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Title

Study of the cytotoxicity and therapeutic virtues of some aromatic and medicinal plants used in traditional Algerian medicine

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Résumé

La présente étude vise à évaluer la cytotoxicité et les vertus thérapeutiques de certaines plantes utilisées en médecine traditionnelle algérienne. Les résultatsobtenusdémontrent que l'extrait aqueux d'*E. spinosus* présente un risque élevé de cytotoxicité, un faible effet antihémolytique et anti-inflammatoire, mais une activité antioxydante très élevée. Cependant, l'extrait aqueux de *B. incrassatum* présente une cytotoxicité non significative ainsi que des activités anti-inflammatoires et antioxydantes élevées. En outre, l'extrait aqueux d'*U. dioica*

présente une cytotoxicité modérée représentée par son effet hémolytique, ses effets anti-inflammatoires et antioxydants élevés.

L'analyse quantitative a révélé la présence de teneurs plus élevées en polyphénols et tanins chez *B. incrassatum* et *U. dioica* contre des teneurs plus élevées en flavonoïdes et tanins chez *E. spinosus*. Cette variation pourrait expliquer en partie la différence observée en termes de cytotoxicité et d'activités biologiques.

Des études complémentaires sont recommandées pour évaluer expérimentalement le potentiel biologique et la toxicité des plantes aromatiques et médicinales utilisées en Algérie.

Mots clés

Buniumincrassatum,*Echinopsspinosus*, *Urticadioica*,cytotoxicité, activité hémolytique, activité anti-hémolytique, activité anti-inflammatoire, activité anti-oxydante, polyphénols, flavonoïdes, tannins.

Abstract

The present study aims to evaluate the cytotoxicity and therapeutic virtues of some plants used in Algerian traditional medicine. The obtained results demonstrate that the aqueous extract of *E. spinosus* presents a high risk of cytotoxicity, a weak antihemolytic and anti-inflammatory effects, buta very high antioxidant activity. However, the aqueous extract of *B. incrassatum*presents a non-significant cytotoxicity along with high anti-inflammatory and antioxidant activities. Besides, the aqueous extract of *U. dioica*presents a moderate cytotoxicity represented byits hemolytic effect, high anti-inflammatory and antioxidant effects.

The quantitative analysis has revealed the presence of higher contents of polyphenols and tannins in *B. incrassatum*and *U. dioica* against higher contents of flavonoids and tannins in *E. spinosus.*This variation could explain partially the difference observed in terms of cytotoxicity and biological activities.

Further studies are recommended to experimentally evaluate the biological potential and toxicity of the used aromatic and medicinal plants in Algeria.

Keywords

Buniumincrassatum, *Echinopsspinosus*, *Urtica dioica*, cytotoxicity, hemolytic activity, anti-hemolytic activity, anti-inflammatory activity, antioxidant activity, polyphenols, flavonoids, tannins.

الملخص

تهدف الدراسة الحالية إلى تقييم السمية الخلوية والخصائص العلاجية لبعض النباتات المستخدمة في الطب التقليدي الجزائري. تظهر النتائج التي تم الحصول عليها أن المستخلص المائي من*Echinopsspinosus*يظهرمخاطر عالية من السمية الخلوية، وتأثير ضعيف مضاد للالتهابات، ولكن النشاط مضاد للأكسدة مرتفع للغاية. من جهةاخرى، فإن المستخلص المائي لـ*Buniumincrassatum*لا يظهرسمية خلوية بينما نشاطه المضاد للالتهابات والمضاد للأكسدةعالي جدا. إلى جانب ذلك، فإن المستخلص المائي لـ*Urticadioica*يظهرسمية خلوية معتدلة تتمثل في تأثيره الانحلالي وتأثيراته العالية المضادة للالتهابات والمضادةللأكسدة.

أظهر التحليل الكمي وجود محتوى عالي من البوليفينول والعفص في*B. incrassatum*و*U. dioica*مقابل محتوى أعلى من الفلافونويد والعفص في*E. spinosus*يمكن أن يفسر هذا الاختلاف جزئيًا الاختلاف الملحوظ من حيث السمية الخلوية والأنشطة البيولوجية.

يوصى بإجراء مزيد من الدراسات لإجراء تقييم تجريبي للإمكانيات البيولوجية والسمية للنباتات العطرية والطبية المستخدمة في الجزائر.

الكلمات الدالة

Buniumincrassatum، *Echinopsspinosus*، *Urticadioica*، السمية الخلوية ، النشاط الانحلالي ، النشاط المضاد للدم النشاط المضاد للالتهابات ، النشاط المضاد للأكسدة ، البوليفينول ، الفلافونويد ، العفص.

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WHO	World Health Organization
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic acid
RNA	Ribonucleicacid
UV	Ultraviolet radiation
AIS	Anti-Inflammatory Steroidal
NSAI	Anti-Inflammatory non-steroidal
Vit	Vitamin
Fig	Figure
Rpm	Rotation per minute
mg/ml	Milligram per milliliter
Abs	Absorbence
M	Mole
mM	Millimole
I	Inhibition
%	Percentage
C°	Temperature in degrees Celsius
Ac	Ascorbic Acid
GA	Gallic acid
Q	Quercitine
Eq	Equivalent
GAE	Gallic acid equivalent
QE	Quercetin equivalent
CE	Cyanidine equivalents
IC_{50}	50% inhibitory concentration
FRAP	Ferric Reducing Antioxidant Power
DPPH	2,2 – diphenyl-1picrylhydrazyl
ABS	Albumin Bovin Serum
H_2SO_4	Sulphuric acid
$AlCl_3$	Aluminum Trichloride
$FeCl_3$	Iron chloride
Na_2CO_3	Sodium carbonate
H_2O_2	Hydrogen peroxide
PBS	Phosphate buffered saline
NaCl	Sodium chloride
TCA	Trichloroacetic acid

NaOH	Sodium hydroxide
$K_3Fe(CN)_6$	Potassium Ferrocianide
H	Hour
µl	Microliter
L	Liter
M	Mass
G	Gram
Mg	Milligram
Min	Minute
Ml	Milliliter
Nm	Nanometer

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Introduction

Since ancient times, humanity has used various remedies found in its environment to treat all kinds of diseases (Lee 2004). There has been a great revival of reliance on herbal product medication to manage traditionally various ailments (Perrino et al. 2021).Traditional medicine encompasses knowledge and practices about theories, beliefs and experiences of different cultures (WHO 2013). According to the World Health Organization (WHO), 80% of the world's population, especially people in developing countries, is dependent on traditional medical practices for some aspect of primary health care (Omwenga et al. 2015).

Today still a majority of the world's population, is treated mainly with traditional herbal remedies (Kharchoufa et al. 2021).Medicinal plants still remain the first reservoir of new drugs, they are considered as a source of essential raw material for the discovery of new molecules needed for the development of future drugs (Chabrier 2010).This source seems inexhaustible roughly 400.000 known plant species have been investigated chemically and pharmacologically, and each species can contain up to several different constituents (Ferhat 2016). Aromatic and medicinal plants have the ability to synthesize many compounds called secondary metabolites and thus constitute an immense reservoir of compounds of great chemical diversity such as polyphenols, flavonoids and tannins, possessing a wide range of biological activities (Hadouchi et al. 2006).

Algeria holds a rich and diversified flora represented by numerous aromatic and medicinal plants, most of which grow spontaneously. In fact, there are around 3,183 plant species in Algeria, most of which have therapeutic properties (Taïbi et al. 2021). The valorization of these plants remains an area of great importance for the country and might offer immense opportunities for sustainable development in the short and medium term. However, this heritage is fragile and the constraints which threaten it are multiple such as deforestation, pollution, degradation of rangelands and desertification among others.

Nonetheless, there is a significant knowledgegap regarding aromatic and medicinal plants uses safety (Kharchoufa et al. 2021).Though the use of medicinal plants can have deleterious effects on health as reported elsewhere (Fall et al. 2011). Indeed, several investigated plants contain toxic substances like some secondary metabolites (Boukandou Mounanga et al. 2015).It has been shown that the toxicity of a given plant depends on various factors, including the strength of secondary metabolites, the quantity consumed, the time of exposure, different parts of the plant (root, oil, leaves, stem bark and seeds), individual body chemistry, climate and soil, and genetic differences within the species (Tülay 2012).

This said it clearly appears that medicinal plants should be used with precautions and toxicology studies conducted to increase the knowledge on the plant or plants preparation given to populations.

For this purpose, the objective of this study is to evaluate the toxicity and/or safety of use of some aromatic and medicinal plants used in Algerian traditional medicine,namelynettle (*Urtica*

*dioica*L.),Pig Nut(*Buniumincrassatum*(Boiss) Batt. et Trab.) and Echinops (*Echinopsspinosus* L.)on the basis of some cytotoxic tests and biological activities such as hemolytic, anti-hemolytic, anti-inflammatory, and antioxidant activities alongside with the analysis of the phytochemical composition.

Hence, this study will make possible to assess the knowledge relating to thegood uses of local aromatic and medicinal plants as well as the dangers linked to their misuse.Consequently, this study constitutes a significant contribution in the development of a national strategy for the standardization of natural products'uses while ensuring optimum quality, efficiency and safety.

Literature review

1. Traditional medicine

Traditional medicine or as it is known as natural medicine or alternative medicine is defined by the World Health Organization (WHO 2013) in the strategic plan for 2014-2023, as « the sum of the knowledge, skills and practices based on the theories, beliefs and experiences of different cultures, whether explainable or not, which are used in the preservation of health, as well as in prevention, diagnosis, the improvement or treatment of physical or mental illnesses».

In reality, traditional medicine is a concept that goes far beyond the field of health to place itself at the broadest socio-cultural, religious, political and economic level,and are used to keep human beings healthy and to prevent, diagnose, treat and cure physical and mental illness (Epelboin 2009).

2. Aromatic and medicinal plants

Herbs represented often the original sources of most drugs. Medicinal plants are all plants that contain bioactive natural substances and compounds that may be used for therapeutic purposes (Chabrier 2010). Medicinal plants and their derived compounds are widely used in traditional medicines around the world even they still increasingly popular in modern society as natural alternatives of conventional treatments (Van Wyk etWink 2018). Approximately 35.000 plant species are used for medicinal purposes (Elqajet al. 2007).

3. Secondary metabolites of aromatic and medicinal plants

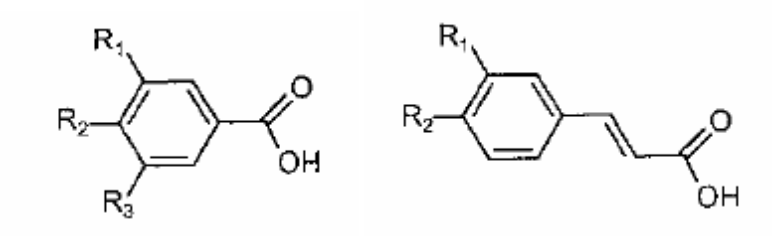
Plants produce various organic compounds often distributed differently among limited taxonomic groups in the plant kingdom. These compounds are responsible for several biological activities of aromatic and medicinal plants (Kone 2018).

3.1. Polyphenols

Polyphenols constitute an important class of natural compounds widely distributed in the plant kingdom. Scientists have identified more than 8.000 of them, ranging from simple molecules to highly complex compounds. Their accumulation in plants varies quantitatively and qualitatively not only among the different plant parts, but also from one plant species to another (Saffidine 2015).

Phenolic compounds or polyphenols are secondary metabolites characterized by the presence of a 6-carbon aromatic ring with free or carbohydrate hydroxyl groups (Nathalie et Jean-Paul. 2006). They possess strong antioxidant properties and have several other specific biological actions that could prevent or treat various diseases (Daiet Mumper2010).

Two classes of phenolic acids can be distinguished derivatives of benzoic acid or hydroxybenzoic acids and cinnamic acid or hydroxycinnamic acids (Fig. 1) (Manach et al. 2004).



1. Hydroxybenzoic acids.

R1=R2=OH, R3= H: Protocatechuic acid.

R1=R2=R3=OH: Gallic acid.

2. Hydroxycinnamic acids.

R1=OH Coumaric acid.

R1=R2=OH Caffeic acid.

R1=OCH3, R2=OH: Ferulic acid.

Figure1. Chemical structures of phenolic acids (Manach et al. 2004).

Modern techniques of isolation of molecules and medical exploration, show that the latter have many therapeutic properties. They have anti-cancer properties (Li et al. 2008), anti-ulcer (Martin et al. 2007), anti-inflammatory (Nowakowska 2007) and antioxidant (Sarni etCheynier 2006).

3.2. Flavonoids

Flavonoids refers to a very wide of natural compounds belonging to the polyphenols' family.They are of particular interest to plant qualities because they contribute to the color and flavor of fruits and vegetables, some of these compounds have biological activities of interest, such as antioxidant actions, and are said to have health-promoting effects, and these include protecting plants from ultraviolet radiation (UV) and pathogens (Fiorucci 2006; Lillo et al. 2008).

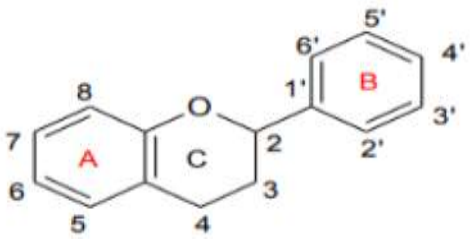


Figure2. Basic structure of flavonoids (Abedini 2013).

Their basic structure is that of a diphenylpropane with 15 carbon atoms (C6-C3-C6) consisting of two aromatic nuclei (or rings), referred to by the letters A and B, linked by an oxygenated heterocycle, referred to by the letter C (Fig. 2)(Liu 2004).

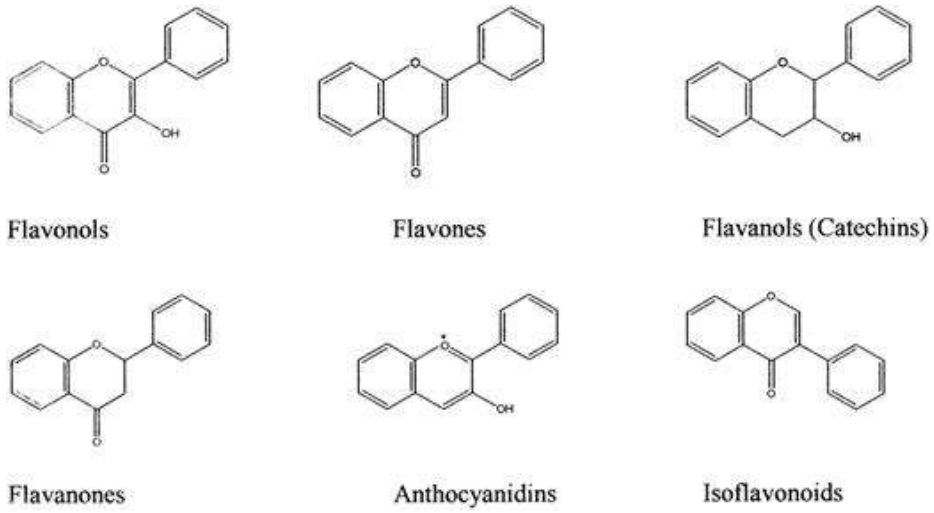


Figure 3. Structures of main classes of dietary flavonoids (Liu 2004).

They are mainly classified in flavones, flavonols, flavanones, flavanols, isoflavone, and anthocyanidins (Fig. 3).

3.3.Tannins

Tannins are also polyphenolic compounds with varying molecular weights (Aguilar et al. 2007). The biological role of tannins in plants is related to its own protection against diseases, insects and herbivorous animals in addition to protection against fungal and bacterial attacks (LahmeretMessai2017).Tannins are divided into four major groups: Gallotannins, Ellagitannins, condensed tannins, and complex tannins (Aguilar et al. 2007)(Fig. 4).

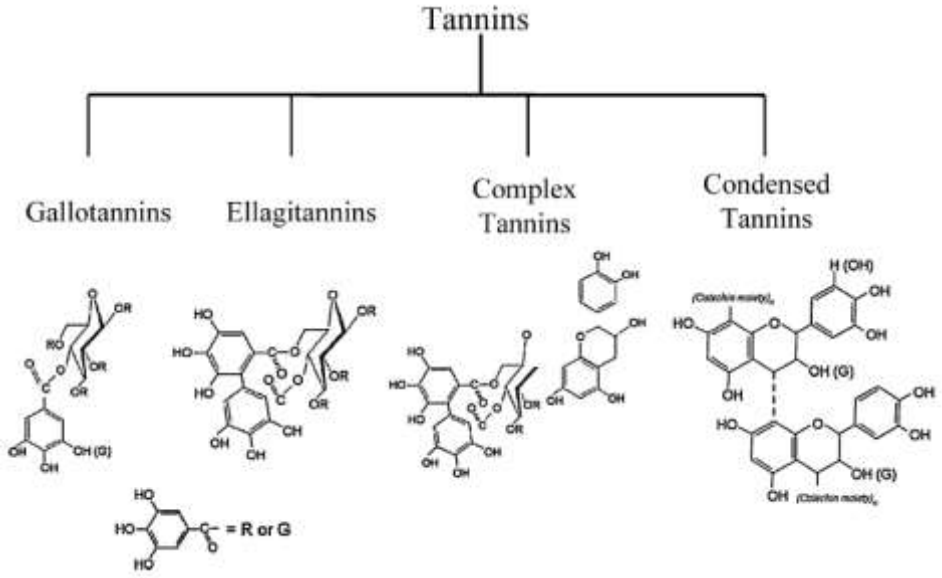


Figure4. Main chemical structures of tannins.

4. Therapeutic properties of aromatic and medicinal plants

Studies of the biological activities of bioactive substances derived from medicinal plants constitute the basis for so-called alternative medicines, active substances used in pharmaceuticals (Hammoudi 2015).

4.1. Antioxidant activity

Oxidative stress is a condition in which cells are no longer able to control the presence of toxic oxygen free radicals in excess. Free radical scavenging is probably the best-studied antioxidant property. Vitamin E (tocopherol), C (ascorbic acid), Q (ubiquinone) or carotenoids the role of antioxidants is to scavenge free radicals and capture individual electrons and convert them into stable molecules or ions (Favier 2003).

Antioxidants are substances or molecules capable of preventing, delaying and reducing the oxidation of substrates in small amounts. Antioxidants are endogenous metabolism as enzymes and an exogenous food source and they play vital role in protection against tissue damage associated with various human diseases (AiteuretAmrani 2017).

4.2. Anti-hemolytic activity

Anti-hemolytic activity is the ability of certain agents to delay or inhibit the lysis of red blood cells, such as folic acid, iron supplements, vitamin B 12, and corticosteroids (AberraneetMehalla, 2019).

4.3. Anti-inflammatory activity

Anti-inflammatory agents are defined as substances that act on the pain and swelling caused by pathogen attack. They block the release or action of certain chemical inflammatory mediators, thereby reducing the sensation of pain and inflammation. They are used when the inflammatory response is abnormally prolonged (chronic inflammation) and causes tissue damage (Diallo 2019). Anti-inflammatory drugs fall into two classes;steroidal (AIS) non-steroidal NSAI.Anti-inflammatory drugs are symptomatic medications, which do not act on the cause of the inflammation. They are indicated when inflammation, a normal process of defense against aggression, becomes bothersome, particularly because of the pain it causes. In fact, AIS also have an analgesic and antipyretic action. If necessary, they can be combined with other anti-inflammatory treatments, such as simple immobilization of the inflamed area (Boulanger 2017).

5. Toxicity of aromatic and medicinal plants

A toxic, from the Greek toxikon which means poison, is any substance foreign to the organism which could interferes in the context of a dose-dependence relationship (Génestal et al. 2009).

A plant is considered toxic if it contains one or more substances harmful to human or animals which cause various disorders and could lead to death. The active principle of a toxic plant can be distributed throughout the plant or preferentially in one or more of its parts (root, berries, leaves…) (BoumediouetAddoun 2017).

Today, the use of medicinal plants continues to grow in all regions of the world. Plants are beneficial to health because they contain active principles responsible for their therapeutic effects but many plants used in phytotherapy are toxic. Some plants are rich in substances that are toxic to humans and their use causes a variety of disorders, sometimes fatal.

Poisoning by plants is a frequent accident in most parts of the world. According to the World Health Organization, millions of calls are made to poison control centers every year. Data from poison control centers have shown that plant poisoning is often the cause of significant morbidity and mortality. North Africa has a long history in herbal medicine and this region is the source of a significant number of medicinal plants that are used for therapeutic purposes worldwide. A fraction of these plants is toxic and can cause serious poisoning(Alami2021).

5.1. Hemolysis

The process of hemolysis is an irreversible phenomenon during which the red blood cells are destroyed and release their contents (Mezzou et al. 2006). Factors specific to the body, such as the state of the membrane, intracellular energy metabolism and the structure of the hemoglobin, regulate the degree of hemolysis. Extracorporeal factors are also important in the process of hemolysisprocess of hemolysis, citing the plasma, the anatomical state of the circulatory system and the state of the phagocytic mononuclear system (Aguilar 2007).

Physiological hemolysis must be differentiated from pathological hemolysis or hyper-hemolysis related to the modification of the three vital factors for the red blood cells. In this case, the erythrocyte membrane; the metabolism energy (integrity of the enzymes involved) and hemoglobin content (Dahmani et al. 2016).

5.2. Inflammation

Inflammation or inflammatory reaction is the response of living, vascularized tissue to an aggression. This process includes (i) a general phenomenon, expressed biologically by the inflammatory syndrome andclinically in a variable way, most often by fever and possibly an alteration of general condition, and (ii) a local phenomenon where the inflammation takes place in the vascularized connective tissue.Tissues without vessels (cartilage, cornea) are unable to develop a complete inflammatory reaction(Russo-Marie et al.1998).

Epithelial tissues do not have an active role in the development of the inflammatory reaction, but they can be altered by the aggression that triggers the inflammation and then be repaired during the terminal phase of the inflammation. Inflammation is a process its goal is to eliminate the pathogen and repair the tissue damage(Autier et al. 2004).

Sometimes inflammation can be harmful because of the aggressiveness of the pathogen, its persistence the site of the inflammation, by abnormalities in the regulation of the inflammatory process, or by inflammatory process, or by quantitative or qualitative abnormality of the cells involved in inflammation (Prin et al. 2009).

The causes of the inflammatory reaction are multiple and represent the pathogens. Thesecauses determine cellular and tissue lesions that will trigger inflammation, contamination bymicro-organisms (bacteria, viruses, parasites, fungi), physical agents trauma, heat, cold, radiationand chemical agents caustics, toxins, venoms, foreign bodies: exogenous or endogenous;vascularization defect, inflammatory reaction secondary to necrosis by ischemia;dysimmuneaggression(abnormal immune response, allergies, autoimmunity) (Weill andBatteux 2003).

Aninflammatory reaction is not synonymous with infection;the same pathogen can cause different inflammatory reactions depending on the host,in particular according to the state of the immune system;several causes may be associated in the triggering of an inflammatory reaction (Zerbato 2010).

6. Description of medicinal plants used in Algerian traditional medicine

6.1. Nettle

Netlle or *Urtica dioïca*L, is a perennial herbaceous plant belonging to the family Urticaceae(Fig. 5) (Beaudoin et Ouellet. 2009). This species known as Horaig or al quaras in Arabic (Feraguena et Boudelloua. 2018) is widespread in the world, from Africa and Europe to Asia and America (Belabbass 2020).

Nettle plants hold several medical properties this is why it is considered since years as potential remedy to treat anemia, lack of energy and digestive stimulatory (Draghi 2005). They are traditionally used for the symptomatic treatment of inflammatory diseases, renal lithiasis and as therapeutic for rheumatic conditions, antiseborrheic, scalp stimulant or deodorant for external use(Delahaye 2015).

Figure 5.*Urtica dioica*L.

The phytochemical studies of *U. dioica*, revealed that this plant contains various secondary metabolites represented mainly by flavonoids, tannins and volatile compounds (Myah et Touati 2020).Indeed, the aerial parts (especially leaves) contain chlorophyll, carotenoids, several vitamins (Vit C, K, B1 and B2 among many others) and essential oils. However, nettle roots contain coumarin, polysaccharides acids and mineral elements (calcium, zinc...) (Ait Haj Said 2016).

6.2. Echinops

Echinops, *Echinops spinosus* L., is a perennial plant herb belonging to the family Asteraceaecommonly ***known in Algeria as 'Taskra', 'Fouga el djemel' or 'Chouk el Djemel'***(Fig. 6)**.** This species ***is a grass native to the dry soils of North and tropical Africa (***Boumarafs 2016).

Figure 6.*Echinops spinosus* L.

E. spinosus has been used traditionally to treat prostate diseases and dysmenorrhea. It has been also used in phytotherapy as a peripheral vasoconstrictor to treat hemorrhoids, varicose veins and varicocele besides various venous and uterine bleeding ***(Bouzabata et al. 2018).***

Phytochemical investigations carried out on this species revealed the presence of polyphenols, flavonoids, tannins, alkaloids, thiophenes polyacetylene and reducing sugars (Gheffour 2015; Boumaraf 2016).

6.3. Pig Nut

Pig Nut or *Bunium incrassatum* L. is a perennial herbaceous plant belonging to the family of Apiaceae(Fig. 7). This species is commonly called 'Talghouda***' in Algeria(Fadel***2018).***Dried tubers and pudding powder of B. incrassatum are considered as astringent and antidiarrheal in traditional pharmacopoeia, and have been found to be useful in the treatment of hemorrhoid inflammation. In addition, this plant is also used to treat bronchitis and cough (***Fadel 2018***).***

Figure 7. *Bunium incrassatum* L.

Phytochemical studies of B. incrassatum revealed the presence of tannins, terpenoids, steroids, reducing compounds,coumarines, oleic acid (Bousetla et al. 2011; ***Gagi et Toubal 2018) and apigenin, chlorogenic acid,*** isoquercitrin, rutin, pantothenic acid, esculin, quinic acid, scopoletin, monoterppenoids and sesquiterpenes. However, the presence of angelicin, diosmin, vitexin, cosmosiin, luteolin, salcolinB, vicenin-2, naringenin, afzelin, kaempferol and orientin was species dependent (Taïbi et al. 2021).

Material and methods

1. Plant material

Three medicinal and aromatic plants namely*Bunium incrassatum*, *Echinops spinosus* and Urtica *dioica* were harvested from the region of Tiaret during spring 2022. The roots of *E. spinosus*and *B. incrassatum*as well as the aerial part of *U. dioica*has been dried, grinded and kept away from light and moisture for further analysis.

2. Human blood samples

Human blood has been collected from healthy no smoker volunteers, who are not taking anti-inflammatory drugs,to evaluate thehemolytic andanti-hemolytic effects of plant extracts.Blood (O positive) was obtained by venipuncture from the volunteers and was collected in heparinized tubes then used directly for analyses.

3. Preparation of plant extract

The extraction was performed according to the protocol of Potel (2002).The plants powderswere extracted in distilled water at the ratio10% hence, 10g of the powder was extracted in 100mL of distilled water during 24hoursby maceration at room temperature then the mixture was filtered using paper of Whatman.The extraction rate expressed in percent was determined according to the following formula:

$$R(\%)= [P1 / P0] \times 100$$

Where :

P1 is the weight of dry extract expressed in grams.

P0 is the initial weight of vegetable powder expressed in grams.

4. Evaluation of the hemolytic activity of plant extracts

To evaluate the possible hemolytic effect of plant extracts, the hemolytic assay wasperformed. For this, 5 mL blood obtained from a human donor by venipuncture in aheparinized tube. The erythrocytes fromthe blood were collected through centrifugation at 1500 rpm for 3 min. The pellet waswashed thrice with sterile phosphate-buffered saline solution (pH 7.2 ± 0.2)by centrifugation at 1500 rpm for 5 min while the supernatant wasdiscarded. Washed erythrocytes were further resuspended in phosphate-buffered solution.

A volume of 1 mL of the erythrocytes'suspension wastreated with 1 mL of plantextracts diluted in the phosphate buffer saline solutionat different concentrations (0.25, 0.50, 0.75and 1 mg/mL).

The mixture was incubated at 37°C in an incubator during 30 min. The mixture was centrifuged later at 1500 rpm for 10 min. The absorbance of the supernatant was recorded at 540 nm.

The percent hemolysis for each extract was calculated using the following formula:

$$\text{Percent hemolysis} = (\text{Abs}_{540nm} \text{ of Sample} - \text{Abs}_{540nm} \text{ of Negative control}) / (\text{Abs}_{540nm} \text{ of Positive control} - \text{Abs}_{540nm} \text{ of Negative control}) \times 100$$

Where water was used as negative control while the phosphate buffered sollution was used as positive control. Each experiment was performed in triplicates for each concentration of the plant extract (Kumar et al. 2011).

4. Evaluation of the anti-hemolytic activity of plant extracts

Anti-hemolytic potential of plant extracts was inspectedby spectrophotometric procedure as described previously by Afsar et al. (2016). Five milliliters of blood from a healthy person were collectedin EDTA vials and centrifuged at 3000 rpmfor 10 min.

Supernatant was removed and pellet was washed thricewith PBS (0.2 M, pH 7.4) and centrifuged at 300 rpm for 10 min before re-suspending in salinesolution (0.9 % NaCl). 0.8 mL of the extracts (0.25, 0.5, 0.75 and 1 mg/mL in PBS) was dispensed to 0.8 mL of erythrocytesuspension and incubated at 37 °C for 5 min.

Next, 0.4 mL of H_2O_2 solution (0.82 M, PBS) was added to the reaction mixture for provoking oxidative degradationof the membrane lipids. Subsequently, after the incubation of the reaction for 3 hours at 37 °C, the sampleswere centrifuged at 3000 rpm for 10 min and the absorbanceof supernatant was noted spectrophotometrically at540 nm.

The relative hemolysis was assessed in comparisonwith the hemolysis in the H_2O_2 treated (negative control),which was set as 100%. For positive controlphosphate buffer saline was used. Each set of experimentswas performed in triplicate and inhibitory activity of differentextract was calculated and expressed as percentinhibition of hemolysis.

5. Evaluation of the *in vitro* anti-inflammatory activity of plant extracts

The anti-inflammatory activity was determined *in vitro* by the thermal denaturation of BSA (Kandikattu et al., 2013). A volume of 1 mL of each extract was mixed with 1 mL of Bovine Serum Albumin solution (0.2%) prepared in Tris-HCl buffer (50 mM, pH 6.6). The mixture was allowed to stand for 15 min at 37° and then heated in a bathwater at 72 °C for 5 min. The absorbance was recorded at 660 nm using a UV-visible spectrophotometer after cooling to room temperature. The experimentwasperformed intriplicate.

Diclofenac sodium was used as a standard. The protective effect of samples against the denaturation of BSA was presented as inhibition percentages calculated using the formula:

$$I\ (\%) = [(\text{Abs control} - \text{Abs sample}) \times 100] / \text{Abs control}$$

Where, I: The inhibition percentage, AS: absorbance of the test sample, and AC: absorbance of control.

6.Evaluation of the antioxidant activity

6.1.DPPH assay

The capability of the extract to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed spectrophotometrically and compared with ascorbic acid. The effect of each extract on DPPH is measured by the procedure described by Hatano (1988).2 mg of extract is dissolved in 10mL of methanol to obtain a concentration of 200µg/mL. Then preparation of the dilutions in dry and sterile tubes as follows:

Table 1. Preparation of the range of extract dilutions.

N° tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C (µg/ml)	20	40	50	60	70	80	100
V extract (µl)	400	800	1000	1200	1400	1600	2000
$V_{methanol}$ (µl)	3600	3200	2800	2600	2400	2000	2000

Then, 250 µL of DPPH was added to each tube. Later, the mixture was incubated in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 517 nm.The ability to scavenge DPPH radical was calculated by the following equation:

$$IC\ (\%) = [(\text{Abs Control} - \text{Abs Sample})]/ (\text{Abs Control})] \times 100$$

Where; Abs Control is the absorbance of DPPH radical methanol; Abs Sample is the absorbance of DPPH radical+sample extract/standard.

6.2. FRAP assay

The FRAP method is based on the reduction of the ferric ion (Fe^{3+}) into a ferrous ion (Fe^{2+}). This method evaluates the reducing power of the compounds. It is performed according to the protocol of (Charif and Louizini 2016).

0.5mL of the extract is mixed with 0.5ml of phosphate buffer solution (0.02M, pH 6.6) and 0.5ml of a [K_3Fe (CN) 6] (1%) potassium ferricyanide solution.

The mixture was incubated in a water bath at 50°C for 20 min. Later, 0.5 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction. The mixture was centrifuged at 3000 rpm for 10 minutes. 1 mL of supernatant recovered is combined with 1 mL of water distilled and 0.5 mL of aqueous $FeCl_3$ solution (0.1%). The mixture was incubated in the dark for 10 min at

room temperature. The absorbance of the reaction medium is measured at 700nm.The blank solution is similarly prepared, by replacing the extract with distilled water.

7. Phytochemical characterization

7.1.Polyphenols

The total phenolic content was determined by the Folin-Ciocalteu method as described by Singleton et al. (1999). The aqueous extract (1 mL) was mixed with 5 mL of Folin-Ciocalteu reagent previously diluted 10-fold with distilled water. After 1 min, 15 mL of Na_2CO_3 (20%) was added to the mixture and the volume was adjusted to 10 mL with distilled water. The solution was kept 2 hours of incubation in dark at room temperature. The absorbance was then measured at 760 nm. The total polyphenol content is calculated from the calibration curve and the results are expressed as mg gallic acid equivalent (GAE)/g dry weight of the extracts.

7.2.Flavonoid

Total flavonoid content was determined following the method described by Acharya et al. (2015). Briefly, 1 mL of the aqueous extract was added to 1 mL of AlCl3 (2%). After 15 min, the absorbance was read at 430 nm. Total flavonoid content is calculated from the calibration curve and the results are expressed as mg quercetin equivalent (QE) /g dry weight of the extracts.

7.3. Tannins

The quantification of tannins was carried out according to the method of Ouerghemmi et al. (2017). An aliquot (50 mL) of extracts was mixed with 3 ml of 4% methanol vanillin solution and 1.5 mL of H_2SO_4. After 15 min, the absorbance was measured at 500 nm. Tannin contents were expressed as mg cyanidine equivalents (CE)/ g dry weight through the calibration cu

Results

1. Hemolytic activity

In general, the rate of hemolysis increased as the concentration of the aqueous plant extract increased in the reaction medium. The lowest rates of hemolysis were recorded in the treatments containing the roots aqueous extract of *Buniumincrassatum*. However, the highest hemolysis rates were recorded in the treatment with the rootsaqueous extract of *Echinopsspinosus*(Fig. 8).

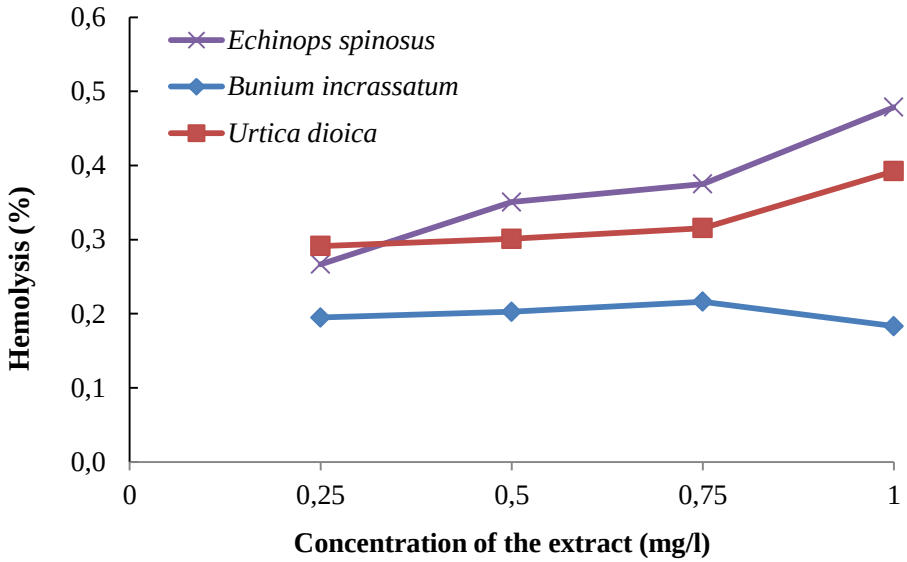


Figure8. Effects of the aqueous plant extracts on the evolution of hemolysis rate.

Although the hemolysis rate remained stable around 0.2% regardless of the tested concentration of the aqueous extract of *B.incrassatum*, the hemolysis rate of the roots aqueous extract of *E.spinosus* increased from 0.27% under the concentration of 0.25 mg/ml, to 0.45% when the concentration of the extract increased to 1 mg/ml.

Similarly, the hemolysis rate of the aqueous extract of *U. dioica* leaves increases from 0.3% under the low concentration of 0.25 mg/ml, to 0.38% under the high concentration at 1 mg/ml.

2. Anti-hemolytic activity

Overall, the rate of anti-hemolysis increased when the concentration of the aqueous plant extract increased in the medium. The highest anti-hemolysis levels were recorded in the treatment with the aqueous leaves extract of *U. dioica*. At the same time, the lowest anti-hemolysis levels were recorded in the reactions containing the roots aqueous extract of *B.incrassatum*and*E. spinosus*(Fig. 9).

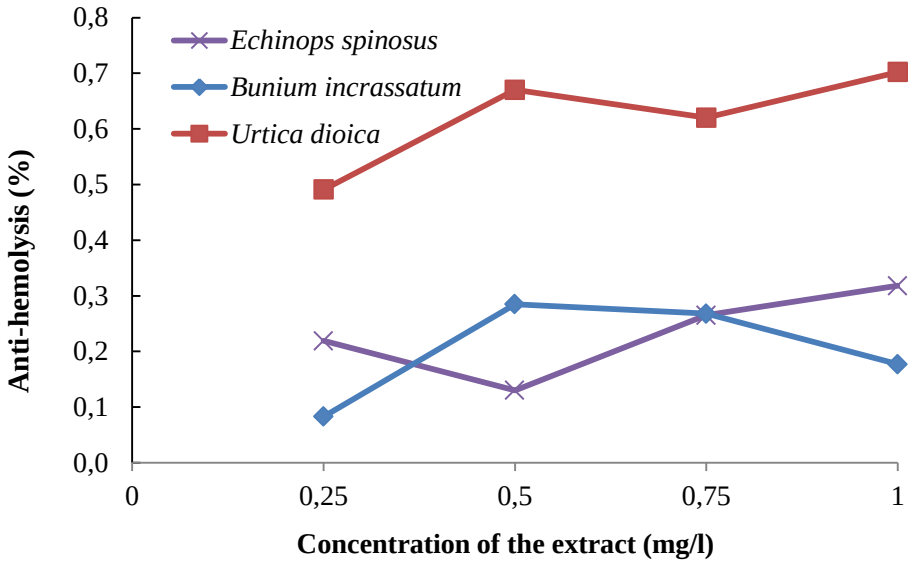


Figure9. Effects of aqueous plant extracts on the evolution of anti-hemolysis rate.

The anti-hemolysis rate ranges between 0.09% and 0.2% without clear tendencyrelated to the increase in the concentration of the aqueous extract of *B.incrassatum*. However, the anti-hemolytic effect of *E.spinosus*increased beyond the concentration of the extract 0.5 mg/L to reach 0.3%under the concentration of 1 mg/L.

Regarding *U. dioica*, a concomitant increase of the anti-hemolytic effect from 0.5% to 0.7% was associated to the increase of the extract concentration in the reaction medium.

3. *In vitro* anti-inflammatory activity

The *in vitro*anti-inflammatory potential increases after the addition of a single concentration of the aqueous plant extract in the reaction medium. The lowest anti-inflammatory levels were recorded in the treatments containing the roots aqueous extract of *E.spinosus*(70%). However, the highest anti-inflammatory rates were recorded in the treatment with the roots aqueous extract of *B.incrassatum*(90%) (Fig. 10).

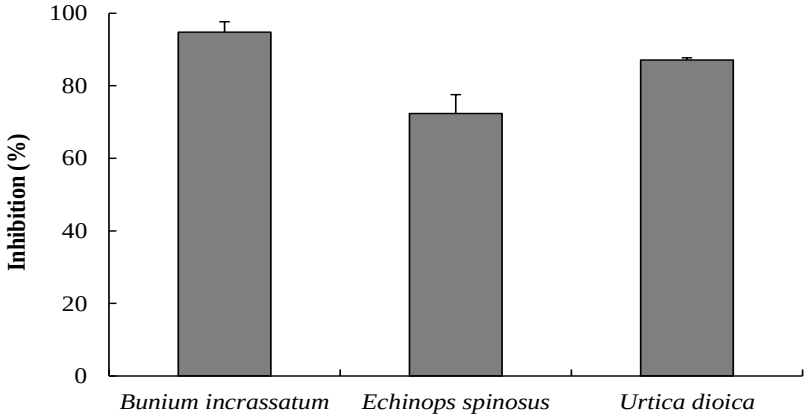


Figure 10.Variation of *in vitro* anti-inflammatory activitybetween the aqueous plant extracts.

Although, the anti-inflammatory rate of the aqueous extract of the leaves of *U. dioica*was around 85%.

4. Antioxidant activity

4.1. DPPH

In general, the DPPH $IC_{50}$was higher in the *E. spinosus* aqueous extract by around 0.036 mg/mL extract followed by the DPPH $IC_{50}$of *B. incrassatum* aqueous extract by 0.035 mg/mL extract. However, the lowest DPPH $IC_{50}$was recorded in the treatments containing the aqueous extract of *U. dioica* leaves (0.024 mg/mL extract) (Fig. 11).

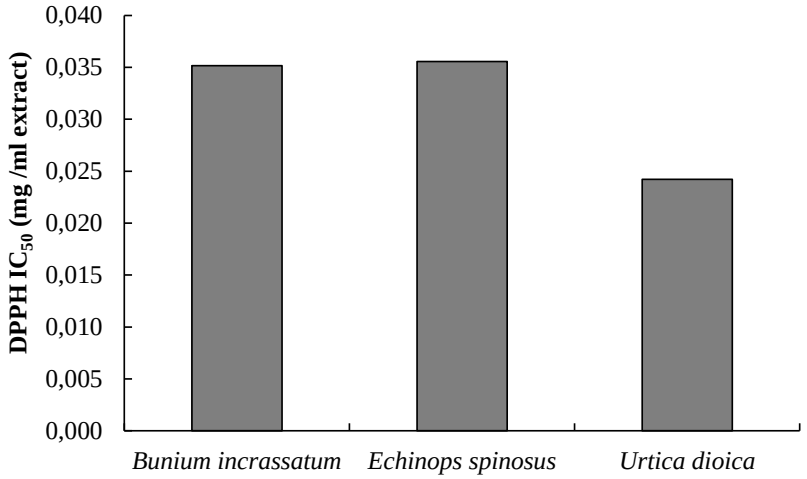


Figure 11. Variation of the DPPH IC50 between the different aqueous plant extracts.

4.2. FRAP

The FRAP efficient concentration (mg/ml) was higher in the roots aqueous extract of *E.spinosus*by arround 12.5 mg/mL extract, followed by the leaves aqueous extract of *U. dioica*by 3.1 mg/mL extract. However, the lowest FRAP efficient concentration (mg/ml) was recorded in the treatments containing the roots aqueous extract of *B.incrassatum* (1.5 mg/mL extract) (Fig. 12).

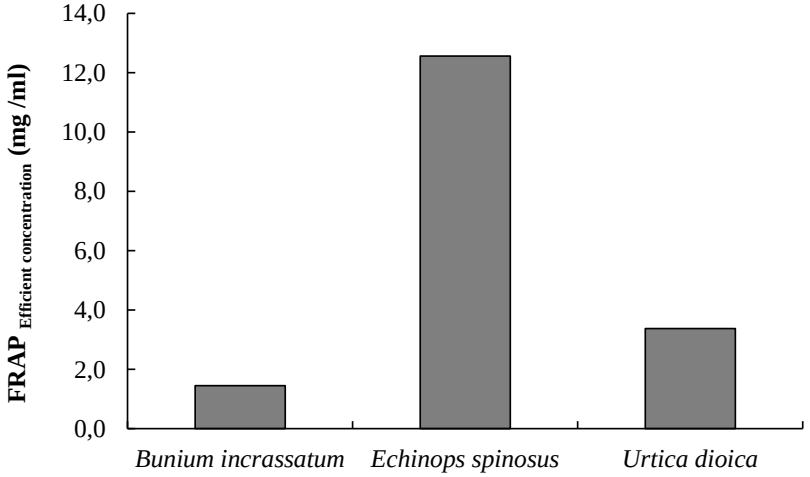


Figure 12.Variation of FRAP efficient concentration between the different aqueous extracts.

5.Phytochemicals content

5.1. Polyphenols

The highest polyphenols content was recorded in the leaves aqueous extract of *U. dioica* by around 34.83 mg GAE/mg extract followed by the roots aqueous extract of *B. incrassatum* by around 32.83 mg GAE/mg extract (Fig. 6). However, the lowest polyphenols content was reported in the roots aqueous extract of *E. spinosus* (32.42mg GAE/mg extract) (Fig. 13).

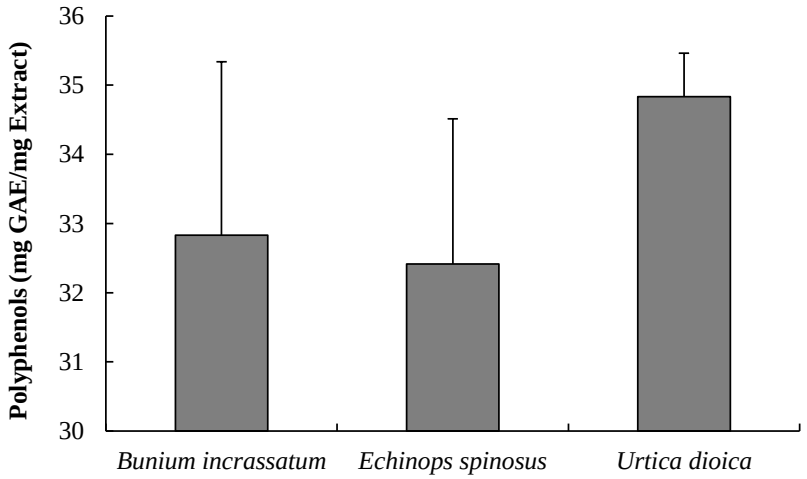


Figure 13.Variation of polyphenols content between the different aqueous plant extracts.

5.2.Flavonoids

The highest flavonoids content was recorded in the roots aqueous extract of *E.spinosus*(55 mg EQ/mg extract), followed by the leaves aqueous extract of *U. dioica*(15 mg EQ/mg extract) (Fig. 14). However, the lowestflavonoids content wasreported in the rootsaqueousextract of *B.incrassatum* (5.2 mg EQ/mg extract).

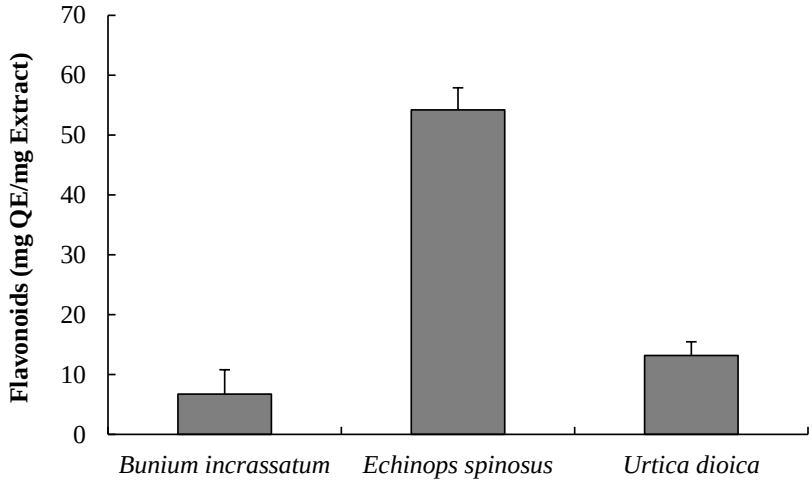


Figure 14. Variation of flavonoids content between the different aqueous plant extracts.

5.3. Tannins

The highest tannins content was recorded in the roots aqueous extract of *E. spinosus* with about 36.85 mg EC/mg extract, followed by the leaves aqueous extract of *U. dioica* with about 34.85 mg EC/mg extract (Fig. 15). However, the lowest polyphenol content was reported in the roots aqueous extract of *B. incrassatum* (34.5 mg EC/mg extract).

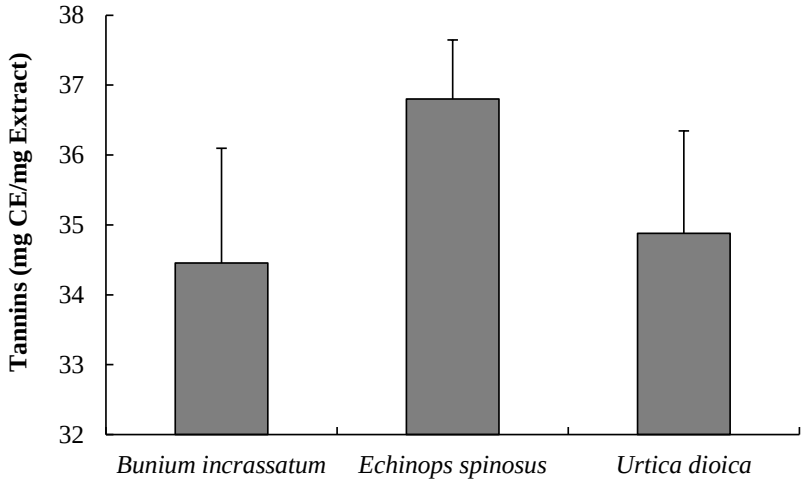


Figure 15. Variation of tannins content between the different aqueous plant extracts.

Discussion

Natural products play a crucial role in prevention and treatment of different human diseases from prehistoric times and antiquity until today. Over the past two decades, much attention has been paid to aromatic and medicinal plants as alternative therapeutic agents due to their natural bioactive compounds that hold several biological activities and pharmacological properties. In recent decades, the most modern scientific researchers have confirmed the validity of the therapeutic virtues of most medicinal plants used in traditional medicines (Carillon 2000). Besides, the traditional ancestral knowledge, passed down from generation to generation, has now become a treasure trove of information that is extremely valuable to researchers in the pharmaceutical industry (Fouché et al. 2000). Medicinal plants are therefore important for pharmaceutical research and the development of drugs, directly as therapeutic agents, but also as raw material for the synthesis of drugs or as a model for pharmaceutically active compounds (Decaux 2002).

Overall, the studied aromatic and medicinal plants have revealed throughout the present study a significant richness in phytochemical compounds along with several biological activities of therapeutic interests. However, *E. spinosus* has demonstrated a significant cytotoxicity. Hemolytic activity was assessed to determine the toxicity of the aqueous extract of the studied plants. The obtained results show that the roots aqueous extract of *E. spinosus* has a high hemolytic potential around 0.5%. Hemolytic activity of *E. spinosus* has already been reported; Kamila et al. (2018) reported hemolytic activity of root aqueous extract of *E. spinosus* by around 5%. The hemolytic activity of *B. incrassatum* was less pronounced than that of *E. spinosus*. Berroukeche et al. (2022) found that *B. incrassatum* ethanolic extract has also significant hemolytic activity on human erythrocytes. However, *U. dioica* induced low hemolysis compared to the roots aqueous extract of *B. incrassatum* and *E. spinosus*.

The anti-hemolytic activity allows to determine the anti-toxicity or the protective effect of the aqueous extract of the studied plants. The obtained results demonstrate that the roots aqueous extract of *E. spinosus* has a low anti-hemolytic capacity which remains around 0.21% and 0.30%. By the same, *B. incrassatum* showed also reduced anti-hemolytic activity just like *E. spinosus*. However, *U. dioica* demonstrated a potent anti-hemolytic effect when compared to the roots aqueous extract of *B. incrassatum* and *E. spinosus.*

In fact, non-immunological extra-corpuscular hemolysis is due to the action of mechanical, physical, chemical, infectious or parasitic phenomena inducing the destruction of red blood cellsand associating or not with the appearance of anemia. They are in intravascular rule and result from morphological or functional disturbances of the erythrocyte membrane. The most common is the deficiency of an enzyme called glucose-6-phosphate dehydrogenase, which causes premature destruction of red blood cells and subsequent hemolytic anemia (Tabbara 1992). Results related to the anti-inflammatory activity demonstrate that all the studied plants

hold an anti-inflammatory potential. El Kolli et al. (2017) found that the inhibition of the denaturation was equal to 49.66 mg/mL throughout *in vitro* anti-inflammatory activity evaluated on a methanolic extract of *B. incrassatum*.

The antioxidant activity makes it possible to mark the potency of the aqueous extracts of the studied plants to capture the free radicals responsible for oxidative stress in the cells. The obtained results show that the roots aqueous extract of *E. spinosus* has a high ability to trap free radicals according to the DPPH test which gives values around 0.035 mg/mL. The antioxidant activity of *E. spinosus* has already been reported previously by Khedher et al. (2014) who worked on the ethanolic extract of the roots of *E. spinosus*. The DPPH IC_{50} value found was 147 µg/mL. The antioxidant activity of *B. incrassatum* was comparable than that of *E. spinosus*. Dehimi et al. (2020) found that the aqueous extract of *B. incrassatum* had no activity even in a higher dose while the methanol extract yielded a DPPH IC_{50} value of 21.18 mg/mL.

The antioxidant activity measured by the FRAP test aid to determine the reductive power of the extract through the transformation of ferrous acid Fe^{+3} into ferric acid Fe^{+2}. The obtained results show that the aqueous extract of the roots of *E. spinosus* has a reducing potential of 12.5 mg/mL. The antioxidant activity FRAP of *E. spinosus* has already been reported by Gheffour et al. (2015) who found a FRAP antioxidant activity of root n-butanol extract and *E. spinosus* of the order of 2.85. The reducing activity of *B. incrassatum* was marked as low 1.5 mg/mL.Dehimi et al. (2020) found that the aqueous extract of *B. incrassatum* recorded an absorbance of the aqueous extract of 0.25 in 6 mg/mL and the methanol extract 0.6 in 6 mg/mL. The *U. dioica* extract showed a low reducing activity of 2.5 mg/mL compared to the ethanolic extract of Kukric et al. (2012) which was found to be around 7.5 mM Fe(II)/g.

Oxidative stress is commonly defined as an imbalance in the equilibrium between the systems of antioxidant defenses and the production of a body's reactive oxygen species (ROS). However, excessive production of these molecules or a lack of antioxidant mechanisms can disrupt this balance, ROS can cause cell damage and induce oxidative damage in proteins, lipids, DNA and RNA molecules. However, these last reactive molecules are controlled by antioxidants, which are substances capable of neutralizing or reducing their damage in the body and are able to maintain non-cytotoxic concentrations of ROS at the cell level. Several types of defenses can be distinguished, such as antioxidants (Hamid et al. 2018). However, plants proveto be powerful sources of natural antioxidants, they constitute a good source of polyphenols and phytochemicals associated to a high antioxidant activity. Polyphenols content was measured in the aqueous extract of the studied plants. The obtained results show that the aqueous extract of *U. dioica* leaves has a high content of polyphenols around 34.83 mg GAE/mg. Similar results have been reported by Ghaima et al. (2013) with a phenolic content of 48.3 mg GAE/g using an extract of ethyl acetate. Kukric et al. (2012) reported in their work a phenolic content of 208.37 g GAE/g using ethanolic extract of the leaves of *U. dioica*. *B. incrassatum* showed a content of 32.83 mg GAE/mL. Based on the work carried by Dehimi et al. (2020), polyphenols content is higher than the reported results indicating an amount of 6.92 µg GAE/mg. *E. spinosus* demonstrated a low

content compared to *U. dioica* and *B. incrassatum*. Similar findings have been already postponed by Khedher et al. (2014) indicating polyphenols content of 19.3 mg GAE/g. Regarding the flavonoid content, the obtained results demonstrate that the aqueous extract of the roots of *E. spinosus* has a high content. According to the work of Khedher et al. (2014), ethanolic extracts of *E. spinosus* is rich in flavonoids (680.1 µg QE/g). however, *U. dioica* has demonstrated a low content of flavonoids. Kukric et al. (2012) showed that the ethanolic extract has a content equal to 20.29 mg QE/g in the leaves. Results report also a low content of flavonoids in *B. incrassatum* aqueous extract. Similar results have been reported by Dehimi et al. (2020) who found 6.91 µg EQ/mg in the aqueous extract. Tannins have a protective effect; these secondary metabolites are secreted to protect the plant from aggressions.

It was found that the aqueous extract of *E. spinosus* showed a very high content followed by *U. dioica* then *B. incrassatum*. Khedher et al. (2014) and Dehimi et al. (2020) found also a low content of tannins in the ethanolic extract from the roots of *E. spinosus* and the aqueous extract of *B. incrassatum* respectively.

Conclusion

Aromatic and medicinal plants are widely used for therapeutic purposes in traditional medicine around the world and in Algeria for the treatment of various diseases. An increasing number of people are turning to traditional medicine, partly because the cost of conventional drugs is quite high, and partly because these drugs may have a limited effect.However, the use of medicinal plants is not without risk. Several cases of toxicities have been reported worldwide due to the mismanagement of medicinal plants.

This work is part of the programme in quest of the valorization of the local flora and medicinal plants, through the evaluation of their toxicity along with their *in vivo* and *in vitro* biological activities.

The obtained results demonstrate that the aqueous extract of *E.spinosus* presents a high risk of cytotoxicity, a weak antihemolytic and anti-inflammatory effects, buta very high antioxidant activity. However, the aqueous extract of *B.incrassatum*presents a non-significant cytotoxicity along with high anti-inflammatory and antioxidant activities.Besides, the aqueous extract of *U. dioica*presents a moderate cytotoxicity represented byits hemolytic effect, high anti-inflammatory and antioxidant effects.

The quantitative analysis has revealed the presence of higher contents of polyphenols and tannins in *B. incrassatum*and *U. dioica*against higher contents of flavonoids and tannins in *E. spinosus.*This variation could explain partially the difference observed in terms of cytotoxicity and biological activities.

Further studies are recommended to experimentally evaluate the biological potential and toxicity of the used aromatic and medicinal plants in Algeria.

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