الجمهورية الجزائرية الديموقراطية الشعبية

وزارة التعليم العالي و البحث العلمي

People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

جـــامعـــة محمد الصديق بن يحي – جيجل-

University of Mohammed Seddik Ben Yahia – Jijel

Faculty of Nature and Life Science

Department of Molecular and

Cellular Biology

كليـة عـــلوم الطـبيعـة و الحـــياة

قســـــم: البيولوجيا الجزيئية و الخلوية

Master Thesis

To obtain the academic master's degree in biology

Speciality: Molecular and Cellular Biology

Theme

The anticancer activity of some medicinal plants of the genus *Ephedra*

Examiner's committee:

Chairperson: Dr. Hanane BOUTENOUNE

Examiner: Dr. Salma HAMMIMED

Supervisor: Mrs. Moufida BENSAM

Presented by:

Aya BENAYAD

Hind BOUDERMINE

Academic year: 2022-2023

Order Number :……..…

Acknowledgements

We would like to thank always and on this occasion, first and foremost, “Allah, who gave us the strength and patience to accomplish this Modest Work.

First of all, We would like to thank our supervisor, Mrs. Moufida BENSAM, for the time she devoted to providing us with the necessary methodological tools to conduct this research. Her demand stimulated our greatly.

We are very honored to thank Dr. Hanane BOUTENOUNE at University of Jijel, for the great privilege she gave us to accepte the presidency of our jury of defense and Dr. Salma HAMMIMED at University of Jijel for agreeing to spend time reviewing and judging this work.

We would also like to thank the teachers of the department of Molecular and Cellular Biology who, throughout the five years of study, have passed on their knowledge without reservation, and all those who have helped us in the realization of this project.

A big thank you also to DYDA for having had the patience to answer our countless questions.

Dedication

Thank Allah (my god) for giving me the ability to write and think, the strength to believe in it, the patience to go to the end of the dream and the happiness to raise my hands to the sky and say "Ya Kayoum"

I dedicate this modest work:

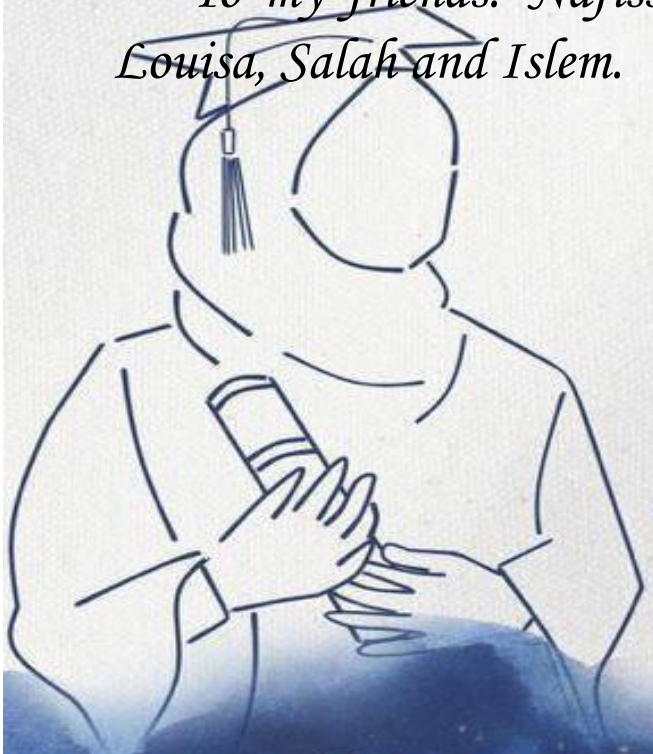
To my dear parents, who have all the credit.

To my Brother Mustapha.

To all my family, uncles and uncles.

To my friend, sister, colleague, and binomial "Hind" who shared with me the difficult moments of this job and to her family.

To my friends: Nafissa, Nassrin, Chiraz, Nour El Houda, Louisa, Salah and Islem.



Benayad Aya

Dedication

In the name of Almighty ALLAH for all the blessings that He continues to perform in our lives, Peace and salvation be upon His Messenger, His family and companions.

I dedicate this modest work:

To my dear parents, who have all the credit.

To my Brother Imad and my Sister Liliya.

To grandparents Ahmed and Nwara.

To my Ant

BOUDERMINE Hind

Summary

List of figures

List of tables

HGF: hepatocyte growth factor.

MAP: mitogen-activated proteins.

TNF: Tumor necrosis factor.

HPV: Human papillomavirus.

RB: Retinoblastoma.

MTT: multi transaction translator.

GCMS: gas chromatography mass spectrometry.

Introduction

It is becoming increasingly clear that plants, ranging from across the plant kingdom, produce a unique diversity of secondary metabolites that can be exploited for the discovery of new drugs, bio-sourced materials, nutraceuticals, or cosmetics. For finding new molecules, plant naturel products are undoubtedly good sources of chemical diversity. It is estimated that over 200,000 primary and secondary metabolites may be present in the plant kingdom (Elhadef et al., 2020)

Medicinal plant is the product of long-term medical practice worldwide, with the advantages of outstanding curative properties and less side effects .Containing many natural products and their derivatives of therapeutic value, medicinal plants are considerate as main source of remedies able to protect human body against diseases Herbal medicine is the oldest form of medicine known to mankind. It was the mainstay of many early civilizations and still the most widely practiced form of medicine in the world today. Ephedra is one of the largest genera of the Ephedraceae family, which is distributed in arid and semiarid regions of the world (Elhadef *et al.*, 2020).

As medicinal plant, enclosing over 50 species, *Ephedra* genus belongs to the family Ephedraceae which in turn represents one of three families in the order Gnetales. *Ephedra* is common in cold and dry places in both the Old and the New Worlds; the Gnetaceae members live in warm and humid tropical/subtropical forests of Asia, Africa, and South America The shrubs, which reach approximately one meter in height, grow in semiarid and desert conditions in both hemispheres across six continents (Elhadef *et al.*, 2020).

Algeria, Due to its bioclimatics zones (humid ,sub humid, semiarid, arid or desert) with cold, wet, dry, mild winters ; and its geographical situation, has a privileged place for exploitation, culture, production and export of medicinal and aromatic plants very diverse in their raw or processes states (Reguieg, 2011).

For years, ephedrine series ((-)-ephedrine, (+) pseudoephedrine, (-)-N-methylephedrine, (+)-N-methylpseudoephedrine, (-) norephedrine, (+)-norpseudoephedrine) were considered to be the main *Ephedra* constituents .Nowadays, at the side of pharmacological effects, there has been considerable research on the phytochemistry and bioactivities of genus *Ephedra*, including their antibacterial and primarily antioxidant activity(Elhadef *et al.*, 2020). From the entire

plant, a wide range of *Ephedra* natural products including alkaloids, tannins, saponins, proanthocyanidins, phenolic acids, flavonoids, and essential oils have been mentioned and the plants-derived polyphenols are of great importance for their biological and pharmacological potential .Numerous chemicals originating from edible plants have been connected to cancer chemoprevention and treatment (Elhadef *et al.*, 2020).

In light of this, our goal is to carry out a bibliographic research on the anticancer activity of two medicinal plants of the genus Ephedra: *E. alata* and *E. feominea.* That's why our dissertation will be divided on three parts. The first chapter discusses *Ephedra* genus. The second chapter discusses the cancer and in the last chapter we will analyze some recent papers that studied the anticancer effect *in vitro* and *in vivo* of the two plants above.

Chapter I
Ephedra genus

I. 1. General Background on *Ephedra* genus

Herbal medicine is the oldest form of medicine known to mankind. It was the mainstay of many early civilizations and still the most widely practiced form of medicine in the world today. *Ephedra* is one of the largest genera of the Ephedraceae family, which is distributed in arid and semiarid regions of the world (Rashed, 2021).Containing approximately fifty species, the *Ephedra* genus was distributed in arid and semi-arid regions of Asia, Europe, Northern Africa and southwestern North America, and South America. The early evolution and diversification of the *Ephedra* have increasingly become clear because of recently reported macrofossils from the Early Cretaceous strata of Asia, Australia, Europe, and Americas . *Ephedra-macrofossils*, especially female cones, provide a historical perspective for the early evolution, taxonomy, and Evidence-Based Complementary and Alternative Medicine (Fig.1) (Elhadef et *al*.,2020).

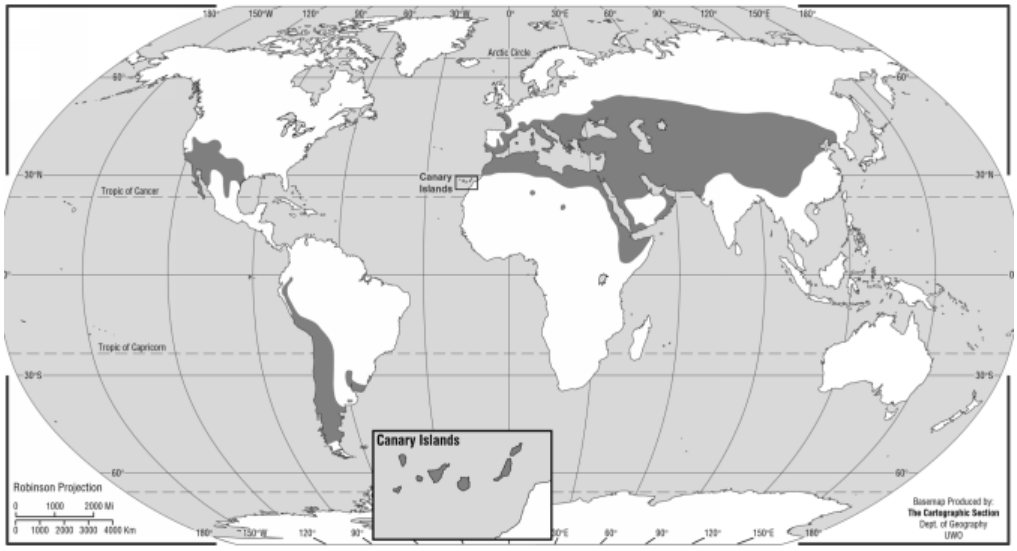


Fig.1. The world wild distribution of *Ephedra* (Cavaney et *al*., 2001).

The Chinese dispensatory written in1569 mentions that *Ephedra* species were valuable as an antipyretic, diaphoretic, circulatory stimulant, and sedative for cough. However, *Ephedra* has been used in traditional Chinese medicine to treat allergies, asthma, lung-congestion, chills, colds, hay fever, coughs, edema, fever, flu, headaches, and nasal congestion. (Al-Snafi, 2017). Ephedra was also traditionally used in Russia for respiratory disorders and rheumatism for many centuries. The Native Americans and Spaniards of the southwestern United States used ephedra for various medicinal purposes, especially venereal diseases (Table 1) (Al-Snafi, 2017).

Table.1. Example of some *Ephedra genus* species

Scientific name	*Arabic name/ common name*	*figure*	*Principal distribution*	*reference*
Ephedra Fragilis	Gnodopsida, Mormam tea	 	Southern Europ, rediterranea	Celedon-Neghme et *al*., 2016 Attardet *et al*., 2009 Caveney et *al*., 2014
Ephedra Sinica	Ma Hung		Liaoning, Jilin, Imer Mongolia Hebei, Shansci, Henan china	Elhadef et *al*., 2020 Zhang et *al*., 2022
Ephedra Antisyphilitica (candellila)	Canatilla, popotillo, tepopete		Northen Mixico, Southern united states of america	Vargas-Piedra *et al*., 2020 González-Juárez *et al*.2020
E-compocta	Camutillo, Samguinaria		Northen Mixico Southern united states of america	illanueva-Almanza *et al*., 2011 González-Juárez *et al*., 2020

I. 2. Chemical composition

Ephedra species are a source of bioactive natural products (González-Juárez et *al*., 2020).The term "primary metabolism" refers to the collection of plant pathways that ensure the fundamental physiological functions shared by the majority of higher plants. These pathways allow for the synthesis of sugars, lipids, enzymes, cell wall components (which are essential to the construction of cells), and cellulosic compounds. All of these substances

contribute to the cellular structure and physiology of tissues and ensure the growth and survival of the plant (calatayud et *al*., 2013).

Kossel is the first to use the term "secondary metabolism" in 1891, and according to his definition, "secondary metabolism" refers to compounds that are only "accidentally" present and are not necessary for the survival of the plant, whereas "primary metabolism" occurs in all vegetative cells that are capable of being divided. Following this, Kossel established a difference between these two categories of metabolites that is still relevant today. Primary, metabolites are molecules with low molecular weight that are present in all plants. There are a few hundreds of them, such as aminated or nuclear acids. However, tens of thousands of secondary metabolic compounds have been discovered that are unique to a particular plant family or species (Bouque, 1997).

Secondary metabolites isolated from soluble extracts in organic solvents obtained from both aerial parts and roots of Ephedra plants are represented by 26 alkaloids, mainly ones with an ephedrine-type framework (compounds 1- 26); 75 phenolic compounds, which include aromatic compounds, flavonoids, lignans and proanthocyanidins (compounds 27–99); and seven amino acid derivatives (compounds 100–106)(González-Juárez et *al*.,2020). Furthermore, in the essential oils of these species, 98 volatile organic compounds (VOCs) were obtained by hydro distillation and about 70 compounds were obtained by extraction with supercritical CO2 (González-Juárez et *al*.,2020).As shown in general studies, the *Ephedra* species were characterized by the alkaloids and phenolic compounds content, such as trans-cinnamic acid, catechin, epicatechin, symplocoside, and favonol-3-*O*-glycosides, and proanthocyanidins (Al-Rimawi et *al*.,2017).

I.2.1. Phenolic compounds

Phenolic compounds are a diverse class of bioactive secondary metabolites and are of high and significant importance. They can be described as compounds that contain a phenol moiety. Phenol itself is a benzene ring that is substituted with a hydroxyl group (Fig 2) (Al Mamari , 2021).

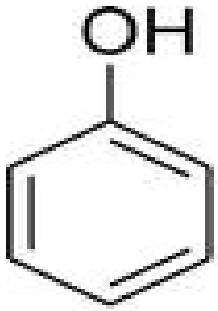


Fig.2. Structure of phenol (Al Mamari , 2021).

Phenolic compounds display a wide range of biological activities. For instance, they are known to exhibit antioxidants, antimicrobial, and anti-inflammatory properties. They are ubiquitous in nature, for example, myricetin (a flavonol) is found in apple ferulic acid (a Hydroxycinnamic acid) and apigenin (a flavone) are found in mango, and Luteolin (a flavone) is found in watermelon and pineapple (Al Mamari, 2021).

I. 2.1.1. Classification of phenolic compounds

Phenolic compounds can generally be classified into simple and polyphenolic compounds (Fig.3) (Al Mamari, 2021).

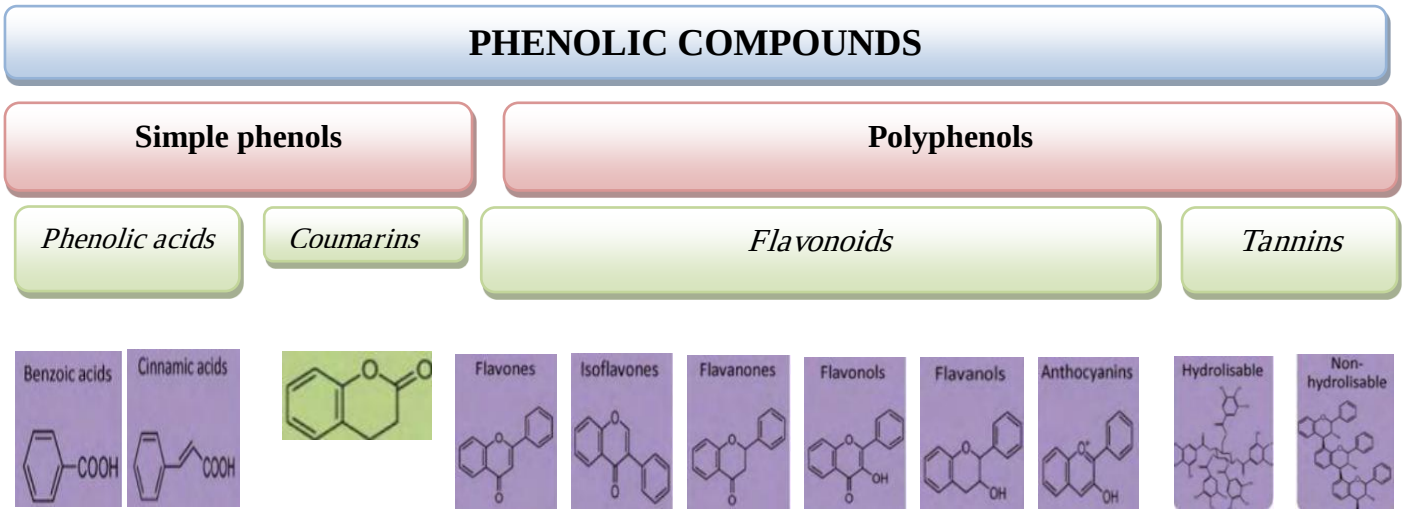


Fig.3. Major classes of phenolic compounds (Hurtado-Fernández et *al*., 2010).

I.2.1.1.1. Simple phenolic

Phenols that contain a carboxylic acid are termed as phenolic acids. If the carboxylic acid functional group is directly bonded to the phenol ring, the phenolic compound is termed as hydroxybenzoic acid. When carboxylic acid functional group and the phenol ring are

separated by two doubly bonded carbons (a C=C bond), phenolic compounds are termed as Hydroxy-cinnamic acids (Al Mamari , 2021).

a. Hydroxybenzoic acids

Hydroxybenzoic acids are benzoic acids substituted with a hydroxyl group. Alternatively, they can be viewed as phenols that are substituted with a carboxylic acid functional group that is directly bonded to the phenol ring (Fig 4) (Al Mamari , 2021).

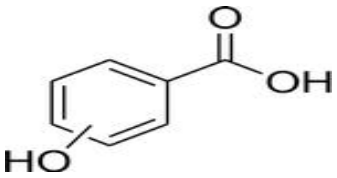


Fig.4. Structures of hydroxyl-substituted benzoic acids (Al Mamari, 2021).

b. Hydroxycinnamic acids

When the carboxylic acid functional group is separated from the phenol ring by a C=C bond, phenolic acids are described ashydroxycinnamic acids (Al Mamari , 2021).

I.2.1.1.2. Coumarins

Hydroxycoumarins are hydroxyl-substituted coumarins. Scopoletin and auraptene are examples of hydroxycoumarins (Al Mamari, 2021).

I.2.1.1.3. Polyphenols

Phenolic compounds that contain more than one phenol unit are considered polyphenol. Polyphenolic compounds have C15 general skeleton representation (Al Mamari, 2021). pomyphenols are classified into:

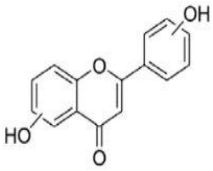
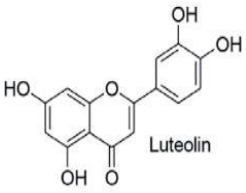
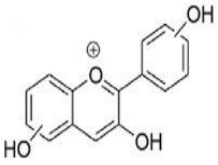
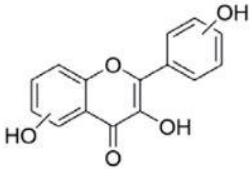
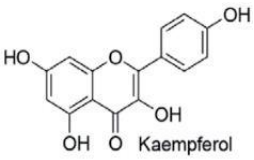
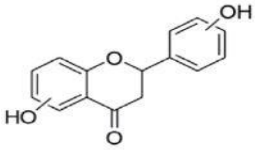
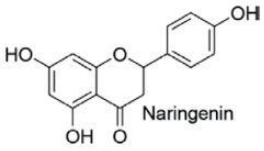
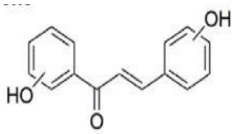
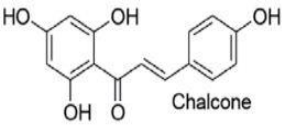
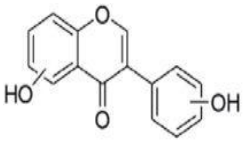
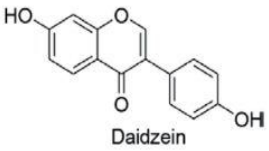
a. Flavonoids

Flavonoids are polyphenolic compounds with the general structure shown below Generally, rings A and C are either mono, di, or trihydroxylated. The O-heterocycle B is usually a pyrone ring as in Luteolin but could also be a pyrlium ring as in delphinidin (Al Mamari, 2021).

Flavonoids are the most common class of secondary me-tabolites within this genus. More than forty flavonoids have been identified from the genus Ephedra, which are clas-sified as flavonols, dihydroflavonols, fla- vonones , flavanols , flavones , and anthocyans . Flavones and their glycosides, as well as flavonols and their 3-O-glycosides constituents, are

the most common flavonoids in Ephedra. The sugar moieties of these flavonoid glycosides are commonly made up of glucose and rhamnose. Formerly, it was believed that most flavonoids in the genus were derivatives of the flavonol aglycones kaemp-ferol, herbacetin or quercetin. Recently, some other aglycones such as apigenin, catechin, luteolin, and hesperetin have been characterized. Most flavonoid glycosides are mono-gly- cosides or di-glycosides (Table 2) (Zhang et al., 2018).

Table.2. Classification of flavonoids (Al Mamari , 2021).

Class	*General structure*	*Example*	*Class*	*General structure*	*Example*
Flavone			**Anthocyanin**		
Flavonol			**Flavanone**		
Chalcone			**Isoflavone**		

Quercetin is an ordinary flavonoid that is pervasive in various types of foods and plant, it is reported to have several beneficial effects on human health such as anti-inflammatory effects, cardiovascular protection and anticancer activity (Fig 5) (Vafadar *et al*., 2020).

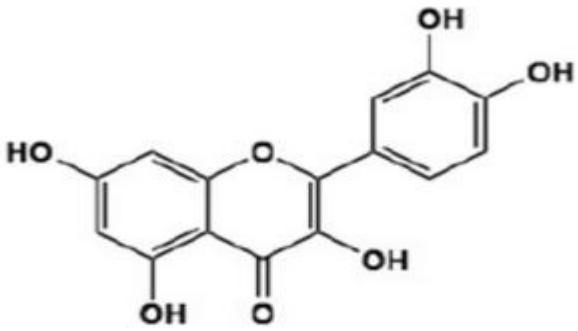


Fig.5. Chemical structure of quercetin (Vafadar *et al*., 2020).

In-vitro studies indicate that quercetin plays an important role in cancer treatment with the ability to act as potential antioxidants and there by inducing numerous molecular pathways such as apoptotic pathway, down-regulation of mutant P53 protein, G1-phase arrest, inhibition of tyrosine kinase, inhibition of heat shock proteins, inhibition of Ras protein expression, and estrogen receptor binding capacity (Rani et *al*., 2015). The P53 is an important tumor suppression protein which activates the Bax and initiate cell death. When human hepatocellular carcinoma cell was treated with 40–120 μ mol/L of quercetin, p53 expression was increased, while down-regulating the anti-apoptotic protein surviving that regulates the caspase activation and Bcl-2 that prevents mitochondrial mediated apoptosis (Rani et *al*., 2015).

Quercetin has revealed an anti-tumor effect by reducing development of blood vessels. In addition, this natural component decreases tumor growth through targeting VEGFR-2-mediated angiogenesis pathway and suppressing the downstream regulatory component AKT in prostate and breast malignancies. The potential of quercetin to inhibit angiogenesis in drug-resistant cells increases its effects on anti-cancer medicines (Lotfi et *al*., 2023).

The Bcl-2 protein family and Bax are controlled by the PI3K/AKT pathway, which has anti-apoptotic properties (a proapoptotic gene). Deregulation of this pathway could be a key event in cancer pathogenesis. Several studies explored the quercetin effect on PI3K/AKT pathway using the HCC1937 PTEN-null cancer cell line, Gulati et al. observed that 25 μM quercetin may decrease active AKT/PKB phosphorylation and dramatically limit cell proliferation of PTEN-null cancer cells (Asgharian et *al*., 2022).

b. Tannins

Tannins are known to bind to and precipitate proteins and amino acids. They are subdivided into three types; hydrolysable, condensed and complex. Hydrolysable tannins can be Gallo-tannins or ellagi-tannins. Gallo-tannins are polyols that are substituted with Gallic acid units. The galloyl units in Gallo-tannins are linked by ester linkages. Commonly, the polyol core is a D-glucose that is substituted with Gallic acid units. Tannicacidis an example of gallo tannins (Al Mamari, 2021).Tannins are also important compounds in *Ephedra*, which mainly exist in the form of condensation. Tannins, mainly proan- thocyanidins, have been proven to be present in many Ephedra plants (e.g., Eurasian *Ephedra: E. przewalskii*, *E. alata*, *E. distachya*, *E. fragilis* and *E.* intermedia; North American Ephedra: *E. californica*, *E. nevadensis*, *E. fasciculata*, *E. trifurca*, *E. torreyana*, and *E. viridis*). At present, the con-densed tannins of procyanidin A-type are the most common in *Ephedra*, containing dimers,

trimers, and tetramers. These com-pounds are made of flavan-3-ol monomer units, which are linked together through C4-C6, C4-C8, or C2-O-C7 bonds (Zhang et al., 2018).

I.2.2. Alkaloid

Beginning of the nineteenth century, MEISNER coined the term " to describe natural compounds acting as bases, such as alcalis (from the Arabic al kaly, base, and the Greek eidos, aspect). An alcalode is an organic, alive, azote substance of plant origin that has a complex structure and has an alkaline character. The alcalodes have a significant pharmacological activity, and their atom of azote is part of a heterocyclic system. As a result, the three categories of alcalodes are true alcalodes, proto-alcalodes, and pseudo-alcalodes (Badiaga , 2011).

The alkaloids *ephedrine, pseudoéphédrine, noréphédrine, norpseudoéphédrine*, methyléphédrine, and methyl *pseudoéphédrine* are found in the species of *ephedra*. The medical significance of *ephedra* is mostly based on *ephedrine's* sympathomimetic properties. The amount of ephedrine in ephedra alata typically ranges from 0.5% to 0.19 %, but the amount of pseudoephedrine is greater than 0.5% (luejinea.pdf). A pyrrolidine alkaloid is isolated from the seeds of *E. foeminea* and *E. foliata* (Fig.6) (Zhang et al., 2018).

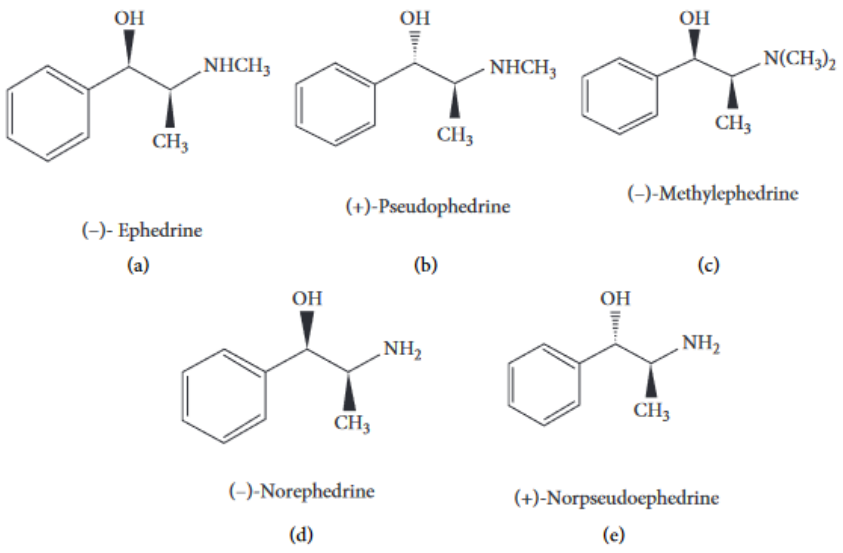


Fig.6. Chemical structures of ephedrine alkaloids (Elhadef et *al*., 2020).

Two additional aspects that illustrate the chemical diversity of *Ephedra* genus are the chemotaxonomy approaches and the use of ephedrine-type alkaloids as building blocks during organic synthesis (González-Juárez et al., 2020).

I.2.2.1. True alkaloids

True alkaloids make up the majority of alkaloids; they are toxic and have a wide range of biological activities. They contain an azote atom and originate from amine-based acids in a cyclic system. They are present in the plant (Fig 7) (Badiaga, 2011).

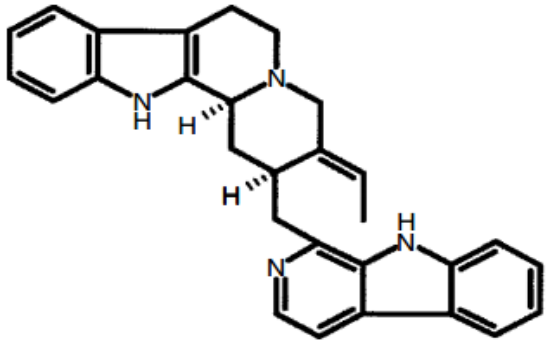


Fig.7. In example of true-alkaloïdes (Aniszewski, 2007).

I.2.2.2. Pseudo-alkaloids

Most frequently exhibit all the characteristics of true alcaloides but are not derived from amino acids. The two main representatives of this class of alkaloids are steroidal alkaloids and purines (Badiaga, 2011).

I.2.2.3. Proto-alkaloids

The azote is not a part of a heterocyclic system like mescaline, making the proto-alcalodes simple amines (Badiaga, 2011).

The *ephedrine* quantity in dietary supplements taken orally is about 20 mg per serving, and doses are taken up to two to three times per day (Elhadef et *al*.**,** 2020**).** It has been shown that labels of dietary supplements do not list *ephedrine* content or incorrectly report the amount of *ephedrine* in these products (Elhadef et *al*., 2020**).** *Ephedrine* has been associated with cardiovascular dysfunction such as myocardial infarction, severe hypertension, myocarditis, and lethal cardiac arrhythmias (Elhadef et *al*., 2020).

I.2.3. Polysaccharides

Large-molecule components in *Ephedra* species have been studied. *Ephedra sinica* contains a high amount of polysaccharides, which range from 3% to 5% of the total dry weight. Generally, the acidic hetero-polysaccharide (ESP-B4) has been isolated from *E.sinica* and shows an immunosuppressive effect (Zhang et al., 2018).

I.3. Pharmacological properties

The pharmacological effect of the different *Ephedra* species depends on the phyto-constituents of each one (Table.3) (Elhdaf et al., 2022).

Table.3. Pharmacological activity of extracts from differents species of the worldwide genus *Ephedra* (published in the period 2015-2023) (Elhdaf et *al*., 2022) (Bensam et *al*., 2023).

Source	Part	Extact	Therapy
Tunisia	Aerial part of E-alata	Ethyl acetate	Antiproliferacive, proapoptotic, and cytocoxic potential
Palestine	Aerial part of E-alata	Decoction	Anticancer
Jordan	Aerial part of E-aphylla	MeOH , methanol,$CHCL_3$, EtOAc, n-Hx, and water	Anti-inflammatory Antiproliferative
Lebanon	Strem of E-campylopoda	EtOH, MeOH and water	Anti-inflammatory Antiproliferative
Iran	Stems and leaves of E-sarcocarpa Aerial parts of Ephedra	Water water	Anticancer Antidiabetic and antihyperlipidemic
Pakistan	Aerial part of E-gerardiana	EtOH70%, EtOAc, n-BuOH, and water	Antiarthritic
Korea	Dried stems and leaves of E-sinica stapf., E-intermedia schrenk, E-equisetina Ephedra	Watet MeOH Water	Antineuroinflammatory Antihyperlipidemic Analgesic Anti-influenza
Japan	E-sinica	Water	Anticancer Analgesic
Taiwan	E-sinica Aphedra	Water Water	Antiproliferative Antipyretic Antitussive
China	Aphedra	Water	Antipyretic and antiasthmatic
Chile	Aerial part of E-chilensis	Hexane, dichloromethene and EtOH	Antiproliferative
Algeria	Aerial part of Ephedra Alata	Ethanol	Anticancer

I.3.1. Anti-inflammatory activity

Ephedrine analogs, principally ephedrine, pseudoephedrine, and ephe-droxan, were found to have strong anti-inflammatory efficacy *in vivo* as early as 1985. The inhibition of prostaglandin E2 biosynthesis was most likely the cause of this anti-inflammatory impact. Ephe-drannin A and Ephe-drannin B were discovered by Iksoo et al. in the *Ephedra* root extracts to have anti-inflammatory properties. They could prevent inflammation caused by Lps(lipopolysaccharides) and reduce the transcription of TNF- and IL-1. They prevented NF-

B from moving and p38 mitogen-activated protein (MAP) kinase from being phosphorylated (Zhang et *al*., 2018).

I.3.2. Antibacterial and antifungal activities

The phenolic compounds isolated from the *Ephedra* herbs exhibit substantial antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria 76. Among *E.strobilacea, E.pachyclada* and *E. Procera*, the *E. strobilacea* showed the highest antimicrobial activity against three microorganisms, including Pseudomonas aeruginosa, a Gram negative bacterium, Staphylococcus aureus, a Gram positive bacterium, and Aspergillus nigra a fungus. The plant metha-nolic extracts of *E.pachyclada* show significant antimicrobial activity against Klebsiella pnemoniae, a Gram negative bacterium, Bacillus subtilis, a Gram positive bacterium and highest antifungal activity against the Candida albicans microorganism. There is a strong relationship among total phenol content and antioxidant, antimicrobial activity, as phenols are very important plant constituents because their hydroxyl groups can scavenge free radicals (Zhang et al., 2018)

I.3.3. Antiviral activity

E. herb extracts can induce the replication of latent human immunodeficiency virus type 1 (HIV-1) in latently infected U1 cells *in vitro*, through NF-KB activation. The ultimate goal is to eliminate the persistent viral reservoirs in individuals infected with HIV-1. NF-KB is an inducible cellular transcription factor that regulates a wide variety of cellular and viral gene expression. The *E. herb* extracts can efficiently induce NF-KB nuclear translocation and activate the NF-KB promoter. Zhang et all these confirmed that ephedrine alkaloids-free *Ephedra Herb* extracts have anti-influenza virus activity by showing inhibition of MDCK cell infected with influenza virus A/WSN/33 (H1N1) (Zhang et *al*.,2018).

I.3.4. Anti-oxidant activity

Evaluation of a becoming increasingly relevant in the field of nutrition as it provides useful information with regard to health promoting and functional quality of raw materials whether they are fruits, vegetables, or medicinal plants. This parameter accounts for the presence of efficient oxygen radical scavengers, such as phenolic compounds. The antioxidant activity of phenolic is mainly due to their redox properties, which make them acting as reducing agents, hydrogen donors, and singlet oxygen quenchers. There are two types of antioxidant assays used to evaluate the antioxidant activity of plant extracts (*ephedra*). The first category measures the potential of plant extracts to reduce ions or oxidants (to act as

reducing agents) like ferric ioncupricion. The main two assays of this antioxidant activity category are FRAP (measures the reduction potential of ferric to ferrous ion), and CUPRAC (measures the reduction of cupric to cuprous ion). The second category of antioxidant activity measures the ability of plant extracts to scavenge free radicals. DPPH and ABTS assays (where DPPH and ABTS are stable free radicals) are the two main examples of this category. These assays are used because they are quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present (Al-Rimawi et *al*., 2017).

I.3.5. Anticancer activity

Many recent studies demonstrated the anticancer effect of several *E. alata* extracts (methanolic, ethanolic, water…). Corina *et al.* (2018), Sioud *et al.* (2020) and Bensam *et al.* (2023) were tested *in vitro* the effect of *E. alata* on different cancer cell lines: MCF-7, 4T1, HepG- 2, Caco-2, A549…They found that this plant inhibit growth of all cancerous cells in a dose and time dependent manner.

I.4. Toxicity

Herbal compositions containing *Ephedra* alkaloids have been widely consumed as dietary supplements for weight loss and energy enhancement González-Juárez., 2020). In 2002, several deaths from cardiac and cerebrovascular events were recorded in previously healthy patients taking "Hydroxycut" products. These adverse events were traced to their *Ephedra* content (*Ephedra Herba*, *Mahuang*) and at the end of 2004, the U.S. Food and Drug Administration (FDA) banned the sale of those products (González-Juárez et *al*., 2020).

The primary pharmacological activities and adverse effects of *Ephedra* species are caused mainly by two active constituents, (-)-*ephedrine* (1) and (+)-*pseudoephedrine* (2), which are potent sympathomimetic drugs. Chronic use can produce hypertension, palpitations, tachycardia, arrhythmia, acute myocardial infarction, cardiac arrest, or sudden death and hemorrhagic and ischemic strokes (González-Juárez et *al*., 2020).

Persky et *al* (2004) studied the cardiovascular effects of (-)-*ephedrine* (1) with eight subjects who received placebo, or different doses of *ephedrine* sulphate (0.25, 0.5 or 1.0 mg/kg) administered orally for seven days. Although systolic blood pressure increases quickly after the *ephedrine* sulphate administration, the increase was nearly abolished by compensatory mechanisms .This compensatory response is very important since regularly, the *Ephedra* alkaloids are consumed in dietary supplements with an approximate quantity of 20 mg to 66 mg/day (González-Juárez et *al*., 2020).

Han et al. (2018) studied the sub chronic toxicity of an *Ephedra Herba* aqueous extract in F344 rats or 13 weeks, during the study, several animals died only in the highest-dose group, indicating that the *Ephedra Herba* aqueous extract is toxic at high doses (González-Juárez et *al*., 2020). These results suggest that Ephedra may contribute to increased blood pressure, causing kidney disorders. The NOAEL (No observed adverse effect level) was determined at 125 mg/kg/day dose (González-Juárez et *al*., 2020).

I.5. *Ephedra alata*

I.5.1. Systematic position

Kingdom: *Plantae*
Phylum: *Tracheophyta*
Division*: Gnetophyta*
Class*: Gnetopsida*
Order*: Ephedrales*
Family*: Ephedraceae*
Genus*: Ephedra*
Species*: Ephedra alata* (Al-Snafi, 2017).

I.5.2. Botanical description

Ephedra alata plant species found mainly in Sinai desert and Eastern Mediterranean coastal region. It is a pharmaceutically important plant, which belongs to the Ephedraceae family of gymnosperms and is known to have a number of medicinal properties (Nadir, 2022).

It is short, evergreen and almost leafless shrubs that grow about 60 to 90 cm high. The stems are green in color, slender, erect or reclining, small ribbed and channeled, about 1.5 mm in diameter and usually terminating in a sharp point. Nodes are 4 to 6 cm apart, and small triangular leaves appear at the stem nodes. The nodes are characteristically reddish brown, and the stems usually branch form the base. They bear minute, yellow-green flowers and fruits, and emit a strong pine-like odor and have an astringent taste (Fig 8) Al-sanafi,2017).

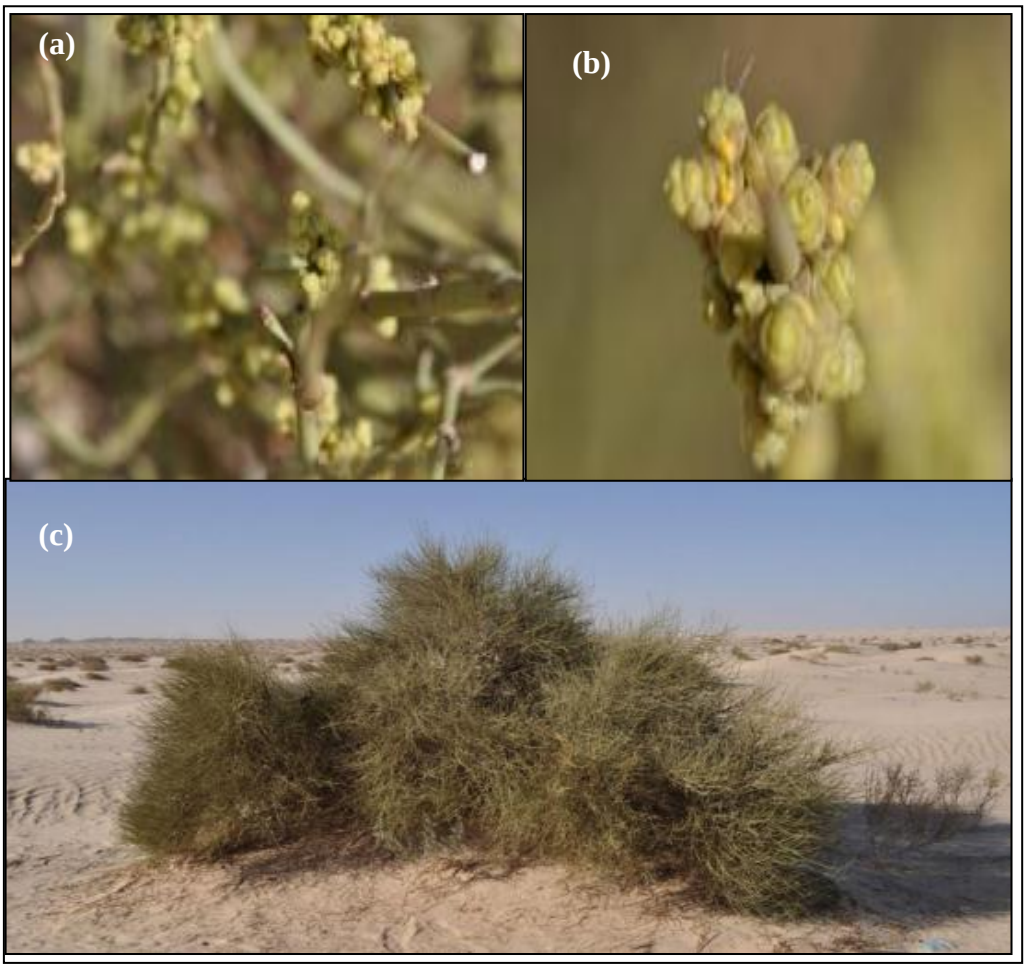


Fig.8. ***Ephedra alata plant*** **.A.** ***Fleur in bloom*** **.B .*Flowering rameau*** **.C.** (Palici., 2016).

I.5.3. Distribution

Ephedra alata is distributed in Africa; Algeria; Egypt, Libyan, Morocco, Tunisia, Mauritania, Chad, Mali andin Asia, Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan and Syria (Al-Snafi, 2017).

I.5.4. Chemical composition

The preliminary phytochemical analyses of *Ephedra alata* indicated the presence of cardiac glycosides, reducing sugars, flavonoids, phenolic compounds and alkaloids .*Ephedra* species contain alkaloids *ephedrine*, *pseudoephedrine*, *norephedrine*, *norpseudoephedrine*, methylephedrine, and methyl *pseudoephedrine*. Beside the E-type alkaloids, ephedroxane, and macrocyclic spermidines called *ephedradine* A-D, which isolated from some Eurasian *Ephedra* species. The total amount of alkaloids isolated from *Ephedra alata* aerial parts was 0.2-0.22%. The amount of ephedrine in *Ephedra alata* was 0.05–0.19%, *pseudoephedrine* > 0.5%, while the amount of tannin was 0.2–0.5% (Al-Sanafi, 2017).

Kittana et *al*., (2017) were performed a phytochemical analysis of *E. alata* aqueous extraction order to identify the mjor classes of phytochemical compounds. They were found that this plant is a natural source of chemical compounds (Table 4).

Table.4. Results of phytochemical analysis of *E. alata* extract (Kittana et *al*., 2017).

Test	Result	Phytochemical compound
Wagner's test	Positive	Alkaloids
Keller-Kilani test	Positive	Glycoside
Alkaline reagent test	Positive	Favonoid
Liebermann test	Positive	Phytosteroid
Folinciocalteu test	Positive	Phenolic compound
KOH test	Positive	Volatile oil
Ferric chloride test	Positive	Tannin

I.5.5. Utilization

In general, plants in the genus *Ephedra* have been used in traditional medicine to treat allergy, bronchial asthma, chills, cold, cough, edema, fever, flu, nasalcongestion, and headache. Phytochemical analysis of *E. alata* indicated the presence of tannins, cardiac glycosides, alkaloids, phenolics, reducing sugars, and flavonoids. Additionally, Ephedra species contain alkaloids such asephedrine, pseudoephedrine, norephedrine norpseudoephedrine, methylephedrine, methylpseudoephedrine, ephedroxane, and ephedradine. Phenolic compounds including chlorogenic acid, rutin, catechin, quercetin, and coumaric acid and various flavonoids have also been isolated from *E. alata*. The total amount of alkaloids isolated from *E. alata* aerial parts was 0.2–0.22% , and the amount of *ephedrine* and pseudoephedrine was 0.05–0.19% and >0.5%, respectively (Al-Snafi, 2017).

I.5.6. pharmacologie propreities

I.5.6.1. Antioxidant effect

The antioxidant activity of *Ephedra alata* was evaluated by 2, 2-diphenyl-1-picryl-hydrazyl hydrate assay. *Ephedra alata* methanolic extract showed high antioxidant activity and powerful oxygen free radical scavenging abilities, the IC50 for the plant was almost equivalent to the Trolox standard antioxidant .Danciu et *al*. (2018) Showed that the Tunisian aerial parts of *E. alata* Decne, extracted with EtOH 70%, have an important antioxidant activity (CUPRAC) which is around 7453.18 µmol Trolox/g. Also, Benabderrahim et *al*.

(2019) found that the antioxidant con-tents, expressed by DPPH and ABTS, of EtOH/water (v/v) extracts of *E. alata* Decne were, respectively, 33.51 ± 0.05 mg TEAC/100 g and 37.86 ± 0.03 mg TEAC/100 g. Mighri et al. (2019) showed that the chloroform fraction of Tunisian aerial parts of *E. alata* exhibited the highest antioxidant activity (TAC and DPPH) compared to methanol extract and butanol, ethyl acetate, and water fractions (Elhadef et *al*.,2020).

I.5.6.2. Hypoglycemic effect

Alcoholic extract of *Ephedra alata* exerted hypoglycemia, one hour after administration to fasting rats. The same extract failed to reduce blood glucose levels in alloxanized rats compared to the positive control, glibenclamide (Rashed, 2021).

I.5.6.3. Antimicrobial effects

Antimicrobial activity of different extracts of *Ephedra alata* stem was investigated against bacteria, yeast and fungi. Four bacteria, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Escherichia coli and four fungi, Aspergillus fumigatus, Penicillium italicum, Syncephalastrum racemosum, and Candida albicans were used as test microorganisms. Acetonitrile extracts exhibited the most potent antimicrobial effect with a broad spectral range. Thin layer chromatographic separation of active constituents in acetonitrile extracts revealed the presence of seven fractions. All fractions showed antimicrobial activities with four fractions having a potent inhibitory effect (Rashed, 2021).

I.5.6.4. Diuretic effect

It was reported that alkaloids from *Ephedra* stem have the function of clearing and regulating the distribution and excretion of water in vivo to exert the diuretic and antioncotic effects, and D-pseudoephedrine shows the strongest pharmacological activity among all the alkaloids. Experiment results showed that urine volume can be extended to two to five times that of pre-dose when anesthetized dogs are intravenously injected with D-pseudoephedrine (0.5–1.0 mg·kg−1), and the pharmacological time of single administration canreach 30–60 min (Rashed, 2021).

I.5.6.5. Analgesic and Hypolipidaemic Effects

The histological study confirmed the biochemical results. According to the results of the analgesic activity, the extract of *E. alata* induced a significant decrease in abdominal writings compared to the control group and the values obtained are very close to those obtained with indomethacin (Rashed, 2021).

I.6. *Ephedra Foeminea*

I.6.1. Systematic position

Super division: *Spermatophyta*

Division: *Gnetophyta*

Family*: Ephedraceae*

Genus: *Ephedra*

Species: *Foeminea*

I.6.2. Botanical description

The tectum is distinctly thinner at the top of the plicae (0.57 ± 0.23 µm in studied pollen) and thicker at its sides and bases (1.20 ± 0.38 µm). The infractectum is granular with a high density of small granules of equal size. The granular infractectum constitutes only a small fraction of the plicae (Fig 9) (Bolinder et *al*., 2015).

Fig.9. Photo of pollen grains around an ovule of Foeminea *and general view.* (Bolinder et *al*., 2015*; Plants of the world online*).

I.6.3. Distribution

Ephedra foeminea is distributed in south eastern Mediterranean from: Italy and Greece; in Middle East from: Jordan, Syria, Iraq. Extends further south to Sinai, Saudi Arabia and Yemen (fig12) (Caveney et *al*., 2001), and from Palestine (Ghadeer et *al*., 2021).

Fig.10. Geographical distribution of *E. foeminea* (Plant in the world online).

I.6.4. Chemical composition

The phytochemical investigations showed that, unlike other *ephedra* plants, *ephedra* alkaloids were absent in *Ephedra foeminea*. This finding is consistent with a previous study which confirmed the absence of potentially hazardous ephedra alkaloids from *Ephedra foeminea* (Al-saraireh et *al*., 2021).

Interestingly, the analysis found that the major component of the plant methanol extract was 4Hpyran-4 one, 2, 3-dihydro-3,5-dihydroxy-6-methyl. This compound has been shown to reduce the growth of colon cancer cells by causing apoptotic cell death through inhibition of NF-κB(Al-saraireh et *al*., 2021).

I.6.5. Utilization

Ephedra foeminea also known as "Qudab" in Arabic (R. Al-Nemi et *al*., 2022).There are several historically medicinal plants in the East Mediterranean region that are utilized in folk medicine to treat a variety of illnesses and problems. According to a published study, *Ephedra foeminea* is the only *Ephedra* species that does not contain the stimulants *ephedrine* and *pseudoephedrine*, which can have negative effects on the heart, blood pressure, and

central nervous system. This makes it a relatively safe therapeutic choice. *Ephedra* is used as a treatment for a variety of ailments in folk medicine, including headaches, edema, fever, flu, allergies, bronchial asthma, chills, colds, and coughs (Al-Nemi et *al*., 2022).

I.6.6. pharmacological propreities

I.6.6.1. Antimicrobial activity

In the current work, aqueous and ethanoic aerial component extracts of *E. foeminea* were tested for possible antibacterial activity against E. coli using the broth microdilution method. The outcomes demonstrated that *E. foeminea* aerial component extracts both exhibited antibacterial activity against the E. coli strain and were both aqueous and ethanoic. Using various types of extracts, antimicrobial activity of some *Ephedra* species has been reported. The active components of the ephedra plant are phenolic chemicals, according to earlier investigations (Ismail et *al*., 2020).

I.6.6.2. Antioxidant activity

The aqueous Ephedra foeminea extract's antioxidant capacity was assessed using the ABTS and DPPH techniques. In terms of trolox equivalents, the antioxidant activity against ABTS radical scavenging was calculated. Ephedra *foeminea's* aqueous extract had a 12.28 mg Trolox/g plant extract scavenging activity for the radical ABTS. With an observed value of 72.8 mg GAE/g plant extract, the overall antioxidant activity as evaluated by DPPH was comparable to that indicated by ABTS. *Ephedra foeminea* aqueous extract demonstrated a DPPH scavenging potential of more than 50% at a low concentration (0.4 mg/mL) (Abu hajleh et *al*., 2022).

I.6.6.3. Antidiabetic activity

The reduction in blood glucose of induced diabetic rats given *Ephedra foeminea* extract on the first day of the experiment and for just four days was used in this study to support the protective effect of the herb. For a period of four weeks, the blood glucose levels of each group were assessed on a weekly basis. Untreated induced diabetic rats showed a substantial progressive increase in blood glucose levels, whereas those treated with metformin and *Ephedra foeminea* extract showed a significant gradual drop in blood glucose levels. In addition, when induced diabetic rats receiving metformin and *Ephedra foeminea* extract were compared to the untreated induced diabetic rats, the glucose concentration was considerably lower (Abu hajleh et *al*., 2022).

Over the course of four weeks, the body weights of each group were assessed weekly. Induced diabetic rats (G2) that were left untreated for the duration of the experiment had a substantial loss in body weight when compared to the first day of the experiment (p 0.05). In comparison to the untreated induced diabetic rats, diabetic rats treated with metformin (G3) and *Ephedra foeminea* extract (G4 and G5) from Day 15 onwards had considerably (p 0.0001) higher body weights (Abu hajleh et *al*., 2022).

I.6.6.4. Anticancer activity

Various plants have been examined for their content of different bioactive compounds and how they affect cell physiological functions, as well as their anti-cancer potential and capacity to prevent cancer cell growth (Greenwell et Rahman, 2015).

Recently, the idea of using *Ephedra* as an alternative to cancer therapy has become popular. This is particularly true of *Ephedra foeminea*, which many cancer patients in the Middle East region utilize because of the belief that it has cancer-curative properties (Mendelovich et *al*., 2017). Based on these observations, limited studies have investigated the effect of crude extracts of various types of *Ephedra foeminea* on different cancer cell lines (Mpingrika et *al*., 2020).The study of Mpingirika et al (2020) showed that extracts and fruit juice of Ephedra foeminea significantly decreased the viability of colon cancer cells (HTC116) and breast cancer cells (MDA-MB-213).

Chapter II

Cancer

II.1. What is cancer

Cancer develops when normal cells in a particular part of the body begin to grow out of control. There are different types of cancers; all types of cancer cells continue to grow, divide and re-divide instead of dying and form new abnormal cells. Some types of cancer cells often travel to other parts of the body through blood circulation or lymph vessels (metastasis), where they begin to grow. For example when a breast cancer cell spread to liver through blood circulation, the cancer is still called as breast cancer, not a liver cancer. Generally cancer cells develop from normal cells due to damage of DNA. Most of the time when ever DNA was damaged, the body is able to repair it, unfortunately in cancer cells, damaged DNA is not repaired. People can also inherit damaged DNA from parents, which accounts for inherited cancers. Many times though, a person's DNA becomes damaged by exposure to something in the environment, like smoking (Sudhakar, 2009).

Cancer generally forms as a solid tumor. Some cancers like leukemia (blood cancer) do not form tumors. Instead, leukemia cells involve the blood and blood forming organs and circulate through other tissues where they grow. Not all tumors are cancerous, some tumors are benign (non-cancerous). Benign tumors do not grow and are not life threatening. Different types of cancer cells can behave differently. The risk of developing many types of cancers can be reduced by changes in lifestyle by quitting smoking and eating low fat diet. If cancer is identified in early stage it is easy to treat and may have better chances for living many years (Sudhakar, 2009).

II.2. History of cancer

Cancer is the second leading cause of death in the world after cardiovascular diseases. Half of men and one third of women in the United States will develop cancer during their lifetimes. Today, millions of cancer people extend their life due to early identification and treatment. Cancer is not a new disease and has afflicted people throughout the world. The word cancer came from a Greek words karkinos to describe carcinoma tumors by a physician Hippocrates (460-370 B.C), but he was not the first to discover this disease. Some of the earliest evidence of human bone cancer was found in mummies in ancient Egypt and in ancient manuscripts dates about 1600 B.C. The world's oldest recorded case of breast cancer hails from ancient Egypt in 1500 B.C and it was recorded that there was no treatment for the cancer, only palliative treatment. According to inscriptions, surface tumors were surgically removed in a similar manner as they are removed today (Sudhakar, 2009).

II.3. Epidemiology

Each year, the American Cancer Society estimates the numbers of new cancer cases and deaths in the United States and compiles the most recent data on population-based cancer occurrence and outcomes using incidence data collected by central cancer registries and mortality data collected by the National Center for Health Statistics. In 2023, 1,958,310 new cancer cases and 609,820 cancer deaths are projected to occur in the United States. Despite the pandemic, and in contrast with other leading causes of death, the cancer death rate continued to decline from 2019 to 2020, contributing to a 33% overall reduction since 1991 and an estimated 3.8 million deaths averted. This progress increasingly reflects advances in treatment, which are particularly evident in the rapid declines in mortality for leukemia, melanoma, and kidney cancer, despite stable/increasing incidence, and accelerated declines for lung cancer. In summary, although cancer mortality rates continue to decline, future progress may be attenuated by rising incidence for breast, prostate, and uterine corpus cancers, which also happen to have the largest racial disparities in mortality (Siegel *et al*., 2022).

Algeria registers nearly 47,050 new cancer cases of all types annually, according to data from the National Cancer Registry of the National Institute of Public Health (INSP) in 2022, with the most common type being breast cancer. In 2023, there are projected to be 58,418 new cases. Among the most common types of cancer in society, the national registry cites colorectal, lung, prostate, bladder and digestive cancer in men. Next is breast cancer, colorectal cancer, thyroid and cervical cancer in women. Cancer of the digestive tract generally remains the national predominant in both sexes (INSP, 2022).

The experts attribute this large proliferation in Algeria, like in some countries of the world, to several factors, including the change in the population's diet, dominated by industrialized foods, to environmental factors, such as pollution, fertilizers that are added to agriculture, as well as other genetic factors. Breast cancer, one of the most common types of cancer according to the INSP, registers more than 14,000 cases annually. This type of cancer has a particularity in Algeria, in that it affects women at an early age within the age of 40, unlike advanced countries where it spreads among women aged 55 and over (INSP, 2022).

II.4. Carcinogenesis

Carcinogenesis represents all the pathological phenomena leading to the transformation of a normal cell into a cancer cell. When a cell reaches this stage, its survival and proliferation

are no longer controlled. Cancer cells also have the properties of migration, invasion, and metastatic development (Farion, 2019).

Normal cells depend on growth signaling of a tightly-regulated cell cycle to controllably proliferate and maintain tissue homeostasis this is disrupted in case of cancer .It is currently appreciated that in cancer cells, the growth and proliferative signaling pathways harbor one or more driving alterations within their compartments giving them a survival edge. Those compartments include growth factors, their receptors or the cytosolic signaling molecules (Fouad and Aane, 2017).

Growth factors in a tumor setting would be growth-promoting, expected to be aberrantly produced in high levels by epithelial or stromal cells acting in an autocrine or paracrine fashion to promote tumor progression, and growth-inhibiting, expected to be shutdown to allow the tumor's escape from braking signals .One of these transforming growth factor (TGF-β), which is a conventional anti-growth ligand. It has been conversely shown to be implicated in tumor progression both by stimulating cancer cell dedifferentiation (Fig.11) (Fouad and Aane, 2017). .

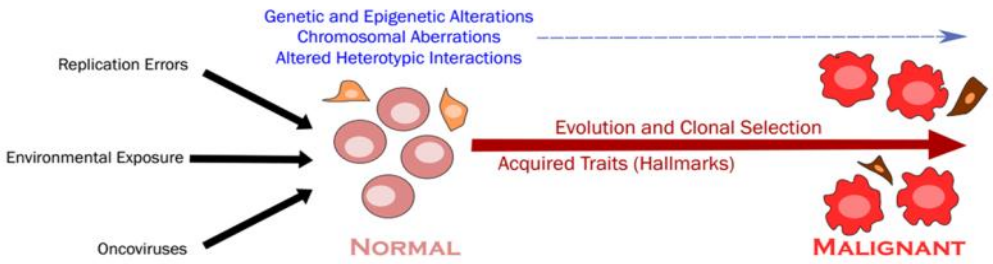


Fig.11. Transformation process (Fouad and Aanei, 2017).

II.4.1. Stage of carcinogenesis

Cancer cells can remain very vulnerable for many years, even decades, and only a few will reach the malignant stage (Béliveau and Gingras, 2007). This process is lengthy and can make it difficult to account for a potentially carcinogenic agent. For example, in an epidemiological case-control study carried out after exposure to the agent under consideration, different biases such as confusion or anamnesis biases may affect the quality of the study because the study is carried out retrospectively (Farion, 2019).

A cancer occurs from a cell that undergoes a mutation or alteration of its genetic or epigenetic heritage, and then transforms through three main stages as shown in (Fig.12).

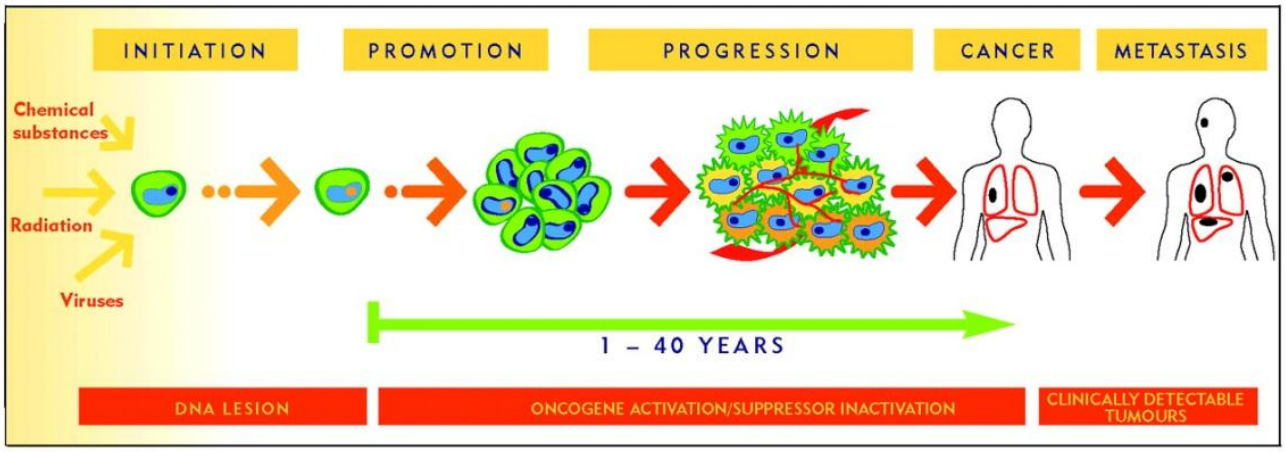


Fig.12. Stage of carcinogenesis (Béliveau and Gingras, 2007).

II.4.1.1. Initiation

Any sudden and permanent modification of hereditary traits by change in the number or quality of genes is called a mutation. The mutations correspond to an irreversible mutagenic impairment, carried out by an initiating carcinogen. This stage represents the initiation phase of the carcinogenesis process (Farion, 2019). Mutations can be:

-In gene: modification of the genotype by addition, substitution by alkylating agents, suppression of base pairs, shift of the reading frame of DNA strands called frameshifts at the time of translation of DNA strands (Farion, 2019);

-chromosomal: breaks and exchanges of genetic material between chromosomes where the mutation corresponds to a change in the position of DNA sequences coding for one or more genes, which correspond to a clastogenic effect (Farion, 2019);

-genomics: changes in the number of chromosomes; which correspond to a so-called "aneugene" effect (Farion, 2019).

Although mutations are genome modifications, they do not necessarily have consequences for the cell phenotype. Indeed, the cell containing the damaged DNA is a cell called «initiated»: its genome is modified, but not necessarily its phenotype. On the other hand, as shown in (Fig.12), an initiated cell can remain dormant for years. The mutagenic properties of an agent can be demonstrated by different types of mutagenesis tests widely used in toxicology. These mutagenesis studies have a key place in the assessment of an

agent's genotoxic potential. It is therefore necessary in an assessment to characterize the "genotoxic" character of the agent under consideration (Farion, 2019).

II.4.1.2. Promotion

The phase of «promotion» corresponds to the stimulation of «initiated» cells, which promotes their proliferation. This epigenetic process can be performed by the agent that caused the DNA damage, or by another agent. Regardless of the type of agent, they are called tumour promoters and the process leads to the formation of pre-neoplastic cells. This is a reversible phenomenon because it can be stopped by apoptosis of formed cells (Farion, 2019).

II.4.1.3. Progression

The progression phase corresponds to the last phase of genetic instability generated during the previous stages. It is manifested by the loss of the differentiation properties of pre-neoplastic cells and the acquisition of uncontrolled multiplication properties by the loss of contact inhibition, which leads to the formation of tumors. This irreversible clinical step leads to the local invasion of the tissues surrounding the tumor, or even the development of metastases in other sites of the body (Farion, 2019).

II.5. Metastasis

The spread of cancer from its tissue of origin and its subsequent growth in other organs is the most life-threatening aspect of the disease. This process is called metastasis, and requires cancer cells to survive and proliferate outside their tissue of origin. The first crucial step in this process is the movement of cancer cells into tissue surrounding the tumour and the vasculature. Metastasis is a dynamic, multifaceted process during which normal cells transform into oncogenic cells that proliferate uncontrollably, evade the immune system, become resistant to programmed cell death, stimulate angiogenesis, acquire invasive potential, survive in the blood stream and establish cancerous growths in distant organs (Fig.13) (Sahia, 2005; Castanedaetall, 2022).

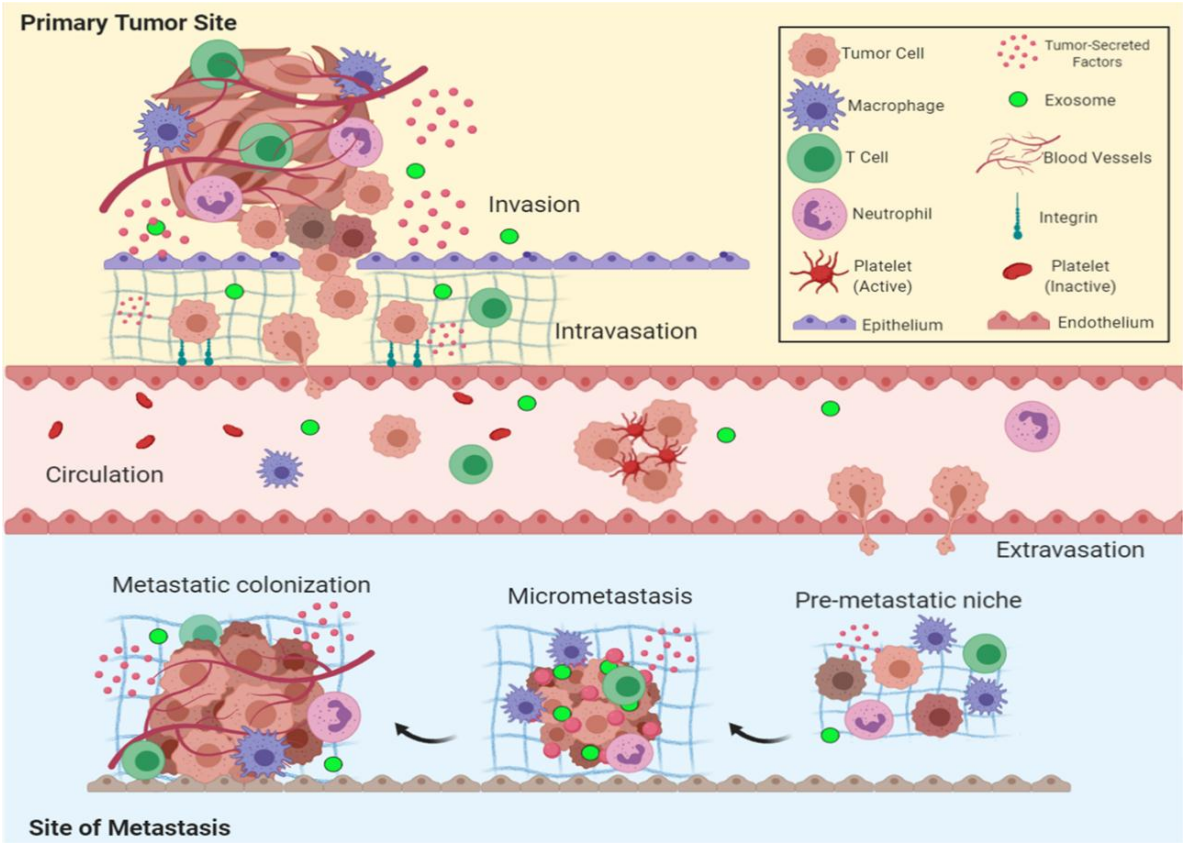


Fig.13. The metastatic cascade: the five key steps of metastasis include invasion, intravasation, circulation, extravasation, and colonization (Fares *et al*., 2020).

II.6. Characteristics of cancer cell

A cancerous cell is differentiated from a healthy cell by morphological, behavioral, biochemical and chromosomal criteria (Table.5) (Chiapolino, 2018).

Table.5. Characteristics of the cancer cell (Chiapolino, 2018).

Criteria	Characteristics
Morphological	- Cells of unequal size: anisocytosis - Cellular gigantisme - Increased nucleocytoplasomic ratio - Irregularty shape hyperchromatic nucleus - Nunerous and large nucleoli - Hyperbasophilic cytoplasm (protein synthesis increased) - Unequal sized nucleus : anisokaryosis
Behavioral	- Immortality

	- Indefinit growth - Loss of dependence on anchoring (may grow in hack of support) - Loss of cantact inhibition - Aggressiveness (destroys normal cells) - Self-sufficiency for growth signals (synthesizes its own growth factors) - Ability to metastasize
Biochemical	- Increased mobility - Membrane modifications: concern the proteins, phospholipids and membrane antigens - Functional modifications: corresponds to maintaining loss or gain by tumor cells of functions characterizing normal cells counterparts
Chromosomal	- Quantitative anomalies: anomalies in the number of chromosomes (aneuploidy, polyploidy) - Qualitative anomalies: anomaly in the structure of the chromosome (addition, deletion, translocation

II.7. Genes of cancer

II.7.1. Oncogenes

These genes that cause normal cells to grow out of control and become cancer cells They are formed by the mutations of certain normal genes of the cell called proto-oncogenes (genes that normally control how often a cell divides and the degree to which it differentiates) (Sudhakar, 2009). An example of such a mode of oncogene activation is that of HER-2, which is seen in about 20% of primary breast cancer cases. Another mechanism of activation is a point mutation that enhances the function of the oncoprotein. Examples include point mutations in the Ras oncogene, seen commonly in lung, colorectal, and pancreatic (but not breast) cancer. Activating point mutations in Ras codons 12,13, and 61 prevent the interaction of p21ras with GTPase-activating protein (GAP), maintaining p21ras in an activated and GTP-bound form and enhancing downstream signaling events such as cell cycle entry/activation (Table.6) (Osborne, 2004).

Table.6. Oncogenes involved in cancer (Weinstein and Joe, 2008).

Targeted oncogene	Cancer type
c-myc	T cell and acute myeloid Leukemia
Bcr-Abl	Leukemia
H-ras	Melanoma
K-ras	Lung
c-myc	Pancreatic h-cell
c-myc	Osteogenic sarcoma
Her-2	Breast
c-myc	Breast
Wnt-1	Breast

II.7.2. Tumor suppressor genes

These are normal genes that control cell division, DNA repair and inform cells when to die. When a tumor suppressor gene doesn't work properly, cells can grow out of control, which can lead to cancer. Scientists identified oncogenes and tumor suppressor genes that are damaged by chemicals or radiation. For example, the discovery of breast cancers genes BRCA1 and BRCA2. Other genes have been discovered that are associated with cancers that run in families, such as thyroid, pancreas, rectum, colon, kidney, ovary and skin cancers (Table.7)(Sudhakar, 2009).

II.7.2.1. P53

The p53 gene product is a significant tumor suppressor that is rendered inactive by deletion or mutation in around 50% of all human malignancies. Additionally, p53 mutations are discovered in patients with the Li-Fraumeni syndrome, which is characterized by a high propensity for cancer. Protein p53 is a transcription factor that links ADN and induces the production of numerous genes involved in controlling cell proliferation, ADN repair, and apoptosis. The levels of p53 protein are under tight control in resting cells, but the protein is quickly induced and activated post-transcriptionally by several signaling pathways. Many stresses, including ADN damage, oxidative stress, and oncogene activation, can activate p53 (Mallette, 2008).

Table.7. Genes that normally suppress tumor growth are inactive during tumorigenesis (Deltour et *al*., 2005).

Genes	Roles	Inactivation by		Main types of cancer associated
		genetic mutations	changes epigenetics	
Cell cycle				
Rb	Inhibition of transcription dependent on E2F	+	+	Retinoblastoma, glioma Colon Cancer
p16 INK4A	CDK 4 and 6 inhibitor	+	+	leukemia, lymphoma, skin and lung cancers
p15 INK4B	CDK 4 and 6 inhibitor	+	+	Leukemia, lymphoma
Maintaining the integrity of the genome				
p53	Gene transcription (cell cycle and apoptosis) and DNA repair	+	+	Cancers of lung, prostate, breast and ovary
BRCA1	Transcription and DNA repair regulation	+	+	Breast and ovary cancers
O6-MGMT	Drug resistance repair	-	+	Glioma, lymphoma, cancers prostate lung
Hmlh1	DNA repair	+	+	Gastric cancers, colon and ovary
Apoptosis				
Caspase 8	Substrates cleavage when activating death receivers	+	+	Medulloblastoma, lung and liver cancers
DAPK	Kinase calcium-dependent phosphorying various substrates	+	+	Lymphoma, glioma, gastric cancers and cervix
Migration, invasion				
E-cadherin	Interaction cell/ cell and extracellular matrix	+	+	Gastric cancers, thyroid and breast
TIMP-3	Metaloprotease inhibitor	+	+	Medulloblastoma and liver cancer

Response to growth factors				
PTEN	Inhibition of the PI3-K route	+	+	Glioblastoma, gastric cancers, breast and thyroid
ER	Proliferation control	+	+	Breast and prostate cancers
Other tumor suppressors				
HIC1	Transcriptional repressor	-	+	Medulloblastoma, glioma, breast and ovary cancers
OVCA1	Homology with DPH2	-	+	Ovary cancers
OVCA1	Interaction and stabilization of microtubules	-	+	Glioma,melanoma lung and breast cancer

II.7.2.2. RB

The retinoblastoma gene (Rb) was found in young children who had eye tumors, and its large domain (amino acids 395-876) allows for its relationship with E2f. The transcription factors proteins E2Fs are linked to the consensus ADN site TTTCGCGC. The E2F gene belongs to a family of heterodimeric transcription factors. Eight members (E2F-1 to E2F-8) make up the family E2F among mammals. Numerous genes involved in the regulation of the cell cycle, including cyclin A, cyclin E, E2F, Cdc2 and Cdc25A, are activated by this family of transcription factors (Mallette, 2008).

II.7.2.3. Control of E2F activity

The degree to which Rb is phosphorylated determines how E2f activity is regulated. When Rb is hypo phosphorylated, it is inactive and inhibits E2f, but when Rb is phosphorylated, E2F is released, allowing the production of genes that allow entry into phase S. The activity of mitogenetic signals convergent toward the cell cycle machinery, which is made up of complexes cyclin D/CDK4 or CDK6 in the early G1 phase and cyclin E/CDK2 in the late G1 phase, results in phosphorylation (Mallette, 2008).

II.7.2.4. Connection of the RB and p53 pathways

Although p53 and Rb may initiate separate tumor-suppressive programs, a number of factors connect these two major pathways. First off, Rb's ability to inhibit E2F activity depends on its level of phosphorylation and, consequently, the activity of the cyclin/CDK complexes. Therefore, the p21-containing kinase inhibitors of these complexes contribute to

the activation of Rb. Alternately, p21 serves as a traditional transcriptional target for p53. Therefore, p53 activation enables control over Rb activity (Mallette, 2008).

II.8. Signaling pathways involved in cancer

Since the early discovery of oncogenes (e.g., MYC, RAS, BRAF) and tumor suppressor genes (e.g., pT53, BRCA1 and PTEN), cancer-associated genetic lesions have been extensively documented. Nowadays, signaling pathways and molecular networks are recognized for their critical roles in executing and controlling important pro-survival and pro-growth cellular processes and are therefore chiefly implicated in the onset of cancer, and also in its potential treatment (Yip and Papa; 2021)

Two pathways in particular, the PI3K/AKT/mTOR signal transduction pathway and the Ras/MAPK pathway, are frequently activated or mutated in cancer. These cascades are highly interconnected in mediating upstream signals from receptor tyrosine kinases (RTKs) to intracellular effector proteins and cell cycle regulators. In addition to the linear signals propagated from the extracellular space into the cytoplasmic and nuclear compartments, the PI3K and MAPK pathways are strongly interconnected through a number of positive and negative feedback loops. As an example, targeted inhibition of mTORC1 through the administration of analogs of rapamycin, also known as rapalogs, can cause MAPK reactivation and can lead to resistance to single mTORC1 inhibition. Consequently, combinatorial targeting of mTOR plus MAPK has been shown to induce a better response to therapies (Yip and Papa, 2021).

II.9. Cancer particularity

II.9.1. Apoptosis

Apoptosis is a ubiquitous mode of cell death that plays an opposite role to mitosis in the regulation of animal cell populations. It intervenes, for example, in the embryonic and fetal development (from the regulation of the cellularity of the blastocyst to the organization of the central nervous system or the genital system), in the development of the immune system and in the homeostasis of hormone-dependent tissues. Tissue homeostasis requires a constant balance between death and cell proliferation. In higher organisms, cellular metabolism is directed by signals (hormones, growth factors) from neigh boring cells: some control its proliferation, others its suicide program. For example, the primary function of hematopoietic growth factors is to prevent the apoptosis of cells capable of recognizing them (Solary et *al*., 1993).

In general, cell suicide is activated to selectively eliminate cells that have become undesirable. These may be damaged or senescent cells (polynuclear accumulated on an inflammatory site), cells recognized as foreign or pre-neoplastic (cytotoxic cells kill their target cells by inducing their apoptosis), cells that are no longer in their place and have lost contact with their micro-environment (epidermal cells that have migrated into the subcutaneous tissue as a result of trauma), or excess cells competing with other vis-à-vis cells to an inhibitor signal **(**Solary et *al*., 1993).

II.9.1.1. Characteristics of the apoptosis

The identification of an apoptotic cell is primarily based on stereotyped morphological criteria dominated by the condensation of nuclear chromatin, the fragmentation of the cell into apoptotic bodies, and their phagocytosis by neigh boring cells (Fig.14). Unlike necrosis, chromatin does not flocculate, mitochondria do not swell, the cell membrane is not permeable to vital dyes and apoptosis does not trigger an inflammatory reaction because lysosomal enzymes are not released. The internucleosomial fragmentation of DNA gives it a characteristic appearance in scale, in an electrophoresis gel. This fragmentation is a central element of the apoptotic process. Its intensity and its precocity vary from cell to cell. The apoptotic process is accompanied by characteristic changes in the plasma membrane, cytoskeleton, transduction signals and nucleus. These changes are most often limited to certain cellular systems and are summarized. Active transcription of non-specific genes and synthesis of new proteins are not always necessary for the apoptotic process **(**Solary et *al*., 1993).

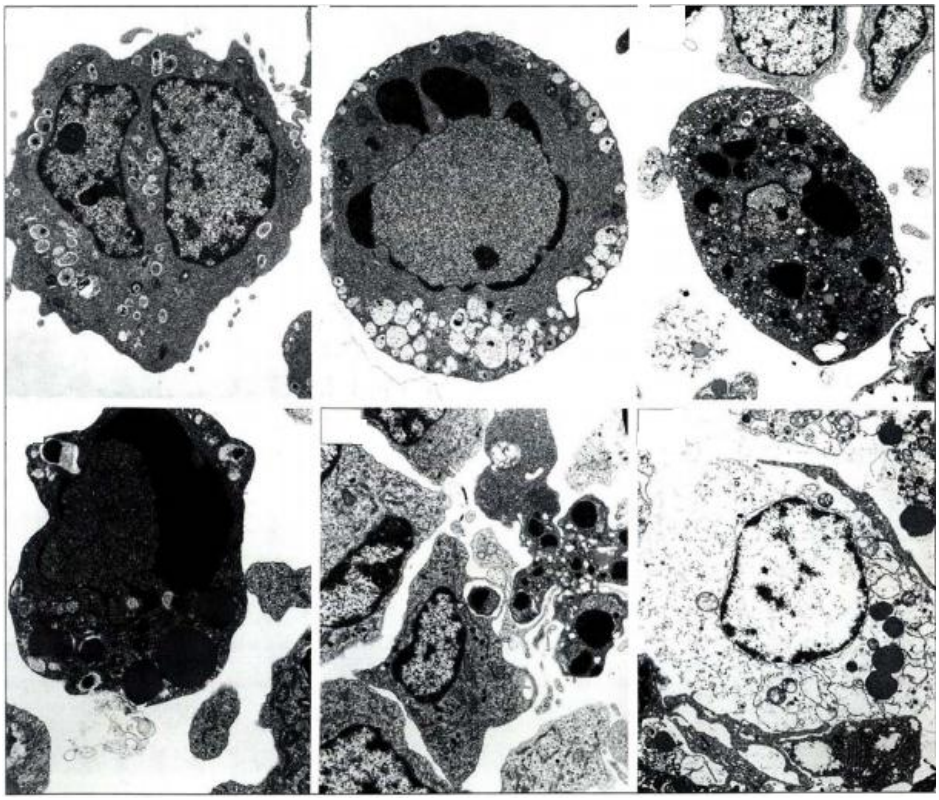


Fig.14. Morphology of apoptosis (Solary et *al*., 1993).

II.9.2. Angiogenesis

Angiogenesis is therefore a complex and strictly regulated process. Due to this complexity, the occurrence of dysfunctions in the mechanism can lead to many pathologies. Insufficient angiogenesis leads to tissue ischemia which can lead, among other things, to strokes, pre-eclampsia or neuropathies. In contrast, uncontrolled angiogenesis is found in diseases such as obesity, age-related macular degeneration (AMD), arthritis and cancer. (Poupard, 2017).

Once vascularized, the tumor has access to oxygen and nutrients to promote its development while allowing it to evacuate catabolic waste. This neovascularization also allows cells from the primary tumor to pass into the bloodstream to colonize neighboring or distant tissues. The onset of these new tumour, called metastases, strongly affects the patient's prognosis. The intra-tumor density of blood vessels has also been associated with the formation of metastases and the survival of patients in various cancers (Poupard, 2017).

II.10. Cancer risk factors

The past few decades have seen significant progress in our understanding of cancer etiology as well as advances in early detection, treatment, and prevention, which have led to

declining cancer mortality in the industrialized world. Despite this progress, certain cancers continue to increase in different parts of the world due, in part, to longer lifespans and changing patterns of cancer risk factors. This includes the first evidence of impacts of the obesity epidemic on cancers. Furthermore, significant gaps in age-adjusted cancer incidence rates for nearly all cancers across different regions of the world suggest that much of cancer risk is due to causes other than unmodifiable intrinsic DNA replication errors common to all humans which we define as the intrinsic risk (Wu *et al*., 2018).

To facilitate the discussion and relate to recent published model-based estimates, separate categories for cancer risk factors are defined below based on their biologic nature, modifiability and use in the literature:

-Unmodifiable intrinsic risk refers to unavoidable spontaneous mutations that arise as a result of random errors in DNA replication related as a characteristic of being human. These unavoidable DNA replication processing errors occur in different organisms at different rates as a species specific, random replication error rate.

-Non-intrinsic risk refers to factors that include: (2a) Modifiable exogenous/external factors (e.g., carcinogens, viruses, xenobiotic) and lifestyle factors (e.g., smoking, hormone therapy, nutrient intake, physical activity) that are exogenous to the host; and (2b) Endogenous factors that are partially modifiable and related to the characteristics of an individual (e.g., immune, metabolism, DNA damage response, hormone levels) and influence key aspects of cell growth control and genome integrity(Table.8) (Wu *et al*., 2018).

Table.8. Three types of cancer risk factors (Wu *et al*., 2018).

Intrinsic risk factors	Non-intrinsic risk factors	
❖ Random errors in DNA replication	Endogenous risk factors ❖ Biologic aging ❖ Genetic susceptibility ❖ DNA repair machinery ❖ Hormones ❖ Growth factors ❖ Inflammation ❖ etc.	Exogenous risk factors ❖ Radiation ❖ Chemical carcinogens ❖ Tumour causing viruses ❖ Bad lifestyles such as smoking, lack of exercise, nutrient imbalance
(Unmodifiable)	**(Partially modifiable)**	**(Modifiable)**

II.11. Epigenetics and cancer

The term epigenetics refers to the study of changes in gene expression that occur during mitosis and/or meiosis but do not result from alterations to the ADN sequence. Setting up the epigenetic program is crucial for correct development and its stable inheritance throughout its lifespan is essential for the maintenance of the tissue and cell specific functions of the organism. For many years, the genetic causes of cancer have hold center stage. However, the recent wealth of information about the molecular mechanisms which, by modulating the chromatin structure, can regulate gene expression has highlighted the predominant role of epigenetic modifications in the initiation and progression of numerous pathologies, including cancer. The nucleosome is the major target of these epigenetic regulation mechanisms. They include a series of tightly interconnected steps which starting with the setting writing of the epigenetic mark till its reading and interpretation will result in long-term gene regulation (Deltour et *al*., 2005).

The major epigenetic changes associated with tumorigenesis are aberrant DNA methylation of CpG islands located in the promoter region of tumor suppressor gene, global genomic hypomethylation and covalent modifications of histone N-terminal tails which are protruding out from the nucleosome core. In sharp contrast with genetic modifications, epigenetic modifications are highly dynamic and reversible. The characterization of specific inhibitors directed against some key epigenetic players has opened a new and promising therapeutic avenue, the epigenetic therapy since some inhibitors are already used in clinical trials (Deltour et *al*., 2005).

II.12. Cancer treatment

Cancer is a devastating disease, which has been one of the main threats to human health .Surgery, radiotherapy, and chemotherapy are the three traditional treatments of cancer, but these methods have certain limitations, such as traumatic, low targeting, serious toxicity, and drug resistance. They often fail to provide long-term survival benefits for patients with advanced solid tumors, according to clinical practice Studies have shown that cancer development and metastasis are highly positively correlated with immunosuppression (yang et al.,2022).

II.12.1. Radiotherapy

The toxicities brought on by radiotherapy, which is a localized kind of treatment, are correlated with the amounts of radiation involved. The first phase of the irritative effects of

radiation generally begins at the end of the second radiation week. Nowadays, particle-linear accelerators are the most common types of treatment equipment used in radiotherapy in order to eradicate cancerous cells (Gross**,** 2011**).**

Radiotherapy uses X-ray or gamma radiation to damage the DNA of rapidly dividing cancer cells beyond repair, causing the cells to undergo apoptosis. Even though healthy cells typically have more efficient DNA repair mechanisms than cancer cells and can thus better withstand the radiation (Buyel,2017).

In radiotherapy, a dose is prescribed over a volume in a number of fractions. The biologically effective dose depends on the total dose as well as the dose given per session and the length of the treatment. The target volumes are determined by the scanner's center point (Gross**,** 2011**).**

II.12.2. Chemotherapy

Chemotherapy is the treatment of cancer with drugs. This approach is advantageous because it can kill residual cancer cells and small, undetectable secondary tumors. Chemotherapy can also be combined with radiotherapy to increase the therapeutic efficacy .One drawback is that the efficacy of chemotherapy depends on the way drugs are distributed in tissues, and poor results are often observed with larger solid tumors due to the limited vascularization, which prevents effective tumor penetration (Buyel,2017).

The active pharmaceutical ingredients (APIs) used for chemotherapy are often small molecules, such as paclitaxel. Such molecules can circulate relatively freely and reach the tumor site (s) even if their precise location is unknown. The first generation of chemotherapeutics were developed to disrupt the metabolism and/or mitotic activity of rapidly dividing cells, whereas the second generation instead targeted signaling components, such as protein kinases or growth factors receptors (Buyel,2017).

II.12.3. Immunotherapy

Immunotherapy harnesses the immune system against cancer, and is the most selective treatment approach and therefore the treatment associated with the least severe side effects (Fig.15). Immunotherapy can take several forms, including the use of vaccines to prevent cancer, as seen with the vaccine against HPV (Human papillomavirus) to prevent cervical cancer, introduction of cytokines to manipulate the immune response, or antibody therapy to target cancer cells in the same way that antibodies normally target pathogens (Buyel,2017).

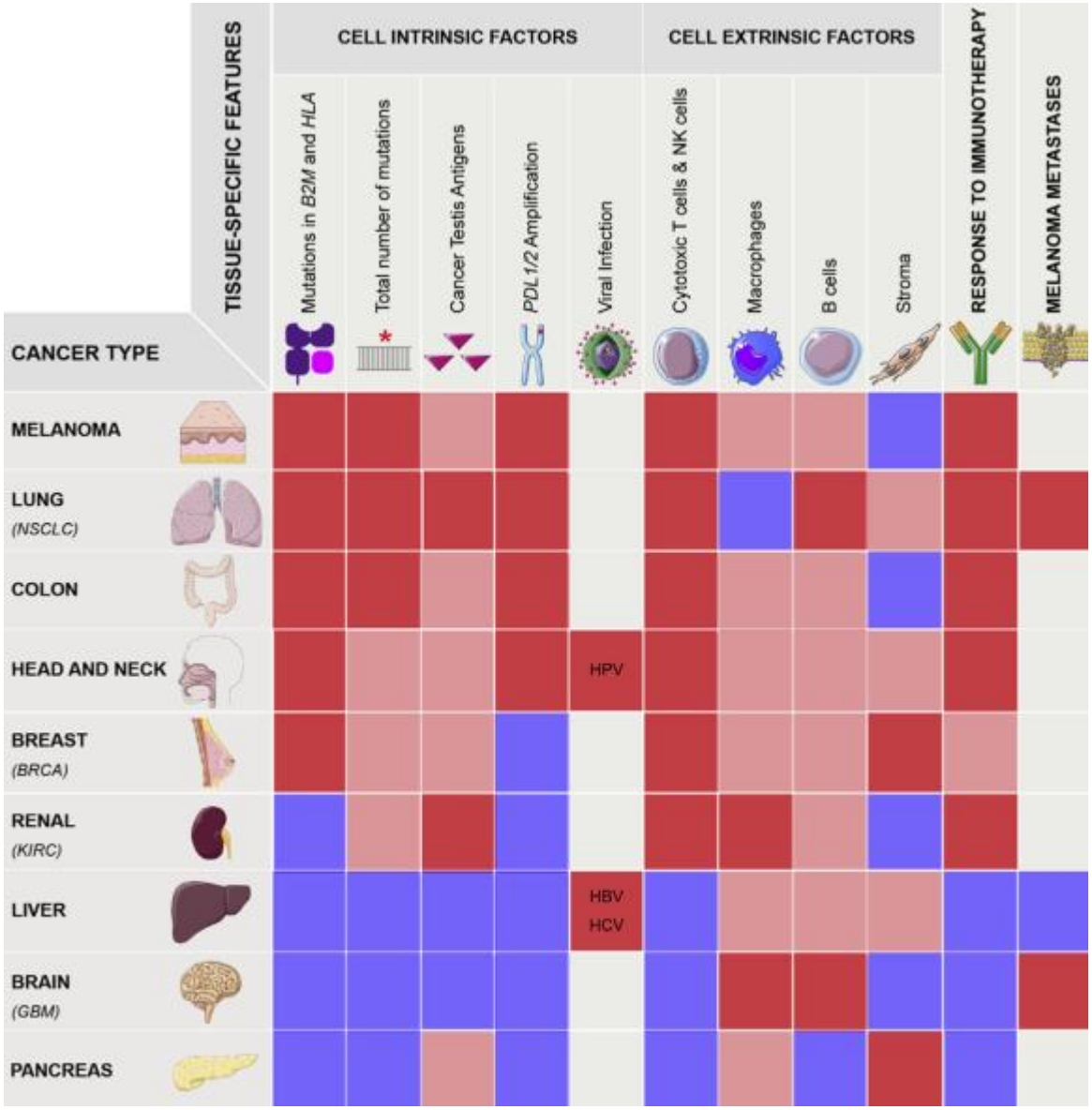


Fig.15. Tissue-specific features affecting immune response across cancer types (Bianchi *et al*., 2020).

In the latter case, monoclonal antibodies (mAbs) are directed against cancer-specifc cell surface structures including receptors and other surface proteins that are overexpressed in tumors, or glycan structures that are more common in cancer cells these tumor-selective targets are collectively described as tumor markers (Buyel,2017).

After binding to cancer cells, the mAbs can elicit antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) through their constant domains, causing natural killer (NK) cells to force the cancer cells into apoptosis (Buyel,2017).

II.12.4. Hormonotherapy

There may be certain misunderstandings when the term hormonotherapy is used. Since it is an anti-hormones treatment rather than a treatment based on hormones, as its name would suggest. Hormone therapy is therefore a treatment option for hormonosensitive tumors. By using this technique, it is possible to block the natural effect of sexual hormones secreted by the human body on the receptors of cancerous cells. The hormone receptors are found on hormone-sensitive cells. The proteins found on the surface of cancerous cells serve as these receptors' representations. They are able to capture the circulating sex hormones in the blood, which promotes the growth of tumors (Léonard, 2020).

II.12.5. Surgery

Ancient surgeons knew that cancer would usually come back after it was removed by surgery. Many people even today consider that many types of cancers are incurable and may delay to consult a doctor in early stage. After anesthesia was invented in 1846, surgeons Bilroth, Handley and Halsted led cancer operations by removing entire tumor together with lymph nodes. Later Paget a surgeon reported that cancer cells were spread from primary tumor to other places through the blood stream (metastasis). Understanding the mechanism(s) of cancer spreading became a key element in recognizing the limitations of cancer surgery. Recently, less invasive ways of destroying tumors without removing them are being studied including liquid nitrogen spray to freeze and kill cancer cells (cryosurgery). Lasers also can be used to cut the tumor tissue of cervix, larynx, liver, rectum, skin and other organ (Sudhakar, 2009).

Chapter III
Articles Analysis

III.1. Phytotherapy interest in the treatment of diseases and cancer

"phyton" and "therapy" are Greek words that mean "plant" and "treatment" respectively. Thus, phyton was defined as the utilization of plants for therapeutic purposes. The use of plants as medicine dates back to the beginning of time. In the beginning, men have always used plants for food, and later, they have used them to treat themselves empirically. The phytotherapist's approach to treat a patient remains traditional, albeit giving the environment a crucial role (Moatti, 1990).

Plants have played an important role in ancient cultures' folklore and they have been used as treatment and prevention for over 5000 years for many diseases, in addition to being used in nutrition and flavors. Drug discovery and development from natural resources have always been of great importance particularly for cancer and infectious diseases. The United States Food and Drug Administration (FDA) found that 62 percent of the new and approved cancer treatments were of natural origin between 1981 and 2002 (Ala'a Mohammad, 2022).

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originate from plant sources. A century ago, most of the few effective drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy). The development of drugs from plants continues, with drug companies engaged in large scale pharmacological screening of herbs (Vickers and Zollman, 1999).

In humans cancer is the second most common reason of disease-related death (Ala'a Mohammad, 2022).

The most effective way for illness prevention in Islam is healthy diet. Prophet Mohammad, Peace Be upon Him (PBUH) said "food is the source of illness; however the diet program is the source of health". Avicenna also had discussed the diet effect on cancer progression. In regard of cancer prevention he said that to prevent its progress, it can be achieved by improving the diet and reinforcing the involved organ by the known effective medications. It is well known nowadays that several chemicals are carcinogenic and obesity is a cause of various diseases including cancer (Zaid et *al.*, 2010).

Chemotherapy resistance is a hard problem to solve as well. According to recent research, phytochemicals have been used to treat cancer since they are relatively low/nontoxic, and have anticancer properties with few side effects, other than standard cancer treatments. Furthermore, Phytochemicals, which are naturally occurring substances found in

plants, are important resources for developing new medications and can also be used to treat cancer Natural remedies, such as using plant-derived medicines to treat cancer, may lessen the negative side effects (Ala'a Mohammad, 2022).

A plant-based products and their phyto constituents are now being used to treat cancer by different modes of action. Traditional herbal medicines are encouraged, recommended, and promoted by the World Health Organization (WHO) in national health-care systems because they are inexpensive, relatively safe, and have growing public trust them. WHO has released monographs on the quality, safety, and efficacy of a number of drugs from plant origin (Ala'a Mohammad, 2022) .*Ephedra* plant has a long history in Traditional Chinese medicine. Many old medical guidebooks and traditional prescriptions recommended it as a folk phyto-medicine. Also *Ephedra* herb contains a range of effective phytochemical compounds. *Ephedra* is used to treat colds, coughs, cardiovascular and immune system illnesses, cancer, microbial infections, and other ailments (Shuang-Man et *al.,* 2020). It has been link to cancer therapy and prevention of cancer metastases (Shuang-Man et *al.,* 2020). However, this unique herb needs more studies and research to investigate its treasures (Ala'a Mohammad, 2022).

Recently, many researches demonstrated the anticancer effect of Ephedra plants *in vitro* and *in vivo.* In this chapter, we chose and highlighted six papers in order to give more details about the anticancer activity of *E. alata* and *E. Foeminea.* We will also discuss the possible molecular mechanism involved in antitumor effect.

III.2. Study outline

We have produced a synthesis of data from researches carried out on the anticancer effect of the two plants mentioned above. We used different databases like Pub Med, Science Direct and Google Scholar. The keywords and terms used in this research are: *E. alata, E. Foeminea,* anticancer effect, cell lines, molecular mechanisms, phenolic compounds, alkaloids, apoptosis and cancer.

Our work has been followed throught three steps. At first, we proceded a bibliographic research according to certain inclusion criteria. Secondly, we analysed the chosen papers and finaly, we did a summary table using obtained data during articles analysis.

III.3. Inclusion criteria

These criteria are defined to identify articles that meet our objective. Items that meet the following criteria are include:

- English publication.

- Original research.

- Publication date from 2017 to 2023.

- Any research related to the chosen theme.

III.4. Analysis of selected articles

The analysis was undertaken emphasizing:

- Study objective.

- The study methodology.

-The main results.

- Discussion and conclusion.

III.5. Results

Information from articles analysis were summarized in the table.9.

Table.9. Classification of the chosen articles for analysis.

Article number	Citation	Title	Objective	Type of study
		Ephedra alata		
01	Corina *et al.*, 2018	Phytochemical characterization and evaluation of the antimicrobial, antiproliferative and proapoptotic potential of *Ephedra alata Decne* hydroalcoholic extract against the MCF-7 breast cancer cell line	To conduct a phytochemical characterization of the hydroalcoholic extract of the aerial part of *Ephedra alata Decne* and to evaluate its antimicrobial, antifungal, antiproliferative, pro-apoptotic, and cytotoxic potential against the MCF-7 breast cancer	*In vitro* study: The cells used in this study were MCF-7 human breast cancer cells
02	Sioud *et al.*, 2020	A new highlight of *Ephedra alata* decne properties as potential adjuvant in combination with Cisplatin to induce cell death of4T1 breast cancer cells *in vitro* and *in vivo*	To test the potential effects of *Ephedra alata Decne* extract towards a 4T1 breast cancer model *in vitro* and *in vivo.*	-*In vitro* study: Murine mammary carcinoma cell line, 4T1 were used. -*In vivo* study: female BALB/c mice were used.
03	Bensam *et al., 2023*	The role of Algerian *Ephedra alata* ethanolic extract in inhibiting the growth of breast cancer cells by inducing apoptosis in a p53-dependent	To evaluate the antioxidant, cytotoxic and apoptotic effects of *Ephedra alata* ethanolic extract (EAEE), against different human cancer cell lines.	*In vitro* study: Three cancer cell lines were used: breast cancer cell line (MCF-7), liver cancer cell line (HepG- 2), and colon cancer line

		pathway		(Caco-2)
Ephedra feominea				
04	Mendelovich *et al.*, 2017	Effect of *Ephedra foeminea* active compounds on cell viability and actin structures in cancer cell lines	To evaluate the effect of different extracts of *E. foeminea:* leaf ethanol extract, water extract and fruit juice on viability of cancer and non-cancer cells	*In vitro* study: Human cell lines used in this study included: MDAMB-231-mammary gland/breast cells, A549 lung carcinomatous cells; HaCaT, keratinocytes from histologically normal skin and epithelial colorectal carcinoma cells
05	Mpingirik *et al.*, 2020	Potential anticancer activity of crude ethanol, athyl acetate, and water extracts of *Ephedra foeminea* on human osteosarcoma U2OS cell viability and migration	To assess the effect of *E. foeminea* ethyl acetate, ethanol, and water extracts on morphology, viability, migration, and gene expression of U2OS osteosarcoma cells	*In vitro* study: The cell line used for this study was the U2OS human osteosarcoma cell line.
06	Al-Saraireh *et al.*, 2021	Phytochemical characterization and anti-cancer properties of extract of *Ephedra foeminea* (Ephedraceae) aerial parts	To evaluate the phytochemical profile of methanol extract of *Ephedra foeminea* and assess its anti-carcer effect on a large set of normal and cancerous cell lines	*In vitro* study: breast (MCF-7), lung (A549), colon (Caco-2), liver (HepG-2) and prostate (PC-3) were used

III.5.1. Plant collection and extract preparation

Corina *et al.* (2018) and Bensam *et al.* (2023) in their investigations were used the ethanol to prepare the extract of dried aerial parts of *E. alata Decne* collected from the southern part of Tunisia (Djerba) and Algerian semi-desert areas respectively. On the other hand, Sioud *et al.* (2020) were prepared a methanolic extract of *Ephedra alata* collected from the Sahara of Tataouine (Tunisia).

Mendelovich *et al.* (2017) used decoction to prepare water extract of leaves from male and female plants of *Ephedra foeminea* collected in the Samaria Mountains in Central Palestine. A fruit juice and an ethanol extract were prepared too. Mpingirika *et al.* (2020) used plant aerial parts (the scale minute leaves and stems) of *E. feominea* collected from Hebron city. The extract was prepared using an analytical grade solvents; deionized water, absolute ethanol, and ethyl acetate and powder of *ephedra foeminea*. Meanwhile, Al-Saraireh *et al.* (2021) were prepared a methanolic extract from the aerial parts of *Ephedra foeminea* collected from South Jordan, Mutah, Al-Karak, Jordan.

III.5.2. Methods used to evaluate the anticancer activity

In all studied articles the MTT assay was used to evaluate the anticancer activity of the chosen plants, except for the research carried out by Mendelovich *et al.* (2017) where XTT assay was used.

III.5.3. Anticancer activity (results and discussion)

The *E. alata* was screened for possible *in vitro* anticancer activity against the MCF-7 human breast cancer cell line. The anti-proliferative activity of *E. alata* at the selected concentrations after a period of incubation of 72 h statistically significant. Results were detected starting from the concentration of 10 µg/mL, with a cell growth inhibition percentage of 19.68 ± 4.2. For the highest tested concentration, namely 30 µg/mL, the growth inhibition percentage was 56.45 ± 3.9 (Fig.16) (Corina *et al.*, 2018).

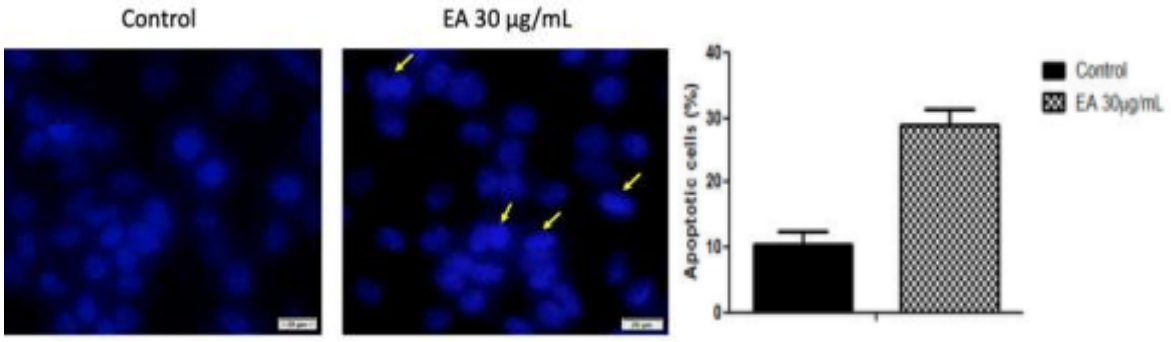


Fig.16. The effect of 30 µg/mL of EA on MCF-7 cells' nuclei after 72 h. Morphological changes distinctive for apoptosis induction are marked with yellow arrows (Corina *et al.*, 2018).

Sioud *et al.* (2020) obtained that *E. alata* reduced the viability of 4T1 breast cancer cells and, in a caspase-dependent way, triggered apoptosis. Smac/Diablo and cytochrome C were released from the intermembrane space of mitochondria into the cytosol during this intentional cell death. The anti-proliferative action significantly slowed the growth of tumors in mice. In this paper, *E. alata* 's potential, for the first time, was emphasized for interacting favorably with an anticancer medication like cisplatin (CDDP). A strong caspase activation, a reduction in anti-apoptotic Bcl-2 proteins, and a redistribution of pro-apoptotic molecules into the cytosol to active effector caspases like caspases-3 and -7 to ultimately cleave PARP protein. All contributed to the fact that the combination of the *E. alata* extract with CDDP increased the cell death of 4T1 breast cancer cells (Fig.17). These event were associate with a strong decrease of breast cancer tumor growth in mice treated with an *E. alata* cisplatin combination and simultaneously with a decrease of hepato-and nephrotoxicities of cisplatin.

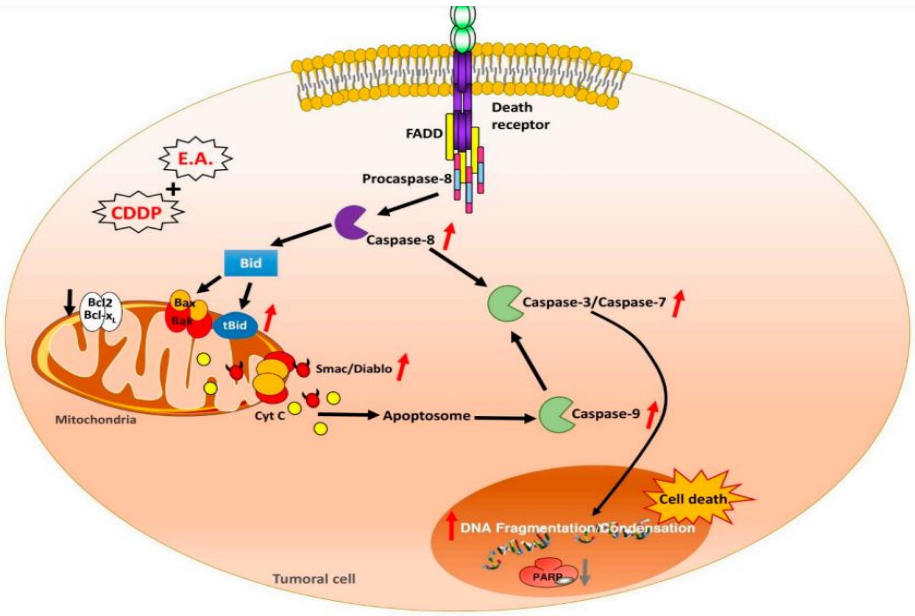


Fig.17. Key regulators modulated by an E.A./CDDP combination in breast cancer cells. E.A./CDDP acts on the intrinsic pathway of apoptosis by decreasing the expression of BCL-2 and BCL-Xl (Sioud *et al.*, 2020).

Bensam *et al.* (2023) found that *E. alata* extract cause death for all human cancer cells used in the study, mainly through apoptosis. The highest significant apoptosis percentage (77.15%) was noted in MCF-7 followed by HepG-2 and Caco-2 cell lines (73.4% and 51.66%, respectively). This confirmed that the most sensitive cancer cell line for treatment with *E. alata* is breast cancer cells. Four genes, Bax, p21, RB1, and TP53, were found to be up-regulated in MCF-7 cells treated with the drugs *E. alata* ethanolic extract or S-FU (anticancer drug) by qRT-PCR analysis. These findings suggest that *E. alata* extract kills breast cancer cells by activating the intrinsic pathways of Bax, p21, and RB1, which leads in apoptosis in a P53-dependent manner (Fig.18).

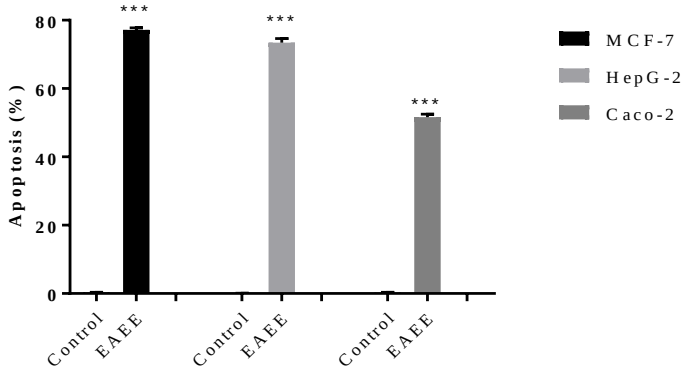


Fig.18. Total percentage of apoptosis in cancer cells. Values are expressed as mean±SEM. Comparison between the control and treated cells was carried out using two-way ANOVA followed by Sidak's multiple comparisons test. ***p <0.001(**Bensam et *al.*, 2023**)

Both *E. foeminea* fruit juice and leaf ethanol extract decreased the viability of cancer cells *in vitro*, but the water extract decreased cytotoxic activity in all cell types. The cytotoxic activity of *E. foeminea* ethanol extract and fruit juice is mediated, at least partially, by induction of apoptosis via a caspase 3-dependent pathway. This cytotoxic activity was enhanced by Taxol, and vice versa, that of Taxol was enhanced by the extract and juice, suggesting that they may act together to enhance cancer cell death. Actin-stained filaments were impacted by *E. foeminea* ethanol leaf extract and fruit juice, whereas tubulin-stained filaments were unaffected. *E. foeminea* ethanol extracts caused changes in the organization of actin filaments, leading to the formation of invadopodia-like structures. And Fruit juice did not lead to the formation of invadopodialike structures, but rather to the formation of what might be large focal adhesion points. Active sub-fractions of *E. foeminea* extracts were found to contain several compounds including trans-sinapyl alcohol and trans-sinapaldehyde derivative (Fig.19) (Mendelovich *et al.*, 2017).

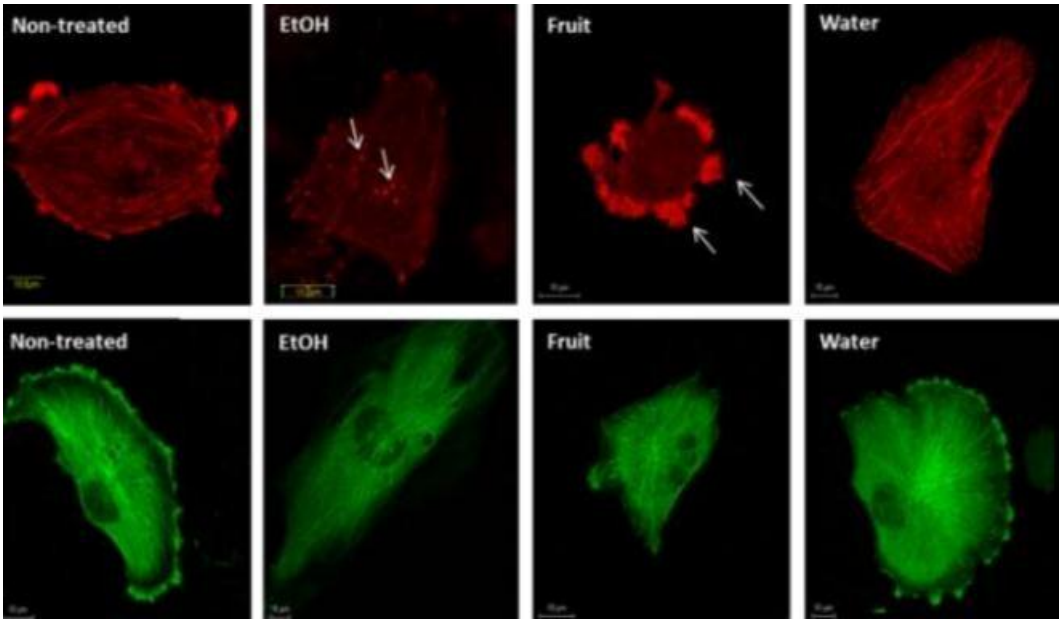


Fig.19. Effects of *E. foeminea* on cell cytoskeleton (Mendelovich *et al.*, 2017).

Mpingirik *et al.* (2020) obtained that *E. foeminea* ethyl acetate, ethanol, and water extracts each significantly decreased U2OS percentage cell viability in a manner dependent on both concentration and time. The least half-maximal inhibitory concentration (IC50) was observed in the water extract after 48 h of incubation (30:761 ± 1:4 µg/mL) followed by the ethyl acetate extract after 72 h incubation (80:35 ± 1:233 µg/mL) and finally the ethanol extract after 48 h incubation (97:499 ± 1:188 µg/mL). On the other hand, both ethanol and

water extracts considerably reduced the steady state mRNA expression of beta- catenin, promoting both cell proliferation and migration in osteosarcoma by regulating target genes (Fig.20).

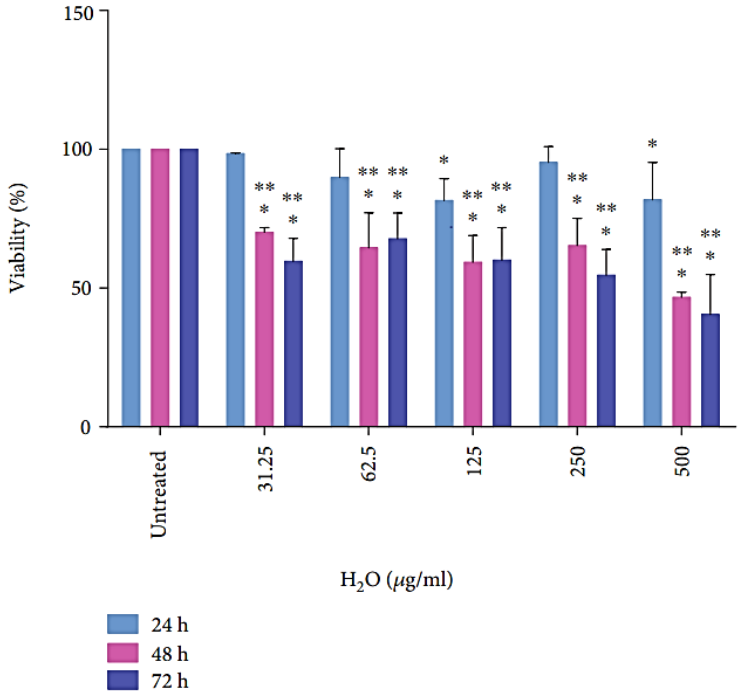


Fig.20. Effect of *E. foeminea* water extracts on U2OS cell viability. ∗∗∗ P ≤ 0:001, ∗∗P ≤ 0:01, and ∗P ≤ 0:05; n = 3(Mpingirik *et al.*, 2020).

The extract of *E. foeminea* showed significant anti- proliferative activity against cancer cell lines, when compared with the positive control, doxorubicin. Cancer cell lines from the liver (HepG-2) and prostate (PC-3) as well as the breast (MCF-7) and lung (A549) and colon (Caco-2) all shown selective and concentration- dependent cytotoxicity. Other cancer cell lines, such as cervical carcinoma (Hela) and human epithelioma (Hep-2) demonstrated weak selectivity; it's interesting to note that non-cancerous cells have minimal or no cytotoxicity (Fig.21) (Al-Saraireh *et al.*, 2021).

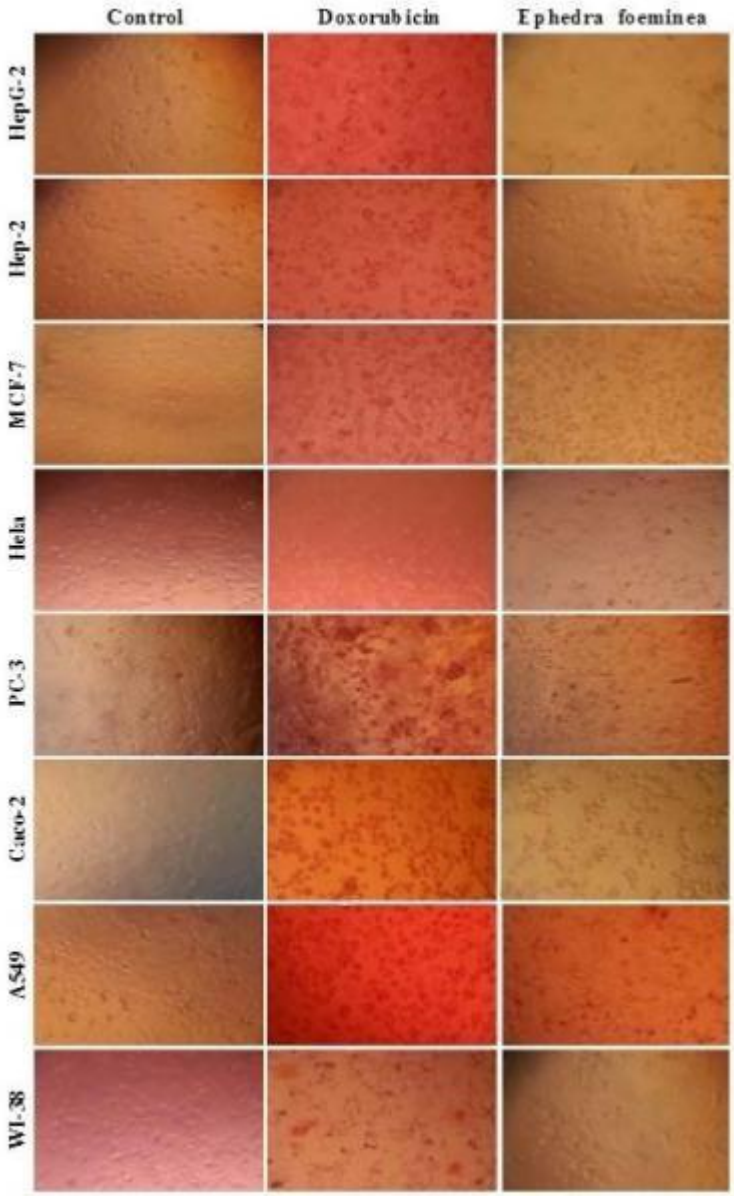


Fig.21. Anticancer impact of *E. foeminea* extract on normal and cancer cell lines. These cell lines were treated with 125 MG/ml of plats extracts and doxorubicin (positive control) for 72 hours **(Al-Saraireh *et al.*, 2021).**

III.6. Conclusion

The findings above demonstrate the potential of *E. alata* extract has important cytotoxic effects on human cancer cell lines (HepG2, Caco-2, and MCF-7 cells). It could be as a chemosensitizer in association with cisplatin to both decrease its nephro- and hepatotoxic effects and to enhance its anticancer activity. *E. foeminea* decreased U2OS cell viability and migratory ability by modulating the expression of critical genes involved in regulating of these processes. Moreover, its fruit juice and leaf ethanol extract both significantly reduce cancer cell viability. This is achieved, at least in part, by inducing

caspase 3-dependent cell death, which may be triggered by sinapyl-related substances present in the ethanol extract of *E. foeminea*. However, the popular folk remedy water extract has no cytotoxic action. *E. foeminea* has an anti-proliferative activity that may be selectively effective against particular cancer cell types.

Conclusion

Plants have traditionally been the principal source of natural product medication development. They have been used as a treatment and prevention for over 5000 years for many diseases, in addition to nutrition and flavors. Furthermore, medicinal plants are essential in drug discovery and development, particularly in cancer and infectious diseases. One of the biggest genera in the Ephedraceae family, which is found throughout the world's arid and semiarid regions, is Ephedra .This genus contains over 50 species which used to treat many diseases in the word.

In humans, cancer is the second most common reason for disease-related death. Several plant-derived medicines have been screened for their anticancer potential. Some have been successfully developed and used for cancer therapy throughout the last several decades.

The results of our bioliographic research and papers analysis about two plants of genus Ephedra: *E. alata* and *E. feominea* showed that they are rich of bioactive molecules that have many biological and pro-apoptotic properties for the treatment of cancer disease. The analysis of some recent articles (from 2017 to 2023) demonstrated an interesting *in vitro* and *in vivo* anticancer effect of both *E. alata* and *E. feominea*.

As a result, some species of *Ephedra* genus may be a source of fresh chemicals and they offer a promising medication source for the development of novel and effective anticancer drugs. However, more researches are required to ascertain their molecular mechanism of action and the ultimate impact of these two plants on the cancer cell especially on animal models.

Abu- hajleh ,M., Khelifat,K .M., Alqaraleh, M., Al-Haraishat, E.A,Al-limoun,M.O., Qarallelh,H.and Al- Dujaili,2022. Antioxydant and anti hyperglycemic effects of ephedra foeminea aqueous extract in streptozoticin-induced diabetic rats . Nutrients,14(11),p.2338.

Al Mamari, H. H. (2021). Phenolic compounds: Classification, chemistry, and updated techniques of analysis and synthesis. Phenolic compounds-chemistry, synthesis, diversity, non-conventional industrial, pharmaceutical and therapeutic applications, 10.

Ala'a Mohammad, A. Y. (2022). In-vitro Anticancer Potential of Jordanian Ephedra Species (Doctoral dissertation, University of Petra).

Al-Nemi, R., Makki, A. A., Sawalha, K., Hajjar, D., & Jaremko, M. (2022). Untargeted metabolomic profiling and antioxidant capacities of different solvent crude extracts of Ephedra foeminea. Metabolites, 12(5), 451.

Al-Rimawi, F., Abu-Lafi, S., Abbadi, J., Alamarneh, A. A., Sawahreh, R. A., & Odeh, I. (2017). Analysis of phenolic and flavonoids of wild Ephedra alata plant extracts by LC/PDA and LC/MS and their antioxidant activity. African Journal of Traditional, Complementary and alternative medicines, 14(2), 130-141.

Al-saraireh, Y. M., Youssef, A. M., Alshammari, F. O., Al-Sarayreh, S. A., Al-Shuneigat, J. M., Alrawashdeh, H. M., & Mahgoub, S. S. (2021). Phytochemical characterization and anti-cancer properties of extract of Ephedra foeminea (Ephedraceae) aerial parts. Tropical Journal of Pharmaceutical Research, 20(8), 1675-1681.

Al-Snafi, A. E. (2017). Therapeutic importance of Ephedra alata and Ephedra foliata-A review. Indo American Journal of Pharmaceutical Sciences, 4(2), 399-406.

Aniszewski, T. (2007). Alkaloids-Secrets of Life: Aklaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier.

APS. (2023, fevrier). SICAN: le plan national anti-cancer 2023-2030 repose sur la prévention. Algérie presse service

Asgharian, P., Tazekand, A.P., Hosseini, K., Forouhandeh, H., Ghasemnejad, T., Ranjbar, M., Hasan, M., Kumar, M., Beirami, S.M., Tarhriz, V. and Soofiyani, S.R., 2022. Potential mechanisms of quercetin in cancer prevention: focus on cellular and molecular targets. *Cancer cell international*, 22(1), p.257.

Attard, E. and Vella, K., 2009. Effects of ephedrine and Ephedra fragilis crude extracts on human peripheral lymphocytes. Pharmacognosy Research, 1(2).Badiaga, M. (2011). Etude ethnobotanique, phytochimique et activités biologiques de Nauclea latifolia Smith, une plante médicinale africaine récoltée au Mali (Doctoral dissertation, Université Blaise Pascal-Clermont-Ferrand II).

Béliveau, R., & Gingras, D. (2007). Role of nutrition in preventing cancer. *Canadian Family Physician*, *53*(11), 1905-1911.

Bensam, M., Rechreche, H., Abdelwahab, A.E., Abu-Serie, M.M. and Ali, S.M., 2023. The role of Algerian Ephedra alata ethanolic extract in inhibiting the growth of breast cancer cells by inducing apoptosis in a p53-dependent pathway. *Saudi Journal of Biological Sciences,* 30(6), p103650.

Bianchi, J. J., Zhao, X., Mays, J. C., & Davoli, T. (2020). Not all cancers are created equal: Tissue specificity in cancer genes and pathways. *Current opinion in cell biology*, *63*, 135-143.

Bolinder, K., Niklas, K.J. and Rydin, C., 2015. Aerodynamics and pollen ultrastructure in Ephedra. American Journal of Botany, 102(3), pp.457-470.

Bouque, V. (1997). Étude de la production de métabolites secondaires par des cultures in vitro de Psoralées (leguminosae) (Doctoral dissertation, Vandoeuvre-les-Nancy, INPL).

Buyel, J. F. (2017). How plants can contribute to the supply of anticancer compounds. *Biotechnology and production of anti-cancer compounds*, 39-72.

Calatayud, P. A., Desneux, N., & Le Gall, P. (2013). Caractéristiques chimiques des plantes. Interactions insectes-plantes, 217-228.

Castanedaetall, M., den Hollander, P., Kuburich, N. A., Rosen, J. M., & Mani, S. A. (2022, October). Mechanisms of cancer metastasis. *In Seminars in cancer biology*. Academic Press, 87, 17–31.

Caveney, S., Charlet, D. A., Freitag, H., Maier-Stolte, M., & Starratt, A. N. (2001). New observations on the secondary chemistry of world Ephedra (Ephedraceae). American journal of botany, 88(7), 1199-1208.

Caveney, S., Charlet, D.A., Freitag, H., Maier-Stolte, M. and Starratt, A.N., 2001. New observations on the secondary chemistry of world Ephedra (Ephedraceae). American journal of botany, 88(7), pp.1199-1208.

Celedón-Neghme, C., Santamaría, L. and González-Teuber, M., 2016. The role of pollination drops in animal pollination in the Mediterranean gymnosperm Ephedra fragilis (Gnetales). Plant Ecology, 217, pp.1545-1552.

Chiapolino, T., 2018. La phytothérapie: une approche intéressante en prévention des cancers (Doctoral dissertation, Université de Lorraine).

Corina,D, ., Muntean, D., Alexa, E., Farcas, C., Oprean, C., Zupko, I., Bor, A., Minda, D., Proks, M., Buda, V. and Hancianu, M., 2018. Phytochemical characterization and evaluation of the antimicrobial, antiproliferative and pro-apoptotic potential of Ephedra alata Decne. hydroalcoholic extract against the MCF-7 breast cancer cell line. *Molecules*, 24(1), p.13.

Deltour, S., Chopin, V., & Leprince, D. (2005). Modifications épigénétiques et cancer. *médecine/sciences*, *21*(4), 405-411.

El Hadj, M. O., Hadj-Mahammed, M., Zabeirou, H., & Chehma, A. (2003). Importance des plantes spontanées médicinales dans la pharmacopée traditionnelle de la région de Ouargla (Sahara Septentrional-Est Algérien). Sciences & Technologie. *C,* Biotechnologies, 73-78.

Elhadef, K., Smaoui, S., Fourati, M., Ben Hlima, H., Chakchouk Mtibaa, A., Sellem, I., ... & Mellouli, L. (2020). A review on worldwide Ephedra history and story: from fossils to natural products mass spectroscopy characterization and biopharmacotherapy potential. Evidence-Based Complementary and Alternative Medicine, 2020,22.

Fares, J., Fares, M. Y., Khachfe, H. H., Salhab, H. A., & Fares, Y. (2020). Molecular principles of metastasis: a hallmark of cancer revisited. *Signal transduction and targeted therapy*, *5*(1), 28.

Farion, N. (2019). Thèse d'exercice pour l'obtention du Diplôme d'État de Docteur en Pharmacie-comparaison des systèmes de classification des agents cancérogènes causes de divergences et proposition d'équivalences.

Fouad, Y. A., & Aanei, C. (2017). Revisiting the hallmarks of cancer. *American journal of cancer research*, *7*(5), 1016-1036.

Ghadeer.O., Abdallah.L., Ahmed.A.M., and Abu-Arra.M., 2020.In vitro inhibition of sheep liver glutathione -s- Transferases activity by different extracts from ephedra aphylla and ephedra foeminea. Palastinia Medical Journal ,6(2),p.3.

González-Juárez, D. E., Escobedo-Moratilla, A., Flores, J., Hidalgo-Figueroa, S., Martínez-Tagüeña, N., Morales-Jiménez, J., ... & Bautista, E. (2020). A review of the Ephedra genus: distribution, ecology, ethnobotany, phytochemistry and pharmacological properties. Molecules, 25(14), 3283.

Green well,M. and, Rahman,P.K.S.M.,2015 Medicinal plants: their use in anticancer treatment. International Journal of pharmaceutical sciences and research,6(10),p.4103.

Gross, E. (2011). Radiothérapie conformationelle du cancer de prostate. *Progrès en urologie*, *21*(11), 801-807.

Hadjadj, K., Daoudi, B. B., & Guerine, L. (2020). Importance thérapeutique de la plante Ephedra alata subsp. alenda dans la médecine traditionnelle pour la population de la région de Guettara (Djelfa, Algérie). Lejeunia, Revue de Botanique.

Hajleh, M.N.A., Khleifat, K.M., Alqaraleh, M., Al-Hraishat, E.A., Al-limoun, M.O., Qaralleh, H. and Al-Dujaili, E.A., 2022. Antioxidant and antihyperglycemic effects of ephedra foeminea aqueous extract in streptozotocin-induced diabetic rats. Nutrients, 14(11), p.2338.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *cell*, *144*(5), 646-674.(ps79).

Hurtado-Fernández, E., Gómez-Romero, M., Carrasco-Pancorbo, A. and Fernández-Gutiérrez, A., 2010. Application and potential of capillary electroseparation methods to determine antioxidant phenolic compounds from plant food material. Journal of Pharmaceutical and Biomedical Analysis, 53(5), pp.1130-1160.

Ismail, S.M., Adwan, G.M. and Jarrar, N.R., 2020. Evaluation of Antimicrobial and Genotoxic Activity of Ephedra foeminea Ethanolic and Aqueous Extracts on Escherichia coli. Jordan Journal of Biological Sciences, 13(1).

Kittana, N., Abu-Rass, H., Sabra, R., Manasra, L., Hanany, H., Jaradat, N., Hussein, F. and Zaid, A.N., 2017. Topical aqueous extract of Ephedra alata can improve wound healing in an animal model. *Chinese Journal of Traumatology*, *20*(02), pp.108-113.

Léonard, S. (2020). *Résistance à l'hormonothérapie et mutations ESR1 dans les cancers du sein* (Doctoral dissertation, Université de Lorraine).

Lotfi, N., Yousefi, Z., Golabi, M., Khalilian, P., Ghezelbash, B., Montazeri, M., Shams, M.H., Baghbadorani, P.Z. and Eskandari, N., 2023. The potential anti-cancer effects of quercetin on blood, prostate and lung cancers: An update. *Frontiers in Immunology*, 14, p.1077531.

Mallette, F. A. (2008). Mécanismes de suppression tumorale impliqués lors de la sénescence induite par les oncogènes.

Mendelovich, M., Shoshan, M., Fridlender, M., Mazuz, M., Namder, D., Nallathambi, R., Selvaraj, G., Kumari, P., Ion, A., Wininger, S. and Nasser, A., 2017. Effect of Ephedra foeminea active compounds on cell viability and actin structures in cancer cell lines. Journal of Medicinal Plants Research, 11(43), pp.690-702.

Moatti, R. (1990). La phytothérapie. *Revue des Deux Mondes*, 80-89.

Mpingirika E.Z., El Hosseiny, A., Bakheit, S.M.S., Arafeh, R. and Amleh, A., 2020. Potential Anticancer Activity of Crude Ethanol, Ethyl Acetate, and Water Extracts of Ephedra foeminea on Human Osteosarcoma U2OS Cell Viability and Migration.

Nadir, D. S. (2022). Aspergillus niger biotic elicitors of secondary pharmaceutical metabolites in medicinal plants (in vitro): a review. plant archives, 22(1), 308-319.

Osborne, C., Wilson, P., & Tripathy, D. (2004). Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications. *The oncologist*, *9*(4), 361-377.

Poupard, N. (2017). *Conception de polysaccharides sulfatés inhibiteurs de l'héparanase pour le traitement de l'angiogénèse tumorale* (Doctoral dissertation, Université de La Rochelle).

Rani, N., Velan, L.P.T., Vijaykumar, S. and Arunachalam, A., 2015. An insight into the potentially old-wonder molecule—quercetin: the perspectives in foresee. Chinese Journal of Integrative Medicine, pp.1-16.

Rashed, K. Phytochemical and biological activities of Ephedra alata: A. review, IJSIT, 10(3), 175-178.

Reguieg, L. (2011). Using medicinal plants in Algeria. *Am J Food Nutr*, *1*(3), 126-127.

Rydin, C., & Bolinder, K. (2015). Moonlight pollination in the gymnosperm Ephedra (Gnetales). Biology letters, 11(4), 20140993.

Sahai, E. (2005). Mechanisms of cancer cell invasion. *Current opinion in genetics & development*, *15*(1), 87-96.

Siegel, R. L., Miller, K. D., Wagle, N. S., & Jemal, A. (2023). Cancer statistics, 2023. *CA: a cancer journal for clinicians*, *73*(1), 17-48.

Sioud, F., Amor, S., Toumia, I.B., Lahmar, A., Aires, V., Chekir-Ghedira, L. and Delmas, D., 2020. A new highlight of ephedra alata decne properties as potential adjuvant in combination with cisplatin to induce cell death of 4T1 breast cancer cells in vitro and in vivo. Cells, 9(2), p.362.

Solary, E., Bertrand, R., & Pommier, Y. (1993). Le rôle de l'apoptose dans la genèse et le traitement du cancer, 9 , 667-75.

Sudhakar, A., 2009. History of cancer, ancient and modern treatment methods. Journal of cancer science & therapy, 1(2), p.1.

Vafadar, A., Shabaninejad, Z., Movahedpour, A., Fallahi, F., Taghavipour, M., Ghasemi, Y., Akbari, M., Shafiee, A., Hajighadimi, S., Moradizarmehri, S. and Razi, E., 2020. Quercetin and cancer: new insights into its therapeutic effects on ovarian cancer cells. Cell & bioscience, 10, pp.1-17.

Vargas-Piedra, G., Valdez-Cepeda, R.D., López-Santos, A., Flores-Hernández, A., Hernández-Quiroz, N.S. and Martínez-Salvador, M., 2020. Current and future potential distribution of the xerophytic shrub candelilla (Euphorbia antisyphilitica) under two climate change scenarios. Forests, 11(5), p.530.

Vickers, A. and Zollman, C., 1999. Herbal medicine. *Bmj*, *319*(7216), pp.1050-1053.

Villanueva-Almanza, L. and Fonseca, R.M., 2011. Revisión taxonómica y distribución geográfica de Ephedra (Ephedraceae) en México. Acta botánica mexicana, (96), pp.79-116.

Weinberg, R. A., & Hanahan, D. (2000). The hallmarks of cancer. *Cell*, *100*(1), 57-70.(ps39).

Weinstein, I. B., & Joe, A. (2008). Oncogene addiction. *Cancer research*, *68*(9), 3077-3080.

Wu, S., Zhu, W., Thompson, P., & Hannun, Y. A. (2018). Evaluating intrinsic and non-intrinsic cancer risk factors. *Nature communications*, *9*(1), 3490.

Yang, L., Ning, Q., & Tang, S. S. (2022). Recent advances and next breakthrough in immunotherapy for cancer treatment. *Journal of Immunology Research*, *2022,9.*

Yip, H. Y. K., & Papa, A. (2021). Signaling pathways in cancer: therapeutic targets, combinatorial treatments, and new developments. *Cells*, *10*(3), 659.

Zaid, H., Rayan, A., Said, O. and Saad, B., 2010. Cancer treatment by Greco-Arab and Islamic herbal medicine. The Open Nutraceuticals Journal, 3(1).

Zhang, B. M., Zhi-Bin, W. A. N. G., Ping, X. I. N., Qiu-Hong, W. A. N. G., He, B. U., & Kuang, H. X. (2018). Phytochemistry and pharmacology of genus Ephedra. Chinese journal of natural medicines, 16(11), 811-828.

Zhang, K., Liu, Z., Abdukeyum, N. and Ling, Y., 2022. Potential Geographical Distribution of Medicinal Plant Ephedra sinica Stapf under Climate Change. Forests, 13(12), p.2149.

- مريم خالد محمد صوالحة. (2018). فصل المركبات الاولية النقية وفحص النشاط الدوائي المضاد للفطريات والبكتيريا والاكسدة من مستخلصات النباتات البرية *الفلسطينية* Ephedra foeminea Smilax aspera (Doctoral dissertation, AL-Quds University).

The anticancer activity of some medicinal plants of the genus Ephedra

Examiner's committee:	***Presented by:***
Chairperson: Dr. Hanane BOUTENNOUNE	Aya BENAYAD
Examiner: Dr. Salma HAMMIMED	Hind BOUDERMINE
Supervisor: Mrs. Moufida BENSAM	

Abstract

Cancer is a disease that is born in part as a result of our life style changes. Nowadays, it is becoming more and more common, with many new cases occurring every day causing more than 19 million patients worldwide and thus many deaths. Chemotherapy and radiation are the most common ways to treat cancer. These treatments include the use of chemicals that may harm an individual's physical and mental health. This can lead to serious side effects and complications. Therefore, we need to find an alternative way to fight cancer that is free of side effects and equally effective. *Ephedra* is likely to be one of these alternative methods. For this reason, the aim of this dissertation was to make an analysis of some of the published articles of the two plants *E. alata* and *E. foeminea in vitro* and *in vivo* in order to find out how these two plants are effective in fighting cancer. The results indicated that the use of these plants in anticancer therapy may be very beneficial as their extracts contains bioactive molecules possessing antiproliferatif effect. Accordingly, These plants may offer a promising drug source for the development of novel and effective anticancer drugs.

Key words: cancer, *Ephedra feominea, Ephedra alata,* anticancer.

Résumé

Le cancer est une maladie qui est née en partie à la suite de changements dans notre mode de vie. De nos jours, elle devient de plus en plus fréquente, de nombreux nouveaux cas survenant chaque jour faisant plus de 19 millions de patients dans le monde et donc de nombreux décès. La chimiothérapie et la radiothérapie sont les moyens les plus courants pour traiter le cancer. Ces traitements comprennent l'utilisation de produits chimiques qui peuvent nuire à la santé physique et mentale d'un individu. Cela peut entraîner des effets secondaires graves et des complications. Par conséquent, nous devons trouver une autre façon de lutter contre le cancer qui soit sans effets secondaires et tout aussi efficace. L'Ephédra est susceptible d'être l'une de ces méthodes alternatives. Pour cette raison, le but de ce mémoire était de faire une analyse de certains articles publiés sur les deux plantes *E. alata* et *E. foeminea in vitro* et *in vivo* afin de découvrir comment ces deux plantes sont efficaces pour lutter contre le cancer. Les résultats ont indiqué que l'utilisation de ces plantes dans la thérapie anticancéreuse peut être très bénéfique, car leurs extraits contiennent des molécules bioactives possédant un effet antiprolifératif. En conséquence, ces plantes peuvent offrir une source de médicaments prometteuse pour le développement de médicaments anticancéreux nouveaux et efficaces.

Les mots clés : cancer, *Ephedra feominea, Ephedra alata,* anticancéreux.

الملخص

السرطان مرض يولد جزئيًا نتيجة لتغييرات أسلوب حياتنا. في الوقت الحاضر أصبح أكثر شيوعًا، مع حدوث العديد من الحالات الجديدة كل يوم. يتسبب في أكثر من 19 مليون مريض في جميع أنحاء العالم وبالتالي العديد من الوفيات. يُعد العلاج الكيميائي والإشعاعي أكثر الطرق شيوعًا لعلاج السرطان. تشمل هذه العلاجات استخدام المواد الكيميائية التي قد تضر بصحة الفرد الجسدية والعقلية. هذا يمكن أن يؤدي إلى آثار جانبية خطيرة ومضاعفات. و لذلك نحن بحاجة إلى إيجاد طريقة بديلة لمكافحة السرطان تكون خالية من الآثار الجانبية وفعالة بنفس القدر. و من المحتمل ان تكون نبات الافيدرا إحدى هذه الطرق البديلة. لهذا السبب كان الهدف من هذه المذكرة هو اجراء تحليل لبعض المقالات المنشورة للنبتتين *E. alata* و *E. feominea* في المختبر و الجسم الحي لمعرفة مدى كفاءة هاتين النبتتين في مكافحة السرطان. أشارت النتائج إلى أن استخدام هذه النباتات في العلاج المضاد للسرطان قد يكون مفيدًا جدًا لأن مستخلصاتها تحتوي على جزيئات نشطة بيولوجيًا لها تأثير مضاد للبروفيراتيف . وفقًا لذلك ، قد توفر مصدرًا دوائيًا واعدًا لتطوير عقاقير جديدة وفعالة مضادة للسرطان.

الكلمات المفتاحية: السرطان ، الإيفيدرا ألاتا، الإيفيدرا فيومينيا، مضاد للسرطان.