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THEME

Phytochemical analysis and biological activity evaluation of the hydromethanolic extract of Fenugreek (*Trigonella foenum-graecum* L.) seeds locally cultivated In El-Oued Region, Algeria

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Thanks be to **Allah**, for the gift of life and knowledge

To my father, rest his soul

To my mother, for her unwavering love and support

To my professors, for their guidance and wisdom

To my friends, for their encouragement

To the difficult days

This work is dedicated to you

TABLES OF CONTENTS

CHAPTER II

Materials and Methods

CHAPTER III

Results and Discussion

ACKNOWLEDGMENT

In the Name of Allah, the Most Gracious, the Most Merciful.

All praise is due to Allah, by Whose grace good deeds are completed and by Whose help hardships are eased. Peace and blessings be upon our ***Prophet Muhammad*** ﷺ*, upon his family, his companions, and all who follow them in righteousness.*

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My faithful friends ***Ritaj****,* ***Kawthar****, and* ***Chaimaa****, companions in both difficulty and joy, and a light in the darkest nights.*

To every hardship endured with patience, and every tear shed in the pursuit of knowledge, I dedicate this work. I pray that Allah accepts it sincerely for His sake, blesses its impact, and makes it a light for us on the Day we meet Him.

Dedication by FEGUIRI Hala

I dedicate this humble work to the pure soul of my beloved mother, Wahiba—may Allah have mercy upon her. You were the heartbeat of my life before I was born, and the light of my path before I could see. Though you are absent from my sight, you remain forever present in my heart. May Allah grant you Paradise and reunite me with you in His mercy and pleasure.

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To all of you, I dedicate the fruit of this effort, praying that Allah makes it sincere for His noble sake, beneficial, and counted among our good deeds on the Day we meet Him.

Dedication by MEDELLEL Aya

ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L., Fabaceae) is a leguminous spice and medicinal plant traditionally cultivated in many regions of the world and in North African countries and Algeria, yet the phytochemical and biological value of locally grown seeds remains insufficiently documented. The aim of this work was to characterise the hydromethanolic extract of *T. foenum-graecum* L. seeds harvested in the El-Oued region (Hassani Abdelkrim, southeast Algeria) and to evaluate its *in-vitro* antioxidant, antidiabetic and anti-Alzheimer potential.

Air-dried, ground seeds were macerated in methanol/water (75:25, v/v) to obtain the crude extract, which was used to determine total phenolic, total flavonoid and condensed tannin contents by the Folin–Ciocalteu, aluminium chloride and vanillin–HCl methods, respectively. The phenolic and bioactive profile was established by ultra-performance liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (UPLC–ESI–MS/MS), and biological activities were assessed through DPPH and ABTS•+ radical-scavenging assays, the o-phenanthroline reducing assay, and inhibition of α-amylase, α-glucosidase and acetylcholinesterase.

The extract was particularly rich in polyphenols (88.57 ± 1.10 mg GAE/g), flavonoids (47.33 ± 3.90 mg QE/g) and condensed tannins (13.62 ± 1.59 mg CE/g), and twenty-two bioactive compounds, including phenolic acids and flavonoid glycosides, were identified by LC–MS/MS out of forty targeted standards. Significant radical-scavenging activity was recorded against DPPH• (IC50 = 77.37 ± 1.80 µg mL^{-1}) and ABTS•+ (IC50 = 29.49 ± 1.11 µg mL^{-1}). Notably, the extract inhibited α-amylase (IC_{50} = 264.03 ± 23.87 µg mL^{-1}) and α-glucosidase (IC_{50} = 245.14 ± 26.63 µg mL^{-1}) more strongly than the reference inhibitor acarbose, and exhibited measurable acetylcholinesterase inhibition (IC_{50} = 237.82 ± 35.25 µg mL^{-1}) compared with galantamine.

These findings demonstrate that locally cultivated El-Oued fenugreek seeds constitute a promising antioxidant, antihyperglycaemic and neuroprotective phytotherapeutic and functional-food resource that could effectively replace imported material; nevertheless, the present results are restricted to in vitro models, and further in vivo, toxicological and clinical investigations are required to confirm efficacy, safety and optimal conditions of use.

Keywords: *Trigonella foenum-graecum* L, hydromethanolic extract, polyphenols, LC–MS/MS, biological activities , antidiabetic activity, acetylcholinesterase inhibition

ABSTRACT IN ARABIC (مُلخص)

الحلبة (*Trigonella foenum-graecum* L.) ، الفصيلة البقولية Fabaceae) نبتة بقولية تُستعمل في آنٍ واحد كتابلٍ وكنباتٍ طبي، وتُزرع تقليديًا في العديد من مناطق العالم و في دول شمال افريقيا ، غير أنّ القيمة الكيميائية النباتية والبيولوجية للبذور المنتَجة محليًا في الجزائر لا تزال غير موثَّقة بصورة كافية. هدفت هذه الدراسة إلى توصيف المستخلص الهيدروميثانولي لبذور *T. foenum-graecum* L. المزروعة بمنطقة الوادي (حساني عبد الكريم، جنوب شرق الجزائر) وإلى تقييم فاعليتها المضادة للأكسدة والمضادة للسكري والمضادة لمرض الزهايمر مخبريًا (*in-vitro*).

جُفِّفت البذور هوائيًا ثم طُحنت ونُقعت في خليطٍ من الميثانول والماء (75:25، حجم/حجم) للحصول على المستخلص الخام، الذي استُعمل لتحديد محتوى الفينولات الكلية والفلافونويدات الكلية والعفصيات المكثَّفة باستخدام طرق فولين–سيوكالتو، وكلوريد الألومنيوم، والفانيلين–حمض كلور الماء على التوالي. وقد أُنجز التشخيص الفينولي والبيولوجي بواسطة كروماتوغرافيا السائلة فائقة الأداء المقترنة بمطيافية الكتلة الترادفية ذات التأيين بالرذاذ الكهربائي (UPLC–ESI–MS/MS)، فيما قُيِّمت الأنشطة البيولوجية باختبارات اقتناص الجذور الحرة DPPH وABTS•+، واختبار الاختزال بالأورثوفينانثرولين، إضافةً إلى تثبيط إنزيمات ألفا-أميلاز و ألفا -غلوكوزيداز والأسيتيل كولين إستراز. أبدى المستخلص غنىً ملحوظًا بعديد الفينولات (1,10± 88,57 ملغ مكافئ حمض الغاليك/غ) والفلافونويدات (3,90 ±47,33 ملغ مكافئ كيرسيتين/غ) والعفصيات المكثَّفة (1,59 ±13,62 ملغ مكافئ كاتيشين/غ)، كما تمّ التعرّف بواسطة تحليل LC–MS/MS على اثنين وعشرين مركّبًا فعّالًا، تشمل أحماضًا فينولية وغليكوزيدات فلافونيدية، من أصل أربعين مركبًا قياسيًا مستهدفًا.

وقد سُجِّل نشاطٌ معتبر لاقتناص الجذور الحرة تجاه DPPH• (التركيز المثبِّط النصفي IC_{50} = 77,37 ± 1,80 ميكروغرام/مل) وABTS•+ (IC_{50} = 29,49 ± 1,11 ميكروغرام/مل). وعلى نحوٍ لافت، ثبَّط المستخلصُ إنزيمَي الألفا-أميلاز (IC_{50} = 264,03 ± 23,87 ميكروغرام/مل) و ألفا - غلوكوزيداز (IC_{50} = 245,14 ± 26,63 ميكروغرام/مل) بفاعلية تفوق المثبِّط المرجعي أكاربوز، وأظهر تثبيطًا ملموسًا لإنزيم الأسيتيل كولين إستراز (IC_{50} = 237,82 ± 35,25 ميكروغرام/مل) مقارنةً بالغالانتامين.

تُبيّن هذه النتائج أنّ بذور الحلبة المزروعة محليًا بمنطقة الوادي تمثّل موردًا واعدًا للعلاج النباتي والأغذية الوظيفية، حيث تتمتّع بذور الحلبة المحلية بخصائص مضادة للأكسدة وخافضة لسكر الدم وواقية للأعصاب، وقادِرة على أن تعويض بذور الحلبة المستوردة؛ غير أنّ النتائج المُستخلصة تبقى محصورةً في نماذج مخبرية، و التي تستوجب إجراء دراساتٍ تكميلية على الكائنات الحية (*in-vivo*) ودراساتٍ سُمّية وسريرية للتحقّق من الفاعلية والسلامة وتحديد الشروط المُثلى للاستعمال.

الكلمات المفتاحية: *Trigonella foenum-graecum* L ، المستخلص الهيدروميثانولي، LC–MS/MS، عديد الفينولات، النشاط المضاد للسكري، تثبيط الأسيتيل كولين إستراز

SUMMARY IN FRENCH (RESUME)

Le fenugrec (*Trigonella foenum-graecum* L., Fabaceae) est une légumineuse à la fois épice et plante médicinale, traditionnellement cultivée dans de nombreuses régions du monde et dans les pays d'Afrique du Nord et l'Algérie, mais la valeur phytochimique et biologique des graines produites localement demeure insuffisamment documentée. L'objectif de ce travail était de caractériser l'extrait hydrométhanolique des graines de *T. foenum-graecum* L. récoltées dans la région d'El-Oued (Hassani Abdelkrim, sud-est de l'Algérie) et d'évaluer son potentiel antioxydant, antidiabétique et anti-Alzheimer *in-vitro*.

Les graines séchées à l'air et broyées ont été macérées dans un mélange méthanol/eau (75:25, v/v) afin d'obtenir l'extrait brut, lequel a été utilisé pour déterminer les teneurs en composés phénoliques totaux, en flavonoïdes totaux et en tanins condensés par les méthodes de Folin–Ciocalteu, au chlorure d'aluminium et à la vanilline–HCl, respectivement. Le profil phénolique et bioactif a été établi par chromatographie liquide à ultra-haute performance couplée à la spectrométrie de masse en tandem avec ionisation par électronébulisation (UPLC–ESI–MS/MS), et les activités biologiques ont été évaluées au moyen des tests de piégeage des radicaux DPPH et ABTS•+, du test réducteur à l'o-phénanthroline ainsi que de l'inhibition de l'α-amylase, de l'α-glucosidase et de l'acétylcholinestérase.

L'extrait s'est révélé particulièrement riche en polyphénols (88,57 ± 1,10 mg EAG/g), en flavonoïdes (47,33 ± 3,90 mg EQ/g) et en tanins condensés (13,62 ± 1,59 mg EC/g), et vingt-deux composés bioactifs, incluant des acides phénoliques et des hétérosides flavoniques, ont été identifiés par LC–MS/MS parmi les quarante standards ciblés. Une activité notable de piégeage des radicaux a été enregistrée vis-à-vis du DPPH• (IC_{50} = 77,37 ± 1,80 µg mL^{-1}) et de l'ABTS•+ (IC_{50} = 29,49 ± 1,11 µg mL^{-1}). De manière remarquable, l'extrait a inhibé l'α-amylase (IC_{50} = 264,03 ± 23,87 µg mL^{-1}) et l'α-glucosidase (IC_{50} = 245,14 ± 26,63 µg mL^{-1}) plus fortement que l'inhibiteur de référence acarbose, et a présenté une inhibition mesurable de l'acétylcholinestérase (IC_{50} = 237,82 ± 35,25 µg mL^{-1}) comparativement à la galantamine.

Ces résultats démontrent que les graines de fenugrec cultivées localement à El-Oued constituent une ressource phytothérapeutique et alimentaire fonctionnelle prometteuse, dotée de propriétés antioxydantes, antihyperglycémiantes et neuroprotectrices, susceptible de remplacer efficacement la matière première importée ; néanmoins, les résultats obtenus se limitent à des modèles in vitro, et des investigations complémentaires in vivo, toxicologiques et cliniques seront nécessaires pour confirmer l'efficacité, l'innocuité et les conditions optimales d'utilisation.

Mots-clés : *Trigonella foenum-graecum* L, extrait hydrométhanolique, polyphénols, LC–MS/MS, activités biologiques, activité antidiabétique, inhibition de l'acétylcholinestérase.

NOMENCLATURE

***Symbol*:**

C Catechin

Fe2+ Ferrous (iron II) ion

Fe3+ Ferric (iron III) ion

FeCl3 Iron (III) chloride

h Hour(s)

HCl Hydrochloric acid

K2S2O8 Potassium persulfate

M Molar

MeOH Methanol

min Minute(s)

mM Millimolar

m/z Mass-to-charge ratio

Na2CO3 Sodium carbonate

nm Nanometre(s)

Q Quercetin

rpm Revolutions per minute

v/v Volume per volume

w/w Weight per weight

µg Microgram

µL Microlitre

µM Micromolar

°C Degrees Celsius

Abbreviations:

ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

Abs Absorbance

AChE Acetylcholinesterase

AKT Protein kinase B

ALP Alkaline phosphatase

ALT Alanine aminotransferase

AMPK AMP-activated protein kinase

ANOVA Analysis of variance

APG IV Angiosperm Phylogeny Group, fourth classification

AST Aspartate aminotransferase

ATP Adenosine triphosphate

BHA Butylated hydroxyanisole

BMI Body mass index

C18 Octadecyl-bonded silica (reversed-phase column)

CAT Catalase

CE Catechin equivalent

CNS Central nervous system

COX-2 Cyclooxygenase-2

CUPRAC Cupric ion reducing antioxidant capacity assay

DAD Diode array detector

DCM Dichloromethane

DMSO Dimethyl sulfoxide

DPPH 2,2-Diphenyl-1-picrylhydrazyl (radical)

DTNB 5,5′-dithiobis(2-nitrobenzoic acid)

EC50 Half maximal effective concentration

EDTA Ethylenediaminetetraacetic acid

ERK Extracellular signal-regulated kinase

ESI Electrospray ionisation

EtOAc Ethyl acetate

FCR Folin–Ciocalteu reagent

FOXO1 Forkhead box transcription factor O1

FRAP Ferric reducing antioxidant power assay

GAE Gallic acid equivalent

GC–MS Gas chromatography–mass spectrometry

GLUT-4 Glucose transporter type 4

GSH-Px Glutathione peroxidase

HbA1c Glycated haemoglobin

HDL High-density lipoprotein

HPLC High-performance liquid chromatography

HPLC–DAD High-performance liquid chromatography coupled with diode array detection

IC50 Half maximal inhibitory concentration

IL-1β Interleukin-1 beta

IL-6 Interleukin-6

IRS-1 Insulin receptor substrate 1

IUCN International Union for Conservation of Nature

LC–MS/MS Liquid chromatography coupled with tandem mass spectrometry

LDL Low-density lipoprotein

LOX Lipoxygenase

MIC Minimum inhibitory concentration

MRM Multiple reaction monitoring

MS/MS Tandem mass spectrometry

MUFA Monounsaturated fatty acids

NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells

PBS Phosphate buffered saline

PEPCK Phosphoenolpyruvate carboxykinase

PI3K Phosphoinositide 3-kinase

PNPG p-Nitrophenyl α-D-glucopyranoside

PUFA Polyunsaturated fatty acids

QE Quercetin equivalent

RCT Randomised controlled trial

SD Standard deviation

SFA Saturated fatty acids

SOD Superoxide dismutase

STZ Streptozotocin

T2DM Type 2 diabetes mellitus

TC Total cholesterol

TCT Total condensed tannin content

TFC Total flavonoid content

TG Triglycerides

TNF-α Tumour necrosis factor alpha

TPC Total phenolic content

Tris-HCl Tris(hydroxymethyl)aminomethane hydrochloride

UPLC Ultra-performance liquid chromatography

UV Ultraviolet

UV–Vis Ultraviolet–visible

LISTE OF FIGURE

LIST OF TABLES

LIST OF EQUATIONS

GENERAL INTRODUCTION

Algeria is the largest country in Africa and the Mediterranean basin, covering an area of approximately 2.38 million km² and stretching from a 1,622 km Mediterranean coastline in the north to the heart of the Sahara in the south. This vast territorial extent, combined with marked latitudinal, altitudinal and edaphic gradients, sustains a remarkable floristic richness: the national flora is currently estimated at about 4,000 vascular plant taxa belonging to 917 genera and 131 families, of which a significant proportion are endemic to North Africa or to the Saharan domain (Belhouala & Benarba, 2021; Yebouk et al., 2020). This biodiversity makes Algeria one of the most floristically diverse countries of the southern Mediterranean rim and a strategic reservoir of plant genetic resources for food, medicinal and industrial uses (Miara et al., 2019).

The bioclimatic zonation of Algeria reflects its geographical position between the temperate Mediterranean and the hyper-arid Sahara. Four main bioclimatic belts can be distinguished — humid and sub-humid in the Tellian Atlas, semi-arid on the high plateaus, arid in the pre-Saharan steppe, and Saharan to hyper-Saharan in the south — with the desert alone covering more than 80 % of the national territory and roughly 31 % of the African continent (Benhouhou et al., 2018; Kouzmine & Fontaine, 2018). Despite extreme aridity, the Algerian Sahara harbours more than 500 species of higher plants, many of which are still routinely employed in domestic phytotherapy by Saharan communities (Hammiche & Maiza, 2006; Ozenda, 2004). The El-Oued region (Souf), located in the north-eastern Algerian Sahara, is a long-standing oasis agro-ecosystem in which traditional knowledge of medicinal and aromatic plants is transmitted across generations and frequently associated with seed crops cultivated under the local *ghout* system, including fenugreek (*Trigonella foenum-graecum* L.) (Boudjelal et al., 2013; Hadjadj et al., 2020).

Plants have been a cornerstone of human medicine since antiquity, and contemporary pharmacology continues to draw heavily on this botanical heritage. The therapeutic value of medicinal species largely resides in their secondary metabolites — bioactive molecules synthesized as defence and signalling compounds rather than for primary growth (Krishnaprabu, 2020). Three pharmacologically prominent classes of these compounds are the phenolics and flavonoids, the alkaloids, and the saponins, all of which are well-documented constituents of fenugreek seeds and have been associated with antioxidant, antidiabetic, hypolipidaemic and neuroprotective effects (Tabasum et al., 2016; Visuvanathan et al., 2022; Wani & Kumar, 2018). The continuous rediscovery of such phytochemicals through modern analytical techniques — particularly liquid chromatography coupled with tandem mass

spectrometry (LC–MS/MS) — supports the historical importance of plants in medicine and reinforces their relevance as a source of novel drug leads (Khalil et al., 2017; Wani & Kumar, 2018).

The renewed scientific interest in plant secondary metabolites is also driven by the search for safer, more affordable alternatives to synthetic pharmaceuticals. Although phytomedicines are generally less potent than pure synthetic molecules, their regular dietary intake within complex matrices can elicit significant long-term physiological benefits, especially in chronic, lifestyle-related disorders such as type 2 diabetes, dyslipidaemia and neurodegenerative diseases (Ksouri et al., 2012; Yadav & Baquer, 2014). Fenugreek epitomizes this dual nature: its seeds are simultaneously a culinary spice, a galactagogue functional food and a recognized nutraceutical raw material, and its hydromethanolic extracts are increasingly used as ingredients in cosmetics, dermatological products and agro-industrial formulations (Wani & Kumar, 2018; Snehlata & Payal, 2012). Such versatility, combined with a long ethnopharmacological record, has placed *T. foenum-graecum* L. among the most thoroughly investigated species of the *Fabaceae* family.

Plants growing in arid and Saharan environments are subjected to a constellation of abiotic stressors — water deficit, high temperatures, soil salinity, intense solar radiation and important diurnal thermal amplitudes — which collectively generate oxidative stress and stimulate the accumulation of phenolic and flavonoid antioxidants (El-Keblawy et al., 2016; Ghanem et al., 2021). Numerous studies have shown that drought and salinity enhance the biosynthesis of secondary metabolites in legumes and other Saharan species, often translating into measurably higher antioxidant capacity in extracts of plants from desert ecotypes compared with the same species cultivated under temperate conditions (Liu et al., 2011; Selmar & Kleinwächter, 2013). It is therefore reasonable to hypothesize that fenugreek seeds locally cultivated in El-Oued — under a hyper-arid climate, sandy soils and traditional irrigation — accumulate distinctive phytochemical profiles whose pharmacological relevance deserves dedicated investigation.

In this work, *Trigonella foenum-graecum* L. was selected for several converging reasons. Fenugreek occupies a central place in the ethnobotany of El-Oued, where its seeds are routinely consumed as a post-partum tonic (the *helba* preparation) and used in domestic decoctions for diabetes, gastrointestinal complaints and respiratory conditions (Boudjelal et al., 2013; Hadjadj et al., 2020). Despite this strong traditional use and despite the global popularity of fenugreek

as a medicinal and functional crop, peer-reviewed studies devoted specifically to the El-Oued ecotype remain scarce, and the chemical fingerprint and bioactive potential of its hydromethanolic seed extract have not yet been systematically characterized. Filling this gap directly contributes to the broader effort of valorizing Saharan plant resources of Algeria and of providing a scientific basis for traditional uses that have so far rested mainly on empirical knowledge.

The general aim of the present study is therefore to characterize the phytochemical composition and to evaluate selected biological activities of the hydromethanolic extract of Trigonella foenum-graecum L. seeds locally cultivated in the El-Oued region (Hassani Abdelkrim, 33°28′ N – 6°53′ E). To this end, cleaned, dried and ground fenugreek seeds were first macerated in a hydromethanolic mixture (methanol/water, 75:25, v/v) in order to prepare the crude extract and to determine its corresponding yield. The total phenolic, total flavonoid and condensed tannin contents of the resulting extract . phenolic profile was established by ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC–ESI–MS/MS). The *in-vitro* antioxidant activity of the extract was subsequently assessed through complementary radical-scavenging and reducing assays — DPPH, ABTS and o-phenanthroline . Finally, the *in-vitro* inhibitory potential of the extract was evaluated against three biomedically relevant enzymes, namely α-amylase, α-glucosidase and acetylcholinesterase, in order to explore its plausible antidiabetic and neuroprotective relevance.

The **first chapter** of this thesis presents a bibliographic review of *T. foenum-graecum* L., covering its botany, geographical distribution, nutritional and phytochemical composition, ethnobotanical uses and reported pharmacological activities.

The **second chapter** describes in detail the experimental procedures applied to the El-Oued seed material, from plant collection and extract preparation to phytochemical quantification, LC–MS/MS profiling and bioactivity assays.

The **third chapter** reports and discusses the results obtained, before drawing a general conclusion and outlining perspectives for the further valorization of fenugreek as a Saharan medicinal and nutraceutical resource.

CHAPTER I

Bibliographic Review on Fenugreek (*Trigonella Foenum-Graecum* L.) Seeds: Botanical, Phytochemical and Biological Activity

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L., Fabaceae) is one of those rare cultivated plants whose value cannot be captured within a single discipline. It is at once a spice, a leafy vegetable, a forage legume, a traditional medicine and a raw material for modern industry, and the same small golden seed therefore appears in scientific literatures as different as agronomy, food technology, ethnopharmacology, medicinal chemistry and clinical nutrition (Ahmad et al., 2016; Saif et al., 2024). This unusual breadth of relevance is what makes a dedicated bibliographic review necessary: before the experimental work on locally cultivated material can be properly interpreted, the available knowledge on the species must be assembled into a single coherent picture.

In the food and culinary sphere, fenugreek seeds are used worldwide as a spice in curries, breads, pickles and cheeses, and the young leaves (*methi*) are consumed as a vegetable, while the characteristic warm, maple-like aroma due to the volatile sotolone, together with galactomannans and proteins, accounts for the technological behaviour of the seed in food matrices (Meghwal & Goswami, 2012; Wani & Kumar, 2018). Closely related to this culinary tradition is the rapidly expanding category of functional foods and nutraceuticals, in which fenugreek now occupies a prominent position: hypoglycaemic and hypolipidaemic dietary supplements, lactation tonics and sport-nutrition formulations exploit, in particular, the bioactivity of 4-hydroxyisoleucine, diosgenin and soluble dietary fibre (Goyal et al., 2016; Visuvanathan et al., 2022). The seed thus illustrates with unusual clarity the continuum that links food and pharmacology in modern nutrition science.

In parallel with these contemporary uses, fenugreek remains one of the most widely cited herbs in the traditional medical systems of the Mediterranean basin, the Middle East, North Africa, the Indian subcontinent and the Horn of Africa, where it is indicated for diabetes, dyspepsia, cough, skin abscesses, postpartum weakness and a number of veterinary conditions (Saif et al., 2024; Sarker et al., 2024). Many of these empirical uses have been progressively rationalised by experimental and clinical work focused on saponins, alkaloids and polyphenols, and a substantial part of the present review is devoted to that rationalisation. Beyond traditional medicine, the seed also has a well-established pharmaceutical and industrial profile: it is a major plant source for the extraction of diosgenin, used in the semi-synthesis of steroidal hormones, and the basis of galactomannan-derived hydrocolloids of growing interest as natural emulsifiers and matrices in drug delivery (Dhull et al., 2022; Haxhiraj et al., 2024).

The relevance of fenugreek extends still further into cosmetic and agronomic uses. Polyphenol- and mucilage-rich preparations are incorporated into hair-care, anti-acne and topical

anti-inflammatory products, while in the field the species is valued as a green-manure and fodder crop whose symbiotic association with *Rhizobium* improves soil nitrogen status. This combination of food, medicinal, industrial and agronomic value, on a plant that also tolerates hot and arid conditions, is precisely what justifies a dedicated bibliographic review and explains why the species has been studied in such different contexts — from the Mediterranean and Indian centres of diversity to the North Africa (Coban et al., 2025; Wani & Kumar, 2018).

2. Taxonomy and Botanical Description

2.1. Morphology and anatomy of the fenugreek plant

Trigonella foenum-graecum L. is an annual herbaceous legume that completes its life cycle within four to five months under most agro-climatic conditions. The mature plant typically reaches a height of 30 to 80 cm — occasionally 100 cm under irrigated, fertile conditions — and forms a single, erect, slightly succulent and finely pubescent stem that branches profusely from the base (Ahmad et al., 2016; Saif et al., 2024). Stems are cylindrical, hollow when mature, and bear alternate, long-petiolate trifoliate leaves typical of the Fabaceae. The three obovate to oblanceolate leaflets are 15–40 mm long, with finely serrate margins and pinnate venation; the lateral leaflets are slightly asymmetric while the terminal leaflet is symmetrical and longer-stalked.

Flowers are borne singly or in pairs in the upper leaf axils. Each papilionaceous flower is short-pedicelled, 7–13 mm long, and shows the classic legume pattern: a five-toothed campanulate calyx, an obovate yellowish-white standard petal, two oblong wing petals, and a curved keel. The corolla is most often pale yellow, occasionally tinted lilac at the base. Pollination is predominantly autogamous, with limited cross-pollination by small bees. After fertilisation, the ovary develops into an elongated, sub-cylindrical pod (sometimes called *legume*), 8–15 cm long, ending in a pointed beak and containing 10–20 seeds arranged in a single row (Saif et al., 2024; Wani & Kumar, 2018).

Seeds are small, hard and rhomboidal, 3–5 mm long, 2–3 mm wide and approximately 2 mm thick. Externally they show a characteristic deep diagonal furrow that almost divides the seed into two unequal halves; this groove separates the radicle from the cotyledons and is a reliable diagnostic feature for the species. Mature seeds are golden-yellow to brownish-amber, with a hard, smooth and slightly rugose seed coat that is rich in mucilaginous galactomannans (Dhull et al., 2022). The endosperm is unusually well-developed for a legume, white and translucent, and accounts for nearly half of the seed mass; the embryo, with its two large cotyledons and short radicle, occupies the remaining volume.

The root system is typical of grain legumes: a slender taproot that may penetrate to 50–80 cm in deep, sandy soils, supplemented by an extensive lateral network in the upper 20 cm. Pinkish, club-shaped nodules colonised by *Rhizobium* species (mainly *Sinorhizobium meliloti*) develop along the lateral roots within three to four weeks after sowing and are responsible for biological nitrogen fixation rates that, under favourable conditions, can reach 70–150 kg N ha^{-1} per cycle (Ahmad et al., 2016).

Several anatomical adaptations explain the species' tolerance to hot, dry environments. The seed coat is reinforced by a multilayered palisade of hard *macrosclereid* cells that delays imbibition and protects the embryo against rapid dehydration; the leaf mesophyll is characterised by abundant calcium-oxalate crystals and a relatively thick cuticle that limits transpiration; and the stomata, predominantly on the abaxial surface, are surrounded by accessory cells typical of xerophytic Fabaceae (Coban et al., 2025; Bakhtiar, Hassandokht, Naghavi, & Mirjalili, 2024).

Diagnostically, *Trigonella foenum-graecum* can be distinguished from related Fabaceae such as *Trigonella caerulea* L. (blue fenugreek) and *Medicago sativa* L. (alfalfa) by the combination of: pale-yellow solitary flowers (vs. dense racemes in alfalfa); long, beaked, single-row pods (vs. spiral pods in *Medicago*); and the deep diagonal furrow on the seed surface, which is unique within the genus *Trigonella* (Ahmad et al., 2016; Dangi et al., 2004).

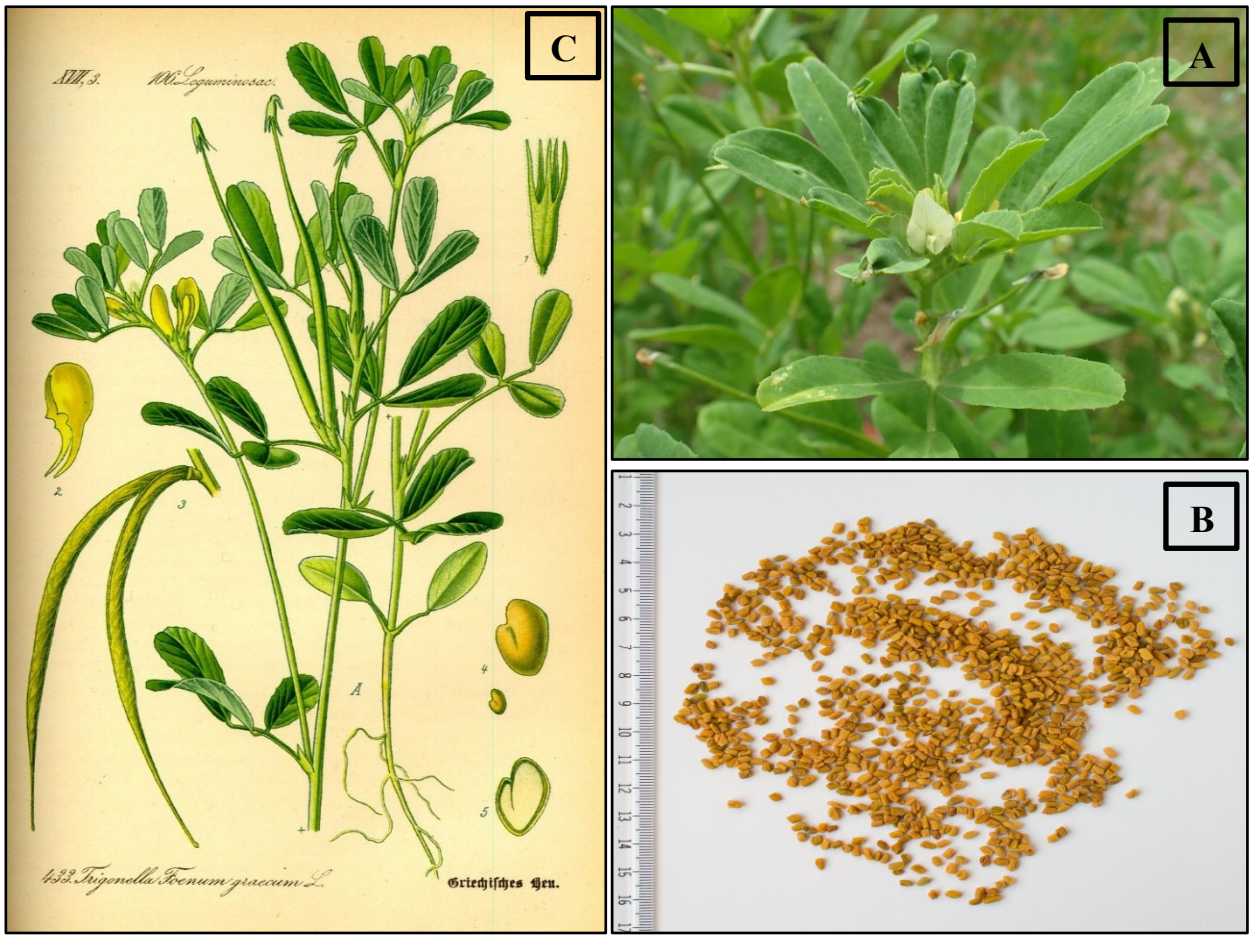

Figure 1. General morphology of fenugreek (*Trigonella foenum-graecum* L., Fabaceae): (A) habit of the plant at the vegetative–early-flowering stage, showing the erect, branched stem, the alternate trifoliate leaves with finely toothed leaflets and the pale-yellow papilionaceous flowers borne in the upper leaf axils; (B) macroscopic close-up of mature seeds (3–5 mm long), rhomboidal to oblong, with a characteristic golden-yellow to amber-brown colour and a deep diagonal furrow that separates the radicle from the cotyledons; (C) labelled botanical plate by O. W. Thomé (1885) showing the habit, an isolated flower (1), banner-petal detail (2), mature pods (3), seeds in face view (4) and seed in longitudinal section (5). (Thomé, 1885 ; Sanjay Acharya, 2014; Ahmad et al. 2016 ; Ziarnek, 2017 ; Saif et al. 2024).

2.2. Taxonomic position and nomenclature

Fenugreek belongs to the genus *Trigonella* L., which comprises approximately 135 species distributed mainly across the Mediterranean basin, southwestern Asia, the Irano-Turanian region and parts of South Africa. The species was formally described by Carl Linnaeus in *Species Plantarum* (1753) and the binomial *Trigonella foenum-graecum* L. has been retained without modification for more than 270 years (Ahmad et al., 2016). Recent molecular phylogenies based

on ITS and chloroplast markers confirm the placement of *Trigonella* within the tribe Trifolieae, alongside *Medicago* and *Melilotus*, and place *T. foenum-graecum* in the section *Foenum-graecum*, which is morphologically defined by its distinctive single-rowed beaked pod and deep-furrowed seed (Bakhtiar, Hassandokht, Naghavi, Rezadoost, et al., 2024; Dangi et al., 2004).

The complete taxonomic hierarchy of the species is shown in Table 1. Additional vernacular names reflect the wide cultural footprint of fenugreek: in English it is fenugreek, methi or Greek hay; in French *fenugrec*; in classical Arabic حُلْبَة (*hulba*) and in Algerian Maghrebi Arabic حلبة (*helba*); in Berber dialects of southern Algeria it is also referred to as *tifidas*; in Hindi/Urdu *methi*; in Persian *shanbalileh*; in Turkish *çemen otu*; and in Mandarin Chinese 葫芦巴 (*húlúbā*).

Table 1. *Taxonomic classification of Trigonella foenum-graecum L.* (Fabaceae) according to the APG IV system. (Angiosperm Phylogeny Group, 2016 ; Ahmad et al. 2016 ; Saif et al. 2024 ; Visuvanathan et al. 2022).

Rank	**Scientific name**
Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta (Angiospermae)
Class	Magnoliopsida (Eudicotyledons)
Order	Fabales
Family	Fabaceae (Leguminosae)
Tribe	Trifolieae
Genus	*Trigonella* L.
Species	***Trigonella foenum-graecum*** *L.*
Common names	Fenugreek, methi, helba, shanbalileh

2.3. Geographic origin, distribution and cultivation

The geographic origin of fenugreek has been debated for more than a century. Vavilov's pioneering surveys placed the centre of origin in the Near East (Mediterranean and southwestern Asia), and modern molecular studies broadly confirm this view: the highest genetic diversity of cultivated fenugreek is found between Turkey, Iran and the eastern Mediterranean, with secondary centres of diversification in India and Ethiopia (Dangi et al., 2004; Rajabihashjin et al., 2022). From these centres, the species spread along ancient trade routes — eastward through the Silk Road to South and East Asia, southward into the Arabian Peninsula and the Horn of Africa, and westward across North Africa to the Iberian Peninsula and southern Europe.

At present, fenugreek is cultivated commercially on every inhabited continent. The largest producers are India (which alone accounts for more than 80 % of world production, with about 200 000 t per year), followed by China, Pakistan, Iran, Turkey, Egypt, Morocco and Algeria, while smaller-scale cultivation is reported in Spain, France, Greece, Ethiopia, Yemen, Saudi Arabia and Sudan (Ahmad et al., 2016; Saif et al., 2024). A schematic distribution of the main production areas, including the Algerian El-Oued site, is shown in Figure 2.

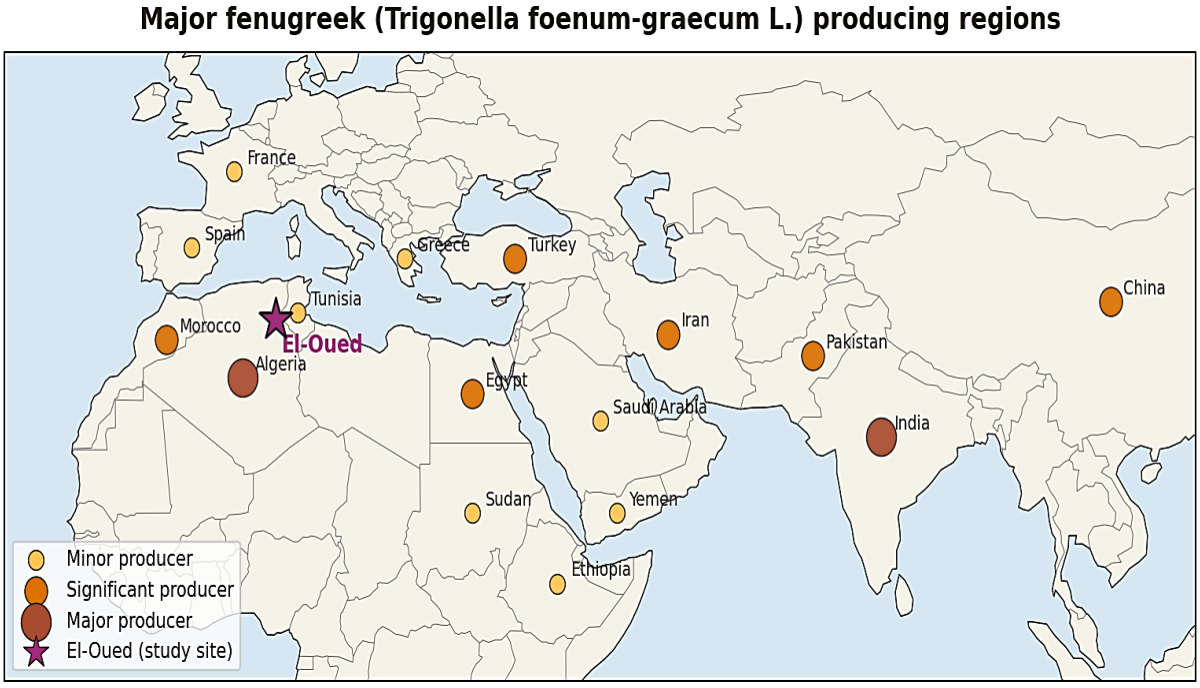

Figure 2. Major fenugreek (*Trigonella foenum-graecum* L.) producing regions across the Mediterranean–Asian belt. Symbol size encodes relative production importance; the magenta star marks the El-Oued region (south-eastern Algeria), where the seeds analysed in the present study were cultivated. *Map drawn from public-domain Natural Earth data* (Feguiri And Medellel, 2026).

Agro-ecologically, fenugreek thrives in regions with mild winters, warm and dry growing seasons, and well-drained soils. Optimum mean temperatures during the vegetative phase are 18–27 °C, with tolerance to short cold spells down to 4 °C and to heat up to 38 °C during ripening (Ahmad et al., 2016; Mekky et al., 2024). The species prefers loamy to sandy-loam soils with neutral to slightly alkaline pH (6.5–8.0), but it is remarkably tolerant of low-fertility, calcareous and even mildly saline substrates — a tolerance that is rare among grain legumes and that explains the success of fenugreek in semi-arid and arid environments.

Table 2. Major fenugreek-producing regions, dominant climate types and main uses.

Region / country	Climate type	Main uses	References
India (Rajasthan, Gujarat)	Semi-arid sub-tropical	Spice, ground seed in curries, methi leaves, nutraceuticals	Petropoulos (2002); Meghwal & Goswami (2012); Wani & Kumar (2018)
China (north-western provinces)	Continental, temperate	Traditional Chinese medicine, fodder	Yao et al. (2020); Visuvanathan et al. (2022)
Iran, Pakistan, Afghanistan	Continental semi-arid	Spice, traditional remedies, sprouts	Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024); Rajabihashjin et al. (2022)
Turkey, Greece	Mediterranean	*Çemen* paste, bakery, herbal teas	Akbari et al. (2019); Mekky et al. (2024)
Egypt, Morocco, Tunisia	Mediterranean–arid transition	Spice, lactation tonic, "*helba*" beverages	Saif et al. (2024); Goyal et al. (2016)
Algeria — El-Oued, Biskra,	**Saharan, hyper-arid with phreatic irrigation**	**Spice, traditional medicine, post-partum tonic, hair care**	Belaïd-Nouira et al. (2012); Atila Uslu et al. (2019); Suleiman et al. (2024)
Ethiopia, Yemen	Tropical highlands / monsoon	Bread (*injera*), porridge, traditional medicine	Petropoulos (2002); Wani & Kumar (2018)
Spain, France, Italy	Mediterranean	Bakery, herbal supplements, livestock feed	Petropoulos (2002); Ahmad et al. (2016)

3. Nutritional Composition of Fenugreek Seeds

Long before its biological activities were investigated by modern science, fenugreek had earned a reputation in traditional kitchens as a particularly *nutritious* spice. Modern analytical work has confirmed this intuition: gram for gram, fenugreek seeds combine a remarkably high protein content with a substantial amount of dietary fibre, a balanced lipid fraction and a wide range of minerals and vitamins. Among grain legumes, only soybean and lupin compete with fenugreek in terms of crude protein, and very few species rival its content in soluble galactomannan-rich gum.

This nutritional richness is one of the reasons fenugreek has been classified as a functional food (Meghwal & Goswami, 2012; Wani & Kumar, 2018; Saif et al., 2024).

3.1. Proximate composition

The proximate composition of fenugreek seeds varies with cultivar, agro-ecological zone and post-harvest treatment, but the published ranges are now sufficiently consistent to allow reliable averages (Table 3). Crude protein typically falls between 22 and 30 % of the seed dry weight, total dietary fibre is unusually high at 25–48 %, with a soluble gum (galactomannan) fraction of 18–28 %, and the lipid content remains modest at 5–10 %. The high fibre fraction is largely responsible for the characteristic mucilaginous behaviour of fenugreek seeds when soaked in water and is mechanistically central to their hypoglycaemic and hypolipidaemic effects (Dhull et al., 2022; Fuller & Stephens, 2015).

Table 3. Typical proximate composition of fenugreek (*Trigonella foenum-graecum* L.) seeds (g/100 g dry weight). Adapted from Meghwal & Goswami (2012), Wani & Kumar (2018), Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024) and Saif et al. (2024).

Constituent	Range (g/100 g DW)	Mean ± SD
Moisture	5.5 – 12.0	8.7 ± 1.6
Crude protein	22.0 – 30.0	26.2 ± 2.4
Crude lipids	5.0 – 10.0	7.1 ± 1.2
Total carbohydrates	45.0 – 58.0	50.4 ± 3.9
Total dietary fibre	25.0 – 48.0	34.7 ± 5.6
1– Soluble fibre (gum)	18.0 – 28.0	23.1 ± 2.8
2 – Insoluble fibre	6.0 – 20.0	11.6 ± 3.4
Ash	3.0 – 5.0	3.8 ± 0.4
Energy (kcal/100 g)	320 – 360	337 ± 12

In comparison, lentils contain about 24 % protein and 12 % total dietary fibre, and chickpeas approximately 19 % protein and 17 % fibre — figures that highlight just how protein- and fibre-dense fenugreek seeds are within the legume family. The lipid profile is dominated by unsaturated fatty acids (see Section 3.3), placing fenugreek among legumes with a favourable nutritional profile from a cardiovascular point of view (Bakhtiar, Hassandokht, Naghavi, & Mirjalili, 2024).

3.2. Mineral and vitamin content

Fenugreek seeds are a notable source of minerals essential for human nutrition. Iron, calcium, magnesium, potassium, phosphorus and zinc are all present in concentrations that, on a 100 g dry-

weight basis, contribute meaningfully to recommended dietary allowances. Iron content is particularly noteworthy — between 30 and 60 mg/100 g — placing fenugreek among the most iron-rich plant foods in routine human diet. Calcium and magnesium contents are also high, partly because of the gypsiferous soils on which the plant is often cultivated (Bakhtiar, Hassandokht, Naghavi, & Mirjalili, 2024; Singh et al., 2025). The vitamin fraction includes appreciable amounts of B-group vitamins (thiamine, riboflavin, niacin, folate) and moderate quantities of vitamin C and provitamin A (β-carotene), the latter being especially abundant in young leaves rather than seeds.

Table 4. Mineral and vitamin contents of fenugreek seeds reported in the literature (per 100 g dry weight).

Element / Vitamin	Range	Mean ± SD	Reference(s)
Iron (mg)	30 – 60	42.3 ± 6.8	Meghwal & Goswami (2012); Singh et al. (2025)
Calcium (mg)	160 – 320	230 ± 38	Bakhtiar et al. (2024a)
Magnesium (mg)	160 – 220	191 ± 18	Wani & Kumar (2018)
Potassium (mg)	780 – 1100	935 ± 90	Bakhtiar et al. (2024a)
Phosphorus (mg)	290 – 450	370 ± 42	Saif et al. (2024)
Zinc (mg)	2.5 – 4.4	3.4 ± 0.5	Singh et al. (2025)
Manganese (mg)	1.1 – 2.6	1.7 ± 0.3	Bakhtiar et al. (2024a)
Sodium (mg)	15 – 70	38 ± 12	Wani & Kumar (2018)
Thiamine (B_1, mg)	0.30 – 0.50	0.41 ± 0.06	Meghwal & Goswami (2012)
Riboflavin (B_2, mg)	0.35 – 0.45	0.39 ± 0.04	Meghwal & Goswami (2012)
Niacin (B_3, mg)	1.6 – 2.5	2.0 ± 0.2	Wani & Kumar (2018)
Folate (B_9, µg)	55 – 110	79 ± 14	Saif et al. (2024)
Vitamin C (mg)	3 – 10	6.1 ± 1.7	Saif et al. (2024)

3.3. Amino acid and fatty acid profiles

Two compositional features of fenugreek deserve particular emphasis. The first is the presence of a *non-protein* amino acid almost unique to this species: 4-hydroxyisoleucine (4-HIL), which represents up to 80 % of the free amino acid pool of the seed and accounts for approximately 0.55 g per 100 g of dry seed. Beyond its abundance, 4-HIL is mechanistically interesting because it stimulates glucose-induced insulin secretion *only* under hyperglycaemic conditions, with virtually no effect at normal blood glucose levels — a glucose-sensitive, "smart" insulinotropic effect that is rare in the plant kingdom (Avalos-Soriano et al., 2016; Yang et al., 2021). The protein-bound amino acid fraction is itself well-balanced, with all eight essential amino acids present in nutritionally relevant amounts; lysine and tryptophan, which are limiting in cereals, are particularly

abundant, supporting the traditional use of fenugreek as a complement to cereal-based meals (Bakhtiar, Hassandokht, Naghavi, Rezadoost, et al., 2024; Saif et al., 2024).

Table 5. Major free and protein-bound amino acids of fenugreek seeds (mg/100 g DW).

Amino acid	Concentration (mg/100 g DW)	Nutritional / pharmacological note	References
4-Hydroxyisoleucine (free)	450 – 700	Glucose-dependent insulin secretagogue; specific to *Trigonella*.	Avalos-Soriano et al. (2016); Fuller & Stephens (2015); Coban et al. (2025)
L-Histidine	600 – 720	Essential; precursor of histamine.	Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024); Saif et al. (2024)
L-Lysine	1500 – 1800	Essential; high vs. cereals.	Wani & Kumar (2018); Meghwal & Goswami (2012)
L-Threonine	850 – 980	Essential; protein synthesis.	Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024); Saif et al. (2024)
L-Methionine	320 – 410	Sulphur-containing essential amino acid.	Wani & Kumar (2018); Meghwal & Goswami (2012)
L-Phenylalanine	1100 – 1300	Essential; precursor of tyrosine.	Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024); Saif et al. (2024)
L-Leucine	1700 – 1950	Essential; muscle protein synthesis.	Wani & Kumar (2018); Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024)
L-Isoleucine	1050 – 1240	Essential; branched-chain amino acid.	Wani & Kumar (2018); Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024)
L-Valine	1180 – 1390	Essential; branched-chain amino acid.	Wani & Kumar (2018); Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024)
L-Arginine	2200 – 2700	Conditionally essential; insulin sensitivity.	Saif et al. (2024); Naghdi Badi et al. (2018)
L-Glutamic acid	4100 – 4900	Most abundant; flavour-active.	Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024); Meghwal & Goswami (2012)
L-Aspartic acid	2300 – 2700	Major non-essential amino acid.	Bakhtiar, Hassandokht, Naghavi & Mirjalili (2024); Meghwal & Goswami (2012)

Amino acid	Concentration (mg/100 g DW)	Nutritional / pharmacological note	References
L-Proline	900 – 1300	Osmoprotectant; abundant in xerophytic plants.	Naghdi Badi et al. (2018); Coban et al. (2025)

The second compositional feature is the lipid profile. Although seed lipids account for only 5–10 % of dry mass, more than 80 % of the fatty acids are unsaturated, with a clear dominance of linoleic acid (C18:2 ω-6) and oleic acid (C18:1 ω-9), followed by linolenic acid (C18:3 ω-3) and palmitic acid (C16:0) (Bakhtiar, Hassandokht, Naghavi, & Mirjalili, 2024). This profile is comparable to that of safflower or soybean and contributes to the cardioprotective reputation of fenugreek consumption.

Table 6. Main fatty acids of fenugreek seed oil (% of total fatty acids)

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Fatty acid	Symbol	Range (% of total FA)	Type	References
Linoleic acid	C18:2 ω-6	34 – 47	PUFA, essential	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024); Saif et al. (2024)
Oleic acid	C18:1 ω-9	14 – 26	MUFA	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024)
Linolenic acid	C18:3 ω-3	6 – 14	PUFA, essential	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Wani & Kumar (2018)
Palmitic acid	C16:0	8 – 12	SFA	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024); Saif et al. (2024)
Stearic acid	C18:0	3 – 5	SFA	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024)
Arachidic acid	C20:0	0.8 – 1.6	SFA	Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Saif et al. (2024)
Behenic acid	C22:0	0.3 – 0.8	SFA	Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Saif et al. (2024)
Total saturated	— SFA	12 – 19	—	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024); Wani & Kumar (2018)

Fatty acid	Symbol	Range (% of total FA)	Type	References
Total mono-unsaturated	— MUFA	14 – 26	—	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024)
Total poly-unsaturated	— PUFA	40 – 60	—	Hamden et al. (2010); Bakhtiar, Hassandokht, Naghavi, Rezadoost & Mirjalili (2024); Mekky et al. (2024); Wani & Kumar (2018); Saif et al. (2024)

Taken together, the proximate, mineral, vitamin, amino-acid and fatty-acid profiles establish fenugreek as a remarkably complete plant-based ingredient. They also already foreshadow several of its biological activities: the very high soluble fibre content underlies its glycaemic-control and lipid-lowering properties; 4-hydroxyisoleucine offers a direct mechanism for glucose-dependent insulin release; and the unsaturated lipid fraction contributes to cardiovascular benefits. With this nutritional baseline in mind, the next section turns to the seed's specialised metabolites, which carry most of the pharmacological weight.

4. Phytochemical Profile of Fenugreek Seeds

4.1. Overview of the major phytochemical classes

The pharmacological reputation of fenugreek rests on a small number of specialised metabolite classes, each represented by chemically diverse but functionally related molecules. Five families dominate the seed metabolome: (i) steroidal saponins, particularly diosgenin and its glycosides; (ii) pyridine alkaloids, primarily trigonelline and minor congeners; (iii) polyphenols, including phenolic acids, flavones, flavonols and condensed tannins; (iv) galactomannan-type soluble fibres; and (v) non-protein amino acids led by 4-hydroxyisoleucine (Chaudhary et al., 2018; Saif et al., 2024; Visuvanathan et al., 2022). Smaller but biologically meaningful contributions come from coumarins, volatile aroma compounds (sotolone), vitamins and trace alkaloids. Table 8 summarises this chemical landscape.

Table 7. Major phytochemical classes of fenugreek seeds, representative compounds and indicative biological roles.

Class	Representative compounds	Indicative biological roles	References
Steroidal saponins	Diosgenin, yamogenin, gitogenin, smilagenin, fenugreekine; glycosides foenugraecin, trigoneoside.	Hypoglycaemic, hypocholesterolaemic, antioxidant, anti-inflammatory.	Wang et al. (2019); Zhang et al. (2020); Tak et al. (2024); Chaudhary et al. (2018)
Pyridine alkaloids	Trigonelline (N-methylnicotinic acid),	Neuroprotective, antioxidant, modulation of glucose metabolism.	Aldholmi et al. (2024); Sekhar et al. (2024); Naghdi

Class	Representative compounds	Indicative biological roles	References
	gentianine, carpaine, choline.		Badi et al. (2018); Goyal et al. (2016)
Phenolic acids	*p*-Coumaric, ferulic, salicylic, gallic, caffeic, sinapic, vanillic acids.	Antioxidant, anti-inflammatory, hepatoprotective.	Bensouici et al. (2023); Sandhu et al. (2020); Yang et al. (2022)
Flavonoids	Apigenin, luteolin, vitexin, isovitexin, orientin, kaempferol, quercetin glycosides.	Antioxidant, antidiabetic, anti-inflammatory.	Khole et al. (2016); Bhatt et al. (2021); Bensouici et al. (2023)
Tannins (condensed)	Procyanidins, polymeric proanthocyanidins.	Antioxidant, astringent, antimicrobial.	Wani & Kumar (2018); Sandhu et al. (2020)
Galactomannans / soluble fibres	1,4-β-D-mannan backbone with 1,6-α-D-galactose substitution.	Hypoglycaemic, hypolipidaemic, prebiotic, satiogenic.	Dhull et al. (2022); Al-Khalifah et al. (2022); Kandhare et al. (2018); Majeed et al. (2018)
Non-protein amino acids	4-Hydroxyisoleucine, hydroxyproline, GABA.	Insulinotropic, neuroactive.	Avalos-Soriano et al. (2016); Fuller & Stephens (2015); Yang et al. (2021)
Volatile / aroma compounds	Sotolone (3-hydroxy-4,5-dimethyl-2(5H)-furanone), 2-methylbutyric acid esters.	Sensory; characteristic body/urine odour after intake.	Mebazaa et al. (2009); Visuvanathan et al. (2022)
Coumarins	Trigocoumarin, fenugrin B.	Anticoagulant, antioxidant.	Acharya et al. (2008); Visuvanathan et al. (2022)
Lectins, vitamins, minerals	See Section 3.	Nutritional and metabolic.	Meghwal & Goswami (2012); Saif et al. (2024); Wani & Kumar (2018)

4.2. Steroidal saponins and diosgenin

Steroidal saponins constitute the most studied class of fenugreek phytochemicals and represent 0.6 to 2.2 % of the seed dry weight. They are amphiphilic glycosides built around a steroidal aglycone — most often diosgenin (Figure 3) — bearing one to four sugar units (mostly D-glucose, D-galactose, L-rhamnose or D-xylose) attached at the C-3 position. Hydrolysis under acidic or enzymatic conditions releases the free sapogenin, which can then be quantified by HPLC or GC–MS (Chaudhary et al., 2018; Wang et al., 2019; Zhang et al., 2020). Diosgenin (3β-hydroxy-(25R)-spirost-5-ene) is by far the most abundant aglycone in fenugreek and remains, even today,

the major industrial precursor for the chemical hemisynthesis of progesterone, cortisone and a wide range of corticosteroid drugs (Tak et al., 2024).

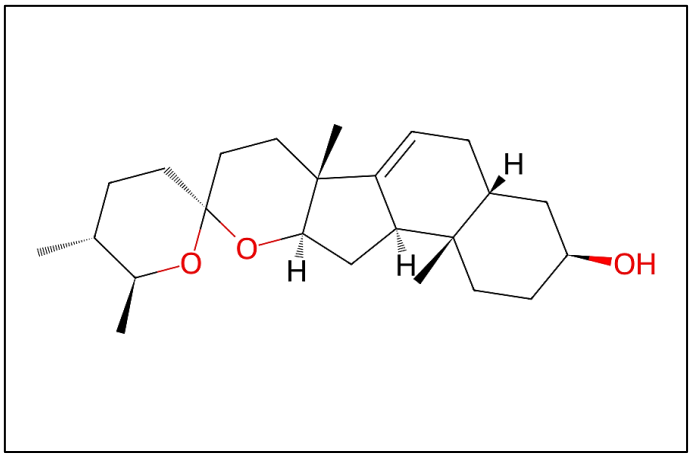

Figure 3. Chemical structure of diosgenin, the major steroidal sapogenin of fenugreek seeds. Diosgenin ($C_{27}H_{42}O_3$; molecular weight 414.6 $g \cdot mol^{-1}$) is the aglycone of glycosides such as foenugraecin and trigoneoside Ib and the industrial precursor of pharmaceutical steroidal hormones (Tak et al., 2024).

Beyond their commercial value, fenugreek saponins are pharmacologically active in their own right. Diosgenin and its glycosides have been shown to inhibit α-glucosidase with IC_{50} values in the 30–80 µM range (Zhang et al., 2020), to lower fasting blood glucose and triglycerides in diabetic and high-fat-diet-fed rodents (Fuller & Stephens, 2015; Tak et al., 2024), and to exert hepatoprotective and chondroprotective effects through the modulation of NF-κB and mitogen-activated protein kinase pathways (Chaudhary et al., 2018). Some glycosides, notably trigoneosides Ia–IIIb, additionally show insulinotropic effects on isolated pancreatic β-cells. Reported diosgenin concentrations vary widely between geographic origins, from 0.1 to 1.4 % of seed dry weight, with Indian and Iranian cultivars often reaching the upper end of the range (Coban et al., 2025; Paramesha et al., 2021).

4.3. Alkaloids: trigonelline and others

Although less abundant than saponins, alkaloids contribute decisively to the bioactivity of fenugreek seeds. Trigonelline (1-methylpyridinium-3-carboxylate; Figure 4) is the dominant alkaloid, accounting for 0.2 – 1.4 % of the seed dry weight, and is in fact one of the most reliable taxonomic markers of the genus *Trigonella* (Aldholmi et al., 2024; Naghdi Badi et al., 2018). Beyond trigonelline, fenugreek seeds contain smaller amounts of choline, gentianine, carpaine and trace amounts of nicotinic acid and stachydrine (Chaudhary et al., 2018; Sekhar et al., 2024).

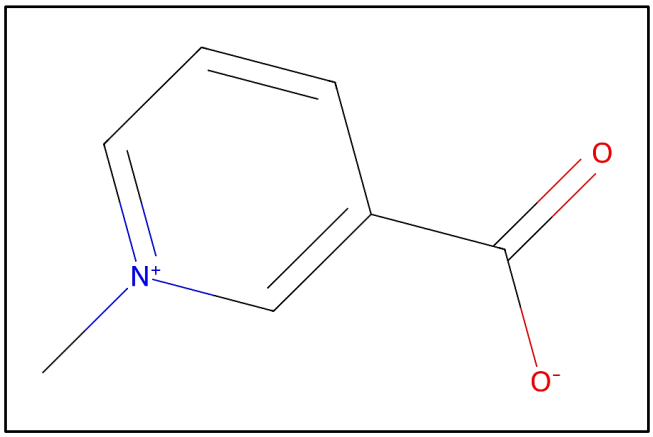

Figure 4. Chemical structure of trigonelline (1-methylpyridinium-3-carboxylate; $C_7H_7NO_2$; 137.14 g·mol^{-1}), the dominant alkaloid of fenugreek seeds, drawn here in its zwitterionic form (Zhou et al. 20212) .

Pharmacologically, trigonelline is one of the molecules behind fenugreek's reputation as an anti-diabetic plant. It improves fasting glycaemia and oral glucose tolerance in streptozotocin-induced diabetic rats, increases hepatic glycogen content, partially restores β-cell function and inhibits aldose reductase, the key enzyme of the polyol pathway implicated in diabetic complications (Aldholmi et al., 2024; Goyal et al., 2016; Sekhar et al., 2024). At the molecular level, trigonelline acts as an antioxidant by scavenging hydroxyl and peroxyl radicals and modulates several key kinases (AMPK, AKT, ERK1/2) involved in glucose and lipid homeostasis. Outside the diabetic context, trigonelline shows neuroprotective effects in models of Parkinson's and Alzheimer's disease and exerts cardioprotective activity against alcohol-induced toxicity (Sekhar et al., 2024).

Table 8. Main alkaloids identified in fenugreek seeds and their reported activities.

Alkaloid	Approx. content	Reported activities	Reference
Trigonelline	0.2 – 1.4 % DW	Antidiabetic, neuroprotective, antioxidant, hypolipidaemic	Visuvanathan et al. (2022)
Choline	0.05 – 0.20 % DW	Lipid metabolism, methyl-donor, hepatoprotective	Snehlata & Payal (2012); Yadav & Baquer (2014)
Gentianine	Traces	Mild CNS effects; anti-inflammatory	Yadav & Baquer (2014); Visuvanathan et al. (2022)
Carpaine	Traces	Cardiotonic in some models	Yadav & Baquer (2014); Wani & Kumar (2018)
Stachydrine	Traces	Cardio- and neuroprotective	Mehrafarin et al. (2011); Petropoulos (2002)

Alkaloid	Approx. content	Reported activities	Reference
Nicotinic acid	Traces	Precursor of trigonelline; lipid-modulating	Meghwal & Goswami (2012); Acharya et al. (2008)

4.4. Polyphenols: phenolic acids, flavonoids and tannins

Polyphenols form the third large family of fenugreek metabolites and are the chemical class most directly responsible for the antioxidant capacity of the hydromethanolic extract studied in this dissertation. Reported total phenolic contents range from 30 to 110 mg gallic acid equivalents (GAE) per gram of dry extract, total flavonoids from 8 to 50 mg quercetin equivalents (QE) per gram, and condensed tannins from 4 to 20 mg catechin equivalents (CE) per gram, with strong dependence on cultivar and extraction solvent (Bensouici et al., 2023; Sandhu et al., 2020; Yang, X., et al., 2022). The hydromethanolic system used in the present study, by combining methanol's ability to disrupt protein–polyphenol complexes with the polarity of water, is recognised as one of the most efficient solvents for total polyphenol recovery in seed matrices (Saif et al., 2024).

Among the phenolic acids, *p*-coumaric, ferulic, salicylic, gallic, caffeic and sinapic acids are routinely detected by HPLC–DAD or LC–MS/MS, in concentrations of 50 µg to several mg per gram of extract (Bensouici et al., 2023; Sandhu et al., 2020). The flavonoid fraction is dominated by C-glycosylflavones — notably vitexin (apigenin-8-C-β-D-glucopyranoside) and isovitexin (apigenin-6-C-β-D-glucopyranoside) — alongside O-glycosides of apigenin, luteolin, kaempferol and quercetin (Bhatt et al., 2021; Khole et al., 2016). Vitexin (Figure 5) is of particular interest because it shows broad antioxidant, anti-inflammatory and antidiabetic activities and is currently being investigated as a multi-target nutraceutical (Khole et al., 2016).

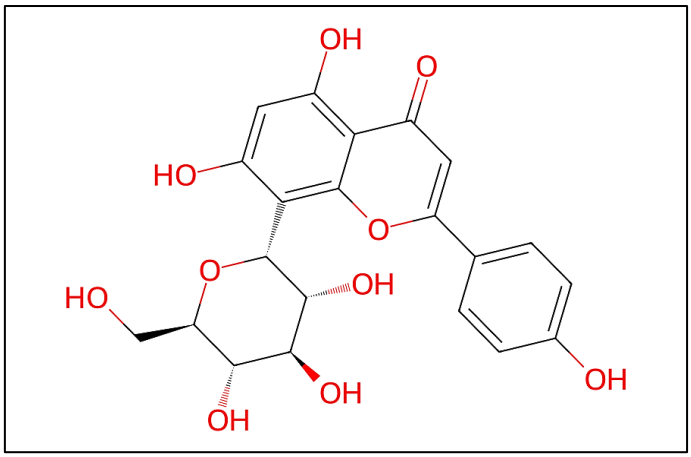

Figure 5. Chemical structure of vitexin (apigenin-8-C-β-D-glucopyranoside; $C_{21}H_{20}O_{10}$; 432.4 $g \cdot mol^{-1}$), one of the major C-glycosylflavones characterised in fenugreek seeds (He et al., 2016).

Table 9. Key phenolic acids and flavonoids reported in fenugreek seeds.

Compound	Class	Indicative content (mg/g)	Activity highlight	References
p-Coumaric acid	Hydroxycinnamic acid	0.3 – 1.6	Antioxidant, anti-inflammatory	Rababah et al. (2011)
Ferulic acid	Hydroxycinnamic acid	0.8 – 3.2	Antioxidant, neuroprotective	Rababah et al. (2011); El Nagar et al. (2023)
Salicylic acid	Hydroxybenzoic acid	0.4 – 2.0	Anti-inflammatory	El Nagar et al. (2023)
Gallic acid	Hydroxybenzoic acid	0.2 – 1.4	Strong antioxidant	Rababah et al. (2011); Neveu et al. (2010) – Phenol-Explorer
Caffeic acid	Hydroxycinnamic acid	0.1 – 0.8	Antioxidant, hepatoprotective	El Nagar et al. (2023); Bouhenni et al. (2019)
Apigenin	Flavone	0.2 – 1.5	Antidiabetic, anti-inflammatory	El Nagar et al. (2023)
Luteolin	Flavone	0.05 – 0.7	Antidiabetic, anti-inflammatory	Kenny et al. (2013)
Vitexin	Flavone C-glycoside	0.4 – 2.8	Antioxidant, antidiabetic	Khenifi et al. (2023); Benayad et al. (2014)
Isovitexin	Flavone C-glycoside	0.2 – 1.9	Antioxidant	Khenifi et al. (2023); Benayad et al. (2014)
Orientin	Flavone C-glycoside	0.1 – 0.9	Antioxidant, cardioprotective	Khenifi et al. (2023); Benayad et al. (2014)
Quercetin (and glycosides)	Flavonol	0.3 – 2.6	Antioxidant, anti-cancer	El Nagar et al. (2023); Naidu et al. (2011)
Kaempferol (and glycosides)	Flavonol	0.2 – 1.5	Anti-inflammatory	El Nagar et al. (2023); Benayad et al. (2014)
Catechin	Flavanol	0.5 – 4.0 mg/g	Antioxidant, antimicrobial	El Nagar et al. (2023); Bouhenni et al. (2019)
Rutin	Flavonol glycoside	0.05 – 0.8 mg/g	Vasoprotective, antioxidant	Hachouf et al. (2023); Belguith-Hadriche et al. (2013)

4.5. Fibres, gums, galactomannans and other constituents

A defining and almost unique feature of fenugreek seeds is their exceptionally high content of soluble galactomannan-type fibre. The endosperm of the seed contains 18–28 % of a

galactomannan polysaccharide built on a 1,4-β-D-mannose backbone substituted at every second residue by 1,6-α-D-galactose units, giving an unusually low mannose-to-galactose ratio of about 1.0–1.2. This structure makes fenugreek gum one of the most water-soluble galactomannans known, with viscosity, gelling and surfactant properties that have been exploited in the food industry as a thickening agent, an emulsion stabiliser and a fat replacer (Dhull et al., 2022; Majeed et al., 2018). Beyond technological functionality, fenugreek galactomannans show prebiotic activity, support beneficial gut microbiota, slow gastric emptying, blunt postprandial glycaemic excursions, lower serum LDL-cholesterol and improve insulin sensitivity in animal and human studies (Bruce-Keller et al., 2020; Kandhare et al., 2018; Wang, W., et al., 2023; Al-Khalifah et al., 2022). The insoluble fibre fraction (cellulose, hemicelluloses, lignin) accounts for 6–20 % of seed dry weight and contributes to faecal bulk and gut transit.

Table 10. Fibre fractions of fenugreek seeds and their physiological effects.

Fibre fraction	Approx. content (% DW)	Physiological effects	References
Soluble galactomannan ("*fenugreek gum*")	18 – 28	Slow gastric emptying, ↓postprandial glucose, ↓LDL-cholesterol, prebiotic	Ribes et al. (1984); Mathern et al. (2009); Srichamroen et al. (2008); Roberts (2011)
Cellulose	6 – 12	Faecal bulking, normal transit	Ribes et al. (1984); Naidu et al. (2011)
Hemicelluloses	3 – 7	Stool weight, fermentation in colon	Ribes et al. (1984); Naidu et al. (2011)
Lignin	1 – 3	Mild antioxidant, sequestration of bile salts	Naidu et al. (2011); Hooda & Jood (2003)
Pectins	< 1	Gel-forming, satiety	Naidu et al. (2011); Madhava Naidu et al. (2011)

Other minor but biologically relevant constituents include coumarins (trigocoumarin, fenugrin B), volatile aroma compounds dominated by sotolone — a furanone responsible for the characteristic *fenugreek* note in maple-syrup imitations and also for the well-known body and urine odour after intake — lectins, and small amounts of phytosterols such as β-sitosterol and stigmasterol (Saif et al., 2024; Sulieman et al., 2024; Visuvanathan et al., 2022).

5. Biological Activities of Fenugreek Seed Extracts

5.1. Antioxidant activity

The antioxidant capacity of fenugreek seeds is one of the most consistently reported activities and is largely attributed to the polyphenol and flavonoid fractions discussed in Section 4.4. Methanolic, ethanolic, hydroethanolic and hydromethanolic extracts have all shown strong dose-dependent radical-scavenging activity in the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, with reported IC_{50} values typically between 30 and 200 µg/mL, comparable to or only modestly weaker than ascorbic acid and Trolox standards (Bensouici et al., 2023; Mekky et al., 2024; Sandhu et al., 2020; Yang, X., et al., 2022). The $ABTS^{\bullet+}$ radical-scavenging assay yields slightly more favourable IC_{50} values (10–60 µg/mL), and the FRAP and CUPRAC reducing-power assays confirm a substantial electron-donating capacity. Fenugreek extracts also display ferrous-ion-chelating activity and inhibit lipid peroxidation in liposome and microsomal systems (Khole et al., 2016).

Mechanistically, the in vitro antioxidant capacity correlates strongly with the total phenolic content (TPC) and, even more, with the total flavonoid content (TFC), with Pearson correlation coefficients above 0.85 in several studies (Bensouici et al., 2023; Sandhu et al., 2020). At the cellular and animal level, fenugreek extracts up-regulate endogenous antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and reduce malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine markers in oxidatively challenged tissues (Atila Uslu et al., 2019; Zargar, 2014).

Table 11. Representative antioxidant activities of fenugreek seed extracts (*in-vitro* assays).

Extract	Assay	Activity (IC_{50} or %)	Reference
Methanolic, Egyptian "Giza 2"	DPPH	$IC_{50} \approx 92$ µg/mL	Mekky et al. (2024)
Methanolic, Indian cv.	DPPH	IC_{50} 60–180 µg/mL	Sandhu et al. (2020)
Methanolic, Algerian (review)	DPPH	IC_{50} 30–110 µg/mL	Bensouici et al. (2023)
Aqueous–ethanol	$ABTS^{\bullet+}$	IC_{50} 12–55 µg/mL	Yang, X., et al. (2022)
Hydroethanolic	FRAP	120–340 µmol Fe^{2+}/g	Bensouici et al. (2023)
Methanolic	β-carotene/linoleate	Inhibition 60–88 %	Bensouici et al. (2023)
Aqueous (in vivo)	SOD, CAT, GSH-Px in rat liver	↑ 30–70 % vs. control	Zargar (2014); Atila Uslu et al. (2019)

5.2. Antidiabetic and antihyperglycaemic effects

The antidiabetic activity of fenugreek is by far the most extensively documented and is the property that best explains its strategic interest as a functional food. Multiple randomised clinical trials and meta-analyses involving patients with type 2 diabetes or pre-diabetes consistently report significant reductions in fasting blood glucose, postprandial glucose and glycated haemoglobin (HbA1c), with effect sizes that compare favourably with those of low-dose oral antidiabetics in mild-to-moderate disease (Geberemeskel et al., 2019; Haxhiraj et al., 2024; Kim et al., 2023; Shabil et al., 2023; Shakil et al., 2024). At the experimental level, hydroalcoholic and aqueous fenugreek extracts have been shown to:

• inhibit the brush-border carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase, thereby slowing post-prandial glucose absorption (Hamden et al., 2010; Bensouici et al., 2023);

• stimulate glucose-induced insulin secretion from pancreatic β-cells via the action of 4-hydroxyisoleucine (Avalos-Soriano et al., 2016);

• enhance peripheral glucose uptake by promoting GLUT-4 translocation to the plasma membrane and by activating the IRS-1/PI3K/AKT pathway (Vijayakumar et al., 2005);

• reduce hepatic gluconeogenesis through AMPK activation and modulation of the FOXO1/PEPCK axis (Luo et al., 2023; Sarker et al., 2024);

• and protect pancreatic β-cells from oxidative damage, which delays the progressive insulin-secretion decline characteristic of long-term type 2 diabetes (Sarker et al., 2024; Tak et al., 2024).

Figure 8 summarises these convergent mechanisms in a single conceptual diagram. The complementary action of saponins, alkaloids, polyphenols and soluble fibres on multiple targets explains why fenugreek behaves as a *multi-target* antidiabetic agent rather than as a selective inhibitor — a property which, in chronic metabolic disease, is increasingly considered desirable.

Table 12. Selected experimental and clinical evidence on the antidiabetic activity of fenugreek seeds.

Study type	Model / population	Main finding	Reference
In-vitro	α-Amylase, α-glucosidase	IC_{50} 80–600 µg/mL; competitive inhibition	Hamden et al. (2010)
In-vitro	Pancreatic β-cells	4-HIL ↑ glucose-induced insulin release	Avalos-Soriano et al. (2016)
In-vivo (rat)	Streptozotocin-diabetic rats	↓Fasting glucose 25–45 %; ↑hepatic glycogen	Vijayakumar et al. (2005); Sarker et al. (2024)

Study type	Model / population	Main finding	Reference
Clinical	Type 2 diabetes (n = 25)	↓Fasting glucose 10–18 %, ↓HbA1c 0.7 %	Geberemeskel et al. (2019)
Meta-analysis	12 RCTs, 1,026 patients	Mean ↓fasting glucose 0.96 mmol/L	Shabil et al. (2023)
Systematic review	10 RCTs	Significant ↓HbA1c, ↓fasting glucose vs. placebo	Haxhiraj et al. (2024); Kim et al. (2023)
Network pharmacology	In silico — T2DM	Multi-target modulation of PI3K/AKT, AMPK	Luo et al. (2023)

5.3. Antihyperlipidaemic and cardioprotective effects

Hyperlipidaemia and diabetes frequently coexist; consistently, fenugreek seeds and their galactomannan-rich fractions lower total cholesterol, LDL-cholesterol and triglycerides, while preserving or modestly increasing HDL-cholesterol. Mechanistically, the soluble fibre binds bile acids in the gut, increases their faecal excretion and forces the liver to up-regulate cholesterol-7α-hydroxylase to replace the lost bile acids, indirectly lowering circulating LDL. In parallel, diosgenin and 4-hydroxyisoleucine modulate the SREBP-1c pathway and reduce hepatic lipogenesis, while phenolic acids inhibit pancreatic lipase (Badiee Gavarti et al., 2025; Mukthamba & Srinivasan, 2017; Wang, W., et al., 2023; Kumar et al., 2014). The cardioprotective effect of fenugreek therefore appears to combine a primary lipid-lowering action with secondary contributions from antioxidant and anti-inflammatory pathways.

Table 13. Antihyperlipidaemic and cardioprotective effects of fenugreek (selected studies).

Model / population	Intervention	Main outcomes	Reference
High-fat diet rats	Fenugreek seed extract 1 g/kg	↓ TC 24 %, ↓ LDL 33 %, ↓ TG 28 %	Kumar et al. (2014)
Myocardial-infarction rats	Dietary seed + garlic	↓ Cardiac lipids; ↑ antioxidant enzymes	Mukthamba & Srinivasan (2017)
Mice fed fenugreek gum	Galactomannan 5 % diet	↓ Serum cholesterol; gut microbiota shift	Wang, W., et al. (2023)
Mild–moderate hypercholesterolaemia (RCT)	Hydrolysed protein 4 g/d, 8 wk	↓ TC, ↓ LDL vs. placebo	Badiee Gavarti et al. (2025)

5.4. Antimicrobial and antifungal activities

Fenugreek extracts and essential oils show broad-spectrum but generally moderate antimicrobial activity. They inhibit common Gram-positive bacteria (*Staphylococcus aureus*, including some methicillin-resistant strains, *Bacillus subtilis*, *Streptococcus mutans*), Gram-

negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and several pathogenic fungi (*Candida albicans*, *Aspergillus niger*, *Fusarium* spp.), with MIC values typically between 0.5 and 8 mg/mL for crude extracts and 0.1–2 mg/mL for partially purified fractions or essential oils (Katnoria et al., 2024; Sethi et al., 2024; Sulieman et al., 2024). The antifungal effect of the essential oil is largely attributable to volatile monoterpenes such as α-pinene, limonene and linalool, while the antibacterial activity of polar extracts is more closely associated with phenolic acids, flavonoids and saponins acting on membrane integrity and quorum-sensing pathways.

Table 14. Selected antimicrobial activities of fenugreek seed preparations.

Pathogen	Preparation	Activity	Reference
Staphylococcus aureus	Methanolic extract	MIC 0.5–4 mg/mL	Sulieman et al. (2024)
Escherichia coli	Aqueous extract	Inhibition zone 8–14 mm	Sethi et al. (2024)
Streptococcus mutans	Ethanolic extract	Inhibition of biofilm formation	Sethi et al. (2024)
Candida albicans	Essential oil	MIC 0.25–1.5 mg/mL	Katnoria et al. (2024)
Aspergillus niger	Essential oil	MIC 0.5–2 mg/mL	Katnoria et al. (2024)
Oral biofilm consortium	Hydroalcoholic gel	Significant biofilm reduction	Sethi et al. (2024)

5.5. Anti-inflammatory and analgesic effects

Anti-inflammatory effects have been demonstrated for crude seed extracts, isolated polyphenols (especially apigenin and luteolin glycosides) and characterised flavonoid glycosides such as quercetin-3-*O*-diglucoside-7-*O*-glucoside (Bhatt et al., 2021). In the carrageenan-induced rat-paw oedema model, petroleum-ether and hydroalcoholic seed extracts inhibit oedema by 30–55 % at 200–400 mg/kg, an effect comparable to that of 10 mg/kg indomethacin, and reduce serum levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) (Panchal et al., 2016). At the molecular level, fenugreek polyphenols inhibit cyclo-oxygenase-2 (COX-2), 5-lipoxygenase and the NF-κB transcription pathway, three checkpoints relevant to the management of chronic inflammatory conditions.

Table 15. Anti-inflammatory and analgesic effects of fenugreek (selected studies).

Model / preparation	Dose / fraction	Main outcome	Reference
Carrageenan-induced paw oedema (rat)	200–400 mg/kg p.o.	Oedema ↓ 30–55 %	Panchal et al. (2016)
LPS-stimulated macrophages	Quercetin-glucoside fraction	TNF-α, IL-6 ↓; NO ↓	Bhatt et al. (2021)
Oral submucous fibrosis (clinical)	Fenugreek topical gel	Pain ↓, mouth opening ↑	Rajasekar & Sindhusha (2023)

5.6. Hepatoprotective and renoprotective effects

Several in vivo studies report a clear hepatoprotective and renoprotective potential. In rodents exposed to thioacetamide, sodium nitrite, alcohol or aluminium chloride, fenugreek extracts attenuate the rise of serum AST, ALT, ALP, urea and creatinine, restore the activity of antioxidant enzymes (SOD, CAT, GSH-Px) and reduce histopathological scores of necrosis and tubular damage (Atila Uslu et al., 2019; Belaïd-Nouira et al., 2012; Zargar, 2014). A randomised double-blind placebo-controlled trial in patients with non-alcoholic fatty liver disease showed that 600 mg/day of a standardised seed extract over three months significantly reduced serum ALT and AST and improved hepatic ultrasound grade (Taghavi & Babaei, 2020). Galactomannan-rich fractions specifically protect the kidney from diabetic nephropathy in streptozotocin-treated rats (Al-Khalifah et al., 2022).

Table 16. Hepatoprotective and renoprotective effects of fenugreek (selected studies).

Toxicity model	Treatment	Main outcome	Reference
Thioacetamide-induced hepatic injury	Aqueous extract 200 mg/kg	↓ ALT/AST 40–60 %; ↑ SOD/CAT	Zargar (2014)
Sodium nitrite intoxication	Seed extract 5–10 % diet	Liver, kidney histology restored	Atila Uslu et al. (2019)
Diabetic nephropathy (STZ rats)	Galactomannan 0.5 g/kg	↓ Urea, creatinine; tubular protection	Al-Khalifah et al. (2022)
Non-alcoholic fatty liver (RCT)	600 mg standardised extract	↓ ALT, ↓ AST, ↑ ultrasound score	Taghavi & Babaei (2020)

5.7. Gastroprotective and anti-ulcer activities

Although less explored than antidiabetic effects, gastroprotective activity has been documented for fenugreek seed mucilage and ethanolic extracts in ethanol-, indomethacin- and aspirin-induced gastric ulcer models. The mucilage forms a viscous protective layer over the gastric mucosa, while flavonoids inhibit H^+/K^+-ATPase and stimulate the synthesis of mucin and

prostaglandin E_2. Reductions of ulcer index of 50–80 % have been reported at doses of 200–400 mg/kg (Goyal et al., 2016; Saif et al., 2024).

5.8. Effects on obesity and metabolic syndrome

Anti-obesity and metabolic-syndrome studies converge on a complementary mechanism to the antidiabetic and antihyperlipidaemic activities. In high-fat-diet-fed rodents, fenugreek seed extracts and standardised galactomannan fractions reduce body-weight gain, visceral adipose mass, fasting insulin, fasting glucose and inflammatory markers, while reshaping the gut microbiota toward a leaner phenotype (Bruce-Keller et al., 2020; Kandhare et al., 2018; Muraki et al., 2012; Yang, J., et al., 2021). 4-Hydroxyisoleucine specifically attenuates macrophage-driven chronic inflammation in adipose tissue and improves whole-body insulin sensitivity. Clinical evidence in humans, although limited, points in the same direction: a recent triple-blind randomised trial showed that fenugreek hydrolysed protein significantly improved the lipid profile of patients with mild-to-moderate hypercholesterolaemia (Badiee Gavarti et al., 2025).

Table 17. Anti-obesity and metabolic-syndrome effects of fenugreek (selected studies).

Model	Intervention	Main outcome	Reference
High-fat-diet mice	Standardised galactomannan extract	↓ Body weight, ↓ FASn, ↓ leptin	Kandhare et al. (2018)
High-fat-diet mice	Whole seed	Gut microbiota normalised; ↓ adiposity	Bruce-Keller et al. (2020)
High-fat-diet rats	Reduced-bitterness fenugreek	↓ Visceral fat, ↓ insulin	Muraki et al. (2012)
High-fat-diet mice	4-Hydroxyisoleucine	↓ Macrophage inflammation	Yang, J., et al. (2021)

5.9. Other activities: neuroprotective, anticancer and immunomodulatory

Beyond metabolic effects, fenugreek extracts and isolated compounds have shown promising activity in three additional areas. (1) **Neuroprotective**: trigonelline and diosgenin protect cortical and hippocampal neurons against amyloid-β- and aluminium-induced damage, restore cholinergic transmission and inhibit acetylcholinesterase in vitro and in vivo, suggesting a potential anti-Alzheimer role (Belaïd-Nouira et al., 2012; Sekhar et al., 2024). (2) **Anticancer**: diosgenin and seed sprout extracts induce apoptosis in MCF-7 breast-cancer, prostate and pancreatic cancer cell lines, modulate p53 and Bcl-2/Bax expression, and reduce tumour growth in xenograft models (Khoja et al., 2022; Shabbeer et al., 2009; Al-Daghri et al., 2012). (3) **Immunomodulatory**: galactomannan fractions act as fermentable substrates for *Bacillus coagulans* and *Lactobacillus*

strains, supporting beneficial gut microbiota and indirectly modulating systemic immunity (Majeed et al., 2018).

Table 18. Selected neuroprotective, anticancer and immunomodulatory studies on fenugreek.

Activity	Model	Main finding	Reference
Neuroprotection	$AlCl_3$-treated rats	Lipid peroxidation ↓; cognitive function ↑	Belaïd-Nouira et al. (2012)
Neuroprotection	Cardiac alcohol toxicity (rat)	Trigonelline restores antioxidant defence	Sekhar et al. (2024)
Anticancer	MCF-7 breast-cancer cells	Cytotoxic IC_{50} 30–80 µg/mL; apoptosis	Khoja et al. (2022)
Anticancer	Pancreatic cancer in vitro	Cell-cycle arrest; ↑ p53	Shabbeer et al. (2009)
Immunomodulation	Probiotic *B. coagulans* fermentation	Galactomannan as effective prebiotic	Majeed et al. (2018)

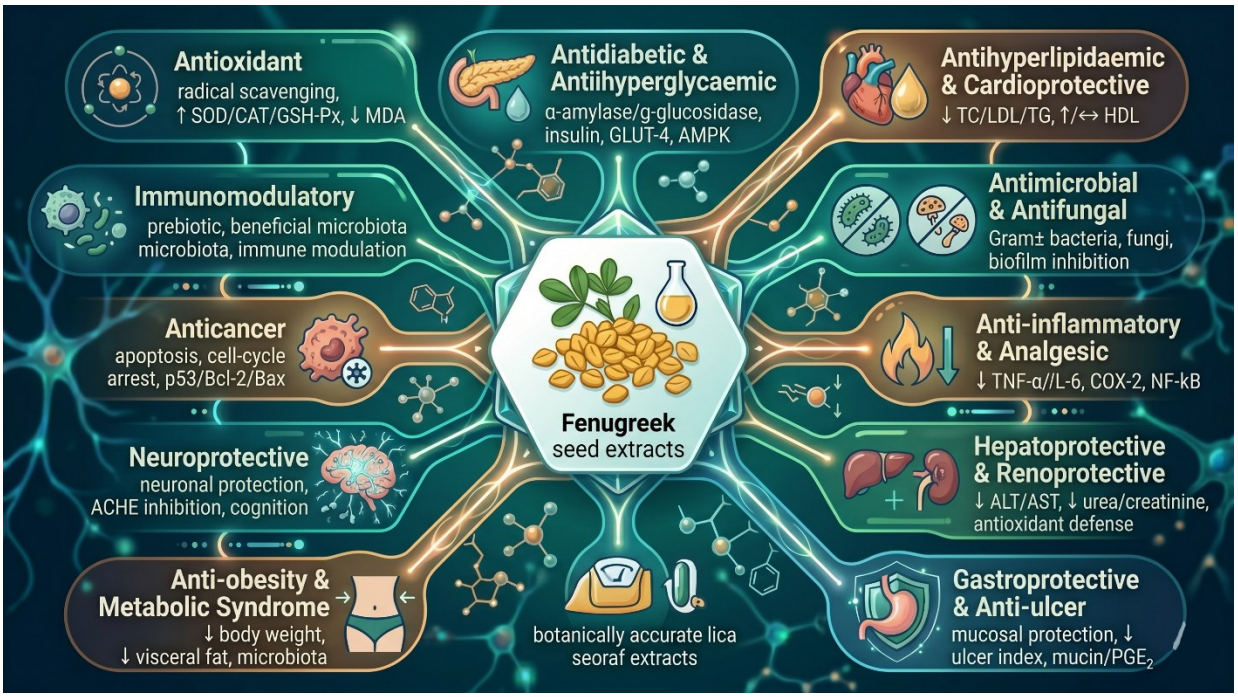

Figure 6. Multifunctional Pharmacological Effects and Mechanisms of Fenugreek (Trigonella foenum-graecum L) Seed Extracts (Feguiri And Medellel, 2026).

6. Ethnobotanical and Traditional Uses of Fenugreek Seeds

No review of fenugreek would be complete without addressing the long ethnobotanical record that links the species to human cultures from the Mediterranean basin to South-East Asia. Beyond its agronomic and pharmacological significance, fenugreek occupies a clear cultural niche, especially in the daily practices of households in North Africa, the Middle East, the Indian subcontinent and the Horn of Africa.

Culinarily, the dried seeds are used whole, cracked or finely ground, often after a brief dry-roasting that mellows their bitterness. They are an essential ingredient of curry powders, of the Egyptian *helba*-based hot drinks, of the Yemeni *hilbeh* paste, of Turkish *çemen* (used to coat *pastırma*), of Ethiopian *injera* batters and of many bakery applications, where the soaked seeds add aroma and improve dough texture (Saif et al., 2024; Wani & Kumar, 2018). Fresh young leaves (*methi*) are eaten as a vegetable in the Indian subcontinent, and the sprouts are an emerging functional-food ingredient used in salads and smoothies (Khoja et al., 2022). The characteristic odour of fenugreek, often described as warm, slightly sweet and reminiscent of maple syrup, is largely due to sotolone, a furanone present in trace amounts in the seeds and concentrated by post-prandial metabolism — a feature responsible for the well-known body and urine odour after intake (Visuvanathan et al., 2022).

Medicinally, fenugreek is one of the most cited herbs in traditional medical systems. Greco-Roman authors (Dioscorides, Pliny) described it as an emollient and a remedy for digestive complaints; classical Arabic medicine (Ibn Sina, Al-Razi) recommended *helba* for fevers, dyspepsia, weakness, lactation support and skin disorders; Ayurvedic compendia (*Charaka Samhita*) prescribed *methi* for diabetes (*madhumeha*), digestive sluggishness and post-partum recovery. Modern ethnobotanical surveys conducted in North African communities — including those of southern Algeria — confirm that contemporary uses still mirror these ancient recommendations: hot infusions or decoctions are taken to relieve abdominal cramps, sore throats and post-partum exhaustion; soaked seeds are applied as poultices on abscesses and burns; and roasted-seed beverages are routinely offered as galactagogue tonics to nursing mothers (Goyal et al., 2016; Saif et al., 2024).

Table 19. Traditional uses of fenugreek seeds and their corresponding modern therapeutic targets.

Traditional indication	Region(s)	Modern validated activity
Diabetes (*madhumeha*, *as-sukkari*)	India, Middle East, North Africa	Antihyperglycaemic, antidiabetic
Hyperlipidaemia / heart weakness	Mediterranean, Middle East	Hypolipidaemic, cardioprotective
Digestive complaints, dyspepsia, constipation	Mediterranean, India	Pro-kinetic, prebiotic, mild laxative
Lactation support (galactagogue)	North Africa, Middle East, India	Enhanced milk production (clinical reports)
Post-partum tonic & convalescence	Algeria, Egypt, Yemen	Anti-anaemic (high Fe), nutritive
Cough and respiratory complaints	Mediterranean	Mucolytic (mucilage), anti-inflammatory
Abscesses, burns, skin lesions (poultice)	Maghreb, Middle East	Anti-inflammatory, mild antimicrobial
Hair loss and dandruff (topical)	Algeria, Morocco, India	Antioxidant, anti-microbial
Veterinary (cattle appetite, milk yield)	North Africa, India	Galactagogue, appetite stimulant

In El-Oued specifically, fenugreek seeds are part of the *helba* preparation that traditionally accompanies childbirth: nursing mothers receive a hot porridge of toasted fenugreek, dates, semolina and olive oil for at least seven days post-partum to "restore body heat" and promote milk production. The same households use fenugreek decoctions for cold-season coughs, mild stomach complaints and as a general tonic during periods of fatigue. A recurrent observation among local users is that *El-Oued seeds* are perceived as more aromatic and more bitter than imported material — a sensory signature most likely linked to higher sotolone, trigonelline and saponin contents under the local pedoclimatic conditions, even though comparative quantitative data are still scarce in the literature (Hammiche et al., 2006) .

CHAPTER II

Materials and Methods

7. Materials and Methods

7.1. Plant Material Collection

Fenugreek (*Trigonella foenum-graecum* L.) seeds locally cultivated in the El-Oued region (Hassani Abdelkrim area, 33°28'33.4" N 6°53'48.7" E, southeast Algeria) were used as plant material. Before use, the seed lot was carefully cleaned to remove foreign matter and damaged seeds, then rinsed with distilled water to eliminate surface impurities. The cleaned seeds were spread in a thin layer and dried at ambient room temperature in the shade, covered with a breathable, dust-free cloth to protect them from light, dust, and insects while allowing adequate air circulation. After complete drying, the seeds were stored in a large, well-sealed glass bottle at room temperature, protected from moisture and direct light, until subsequent use in the extraction procedures.

7.2. Preparation of the Extract and Fractionation of Plant Material

Fenugreek (*Trigonella foenum-graecum* L.) seeds (200 g) were ground using an electric grinder to obtain a coarsely pulverized material (medium granulometry) suitable for solvent extraction. The resulting seed powder was macerated in 800 mL of a hydroalcoholic methanol/water solution (75:25, v/v) for 24 hours at room temperature with occasional stirring. This procedure was repeated three times, renewing the solvent every 24 hours, and the combined macerates were subsequently filtered. At a later stage, the hydroalcoholic filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain the crude dried extract.

7.3. Phytochemical Studies

7.3.1. Determination of the Extraction Yield

The extraction yield of the hydromethanolic extract of *Trigonella foenum-graecum* L.) seeds, cultivated in the El Oued region, was determined gravimetrically. Following the three successive 24-hour maceration cycles in methanol–water (75:25, v/v), the combined filtrates were concentrated to dryness under reduced pressure using a rotary evaporator at 40 °C until a constant mass was attained. The resulting crude dried extract was carefully recovered into a pre-weighed glass container and weighed on an analytical balance. The initial mass of the shade-dried, ground seed material subjected to extraction was recorded prior to maceration on the same balance and served as the reference basis for the calculation.

The extraction yield was expressed as a percentage on a weight-by-weight basis (%, w/w), relative to the initial dry weight of the plant material, and was calculated according to the following equation:

$$\text{Extraction yield } (\%, w/w) = \frac{m_{\text{extract}}}{m_{\text{sample}}} \times 100 \quad \text{(Equation 01)}$$

where:

***m*_extract** is the mass of the crude dried hydromethanolic extract obtained after complete solvent removal under reduced pressure (g);

***m*_sample** is the initial mass of the shade-dried, ground *Trigonella foenum-graecum* L. seeds subjected to maceration (g).

7.3.2. Determination of Total Phenolics

The total phenolic content of the hydromethanolic extract of *Trigonella foenum-graecum* L. seeds was determined using the Folin–Ciocalteu colorimetric method as described by Li et al. (2007), with slight modifications. An aliquot of 0.2 mL of the appropriately diluted extract was mixed with 1.0 mL of Folin–Ciocalteu reagent (10%, v/v) and allowed to stand for 5 minutes at room temperature. Subsequently, 0.8 mL of a 7.5% (w/v) Na_2CO_3 solution was added, and the mixture was incubated in the dark at room temperature for 40 minutes. The absorbance was then measured at 765 nm against a reagent blank using a UV–Visible spectrophotometer.

Total phenolic content was quantified using a gallic acid calibration curve and expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg extract), according to the linear regression equation $y = 0.006x + 0.0007$ ($R^2 = 0.99$).

7.3.3. Determination of Total Flavonoid

Total flavonoid content of the *Trigonella foenum-graecum* L. seeds extract was determined according to the aluminum chloride colorimetric method described by Mbaebie et al. (2012), with slight modifications. Equal volumes of the appropriately diluted extract and aluminum chloride solution (0.2%, w/v in methanol) were mixed thoroughly and allowed to incubate for 60 minutes at room temperature. After incubation, the absorbance of the reaction mixture was measured at 415 nm against a reagent blank using a UV–Visible spectrophotometer.

Total flavonoid content was calculated from a quercetin calibration curve and expressed as micrograms of quercetin equivalents per milligram of dry extract (µg QE/mg extract), according to the linear regression equation $y = 0.005x + 0.0402$ ($R^2 = 0.99$).

7.3.4. Determination of Tannins Content

Total condensed tannins were determined according to the vanillin–HCl method described by Kokoska et al. (2008) and Muthukrishnan et al. (2018), with slight modifications. In stoppered (coated) test tubes, 0.5 mL of the appropriately diluted extract was mixed with 1.5 mL of concentrated HCl and 3.0 mL of vanillin reagent (4%, w/v, prepared in methanol). The reaction mixtures were incubated at 20 °C for the specified reaction time, and the absorbance was then measured at 500 nm against a reagent blank using a UV–Visible spectrophotometer.

The total condensed tannin content was expressed as micrograms of catechin equivalents per milligram of dry extract (µg C/mg extract), based on the linear regression equation obtained from the catechin calibration curve $y = 0.0036x + 0.0249$ ($R^2 = 0.996$).

7.3.5. LC-MS/MS Analysis

The phenolic profile of the hydromethanolic extract of *Trigonella foenum-graecum* L. seeds was analyzed by ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC–ESI–MS/MS). The analyses were performed using a Shimadzu 8040 triple quadrupole mass spectrometer equipped with UFMS technology and coupled to a Nexera XR LC-20AD binary pump system. Chromatographic separation was achieved on a Restek Ultra C18 reversed-phase column (150 × 4.6 mm, 3 µm particle size), operated at ambient column temperature.

The mobile phase consisted of solvent A (water containing 0.1% formic acid) and solvent B (methanol). Elution was carried out at a constant flow rate of 0.2 mL/min using the following gradient program: 0.0–0.2 min, 98% A; 0.2–2.5 min, linear change to 25% A; 2.5–4.0 min, 0% A; 4.0–10.0 min, 0% A; 10.0–10.01 min, return to 98% A; and 10.1–15.0 min, 98% A for column re-equilibration. The injection volume was 5 µL of the filtered hydromethanolic extract.

Mass spectrometric detection was performed using an electrospray ionization (ESI) source operated in both positive and negative ionization modes. The general source conditions were: collision-induced dissociation (CID) gas pressure 230 kPa, conversion dynode voltage −6.0 kV, desolvation line (DL) temperature 250 °C, nebulizing gas flow 3.0 L/min, heat block temperature 400 °C, and drying gas flow 10.0 L/min. Data acquisition was carried out in multiple reaction monitoring (MRM) mode, with precursor/product ion transitions and collision energies optimized for each standard compound and applied under identical chromatographic conditions to the fenugreek seed extract.

7.4. *In-Vitro* Antioxidant Activity

7.4.1. DPPH Free Radical Scavenging Assay

The putative radical-scavenging activity of *Trigonella foenum-graecum* L seeds extract was evaluated against $DPPH^{\bullet}$ free radicals using the method described by (Jafri et al., 2017). Equal amounts of samples and 0.1mM of $DPPH^{\bullet}$ solution were blended and placed in the dark for 30min at ambient temperature. The absorbance of the solutions was determined at a wavelength of 517 nm, Trolox and Ascorbic acid were used as standards in this assay, and the radical scavenging potential of $DPPH^{\bullet}$ is determined as IC_{50} values calculated according to the below equation:

$$Inhibition\ Percentage = [1-(Abs/Abc)] \times 100 \quad \text{(Equation 02)}$$

Ab_S: absorbance of the sample or standard solution.

Ab_C: absorbance of control solution.

7.4.2. ABTS Cation Radical Scavenging Assay

The test was conducted as described earlier (Re et al., 1999), 4 mL of ABTS (7mM) solution were mixed with 4 mL 2.45 mM $K_2S_2O_8$ solution and stored for 16 hours at room temperature. Prior to testing the ABTS solution was concentration with ethanol till the absorbance value at 734 nm came out to be 0.703 ± 0. 025. Volumes of 950 µL of $ABTS^{\bullet+}$ solutions were mixed with 50µL different concentrations of *Trigonella foenum-graecum* L seeds samples. Ethanol was used as negative control, while Trolox and Ascorbic acid were used as standard. The graph of the percentage scavenging effect with sample concentration was used to obtain the IC_{50} values.

$$ABTS^{\bullet+}\ scavenging\ power\ (\%) = [(Ab_0 - Ab_1)/Ab_0] \times 100 \quad \text{(Equation 03)}$$

Ab_0: absorbance of $ABTS^{\bullet+}$ solution .

Ab_1: absorbance of $ABTS^{\bullet+}$ standard or extract.

7.4.3. Phenanthroline Antioxidant Assay

The antioxidant activity of the hydromethanolic extract of *Trigonella foenum-graecum* L. seeds was evaluated using the o-phenanthroline assay, following the iron(III)–phenanthroline total antioxidant capacity method described by Özyürek et al. (2007) and further discussed by Apak and co-workers, with slight modifications to adapt it to plant extracts. Briefly, an appropriate volume of the extract (or standard) was mixed in a test tube with phosphate buffer (0.1 M, pH 7.4), o-phenanthroline solution (5 mM), and ferrous sulfate solution (5 mM) to obtain a final reaction mixture volume of 3.0 mL. The mixture was incubated at 37 °C for 30 minutes, and the formation of the Fe(II)–o-phenanthroline complex was monitored by measuring the absorbance at 510 nm against a reagent blank using a UV–Visible spectrophotometer.

The assay was performed at different concentrations of the hydromethanolic extract of *T. foenum-graecum* seeds to establish a concentration–response curve, while Trolox and Ascorbic acid were used as standard. Antioxidant activity was expressed as the percentage inhibition of the control reaction, calculated according to the following equation, commonly used in spectrophotometric antioxidant assays:

$$\text{Inhibition Percentage} = \left[1 - \left(\frac{\text{Abs}_S}{\text{Abs}_C}\right)\right] \times 100 \quad \text{(Equation 04)}$$

where Abs_S is the absorbance of the reaction mixture containing the sample or standard solution and Abs_C is the absorbance of the control reaction (without extract or standard). The IC_{50} value (concentration providing 50% inhibition) can be determined from the plot of inhibition percentage versus sample concentration by linear regression.

7.5. Enzyme inhibitory activities

7.5.1. Evaluation α-amylase inhibitory activity

α-amylase inhibitory activity was determined using the technique described by the Marmouzi study (Marmouzi et al., 2018).This involved incubating various concentrations of *Trigonella foenum-graecum* L. seeds extract (25 µL) with the α-amylase enzyme in a phosphate buffer (50 µL) and starch solution (50 µL) at 37°C. After 10 minutes, the reaction was stopped by adding 25 µL of 1 M hydrochloric acid and 100 µL of IPI (iodine-potassium iodide) solution. Following that, the absorbance was measured at 630 nm. As a positive control, acarbose was used. The inhibitory % activity was calculated in the following way (Equation 05):

$$Inhibition\ (\%) = (1 - (Absorbance_{sample}/Absorbance_{control})) \times 100 \quad \text{(Equation 05)}$$

The results were represented as IC_{50} values, which show the fraction concentration necessary to block 50% of enzyme activity.

7.5.2. Evaluation of α-glucosidase inhibitory activity

To determine the inhibitory capacity of α-glucosidase using *Trigonella foenum-graecum* L. seeds extract as the test sample. Methodologies established by Marmouzi (Marmouzi et al., 2018) were utilised, with p-nitrophenyl-α-D-glucopyranoside as the substrate and a 96-well microplate format. A mixture of 50 µL of the extract, 50 µL of glutathione, and 50 µL of α-glucosidase solution in phosphate buffer (pH 6.8), and PNPG (50 µL) was prepared and incubated for 15 minutes. The process was then stopped by adding 50 µL of 0.2 M Na_2CO_3, and the absorbance at 400 nm was

measured. Acarbose was employed as a positive control. The inhibitory percentage activity was estimated using the equation below (Equation 06):

$$Inhibition\ (\%) = (1 - (Absorbance_{sample}/Absorbance_{control})) \times 100 \quad \text{(Equation 06)}$$

The results were subsequently expressed as IC_{50} values.

7.5.3. Evaluation of acetylcholinesterase inhibition activity

The capacity of *T. foenum-graecum* seeds to inhibit acetylcholinesterase was analysed through the method of Ellman et al (Ellman et al., 1961). The procedure involved mixing 300 µL of 50 mM Tris-HCl buffer (pH 8), 30 µL of AChE solutions, and 100 µL of the sample. Following a 15-minute incubation, 130 µL of acetylthiocholine iodide and 440 µL of DTNB (3 mM) were added to end the reaction, and the absorbance was determined at 412 nm. Galantamine was used as the standard, and IC_{50} values were utilised to report the results. The inhibition potency was calculated using the equation below (Equation 07):

$$\%Inhibition = [(A-B)/A] \times 100 \quad \text{(Equation 07)}$$

A is AChE capacity in the absence of an inhibitor, B is AChE capacity in the presence of an inhibitor.

7.6. Statistical Analysis

All experiments, including phytochemical quantification, *in vitro* antioxidant assays and enzyme inhibitory tests (α-amylase, α-glucosidase and acetylcholinesterase), were performed in triplicate (n = 3). Results are expressed as mean ± standard deviation (SD). IC_{50} ($A_{0.5}$, where applicable) values were obtained by linear regression from concentration–response curves.

CHAPTER III

Results and Discussion

10. General Conclusion And Recommendations

The present work was undertaken to characterize, in a systematic manner, the phytochemical composition and the in vitro biological potential of the hydromethanolic extract obtained from fenugreek (*Trigonella foenum-graecum* L.) seeds locally cultivated in the El-Oued region of southeastern Algeria. The extraction procedure provided a satisfactory yield, and the resulting extract proved to be particularly rich in phenolic compounds, flavonoids and condensed tannins, reflecting a substantial reservoir of bioactive secondary metabolites. The LC–MS/MS profile further confirmed the chemical complexity of these seeds, revealing a wide spectrum of constituents that included free amino acids, flavan-3-ols, hydroxycinnamic and hydroxybenzoic acids, stilbenes, secoiridoids, monoterpene phenols, carotenoids, methoxylated flavones and pentacyclic triterpene acids, which together provide a credible molecular basis for the pharmacological behaviour subsequently observed.

From a biological standpoint, the hydromethanolic extract displayed a coherent and reproducible spectrum of in vitro activities. It exhibited a marked free-radical-scavenging capacity in the DPPH and ABTS systems, together with a meaningful reducing power in the o-phenanthroline assay, confirming a robust antioxidant profile fully consistent with its high content of phenolic and flavonoid constituents. More importantly, the extract demonstrated a potent antidiabetic potential through the inhibition of the carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase, which appeared more efficient than the reference drug acarbose, as well as a tangible anti-Alzheimer potential through the inhibition of acetylcholinesterase. Taken together, these findings indicate that the seeds cultivated in El-Oued can effectively replace imported fenugreek in terms of biological efficacy and therefore deserve to be considered as a strategic local resource rather than a marginal traditional crop.

On the basis of these encouraging findings, it is recommended that the in vitro evidence assembled here be extended through a coordinated programme of in vivo investigations in suitable animal models, followed by carefully designed clinical studies. Such work is indispensable to translate the observed antioxidant, antidiabetic and anti-Alzheimer activities into reliable therapeutic or nutraceutical applications, and it should specifically address the determination of safe and effective dosage ranges, possible contraindications, the identification of population subgroups that should avoid fenugreek—notably pregnant women, individuals on hypoglycaemic or anticoagulant therapy, and patients with known legume hypersensitivity—and the systematic monitoring of potential adverse effects under prolonged consumption.

In parallel, the scientific community is encouraged to invest in technological and formulation strategies capable of mitigating the well-known sensory and morphological drawbacks of fenugreek seeds. In particular, research efforts should target processing approaches—such as solvent fractionation, microencapsulation, debittering or the selective removal of odorant sotolone-type compounds—that allow the consumption of fenugreek without generating the strong and persistent body and urine odour traditionally associated with its intake, since this olfactory side-effect represents a major obstacle to consumer acceptance and to regular dietary use. Complementary work should also address the technological and commercial limitations linked to the small size of local seeds and to the abundant pale awns that diminish their visual quality and market attractiveness, through improved post-harvest cleaning, calibration and surface treatment. Building on these advances, it would be highly relevant to develop and standardize a dietary supplement or functional food product based exclusively on locally cultivated El-Oued fenugreek seeds, designed to fully exploit their demonstrated antioxidant, antidiabetic and neuroprotective potential while ensuring chemical standardization, microbiological safety and sensory acceptability.

Beyond their scientific significance, these results carry a clear socio-economic dimension. By documenting that locally cultivated fenugreek matches, and in several assays appears to exceed, the bioactivity commonly attributed in the literature to imported material, the study provides an evidence-based argument for paying greater attention to indigenous fenugreek production. Promoting the cultivation, processing and valorization of El-Oued fenugreek could contribute to strengthening the national economy by reducing dependence on imported fenugreek, lowering the import bill of foreign seeds that currently dominate the domestic market, and generating new opportunities along the entire value chain, from agricultural production to the processing and export of standardized fenugreek-based ingredients and finished products.

Taken together, the conclusions and recommendations formulated above underline both the scientific and the socio-economic relevance of valorizing Algerian local fenugreek and open several promising avenues for future research. Pursuing this line of work will require a genuinely multidisciplinary collaboration involving plant biology, phytochemistry, pharmacology, nutrition, food technology and agricultural economics, so that the chemical, biological, agronomic and commercial dimensions of the species can be addressed in an integrated manner. From an applied perspective, the present study can serve as a methodological reference and as a starting point for broader projects devoted to the characterization and valorization of Algerian medicinal and aromatic plants, with the ultimate goal of transforming the rich but still under-exploited national phytogenetic heritage into innovative, evidence-based products contributing to public health and to sustainable economic development.

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INTERNATIONAL SCIENTIFIC COMMUNICATIONS

Phytochemical Analysis and Biological Activity Evaluation of the Hydromethanolic Extract of Fenugreek *(Trigonella foenum-graecum L.)* Seeds Locally Cultivated in El-Oued Region, Algeria

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INTRODUCTION

- Trigonella foenum-graecum L. (fenugreek) is a widely used medicinal plant across North Africa, valued for its therapeutic potential.
- Fenugreek seeds are rich in bioactive secondary metabolites including phenolics, flavonoids, and tannins with documented pharmacological activities.
- The El-Oued region possesses unique edaphoclimatic conditions that may influence the phytochemical composition of locally cultivated varieties.
- **Objective: Comprehensive phytochemical characterization and evaluation of the antioxidant and antidiabetic activities of the hydromethanolic extract.**

MATERIALS & METHODS

Trigonella foenum-graecum L. seeds — El-Oued, Algeria

1 Plant Material
Fenugreek seeds (*Trigonella foenum-graecum* L.) Locally cultivated in El-Oued, Algeria

2 Extraction
Maceration in MeOH:H_2O (75:25, v/v) Room temperature • 48 hours

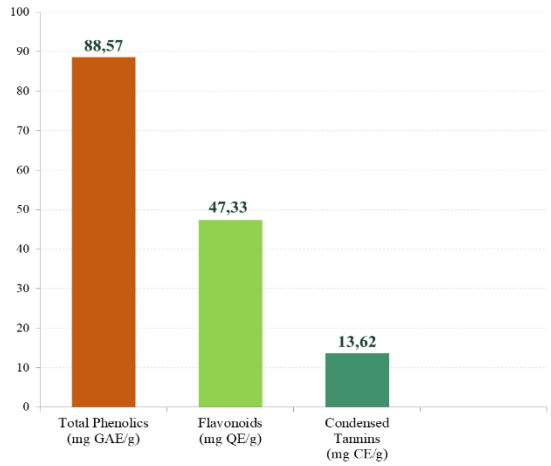
13.38%
Extraction Yield

3 Analysis
LC-MS profiling Phytochemical quantification

4 Bioactivity Testing
Antioxidant assays (DPPH, ABTS, PHE) α-Amylase inhibition assay

PHYTOCHEMICAL PROFILE

Quantitative Analysis of Bioactive Compounds



LC-MS PROFILING

6
Major Compounds identified

27
Flavonoid

	Compound (HPLC)	Class
1	Protocatechuic acid	Phenolic acid
2	Vanillic acid	Phenolic acid
3	Luteolin	Flavonoid
4	Apigenin	Flavonoid
5	Kaempferol	Flavonoid
6	Quercetin	Flavonoid

ANTIOXIDANT ACTIVITY

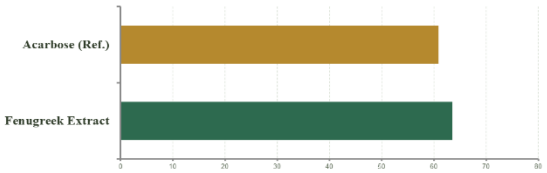
77.365 µg/ml
DPPH
IC_{50}

29.493 mg/g
ABTS
IC_{50}

42.033 mg /g
PHE
Phenanthroline Antioxidant

ANTIDIABETIC ACTIVITY

α-Amylase Inhibition — IC_{50} Comparison (µg/mL)



CONCLUSION

- The hydromethanolic extract of fenugreek seeds from El-Oued demonstrated potent antioxidant activity across three complementary assays, confirming its strong free-radical scavenging and reducing potential.
- Notable α-amylase inhibitory activity (IC_{50} = 245.14 ± 26.63 µg/mL) was comparable to the reference drug acarbose (IC_{50} = 496.15 ± 16.97 µg/mL), confirming significant antidiabetic potential for glycemic control applications.
- LC-MS profiling revealed a rich and diverse polyphenolic composition (6 identified compounds, 27 flavonoid), strongly supporting the observed bioactivities.
- **These findings position fenugreek seeds from El-Oued as a promising natural source for pharmaceutical, nutraceutical, and functional food development. Fenugreek seeds from El-Oued represent a promising local natural resource for pharmaceutical, nutraceutical, and functional food development, reducing import dependency while promoting regional agricultural sustainability.**
- .

SA-W&P

CERTIFICATE

Of Participation

The chairman of the First International Conference on Sustainable Agriculture: Water Treatment and Plant Protection (SA-W&P-2026), Faculty of Life and Natural Sciences, University of El Oued, certifies that :

FEGUIRI Hala

Has participated with a **Poster** communication entitled:

Phytochemical Analysis And Biological Activity Evaluation Of The Hydromethanolic Extract Of Fenugreek (*Trigonella Foenum-Graecum* L.) Seeds Locally Cultivated In El-Oued Region, Algeria

Co-authors: CHEERADA Nezar , MEDLEL Aya

Dr. MEKHADMI Nour Elhouda
Seminar President

Chairman of the conference
Dr. Mekhadmi Nourelhouda

Dean of faculty
Dr. ZAATER Abdelmalek

SA-W&P

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Co-authors: CHEERADA Nezar , FEGUIRI Hala

Dr. MEKHADMI Nour Elhouda
Seminar President

Chairman of the conference
Dr. Mekhadmi Nourelhouda

Dean of faculty
Dr. ZAATER Abdelmalek

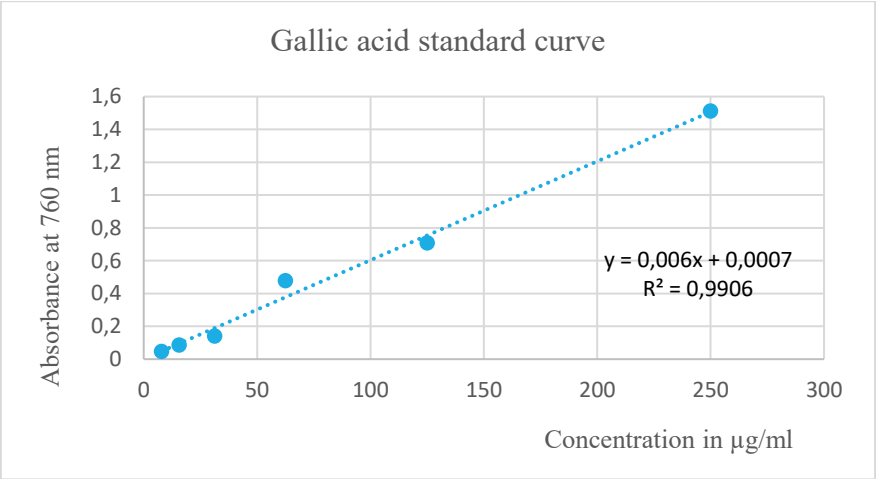
APPENDICES

Appendix 01. « LC-MS/MS analysis characterizations »

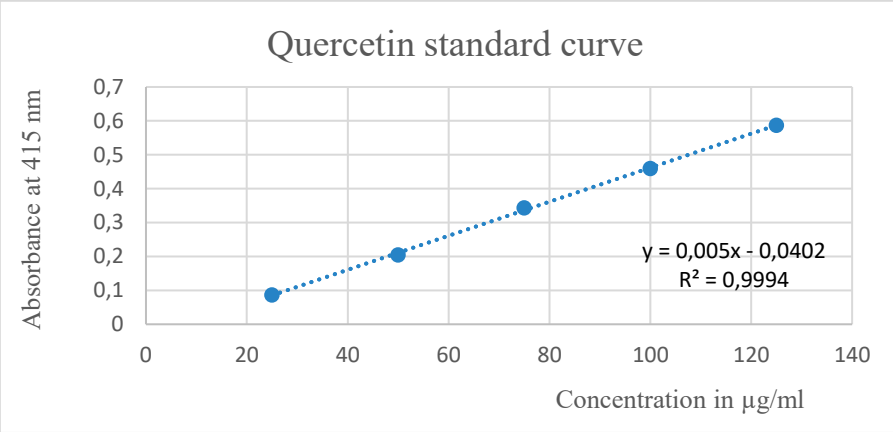
<table>
<tr><th>LC CONDITIONS</th><th>MS CONDITIONS (Mass Spectrometer)</th></tr>
<tr><td>
Mobile Phase A: Water + 0.1 % Formic Acid
Mobile Phase B: Methanol
Column: Restek Ultra C18 — 150 × 4.6 mm, 3 µm
Flow rate: 0.20 mL/min
Injection volume: 5 µL

<table>
<tr><th>Gradient</th><th>Time (min)</th><th>Flow (mL/min)</th><th>% A</th></tr>
<tr><td></td><td>0.00 – 0.20</td><td>0.20</td><td>98</td></tr>
<tr><td></td><td>0.20 – 2.50</td><td>0.20</td><td>25</td></tr>
<tr><td></td><td>2.50 – 4.00</td><td>0.20</td><td>0</td></tr>
<tr><td></td><td>4.00 – 10.00</td><td>0.20</td><td>0</td></tr>
<tr><td></td><td>10.00 – 10.01</td><td>0.20</td><td>98</td></tr>
<tr><td></td><td>10.01 – 15.00</td><td>0.20</td><td>98</td></tr>
</table>
</td><td>
System: Shimadzu UPLC-ESI-MS/MS 8040 (UFMS technology)
Binary pump: Nexera XR LC-20AD
Ion source: Electrospray Ionization (ESI)
Ionization modes: Positive and Negative
CID gas: 230 kPa
Conversion dynode: −6.00 kV
DL temperature: 250 °C
Heat-block temperature: 400 °C
Nebulizing gas flow: 3.00 L/min
Drying gas flow: 10 L/min
Acquisition: Multiple Reaction Monitoring (MRM)
Definitions: ILMER at BVU
</td></tr>
<tr><td colspan="2">ENVIRONMENTAL CONDITIONS
Temperature: (22.0 ± 5.0) °C
Relative Humidity: (50 ± 15) % rh</td></tr>
</table>

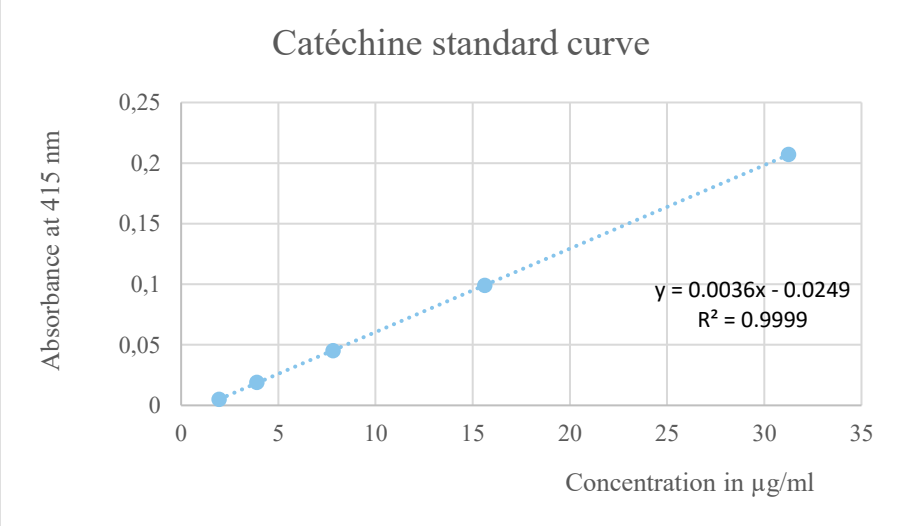
Appendix 02. « Standard curves »



A. Gallic acid standard curve



B. Quercetin standard curve



C. Catéchine standard curve