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Ministry of Higher Education and Scientific Research
Al-Mustansiriyah University
College of Pharmacy

Phytochemical Investigation of Horsetail (*Equisetum arvense* L.) grown in Iraq

A thesis

Submitted to the Department of Pharmacognosy and Medicinal Plants and the Committee of Graduate Studies of the College of Pharmacy/Al-Mustansiriyah University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacy (Pharmacognosy and Medicinal Plants)

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بِسْــمِ اللَّهِ الرَّحْمَـنِ الرَّحِيــم

وَ قُل رَّبِّ زِدنـي عِلماً

صَدق اللّهُ العَظيمْ

Dedication

To …

All My Family

My Dear Parents

My Brother, Sisters

Especially My Beloved Wife

Who offered me Love & Care As Well As the Inspiration Necessary to Shape My Academic Life the Way it is now

Haider

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I pray to God to enable me showing them my graceful gratitude.

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Abbreviation	Meaning
Conc	Concentration
EtOH	Ethanol
GC/MS	Gas Chromatography/ Mass Spectroscopy
G/I	Growth/Inhibition
G	Gram
Hr	Hour
OD	Optical Density
M/Z	Mass/Charge
min	Minute
No	Number
R_f	Retardation factor
SPP	Species
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
PBS	Phosphate Buffer Solution

Abstract

Equisetum arvense is a well-known medicinal plant, with a long history in folklore medicine in many areas around the world as a therapeutic agent in many disorders especially that are related to kidney and urinary tracts.

An investigational study was performed to assess the phytochemical constituents of the plant, many techniques were used in this research, such as TLC, HPLC, and GC/MS, results reveals the presence of the alkaloid Nicotine, the flavonoid Quercetin, and some flavonoid glycosides such as Isoquercitrin.

Furthermore, a cytotoxic study was performed to assess the effect of different plant extracts on the growth of viable human cervical carcinoma cells (HeLa cells), in this research we used Ethyl acetate, Ethanol, and Aqueous extracts, results shows that the different plant extracts are associated with different cytotoxic action, the highest growth inhibition rate was observed with the Ethyl acetate extract, followed by the Ethanol extract, the least one is the Aqueous extract.

Chapter One

1. Introduction

1.1Back ground

For all the past centuries, there was no any difference between pharmacy and pharmacognosy, which deal with medicines that are derived or from a natural origin which are in most cases are plants, with a small participation of fungus and animals. All ancient communities have their own special and customized pharmacognosy and natural medicines specified for their common plants, some of them were difficult to be investigated at all.[1]

Investigations of traditional herbal medicines does not provide an over view about the development history of medicines only, it also provides a fascinating insight for our capability in developing a wide range of civilized habits.[2]

The unique role of the natural product in the management of disorders and diseases has been a typical example by their presence almost all departments of disorders management. As examples, the medicine in west is with an origin in ancient Iraq (Mesopotamia) and ancient Egypt.[3]

The term 'pharmacognosy' was known for the first time in the early nineteenth century to refer to medicinal plants; it is derived from two words, both of a Greek origin pharmakon, 'a drug', the second is gignosco, 'to acquire a knowledge of'. The human beings that lived the ancient era in all the inhabited areas of the earth discovered in an independent manner the inherent stimulating effects of a wide variety of remedies especially that are of a vegetative source. The unmet needs with ordinary medicines and the desire for a life style more ‘natural’ lead to an increased use of complementary and alternative medicine (CAM) all over the developed world. [4]

The utilization of these remedies is extensive, in an increased manner and complex at the same time. Recent studies shows that 20–33% of the United Kingdom population referred to routinely use CAM either alone or in combination to orthodox or ordinary medicine and treatments. In the United Kingdom, utilization is actually more frequent in CAM that are available as over-the-counter medicines compared to that are restricted as Prescription only medication.[5]

Employees and students in the healthcare field also commonly use such products. 43% of students at a University School of Pharmacy claimed to use at least one type of CAM over the past 12 months. [6]

Generally, drugs that are of a natural origin provide four crucial and highly appreciated roles in the modern strategy of disorders' management that makes their presence in the common therapeutic guidelines is logic, namely:

1- Acts as very useful natural medicines.

2- Support us with the basic compounds characterized by less toxicity and more effectiveness molecules.

3- Discovery of pharmacologically active prototypes to the development of novel and superior drugs.

4- Use the inactive natural compounds as a template to be modified by special chemical or biological methods to synthesize potent pharmacological active agents. [7].

The European pharmacopeia stated that *Equisetum arvense* plant is an official medicinal plant for a variety of disorders mainly that are affecting the urinary system.[8]

Equisetum arvense plant, according to Carl Linnaeus system of classification, is classified as shown following in (Table 1.1)

Kingdom	**Plantae**
Division	Pteridophyta
Class	Equisetopsida
Order	Equisetales
Family	Equisetaceae
Genus	Equisetum
Species	E. arvense

Table 1.1: Classification of Equisetum arvense

1.2-Family Equisetaceae

Equisetaceae, also sometimes named as horsetail family, is the only extinct family of the order Equisetales, with one surviving genus, *Equisetum*, which comprises about twenty species.

All available horsetails nowadays are belonging to Eqisetum genus Equisetum. Also some fossils are found that did not classify in the new classification system. Equisetites is a "catch all taxon" unifying all different types of larger horsetails that belong to the Mesozic era. [9]

1.3-Genus Equisetum

Horsetails (Equisetum L.) are the only living of the previously more diverse class Equitopsida, they are free-sporing plants, which are characterized by an each node has articulate stems with a whorls of leaves carried on. With regard to the found fossils, the early forms that might be classified as Equisetopsida lived the first

time in the late Devonian geological period, after that Equisetopsida was at its maximum diversity in the Carboniferous period. After major extinction periods occurred in the late Jurassic and early Permian periods. Beginning from the early Cenozoic period, all knows Equisetopsida were herbaceous in their form that are difficult to distinguish them from the nowadays living Horsetails.[10]

The discovered fossils that are related to the nowadays living Horsetails were living in the Permian or possibly in the Carboniferous periods. Some researchers believed that Equiestum could be the oldest forms of living vascular genus on the planet. [11]

The most important species medicinally is the *Equisetum arvense*, it is prescribed in plants monographs and other references and it is used in folklore medicine in many countries.

The genus Equisetum has the following species as shown in table 1.2.

Generic Name	Common Name
Equisetum arvense	Field Horsetail, Common Horsetail or Mare's tail
Equisetum bogotense	Andean Horsetail
Equisetum diffusum	Himalayan Horsetail
Equisetum fluviatile	Water Horsetail
Equisetum palustre	Marsh Horsetail
Equisetum pratense	Meadow Horsetail, Shade Horsetail, Shady Horsetail
Equisetum sylvaticurn	Wood Horsetail
Equisetum telmateia	Great Horsetail, Northern Giant Horsetail
Equisetum giganteum	Southern Giant Horsetail or Giant Horsetail
Equisetum myriochaetum	Mexican Giant Horsetail
Equisetum hyemale	Rough Horsetail, Scouring rush Horsetail
Equisetum laevigatum	Smooth Horsetail
Equisetum ramosissimum	Branched Horsetail
Equisetum scirpoides	Dwarf Horsetail
Equisetum variegatum	Variegated Horsetail
Equisetum californicum	
Equisetum robustum	
Equisetum trachyodon	
Equisetum thermal	

Table 1.2: Species of the genus Equisetum

1.4-Equisetum arvense:

Equisetum arvense (Horsetail) is a perennial plant that lives in or close to watery areas like marshes, or rivers. It grows in the northern part of the earth that are characterized by a mild-temperature weather, It's the best areas where it could grow its root in water or in clay soil, its name has a Latin origin, from the word sequus which means "horse" and the word seta which means "bristle" Its conventional use as an herbal medicine is referred in several handbooks of phytotherapy [12]

1.5-Morphology:

1.5.1 –Macroscopical

E. arvense is a perennial herb with up to 0.5 m high. The stem is branching and evergreen. The root is fibrous and dark in color (brown to black).the rhizome of the plant is long and its width is up to 0.2 cm. The leaves are renewed each year and arrange in whorls shape. The color of the leaves is darkening in color as going to the margin of the leaves being brown in color at the end of the leave. The distance between each teeth of the leave is up to 0.3 cm.[13]

During March and April the plant is red brown in color with a large number of leaves with emerging of three or more leaves from one node. Green spore powder is usually sprinkled from the brown leaves. In summer and specifically in June the stem is elongated up to 140 mm in high with a large number of rough and square branches Figure 1. [14]

Figure 1.1: *Equisetum arvense* whole plant

1.5.2-Further Description of the plant:

The structure of the fertile stems do not contain chlorophyll, therefore they are brown in color, short-lived in the season of spring, also are lacking to branches, 6-30cm tall. First segment of a branch is longer than the corresponding stem sheath. Plants are very variable.

Sterile stems are greenish, erect or ascending, about 10-50cm tall. Very variable, much branched. Grow throughout the summer; generally taller than fertile stems. The central cavity is about 1/3-2/3 diameter of stem. [15]

The leaves' Sheaths with 4-14 teeth, leaves have been reduced to sheaths around the stems and branches. (this property is shared within all species) as shown in figure 1.2

Figure1.2: Branches of Equisetum arvense

1.5.3- Microscopical

The Powdered dried aerial parts of the plant show following characteristic under microscope

-Bounded pitted xylem vessels as shown in Figure 1.3.

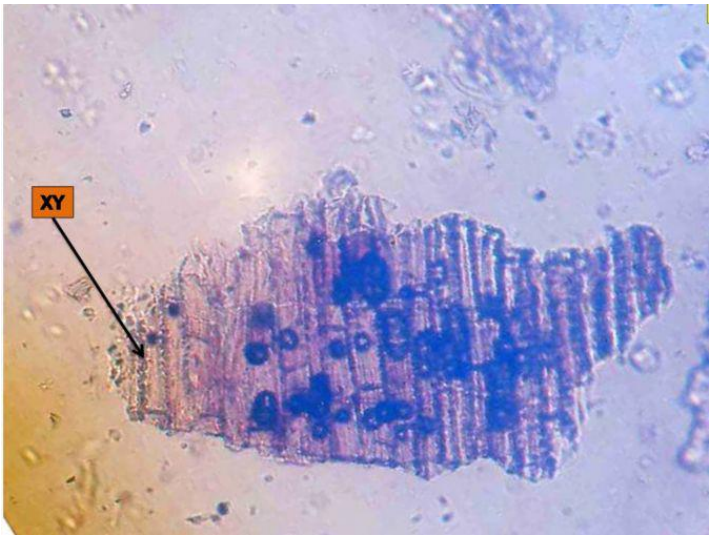


Figure 1.3: Xylem of *E arvense* under microscope

Cortex is seen with about 7 or 8 strata of cells Figure 1.4.

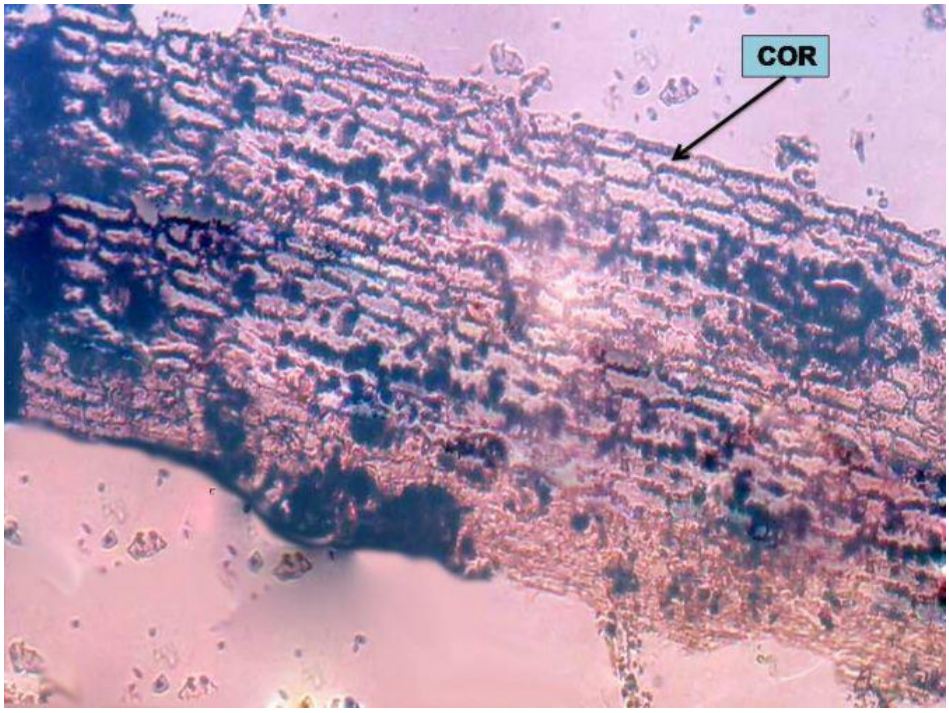


Figure 1.4: Cortex of *E. arvense*

Viable parenchymatous cells are also visible under microscope (Figure 1.5).

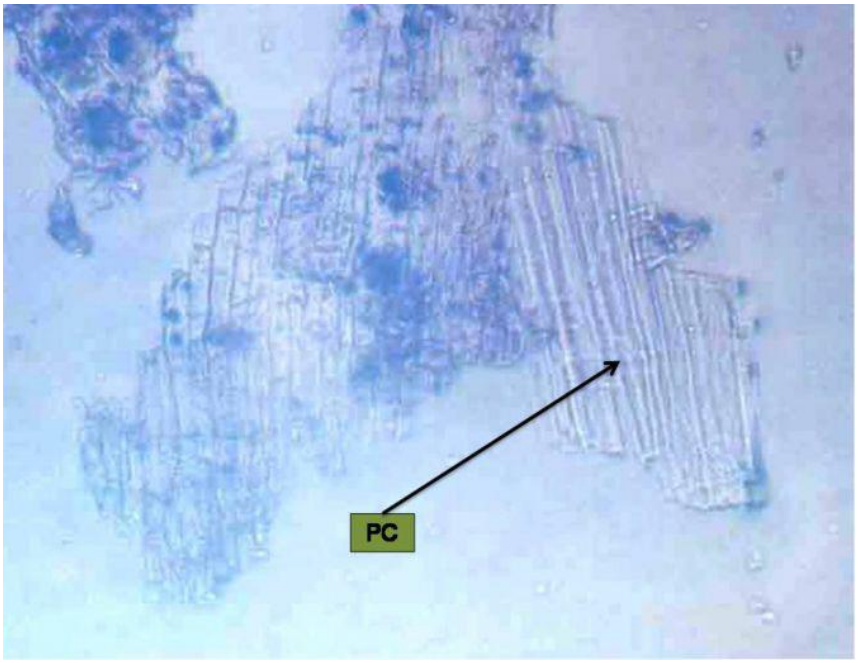


Figure 1.5: Parenchymatous cells of *E. arvense*

Stomata are obviously seen when investigated by microscope. Their function mainly is to facilitate the exchange of carbon dioxide and water between plant tissues and the outer environment.[16].

They are of diacytic type in which a stoma is accompanied by two subsidiary cells, the long axis of which is at right angles to that of the stoma. This type of stoma is also, called the Labiatae type as it is also found in many plants of the family Labiatae such as vasaka, tulsi, spearmint and peppermint). [17]

Guard cells are about to be invisible due to the subsidiary cells that lie over them. They are partially covered with silica on their surface that giving Horsetail a feeling of roughness and naming it commonly as scouring rushes. Silica granules are seen distributed in a diffused manner in all microscopical slides. [18]

These also are seen covering the surface of guard cells surface in stomata as shown in Figure 1.6.

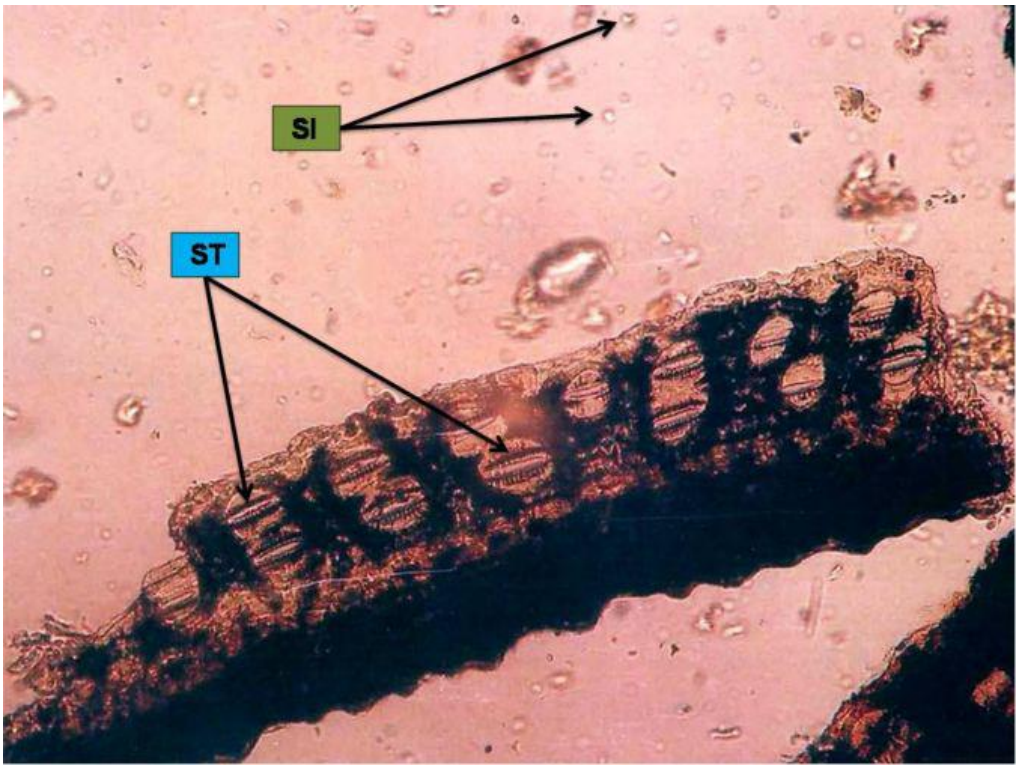


Figure 1.6: stomata of *E. arvense*

Xylem vessel is complex tissue system its role is to conduct water, food and other material in plants. The vessels have no cytoplasm. It is non-viable, but made from living cells. Cells are coordinated in a tube-like form, by joining end to end with the disappearance of the cell wall.[19]

Vessels are synthesized from lignin. Their central cavity and cell wall are lignified. Vessel subunits are connected with each other via holes in the joint walls. Lignin is a rigid polymer made from organic monomers. It gives rigidity and long lives to cell walls. And due to its properties and presence in xylem vessels it makes can hold up.[20]

Cortex is the almost outer layer of the root and stem of plants. It is located between the epidermis and endodermis. Mainly, it is composed of differentiated cells.

Cortex's function is transportation of nutrients and other contents toward the central major cylinder of the stem or root by simple diffusion, also some believe that it is a store for starch.[21]

The parenchyma composed of aggregation of cells which share a close diameter and shape, that is, expand on all sides similarly. Ideal parenchymatous cells are shaped ovally, spherically or polytonally. Their walls are thin and made of cellulose. They are usually living. Parenchymatous tissue is of universal occurrence in all the soft parts of plants. It is responsible for the storage of food in plant. [17].

Stomata are minute openings usually found in the epidermis of the leaves as in Digitalis, Senna, etc., or in young green stems as in Ephedra, in flower as in clove and in fruit as in fennel, orange peel. These openings are surrounded with a pair of kidney-shaped cells called guard cells. [22].

The term 'stoma' is often applied to the stomatal arrangement, which consists of slit like opening along with the guard cells. The guard cells are surrounded by epidermal cells which are called neighboring cells or

subsidiary cells. These, in many cases, as in Digitalis resemble the other epidermal cells, but in large number of plants they differ in size, arrangement and shape from the other epidermal cells.[17][23]

It is believed that Horsetail contains the highest ratio of silica among all other plants. In the ancient era, horsetail was used for polishing pots and for this it is commonly called “scouring rushes”. [24]

Horsetail is widely used in medicinally agents as a supplement for silica, due to the fact that silica composes about 25% (w/w) of the dried plant. Scientists suppose that some of the pharmacological activity (antibacterial, antiseptic and astringent) of Horsetail is attributed to silica that is present in that high concentration. [25]

In regard to plant, silica is believed to be of high benefit, it increases plant immunity against fungal infections, it alleviates or decreases toxicity of metals to plant, it also has significant positive action on both reproductive system and balance of nutrients and electrolyte, some studies reveal that silica also has a role in accumulation of phenolic compounds in parts that are rich in silica.[26]

Silica is found dissolved in many plants in the places of water evaporations but it is not pharmacologically active in all these plants, its properties activity is determined by the structure or by the crystal form of the silica in the plant. It is valuable here to mention that the mechanism by which the silica is accumulated in the plant is still not well understood or studied despite the various theories that are proposed to explain it.[24].

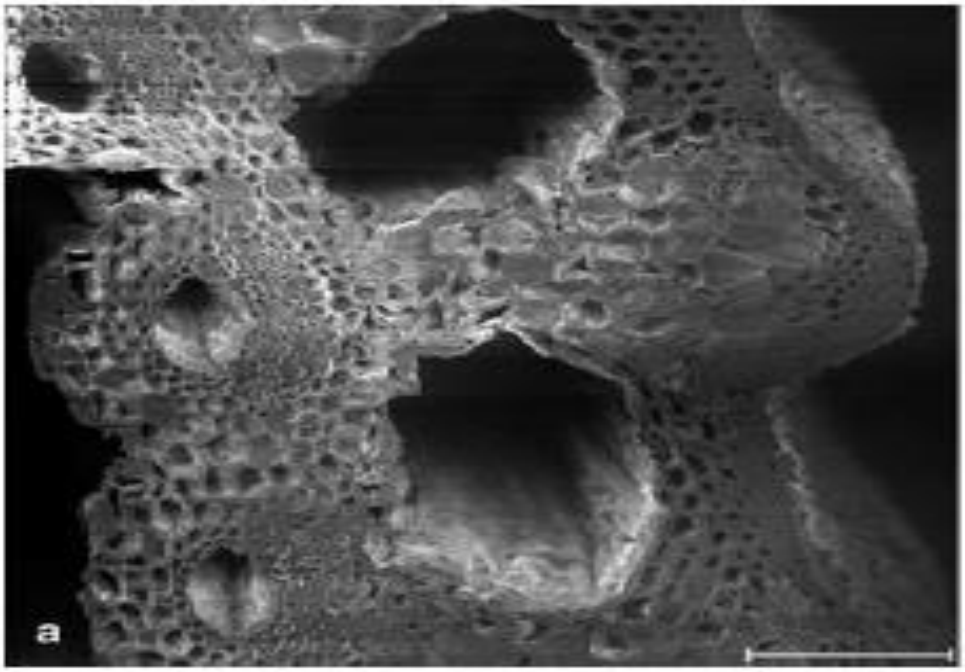


Figure 1.7: A cross section through the stem of Horsetail.[25]

Figure 1.8: Distribution of silica in stem of Horsetail.[25]

1.6-Herbal Preparations:

The method of preparation [9] is given in table 1.3

Preparation	Notes
Comminuted herbal powder	
Expressed juice	1 : 1.6-2.0)
Liquid extract (1 : 4 - 5)	Extraction solvent: ethanol 31.5 % (m/m)
Liquid extract (1 : 5)	Extraction solvent: ethanol 96 %(V/V)/water/sweet wine (16.5/13.5/70) (m/m)
Liquid extract (1 : 5.5)	Extraction solvent: sweet wine/ethanol 96 % (V/V) (91/9) (m/m)
Dry extract (4 - 7 : 1)	Extraction solvent: water
Dry extract (7.5 - 10.5 : 1)	Extraction solvent: ethanol 70 % (V/V)

Table 1.3: Methods of preparation

1.7: Pharmacological actions of *Equisetum arvense*:[27,28]

1. Analgesic
2. Anti-inflammatory.
3. Antioxidant.
4. Antimicrobial.
5. Antidiabetic.
6. Anemia.
7. Anti-haemorrhagic.
8. Astringent.
9. Diuretic
10. Urolithiasis management
11. Neuroprotective agent
12. Hepatoprotective agent
13. Treatment of Osteoporosis
14. Cosmetic use for nails and hair.

1.8- Method of Administration:

Utilization of these herbal products is restricted to Adolescents over 12 years of age, and to adults, each single dose is illustrated as shown below table 1.4:

Type of Herbal Preparation	Single use dose
Comminuted herbal substance for tea preparation	2 - 3 g herbal substance into 250 ml boiling water
Expressed juice from fresh herb (1 : 1.6-2.0)	20 ml
Liquid extract (1 : 4 - 5) extraction solvent ethanol 31.5 % (m/m)	20 drops
Liquid extract (1 : 5) extraction solvent: ethanol 96 % (V/V) /water/ sweet wine (16.5/13.5/70) (m/m)	30 - 40 drops
liquid extract (1 : 5.5) extraction solvent: sweet wine/ethanol 96 % (V/V) (91/9) (m/m)	25 drops
Dry extract (4 - 7 : 1) extraction solvent: water	185 mg
Dry extract (7.5 - 10.5 : 1) extraction solvent: ethanol 70 % (V/V)	200 - 225 mg

Table 1.4: Method of Administration

- Daily dose: 3 single doses from the mentioned above.
- Maximum daily dose: 4 single doses [9]

1.9-New approaches in using *E. arvense*:

1.9.1Antidiabetic activity:

Diabetes management is one of the global obstacles, most of international pharmaceutical companies spend billions annually in the field of discovery and development of new hypoglycemic agents, at the same time too many herbal products have a prominent antidiabetic effect, but they are still not invested adequately.[29]

Equisetum arvense extract when administered orally produces a significant hypoglycemic effects[30], a study compared the activity of the orally administered methanolic extract of *Equisetum arvense* with Glibenclamide (a standard antidiabetic medicine), results revealed that there is no any significant difference in activity between the standard antidiabetic agent and the extract of *Equisetum arvense*.[31]

Other studies shows that extract of Equisetum arvense has the ability to regenerate beta cell of pancreas in Streptozocin-indeced diabetic rats.[32]

1.9.2-Effect on osteoporosis:

Osteoporosis is a common disorder in elderly, it is characterized by decreased bone hardness, which in turn increases the risk of bone fractures, and it is more common in women than in men. It is caused by imbalance in bone minerals, hormonal changes, and may be malnutrition. [33]

Several studies suggest that silica has a crucial role in the treatment of osteoporosis, a study reveals that routine utilization of silica is accompanied by a significantly decreased rate of bone injuries in horses.[34]

Since *Equisetum arvense*, as mentioned before, is known to contain a high amount of silica, and considered as the plant that contains the

highest amount of silica in the plant kingdom, for this reason its use in the treatment of osteoporosis is highly beneficial, it has an important role in normalization of bone mineral state, bone calcification, thus decreasing risk of bone fractures and increasing bone healing rate.[35][36]

1.9.3-Effect on wound healing:

Wounds are physical injury, it is caused by a direct rupture, cut, or scratch of the outer dermal tissue, wounds are usually accompanied by an inflammation in the injured area, wound healing is a challenge to all surgeons and many therapeutic options are introduced to increase wounds healing rate.[37][38]

A study investigate the effect of *Equisetum arvense* ointment on wound healing rate in rats reveals that plant extract ointment significantly increased wounds healing rate with a healing rate of about 92% faster compared to control group, as shown in table 1.5 [39]

These effects might be attributed to the Sitosterol content of the plant which is known to be an effective wound healing agent.[40]

	Healing Status (REEDA Scale, 0-15)			Pain (VAS, 0-10)		
Group	Baseline	(5 ± 1) d after intervention	(10 ± 1)d after delivery	Baseline	(5 ± 1) d after intervention	(10 ± 1)d after delivery
Horsetail	5.0 ±1.6	2.9 ± 1.4	0.8 ± 1.3	5.7 ± 2.4	4.9±2.4	0.8±1.7
Placebo	4.1 ± 1.6	4.0 ± 1.4	3.5 ± 1.6	5.3 ± 2.2	7.1±2.4	4.6±2.6
Mean difference (95% CI)	0.9 (0.3 to 1.6)	-1.1(-1.7- -0.6)	-2.6 (-3.2- -2.1)	0.4(-0.5-1.3)	-2.3(-3.2- -1.3)	-3.8(-4.7–3.0)
P Value	0.003 ŧ [d]	P < 0.001	P < 0.001	0.526 ≠ [e]	P < 0.001	P < 0.001

[a] Abbreviations: VAS, visual analogue scale; REEDA, redness edema ecchymosis discharge approximation; CI, confidence interval.
[b] Data indicate Mean ± Standard Deviation.
[c] Number of participants was 54 in each group at baseline and (5 ± 1) after intervention, and (10 ± 1) after intervention 54 in each group for pain score and 53 in each group for pain score; for REEDA score Mean difference for the baseline comparison and (5 ± 1) after intervention using independent t-test.
[d] ≠: means whitney
[e] ŧ: Independent t-test.

Table 1.5: Comparison of healing status and pain between the study group and control

A clinical trial on 108 women with episiotomy procedure was performed to investigate the plant extract ointment effects on healing rate and pain, results show that Horsetail 3% ointment is significantly decreases wound healing period and decrease pain sensation with a side effects quiet resemble that of placebo group as shown in tables 1.5 and 1.6. [41]

	Adverse Events During the 10-Day	Placebo Group	Horse Tail Group
1	Nausea	2	2
2	Vomiting	1	1
3	Diarrhea	2	1
4	Urination frequency	1	2
5	Fever	5	3
6	Difficulty in walking	7	8
7	Paresis	1	3
8	Skin Problems	3	1

Table 1.6: Adverse effect during the wound healing study

1.9.4-Anxiolytic effects:

Anxiety and especially in recent years is considered as a common disorder, affects population globally, there is a lot of medications that are known to be efficacious in reducing anxiety and alleviate its symptoms but unfortunately most of them have a wide range of adverse effects that lead to many safety concerns regarding the use of such agents.[42]

The anxiolytic effects of *Equisetum arvense* was studied in compasrison with diazipam (a benzodiazepine that is known to be a standards therapy for anxiety), methanolic extract produces a significant anxiolytic effect in rats slightly less than that of diazepam., it alleviate all symptoms of

anxiety at the same time no safety concerns of adverse events were seen during the study period.[43]

1.9.5-Immunomoduation effect:

Inflammatory diseases is considered as an increasingly medicinally challenge, treatment options of such diseases (such as steroids) is seldom to be curative, with a high incidence of adverse events, at the same time newer agents are highly expensive (such as monoclonal antibodies).

T cell activation is known to be involved to a great extent in the inflammatory pathway of many diseases, such as asthma [44], and many other serious disorders such as crohn's disease and ulcerative colitis [45], therefore T cell antagonism is accompanied with alleviation of these disorders.

Regarding to *Equisetum arvense*, extract is believed to inhibit T cell proliferation in a dose-dependent manner, The effect is thought to be via its ability to inhibit the activation pathway of lymphocyte, by interfering with CD69 and interlukin2. In addition to that, utilization of Equisetum arvenseis shown to decrease generation of IFN-γ and TNF-α.[46]

1.9.6-Cognitive and gynecological uses:

A study investigates the action of the hydroalcoholic extract of E.arvense, reveals the ability of the plant to improve cognitive properties and a significant free radical scavenger activity in rats.[47] .It is also reported to alleviate post-menopausal symptoms in women [48]; and as a vasorelaxant.[49]

1.9.7- Cytotoxic activity:

It is considered as one of the major of death around the world with estimated with more than seven million and a half death annually which compromise about 13% from all mortalities estimated in 2008.

Mortalities caused by cancers are with an increasingly manner and they are expected to reach about eleven millions in 2030. [50].

The risk of cancer affection can be modified or altered through advising population to avoid the major knows risk factors, such as smoking, obesity, inadequate fresh food, inadequate aerobic exercises, alcohol abuse, and the diseases that are transmitted via sexual means. [51]

Cancer patients are usually treated by chemotherapeutics, radiation management, surgical approaches, and recently discovered and used the mono clonal antibodies and immunotherapy agents, some patients may require a combinations of those approaches according to type and site of affection, the state at which the disease is progressed and the patient status.

Options used are depending on the type of cancer and location, disease stage, as well as the general health status of the patient. In general, cancer is known as a deadly disease due to its poor prognosis.[52]

For a very long period herbal preparations or medicines of a natural origin were the major source of remedies that are used to deal with or treat man illness. In modern medicine, Natural products have an essential role in the field of drug developing and drug inventions, many compounds that are derived from plants have a crucial participation in fighting cancer.[53].

The use of herbal preparation and remedies of a natural origin in management of cancer belongs to the ancient eras. Researchers believed that there are many strong enough clues that many herbs, fresh food (vegetables and fruits) can be considered as a prophylactic agents against a lot of kinds of cancers, many of them are studied extensively for their expected anti-cancer activity, that is believed to be due to containing a high amounts of vitamins (most of vitamins), fibers, and minerals, and at the same time they can be considered as side effects free, making them

the optimum option in decreasing the risk of cancer affection and achieve and maintain better health status.[54][55].

Regarding *Equisetum arvense*, different types of extracts can inhibit cellular growth; this variation is controlled by the type of the cell line, type and procedure of extraction, and on the concentration incubated with cells. A notable antiproliferative activity (on multiple cell lines and on human leukemia cells) is seen with ethyl acetate extract of the plant, this cytotoxic action is believed to be in a dose dependant manner.[56]

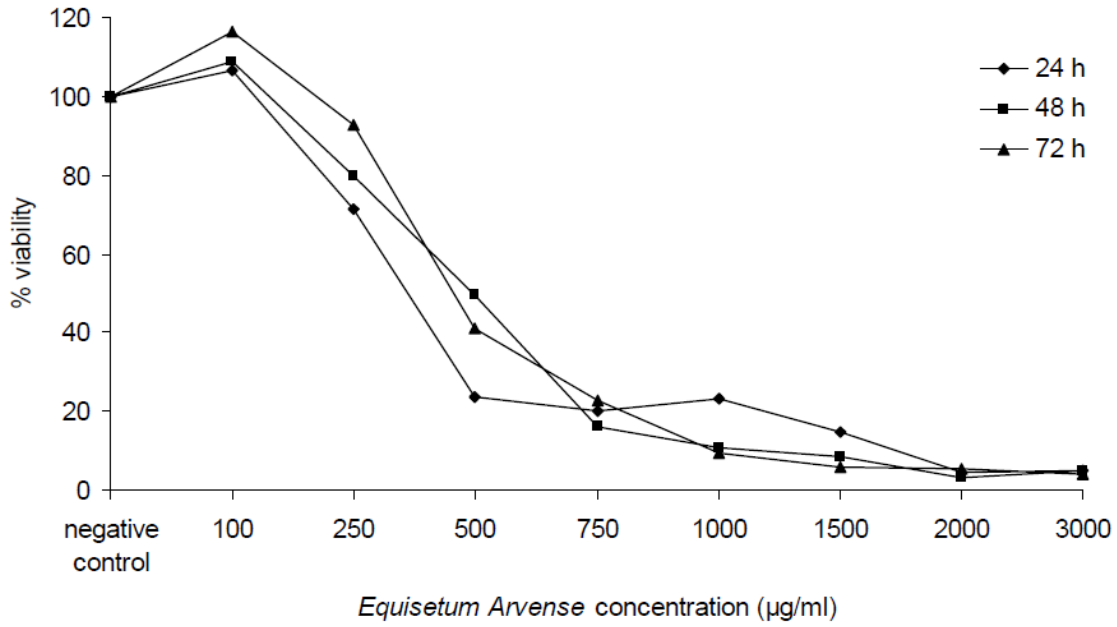


Figure1.9: Dose dependant cytotoxic activity of *E. arvense*

1.10-Phytochemical constituents of *Equisetum arvense*

1.10.1-Flavonoids and their Glycosides:

Flavonoids, a term usually used to refer to a wide range of naturally occurring compounds that have (C6-C3-C6) base unit, or what is called phenylbenzopyran structure. Flavonoids are classified according to the site of attachment of the benzopyrano moiety to the aromatic ring into three types:

a. Flavonoids: these compounds share the basic structure of (2-phenyl benzopyrans) as shown in Figure 1.10

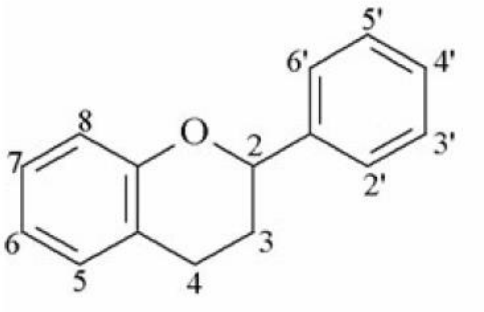


Figure 1.10: Basic structure of flavonoids

Compounds belong to this type are sub-classified according to the degree of oxidation and saturation in ring C to the following: as shown in figure 1.11

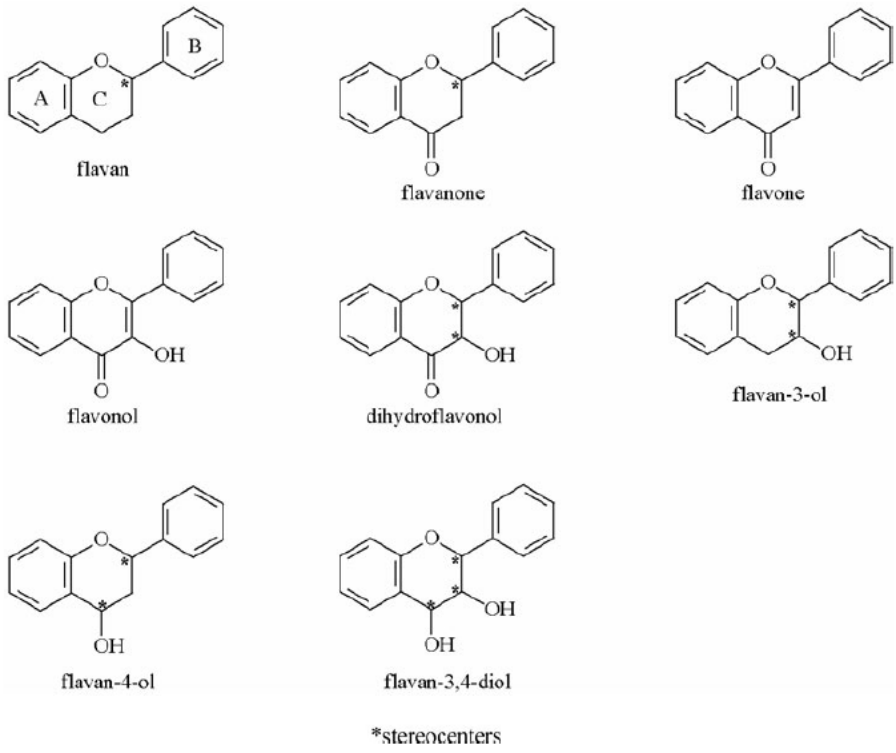


Figure 1.11: Types of flavonoids

b. Isoflavonoids:

Compounds belong to this type have the basic structure of (3-phenylbenzopyran) as shown in Figure 1.12:

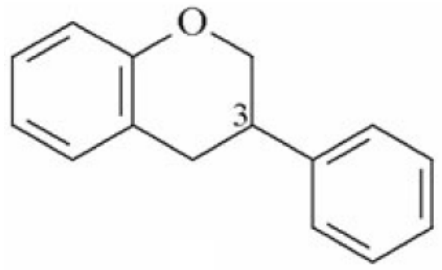


Figure 1.12: Basic structure of isoflavon

Compounds in this class are sub-classified into the following classes: as shown in figure 1.13:

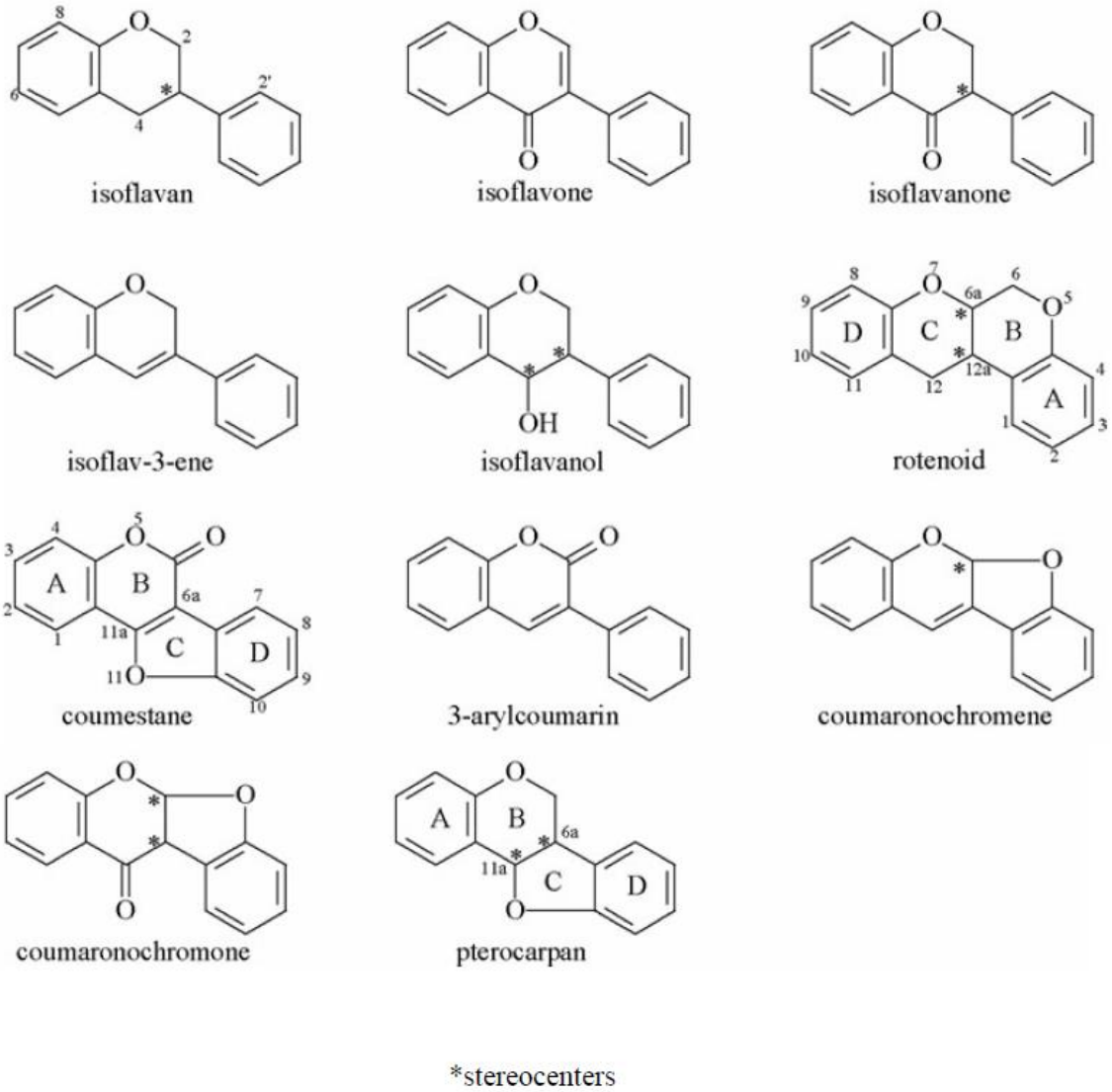


Figure 1.13: Classes of Isoflavon

c. Neoflavonoids: these compounds have the basic structure of (4-phenylbenzopyrans): as shown in figure 1.14

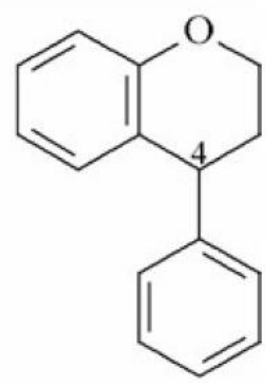


Figure 1.14: Basic structure of Neoflavon

They are structurally related to Isoflavonoids and flavonoids, and sub-classified into the following structures as shown in figure 1.15:

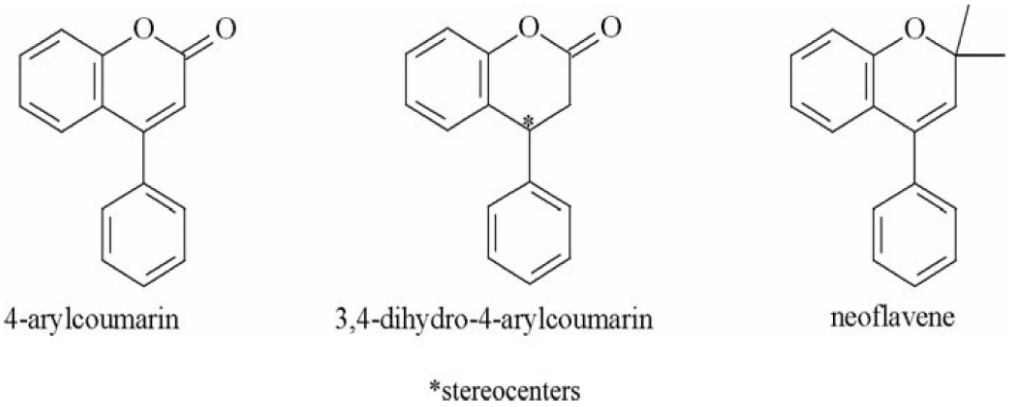


Figure 1.15: Relation of neoflavonoids to flavonoids

Among the three types above, compounds belong to the first class are considered as the major naturally occurring flavonoids.[57].

Flavonoids are biosynthesized from a phenyl propane-derived unit (C6-C3), the later source or origin is from shikimate (from the amino acid phenylalanine) and an additional six carbons unit which is obtained from poly ketide biosynthetic pathway. This poly ketide unit is synthesized from 3 units of malonyl-CoA, that are combined with the phenyl propane-

derived unit (which is found as a CoA thioester) to generate the triketide molecule as a starter

So that flavonoids are biosynthesized by mixed pathways, including of fragments derived or obtained from both polyketide and shikimic acid pathways.[58]**,** as shown in Figure 1.16

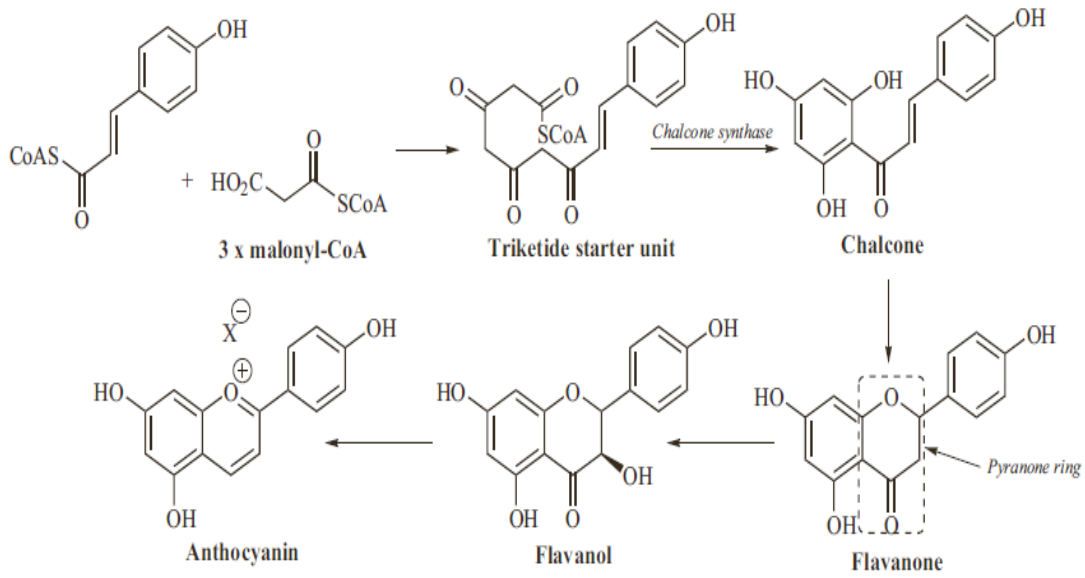


Figure 1.16: Biosynthesis of flavonoids

All previous compounds are illustrated as the major constituents in the *Equisetum arvense* plant. Major constituent in ethanolic extract of the plant is(quercetin3-O-glucoside) which is also known as (isoquercitrin), other glycosides are available is considerable amounts such as (kaempferol 3-O-glycoside) and (apigenin 5-O-glucoside). [59]

Quercetin is available in high concentrations, about 50 % of all total flavonoids in plant, but as the weather changes into summer and becomes hotter, the quercetin amounts decreases rapidly.[60]

The importance of the flavonoids is their health benefit by acting as a free radicals scavenger converting them into much more ‘stable radical’

which in turn would undergo reaction with another ‘flavonoid radical’ to yield two non-radicals.[7]

1.10.2. Caffeic acid ester (about one percent):

These compounds are including chlorogenic acid, and dicoffeoyl-meso-tartaric acid, their role in plant is thought to be a protective agent against pests or microbes, while their pharmacological actions are antibacterial, and antiviral.[61]

1.10.3. Silicic acid (about five percent): it has role in regulating metabolism of different classes of secondary metabolites especially phenolic compounds, new studies shows that silicon-treated plants produces significantly higher amounts of flavonoids than their intact correspondent. [26]

Some European clinical studies reported that bone fractures heal significantly faster when *Equisetum arvense* is used because of its high content of silica, also for the same reason it shows to decrease the incidence of osteoporosis more greatly when *Equisetum arvense* is routinely used. [13].

1.10.4. Alkaloids:

The term Alkaloid is derived from the word alkaline; it previously was referred to any Nitrogen-containing compound. They are biosynthesized by many beings, including bacteria, fungi, plants, and even animals. Many of them have a pharmacological action and indicated to many therapeutic fields, such as caffeine, cocaine, nicotine, ephedrine and many others.[62]

It is difficult to find a specific definition for the term ‘alkaloid’ (alkali-like) due to that there is no clear-cut border between alkaloid itself and

other naturally occurring complex amines, the following is the most accepted classification for alkaloids:

1- Typical alkaloids are defined as that extracted from plants, alkaline, and has at least a nitrogen atom (usually in a heterocyclic ring) in addition to that, usually they have a marked pharmacological use either on human or on animals.

2- Proto-alkaloid: sometimes called 'amino-alkaloid' is a term occaisionally applied to refer to compounds such as colchicine and ephedrine in which one or more of the features mentioned above of the typical alkaloids is absent.

3- Other alkaloids: they aren't fit with conventional or general alkaloids definitions, they are chemically synthesized, not extracted from any natural source, at the same time they have a strong correlation to the naturally occurring alkaloids (such as the anticholinergic agent homatropine). [63]

In Pharmacognosy practice, these compounds are found in plants and gives positive results with the standard qualitative tests specified for alkaloids, and frequently in plant researched these tests alone are used to identify a plant as 'alkaloid-containing [64]

Alkaloids are reported to be present in *E. arvense [65]* , are mainly from the Pyridine alkaloid class, nicotine and palustrine are the main alkaloids reported to be present in *Equisetum arvense* [15], Figure 1.17 shows the biosynthetic pathway of nicotine in the plant.

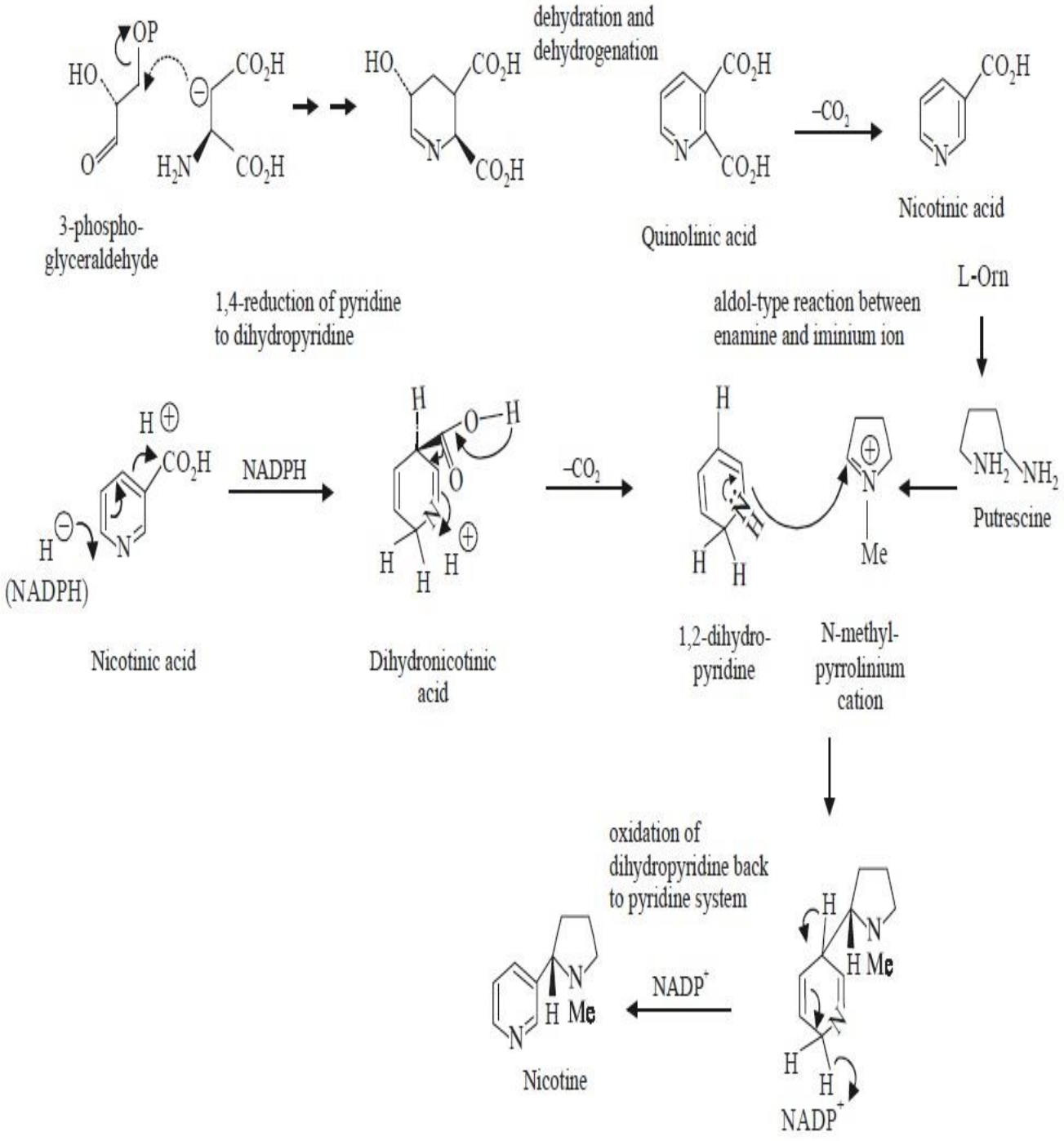


Figure 1.17: Biosynthesis of alkaloids of *E. arvense*

1.10.5. Triterpinoids:

Naturally occurring secondary metabolites, have a crucial role in regulation of plant interaction with its media or environment, they may have a defense role to the plant, to prevent animals from feeding on the plant, or may have an attractant role in the plant, many of them have a pharmacological activity for animal and humans.[66]

Among phytochemicals that are reported to be found in Equisetum arvense that belong to this class, (Isobauerenol, taraxerol, germanicol, ursolic acid, oleanolic acid, betulinic acid), Betulinic acid is pentacyclic compound with a well-known anticancer activity.[13].

1.11-Aims of study

According to the mentioned above about the uses and activity of this plant we aim to investigate the Iraqi Horse tail plant (*Equisetum arvense*) for the presence of medicinally active constituents that have potential to be curative agents for several diseases. Furthermore, the cytotoxic activity of the plant is assayed to investigate the plant's activity to inhibit the cellular growth of human cancer cells.

It was reported that this plant is widely used in Europe and other countries. The medicinal plant *Equisetum arvense* is wild and exists in abundance in Iraq; no study was conducted on the phytochemical constituent of this plant.

Chapter Two

2-Materials and methods

2.1-Genaral Instruments

- Rotary Evaporator: Evaporator of the extract was performed under reduced pressure using Buchi Rotatory Evaporator attached to a vacuum pump.

- GCMS-QP2010 ultra: SHIMADAZU/ Gas chromatography GC 2010 Plus Mass Spectroscopy was carried out in College of Sciences – Chemistry Department – Almustansiriya University.

- HPTLC: Instrument and tools for all steps of modern thin-layer chromatography (Switzerland). Eike Reich/CAMAG - Laboratory.

HPTLC analysis was carried out in Baghdad College of pharmacy.

-HPLC: Schimadzu apparatus, performed in Science and Technology Ministry.

-Incubator for Cytotoxic activity test.

- Water bath Memmert/Germany.

All chemicals used were analytical grade (the highest available purity of the substance) unless otherwise specified. They are listed with their suppliers in table 2.1

Chemical	Supplier
Aceton	Scharlau/Spain
Ammonia	Scharlau/Spain
Chloroform	Scharlau/Spain
Ethanol	Analyt GCC/U.K
Ethyl acetate	GCC/ U.K
Hydrochloric acid	BDH – England
Methanol	Scharlau/Spain
MTT Dye	Sigma aldrich
Potassium hydroxide	FLUKA-Switzerland
Anhydrous Sodium sulphate	FLUKA-Switzerland
RPMI 1640	Sigma aldrich

Table 2.1: Chemical used with their suppliers

2.2-Plant Material

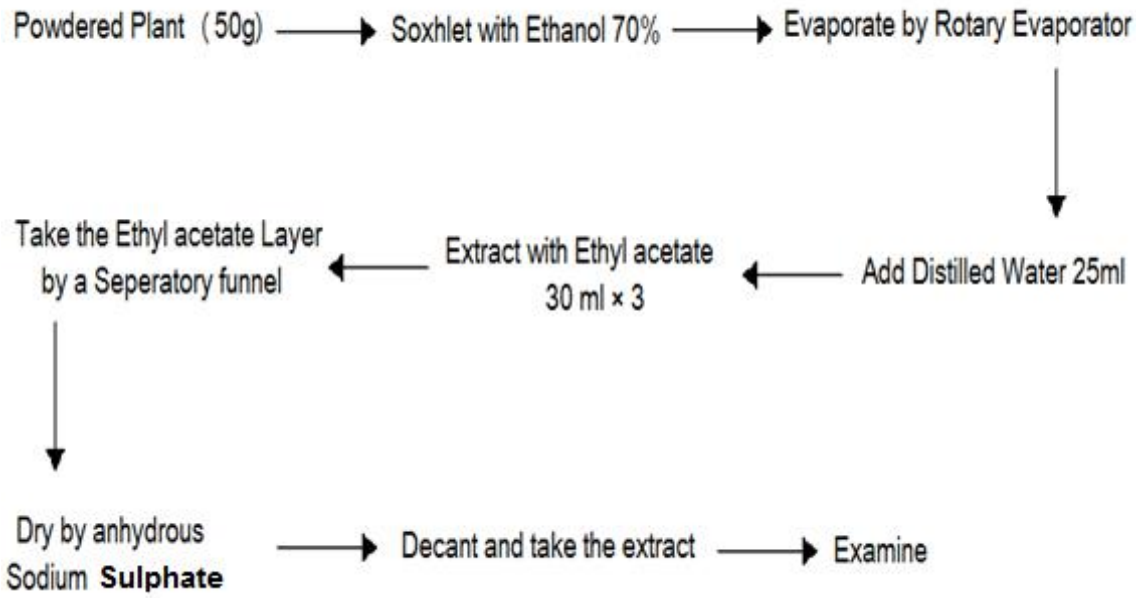
Plant material was collected from Mkeshefa town in Salahaddin province, and was authenticated by the Iraqi National Herbs Center in Abughreb.

It was collected during June, and then it was dried in shade at room temperature.

2.3-Extraction of Flavonoids:

Powdered (whole plant50 g) was extracted by Soxhlet apparatus with ethanol (70%, 200 mL) till exhaustion. The extract was concentrated by

evaporation under vacuum by using Rotary evaporator. Then mix with distilled water (25mL), and extracted with ethyl acetate (30mL×3), the upper layer (ethyl acetate layer) were separated by a seperatory funnel then it was dried by using anhydrous sodium sulphate, and labeled as Ethyl acetate extract (as shown in scheme 2.1)



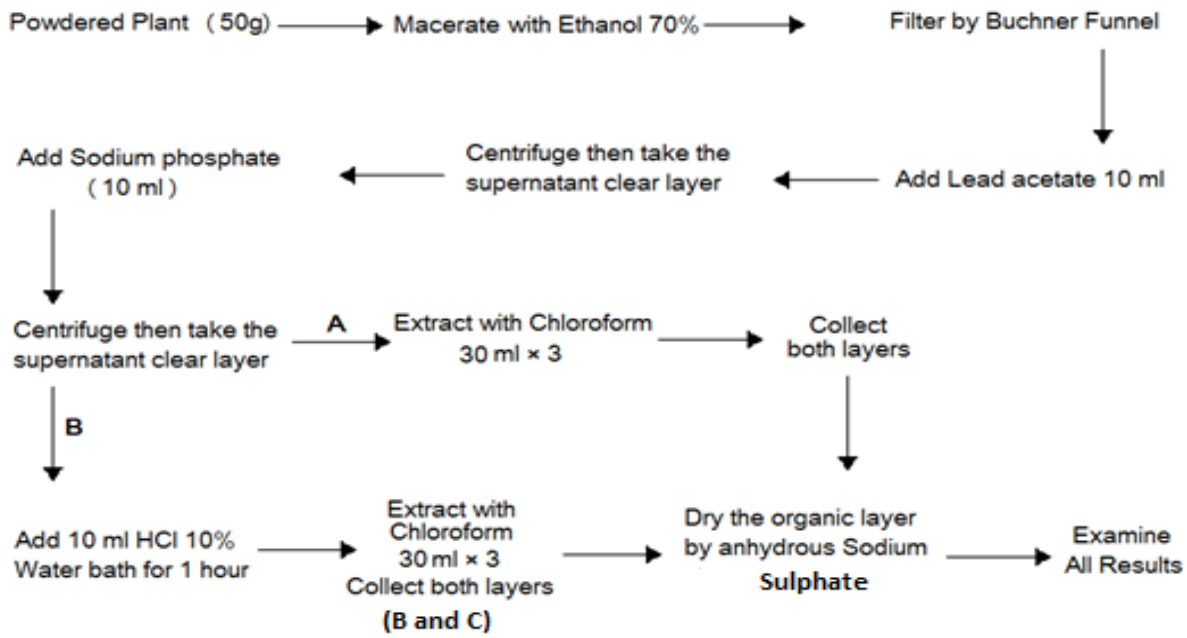
Scheme 2.1: Extraction of flavonoids

2.4-Extraction of Glycosides:

Powdered plant (50 gm) in was macerated in ethanol (200 mL of 70%) for 24hr. The macerate then filtered through filter paper by Buchner funnel to get clear filtrate. The alcoholic extract (150 mL) was introduce in a conical flask, and then lead sub acetate (10 mL) was added, mixed with solution and allowed to settle for about two min. The extract was centrifuged for 10 minutes on 3000 rpm. The supernatant was taken into a flask and sodium phosphate (10 mL of 10%) was added with shaking thoroughly and centrifuge.

Decant the supernatant and divide the mixture into two fractions; first fraction is labeled as fraction A.). The second fraction B was divided into two portion one of them is the glycosides and the other is converted to aglycone by hydrolysis by addition of HCl (10%, 30 mL) and boil in a

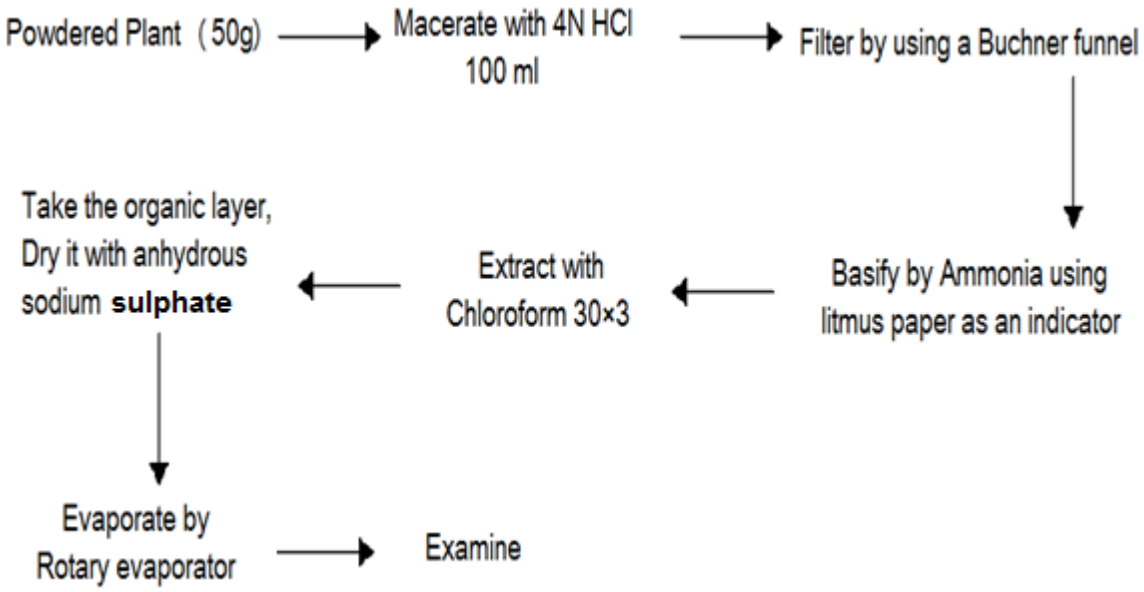
water bath for an hour. The resultant aglycone was extracted with Chloroform (3×30 mL). The combined chloroform layer was dried with anhydrous sodium sulphate and labeled as fraction C. as shown in scheme 2.2.



Scheme 2.2 : extraction of glycosides and their aglycons

2.5-Extraction of Alkaloids:

Powdered plant (50 g.) was macerated in 2N Hydrochloric acid (100 mL.) and stirred gently by a magnetic stirrer. The extract was filtered by a Buchner funnel, and the alkaloids salts were basify with ammonia solution to free the basic alkaloid using pH paper. The free alkaloid was then extracted with chloroform (3×30 mL). The combined organic layers, was dry with anhydrous sodium sulphate then evaporated by a rotary evaporator to yield a sticky mass which was analyzed for the presence of alkaloids using GC /MS analysis. Scheme 2.3 illustrates the above procedure.



Scheme 2.3: Extraction of Alkaloid

2.6-Phytochemical Screening:

Multiple tests were performed in order to assess the presence of major classes of secondary metabolites:

a. **Test for Flavonoids:**

   Ethanolic KOH (2 mL) was added to 1 mL of ethanolic extract of the plant, Yellow color indicates the presence of flavonoids.

b. **Test for Alkaloids:**

   By Dragendroff's reagent, apply, by using a capillary tube, some spots of extract on a filter paper, spray it with the freshly prepared Dragndroff's reagent.

   Brown orange color after exposure of extract to Dragendroff's reagent indicates the presence of alkaloids.

c. **Test for Glycosides:**

   Since a glycoside molecules is composed of two moieties, a glycone and an aglycone, each will gives its specific results after

hydrolysis by acid and heat, thus, we obtained a flavonoid and a sugar part which is confirmed by benedict test.

2.7-Thin Layer Chromatography (TLC):

It is a simple and accurate method of separation and identification of phytochemicals, it is performed for most steps in research in order to get an overview for the present substances in the plant.

2.8-High Performance Thin Layer Chromatography (HPTLC):

It is the most sophisticated TLC technique, it compromise a fully automated system for all steps of chromatography process, in addition to a computerized system of scanning and measuring R_f values for the inspected samples.

In this research; Ethanolic extract was investigated for the presence of possible phytochemicals, Quercetin standard was used in the analysis.

2.9-High Performance Liquid Chromatography (HPLC):

Column: phenomenex C-18, 3 micrometer particle size (50×2.0 mm I.D) column.

Mobile phase: linear gradient of solvent A 0.1% formic acid: solvent B was (6:3:1, v/v) of acetonitrile: methanol: 0.1% formic acid, gradient program from 0%B to 100%B for 10 minutes, Flow rate 1.2 mL/min

2.10-Gas Chromatography/Mass Spectroscopy (GC/MS):

GC Ms was performed by using Shimadzu apparatus at the college of science-almustansiriyah University. obvious from its name that is compromises two complementary processes, the first (GC) is involved in separation of sample contents, then the separated contents passed sequentially according to their separation rate into

the second part of the system (MS), in which each of the separated compounds.

In this research, this analysis technique is performed in order to investigate the presence of alkaloids in plant extract.

GC/MS was shimadzu apparatus GCMS-QP2010Ultra. Injection Volume was 0.50.Column Oven Temp was 70.0 °C. Injection Temp was 240.00 °C. Injection Mode was Splitless. Sampling Time was1.00 min. Flow Control Mode was Pressure. Pressure was 100.0 kPa. Total Flow was 19.9 mL/min Column Flow was 1.53 mL/min. Linear Velocity was 45.4 cm/sec. Purge Flow was 3.0 mL/min. Split Ratio was 10.

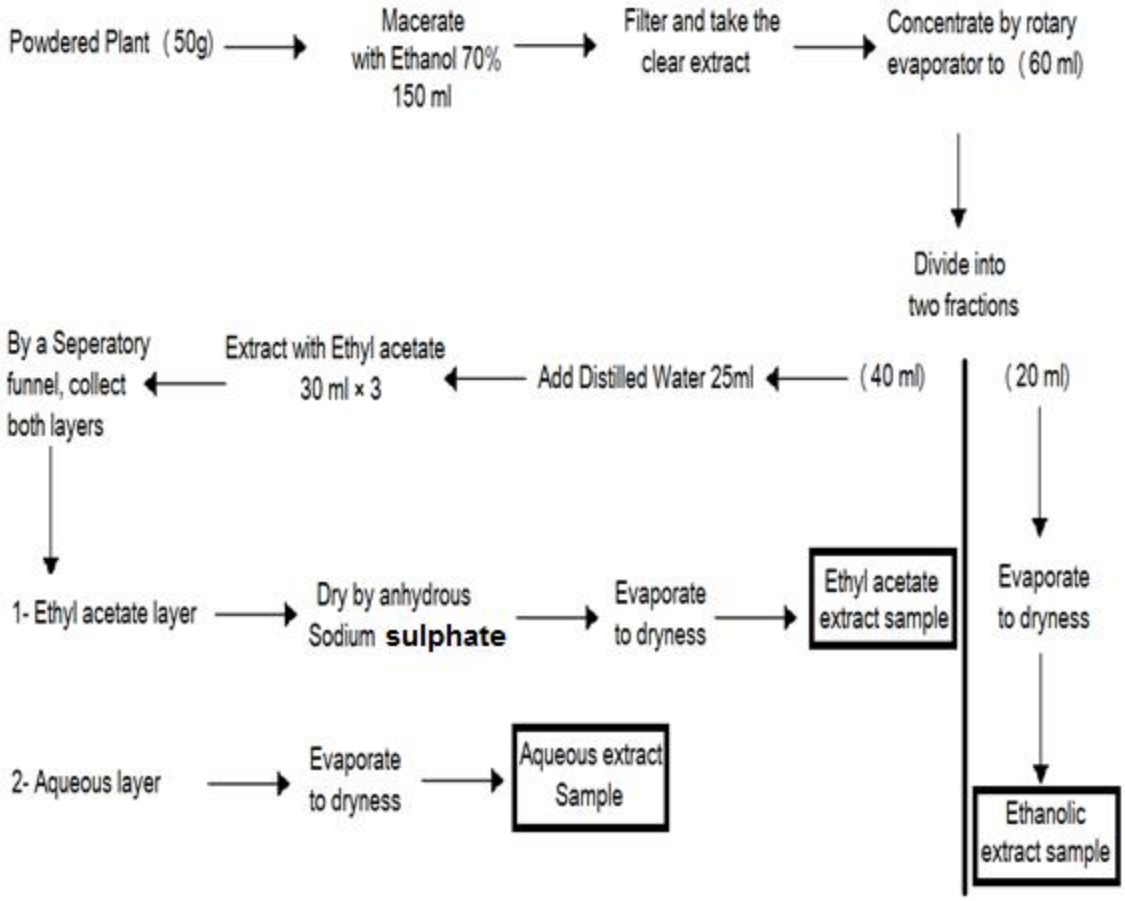
2.11-Extraction of possible Cytotoxic ingredients:

In order to assay the cytotoxic activity of the plant active compounds, three different types of extracts were used; ethyl acetate, Ethanol and aqueous.

50 g of the dried powdered plant is macerated with 150 ml of 70% ethanol for 24 hour with occasional shaking on a magnetic stirrer. Then filtered to get rid of plant ashes, then the filtrate was concentrated to 60 ml., and divided into two fractions; the first (20 mL.) is evaporated by rotary evaporator to dryness and taken as the Ethanol extract. The other fraction (40 mL.) is taken and 25 mL. of distilled water is added, then extracted by ethyl acetate (30 mL×3), both layer were collected, the ethyl acetate layer was dried by anhydrous sodium sulphate (2 g.) then both layers were evaporated to dryness and collected as ethyl acetate and aqueous samples.

All the three dried extracts were dissolved in Roswell Park Memorial Institute (RPMI) 1640 as medium in a conc. of (25 µg/mL) and incubated with viable human HeLa cells (human

cervical carcinoma cells) for 24 hours, then viability or growth inhibition was measured by MTT method (Scheme 2.4)



Scheme 2.4: Fraction used for cytotoxicity study

After completion of the incubation period, the cytotoxicity of each sample and the percentage of survived viable cells were estimated. MTT assay method was used in order to estimate the growth inhibition rate of the sample, MTT dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dissolved in PBS is added to wells which is reduced by the action of mitochondrial dehydrogenases and reductases into formazan dye, the latter's amount is measured via the colorimetric method by measuring the optical density (OD) of each well, since dehydrogenases and reductases enzymes are limited only to viable cells

rather than cells that were killed by the action of drug, thus the optical density is proportional directly to the survived viable cells.

Chapter Three

3-Results and Discussion

3.1-Isolation of Nicotine:

TLC and Dragendroff's tests show the presence of an alkaloid with an Rf value which is similar to that of alkaloids of this genus as stated in the Plant drug analysis [67] as shown in figure 3.1

Spray reagent Dragendorrf

Figure 3.1: TLC for nicotine alkaloid

Further investigation for the alkaloids of the plant was performed by an advanced method that can separate each compound in the sample and identify them individually, we used the Gas Chromatography/Mass

Spectroscopy technique, and the results were shown in figure 3.2

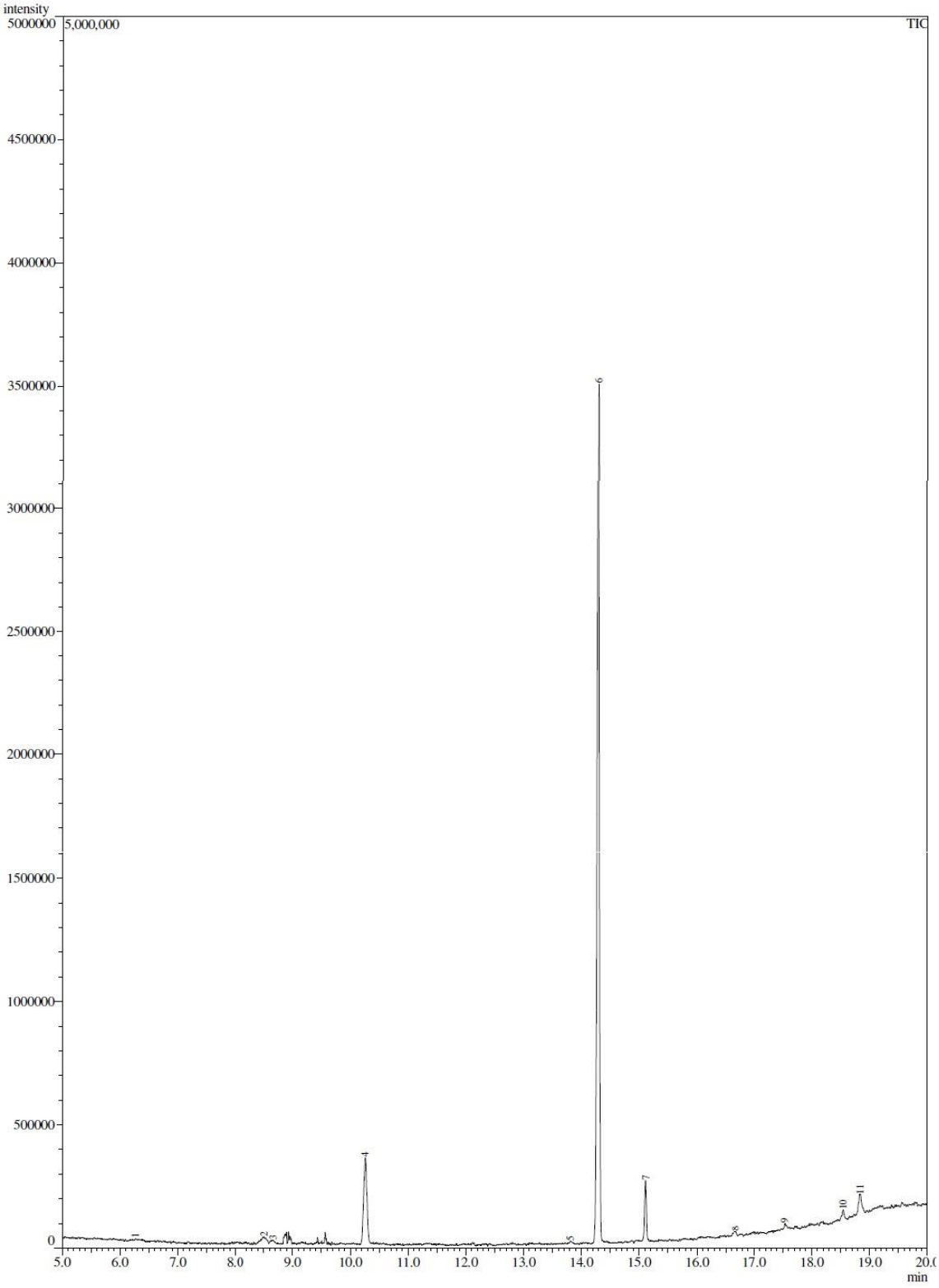


Figure 3.2: GC/MS analysis of the extracted alkaloid

Regarding the previous chart, peak 6 75%, the most intense peak, belongs to a compound that has the following fragmentation pattern: Figure 3.3

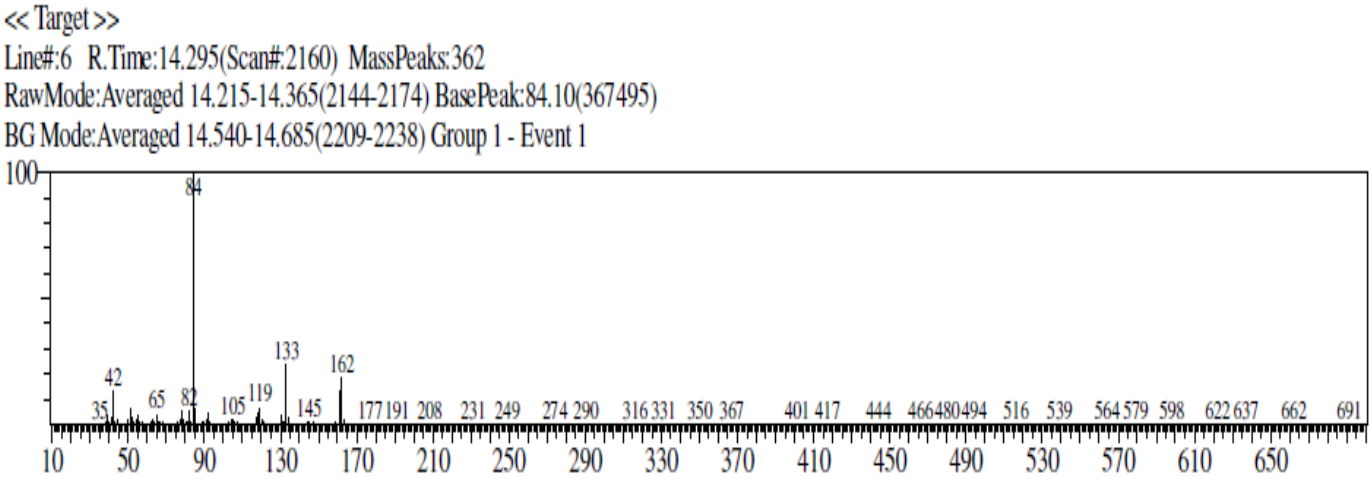


Figure 3.3: Fragments of nicotine

The above data, when compared to the data base of the (GC/MS) device, it shows that those data belong to the alkaloid Nicotine, as the following:Figure 3.4

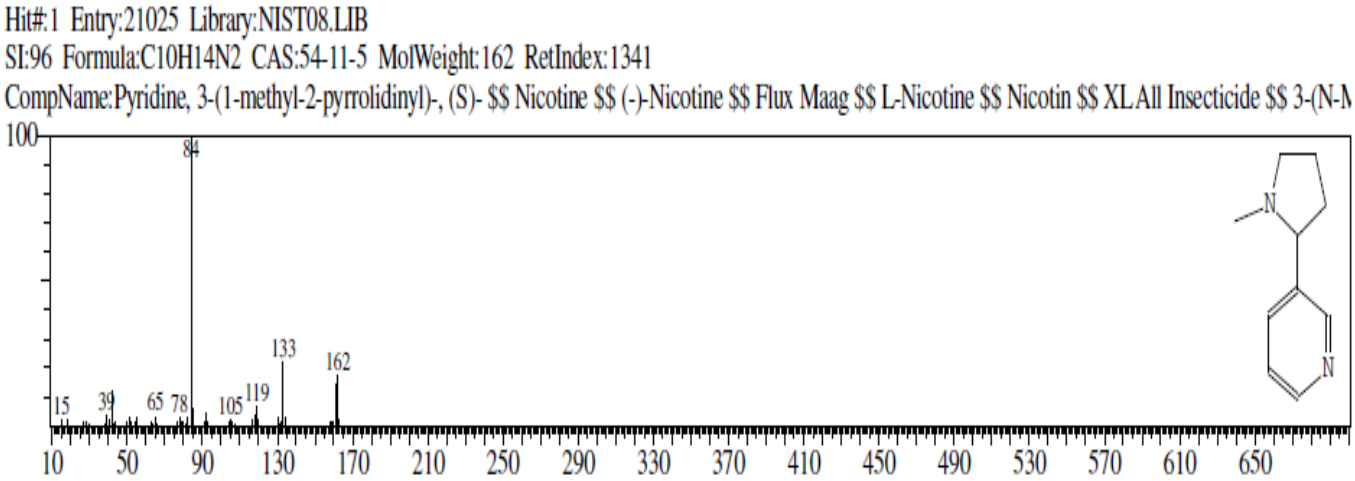


Figure 3.4: Data base for nicotine

The base peak has an m/z value =84 and explained by the cleavage of the two nitrogenous heterocycles, which is confirmed by the adjacent peak 78 as follows [68]: Figure 3.5

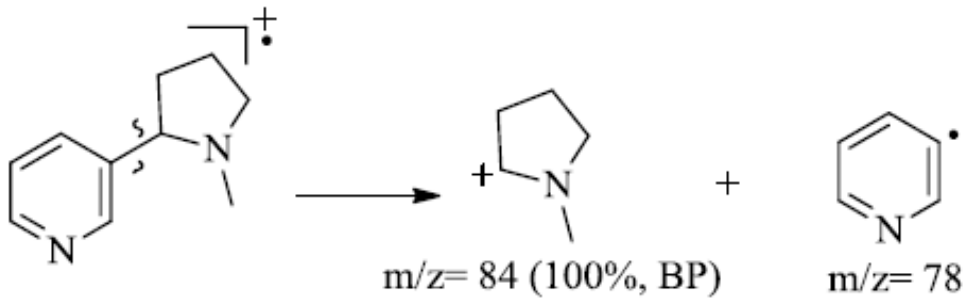


Figure 3.5: Fragmentation pattern of nicotine as reported in the literature

Regarding the second peak in intensity, the 133, its explanation is as follows: Figure 3.6

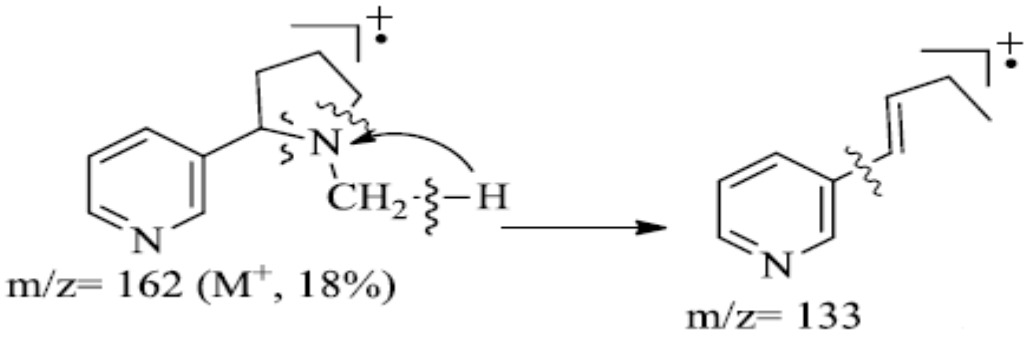


Figure 3.6: Interpretation of nicotine fragments

The alkaloid nicotine is reported to be found in *Equisetum* arvense[69], but this research shows that Nicotine in the Iraqi plant is found in higher concentrations, that is might be due to that the Iraqi soil is rich in nitrogenous compounds, which will lead to higher amounts of nitrogenous compounds (alkaloids) than that grown in other areas.[70]

For more investigation, TLC technique was used by using three different solvents systems and compared to the reported one.[71]. As shown in table 3.1

Solvent System	Reported R_f of Nicotine	Observed R_f	TLC Plate
1. Ethyl acetate: methanol: NH_4OH (85:10:3)	0.8	0.8	
2. Methanol :NH_4OH (100:1)	0.9	0.9	
3. Isopropanol: acetic acid: H_2O (60:20:20)	0.2	0.2	

Table 3.1: TLC for nicotine in three solvent system

3.2-Isolation of Flavonoids:

Multiple Flavonoids are reported to be found in *Equisetum arvense* plant, quercetin is one of them. Fraction C was investigated for the presence of flavonoids together with ethyl acetate-enriched extract was compared to a standards quercetin by TLC technique, in addition to that, plant extract was investigated by TLC in three different solvent systems and compared to the reported one[72], all tests revealed the presence of quercetin free flavonoids as shown in Table 3.2

Solvent System	Reported R_f of Quercetin	Observed R_f	R_f of Standard Quercetin
1. Chloroform: Acetone: Formic acid (75:16.5:8.5)	0.43	0.45	0.45
2. Chloroform: Methanol (90:10)	0.39	0.42	0.41
3. Toluene: Chloroform: Acetone (40:25:35).	0.70	0.71	0.70

Table 3.2: R_f values for flavonoids

3.3-Isolation of Glycosides:

Glycosides are reported to be found in *equisetum arvense*, mainly those which are having flavonoid aglycones. [73].

Among those flavonoid glycosides, rutin was investigated in fraction A by TLC and compared to standard rutin, results was as shown in table 3.3

Solvent System[74]	R_f of Standard Rutin	Observed R_f of sample
1. chloroform: acetone: formic acid(75:16.5:8.5)	0.30	0.30
2. n.butanol: glacial acetic acid:water (40: 10:50)	0.53	0.55

Table 3.3: Rf values for flavonoids glycosides

HPLC technique was used to investigate the presence of some other flavonoid glycosides (Table 3.4)

Compound	Retention time of standard	Retention time of sample
1. Apigenin-5-O-glucoside	1.37	1.38
2.Kaempferol-3-O-glucoside	2.75	2.74
3.Isoquercetrin	3.74	3.78

Table 3.4: Retention time of flavonoid glycosides

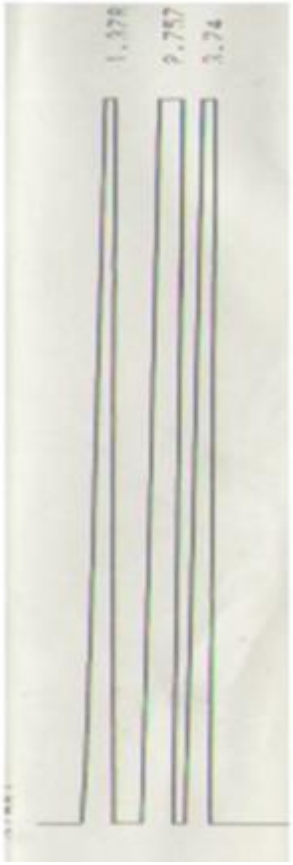
	
HPLC results of plant extract	HPLC result of standards of flavonoid glycosides

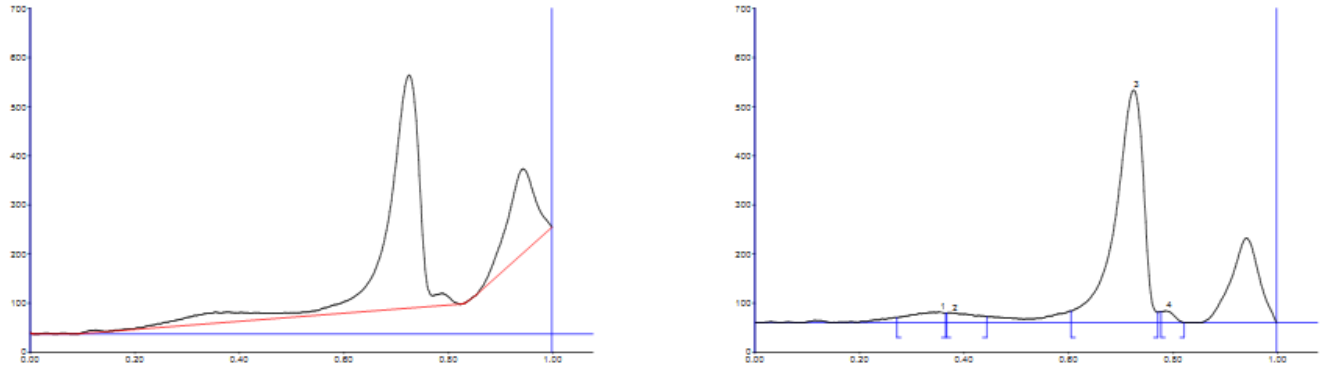
Table 3.5: HPLC Results of plant extract versus standards

All the three above glycosides are reported to be found in Equisetum arvense plant [75][76]

3.4- HPTLC results

Quercetin standard was used versus plant ethanol extract, results confirmed the presence of the flavonoid quercetin as shown in the next figures:

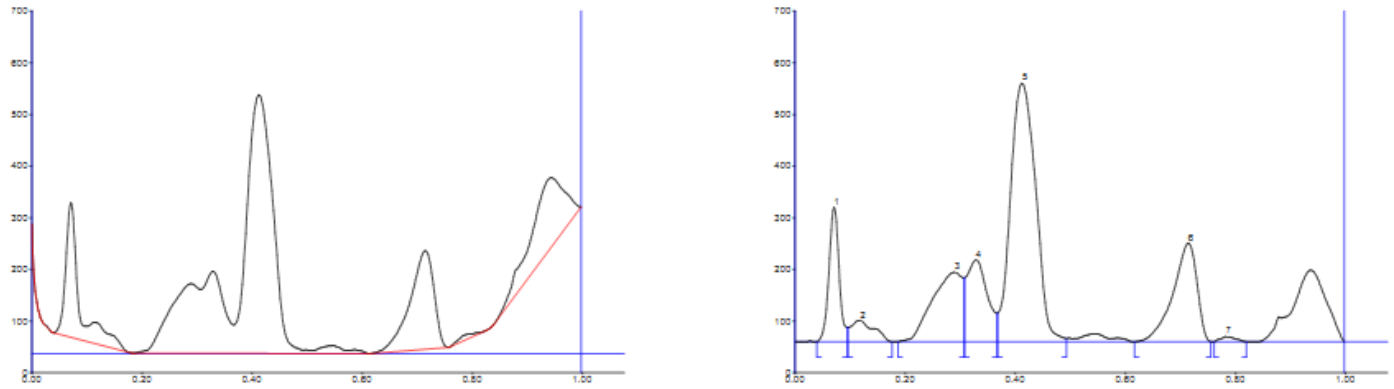
Track 1, ID: Standard1



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.27	10.9	0.36	22.3	4.11	0.37	19.9	1107.5	5.09	unknown *
2	0.37	20.1	0.38	20.7	3.82	0.44	12.6	870.3	4.00	unknown *
3	0.61	24.0	0.73	474.3	87.51	0.77	21.4	19348.1	88.86	unknown *
4	0.78	22.9	0.79	24.8	4.57	0.82	0.1	447.4	2.05	unknown *

Figure 3.7: HPTLC chart of Quercetin standard

Track 2, ID: Plant extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.0	0.07	260.5	20.08	0.10	28.2	3669.3	9.44	unknown *
2	0.10	28.2	0.12	41.4	3.19	0.18	0.1	1309.9	3.37	unknown *
3	0.19	0.6	0.29	134.8	10.39	0.31	123.8	5586.2	14.37	unknown *
4	0.31	124.3	0.33	158.8	12.24	0.37	55.4	4510.8	11.60	unknown *
5	0.37	55.9	0.41	500.5	38.58	0.49	6.7	17309.7	44.52	unknown *
6	0.62	0.5	0.72	190.8	14.71	0.76	0.3	6279.6	16.15	unknown *
7	0.76	0.3	0.78	10.5	0.81	0.82	0.6	216.2	0.56	unknown *

Figure 3.8: HPTLC of plant extract

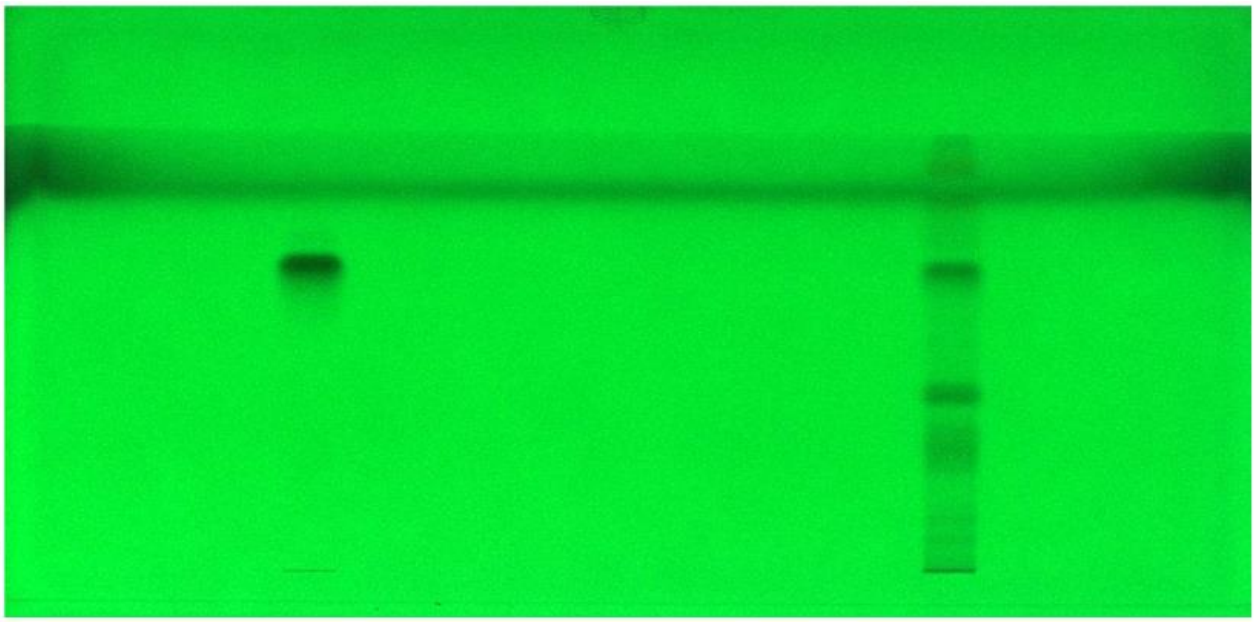


Figure 3.9: HPTLC plate under UV 256 nm

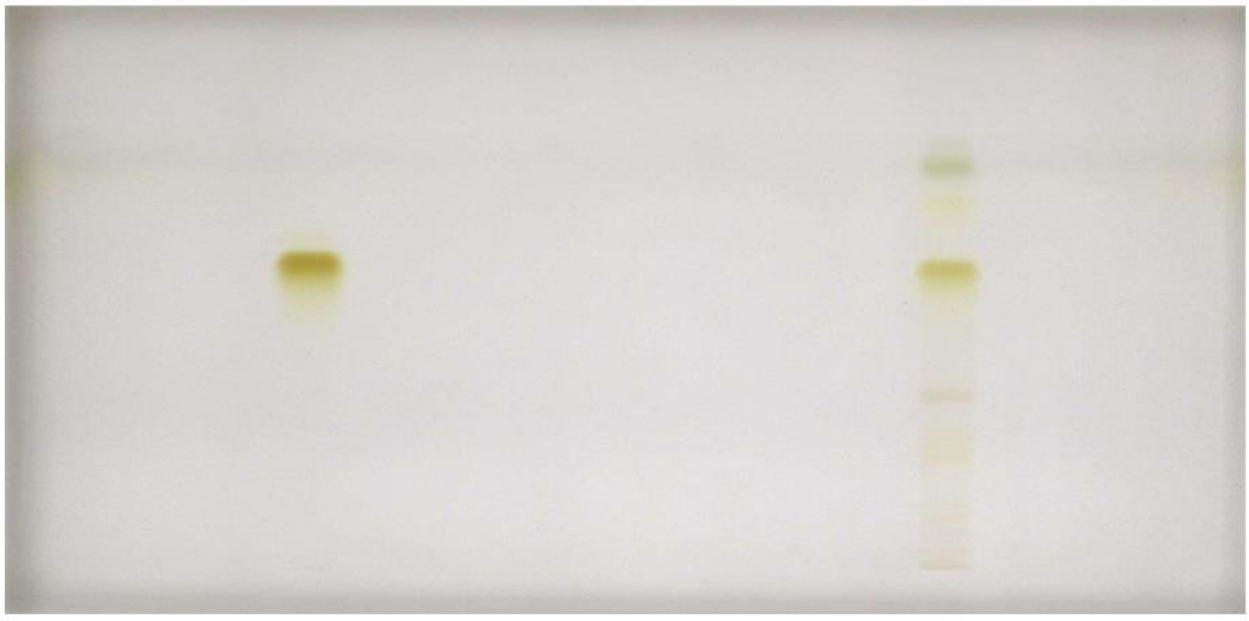


Figure 3.10: HPTLC plate under white light

3.5-Cytotoxic activity:

Three different plant extracts were investigated for their cytotoxic activity; all of them show a detectable activity of cell growth inhibition for the human cervical carcinoma cells (HeLa cells).

Ethyl acetate, Ethanolic, and aqueous extracts show the ability of plant extract to inhibit cell growth as follows:

1- Ethyl acetate extract:

   Upon measuring the Optical Density (OD) value for the wells incubated with ethyl acetate extract, the average value of all readings was 0.2622 as shown in figure 3.11

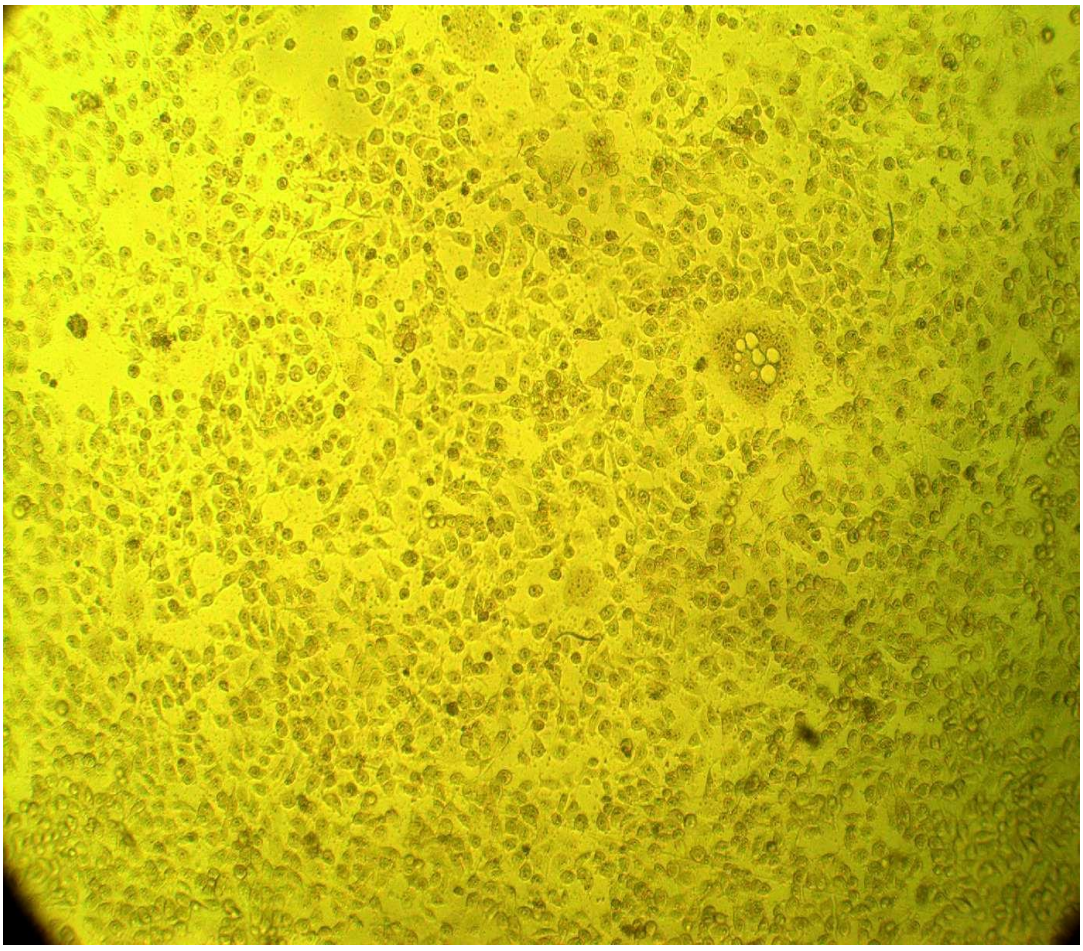


Figure 3.11: Cytotoxicity of Ethyl acetate layer

2- Ethanolic extracts:

Upon measuring the Optical Density (OD) value for the wells incubated with Ethanolic extract, the average of all readings was 0.352 as shown in 3.12 and 3.13

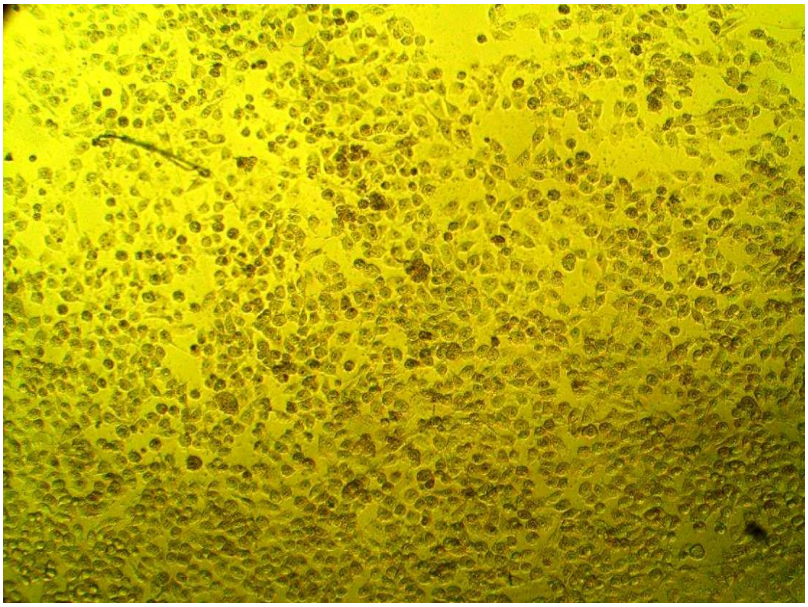


Figure 3.12: Cytotoxicity of Ethanol extract

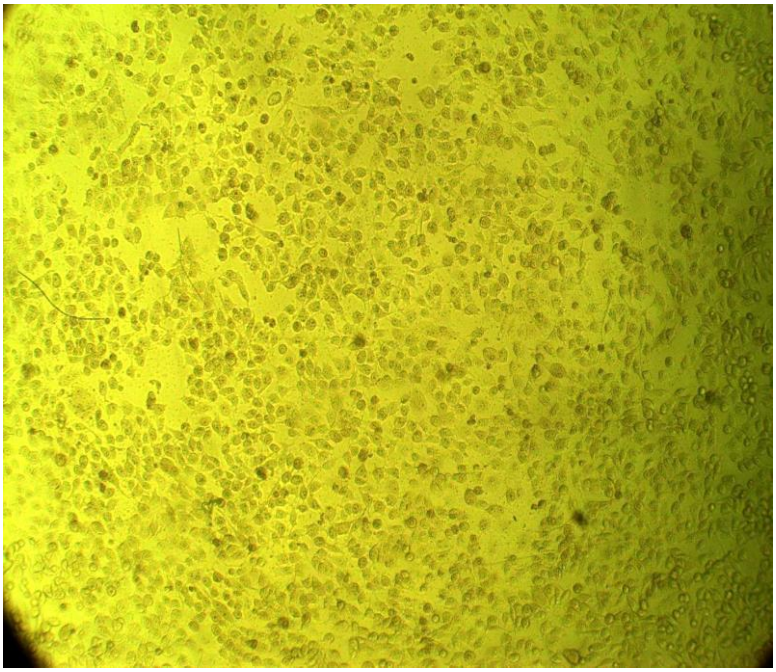


Figure 3.13: Cytotoxicity of Ethanol extract 2

3- Aqueous extract:

Upon measuring the Optical Density value for the wells incubated with aqueous extract, the average of all readings was 0.3902. as shown in Figure 3.14, and 3.15.

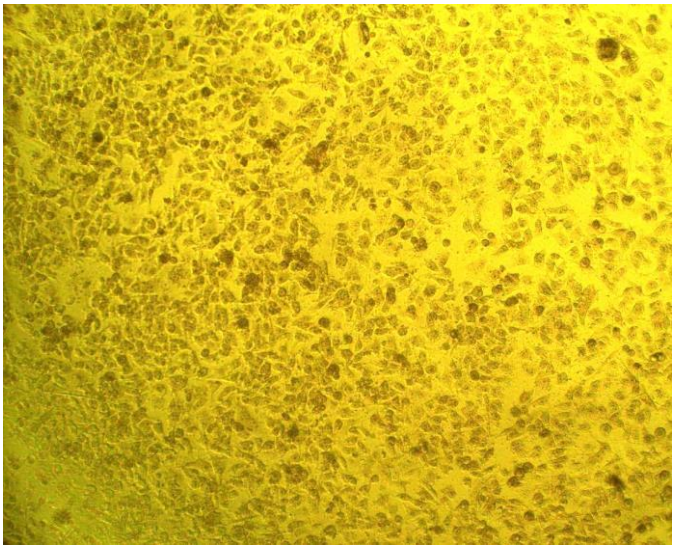


Figure 3.14: Cytotoxicity of aqueous extract 1

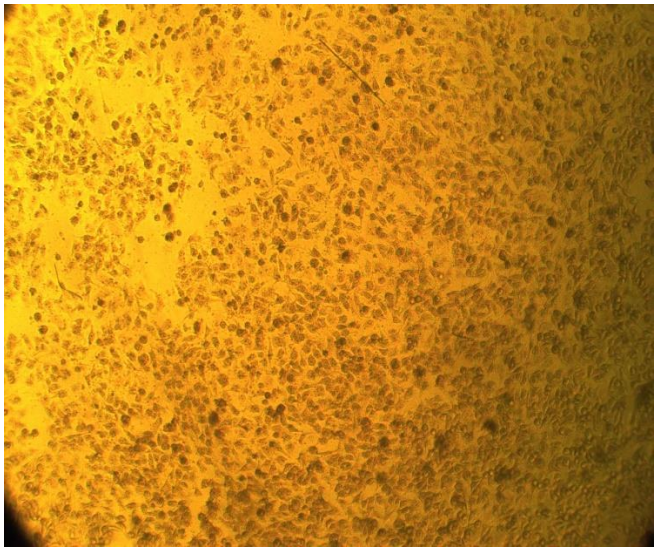


Figure 3.15: Cytoxicity of aqueous extract 2

4- Control wells:

Upon measuring the Optical Density value for the control wells that were incubated alone without any extract, the average of all readings was 0.407.figure 3.16, 3.17 and 3.18

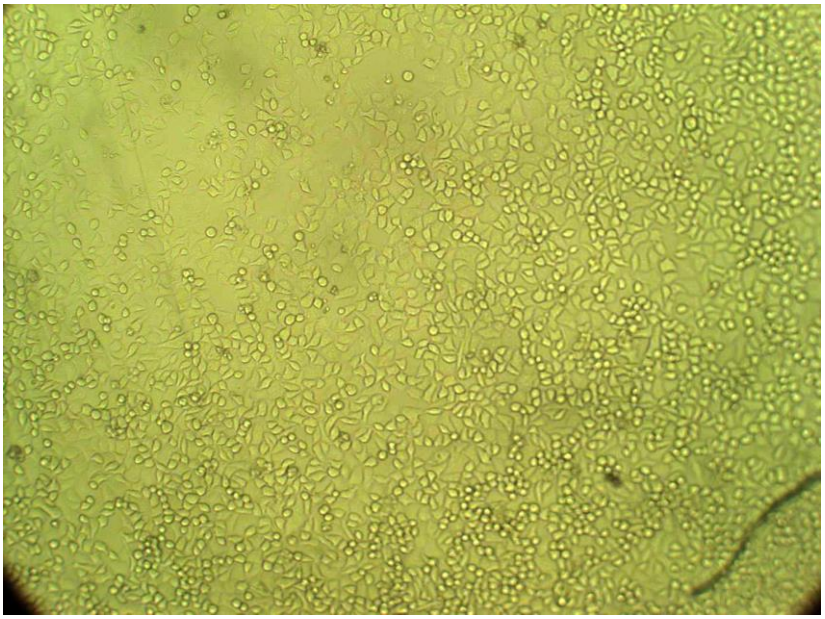


Figure 3.16: Control 1

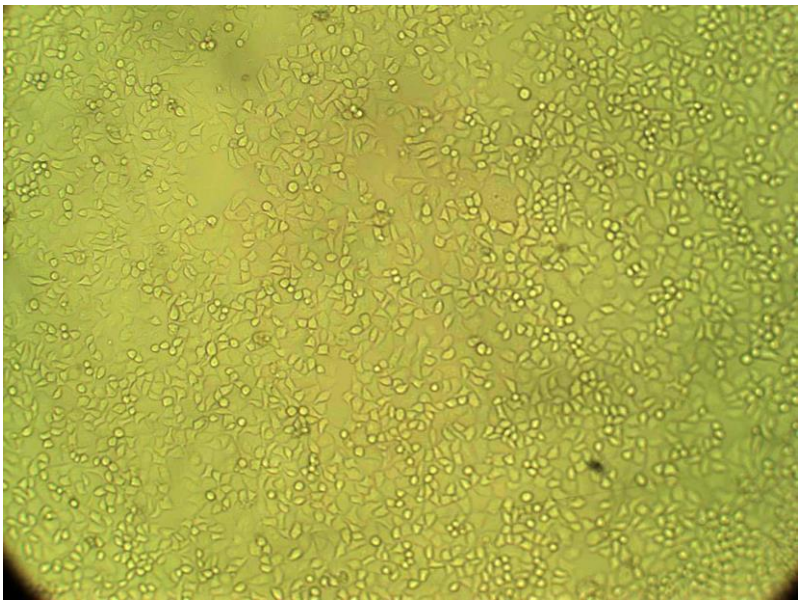


Figure 3.17: Control 2

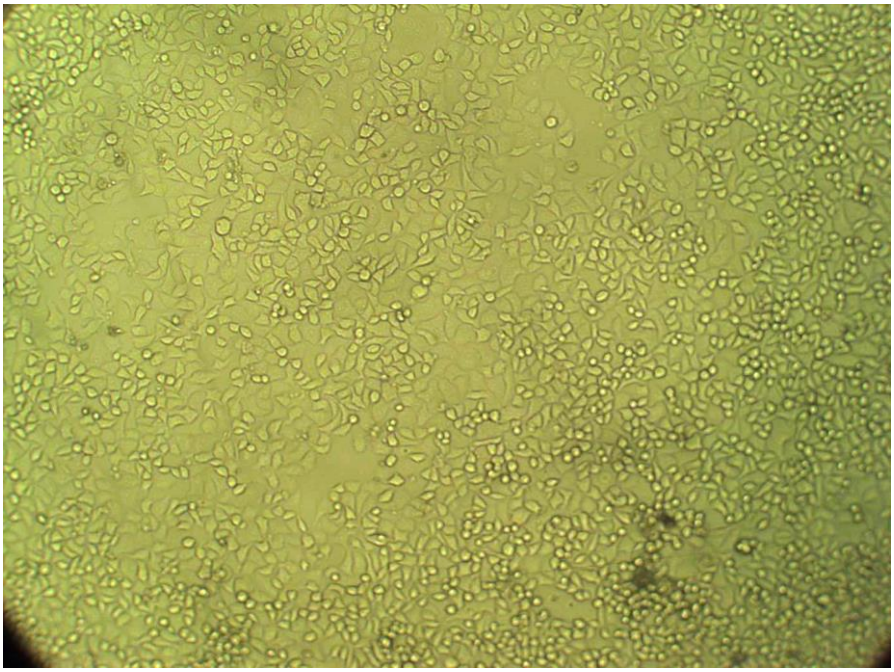


Figure 3.18: Control 3

Calculations:

To estimate the activity of each extract separately, the viable survived cells percentage after incubation is calculated by the following equation, the Optical Density (OD) value of each extract is divided by that of the control wells and multiplied by 100:

1- Ethyl acetate extract:

   0.2622 / 0.407 × 100 = 64.4 % of cells survived.

   Therefore, 35.57 % of cells died, which represent the percentage of cellular growth inhibition.

2- Ethanolic Extract:

   0.352 / 0.407 × 100 = 84.48 % of cells survived.

   Therefore, 13.51 % of cells died, which represent the percentage of cellular growth inhibition.

3- Aqueous extract:

0.3902 / 0.407 × 100 = 95.87 % of cells survived.

Therefore, 4.12 % of cells died, which represent the percentage of cellular growth inhibition.

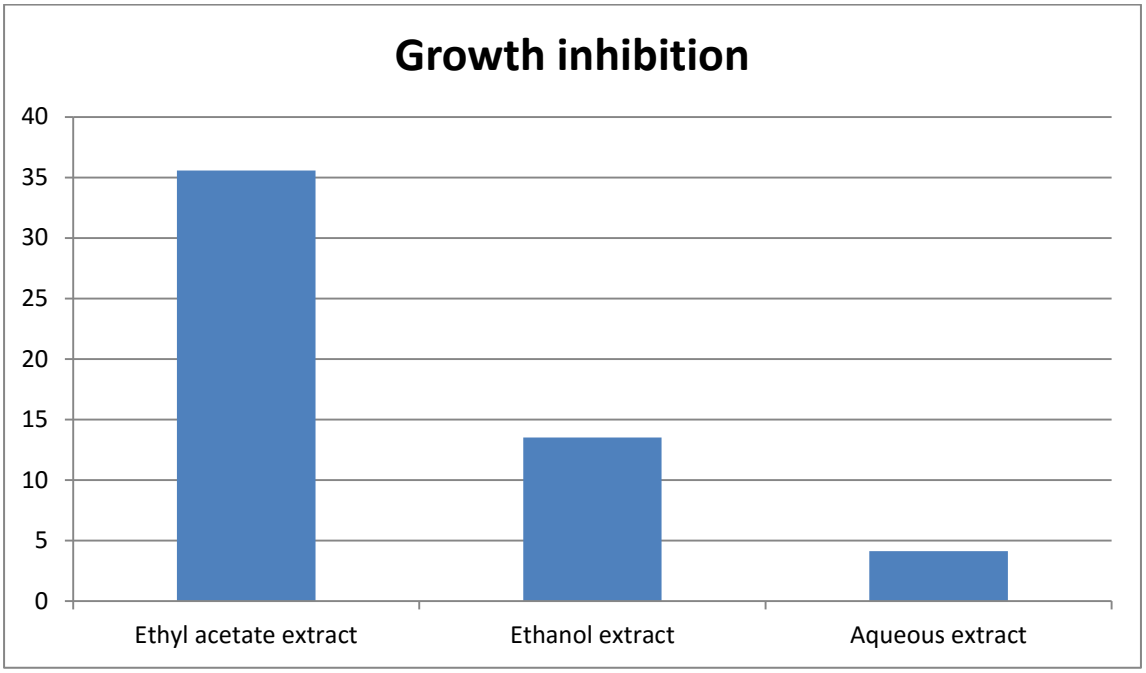


Figure 3.19: Schematic comparison of different cytotoxicity effect

The optical density measured is related to the amount of formazan formed by the action of dehydrogenase enzymes that are limited to viable cells only, the amount of formazan is directly proportional to the extent of viable survived cells in wells figure 3.20.

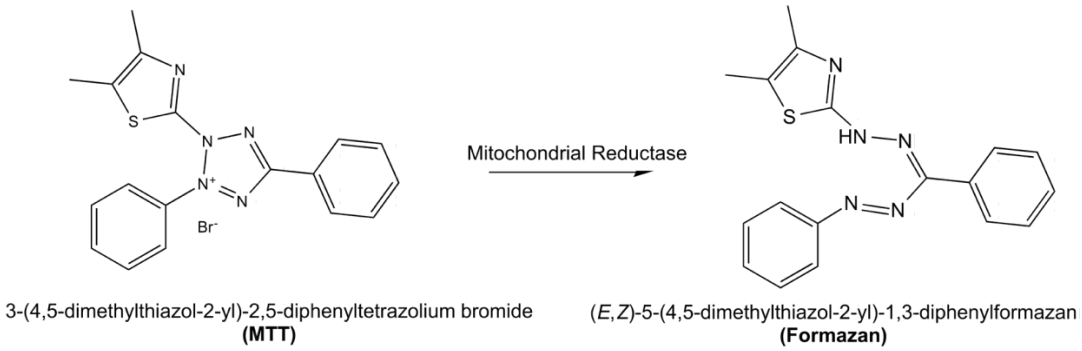


Figure 3.20: Conversion of MTT into Formazan

Cytotoxic activity of the plant is known to be attributed mainly to flavonoids and to lesser extent to their glycosides.[77].

Ethyl acetate extract is reported to be the one that has the most potent anticancer effect against different cell lines [78]

Ethyl acetate is known to be the best enriching solvent for flavonoids [79], therefore the highest amount of flavonoids are present in ethyl acetate extract, thus the highest percentage of cellular growth inhibition was observed in the ethyl acetate extract.

Ethanol solubilize both the free flavonoids and their glycosides, this explain the reduced cytotoxic activity when compared with the ethyl acetate extract.

Regarding the aqueous extract, which was partitioned with Ethyl acetate, contains the least amount of flavonoids (especially free aglycones) for this reason, aqueous extract cytotoxicity was the least when compared to ethyl acetate and Ethanol extract.

HPLC analysis of the ethyl acetate layer reveals the presence of iso-quercetrine flavonoid (retention time 3.7) as a major component of the ethyl acetate extract as compared with standard.

4-Conclusion

Phytochemical study of Iraqi Horsetail (Equisetum arvense) reveals the presence of the alkaloid nicotine as shown in GC/MS analysis. The flavonoid quercetin, and flavonoid glycosides (Apigenin-5-O-glucoside, Kaempferol-3-O-glucoside, and Quercetin-3-O-glucoside) were found in the plant as shown by HPLC, Quercetin flavonoid was confirmed to be present in the plant.

Cytotoxic study for plant extracts reveals the ability of plant to inhibit the cellular growth of HeLa cell line with different extents, the inhibition rate rank was observed in Ethyl acetate extract>Ethanol extract>Aqueous extract.

5-Recommendation

As a researcher, I would like to increase the awareness of the medical society to the importance of plants and herbs, a lot of indications could be managed more safely by using natural products compared to conventional medicines.

Regarding the Iraqi Horsetail (Equisetum arvense), an extensive chemical, pharmacological, toxicological and pharmacokinetic studies are required to assure the ability to introduce a new anticancer agent.

I suggest the use of plant extract as a Nicotine replacement therapy in individuals that are planning for smoking cessation.

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الخلاصة

ان نبات كنباث الحقول (ذنب الخيل) من النباتات الطبية المعروفة والتي لها تاريخ طويل من الاستعمال في الطب التقليدي في مختلف انحاء العالم لعلاج امراض متعددة اهمها تلك الامراض المتعلقة بالكليتين والمجاري البولية, تم اجراء دراسة لمعرفة واثبات وجود المركبات الكيميائية في النبات, وتم توثيق وجود القلويد نيكوتين, الفلافونويد كويرستين, وبعض الفلافونويد كلايكوسايد واهمها آيزكويرسترين.

تم اجراء دراسة لمعرفة نشاط النبات على النمو الخلوي لخلايا سرطان عنق الرحم, تم استخدام ثلاث مستخلصات مختلفة للنبات, واظهرت النتائج ان الثلاث انواع من المستخلصات سببت ايقاف للنمو الخلوي للخلايا السرطانية بنسب مختلفة.

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

الجامعة المستنصرية

كلية الصيدلة

دراسة كيمونباتية لنبات ذنب الخيل العراقي

رسالة مقدمة إلى فرع العقاقير والنباتات الطبية والى لجنة الدراسات العليا في كلية الصيدلة/الجامعة المستنصرية كجزء من متطلبات الحصول على شهادة الماجستير في علوم الصيدلة (فرع العقاقير والنباتات الطبية)

من قبل

الصيدلاني

حيدر محمد بديع البدري

(بكالوريوس صيدلة 2011)

بإشراف

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