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Topic

Toxicological, phytochemical study and biological activities of a medicinal Plant: *Ephedra alata*

Presented by:

ANTEUR Nour El Houda

REZKALLAH Malak

Board of the jury :

Mr. ROUABHI Rachid	**Professor**	**Supervisor**	**U. Larbi Tebessi**
Mm. BOUSSEKIN Samira	**CMB**	**President**	**U. Larbi Tebessi**
Mr. GASMI Salim	**CMA**	**Examiner**	**U. Larbi Tebessi**

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بسم الله الرحمن الرحيم

Abstract

Ephedra is a medicinal plant that has been used in traditional medicine to treat a variety of ailments. The goal of this study is to identify the toxicological, biological effect of the "***Ephedra alata***" as well as evaluate and analyze the biological activity of "***Ephedra alata***". The current research is part of a larger effort to increase the value of a medicinal plant. ***Ephedra alata*** is a pharmacologically significant plant that is well-known for its drought resistance. Polyphenols, flavonoids, tannins, alkaloids, saponosides, reducing sugars, and terpenoids were discovered in the aerial section of the plant during the phytochemical investigation.According to the ethnobotanical survey, the plant's pharmacological significance appears to be overlooked by a huge portion of the population, although scientific studies in pharmacology demonstrate that "***Ephedra alata***" has a significant therapeutic value and can be used to treat a variety of disorders.

Keywords: *Epehdra alata*, biological, therapeutical and toxicological effect, secondary metabolites, medicinal plants.

Résumé

L'*Ephédra* est une plante médicinale qui a été utilisée en médecine traditionnelle pour traiter une variété de maux. Le but de cette étude est d'étudier l'effet toxicologique et biologique de l'***Ephédra alata*** ainsi que d'évaluer et d'analyser l'activité biologique de l'***Ephédra alata*** La recherche actuelle fait partie d'un effort plus large visant à augmenter la valeur d'une plante médicinale. ***Ephédra alata*** est une plante pharmacologiquement importante qui est bien connue pour sa résistance à la sécheresse. Des polyphénols, des flavonoïdes, des tanins, des alcaloïdes, des saponosides, des sucres réducteurs et des terpénoïdes ont été découverts dans la section aérienne de la plante au cours de l'étude phytochimique. Selon l'enquête ethnobotanique, l'importance pharmacologique de la plante semble être négligée par une grande partie de la population algérienne, bien que des études scientifiques en pharmacologie démontrent que *l'****Ephédra alata*** a une valeur thérapeutique significative et peut être utilisé pour traiter une variété de troubles.

Mots-clés: *Epehdra alata,* effet biologique, thérapeutique et toxicologique, métabolites secondaires, plante médicinale.

الملخص

الإيفيدرا هو نبات طبي تم استخدامه في الطب التقليدي لعلاج مجموعة متنوعة من الأمراض. الهدف من هذه الدراسة هو دراسة التأثير السمي و البيولوجي للإيفيدرا ألاتا وكذلك تقييم و تحليل النشاط البيولوجي ل***Ephedra alata***البحث الحالي هو جزء من جهد أكبر لزيادة قيمة النبات الطبي . ***Ephedra*** *alata*هو نبات مهم من الناحية الدوائية معروف بمقاومته للجفاف. تم اكتشاف البوليفينول و الفلافونويد و العفص و القلويدات و السابونوسيدات و السكريات المختزلة و التربينويدات في القسم الجوي من النبات أثناء التحقيق الكيميائي النباتي وفقا للمسح العرقي النباتي ، يبدو أن الأهمية الدوائية للنبات يتم تجاهلها من قبل جزء كبير من السكان ، على الرغم من أن الدراسات العلمية في علم الأدوية تظهر أن ***Ephedra alata*****له** قيمة علاجية كبيرة و يمكن استخدامه لعلاج مجموعة متنوعة من الاضطرابات.

الكلمات المفتاحية : *Epehdra alata*,التأثير البيولوجي و العلاجي ، السمية ،الاعشاب الطبية.

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Dedication

With all the love and sincere feelings this work is dedicated first of all to the two persons that brought me to this world: my Father Ridha and my Mother Aziza thanks for being such a caring, loving, supportive parents. I am beyond happy for where I am today and I will always look up to you and learn from you, blessed to share this life with you.

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To the entire promotion of the master 2 Pharmaco-Toxicology

Last but not least; Thanks to everyone who has been a part of my life and has brought joy into my life one day, without forgetting Korean dramas and shows that never failed to lift up my spirit.

...Nour El Houda

Dedication

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Last but not least, i dedicate this work to myself, for being able to get through all the bad things and be who i am today.I love you all and i'm so lucky to have you.

...Malak

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B.P: Blood pressure

BCC: Business Communication Company

CLIA: Clinical Laboratory Improvement Amendments

DNA:Deoxyribonucleic acid

DPPH: 2, 2-diphenyl-1-picrylhydrazyl

E. coli: Escherichia Coli

E.alata: Ephedra Alata

EOR: Chemical enhanced oil recovery

FDA: Food and drug administration

FLAV: Flavonoids

FRAP: Ferric reducing ability power

G: Gram

GM: Genetic Modified

Hmps: Hydrophobically Modified Polymers

IM: intramuscular

IP: Intraperitoneal

IV: Intravenous

Kg: Kilogram

Km: Kilometers

LD50: Lethal Dose

MAO: Monoamine oxydase inhibitors

MeOH: Methanol

Nm: Nanometre

NOAEL: No Observed Adverse Effect Level

NS: Nervous system

PCs: Phenolic compounds

Ph A :Phenolic Acids

PPT: Polyphenols

ROS: Reactive oxygen species

S. aureus : Staphylococcus aureus

SC: Subcutaneous

TFC: Total Flavonoids Content

TM; Traditional Medicine

TPC: Total Phenolic Content

UK: United KIngdom

UV: Ultra Violet

WHO: World Health Organization

Medicinal plants have important contributions in the healthcare system.Use of herbal medicines represents a long history of human interactions with the environment.

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A number of modern drugs currently in use have been obtained through medicinal plants.

Despite the profound therapeutic advantages possessed by some of the medicinal plants, some constituents of medicinal plants have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic. This raises concern about the potential toxic effects resulting from the short-term and long-term use of such medicinal plants. Therefore, evaluating the toxicity effects of any medicinal plants extracts intended to be used in humans and animals is of greatest significance.

Chapter 01:

Medicinal plants and phytotherapy

1.1. Introduction :

Medicinal plants have played a critical role in the maintenance of human health and the survival of humanity for a long time. These plants provide an enormous reservoir of potential chemicals attributable to secondary metabolites, which have the benefit of having a wide range of chemical structures and biological functions **(Messadia, 2017).**

Many procedures, like as maceration, decoction, and infusion, is used to extract raw, natural extracts of these compounds from plants. These compounds, which are utilized in traditional medicine, can be sources of novel medications **(Karmakar et *al.*, 2011).**

Plants are a reservoir of bioactive molecules that have yet to be chemically and biologically explored, according to one estimate. More than 300,000 species have been described around the world, and 15% of them have been studied phytochemically, including 6% for their biological activities, making plants a reservoir of bioactive molecules that have yet to be chemically and biologically explored. **(Negri and Tabach, 2013).**Algeria has a wide range of climatic and taxonomic diversity, which results in a wide range of plant diversity. The Algerian flora is estimated to number over 3152 species from various botanical families. 15 percent of these species are indigenous to the area. Herbal medicines (phytomedicines) are slowly but steadily gaining traction in Algeria's pharmaceutical sector **(Quezel and Santa, 1963)**.

Indeed, the search for bioactive molecules in plants can be done using a variety of strategies, including an ethnopharmacological approach that relies on traditional medicine knowledge, a chemotaxonomic approach that focuses on taxa known to contain specific secondary metabolites, or a combination of the two. In this regard, we were interested in evaluating the biological activities, toxicological and phytochemical characterization of *Ephedra alata*, a plant species of the *Ephedraceae* family that grows in Algeria's dry regions. This plant has a long history of usage in traditional medicine as a natural cure for a variety of ailments.

1.2. History of medicinal plants use by humans :

A medicinal plant's definition is straightforward. It is, in fact, a plant used to prevent, treat, or relieve a variety of maladies “plant medications”, at least some of which have therapeutic effects, make up the medicinal plants. **(Aribi and Hasasni, 2018).** Plants have been the primary source of treatments for many diseases for thousands of years.

Phytotherapy became less popular as modern medicine advanced at the end of the nineteenth century, and "wonder" drugs (antibiotics, etc.) were discovered. However, due to the adverse effects of synthetic medications, the utilization of plants for therapeutic purposes has seen an indisputable revival of interest since the 1970s. Herbal medicine was not formally acknowledged as a separate medicine by the Ministry of Health until the late 1980s. These are plants that have traditionally been used in medicine. Their action is based on chemical molecules (primary or secondary metabolites) or synergy between the various chemicals present. **(Aribi and Hasasni, 2018) .**According to the World Health Organization, 80 percent of the world's population particularly in impoverished nations uses herbal medicine for treatment. Traditional practitioners' knowledge is becoming increasingly rare and is on the verge of extinction. This is why ethnobotany and ethnopharmacology are attempting to identify active plants all around the world, whose qualities and uses are still being defined and validated by current researches. **(Hajjaj, 2017).**

In the lack of a contemporary medical system, medicinal plant use for therapeutic purposes is common in several parts of the world, particularly in underdeveloped countries .The plant's richness of active chemicals provides it outstanding pharmacological characteristics, which could explain its wide range of medicinal indications and use in traditional medicine. In Africa, herbal remedies are still widely used in health treatment by many people. In Algeria, as an example *ephedra alata* is used in herbal tea and inhalation to treat influenza, whooping cough, and general weakness, as well as in nasal drops to treat colds; **(Chouikh, 2020)** As a result, these medicinal plants are widely used, and they can also be valuable sources of novel compounds with high therapeutic potential and low costs. The widespread use of these medicinal herbs necessitates the concept of toxicity, which is defined as the set of detrimental effects generated by a drug introduced at a single relatively high dose or at modest doses repeated over time on a living organism ; The toxicity of a chemical is determined using a series of pharmacological assays.**(Kaboua et *al.*, 2021).**

Plants with therapeutic properties are used in a variety of fields, medical plants have recently come to take a significant place at the level of different sectors, including research, agriculture, industry, medicine, and the environment, due to their economic, social, medicinal, ecological, and cultural relevance.**(Aribi and Hasasni, 2018).**

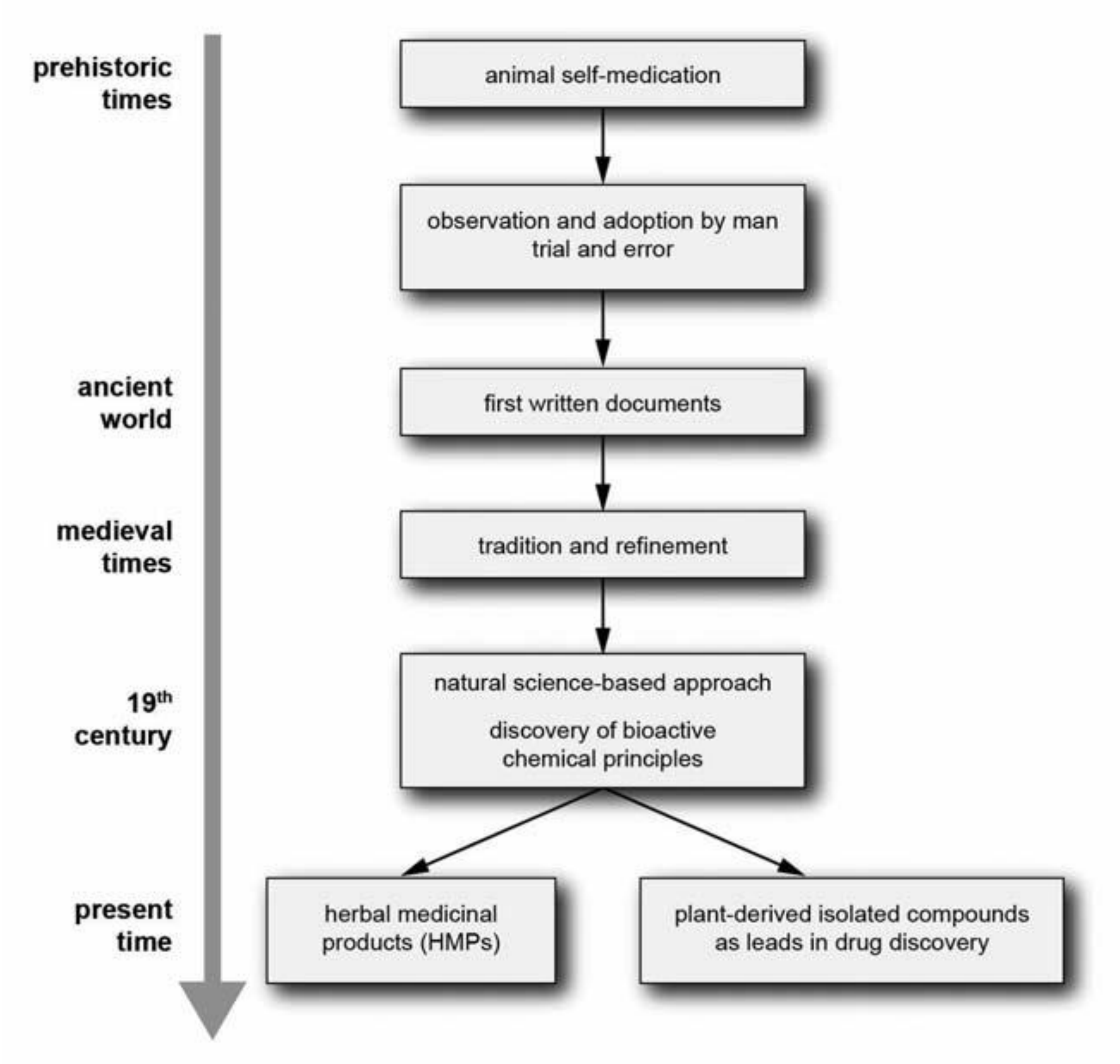


Figure 01: The history of herbal remedies **(Fürst and Zündorf, 2015)**

1.3. An overview of the system of traditional medicine :

Algeria, with a total size of about 2.4 million km2 and 1200 kilometers of coastline, is the largest country in the Mediterranean basin, Africa, and the Arab region. Algeria has a diverse climate and a diverse flora with 4,000 species, 917 genera, and 131 families. Algeria also has a significant and diverse cultural diversity, owing to its historic history as one of the world's original cradles of Homo sapiens and civilization. Although several studies have been carried out to document local knowledge about the use of medicinal plants to treat various diseases, more ethnobotanical research into Algerian ancestral ethnomedicinal knowledge is needed. **(Belhouala and Benarba, 2021).**

Algeria, with around 3150 species of vascular plants and a rate of endemism of about 10% of the total number of species, provides a significant plant genetic reservoir due to the richness and original diversity of its floran ; From a phytochemical and biological standpoint, however, this floristic potential has yet to be fully explored **(Aissani , 2022).**

Medicinal plants are still regarded as essential and promising sources of medications for treating a variety of ailments. Oral transmission of their therapeutic uses, vernacular names, ways of preparation, and routes of administration formed a local ancestral knowledge defining each people or ethnic group residing in a given area .Furthermore, plants or natural products utilized in clinical practice. **(Belhouala and Benarba, 2021)** They have been employed as key sources of medications not only when plant constituents are used directly as medicinal agents, but also as a raw material for the production of pharmaceuticals pharmacologically active chemicals, or as a model for pharmacologically active molecules. The “Table 1” depicts various active chemicals that accumulate in plants. Medicines are primarily derived from these sources.

Table 1: Example of some active ingredients used in pharmacology with their sources plants and their therapeutic roles **(Iserin, 2001; Raven, 2017).**

Secondary metabolite	**The plant source**	**Role**
Phytoestrogens	Soybeans	manage the symptoms of menopause
Paclitaxel (taxol)	yew	used as an anti-cancer drug
Quinine	Cinchona	fight against malaria
digoxin	Digitalis lanata	heals the heart
Ephedrine	Plants of the genus Ephedra	Against colds

Morphine	Opium poppy	Analgesic
Tubocurarine	Curare	muscle relaxant

As a result, medicinal plants might be defined as plants that are part of the Pharmacopoeia, and which are utilized for their disease-prevention or cure capabilities in humans or animals. Plants, by another definition, are a group of medicines plants in which at least a portion of their organs (bark, leaf, root, etc.) possesses medicinal capabilities due to their abundance of secondary metabolites. **(Belhadj, 2019).**

People are becoming disillusioned with modern medicine and losing faith in it, hence traditional medicine is spreading rapidly in certain developed countries. Almost half of the population in developed countries uses traditional medicine, either because they believe it is risk-free and more natural, or because they believe it is more effective. Although traditional medicine is recognized in many developing and developed countries, it is not always made a priority in their healthcare systems. However, a few nations, such as China and India, have progressed to the point where Traditional medicine can be used as a kind of healthcare. **(Medikizela, 2014).**

TM which lacks a scientific foundation contributes greatly to healthcare but rarely collaborates with modern medicine. Another issue with TM is that its methods and training are generally kept secret, and it does not keep up with scientific and technological progress. Until the late 1980s, the developed world paid little attention to indigenous traditional knowledge and provided little assistance to developing countries in the preservation, collection, and systematization of the knowledge. However, there has recently been an increase in interest in natural products, which has led to a greater respect for indigenous people and their customs and beliefs. **(Medikizela, 2014).**

1.4. Conventional and modern approach for medicinal plant authentication :

Authentication of medicinal plants is crucial, particularly when the plant material is to be utilized directly as a medical therapy or as a botanical supplement. Authentication should take place at all phases of the manufacturing process, from raw material to completed product, to ensure the ultimate protection of consumers. Unfortunately, there is no single, superior approach for ensuring a product's 100% authenticity. Instead, achieving this goal necessitates a combination of approaches applied correctly and to the appropriate tissue.

For the security of the public and industry, reliable authentication techniques for plant material are essential. They can assist prevent errors caused by material misidentification at any point in the manufacturing process, from raw material collection to finished product delivery. They can also be used to detect and prevent fraudulent activity by unscrupulous persons. Unfortunately, no one authentication process can be used for all medicinal plants; instead, a combination of technology and behaviors is required.**(Techen et *al.*, 2004).**

Drugs are desperately needed. The truth is that novel medicines are sorely needed in modern medicine. A new drug must go through a lengthy research and development process before it can be manufactured, and the expense is significant. Furthermore, rising drug resistance has rendered some antibiotics and other life-saving treatments worthless, owing in part to medication overuse. Both of these trends indicate that scientists and pharmaceutical corporations are scrambling to find new medication sources and are increasingly resorting to traditional medicine. A few notable victories have reignited interest in traditional medicine as a source of extremely successful and profitable pharmaceuticals. **(Shetty, 2010).**

Traditional medicine's mainstreaming — merging its expertise into modern healthcare and ensuring that it fulfills modern safety and efficacy requirements it is a difficult and ongoing effort. Conservationists are increasingly concerned that the booming traditional medicine business is endangering biodiversity by overharvesting medicinal plants or increasing the usage of body parts from endangered animals like tigers, rhinos, and elephants. Aside from natural resource sustainability, combining traditional and contemporary medicine confronts various problems due to significant variations in how each is practiced, evaluated, and controlled (see table 2). **(Shetty , 2010).**

Table 2: Key differences between traditional and modern medicine **(Shetty , 2010).**

	Traditional medicine	Modern medicine
Knowledge protection	Open access	Closed, patent-protected
Formulation	Ad hoc during consultation with the patient	Pre-determined, and once tested in clinical trials cannot be changed

		unless re-tested
Regulation	Virtually none, though some countries are trying to introduce rules and standardisation	Extremely tight, to the point that bringing drugs to market now costs billions of dollars
Testing	No formal testing as knowledge of the effectiveness is handed down through generations	Rigorous trials that happen in different phases, first testing for safety, then efficacy
Dosage	Unfixed: the amount of medicine given might be roughly similar, but the active ingredient (which is what dosage really is) can vary hugely	Fixed doses that tend to vary only slightly with age or weight, or disease severity
Consulataion	Lengthy, and the patient is asked about a wider range of questions than just their symptoms	Consultations in both primary and secondary care tend to be brief and focused, especially as national health systems come under strain
Training	Both systems of medicine require lengthy training over many years but with traditional medicine, knowledge is often passed one-to-one through families, and practitioners are often born into a family of healers	Often vocational: health professionals go through formal training in schools and universities

Chemical components of herbal medicine or their preparations can be examined using modern analytical technologies. Reflecting the typical curves of complicated chemical components, as well as the overall influence of herbal medicine active substances. Modern analytical technology (chromatography, spectroscopy, mass spectrometry) and chemometrics approaches (unsupervised, supervised), as well as their benefits. **(Liu et *al.*, 2022).**

When numerous distinct individual species were crushed and put together in a proprietary pharmaceutical, evaluation became even more challenging. Before beginning the investigation, scientific approaches should be used to confirm the method of evaluating pharmaceuticals by veteran collector's experience. **(Sudha Revathy, 2012)** see figure 2.

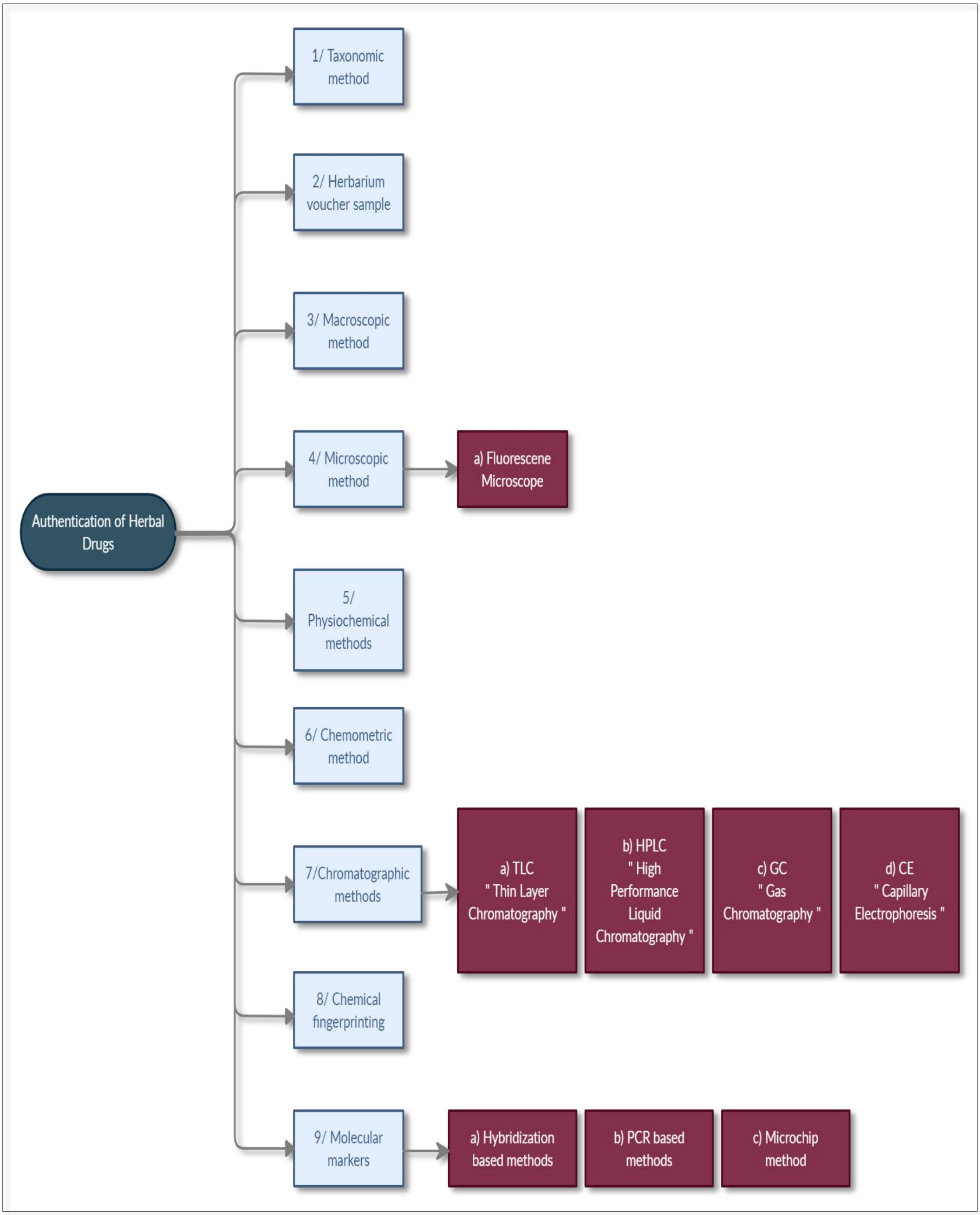


Figure 2: Authentication of Herbal Drugs techniques

When taken by humans, these substances have the potential to interact with human protein targets or change the growth of commensal, pathogenic, or parasitic organisms living inside the human body, affecting human health and disease. A prescription in many traditional herbal medicine systems generally consists of many components blended in a certain ratio in a single formula, and each item in isolation may lack therapeutic effects present in the holistic formulation, a phenomenon known as the combinatorial effect. Pharmacological efficacy could be boosted by the simultaneous action of many drugs targeting multiple sites, or by synergistic action on a single site, according to the theory. **(Li and Weng, 2017)**It is critical that the company purchasing plant materials take all required precautions to ensure that the material is pure. One step is harvester training, followed by the collection and preservation of voucher samples. This is no easy undertaking because the amount of material gathered in a single year is sometimes measured in tons rather than tens or hundreds of kg **(Techen et *al.*, 2004)** .Voucher specimens and macroscopic samples must be kept in optimum conditions, which necessitate the use of space-intensive storage facilities. Microscopic approaches may not allow identification of some plants to the species level, and both methods of identification are not particularly sensitive to recognition of chemotypes or ecotypes. A trained individual must execute the identification, which frequently requires access to a herbaria or a photographic reference library.DNA sequences and genetic profiles have the added benefit of being able to be recorded and transferred in an electronic format, allowing for more independent comparisons with authorized sources and identification verification. Molecular methods of identification can detect minute intra-specific differences, down to variances between and within plant populations. DNA sequence analysis requires a trained individual, while DNA sequencing requires a computer program. **(Techen et al., 2004).**

1.5. General informations about phytotherapy :

Scientific study has succeeded in replacing fragmentary folk wisdom from prior eras with the light of order, and development throughout the past two centuries. The average lifespan has increased by several decades. Many diseases that were formerly fatal are now curable or have been totally eradicated. The ever-improving fruits of scientific labors help to lessen pain and suffering, which are nevertheless all too widespread. **(Abdulla, 1999)**.The pharmaceutical and agricultural industries, natural goods are becoming more important. A variety of chemical compounds developed from organic synthesis necessitate pharmacovigilance due to their unfavorable side effects. **(Sahli, 2017).**

Phytotherapy is most likely the most economically successful and commonly used branch of alternative or supplemental medicine. These are herbal medicine tablets, powders, and elixirs that are offered as 'nutritional supplements' in most countries through health shops and pharmacies. Whole-plant preparations such as raspberry-leaf tea (*Rubus idaeus*) or extract of horse-chestnut seed (*Aeschlus hippocastanium*) are believed to be more effective than the sum of their parts through the (Scantily supported) mechanism of'synergy.' Phytotherapy is distinguished from the appropriation and synthetic production of plant active chemicals by mainstream medicine by its concentration on whole-plant material rather than specific chemical elements. **(Abdulla, 1999)**

New techniques, in particular the consideration of the habitat in which plant species develop, have demonstrated their effectiveness. Indeed, environmental limits drive adaptive responses in specific types of plants, including the buildup of secondary metabolism molecules that have been found to be active against several plant and human illnesses. This characteristic can be seen in *halophyte* and *xerophyte* plants. These are plants that thrive in abiotic environments.In many situations, their virtues are linked to their ability to withstand oxidative stress, which is caused by an excessive buildup of oxidants and disrupts plant development. As a result, these plants generate a strong antioxidant system. **(Sahli, 2017).**

Herbal remedies have also largely evolved in the last decades in many aspects.

(Figure 3) **(Fürst and Zündorf, 2015).**

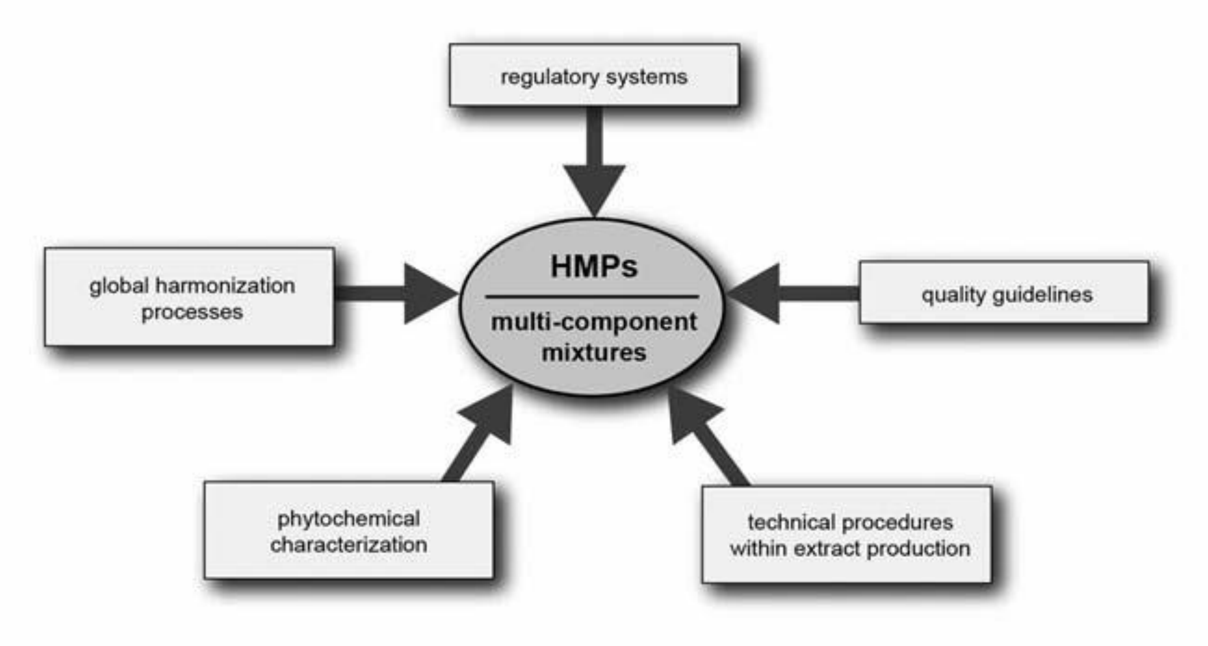


Figure 03: Factors that have contributed to the evolution of HMPs

(Fürst and Zündorf, 2015)

The attractiveness of self-medication appears to be the final motive for the phytotherapy craze, as it is for many other fields of alternative medicine. People are eager to determine for themselves what they put into their bodies in an age of choice; empowerment, ease, and the decline of unchallenged authority (as evinced by the recent fuss about genetically modified [GM] foods). Somehow, people who wouldn't trust their hair, houses, or vehicles to anybody except experts feel qualified to deal with the human body's immensely complicated chemistry, physics, and biology. Worse, many people who use herbal supplements are hesitant to discuss them with their regular doctors. Another key factor is that, as [Dr] Elizabeth Williams, editor of the journal Phytotherapy Research at the University of London, UK, says, ".people still believe that 'natural' equals good and safe despite plenty of evidence to the contrary" Most researchers caught up in this surprise anti-science wave share this viewpoint. "People seem to be able to have contradictory opinions at the same time," Williams continues. "For example, Nicotine is unhealthy but other natural compounds are helpful." "Despite the fact that they are aware of harmful plants, they appear to believe that herbs are beneficial." This circumstance could be explained by the fact that herbs are connected with healthy eating and cooking, according to Williams. **(Abdulla, 1999).**

Because of new or enhanced methodologies, technological procedures for extract extraction have advanced, and phytochemical characterization has vastly improved. Furthermore, many nations have started harmonization initiatives to improve the quality of herbal treatments.

Pharmaceutical quality can range from uncontrolled analytical mixes to high-tech specific extracts based on complex extraction, enrichment, and standardization techniques.

Manufacturers work in an appealing field from an economic standpoint. The global botanical and plant-derived medicine market was estimated at US$21.4 billion in 2011, according to the 2013 BCC (Business Communication Company, Inc.) Research Report BIO022F "Botanical and Plant-Derived Drugs: Global Markets," and is predicted to climb to 26.6 billion in 2017. This demonstrates the high level of interest in these items among customers and patients.

Herbal medicines are extremely popular. **(Fürst and Zündorf, 2015).**

Chapter 2:

Historical and Biological studies of

"*Ephedra alata* "

1.1. Description

Ephedra is a genus of a non-flowering seed plants belonging to the Gnetales, the closest living relative of the Angiosperms. *Ephedra alata* Decne belongs to the" *Ephedraceae*" Dum **(Chouitah, 2019)**

It is one of the rare bushes in Saharan areas (FIG. 1), and it is also a nanophanerophyte in the RAUNKIAER sense (1934). It's a stiff, green yellow, thickly branching perennial shrub that grows 40-100 cm tall and is typically broader than tall. The twigs are articulated and have scales that are decreased and opposing at the nodes of the leaves. Small cones are formed by unisexual blooms. Male and female flowers normally stand on separate feet. Individuals carrying two types of flowers have been seen in some circumstances. **(Hadjadj et *al.*, 20)**

(See figure 04)

Figure 04: Shrub of Ephedra alata **(Chaieb et al., 2008**)

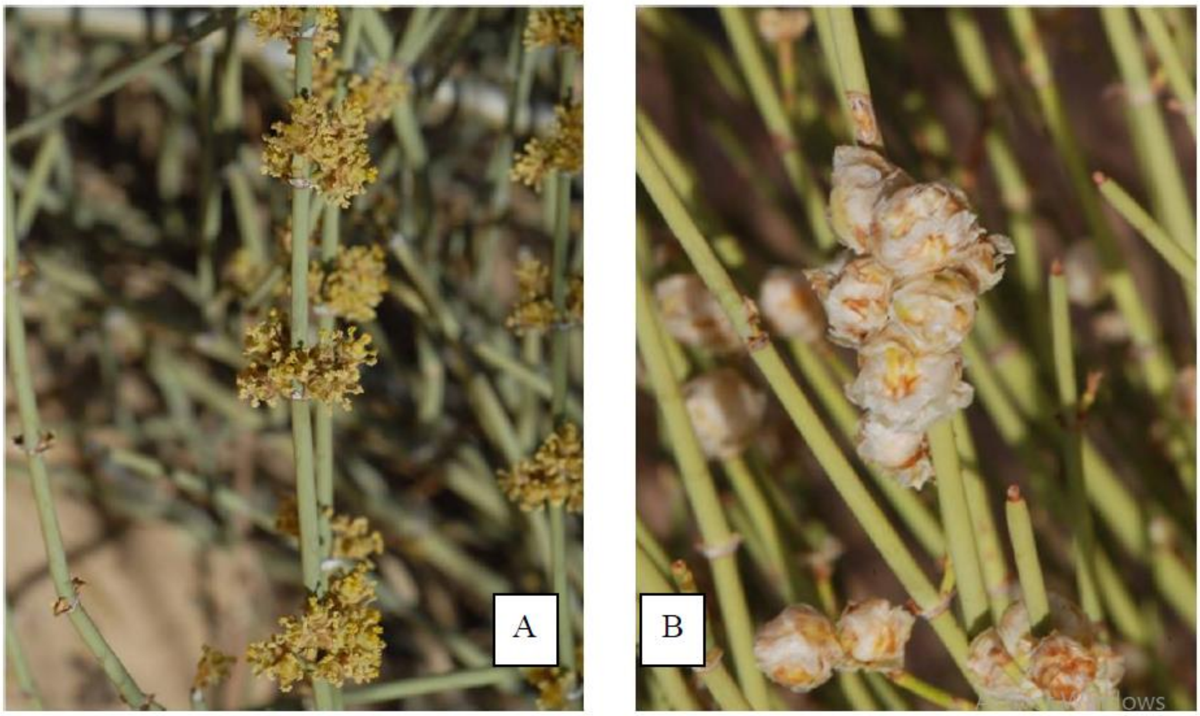


Figure 05: Flowering branches of Ephedra alata **(Chaieb et *al.*, 2008**)

A: Male flowers of E. alata **B:** Female flowers of E. alata

The plant is an outstanding aeolian sand fixer species in terms of ecology. However, the high quality of its coal wood, which is prized by Saharan people, exposes it to deforestation **(Hadjadj et *al.*, 2020).**

Ephedra has a long history of therapeutic usage, thanks to the presence of several alkaloids, including ephedrine. *Ephedra* has been used in medicine since at least 2700 B.C., when the Chinese employed it to cure asthma, cough, and bronchitis (*Ephedra sinica Stapf*) to treat bronchitis, asthma, and cough.The *ephedra* plant has a strong scent and a harsh flavor. The dried stem is the component of the plant that is most commonly used for its medicinal properties. It is commonly found in weight reduction and energy solutions and is available in bulk herb, capsules, and hydro-alcoholic extract. *Ephedra* is recommended for respiratory tract illnesses with moderate bronchospasms.**(Chebouat et *al.*, 2016)** The phytochemical composition of distinct ephedra species is not fully understood. Alkaloids, amino acids and derivatives, volatile chemicals, and phenolic compounds are all secondary metabolites found in *Ephedra* species. **(Ozenda, 1983).**

In this context, extensive scientific study on bioactive chemicals, particularly polyphenols (PP), has led to their usage in the food, cosmetics, and pharmaceutical industries **(Pietta et *al.*, 2000).** The evaluation of phytotherapeutic qualities such as antibacterial activity and antioxidant activity is deemed crucial and beneficial **(Alouane, 2009).**

Ephedra alata subsp. alenda is well-known in Algeria for its use in traditional medicine. The people of the Region of Ourgla use it to treat influenza, pertussis, weakness, and colds. The plant is used to treat abortions, cancer, diabetes, cough, stomach ulcer, flu, intestinal gas, obesity, and renal and heart failure in the El Oued region. For the Tuareg community of the Illizi region, cancer is only treated with Ephedra alata subsp. alenda. **(Hadjadj et *al.*, 2020).**

Ephedra alata is high in antioxidants since it contains phenolic components. *Ephedra* is said to contain phenolic substances such as trans-cinnamic acid, catechin, syringin, epicatechin, symplocoside, kaempferol 3-O-rhamnosid 7-O-glucoside, and isovitexin 2-O-rhamnoside, all of which contribute to the plant's antioxidant action. Plant secondary metabolites known as phenolic compounds play a crucial role in disease resistance and pest prevention. **(Al-Rimawi et *al.*, 2017).**

1.1.1. Distribution and Ecology :

The *Ephedraceae* family, which comprises the genus “*Ephedra alata”*, has roughly 40 species worldwide. Because species in this genus can thrive in semi-arid and desert environments, they can be found on all six continents. The latter thrives in sandy soils, dry slopes, and mountain sides, and can be found in China, India, Egypt, the Middle East, Europe, and the Americas. **(Digheche and Khalfallah, 2019)**

The genus is native to temperate and subtropical latitudes in Europe, Asia, and North America, with populations particularly strong in northern and western China, northern India, and Spain. The Rocky Mountains in the United States are home to ephedra plants. In Africa, Ephedra alata is found in Algeria, Egypt, Libya, Morocco, Tunisia, Mauritania, Chad, and Mali; in Asia, it is found in Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan, and Syria. **(Al-Snafi, 2017)**

Despite the presence of similar appropriate habitats in southern Africa and Australia, no species has been found. There are no known fossils in these regions, and none that are wellauthenticated. **(Chenini and Boumegouas, 2020)** As we can see in the map below the distribution of the *ephedra* plant across the world. (Figure 04)

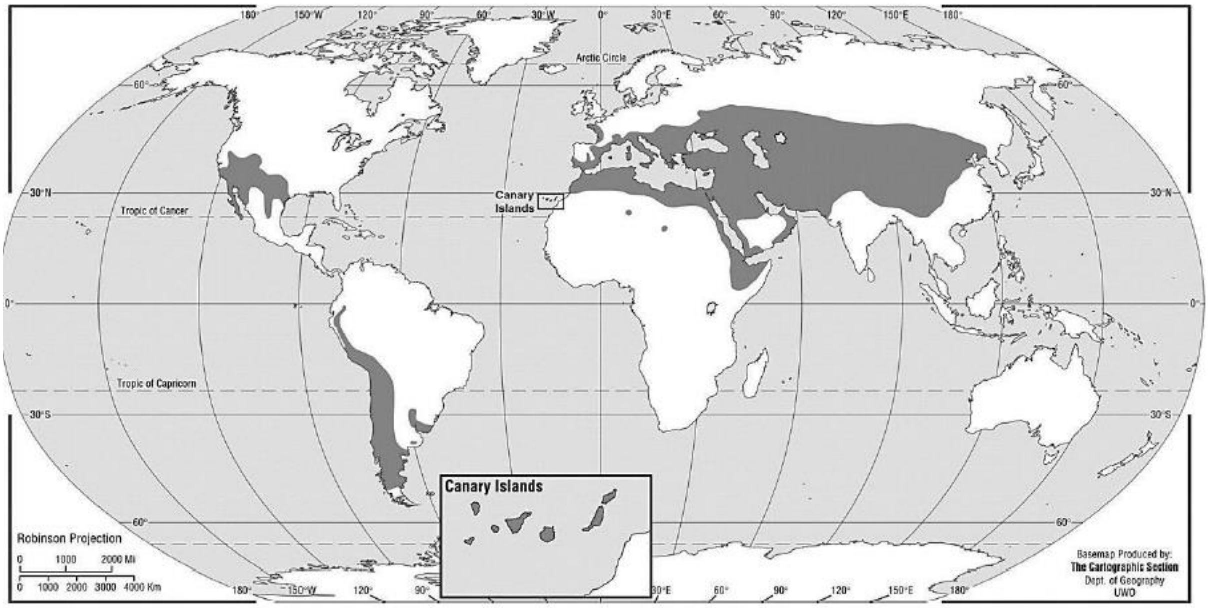


Figure 06: Geographical distribution of *Ephedra* in the world **(Caveney et *al.*, 2001)**.

1.1.2. Taxonomy :

Figure 07: Taxonomic traits of the genus *Ephedra* **(Ozenda , 1991)**

Kingdom: Plantae; **Phylum**: Tracheophyta;

Division: Gnetophyta; **Class**: Gnetopsida; **Order**: Ephedrales;

Family: *Ephedraceae*; **Genus**: *Ephedra*; **Species**:

Ephedra alata and *Ephedra foliata*

Common names:

Ephedra alata: Arabic: Alanda, Alanda Mujanaa**;**

Theel maiz, ephedra, Anab bahar, Ather, jashia**;**

English: Ephedra.

Ephedra foliata: **Arabic:** *Alanda warakia*, fedr waraki,

Al-Kuood al-waraki; **English**: Shrubby horsetail.

Table 03: Classification of *Ephedra alata* alenda according to **(Ozenda, 1991)**.

Reign	Plantae
Embranchment	Spermaphytes
Sub-branch	Gymnosperms
Class	Gnetopsida
Order	Ephedral
Family	*Ephedraceae*
Gender	Ephedra
Species	*Ephedra alata*

1.1.3. Subspecies :

There are three recognized subspecies:

- *Ephedra alata* subsp. *alata* (syn.: *Ephedra altissima* Bové, 1834);
- *Ephedra alata* subsp. *alenda* (Stapf) Trab. 1905 (syn.: *Ephedra alenda* (Stapf) Andr,1931);
- *Ephedra alata* subsp. *monjauzeana* (Dubuis and Faurel 1957)

For their distribution it is shown that:

- Subsp. *alata* is native to Algeria, Chad, Iraq, Lebanon, Libya, Mali, Mauritania, Palestine, Saudi Arabia, Sinai, and Syria.
- Subsp. *alenda* is native to Africa: Algeria, Egypt, Libya, Mauritania, Morocco, Tunisia, and Western Sahara.
- Subsp. *monjauzeana* is native to Algeria only. **(Bell and Bachman , 2011)**

1.1.4. Distribution and Habitat :

A thick clump-forming xerophytic shrub. In desert areas, it is typically found along Wadis, in Wadi-beds, or on changing sand dunes on sandy calcareous soil, gravely/rocky soil, or clay soil. Haloxylon is frequently present. A major component of «vegétation contractée" on occasion. Because it possesses wing-like bracts, dispores are distributed by the wind. From March until May, the plant is in full bloom. **(Bell and Bachman, 2011)**

In Algeria most of the studies were conducted and the samples were taken from the following cities:

- To the north by the city of Oum el Bouaghi.
- To the east by the city of Tébessa.
- To the west by the city of Batna.
- To the southwest by the city of Biskra.
- To the south by the city of El Oued.
- To the south by the city of El Khenchla.**(Rehouma and Guemari , 2018)**

1.1.5. Uses :

For the traditional uses *Ephedra* species were valued as antipyretics, diaphoretics, circulatory stimulants, and cough sedatives, according to a Chinese dispensatory written in

1569. Traditional Chinese medicine, on the other hand, has utilized Ephedra to treat allergies, asthma, lung congestion, chills, colds, hay fever, coughs, edema, fever, flu, headaches, and nasal congestion. For many generations, the plant has also been utilized in Russia to treat respiratory problems and rheumatism. *Ephedra* was utilized by Native Americans and Spaniards in the Southwest for a variety of ailments, particularly sexual illnesses. Yamanashi was the first to discover an active principle, in 1885. Nagai called the alkaloid ephedrine after obtaining it in pure form in 1887. The medication was shown to be poisonous, mydriatic, and sympathomimetic during pharmacological testing. (See figure 08)

Figure 08: Ephedra alata plant **(Mater, 2015)**

This species is widely utilized in traditional medicine, particularly in Arab nations, where young stems are employed **(Abourashed et *al.*, 2003)**. These stems are normally boiled in water for around 30 minutes before being eaten as a hot tea at a dose of 1.5 to 9 g of grass.**(Jaradat et *al.*, 2015)**

E.alata is employed as a depurative, hypotensive, antiasthmatic, and astringent agent in Egyptian traditional medicine. *Ephedra* is one of the most common rangeland plants in Saudi Arabia. Many animals have been drawn by its pleasant perfume and have used it as pasture.*E.alata* is used to treat diabetes in Morocco. *Ephedra* is used vaginally to induce abortion; indeed, the women of the Sahara would eat the crushed stems of *E.alata* fried in butter to induce abortion.In Algeria, *E. alata* is used in herbal tea and inhalation for influenza, pertussis, and general weakness, as well as nasal drops for colds. The aerial section of the *Ephedra alata* species is known in Asia as Ma-huang and is used to treat bronchial asthma, colds, flu, fever, chills, rhinitis, nasal congestion, edema, headache, arthralgia, and as a diaphoretic, anti allergenic, and antitussive, and to manufacture clandestinely a street drug, methamphetamine 'd-desoxy-ephedrine' .**(Messadia , 2017)**

1.1.6. Pharmacological effects :

The phytoconstituents in different *Ephedra* species have varied pharmacological effects. The alkaloids and phenolic compounds content of *Ephedra* species were studied in general research, such as trans-cinnamic acid, catechin, epicatechin, symplocoside, and flavonol-3-O-glycosides, and proanthocyanidins. **(Jaradat et *al.*, 2021)**

Many phyto-constituents, including alkaloids, tannins (primarily pronthocyanidins), phenolic acid saponins, flavonoids (vicenine II, leucine III, kaempferol 3-rhamnoside, quercetin 3-rhamnoside, and herbacetin 7-O-glucoside are flavonoids isolated from *Ephedra alata*), and essential oils, have natural origins in Ephedra alata Ephedrine alkaloids, proto-alkaloid types generated from phenylalanine, are mostly responsible for their biological features. Note that (-) ephedrine and (+) pseudoephredrine are the most common alkaloids in the dried plant, accounting for around 80% of the total alkaloid content. **(Rehouma and Guemari, 2018)**

Oxidation is a natural process that damages cell membranes and other structures in the body, including cellular proteins, lipids, and DNA molecules. When oxygen is rapidly digested, it produces unstable molecules known as free radicals, which steal electrons from other molecules, causing DNA damage and cell death. However, over time, the damage produced by an excessive upload of free radicals may become irreversible, leading to ailments such as heart disease and liver disease. With some cancers, particularly oral, stomach, esophageal, and bowel cancers The market for traditional, complementary/alternative, and herbal medicines is continuously rising worldwide, according to data collected from a World Health Organization (WHO) global study.In reality, the use of phytopharmaceuticals and nutraceutical goods is increasing all the time.Many people are now adopting these natural formulations in various national healthcare centers for the treatment or prevention of numerous ailments and health conditions. **(Jaradat et *al.*, 2021)**

1.1.7. Toxicological effects:

Ephedra species with both positive and negative effects **(Ma et *al.*, 2007)**. Tachycardia, hypertension, hyperperspiration, bronchodilation, agitation, and mydriasis can all be symptoms of this. *Ephedra* use has also been linked to gastrointestinal and psychological side effects **(Peters et *al.*, 2005)**. These side effects may explain why *Ephedra* is only suggested for acute circumstances in traditional Chinese medicine and is not recommended for long-term use. **(Chen et *al.*, 2010)**.

1.1.8. The antidote effects of drugs extracted from Ephedra Alata :

Ephedrine, the major active ingredient in *Ephedra*, has been used to treatasthma in traditional medicine because it acts as a bronchodilator. Common synthetic bronchodilators had essentially the same side effects as ephedrine at the time it was regularly prescribed for this purpose, which would no longer be the case for succeeding generations of asthmatic medicines. *Ephedrine* is no longer used for this purpose. Another chemical found in substantial proportions in *Ephedra*, pseudoephedrine, is still used in the manufacture of over-the-counter nasal decongestants today. It is the sole use of over-the-counter products that is approved, and dosages are limited. Ephedrine is also used intravenously in obstetrics to prevent blood pressure dips during difficult deliveries, when an epidural is administered, or when a caesarean section is performed. According to a more recent study, the ephedrine-caffeine combination can certainly improve sports performance, particularly by reducing the impression of physical weariness, but the risk of such a combination is extremely high.

(Site 01)

1.1.9. medical contraindication :

- Like synthetic ephedrine, *Ephedra* is contraindicated in people who suffer from :
  - Coronary thrombosis;
  - Diabetes;
  - Glaucoma;
  - Heart disease;
  - High blood pressure;
  - Disorders of cerebral circulation;
  - Adrenal disorders;
  - Thyroid disorders;
  - ¨Prostatic hyperplasia;
  - Depression, anxiety, agitation;
  - Difficulty urinating or prostate hyperplasia.
- *Ephedra* is also contraindicated in:
  - People on treatment with monoamine oxidase inhibitors (family of antidepressants);

- Children under 6 years of age;
- Pregnant and breastfeeding women

(Site 02)

2.1. Secondary metabolites :

Secondary metabolites are complex organic molecules synthesized and accumulated in small amounts by autotrophic plants. They are divided mainly into three main families: Polyphenols, terpenes, alkaloids **(Lutge et al., 2002; Abderrazak and Joël, 2007**). They have very different functions, such as Protection from the attack of pathogens or herbivores. Pollinator attraction. They participate in allelopathic responses "competition between plants for germination and growth". They are molecules that are also very useful for humans, as dyes, flavourings, antibiotics, herbicides, drugs etc.

Ephedra species' chemical constituents have piqued researchers' interest for decades because to the presence of ephedrine-type alkaloids and their pharmacological characteristics. Other chemical compounds, including as phenolic and amino acid derivatives, have also proven appealing and have offered evidence-based support for the *Ephedra* species' ethnomedical usage. **(Rashed, 2021).**

Research results demonstrated that *Ephedra alata* extract is rich in powerful falvonoid glycosidic substances. These findings come to the fact that *Ephedra alata* is a natural source of strong antioxidants that may help to prevent a variety of diseases and could be employed in food, cosmetics, and pharmaceutical industries. **(Rimawi et *al.*, 2017)** Ephedrine and pseudoephedrine, which are present in the stem of *Ephedra alata*, are used to treat inflammatory disorders, asthma, and viral infections. **(Peters et al., 2015).**

This plant also has antimicrobial and cancer-fighting abilities. **(Rimawi et *al.*, 2017)** . It also has been shown to have phenolic compounds such as trans-cinnamic acid, catechin, syringin, epicatechin, symplocoside, kaempferol 3-O-rhamnoside 7-O-glucoside, as well as isovitexin 2-O-rhamnoside**. (Amakura et *al.*, 2013)** A wide range of *E. alata* natural compounds have been noted from the entire plant, including alkaloids, tannins, saponins, proanthocyanidins, phenolic acids, flavonoids, and essential oils, and the plant-derived polyphenols are of major relevance for their possible antioxidant property.**(Hegazi and El-Lamey, 2011; Moussaoui et *al.*, 2010; Nawwar et *al.*, 1985).**

E. alata had the highest TPC and TFC levels of the Ephedra species studied E. foeminea had the lowest TPC, but E. fragilis had the lowest TFC. A high TPC value was associated with a high TFC value, depending on the species, whilst a low TPC value was associated with a low TFC value. **(Ibragic and Sofić, 2015) (See table)**

Table 04: TPC, TFC and TAC in *Ephedra* species **(Ibragic and Sofić, 2015)**

Ephedra species	TPC (mg GAE/g dry weight)	TFC (mg QE/g dry weight à	TAC (mg/g dry weight)
Ephedra alata	53.3±0.1	2.8±0.0	n.a.
Ephedra foliata	52.6 ±0.1	2.5±0.0	n.a.
Ephedra distachya subsp.*Helvetica*	27.0±0.4	2.1±0.3	15.8±2.0
Ephedra major	26.2±0.4	1.3±0.2	14.8±1.9
Ephedra altissima	16.4±0.1	2.0±0.0	n.a.
Ephedra fragilis	7.7±0.1	0.5±0.2	0.2±0.0
Ephedra foeminea	6.8±0.4	0.6±0.2	0.1±0.0

N.A: Not analyzed due to insufficient plant material; TPC: Total phenolics content; TFC: Total flavonoids content; TAC: Total alkaloids content.

Table 05: Phytochemical screening of *E. alata* aqueous and methanolic extracts **(Benarba et *al.*, 2021)**

Phytochemical	**Aqueous extract**	**Methanolic extract**
Quinones	++	++
Coumarins	-	++
Anthraquinones	+	-
Steroid	+	++
Phyto-steroids	+	++
Phenols	++	++
Terpenoids	++	++
Phlobotannins	-	-

Glucosides	-	-
Flavonoids	+	+
Saponins	+	+
Cardiac glycoside	+	+
Resin	-	-
Reducing sugars	++	+
Proteins / amino acid	-	+
Anthocyanin	++	++
Iridoids	-	-

+ Present, ++ Highly present, - Absent

The total phenolic and flavonoid content of *E. alata* aqueous extract was found to be the highest.The methanolic extract was reported to have the highest tannin amount. The methanolic extract, on the other hand, had the lowest phenolic and flavonoid levels. **(Benarba et *al.*, 2021)**

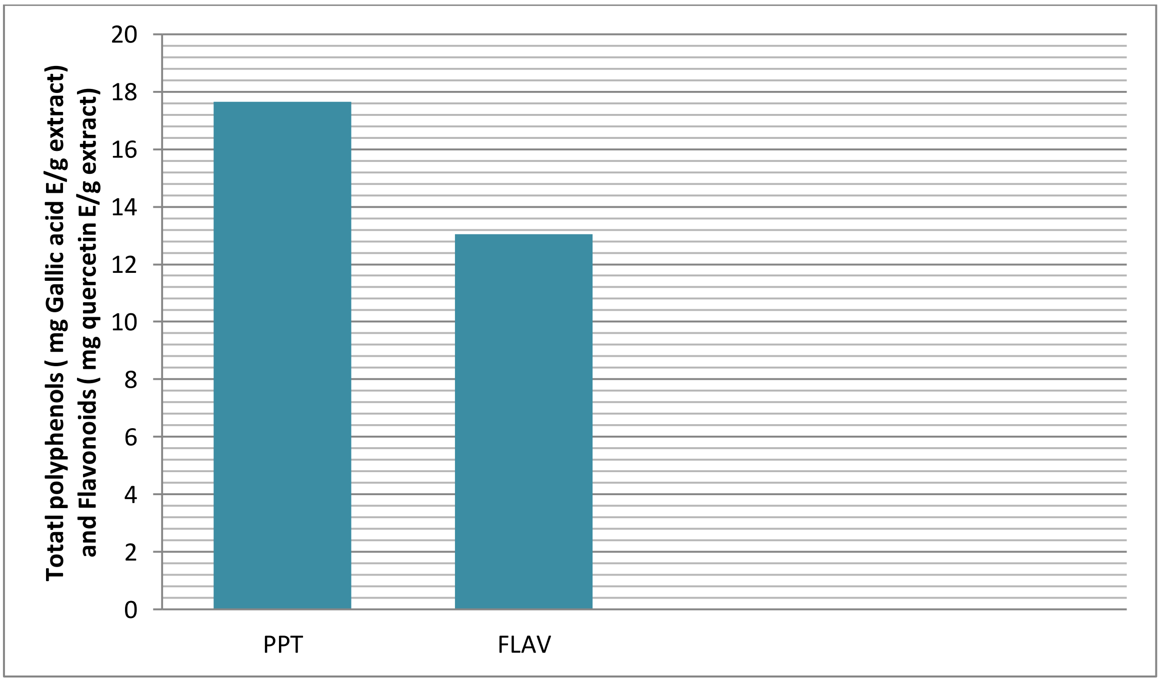


Figure 09: Polyphenols contents and Flavonoids in methanol extract of *Ephedra alata*

PPT: polyphenols **FLAV:** flavonoids

(Chouikh, 2020)

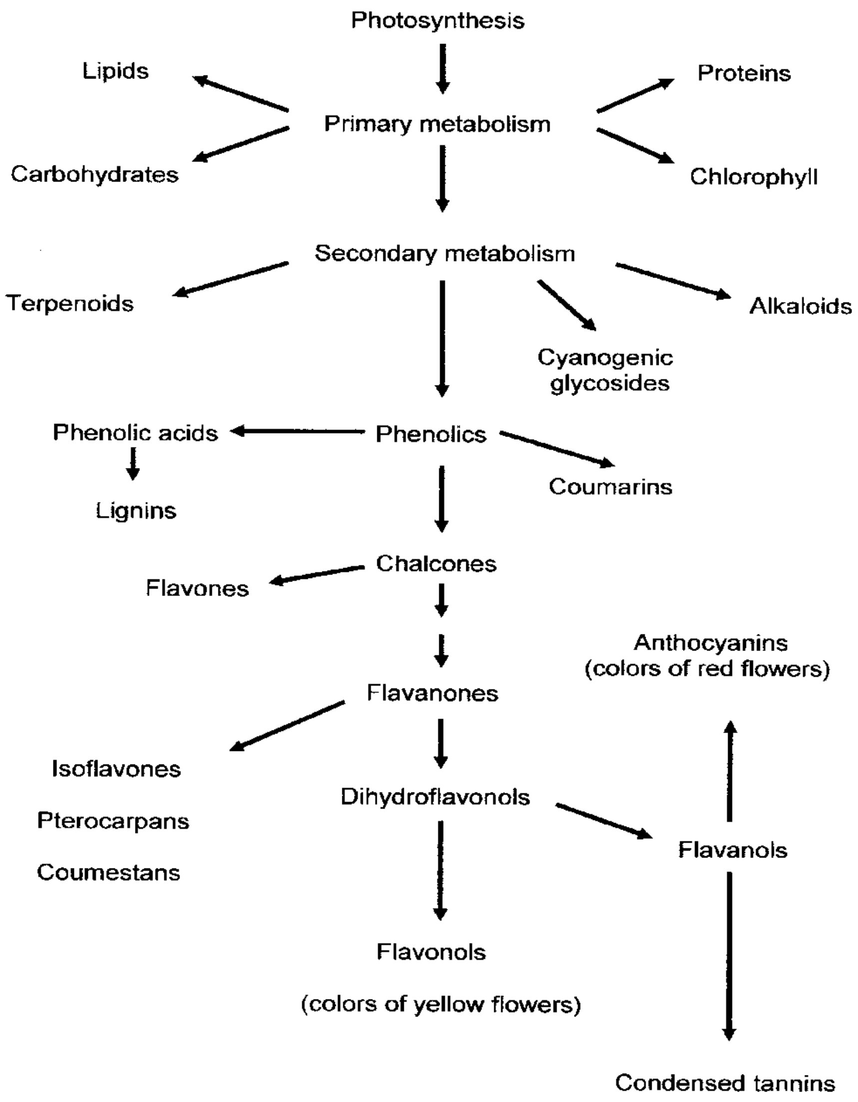


Figure 10: Inter-relationships between the primary and secondary metabolism in plants.

(**Giada and Maria, 2013)**

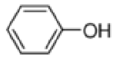
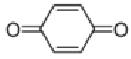
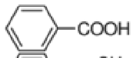
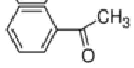
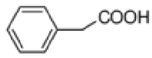
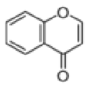
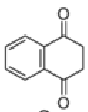
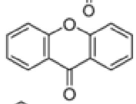
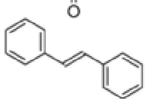
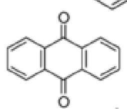
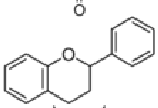
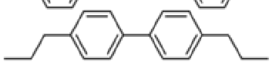
2.2 Phenolic Compounds :

Phenolic compounds (PCs) are phytochemicals found among almost all plant tissues, including fruits and veggies. The shikimic acid and phenylpropanoid pathways release them as secondary metabolites... **(Laura de la Rosa et *al.*, 2019)**. They are the most abundant secondary metabolites in the plant. **(Ayad and Akkal, 2019)**. Phenolic compounds are a type of small molecules with at minimum one phenol unit in their formula. They are categorized into subgroups based on their chemical structures, such like phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbens, and curcuminoids. **(Ren et *al.*, 2019)**.

These are the most common PCs, and they are also an important component of everyday dietary antioxidants in individuals around the globe. Phenolic acids (benzoic and cinnamic acid derivatives) and flavonoids are the most prevalent phenolic components in the nutrition, representing almost 60% and 30% of total dietary phenolic compounds, respectively. **(Rupasinghe et *al.*, 2014)**

Briefly distributed (as simple phenols, pyrocatechol, hydroquinone, resorcinol, Aldehydes derived from benzoic acids that are components of essential oils, such as vanillin), Widely distributed (as flavonoids and their derivatives, coumarins, and phenolic acids, such as benzoic and cinnamic acid and their derivatives) Polymers (as polymers derived from phenolic acids, such (tannin and lignin) **(Kiokias and Oreopoulou , 2021)**

Table 06: Classification-of-families-of-phenolic-compoundsand their sources **(Garcia-Salas et al, 2010)**

Carbon numbers	***Class***	***Basic structure***	***Sources***
C_6	Simple phenols		
	Benzoquinones		
C_6-C_1	Benzoic acid		Cranberry, cereals
C_6-C_2	Acetophenones		Apple, apricot, banana, cauliflower
	Phenylacetic acid		
C_6-C_3	Cinnamic acid		Carrot, citrus, tomato, spinach, peaches, cereal, pears, eggplant
	Phenylpropene		
	Coumarins		Carrot, celery, citrus, parsley
	Chromones		
C_6-C_4	Naphthoquinones		Nuts
C_6-C_1-C_6	Xanthones		Mango, Mangosteen
C_6-C_2-C_6	Stilbenes		Grapes
	Anthraquinones		
C_6-C_3-C_6	Flavonoids		Widely distributed
$(C_6$-$C_3)_2$	Lignans, neolignans	 	Sesame, rye, wheat, flax
$(C_6$-$C_1)_n$	Hydrolysable tannins	Heterogeneous polymer composed of phenolic acids and simple sugars	Pomegranate, raspberry
$(C_6$-$C_3)_n$	Lignins	Highly crosslinked aromatic polymer	

Because of their hydrophobic benzenoid rings and hydrogen-bonding capability of the phenolic hydroxyl groups, phenolic structures have the capacity to interact strongly with proteins. This ability to suppress several enzymes involved in radical formation, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase, and xanthine oxidase, gives phenolics the opportunity to govern as antioxidants. **(Pereira et *al.,* 2009)** Look at (figure 11)

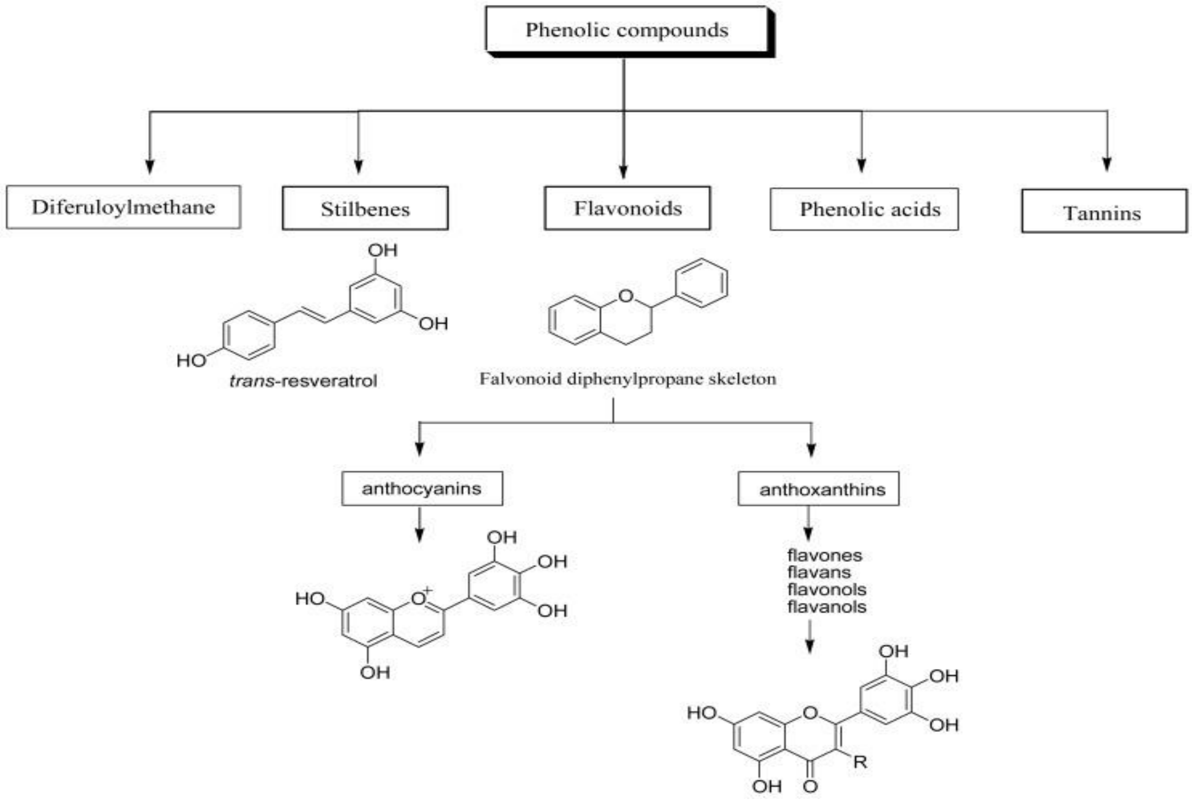


Figure 11: Classification of dietary polyphenols **(Han et *al.,* 2007)**

2.3. Flavonoils :

2.3.1. Definition :

Flavonoids are little substances that plants create spontaneously as secondary metabolites in response to a variety of biotic and abiotic conditions. These chemical compounds have a wide range of known health-promoting actions.**(Doughari ,2012)**.Their biological functions are diverse: From flower coloring to participation in the plant organism's growth processes and disease defense **(Havsteen , 2002)** .These chemicals are found in practically all plant species, mainly as a result of their UV-blocking characteristics, providing protection to the plant. **(Bruneton, 1993)**

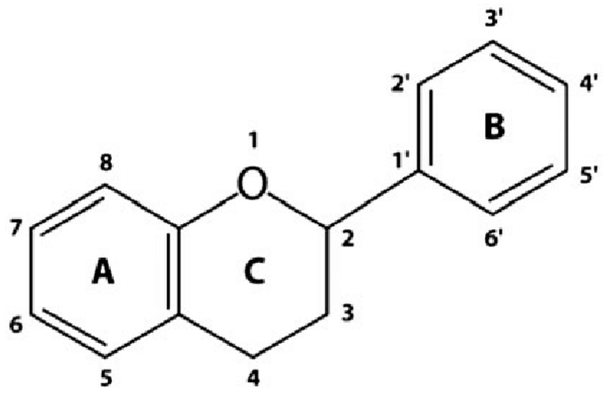


Figure 12: basic structure of flavonoids (**Alkhaldy et *al.*, 2018)**

2.3.2. Structure and classification :

Every flavonoid structure has a flavan core that can be determined. It is made up of 15 carbon atoms that form two aromatic rings (known as A and B) bonded by a three-carbon chain. The connecting carbon chain is a portion of a heterocyclic center ring (coded as C) present in most flavonoids, with one exception: the carbon chain between the A and B rings in chalcones is linear. **(Beecher, 2003)**

The flavonoid family contains almost 6000 low-molecular-weight phenolic chemicals; which are flavan derivatives **(Harborne and williams, 2000),** Flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, and isoflavones are the major sub-classes of flavonoids, whereas dihydroflavonols, flavan-3,4-diols , coumarins, chalcones, dihydrochalcones, and aurones are minor components . Multiple substituents can be added to the flavonoid skeleton. Hydroxyl groups are normally found at positions 4', 5', and 7. Sugars are numerous, and the vast of flavonoids are present as glycosides in nature. Other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic, whilst sugars and hydroxyl groups augment its water solubility. **(Crozier, 2009)** "See Figure 13".

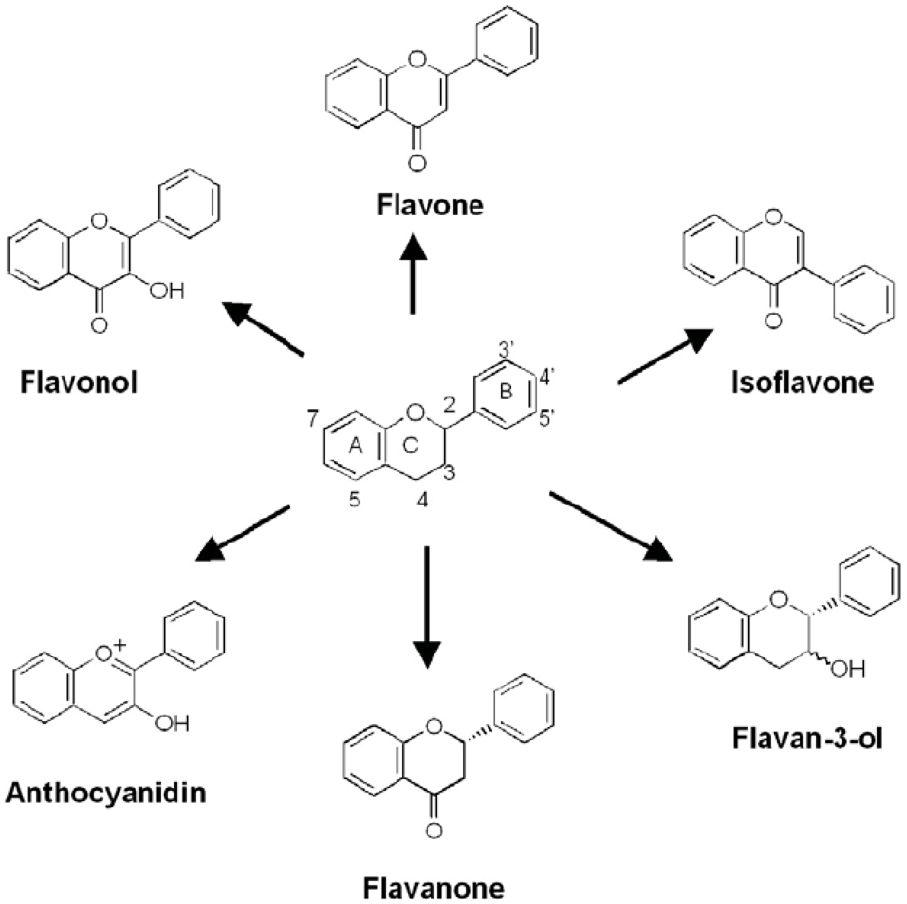


Figure 13: basic structure of flavonoids subclasses (**Nishiumi et *al.*, 2011)**

2.3.3. Natural sources :

Lavones are found as glucosides in leaves, flowers, and fruits and their primary sources are green leafy spices such as parsley. The flavone luteolin has to be the most frequent.All citrus fruits contain flavanones [esperitin, naringenin, and eriodictyol are examples of this class of flavonoids]. Citrus juice and peel have a bitter taste due to these compounds. Naringin and eriodictyol are also plentiful in almonds.Anthocyanins are pigments that give flowers and fruits its color. **(Tzanova, 2020)**

2.4. Phenolic acids :

2.4.1. Definition:

Phenolic acids (Ph A) are aromatic secondary plant metabolites that are found all over the world. Phenolic acids are non-flavonoid phenolic chemicals that can be found in free, conjugated-soluble, and insoluble-bound forms in plants. Recent interest in Ph A arises from their ability to protect against oxidative damage illnesses by intake of fruits and vegetables (coronary heart disease, stroke, and cancers). **(Rebecca and Robbins, 2003).**

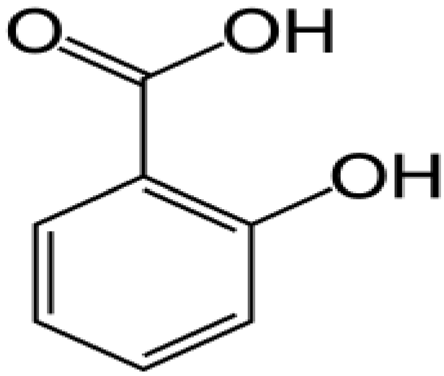


Figure 14: basic structure of phenolic acids **(Site2)**

2.4.2. Structure and classification:

Phenolic acids are categorised according to their chemical structure:

- C6-C1 structure Hydroxybenzoic acids : derived from benzoic acid.
  - Gallic acid (a trihydroxy derivative) has been linked to tea antioxidant activity,
  - Vanilic acid is a well-known methoxy-hydroxy derivative that serves as a flavoring ingredient.

- C6-C3 structure hydroxycinnamic acids:which are prevalent in plant sources, derived from cinnamic acid.
  - P-coumaric (4-hydroxy derivative),
  - Caffeic (3, 4-dihydroxy derivative)
  - Ferulic (3-methoxy, 4-hydroxy derivative)
  - Rosmarinic acid (a caffeic acid ester with 3, 4-dihydroxyphenyl lactic acid)

  (Kumar and Goel, 2019); (Kiokias and Oreopoulou, 2021)

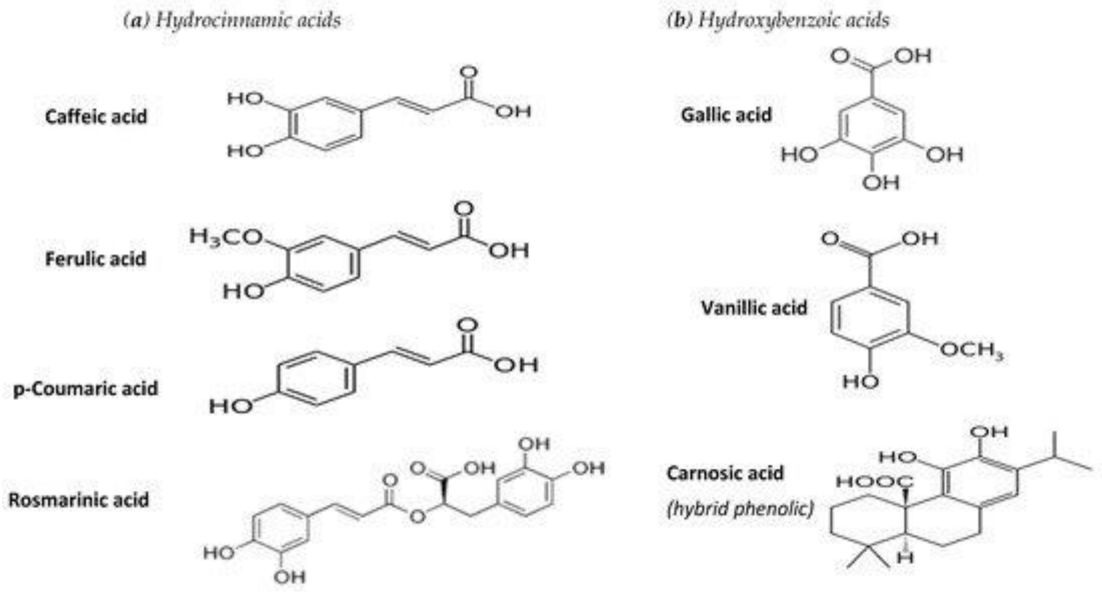


Figure 15: Chemical structure of phenolic acids classes **(Kiokias and Oreopoulou, 2021)**

2.5. Tannins:

2.5.1. Definition :

Tannin is a phenolic compound that precipitates proteins from their aqueous solutions. Tannins are present in large quantities in trees, barks, roots, leaves and fruits. They are placed in the vacuoles of cells. In therapy, tannins have antiseptic and bactericidal activities, they have antioxidant properties and prevent the development of microbesTannins are used as vasoconstrictor and haemostatic antidiarrheals,but especially as venous protectors in the treatment of varicose veins and hemorrhoids.**(Atanu et *al.*, 2019).**

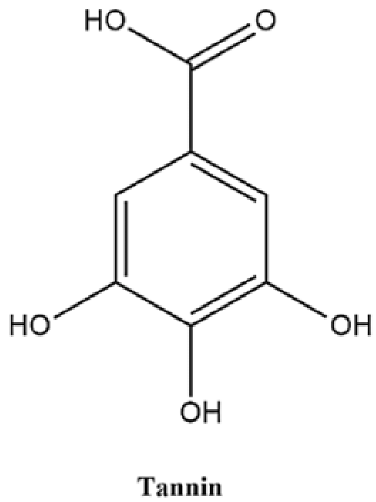


Figure16: basic structure of tannin **(Kavitha et *al.*, 2020)**

2.5.2. Classification :

Tannins are divided into two groups. One type of hydrolysable polyhydric alcohol is one in which the hydroxyl groups are partially or totally esterified by Gallic acid or other related substances. Tannins, which are generated by the condensation of phenolic compounds, make up the other category. These are known as condensed tannins because they are not hydrolysable. **(Ghosh and Debosree, 2015)** 'Look at Figure 17'.

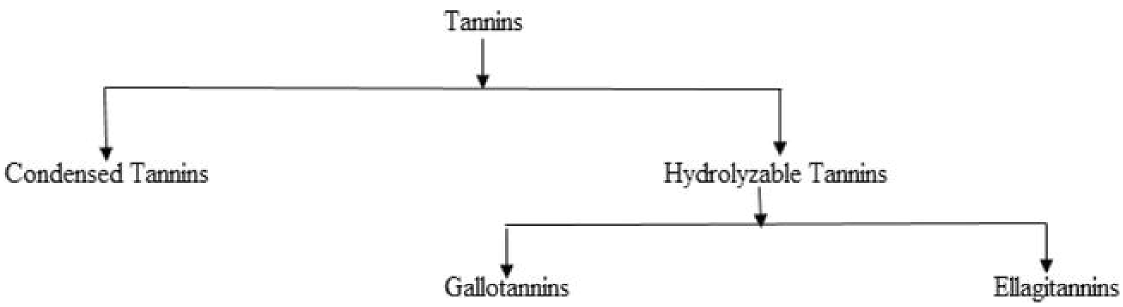


Figure17: classification of tannin **(Kumar et al., 2019)**

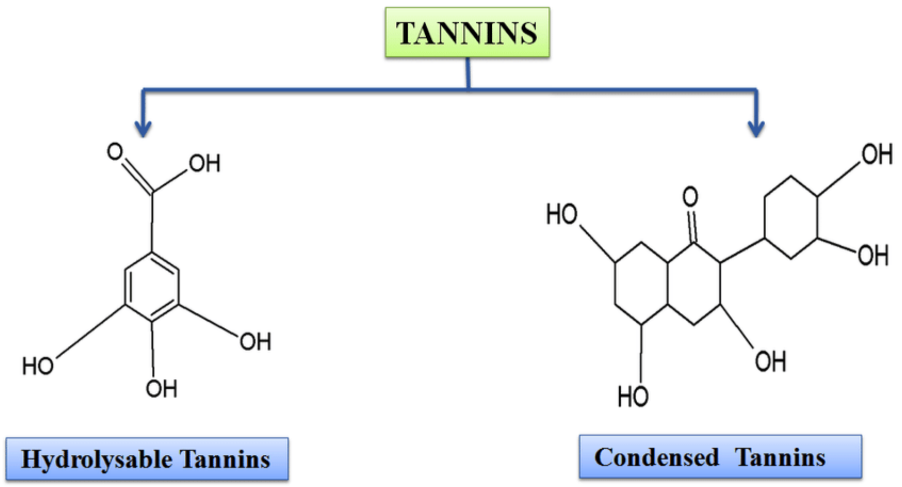


Figure18: Types of tannins and their basic structures **(Ghosh and Debosree, 2015).**

2.6. Alkaloids:

2.6.1. Definition :

They are nitrogenous organic substances of vegetable origin, of alkaline character and complex structure (heterocyclic nucleus), they are found in several families of plants, most alkaloids are soluble in water and alcohol and have a bitter taste. and some are highly toxic. Some alkaloids are used as a defense against microbial infections (nicotine, caffeine, morphine, lupinin). Also as cancer drugs (**Vincristine, Vinblastine and Verpoorte, 2005).**

2.6.2. Structures and classification:

Various characteristics, such as natural origins or chemical nature, are used to classify them. The distribution of alkaloids according to their principal structure, the principal C-N skeleton, is the most correct and common classification. **(Bribi, 2018).**

Alkaloids have many different chemical structures. There are around 20000 alkaloids recorded, the majority of these have been isolated from plants. Microorganisms, marine organisms such as algae, dinoflagellates, and puffer fish, and terrestrial animals such as insects, salamanders, and toads were all found to contain alkaloids. **(Katerova and Zornitsaet *al.*, 2012)**

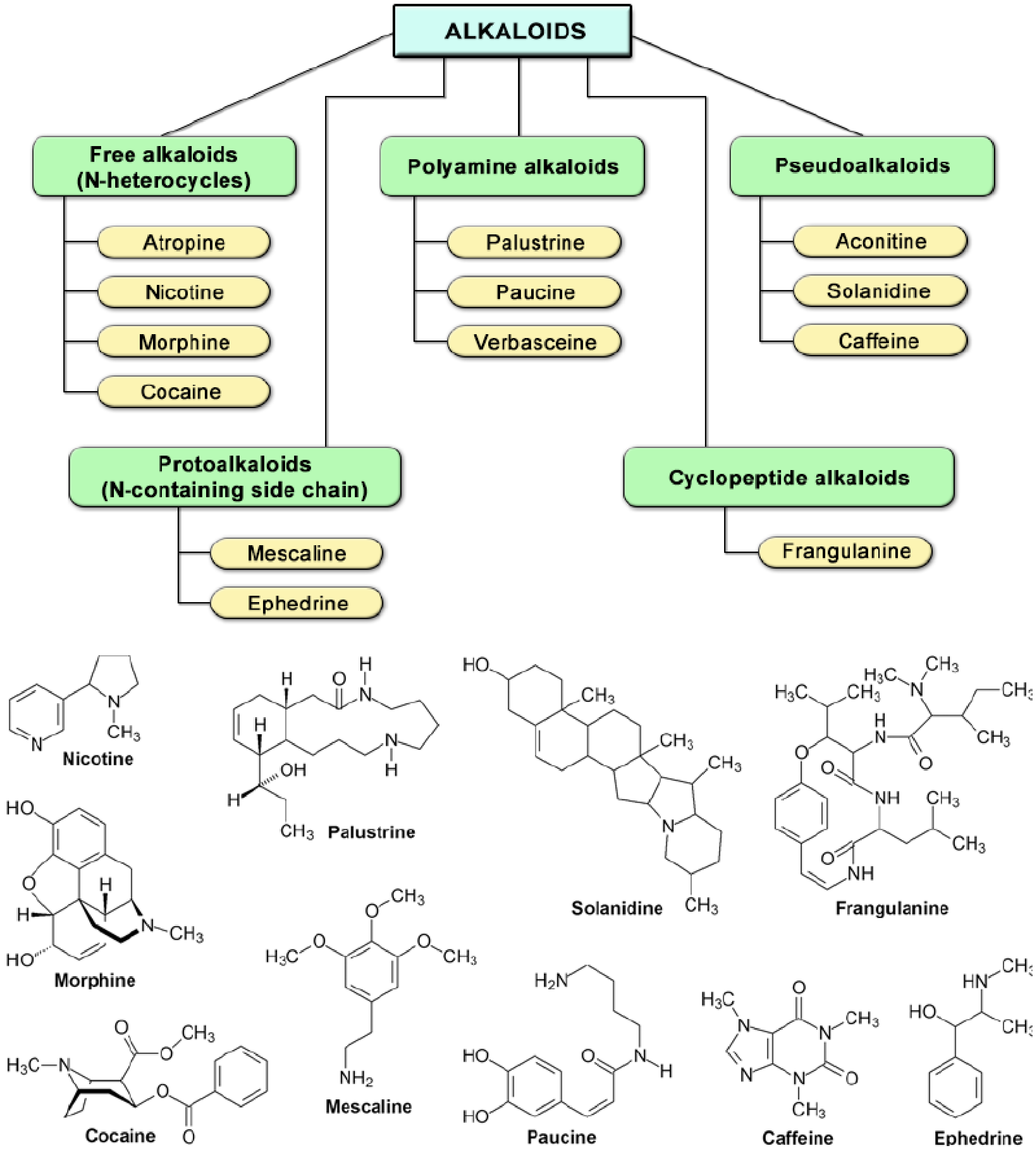


Figure19: Major-alkaloid-subgroups-and-chemical-structures-of-some-representatives

(Katerova et *al.*, 2012)

2.7. Steroids:

Any of a group of natural or artificial organic substances with a molecular structure of 17 carbon atoms arranged in four rings is known as a steroid. In biology, chemistry, and medicine, steroids are fundamental. The steroid family contains all vertebrate sex hormones, adrenal cortical hormones, bile acids, and sterols, along with insect molting hormones and a variety of other physiologically active compounds. Various types of steroids are often differentiated from one another by names that refer to their biological origin, for example phytosterols from plants or to some important physiological function, such as:

- Progesterones (promoting gestation)
- Androgens (favouring development of masculine characteristics)
- Cardiotonic steroids (facilitating proper heart function) **(Clayton et *al.*, 2021).**

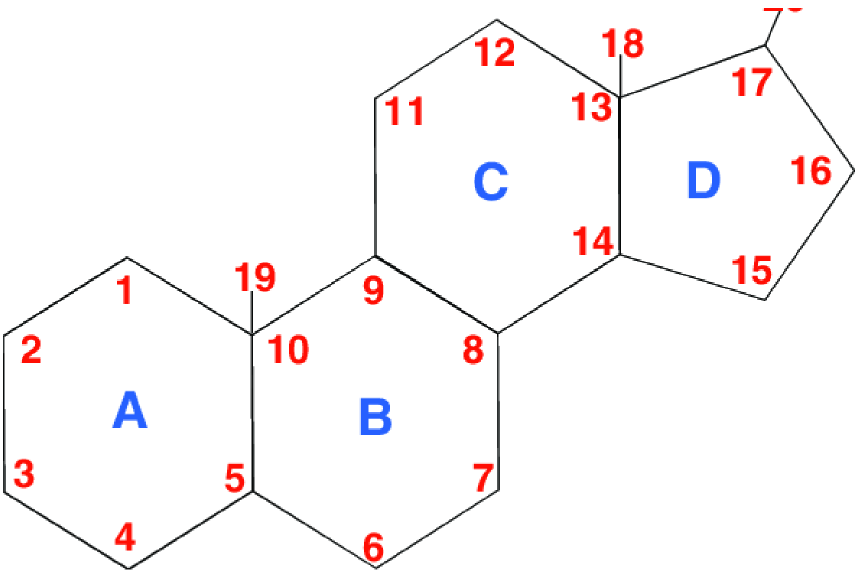


Figure 20: Basic steroid structure **(Greaves et al., 2014)**

- Phytosterols have a chemical structure similar to cholesterol and can only be found in plant-based foods such vegetables, fruits, seeds, and vegetable oils. They are categorized into two kinds: sterols and stanols. Sterols have unsaturated aromatic rings, whilst stanols have saturated rings.

They can act in the intestine to lower cholesterol absorption by displacing cholesterol from intestinal micelles. **(Pizzorno, 2016).**

2.8. Saponin:

Saponins are a wide group of amphiphilic steroids and triterpene glycosides present in plants and even some marine creatures. Saponins exhibit a wide range of biological and pharmacological activities by expressing a huge diversity of structures on both sugar chains and aglycones, and serve as significant active ingredients in folk medicines, particularly in traditional Chinese medicine.**(Yang et *al.,* 2021).**

They are responsible for giving a bitter taste and astringency to plant materials containing a high concentration of saponins due to their hemolytic activity and foaming capabilities.**(Mohan et *al.,* 2016)** look at the following saponin's structure (Figure21).

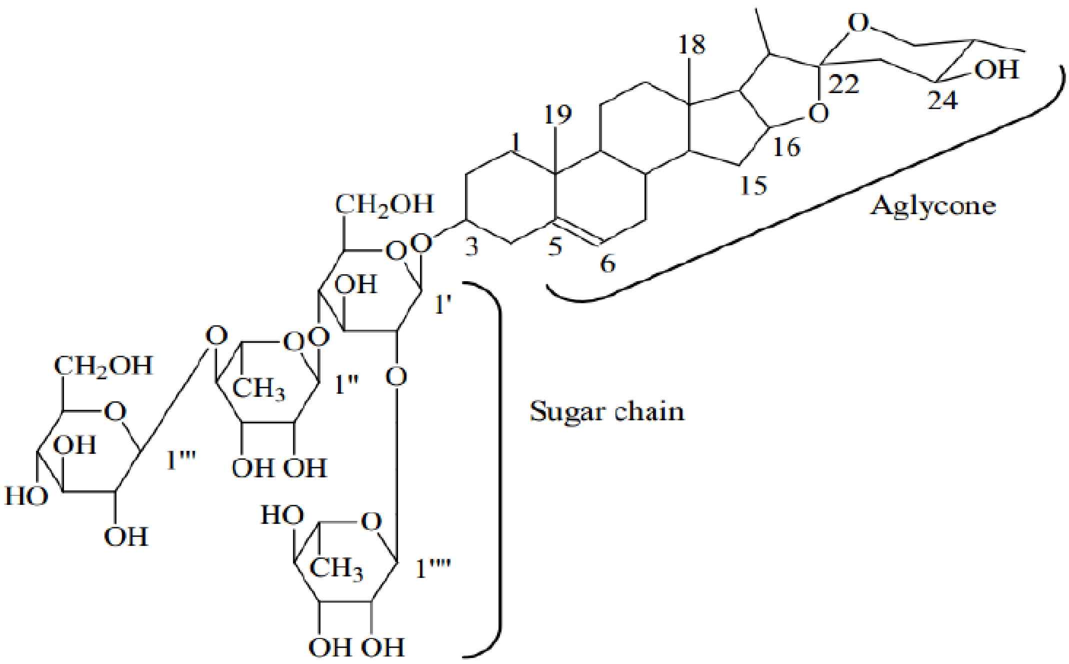


Figure 21: Structure-of-saponin **(Moghimipour and Handali, 2015)**

3. Biological activity of Ephedra Alata :

3.1. Antibacterial activities :

Ephedra alata extract had modest antibacterial activity against three bacterial strains and a considerable inhibitory effect against methicillin resistance in *S. aureus*. *E. coli* extracts contained alkaloids such as ephedrine and pseudoephedrine, lignans, flavonoids, and other phenolic compounds, but the chemicals responsible for antibacterial activity have yet to be discovered**.(Palici et *al.*, 2015)**.

A research was conducted in kebeli (a Tunisian-south located city) in March 2015 it showed that *Ephedra alata* Subsp *alenda* is shown to have antibacterial properties, particularly against gram-positive and gram-negative bacteria. The MeOH extracts all demonstrated significant antibacterial action against the strains. For a time, Gram (-) bacteria's resistance can be explained by the presence of an outer membrane wrapping the cell wall that appears to impede the diffusion of hydrophobic chemicals via its lipopolysaccharide coating. The absence of a barrier at the Gram (+) aids in maintaining direct contact between essential oils and hydrophobic components and the cell membrane's phospholipid bilayers. This results in a rise in ion permeability and intracellular component leakage, as well as a reduction in bacterial enzyme systems. The presence of linalool and -terpineol in the essential oil could explain its antibacterial properties. **(Jerbi et *al.*, 2016).**

3.2. Antioxidant activity and mechanism :

3.2.1. General information about oxidative stress:

Our cellular and tissue organizations can be subjected to many metabolic attacks (exposure to xenobiotics, deprivation of a hormonal factor or factor of growth), physical (trauma, irradiation, hyper or hypothermic) and chemical (acidosis, toxins) and the majority of these aggressions lead to the appearance of what we called oxidative stress, which is due to the exaggeration of the production of free radicals **(Walker et *al.*, 1982)**.

This phenomenon causes serious and immediate damage to all cellular constituents (DNA, proteins, and lipids) leading to structural and functional modifications, which are the basis of various diseases such as atherosclerosis, cancer, Alzheimer's or Parkinson's disease. **(Favier, 2003).**

3.2.2. Free radicals :

- **Definition:**

Free radicals are chemical species, possibly unstable atoms or molecules characterized by an extremely unstable single electron on their outer orbitals, these compounds can react with the most stable molecules to pair their electrons. They can create bonds with the oxygen or nitrogen atom, hence the denomination of reactive oxygen species (ROS).

- **Classification :**
  - Reactive Oxygen Species (ROS)
  - ROS are an important class produced in living systems. There are primary ROS (radicals), which are the species playing a particular role in physiology such as: Superoxide anion ($O2•^-$), hydroxyl radical (•OH), hydroperoxyl radical (HO2•).
  - Peroxyl radical (RO2•), alkoxyl radical (RO•) and secondary ROS which are non-radical species and can be radical precursors **(Favier, 2003)**
  - Reactive species of nitrogen origin
  - Similarly, they include radical species such as nitric oxide (•NO) **(Rousselot et *al.*, 2003)** and non-radical species such as: the anion peroxynitrite (ONOO -) which is among the precursors of free radicals.
- **Origin of production :**

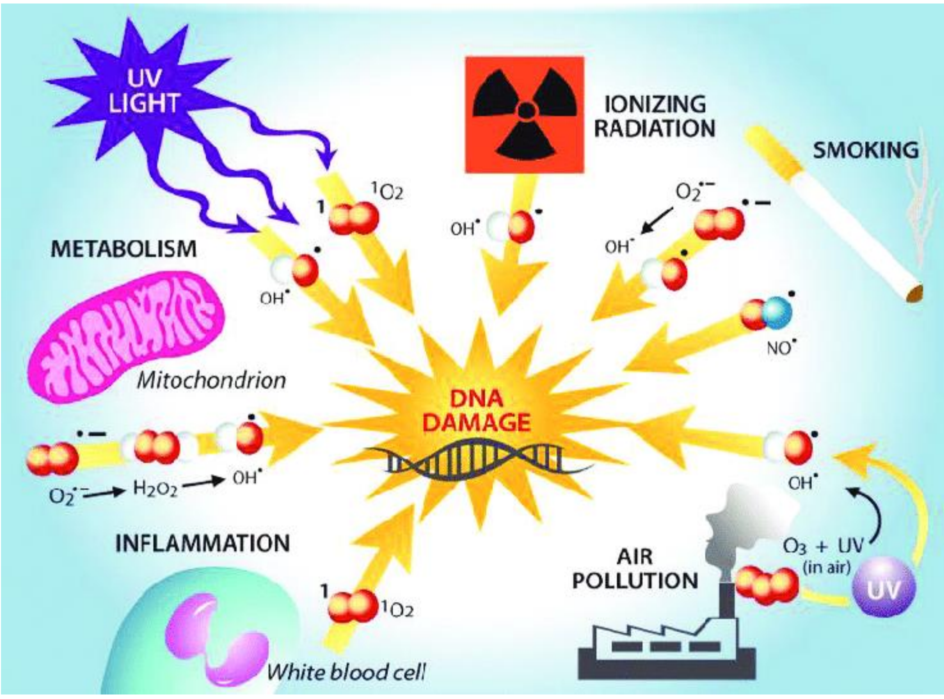


Figure 22: sources of free radicals **(Perez et al., 2017)**

Harmful free radicals are produced in is identified as it shown in the next figure, far several mechanisms and systems responsible .the body during normal metabolism. Increased oxygen consumption increases the production of radicals **(Gauche and Hausswirth, 2006)**. So harmful free radicals are produced in is identified as it shown in the next figure, far several mechanisms and systems responsible.

3.2.3 Antioxidants:

3.2.3.1 Definition :

The body produces free radicals, but it also protects itself with the greatest care thanks to molecules called antioxidants. Antioxidants are substances capable of neutralizing or reducing the damage caused by free radicals in the body and helps maintain non-cytotoxic concentrations of ROS at the cell level.

3.2.3.2 Antioxidant types :

There are two types: enzymatic antioxidants and non-enzymatic antioxidants

- Enzymatic antioxidants (endogenous) :
  These are enzymes or antioxidant proteins (Superoxides, dismutase, Catalase and Glutathione peroxidase) which are produced by our body using certain elements minerals from food and they can also be specific factors such as (glutathione, alpha-lipoic acid, uric acid, and coenzyme). They are present in permanently in the body but their quantities decrease with age. **(Mika et *al.*, 2004)**
- Non-enzymatic (exogenous) antioxidants :
  These are components that are not synthesized by the body and are provided by food, such as:
  -Certain vitamins: A, C, E and also B2 (riboflavin).
  -Compounds of fruits and vegetables: carotenoids, polyphenols, in particular flavonoids.
  - Trace foods; which are cofactors of the enzymes involved in the systems Endogenous antioxidants like selenium, zinc and manganese.

3.2.3.3 Mechanism of antioxidants :

To limit the deleterious effects of radical and non-radical reactive species and their pathophysiological consequences, the intracellular redox state is balanced by antioxidant systems that can defend against the free radical in different ways, Their mechanisms of action are diverse, they can act : either by direct trapping of EOR, or by deactivation of radicals by covalent addition reaction, or by reduction of radicals or peroxides, or by chelation of transition metals which has the effect of slowing down Fenton reactions. **(Favier , 2006)**.

The following table represents the non-enzymatic antioxidants, their modes of action, their interests and their main food sources. (Table 07)

Table 07: Mode of action of some non-enzymatic antioxidants **(Goudable and Favier, 1997) ;(Haleng et *al.*, 2007); (Karmella and Christine, 2011); (Anderson et *al.*, 1996).**

Nature of antioxidant	Mode of action	Prevention against	Food sources
Carotenoids	It is a precursor of vitamin A, reacts with radicals $O2^{\bullet-}$, $ROO^{\bullet}$, $HO^{\bullet}$, either by abstraction of hydrogen, either by transfer electron, either by adding the root	Lung cancer ; cardiovascular diseases	Carrot , sweet potato ,broccoli,spinach,apricot
Flavonoids	act: either by direct capture of reactive oxygen species, or by chelation of transition metals such as iron copper, or by inhibiting the activity of certain enzymes responsible	Cancer	Blueberry, cherry, cranberry, blackberry, black currant, plum, grape red

	for the production of ROS such as xanthine oxidase		
Coumarins	scavenge hydroxyl, superoxide and peroxyl radicals, and the prevention of membrane lipid peroxidation	lymphedema	Tonka bean, corn, cinnamon
Selenium	neutralizes toxic metals	Prostate, colon and lung cancers	onion, garlic, poultry, meat

3.2.3.4 Methods for evaluating antioxidant properties in vitro :

There are several spectrometric methods to assess the antioxidant capacity, most of these methods are based on measuring the consumption of previously formed free radicals such as ROO• peroxides.

Among the most used tests, we will present those commonly cited: the DPPH (Diphenyl Picrylhydrazyl) method and the FRAP (Ferric Reducing Antioxidant Power) method.

- **The DPPH method**

2,2-Diphenyl-2-picrylhydrazyl is a stable purplish-colored free radical that absorbs at 517 nm. In the presence of antiradical compounds, the DPPH• radical is reduced and changes color by turning yellow. The measured absorbances are used to calculate the percentage inhibition of the DPPH• radical, which is proportional to the antiradical power of the sample. This method is based on measuring the ability of antioxidants to scavenge the DPPH• radical. **(Parejo et *al.*, 2003)**

- **FRAP test (Ferric reducing ability power)**

The FRAP method can be a good method for calculating the antioxidant power of an extract by evaluating its ferric cation reduction power.

This technique consists in reducing the ferric tripyridyltriazine complex [(Fe(III)-TPTZ] of yellow color to a ferrous complex [(Fe(II)-TPTZ] of blue color, under the action of an antioxidant by a transfer of The variation in color is measured at 700 nm **(Lamien-Meda et *al.*, 2008)**.

3.3. Antifungal activities :

The presence of cis-3,4-methanoloprlin, citronellol, and heptadecane, which have been registered as antibacterial compounds and identified in *Ephedra*, has previously been attributed to the antifungal potential of *Ephedra* plants in vitro and in vivo.

(Gueham and Gueham, 2017)

Chapter 3:

Evaluation of “*Ephedra alata*’s” Toxicity

1. Qualitative and Quantitative phytochemical screening overview

Phytochemical screening is a key step that leads to the isolation of certain compounds. Conclusions were reached utilizing several organic solvents such as ethanol, chloroform, and acetone, as well as aqueous extracts of *Ephedra* leaves (*Ephedra alata*), Tannins, Saponins, Flavonoids, Cardiac glycosides, and Alkaloids) previously Steroids; Terpenoids and Anthraquinones not present in certain crude extracts were evaluated. Furthermore, the results in different studies showed that the ethanolic extract of leaves had a high number of chemical components (87%) while the aqueous crude extract was intermediate (82%) and the chloroform (79%) and acetone extracts (77%) were the least. **(Edrah et *al.*, 2016)**

A few steps must be followed to get the full process done without any wrong outcomings which are:

- Plant collection (Source of plant material)
- Preparation of leave extracts
- Qualitative Phytochemical Analysis
- Quantitative phytochemical analysis
- Extraction of Plant Materials
- Results and Discussion
- Screening of Phytochemicals
- Conclusion

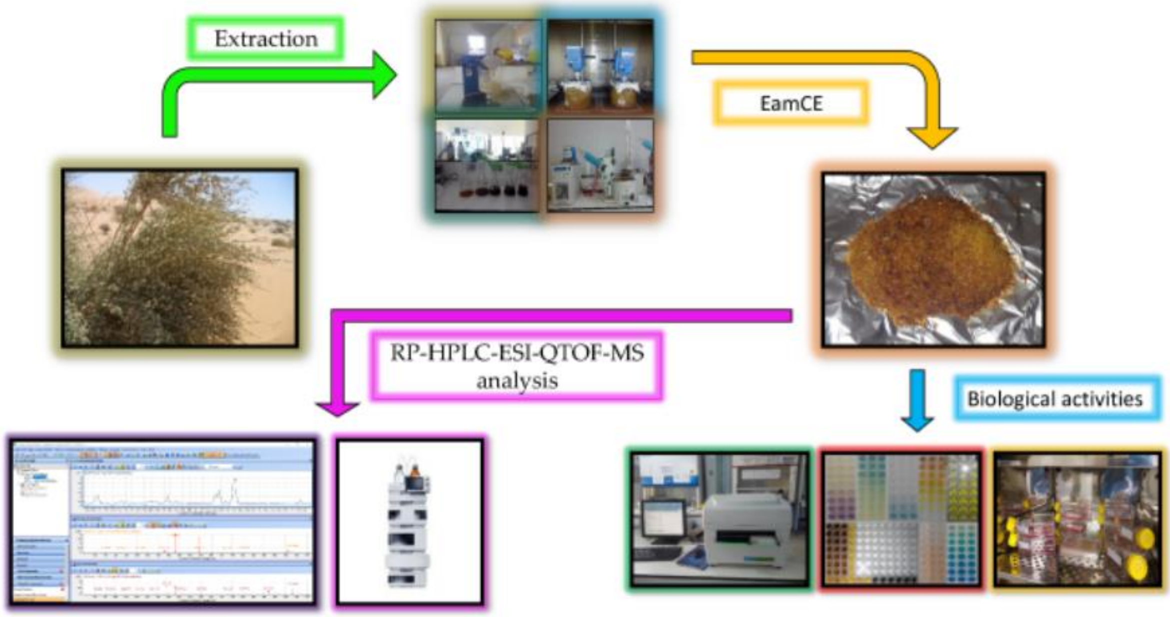


Figure23: Graphic abstract for qualitative and quantitative phytochemical screening process **(Khattabi et *al.*, 2022)**

These are basically the regular steps that need to be done in order to go through a phytochemical screening for any medicinal plant for any type of research. Phytochemical screening might be coupled with an ethnopharmacological investigation or a survey if it was about *E.alata*'s effect on a certain population that has a certain illness (Cancer, Diabetes, asthma…etc).

<u>Ethnopharmacological inquiry:</u> The ethnopharmacological survey is critical for understanding medicinal plants and their applications. In locations where the use of plants is always of major importance, having a comprehensive understanding of how to utilize plants against a certain disease is quite realistic.Ethnopharmacological knowledge must be documented in order to investigate and comprehend human-plant relationships, to apply general policies on natural resource usage, and to estimate possible livelihoods and monetary rewards. **(Gueham and Gueham, 2017)**

2. Toxicity of medicinal plants :

Galen, a Greek pharmacist and physician, is credited with being the first to report on the toxicity of plants, demonstrating that herbs can contain both medicinally useful and hazardous elements.**(Ifeoma and Oluwakanyinsola ,2013)**

It has long been believed that if a medicine succeeds, it will have adverse effects. As a result, medicinal herbs are either worthless or have health effects as drugs. Herbal remedies, on the other hand, are commonly considered to be safe and reliable. Therefore, individuals turn to herbal therapy every year because they claim plant medicines are free of harmful effects.**(Nasri and Shirzad , 2013).**

Synthetic medications are mainly composed of just one chemical, whereas medicinal plants might have a complex mixture of 400 or more. It's quite simple to figure out the function and adverse effects of a single molecule, but scientists will never be able to map all of the complex relationship and interactions that could occur between all of the chemicals found in a plant. **(Philomena G, 2011).**

It has been recommended that instead of using the term "side effects" for plants, we use the terms "indications" and "contraindications."**(Nasri and Shirzad, 2013).**

In several situations, herbalists would focus on supporting secondary systems and functions affected by the primary ailment rather than treating the primary presenting illness with drugs. Since herbals have therapeutic properties, this permits the body to recover. **(Philomen, 2011).**

Numerous common foods contain potentially dangerous compounds, such as cyanogenic glycosides found in many fruit seeds, lectins found in many pulses, including soya and red kidney beans, and Solanaceae alkaloids. Yet, these foods are often considered to be safe. Furthermore, excessive amounts of both water and oxygen can kill; therefore quantity is frequently a factor to be considered. **(Haq, 2004).**

In practice, meanwhile, three types of herbs can be defined from a safety standpoint. There are a few herbs in the first group that have near pharmaceutical quantities of toxic components and should not be consumed internally by unprepared people unless in *homeopathic potencies, Atropa belladonna, Arnica spp*...Are few examples **(Mcrae, 1996).**

Herbs in the second group have very strong effects, frequently producing nausea or vomiting. They are absolutely safe when used under the right circumstances. *Lobelia* and *Eonymus* spp. are two examples. **(Philomena, 2011).**

Last, there is a group of herbs that have been linked to certain types of toxicity. The hepatotoxicity of plants containing pyrrolizidine alkaloids, such as Comfrey, is perhaps the most well-known. *Dryopteris*, *Viscum*, and *Corynanthe* are other examples. **(Nasri and Shirzad H, 2013).**

Table 08: Side effects/ adverse reactions & interactionsof some medicinal plants **(Haq, 2004)**

Medicinal plant	Side effect/ad. reaction & interaction
Liquorice	Glycyrrhizinic acid promotes sodium and water retention & potassium depletion- risky for people with B.P, Kidney aI1c1 Heart diseases.
Ephedra	Insomnia, restlessness, irritability. Can cause toxic reactions when taken with some drugs
Aloe vera	Aloe latex could result in potassium deficiency thus endangering electrolyte imbalance.
Ginkgo biloba	Potent inhibitor of platelet-activating factor-long term use associated with increased bleeding time.Adverse reactions including hypertension, leucopoenia, thrombo penia and hallucinations
Sylibum marianum	Cerebral hemorrhage, hepatic coma and neuropathy.
Ginseng	May create blood clotting problem. Potential medication interaction with Warfarin, Digoxin, Anti diabetic and other drugs.
Comferey	Hepato-toxic.
Acconite	Acute poisoning
Ispaghula	Bronchospasm, asthma and intestinal obstruction.
St.john's wort	Significant interaction with lndinavir-a protease inhibitor and, some other drugs.
Ginger .ginseng Feverfew.Ginkgo bilob	Delay blood clotting time- interaction with Aspirin & Warfarin.

Ecologically, a number of alkaloids act as feeding deterrents in the environment by exerting agonistic or agonistic activity on neurotransmitter systems. Similarly, some lipid soluble terpenes have been demonstrated to inhibit mammalian cholinesterase, while others interact with vertebrates' GABAergic system. Saponins are also powerful surfactants that can break lipid-rich cellular membranes in human erythrocytes and bacteria, explaining their antimicrobial effects. **. (Ifeoma and Oluwakanyinsola, 2013).**

2.1 Toxicity of Ephedra:

Since antiquity, the plant has been administered in traditional Chinese medicine to treat respiratory disorders. The medicine was marketed as a natural, safe weight-loss, body-building, and mood-improvement supplement. Due to its use in dieting formulations and products claiming to give a "legal high," the drug has recently become increasingly contentious, posing major health risks. Since 1990, 15 deaths have been connected to the use of ephedra-based medications, necessitating regulatory restrictions on the duration and quantity of such treatments, according to the FDA (Food and drug administration). **(Haq I, 2004).**

Restlessness, irritability, hypertension, and cardiac rhythm abnormalities are just a few of the adverse affects of ephedra.. When consumed with Monoamine oxydase inhibitors (MAO), ergot alkaloid derivatives or oxytocin, cardiac glycosides, halothane, or guanethedine, the alkaloid ephedrine found in ephedra herb can induce serious toxic reactions ranging from liver damage to severe high blood pressure and heart issues.**(Cupp, 2000)** ; so the plant should not be used with any MAO-inhibitors (like those found in some antidepressants) or heart or blood pressure drugs due to its unpleasant responses.

3. Goals of toxicity testing of herbal drugs :

The primary goal of any herbal medicine's toxicological evaluation is to identify side effects and quantify the exposure levels at which they develop. Of any herbal medicine's toxicological evaluation is to identify side effects and quantify the exposure levels at which they develop. The nature and degree of the adverse effect, as well as the exposure level at which the impact is detected, are two key elements to consider when evaluating the safety of any herbal medicine. Toxicity testing can show some of the risks connected with herbal usage, particularly in vulnerable communities.**(Ifeoma and Oluwakanyinsola and ,2013).**

The detection of poisonous plant extracts or compounds produced by them in the early (pre-clinical) and late (clinical) stages of drug discovery and development from plant sources is an equally essential goal of toxicity testing. This will make it easier to identify toxicants that can be rejected or adjusted during the process, as well as allow for a more thorough study of safer, more promising alternatives.**(Gamaniel , 2000).**

3.1 Pre-clinical toxicity testing of herbs :

Pre-clinical toxicological screening aims to:

- Discover harmful doses (including dose-response relationships);

- Define the optimal starting dose and aid dose escalation strategy for the first human trials, as well as the maximum human dose, among other things.

Furthermore, pre-clinical toxicity testing is essential to determine the "No Observed Adverse Effect Level" (NOAEL), which is required to begin clinical trials of experimental drugs.**(Akinmayowa, 2020).**

4. General tests :

The Organization for Economic Cooperation and Development has unified standard criteria for conducting animal toxicity studies as part of ongoing efforts to coordinate test guidelines internationally.

Certain significant considerations must be taken into account before doing an animal safety research of a herb or its product are:

- Preparation of test substance: Herbal products can be produced as capsules, pills, serums, lotions, and pastes, among other dosage forms. The product should be quantitatively standardized and administered according to its intended usage in humans for proper delivery of a pre-defined dose.

- Animal welfare considerations: When an animal exhibits escalating clinical symptoms that lead to further deterioration in state, it is necessary to make an informed choice about whether or not to mercifully terminate the animal.
- Animals: Animal toxicity testing utilizes a variety of rodent and non-rodent species. All animals should be housed in suitable environments and fed appropriately in accordance with appropriate standards.
- Regulatory requirements.

  (Ifeoma and Oluwakanyinsola, 2013).

4.1 Different types of toxicity studies:

The assessment of toxicity is based on adequate qualitative (non-measurable) or quantitative (measurable) studies. There are several types of studies that allow us to assess the effects of toxic substances. They can be divided into four categories :

- Experimental studies in vivo, which use animals (eg rabbit, rat and mouse);
- In vitro studies, carried out on tissue cultures or cells;
- Theoretical studies by modeling (eg structure-activity) **(Gilles, 2004).**

5. Toxicity

5.1 Definition:

Toxicity is described as the quantity or level of toxicity required for a material to be poisonous. Toxicity is determined by the amount and concentration of the substance used the frequency of usage, the interactions of the person receiving the substance, and the person's particular reaction. Toxicity can be local or systemic. It can either be reversible or not. It may be acute, subacute, or chronic in type. **(Buckle, 2014).**

5.2. Routes of exposure to toxic products :

- Respiratory route (inhalation) : nose, throat and lungs (the respiratory system).
- Dermal route (skin) : by direct contact.
- Digestive route : stomach and intestines (collectively the gastro-intestinal tract).
- Other routes : There are other routes of entry, called parenteral routes, which are generally less important: intravenous (IV), subcutaneous (SC), intraperitoneal (IP) and intramuscular (IM) injections.

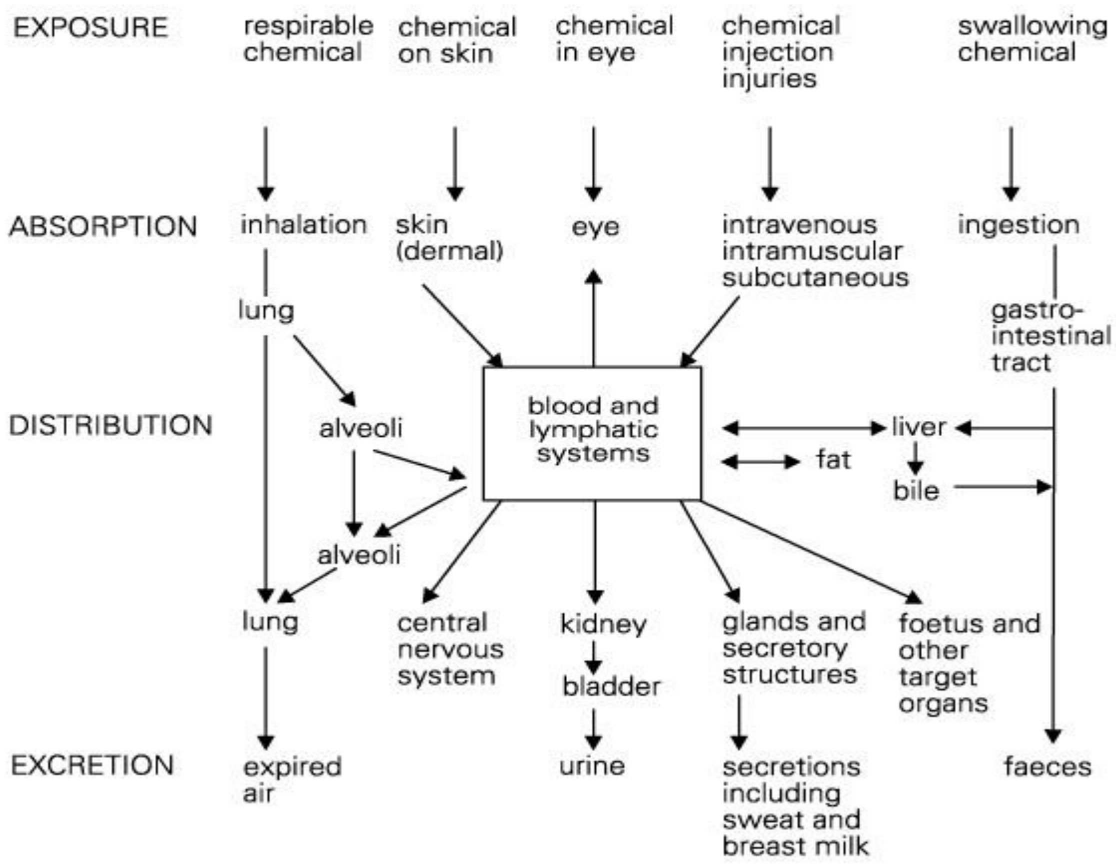


Figure 24: Routes of Exposure, Absorption, Distribution and Excretion of Toxic Chemicals

(Site1)

5.3.Target tissue toxicity:

Chemicals that might produce harmful effects or disease states in certain organs of the body are known as target organ toxins. Toxins have varied effects on different organs in the body due to their distinct cell structures.

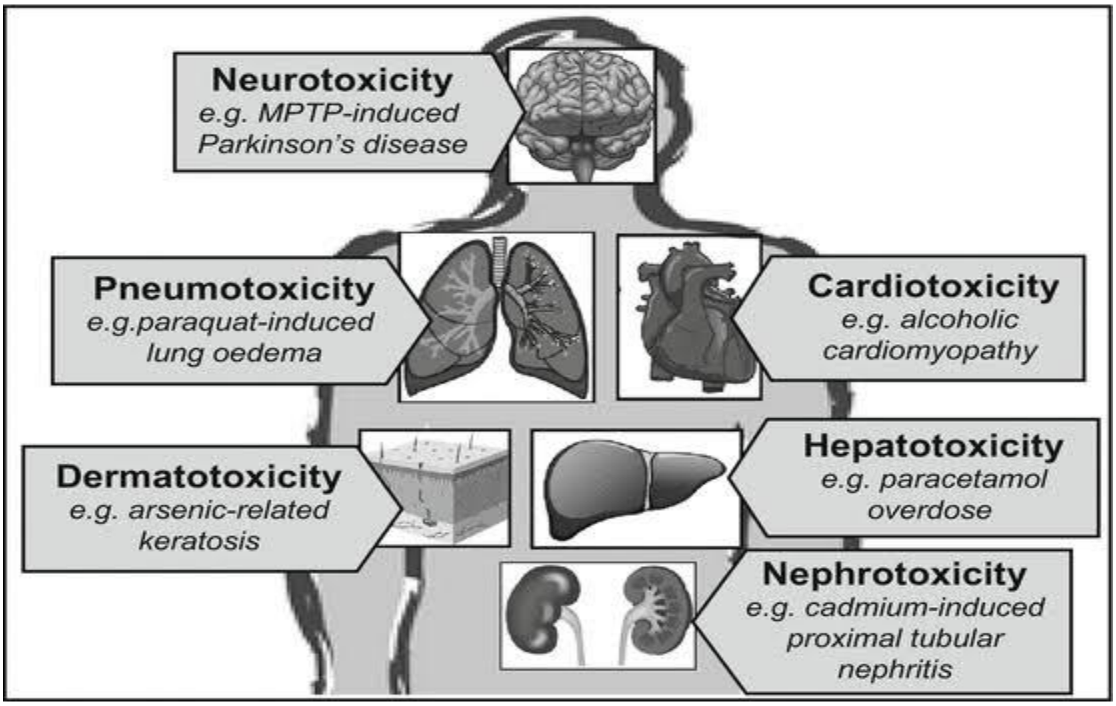


Figure 25: Target tissue toxicity **(Burcham, 2014)**

5.3.1. Hepatotoxicity:

It is liver damage. Like the heart and lungs, the liver is the largest and most complex organ in the body and is involved in the metabolism of nutrients and most xenobiotics. It is a target for many toxins because of its high blood flow and its location in relation to the blood circulation

Main toxic effects observed on the liver:

- Steatosis: It corresponds to the invasion of the tissue by fat. The toxins act by blocking the elimination of hepatic triglycerides in the blood
- Necrosis: It supposes the destruction of hepatocytes and generally corresponds to acute lesions
- Cholestasis: Decreased or stopped bile flow by modification of biliary excretion
- Cirrhosis: Presence of collagen infiltrations in the hepatic mass.
- Hepatitis: Clinical manifestations of inflammation of the liver.**(Lu , 1992).**

5.3.2. Nephrotoxicity:

The toxic effect on the kidney. The kidney plays a vital role in maintaining the water, electrolyte and acid-base balance of the body and helps to rid the blood of its impurities, and in particular of certain toxins **(Gilles , 2004).** Renal damage mainly concerns the glomerulus by reducing filtration, but also the proximal tubules which concentrate toxic substances due to their high absorption and secretion activity. **(Lu, 1992).**

5.3.3 Neurotoxicity:

The toxic effect on the nervous system (NS). The NS is a set of cells, specialized or not, whose fundamental unit is the neuron. Neurons ensure transfer of information (nerve impulses) from one part of the body to another, in order to ensure the internal functioning of the organism and its relations with the external environment. **(Gilles, 2004).**

5.3.4 Hematotoxicity:

Blood is a vital tissue since it is the body's primary mode of transportation. Many medicines and xenobiotics are transported through it. Because all foreign substances are transported by the bloodstream, all cellular and non-cellular components are first exposed to high levels of hazardous compounds**. (Merghem, 2015).**

5.4. Different forms of toxicity:

Humans are constantly exposed to acute, subacute or even chronic toxicity.

5.4.1 Acute toxicity:

Acute toxicity refers to the harmful effects that occur when a single dosage of a chemical is administered or repeated doses are given within 24 hours, or a four-hour inhalation exposure.

Because of the low cost, availability of the animals, and the fact that toxicological reference data for many chemicals in these species are available, all acute toxicity experiments are conducted on rats or mice. Furthermore, these animals may have a metabolism and pharmacodynamics of metabolites that are humanlike. **(Loomis and Hayes, 1996).**

Acute toxicity tests are widely performed to evaluate a drug's or chemical's Lethal Dose 50 (LD50) (LD50 is the dose of a substance that may be predicted to kill 50% of the samples analysed).The lower the LD50 number, the more toxic the substance is in general. Furthermore, the lower the toxicity, the higher the LD50 value. **(Dawoud and Shayoub ,2015).**

Table 09: Hodge and Sterner toxicity scale **(Fayez et al., 2019)**

Toxicity rating	Commonly used term	LD50
1	Extremely toxic	Less than 1 mg/kg
2	Highly toxic	1-50 mg/kg
3	Moderately toxic	50-500 mg/kg
4	Slightly toxic	500-5000 mg/kg
5	Practically non toxic	>5000 mg/kg

5.4.2 Sub-acute toxicity:

Repeated sub-lethal dosages of medication are administered for a period of 14 to 21 days in sub-acute toxicity trials. Sub acute toxicity studies are performed to investigate the influence of a medicine on blood biochemical and hematological markers, as well as histopathological alterations.The drug to be evaluated is given to numerous groups of animals on a daily basis at various dosage levels. At least three test groups and one control group should be employed in these situations. **(Merghem, 2015).**

5.4.3. Chronic toxicity:

Chronic toxicity refers to the negative consequences that occur when experimental animals are exposed to a toxin on a regular basis. Drugs are administered in varying doses for a period of 90 days to over a year in long-term toxicity tests to establish their carcinogenic and mutagenic potential.**(Belguet , 2010).**

5.5 Biomarkers of toxicity:

Biomarkers are biochemical patterns which are used to track biological responses to toxins or other stimuli. With the insight that biomarkers within a biological system fluctuate in response to changes in that system, researchers can assess and predict toxicological consequences. Biomarkers may be used to determine not just the toxicity of a chemical, but also the processes by which it creates toxic effects.**(Kishor and Sahu, 2016).**

Biomarkers will allow us to assess potential harmful exposures and forecast probable impacts, allowing us to make decisions that are more protective of human health.

They are typically defined as:

- Exposure biomarkers : a foreign substance or its metabolite, or the result of a xenobiotic agent's interaction with a target molecule or cell, that is detected in a compartment within an organism.
- Effect biomarkers :a detectable biochemical, physiological, behavioral, or other change within an organism that can be linked to an existing or potential health impairment or illness, depending on its size.
- Susceptibility biomarkers:an indicator of an organism's capacity to respond to the challenge of exposure to a certain xenobiotic chemical, whether innate or acquired.

(FDA, 2011; WHO, 1993).

Table 10: Ten new protein analyte biomarkers approved by the US Food and Drug Administration (FDA) under Clinical Laboratory Improvement Amendments (CLIA) regulations since 1993.**(Kumar et *al.*,2006)**

- Cancer antigen 199
- B-type natriuretic peptide (BNP)
- Cancer antigen 27.29/153
- Lipoprotein (a) (Lp (a))
- Transferrin receptor
- Insulin-like growth factor (IGF-II)
- Troponin T, cardiac
- Troponin-I, cardiac
- Interleukin-2 receptor
- Insulin-like growth factor-binding protein (IGFBP)

5.5.1. Factors affecting performance of a biomarker:

Table 11: Factors affecting the performance of biomarkers and biomarkers-based assays.

(Kumar et *al.*, 2006).

- Experimental designing
- . Quality and source of cancer specimen
- . Progressive biological heterogeneity
- . Pre-analytical factors such as age, sex, dietary status, smoke exposure, use of

tobacco, geographical and environmental factors.

- . Analytical factors like errors during sample collection and processing, dilution errors, purity of reference standards, cross contamination of selected biomarker, fluctuations in temperature and instrument performance, contamination in purity of chemicals, and calculation errors.
- . Social and economical issues.

5.5.2. Characteristics of an ideal biomarker:

- Very precise for a certain illness (and much better, organ specific), as well as reproducible and standardizable across various clinical laboratories.
- Detect nearly all abnormal cells, preferably earlier enough.
- The advantages derived from the usage of an ideal biomarker should be accessible to men and women of all ages and ethnic origins.
- The detection mechanism should be low-cost.

5.5.3 Examples of biomarkers:

Table 10: Examples of Biomarkers of Exposure from Different Categories **(FDA, 2011).**

Exposure Biomarker Category	Biological Matrix	Example Analytes	External stressor
Chemical	Breath	Styrene (unmetabolized)	Styrene
	Feces	Bisphenol (unmetabolized)	Bisphenol A
	Serum/urine	8:2 8:2 DiPap	8:2 8:2 Dipap
Metabolite	Blood	Styrene Oxide	Styrene
	Urine	BPA monoglucoronide	Bisphenol A
	Serum/urine	PFOA	8:2 8:2 DiPap , other fleorinated alkyl acids
Endogenous surrogate	Urine/blood	Testosterone	Bisphenol A, other endocrine active compounds

	Plasma	Butyrylcholinesterase inhibition	Toxicity due to acetylcholinesterase inhibition

Table11: Examples of Biomarkers of Effects from Different Categories **(FDA, 2011)**

Exposure Biomarker Category	Biological Matrix	Example Analytes	External stressor
Bioindicator	Red blood cells	Acetylcholinesterase inhibition	Toxicity due to acetylcholinesterase inhibition
	Blood	Maternal T4/T3	Neurological deficiency in offspring
Undetermined consequenece	Blood/urine	Malondialdehyde	Oxidative stress
	Serum/urine	8-OHdG	Oxidative stress
Exogenous surrogate	Bloood	Lead	Neurological deficiency
	Urine	3-PBA	Toxicity due modulation of neuronal sodium channels
	Urine	Paranitrophenol	Toxicity due to acetylcholinesterase inhibition

3-PBA: 3-phenoxybenzioc acid / 8-OHdG: 8-hydroxy-2'-deoxyguanosine / Acetylcholinesterase: AChE / DiPap: Polyfluoroalkyl phosphate ester / PFOA: Perfluorooctanoic acid.

Conclusion

Conclusion

Through direct use and the development of traditional therapies, herbal medicine has had a huge impact on the health of millions of people throughout the world. According to available information, traditional medicine is used by 60% of the world's population, while traditional medicine and herbal drugs are used by 80% of individuals in developing nations for their main health care needs. The continued use of herbal medicine has been attributed to affordability, ease of access, cheaper prices, historical acceptance, lack of access to conventional care in certain areas, and reports of few or no side effects.

The Ephedra alata plant extract included a combination of phytochemicals such as cardiac glycosides, flavonoids, phenolic compounds, and alkaloids; according to the phytochemical screening.The plant has potent antioxidant activity. The plant can be an excellent choice for biological and chemical analysis, and can be further subjected for the isolation of therapeutically active compounds with anticancer potency.

Unlike most other herbal treatments, ephedra has a health risk that is aggravated by use and/or misuse. The Food and Drug Administration (FDA) declared Ephedra alkaloids-containing food supplements to be an unacceptable health risk in 2004. The FDA has prohibited all over-the-counter drugs that contain ephedrine. Ephedra alkaloids-containing dietary supplement items have been connected to several negative cardiovascular and cerebrovascular outcomes. Stroke, chest discomfort, convulsions, insomnia, nausea and dizziness were all described as Ephedra Alata health risks. Ephedra is only indicated for acute situations in traditional Chinese medicine because of these side effects. It is not recommended for long-term use. In tthe end, as pharmaco-toxicologists, we should know that the dose makes the poison.

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