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THEME

Contribution to the phytochemical study and evaluation of the biological potential of *Salsola tetragona* Delile. growing in Oued Souf region.

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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

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CHAPTER II

Investigating the antioxidant potential and the inhibitory activity of α-amylase, α-glucosidase, and acetylcholinesterase of different fractions from *Salsola tetragona* Delile

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SUMMARY

This study was conducted on the plant *Salsola tetragona* Delile, which is a medicinal desert plant belonging to the *Amaranthaceae* family, spreading in the saline areas located in the northeast of the Oued Souf region. The aim was to determine its nutritional value and assess the secondary metabolite products, as well as understand the chemical composition of this plant and try to determine the extent of biological effectiveness that characterizes it. This is in order to verify what has been mentioned regarding its use in traditional medicine, as well as an attempt to exploit it in the pharmaceutical field.

Chemical analyses of the nutritional value showed that the biomass of the aerial part of *S.tetragona* plant is characterized by a considerable mineral content, distinguished by the abundance of most elements, as it contains an important moisture content indicating its ability to withstand harsh desert growing conditions. It also contains average rates of carbohydrates, lipids and proteins. The qualitative phytochemical examination of the crude extract of the aerial part of the *S. tetragona* plant, showed that it contains most secondary metabolites including alkaloids, coumarins, mucilages, phenols, saponins, sterols and terpenes. Quantitatively, the quantitative estimation of the crude extract and the five fractions derived from it, which are the n-hexane, dichloromethane, ethyl acetate, n-butanol and the remaining aqueous fractions, showed the presence of considerable amounts of phenols, flavonoids, tannins and terpenes which were relatively close in all fractions. Meanwhile, LC-HR/MS analysis of the crude hydromethanolic extract of *S. tetragona* plant revealed the presence of 16 phenolic compounds, 11 of which appear for the first time in the *Salsola* genus, including salicylic acid, ascorbic acid and naringenin as the most abundant elements. While GC/MS analysis of the n-hexane fraction revealed the presence of 7 compounds, 5 of which appear for the first time in this genus, 3 compounds were distinguished by the highest abundance which are hexadecanoic acid methyl ester (23.48%), bis-(2-ethylhexyl) phthalate (21.98%), 8,11-octadecadienoic acid methyl ester (18.49%).

The antioxidant capacity was evaluated using DPPH•, FRAP, ABTS•+, CUPRAC, anti-hemolysis and metal chelating assays. The dichloromethane fraction showed superiority in the DPPH• and ABTS•+ assays, while the ethyl acetate fraction was superior in the CUPRAC assay. The hexane fraction had the greatest ability to chelate Fe^{2+} ions in the metal chelating assay, and also had the highest ability to protect red blood cells from hemolysis in the anti-hemolysis assay. In the anti-inflammatory assay, the ethyl acetate fraction showed strong inhibitory activity against inflammatory protein denaturation, with an IC_{50} value of 13 ±5 µg/mL, superior to that of the standard Aspirin®. Regarding the fractions' activity in inhibiting bacterial growth, all fractions showed moderate antibacterial activity against both *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains.

For antidiabetic activity, the ethyl acetate and n-butanol fractions showed strong inhibition of α-amylase and α-glucosidase enzymes, with an IC_{50} of 70 µg/ml for both, indicating good potential for developing this plant for treating type 2 diabetes. For acetylcholinesterase enzyme inhibition related to Alzheimer's disease, the n-butanol fraction showed the highest inhibitory activity with an IC_{50} of 30 ± 0.30 µg/ml, suggesting its potential for alleviating symptoms of this disease.

Regarding cytotoxicity testing against MCF7 breast cancer cells, the dichloromethane fraction showed high efficacy, outperforming the other fractions with the highest inhibition value of IC_{50} = 98 µg/mL. It and the ethyl acetate fraction also showed significant activity in the anti-SARS-CoV2 assay.

Keywords: *Salsola tetragona* Delile, LC-HR/MS, GC/MS, biological activities, MCF7 breast cancer cell line, anti-SARS-CoV2, antidiabetic, anti-Alzheimer's disease.

SUMMARY IN ARABIC (مُلخص)

أجريت هذه الدراسة على نبات *Salsola tetragona* Delile وهو نبات، طبي صحراوي ينتمي الى العائلة القُطَيفية (*Amaranthaceae*) ينتشر في المناطق المالحة الواقعة في الشـمال الشـرقي لمنطقة واد سـوف، وذلك قصـد تحديد القيمة الغذائية وتثمين نواتج الأيض الثانوي ومعرفة التركيب الكيميائي لهذا النبات ومحاولة تحديد مدى الفعالية البيولوجية التي يتميز بها وذلك في إطار التحقق من صـحة ما جاء في اسـتخدامه في الطب التقليدي وكذا محاولة اسـتغلاله في المجال الصيدلاني.

أظهرت التحاليل الكيميائية للقيمة الغذائية أن الكتلة الحيوية للجزء الهوائي لنبات *S.tetragona* تتميز بمحتوى معدني معتبر، مُميز بوفرة أغلب العناصـر ، كما تحتوي على منسـوب رطوبة مهم يدلّ عن قدرتها على تحمّل ظروف نموها الصـحراوية القاسـية. كما تحتوي على نسـب متوسـطة من الكربوهيدرات واللبيدات والبروتينات. أسـفر الفحص الفيتوكيميائي النوعي للمسـتخلص الخام للجزء الهوائي لنبات *S. tetragona*، احتوائه على أغلب نواتج الأيض الثانوي بما فيها القلويدات، الكومارينات، الصمغ، الفينولات، الصابونينات، الستيرولات والتربينات. أما كميا، فقد أفاد التقدير الكمي للمستخلص الخام والكسور الخمسة المشتقة منه وهم كسر الهكسان وثنائي كلو الميثان وكسر أسيتات الإثيل والبوتانول والكسر المائي المتبقي عن وجود كميات معتبرة من الفينولات والفلافونويدات والتانينات والتي كانت متقاربة عند جميع الكسور تقريبا. في حين أسفر تحليل LC-HR/MS للمستخلص الهيدروميثانولي الخام لنبات *S. tetragona*عن وجود 16 مركب فينولي 11 مركب منها تظهر لأول مرة في جنس *Salsola*منها حمض الساليسيليك وحمض الأسكوربيك والنارنجين كأكثر العناصر وفرة. في حين كشف تحليل الـ GC/MS لكسـر الهكسـان عن وجود 7 مركبات، 5 منها تظهر لأول مرة في هذا الجنس حيث تميزت 3 مركبات بأكثر نسـبة وفرة وهـي(23.48%) 8,11- hexadecanoic acid methyl ester , (21.98%) bis-(2-ethylhexyl) phthalate (18.49%) octadecadienoic acid methyl ester).

تم تقييم القدرة المضادة للأكسدة باستخدام الاختبارات التالية: DPPH•، FRAP، $ABTS^{\bullet+}$ ، CUPRAC، Anti-hemolysis و Metal chelating assays حيث أظهر كسر ثنائي كلور الميثان تفوقه في كل من اختبار كسح الجذر الحر DPPH• و$ABTS^{\bullet+}$ في حين تفوق كسر أسيتات الإثيل في اختبار اختزال أيون النحاس الثنائي (CUPRAC) وكان لكسر الهكسان قدرة الخلب الكبرى لجزيئات الحديد الثنائي Fe^{2+} الحرة في اختبار Metal chelating assays كما كانت له أعلى قدرة في حماية كريات الدم الحمراء من التحلل في اختبار Anti-hemolysis.ومن خلال اختبار مضادات الالتهاب أظهرت كسر أسيتات الإثيل نشاطًا تثبيطيا قويا لتمسخ البروتين الالتهابي، بقيمة بلغت IC_{50} = 13 ±5 µg/mL، متفوقا بذلك على الشاهد Aspirin®.أما عن دراسة نشاط الكسور في تثبيط نمو البكتيريا، فقد كان لجميع الكسور نشاطية مضادة للبكتيريا متوسطة ضد كل من سلالة *Escherichia, coli Staphylococcus aureus, Pseudomonas aeruginosa* و *Klebsiella pneumoniae*.

وفيما يخص النّشاط المضاد للسكري فقد أظهر كسر أسيتات الإثيل وكسر البوتانول تثبيطًا قويًا لانزيم α-amylase وα-glucosidase بقيمة IC_{50} = 70 µg/ml لكل منهما في كلا الاختبارين مما ينبئ جيّدا بإمكانية تطوير استخدام هذا النبات في علاج داء السكري من النوع الثاني. أما عن نشاط تثبيط إنزيم الـ Acetylcholinesterase المتعلق بمرض الزهايمر فقد أظهر كسر البوتانول أعلى نشاط تثبيطي بقيمة $IC_{50} = 30 \pm 0.30$ µg/ml مما يفيد بإمكانية استغلاله في تخفيف الأعراض المتعلقة بهذا المرض. وفيما يخص اختبار السمية الخلوية ضد خط خلايا سرطان الثدي MCF7، فقد كان لكسر ثنائي كلورالميثان فعالية عالية حيث أظهر تفوقًه على بقية الكسور الأخرى مسجل أعلى قيمة تثبيط قدرت بـ IC_{50} = 98 µg/mL كما أظهر الأخير مع كسر أسيتات الإثيل نشاطا مهما في اختبار anti-SARS-CoV2.

الكلمات المفتاحية: *Salsola tetragona* Delile، LC-HR/MS، GC/MS، الفعالية البيولوجية، خط خلايا سـرطان الثدي MCF7، anti-SARS-CoV2، مضاد مرض السكري، مضاد مرض الزهايمر.

SUMMARY IN FRENCH (Résumé)

Cette étude a été menée sur la plante *Salsola tetragona* Delile., une plante médicinale du désert appartenant à la famille des *Amaranthacées*, répandue dans les zones salées du nord-est de la région de Oued Souf, dans le but de déterminer la valeur nutritive et de valoriser les métabolites secondaires et de connaître la composition chimique de cette plante ainsi que de tenter de déterminer l'étendue de l'efficacité biologique dont elle fait preuve, et ce dans le cadre de la vérification de la validité de son utilisation en médecine traditionnelle ainsi que de la tentative de son exploitation dans le domaine pharmaceutique.

Les analyses chimiques de la valeur nutritionnelle ont montré que la biomasse de la partie aérienne de la plante *S. tetragona* se caractérise par une teneur minérale considérable, avec une abondance de la plupart des éléments. Elle contient également un taux d'humidité important, indiquant sa capacité à tolérer les conditions désertiques difficiles dans lesquelles elle pousse. Elle contient également des proportions moyennes de glucides, lipides et protéines. L'examen phytochimique qualitatif de l'extrait brut de la partie aérienne de *S. tetragona* a révélé la présence de la plupart des métabolites secondaires, dont des alcaloïdes, des coumarines, des mucilages, des phénols, des saponines, des stérols et des terpènes. Quantitativement, l'estimation quantitative de l'extrait brut et des cinq fractions dérivées (n-hexane, dichlorométhane, acétate d'éthyle, n-butanol et fraction aqueuse résiduelle) a révélé des quantités importantes de phénols, flavonoïdes, tanins et terpènes, approximativement similaires dans toutes les fractions. L'analyse LC-HR/MS de l'extrait brut hydrométhanolique de *S. tetragona* a révélé la présence de 16 composés phénoliques, dont 11 sont rapportés pour la première fois dans le genre *Salsola*, tels que l'acide salicylique et l'acide ascorbique, la naringénine étant la plus abondante. L'analyse GC/MS de la fraction n-hexane a révélé 7 composés, dont 5 nouveaux dans ce genre, avec 3 composés majeurs : hexadecanoic acid methyl ester (23,48%), bis-(2-ethylhexyl) phthalate (21,98%), 8,11-octadecadienoic acid methyl ester (18,49%).

Le potentiel antioxydant a été évalué en utilisant les tests suivants : DPPH•, FRAP, ABTS•+, CUPRAC, Anti-hémolyse et Chélation des métaux. La fraction dichlorométhane s'est avérée supérieure dans les tests de piégeage des radicaux DPPH• et ABTS•+, tandis que la fraction acétate d'éthyle était supérieure dans le test de réduction de l'ion cuivrique (CUPRAC). La fraction n-hexane avait la plus grande capacité de chélation des molécules de fer ferreux Fe^{2+} libres dans le test de chélation des métaux, ainsi que la plus grande capacité de protection des globules rouges contre l'hémolyse dans le test Anti-hémolyse. Dans le test anti-inflammatoire, la fraction acétate d'éthyle a montré une forte activité inhibitrice de la protéine inflammatoire, avec une valeur IC_{50} = 13 ±5 µg/mL, supérieure au témoin Aspirine®. Concernant l'étude de l'activité des fractions dans l'inhibition de la croissance bactérienne, toutes les fractions ont montré une activité antibactérienne modérée contre *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* et *Klebsiella pneumoniae*

Concernant l'activité antidiabétique, les fractions acétate d'éthyle et n-butanol ont montré une forte inhibition des enzymes α-amylase et α-glucosidase avec des valeurs IC_{50} = 70 µg/ml pour les deux dans les deux tests, ce qui est de bon augure pour le développement de l'utilisation de cette plante dans le traitement du diabète de type II. Quant à l'activité inhibitrice de l'enzyme acétylcholinestérase liée à la maladie d'Alzheimer, la fraction n-butanol a montré la plus forte activité inhibitrice avec une valeur IC_{50} = 30 ± 0,30 µg/ml, indiquant la possibilité d'exploiter cette plante pour atténuer les symptômes de cette maladie. Concernant le test de cytotoxicité contre la lignée cellulaire du cancer du sein MCF7, la fraction dichlorométhane s'est avérée très efficace, se révélant supérieure aux autres fractions avec la plus forte valeur d'inhibition IC_{50} = 98 µg/mL. Cette dernière ainsi que la fraction acétate d'éthyle ont également montré une activité importante dans le test anti-SARS-CoV2.

Mots-clés : *Salsola tetragona* Delile, LC-HR/MS, GC/MS, activité biologique, lignée cellulaire du cancer du sein MCF7, anti-SARS-CoV2, antidiabétique, anti-Alzheimer.

NOMENCLATURE

***SYMBOL*:**

CAT: Catechin

C: Carbon

Ca: Calcium

CFU: Colony forming units

Cl: Chlorine

CO_2: Carbon dioxide

Fe: Iron

$FeCl_2$: Ferrous chloride

$FeCl_3$: Iron (III) chloride, a chemical compound

h: Hours

H_2O: Water

K: Potassium

$K_2S_2O_8$: Potassium persulfate

$K_3[Fe(CN)_6]$: Potassium ferricyanide, a chemical compound

M: Molar

MeOH: Methanol

Mg: Magnesium

MgO : Magnesium oxide

Min : Minutes

mM : Millimolar

µL: Microliter

µl: Microliter

µM: Micromola

Na : Sodium

Na_2CO_3: Sodium carbonate

Na_2O: Sodium oxide

Nm: Nanometers

O: Oxygen

Q: Quercetin

Rpm: Revolutions per minute

°C: Degrees Celsius

Si : Silicon

SiO_2 : Silicon dioxide

S : Sulfur

Cao: Calcium oxide

Abbreviations:

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

Abs: Absorbance

AChE: Acetylcholinesterase

ATCC: American Type Culture Collection

BSA: Bovine serum albumin

BHA: Butylated hydroxyanisole

BSD: Backscattered electron detector

CBB: Coomassie brilliant blue

CCD-1079Sk: Human healthy skin fibroblasts cell line

Chl: Chlorophyll

CE : *Salsola tetragona* crude extract

CUPRAC: Cupric ion reducing antioxidant capacity assay

DMEM: Dulbecco's Modified Eagle's Medium

DMSO: Dimethyl sulfoxide

DPPH: 1,1-Diphenyl-2-picrylhydrazyl

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

DW: Dry weight

EC_{50}: Effective concentration 50%

EDTA: Ethylenediaminetetraacetic acid

EDX: Energy dispersive X-ray spectroscopy

F12: Ham's F12 nutrient mixture

FBS: Fetal Bovine Serum

FCR: Folin-Ciocalteu reagent

FRAP: Ferric reducing antioxidant power assay

FW: Fresh weight

GAE: Gallic acid equivalent

GC/MS: Gas Chromatography / Mass Spectrometry

Hly_{50}: Hemolysis 50%; concentration that causes 50% hemolysis

HRP: Horseradish peroxidas

IC_{50}: The half maximal inhibitory concentration

IPI: Iodine potassium iodide

LC-HR/MS: Liquid chromatography/ high resolution mass spectrometry

MCF-7: Michigan cancer foundation-7

MIC: Minimum inhibitory concentration

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

PBS: Phosphate buffered saline

PNPG: P-Nitrophenyl α-D-glucopyranoside

QE: Quercetin equivalent

RBD: Receptor binding domain

RT: Retention time

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

SEM: Scanning electron microscope

ACE2: Angiotensin converting enzyme 2

St.DCM: *S. tetragona* dichloromethane fraction

St.EtOAc: *S. tetragona* ethyl acetate fraction

St.Hex: *S. tetragona* hexane fraction

St.n-BuOH: *S. tetragona* n-butanol fraction

STD: *S. tetragona* dichloromethane fraction

STE: *S. tetragona* ethyl acetate fraction

STB: *S. tetragona* n-butanol fraction

STH: *S. tetragona* hexane fraction

STW: *S. tetragona* remaining aqueous fraction

TCA: Trichloroacetic acid

TCT: Total condensed tannin content

TFC: Total flavonoid content

TPC: Total phenolic content

Tris-HCl: Tris(hydroxymethyl)aminomethane hydrochloride

USA: United States of America

VA: Virginia

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LIST OF EQUATIONS

CHAPTER I: Phytochemical profiling of *Salsola tetragona* Delile by LC-HR/MS, GC/MS, and investigation of the antioxidant, anti-inflammatory, cytotoxic, antibacterial and anti-SARS-CoV-2 activities

CHAPTER II: Investigating the antioxidant potential and the inhibitory activity of α-amylase, α-glucosidase, and acetylcholinesterase of different fractions from *Salsola tetragona* Delile

GENERAL INTRODUCTION

Algeria is the largest country in the Mediterranean basin, Africa, and the Arab region with a total area of almost 2.4 million km2 and 1,600 of coastline. In addition to a diversified climate, Algeria is characterized by a rich flora consisting of 4,000 taxa, 917 genera, and 131 families (Belhouala & Benarba, 2021).

Algeria's geographical location and vast area provide a diverse climate that is favorable for the growth and development of medicinal and aromatic herbs. The northern region, collectively known as the hill, has a Mediterranean climate, while the high plateaus or high plains have a semi-arid climate. The desert region, which covers more than 80% of the total area and 31% of Africa, has an arid climate (Azzi et al., 2012; Kouzmine & Fontaine, 2018). The Algerian Sahara is home to more than 500 species of higher plants, some of which are still used today by the population as medicinal plants(Hemmami et al., 2023; Ozenda, 2004).

Since ancient times, plants have been a vital source of medicinal compounds for humans. A vast array of bioactive molecules known as secondary metabolites are produced by plants as a defense mechanism against external threats or to attract pollinators. Humans have harnessed these metabolites for their therapeutic potential in treating various diseases (Krishnaprabu, 2020). Three major classes of medicinally useful compounds obtained from plants are alkaloids, saponins, and phenolics (Tabasum et al., 2016). As scientific research continues to verify the traditional medicinal uses of plant extracts (Noor et al., 2022), many more medicinal plants and herbs remain to be fully explored for their bioactive constituents and therapeutic efficacy. With sophisticated screening techniques and increasing interest in natural product drug discovery, plant-derived compounds will likely continue providing novel drug leads, supporting the historical and ongoing importance of plants in human medicine.

Recent studies have focused on identifying phytochemicals from natural sources that have proven medicinal benefits and can serve as alternatives to synthetic pharmaceuticals. Secondary metabolites produced by plants have been a prime target for such research. Many of these plant-derived compounds have been used historically and continue to be utilized today for managing various diseases and disorders. Over time, this has resulted in numerous plant-based medicines, either in their original natural form or as pharmacologically modified derivatives obtained through exploiting plant metabolites. Generally, these phytomedicines tend to have lower potency than synthetic drugs. However, since they are often consumed regularly in dietary quantities, they can elicit significant long-term physiological effects (Ksouri et al., 2012). In addition to their medicinal properties, plant secondary metabolites also have nutritional

importance due to their widespread use as flavoring agents, food preservatives, fibers, and oils. They additionally have applications as adhesives, waxes, fragrances, herbicides, and insecticides (Tabasum et al., 2016). In summary, phytochemicals from medicinal plants offer a logic-based, proven alternative to synthetic pharmaceuticals for preventive and therapeutic healthcare. Their regular dietary intake in natural forms may provide sustained physiological benefits. Besides medicinal properties, plant secondary metabolites also have nutritional and commercial applications.

Desert plants are rich sources of secondary metabolites, in large part due to the extreme environmental stresses they endure in arid habitats. These plants face challenges including scarce and unpredictable rainfall, high temperatures, and high soil salinity (El-Keblawy et al., 2016). Such conditions generate oxidative stress and increase free radical production. As survival strategies, desert plants biosynthesize diverse antioxidant compounds like phenolics and flavonoids to counteract oxidative damage during different life stages (Ghanem et al., 2021). Halophytes, for example, are naturally salt-tolerant plants containing an array of bioactive phytochemicals with medicinal and nutritional value. They represent promising sources of functional food ingredients such as vitamins, polyunsaturated fatty acids, flavonoids, and tannins (Ksouri et al., 2012). Multiple studies have demonstrated that drought stress enhances secondary metabolite accumulation in various plant species tissues (Liu et al., 2011). In summary, the harsh desert climate promotes the synthesis of phytochemical defenses in adaptive plant species, rendering them rich potential sources of nutraceuticals and pharmaceuticals. Further research on desert plant metabolites could uncover new functional food additives and drug leads.

In this research, the plant *Salsola tetragona* Delile (*Amaranthaceae*), which is widespread in the northeast region of the state of Oued Souf, was selected. The initial selection was based primarily on the lack of research and studies conducted on this species, despite its presence in the region, and secondly on the results of an exploratory study and survey that we conducted, which covered almost all segments of society of different ages, especially the elderly. After analyzing the results, it was found that this plant has uses in traditional medicine, as it has been and is still used in some areas as an antidiabetic, anti-ulcer, gastrointestinal pain, hypotensive, and kidney disease medicine. It is also used as a laxative. From this, we decided to conduct this study in an attempt to prove what traditional folk medicine brought, and our pursuit to valorize plant resources of the Algerian desert.

The purpose of this work is to examine the nutritive value and the phytochemical composition of the *Salsola tetragona* Delile specie from the *Amaranthaceae* family growing in the Oued Souf region and identify the main compounds in the aerial parts by using LC-HR/MS and GC/MS analysis. as well as evaluate the biological activity of their extracts as an antioxidant, antibacterial, anti-inflammatory, antiviral, and anti-cancer potential, as well as anti-diabetic and anti-Alzheimer properties. The aim is to exploit these natural products in various fields such as food, pharmaceutical industries, or cosmetics. This thesis consists of two chapters contained in two articles:

Chapter 1: Phytochemical Profiling of *Salsola tetragona* Delile by LC-HR/MS, GC/MS, and Investigation of the Antioxidant, Anti-inflammatory, Cytotoxic, Antibacterial and Anti-SARS-CoV-2 Activities.

Chapter 2: Investigating the Antioxidant Potential and the Inhibitory Activity Against α-Amylase, α-Glucosidase, and Acetylcholinesterase of Different Fractions from *Salsola tetragona* Delile.

CHAPTER I

Phytochemical profiling of *Salsola tetragona* Delile by LC-HR/MS, GC/MS, and investigation of the antioxidant, anti-inflammatory, cytotoxic, antibacterial and anti-SARS-CoV-2 activities

1.1. Introduction

The *Salsola* L. genus, also known as Russian thistle or Saltwort, is a halophyte plant that belongs to the *Amaranthaceae* family. It is a large genus consisting of semi-dwarf to dwarf shrubs and woody tree species. The name of the genus comes from the Latin word "salsus," which means "salty," referring to the plant's salt-tolerant nature. *Salsola* is a cosmopolitan group of plants that are distributed and naturalized worldwide. Over 64 species have been reported, which are widespread in arid and semi-arid regions of Central Asia, the Middle East, Africa, and Europe (Hanif et al., 2018; Murshid et al., 2022).

Salsola species have a variety of features that make them a potential forage species in semi-arid to dry settings along sea beaches. These features include extensive seed production and resistance to extreme climatic conditions, including high temperatures and extended drought conditions. These plants typically grow on flat, generally dry and/or slightly saline soils, with some species occurring in salt marshes (ElNaggar et al., 2022).

The *Salsola* genus is rich in various classes of Phyto-constituents, such as flavonoids, phenolic compounds, nitrogenous compounds, saponins, triterpenes, sterols, volatile constituents, lignans, coumarins, and cardiac glycosides. Moreover, it shows different biological activities, including Alzheimer, antihypertensive, antioxidant, antidepressant, anti-analgesic, anti-inflammatory, antiviral, antibacterial, anticancer, cardioprotective, and hepatoprotective activities. The genus *Salsola* is frequently overlooked, and few people are aware of its significance. The majority of studies focus on pollen morphology and species identification, while little research has looked at its phytochemical makeup or biological effects(Ahmad et al., 2008; Munir et al., 2014; Tundis et al., 2008).

Salsola tetragona Delile is native to the deserts of Algeria, Tunisia, Morocco, Libya, and Mauritania (Ozenda, 1977). In Algeria, this species is relatively common in the Northern and Western Sahara sectors. The plant grows in stony and loamy saline desert pastures, sebkhas, and slightly saline steppes in desert regions; this specie is well-adapted to survive in harsh desert environments and has been previously used in traditional medicine in these regions (Chehma, 2006). The leaves and aerial parts of *S. tetragona* have a significant role in conventional medicine. In southeastern Algeria, it treats indigestion, constipation, abdominal and gastric pain, hypertension, kidney disease, and diabetes (Lakhdari et al., 2016). The powder or decoction of the leaves of *S. tetragona* is used to alleviate gastrointestinal pains, gastric pains, intestinal worms, microbial infections, cancer, and arrhythmia (Daoud et al., 2016; Ghourri et al., 2012). In Morocco, *S. tetragona* is used to treat diabetes, and the parts used for this purpose include the plant's leaves,

roots, bark, and fruit. These remedies are often administered in powder form or decoction (Ghourri et al., 2013).

Considering the potential of this genus, it is important to reveal the phytochemical characters of *S. tetragona*. The purpose of this work is to quantify the phenolic contents and identify the main phenolic compounds present in the crude extract of the aerial part using LC-HR/MS analysis. The unexplored biological activity of the extract, together with its fractions will be evaluated for antioxidant, antibacterial, anti-inflammatory, antiviral, and anti-cancer potentials.

1.2. Materials and methods

1.2.1. Plant material collection

The aerial parts of *S. tetragona* Delile, *Amaranthaceae*, were collected in May 2020 from the El-Magrane region (El-Oued, southeast Algeria 33°48'39" N 6°55'19.5" E). This plant was identified by Pr. Youcef Halis (Director Research. Technical Research Center on Arid Regions, Algeria). A voucher specimen (LOST.St07/09) is kept in the herbarium of the Faculty of Life and Natural Sciences, El-Oued University.The plant was washed well and parched at room temperature for 20 days, in conditions away from moisture, light, dust, and dirt, with adequate ventilation. After drying, it was crushed, and the powder was stored in a closed glass container.

1.2.2. Determination of the nutritive value

The *Amaranthaceae* family includes many wild herbs that are important in traditional medicine and human or animal diets. In this regard, this study aims to identify the nature and characteristics of one of the plants belonging to this family. through Investigating the nutritional value of the dry plant material of *S. tetragona* belonging to the genus *Salsola* and included in this family and that is through was determined by determining the following criteria:

1.2.2.1. Determination of water content

Water content or moisture content means the percentage of water to the solid in the fresh plant matter. It was determined using a drying method (Maatallah Zaier et al., 2020), in which the fresh plant material of *S.tetragona* was dried in an incubator at 60°C. After every 24 hours, the plant material is weighed until the weight is stable. The percentage of water content was calculated according to the equation 01:

$$\text{Water content (\%)} = \frac{W1-W2}{W1-W3} \times 100 \quad \text{(Equation 01)}$$

W_1: Weight of empty container and sample before drying.

W_2: Weight of empty container and sample after drying.

W_3: Weight of empty container.

1.2.2.2. Determination of photosynthetic pigments content

Chlorophyll a, chlorophyll b, and carotenoids were determined by mixing 0.1 g of fresh plant material in 10 mL of acetone (80%) at 4°C for 24 hours. After centrifugation for 10 minutes at 5000 x g the absorbance of the supernatant was read at wavelengths; 663, 645, and 470 nm using a spectrophotometer (Nayek et al., 2014). By applying the following mathematical relationships, the content of chlorophyll a, chlorophyll b, and carotenoids:

$$\text{Chl a (mg/mL)} = 12.25 \times Abs_{663} - 2.79 \times Abs_{649} \text{ (Equation 02)}$$

$$\text{Chl b (mg/mL)} = 21.5 \times Abs_{649} - 5.1 \times Abs_{663} \text{ (Equation 03)}$$

$$\text{Carotenoids (mg/mL)} = (1000 \times Abs_{470} - 1.82 \times \text{Chl a} - 104.96 \times \text{Chl b})/198 \text{ (Equation 04)}$$

1.2.2.3. Estimation of carbohydrates, lipids, and proteins content

Primary metabolites were estimated from the dry matter of the plant *S. tetragona*, according to the method of (Shibko et al., 1967). 0.5 g was soaked in 5 mL of trichloroacetic acid (20%). The mixture was shaken in the vortex for five minutes and then separated by centrifugation (3000 rpm/10 minutes). The supernatant was exploited for carbohydrate determination, and the precipitate was added to 2 mL of chloroform/methanol (1/1 V). Then the separation was further centrifuged (3000 rpm/15 minutes). The second supernatant was used to estimate the lipids content, and the precipitate was added to it 5 mL of sodium hydroxide (0.1 N) and this solution was used to estimate the total proteins.

1.2.2.3.1. Carbohydrate content determination

The first supernatant was used for determination of carbohydrate content, using the phenol–sulfuric acid method described by Dubois et al. (1951). The principle of this method is that the reaction of carbohydrates with concentrated sulfuric acid produces furfural derivatives. This leads to an additional reaction between furfural derivatives and phenol, which leads to the appearance of a color that can be determined by measuring the absorbance. The steps for this method are as follows: 2 mL of an aliquot of the carbohydrate solution was mixed with 1 mL of a 5% aqueous solution of phenol, and then 5 mL of concentrated sulfuric acid was added. The mixture was shaken and incubated for 10 minutes, then the absorbance was measured at 490 nm. The glucose was used

to determine the standard curve (Appendix 02). The carbohydrate content was expressed as: µg glucose/mg dry matter.

1.2.2.3.2. Lipid content determination

The second supernatant was used to estimate the lipid content, using the method of (Goldsworthy et al., 1972). This method is based on the reagent sulfo-phospho-vanillin and concentrated sulfuric acid. First, the sulfo-phospho-vanillin reagent was prepared by dissolving 76 g of vanillin in 11 mL of distilled water, then 39 mL of phosphoric acid (85%) was added to the mixture and shaken well to completely dissolve the vanillin. The steps for the determination are as follows: 0.1 mL of an aliquot of lipid solution was mixed with 1 mL of concentrated sulfuric acid, and then the mixture was placed for 10 minutes in a water bath at 100°C. After cooling, a volume of 0.15 mL was taken from the mixture and 1.5 ml of sulfo-phospho-vanillin reagent was added to it. The mixture was shaken and incubated for 30 minutes, then the absorbance was measured at 530 nm. Soya oil was used to determine the standard curve (Appendix 02). The lipid content was expressed as µg soybean oil/mg dry matter.

1.2.2.3.3. Protein content determination

The protein concentration is determined according to the method of (Bradford, 1976) which uses Coomassie blue (G 250) as a reagent. The amine (-NH_2) groups of proteins react with a reagent based on orthophosphoric acid, ethanol and Coomassie brilliant blue to form a blue complex. The appearance of this color reflects the degree of ionization of the acid medium and the intensity establishes the concentration of proteins in the sample.

Coomassie brilliant blue reagent was prepared by dissolving 100 mg of CBB G-250 in 50 mL of ethanol, then 100 mL of phosphoric acid (85%) was added to the mixture, then distilled water to a volume of one liter was added to the mixture. The protein content of samples was determined by mixing a volume of 0.2 mL of the third sample supernatant with 1.8 mL of Coomassie brilliant blue reagent and then left the mixture for 5 minutes before reading the absorbance at wavelength 546 nm. BSA was used as a standard compound (Appendix 02). The protein content was expressed as: µg BSA/mg dry matter.

1.2.2.4. Ash estimation

Fresh plant material consists of water, minerals and organic matter. Mineral matter (ash) includes all the minerals and inorganic elements stored in the plant. By burning completely dry

plant matter in a muffle furnace at 550°C, raw ash was determined. Exploiting the weights of the samples before and after burning, the ash percentage was calculated using the following formula:

$$\text{Ash content (\%)} = \frac{W1-W2}{W1-W3} \times 100 \text{ (Equation 05)}$$

1.2.2.5. Determination of mineral nutrients by EDX Analysis

Scanning electron microscopy makes it possible to scan part of the surface of the sample using an electron beam with a diameter of a few nanometers. This method allows visualization of morphological features with high magnification and increased depth of field. In addition, an interaction is created between the electron beam and the atomic envelopes of the elements of the material to be analyzed. During scanning, X-ray fluorescence radiation is created which can be recorded by an energy dispersive spectroscopy (EDX) and used for analysis (Djekic & Ćirić, 2022).

The essential chemical elements of dry plant matter of *S. tetragona* were determined by the Phenom Pro Desktop scanning electron microscope (SEM). with an optical magnification range of 20-134×, an electron magnification range of 160-150,000×, maximum digital zoom of 12×, acceleration voltages of 5, 10 and 15 kV, a backscattered electron detector. Backscattered electron detector (BSD) and energy dispersive spectroscopy (EDX), with a nominal resolution of 6 nm or less. The microscope is equipped with a temperature-controlled sample holder (temperature range -25°C to 50°C).

The results of the (SEM) analysis attached to the energy dispersive spectroscopy (EDX) analysis are shown as shown in Appendix 01. However, in this study Table 02 was presented showing the elements and oxides present in the sample.

1.2.3. Phytochemical studies

1.2.3.1. Preparation of the extract and fractionation of plant material

The aerial part of *S. tetragona* (200 g) was macerated in a hydro-alcoholic solution of 600 mL Methanol / Water (80: 20: v / v) for 24 h at room temperature. This operation was repeated three times with renewal solvent every 24 hours. At a later stage, the hydroalcoholic extract has been filtered and concentrated in Rotary Evaporator at a temperature equal to 40 °C in order to obtain the crude dried extract (10%).

The crude extract was dissolved in distilled water, filtered, and subjected to fractionation using different solvents of increasing polarity, starting with n-hexane (STH) (600 ml x 3), dichloromethane (STD) (600 ml x 3), ethyl acetate (STE) (600 ml x 3) and then n-butanol (STB)

(600 ml x 3). All fractions including the aqueous phase were dried by rotavapor at 38-40°C. The five organic fractions were concentrated to provide dryness to extracts; n-hexane fraction (1.6%) dichloromethane fraction (%1.1) ethyl acetate fraction (0.9%) and butanol fraction (1.2%) and the remaining aqueous fraction (%4.9) and they were stored at 4 °C in the dark, in a refrigerator until use.

1.2.3.2. Preliminary phytochemical screening

Phytochemical screening was conducted according to reported methods (Matos, 1997). a qualitative analysis of *S. tetragona*, where then detection of phenols, alkaloids, coumarins, mucilage, saponins, tannins, sterols, terpenes, and oils.

1.2.3.3. Determination of total phenolic

The total phenolic content (TPC) of the extract from *S. tetragona* and its fractions was determined through the utilization Folin-Ciocalteau method (Li et al., 2007). A 0.2 mL of the sample was combined with 1 mL of 10% Folin-Ciocalteau reagent and allowed to incubate for 5 minutes. Subsequently, 0.8 mL of a 7.5% Na_2CO_3 solution was introduced into the mixture and left to incubate for a span of 40 minutes in the obscurity of ambient temperature. The measurement of the absorbance of the mixture was done at 765 nm, and the quantification of total phenolic content was determined in terms of the comparable measure of gallic acid, expressed in micrograms per milligram of extract, employing the equation y= 0.006x+0.0007, where R^2=0.99.

1.2.3.4. Determination of total flavonoid

According to the reported method (Mbaebie et al., 2012), equal volumes of sample and aluminum chloride solution (0.2 %) were mixed. The mixtures were subjected to an hour-long incubation at ambient temperature after thorough agitation. Subsequently, the absorbance was measured at 415 nm, and the quantification of Total Flavonoid Content (TFC) was expressed in micrograms of quercetin equivalents per milligram of dry extract, utilizing the equation y=0.005x+0.0402, where R^2 =0.99.

1.2.3.5. Determination of tannins content

The reported method (Kokoska et al., 2008; Muthukrishnan et al., 2018) were employed to measure the total condensed tannins. In coated test tubes, 0.5 mL of the extracts were combined with 1.5 mL of strong HCl and 3 mL of vanillin reagent (4%, w/v in methanol). The tubes were incubated at 20 °C followed by reading absorption at 500 nm absorption was observed. The TCT

amount was expressed in µg of catechin (CAT) equivalents per milligram of dry extract. Based on a regression equation of catechin's calibration curve (y= 0.0036x + 0.0249, R^2=0.996).

1.2.3.6. LC-HR/MS analysis

LC-HR/MS, which stands for liquid chromatography-high resolution mass spectrometry, was used to analyze the phenolic compounds of the hydromethanolic extracts of *S. tetragona*. LC–MS analysis of the 65 phenolic standards was conducted with a Thermo Orbitrap Q-Exactive. A C18 column (150 x 3 mm x 5 µ) was used, maintained at 30°C. The mobile phases used were water with 1.0% (v/v) formic acid (A) and methanol with 1.0% (v/v) formic acid (B), with a gradient of 50% B (initial–1.0 min), 100% B (1.0–3.0 min), 100% B (3.0–6.0 min), 50% B (6.0–7.0 min), 100% B (7.0-15.0 min), and 10% B (15.1–18.0 min). The flow rate was 0.35 mL/min, and an injection volume of 1 µL was used. MS detection was conducted with a positive and negative ion ESI–MS. The optimized analytical parameters were as follows: Mass Scan Cycles: 100-900 m/z, Sheath gas flow rate: 45, Aux gas flow rate: 10, Spray voltage (kV): 3.80, Capillary temp. (°C): 320, Aux gas heater temp (°C): 320, S-lens RF level: 50.0

1.2.3.7. GC/MS analysis

The chemical content of the n-hexane fraction of S. *tetragona* was determined by converting it to methyl ester derivatives (Jacob et al., 1985). A certain amount of the n-hexane fraction of the plant was taken and 2 mL of 0.5 N NaOH was added to it. It was mixed carefully until dissolved in a 50°C water bath. Then, 2 mL of BF_3:CH_3OH was added to it. Mixture for 2-3 minutes at 80°C boiled and allowed to cool. The volume was made up to 25 mL with saturated NaCl. Then, liquid-liquid extraction was performed with hexane. GC-MS device was used to characterize the chemical components of fatty acid methyl esters diluted to different concentrations.

1.2.4. *In vitro* antioxidant activity

1.2.4.1. DPPH free radical scavenging assay

The putative radical-scavenging activity of *S. tetragona* extract and fractions 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, α-tocopherol and BHA 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:

$$Inhibition\ Percentage = [1-(Abs/Abc)] \times 100 \quad \text{(Equation 06)}$$

Ab_S: absorbance of the sample or standard solution.

Ab_C: absorbance of control solution.

1.2.4.2. 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 plant samples. Ethanol was used as negative control, while α-tocopherol and BHA were used as standard. The graph of the percentage scavenging effect with sample concentration was used to obtain the IC_{50} values.

$$ABTS^{\bullet+} \text{ scavenging power } (\%) = [(Ab_0 - Ab_1)/ Ab_0] \times 100 \quad \text{(Equation 07)}$$

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

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

1.2.4.3. Cupric reducing antioxidant activity (CUPRAC)

The procedure of Apak et al (Apak et al., 2004) was used to determine the antioxidant capacity for reducing cupric ions. The absorbance of the plant extracts was measured and compared with the absorbance of the antioxidant standards BHA and α-tocopherol.

1.2.4.4. Metal chelating assay

With some modifications, the Ferrin method (Decker & Welch, 1990) was used to measure the extract and fractions ability to chelate and inhibit free Fe^{2+} in the solution. Volumes of 80 µl of different samples concentrations were added to 40 µl (0.2 mM) of ferrous chloride ($FeCl_2$). The reaction began by the addition of 80 µl (0.5 mM) of Ferene iron reagent. At ambient temperature, the mixture was stirred hard for 10 minutes. The absorbance was measured at 593 nm after the mixture had reached equilibrium. Results were expressed as IC_{50} (µg/mL) using EDTA as the antioxidant standard and calculated by using Equation 08:

$$\text{Metal chelating activity } (\%) = [(Ab_c - Ab_s)/ Ab_c] \times 100 \quad \text{(Equation 08)}$$

Ab_c: control solution absorption.

Ab_s: chelator with a (sample or standard) absorption.

1.2.5. *In Vitro* anti-inflammatory capacities

The method of Gheraissa et al. (2022) was used with few modifications to explore the anti-inflammatory potential of *S. tetragona* samples *in-vitro*. Human albumin (5%), in a volume of 1 mL, was mixed with 1 mL of different samples concentrations and 20 µg/mL of 1N HCl. Tubes were incubated at 27 °C for 15 minutes, then heated in a water bath for 10 minutes at 70 °C. The absorbance was measured at 660 nm spectrophotometrically after cooling to ambient temperature. Aspirin® was used as a standard. Results were expressed as IC_{50} values calculated as follows Equation 09:

$$Percentage\ inhibition\ (\%) = [(Ab_c - Ab_s)/ Ab_c] \times 100 \quad \text{(Equation 09)}$$

Ab_c: control solution absorption.

Ab_s: sample or standard absorption.

1.2.6. Antibacterial activity

The bacterial strains utilized in this study included *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 27853). These reference strains were acquired from the Pharmaceutical Microbiology Department at Bezmialem Vakıf University. A sterile 96-well microplate was used in the antibacterial susceptibility test based on the microdilution method (Matuschek et al., 2022). From Muller Broth 50 µl were added to each well of the microplate. Ampicillin were used as control. Serial dilution of the *S. tetragona* curd extract and its various fractions was performed starting from a concentration of 1000 µg/mL (the dilution concentrations to be obtained are 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8 and 3.9 µg/mL) respectively. A stock solution was prepared by adding Muller broth from the prepared bacterial strains to 1 x 10^8 (CFU/mL) cells per milliliter. 10 µL of the prepared bacterial stock solution was added to all wells and the microplates were incubated at 37^{O}C for 24 hours in a CO_2 environment. At the end of the incubation, the sensitivity expressed as MIC values of the tested extract and fractions were compared with Ampicillin.

1.2.7. Cytotoxic activity

Human healthy skin fibroblasts CCD-1079Sk (CRL-2097™) and human breast cancer cells MCF7 (HTB-22™) were acquired from American Type Culture Collection (ATCC, VA, USA). In addition to 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, USA), the cancer cell lines were cultured in DMEM-F12 medium. The process of sub-culturing was carried out at intervals of 2-3 days. Before treatment, a total of 10^4 cells/well were seeded and incubated for 24 h in a 96-well. The cells were treated with *S. tetragona* extracts (treatment concentrations: 0-250

µg/ml or 0-260 µ/ml, according to the solubility) for 24 h, and their cytotoxic activities were analyzed by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay (Mosmann, 1983). The negative control/vehicle control was cell culture medium containing same amount of vehicle (0.1% DMSO). After 24 h treatment, a volume of 20 µL MTT solution (5 mg/mL in PBS) was introduced into each well, followed by a period of incubation in darkness for 3 hours at a temperature of 37°C, in an environment characterized by 5% CO_2 and 92% humidity. The solution was then carefully removed and the resultant formazan crystals were dissolved within 100 µL of DMSO. The absorbance was subsequently recorded at a wavelength of 590 nm. The determination of cell viability was accomplished through the division of the absorbance values obtained from the experimental groups by those originating from the solvent control cells. IC_{50} values were calculated using the GraphPad Prism program, and the concentration-dependent % of cell viability data was calculated according to the vehicle control samples.

1.2.8. Cell migration assay

STD and STE extract treatments with concentrations lower than the IC_{50} values were chosen according to the cell viability assay (100 µg/mL and 50 µg/mL for STD; 150 µg/mL and 75 µg/mL for STE). The CytoSelect™ 24-well Wound Healing Assay Kit (Cell Biolabs Inc, USA) was used for evaluating the migration rate of the MCF7 cells. The inserts were placed inside every well of a 24-well plate using sterile forceps, all aligned in the same direction. 500 µl of medium containing $2x10^5$ cells was added to the gaps of the inserts. After 24 h incubation, the inserts were removed, and the images of each well were taken before treatment. After taking the images, STD and STE extract treatments were done (100 µg/mL and 50 µg/mL for STD; 150 µg/mL and 75 µg/mL for STE). After 24 h treatment the images of the same area of the wounds were taken. For calculating the wound healing percentage, the program ImageJ (National Institutes of Health, USA) was used, and a comparison was made between the treatment groups with the vehicle control group. The calculation of the wound healing percentage was done according to the kit manufacturer's protocol.

1.2.9. Anti-Sars-Cov-2 activity

For evaluating the anti-SARS-CoV-2 activity, the protocol of the SARS-CoV-2 Spike-ACE2 Interaction Inhibitor Screening Assay Kit (Cayman Chemical, USA) was used. A recombinant rabbit Fc-tagged SARS-CoV-2 spike S1 RBD that binds to a plate precoated with a mouse anti-rabbit antibody is used and a recombinant His-tagged ACE2 protein binds to the spike RBD. This complex is detected with an HRP-conjugated anti-His antibody and the absorbance is read at 450 nm. A control is used for competition of the SARS-CoV-2 spike RBD-ACE2 interaction. STD and

STE concentrations (0-50 µg-mL) were decided according to the cytotoxicity assay. The activity percentage was calculated according to the given formula:

% Activity = (corrected inhibitor activity) / (corrected 100% initial activity) x 100 (Equation 10)

1.2.10. Statistical analysis

Quantitative estimates and the antioxidant, anti-inflammatory, antiviral, cytotoxic, and cell migration activities were carried out in triplicates. The results obtained have been included as mean ± SD (standard deviation). One-way ANOVA and the Tukey test were used to assess statistical differences or by the statistical test of student's (*t*-test) to evaluate means differences, and P values < 0.05 were considered significant.

1.3. Results

1.3.1. Nutritive value

Analysis of nutritional components in *S. tetragona* (Table 01) showed the plant contains substantial moisture, photosynthetic pigments, and mineral content. The moisture content was 37.56±1.57%, indicating a high water composition. Photosynthetic pigments were also abundant, including chlorophyll a at 9.44±6.36 mg/g, chlorophyll b at 1.96 mg/g, and carotene at 0.16±0.18 mg/g. These pigment levels suggest S. tetragona has effective photosynthetic capacity. Additionally, the ash content of 27.60±1.15% implies significant mineral composition, which may provide essential dietary minerals. Taken together, these nutritional components demonstrate that *S. tetragona* contains valuable moisture, photosynthetic compounds, and minerals, conferring potential nutritional benefits.

Table 01. Moisture, photosynthetic pigment, and ash content of the aerial part of *S. tetragona*

	Moisture content (%)	Ch a (mg/g)	Ch b (mg/g)	Carotene (mg/g)	Ash (%)
S. tetragona	37.56±1.57	9.44±6.36	1.96±0.00	0.16±0.18	27.60±1.15

Chl a, Chlorophyll a. Chl b, Chlorophyll b

Based on the EDX analysis results presented in Table 02, the major elemental composition of the dry aerial part of *S. tetragona* includes oxygen (O), carbon (C), calcium (Ca), sodium (Na), silicon (Si), chlorine (Cl), magnesium (Mg), potassium (K), and sulfur (S). Oxygen made up 30.86% of the dry weight, carbon 42.36%, calcium 1.56%, sodium 1.40%, silicon 0.89%, chlorine 2.85%, magnesium 1.11%, potassium 1.95%, and sulfur 1.09%.

The weight percentage of the oxides of the main elements detected in the EDX analysis are as follows. Calcium oxide (CaO) constituted 3.78% of the dry weight, sodium oxide (Na_2O) 4.08%, and silicon dioxide (SiO_2) 2.81%.

The EDX analysis reveals that the predominant elemental composition of the *S. tetragona* dry aerial parts is oxygen and carbon, as expected for organic plant matter. Other major elements include calcium, sodium, chlorine, potassium, and silicon, along with smaller amounts of magnesium and sulfur.

Table 02. EDX analysis for the dry matter of the aerial part of *S. tetragona*.

	Weight of elements (%)										
	O	**C**	**Ca**	**Na**	**Si**	**Al**	**Cl**	**Mg**	**K**	**S**	**Fe**
S. tetragona	30.86	42.36	1.56	1.40	0.89	/	2.85	1.11	1.95	1.09	/
	Weight of oxide (%)										
	CaO		**Na_2O**		**SiO_2**		**Al_2O_3**	**MgO**		**SO_3**	**Fe_2O_3**
S. tetragona	3.78		4.08		2.81		/	2.96		/	/

Table 03 shows that the studied plant has a clear difference in the chemical content of nutrients (carbohydrates, lipids, and proteins). The analysis in this study revealed a medium protein content of 6.98 mg/g ± 0.06, also a moderate carbohydrate level of 4.037 ± 0.14vmg/g, and a low-fat content of 1.78 mg/g ± 0.13 in the dry matter.

Table 03. Content of carbohydrates, lipids, and proteins in dry matter of *S. tetragona*

	Protein (mg/g)	**Carbohydrate (mg/g)**	**Fats (mg/g)**
S. tetragona	6,98±0,06	4,037±0,14	1,78±0,13

1.3.2. Phytochemical studies

1.3.2.1. Preliminary phytochemical screening

The standard references of phytochemical screening were used to identify the preliminary phytochemicals in *S. tetragona* hydromethanolic extract by color reaction with various reagents (Gul et al., 2017). Table 04 displays the results of analyses for the presence or absence of phytoconstituents. The hydromethanolic extract reacted positively with the reagents identifying the presence of alkaloids, coumarins, mucilage, phenols, saponins, sterols, tannins, and terpenes, with the absence of oils.

Table 04. Preliminary phytochemical screening of *S. tetragona* hydromethanolic extract.

Compounds	Observation
Alkaloids	(+)
Coumarins	(+)
Mucilage	(+)
Phenols	(+)
Tannins	(+)
Saponins	(+)
Sterols and terpenes	(+)
Oils	(-)

The presence of phytochemical compounds is indicated by the plus sign (+), while their absence is indicated by the minus sign (-).

1.3.2.2. Phenolic profile of *S. tetragona* crude extract by LC-MS

The hydromethanolic extract of *S. tetragona* was analyzed using LC/HR-MS to identify and estimate phenolic and flavonoid compounds (Table 05). The analysis revealed the existence of 16 phenolic compounds in *S. tetragona*, 11 of which were detected for the first time in the genus *Salsola*. The dominant compound in the extract was salicylic acid (274 mg/kg extract), followed by ascorbic acid (159.60 mg/kg extract), naringin (33.60 mg/kg extract), gypsogenic acid (16.50 mg/kg extract), dihydrocaffeic acid (15.25 mg/kg extract), and 3'-O-methyl quercetin (13.50 mg/kg extract).

Table 05. Phenolic compounds of the hydromethanolic extract of *S. tetragona* by LC/HR-MS.

Phenolic standards	(mg analyte/ kg extract)	U%
Ascorbic acid	159.60	3.94
Chlorogenic acid	4.30	3.58
(+)-*trans* taxifolin	0.25	3.35
Naringin	33.60	4.20
Hyperoside	4.40	3.46
Ellagic acid	3.60	4.20
Quercitrin	7.10	3.78
Quercetin	0.50	2.95
Salicylic acid	274.00	1.89
Naringenin	3.30	4.20
3'-*O*-Methyl quercetin	13.50	3.58
Caffeic asit phenethyl ester	0.10	3.13
Gypsogenic acid	16.50	3.34
Pyrogallol	6.80	3.74

Dihydrocaffeic acid	15.25	0.86
Chrysoeriol	1.30	2.08

1.3.2.3. Fatty acid profile of *S. tetragona* n-hexane fraction by GC-MS

The gas chromatography/mass spectrometry (GC/MS) analysis of the n-hexane fraction of *S. tetragona* identified several fatty acid methyl esters and other lipid-derived compounds (Table 06). The major constituents were hexadecanoic acid methyl ester (23.48%), bis-(2-ethylhexyl) phthalate (21.98%), 8,11-octadecadienoic acid methyl ester (18.49%), and 6,9,12,15-docosatetraenoic acid methyl ester (12.66%), which together accounted for over 75% of the total area. Additional fatty acid methyl esters detected include tetradecanoic acid methyl ester (2.38%) and heptadecanoic acid 16-methyl- methyl ester (2.58%). The diversity of lipid-derived compounds detected demonstrates that the n-hexane fraction of *S. tetragona* contains mostly nonpolar metabolites.

Table 06. Fatty acids composition of the *S. tetragona* n-hexane fraction.

Compounds	Molecule Formula	RT (min)	Area %
1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	16.15	6.98
Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	18.49	2.38
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	21.92	23.48
8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	24.60	18.49
6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	24.69	12.66
Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	25.07	2.58
Bis-(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	30.89	21.98
		Total	**88.55**

1.3.2.4. Estimation of Total Phenolic Content (TPC)

The total phenolic content of the *S. tetragona* crude extract and the five fractions was expressed as gallic acid equivalents and is presented in Table 07. The results showed that the total phenolic content of the fractions ranged from 15.65 to 46.55 µg GAE/mg. The crude extract had the highest concentration of polyphenols among the fractions (46.55 ± 0.10 µg GAE/mg), followed by the ethyl acetate fraction (39.76 ± 0.25 µg GAE/mg). The aqueous fraction (15.65 ± 0.17 µg GAE/mg) had the lowest amounts of total phenolic compounds among the five fractions.

1.3.2.5. Estimation of total flavonoid content (TFC)

The total flavonoid content of the hydromethanolic extract and its fractions of *S. tetragona* were expressed as quercetin (Q) equivalents (µg/mg) and are presented in Table 07. The ethyl acetate fraction contained the highest amount of flavonoids (15.77 ± 0.09 µg QE/mg), followed by the crude extract (13.17 ± 0.11 µg QE/mg).

1.3.2.6. Estimation of total condensed tannins content (TCT)

The quantitative content of condensed tannins in the *S. tetragona* extract and its fractions were estimated using catechin © and expressed in microgram equivalents per milligram of the extract (Table 07). The n-butanol fraction had the highest concentration of condensed tannins (13.61 ± 0.16 µg CE/mg), followed by the crude extract (11.33 ± 0.43 µg CE/mg). The remaining fractions had similar concentrations of condensed tannins.

Table 07. Values of total polyphenol, flavonoid, and condensed tannins of *S. tetragona* crude extract and its fractions.

Samples	TPC (µg GAE/mg ED)	TFC (µg QE/mg ED)	TCT (µg CE/mg ED)
CE	46.55 ± 0.10^{a}	13.17 ± 0.11^{b}	11.33 ± 0.43^{b}
STH	26.41 ± 0.18^{d}	11.17 ± 0.23^{c}	3.97 ± 0.41^{c}
STD	35.65 ± 0.22^{c}	11.57 ± 0.41^{c}	3.48 ± 0.22^{c}
STE	39.76 ± 0.25^{b}	15.77 ± 0.09^{a}	3.87 ± 0.08^{c}
STB	25.62 ± 0.14^{d}	13.24 ± 0.05^{b}	13.61 ± 0.16^{a}
STW	15.65 ± 0.17^{e}	8.04 ± 0.01^{d}	2.52 ± 0.14^{d}

The values are given as mean ± SD (n=3). $^{a\text{-}e}$Means with distinct letters in each column differ substantially ($p < 0.05$). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

1.3.3. *In vitro* antioxidant activity

Table 08 illustrates the antioxidant power of *S. tetragona* extract and its fractions. The results were significant and were expressed by IC_{50} values, where the dichloromethane fraction expressed the highest antioxidant activity in the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ tests, with IC_{50} = 62.54±0.48 µg/mL, IC_{50} = 19.11±0.70 µg/mL respectively, the ethyl acetate fraction was superior in the CUPRAC test, with an IC_{50} of 116.74±2.39 µg/mL, while the hexane fraction was the best in the metal chelating

activity, with an IC_{50} = 95.13±0.69 µg/mL. The results were compared to α-tocopherol, BHA and EDTA for the metal chelation test. The antioxidant activity with higher IC_{50} values is less potent.

Table 08. *In vitro* antioxidant activity of the different fractions of *S. tetragona*.

		DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	CUPRAC $A_{0.5}$(µg/mL)	Metal Chelating IC_{50} (µg/mL)
Samples	**CE**	478.30±0.66^{f}	64.37±0.61^{g}	306.87±5.01^{e}	405.79±4.05^{c}
	STH	815.12±8.71^{g}	36.58±0.89^{f}	479.63±7.42^{f}	95.13±0.69^{b}
	STD	62.54±0.48^{c}	19.11±0.70^{b}	203.84±1.35^{d}	4624.27±4,10^{f}
	STE	79.02±0.83^{d}	21.53±0.54^{c}	116.74±2.39^{b}	3250.19±4.40^{e}
	STB	258.39±2.54^{e}	31.80±0.42^{e}	133.51±2.88^{c}	1945.27±4.33^{d}
	STW	1449.50±10.02^{h}	104.91±9.23^{h}	1007.75±2.47^{g}	3250.19±4.40^{e}
Standards	**BHA**	28.59±0.06^{a}	7.23±0.01^{a}	24.49±0.19^{a}	-
	***α*-tocopherol**	36.35±0.24^{b}	27.70±0.28^{d}	134.53±0.19^{c}	-
	EDTA	-	-	-	26.85±1.50^{a}

The values are given as mean ± SD (n=3). $^{a-h}$Means with distinct letters in each column differ substantially ($p < 0.05$). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction; BHA, Butylated hydroxyanisole.

1.3.4. *In vitro* anti-inflammatory capacities

The anti-inflammatory activity of *S. tetragona* was evaluated by the albumin denaturation assay at different concentrations. The results are given in terms of IC_{50} (listed in Table 09), which is the concentration that protects 50% of the albumin against denaturation. According to the obtained results, it was found that the crude extract and its fractions had a very high anti-inflammatory activity, where both the ethyl acetate and the aqueous fractions exceeded the positive control Aspirin®, with an IC_{50} value of 13 ±5 µg/mL, 40 ±7 µg/mL respectively.

Table 09. IC_{50} values of albumin denaturation assay of hydromethanolic extract of *S. tetragona* and its fractions.

Samples	CE	STH	STD	STE	STB	STW	Aspirin®
IC_{50} (µg/mL)	636 ±10^{e}	1189 ±100^{f}	1500 ±150^{g}	13 ±5^{a}	120 ±20^{d}	40 ±7^{b}	94 ±12^{c}

The values are given as mean ± SD (n=3). $^{a-g}$Means with distinct letters in each row differ substantially ($p < 0.05$). Abbreviations**:** CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

1.3.5. Antibacterial Activity

The antibacterial potential of *S. tetragona* fractions was evaluated against four bacterial strains: *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*. Six different *S. tetragona* fractions were tested, including the crude extract (CE) and five solvent fractions: n-hexane (STH), dichloromethane (STD), ethyl acetate (STE),

n-butanol (STB), and water (STW). The minimum inhibitory concentration (MIC) was determined for each extract-bacteria combination using the standardized broth microdilution method in Muller Hinton medium. The results (Table 10) of the *S. tetragona* fractions exhibited moderate antibacterial activity across the panel of bacteria, with MIC values ranging from 125-500 µg/mL. The crude extract displayed the lowest MIC of 125 µg/mL against *E. coli*, indicating it has the strongest antibacterial potency amongst the samples tested. The n-butanol fraction (STB) also showed the lowest MIC of 125 µg/mL against the *P. aeruginosa* strain. The n-hexane (STH), dichloromethane (STD), ethyl acetate (STE) and water (STW) fractions had similar MICs of 250-500 µg/mL against all bacterial strains.

Table 10. *S. tetragona* extracts antibacterial activity (MICs).

Bacterial strains	**MIC(µg/mL)**						
	CE	STH	STD	STE	STB	STW	Ampicillin
***E.coli* ATCC 25922**	125	250	250	250	250	250	7.8
***S.aureus* ATCC 29213**	250	250	250	250	250	250	3.9
***K. pneumoniae* ATCC 13883**	250	500	250	250	500	500	31.25
***P. aeruginosa* ATCC 27853**	250	500	250	500	125	500	15.62

Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

1.3.6. Cytotoxic Activity

According to the MTT test results (Figure 01 and Figure 02), STD and STE were the most active against the cell viability of CCD-1079Sk and MCF7 cell lines, showing cytotoxicity in a concentration-dependent manner. Additionally, the extracts were more potent against the normal cell line when compared to the cancer cell line. STH and STM showed slight inhibitor effects, while STB and STW were not cytotoxic at the studied concentration range. The IC_{50} values can be found in Table 11.

Table 11. IC_{50} values of *S. tetragona* extracts estimated in the MTT cytotoxicity assay.

Cell Lines	**STB**	**STD**	**STE**	**STH**	**STM**	**STW**
MCF7	>250 µg/mL	**98 µg/mL**	>250 µg/mL	>250 µg/mL	>250 µg/mL	>250 µg/mL
CCD-1079Sk	>250 µg/mL	**64 µg/mL**	**186 µg/mL**	>250 µg/mL	>250 µg/mL	>250 µg/mL

Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. Tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

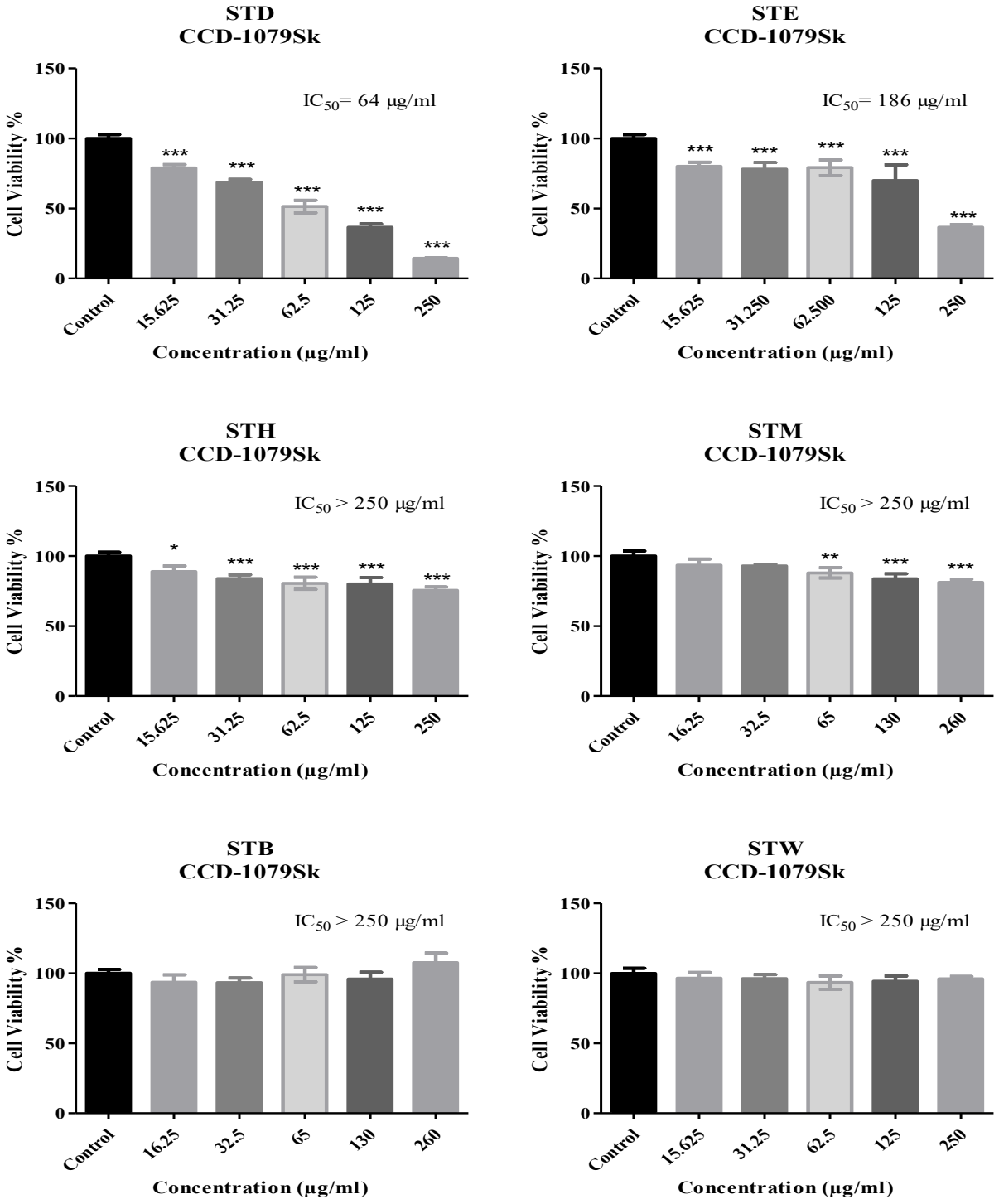

Figure 01. MTT assay results affect STD, STE, STH, STM, STB, and STW on CCD-1079Sk cell viability following 24 h treatment.

Treatment concentrations: 0-250 µg/ml, 0-260 µg/ml. Data are expressed as mean= SD, *p <0.05, **p <0.01, ***p <0.001versus the control group. Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona n*-butanol fraction; STW, *S. tetragona* remaining aqueous fraction; MTT, 3-(4,5-Dimethylthiazoli-2-yl)-2,5-Diphenyltetrazolium Bromide.

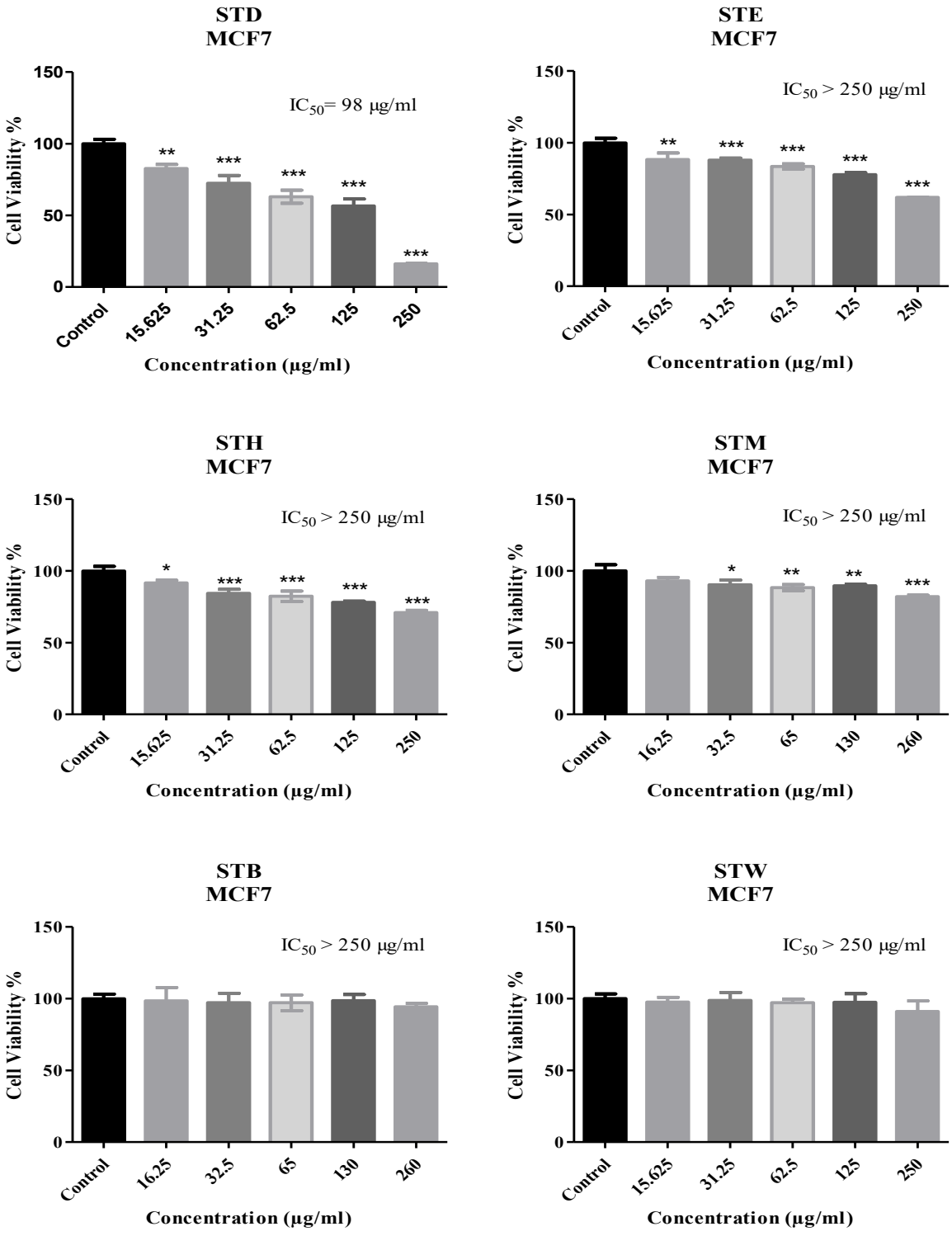

Figure 02. MTT assay results affect STD, STE, STH, STM, STB, and STW on MCF7 cell viability following 24 h treatment.

Treatment concentrations: 0-250 µg/ml, 0-260 µ/ml. Data are expressed as mean= SD, *p<0.05, **p<0.01, ***p<0.001 versus the control group. MTT: (3-(4,5-Dimethylthiazoli-2-yl)-2,5-Diphenyltetrazolium Bromide). Abbreviations: CE, *S. tetragona* crude extract; STH, *S. tetragona* hexane fraction; STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction; STB, *S. tetragona* n-butanol fraction; STW, *S. tetragona* remaining aqueous fraction.

1.3.7. Cell Migration Assay

After 24 h treatment with STD, the cell migration rate of the MCF7 cells was decreased after treatment with 25 µg/mL and 50 µg/mL significantly ($p < 0.05$) compared to the vehicle control group. STE also inhibited cell migration after treatment with 75 µg/mL and 150 µg/mL concentrations, although not significant (Figure 03).

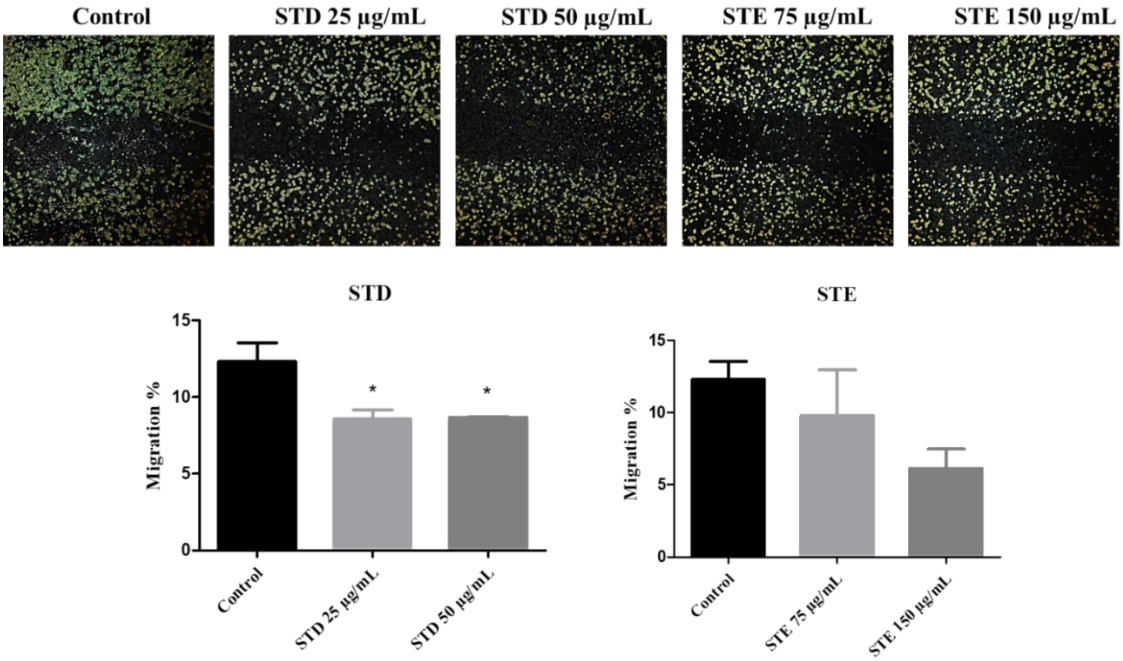

Figure 03. Effects of STD and STE on cell migration of MCF7 cells.

Treatment concentrations for STD: 250 µg/mL and 500 µg/mL. Treatment concentrations for STE: 75 µg/mL and 150 µg/mL. Data are expressed as mean= SD, *$p < 0.05$ versus the control group. Abbreviations: STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction.

1.3.8. Anti-Sars-Cov-2 Activity

The interaction percentage of ACE2 and spike protein was decreased significantly ($p < 0.05$) after STD exposure in the highest studied concentrations of STD (25 µg/mL and 50 µg/mL), while STE also had inhibitory activity in all concentrations, although insignificant. The results are given in Figure 04.

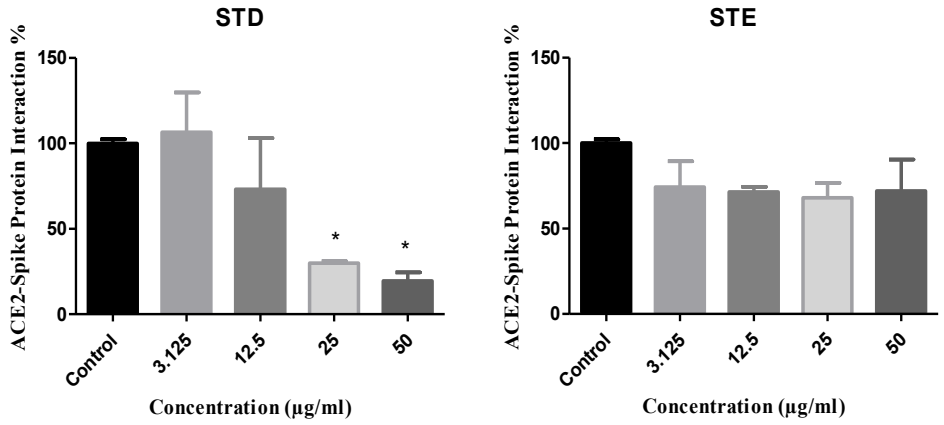

Figure 04. Effects of STD and STE on ACE2-Spike Protein Interaction.

Treatment concentrations: 0-50 µg/ml. Data are expressed as mean= SD, *p <0.05 versus the control group. Abbreviations**:** STD, *S. tetragona* dichloromethane fraction; STE, *S. tetragona* ethyl acetate fraction.

1.4. Discussion

The *Amaranthaceae* family contains many nutritious plant species, including several that are grown specifically for their nutritional benefits. Some examples include *Amaranthus* spp. - The leaves and seeds are high in protein, vitamins, minerals, and antioxidants. *Amaranth* has been shown to have high nutritional quality (Dinssa et al., 2018; Sogbohossou & Achigan-Dako, 2014). *Chenopodium quinoa* - A complete protein containing essential amino acids. High in fiber, magnesium, vitamin B2, iron, and antioxidants. Considered a nutritionally dense pseudocereal (James, 2009). *Beta vulgaris* - Rich in nitrates, antioxidants, folate, and manganese. Beetroots and leaves provide important micronutrients (Wruss et al., 2015). *Spinacia oleracea* - Excellent source of vitamins K, A, C, magnesium, iron, and folate. Widely considered one of the most nutrient-dense vegetables (Boz & Koelsch Sand, 2020). *Beta vulgaris* subsp. cicla - High in vitamins K, A, C, magnesium, potassium, and iron. Leaves and stalks are nutritious and often consumed (Gamba et al., 2021). Based on what was mentioned previously, and although our work focuses on secondary metabolites, the desert plant *S. tetragona* is characterized by being pastoral and sought by camels as a secondary food. A study of the nutritional value was added by analyzing the approximate compositions of the biomass of this plant, in an effort to include this plant in the diet. For humans or its value for animals.

The nutritional analysis conducted in this study provides novel compositional data on the desert medicinal plant *S. tetragona*. The quantitative assessment revealed that *S. tetragona* contains substantial moisture (37.56%), photosynthetic pigments (9.44 mg/g chlorophyll a, 1.96 mg/g chlorophyll b, 0.16 mg/g carotenoids), and ash mineral content (27.60%). The moisture level was higher compared to other *Salsola* species, such as *S. imbricata* (14.7%) and *S. tragus* (14.4%) It also outperformed them in terms of ash value, which was estimated at 6.65% and 7.95%, respectively (Munir et al., 2014). The pigment concentrations imply effective photosynthetic capacity, likely an adaptive mechanism to maximize photosynthesis despite harsh desert conditions. *S. tetragona's* high ash content suggests significant mineral composition, which was further confirmed by EDX analysis detecting predominantly O, C, Ca, Na, Si, Cl, Mg, K, and S. The results were somewhat similar to *Salsola tomentosa* and *Salsola rigida*, with some differences in the values of some minerals (Towhidi et al., 2011). Furthermore, *S. tetragona* exhibited notable levels of proteins (6.98 mg/g), carbohydrates (4.04 mg/g), and lipids (1.78 mg/g) in the dry matter. *S. tetragona* has moderately lower quantities of these primary metabolites. Overall, these results demonstrate that *S. tetragona*, similar to other *Salsola* species, is nutritionally rich in moisture, photosynthetic compounds, minerals, and macromolecules. The data suggests potential nutritional value as a functional food or animal feed source suited to desert environments.

The current study is the first to identify and characterize the phytochemical constituents of *S. tetragona* and evaluate their biological activity, including their potential as an antioxidant, anti-inflammatory, cytotoxic, and anti-viral.

Polyphenols, flavonoids, and tannins are important secondary metabolites that have been shown to possess various biological activities, such as antioxidant, anti-inflammatory, and anticancer properties (Murshid et al., 2022). The presence and quantity of these compounds in plants can be influenced by several factors, such as genetic makeup, environmental conditions, and plant age (Boulaaba et al., 2019).

By comparing the polyphenols and flavonoids content of the crude extract of *Salsola tetragona*, which is estimated at 46.55 ± 0.10 mg GAE/g ED and 13.17 ± 0.11 mg QE/g ED, respectively, with the content of polyphenols and flavonoids in the plant species belonging to the same genus, it was found that the plant contains an average amount. The plant excels in the quantitative content of polyphenols and flavonoids over the species *Salsola imbricata*, *Salsola kali*, and *Salsola tomentosa*, where the quantitative content of polyphenols was estimated at 2.60 mg GAE/g ED; 6.97±0.1 mg QE/g ED; 31.73 ± 0.09 mg GAE/g ED and flavonoids estimated at 0.571 mg QE/100 g ED; 13.6 ±0.2 mg GAE/g ED; 4.56 ± 0.2 mg QE/g ED, respectively(Aniss et al., 2014; Mohammadi et al., 2016; Shehab & Abu-Gharbieh, 2014). However, *Salsola cyclophylla* and

Salsola vermiculata outperformed *S. tetragona*. The quantitative content of polyphenols and flavonoids was as follows (126.6 ± 0.81mg GAE/g ED; 20.5± 1.02 mg RE/g ED) and (135.2 mg GAE/g ED; 18.2 mg QE/g ED). Respectively(Al-Omar et al., 2020; Amin et al., 2022).

Through the results of the LC / HR-MS analysis, it was found that there are different phenolic compounds, five of which were present in *Salsola kali*, *Salsola grandis*, and *Salsola cyclophylla*, namely hyperoside, quercitrin, naringenin, quercetin, and salicylic acid. Quercetin was also found in *Salsola collina* Pall, *Salsola imbricata*, *Salsola nvermiculata*, *Salsola tetrandra*, and *Salsola grandis* (ElNaggar et al., 2022). Additionally, eleven phenolic compounds were detected for the first time in the genus *Salsola*, including ascorbic acid, (+)-trans taxifolin, naringin, ellagic acid, 3'-O-methyl quercetin, caffeic acid phenethyl ester (CAPE), gypsogenic acid, pyrogallol, dihydrocaffeic acid, and chrysoeriol.

The major phenolic compounds of the crude extract of *S. tetragona* were identified using LC-HR/MS analysis as salicylic acid (274.00 mg/kg extract), ascorbic acid (159.60 mg/kg extract), and naringin (33.60 mg/kg extract). The results demonstrated a significant relationship between the quantity and quality of these phenolic compounds and their biological activity. This suggests that the effectiveness of the crude extract and its fractions in the studied assays may be influenced by the presence and concentration of these compounds (Chemsa et al., 2018). However, it is important to note that the biological activity of these compounds is not solely dependent on their polyphenolic nature but rather may also be influenced by their specific structures, functional groups, and potential synergistic effects among compounds (Ahmed et al., 2012).

As for the results of the gas chromatography/mass spectrometry (GC/MS) analysis carried out on the n-hexane fraction of the extract of the *S. tetragona* plant, 7 compounds were detected. The major constituents were hexadecanoic acid methyl ester (23.48%), 8,11-octadecadienoic acid methyl ester (18.49%), bis-(2-ethylhexyl) phthalate (21.98%), and 6,9,12,15-docosatetraenoic acid methyl ester (12.66%), which together accounted for over 75% of the total area. 5 of which appear for the first time in the genus *Salsola*, while tetradecanoic acid methyl ester appeared before in the aerial part of the *S. tetrandra* plant (ElNaggar et al., 2022). Heptadecanoic acid 16-methyl-, methyl ester (margaric acid) which previously appeared in the aerial parts of *Salasola terrandra* Forssk. was also detected (Murshid et al., 2022).

According to the results of the DPPH scavenging test, the crude extract showed weak activity when compared to the standard compounds BHA and α-tocopherol. The IC_{50} value of the crude extract was 478.30±0.66 µg/mL, which was better than the activity value achieved by the hydroethanolic extract of *S. cyclophylla* according to the study conducted by Mohammed et al. (Mohammed et al., 2021), which recorded a scavenging ability with an IC_{50} value of 1350±0.16

µg/mL. While it was lower than the value of DPPH anti-free radical activity achieved by the methanolic extract of *S. villosa* IC_{50} = 290.7 µg/mL according to the study conducted by (Amin et al., 2022). However, some fractions of *S. tetragona* extract dichloromethane (STD) and ethyl acetate (STE) recorded significant antioxidant activity with IC_{50} values of 62.54±0.48 µg/mL and 79.02±0.83 µg/mL, respectively. And by comparing the results of ABTS•+ free radical scavenging, it was found that the extracts of *S. tetragona* plant possess strong activity, as the crude extract recorded CE (EC_{50} = 64.37 ± 0.61 µg /ml), outperforming the activity recorded by the plant *Salsola imbricata* (EC_{50} = 137.7 ± 1.2 mg/ml and *Salsola kali* (EC_{50} = 457.66 µg/mL) (Ajaib & Shah, 2023). For the metal Chelating assay, the reductive activity of the crude extract *S. tetragona* (IC_{50} = 405.79 ±4.05 µg/ml). Better than *Salsola nitraria* (IC_{50} = 4450 ± 0.00 µg/ml) and *Salsola halophila* (IC_{50} = 2840 ± 0.00 µg/mL) both studied by (Şirin & Aslım, 2018).

The capacity of DPPH free radical scavenging by the crude extract and fractions related to the presence of antioxidant molecules such as ascorbic acid, quercetin, chlorogenic acid, naringin, ellagic acid, and others. These compounds can neutralize the free radical by giving hydrogen (Oluwole et al., 2022). The radical's reactivity capacity is attributed to the type and amount of these phenolic compounds and their ability to scavenge $DPPH^{\bullet}$, an important indicator of the extract's ability to prevent the formation of $ABTS^{\bullet+}$. This appeared when the extract's power was very high in removing the $ABTS^{\bullet+}$ radical (Gulcin et al., 2004). So, at higher concentrations, the plant extracts may help treat pathological damage caused by free radicals. The ability of metal ions to form chelates is a vital antioxidant mechanism as it reduces the concentration of transition metals, which are known to accelerate lipid peroxidation (Prior et al., 2005). Chelating agents can disrupt ferrozine-Fe^{+2} complexes, resulting in a decrease in the red color of the complex. The *n*-hexane fraction of *S. tetragona* (STH) exhibited high metal chelating activity, which varied among the other fractions. The CUPRAC assay is based on the reduction of an oxidant through electron transfer, which can be observed as a change in color (Lekouaghet et al., 2020). In this particular test, the extracts of *S. tetragona* showed significant antioxidant action.

Protein denaturation is the process whereby proteins undergo structural unraveling of their secondary and tertiary conformations as a consequence of external perturbation, such as heat, acidity, basicity, concentrated inorganic salt, or organic solvent (Anoop & Bindu, 2015). This process is a major contributor to inflammation, as it occurs in various inflammatory diseases (Osman et al., 2016). Protein denaturation is caused by the disruption of electrostatic, hydrophobic, disulfide, and hydrogen bonds that maintain the three-dimensional structure of proteins (Chirisa & Mukanganyama, 2016). Some phenolic compounds, such as flavonoids and phenolic acids, have been shown to bind to plasma proteins and protect these bonds from being broken (Kurlbaum &

Hogger, 2011). This may explain the potent anti-inflammatory activity of various fractions and the crude hydromethanolic extract of *S. tetragona*, which contains bioflavonoids such as chlorogenic acid, quercetin, and naringenin(Chen & Wu, 2014; Oluwole et al., 2022).

For the antibacterial activity of *S. tetragona* fractions was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Four bacteria commonly responsible for superficial and systemic infections. The results showed that the fractions had moderately successful ability to stop the growth of both gram-positive and gram-negative bacteria. Most of the fractions had roughly equal effectiveness. The antibacterial activity of the crude extract and five fractions of *S. tetragona*, is almost better than that of its close relatives of the same genus such as *Salsola vermiculata*, which recorded an MIC value of 85 ± 2.2 mg/ml against *Escherichia coli* and did not show efficacy with *Staphylococcus aureus* or *S. cyclophylla*, whose MICs value for *Escherichia coli* and *Staphylococcus aureus* was 79 ± 5.1 mg/ml and 75 ± 3.5 mg/ml, respectively (Mohammed et al., 2019). In study conducted by Shahzad et al. (2022), the extracts of *S. bryosoma* were tested against the bacteria *K. pneumoniae* and *P. aeruginosa*. The n-hexane extract of *S. bryosoma* had the lowest minimum inhibitory concentration (MIC) at 1.56 µg/mL against *P. aeruginosa*, in comparison, the crude extract, n-hexane, dichloromethane, ethyl acetate, n-butanol, and remaining aqueous fractions of *S. tetragona* all had higher MICs ranging from 125-500 µg/mL. When tested against *K. pneumoniae*, the hexane and ethanolic extracts of *S. bryosoma* had lower MICs of 50 µg/mL compared to the higher MICs of 125-500 µg/mL exhibited by the various *S. tetragona* fractions. Meanwhile, in another study conducted by Al-Omar et al. (2021) on the plant *S. vermiculata*, the ethanolic extract of this plant showed less activity against the bacteria of *Klebsiella pneumoniae* compared to all fractions of *S. tertagona*, where it recorded a MIC value estimated at 35mg/ml. The main reason for the antibacterial activity recorded in *S. tetragona* was attributed to the presence of secondary metabolites belonging to polyphenols (Bouarab-Chibane et al., 2019) and flavonoids (naringenin and quercetin) in these fractions, which can damage the plasma membranes of the tested bacteria (Oluwole et al., 2022).

The cytotoxicity of *S. tetragona* extracts against human skin cells, and human breast cancer cells show the potential possibility of especially dichloromethane and ethyl acetate fractions (STD and STE) for further anticancer drug development investigation along with the anti-migration effects of the extracts. Although the extracts were more potent against the normal skin cells when compared to the cancer cells, further experiments should be done in other cell lines to conclude their precise cytotoxic effects. Since the total phenolic content (TPC) of STD and STE was observed to be higher compared to the other fractions, this may be the underlying reason for their

higher potency. Phenolic compounds are known for their therapeutic effects on serious diseases like cancer. The different fractions are not observed to be toxic to the cells, which suggests they could be further analyzed for various therapeutic areas besides cancer.

Steps for entry of SARS-CoV-2 into host cells include binding of glycoprotein (S) to its receptor, angiotensin-converting enzyme 2 (ACE2), and subsequent membrane fusion. Thus, one strategy to prevent virus entry is to find compounds that can bind to the S protein, preventing membrane adhesion, the interaction between the SARS-CoV-2 protein and angiotensin-converting enzyme 2 (ACE2) (Heleno et al., 2023; Praharaj et al., 2022). From the results shown in Figure 4, it was found that STD and STE extracts had an inhibitory effect on ACE2-Spike Protein Interaction, where STD extract had better activity than STE. STD was 20% less effective in inhibition tests at a concentration of 50 µg/ml. Also, referring to previous studies, several plant-based compounds have been explored for their potential antiviral activity against SARS-CoV-2 (Islam et al., 2021). Plant secondary metabolites, especially flavonoids, alkaloids, and phenolic acids, showed antiviral activity against several samples of the Coronaviridae family (Campos et al., 2023). It has also been reported through a pre-docking study that 17 of the 18 chemical components of the oils inhibit the binding of ACE-2 to a virus-protein, and these 17 compounds account for 99.4% of all essential oils (Ankri & Mirelman, 1999). In an in-silico study (Arokiyaraj et al., 2020), the researchers suggested that the polyphenols found in *Geranii herba* may have interactions beyond the active sites of the SARS-CoV-2 protein. These polyphenols can interact with cell surface receptor-regulatory protein 78 (GRP78) to regulate cell signaling, triggering endoplasmic reticulum (ER) stress and other processes. Moreover, it may directly or indirectly interact with Cys/its pair of major proteases, thus inhibiting viral replication. As a result, polyphenols can disrupt the pathogenesis of viral disease at different stages (de Oliveira et al., 2022). DNA-binding alkaloids, such as berberine and tetrandrine, have also been found to inhibit viral replication in cells. Flavonoid glucosides, such as isoquercetin and isoorientin, have been identified in *Livistona decipiens* extracts as potential inhibitors of SARS-CoV-2 replication. Other studies have investigated the antiviral activities of plant secondary metabolites, including flavonoids, phenols, tannins, terpenoids, proanthocyanidins, lignins, thiosulfonates, steroids, and polysaccharides, against SARS-CoV-2 and other viruses (Aloufi et al., 2022; Behl et al., 2021; El Gizawy et al., 2021; Selim et al., 2022). These results indicate that plant secondary metabolites have the potential to be developed into natural remedies against COVID-19. The results presented in this study provide evidence to suggest that some compounds in *S. tetragona* extracts, particularly chalcone and flavones, have the potential to impair binding between the viral spike protein and its

ACE2 receptor. This binding inhibition is a critical mechanism involved in the entry and replication of SARS-CoV-2 viruses. However, further research is needed to evaluate their efficacy and safety *in vivo*.

1.5. Conclusion

The results of this study suggest that the fractions of crude hydromethanolic extract of *S. tetragona* have significant *in vitro* antioxidant, anti-inflammatory, antibacterial, cytotoxic, and anti-SARS-CoV2 effects. The nutritional value of this salt plant was moderate to somewhat weak and the qualitative and quantitative composition of phenolic, flavonoid, and tannin in the fractions is varied. The LC/HR-MS and GC/MS analysis of the hydromethanolic extract and n-hexane fraction of *S. tetragona* revealed the presence of several phenolics and fatty acids compounds. Based on the results, the dichloromethane (STD) and ethyl acetate fractions (STE) of the *S. tetragona* can be considered a source of bioactive substances. Further phytochemical analysis of the extract is needed to identify the specific compounds responsible for these biological and pharmacological effects and to evaluate the in vivo effects of these compounds on the immune system and disease prevention.

CHAPTER II

Investigating the antioxidant potential and the inhibitory activity of α-amylase, α-glucosidase, and acetylcholinesterase of different fractions from *Salsola tetragona* Delile

2.1. Introduction

Plants, particularly herbs, have been utilised in medicine and therapy for an extended period and in various forms as they serve as the primary source of medication. With the advancement of science, the therapeutic properties of plants have gained significant attention and importance worldwide. These properties include anti-inflammatory, anti-cancer, anti-malarial, anti-ulcer, anti-microbial, antioxidant effects, and various nutraceutical properties (Krishnaiah et al., 2011). Studies have focused on the beneficial roles of phytochemicals present in plants and their ability to promote overall health of the many secondary metabolites in plants, phenolics, and flavonoids are particularly notable for their ability to provide these health benefits (Fereidon & Ambigaipalan, 2015). New drugs have been developed using plant-based materials or plant precursors, making a comprehensive evaluation of plants crucial. As societies become increasingly focused on wellness and diet, natural plant products are considered viable alternatives to synthetic drugs. New plant-based products or Phytomedicine are deemed valuable resources that may replace synthetic medicines in light of these developments.

One such therapeutic plant includes *Salsola tetragona* Delile. This plant belongs to the genus *Salsola* (comprises over 120), family *Amaranthaceae*, which species found in arid and semi-arid regions of Asia, Europe, and Africa (Singh, 2019). This medicinal plant's leaves and aerial parts have been traditionally used to treat various ailments, including indigestion, constipation, abdominal and gastric pain, hypertension, kidney disease, and diabetes (Lakhdari et al., 2016). The powder or decoction of *S. tetragona* leaves can alleviate gastrointestinal pains, gastric pains, intestinal worms, microbial infections, cancer, and arrhythmia and can be used as a purgative (Daoud et al., 2016). *S. tetragona* is commonly used as a treatment for diabetes, with the leaves, roots, bark, and fruit being used for this purpose, typically in powder form or as a decoction(Ghourri et al., 2013). These findings suggest that *S. tetragona* may have potential therapeutic effects against diabetes and other related diseases.

This investigation aims to determine the characteristics of *S. tetragona* as anti-diabetic and anti-Alzheimer properties by assessing its inhibitory effects on enzymes (α-Amylase, α-Glucosidase, and Acetylcholinesterase) associated with these diseases. Additionally, this study aims to evaluate the antioxidant activity as well as the polyphenol and flavonoid content in four fractions of the aerial portion of *S. tetragona*: n-hexane (St.Hex), dichloromethane (St.DCM), ethyl acetate (St.EtOAc), and n-butanol (St.n-BuOH)**.** This study represents the initial examination of this plant, with the ultimate aim of contributing to the utilization of plants in the creation of novel medications and functional food ingredients to benefit human health.

2.2. Materials and methods

2.2.1. Chemicals and reagents

The chemicals and reagents used in this research are of analytical grade. n-hexane (Fisher Scientific), dichloromethane (Fischer Scientific), ethyl acetate (Fischer Scientific), and n-butanol (Fisher Scientific) were purchased from the local vendor Rouiba, Algeria. Folin‑Ciocalteu phenol reagent (FCR; Sigma Aldrich), α-glucosidase, α-amylase, and acetylcholinesterase, Acarbose, and Galantamine (Sigma-Aldrich, St. Louis) were also purchased from Algeria, Algeria. Chemicals and reagents such as gallic acid, quercetin, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Butylated hydroxyanisole (BHA), α-Tocopherol and Na_2CO_3, $AlCl_3$, C_2H_3O, Al $(NO_3)_3$, $FeCl_3$, and $K_3[Fe(CN)_6$ were of analytical grade (Sigma Aldrich, St. Louis) and purchased from Algeria, Algeria.

2.2.2. Plant material collection

S. tetragona aerial portion was collected on May 15th, 2020, from the El-Magrane region (El-Oued, southeast Algeria, 33°48'39" N 6°55'19.5" E) under the supervision of Pr. Youcef Halis, Director Research. Technical Research Center on Arid Regions, Algeria. A voucher specimen (LOST.St07/09) was deposited at the herbarium of the Faculty of Life and Natural Sciences, El-Oued University. The specimen was meticulously cleaned and underwent a process of desiccation at ambient temperature for 20 days, under conditions that ensured protection from moisture, light exposure, dust, and other contaminants. Adequate ventilation was maintained throughout the process. Upon completion of the drying process, the specimen was pulverized and stored in an airtight glass container to preserve its integrity.

2.2.3. Preparation of the extract and fractionation of plant material

The dried powder of *S. tetragona* (200g) was subjected to maceration at ambient temperature utilizing a solvent mixture of MeOH: H_2O (70:30, V/V) at a volume of 600 mL. This process was repeated thrice, with solvent regeneration at 24-hour intervals. Following filtration, the extract solutions were concentrated via rotary evaporation at a temperature not exceeding 40°C, and distilled water (800 mL) was added. The resulting solution was subsequently subjected to liquid-liquid extraction utilizing organic solvents of varying polarities, including n-hexane, dichloromethane, ethyl acetate, and n-butanol (3x600 mL for each solvent).

2.2.4. Determination of TPC (total phenolic contents)

The TPC of the samples was detected using the Folin-Ciocalteu reagent (FCR) and represented as µg of gallic acid equivalents (Li et al., 2007). The principle of this assay is to produce the Molybdenum-tungsten blue by reducing the (FCR) in the presence of phenolic compounds. The phenolic amount was determined using the standard gallic acid graph (Abs = 0.006 µg gallic acid + 0.0007, $r^2 = 0.999$).

2.2.5. Determination of TFC (total flavonoid contents)

The TFC of the samples was determined using a colorimetric method (Chekroun-Bechlaghem et al., 2019) and represented as µg of quercetin equivalents. This method involved the reaction between aluminium nitrate and potassium acetate, which resulted in the formation of a yellow complex. the flavonoid amount was determined using the standard quercetin graph (Abs = 0.005 µg quercetin + 0.0402, $r^2 = 0.999$).

2.2.6. *In vitro* antioxidant activity

DPPH Scavenging assay: The DPPH• radical scavenging activity of fractions was determined utilising a modified protocol(Brand-Williams et al., 1995). 1 ml at varying concentrations of *S. tetragona* fractions was added to 1 ml of 0.1×10^{-3} M DPPH (methanol solution). At 517 nm, the absorbance was measured after the mixture was incubated for 15 min at ambient temperature. The following equation determined the percentage of inhibition (Equation 11):

$$\%inhibition = (Absorbance_{control} - Absorbance_{sample}) / Absorbance_{control} \times 100 \quad \text{(Equation 11)}$$

In the DPPH test, α-Tocopherol and BHA were employed as reference standards for comparison. The IC_{50} (µg/ml), or the lowest concentration capable of decreasing DPPH• radicals by 50%, was calculated using the calibration curve's regression equation and used to express and compare the results.

Ferric reducing capacity: The reducing potential of *S. tetragona* fractions (St.Hex, St.DCM, St.EtOAc, St.n-BuOH) was evaluated by determining their ability to reduce $FeCl_3$ solution (Saague et al., 2019). The same volume (2.5 mL) of each fraction and 200 mM PBS (phosphate buffer solution pH 6.6) and 1% $K_3[Fe(CN)_6]$ were mixed and then incubated for 20 min at 50 °C. After adding 2.5 mL of 10% TCA, the solution was centrifuged for 10 min at 650 rpm, and 5 mL of the filtrate was mixed with an equal volume of distilled water and 1 mL of 0.1% $FeCl_3$. At 700 nm, the optical density was measured, and ascorbic acid was used as a standard. The results were compared to its EC_{50} value (effective concentration that gives an absorption of 0.5 at 700 nm).

Anti-hemolysis activity: The anti-hemolytic properties of the fractions were assessed *in vitro* using the protocol outlined by Afsar and others (Afsar et al., 2016) with certain modifications. The extract (2ml) was incubated for 5 minutes at 37°C with 40µl of a 10% human red blood cell suspension. Following this incubation time, the mixture was treated with 40 µL of 30 µM H_2O_2, 40 µL of 80 mM $FeCl_3$, and 40 µL of 50 mM ascorbic acid and incubated for an additional hour at 37°C. The mixture was then centrifuged at 700 revolutions per minute for 5 minutes. The absorbance at 540 nm was measured, and the percentage of hemolysis was estimated using the following equation (Equation 12):

$$\%of\ Hemolysis = [Absorbance_{control} / Absorbance_{sample}] \times 100 \quad \text{(Equation 12)}$$

In this experiment, ascorbic acid was used as the standard. The Hly_{50} value, which shows the concentration at which 50% of red blood cells were lysed, was used to express the results.

2.2.7. 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 *S. tetragona* extracts (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 13):

$$Inhibition\ (\%) = (1 - (Absorbance_{sample}/Absorbance_{control})) \times 100 \quad \text{(Equation 13)}$$

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

2.2.8. Evaluation of α-glucosidase inhibitory activity

To determine the inhibitory capacity of α-glucosidase using *S. tetragona* extracts 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 14):

$$Inhibition\ (\%) = (1 - (Absorbance_{sample}/Absorbance_{control})) \times 100 \quad \text{(Equation 14)}$$

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

2.2.9. Evaluation of acetylcholinesterase inhibition activity

The capacity of *S. tetragona* fractions 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 15):

$$\%Inhibition = [(A-B)/A] \times 100 \quad \text{(Equation 15)}$$

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

2.2.10. Statistical analysis

Quantitative estimates, enzyme inhibitory activity assays, and antioxidant ability were carried out in triplicates. The results obtained have been included as mean ± SD (standard deviation). One-way ANOVA with post hoc Tukey's (HSD) test was used to assess statistical differences. And p values < 0.05 were as significant.

2.3. Results and discussion

2.3.1. TPC and TFC in plant fractions

The results of the TPC (total phenolic content) of the *S. tetragona* fractions were quantified and expressed as gallic acid (GA) equivalents (presented in Table 12). The data indicated that the ethyl acetate fraction **(**St.EtOA**)** exhibited the highest quantitative content ($p < 0.05$) of polyphenols at 52.76 ± 0.25 µg GAE/mg, followed by the dichloromethane fraction (St.DCM) with 45.65 ± 0.22 µg GAE/mg, the n-hexane fraction (St.Hex) with 36.41 ± 0.18 µg GAE/mg and finally, the n-butanol fraction (St.n-BuOH) with 35.62 ± 0.14 µg GAE/mg.

Regarding the quantitative total flavonoid content (TFC), the results were quantified and expressed as quercetin (Q) equivalents and presented in Table 12. The values were relatively similar, with slight

variations between the fractions. St.EtOA fraction recorded the highest value ($p < 0.05$) at 22.77 ± 0.09 µg QE/mg ED, followed by St.n-BuOH fraction with 17.24 ± 0.05 µg QE/mg ED, St.DCM fraction with 15.57 ± 0.41 µg QE/mg ED and finally, St.Hex fraction with 15.17 ± 0.23 µg QE/mg ED.The quantitative analysis of phenols and flavonoids in *S. tetragona* can be explained by various factors due to the absence of any previous study of this plant. These include internal factors such as genetic makeup, developmental stage, hormonal signals, nutrient availability, and abiotic stress, and external factors such as light intensity and quality, temperature, water availability, air pollution, soil quality, pathogens, and pollinators(Šamec et al., 2021).

The slight variations observed in the quantified phenol and flavonoid content in the fractions of *S. tetragona* extracts can be attributed to the solvents' polarity used during the extraction process. Different solvents remove different substances from the plant matrix in various ways due to the varying degree of polarity of phenols, flavonoids, and other secondary metabolites (Chaves et al., 2020; Kim & Lee, 2002).

The quantitative assessment of flavonoids revealed that the n-hexane fraction, despite its low polarity, had similar flavonoid content to the other fractions with relatively high polar solvents. This is because solvents with low polarity can extract some types of flavonoids, particularly isoflavonoids, which have lower polarity compared to other flavonoids. Therefore, although flavonoids are generally considered to have high polarity, the presence of flavonoids in less polar solvents can be attributed to the extraction of isoflavonoids known for their low polarity (ElNaggar et al., 2022; Murshid et al., 2022; Selim et al., 2023).

Table 12. Values of total polyphenol, flavonoid, and *In vitro* antioxidant capacity of the various fractions of *S. tetragona.*

Plant extract fractions	**TPC (µg GAE/mg ED)**	**TFC (µg QE/mg ED)**	**DPPH**	**Anti-Hemolysis**	**FRAP**
			IC_{50} (µg/ml)	Hly_{50} (µg/ml)	$EC_{0,5}$(µg/ml)
St.Hex	36.41 ± 0.18^{c}	15.17 ± 0.23^{c}	815 ± 8.20^{e}	200 ± 2.10^{b}	1780 ± 70^{d}
St.DCM	45.65 ± 0.22^{b}	15.57 ± 0.41^{c}	62 ± 4.10^{a}	280 ± 4.40^{c}	1010 ± 20^{b}
St.EtOAc	52.76 ± 0.25^{a}	22.77 ± 0.09^{a}	79 ± 8.20^{b}	390 ± 1.30^{d}	910 ± 30^{b}
St.n-BuOH	35.62 ± 0.14^{c}	17.24 ± 0.05^{b}	258 ± 2.40^{c}	340 ± 5.20^{d}	1360 ± 60^{c}
Ascorbic Acid	-	-	-	110 ± 6.60^{a}	64 ± 10^{a}
***α*-tocopherol**	-	-	363 ± 2.30^{d}	-	-
BHA	-	-	285 ± 6.40^{c}	-	-

Note: the values are given as mean ± SD (n=3). [a-d]Means with distinct letters in each column differ substantially ($p < 0.05$). Abbreviations: St.Hex, *S. tetragona* n-hexane fraction; St.DCM, *S. tetragona* dichloromethane fraction; St.EtOAc, *S. tetragona* ethyl acetate fraction; St.n-BuOH, *S. tetragona* n-butanol fraction; BHA, Butylated hydroxyanisole.

2.3.2. *In vitro* antioxidant activity

Three commonly used assay methods -DPPH, anti-Hemolysis, and FRAP- were utilised to assess the antioxidant activity of four *S. tetragona* plant fractions. According to the study's findings (Table 12), the plant extract fractions showed significant antioxidant activity. The DPPH test is excellent for determining how healthy antioxidants are at scavenging free radicals. This assay is based on the ability of the antioxidants to reverse and stabilise the DPPH• radical, causing the solution to turn purple to yellow (Gülçin, 2006). With an IC_{50} value of 62±4.10 µg/ml, the dichloromethane fraction (St.DCM) of the plant extract had the most significate ($p < 0.05$) activity in the DPPH test. The anti-hemolysis assay measures the antioxidant activity by evaluating how well the antioxidants defend against hemolysis of red blood cells (rupture). This test is based on antioxidants' capacity to guard red blood cells against oxidative damage from hydrogen peroxide (H_2O_2), including phenols, alkaloids, terpenes, and tannins (Shabbir et al., 2013). The hexane fraction (St.Hex) of the plant demonstrated superior performance in the anti-hemolysis test ($p < 0.05$) (IC_{50} =200 ±2.10 µg/ml). The FRAP test assesses antioxidant activity by determining the antioxidant's capacity to decrease the (Fe3+) to (Fe2+);(Gülçin et al., 2012) in this test, the ethyl acetate fraction **(**St.EtOA**)** of the *S. tetragona* exhibited the most significant antioxidant activity with an (IC_{50} =910 ±30 µg/ml) ($p < 0.05$). The current study demonstrates that the fractions of *S. tetragona* plant extract possess significant antioxidant activity as evaluated by DPPH, FRAP, and anti-Hemolysis assays and suggests that the antioxidant capacity of the fractions may be due to the kind and amount of phenolic and flavonoid bio compounds present.

2.3.3. Inhibitory activities of α-amylase and α-glucosidase

The antidiabetic potential of the fractions from *S. tetragona* was evaluated by inhibiting Carbohydrate Metabolism enzymes (α-amylase and α-glucosidase). The results (Table 13) were quantified using IC_{50} values. Overall, the data suggest *S. tetragona* possesses significant antidiabetic activity, which aligns with traditional medicinal uses (Ghourri et al., 2013).

The inhibitory impacts of various fractions on the alpha-amylase enzyme were evaluated. Results indicated that the n-butanol fraction (St.n-BuOH) and the ethyl acetate fraction **(**St.EtOA**)** displayed the highest degree of inhibition ($p < 0.05$), with an estimated IC_{50} value of 70 ±1.80 µg/ml, 70 ± 1.30 µg/ml respectively. This inhibitory capacity was comparable to that of the acarbose control, which had an activity level of 62 ± 2.80 µg/ml. At the same time, dichloromethane (St.DCM) and hexane

(St.Hex) fractions displayed lower inhibitory activity ($p < 0.05$), with IC_{50} values of 124 ± 5.10 µg/ml and 130±0.40 µg/ml, respectively. In regards to the inhibitory properties of the α-glucosidase enzyme, the ethyl acetate and dichloromethane fractions exhibited comparable inhibitory activity ($p < 0.05$), with an IC_{50} value of 70 ± 1.80 µg/ml. This value is significant to the acarbose control, which had an IC_{50} value of 60 ± 1 µg/ml. The n-hexane and dichloromethane fractions' inhibitory activity was similar ($p < 0.05$), with IC_{50} values of 130 ± 1.20 µg/ml and 132 ± 3.20 µg/ml, respectively.

The inhibitory effect of *S. tetragona* fractions is significantly attributed to active natural compounds in both quantitative and qualitative amounts. Several studies have shown that polyphenols and flavonoids inhibit α-amylase and α-glucosidase enzymes, essential in carbohydrate digestion. The inhibition of these enzymes by flavonoids and polyphenols is thought to occur through binding these compounds to the enzyme's active site. This binding prevents substrate binding and enzymatic activity. Studies have identified several types of flavonoids and polyphenols that have been shown to inhibit these enzymes, such as catechins, procyanidins, quercetin, kaempferol, myricetin, curcumin, gallic acid, ellagic acid, naringenin, apigenin, and luteolin (Meng et al., 2016).

One research (Lo Piparo et al., 2008) investigated the relationships between flavonoids and human α-amylase to comprehend the molecular needs for enzyme inhibition better. The study discovered that the quantity of hydroxyl radical on the B ring of the flavonoid structure correlates with inhibitory ability. The interaction occurs through hydrogen bonds between the hydroxyl radical in position R6 or R7 of ring A and position R4 or R5 of the ring B of the polyphenol ligands and the catalytic residues of the binding site. A conjugated π-system is also produced, stabilizing the contact with the active site. These findings are consistent with the postulated mode of action for acarbose (Svensson et al., 2004).

Evidence suggests that the type of solvent used to extract compounds from plants can affect the effectiveness of inhibition digest carbohydrates enzymes (α-amylase, α-glucosidase). Different solvents can selectively extract different types of compounds, and some solvents may be more effective at extracting compounds that inhibit the enzymes in question (Wang et al., 2019). This explains the differential inhibitory capacity between the various fractions of *S. tetragona* hydromethanolic extract.

Table 13. Inhibitory Effects of *S. tetragona* fractions on α-glucosidase, α-amylase, and acetylcholinesterase (AChE).

Plant extract fractions	Inhibition (IC_{50} µg/ml)		
	α-Glucosidase	α-Amylase	AChE
St.Hex	130 ± 1.20^{c}	130 ± 0.40^{d}	63 ± 0.60^{c}
St.DCM	132 ± 3.20^{c}	124 ± 5.10^{c}	60 ± 0.10^{c}
St.EtOAc	70 ± 1.80^{b}	70 ± 1.30^{b}	30 ± 0.30^{a}
St.n-BuOH	70 ± 1.80^{b}	70 ± 1.80^{b}	32 ± 0.60^{b}
Acarbose	60 ± 1^{a}	62 ± 2.80^{a}	-
Galantamine	-	-	28 ± 0.30^{a}

Note: the values are given as mean± SD (n=3). $^{a\text{-}d}$Means with distinct letters in each column differ substantially ($p < 0.05$). Abbreviations: St.Hex, *S. tetragona* n-hexane fraction; St.DCM, *S. tetragona* dichloromethane fraction; St.EtOAc, *S. tetragona* ethyl acetate fraction; St.n-BuOH, *S. tetragona* n-butanol fraction.

2.3.4. Acetylcholinesterase inhibition activity

The acetylcholinesterase (AChE) enzyme inhibition assay results, presented in Table 13, indicate that the fractions derived from *S. tetragona* exhibit a potent inhibitory capacity compared to the reference compound, galantamine. The ethyl acetate fraction **(**St.EtOA**)** showed the most significate levels of inhibition ($p < 0.05$), with an IC_{50} value of 30 ± 0.30 µg/ml, which is comparable to and slightly less than the inhibitory potency of galantamine, 28 ± 0.30µg/ml. The n-butanol (St.n-BuOH) fraction exhibited a somewhat lower inhibitory capacity ($p < 0.05$), with an IC_{50} value of 32 ± 0.60 µg/ml. The dichloromethane (St.DCM) and hexane (St.Hex) fractions also displayed inhibitory capacity, with IC_{50} values of 60 ± 0.10 µg/ml and 63 ± 0.60 µg/ml ($p < 0.05$), respectively.

It is believed that the inhibiting properties of *S. tetragona* plant fractions are attributed to their active compound's contents, particularly polyphenols, and flavonoids. These compounds have been shown to exert an inhibitory influence on acetylcholinesterase (AChE) through various mechanisms, which differ from the known inhibitor, galantamine. Specifically, polyphenols such as gallic acid and quercetin have been found to exert a mixed-type inhibition on acetylcholinesterase, which is characterised by binding to both the active site and non-active site of the enzyme, resulting in uncompetitive and non-competitive inhibition (Liao et al., 2022). Flavonoids, including kaempferol and luteolin, have been found to exert a non-competitive inhibition on acetylcholinesterase, characterised by binding to a location other than the enzyme's active (Dhananjayan et al., 2013) In contrast, galantamine, on the other hand, is a reversible competitive inhibitor of acetylcholinesterase that binds to the enzyme's active site, obstructing substrate binding and decreasing enzyme activity (Greenwood, 1998).

Table 14. Pearson's correlation (R) and significance levels (*p*-values< 0.05) between total phenolic, and flavonoid content of various fractions of *S.tetragona* and their biological activity.

		DPPH	**FRAP**	**Anti-Hemolysis activity**	**α-Amylase inhibition activity**	**α-Glucosidase inhibition activity**	**AChE inhibition activity**
		n-Hexane fraction					
TPC (µg GAE/mg ED)	R	0.561	0.617	0.320	0.409	0.393	0.396
	P	0.216	0.154	0.102	0.182	0.298	0.368
TFC (µg QE/mg ED)	R	0.541	0.511	0.333	0.367	0.343	0.306
	P	0.247	0.113	0.202	0.292	0.398	0.452
		Dichloromethane fraction					
TPC (µg GAE/mg ED)	R	**0.971**	**0.752**	0.411	0.399	0.412	0.396
	P	0.147	0.213	0.102	0.392	0.118	0.292
TFC (µg QE/mg ED)	R	**0.804**	**0.711**	0.401	0.372	0.403	0.376
	P	0.247	0.113	0.202	0.192	0.198	0.252
		Ethyl acetate fraction					
TPC (µg GAE/mg ED)	R	**0.801**	**0.731**	0.520	**0.712**	**0.703**	**0.686**
	P	0.247	0.113	0.202	0.192	0.198	0.252
TFC (µg QE/mg ED)	R	0.761	0.625	0.380	0.402	0.370	0.346
	P	0.347	0.213	0.202	0.402	0.198	0.252
		n-Butanol fraction					
TPC (µg GAE/mg ED)	R	**0.722**	**0.603**	0.360	**0.772**	**0.661**	0.537
	P	0.287	0.134	0.252	0.106	0.178	0.202
TFC (µg QE/mg ED)	R	0.641	0.521	0.364	0.358	0.303	0.402
	P	0.147	0.153	0.262	0.425	0.432	0.352

R: Pearson correlation coefficient., P: p-value (Significant)., AChE: acetylcholinesterase, the dark color of the numbers for the correlation coefficient, R, indicates the strength of the positive correlation between the two variables.

2.4. Conclusion

This study aimed to discover the underlying phytochemical content, antioxidant activity, and inhibitory enzyme properties of *S. tetragona* ariel part fractions. *In vitro* experiments revealed that *S. tetragona* fractions have inhibitory effects on enzymes connected to diabetes and Alzheimer's disease. Furthermore, the study discovered a significant presence of total phenolic and flavonoids in *S. tetragona*, which enhances its antioxidant and enzyme-inhibitory activities. These findings support the traditional usage of *S. tetragona* in the treatment of diabetes and open the door to the development of pharmaceuticals for diabetes and associated disorders. Nonetheless, it is necessary to conduct additional in vivo research to fully comprehend the mechanisms of antioxidant and enzyme inhibition, the degree to which the substances are absorbed and utilised by the body, and their metabolic pathway.

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GENERAL CONCLUSION AND RECOMMENDATIONS

Salsola tetragona Delile, a medicinal plant used traditionally in North Africa, contains a diverse array of bioactive phytochemicals and exhibits promising pharmacological activities that could be harnessed for therapeutic applications.

The nutritional value of the *S. tetragona* plant was moderate to weak due to the environment in which it lives and tries to adapt to it. The qualitative phytochemical screening of the crude extract of the aerial part of *S. tetragona* revealed that it contains most secondary metabolites including alkaloids, coumarins, mucilage, phenols, saponins, sterols, and terpenes. Quantitatively, the quantitative estimation of the crude extract and its five derived fractions showed the presence of moderate amounts of phenols, flavonoids, and tannins.

LC-HR/MS analysis of the hydromethanolic extract identified 16 phenolic compounds, including phenolic acids, flavonoids, and other polyphenols. 10 of which were detected for the first time in the genus *Salsola*, namely ascorbic acid, (+)-trans taxifolin, naringin, ellagic acid, 3'-O-Methyl quercetin, caffeic acid phenethyl ester, gypsogenic acid, pyrogallol, dihydrocaffeic acid and chrysoeriol.

GC/MS analysis of the n-hexane fraction of *S. tetragona* revealed the presence of 7 compounds, 5 of which were detected for the first time in the *Salsola* genus. The extract was found to contain primarily nonpolar lipid-derived compounds. The major constituents identified were fatty acid methyl esters such as methyl hexadecanoate and methyl octadecadienoate, which accounted for over 40% of the total extract. Additional compounds included phthalate esters and methyl docosatetraenoate. The four most abundant lipids (methyl hexadecanoate, methyl octadecadienoate, phthalate esters, and methyl docosatetraenoate) comprised over 75% of the total n-hexane extract of *S. tetragona*.

The crude extract and solvent fractions displayed significant antioxidant capacities in DPPH, ABTS, FRAP, CUPRAC, anti-hemolysis, and metal chelating assays. The antioxidant effects correlated with high concentrations of phenolics and flavonoids. The dichloromethane and ethyl acetate fractions were the most potent.

Potent anti-inflammatory effects were observed, with the ethyl acetate fraction outperforming the standard Aspirin® in an albumin denaturation assay. All fractions exhibited positive antibacterial activities against *Escherichia coli*, *Staphylococcus aureus, Klebsiella pneumoniae*, and Pseudomonas aeruginosa strains.

The ethyl acetate and dichloromethane fractions showed selective cytotoxicity against breast cancer MCF7 cells over normal fibroblasts. They also inhibited cancer cell migration and ACE2-Spike protein interactions, suggesting anti-cancer and potential anti-SARS-CoV-2 effects.

The plant fractions effectively inhibited enzymes linked to diabetes (α-amylase, α-glucosidase) and Alzheimer's disease (acetylcholinesterase), with potencies matching or exceeding standard drug controls. The n-butanol and ethyl acetate fractions were most effective.

Finally, *S. tetragona* contains a wealth of promising bioactive phytochemicals that likely contribute to its antioxidant, anti-inflammatory, antimicrobial, anti-cancer, anti-diabetic, and anti-viral pharmacological activities. Further research should explore its potential for drug development and as a functional food ingredient. Further studies are required to isolate and identify the specific bioactive compounds responsible for the antioxidant, anti-inflammatory, antibacterial, anti-diabetic, anti-Alzheimer's, and anti-viral effects of *Salsola tetragona* observed in preliminary investigations. Pharmacological effects, bioavailability, safety, and mechanisms of action of these compounds need to be evaluated through in vivo studies and human clinical trials. Optimal extraction methods and synergistic effects of phytochemicals also need examination. Overall, more research is warranted to validate the traditional medicinal uses of *S. tetragona* and develop evidence-based herbal remedies.

REFERENCES

1. Afsar, T., Razak, S., Khan, M. R., Mawash, S., Almajwal, A., Shabir, M., & Haq, I. U. (2016). Evaluation of antioxidant, anti-hemolytic and anticancer activity of various solvent extracts of Acacia hydaspica R. Parker aerial parts. *BMC complementary and alternative medicine*, *16*(1), 1-16. https://doi.org/10.1186/s12906-016-1240-8

2. Ahmad, Z., Mehmood, S., Fatima, I., Malik, A., Ifzal, R., Afza, N., Iqbal, L., Latif, M., & Nizami, T. A. (2008). Structural determination of salsolins A and B, new antioxidant polyoxygenated triterpenes from Salsola baryosma, by 1D and 2D NMR spectroscopy. *Magn Reson Chem*, *46*(1), 94-98. https://doi.org/10.1002/mrc.2119

3. Ahmed, A. S., Elgorashi, E. E., Moodley, N., McGaw, L. J., Naidoo, V., & Eloff, J. N. (2012). The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African Bauhinia species used traditionally to treat diarrhoea. *J Ethnopharmacol*, *143*(3), 826-839. https://doi.org/10.1016/j.jep.2012.08.004

4. Ajaib, M., & Shah, S. (2023). Chemical Characterization and Pharmacology of Salsola imbricata Forssk. In *Bioactives and Pharmacology of Medicinal Plants* (pp. 323-331). Apple Academic Press. https://doi.org/10.1201/9781003281658

5. Al-Omar, M. S., Mohammed, H. A., Mohammed, S. A., Abd-Elmoniem, E., Kandil, Y. I., Eldeeb, H. M., Chigurupati, S., Sulaiman, G. M., Al-Khurayyif, H. K., & Almansour, B. S. (2020). Anti-microbial, anti-oxidant, and α-amylase inhibitory activity of traditionally-used medicinal herbs: a comparative analyses of pharmacology, and phytoconstituents of regional halophytic plants' diaspora. *Molecules*, *25*(22), 5457. https://doi.org/10.1080/0972060X.2019.1574611

6. Al-Omar, M. S., Sajid, M. S. M., Alnasyan, N. S., Almansour, B. S., Alruthaya, R. M., Khan, R. A., Mohammed, S. A. A., Al-Damigh, A. M., & Mohammed, H. A. (2021). The halophytic plant, Suaeda vermiculata Forssk extracts reduce the inflamed paw edema and exert potential antimicrobial activity. *Pakistan Journal of Botany*, *53*(1). https://doi.org/10.30848/pjb2021-1(9)

7. Aloufi, B. H., Snoussi, M., & Sulieman, A. M. E. (2022). Antiviral efficacy of selected natural phytochemicals against SARS-CoV-2 spike glycoprotein using structure-based drug designing. *Molecules*, *27*(8), 2401. https://doi.org/10.3390/molecules27082401

8. Amin, E., Abdel-Bakky, M. S., Mohammed, H. A., Chigrupati, S., Qureshi, K. A., & Hassan, M. H. (2022). Phytochemical Analysis and Evaluation of the Antioxidant and Antimicrobial Activities of Five Halophytes from Qassim Flora. *Polish Journal of Environmental Studies*, *31*(4). https://doi.org/10.15244/pjoes/145608

9. Aniss, H. A., Said, A. E. M., El Sayed, I. H., & Adly, C. (2014). Amelioration of adriamycin-induced cardiotoxicity by Salsola kali aqueous extract is mediated by lowering oxidative stress. *Redox Report*, *19*(4), 170-178. https://doi.org/10.1179/1351000214Y.0000000088

10. Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes and infection*, *1*(2), 125-129. https://doi.org/10.1016/s1286-4579(99)80003-3

11. Anoop, M., & Bindu, A. R. (2015). In-vitro Anti-inflammatory Activity Studies on Syzygium zeylanicum (L.) DC Leaves. *International Journal of pharma research & review*, *4*.

12. Apak, R., Guclu, K., Ozyurek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J Agric Food Chem*, *52*(26), 7970-7981. https://doi.org/10.1021/jf048741x

13. Arokiyaraj, S., Stalin, A., Kannan, B. S., & Shin, H. (2020). Geranii Herba as a potential inhibitor of SARS-CoV-2 main 3CLpro, spike RBD, and regulation of unfolded protein response: an in silico approach. *Antibiotics*, *9*(12), 863. https://doi.org/10.3390/antibiotics9120863

14. Azzi, R., Rabah, D., Lahfa, F., Sek, F., Benmehdi, H., & Belkacem, N. (2012). Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. *Journal of Medicinal Plants Research 6*, 2041-2050. https://doi.org/10.5897/JMPR11.1796

15. Behl, T., Rocchetti, G., Chadha, S., Zengin, G., Bungau, S., Kumar, A., Mehta, V., Uddin, M. S., Khullar, G., & Setia, D. (2021). Phytochemicals from plant foods as potential source of antiviral agents: An overview. *Pharmaceuticals*, *14*(4), 381. https://doi.org/10.3390/ph14040381

16. Belhouala, K., & Benarba, B. (2021). Medicinal plants used by traditional healers in Algeria: A multiregional ethnobotanical study. *Frontiers in pharmacology*, *12*, 760492. https://doi.org/10.3389/fphar.2021.760492

17. Bouarab-Chibane, L., Forquet, V., Lanteri, P., Clement, Y., Leonard-Akkari, L., Oulahal, N., Degraeve, P., & Bordes, C. (2019). Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure-Activity Relationship) Models. *Front Microbiol*, *10*, 829. https://doi.org/10.3389/fmicb.2019.00829

18. Boulaaba, M., Medini, F., Hajlaoui, H., Mkadmini, K., Falleh, H., Ksouri, R., Isoda, H., Smaoui, A., & Abdelly, C. (2019). Biological activities and phytochemical analysis of phenolic extracts from Salsola kali L.. Role of endogenous factors in the selection of the best plant extracts. *South African Journal of Botany*, *123*, 193-199. https://doi.org/10.1016/j.sajb.2019.03.003

19. Boz, Z., & Koelsch Sand, C. (2020). A systematic analysis of the overall nutritional contribution of food loss and waste in tomatoes, spinach, and kidney beans as a function of processing. *Journal of Food Process Engineering*, *43*(11), e13509. https://doi.org/10.1111/jfpe.13509

20. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

21. Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

22. Campos, M. F., Mendonça, S. C., Peñaloza, E. M. C., de Oliveira, B. A., Rosa, A. S., Leitão, G. G., Tucci, A. R., Ferreira, V. N. S., Oliveira, T. K. F., & Miranda, M. D. (2023). Anti-SARS-CoV-2 Activity of Ampelozizyphus amazonicus (Saracura-Mirá): Focus on the Modulation of the Spike-ACE2 Interaction by Chemically Characterized Bark Extracts by LC-DAD-APCI-MS/MS. *Molecules*, *28*(7), 3159. https://doi.org/10.3390/molecules28073159

23. Chaves, J. O., De Souza, M. C., Da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A. P. d. F., Forster-Carneiro, T., Vázquez-Espinosa, M., González-de-Peredo, A. V., & Barbero, G. F. (2020). Extraction of flavonoids from natural sources using modern techniques. *Frontiers in Chemistry*, *8*, 507887. https://doi.org/10.3389/fchem.2020.507887

24. Chehma, A. (2006). *Catalogue des plantes spontanées du Sahara septentrional algérien*. Éditions universitaires européennes.

25. Chekroun-Bechlaghem, N., Belyagoubi-Benhammou, N., Belyagoubi, L., Gismondi, A., Nanni, V., Di Marco, G., Canuti, L., Canini, A., El Haci, I., & Atik Bekkara, F. (2019). Phytochemical analysis and antioxidant activity of Tamarix africana, Arthrocnemum macrostachyum and Suaeda fruticosa, three halophyte species from Algeria. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *153*(6), 843-852. https://doi.org/10.1080/11263504.2018.1555191

26. Chemsa, A. E., Zellagui, A., Ozturk, M., Erol, E., Ceylan, O., Duru, M. E., & Lahouel, M. (2018). Chemical composition, antioxidant, anticholinesterase, antimicrobial and antibiofilm activities of essential oil and methanolic extract of Anthemis stiparum subsp. sabulicola (Pomel) Oberpr. *Microb Pathog*, *119*, 233-240. https://doi.org/10.1016/j.micpath.2018.04.033

27. Chen, W.-P., & Wu, L.-D. (2014). Chlorogenic acid suppresses interleukin-1β-induced inflammatory mediators in human chondrocytes. *International journal of clinical and experimental pathology*, *7*(12), 8797.

28. Chirisa, E., & Mukanganyama, S. (2016). Evaluation ofin VitroAnti-inflammatory and Antioxidant Activity of Selected Zimbabwean Plant Extracts. *Journal of Herbs, Spices & Medicinal Plants*, *22*(2), 157-172. https://doi.org/10.1080/10496475.2015.1134745

29. Daoud, S., Elbrik, K., Tachbibi, N., Bouqbis, L., Brakez, M., & Harrouni, M. C. (2016). The Potential Use of Halophytes for the Development of Marginal Dry Areas in Morocco. 141-156. https://doi.org/10.1016/B978-0-12-801854-5.00009-1

30. de Oliveira, J. R., Antunes, B. S., do Nascimento, G. O., Kawall, J. C. d. S., Oliveira, J. V. B., Silva, K. G. d. S., Costa, M. A. d. T., & Oliveira, C. R. (2022). Antiviral activity of

medicinal plant-derived products against SARS-CoV-2. *Experimental Biology and Medicine*, *247*(20), 1797-1809. https://doi.org/10.1177/15353702221108915

31. Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J Agric Food Chem*, *38*(3), 674-677. https://doi.org/10.1016/B978-0-12-801854-5.00009-1

32. Dhananjayan, K., Sumathy, A., & Palanisamy, S. (2013). Molecular docking studies and in-vitro acetylcholinesterase inhibition by terpenoids and flavonoids. *Asian Journal of Research in Chemistry*, *6*(11), 1011-1017.

33. Dinssa, F., Yang, R., Ledesma, D., Mbwambo, O., & Hanson, P. (2018). Effect of leaf harvest on grain yield and nutrient content of diverse amaranth entries. *Scientia Horticulturae*, *236*, 146-157. https://doi.org/10.1016/j.scienta.2018.03.028

34. Djekic, L., & Ćirić, A. (2022). Chapter 2 - Micro- and nanoscale drug delivery systems based on xanthan gum hydrogels. In S. Jana & S. Jana (Eds.), *Micro- and Nanoengineered Gum-Based Biomaterials for Drug Delivery and Biomedical Applications* (pp. 35-76). Elsevier. https://doi.org/10.1016/B978-0-323-90986-0.00007-8

35. Dubois, M., Gilles, K., Hamilton, J. K., Rebers, P. A., & Smith, F. (1951). A Colorimetric Method for the Determination of Sugars. *Nature*, *168*(4265), 167-167. https://doi.org/10.1038/168167a0

36. El-Keblawy, A., Gairola, S., & Bhatt, A. (2016). Maternal habitat affects germination requirements of *Anabasis setifera*, a succulent shrub of the Arabian deserts. *Acta Botanica Brasilica*, *30*, 35-40. https://doi.org/10.1590/0102-33062015abb0212

37. El Gizawy, H. A., Boshra, S. A., Mostafa, A., Mahmoud, S. H., Ismail, M. I., Alsfouk, A. A., Taher, A. T., & Al-Karmalawy, A. A. (2021). Pimenta dioica (L.) Merr. bioactive constituents exert anti-SARS-CoV-2 and anti-inflammatory activities: molecular docking and dynamics, in vitro, and in vivo studies. *Molecules*, *26*(19), 5844. https://doi.org/10.3390/molecules26195844

38. Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

39. ElNaggar, M. H., Eldehna, W. M., Abourehab, M. A., & Abdel Bar, F. M. (2022). The old world salsola as a source of valuable secondary metabolites endowed with diverse pharmacological activities: a review. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *37*(1), 2036-2062. https://doi.org/10.1080/14756366.2022.2102005

40. Fereidon, S., & Ambigaipalan, P. (2015). Phenolics and polypenolics in foods, beverages and spices: Antioxidant activity and health effects. *Journal of Functional Foods*, *18*, 820-897. https://doi.org/10.1016/j.jff.2015.06.018

41. Gamba, M., Raguindin, P. F., Asllanaj, E., Merlo, F., Glisic, M., Minder, B., Bussler, W., Metzger, B., Kern, H., & Muka, T. (2021). Bioactive compounds and nutritional composition of Swiss chard (Beta vulgaris L. var. cicla and flavescens): a systematic review. *Critical Reviews in Food Science and Nutrition*, *61*(20), 3465-3480. https://doi.org/10.1080/10408398.2020.1799326

42. Ghanem, A.-M. F. M., Mohamed, E., Kasem, A. M. M. A., & El-Ghamery, A. A. (2021). Differential Salt Tolerance Strategies in Three Halophytes from the Same Ecological Habitat: Augmentation of Antioxidant Enzymes and Compounds. *Plants*, *10*(6), 1100. https://doi.org/10.3390/plants10061100

43. Gheraissa, N., Chemsa, A. E., Elsharkawy, E., & Cherrada, N. (2022). Phenolic compound profile, and evaluation of biological properties of Bassia muricata (L.) Asch. aerial part. *International Journal of Secondary Metabolite*, *Vol. 9, No. 3*, 335-347. https://doi.org/10.21448/ijsm.1080537

44. Ghourri, M., Zidane, L., & Douira, A. (2013). Usage des plantes médicinales dans le traitement du Diabète Au Sahara marocain (Tan-Tan). *Journal of Animal &Plant Sciences*, *17*(1), 2388-2411.

45. Ghourri, M., Zidane, L., ROCHDİ, A., Fadli, M., & DOUİRA, A. (2012). Etude floristique et ethnobotanique des plantes médicinales de la ville d'El Ouatia (Maroc Saharien). *Kastamonu University Journal of Forestry Faculty*, *12*(2), 218-235.

46. Goldsworthy, G. J., Mordue, W., & Guthkelch, J. (1972). Studies on insect adipokinetic hormones. *General and Comparative Endocrinology*, *18*(3), 545-551. https://doi.org/10.1016/0016-6480(72)90034-2

47. Greenwood, D. (1998). Galantamine. A novel treatment for Alzheimer's disease. *Journal of Pharmacy and Pharmacology*, *50*(Supplement_9), 20-20. https://doi.org/10.1111/j.2042-7158.1998.tb02220.x

48. Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. *The scientific world journal*, *2017*. https://doi.org/10.1155/2017/5873648

49. Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, *217*(2-3), 213-220. https://doi.org/10.1016/j.tox.2005.09.011

50. Gülçin, İ., Elmastaş, M., & Aboul-Enein, H. Y. (2012). Antioxidant activity of clove oil–A powerful antioxidant source. *Arabian Journal of Chemistry*, *5*(4), 489-499. https://doi.org/10.1016/j.arabjc.2010.09.016

51. Gulcin, I., Kufrevioglu, O. I., Oktay, M., & Buyukokuroglu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.) [Research

Support, Non-U.S. Gov't]. *J Ethnopharmacol*, *90*(2-3), 205-215. https://doi.org/10.1016/j.jep.2003.09.028

52. Hanif, Z., Ali, H. H., Rasool, G., Tanveer, A., & Chauhan, B. S. (2018). Genus Salsola: its benefits, uses, environmental perspectives and future aspects-a review. *Journal of Rangeland Science*, *8*(3), 315-328.

53. Heleno, S. A., Carocho, M., Reis, F. S., Pires, T. C., Pintado, M., Ferreira, I. C., & Barros, L. (2023). Plant Extracts and SARS-CoV-2: Research and Applications. *Life*, *13*(2), 386. https://doi.org/10.3390/life13020386

54. Hemmami, H., Seghir, B. B., Zeghoud, S., Ben Amor, I., Kouadri, I., Rebiai, A., Zaater, A., Messaoudi, M., Benchikha, N., & Sawicka, B. (2023). Desert Endemic Plants in Algeria: A Review on Traditional Uses, Phytochemistry, Polyphenolic Compounds and Pharmacological Activities. *Molecules*, *28*(4), 1834.

55. Islam, S. S., Midya, S., Sinha, S., & Saadi, S. M. A. I. (2021). Natural medicinal plant products as an immune-boosters: A possible role to lessen the impact of Covid-19. *Case Studies in Chemical and Environmental Engineering*, *4*, 100105. https://doi.org/10.1016/j.cscee.2021.100105

56. Jacob, J. N., Shashoua, V. E., Campbell, A., & Baldessarini, R. J. (1985). . gamma.-Aminobutyric acid esters. 2. Synthesis, brain uptake, and pharmacological properties of lipid esters of. gamma.-aminobutyric acid. *Journal of medicinal chemistry*, *28*(1), 106-110. https://doi.org/10.1021/jm00379a019

57. Jafri, L., Saleem, S., Ihsan ul, H., Ullah, N., & Mirza, B. (2017). In vitro assessment of antioxidant potential and determination of polyphenolic compounds of Hedera nepalensis K. Koch. *Arabian Journal of Chemistry*, *10*, S3699-S3706. https://doi.org/10.1016/j.arabjc.2014.05.002

58. James, L. E. A. (2009). Quinoa (Chenopodium quinoa Willd.): composition, chemistry, nutritional, and functional properties. *Advances in food and nutrition research*, *58*, 1-31. https://doi.org/10.1016/S1043-4526(09)58001-1

59. Kim, D. O., & Lee, C. Y. (2002). Extraction and isolation of polyphenolics. *Current protocols in food analytical chemistry*, *6*(1), I1. 2.1-I1. 2.12. https://doi.org/10.1002/0471142913.fai0102s06

60. Kokoska, L., Havlik, J., Valterova, I., Sovova, H., Sajfrtova, M., & Jankovska, I. (2008). Comparison of chemical composition and antibacterial activity of Nigella sativa seed essential oils obtained by different extraction methods. *Journal of food protection*, *71*(12), 2475-2480. https://doi.org/10.4315/0362-028X-71.12.2475

61. Kouzmine, Y., & Fontaine, J. (2018). Démographie et urbanisation au Sahara algérien à l'aube du XXIe siècle. *Les Cahiers d'EMAM. Études sur le Monde Arabe et la Méditerranée*(30).

62. Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing*, *89*(3), 217-233. https://doi.org/10.1016/j.fbp.2010.04.008

63. Krishnaprabu, S. (2020). Therapeutic potential of medicinal plants: A review. *Journal of Pharmacognosy and Phytochemistry*, *9*(2), 2228-2233. https://doi.org/10.22271/phyto.2020.v9.i2ak.11184

64. Ksouri, R., Ksouri, W. M., Jallali, I., Debez, A., Magné, C., Hiroko, I., & Abdelly, C. (2012). Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Critical reviews in biotechnology*, *32*(4), 289-326. https://doi.org/10.3109/07388551.2011.630647

65. Kurlbaum, M., & Hogger, P. (2011). Plasma protein binding of polyphenols from maritime pine bark extract (USP). *J Pharm Biomed Anal*, *54*(1), 127-132. https://doi.org/10.1016/j.jpba.2010.07.038

66. Lakhdari, W., Dehliz, A., Acheuk, F., Mlik, R., Hammi, H., DOUMANDJI-MITICHE, B., Gheriani, S., Berrekbia, M., Guermit, K., & Chergui, S. (2016). Ethnobotanical study of some plants used in traditional medicine in the region of Oued Righ (Algerian Sahara).

67. Lekouaghet, A., Boutefnouchet, A., Bensuici, C., Gali, L., Ghenaiet, K., & Tichati, L. (2020). In vitro evaluation of antioxidant and anti-inflammatory activities of the hydroalcoholic extract and its fractions from Leuzea conifera L. roots. *South African Journal of Botany*, *132*, 103-107. https://doi.org/10.1016/j.sajb.2020.03.042

68. Li, H.-B., Cheng, K.-W., Wong, C.-C., Fan, K.-W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, *102*(3), 771-776. https://doi.org/10.1016/j.sajb.2020.03.042

69. Liao, Y., Mai, X., Wu, X., Hu, X., Luo, X., & Zhang, G. (2022). Exploring the Inhibition of Quercetin on Acetylcholinesterase by Multispectroscopic and In Silico Approaches and Evaluation of Its Neuroprotective Effects on PC12 Cells. *Molecules*, *27*(22), 7971. https://doi.org/10.3390/molecules27227971

70. Liu, H., Wang, X., Wang, D., Zou, Z., & Liang, Z. (2011). Effect of drought stress on growth and accumulation of active constituents in Salvia miltiorrhiza Bunge. *Industrial Crops and Products*, *33*(1), 84-88. https://doi.org/10.1016/j.indcrop.2010.09.006

71. Lo Piparo, E., Scheib, H., Frei, N., Williamson, G., Grigorov, M., & Chou, C. J. (2008). Flavonoids for controlling starch digestion: structural requirements for inhibiting human α-

amylase. *Journal of medicinal chemistry*, *51*(12), 3555-3561. https://doi.org/10.1021/jm800115x

72. Maatallah Zaier, M., Ciudad-Mulero, M., Cámara, M., Pereira, C., Ferreira, I. C. F. R., Achour, L., Kacem, A., & Morales, P. (2020). Revalorization of Tunisian wild Amaranthaceae halophytes: Nutritional composition variation at two different phenotypes stages. *Journal of Food Composition and Analysis*, *89*, 103463. https://doi.org/10.1016/j.jfca.2020.103463

73. Marmouzi, I., Tamsouri, N., El Hamdani, M., Attar, A., Kharbach, M., Alami, R., El Jemli, M., Cherrah, Y., Ebada, S. S., & Faouzi, M. E. A. (2018). Pharmacological and chemical properties of some marine echinoderms. *Revista Brasileira de Farmacognosia*, *28*, 575-581. https://doi.org/10.1016/j.bjp.2018.05.015

74. Matos, F. (1997). Introduction to experimental phytochemistry. *São Paulo, Brazil: UFC Editorial*.

75. Matuschek, E., Longshaw, C., Takemura, M., Yamano, Y., & Kahlmeter, G. (2022). Cefiderocol: EUCAST criteria for disc diffusion and broth microdilution for antimicrobial susceptibility testing. *Journal of Antimicrobial Chemotherapy*, *77*(6), 1662-1669.

76. Mbaebie, B. O., Edeoga, H. O., & Afolayan, A. J. (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), 118-124. https://doi.org/10.1016/S2221-1691(11)60204-9

77. Meng, Y., Su, A., Yuan, S., Zhao, H., Tan, S., Hu, C., Deng, H., & Guo, Y. (2016). Evaluation of total flavonoids, myricetin, and quercetin from Hovenia dulcis Thunb. as inhibitors of α-amylase and α-glucosidase. *Plant foods for human nutrition*, *71*(4), 444-449. https://doi.org/10.1007/s11130-016-0581-2

78. Mohammadi, M., Alaei, M., & Bajalan, I. (2016). Phytochemical screening, total phenolic and flavonoid contents and antioxidant activity of Anabasis setifera and Salsola tomentosa extracted with different extraction methods and solvents. *Oriental Pharmacy and Experimental Medicine*, *16*, 31-35.

79. Mohammed, H. A., Al-Omar, M. S., Aly, M. S. A., & Hegazy, M. M. (2019). Essential Oil Constituents and Biological Activities of the Halophytic Plants, Suaeda Vermiculata Forssk and Salsola Cyclophylla Bakera Growing in Saudi Arabia. *Journal of Essential Oil Bearing Plants*, *22*(1), 82-93. https://doi.org/10.1080/0972060x.2019.1574611

80. Mohammed, H. A., Al-Omar, M. S., Mohammed, S. A., Alhowail, A. H., Eldeeb, H. M., Sajid, M. S., Abd-Elmoniem, E. M., Alghulayqeh, O. A., Kandil, Y. I., & Khan, R. A. (2021). Phytochemical analysis, pharmacological and safety evaluations of halophytic plant, Salsola cyclophylla. *Molecules*, *26*(8), 2384. https://doi.org/10.3390/molecules26082384

81. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods*, *65*(1-2), 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

82. Munir, U., Perveen, A., & Qamarunnisa, S. (2014). Comparative Pharmacognostic evaluation of some species of the genera Suaeda and Salsola leaf (Chenopodiaceae). *Pakistan journal of pharmaceutical sciences*, *27*(5).

83. Murshid, S. S., Atoum, D., Abou-Hussein, D. R., Abdallah, H. M., Hareeri, R. H., Almukadi, H., & Edrada-Ebel, R. (2022). Genus Salsola: Chemistry, Biological Activities and Future Prospective—A Review. *Plants*, *11*(6), 714. https://doi.org/10.3390/plants11060714

84. Muthukrishnan, S., Kumar, T. S., Gangaprasad, A., Maggi, F., & Rao, M. V. (2018). Phytochemical analysis, antioxidant and antimicrobial activity of wild and *in vitro* derived plants of *Ceropegia thwaitesii* Hook – An endemic species from Western Ghats, India. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 621-630. https://doi.org/10.1016/j.jgeb.2018.06.003

85. Nayek, S., Choudhury, I., Haque, Nishika, J., & Roy, S. (2014). Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. *Research Journal of Chemical Sciences*, *4*, 2231-2606. https://doi.org/10.1055/s-0033-1340072

86. Noor, F., Tahir Ul Qamar, M., Ashfaq, U. A., Albutti, A., Alwashmi, A. S. S., & Aljasir, M. A. (2022). Network Pharmacology Approach for Medicinal Plants: Review and Assessment. *Pharmaceuticals (Basel)*, *15*(5). https://doi.org/10.3390/ph15050572

87. Oluwole, O., Fernando, W. B., Lumanlan, J., Ademuyiwa, O., & Jayasena, V. (2022). Role of phenolic acid, tannins, stilbenes, lignans and flavonoids in human health – a review. *International Journal of Food Science & Technology*, *57*(10), 6326-6335. https://doi.org/10.1111/ijfs.15936

88. Osman, N. I., Sidik, N. J., Awal, A., Adam, N. A., & Rezali, N. I. (2016). In vitro xanthine oxidase and albumin denaturation inhibition assay of Barringtonia racemosa L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. *J Intercult Ethnopharmacol*, *5*(4), 343-349. https://doi.org/10.5455/jice.20160731025522

89. Ozenda, P. (1977). Flora of the Sahara. *Flora of the Sahara.*(ed. 2), 231-235.

90. Ozenda, P. (2004). *Flore et végétation du Sahara 3 édition* (CNRS, Paris ed.).

91. Praharaj, M. R., Garg, P., Kesarwani, V., Topno, N. A., Khan, R. I. N., Sharma, S., Panigrahi, M., Mishra, B., Mishra, B., & Kumar, G. S. (2022). SARS-CoV-2 spike glycoprotein and ACE2 interaction reveals modulation of viral entry in wild and domestic animals. *Frontiers in Medicine*, *8*, 775572. https://doi.org/10.3389/fmed.2021.775572

92. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements [Review]. *J Agric Food Chem*, *53*(10), 4290-4302. https://doi.org/10.1021/jf0502698

93. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

94. Saague, P. W. K., Moukette Moukette, B., Njimou, J. R., Biapa, P. C. N., Nzufo Tankeu, F., Moor, V. J. A., Pieme, C. A., & Ngogang, J. Y. (2019). Phenolic Compounds from Water-Ethanol Extracts of Tetrapleura tetraptera Produced in Cameroon, as Potential Protectors against In Vivo CC-Induced Liver Injuries. *The scientific world journal*, *2019*. https://doi.org/10.1155/2019/5236851

95. Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., & Salopek-Sondi, B. (2021). The role of polyphenols in abiotic stress response: The influence of molecular structure. *Plants*, *10*(1), 118. https://doi.org/10.3390/plants10010118

96. Selim, D. A., Shawky, E., Ghareeb, D. A., Abdulmalek, S. A., & El-Khair, R. M. A. (2023). Comparative metabolomics of the different fractions of two saltwort (Salsola L.) species in relation to their anti-inflammatory activity. *Food Bioscience*, *51*, 102306. https://doi.org/10.1016/j.fbio.2022.102306

97. Selim, S., Almuhayawi, M. S., Alharbi, M. T., Al Jaouni, S. K., Alharthi, A., Abdel-Wahab, B. A., Ibrahim, M. A., Alsuhaibani, A. M., Warrad, M., & Rashed, K. (2022). Insights into the antimicrobial, antioxidant, anti-sars-cov-2 and cytotoxic activities of Pistacia lentiscus bark and phytochemical profile; in silico and in vitro study. *Antioxidants*, *11*(5), 930. https://doi.org/10.3390/antiox11050930

98. Shabbir, M., Khan, M. R., & Saeed, N. (2013). Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. *BMC complementary and alternative medicine*, *13*, 1-13. https://doi.org/10.1186/1472-6882-13-143

99. Shahzad, M. I., Anwar, S., Aslam, J., Manzoor, A., Ashraf, H., Saba, N., Naseer, M., Rani, U., Aslam, Z., & Kamran, Z. (2022). Antibacterial and Antibiofilm Activities from Extracts of Selected Cholistani Plants. *FRONTIERS IN CHEMICAL SCIENCES*, *3*(2), 32-45. https://doi.org/10.52700/fcs.v3i1.50

100. Shehab, N. G., & Abu-Gharbieh, E. (2014). Phenolic profiling and evaluation of contraceptive effect of the ethanolic extract of Salsola imbricata Forssk. in male albino rats. *Evidence-Based Complementary and Alternative Medicine*, *2014*. https://doi.org/10.1155/2014/695291

101. Shibko, S., Koivistoinen, P., Tratnyek, C. A., Newhall, A. R., & Friedman, L. (1967). A method for sequential quantitative separation and determination of protein, RNA, DNA,

lipid, and glycogen from a single rat liver homogenate or from a subcellular fraction. *Analytical biochemistry*, *19*(3), 514-528. https://doi.org/10.1016/0003-2697(67)90242-4

102. Singh, G. (2019). *Plant systematics: an integrated approach*. CRC Press.

103. Şirin, S., & Aslım, B. (2018). Determination of antioxidant capacity, phenolic acid composition and antiproliferative effect associated with phenylalanine ammonia lyase (PAL) activity in some plants naturally growing under salt stress. *Medicinal Chemistry Research*, *28*(3), 229-238. https://doi.org/10.1007/s00044-018-2278-6

104. Sogbohossou, O. E., & Achigan-Dako, E. G. (2014). Phenetic differentiation and use-type delimitation in Amaranthus spp. from worldwide origins. *Scientia Horticulturae*, *178*, 31-42. https://doi.org/10.1016/j.scienta.2014.08.003

105. Svensson, B., Fukuda, K., Nielsen, P. K., & Bønsager, B. C. (2004). Proteinaceous α-amylase inhibitors. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1696*(2), 145-156. https://doi.org/10.1016/j.bbapap.2003.07.004

106. Tabasum, S., Khare, S., & Jain, K. (2016). Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of *Abrus precatorius* L. Seeds. *Asian Journal of Pharmaceutical and Clinical Research*, *9*, 371-374.

107. Towhidi, A., Saberifar, T., & Dirandeh, E. (2011). Nutritive value of some herbage for dromedary camels in the central arid zone of Iran. *Tropical Animal Health and Production*, *43*, 617-622. https://doi.org/10.1007/s11250-010-9741-9

108. Tundis, R., Loizzo, M. R., Bonesi, M., Menichini, F., Statti, G. A., & Menichini, F. (2008). In vitro cytotoxic activity of Salsola oppositifolia Desf.(Amaranthaceae) in a panel of tumour cell lines. *Zeitschrift für Naturforschung C*, *63*(5-6), 347-354. https://doi.org/10.1515/znc-2008-5-607

109. Wang, L., Clardy, A., Hui, D., Gao, A., & Wu, Y. (2019). Antioxidant and antidiabetic properties of Chinese and Indian bitter melons (Momordica charantia L.). *Food Bioscience*, *29*, 73-80. https://doi.org/10.1016/j.fbio.2019.03.010

110. Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., Höglinger, O., & Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, *42*, 46-55. https://doi.org/10.1016/j.jfca.2015.03.005

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Phytochemical profiling of *Salsola tetragona* Delile by LC-HR/MS and investigation of the antioxidant, anti-inflammatory, cytotoxic, antibacterial and anti-SARS-CoV-2 activities

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ABSTRACT

This study aimed to investigate the phytochemical composition and biological activity of *Salsola tetragona* Delile. (Amaranthaceae), a medicinal plant. The study evaluated the antioxidant potential of the crude extract and five fractions of *S. tetragona* using DPPH•, ABTS•+, CUPRAC, and metal chelating assays. The anti-inflammatory activity was determined using a protein denaturation assay, and the antibacterial activity was determined by the Minimum inhibitory concentrations (MICs) for the growth of *Escherichia coli* and *Staphylococcus aureus* strains. The MTT test and an in vitro scratch assay evaluated the effects on cell viability and cell migration. The potential anti-SARS-CoV-2 activity was assessed by analyzing the effects on the interaction between ACE2 and Spike protein. The bioactive compounds present in the plant were identified using LC-HR/MS analysis. The crude hydromethanolic extract (STM) and five fractions of *S. tetragona*, n-hexane (STH), dichloromethane (STD), ethyl acetate (STE), n-butanol (STB), and aqueous (STW) showed significant antioxidant activity in four different tests. In the anti-inflammatory assay, the ethyl acetate fraction exhibited significantly higher activity than Aspirin® (IC_{50} = 13 ± 5 µg/mL). The crude extract and its fractions showed positive antibacterial activity with similar MICs. In the cytotoxicity assay against the breast cancer cell line MCF7, the dichloromethane fractions (STD) were very effective and demonstrated superiority over the other fractions (IC_{50} = 98 µg/mL). Moreover, the potential of the extract and fractions as anti-SARS-CoV-2, the ethyl acetate, and dichloromethane fractions demonstrated important activity in this test. LC-HR/MS analysis identified 16 different phenolic compounds, Eleven of which had not been previously reported in the genus *Salsola*. The results suggest that the extracts of *S. tetragona* have the potential to become new sources for developing plant-based therapies for managing a range of diseases.

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1. Introduction

The *Salsola* L. genus, also known as Russian thistle or Saltwort, is a halophyte plant that belongs to the Amaranthaceae family. It is a large genus consisting of semi-dwarf to dwarf shrubs and woody tree species. The name of the genus comes from the Latin word

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Antioxidant potentials and inhibitory activities of α-amylase, α-glucosidase, and acetylcholinesterase of different fractions from *Salsola tetragona* Delile

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ABSTRACT

The medicinal use of *Salsola tetragona* Delile (Amaranthaceae) aerial parts is a longstanding tradition. This study delved into the plant's potential as an antioxidant, anti-diabetic, and anti-Alzheimer agent. The aerial portion extracted and evaluated four fractions (n-hexane, dichloromethane, ethyl acetate, n-butanol) for their antioxidant activity using DPPH, FRAP, and anti-hemolysis tests, as well as the inhibitory activity of cholinesterase and carbohydrate digesting enzymes. The results showed that the dichloromethane fraction (St.DCM) of *S. tetragona* had a significant ability to scavenge DPPH• radicals. The ethyl acetate fraction (St.EtOAc) had the best reduction power test activity compared to other solvent fractions. The n-hexane fraction (St.Hex) was most effective in the anti-hemolysis test. The ethyl acetate fraction also had inhibitory activities ($p < .05$) with IC_{50} values of 70 ± 1.80 µg/ml for α-glycosidase, equivalent to the n-butanol fraction (St. n-BuOH), which had very significant activity ($p < .05$) in the α-amylase inhibition test with an IC_{50} of 64 ± 1.80 µg/ml. The ethyl acetate fraction also had a considerable concentration of total phenolic compounds and flavonoids and exhibited significant ($p < .05$) inhibitory effects on acetylcholinesterase with an IC_{50} of 30 ± 0.30 µg/ml. Therefore, the aerial parts of *S. tetragona* contained high levels of biologically active compounds, making it a potential source for developing new plant-based pharmaceuticals and nutraceuticals to enhance human health.

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Introduction

Plants, particularly herbs, have been utilized in medicine and therapy for an extended period and in various forms as they serve as the primary source of medication. With the advancement of science, the

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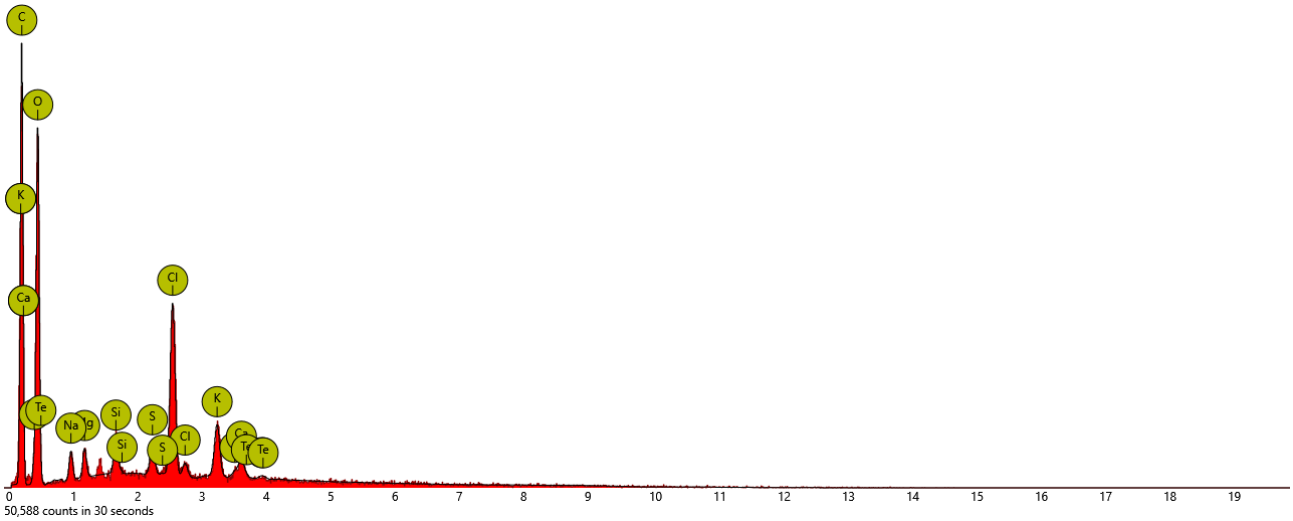
APPENDICES

Appendix 01. « EDX analysis characterizations »

FOV: 373 µm, Mode: 15kV - Image, Detector: SED

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
C	58.45	47.18		
O	34.89	37.52		
Cl	2.88	6.86		
K	1.08	2.85		
Ca	0.45	1.22	**CaO**	2.64
S	0.51	1.09		
Na	0.69	1.06	**Na_2O**	2.21
Mg	0.54	0.89	**MgO**	2.26
Si	0.45	0.85	**SiO_2**	2.81
Con, concentration. Wt, weight.				



A. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *S. tetragona.*

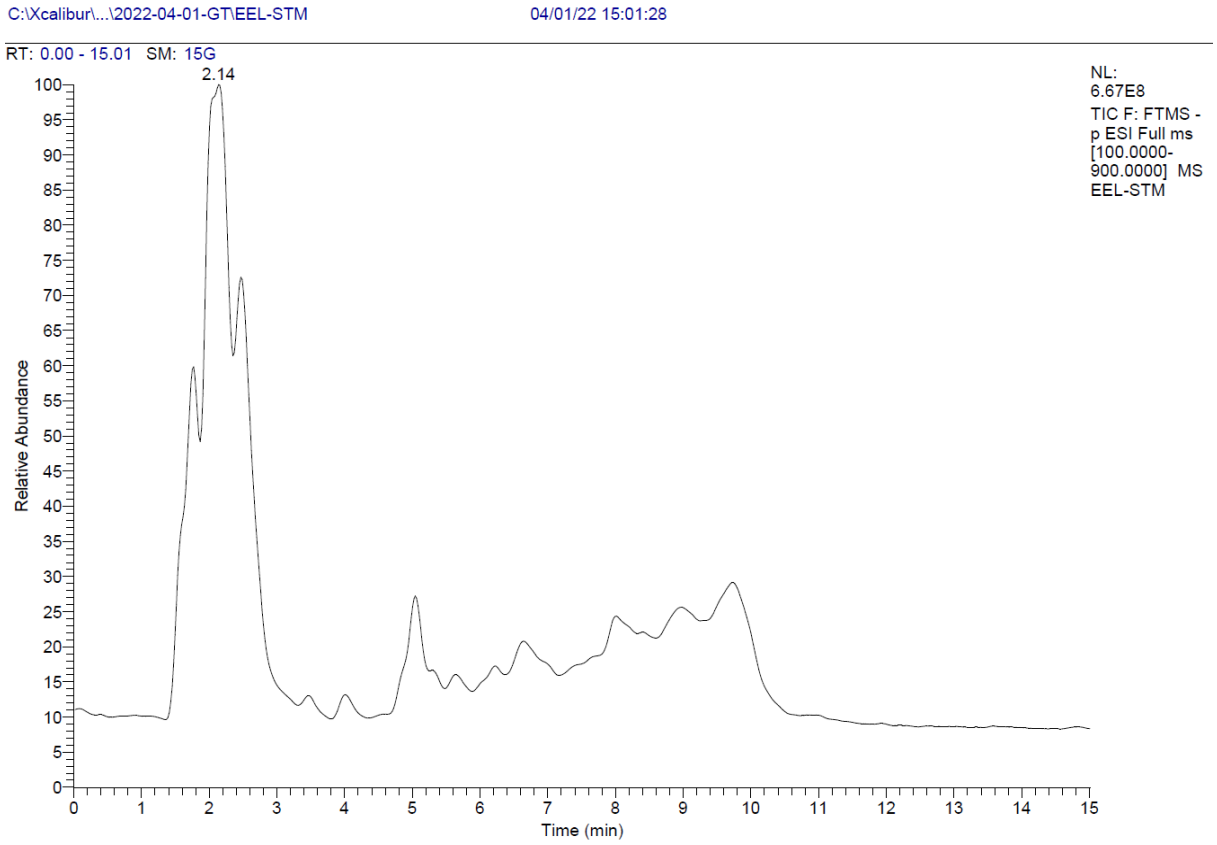
Appendix 02. « LC-MS and GC/MS analysis characterizations »

A. LC-MS conditions

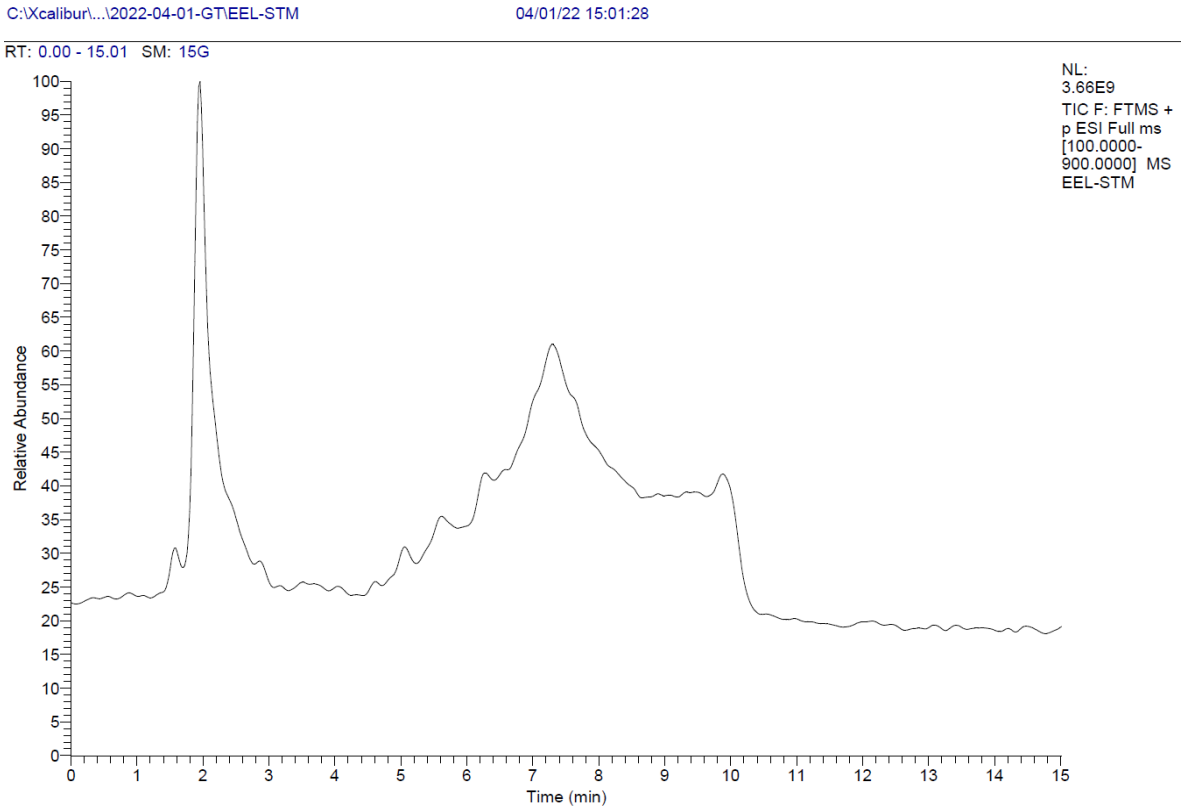
HPLC CONDITIONS	MS CONDITIONS (Mass Spectrometer)
Mobile Phase A: 1% Formic Acid- H2O Mobile Phase B: 1% Formic Acid- MeOH Column: Troyasil C18 HS –150 x 3 mm 5 µ Gradient Time Flow (mL/min) % B 0.00 0.35 50 1.00 0.35 50 3.00 0.35 100 6.00 0.35 100 7.00 0.35 50 15.00 0.35 50	System**:** Thermo Orbitrap Q-Exactive Ion Source: ESI Mass Scanning Range: 100-900 m/z Sheath gas flow rate: 45 Aux gas flow rate: 10 Spray voltage (kV): 3.80 Capillary temp. (°C): 320 Aux gas heater temp (°C): 320 S-lens RF level: 50.0 Definitions: ILMER at BVU
ENVIRONMENTAL CONDITIONS Temperature: (22.0 ± 5.0) °C Relative Humidity: (50 ± 15) % rh	

B. Phenolic standards used for LC-MS analysis

Ascorbic acid	Hesperidin	Kaempferol
(-)-Epigallocatechin	Rutin	3'-O-methyl quercetin
(-)-Epigallocatechin gallate	Syringic acid	Apigenin
(+)-Catechin	Rosmarinic acid	Hispidulin
Chlorogenic acid	Hyperoside	Isosakuranetin
Fumaric acid	Dihydrokaempferol	Penduletin
(-)-Epicatechin	Oleuropein	Glycyrrhizic acid
(-)-Epicatechin gallate	Apigenin 7-glucoside	Sinensetin
Verbascoside	Ellagic acid	Caffeic asit phenethyl ester
Chicoric acid	Quercitrin	Rhamnocitrin
Orientin	Myricetin	Chrysin
Caffeic acid	Nepetin-7-glucoside	Acacetin
Caffeine	Scutellarein	Quillaic acid
(+)-*trans* taxifolin	Quercetin	Sarsasapogenin
Luteolin-7-rutinoside	Herniarin	Gypsogenic acid
Vanillic acid	Salicylic acid	Emodin
Naringin	Naringenin	Shatavarin
Sinapinic acid	Luteolin	Hederagenin
Luteolin 7-glucoside	Nepetin	(-)- caryophyllene oxide
p-Coumaric acid	Genistein	Kaempferol



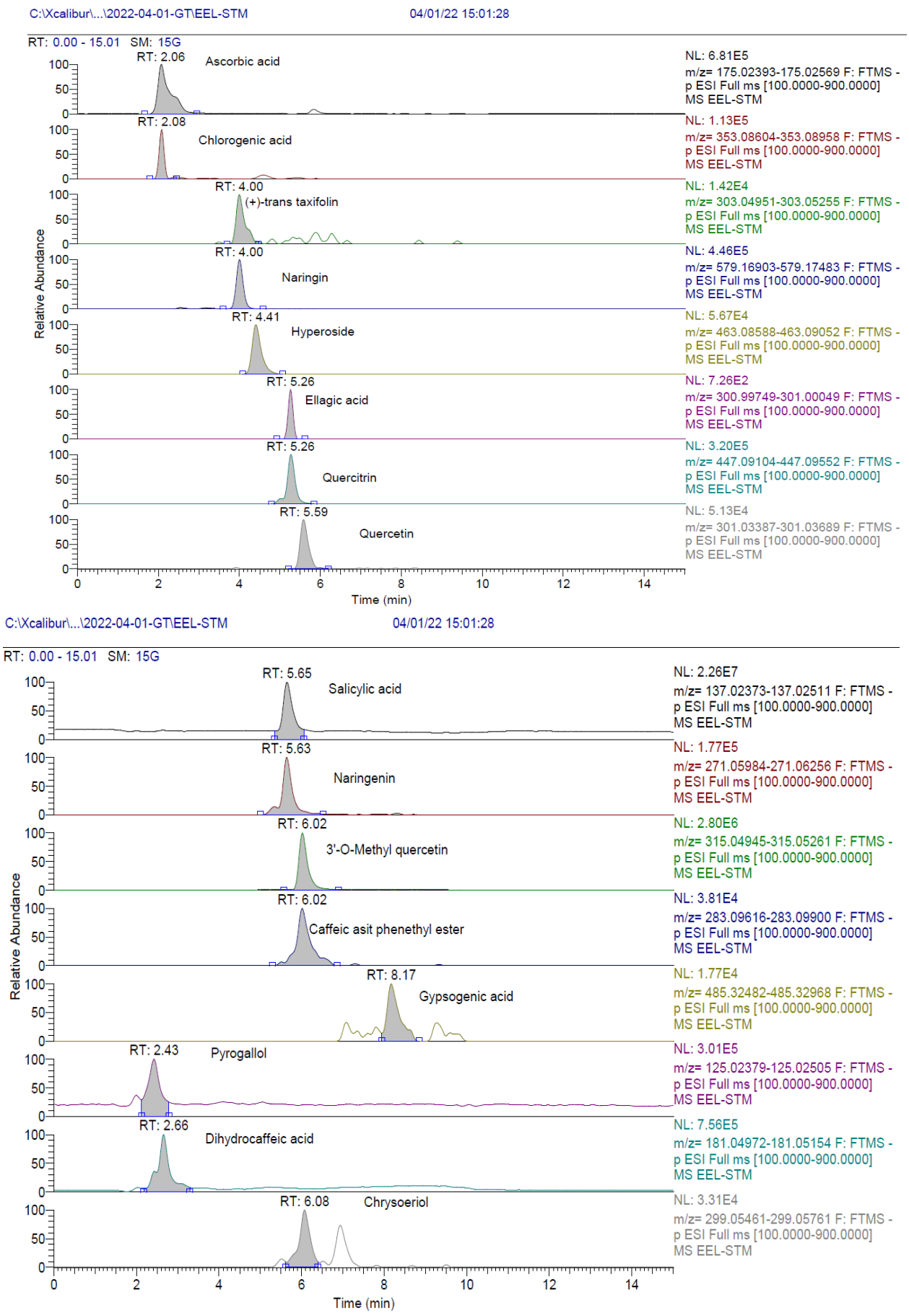
C. HRMS negative chromatogram of the hydromethanolic extract of *S. tetragona*



D. HRMS positive chromatogram of the hydromethanolic extract of *S. tetragona*

E. The quantity of phenolics (mg/kg extract) in hydromethanolic extract of *S. tetragona* determined by LC-HRMS.

Rt	*m/z*	Ionization mode	Phenolic standards	Molecular Formula	(mg analyte/ kg extract)	U%
2.06	175.0248	Positive	Ascorbic acid	$C_6H_8O_6$	159.60	3.94
2.08	353.0878	Positive	Chlorogenic acid	$C_{16}H_{18}O_9$	4.30	3.58
2.43	125.0239	Positive	Pyrogallol	$C_6H_6O_3$	6.80	3.74
2.66	181.0509	Positive	Dihydrocaffeic acid	$C_9H_{10}O_4$	15.25	0.86
4.00	447.0933	Positive	(+)-*trans* taxifolin	$C_{21}H_{20}O_{11}$	0.25	3.35
4.00	593.1512	Positive	Naringin	$C_{27}H_{30}O_{15}$	33.60	4.20
4.41	197.0456	Positive	Hyperoside	$C_9H_{10}O_5$	4.40	3.46
5.26	287.0561	Positive	Ellagic acid	$C_{15}H_{12}O_6$	3.60	4.20
5.26	431.0984	Positive	Quercitrin	$C_{21}H_{20}O_{10}$	7.10	3.78
5.59	317.0303	Positive	Quercetin	$C_{15}H_{10}O_8$	0.50	2.95
5.63	301.0354	Positive	Naringenin	$C_{15}H_{10}O_7$	3.30	4.20
5.65	479.1184	Positive	Salicylic acid	$C_{22}H_{22}O_{12}$	274.00	1.89
6.02	316.2632	Positive	3'-*O*-Methyl quercetin	$C_{16}H_{12}O_7$	13.50	3.58
6.02	284.3110	Positive	Caffeic asit phenethyl ester	$C_{17}H_{16}O_4$	0.10	3.13
6.08	299.0565	Positive	Chrysoeriol	$C_{16}H_{12}O_6$	1.30	2.08
8.17	485.3250	Positive	Gypsogenic acid	$C_{30}H_{46}O_5$	16.50	3.34



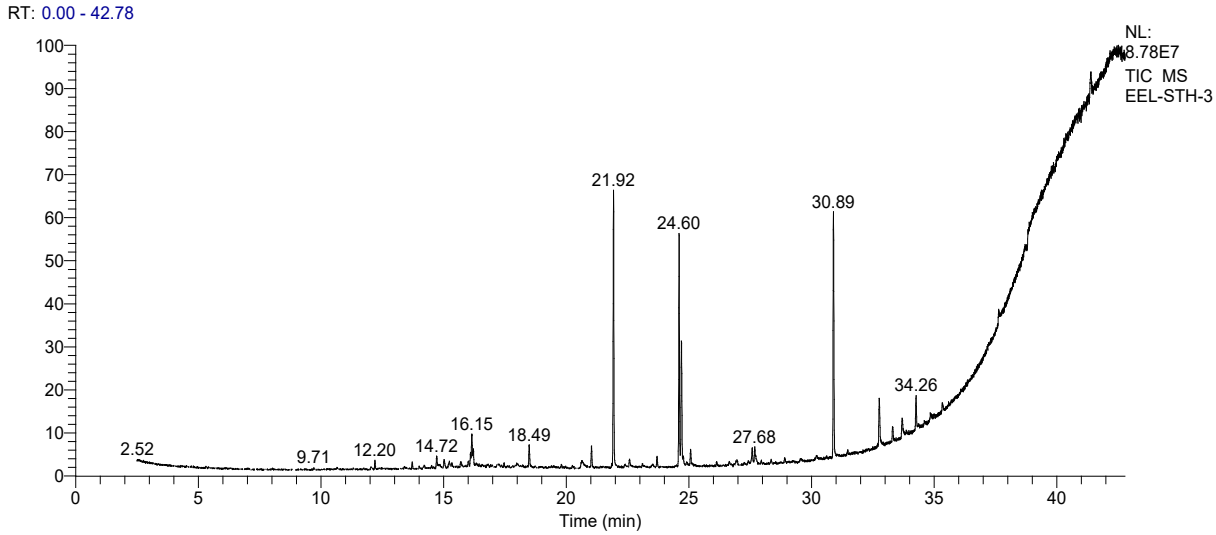
F. LC-HRMS chromatograms of the hydromethanolic extract of *S. tetragona*

G. GC conditions

System: Thermo Scientific Trace 1310 GC	**Oven Temperature Program** Rate: 6.00 Temp 80°C Hold Time: 1.00 min Temp 300°C Hold Time: 5.00 min
Carrier Gas: Helium	
Flow Rate of Carrier Gas: 1 mL/min	
Injector Temperature: 220°C	
Injection Volume: 0.5 µL	
Split Ratio: 50:1	
Column: TG-5MS 0.25µm	

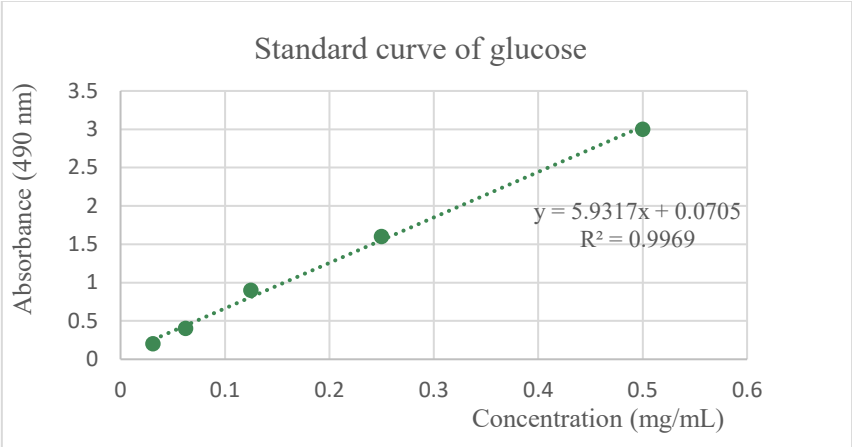
H. MS conditions

System: Thermo Scientific TSQ 9000 MS
Mass Scanning Range: 29-650 m/z
Ion Source Temperature: 280°C
Ionization Mode: (EI)
Definitions: ILMER Library at BVU

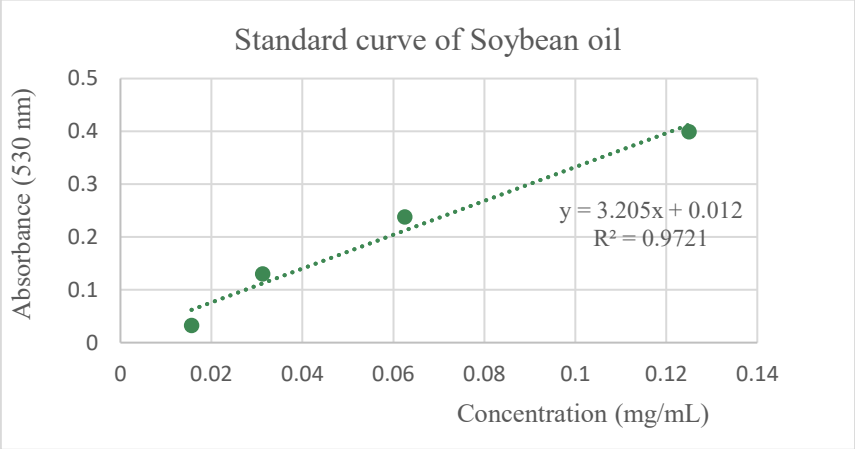


I. GC-MS chromatogram of the *n-hexane fractions* of *S. tetragona.*

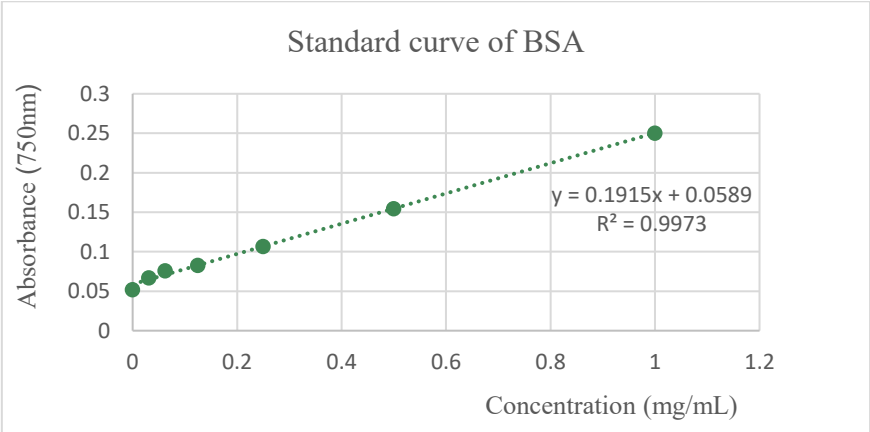
Appendix 03. « Standard curves »



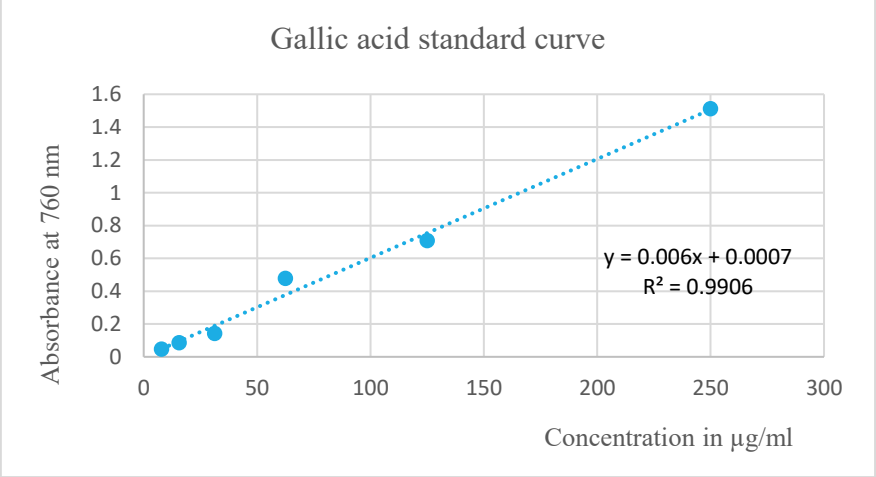
A. Standard curve of glucose for determination of carbohydrate content in *S. tetragona*



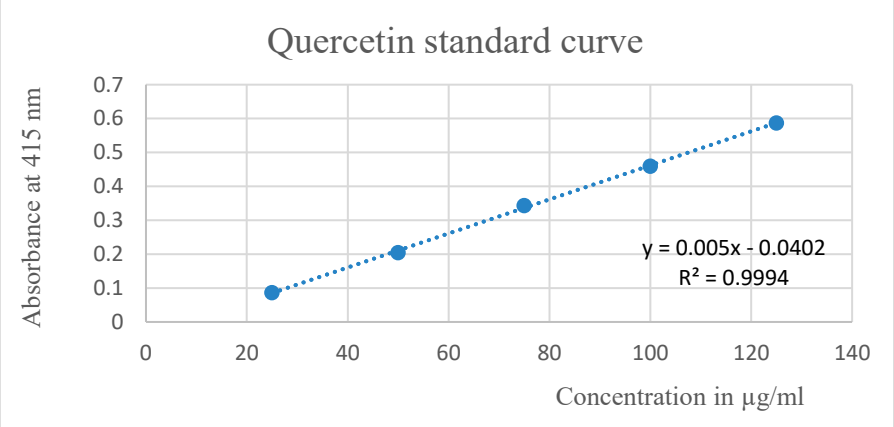
B. Standard curve of Soybean oil for determination of lipid content in *S. tetragona*



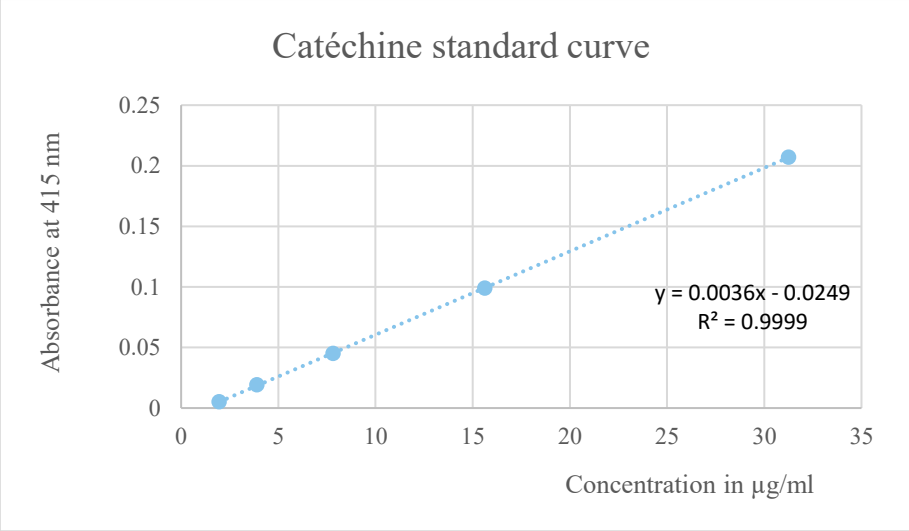
C. Standard curve of BSA for determination of protein content in *S. tetragona*



D. Gallic acid standard curve



E. Quercetin standard curve



F. Catéchine standard curve

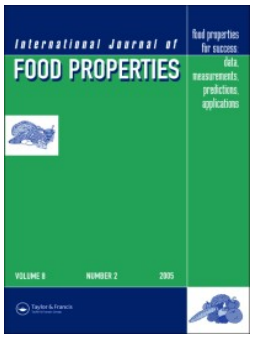
Appendix 04. « Communications and Publications »

A. International Publications

Gheraissa, N., Chemsa, A. E., Elsharkawy, E., & **Cherrada, N.** (2022). Phenolic compound profile, and evaluation of biological properties of *Bassia muricata* (L.) Asch. aerial part. *International Journal of Secondary Metabolite, Vol. 9, No. 3*, 335-347. https://doi.org/10.21448/ijsm.1080537

molecules

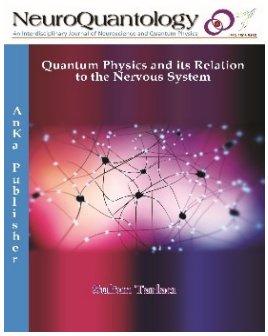
Gheraissa, N., Chemsa, A. E., **Cherrada, N**., Erol, E., Elsharkawy, E. R., Ghemam-Amara, D., ... & AbdelKader, M. S. (2023). Biochemical Profile and In Vitro Therapeutic Properties of Two Euhalophytes, *Halocnemum strobilaceum* Pall. and *Suaeda fruticosa* (L.) Forske., Grown in the Sabkha Ecosystem in the Algerian Sahara. Molecules, 28(8), 3580. https://doi.org/10.3390/molecules28083580



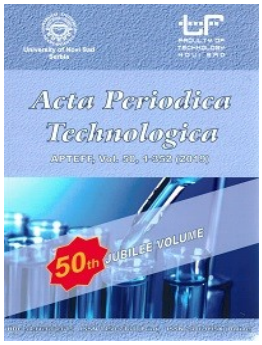
Cherrada, N., Chemsa, A. E., Gheraissa, N., Djilani, G. A., El Manawaty, M. A., Rebiai, A., ... & Awuchi, C. G. (2023). Antioxidant potentials and inhibitory activities of α-amylase, α-glucosidase, and acetylcholinesterase of different fractions from *Salsola tetragona* Delile. International Journal of Food Properties, 26(1), 1787-1796. https://doi.org/10.1080/10942912.2023.2230385

Cherrada, N., Chemsa, A. E., Erol, E., Akyildiz, A. G., Dinc, H. O., Gheraissa, N., ... & Messaoudi, M. (2023). Phytochemical profiling of *Salsola tetragona* Delile by LC-HR/MS and investigation of the antioxidant, anti-inflammatory, cytotoxic, antibacterial and anti-SARS-CoV-2 activities. Saudi Pharmaceutical, 31(9), 101731. https://doi.org/10.1016/j.jsps.2023.101731

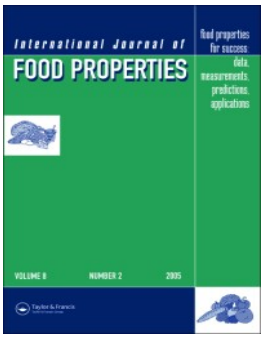
Gheraissa, N., Chemsa, A. E., **Cherrada, N**., Ebru, E., & Elsharkawy, E. R. (2023). *Anabasis oropediorum* Maire. as a health-promoting source: Phytochemical content, in vitro antioxidant, antidiabetic, antibacterial, and anti-inflammatory potential. JOURNAL OF RESEARCH IN PHARMACY, 27(5). https://doi.org/10.29228/jrp.474



Benamor, B., Gheraissa, N., Djellab, K., Haddad, A. A., Retim, L., **Cherrada, N**., Chemsa, A. E., Chala, A. (2023). Exploring the biochemical potential of male date palm (*phoenix dactylifera* L.) leaflets from Biskra region, Algeria: a comprehensive analysis of flavonoids, phenols, proteins, and carbohydrates. NeuroQuantology, 21(7),121-130. https://doi.org/10.48047/nq.2023.21.7.nq23014



Kaddour, A., Chemsa, A., Laouedj, H., Amara, D., Moussaoui, Y., **Cherrada, N**., & Zaater, A. (2023). Biological activities of *Mentha spicata* L. extracts growing in different geographical regions of Algeria. Acta Periodica Technologica (54), 177-186. https://doi.org/10.2298/apt2354177k



Cherrada, N., Elkhalifa Chemsa, A., Gheraissa, N., Zaater, A., Benamor, B., Ghania, A., ... & Teferi Asres, D. (2024). Antidiabetic medicinal plants from the Chenopodiaceae family: a comprehensive overview. International Journal of Food Properties, 27(1), 194-213. https://doi.org/10.1080/10942912.2023.2301576

B. International Scientific Communications

Cherrada, N., Gheraissa, N., Chemsa, A. E., Alia, Z. In-*vitro* sun protection factor evaluation and antioxidant, antibacterial activity of methanol and hexane extracts of *Salsola tetragona* Del. International Conference on the Valorization of Alternative Plants and Degraded and Marginal Lands (VPADM, 2022), 10-11 May, 2022. Echahid Hamma Lakhdar University, El-Oued, Algeria.

Cherrada, N., Chemsa, A. E., Gheraissa, N. In-*vitro* sun protection factor evaluation and antioxidant, antibacterial activity of aqueous and n-hexane fractions extracts of *Salsola tetragona* Del. The First International Congress of Innovations in Chemistry for Therapeutic Aims. 23-24 October, 2022. Larbi Ben M'hidi University, Oum El Bouaghi, Algeria.

Cherrada, N., Chemsa, A. E., Gheraissa, N. Investigating the chemical composition and antioxidant properties of *Centaurea furfuracea* (essential oil and methanol extract). International Webinar on Promotion and Exploitation of Plants of Ecological and Economic Interest. 15-16 March, 2023, Abbes Laghrour University, Khenchela, Algérie.

Cherrada, N., Chouikh, A., Chemsa, A. E., Gheraissa, N. HPLC analysis and antioxidant activity of methanolic extract of *Cistanche tinctoria (*Desf.) Beck. (*Orobanchaceae*). The, First International Seminar on Catalysis, Chemical Engineering & Green Chemistry (CaCEG-2023). 22-23 February, 2023. Echahid Hamma Lakhdar University, El-Oued, Algeria.


People's Democratic Republic of Algeria
Ministry of Higher Education & Scientific Research
University of Echahid Hamma Lakhdar El Oued, Algeria
Faculty of Natural and Life Sciences
1st International Seminar on Valorization of Bioresources in Environment & Health
CERTIFICATE OF PARTICIPATION
This Certifies That
Mr cherrada Nezar
University of El-Oued, Faculty of Natural Science and Life, 39000 El Oued, Algeria
Presented an Oral at the 1st International Seminar on Valorization of Bioresources in Environment & Health (VBEH'23) "Organized on May 10-11, 2023 at the University of Echahid Hamma Lakhdar - El Oued, Algeria.
Entitled "Evaluation of In-Vitro Antioxidant and Antibacterial Activity of Methanol Extract from Anabasis Articulata (Forsk)"
Co-authors: Ahmed Khalifa CHEMSA, Noura GHERAISSA.
President of the Seminar

Cherrada, N., Chemsa, A. E., Gheraissa, N., Ghania, A., Bouras, Y. Evaluation of *In-vitro* antioxidant and antibacterial activity of methanol extract from *Anabasis articulata* (Forsk). 1st International Conference on: Valorization of Bioresources in the Environment and Health (VBEH 23). 10-11, May, 2023. Echahid Hamma Lakhdar University, El-Oued, Algeria.


Democratic People's Republic of Algeria
Ministry of Higher Education and Scientific Research
University of El Oued
Faculty of Natural and Life Sciences
Certificate of participation
We would like to gratefully appreciate to
Cherrada Nezar
Presented a Poster at the "The Second Edition International Pluridisciplinary PhD Meeting (IPPM'23) Artificial Intelligence (AI) Revolution: Challenges, Prospects and Ethical Aspects. Which was held on 11-13, December 2023.
Entitled: Leveraging AI to Unlock the Therapeutic Potential of Medicinal Plants
Co-authors:
Chairman of the conference
Dean of the Faculty

Cherrada, N., Chemsa, A. E., Gheraissa, N. Leveraging AI to Unlock the Therapeutic Potential of Medicinal Plants. The Second Edition International Pluridisciplinary PhD Meeting (IPPM'23) Artificial Intelligence (AI) Revolution: Challenges, Prospects and Ethical Aspects. Which was held on 11-13, December 2023. Echahid Hamma Lakhdar University, El- Oued, Algeria.

C. National Scientific Communications


ATTESTATION DE PARTICIPATION
Le comité scientifique du 1er Webinaire national Natural Products and Bioactive Compounds, an issue of sustainable development atteste que :
CHERRADA Nezar
A participé au Webinaire, le 11 Mars 2023 et a présenté un e-poster intitulé :
HPLC analysis and antioxidant activity of methanolic extract of *Calligonum comosum* L'her. (Polygonaceae)
Co-auteurs : Chemsa A.k, Gheraissa N.
Le Président du Comité Scientifique
La Doyenne de la Faculté SNV/STU

Cherrada, N., Chemsa, A. E., Gheraissa, N. HPLC analysis and antioxidant activity of methanolic extract of *Calligonum comosum* L'her. (Polygonaceae). 1ST Webinaire National of Natural Products and Bioactive Compounds, an Issue of Sustainable Development. 11 March , 2023. Abou Bekr Belkaid University, Tlemcen, Algeria.


1er Séminaire National sur les Substances Bioactives (SBio-2023)
14 Mars 2023
ATTESTATION DE PARTICIPATION

Cherrada, N., Chemsa, A. E., Gheraissa, N., Ghania, A., Meddour, S. HPLC analysis and antioxidant activity of methanolic extract of *Salsola tetragona* Del. National Seminar on Bioactive Substances. 14 March 2023. Ghardaïa University, Algeria.

Attestation de participation

CHERRADA Nezar

HPLC analysis and antioxidant activity of methanolic extract of *Anabasis Articulata* (Forsk.) (Chenopodiaceae)

Co-auteurs : Chemsa AE., Gheraissa N., Ghemam AD., Ghania A.

Cherrada, N., Chemsa, A. E., Gheraissa, N., Gemam Amara, D., Ghania, A. HPLC analysis and antioxidant activity of methanolic extract of *Anabasis articulata* (Forsk) (*Chenopodiaceae*). National Seminar in Toxicology and Phyto- Aromatherapy.02 December, 2022. University of Relizane, Algeria.

Attestation de Participation

Cherrada, N., Chemsa, A. E., Gheraissa, N. Assessment of phytochemicals analysis, antioxidant power, and anti-inflammatory activity of S*alsola tetragona* Del aqueous extract. 1ST National Seminar on Agriculture and Sustainable Development in Semi-Arid Zones (ADDZSA-2022). 15-16, November, 2022 Mohamed Chérif Messaadia University - Souk Ahras, Algeria.

People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Higher School of Saharan Agriculture – El Oued

CERTIFICATE OF PARTICIPATION

The certificate is awarded to

Nezar CHERRADA

For successful participation in the " First National Conference on Saharan Agriculture Challenges and Possibilities " 26 - 27, November 2023 at Higher school of Saharan Agriculture – El Oued.

With a communication entitled " Valorization of Quinoa Cultivation in the Oued Souf Region of Algeria"

Co-author: Ahmed Elkhalifa CHEMSA, Noura GHERAISSA

President of Conference

President of organizing committee

Cherrada, N., Chemsa, A. E., Gheraissa, N. Valorization of Quinoa Cultivation in the Oued Souf Region of Algeria. First National Conference on Saharan Agriculture Challenges and Possibilities" 26-27, November 2023 at Higher School of Saharan Agriculture - El Oued.

Appendix 05. « Patents »

A. An anti-Alzheimer compound extracted from the *Salsola tetragona* plant.

Brevet d'invention Brevet classique DZ2023/000419 received at 26/04/2023 08:30:39 AM by nbouaired.harsi

INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

inapi
Institut National Algérien de la Propriété Industrielle

RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*
Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - **INVENTEUR[S] :** *Nom, Prénom, Adresse*
CHERRADA Nezar, Cité el-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Boite postale No:1045.39001.EL-OUED,Algerie.

[54] - **TITRE DE L'INVENTION** :
Un composé anti-Alzheimer extrait de la planteSalsola tetragona del.

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
230419	26 AVR. 2023	08:30

N° de la demande internationale et date internationale de dépôt

Visa

inapi
Service Dépôt
DZP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le : 22 مارس 2023

Signature et cachet
Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
- ☐ Document de priorité
- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

Les demandes doivent être remises ou adressées par pli postal recommandé avec demande d'avis de réception, à l'Institut National Algérien de la Propriété Industrielle (INAPI) dont les coordonnées sont indiquées ci-dessous.

Le paiement des taxes exigibles peut être effectué soit directement auprès de la caisse de l'INAPI soit par virement bancaire au compte: BEA 12 Avenue AMIROUCHE, Alger· n° 00200012120326418071

Coordonnées de l'INAPI :

Adresse : 42, rue Larbi BEN M'HIDI, 3ème étage, B.P. 403 Alger Gare
Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

B. An anti-inflammatory compound extracted from the plant *Salsola tetragona* del.

INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - DEPOSANT[S] : *Nom, Prénom, [dénomination], et Adresse complète*
Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - INVENTEUR[S] : *Nom, Prénom, Adresse*
CHERRADA Nezar, Cité el-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Boite postale No:1045.39001.EL-OUED,Algerie.

[54] - TITRE DE L'INVENTION :
Un composé anti-inflammatoire extrait de la plante Salsola tetragona del.

[30] – REVENDICATION DE PRIORITE (S)

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
230420	26 AVR. 2023	08:31

N° de la demande internationale et date internationale de dépôt

Visa

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DZP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n°	**du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*	*Date du pouvoir*

Le préposé à la réception	Fait à :	le : 22 مارس 2023
	مديـــر الجامعـــة أ.د عمـــر فرحـــاتي	Signature et cachet Qualité du signataire pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

☐	Copie de la demande internationale	☐	Abrégé descriptif
☐	Mémoire descriptif en langue nationale	☐	Pouvoir
☐	Mémoire descriptif original en langue française	☐	Document de priorité
☐	Mémoire descriptif duplicata en langue française	☐	Cession de priorité
☐	Dessin(s) original (aux) Planche(s)	☐	Titre ou justification du paiement de taxes
☐	Dessin(s) duplicata (aux) Planche(s)		

Les demandes doivent être remises ou adressées par pli postal recommandé avec demande d'avis de réception, à l'Institut National Algérien de la Propriété Industrielle (INAPI) dont les coordonnées sont indiquées ci-dessous.

Le paiement des taxes exigibles peut être effectué soit directement auprès de la caisse de l'INAPI soit par virement bancaire au compte: BEA 12 Avenue AMIROUCHE, Alger· n° 00200012120326418071

Coordonnées de l'INAPI :

Adresse : 42, rue Larbi BEN M'HIDI, 3ème étage, B.P. 403 Alger Gare
Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

C. An anti-diabetic compound extracted from the plant *Salsola tetragona* del.

INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

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Institut National Algérien de la Propriété Industrielle

RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - DEPOSANT[S] : *Nom, Prénom, [dénomination], et Adresse complète*
Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - INVENTEUR[S] : *Nom, Prénom, Adresse*
CHERRADA Nezar, Cité el-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Boite postale No:1045.39001.EL-OUED,Algerie.

[54] - TITRE DE L'INVENTION :
Un composé anti-diabétique extrait de la plante Salsola tetragona del.

[30] – REVENDICATION DE PRIORITE (S)

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
230421	26 AVR. 2023	08:32

N° de la demande internationale et date internationale de dépôt

Visa

inapi
BOUCHEL El Hafsi
Service Dépôt
DTP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le 22 مارس 2023

Signature et cachet

Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
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- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

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Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

D. An anti-breast cancer compound extracted from the plant *Salsola tetragona* del.

Brevet classique Brevet classique P/DZ/2023/000693 received at 04/06/2023 11:34:42 AM by r.boulekfouf:BOULEKFOUF RYAD

المعهد الوطني الجزائري للملكية الصناعية
INSTITUT NATIONAL ALGÉRIEN DE LA PROPRIÉTÉ INDUSTRIELLE

الجمهورية الجزائرية الديمقراطية الشعبية
RÉPUBLIQUE ALGÉRIENNE DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - **INVENTEUR[S]** : *Nom, Prénom, Adresse*

CHERRADA Nezar, Cité El-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Cité El-Nadhour,Boite postale No:1045.39001.EL-OUED,Algerie.
GHEMAM AMARA Djilani, Cité el-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
BOURAS Yacine, Cité El-Izdihar,El- BAYADA39007,EL-OUED,Algerie.
DOUDI Dalal,Cité El-Nadhour,Boite postale No:1045.39001.EL-OUED,Algerie.

[54] - **TITRE DE L'INVENTION** :

Composé anti-cancer du sein extrait de la planteSalsola tetragona del.

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
230693	04 JUIN 2023	11.34

N° de la demande internationale et date internationale de dépôt

Visa

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BOULEKFOUF El Hafsi
Service Dépôt
DZP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le :

Signature et cachet

Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
- ☐ Document de priorité
- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

Les demandes doivent être remises ou adressées par pli postal recommandé avec demande d'avis de réception, à l'Institut National Algérien de la Propriété Industrielle (INAPI) dont les coordonnées sont indiquées ci-dessous.

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Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
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A NE PAS PLIER

* Cocher les cases correspondantes.

E. An anti-inflammatory compound extracted from the *Syzygium aromaticum* plant

المعهد الوطني الجزائري للملكية الصناعية
INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

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الجمهورية الجزائرية الديمقراطية الشعبية
RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - **INVENTEUR[S]** : *Nom, Prénom, Adresse*

BOURAS Yacine, Cité El-Izdihar,El- BAYADA39007,EL-OUED,Algerie.
CHERRADA Nezar, Cité El-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
ZAATER Abdelmalek,Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Cité El-Nadhour,Boite postale No:1045.39001.EL-OUED,Algerie.
CHOUIKH Atef, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHEMAM AMARA Djilani, Cité el-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.

[54] - **TITRE DE L'INVENTION** :

Composé anti-inflammatoire extrait de la plante Syzygium aromaticum

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
230777	12 JUIN 2023	10.47

N° de la demande internationale et date internationale de dépôt

Visa

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DZP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le :

Signature et cachet
Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
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- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

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Coordonnées de l'INAPI :

Adresse : 42, rue Larbi BEN M'HIDI, 3ème étage, B.P. 403 Alger Gare
Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

F. Natural anti-diabetic compound based on *Salsola foetida* del plant extract

المعهد الوطني الجزائري للملكية الصناعية
INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

الجمهورية الجزائرية الديمقراطية الشعبية
RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - **INVENTEUR[S]** : *Nom, Prénom, Adresse*

CHERRADA Nezar, Cité El-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
BOURAS Yacine, Cité El-Izdihar,El- BAYADA39007,EL-OUED,Algerie.
ZAATER Abdelmalek,Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Cité El-Nadhour,Boite postale No:1045.39001.EL-OUED,Algerie.
BENAMOR BILAL,University of Mohamed Khider, BP 145 RP, 07000 Biskra, Algeria.

[54] - **TITRE DE L'INVENTION** :

Naturel composé anti-diabétique à base de extrait de plante de *Salsola foetida* del

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
231123	06 JUIL. 2023	09:43

N° de la demande internationale et date internationale de dépôt

Visa

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BOULEKFOUF El Hafsi
Service Dépôt
DZP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le :

Signature et cachet
Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
- ☐ Document de priorité
- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

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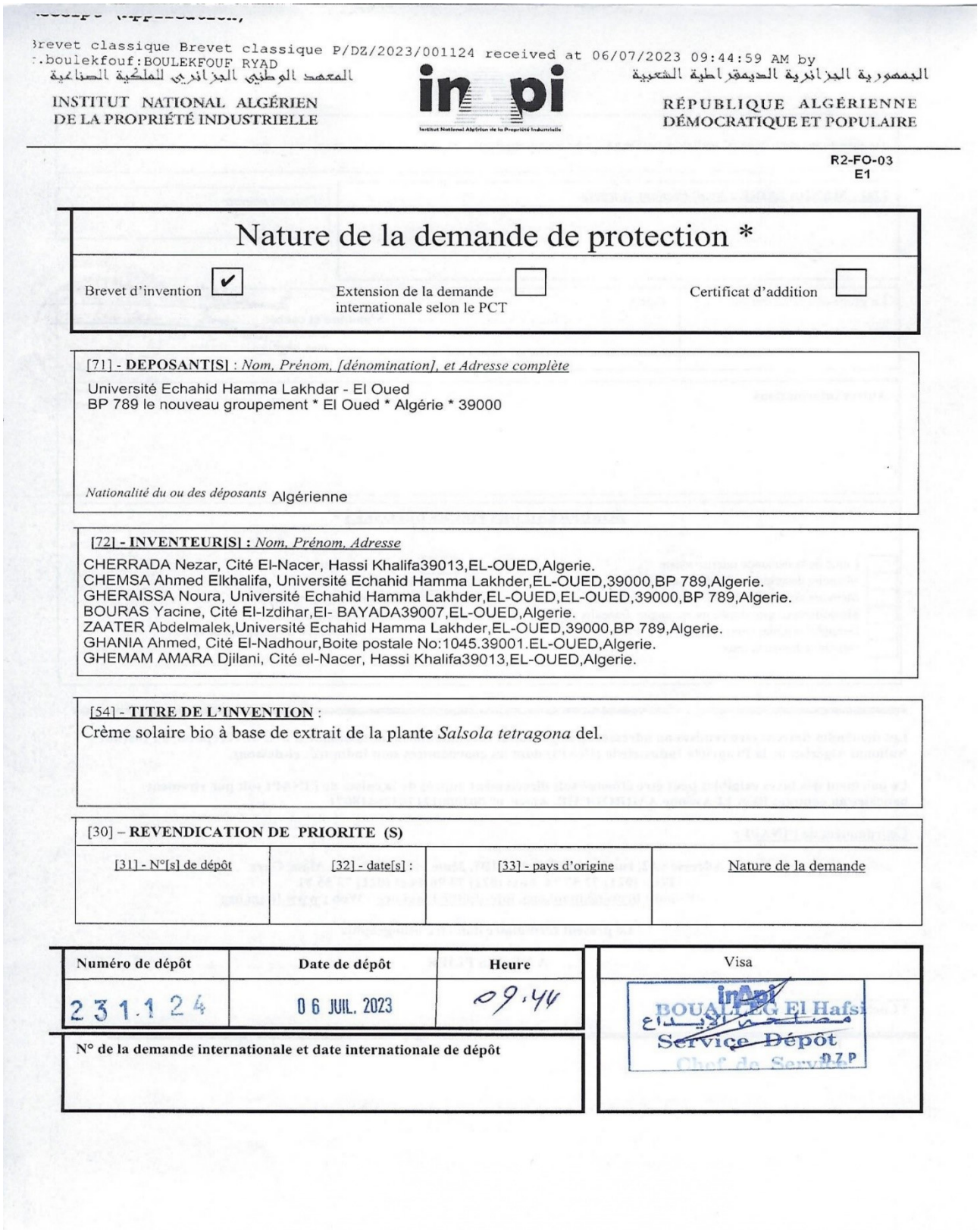
Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

G. Organic sun cream based on an extract of the plant *Salsola tetragona* del



Brevet classique Brevet classique P/DZ/2023/001124 received at 06/07/2023 09:44:59 AM by
.boulekfouf:BOULEKFOUF RYAD

INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

المعهد الوطني الجزائري للملكية الصناعية

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الجمهورية الجزائرية الديمقراطية الشعبية

RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - **INVENTEUR[S] :** *Nom, Prénom, Adresse*

CHERRADA Nezar, Cité El-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
BOURAS Yacine, Cité El-Izdihar,El- BAYADA39007,EL-OUED,Algerie.
ZAATER Abdelmalek,Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Cité El-Nadhour,Boite postale No:1045.39001.EL-OUED,Algerie.
GHEMAM AMARA Djilani, Cité el-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.

[54] - **TITRE DE L'INVENTION** :

Crème solaire bio à base de extrait de la plante *Salsola tetragona* del.

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
231124	06 JUIL. 2023	09,46

N° de la demande internationale et date internationale de dépôt

Visa

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BOUALLEG El Hafsi
Service Dépôt
DZP
Chef de Service

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le :

Signature et cachet
Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
- ☐ Document de priorité
- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

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Coordonnées de l'INAPI :

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Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

H. A natural anti-inflammatory compound extracted from *Seuda fructosa* plant

Brevet classique Brevet classique P/DZ/2023/001126 received at 06/07/2023 09:46:59 AM by
:.boulekfouf:BOULEKFOUF RYAD

المعهد الوطني الجزائري للملكية الصناعية
INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

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الجمهورية الجزائرية الديمقراطية الشعبية
RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ Extension de la demande internationale selon le PCT ☐ Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*

Université Echahid Hamma Lakhdar - El Oued
BP 789 le nouveau groupement * El Oued * Algérie * 39000

Nationalité du ou des déposants Algérienne

[72] - **INVENTEUR[S] :** *Nom, Prénom, Adresse*

CHERRADA Nezar, Cité El-Nacer, Hassi Khalifa39013,EL-OUED,Algerie.
CHEMSA Ahmed Elkhalifa, Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHERAISSA Noura, Université Echahid Hamma Lakhder,EL-OUED,EL-OUED,39000,BP 789,Algerie.
BOURAS Yacine, Cité El-Izdihar,El- BAYADA39007,EL-OUED,Algerie.
ZAATER Abdelmalek,Université Echahid Hamma Lakhder,EL-OUED,39000,BP 789,Algerie.
GHANIA Ahmed, Cité El-Nadhour,Boite postale No:1045.39001.EL-OUED,Algerie.
BENAMOR BILAL,University of Mohamed Khider, BP 145 RP, 07000 Biskra, Algeria.

[54] - **TITRE DE L'INVENTION** :

Naturel composé anti-inflammatoire extrait de plante de *Sueada fructosa*

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
231126	06 JUIL. 2023	09:46

N° de la demande internationale et date internationale de dépôt

Visa

inapi
Service Dépôt
DZP

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : le :

Signature et cachet
Qualité du signataire
pour les personnes morales

Autres informations

BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
- ☐ Document de priorité
- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

Les demandes doivent être remises ou adressées par pli postal recommandé avec demande d'avis de réception, à l'Institut National Algérien de la Propriété Industrielle (INAPI) dont les coordonnées sont indiquées ci-dessous.

Le paiement des taxes exigibles peut être effectué soit directement auprès de la caisse de l'INAPI soit par virement bancaire au compte: BEA 12 Avenue AMIROUCHE, Alger· n° 00200012120326418071

Coordonnées de l'INAPI :

Adresse : 42, rue Larbi BEN M'HIDI, 3ème étage, B.P. 403 Alger Gare
Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.org, info-dpitt@inapi.org - Web : www.inapi.org

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

Appendix 06. « Card of Identification of *S. tetragona* plant growing in Oued souf region »

A. Morphological description

The *Salsola tetragona* Del., called belbel in southern Algeria, is a Saharan plant belonging to the genus *Salsola*, which comprises 120 species and is included in the *Amaranthaceae* family.

S. tetragona. are perennial shrubs (ATIA et al., 2014) 20-50 cm tall, sometimes reaching 1m, grayish-yellow, highly branched from the base, with thick, subtetragonal, fragile branchlets and turions that easily disarticulate.

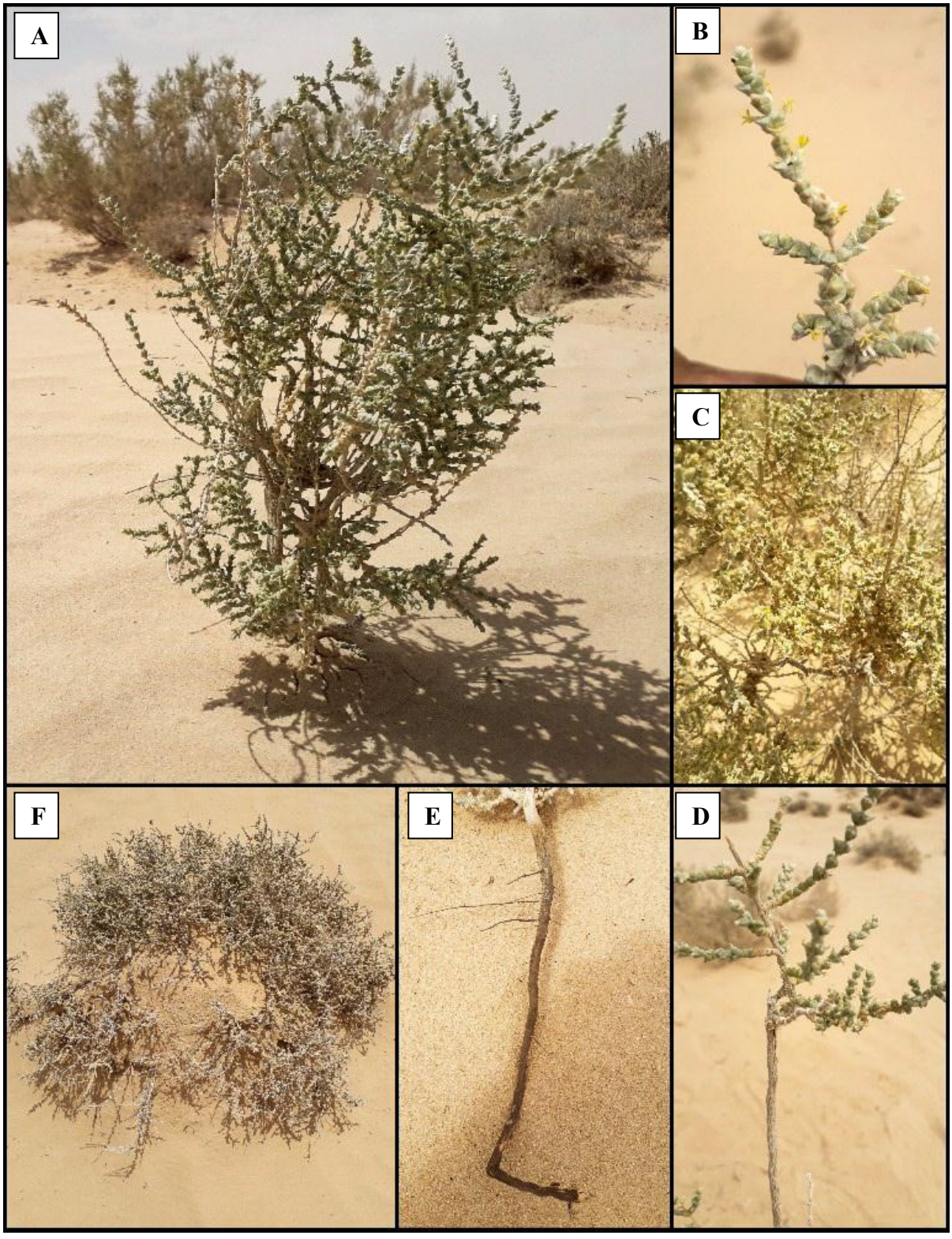
Branchlets are entirely covered by imbricated and strongly keeled leaves, with short internodes (1-2, rarely 3-5 mm), cylindrical (without leaves), covered with short (0.15-0.3 mm), dense, spreading hairs, much longer under the leaf insertion. Leaves are opposite, squamiform, highly imbricated, fleshy, obtuse, villous, 2.25 mm long, 2.5-3 mm wide.

Flowers are solitary, hermaphroditic, with 2 bracteoles in the axils of the leaves, with a perianth of 5 sepals, 5 stamens with linear, flattened filaments, inserted on a poorly developed disc and lacking staminodes; anthers are exserted, yellow, sagittate, apiculate at the apex by the extension of the connective.

Ovary is ovoid, attenuated into a short and thick style, with 2 subulate stigmas, papillose on the inner face. Fruiting perianth is accrescent-indurated with sepals fused at the base, conical, connivent at the apex, bearing an unequal transverse wing on the back (sometimes absent in the inner sepals), purple, membranous, forming a crown 6-10 mm in diameter.

The fruit is an achene included in the fruiting perianth, with a membranous pericarp, barely papery at the top, separable.

Seed is subglobose, depressed, horizontal, with a smooth, membranous tegument, 2.5 mm in diameter, exalbuminous, non-rostrate, with a green, snail-coiled embryo. *S. tetragona*. flowers from May to April and after rains in the central and western Sahara.



Different parts of the aerial part of the *S. tetragona* plant. A: aerial part complete flowering time. **B**: flowers. **C**: intertwined branched stems. **D**: A single plant stem showing the gray color of the stem. **E**: is the root of the plant. **F**: Picture of a plant in winter (Cherrada, 2024) .

B. Phylogenetic Classification

Family: Amaranthaceae

Subfamily: Salsoloideae

Tribe: Salsoleae

Genus: *Salsola*

Species: *Salsola tetragona* Delile.

Synonym(s) Homotypic: *Caroxylon tetragonum* (Delile) Moq. *Halogeton tetragonus* (Delile) Moq. *Salsola diplantha* Botsch .*Salsola pachoi* Volkens & Asch.

Vernacular names: Belbal

C. Geographical distribution

In Algeria, this specie grows in the Northern and Western Sahara. It is found in salty area, sabkhas, and slightly salty steppes.

D. Characteristics of the Oued souf region

The Oued Souf region is located in the southeastern part of Algeria, in the eastern part of the Sahara Desert. Its territory extends between latitudes 31° - 34° north and longitudes 6° - 8° east, with an area of 82,800 km2. The northern boundary of Oued Souf ends at the Salty Lines region (Chott Melghigh and Chott Merouane), while the southern boundary is marked by the red sand dunes of Ouargla Province. The eastern boundary reaches the salty areas of Tunisia (Chott el Djerid and Chott el Gharsa), and the western boundary ends at the flat lands of the Oued Righ and Touggourt regions.

The region is characterized by the appearance of sand dunes that cover three-quarters of the total area, interspersed with depressions and valleys. Oued Souf is also considered the lowest point in the Great Eastern Erg. The Oued Souf region is dominated by a dry climate characterized by high temperatures in the summer and low temperatures in the winter. The humidity and rainfall in Souf are low, not exceeding 100 mm per year. One of the most important characteristics of rainfall in the region is its irregular distribution throughout the year.