5	8,593
M. peregrina extract	—	162,596

The following regression equation was obtained based on peak area vs. concentration:

$y = 50401x – 3024$

Where:

- y = peak area
- x = concentration (ppm)

Quantitative Results

The measured peak area for rutin in the diluted solution of the plant extract was 162,596, which corresponds to a concentration of 3.286 ppm, based on the calibration curve equation ($y = 50401x – 3024$). Considering the total dilution factor of 10, the final concentration of rutin in the original extract was calculated as:

Final Rutin Concentration=3.286 ppm×10=32.86 ppm

This quantification highlights the presence of rutin as a significant flavonoid in *M. peregrina*, reinforcing its phytochemical and pharmacological value.

4.3.5 Quantification of Chlorogenic Acid

The results, including the standard calibration curve, regression equation, and quantitative data, are presented in **Table (4-10)** and **Figure (4-6).**

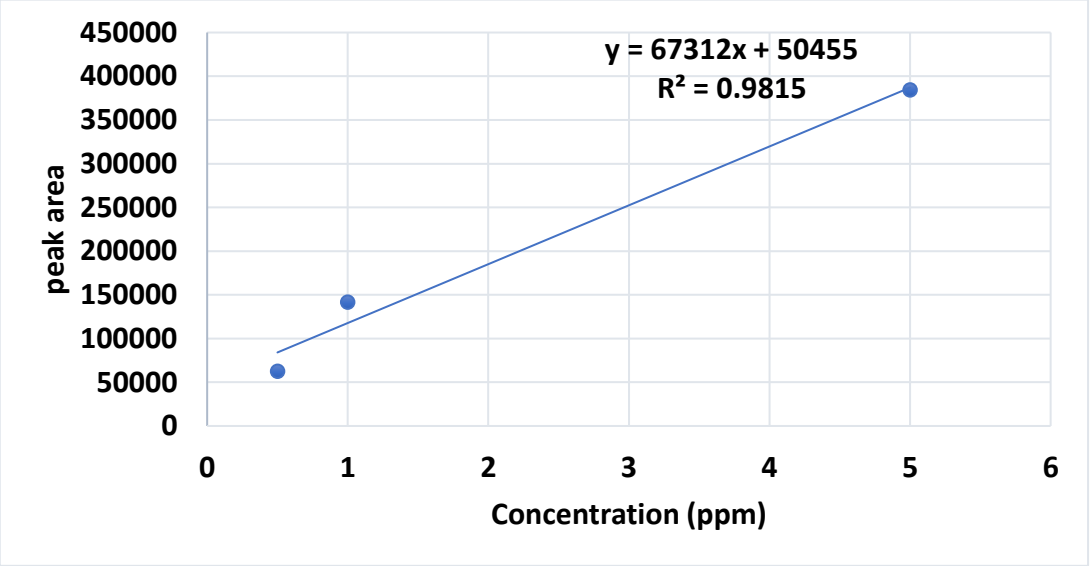


Figure (4-6): Calibration curve for chlorogenic acid Standard

Table (4-10): Concentration and Peak Area for Chlorogenic acid and in *M. peregrina* Extract

Sample	Concentration (ppm)	Area
Chlorogenic acid standard	5	384347
Chlorogenic acid standard	1	141758
Chlorogenic acid standard	0.5	62785
M. peregrina extract	---	129648.4

The following regression equation was obtained based on peak area vs. concentration:

y = 67312x –50455

Where:

- y = peak area

- x = concentration (ppm)

Quantitative Results

The chromatographic analysis of the diluted sample yielded a peak area of 129,648.4 for chlorogenic acid. which corresponds to a concentration of 1.176 ppm, based on the calibration equation (y = 67312x –50455). Considering the total dilution factor of 5, the final concentration of chlorogenic acid in the original extract was calculated as:

Final Chlorogenic Acid Concentration = 1.176 ppm × 5 = 5.88 ppm

4.4 Experimental Animal model

4.4. 1 Changes in body weight

At baseline, no significant difference in the mean BW was observed between study groups (F= 0.171, P = 0.914).

At the end of the experiment (10 days), significant changes in BW were observed in Ct and D2X study groups. In Ct group, BW significantly increased (T = 4.2, P = 0.025), whereas the mean BW values were significantly decreased in D2X (T = -6.9, P = 0.006). However, there were no significant differences in the mean final BW in Dx or in D3X as shown in Table (4-11).

Table (4-11): The means BW changes at baseline and end of the experiment (10 days).

Body Weight (gram)						
Group	**Baseline**		**End**		**T**	**P**
	Mean	**± SD**	**Mean**	**± SD**		
Ct	216.3	8.9	222.5	8.6	4.2	0.025
Dx	215.8	11.6	214.3	12.8	-1.7	0.181
D2x	216.8	6.3	212.8	7	-6.9	0.006
D3x	222.3	19.7	218.3	16.9	-1.7	0.182
F	0.171		0.401			
P	0.914		0.755			

Abbreviations: Ct, healthy control rats. Dx, healthy animals, were orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina*. D2x, healthy animals, was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina*. D3x, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina*

NOTE: F: variation between sample means/variation of study groups for ANOVA

test at the end of experiment. T-test, Paired t-test is used to determine if there is any significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (*n* = 8 for each group).

4.4.2 Changes in INR values before and after warfarin administration

There was a significant difference in the mean INR values between study groups before (F = 4.901, P = 0.011) and after (F = 20.05, P <0.001) warfarin administration. Before warfarin administration, mean INR in D2x group was insignificantly lower than in control group and other experimental groups (P > 0.05) as presented in Table (4-11).

After 24hrs of warfarin administration, Post-hoc multiple comparisons by Dunnett's test indicated that the mean INR values for all study groups were changed. All extract-administrated groups (Dx, D2x, and D3x) have shown significant lower mean INR values than in Ct group as presented in Table (4-11). These findings indicated that the plant extract, irrespective of the dose- dependent association, has a potential coagulating effect

Table (4-12): Final means differences in INR values between study groups before and after 24 h of warfarin administration (n=6).

INR (Score)						
Group	**EBW**		**EAW***		**T**	**P**
	Mean	**± SD**	**Mean**	**± SD**		
Ct	0.912	0.012	7.5	1.5	-29.36	< .00001
Dx	0.913	0.043	5.8	0.2	36.08	< .00001
D2x	0.833	0.052	5.8	0.7	37.16	< .00001
D3x	0.917	0.041	5.6	3.9	34.81	< .00001
F	4.901		20.05			
P	0.011		<0.001			

Abbreviations: **INR** , international normalized ratio ; **EBW**, PT values at the end of experiment (10 days) before Warfarin (W) administration , **EAW***, PT values at the end of experiment (10 days) after Warfarin administration ; **Ct**, healthy control rats. **Dx**, healthy animals , was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina.* **D2x**, healthy animals , was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina*. **D3x,** healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina.*

NOTE: F: variation between sample means/variation of study groups for ANOVA test at the end of experiment. T-test, Paired t-test is used to determine if there is any significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (n = 8 for each group).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$when compared with Ct group by post-hoc multiple comparisons (Dunnett's Multiple Comparisons Test).

4.4.3 Changes in prothrombin time before and after warfarin administration

There was a significant difference in the mean PT values between study groups after 24hrs of warfarin administration (F = 27.14, P = <0.001). Post-hoc multiple comparisons by Dunnett's test indicated that the mean PT values for (Dx, D2x, and D3x) was significantly lower than in control group (Ct) group as presented in **Table (4-13).** At day 10 of extracts administration, but before warfarin administration, there was no significant difference in mean PT values between study groups (F = 2.36, P = 0.123).

Table (4-13): Final means differences in serum PT values between study groups before and after 24 h of warfarin administration (n=8).

PT (Second)						
Group	**EBW**		**EAW***		**T**	**P**
	Mean	**± SD**	**Mean**	**± SD**		
Ct	11	0.14	94	3.5	42.9	0.00003
Dx	10.7	0.4	68.8	1.7	57.62	0.00001***
D2x	10.3	0.3	69.7	6.2	15.85	0.00054***
D3x	10.3	0.6	65.9	4.7	21.35	0.00022***
F	2.36		27.14			
P	0.123		<0.001			

Abbreviations: **PT,** prothrombin time (seconds); **EBW**, PT values at the end of experiment (10 days) before Warfarin (W) administration, EAW*, PT values at the end of experiment (10 days) after Warfarin administration; **Ct**, healthy control rats. **Dx**, healthy animals, was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina.* **D2x**, healthy animals , was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina* . **D3x**, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina.*

NOTE: F: variation between sample means/variation of study groups for ANOVA test at the end of experiment. T-test, Paired t-test is used to determine if there is any

significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (n = 8 for each group).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$when compared with Ct group by post-hoc multiple comparisons (Dunnett's Multiple Comparisons Test).

4.4.4 Changes in Thrombomodulin (TM)

At the end of the experiment (10 days), there was no significant difference in TM levels between study groups (F= 1.14, P = 0.36). After 24 hours of Warfarin administration, significant changes in mean TM levels were observed between study groups (F= 3.47, P = 0.035). Paired T-test has shown that mean TM levels was significantly increased in Dx group after warfarin administration (T= 3.53, P = 0.017). Post-hoc multiple comparisons by Dunnett's Multiple Comparisons Test indicated that the mean TM levels for (D3x) was the highest mean levels and it significantly different from Dx (P = 0.026) as presented in Table 4.13.

Table 4.14. Final means differences in serum TM Levels (ng/dL) between treated animal groups before and after 24 h of warfarin administration (n=6).

TM (ng/dL)						
Group	**EBW**		**EAW***		**T**	**P**
	Mean	**± SD**	**Mean**	**± SD**		
Ct	5.1	0.71	4.8	0.8	-0.7	0.514
Dx	3.9	0.49	5.2	1.1	3.53	*0.017
D2x	4.2	1.1	4.3	0.8	0.324	0.759
D3x	4.8	1.9	6.3	1.3	1.003	0.362
F	1.14		3.47			
P	0.36		0.035			

Abbreviations: **TM**, thrombomodulin ; **EBW**, PT values at the end of experiment (10 days) before Warfarin (W) administration , **EAW***, PT values at the end of experiment (10 days) after Warfarin administration ; **Ct**, healthy control rats. **Dx**, healthy animals , was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina0* **D2x**, healthy animals , was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina.* **D3x**, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina*

NOTE: F: variation between sample means/variation of study groups for ANOVA test at the end of experiment. T-test, Paired t-test is used to determine if there is any significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (n = 6 for each group).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$when compared with Dx group and D3X group by post-hoc multiple comparisons (Dunnett's Multiple Comparisons Test).

4.4.5 The correlation between mean PT values and serum TM levels before and after administration of warfarin.

Before warfarin administration and after 10 days of extracts administration, no significant correlation between mean PT and TM values was observed. After warfarin administration **(EAW*)**, significant positive correlation was noted between PT and TM levels in D2x group only (r = 0.9041, T= 4.23, P = 0.0134) as shown in **Table (4.15)**

Table.(4.15). The correlation between mean PT values and serum TM levels in different study groups before and after administration of warfarin.

Stage	Test	Group			
		Ct	**Dx**	**D2x**	**D3x**
EBW	**R**	0.768	0.633	**-0.3707**	**-0.6015**
	T	2.401	1.634	**-0.7983**	**-1.5059**
	P	0.074	0.178	0.4694	0.2066
EAW*	**R**	**-0.4574**	**-0.2256**	**0.9041**	**-0.13**
	T	**-1.0287**	**-0.4631**	**4.2303**	**-0.262**
	P	0.3617	0.6673	*0.0134	0.806

Abbreviations: **PT**, prothrombin time (seconds); **EBW**, PT values at the end of experiment (10 days) before Warfarin (W) administration, **EAW***, PT values at the end of experiment (10 days) after Warfarin administration; **Ct**, healthy control rats, **Dx**, healthy animals, was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina.* **D2x**, healthy animals, was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina.* **D3x**, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina.*

NOTE: r , coefficient correlation between study groups T-test, statistical test for the correlation coefficient. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ when r test refers to is significant correlation between PT and TM mean values.

4.5 Assessment of the effect of administration Plant extract on kidney function

4.5.1. Changes in Serum Cr level

Findings revealed a significant change in mean Cr levels at the end of plant extract administration period of 10 successive days, before warfarin administration (F=3.393, P = 0.0243). At all treatment doses, these groups showed a significant increase in Cr levels when compared with the control study group (P <0.001). The highest significant change was observed in D3X study group. While at the period of treatment and after warfarin administration (**EAW*), No s**ignificant changes between the study groups (F=2.292, P = 0.109) were observed (Table 4-15).

Table (4-16): Final means differences in serum creatinine Levels (mg/dL) between treated animal groups before and after 10 days of extracts administration (n=8).

Cr (mg/dL)						
Group	**Baseline**		**End**		**T**	**P**
	Mean	**± SD**	**Mean**	**± SD**		
Ct	0.69	0.05	0.74	0.034	0.87	0.424
Dx	0.79***	0.07	0.84	0.06	1.754	0.139
D2x	0.78***	0.06	0.75	0.07	-0.984	0.370
D3x	0.81***	0.08	0.77	0.12	1.003	0.362
F	3.893		2.292			
P	0.0243		0.109			

Abbreviations: **Cr**, creatinine, prothrombin time (seconds); **EBW**, PT values at the end of experiment (10 days) before Warfarin (W) administration, EAW*, PT values at the end of experiment (10 days) after Warfarin administration; **Ct**, healthy control rats, **Dx**, healthy animals, was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina.* **D2x**, healthy animals, was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina.*

D3x, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina.*

NOTE: F: variation between sample means/variation of study groups for ANOVA test at the end of experiment. T-test, Paired t-test is used to determine if there is any significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (n = 6 for each group).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$when compared with Ct by post-hoc multiple comparisons (Dunnett's Multiple Comparisons Test).

4.5.2. Changes in Serum urea level

Findings revealed that serum levels of urea change during the study period. During the period of treatment before warfarin administration (EBA) showed no significant difference between study groups (F=2.997, P = 0.055). At the stage after warfarin administration (EAW), has shown significant change in urea levels between different treatment groups (F = 5.116, P = 0.0087). Post-hoc multiple comparisons by Dunnett's Multiple Comparisons Test potentially linked to significant decrease of urea levels in D2x when compared with Ct and Dx, P = 0.0154; P = 0.0137, respectively. Finally, significant increase in mean urea levels was observed at the end of EAW* stage in Dx group (T = 4.015, P = 0.0154).

Table (4.17). Final means differences in serum urea levels (mg/dL) between treated animal groups before and after 10 days of extracts administration (n=6).

Urea (mg/dL)						
Group	**EBW**		**EAW***		**T**	
	Mean	**± SD**	**Mean**	**± SD**		
Ct	36.83	2.46	40.33	2.8	2.55	0.051
Dx	33.25	3.99	40.45	4.4	4.015	*0.015
D2x	36.33	3.72	*33.13	4.7	-0.194	0.111
D3x	39.42	3.92	38.58	2.3	-0.366	0.729
F	2.997		5.116			
P	0.055		0.0087			

Abbreviations: **EBW**, U values at the end of experiment (10 days) before Warfarin (W) administration, **EAW***, U values at the end of experiment (10 days) after Warfarin administration; **Ct**, healthy control rats, **Dx**, healthy animals, was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina.* **D2x**, healthy animals, was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of

M. peregrina. **D3x**, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina.*

NOTE: F: variation between sample means/variation of study groups for ANOVA test at the end of experiment. T-test, Paired t-test is used to determine if there is any significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (n = 6 for each group).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$when compared with Ct and Dx groups by post-hoc multiple comparisons (Dunnett's Multiple Comparisons Test).

4.5.3 Changes in Serum ALT levels

At the end of stage EBA, ANOVA test has shown significant mean serum ALT difference between study groups (F=3.761, P = 0.0273). Mean ALT levels were significantly lower in D2x and D3X study groups than in Ct group. At EAW* stage, Mean ALT levels were significantly elevated (108,2 ± 37.7 vs 67.38 ± 18.6, T= 3.186, P= 0,024). Compared with the changes in Ct group, all plant extract groups have shown significant lower mean ALT levels than in Ct group (Dx, p=0.0026; D2x, p=0.00026; D3x, p=0.0047, respectively). Finally, warfarin administration significantly elevat ed ALT levels in Ct (T = 3.186, P = 0.024) and DX3 (T = 4.207, P = 0.008). However, the effect of plant extract, irrespective of the dose- dependent association, has potential ameliorating effect against warfarin administration as noted by changes in ALT levels Table 4.18.

Table (4.18). Final means differences in serum ALT levels (IU/L) between animal groups before and after warfarin administration (n=8).

Serum ALT Levels (IU/L)						
Group	**EBW**		**EAW***		**T**	**P**
	Mean	**± SD**	**Mean**	**± SD**		
Ct	67.38	18.6	108.2	37.7	3.186	*0.024
Dx	57.53	10.5	**59.6	3.9	0.464	0.662
D2x	49.86	3.72	***47.78	7.4	0.502	0.637
D3x	47.76	5.7	**62.6	12.8	4.207	*0.008
F	3.761		10.24			
P	0.0273		0.0003			

Abbreviations: **ALT**, alanine aminotransferase; **EBW**, PT values at the end of experiment (10 days) before Warfarin (W) administration, EAW*, PT values at the end of experiment (10 days) after Warfarin administration; **Ct**, healthy control rats, **Dx**, healthy animals, was orally administered for 10 days at doses of 500 mg/kg of leaves extract of *M. peregrina.* **D2x**, healthy animals, was orally administered for 10 days at doses of 1000 mg/kg of leaves extract of *M. peregrina.* D3x, healthy animals, was orally administered for 10 days at doses of 1500 mg/kg of leaves extract of *M. peregrina.*

NOTE: F: variation between sample means/variation of study groups for ANOVA test at the end of experiment. T-test, Paired t-test is used to determine if there is any significant difference in BW at baseline and end of the experiment for the same study. Results are represented as Mean ± SD (n = 6 for each group).

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$when compared with Ct and Dx groups by post-hoc multiple comparisons (Dunnett's Multiple Comparisons Test).

Chapter Five
Discussion and Conclusion

Discussion

5.1 Total Phenolic Content (TPC) & Phytochemical Relevance

Phenolics from natural sources serve as key natural antioxidants, acting via radical scavenging, hydrogen donation, and metal chelation (Kumar & Goel, 2019). In this study, the high TPC provides a chemical basis for downstream functional assays (DPPH, UHPLC profiling) and is analogous to phenolic levels observed in the similar species *M. oleifera* extracts (Platzer et al., 2022).

These findings are consistent with the genus's reputation for rich polyphenolic chemistry and agree with prior work on *M. peregrina* showing substantial antioxidant potency (Abdullah et al., 2024).

5.2 Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging effects of the prepared extract observed in this study confirmed previous findings reported by Dehshahri et al., (2012) and El-Sherbiny et al., (2024), which revealed *M. peregrina* extract to perform a strong, dose-dependent radical-quenching capacity.

5.3 UHPLC–ESI–QTOF–MS/MS profiling

The two main flavonoids, **rutin** or **chlorogenic acid,** determined in the prepared extract justified the observed antioxidants effects in this study. Findings of the study performed by Chakraborty et al., (2025) showed these flavonoids to have significant anti-inflammatory, antioxidant, as well as the vascular modulatory roles.

5.4 Animals Body weight Variations

The observed animals BW reduction suggests a potential metabolic response. Previously, rutin or chlorogenic acid were investigated and found to suppress appetite, enhance fatty-acid oxidation, and activate AMP-activated protein kinase (AMPK), leading to mild weight reduction in rodents (Alkhudhayri et al., 2021). These findings agree with prior reports that suggested *Moringa* polyphenols may improve lipid metabolism and reduce adiposity (Alkhudhayri et al., 2021).

5.5 Animals serum PT, INR, and TM Changes

This study findings revealed that animal groups administrated with warfarin alone have lower INR and PT values, compared with the recorded values in animal groups that were administrated a combination of warfarin and *M. peregrina* leaves extract.

Similarly, a notable coagulant effect of *M. peregrina* extract recorded in the current study was previously reported for the species *M. oleifera.* Systematic comparison of over a hundred natural coagulants has shown that those of *M. oleifera* trees are the most effective (Ferreira et al., 2021; Ebhohon & Miller, 2022). Moreover, *M. oleifera* leaf extract has been reported to induce clot formation (Ebhohon & Miller, 2022). Furthermore, *M. oleifera* seed extracts are recently used a milk coagulant in buffalo milk cheese production (Y. Yang et al., 2025).

Worth mentioning that *M. peregrina* seed proteins have broadly similar surface charges and amino acid compositions to *M. oleifera* seed proteins (Nouhi et al., 2019), therefore expected to have similar coagulation effects.

For better estimation concerning the medicinal impact of the plant extract on coagulation process, the changes in soluble TM levels were monitored. Findings show that changes in the INR and PT values among the study groups were accompanied by stability in serum TM levels, except for highest extract dose administrated animals, that showed higher mean TM levels than in warfarin -administrated groups.

Nevertheless, prior studies have shown conflicting results regarding soluble TM concentration and the risk of CVD. Salomaa & Wu, (1999) showed a decreased risk for CVD in previously healthy patients with high levels of TM. In contrast, many other studies have found that TM concentration can predict CVD progression (Shlipak et al., 2008).

In this context, warfarin dose used in the current study did not change TM concentration significantly. Therefore, it is not reasonable to link the design of the current study to the bleeding effect of warfarin. Subsequently, the model here is more consistent pattern of changes in coagulation process rather than demonstrating a bleeding effect of warfarin. It has demonstrated that the various sized TM molecular subspecies retain functional activity. Thus, it is possible that these smaller TM species have different biological activity in vivo **(**Ohlin et al., 2005). Consequently, our findings were consistent

with a prior clinical study that observed normal thrombin generation in the presence of TM in patients with cirrhosis, including those with high INR value (Ferreira et al., 2021).

5.6 Renal Markers: Serum levels of Creatinine, and Urea

Serum U levels were unchanged at baseline, but diverged post-warfarin, with D2X showing lower U levels than Ct and Dx. These results suggest transient, subclinical renal functional shifts rather than overt nephrotoxicity.

The normalization of Cr levels following warfarin argues against progressive renal injury. Mechanistically, the nephroprotective potential of major *M. peregrina* constituents, particularly the flavonoid rutin and the phenolic chlorogenic acid—offers a biochemical explanation for the absence of sustained renal impairment.

Multiple recent animal studies and reviews report that rutin lowers serum creatinine and blood urea nitrogen (BUN) in models of drug- or toxin-induced kidney injury (LPS-acetaminophen-, or chemical-induced nephropathies), an effect attributed to rutin's antioxidant, anti-inflammatory, and anti-fibrotic actions (Rahmani et al., 2023).

Likewise, chlorogenic acid has been shown to attenuate streptozotocin-induced diabetic nephropathy and other models of renal injury, reducing serum creatinine and BUN while inhibiting oxidative stress and inflammatory signaling (Bao et al., 2018). These preclinical data indicate that rutin or chlorogenic acid can protect glomerular and tubular function, normalize serum creatinine and urea, and thereby help explain the modest and reversible renal biomarker fluctuations observed after *M. peregrina* extract administration in the present study.

5.7 Hepatic Marker: serum levels of ALT

Findings revealed a hepatoprotective effect of *M. peregrina* extract, possibly mediated by its phenols content, namely rutin or chlorogenic acid. The latter are known to possess hepatoprotective via antioxidant activities.

Previously, rutin or chlorogenic acid has been shown to significantly decrease serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in animal models treated with acetaminophen to induced hepatotoxicity (Tan et al., 2016 and Yan et al., 2020).

The attenuation of ALT rise in *M. peregrina*-treated groups in the current study is therefore consistent with these known properties of rutin or chlorogenic acid. Collectively, these findings support the hypothesis that the hepatoprotective effect observed following *M. peregrina* extract administration is mediated, at least in part, through its phenolic constituents' antioxidant and cytoprotective actions.

5.8 Integrated Interpretation & Clinical Implications

The observed effect of the *M. peregrina* leaves extract as an effective coagulant was evident through a significant retraction of INR and PT values after warfarin administration as monitored in experimental groups. These findings suggest that *M. peregrina* significantly decreases the anticoagulant effect of warfarin might be throughout changing its pharmacodynamic or/ and pharmacokinetic mechanism. Therefore, *M. peregrina* extract is liable to be a part of interventional therapy for the risk of bleeding associated with warfarin misuse.

From a translational standpoint, patients using *M. peregrina* (e.g. as an herbal infusion, supplement) while on warfarin or other vitamin K antagonists may be at risk of unexpected coagulation. Clinicians should monitor INR and PT changes more frequently and inquire about herbal use.

5.9 Limitations & Future Directions

- **No direct pharmacokinetic assays** (warfarin plasma levels, hepatic CYP enzyme activity) were conducted; mechanistic claims remain inferential.
- **Limited coagulation endpoints**: aPTT, fibrinogen, platelet aggregation, protein C assays would enrich mechanistic clarity.
- **Short-term exposure**: long-term effects and adaptive responses are unknown.
- **Inter-species differences**: rat CYP expression differs from humans, limiting direct translation.
- **Extract standardization**: batch variation in phytochemical composition may affect reproducibility.

Future work should focus on pharmacokinetic interaction studies, extended coagulation panels, histopathology of organs (liver, kidney, endothelium), purified compound testing (rutin, chlorogenic acid), and controlled human or ex vivo studies of *M. peregrina*–warfarin interaction.

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