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بسم الله الرحمن الرحيم

I dedicate this

research work

To

My Beloved Father And Loving Mother For Their Love And

Support Most Precious For Me In This World, My Family

All My Teachers

All My Friends And Colleagues

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Résumé

La présente étude vise à caractériser le profil chimique des composés phénoliques et certaines activités biologiques *d'Astragalus gombiformis* Pomel de la famille Fabaceae.

L'étude phytochimique des extraits *d'A. gombiformis* Pomel par LC-ESI-MS a révélé la présence de dix sept composés phenoliques dont le Cirsiliol ou Diméthoxyflavone (DMF) est représentée le majore métabolite dans cette plante.

L'activité antioxydante a été évaluée par des tests chimiques (DPPH, ABTS, CUPRAC, GOR et β-carotène). Les résultats obtenus ont montré que:

- La fraction butanolique des fleurs *d'A. gombiformis* a montré la meilleure activité anti radicalaire par les tests : DPPH, ABTS.
- Les extraits des fleurs et de la plante entière ont montré la meilleure activité par le test CUPRAC.
- L'extrait de la plante entière a montré la meilleure activité par le test du β-carotène et une faible activité avec GOR.

Tous les extraits *d'A. gombiformis* sont totalement inefficaces en tant qu'inhibiteurs de la tyrosinase par contre l'extrait de plante entière a montré une efficacité importante contre l'enzyme urease.

L'activité antidiabétique a été évaluée par la méthode d'inhibition de l'α-amylase et α-glucosidase dont les extraits de la plante entière, fleurs et feuilles ont montré une activité antidiabétique la plus importante.

Les extraits butanoliques de la plante entière, les fleurs et les feuilles ont un effet positif vis-à-vis toutes les souches bactériennes testées

Les résultats ont montré qu'*A. gombiformis* a une forte capacité d'absorption des rayonnements UV et une activité anti-inflammatoire significative.

De plus, *A. gombiformis* a une cytotoxicité efficace contre *Artemia salina* mais un faible effet hémolytique contre les érythrocytes humains.

Les différents tests pharmacologiques des extraits *d'A. gombiformis* réalisés *in vitro* montrent qu'ils possèdent des propriétés biologiques importantes: Antioxydante, Antidiabétique, Anti-Alzheimer, Photoprotectrice, Anti-inflammatoire et Antibactérienne.

Mots clés : *Astragalus gombiformis* Pomel, Composés bioactive, LC-ESI-MS, Activités biologiques.

Abstract

The Present study aims to characterize the chemical profile of phenolic compounds and some biological activities of *Astragalus gombiformis* Pomel of Fabaceae family

The phytochemical study of *A. gombiformis* extracts by LC-ESI-MS revealed the presence of 17 phenolic compounds of which Cirsiliol or Diméthoxyflavone (DMF) was detected as the main compounds in this plant.

The Antioxidant activity was evaluated through chemical tests: DPPH, ABTS, CUPRAC, GOR and β-carotene. The results obtained showed that:

- The butanolic fraction of the flowers showed the best antiradical activity with the DPPH and ABTS tests.
- The flowers and whole plant extracts showed the best activity with the CUPRAC test.
- The whole plant extract showed the best activity with the β-carotene test and Low activity with GOR.

All *A. gombiformis* extracts are completely ineffective as tyrosine's inhibitors. However, the whole plant extract is the most effective against the urease enzyme.

The antidiabetic activity was evaluated by the inhibition method of *α-amylase* and α-*glucosidase.* The results showed that the whole plant extract, flowers, and leaves have an important anti-diabetic activity.

The butanolic extracts of the whole plant, the flowers, and the leaves have a positive effect on all the bacterial strains tested

The results showed that *A. gombiformis* had a strong absorption capacity of UV and a significant anti-inflammatory activity.

In addition, *A. gombiformis* has effective cytotoxicity against *Artemia* salina but a weak hemolytic effect against human erythrocytes.

The various pharmacological tests carried out *in vitro* show that *A. gombiformis* have important properties: Antioxidant, Antidiabetic, Anti-Alzheimer, Photoprotective, Anti-inflammatory, and Antibacterial.

Keywords: *Astragalus gombiformis* Pomel, Bioactive compounds, LC-ESI-MS, Biological activities.

الملخص

تهدف الدراسة الحالية إلى توصيف المظهر الكيميائية للمركبات الفينولية وبعض الأنشطة البيولوجية لنبات

Astragalus gombiformis Pomel .

كشفت الدراسة الكيميائية النباتية بواسطة LC-ESI-MS لمستخلصات *A. gombiformis* عن وجود 17 مركبا فينوليا حيث يمثل Cirsiliol ou Diméthoxyflavone (DMF) المركب الرئيسي لهذا النبات.

تم تقييم النشاط المضاد للأكسدة من خلال الاختبارات الكيميائية DPPH و ABTS و CUPRAC و GOR وبيتا كاروتين. أظهرت النتائج التي تم الحصول عليها ما يلي:

- ✓ أظهرالجزء البوتانولي لأزهار *A. gombiformis* أفضل نشاط مضاد للجذور الحرة مع اختبار DPPH و ABTS
- ✓ أظهرت مستخلصات الأزهار والنبات كله أفضل نشاط مع اختبار CUPRAC.
- ✓ أظهر المستخلص النبات بأكمله أفضل نشاطا في اختبار β-Carotene ونشاط منخفض مع GOR.

جميع مستخلصات *A. gombiformis* غير فعالة تمامًا كمثبطات لإنزيم التيروزيناز بينما المستخلص النباتي بأكمله كان الأكثر فعالية ضد إنزيم اليورياز.

تم تقييم النشاط المضاد لمرض السكر من خلال طريقة تثبيط α-amylase و α-glucosidase. أظهرت النتائج أن مستخلص النبات الكلي متبوع بمستخلص الأزهار والأوراق لهم نشاط مضاد لمرض السكر.

أظهرت النتائج أن المستخلصات البوتانولية للنبات كله الزهور، والأوراق لها تأثير إيجابي على جميع السلالات البكتيرية المختبرة.

أظهرت النتائج أن نبات *A. gombiformis* له قدرة امتصاص قوية للأشعة الفوق البنفسجية بالاظافة الي نشاط كبير مضاد للالتهابات.

بالإضافة إلى ذلك، لدى *A. gombiformis* سمية خلوية قوية ضد *Artemia salina* وتأثير انحلالي ضعيف ضد كريات الدم الحمراء البشرية.

تبين الاختبارات الفرمكولوجية المختلفة التي أجريت في المختبر للمستخلصات نبات *A. gombiformis* أن له خصائص هامة: مضادات الأكسدة ، مضادات السكر ، مضادة للزهايمر ، الحماية من الضوء ، مضادة للالتهابات ومضاد للبكتيريا

الكلمات المفتاحية: *Astragalus gombiformis* Pomel ، المركبات النشطة، LC-ESI-MS ، الأنشطة البيولوجية

Abbreviations list

- ***µg*:** Microgram.
- **A0.5**: Concentration indicating 0.50 of absorbance.
- **ABTS:** 2,2`-azinobis- (3-ethylbenzothiazoline-6-sulfonic).
- **AChE :** Acetylcholinesterase.
- **AD** : Alzheimer's disease.
- **ATCC**: American Type Culture Collection.
- **BChE** : Butyrylcholinesterase.
- **BHA** : Butyl-hydroxy-anisole.
- **BHT** : Butyl-hydroxy-toluene.
- **CFU**: Colony-forming unit
- **CUPRAC**: Cupric Reducing Antioxidant Capacity.
- **DMSO**: Dimethylsulfoxide.
- **DPPH**: 1,1-Diphenyl-2-PicrylHydrazyl.
- **DTNB**: 5, 5'-dithiobis nitrobenzoic acid.
- **EC 50:** Half maximal effective concentration
- **EDTA** : Ethylenediaminetetraacetic acid.
- **FCR:** The Folin-Ciocalteu reagent
- **GNT** : Gentamicin.
- **H_2O_2** : Hydrogen peroxide.
- **HPLC**: High Performance Liquid Chromatography.
- **IC50** : inhibition concentration of 50%.
- **LC / MS**: Liquid chromatography coupled with mass spectrometry.
- **MH** : Mueller Hinton.
- **MIC** : Minimum Inhibitory Concentration.
- **NA** : Not active.
- **NCCLS**: National Committee for Clinical Laboratory Standard.
- **$O2^{\cdot-}$** : Superoxide radicals.
- **OH** : Hydroxyl.
- **PDA**: Potato dextrose agar.
- **R**: Resistance.
- **$RO^{\bullet}$:** Peroxyls.
- **ROS** : Reactive oxygen species.
- **Rt** : Retention time.

- **SPF** : Sun protection factor.
- **UV**: Ultra-Violet.
- **λmax**: maximum wavelength.

List of tables

List of Figures

Contents

General Introduction

"Every new beginning comes from some other beginning's end"

- Seneca-

Natural medicines derived from medicinal plants have sparked increased attention in recent years as a source of bioactive chemicals treating various human diseases, this interest is due to apprehension about the side effects of synthetic molecules **(Gulçin, 2020).** The Algerian natural surface is characterized by its diversity: Mediterranean, saharan, and paleo-tropical **(Hallimi, 2004)** and contains more than 3000 species belonging to several botanical families. These species are mostly spontaneous with a high number of endemic species **(Ozenda, 1997).**

The Algerian sahara is the world's largest desert covering nearly eight million km^2 and characterized by an ecosystem marked by harsh and restrictive edapho-climatic conditions. This has made the Saharan flora in demand by researchers due to its adaptation to the dry climate and slightly salty soil, which stimulate plants to produce more bioactive compounds compared with the other flora **(Trabut and Mares, 1906).**

The study of medicinal plants reveals locally important species that are often useful for discovering new bioactive products **(Tiwari and Rana, 2015; Nieto, 2020)**. In line with this, several studies have been carried out on the characteristics of several species of *Astragalus*, the largest genus of the Fabaceae family that includes more than 3000 species and represents one of the most important legumes widely used in the food and pharmaceutical industries due to its strong antioxidant capacity, which is attributed to the presence of many bioactive secondary metabolites **(Heywood, 1978).** This genus is distributed in Mediterranean climatic regions in Europe and North Africa **(Davis, 1982),** where fifteen species have been found in the Sahara desert of Algeria, as well as ten species endemic to Morocco and Tunisia **(Mahmoudia et al., 2021; Ozenda, 1991).**

Legumes, including their perennial species, are an excellent source of many essential nutrients, including minerals, vitamins, antioxidants, fibers, and phytochemicals **(Muzquiz et al., 2012).** The composition of nutritive and bioactive compounds of Fabaceae has been investigated for functional properties, and this bean family is reported to possess many health benefits **(Messina, 1999).**

Various species of *Astragalus* are used in traditional and modern medicine due to their biological effects, such as antioxidant, antibacterial, and antiviral **(Rios, 1997).** In Turkey, for instance, the roots of *Astragalus* species are widely used as a remedy for leukemia and wound healing purposes **(Yesilada et al., 2005).** However, in China, *A. mongholicus* Bunge and *A.*

membranaceus Bunge are among the most popular medicinal plants commonly used as an adjunct in cancer chemotherapy **(Lei et al., 2003; Yin et al., 2006).**

Several products of *Astragalus*, such as gum tragacanth, are used in the preparation of pharmaceuticals and as thickening agents in food products **(Zarre, 2000).** Many bioactive chemicals from this genus are useful in the treatment of cancer cells, **(Somer and Calı¸skan, 2007)** such as saponins isolated from *A. corniculatus* M. Bieb, which exhibited a strong antineoplastic effect against myeloid tumors in hamsters **(Krasteva et al., 2004).**

Several *Astragalus* species are widespread in North Africa, including *Astragalus gombiformis* Pomel, which grows in arid regions. This species is traditionally used in cases of snake and scorpion bites, probably due to the presence of active products that act against scorpion envenomation **(El Rhaffari and Zaid, 2002).** This species also contains several pharmacologically active compounds, such as phenolics and polysaccharides with immunostimulatory effects **(Teyeb et al., 2011).**

With multiple biological activities, *Astragalus* species contain natural antioxidants compounds attracts the attention of several research teams for its role against numerous skin illness. The skin is the most regenerative organ in the body, representing a barrier with important roles, such as the regulation of water balance, thermogenesis, and permeability; hence, it is considered a natural defense mechanism against microbial infections **(Mancini and Lawley, 2001; Afaq, 2011).** However, this protective barrier can be continuously damaged by several environmental factors and biological insults. For example, excessive exposure to solar radiation without protection allows UV radiation to penetrate the skin, in particular, UV-A and UV-B, which stimulate the generation of reactive oxygen species (ROS) such as (O_2), (OH), and (H_2O_2) **(Pillai et al., 2005).**

Excessive ROS production may contribute to the ineffectiveness of natural antioxidant systems by disrupting cell homeostasis, leading to oxidative stress, and damaging proteins, lipids, and nucleic acids **(Mahjoub and Masrour-Roudsari, 2012).** It is widely accepted that oxidative stress is a major factor responsible for the initiation or progression of several illnesses, such as skin elasticity, wrinkles, alterations in elastic fibers, and collagen color.

Our skin serves as a vital barrier against various aggressions. As a result, it has become a more popular topic, hyperpigmentation and its treatment are the most researched skin

disorders in dermo-cosmetics. These hyperpigmentations are caused by a malfunction of melanins synthesis, which are the pigments responsible for skin color. As a result, depigmenting products that block melanogenesis are used to treat these hyperpigmentations. On this purpose, the development of these products necessitates a thorough knowledge of melanin formation and the regulatory mechanisms of melanogenesis.

Several methods can be used to stop melanogenesis: blocking melanin production at a given stage, blocking melanin transfer, or destroying melanocytes. For our part, we have selected to use the first method, by developing inhibitors of tyrosinase, an enzyme involved in the melanins synthesis.

These inhibitors are polyphenols belonging to secondary metabolites present in medicinal plants. They can help to reduce the risk of cardiovascular disease, diabetes mellitus, oxidative hemolysis, inflammatory and neurodegenerative diseases **(Rinnerthaler et al., 2015).**

The use of anti-inflammatory drugs to reduce pain during inflammation presents risks of gastrointestinal and cardiovascular toxicity. For this reason, it is necessary to discover other anti-inflammatory drugs with fewer side effects to be used particularly in the treatment of chronic inflammation. So the search for new therapeutic agents from natural products gives a great chance to discover an effective medication against many diseases, by the direct therapeutic effect, after a semi-synthetic modification or by a new synthesis of a molecular model from natural products **(Cragg et al., 1997).**

Epidemiological studies show that the consumption of legumes plants is associated with the reduction of chronic diseases **(Dastmalchi et al., 2008),** one of the main public health problems is Alzheimer's disease (AD), which affects 24 million people around the world (100,000 cases in Algeria), 20% of whom are over 85 years old. Every seven seconds, a new case is diagnosed; this frequency is on increasing more and more due to the aging of the population.

Currently, there is no cure for AD. Only the strategies to alleviate the symptoms of AD are proposed by the increase the level of acetylcholine in the brain using the inhibitor of acetylcholinesterase, the enzyme responsible for the degradation of this neurotransmitter.

Several studies investigate the search for new molecules from plants implicated in diabetic treatment. The prevalence of diabetes was estimated at 2.8% in 2000 and may reach 4.4% in 2030. In which it is higher in women compared to men **(Wild et al., 2004).**

A therapeutic approach to decrease hyperglycemia is to reduce the digestion and absorption of ingested carbohydrates **(Khacheba, 2014)** by using inhibitors of enzymes implicated in the lysis of carbohydrates to simple glucose (such as α-amylase and/or α-glucosidase) causing a reduction of glucose concentration and, consequently, dulling of postprandial plasma glucose **(Rhabasa-Lhoret and Chiasson, 2004).**

Several types of research have recently concentrated on the extraction of natural antioxidants from medicinal plants at a low price that can replace synthetic compounds such BHA (Butyl-hydroxy-anisole) and BHT (Butyl-hydroxy-toluene) which could be carcinogenic and even toxic to the consumer. Besides antioxidant activity, plant extracts have been found to have antibacterial effects towards various pathogenic bacteria **(Karou et al., 2005)** by blocking enzymes involved in bacterial persistence resulting in many illnesses such as urease.

On the other hand, urease, an interesting enzyme responsible for urea hydrolysis to produce the ammonia and plays an important role in the persistence of *helicobacter pylori* (H. pylori) in the acidic environment of the stomach, resulting in gastrointestinal illnesses such as gastritis, duodenal ulcers, and gastric cancer **(Devesa et al., 1998; Howson et al., 1986).** It has previously been demonstrated that urease risks the bacteria's existence **(Michetti, 1998).**

Moreover, other diseases caused by urease activity include urinary stones, pyelonephritis (inflammation of the kidneys) **(Upadhyay, 2012),** Despite comprehensive researches performed on inhibitors of urease and its mechanisms actions, only a few of them are promising.

According to the researches, the majority of medical treatments and antibiotics used to treat the mentioned diseases not only has negative consequences, but also cause bacteria to be resistant to them. Furthermore, the use of several chemicals, particularly synthetic ones, for controlling and regulating urease function has been prohibited due to their toxicity and low stability in the natural environment. As a result, the scientific researchers looking for natural compounds used to develop new drugs with higher efficiency, stability, and less toxicity.

In the framework of valorization of the Algerian natural surface and to develop new products, we are interested in this work by *Astragalus gombiformis* Pomel that belongs to the *leguminosae* plant obtained from arid zoon (El Oued- Algeria).In order to have an overall picture of identification, quantification and distribution of phenolic compounds present in different areal organs from *A. gombiformis* and further elucidate the similarities and differences in chemical constituent between the whole plant and each organ separately using liquid chromatography mass spectrometryanalysis (LC-ESI-MS). Moreover, this work aimed also to determine the antioxidant potential, α-amylase and α-glycosidase inhibitory assay, cholinesterase inhibitory activity, anti-inflammatory effect, antibacterial and photoprotective activities of *A. gombiformis* extracts.

Furthermore, the biological and chemical research of medicinal plants is focused on the extraction, quantification identification for the discovery of new natural bioactive products. Despite the active potential of these plants, they may be toxic to human health by the lowest concentration. For this purpose, the present study also aimed to:

- Evaluate the toxicity effect against brine shrimp (*Artemia salina*) and erythrocyte cells from healthy blood to assess the possibility of its use as an alternative remedy, more secure and an effective source for human health.

First Part Species under investigation

"Absence of evidence is not evidence of absence"

- Sir Martin Rees

Chapter 1 Fabaceae family

1. Introduction

For several years, the use of medicinal plants or herbal preparations has enjoyed increasing success as a promising source of natural substances, innovative and original compositions. These natural products isolated from crude extracts of plants have been used for a long time in the pharmaceutical industries. Thus, it is estimated that 80% of the world's population depends mainly on traditional medicine using medicinal plants due to their primary key role in health care **(Who, 2012).**

The selection of plant material is the first step in the phytochemical study. The following criteria can guide a phytochemist when harvesting plants:

- Traditional uses of plants by local people;
- Observation of plants in their natural environment;
- Botanical and chemotaxonomic aspects;

Before undertaking the actual phytochemical study, the researcher must still learn about any previous scientific work (phytochemical, ethnobotanical studies, etc).

2. Fabaceae Family

The Fabacea*e* family (Eg: Legumes) is one of the most important in the plant kingdom **(Ozenda, 1991),** commonly called Fabales, with around 630 genera and 18,000 species, widespread throughout the world **(Judd et al., 2002).** In Algeria, 53 genus and 339 species are recorded **(Quezel and Santa, 1963).** Specialists agree to classify this superfamily into three groups, some researchers make all of these three groups into a family named "*Leguminosae* or *Fabale*" and divide it into three sub-families "*Cesalpinioideae*, *Mimosoideae* and *Papilionoideae* = *Faboideae*" **(Spichiger et al., 2004; Marouf and Reynaud, 2007).** The first two, *Caesalpiniaceae* and *Mimosaceae* mainly include tropical and subtropical bushes and trees such as: *Mimosa, Acacia.* The third, the *Papilionaceae* has globally herbaceous, cosmopolitan plants, it is particularly well represented in temperate, arid and semi-arid zones such as clovers, peas, beans **(Ozenda, 1991).**

2.1.Importance of Fabaceae

The major's spontaneous or cultivated Fabaceae, for their properties, have a great economic and medical interest. They provide many food substances, medicinal and pharmacological natural molecules, a super-quality of woods for construction, etc.

On all over the agricultural world, the Herbaceous Fabaceae grown in temperate regions have an important role as a green manure and soil insurance to reduce the erosion **(Chen et al., 1995).** On the industrial level, Fabaceae plants provide us with wood used in cabinetmaking.

Furthermore, the *Indigofera tinctoria* plant produces the indigo (a blue coloring matter) campeche wood. *Hematoxylon campechianum*, grown in India, provides a red coloring matter. From a pharmaceutical standpoint, the Fabaceae family gives a high number of species appearing in the pharmacopoeias and provides several products that can be used in therapeutic **(Pant et al., 1968; Bézanger-beauquesne et al., 1986).**

3. *Astragalus* genus

Among the 730 genus of the Fabaceae family, we find in Algeria about 53 genus and 339 species **(Quezel, 1991).** In our work, we interested about the *Astragalus* genus which is considered as the largest flowering genus with about 3000 species, 10 of theme are endemic in Morocco, Tunisia, and Algeria **(Ozenda, 1991).** According to the bibliography, this genus shows a high concentration of saponins, and particular the cycloartane and olenane skeletons. Phenolic compounds and polysaccharides are also mentioned **(Yalçın et al., 2012).**

3.1.The use of *Astragalus* genus in traditional medicine

Species of *Astragalus* genus are used in traditional medicine around the world as herbal remedies for stomach ulcers, cough, chronic bronchitis, hypertension, gynecological disorders, diabetes, stings and poisonous scorpions **(Bellakhdar, 1997).**

The fresh roots of *Astragalus lusitanicus* Lam are used as a poultice in diseases of the knee and elbow (swelling, arthritis, and dislocation) **(Bellakhdar, 1997).**

In Algeria, the *Astragalus gyzensis* Bunge species is used to relieve snakebites **(Kherraze et al., 2010).** While the decoction of the leaves and roots of *Astragalus tenuifolius*

desf in Morocco is used against fatigue and helminthiasis **(El Rhaffari and Zaid, 2002).**

Moreover, *Astragalus membranous* is a traditional Chinese herb well known for the treatment of neurodegenerative diseases, it is among the most popular tonic herbs **(Iserin, 2001).**

3.2.Geographical distribution of *Astragalus* genus

The *Astragalus* genus is the most abundant of the Fabaceae family with around 2,500 species and with more than 250 taxonomic sections worldwide **(Maassoumi, 1998; Wojciechowski et al., 1999).** This genus is widely distributed throughout temperate and arid regions of the world and mainly localized in Asia (1500 species), North America (500 species), South America (150 species), and Europe (120 species) **(Podlech, 2008).** In the countries of the mediterranean basin, 500 species have been described, of which about fifty in North Africa (**Podlech, 1986; Raynaud, 1982).**

However, the center of origin and biodiversity of the *Astragalus* genus is Eurasia, particularly the mountainous parts of southwestern and south-central Asia **(Lock and Schrire, 2005; Podlech, 1986).**

The *Astragalus* species growing in North Africa are Saharan mediterranean or Arab plants. They are represented by more than 50 species delimited in several sections, 15 are found in the Sahara of Algeria **(Ozenda, 1991).**

In Algeria, the genus *Astragalus* is distributed in the pre-Saharan zone and associated with desertification in arid zones due to overgrazing **(Hirche et al., 2010).**

Chapter 2 *Astragalus gombiformis* Pomel.

1. Introduction

A. gombiformis Pomel is locally named Tafilalet or Foulet El Ibel, is grown in the arid and semi-arid regions of south Algeria such as Biskra, El Oued, Djelfa and Boussaâda. This species contains several of pharmacologically active components, such as phenolics and polysaccharides with hepatoprotective, immunostimulatory and antiviral effects **(Ríos and Waterman, 1997).**

2. Botanical description of *A. gombiformis*

The *A. gombiformis* plant **(Figure 1)** is caracterised by:

a. Stems and leaves are branch out in all directions **(Figure 2).**

Figure 1: *A. gombiformis* plant.

Figure 2: Stems and leaves of *A. gombiformis* plant.

a. Yellow Flowers **(Figure 3).**

Figure 3: Flowers of *A. gombiformis* plant.

a. Fruits (Pods with Seeds) covered with short woolly hairs (**Figure 4 and 5).**

Figure 4: Pods of *A. gombiformis* plant.

Figure 5: Seeds of *A. gombiformis* plant.

3. Phytochemistry and pharmacological properties of *A. gombiformis* specie

All plant parts of *A. gombiformis* have interesting phenol flavonoids, alkaloids, and proanthocyanidols which contribute to the plant's rich chemical composition. Tannins were found in all organs except the roots. Saponosides were present only in stems and leaves. These properties depend also on other factors such as environmental conditions, especially light conditions, and phenological stage **(Ksouri et al., 2008)**.

According to **Teyeb et al. (2011),** the GC/MS analysis of *A. gombiformis* ether extract has detected the presence of twenty-seven volatile compounds. These compounds are mainly alkanes and fatty acids. For the leaves, 9-octadecenoic acid was detected as the major compound with percentages exceeding 22 %.

In seeds, the most abundant molecule is the 9, 12, 15-octadecatrienoic acid, representing 50.58 %. The butylated hydroxytoluene (BHT) was detected in all organs and represents more than 10 % in fruits, stems, and roots. N-Hexadecanoic acid is present in all investigated organs, in particular in stems, with amounts of 31.05 %.

For the pharmacological properties, this species possesses interesting antioxidant and antibacterial activities. *A. gombiformis* seems to have an antibacterial activity potentially useful for the development of new antibiotics (**Teyeb et al., 2012).**

Second part Experimental part

"Every experiment proves something. If it doesn't prove what you wanted it to prove, it proves something else"

- Prof. Anon

Chapter 1 Material and Methods

1. The main objective and the experimental realization place of the study

This work aims to investigate the identification, quantification, and distribution of phenolic compounds present in different areal organs of *A. gombiformis* and further elucidate the similarities and differences in chemical constituents between the whole plant and each organ separately using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS) analysis.

Moreover, this work aimed also to determine the antioxidant potential, α-amylase and α-glycosidase inhibitory assay, cholinesterase inhibitory activities, anti-inflammatory effect, photoprotective effect, antibacterial activities, and the cytotoxicity *of A. gombiformis* extracts.

The maceration was realized in the laboratory of bioactive molecules and applications, Larbi Tebessi University, Tebessa. The extraction and biological activities were investigated at biochemistry laboratory in biotechnology research center (C.R.B.T), Constantine, and liquid chromatography-electrospray ionization–tandem mass spectrometry (LC–ESI–MS) analysis was performed at drylands and oases cropping laboratory institute of arid regions, médenine, Tunisia.

2. Reagents and Standards

2.1. Chemicals and reagents for extraction and spectrophotometric determinations of biochemical activities

The solvents utilized were obtained from PROLAB, MERK EUROLAB. Folin-Ciocalteu, sodium carbonate (Na_2CO_3), aluminum nitrate ($Al(NO_3)$), potassium acetate (CH_3CO_2K), sulfuric acid (H_2SO_4), acetic acid ($C_2H_4O_2$), dimethyl sulfoxide (DMSO), vanillin, and quillaja saponaria of highest purity (99.0%) were purchased from Sigma-Aldrich Chemie (St. Louis, MO, USA) and Fluka Chemie GmbH (Buchs, Switzerland). α-glucosidase (Saccharomyces cerevisiae), α-amylase (procaine pancreas) and 3,5, di-nitro salicylic acid (DNS) were purchased from Sigma-Aldrich, Bangalore. P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate ($Na_2\ CO_3$), sodium dihydrogen phosphate, and di-sodium hydrogen phosphate were purchased from Hi-Media, Mumbai.

2.2. Reagents for LC-ESI-MS Analysis

The following standards were obtained from Sigma Chemical Co (St. Louis, MO, USA) at the highest purity available: quinic acid, gallic acid, protocatchuic acid, catechin (+), caffeic acid, syringic acid, 1,3-di-*O*-caffeoyquinic acid, epicatechin, p-coumaric acid, rutin, *trans*-ferulic acid, hyperoside (quercetin-3-*O* galactoside), luteolin-7- *O*-glucoside, 3,4-di-*O*-caffeoyquinic acid, naringin, rosmarinic acid, 4,5-di-*O*-caffeoyquinic acid, quercetin (quercetin-3-*O*-rhamonoside), apigenin-7-*O*-glucoside, *O*-coumaric acid, salvianolic acid, kaempferol, quercetin, *trans* cinnamic, silymarin, naringenin, apigenin, luteolin, cirsiliol, cirsilineol, acacetin.

3. Plant Materiel

3.1.Collection of plant material

A. gombiformis plants were selected basis on an intensive review of ethno-pharmacological and chemical information from the world. The aerial parts were collected from the Saharan region (EL Oued -Algeria) in April 2019 at the end of the flowering phase and the beginning of the fruiting phase (pods formation).

After drying away from direct sunlight, the different organs of the aerial parts (stems, flowers, leaves, pods, and seeds) were separated and cleaned, air-dried, grounded, and stored in paper bags until use.

3.2.Geographical location of the plant in Algeria

The vegetal substrate (***Astragalus gombiformis* Pomel**) selected for this study is an herbaceous plant growing in Saharan rangelands especially EL Oued region (The Nakhla commune) **(Figure 6).** This place is located in the southeast of Algeria (Latitude: 33.3683, Longitude: 6.8674, 33°- 22′ 6″ North, 6°- 52′ 3″ East. The climate in this region is dry, with a three-month rainy season. The average annual precipitation is 75mm and typically falls between november and december (weak, irregular and vary from year to year). The average temperature varies between 11°C in January and 32.5°C in July (July). The majority of the soils in this area are arid, brown, and saline **(Khezzani and Bouchemal, 2016).**

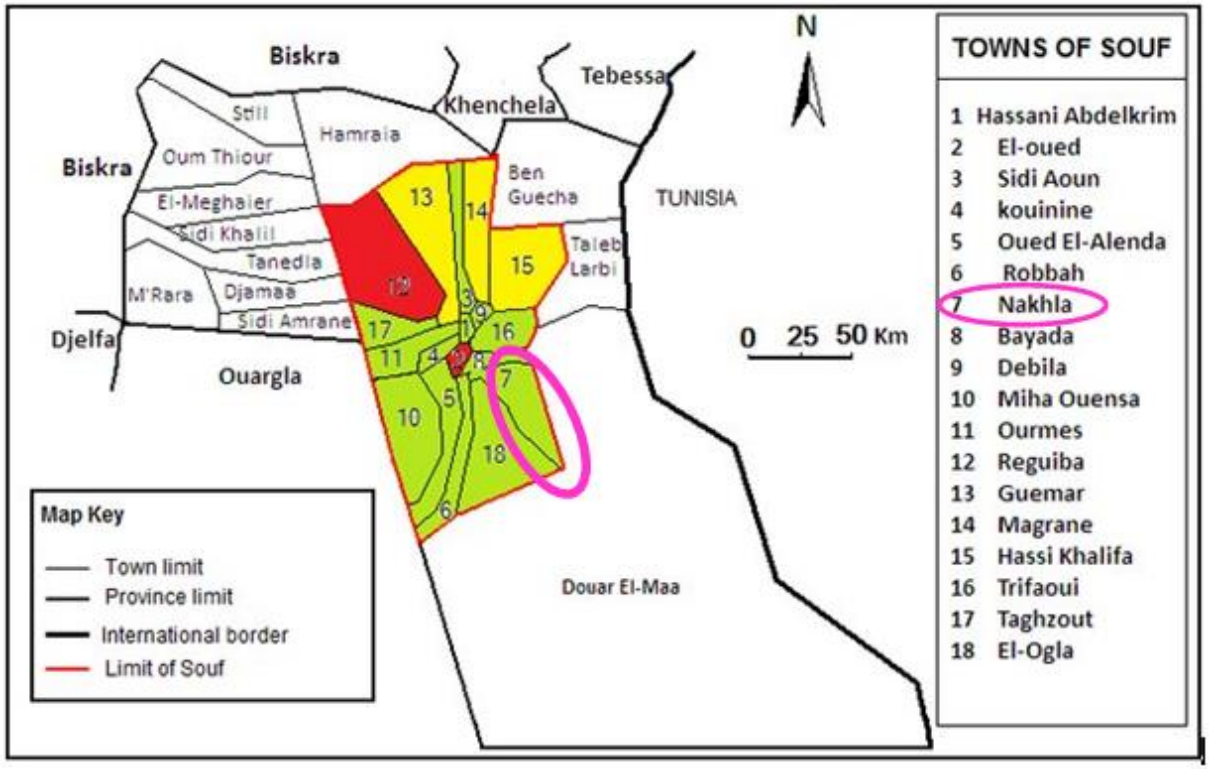


Figure 6: Geographical location of the collection area of *A. gombiformis* (Nakhla-wilaya of Oued Souf) **(Khezzani and Bouchemal, 2016).**

4. Maceration and extraction

The extraction was realized according to **Bensouici et al. (2019)** method **(Figure 7).** The whole plant and each organ separate of *A. gombiformis* (stems, flowers, leaves, pods and seeds**)** were extracted with ethanol-water (70:30 v/v). After concentration under reduced pressure, the residue was suspended in water and extracted successively with petroleum ether and butanol respectively. The butanolic fractions were the objective of our study. The chlorophyll was removed using activated charcoal and at vacuum filtration system **(Tzima et al., 2020).**

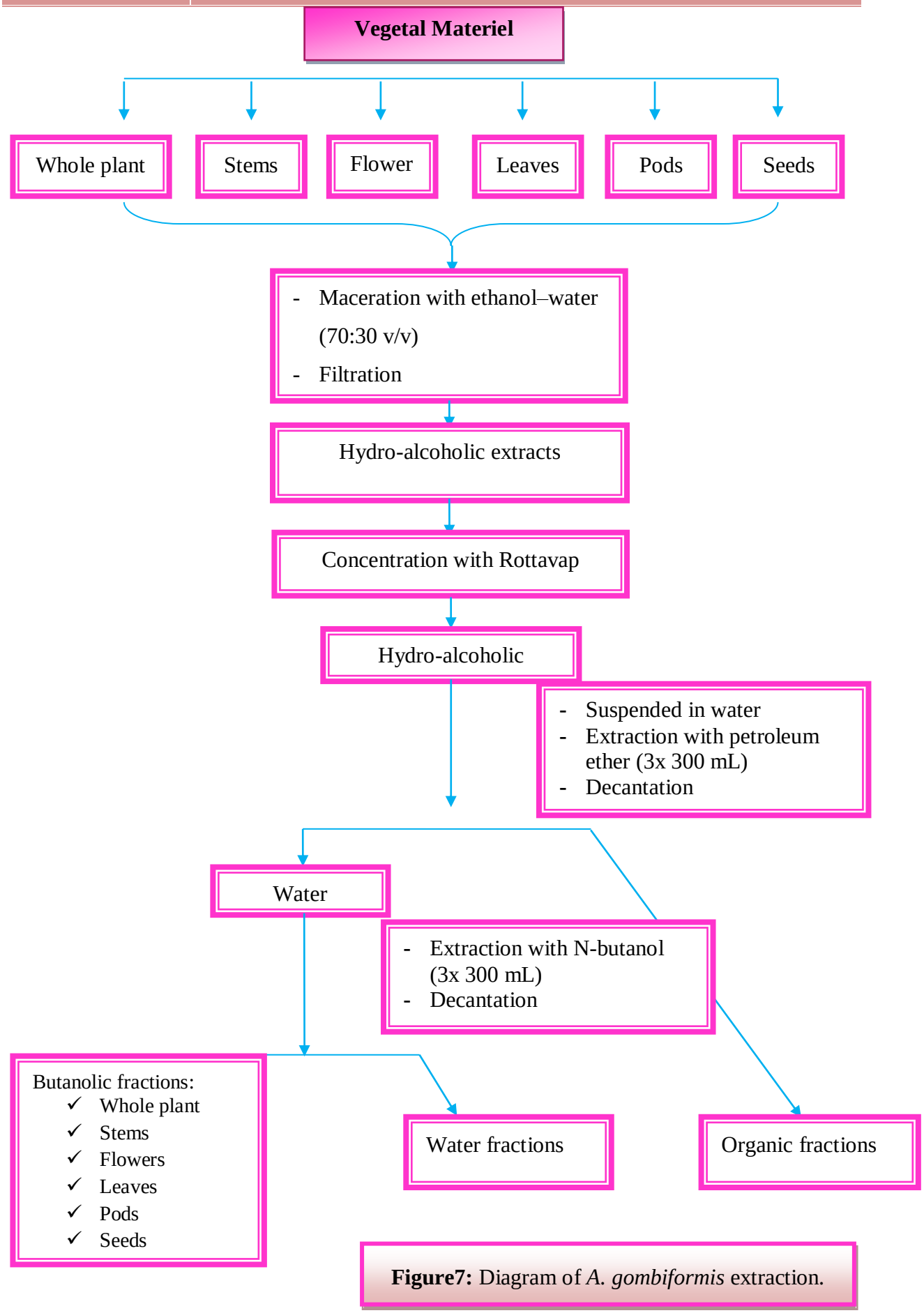


Figure7: Diagram of *A. gombiformis* extraction.

5. The quantitative and qualitative study of *A. gombiformis* extracts

5.1.Phytochemical screening

Phytochemical screening is the principal means to demonstrate the presence of chemical groups present in a given drug and particularly plants. This preliminary test is based on qualitative analysis, such as the production of insoluble complexes by precipitation processes or the development of color complexes by coloring reactions **(Mujeeb et al., 2014).**

5.1.1.Alkaloids

Precipitation reactions using Dragendorff's reagent are applied to highlight the presence of alkaloids in different parts of plants. 50 mL of H_2SO_4 (1/10) dilute in distilled water was added to 10 g of dry vegetable powder. After 24h of maceration, few drops of dragendorff reagent are added to 1mL of the filtrate. The orange precipitate indicates the presence of alkaloids **(Harborne, 2005).**

5.1.2.Tannins

Tannins were detected by adding a few drops of iron chloride ($FeCl_3$, 2%) to 5 mL of each sample. According to **Trease and Evans (1987),** the presence of tannins was detected by the production of a dark blue-colored precipitate.

5.1.3.Flavonoids

1 mL of the each extract was mixed with a solution of NaOH. The mixture obtained was left for 3 min. Intense yellow coloring implies the presence of flavonoids.

Adding a few drops of a dilute acid solution allows the color to persist **(Karumi et al., 2004).**

5.1.4.Saponins

The presence of saponins was determined using the method outlined by **Karumi et al. (2004)**. A combination comprising 5 mL of plant extracts in 10 mL distilled water was vigorously shaken for 2 min. The presence of saponins was revealed by the development of foam.

5.2.Determination of total bioactive compounds

5.2.1.Total phenolic content (TPC)

The total phenolic content of the butanolic fractions was quantified spectrophotometrically using the modified Folin–Ciocalteu technique **(Le et al., 2007).**

Principle

The Folin-Ciocalteu reagent (FCR) is a combination of phosphotungstic and phosphomolybdic acids that is reduced during the oxidation of phenols. The blue coloration produced is proportional to the polyphenol content and has a maximum absorption of around 750 -765 nm.

Method

20 μL of the extract was mixed with 100 μL Folin–Ciocalteu reagent (diluted ten-fold) and 75 μL (75 g/L) sodium carbonate. After incubation of 2h in darkness at room temperature, the absorbance was measured at 740 nm in the microplate reader.

The result was expressed as micrograms of gallic acid (standard) equivalents per milligrams of extract (μg GAE/mg) **(Müller et al., 2010).**

5.2.2.Total flavonoids content (TFC)

Topçu et al. (2007) method was used to determine total flavonoids content.

Principle

Flavonoids form a yellow complexes by metals chelating (iron and aluminum), and lose electrons to unite with two oxygen atoms of the phenolic molecule acting as an electron donor.

Method

50 μL of the extract were mixed to10 μL of 10% aluminum nitrate, 10 μL of 1M potassium acetate, and 130 μL of methanol. After 40 min incubation at room temperature, the absorbance was measured spectrophotometrically at 415 nm.

Quercetin was used as reference (standard) and the result was expressed as microgram quercetin equivalents per milligram of extract (*µg* QE/mg) (**Haşim et al., 2017).**

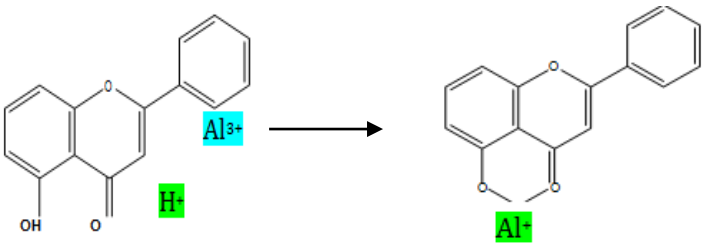


Figure 8: Mechanism of the interaction of aluminum chloride with flavonoids **(Topçu et al., 2007).**

5.2.3.Total saponins (TS)

The vanillin-sulfuric acid technique of **Shiau et al. (2009)** was used to assess the total saponins content. Vanillin (8%, w/v) and sulfurique acid (72%, w/v) were added to the extract. The absorbance was measured at 538 nm after 10 min of incubation at 60 °C.

Quillaja saponins (*Quillaja Saponaria*) is used as a reference standard **(Kamyab et al., 2020)** and the total saponin content is expressed in quillaja saponins equivalents (µg QSE/ mg).

5.3.Method of phenolic compounds quantification by Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry (LC–ESI–MS) Analysis

The phenolic profile was determined via LC–ESI–MS analysis using a Shimadzu UFLC XR system (Kyoto, Japan), equipped with SIL-20AXR auto-sampler, CTO-20 AC column oven, LC-20ADXR binary pump, and quadripole 2020 detector system. For analysis, an Aquasil C18 column (Thermo Electron, Dreieich, Germany) (150mm×3 mm, 3µm) preceded by an Aquasil C18 guard column (10mm×3mm, 3 µm, Thermo Electron) was used.

The mobile phase was composed of A (0.2% acetic acid in 5% MeOH and 95% H_2O, *v*/*v*) and B (0.2% acetic acid in 50% CAN and 50% H_2O, *v*/*v*) with a linear gradient elution: 0–45 min, 10–100% B; 45–55 min, 100% B. Re-equilibrate duration was 5 min between individual runs.

The injection volume was 20 µL, the flow rate of the mobile phase was 0.4 mL/min, and the temperature of the column was maintained at 40 °C. Spectra were monitored in selected-ion-monitoring (SIM) mode and processed using Shimadzu LabSolutions LC–MS software.

The mass spectrometer was operated in negative ion mode with a capillary voltage of 3.5 V, a nebulizing gas flow of 1.5 L/min, a dry gas flow rate of 12 L/min, a dissolving line (DL) temperature of 250 °C, a block source temperature of 400 °C, a voltage detector of 1.2 V, and the full scan spectra from 50 to 2000 m/z.

Figure 9: LC–ESI–MS technique device.

6. Evaluation of biological activities of *A. gombiformis* extract

6.1.Determination of antioxidant activity

6.1.1.DPPH free radical-scavenging assay

The activity of DPPH was measured according to the method described by **Blois (1958).**

Principle

The reduction of purple DPPH (2,2-diphenyl-1-picrylhydrazyl) to 2,2-diphenyl-picrylhydrazine yellow is the principle of this technique. DPPH absorbs at 517 nm. However, its absorption is minimized under the reducing effect of an antioxidant.

Method

Briefly, a 0.4 mM of DPPH solution was prepared in methanol, 160 µL of this solution was added to 40 µL of sample diluted in methanol at different concentrations. After 30 min of incubation in the dark, the absorbance was measured at 517 nm.

The results were given as 50% inhibition concentration (IC50) and compared with BHA, BHT, α-Tocopherol, Ascorbic acid, and Tannic acid **(Benouchenne et al., 2020).** The low absorbance value indicates a higher free radical scavenging activity.

The following equation was used to calculate the capacity to trap the DPPH radical.

$$Inhibition\ \% = \frac{A_{White-}\ A_{extract}}{A_{white}} \times 100$$

$A_{White=}$ the absorbance of the reaction containing only the reagents .
$A_{extract=}$ the absorbance of the reaction containing the reagents and the extract.

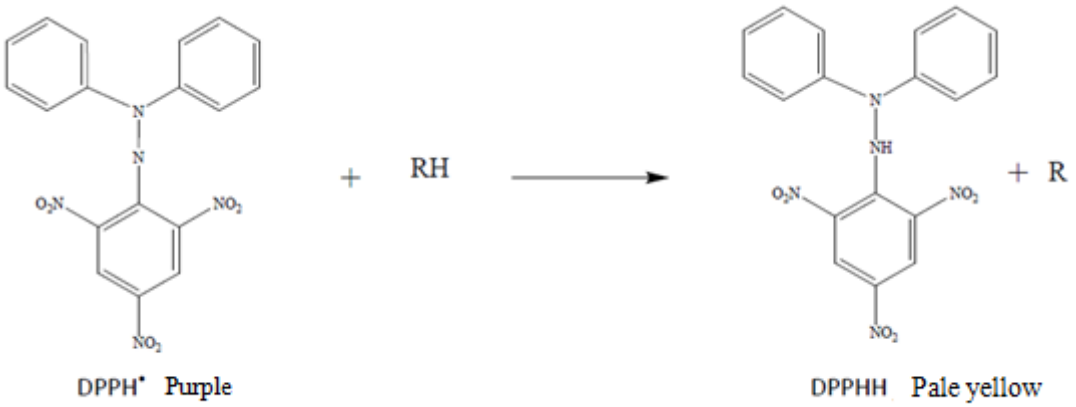


Figure 10: Transformation of the DPPH• radical into DPPHH **(Parejo et al., 2003).**

6.1.2.ABTS cation radical assay

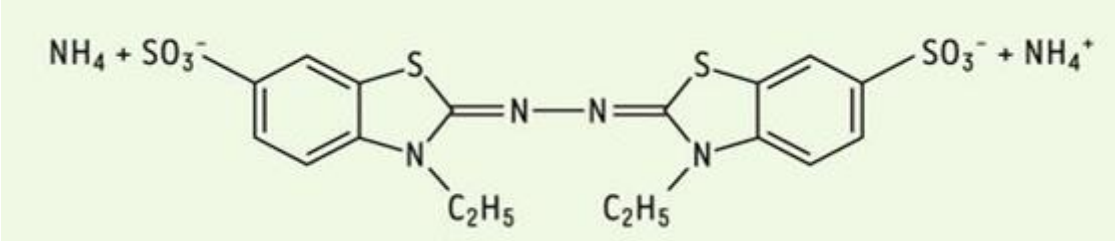
The trapping activity of $ABTS^{\bullet+}$ was measured spectrophotometrically using the technique of **Re et al. (1999)** with minor modifications. 7 mM ABTS in water and 2.45 mM potassium persulfate were used to prepare $ABTS^{\bullet+}$ which was then stored in the dark at room temperature for 12 hours.

The oxidation of ABTS begins almost immediately, although absorption is not maximal until 6 hours later. The cation radical is stable at this form for more than 2 days in the dark at room temperature. Before the use, $ABTS^{\bullet+}$ solution was diluted with ethanol until an absorbance of 0.708 ± 0.025 at 734 nm. Then, 160 µL of the $ABTS^{\bullet+}$ solution was added to 40 µL of the extract solution dissolved in ethanol at different concentrations.

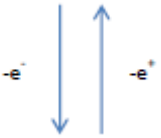
After 10 min, the absorbance was measured at 734 nm using a 96 well microplate reader. Percentages of inhibition of each concentration were calculated relative to a white absorbance of ethanol. The following equation was used to calculate $ABTS^{\bullet+}$ trapping.

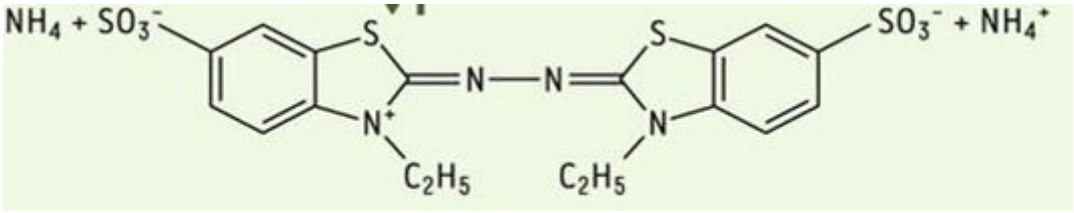
$$Inhibition\ \% = \frac{A_{White-}\ A_{extract}}{A_{white}} \times 100$$

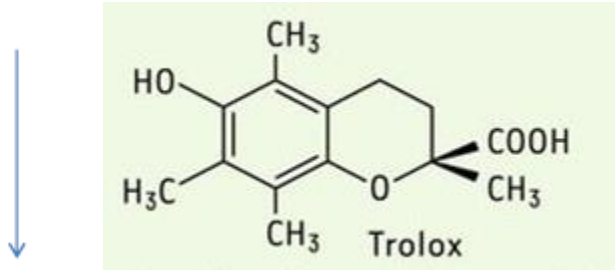
$A_{White=}$ the absorbance of the ethanol
$A_{extract=}$ the absorbance of the reaction containing the reagents and the extract.



ABTS: 2,2'-Azino bis- (3-ethylbenzothiazolin-6-sulfonic acid) ammonium salt







(or antioxidant to be tested, donor of H •)

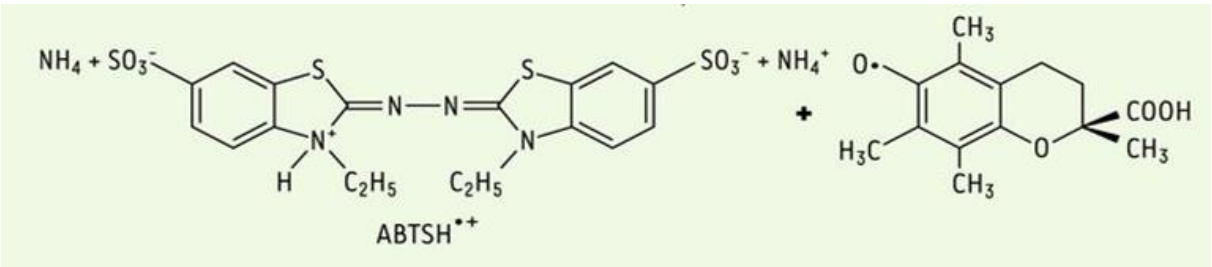


Figure 11: Formation and trapping of the ABTS$^{\bullet+}$ radical by an H$^{\bullet}$ donor antioxidant (**Re et al.,1999)**

6.1.3.Copper Reduction Antioxidant Capacity Test (CUPRAC)

The reduction of copper was determined by the CUPRAC method described by **Apak et al. (2004).**

Principle

The CUPRAC (cupric ion reducing antioxidant capacity) technique is based on measuring the decreased absorbance of the Neocuproene (NC), copper (Cu^{+2}) Nc_2-Cu^{+2} complex.

The copper-neocuproene complex is reduced in the presence of an antioxidant, this reaction measured spectrophotometrically at 450 nm **(Apak et al., 2004).**

Method

The solution was prepared by mixing 50 µL of Cu (II) (10 mM), 50 µL of neocuprine (7.5 mM), and 60 µL of NH_4Ac buffer solution (1 M, pH = 7.0). Different concentrations of extracts were added to the initial mixture to obtain the final volume of 200 µL in each well of the microplate.

After 1h, the absorbance was measured at 450 nm. The reducing capacity of the extracts was compared to those of α-tocopherol and BHT. The results were calculated as A0.5 (μg / mL) corresponding to the concentration indicating 0.50 absorbance.

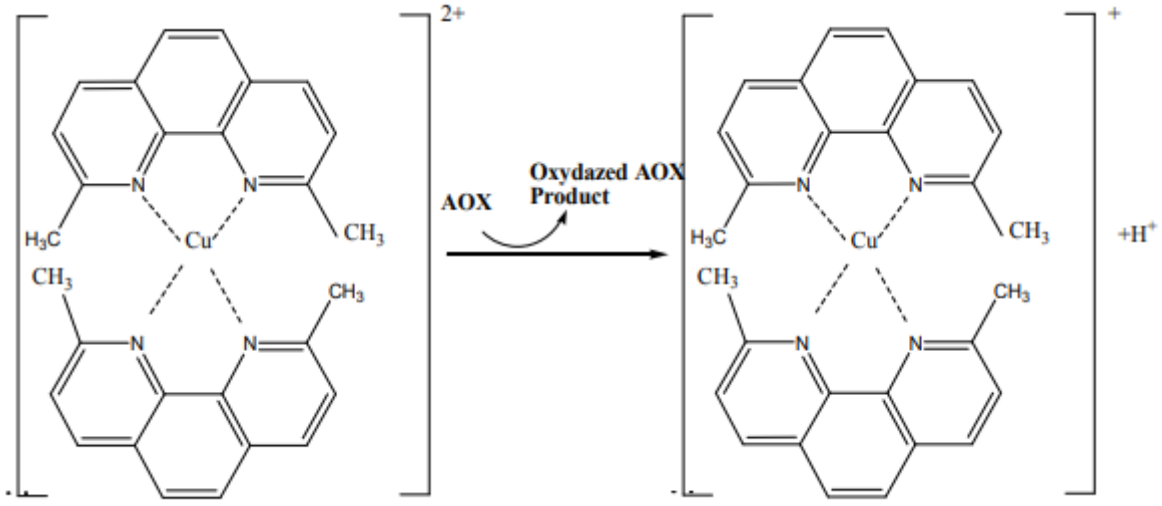


Figure 12: Reduction of the chromogenic complex of Cu^{+2}-Nc **(Apak et al., 2004).**

6.1.4.GOR scavenging assay

The Galvinoxyl radical (GOR) scavenging assay activity was determined according to **Shi et al. (2001)** method.

Principle

Galvinoxyl is a free radical, stable, or hydrogen acceptor with a dark yellow color and high absorbance that is used as a probe to study free radical reactions and as an inhibitor of free radical polymerization.

This radical loses the native color cause of its interaction with antioxidant substances, which transfer electrons or protons to it. The reduced form of galvinoxyl gives the solution a light yellow color with low absorbance. The color change is measured at 428 nm.

Method

160 µL of 0.1 mM methanolic solution of galvinoxyl was mixed with 40 µL of different concentrations of *A. gombiformis* extracts. After 120 min of incubation in dark at room temperature, the absorbance was measured at 428 nm. Galvinoxyl solution in methanol was used as a control. BHA and BHT were used as standards .The following equation was used to calculate the scavenging of galvinoxyl radical:

$$Inhibition\ \% = \frac{A_{control-}\ A_{extract}}{A_{control}} \times 100$$

6.1.5.B-carotene bleaching activity

The β-carotene bleaching activity of *A. gombiformis* extracts was evaluated using the β-carotene-linoleic acid model system **(Marco, 1968).**

Method

0.5 mg of β-carotene dissolved in 1 mL of chloroform was added to 25 µL of linoleic acid and 200 mg of Tween 40 to obtain an emulsifying mixture. After evaporating the chloroform, 100 mL of distilled water saturated with oxygen was added with strong agitation. The absorbance of the β-carotene solution should be between 0.8 and 0.9 nm.

A volume of 160 µL of this prepared solution was added to 40 µL of extract at the different concentrations. Absorbance was measured at 470 nm using a microplate reader. BHA and α-tocopherol were used as standards.

6.2.Antidiabetic activities

The enzyme inhibitors of carbohydrate digesting play an important role in regulating hyperglycemia by inhibiting glucose absorption in the intestine **(Wu et al., 2012; Kazeem et al., 2013; Poongunran et al., 2015; Sulistiyan et al., 2016).** As a result, the main objective of this work was to investigate the in vitro inhibitory effect of *A. gombiformis* extracts against α-amylase and α-glucosidase**.**

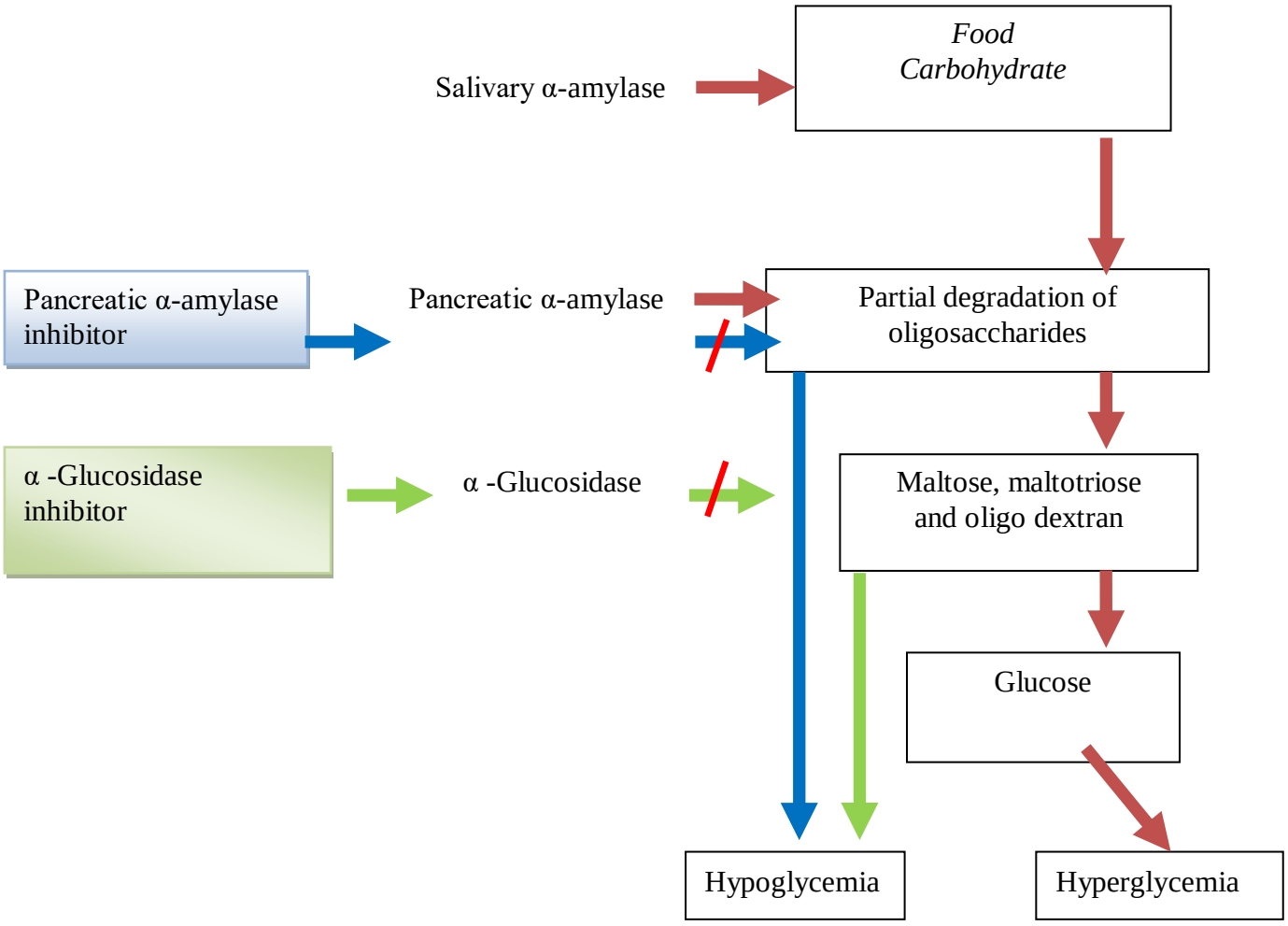


Figure 13: Carbohydrate metabolism.

6.2.1. A-amylase inhibitory assay

Screening of plant material for α-amylase inhibitors was carried out in a microplate according to **Behvar et al. (2018).**

Method

Based on the starch-iodine test **(P et al., 2011),** the assay mixture was composed of 50 µL (1U α amylase solution) and 25µL extract. After the first incubation for 10 min at 37°C, 50 µL of 0.1% soluble starch was added to each reaction well and incubated for 15 min at 37°C. 25 µL HCl (1M) was added to stop the enzymatic reaction, followed by the addition of 100 µL of IKI (iodine reagent 5 mM of I2 and 5 mM of KI).

The color change was noted and the absorbance was read at 620 nm on a microplate reader. The control reaction representing 100% enzyme activity did not contain any plant extract to eliminate the absorbance produced by plant extract; appropriate extract controls without the enzyme were also included.

The acarbose was used as positive control **(Taslimi and Gulçin, 2017 ; Taslimi, et al., 2017 ; Gulçin et al., 2018 ; Taslimi et al., 2018).** A dark-blue color indicates the presence of starch. The yellow color indicates the absence of starch while the brownish color indicates partially degraded starch in the reaction mixture.

The starch added to the enzyme assay mixture is not degraded in the presence of inhibitors from the extracts and gives a dark-blue color complex whereas no color complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase. The percentages of inhibition were calculated by the following formula:

$$\%INH=1-[(A_c-A_e)-(A_s-A_b)/(A_c-A_e)]$$

Ac = Absorbance [Starch + IKI + HCl + Extract solvent volume + Enzyme buffer volume]

Ae = Absorbance [Enzyme + Starch + IKI + HCL + Vol of extract solvent]

As = Absorbance [Enzyme + Extract + Starch + IKI + HCl]

Ab = Absorbance [Extract + IKI + 125µl of buffer]

	1	2	3	4	5	6	7	8	9	10	11	12
A	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
B	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
C	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
D	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
E	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
F	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
G	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
H	Ae	Ae	Ae	Ae	Ae	Ae	Ac	Ac	Ac	Ac	Ac	Ac

Figure 14: Microplate of alpha-amylase inhibitory activity.

6.2.2. A- glucosidase inhibitory assay

The α-glucosidase inhibitory activity was determined by the method of **Nampoothiri et al. (2011).**

Principle

Alpha-glucosidase (α-D-glucoside glucohydrolase; EC 3.2.1.20) is an enzyme that hydrolyzes the α-1, 4-glycosidics bonds from the non-reducing end of oligosaccharides and polysaccharides, releasing glucose that may be absorbed by the intestine **(Sulistiyani et al., 2016),** the basic idea of this essay is to combine the extracts with the enzyme glucosidase and its substrate, then measure the absorbance at 405 nm to estimate the enzyme inhibit activity. The used standard is acarbose.

Method

50 µL of the extract solution dissolved in methanol at different concentrations (15.62-1000 µg / mL) were added to 100 µL of the enzyme solution (0.1 U / mL) and 50 µL of the substrate solution (5 mM p- nitrophenyl- α-D-glucopyranoside in 100 mM sodium phosphate buffer, pH 6.9).

After incubation for 5 minutes at 37° C, the absorbance was determined at 510 nm every 10 minutes, for 30 minutes using a 96-well microplate reader. Acarbose was used as a positive control.

✓ Calculation of inhibition percentages:

The percentages of inhibition were calculated by the following formula:

$$I\ \% = [(Ac-At)/Ac] \times 100$$

Ac: Absorbance of the control.

At: Absorbance of the test performed.

✓ Calculation of IC 50:

The IC50 was calculated graphically by linear regressions of the graphs of percentages of inhibition as a function of different concentrations of the extracts tested. Each test was performed in triplicate and the results were calculated by the mean of three tests.

6.3.Anti-Alzheimer activities

Cholinesterase inhibitory of the areal parts of *A. gombiformis* was evaluated using the spectrophotometric method developed by **Ellman et al. (1961).** AChE from electric eel and BChE from horse serum were utilized, while acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates. The cholinesterase activity was measured using DTNB [5,5-dithio-bis (2-nitrobenzoic acid).

Method

Briefly, 150 µL of sodium phosphate buffer (100 Mm, pH8.0), 10 µL of the extract dissolved in ethanol at various concentrations and a volume of 20 µL AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) were mixed and incubated for 15 min at 250°C before adding 10 µL DTNB (0.5 mM). The reaction was then started by adding 20 µL acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM).

The hydrolyses of acetylthiocholine by AChE or butyrylcholine by BChE produce thiocholine. This latter will react with 5, 5-dithio-bis (2-nitrobenzoïque (DTNB) to produce the yellow color of methyl 5-thio-2-nitrobenzoate anion.

The reaction is followed spectrophotometrically at 412 nm, using a 96-well microplate reader (SpectraMax PC340, Molecular Devices, USA). PRO Softmax V5.2 software was used to analyze the measurements and results.

The percent inhibition of AChE or BChE was determined by comparing the reaction rates of samples with the control (ethanol in phosphate buffer, pH 8) using the formula (E - S) / E x100, where E represents the enzyme's activity without the test sample and S indicates the enzyme's activity with the test sample. The experiments were carried out in triplicate.

Galantamine was used as a reference compound and results were given as 50% inhibition concentration (IC50) (**Taslimi et al., 2018; Cakmak et al., 2019; Taslimi et al., 2020).**

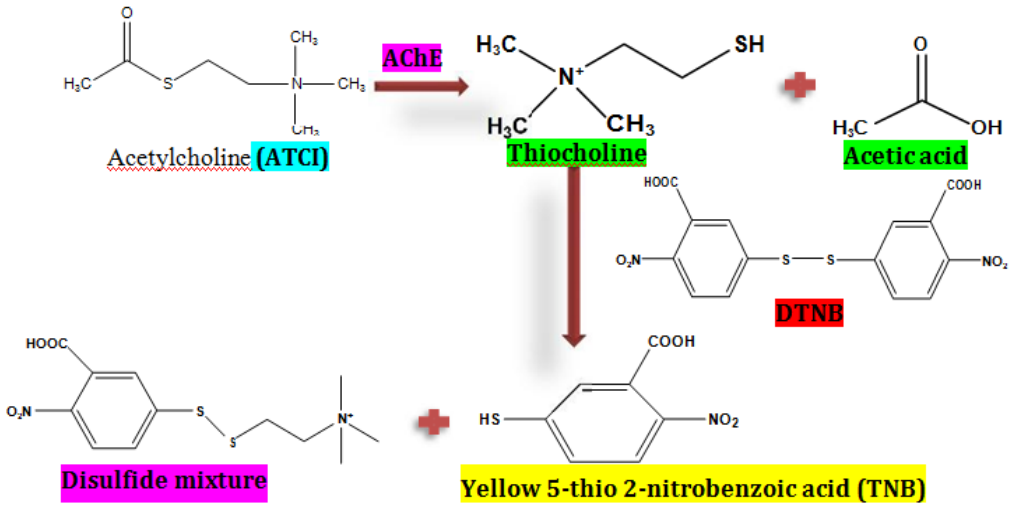


Figure 15: Principle of Ellman's reaction **(Ellman et al., 1961).**

6.4.Anti-tyrosinase activity

The tyrosinase activity is determined according to the method described by **Ebru Deveci et al. (2018).**

Method

This method consists of mixing 150 µL (PH 6.8) with 10 µL of extract, then 20 µL of enzyme, after incubation for 10 minutes at 37 ° C, 20 µL of L-DOPA is added and incubate the mixture for 10 minutes at 37° C. The absorbance reading is taken at 475 nm.

6.5.Urease Inhibitory Assay

Urease Inhibitory activity was performed according to **Muhammad et al. (2018).**

Method

10 µL of various concentrations was added to 25 µL of urease enzyme solution and 50 µL substrate solution, using 0.2553 g Urea in 25mL phosphate buffer solution (3 mM, pH4.5). The mixture was incubated for 15 min at 30°C, and 40 µL of phenol reagent containing a mixture of 1% phenol, 0.005% of sodium nitroprussside, and appropriate amount of alkaline (NaOH) reagents were added to each of the well.

After 50 min, the absorbance of the solution was measured at 630 nm by the use of 96-well microplate reader. The urease inhibitory activity was calculated using the following equation:

$$\% \text{ Inhibition} = [(\text{AbsCN} - \text{AbsExt})/\text{AbsCN}] \times 100$$

- AbsCN is the absorbance of the control reaction
- AbsExt is the absorbance of the extract.

The results were given as IC50 value (µg/mL) corresponding the concentration of 50% inhibition. Thiourea (CH_4N_2S) was used as positive control.

6.6.Anti-inflammatory activity

In vitro anti-inflammatory activity was tested via the protein denaturation method using bovine serum albumin (BSA) as described by **Karthik et al. (2013).**

Method

To 0.5 mL of different concentrations of the extract or reference compounds (ketoprofen and diclofenac), 0.5 mL of BSA (0.2% w/v) in Tris-HCl buffer (pH 6.8) was added. Tightly closed tubs were incubated in the oven at 37 °C for 15 min and then heated in a water bath at 70 °C for 5 min.

The absorbance of turbidity was taken at 660 nm. The percentage inhibition of protein denaturation was determined as the following equation and each experiment was released in triplicate. The results were expressed as means ± SD of three parallel measurements and compared with reference compounds.

$$\% I = [[Ac-(As-Aw)]/Ac] \times 100.$$

As: Absorbance of samples: 0.5 ml extract + 0.5 ml BSA

Aw: Absorbance of white 0.5 ml extract + 0.5 ml Tris-HCL (pH: 6.8)

Ac: absorbance of control: 0.5 H2O + 0.5 ml BSA (The control represent 100% of proteins denaturation).

6.7.Photoprotective activity

The Sun Protection Factor (SPF) is a measure of sunscreen capacity against UVB-induced sunburn. The sun protection factor is the ratio between the minimum erythemal dose on skin protected by a sunscreen product and the minimum erythemal dose on the same unprotected skin **(Wolf et al., 2001).**

The high sun protection factor indicates the effectiveness of the product in preventing sunburn.

SPF = MED skin protected / MED unprotected skin

In vitro methods are generally two types. First is the measuring of UV transmission solar by quartz slabs films, and second is the spectrophotometric study of dilute solutions to determine the absorption properties of agents in sunscreens **(Pissavini et al., 2003).**

The *A. gombiformis* extract was analyzed for *in vitro* sun protection factor (SPF). The absorption characteristics were established based on the spectrophotometric analysis of the diluted solution **(Maske et al., 2013).**

The extract was diluted in absolute methanol and the absorbance values were determined between 290 and 320 nm, at 5 nm intervals, by using 1 cm quartz cuvettes in a UV_Vis spectrophotometer SHIMADZU UV-1700. Methanol taking as white SPF and the value was calculated using the application to the equation developed by **Mansur et al. (1986).**

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where EE(λ) is the erythemal effect spectrum

I(λ) is the solar intensity spectrum

Abs(λ) is the absorbance

CF is the correction factor (CF=10).

The values of EE (λ) × I(λ) are constant determined by **Sayre et al. (1979).**

Table 1: Normal product function used in the calculation of SPF **(Sayre et al., 1979).**

Wavelength ƛ(nm)	EE(ƛ ×I(ƛ) (standards)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180
Total	1

The photoprotective activity of *A. gombiformis* the extracts was evaluated according to Table 2.

Table 2: Protection categories displayed on solar products according to the protection factors measured **(Commission of the European Communities, 2006).**

Indicated Category	Indicated Protection Factor	Measured Sun Protection Factor	Recommended Minimum UVA Protection Factor	Recommended Minimum Critical Wavelength
Weak protection	6	6-9.9	1/3 of the sun protection factor indicated on the label	370 nm
	10	10-14.9		
Medium protection	15	15-19.9		
	20	20-24.9		
	25	25-29.9		
High protection	30	30-49.9		
	30	50-59.9		
Very high protection	50+	60≤		

6.8.Cytotoxic effect

The Toxicity essay of *A. gombiformis* extract was carried out against 2 types of eukaryotic cells: *Artemia salina* and human Erythrocytes.

6.8.1.Brine Shrimp Lethality Test (BST)

According to the method described by **Meyer et al. (1982).** The cytotoxicity of *A. gombiformis* was carried out against *Artemia salina.*

Method

200 mg of Artemia salina eggs were placed in a becher glass containing one liter of lukewarm seawater into an illuminated incubator at 30°C.

After 48 hours, by using pasteur pipette, the phototropic brine shrimps were collected from the illuminated compartment. The fraction in various concentrations (0.5, 1, 2, 4 mg/mL) was prepared in dimethyl sulfoxide (DMSO)-seawater (4%). 100 µL of each concentration was transferred into vial tubes containing 4.9 mL of seawater and 10 larvae of brine shrimps. The vials were incubated under illumination.

After 24 hours, the survived shrimps were counted and the percentage of mortality (death) at each dose and also the control (blank) were determined.

6.8.2.Oxidative Hemolysis Inhibition Assay (OxHLIA)

The hemolysis inhibition assay (OxHLIA) was used to investigate the toxicity of the plant tested **(Kumar et al., 2011; Takebayashi et al., 2010).**

Method

First, in tubes containing heparin, five milliliters of blood was taken from a healthy person and used to prepare a suspension of erythrocytes. The collected blood was centrifuged at 1500×*g* for 3 min. Plasma was removed, and the pellet was washed many times with a sterile phosphate buffer saline (PBS; Ph 7.2).

Then, the erythrocytes were resuspended by gentle shaking in a normal saline suspension (0.5%). A total of 500 µL of the different concentrations of the test extract (125, 250, 500, and 1000 µg/mL) prepared in PBS was mixed with 500 µL of the cell suspension.

The resulting mixtures were incubated at 37 °C for 30 min and centrifuged at 1500× *g* for 5 min. The absorbance was measured spectrophotometrically at 540 nm. Finally, the following formula was used to determine the percentage of hemolysis:

$$\%H = [(AE - AN)/(AP - AN)] \times 100$$

AE: the absorbance of the extract;

AP: the absorbance of the positive control (phosphate buffer saline);

AN: the absorbance of the negative control (distilled water).

6.9.*In vitro* evaluation of antibacterial activity

The antibacterial activity of *A. gombiformis* extracts was evaluated using two methods: disk diffusion on solid medium and dilutions method (on solid medium). Only extracts that exhibited activity by using the disk method were tested using the dilution method to determine the minimum inhibitory concentration (MIC).

6.9.1.Bacterial strains tested

A wide range of bacterial strains were utilized in this study, these strains are outlined in Table 3.

Table 3: Bacterial strains used throughout this project.

N°	*Strains*	*Code*
1	***Acinetobacter baumanii***	*HS*
2	***Pseudomonas aeruginosa***	*(ATCC27853)*
3	***Escherichia coli***	*(ATCC25922)*
4	***Bacillus cereus***	*HS*
5	***Staphylococcus aureus***	*(ATCC291)*
6	***Staphylococcus aureus***	*(ATCC43300)*

(*ATCC*): American Type Culture Collection

(HS): Hospital strains

The reference strains were obtained from the Pasteur Institute (Algeria) while the clinical strains were isolated from patients in the bacteriology laboratory of Bodhiaf hospital, Oum el Bouaghi.

6.9.2.Disc diffusion technique on solid medium

This method is based on the migratory capacity of the extracts on a solid medium inside petri dish. This method allows us to demonstrate the antibacterial effect of extracts on bacteria, as well as to determine the resistance or sensitivity of these bacteria to these extracts.

Method

The method of disk diffusion applied is described by **Hayes and Markovic (2002).**

A bacterial suspension was produced from an 18-hour culture. The Turbidity of the suspensions was adjusted to 0.5 McFarland (10^8 CFU/mL for bacteria and 10^6 CFU/mL for yeasts) using Densi-La-Meter or Densimat.

Prepared microbial inocula were seeded or swabbed on the surface of on Mueller Hinton medium. Then, discs of 6 mm in diameter were previously impregnated with 10 µL of extracts were transferred to the inoculated plats.

The control disks were impregnated with 10 µL of pure DMSO. Gentamycin was used as a positive control. The plats were finally incubated for 24 hours at 37 ° C.

For each concentration of extract, three repetitions were conducted. The measurement was made by measuring the diameters of the inhibitory zones in millimeters **(Kumar et al., 2009)**.

6.9.3. Dilution method

Principal

This technique allows testing the extract directly in the culture medium. All bacterial strains may be examined at the same time and on the same plat using this method allowing researchers to investigate the behavior of the extracts against all strains under the same conditions.

Method

The Minimum Inhibitory Concentration (MIC) was determined by the method described by **Allegrini et al. (1973).**

The extracts were prepared in DMSO (dimethyl sulfoxide) using the double dilution technique with a geometric progression of ½ to obtain 6 concentrations from 20 mg/mL to 0.312 *mg*/mL.

A volume of 1 mL of each dilution was mixed with 19 mL of Mueller Hinton medium in a sterile petri dish. These dishes were shaken and held until the agar solidified. Inoculation is done by depositing a spot that contains 10^4 CFU / mL.

The dishes were then incubated for 24 hours at 37 ° C. Control dishes containing only MH were used as negative controls.

The MIC, in general, is the lowest concentration of antimicrobial capable of inhibiting any visible growth after an incubation time of 18 to 24 hours.

7. Statistical analysis

All determinations were carried out in triplicate of each sample. The results reported as mean ± standard deviation (SD) were calculated from the data obtained. The analysis of variance of bioassays were the subject of one-way analysis of variance (ANOVA) using the PRISM GRAPHPAD V: 5.00 (Trial) followed by Tukey test. The level of significance was fixed at $p<0.05$.

Chapter 2 Results and discussion

"The joy of discovery is certainly the liveliest that the mind of man can ever feel"

- Claude Bernard

1. The quantitative and qualitative study of *A. gombiformis* extracts

1.1.Phytochemical screening

The presence or absence of secondary metabolites was verified through the phytochemical screening. **Table 4** shows the major constituents in different extracts. Flavonoids and tannins are present in all extracts. Flavonoids are mainly found in the whole plant extract, leaves, flowers, and rarely in the stems, pods extracts. Saponins are found particularly in leaves and almost do not exist in the rest of the plant. For the alkaloids, seeds have a relatively high concentration compared to the other extracts. All these results are significantly different. **Teyeb et al. (2013)** found similar results by comparing the presence of secondary metabolites in wild and cultivated *A. gombiformis* plants growing in Tunisia.

Table 4: Phytochemical screening of *A. gombiformis* extracts.

Treatments	Flavonoids	Tannins	Saponins	Alkaloids
Whole plant	++++	++	++	+
Seeds	++	+	-	++++
Leaves	++	+	+++	+
Pod	+	+	-	-
Stems	+	+	-	-
Flowers	++++	+	+	++

(-) Not founded, (+) low concentration, (++) medium concentration, (+++) high concentration, (++++) very high concentration

1.2.Determination of total bioactive compounds

The total phenolic content (TPC) of *A. gombiformis* extracts was estimated according to the calibration curve prepared from gallic acid ($y = 0.0034x + 0.1044$, $R^2 = 0.997$) **(Figure 16).** The total flavonoids content (TFC) was calculated following the calibration curve prepared from quercetin ($y = 0.0048x$, $R^2 = 0.997$) **(Figure 17).** While the total saponins content (TSC) was determined by the calibration curve prepared by quillaja saponins and presented in **Figure 18** ($y=0.0161x+0.033$, $R^2 =0.950$).

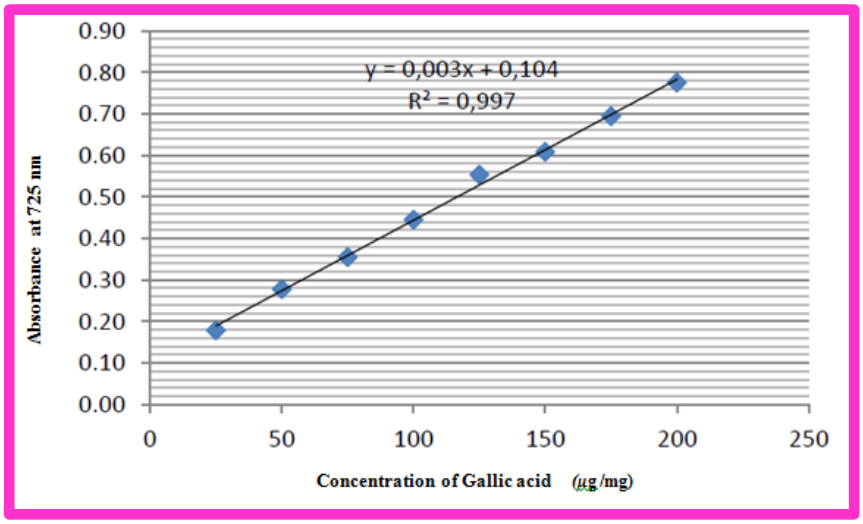


Figure 16: Gallic acid calibration curve for the determination of total phenolic content.

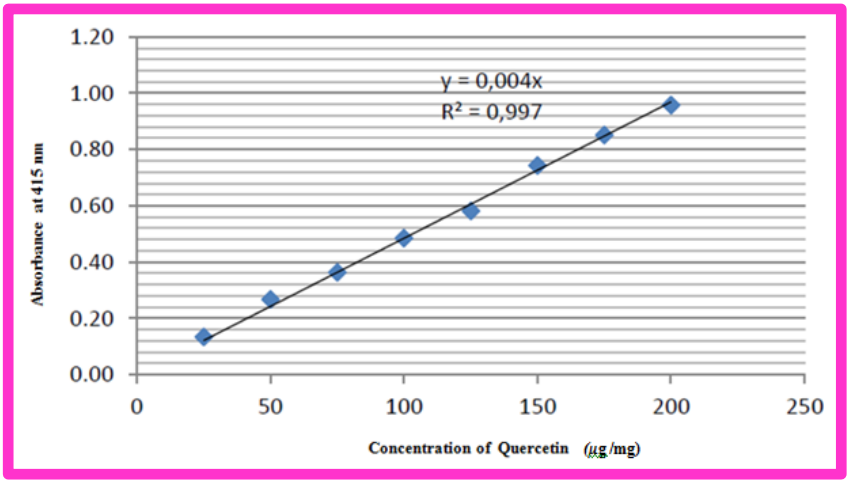


Figure 17: Quercetin calibration curve for the determination of total flavonoids content.

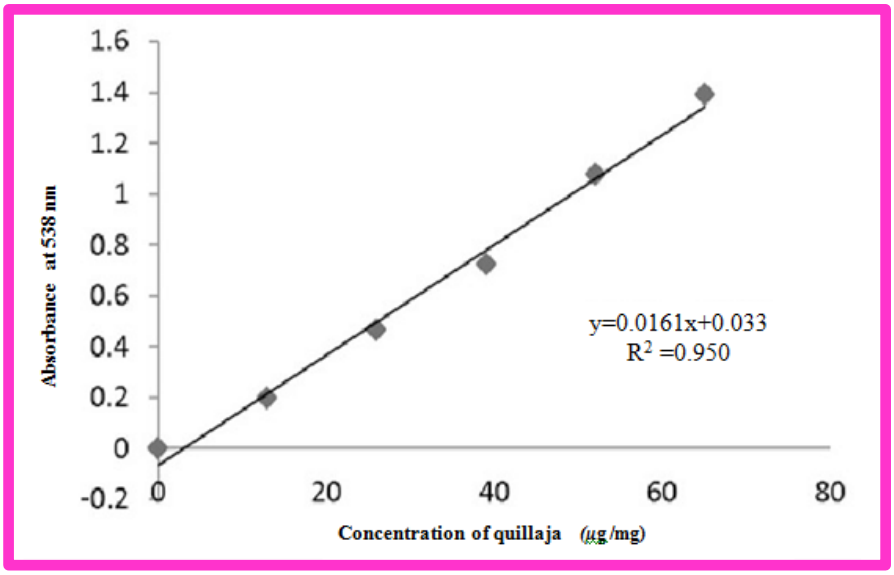


Figure 18: Quillaja saponins curve for the determination of total saponins content.

As shown in **Figure 19,** there is a large variation in TPC content in different extracts examined. The TPC of butanolic extract from the flowers (110.56±19.62 µg GAE /mg) was found to be higher than the other part's extracts, followed by the whole plate, leaves, stems, seeds and pods with values of (92.8±1.69; 92.52±7.90; 83.21±15.34; 52.23±2.29 and 17.43±3.38 µg GAE /mg, respectively).

The same trend was observed with TFC distribution, the highest content was registered in butanolic extract of flowers (94.65±10.43 µg QE/mg), followed by the whole plant, leaves, stems, seeds and pods by values of 63.2±1.56; 58.33±2.80; 40.20±4.30; 33.47±2.65 and 26.31±1.27 µg QE/mg.

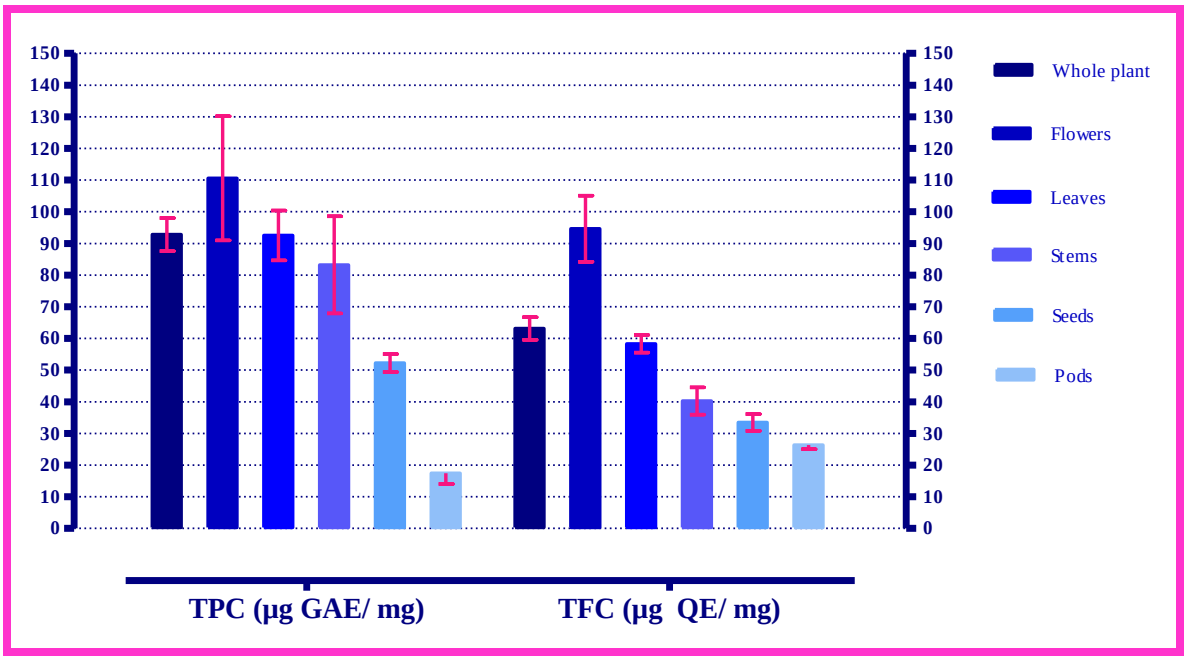


Figure 19: Total phenolic (TPC) and flavonoids (TPC) compounds of *A. gombiformis* extracts. Bars represent standard deviation

The whole extract of *A. gombiformis* presented a TPC of 92.8±1.69 µg GAE/mg, mainly due to the TFC (63.2±1.56 µg QE/ mg), which was composed by low amounts of TSC and presented especially in leaves compared to the other parts (11.33±2.34 µg QSE/ mg), as shown in **Figure 20.**

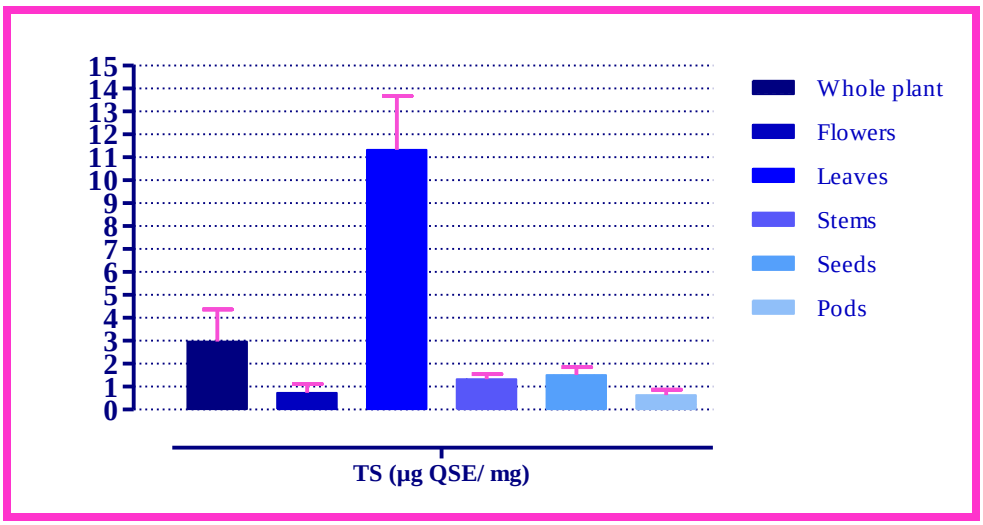


Figure 20: Total saponins content of *A. gombiformis* extracts. Bars represent standard deviation.

A. gombiformis is a medicinal plant species endemic to Algeria with interesting chemical composition. It is commonly used in traditional medicine in North Africa for the treatment of a wide range of illnesses. This specie is well known for its richness in phenolic compounds and flavonoids **(Jing et al., 2011),** which are one of the most numerous classes of secondary metabolites of great diversity in structure and properties (**Rakib et al., 2010).**

Based on this study, the phytochemical screening of butanolic extracts of the whole plant and flowers have considerable concentrations of phenolic compounds and flavonoids compared to all organs extracts.

The TPC of the whole plant (92.8 ± 1.69 µg GAE/ mg) was higher compared to the methanolic extract (9.19± 0.27 mg GAE/g DM) of the aerial parts of *A. gombiformis* growing in Tunisia results **(Teyeb et el., 2012)**. The same trend was registered by **Sevil and Onur (2019)** for the methanolic extract of *Astragalus argaeus* Boiss from Turkey (10.4±0.3 mg GAE/g DM). The TPC results of this study are higher than those obtained by **Bronislava et al. (2018)** from *A. glycyphyllos* extract of leaves and flowers (25.99 and 23.71 mg GAE/g DM).

The TFC results of this study are higher compared to the TFC of *A.argaeus* in the variation of methanolic extract (5.88 ± 0.1 mg QE/g DM) of **Sevil and Onur (2019).** Moreover, TFC is relatively higher compared to flavonoid content in leaves (21mg RE/g) and flowers (16.71 mg RE/g) extract of *A. glycyphyllos* **(Lobanova, 2011).**

The high phenolic contents in *A. gombiformis* increase their nutritional and therapeutic values. Overall, we can conclude that distribution of TPC and TFC are organ-dependent, and the variation of results due to several factors, such as ecological condition and climate, genotypic and environmental stress within geographical positions of herbal tested **(Lobanova, 2011).**

It is not necessary to compare our data directly with the results of literature due to the differences of species, maturity, plant part, and the heterogeneity of the plant tested.

Moreover, the TS content is low compared to phenolic compound with specific distribution in the leaves part by concentration of 11.33±2.34 (µg QSE/ mg) (**Figure 20).** Several factors have been reported to influence the TSC of plants, including variety and degree of plant maturation **(Benchikh et al., 2017)** and the extraction conditions **(Saci et al., 2017; Saci et al., 2020).**

1.3.Identification and quantification of phenolic and flavonoids contents

The quantitative analysis results of major phenolic compounds identified in different *A. gombiformis* extract are summarized in **Table 5**.

Table 5: Phytochemicals identified in extract of *A. gombiformis* by LC-ESI-MS

	Analyte	Rt	M/h	Butanolic fractions Concentration (ppm)					
				TPC: 38.5 Seeds	TPC:46.176 Stems	TPC: 99.304 Flowers	TPC:28.419 Pods	TPC:78.355 Leaves	TPC:121.81 Whole plant
1	Quinic Acid	2.017	191.00	2.268	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	3.859	6.16
2	Gallic Acid	4.317	169.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	1.16
3	Protocatchuic Acid	-	153.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
4	Catechin (+)	-	289.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
5	Caffeic Acid	-	179.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
6	Syringic Acid	-	197.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
7	1,3-Di-O-Caffeoyquinic Acid	-	515.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
8	Epicatechin	-	289.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
9	*P*-Coumaric Acid	22.283	163.00	4.370	2.039	5.082	7.341	6.069	8.99
10	Rutin	25.256	609.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	4.12
11	T*rans* Ferulic Acid	24.550	193.00	N.D.(Peak)	0.578	N.D.(Peak)	N.D.(Peak)	0.217	2.34
12	Hyperoside (Quercetin-3-O-Galactoside	25.829	463.00	4.744	0.698	2.622	1.002	1.221	2.15
13	Luteolin-7-O-Glucoside	-	447.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
14	3,4-Di-O-Caffeoyquinic Acid	-	515.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
15	Naringin	-	579.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
16	Rosmarinic Acid	27.876	359.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	4.45
17	4,5-Di-O-Caffeoyquinic Acid	-	515.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
18	Quercetrin (Quercetin-3-O-Rhamonoside)	28.050	447.00	0.684	0.285	8.352	5.454	0.629	14.01
19	Apegenin-7-O-Glucoside	28.028	431.00	N.D.(Peak)	0.873	0.977	0.376	1.281	1.87
20	O-Coumaric Acid	-	163.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
21	Salviolinic Acid	-	717.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
22	Kampherol	33.350	285.00	0.006	2.159	1.531	0.291ab	0.781	10.05
23	Quercetin	-	301.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
24	Trans Cinnamic	-	147.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
25	Silymarin	35.398	481.00	N.D.(Peak)	N.D.(Peak)	14.765	N.D.(Peak)	N.D.(Peak)	14.76
26	Naringenin	35.083	271.00	0.269	0.277	N.D.(Peak)	0.268	N.D.(Peak)	1.29
27	Apegenin	35.717	269.00	0.357	N.D.(Peak)	0.335	0.101	0.463	1.41
28	Luteolin	36.283	285.00	N.D.(Peak)	N.D.(Peak)	0.872	1.326	0.776	1.99
29	Cirsiliol	36.975	329.00	24.915	38.774	64.768	11.538	62.337	44.46
30	Cirsilineol	40.139	343.00	1.187	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	1.68
31	Acacetin	42.117	283.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	0.722	0.722	0.92

The data are presented as mean ± SD of three parallel measurements (n = 3); Rt: retention

Following the total phenolic and flavonoids content results, the LC-ESI-MS analysis confirms that flavonoids contents in this plant represent more than 50% of the polyphenolic fraction. The Cirsiliol or Dimethoxyflavone (DMF) is represented the major flavonoids in this plant.

As shown in **Figure 21,** dimethoxyflavone is flavone substituted by methoxy groups at positions 6 and 7 and hydroxy groups at positions 5, 3' and 4' respectively. The highest concentration of this metabolite is fawned in flowers by a value of 64.768 ppm, followed by leaves, whole plant, stems seeds and pods (62.337; 44.46; 38.774; 24.915 and 11.538 ppm).

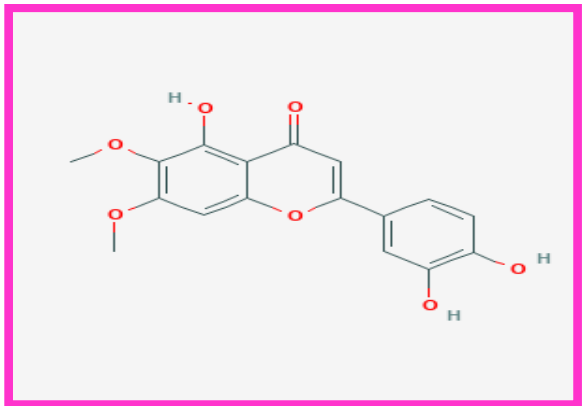


Figure 21: Chemical structure of major flavonoid identified in *A. gombiformis* **(Cirsilio: dimethoxyflavone**

The Analytical characteristics of thirty-one phenolic standards were assessed and presented in **Table 11** (Appendix).

By comparing the results of LC-ESI-MS analysis with the reference compounds referring to their retention times, the characterization of phenolic compounds of *A. gombiformis* revealed the presence of 17 compounds, of which five were detected as main compounds **[quercetin-3-o-rhamonoside(18), kampherol(22), cirsiliol(29), p-coumaric acid(9) and hyperoside(12**)].

In comparison to each organ individually, the total plant extract has detected a large number of phenolic compounds as showing in **Figure 22.**

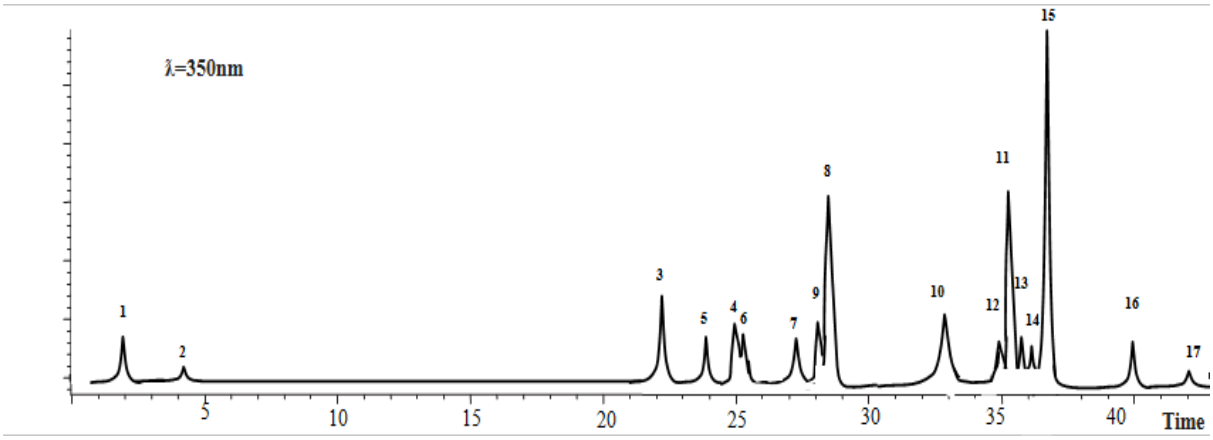


Figure 22: Total ion chromatograms (TIC) of the active extract of *A. gombiformis* (whole plant) obtained by LC-ESI-MS analysis in negative ionization mode.

The different peaks in the chromatogram represent the following:

1: Quinic acid; 2:Gallic acid; 3: *P*-coumaric acid; 4: Rutin; 5: *Trans*-ferulic acid; 6:Hyperoside (quercetin-3-*O*-galactoside) ; 7: Rosmarinic acid; 8: Quercitrin (quercetin-3-*O*-rhamnoside); 9: Apigenin-7-*O*-glucoside; 10: Kaempferol; 11: Silymarin; 12: Naringenin; 13:Apigenin; 14: Luteolin; 15: Cirsiliol; 16: Cirsilineol; 17: Acacetin.

The highest content of **quercetrin (quercetin-3-o-rhamonoside), kampherol**, and **p-coumaric acid** was detected in butanolic extract of the whole plant (14.01; 10.05 and 8.99 ppm). Whereas **hyperoside (quercetin-3-o-galactoside)** was detected by significant concentration in seeds fraction compared to the other extracts (4.744 ppm).

Moreover, this analysis showed the presence of **quinic acid (1)** in whole plant, stems and pod (6.16; 2.268 and 3.859 ppm) while **Apegenin-7-o-glucoside (19)** in whole plant, leaves, flowers, seeds and pod (1.87; 0.873; 0.977; 0.376, and 1.281 ppm).

Apegenin (27) was detected in whole plant, stems, flowers, seeds, and pod (1.41; 0.357; 0.335; 0.101, and 0.463 ppm), and **Luteolin (28)** in whole plant, flowers, seeds, and pod (1.99; 0.872; 1.326 and 0.776 ppm). While **gallic acid, rutin, rosmarinic acid** was founded just in the whole plant extract. All this results are significant difference in concentrations ($p<0.05$).

Silymarin was detected by the same concentration in flowers and whole plant extracts (14.76 ppm), while ***Trans* ferulic acid (11), Naringenin (27), Cirsilineol (30), Acacetin (31**) were found as traces with the lowest values (<2 ppm).

The identification and quantification of phenolic compounds and flavonoids were well documented in previous studies **(Yasinov and Khaitov, 1988; Yasinov et al., 1983)** as well as their isolation was carried out by chemical and spectroscopic chromatographic methods in order to elucidate their structure.

In order to confirm the above screening phytochemical and total bioactive content results, a complete liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI–MS) analysis was optimized and validated to quantify 31 phytochemical fingerprint compounds (phenolic and flavonoid compounds) **(Susamci et al., 2017).**

These results of the whole plant extract indicated a relatively higher number of compounds (17) compared to stems, flowers, leaves, pods, and seeds which have detected the presence of just 12 compounds, with low concentrations.

The emergence of new compounds from the total plant extract that was not previously recorded by LC-ESI–MS of the five separate organs could be explained by the synergistic effect between compounds presented in each organ. This interaction may cause the synthesis of new derived molecules that was demonstrated by the present analysis.

Moreover, this finding can be clarified also by the presence of these new molecules in each organ, but with low concentrations not detectable by LC-ESI–MS. So, the combination of the whole plant resulted in to increase in their concentrations and therefore to their emergences in the LC-ESI–MS analysis.

According to the literature and also based on our results, we may consider the *A. gombiformis* plant as a potential source of bioactive molecules with dual action in pharmacological development. Previous experiments on *Astragalus* genus have shown the presence of major similar phenolic compounds obtained from our study such as **hyperoside, naringenin, quercetin, kaempferol, apigenin, p-coumaric acid, rosmarinic acids** from *A. schizopterus* methanol extract **(Haşimi et al., 2017).**

Similarly, the major phenolic compounds obtained such as **kaempferol, quercetin, rutin, rosmarinic acid, calycosin, ononin, formononetin** was detected in *Astragalus* species by using HPLC-DAD and HPLC-MS/MS **(Qi et al., 2008; Yunfei et al., 2008; Montoro et al., 2012; Zhang et al., 2013).**

The variation in chemical composition depended on the plant's part, the species, the maturity and vegetation period **(Lobanova, 2011; Platikanov et al., 2005).**

Moreover, the characterization of phenolic compounds in *Astragalus quisqualis* and *Astragalus kabadianus* was well documented **(Yasinov et al., 1983; Yasinov and Khaitov, 1988).** Eight phenolic compounds were isolated and purified from *Astragalus taipaishanensis* as well as their structures have been elucidated by ESI-MS, HR-ESI-MS, 1D-NMR and 2D-NMR in the form of **7,2′-dihydroxy-3′, 4′-dimethoxy isoflavane, formononetin, isoliquiritigenin, quercetin, kaempferol, ononin, p-hydroxybenzoic acid and vanillic acid (Pu et al., 2015).**

The variation is also related to several factors, such as ecological and climate conditions, genotypes, and the environmental stress within geographical positions of plant material tested **(Lobanova, 2011).**

Our findings of butanolic extract from the whole plant are in agreement with **Sevil and Onur (2019)** who found almost the same compounds from *Astragalus taxa* such as **rutin, chlorogenic acid, kaempferol, ferulic acid, and syringic acid**. Anyway, **Jun et al (2012)** also has documented the presence of thirty phenolic compounds in *A.membranaceus* by using high-performance liquid chromatography (HPLC).

According to **Mollaei et al (2020),** the environmental factors had a significant effect on the essential oil content and antioxidant activity of *Mentha pulegium* L. Besides. **Mehalaine and Chenchouni (2020)** extensively explained how edaphic variables and climatic factors influenced the accumulation of essential oils in wild plants in North Africa (**Mehalaine and Chenchouni, 2021).** Based on the literature and the present results, we can consider *A. gombiformis* as an important plant with potential for biomedical applications due to its secondary metabolits that is dependent on the local ecological conditions.

2. Antioxidant activity

In this study, the butanolic fractions of the areal part of *A. gombiformis* were tested for its antioxidant activity with more than one method (**DPPH** scavenging, **ABTS** radicals scavenging activity, **CUPRAC** method, **β-carotene** bleaching and galvinoxyl assay **GOR**) in order to take into consideration the nature of bioactive molecules through their various mechanisms actions and the capacity of radical scavenging **(Li et al., 2008)** and compared the results with several reference standards with the aim to achieve more informative and arguably necessary results.

2.1.Antiradical activity with DPPH of *A. gombiformis* extracts

The percentage of inhibition for each concentration as well as the IC50 (concentration capable of trapping 50 percent of DPPH radicals in the medium) are used to illustrate the DPPH scavenging assay by microplate method (**Figure 23).**

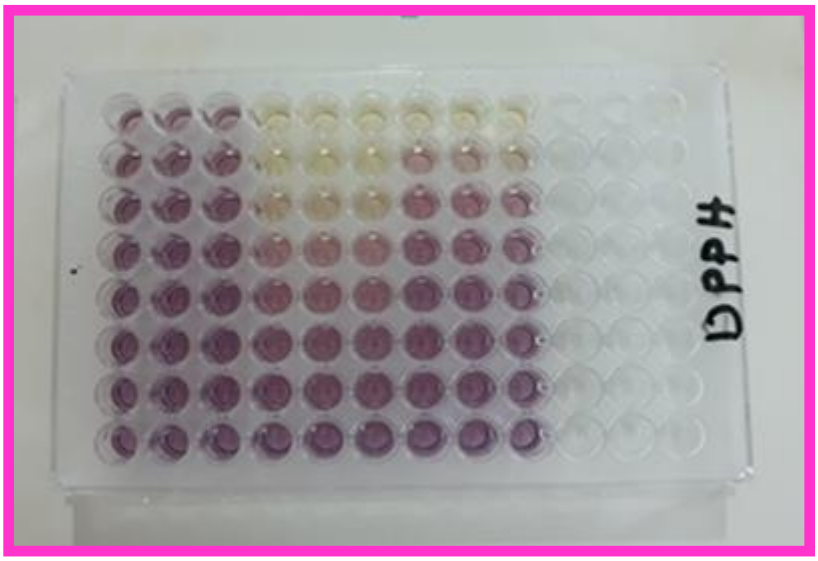


Figure 23: DPPH results on the microplate.

The butanolic extract from the flowers exhibited the highest DPPH radical scavenging activity with a percentage inhibition of 85.52 at 200 µg/mL. While the positive standards, BHT, BHA, and α-Tocopherol, showed a percent inhibition of 94.98%, 87.13%, and 89.45% respectively at the same concentration (**Figure 24).**

The flowers fraction have demonstrated a strong IC50 (16.43±0.46 µg/mL) closer to that of BHT, α-tocopherol, and ascorbic acid (IC50: 12.99±0.41, 13.02±5.17 and

13.94±2.81 µg/mL) respectively, and less than BHA (IC50: 6.14±0.41 µg/mL), followed by the whole plant and leaves (IC50: 56.9±4.56; 147.82±0.28 µg/mL) with a percent inhibition of 77.9% and 54.33% at 200 µg/mL). But the stems, seeds, and pod extracts demonstrated a low activity at the same dose (IC50: 219.12±4.14, 244.63±1.94, and 306.43±2.48 µg/mL) (**Figure 25).**

The flowers fraction was exhibited the best antioxidant activity by scavenging free radicals, which appears to be strongly correlated with total phenolic content in almost all tests. Based on this results, the highest activity in flowers, whole plant, and leaves using the DPPH method are agree with a study previously reported by **Bronislava et al. (2018),** *Astragalus. cicer* leaves exhibited a particularly high antioxidant activity (128.6 µg/mL) close to our results of leaves extract (IC50: 147.82±0.28 µg/mL).

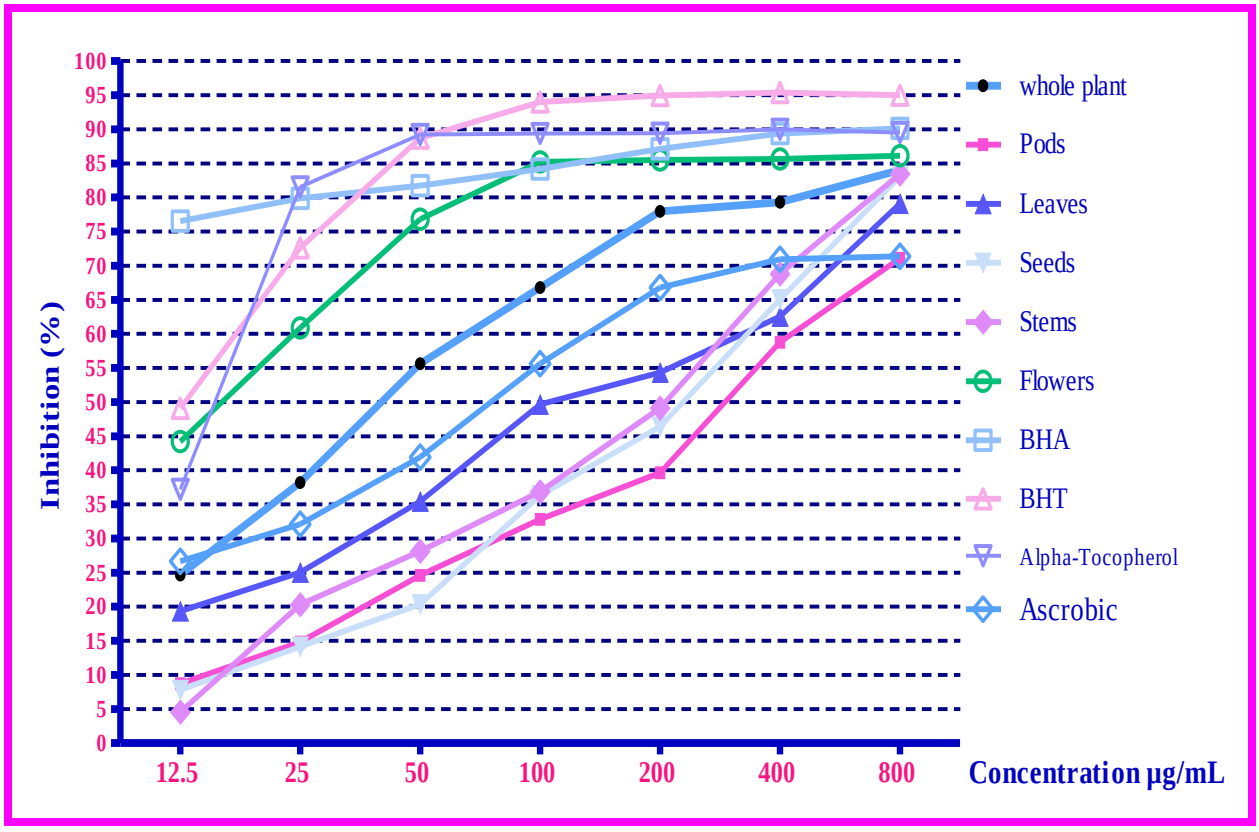


Figure 24: Inhibition of the DPPH radical by *A. gombiformis* extracts and standards.

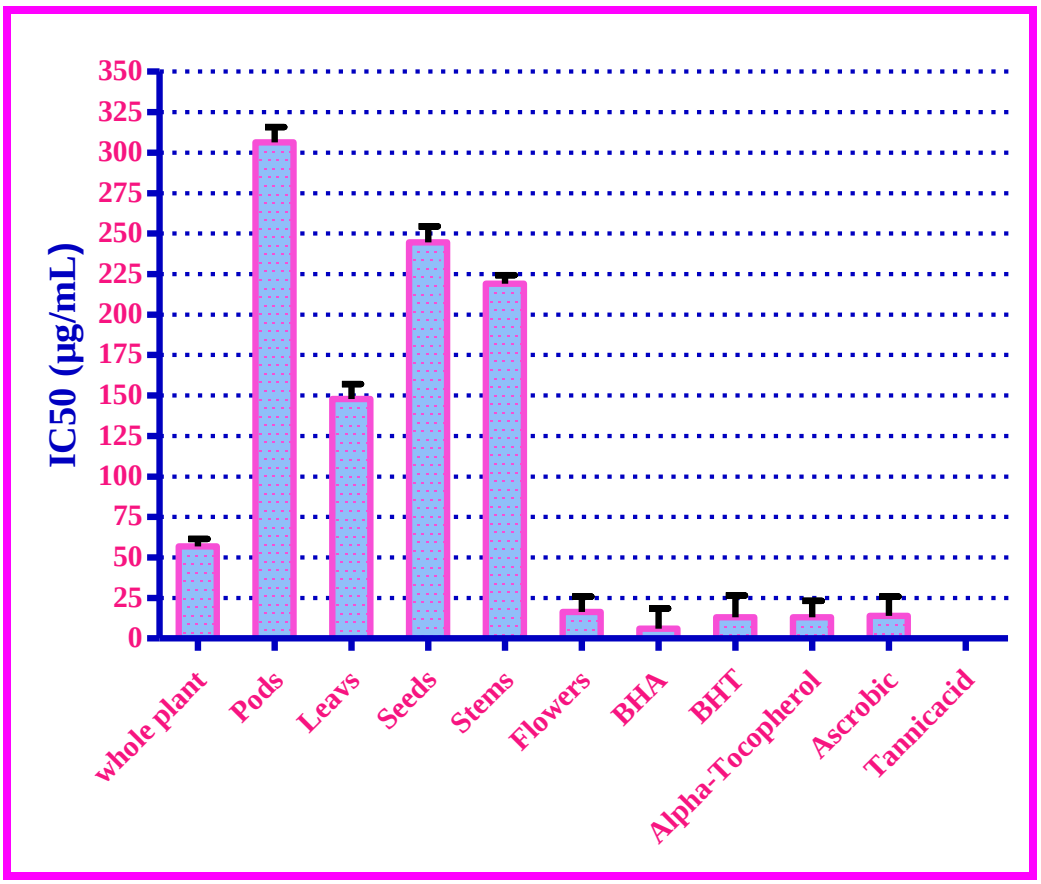


Figure 25: IC50 of *A. gombiformis* extracts and standards (by DPPH method).

2.2.ABTS cation radical Assay

The results of the antioxidant activity using ABTS cation radical assay in microplate is represented in **Figure 26.**

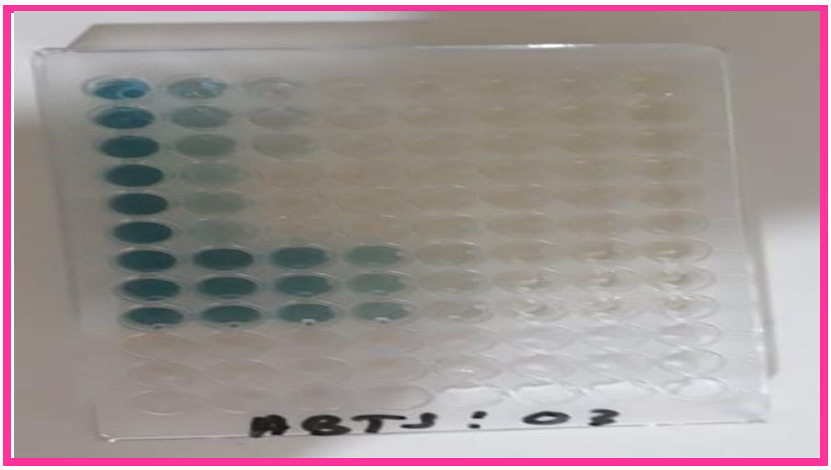


Figure 26: ABTS results on the microplate.

The analysis data of the ABTS assay showed that the same fraction of flowers of *A. gombiformis* gives the best activity with a percentage inhibition of 86.71% at 800 µg/mL **(Figure 27)**.

Compared with standards, the IC50 of flowers (IC50: 16.13±0.35 µg/mL) are very closer to α-tocopherol (IC50: 7.59±0.53 µg/mL) and moderate activity compared with BHT, BHA, ascorbic acid, and tannic acid (IC50:1.81±0.10, 1.29±0.30 1.74±0.10 and 1.01±0.16 µg/mL, respectively). Followed by whole plant, leaves, seeds, stems and pods (IC50: 18.90±4.56; 143.45±4.06; 241.87±1.33; 257.55±396; 266.95±2.26 µg/mL)**(Figure 28).**

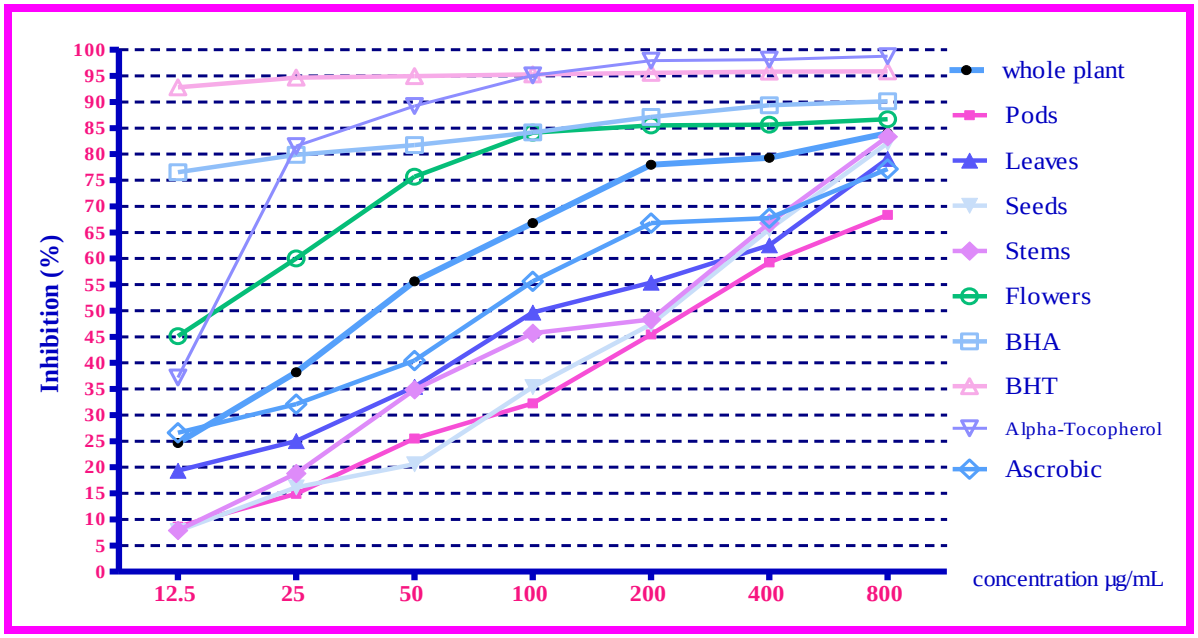


Figure 27: Inhibition of the ABTS radical by *A. gombiformis* extracts and standards.

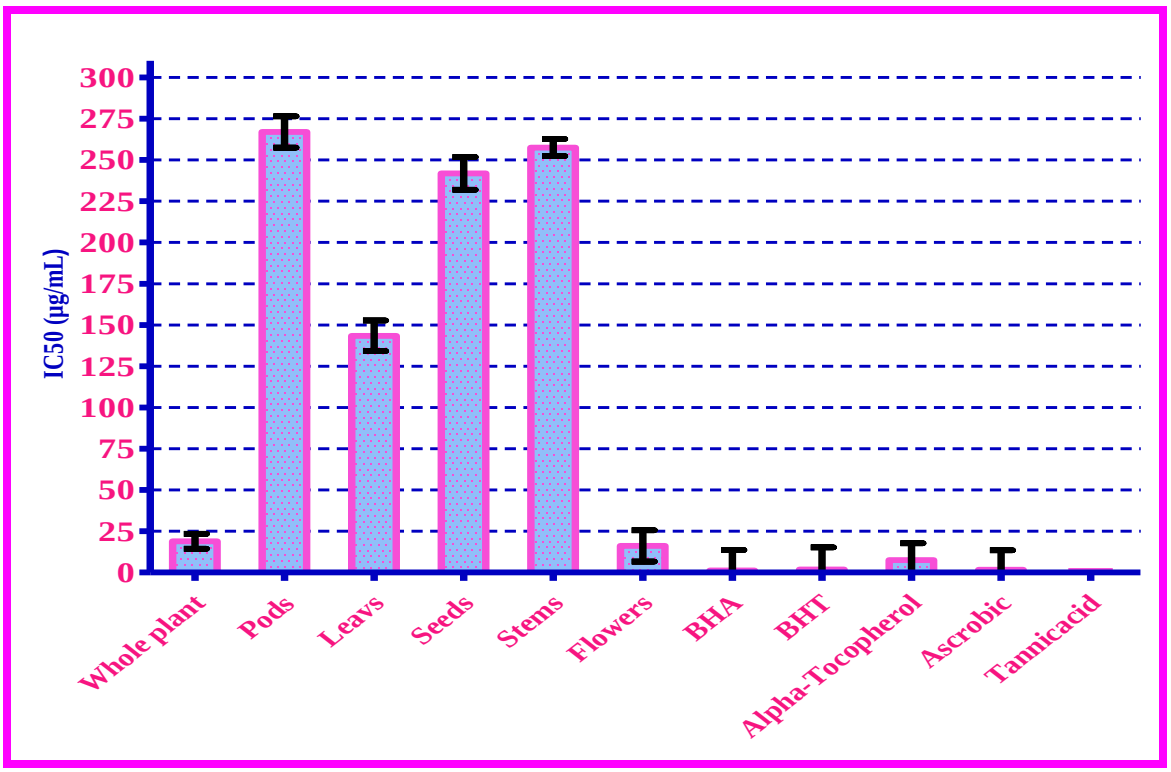


Figure 28: IC50 of *A. gombiformis* extracts and standards (by ABTS method).

Moreover, the half-maximal inhibitory concentration registered in the ABTS assay of flowers was founded to be lowest compared by the methanolic extract from flowers of *A.membranaceus var.mongholicus* (22.02 *µg*/mL) **(Yuan et al., 2019).**

On contrary, *A.cicer* seeds revealed a strong antioxidant capacity with 67.2% inhibition compared with our results (306.43±2.48; 241.87±1.33 µg/mL), due to the action and the different concentration of antioxidant metabolites present in the corresponding medicinal plants **(Bronislava et al., 2018)**. Indeed, whole plant, leaves and flowers extracts that are richer with these compounds were generally more actives.

According to literature, **Tepavˇcevic et al. (2010)** have documented that the ABTS scavenging activity correlated well with total polyphenolic content. Many researchers have found that the medicinal plants with high amounts of flavonoids and phenols have potent antioxidant actions **(Da Silva et al., 2006; Ksouri et al., 2009; Falleh et al., 2011; Dehshiri et al., 2013).**

2.3.Copper Reduction Antioxidant Capacity Test (CUPRAC)

The copper reduction antioxidant capacity test (CUPRAC) findings using the microplate method are provided in the **Figure 29.**

Figure 29: CUPRAC results on the microplate.

The potential to reduce copper increases with the concentration of the extracts rises. At a concentration of 800 µg/ mL, flowers and whole plant extracts had a significant CUPRAC activity (OD: 3.60) which was very close to that of BHA and BHT (OD: 3.92 and 3.40 respectively).

Followed by leaves fraction (OD 1.65 µg/mL), stems (OD: 1.12 µg/mL), pods extract (OD: 0.940 µg/mL), and seeds fraction (OD: 0.65 µg/mL) (**Figure 30).**

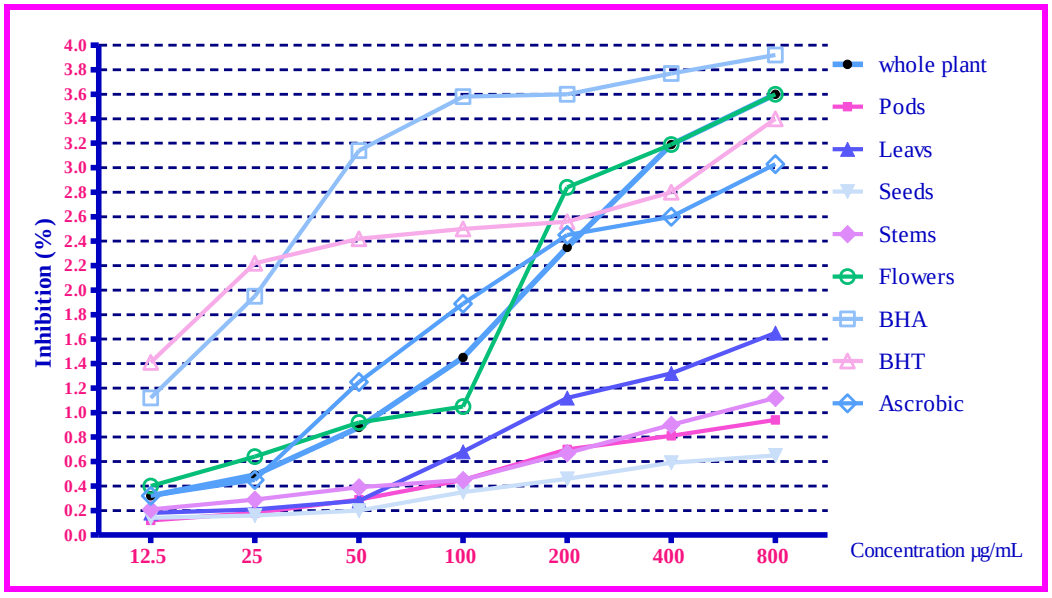


Figure 30: Copper reduction antioxidant capacity test (CUPRAC) of *A. gombiformis* (OD)

The butanolic extract of the flowers showed better CUPRAC reduction (A0.50: 17.89 µg/mL) compared to the other extracts, followed by the whole plant fraction (A0.50: 24.58 µg/mL), the fraction of leaves (A0.50: 88.00 µg/mL), fraction of pods (A0.50: 105.00 µg/mL), fraction stems (A0.50: 170.0 µg/mL) and fraction of seeds (A0.50: 175.00 µg/ mL). On the other hand, this capacity is lower than that of the positive BHA and BHT standards (A0.50: 5.35 ± 0.71 and 8.97 ± 3.94 µg/mL, respectively) **(Figure 31**).

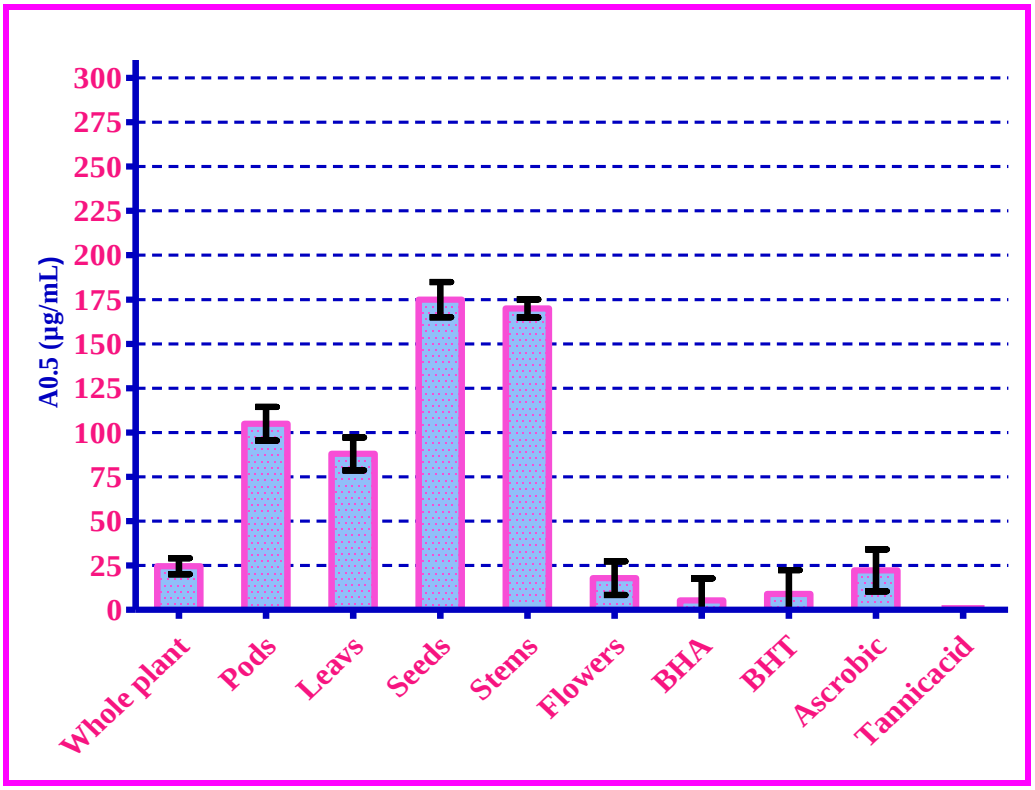


Figure 31: A0.5 of *A. gombiformis* extracts and standards (by CUPRAC method).

2.4.β-carotene bleaching assay

The *β*-carotene bleaching assay was accessed by the microplate method (**Figure 32).** The percentage of inhibition of all fractions tested was lower than those of BHA 95% and -tocopherol (94%) at 800 µg / mL **(Figure 33).**

Figure 32: β-carotene bleaching assay results on the microplate.

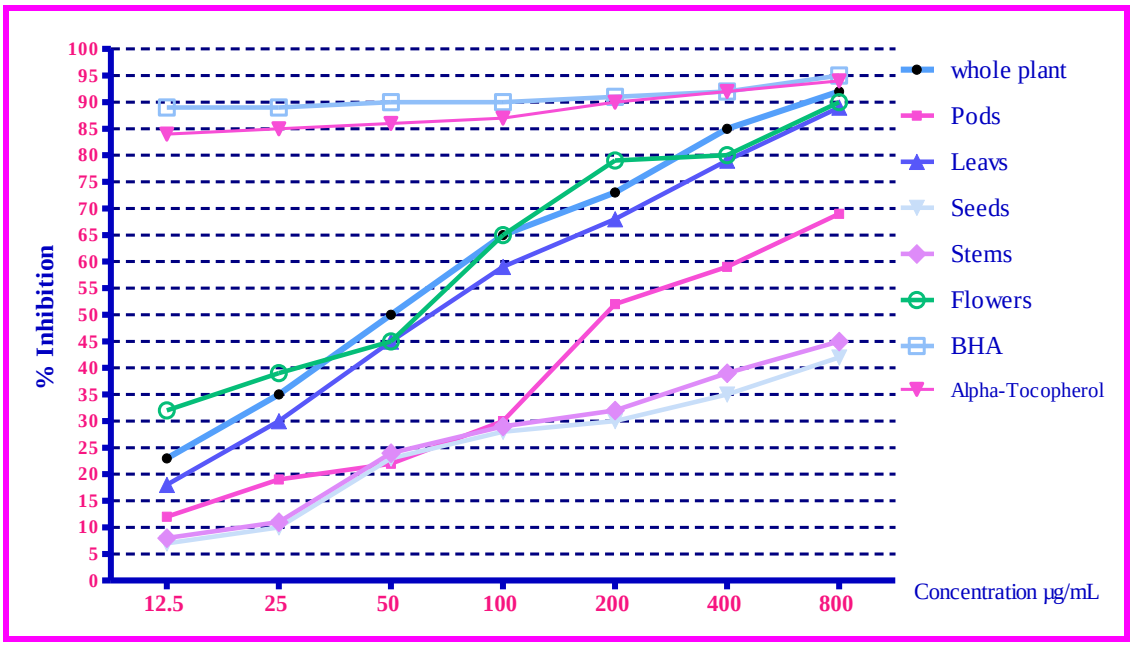


Figure 33: Percentage of Inhibition using *β*-carotene bleaching by *A. gombiformis* extracts and standards.

All results observed of the *A. gombiformis* extracts are lower than the standard BHA (IC50: 1.87±0.04 µg/mL) and α-tocopherol (IC50: 2.10±0.08 µg/ mL) **(Figure 34)** and more particularly seeds and stems (IC50: 666.67±2.21 and 569.23±2.21 µg/mL). Compared to the other antioxidant methods, the whole plant extract (IC50: 58.44± 4.56 µg/mL) has the best antioxidant potential than flowers extract (IC50: 62.50±4.56 µg/*mL*) which was typically ranked first.

This difference between the results of the methods could explain by the nature and concentration of phenolic compounds present in each part of the plant, such as phenolic acids including **gallic acid** and its derivatives, **coumaric acid,** and **flavonoids** such as **luteolin**.

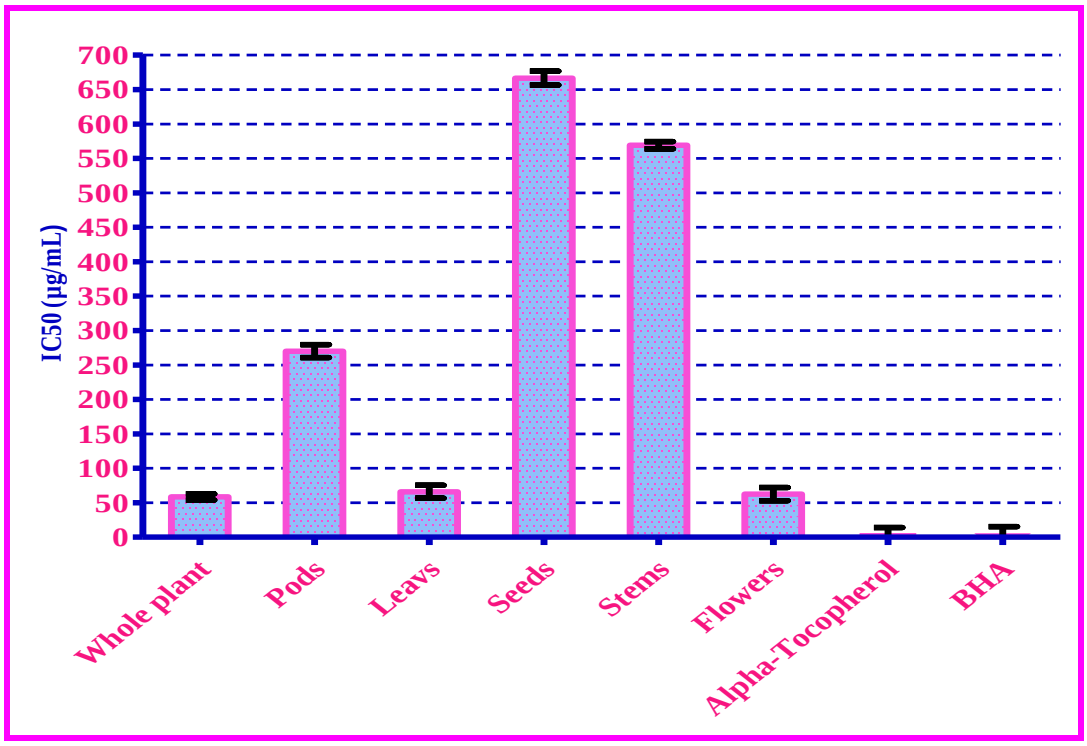


Figure 34: IC50 of *A. gombiformis* extracts and standards (***β*-carotene bleaching assay**).

2.5.Galvinoxyl assay (GOR)

The galvinoxyl radical assay was investigated by microplate method **(Figure 35).**

Figure 35: Galvinoxyl assay (GOR) results on the microplate.

Based on the results obtained in the **Figure 36**, a weak activity of all parts tested as

well as the whole plant extract of *A. gombiformis* was observed with galvinoxyl radical (GOR) by values of IC50 ranging from 489.23±1.09 to 655.51±1.92 µg/mL.

All value of IC50 of galvinoxyl assay (GOR) of the *A. gombiformis* extracts are lower than the standard BHA (IC50 = 5.38 ± 0.04 μg / mL) and BHT (IC50 = 3.38 ± 0.08μg / mL).

The results of the various methods used to evaluate the antioxidant activity of the *A.gomiformis* plant revealed that the flowers, whole plant and leaves extracts expressed a strong antioxidant activity compared to the other organs parts of the plant.

These results are relatively correlated with the total phenolic and flavonoids due to their ability as an inhibitor of free radicals. This important antioxidant activity is dose-dependent and proportional to the level of phenolic compounds present in the different plant organs.

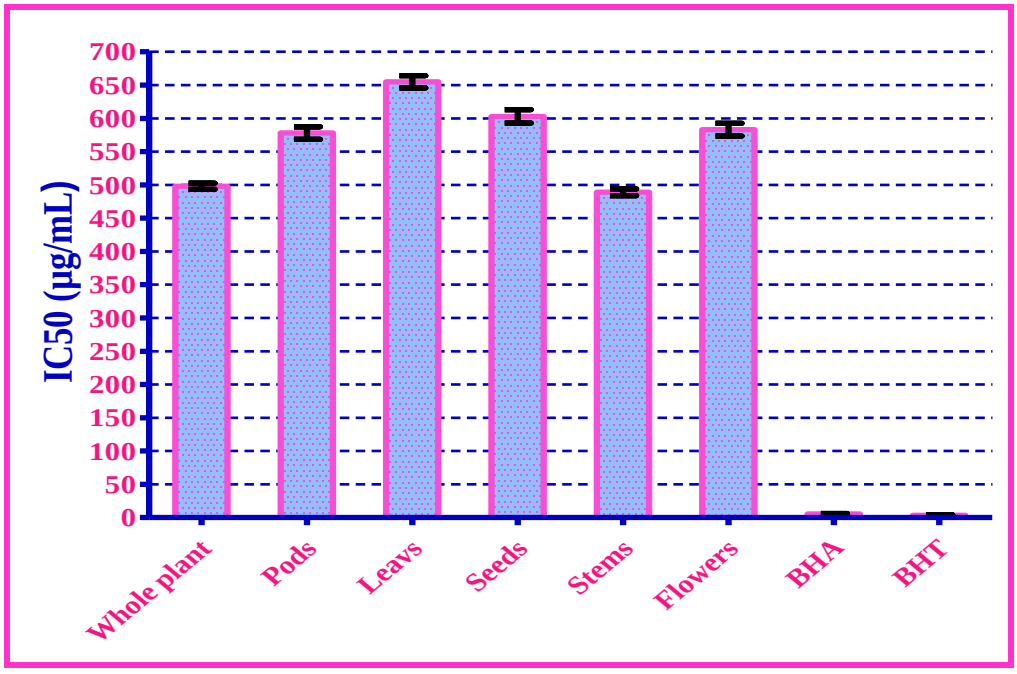


Figure 36: IC50 of *A. gombiformis* extracts and standards (Galvinoxyl assay (GOR).

3. Anti-tyrosinase activity

Anti-tyrosinase activity was realized by the microplate method **(Figure 37).** The results are expressed as IC5O in **Table 6**. The IC50 value is the concentration that inhibits 50% of the enzyme.

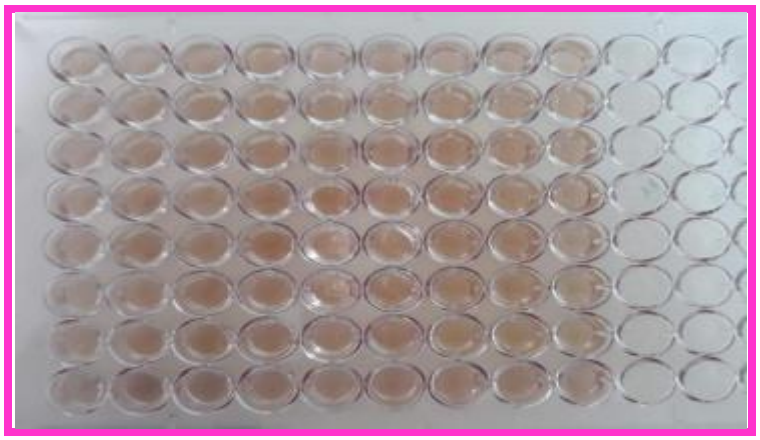


Figure 37: Results of anti-tyrosinase activity on the microplate.

Table 6: Results of anti-tyrosinase activity of *A. gombiformis* extracts.

	% Inhibition							
Extracts µg/mL	3.125	6.25	12.5	25	50	100	200	IC_{50}
Whole plant	NA	NA	NA	NA	NA	NA	NA	/
Pods	NA	NA	NA	NA	NA	NA	NA	/
Leaves	NA	NA	NA	NA	NA	NA	NA	/
Seeds	NA	NA	NA	NA	NA	NA	NA	/
Stems	NA	NA	NA	NA	NA	NA	NA	/
Flowers	NA	NA	NA	NA	NA	NA	NA	/
Kojic acid	6.91±0.75	19.22±0.75	36.29±2.92	49.46±2.24	58.32±0.37	64.36±0.65	66.95±2.24	25.23±0.78

NA: NO ABSORBANCE

Tyrosinase is an enzyme that plays a major role in the production of melanin or skin colors. This enzyme is known to be inhibited by several polyphenols.

The findings demonstrate that the six extracts of *A. gombiformis* are completely ineffectual as tyrosine's inhibitors. These results indicating that the phenolic compounds in this species do not affect human pigmentation or melanin production.

4. Urease inhibitory assay

Herein, the urease enzyme inhibitory activity of six natural extracts of *A. gombiformis* was evaluated by using microplate method **(Figure 38)**. Three extracts were elucidated as the most potent (the butanolic fraction from flowers, whole plant, and leaves).

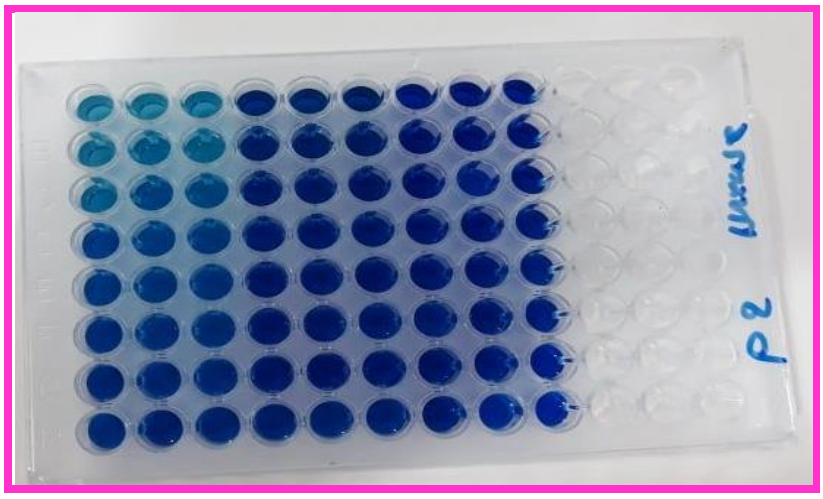


Figure 38: Results of anti-urease activity on the microplate.

As it is presented in **Figure 39,** the whole plant extract with an IC50 value of 65.00µg/mL (even lower than that of thiourea (CH_4N_2S), the positive control, with the IC50: 11.57±0.68 µg/mL) is the most effective extract followed by flowers and leaves with IC50 values of 95 and 110 µg/mL, respectively.

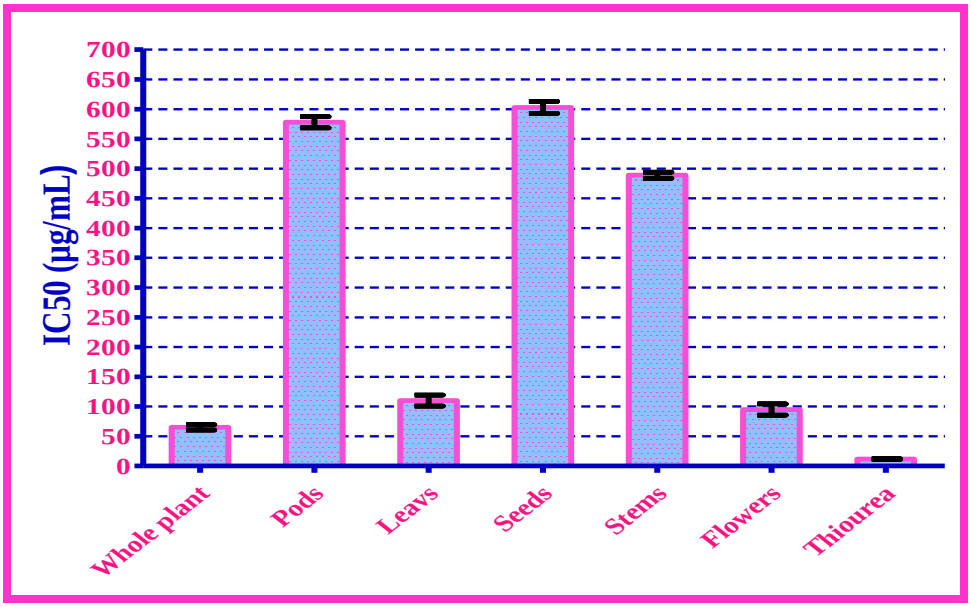


Figure 39: Results of anti-urease activity of *A. gombiformis* extracts.

The results obtained are strongly correlated with total phenolic content in the plant extract and particularly the whole plant extract, flowers and leaves.

According to **Shabnam Mahernia et al. (2015)**, the *Matricaria inodora* plant exhibited a strong anti-urease activity due to the flavonoids contain such as **hydrolyzable tannins, gallo-tannins, volatile oil, flavonoids, anthocyanin, gallic acid, quercetin, and kaempferol** which act as anti-inflammatory operators and are used to relieve indigestion, promote appetite, and also for the treatment of stomach ulcers to help prevent food poisoning and treat swollen liver, and spleen **(Evans, 2009).**

5. *In vitro* evaluation of antidiabetic activity

5.1.A-amylase inhibitory assay

The evaluation of *the A. gombiformis* effect against α-amylase inhibitory assay was not been investigated before worldwide. For this objective, the anti-diabetic activity of this plant will be of major importance by using the microplate method **(Figure 40).**

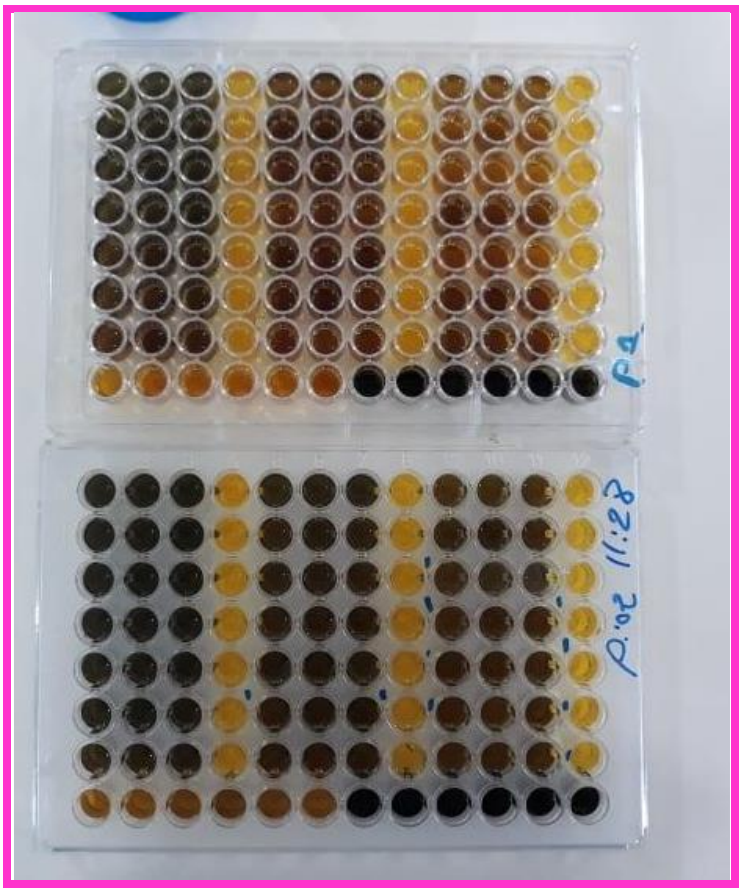


Figure 40: The α-amylase inhibitory assay on the microplate.

As shown in **Figure 41**, the results of α-amylase inhibitory obtained from different concentrations of acarbose and extracts of *A. gombiformis* is represented as IC50. The high half maximal inhibitory concentration were registered in the whole plant extract (IC50: 53.86±0.06 µg/mL), followed by flowers and Leaves (76.41±3.72 and 74.61±3.68 µg/mL).No significant difference between the both organs ($p<0.005$).

The IC50: 88.13±1.81 and 83.81±1.74 for the pods and stems are founded to be higher than acarbose used as a standard. (IC50: 275.43±1.00 µg/mL).

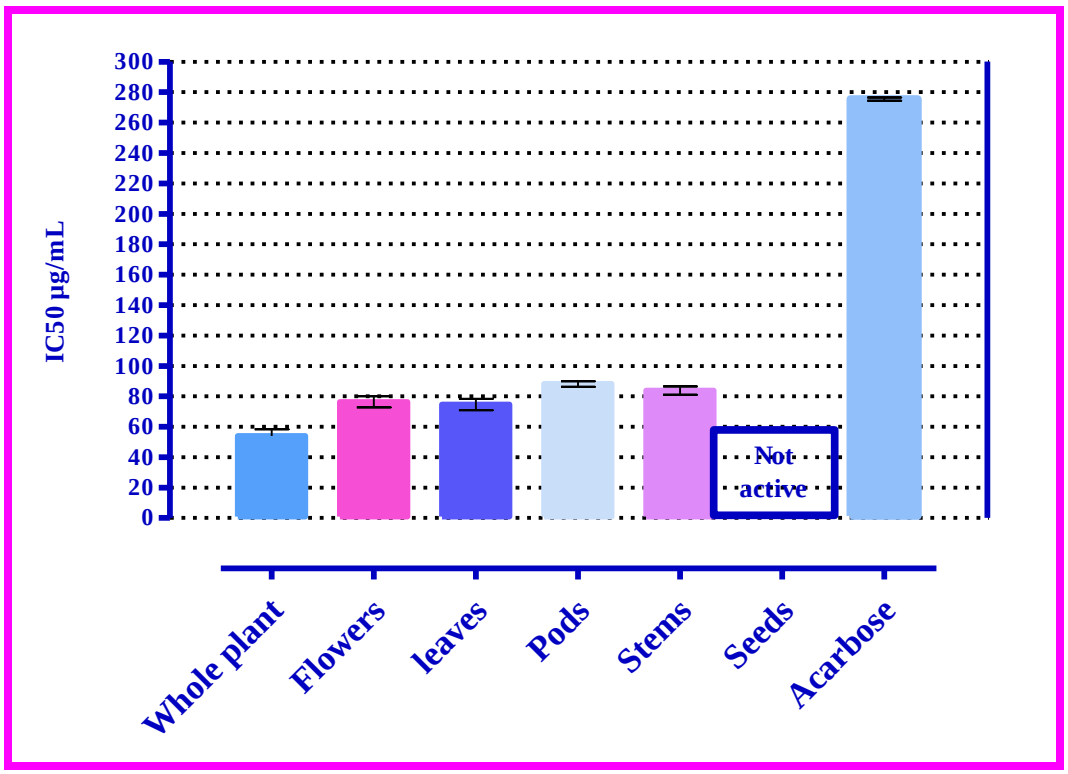


Figure 41: Alpha-amylase inhibitory assay of different organs of *A. gombiformis.*

5.2. A-glucosidase inhibitory activity

The potential of *A. gombiformis* plant to inhibit carbohydrate-hydrolyzing enzymes was assessed in this investigation. As showing in **Figure 42**, the results obtained are expressed as IC50 inhibition of α-glucosidase by acarbose, and *A. gombiformis* extracts.

It was observed that the inhibitory activity for the *α*-glucosidase enzyme was maximum by the whole plant extract (IC50: 23.9±2.3 µg/ml) followed by leaves fraction (IC50: 30.4±1.9 µg/mL), and flowers (IC50: 33.35±1.9 µg/mL). At all concentrations, the other fractions exhibited a modest activity.

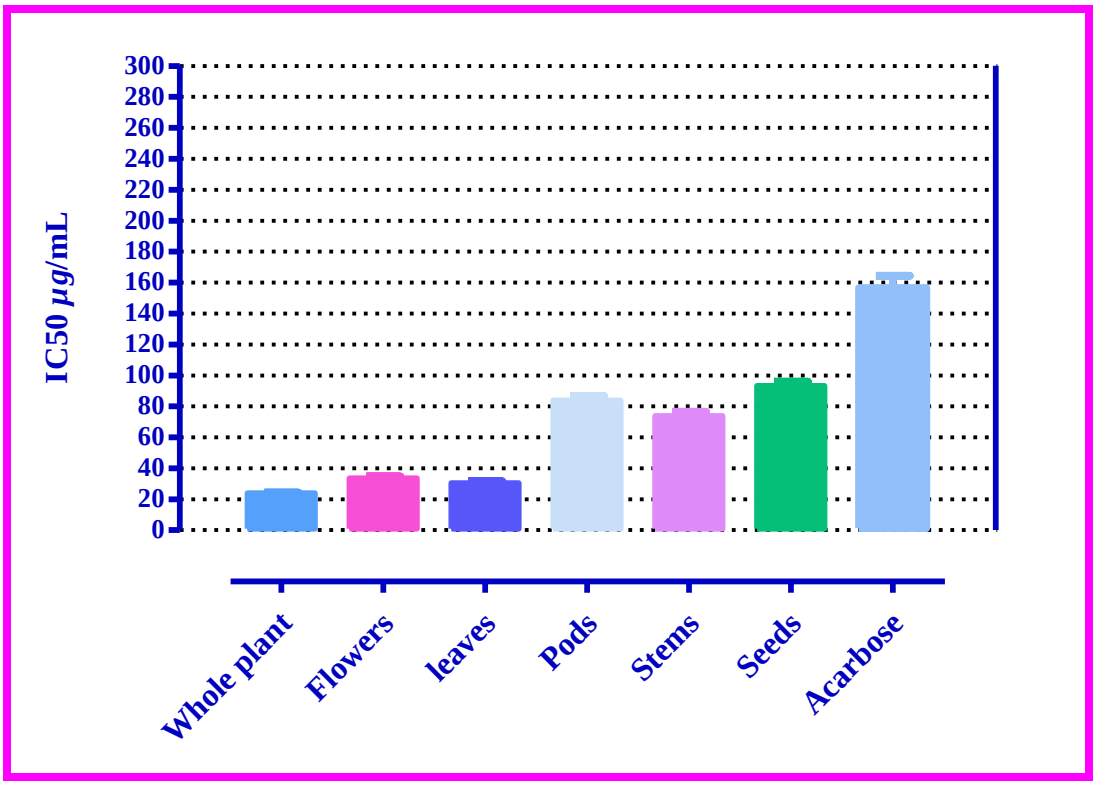


Figure 42: Alpha-glucosidase inhibitory assay of different organs of *A .gombiformis.*

This finding confirmed that the anti-diabetic property of *A. gombiformis* is related to enzymatic inhibition.

Diabetes mellitus is one of the chronic diseases caused by metabolic disorders **(Pallavi et al., 2015)** which are characterized by high levels of glucose in the blood resulting from the poor production and action of insulin **(Kojo et al., 2013).** This disease is treated either by injecting insulin or by oral anti-diabetic drugs **(Cheribet et al., 2019).**

The alpha-amylase and alpha-glucosidase inhibitors are considered as actives principles in oral antidiabetic drugs; they produce a delay in the release of glucose from complex carbohydrates as well as glucose absorption leading to lower plasma glucose levels.

In current research on anti-diabetic drugs, a good activity was founded by *A. gombiformis* extract against alpha-amylase inhibitor assay. Similar to our results,

A. ponticus.pall reported a high α-amylase and α-glucosidase inhibitory potential in leaves and roots extract **(Arumugam et al., 2018).**

From literature, **Kojo et al. (2013) and Zang et al. (2011)** have been well noted that *A. membranaceus* has a long history for the treatment of diabetes, the decoction of this herb reduces fasting blood glucose and homeostatic model assessment (HOMA) levels in type 2 diabetes mellitus patients.

Several researchers have indicated the presence of a positive correlation between the total polyphenols, total flavonoids, and the capacity to inhibit α-amylase **(Ramkumar et al., 2010).** Phenolic compounds are known for their ability to inhibit the activities of carbohydrate-hydrolyzing enzymes due to their ability to bind to proteins **(Shobana et al., 2009).**

In addition, flavonoids have a high *in vitro* and *in vivo* inhibitory potential of α-glucosidase **(Adefegha and Oboh, 2012).** In our study, LC-ESI-MS identification of phenolic compounds in whole plant extract as well as the flowers and leaves was showed the highest flavonoids content; this could explain the strong antidiabetic effect of the plant.

On the other hand, only the seed extract didn't exhibit any α-amylase inhibitory activity at 400 µg/mL, this finding may be explained by the presence of other bioactive compounds in whole plant extract, flowers, leaves, stems, and pods that distinguished by strong α-amylase inhibitory and was not identified in our study.

According to our literature survey, several researchers documented that polysaccharides, saponins in *Astragalus* are the major chemical constituents demonstrating biological activity to diabetes mellitus **(Who, 1999; Thorone, 2003),** which reinforces and confirms the hypothesis of the presence of other compounds which have not been detected by LC-ESI-MS.

6. *In vitro* evaluation of anti-Alzheimer activity

In biochemistry, Cholinesterase is an enzyme that catalyzes the hydrolysis of a choline ester (acetylcholine, butyrylcholine) into choline and acetic acid. This reaction is required in physiology for cholinergic receptors to return to their resting state after being activated.

This study was evaluated the capacity of *A. gombiformis* as an inhibitors enzyme of the cholinesterase family that used as a control and treatment to fight against Alzheimer′s disease **(Hebert et al., 1995).**

6.1.Butyrycholinesterase inhibitory activity

This assay was performed by the microplate method **(Figure 43).** The results of fractions tested were compared by the positive control (Galanthamine). The butanolic extract obtained from the whole plant exhibited the highest inhibitory effect against BChE (IC50: 44.09±0.1 ug/mL) with 72% of inhibition at 200 ug compared to the other parts.

Followed by the leaves extract (165.54±3.49 ug/mL) with a percentage ct of inhibition of 57% at 200 ug. Moreover, a weak inhibitory effect was detected by the stems, flowers, pod, and grains (IC50: <200 ug/mL) **Table 7.**

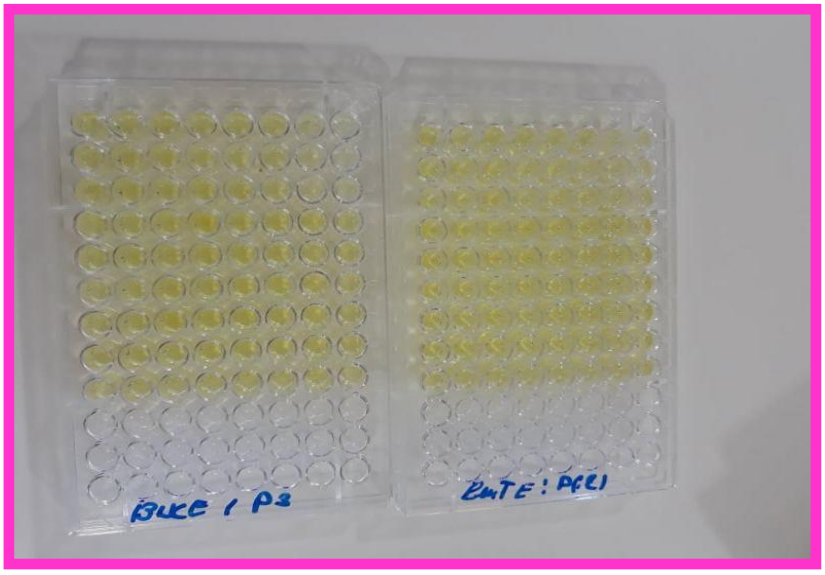


Figure 43: Butyrycholinesterase inhibitory activity on the microplate.

Table 7: Butyrycholinesterase activity (BchE) of *A. gombiformis* fractions

Extracts	BchE Inhibitory activity							
µg/mL	3.125	6.25	12.5	25	50	100	200	IC50
Whole plant	NA	NA	NA	NA	46.65±1.23	65.15±0.9	72.03±1.16	44.09±0.1
seeds	NA	NA	NA	NA	NA	5.69±1,45	40.80±3,53	>200
Leavs	NA	NA	NA	NA	1.96±2.91	39.72±1.45	57.10±2,08	165.54±3.49
pod	NA	NA	NA	NA	NA	19.47±0.95	44.16±3.94	>200
Stems	NA	NA	NA	NA	NA	4.97 ±4.15	38.41±1.45	>200
Flowers	NA	NA	NA	NA	NA	4.83±1.05	34.78±0.96	>200
Galantamine	35.9±2.28	43.8±0.0	68.5±0.31	80.7±0.41	85.8 ±1.63	91.8 ±0.20	94.8±0.34	6.27±1.15

IC50 values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expessed es Mean ± SD (n=3) NT: not absorbance. b: reference compounds

6.2.Acetylcholinesterase inhibitory activity

This activity was performed by the microplate method **(Figure 44),** The results obtained from the different concentrations of the six *A. gombiformis* extracts as well as the reference compound are shown in **Table 8.** The whole plant extract showed low activity against acetylcholinesterase (IC50 > 200 µg/mL), while the other extracts were inactive for all concentrations.

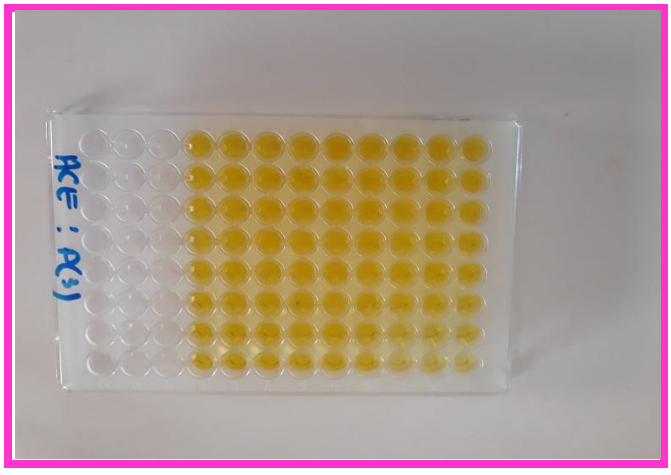


Figure 44: Acetylcholinesterase inhibitory activity on the microplate.

Table 8: Anticholinesterase activity (AchE) of *A. gombiformis* fractions

Extracts µg/mL	AchE Inhibitory activity							
	3.125	6.25	12.5	25	50	100	200	IC50
Whole plant	NA	NA	NA	NA	NA	6.03±1,45	22.14±3,53	>200
seeds	NA	NA	NA	NA	NA	NA	NA	//
Leavs	NA	NA	NA	NA	NA	NA	NA	//
Pod	NA	NA	NA	NA	NA	NA	NA	//
Stems	NA	NA	NA	NA	NA	NA	NA	//
Flowers	NA	NA	NA	NA	NA	NA	NA	//
Galantamine	35.9±2.28	43.8±0.0	68.5±0.31	80.7±0.41	85.8±1.63	91.8±0.20	94.8±0.34	6.27±1.15

IC50 values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expessed es Mean ± SD (n=3) NT: not absorbance. b: reference compounds

Alzheimer's disease (AD) or dementia disease, which affects around 10% of the population over the age of 65-year old, is caused by alteration of neurons which cause in decreasing of the neurotransmitter levels and lead to blocking the cholinergic transmission producing decline the cognitive function of patients **(Choi et al., 2012).**

The inhibitors of the cholinesterase family are the major advances in the treatment of Alzheimer's disease **(Ezio, 2004; Melkinova, 2007).**

Through our study, only the whole plant extract and leaves fraction were showed a considerable activity, According to our literature survey, *A. schizopterus* did not exhibit any enzyme inhibitory activity against AChE and BChE **(Haşimi et al., 2017),** while *A. leporinus Boiss. var. hirsutus* extract showed strong inhibitory by a values of IC50: 46.96± 4.06 and 66.15± 4.08 µg/ml against acetyl- and butyryl-cholinesterase. Furthermore, these findings contradict those of **Hassen et al. (2013)** whose results of *A. gombiformis* growing in Tunisia have shown a high anticholinesterase activity with an IC50 of 110 µg/ml.

This observation suggests the implication of other bioactive molecules than phenolic compounds in the inhibition of these enzymes and their interaction with secondary metabolites in the extract as well as their concentrations.

7. Photoprotective activity

The photoprotective activity of *A. gombiformis* extracts and the five separate organs was evaluated by calculating the sun protection factor (SPF). The value of the SPF corresponding to each extract is illustrated in **Figure 45.**

An excellent capacity to absorb UV radiation was registered by the whole plant extract of *A. gombiformis* (SPF: 37.78±0.85) followed by flowers and leaves extracts (SPF: 35.45±1.03 and 30.21± 1.50). while the other extracts showed a low SPF value (less than 15)

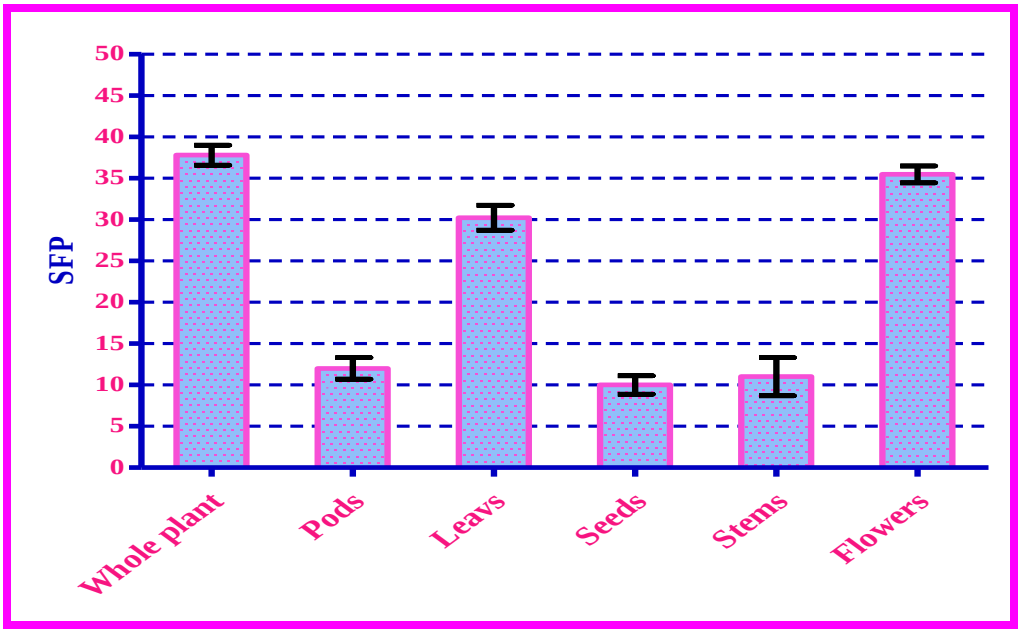


Figure 45: Values of sun protection factor of *A. gombiformis* extracts.

The deleterious effects of exposure to ultraviolet (UV) radiation on the skin have become more apparent. Numerous sunscreen and skincare products have therefore been developed to help to reduce the occurrence of sunburn, photoaging, as well as skin carcinogenesis. In this vein, this study has stimulated research on using new natural sources of effective skin-protecting compounds.

The photoprotective activity of *A. gombiformis* has not been previously investigated. Therefore, the data presented in this study represent an original contribution to the literature. According to the results, the SPF ranges from 10±1.12 to 37.78±0.85. The findings demonstrate that all extracts and fractions have a significant level of photoprotective activity.

According to reports of Napagoda **et al. (2016)**, UV-B rays absorbed by the skin have led to the formation of damage caused by free radicals such (O_2, ROO, OH, HO_2). As a result,

examining the most effective extracts for antioxidant activity is important for the development of more effective sunscreens.

The presence of phenolic and flavonoids compounds is typically responsible for these essential sun protection properties. Flavonoids including **quercetin, kaempferol, galanin, and apigenin** have substantial photoprotective benefits due to their antioxidant properties and UV absorption capability **(Stevanato et al., 2014; Saewan and Jimtaisong, 2013).**

Several researchers have recently correlated plant extracts' photoprotective activity to their flavonoid concentration **(Souza et al., 2015; De-Oliveira-Junior et al., 2013).**

Skin-protecting products having SPF values greater than 30 are considered to be effective UV-radiation filters **(Ratnasooriya, et al., 2016).** The presence of **rosmarinic acid** in the whole plant extract, which has been considered as a photoprotective agent against UV and other ionizing radiations might explain these results (**Sánchez-Campillo et al., 2009).** It has a high protective effect against the unfavorable influence of methylparaben and propylparaben on collagen in human skin fibroblasts (**Matwiejczuk et al., 2020).** This attenuates cell damage induced by UV-B radiation via enhancing the antioxidant defense system in human HaCaT cells **(Fernando et al., 2016).**

As a result, *A. gombiformis* extract can be used as a sun protection agent in sunscreen formulations to prevent sunburn. Our findings are in agreement with many results of crude extracts made from *Astragalus* spp, which are well-known for their medicinal properties and applications.

According to **Curnow and Owen (2016)**, *Astragalus membranaceus* and *Althea officinalis* are considered natural sources for UV-protecting dermatological formulations. We may also conclude that this photoprotective capacity may be due to the climate conditions of the Saharan region of *A. gombiformis*, as daily exposure to the sun leads to the production of more bioactive compounds to protect the plant from UV damage.

Several studies have revealed that the significant abilities of UV absorption are associated with the chemical constituents, especially flavonoids, and phenolics (**Martínez et al., 2012).**

Based on the results of this work, there is a strong correlation between total phenolic contents and the photoprotective activity of *A. gombiformis* extract. According to the literature, these compounds are considered excellent sun filters with significant photoprotective effects **(Saewan and Jimtaisong, 2013; De-Oliveira-Junior et al., 2017).**

Moreover, the presence of cyclic and aromatic hydrocarbons offers the ability to absorb ultraviolet light with wavelengths ranging between 240–285 nm and 300–550 nm **(Korac and Khambholja, 2011).**

8. Anti-inflammatory activity

As presented in **Figures 46 and 47.** The extracts of *A. gombiformis*, as compared to the references molecules (diclofenac and ketoprofen), had shown a concentration-dependent inhibitory activity against the protein denaturation induced by the high temperature.

At 500 µg/mL, a significant anti-inflammatory effect was obtained by the whole plant fraction with a percentage of inhibition of 75.38%, which was close to inhibition scores of diclofenac (99.23%) and ketoprofen (73.47%) at 250 µg/mL.

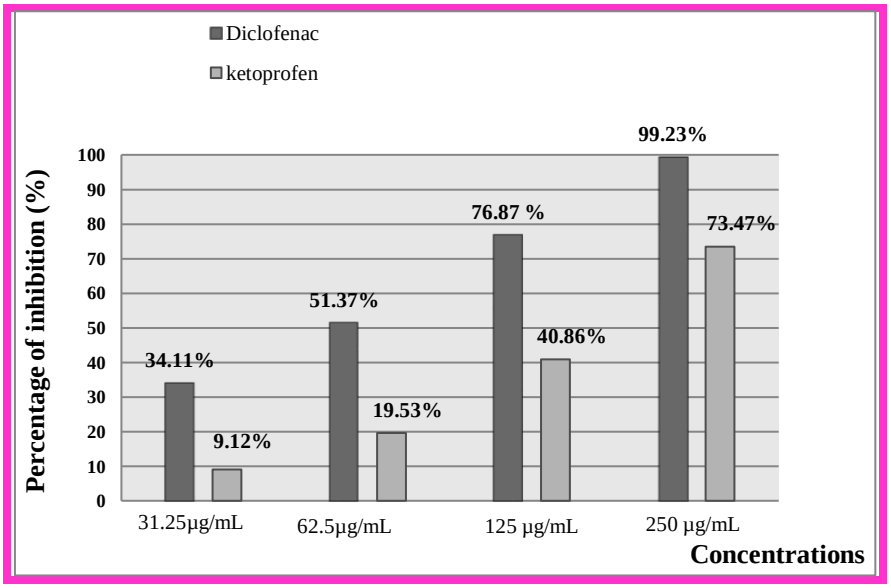


Figure 46: *In vitro* anti-inflammatory effect of diclofenac (Standard 1) and ketoprofen (Standard 2).

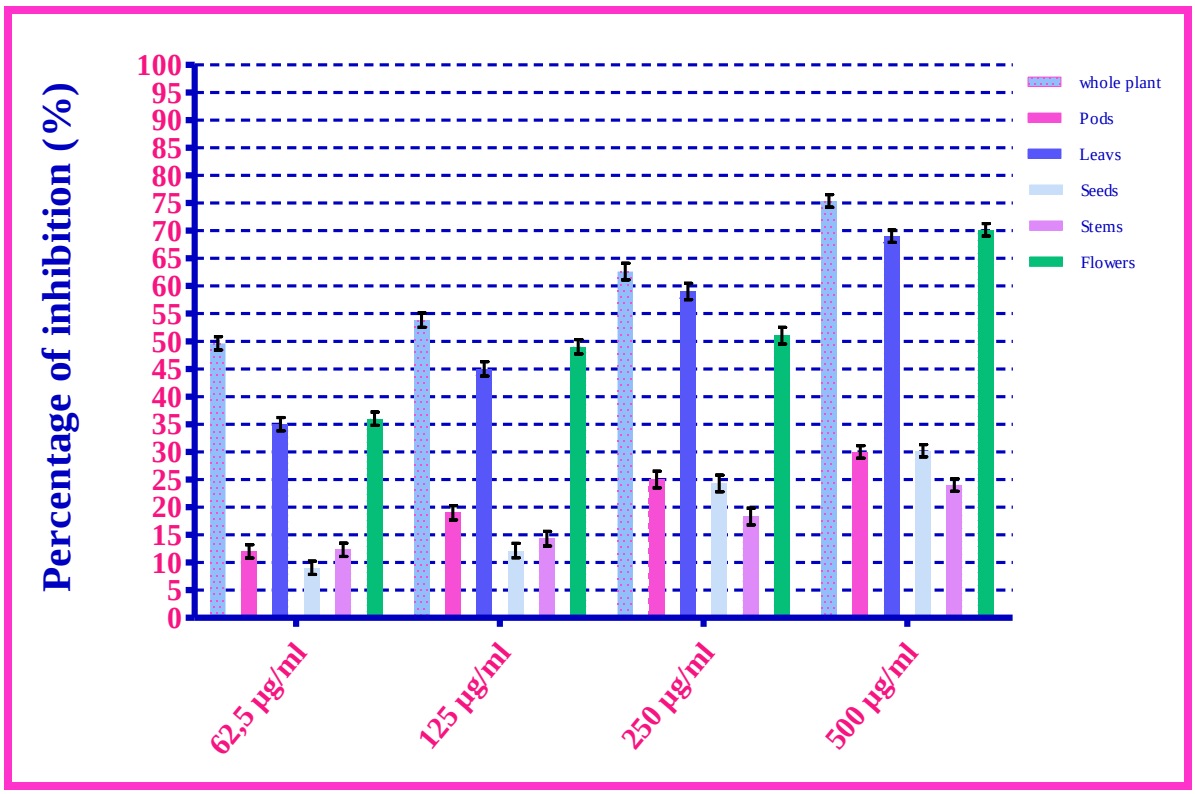


Figure 47: *In vitro* anti-inflammatory effect of *A. gombiformis* extracts.

The protein denaturation technique was used to test the anti-inflammatory properties of *A. gombiformis* extracts. The main mechanism of this denaturation is the alteration of electrostatic, hydrophobic, hydrogen, and disulfide bonds that stabilize the three-dimensional structure of proteins (**Mizushima and Kobayashi, 1968; Barros et al., 2008).**

Most proteins lose their biological activities after denaturation, lead to the formation of autoantigens, which induce autoimmune dysfunctions such as rheumatic and inflammatory diseases. Therefore, agents which inhibit protein denaturation are considered to be effective anti-arthritis and anti-inflammatory drugs **(Mouffouk et al., 2018).**

The results of *in vitro* anti-inflammatory activity revealed that the whole plant extract has a high capacity to preserve protein three-dimensional structure. These findings are in agreement with the results obtained for diclofenac, which is used as a standard by **Mouffouk et al. (2020)** and possesses an important anti-inflammatory effect (inhibition = 86.72%) at the same concentration as that in which *A. gombiformis* extract were tested (500 µg/mL)**.**

The Anti-inflammatory efficacy was shown to be highly correlated with phenolic chemicals and flavonoids previously discovered using the LC–ESI–MS method. Nevertheless, this anti-inflammatory capacity exhibited by this plant extract could be attributed to the

presence of the main bioactive molecules that have not been detected via, LC–ESI-MS analysis, such as fatty acids, carotenoids, and steroids. This hypothesis can be confirmed through the *in vitro* anti-inflammatory activity of cycloartane type saponins from *Astragalus* species that was investigated by **Nalbantsoy et al. (2012).**

9. The cytotoxic effect

9.1.Brine shrimp lethality bioassay (BSLB)

The brine shrimp lethality assay (BSLA) is a simple and inexpensive bioassay used for testing the efficacy and cytotoxicity of phytochemical present in the plant extracts. The present study determined that the extent of lethality was directly proportional to the concentration of the extract. After 24 h of observation all the shrimp were survived in the control treated with DMSO **Figure 48.**

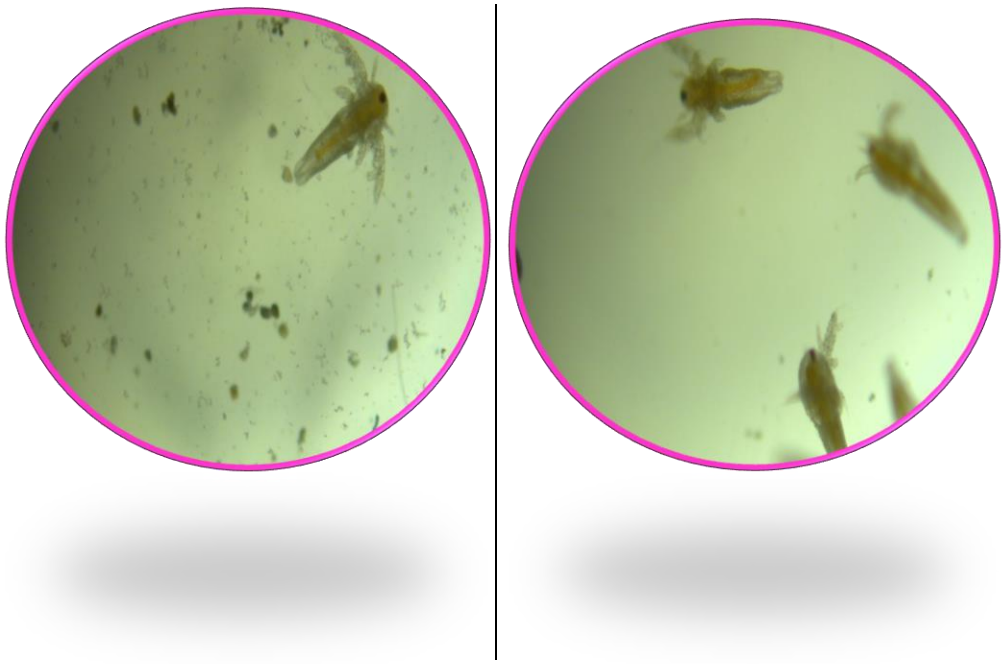


Figure 48: Fully grown Brine shrimps.

As showing in **Figure 49,** a significant cytotoxic effect was registered by leaves, flowers, and the whole-plant extract with a DC50 of 40.23±1.1; 40±1.2 and 44.7±1.76 µg/mL. This finding is very low compared with the standard tested $K_2Cr_2O_7$ which confirms that *the A. gombiformis* plant has no strong toxicity against the cells membranes.

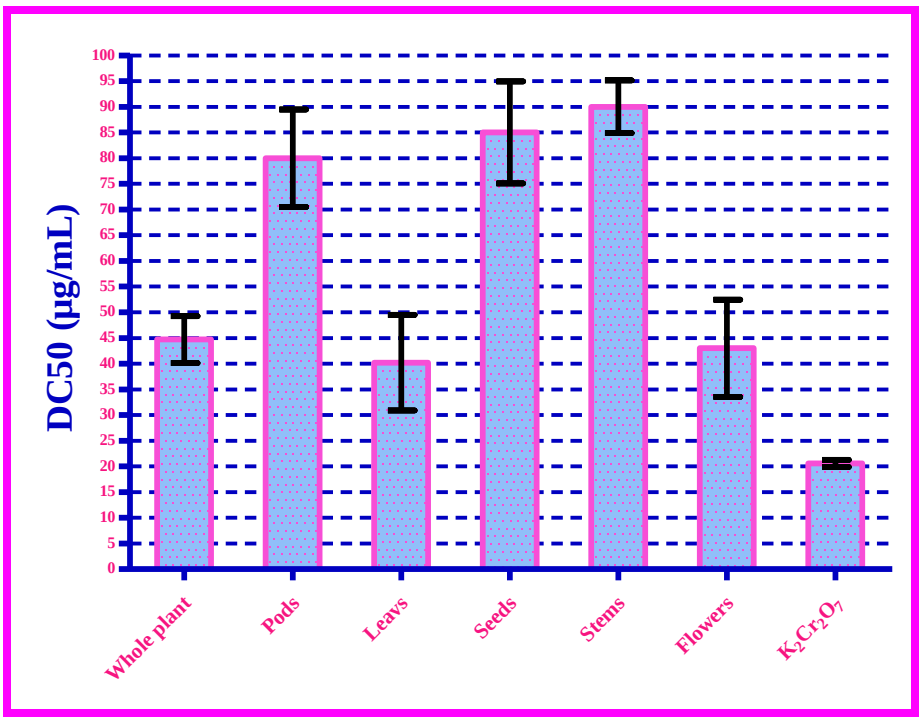


Figure 49: DC50 of brine shrimp lethality assay by *A. gombiformis* extracts and standard.

Brine shrimp bioassay was used to elucidate the possible toxicity of bioactive compounds in extracts which are generally toxic in low doses **(Mclaughlin and Rogers, 1998).** According to **Mouffouk et al. (2020)**, the secondary metabolites present in *Noneavesicaria* extract induced direct damage on membrane integrity by causing cell lysis. Therefore, these compounds may likely have cytotoxic effects, something that may also apply to *A. gombiformis.*

However, previous studies have shown that plant extract lethality against brine shrimp with a value of DC50 below 100 g/mL is reasonably correlated with cytotoxic and antitumor properties and may constitute potential antitumor and anticancer agents (**Moshi et al., 2010).**

Therefore, the cytotoxicity observed in this study could be related to the chemical profile of the tested plant and its metabolite content, such as saponins, which were detected previously through phytochemical screening and which are well known as antitumor agents with cytotoxic effects and antiproliferative potential **(Ayaz et al., 2016).**

Despite the low cytotoxic effect of the plant against *Artemia Salina,* it remains a plant with low side effects whose *Astragalus* genus is mostly used as fodder crop for both livestock and wild animals in dry regions as well as in industrial foods as a tea flavoring agent, coffee

substitute, and source of natural gum, and in cosmetics and pharmaceutical medicines **(Mahmoudia et al., 2021).**

9.2.The OxHLIA Oxidative Hemolysis Inhibition Assay

The results the OxHLIA *assay* of *A. gombiformis* extracts is represented in **Figure 50.** All the extracts exhibited a low hemolytic effect toward human erythrocytes. However, the hemolytic activity of these extracts was dose-dependent and increased with the increase in extract concentration.

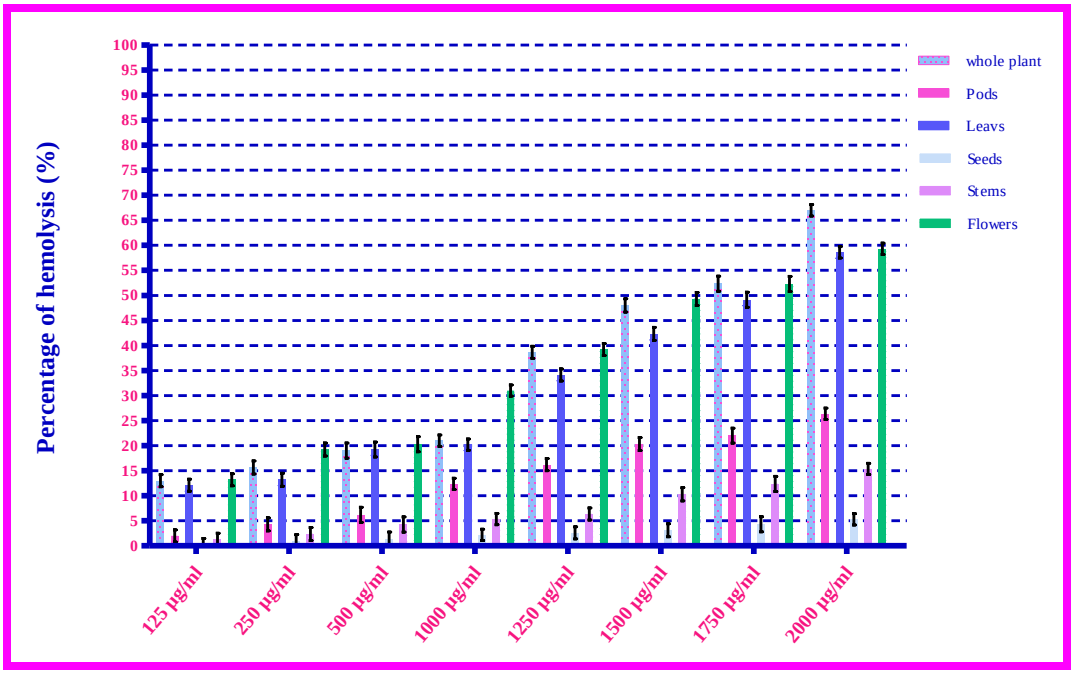


Figure 50: Percentage of hemolysis of *A. gombiformis* extracts.

The highest value of hemolytic effects was registered by the whole plant extract with EC50 of 1643.78 µg/mL, which was calculated according to the linear equation: $y = 0.027x + 5.618$ (R^2=0.923) (**Figure 51).**

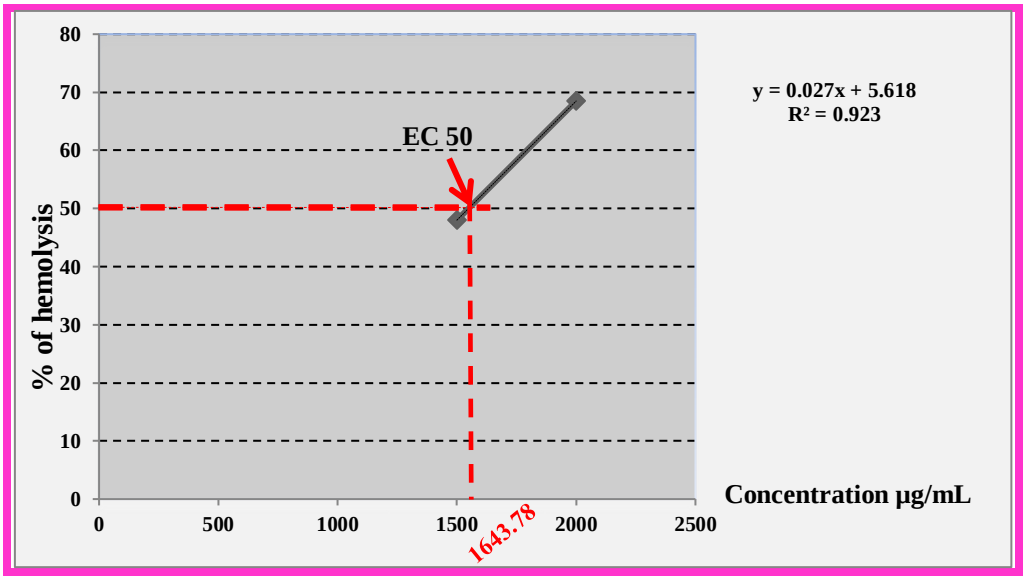


Figure 51: Linear equation of the curve to determine the EC50 of *A. gombiformis* butanolic extract.

The findings regarding OxHLIA activity are related to the chemical composition of the butanolic extract obtained from *A. gombiformis*, such as saponins. These compounds are well-known for their capacity to alter membranes by causing hole formation or the permeabilization of the erythrocyte membrane due to their amphiphilic properties (**Sharma et al., 2012).**

Hemolysis can also be induced by the presence of other metabolites such as alkaloids or phenolic compounds (**Voutquenne et al., 2002)**. Several investigations have demonstrated that saponins can cause erythrocytes to lyse (**Sharma et al., 2012; Milla et al., 2021)**, such as **iridoids**, which constitute the main cause of the hemolysis observed when using the n-BuOH and EtOAc extracts of *S. stellata*. In addition, iridoid glycosides cause hemolytic anemia and a decrease in red blood cells and hemoglobin. Accordingly, based on the results we obtained through phytochemistry screening, we may conclude that this ability is due to the presence of saponins **(Zhang et al., 2018),** which could be isolated and utilized as a food ingredient flavor enhancer and anti-yeast agent according to **Golmohammadi (2013).**

Indeed, some species of *Astragalus* have been used as a source of many stabilizers and commercial thickening agents, such as Tragacanth (E413). From a nutritional point of view, *Astragalu*s seeds are an important source of protein, carbohydrates, polyunsaturated fatty acids (PUFA), microelements, and vitamins, which are the most substantial nutrients for human beings (**Mahmoudia et al., 2021)**.

10.*In vitro* evaluation of antibacterial activity

10.1.Disks diffusion technique on solid medium

The effects of *A. gombiformis* extracts on the growth of the following bacterial strains: *Bacillus cereus*, *Staphylococcus aureus* (*ATCC*291), *Staphylococcus aureus* (*ATCC*43300), *Pseudomonas aeruginosa* (*ATCC*27853), *Escherichia coli* (*ATCC*25922), and *Acinetobacter baumanii* are shown in **Table 9.**

The antibacterial activities of extracts were evaluated by the diameter of the inhibition zone around the disk.

For the interpretation of antibacterial assay results, we adopted the following scale of measurement according to **Ponce et al. (2003).**

- The diameter < 8 mm: the strain is resistant to the plant extract tested.
- The diameter 8< D <14 mm: the strain is sensitive
- The diameter is between 14< D <19 mm: the strain is very sensitive
- The diameter > 20 mm: the strain is extremely sensitive

The results showed that the butanolic extracts of the whole plant, flowers, and leaves have a positive effect against all the bacterial strains tested with diameters of inhibitions range between 10±1.00 and 27.33±1.52 mm.

Staphylococcus aureus (*ATCC*291) and ***Staphylococcus aureus*** (*ATCC*43300) are extremely sensitive to:

- The butanolic fraction of the whole plant 24±2.01 and 27.33±1.52.
- The butanolic fraction of flowers 20±1.00 and 22.1±2.00.
- The butanolic fraction of leaves 20±1.00 20.5±1.10.

Escherichia coli (ATCC 25922) **is:**

- Extremely sensitive to whole plant extract (20 ± 1.53 mm at 20 mg / ml).
- Very sensitive to the butanolic fraction of flowers (18.10±2.2 mm at 20 mg / ml).
- Sensitive to seeds and Leaves extracts (13.12±1.10 and 10.12±1.10 mm).

***Pseudomonas aeruginosa* (*ATCC* 27853)** is:

- Very sensitive to whole plant 16.45±1.51mm and flowers 15.15±1.58mm at 20 mg / ml.
- Sensitive to leaves fraction 14 ±1.00 mm.

Acinetobacter baumanii is sensitive to:

The butanolic fractions of the whole plant, flowers, and leaves (13±1.00; 10±1.00 and 10.33 ±1.12 respectively at 20mg/mL).

Bacillus cereus is sensitive to:

The butanolic fraction of the whole plant and flowers (10 ± 0.00 mm) and leaves extract (9 ± 1.00).

On the other hand, the seeds fraction has a weak effect against ***Bacillus cereus***, ***Staphylococcus aureus*** (*ATCC* 291) and ***Staphylococcus aureus*** (*ATCC* 43300) (9.5±1.12; 9.2±2.00 and 9±1.00 mm respectively at 20 mg / mL).

The pods fraction has a weak effect (10.33 ± 2.51 mm) only against ***Escherichia coli*** (*ATCC* 25922), while stems fraction showed no activity against the strains tested.

The negative control (DMSO) had no effect against all the bacteria, while the positive control **(gentamicin)** revealed that all strains tested were **gentamicin**-sensitive.

Table 9: Diameters of inhibition zones (mm) against the bacterial strains tested by *A. gombiformis* extracts.

Bacterial strains	Extracts of *A. gombiformis* plant 20mg/ mL	Diameters of inhibition zones (mm)	GNT 20µg
Acinetobacter baumanii	Whole Plant	13±1.00	14.14±1.03
	Seeds	R	
	Leavs	10.33 ±1.12	
	Pod	R	
	Stems	R	
	Flowers	10±1.00	
Pseudomonas aeruginosa (ATCC 27853)	Whole Plant	16.45±1.51	23.45±1.10
	Seeds	R	
	Leavs	14 ±1.00	
	Pod	R	
	Stems	R	
	Flowers	15.15±1.58	
Escherichia coli (ATCC 25922)	Whole Plant	20 ± 1.53	30.1±1.1
	Seeds	13.12±1.10	
	Leavs	10.12±1.10	
	Pod	9±1.00	
	Stems	R	
	Flowers	18.10±2.2	
Bacillus cereus	Whole Plant	12.12±2.03	23.12±1.03
	Seeds	9.5±1.12	
	Leavs	10.12±1.10	
	Pod	R	
	Stems	R	
	Flowers	11.45±1.2	
Staphylococcus aureus (ATCC 291)	Whole Plant	24±2.01	21.13±2.00
	Seeds	9.2±2.00	
	Leavs	20±1.00	
	Pod	R	
	Stems	R	
	Flowers	20±1.00	
Staphylococcus aureus (ATCC 43300)	Whole Plant	27.33±1.52	23.46±1.02
	Seeds	9±1.00	
	Leavs	20.5±1.10	
	Pod	R	
	Stems	R	
	Flowers	22.1±2.00	

GTN: gentamicin R: RESISTANCE

10.2.Dilution method

The solid medium dilution method was used in order to determine the minimum inhibitory concentration; the results are shown in **Table 10.**

Table 10: Minimum inhibitory concentration (MIC) of the *A. gombiformis* extracts

	The minimum inhibitory concentration (MIC)					
	Whole Plant	Seeds	Leavs	Pod	Stems	Flowers
Acinetobacter baumanii	-	-	-	-	-	-
Pseudomonas aeruginosa (ATCC27853)	5	-	-	-	-	-
Escherichia coli (ATCC25922)	1.25	-	5	-	-	1.25
Bacillus cereus	-	-	-	-	-	-
Staphylococcus aureus (ATCC291)	2.5	-	5	-	-	1.25
Staphylococcus aureus (ATCC43300)	1.25	-	5	-	-	-

The antibacterial screening of *A. gombiformis* extracts against the bacterial strains tested showed different MIC values ranging from 1.25 to 5 mg / mL.

The inhibitory effect of the butanolic fraction of the whole plant started at 1.25 mg / mL against ***Escherichia coli*** (ATCC25922) and ***Staphylococcus aureus*** (ATCC43300), 2.5mg/mL against ***Staphylococcus aureus*** (ATCC291), and 5 mg/mL against ***Pseudomonas aeruginosa.***

While the flower extract stopped bacterial growth at a concentration of 1.25 mg/mL against ***Escherichia coli*** (ATCC25922) and ***Staphylococcus aureus*** (ATCC291).

The inhibitory effect of the flower extracts starting at concentrations of 5 mg / mL and 10 mg / mL against ***Staphylococcus aureus*** (ATCC43300) and ***Pseudomonas aeruginosa,*** respectively.

For the leaves fraction, the MIC was found at 5 mg / mL against ***Staphylococcus aureus*** (ATCC43300), ***Escherichia coli*** (ATCC25922) and ***Staphylococcus aureus (ATCC291).***

This findings are in agreement with **Tayeb et al. (2012)** study who found that the aqueous roots extract of *A. gombiformis* from Tunisia have MIC values of 233, 310, and 1250 μg/ml, respectively, against *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Salmonella typhimurium*, while the methanolic extract from roots showed also a MIC value of 1250 μg/ml.

Almost same in a research by **Türker et al. (2009),** aqueous, methanolic, and ethanolic extracts of *A. gymnolobus* Fisch demonstrated inhibitory zones against *Aeromonas hydrophila* with diameters of 7.75, 8.50, and 7.25 mm, respectively. **Jassbi et al. (2002)** presented another example of active chemicals from *Astragalus* species, stating that isolates from *A. brachystachys* have antibacterial activity against *Bacillus subtilis.*

According to **Brantner et al. (1996),** phenolic substances, flavonoids, and steroids could decrease bacterial growth. Lipophilic flavonoids have been demonstrated to destroy microorganism membranes by increasing the fluidity of membrane lipids.

Polyphenols, such as tannins and flavonoids such as epigallocatechin, catechin, myricetin, quercetin **(Shan et al., 2007),** and luteolin **(Askun et al., 2009)** are important antibacterial substances. The presence of phenolic compounds, as well as the presence of different secondary metabolites, the position and number of hydroxyl groups, all influence antimicrobial activity **(Falleh et al., 2008).**

A. gombiformis seems to have an effective antibacterial activity useful for the discovery of new antibiotics. Among the Fabaceae family, methanolic extracts of aerial parts of other Astragalus species such as *A. ponticus, A. microcephalus, A. macrocephalus, A. erinaceus, and A. argyroides* have been been tested for their antibacterial activity but, at 300 µg/disk, were found inactive against the tested bacteria **(Adigüzel et al., 2009).**

Antimicrobial resistance has steadily increased **(Stahl, 2006).** Many investigations on plant antibacterial properties have been conducted in order to identify novel therapeutic prospects **(Kudi et al., 1999; Mothana and Lindequist, 2005; Palombo and Semple, 2001).** Several commonly used antibiotics, including daptomycin and teicoplanin, were developed from plants **(Cragg et al., 1997).** In this regard, our findings may be useful to efforts aimed at the creation of natural antibacterial medicines.

Conclusion

"This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning"

- Winston Churchill

Conclusion

In recent years, many researchers have been interested in the bioactive compounds isolated from plant extracts, which are considered to be real treatments with maximum benefit against many human illnesses.

The main objective of our research is to investigate the phytochemical constituents to find the maximum accumulation of these substances in the different parts of the plants by measuring the antioxidant potential and its inhibitor effects of enzymes related to chronic illnesses.

The present study reported for the first time an extensive evaluation of biological activities of *Astragalus gombiformis* Pomel, an endemic species from Algeria. Phytochemical screening of stems, flowers, leaves, pods and seeds, and the whole plant revealed the presence of various types of secondary metabolites with interesting pharmacological activities such as saponins, tannins, flavonoids, and alkaloids.

The butanolic extracts of the whole plant as well as the 5 organs were subjected to qualitative estimation by LC-ESI-MS which demonstrated a richness of the plant in phenolic compounds. Based on the results, we can say that it is an important source of phenolic and flavonoids compounds.

Evaluation of antioxidant activity by six methods (**DPPH, ABTS, CUPRAC, GOR and *β*-carotene**) demonstrated that the butanolic extract of the whole plant is more active compared to other extracts.

The results of antidiabetic activity by the α-amylase and α-glucosdase method showed that the butanolic fraction of flowers and the whole plant has a very strong inhibitory activity

On the other hand, the *in vitro* anti-Alzheimer's activity was investigated by inhibition of acetylcholinesterase and butyrylcholinesterase, the results indicated that the whole plant and leaves fraction showed moderate activity against BChE and AChE.

These results showed that *Astragalus gombiformis* would be suggested as a potential source of natural antioxidants, which can replace synthetic antioxidants in cosmetics, pharmaceuticals, and food. In addition, the results revealed that *Astragalus gombiformis* flowers can be exploited in the discovery of natural bioactive products for the treatment of diabetes mellitus and Alzheimer's disease.

Conclusion

The results of the photoprotective activity showed an excellent capacity to absorb UV radiation exhibited by the whole plant extract of *A. gombiformis* followed by flowers and leaves

Whereas, *A. gombiformis* extract showed a good efficiency to inhibit thermally-induced protein denaturation in a dose-dependent manner. Moreover, the plant extract showed high potential therapeutic application as shown in the cytotoxic test. Economically, it is advised to use the whole plant to give greater returns compared to each organ separately, because of the considerable concentration of the phenolic compounds identified by LC-MS analysis in the whole plant.

Taken together, the high contents of bioactive compounds play more significant roles as novel raw materials for functional foods and promising chemical additives in the food and pharmaceutical industry.

Based on the above findings, *A. gombiformis* plant can be considered as an important source of bioactive components in therapeutic medicine, as well as, in cosmetics applications as a photoprotective factor with anti-inflammatory effect and antioxidant response induced by UV radiation.

These findings are preliminary, and it would be great to evaluate the activity of highly purified fractions and identify the important molecules that underlie the varied activities found in various extracts using more efficient techniques. In any case, it is important to highlight that the tests were performed *in vitro*. It is thus mandatory to confirm these findings by *in vivo* studies to obtain useful information for eventual therapeutic or dietary interventions.

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Appendix

Table 11: Analytical characteristics of selected phenolic compounds.

D#	Analyte	% Purity	Ret. Time	([M-H]-)	RSD curve calibration	(R2)	Linear range µg/mL))	LOD (µg/mL)	LOQ (µg/mL)
1	Quinic acid	98	2,028	191.00	14.320	0.9952	0.05–7.5	0.616	1.867
2	Gallic acid	97.4	4,317	169.00	10.548	0.9999	0.05–7.6	0.102	0.308
3	Protocatchuic acid	98	--	153.00	17.688	0.9995	0.10–7.5	0.122	0.369
4	Catechin (+)	99	12,606	289.00	10.700	0.9993	0.05–5.0	0.063	0.191
5	Caffeic acid	98	16,167	179.00	8.102	0.9993	0.05–1.0	0.384	1.165
6	Syringic acid	98	18,033	197.00	10.861	0.9965	0.05–5.0	0.373	1.131
7	1,3-di-O-caffeoyquinic acid	>90	19,031	515.00	16.843	0.9981	0.05–20.0	0.156	0.474
8	Epicatechin	98	18,604	289.00	9.921	0.9988	0.05–20.0	0.155	0.468
9	*p*-coumaric acid	98	22,350	163.00	5.560	0.9981	0.05–7.5	0.337	1.022
10	Rutin	98	25,256	609.00	11.809	0.9991	0.05–20.0	0.172	0.521
11	*trans*-ferulic acid	99	24,550	193.00	5.231	0.9982	0.05–7.5	0.624	1.890
12	Hyperoside (quercetin-3-O-galactoside)	98	25,712	463.00	10.851	0.9964	0.05–20.0	0.115	0.349
13	Luteolin-7-O-glucoside	97	25,949	447.00	16.329	0.9983	0.05–15.0	0.092	0.280
14	3,4-di-O-caffeoyquinic acid	>90	26,263	515.00	15.366	0.9981	0.05–5.0	0.697	2.114
15	Naringin	95	27,384	579.00	13.362	0.9973	0.05–5.0	0.001	0.002
16	Rosmarinic acid	98	27,876	359.00	8.618	0.9995	0.05–15.0	0.115	0.454
17	4,5-di-O-caffeoyquinic acid	>90	28,196	515.00	17.059	0.9968	0.05–5.0	0.166	0.504
18	Quercitrin (quercetin-3-O-rhamnoside)	91.4	28,223	447.00	10.970	0.9996	0.05–5.0	0.171	0.520
19	Apigenin-7-O-glucoside	98	28,336	431.00	12.817	0.9989	0.05–2.0	0.821	2.489
20	*O*-coumaric acid	≥ 90	27,661	163.00	7.064	0.9984	0.05–7.5	0.203	0.617
21	Salvianolic acid	≥ 95	29,474	717.00	13.123	0.9976	0.05–2.0	0.143	0.432
22	Kaempferol	97	33,269	285.00	12.466	0.9985	0.05–5.0	0.148	0.450
23	Quercetin	98	33,264	301.00	11.756	0.9985	0.05–2.0	0.233	0.705
24	*trans* cinnamic	99	--	147.00	16.931	0.9958	0.05–7.5	0.887	2.688
25	Silymarin	>95	35,073	481.00	13.218	0.9952	0.05–20.0	0.051	0.154
26	Naringenin	95	35,286	271.00	10.058	0.9970	0.05–2.0	0.115	0.349
27	Apigenin	>95	35,847	269.00	11.067	0.9981	0.05–1.0	0.068	0.206
28	Luteolin	97	36,244	285.00	12.376	0.9973	0.05–5.0	0.516	1.565
29	Cirsiliol	95	36,585	329.00	12.911	0.9982	0.05–5.0	0.030	0.090
30	Cirsilineol	95	40,037	343.00	6.743	0.9977	0.05–2.0	0.181	0.548
31	Acacetin	≥99	42,087	283.00	20.134	0.9987	0.10–7.5	0.085	0.258

LOD and LOQ: limit of detection and limit of quantification, respectively.

Appendix

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Investigation of Photoprotective, Anti-Inflammatory, Antioxidant Capacities and LC–ESI–MS Phenolic Profile of *Astragalus gombiformis* Pomel

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Abstract: Plant-derived compounds have recently been gaining popularity as skincare factors due to their ability to absorb ultraviolet radiations and their anti-inflammatory, and antioxidant properties. In this light, this work aimed to evaluate in vitro the pharmacological activities of the butanolic extract prepared from the aerial parts of *Astragalus gombiformis* Pomel, an endemic species to southern Algeria. The sun protection factor was used to assess the photoprotective effect (SPF), the protein denaturation method to determine the anti-inflammatory activity, and brine shrimp nauplii and OxHLIA assay, respectively, to assess the cytotoxicity and antioxidant capacity of *A. gombiformis*. In addition, LC–ESI–MS analysis was employed for the characterization of the phenolic constituents of *A. gombiformis*. The results showed that *A. gombiformis* had high capacity for absorbing UV radiations with an SPF of 37.78 ± 0.85 and significant anti-inflammatory activity with a percentage inhibition of 75.38% which is close to that of diclofenac and ketoprofen. In addition, *A. gombiformis* was found to have effective cytotoxicity against *Artemia nauplii* with a DC50 value of about 44.7 µg/mL, but a weak hemolytic effect against human erythrocytes. LC–ESI–MS results detected the presence of 17 phenolic compounds with a predominance of cirsiliol, silymarin, quercitrin (quercetin-3-O-rhamnoside), and kaempferol. Taken together, these results suggest that *A. gombiformis* extract could be used as a skincare agent in cosmetic formulations, providing excellent antioxidant and anti-inflammatory protection, allowing the treatment of skin conditions, as well as a pharmaceutical agent with multidimensional applications.

Keywords: *Astragalus gombiformis* Pomel; photoprotective effect; anti-inflammatory activity; antioxidative activity; cytotoxicity; phenolic compounds; LC–ESI–MS

1. Introduction

The skin is the most regenerative organ in the body, representing a barrier with important roles, such as the regulation of water balance, thermogenesis, and permeability;

Appendix

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A comparative study on chemical profile and biological activities of aerial parts (stems, flowers, leaves, pods and seeds) of *Astragalus gombiformis*

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ABSTRACT

The present work aims to characterize the chemical profile of phenolic compounds and some biological acti ties of *Astragalus gombiformis*. The butanolic fractions of five aerial organs (stems, flowers, leaves, pods a seeds) were quantified and identified by LC–MS analysis. The results were revealed the presence of 13 phe lic compounds (quinic acid, p-coumaric acid, transfrulic acid, Hyperoside (quercetin-3-o-galactosi Quercetrin (quercetin-3-o-rhamonoside), Apegenin-7-o-glucoside, kampherol, Naringenin, Apegenin, Luteol Cirsiliol, Cirsilineol and Acacetin. In terms of biological activities, the anticoxidant, α-amylase inhibitory a anticholinesterase were determined. The butanolic extract from flowers showed the highest antioxidant act ity in DPPH and ABTS(IC50: 16, 43 ± 0, 46 and 16, 13 ± 0, 35 μg/mL) very closer with standards tested a week activity with Galvinoxyl radical (GOR) (IC50: 583, 95 ± 2, 20 μg/mL.), while other organ's extract hibited moderate antioxidant activity. The leaves extract was found to exhibit the highest inhibitory eff against BChE (IC50: 165,54 ± 3,49 μg/mL) compared by the other parts which give a weak inhibitory eff at 200 μg/mL. A significant alpha-amylase inhibitory activity was displayed by seeds, leaves, pods and ste extracts (IC50:76.41 ± 3.72; 74.61 ± 3.68; 88.13 ± 1.81 and 83.81 ± 1.74 μg/mL) more the standard us Based on these results, it is right to conclude that *A. gombiformis* is important source of the natural a diabetic, antioxidants and anti-Alzheimer's disease.

1. Introduction

During the past few years, increasing interest has focused in natural products from medicinal plants that represent important source of bioactive compounds for treating various human diseases(Ak and Gulçin, 2008; Gulçin, 2009; Gulçin, 2010; Gulçin, 2012; Gulçin, 2020). With multiple biological activities, many plants contain natural antioxidants compounds attracts the attention of several research teams for its role against numerous illness. The *Astragalus* genus is member of the *Fabaceae* family, is commonly used as forage for livestock and by wild animals as well as in food and medicines (Ríos and Waterman, 1997; Zarre-Mobarakeh, 2000). In china, the roots of *A. membranaceus* have a long history in traditional chinese medicine (Yin et al., 2006) and especially for antidiabetic treatments (Kojo al., 2013). Moreover, leaves and roots of *A. glycyphyllos* are wide used to their purifying, refreshing, diuretic and many other propert (Ionkova, 2008; Pistelli, 2002; Godevac et al., 2008; Guarino et a 2008).In Anatolia, *A.trojanus* aqueous extract is traditionally us against leukemia (Bedir et al., 2001). Many researches on *Astragu* genus have been demonstrated the presence of different seconda metabolites such as *A. trojanus*, *A. microcephalus*, *A. zahlbruckn* (Calis et al., 2001; Bedir et al., 1998, 1999). In the framework of v orization of the Algerian natural surface and to develop new produc we are interested in this work by *Astragalus gombiformis* Pomel t belongs to the *leguminosae* plant obtained from arid zoon (El Oue Algeria).In order to have an overall picture of distribution, identifi

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