Summary

This research was conducted to study the biological activity of *Fumaria officinalis* plant extract on laboratory animals.

The herb *Fumaria officinalis* is a wide growing plant, has a wide reputation among herb- experts in Iraq, it's a folk traditions

Few photochemical investigation of this plant had been reported previously but non on the Iraqi plant, thus indicating that a research on this plant might be of value

Chemical detection was done to identify the active compound present in it. Two kinds of extracts were prepared from plant powder of *Fumaria officinalis*, that are aqueous and ethanolic extract, these two different extract of *Fumaria officinalis* powdered plant were tested for their *in-vitro* antimicrobial activity .

In a screening program from the Iraqi alkaloid containing plants and chemical investigation showed that *Fumaria officinalis* contain different active compounds, the main active compound is the alkaloid, it was found that this plant is rich in alkaloid.

Microorganisms, tested in this study were; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Trichophyton rubrum, Trichophyton mentagrophytes, and Trichophyton tonsurans.*

In –vitro tests showed that the best extract of *Fumaria officinalis* with highest antibacterial and antifungal activity is the ethanolic extract at a different concentrate.

In contrast *in- vitro* showed that aqueous extract of *Fumaria officinalis* has a great antihistaminic activity rather than ethanolic extract.

Also *in-vitro* studies of the values of liver enzyme (GPT, GOT, ALP, and BILL), theses values was varies due to effect of ethanolic extract of *Fumaria officinalis*

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1.1 Introduction

Drugs presently used for the Management of pain and inflammatory condition are narcotics (e.g., opiods), non-narcotic (e.g., Salicylates) or corticosteroids (e.g., hydrocortisone). All of these drugs caused well known side and toxic effects. Moreover synthetic drugs are very extensive to develop.

On the other hand, many medicines of herbal origin have been used long ago without any adverse effect. It is therefore essential that efforts be made to develop cheaper drugs. Medicinal plants and herbal medicine are one of the current areas of investigation that possess all the hallmarks of modern biomedical science. This necessitates efforts in order to identify plants that have potential for medical cure.

In herbal medicine the term herbs is used loosely to refer not only herbaceous plant but also to bark, roots, leaves, seed, flower, and fruit of tree, shrubs, and woody vines; and extract of the same that are valued for their savory, aromatic, or medicinal qualities. The botanical term herb refers to seed-producing plant with non woody streams that die down at the end of the growing season.

The plant have played a significant role in maintaining human health and improving the quality of human life for thousands of yeas, and have served humans well as valuable components of seasonings beverages, cosmetics, dyes and medicine.

Fumaria officinalis or earth smoke has been used in popular medicine for its antiallergic activity, antimicrobial, and antitumor agents.

Because of the importance of this plant that leads to these properties, this study was aimed to:

1- Preparation of ethanolic and aqueous extract of *Fumaria officinalis*.

2- Detection of some active compounds in *Fumaria officinalis* alcoholic and aqueous extracts.

4- Determine the antimicrobial activity of *Fumaria officinalis* extract against some bacteria and fungal strains.

5. Detection the cytotoxic effect of crude extract *Fumaria officinalis* on normal cell (mouse embryo fibroblast) and myloma cell-line.

6. Allergy assay on laboratory animals.

Literature Review

2.1 History of Medicinal Plants

Herbal medicine is among the oldest of medical techniques. The oldest evidence of herbalism appears to date back 60.00 years. As recounted by Griggs, a Neanderthal grave in Iraq contains the pollens of seven plants including, *Althea*, *Ephedra* species. These herbs, together with two of five other plants in the grave, have tremendous medical significant even in modern-day herbalism Marsden *et al*., (1998).

Of these seven species are still used for medicine in dozens of different ways by the local people, for example, the mucilaginous root of the marsh mallow from *Althea* genus yield a soothing and healing remedy for irritant throats and disordered intestinal tracts; the deep dark-blue flowered grape hyacinth of *Muscari* genus is valued as a diuretic; and the shrub *Ephedra* used for asthma and cardiac stimulant a usage confirmed by modern science when nerve-stimulated ephedrine was extracted from it Hobhouse *et al*., (1981).

From history we have known that the people in the past wrote excellent work for melioration of human suffering due to ailments. Medicinal plant in Iraq can be traced back to the Sumerian period (3000-1970 B.C.) and then to the Babylonian. The plants that were used for the cure of diseases were not utilized at that time as were drug as used today but were used as magic medicine from the people of civilization Hobhouse *et al*., (1981).

The Arab Muslims have a great interest in medicinal plants throughout beginning the use of seeds and fruits of plants as a drug like *Atropa belladonna* (Al-Rawi and Chakravaty *et al*., 1964).

2.2 History of the Family *Fumariaceae* and the Genus *Fumaria*

Fumariodeae was considered as subfamily of the pavaveraceae family, order Rhoeadales, until 1956 when it was reported as a separated family of the same order and therefore the name was changed to *Fumaria* ceae, it is also called the fumitory family.

Fumaria cea include number of ornamentals horticultural inters and some plant of medical values which were highly esteemed by Greek and Romans, on account of their diuretic and alterative properties.

Formerly Jones in 1908 held it to be derived from the Greek name fumitory, Also according to Lindley and Moore (1870); they explained that the juice of plant brought tears to the eyes in such away that the site became dimmed as that caused by smoke hence it is used for eye infections.

Fumaria nomenclatural according to Gilbert Carter in 1964 is ultimately derived from Medieval Latin “fumur terrae” which means smoke of earth, (Investigation of the alkaloids of *Fumaria* wildly grown in Iraq).

Wren 1965 reported that *Fumaria officinalis* some times also called earth smoke and this name probably came from the appearance of the foliage on a dewy morning or possibly its smoky odor, It is interesting to note that medicinal use of *Fumaria* reported in other country is the same as that found in Iraq.

Fumaria is described as diuretic, hepatic, obstruction remover, aperients and expellant of the hormones, it is also considered as laxative, in dyspepsia, depending upon turbidity of the intestine, the seeds are used in India by the mandus as a remedy for pain (Investigation of the alkaloids of *Fumaria* wildly grown in Iraq).

2.3 Botanical Distribution

Fumaria ceae, as family, is composed of 17 genera and about 400 species chiefly distributed in the temperate and subtemperate regions of northern hemisphere (Meikie, 1977), *Fumaria* species are chiefly found in Europe, and Mediterranean region, extending to Mongolia and North West India, very few are found in eastern Asia. Elsewhere, it is found in the Atlantic islands and mountains of tropical Africa, it occurs as weeds outside these areas. In Iraq *Fumaria* family (*Fumaria ceae*) is represented by three genera namely: *Fumaria* , Corydalis and Hypecoum. The first one Includes annual herb and so as the third, while the second include perennial herb. *Fumaria* is mostly found in the middle and northern part of the country and in the North West sector of the desert part.

It is found in: Sinjar, Maryaqub, Amadia, Aqra, Ain kibrit, Shaqlawa, Mosul, Baghdad, Jadrya, Rustamia, Zafarania, Babylon, Amara, and Basra.

Fumaria as a genera include about sixty species, 14 of them found in Turkey, 8 are found in Iran, 9 in Cypres, 33 in Europe, 10 of them are found in Britain (Tutin, 1964).

In Iraq six species are found namely:

Fumria cilicica, Fumaria bracteasa, Fumaria vaillantii, Fumaria parviflora, Fumaria asepala, and Fumaria officinalis.

2.4 Botanical Description

*Fumaria*ceae plant is annual or perennial herbs with brittle stems and colorless watery juice, erect, diffuse, scrambling or sometimes scandent. Leaves alternate, rarely apposite, commonly bi-ortri- pinnatisect or compoundly ternate.

Flower is racemes or spikes. Sepals 2, caduceus, rarely absent. petals 4, in two dissimilar pairs, one or both of the outer pairs saccate or spurred at the base, the two inner narrower and frequently epically coherent. The genus

Fumaria is glabrous, alternate leaves, the plant frequently climbing by means of prehensile cirrhoses petioles.

The flower is pink or white, the upper petal alone spurred at the base, in the leaf opposed bracteates racemes, upper and lower petals with a low, gibbous median keel, the laterals fused at the tip with the midribs developed into two bundles, ovules solitary, one on each placenta. The Fruit is an indehiscent nutlet, often rogues when dry. The medicinal parts of the *Fumaria officinalis* are the dried herb and the aerial part of the fresh flower plant (Investigation of the alkaloids of *Fumaria* wildly grown in Iraq).

2.5 Pharmacological Activities of *Fumaria*

Literature survey revealed that *Fumaria* plants have different pharmacological activities.

Some reported that isoquinolin alkaloids were increasing the arterial pressure, the amplitude of cardiac contraction, and the frequency of respiration in anesthetized cat. (Sarker, 1948) reported that sanguinarine; inhibit oxidative decarboxylation of pyruvic acid by inactivation, inhibition of sulfhdryl groups of enzyme.

It is also incorporated into expectorant mixture. (Nistry and Papeu, 1972) reported that subcutaneous administration of some isoquinolin alkaloid produced convulsions and a decrease in the spinal cord acetylcholine content.

Experimental work by (Rynier, 1977) found that *Fumaria* extract increase the volume of bile secretion when administered intraduodenally to anesthetized rats with hypocholeresis. (Rynier, 1977) also found that *Fumaria* extract showed both antispasmodic and spasmolytic activity.

Gorbunov *et al*., (1980) reported that *Fumaria officinalis* alkaloid showed cardiovascular activity, by decreasing or preventing myocardial ischemia caused by occlusion of the coronary artery.

2.6 Alkaloids of *Fumaria*

Most of the papaveraceae alkaloids in the genus *Fumaria* are include the following groups of alkaloid (Novak and Reiniger, 1984).

*Isoquinolines

*Benzylisoquinolines

*Protoberberines

*Tetrahydroprotoberberines

*Phthaliisoquinolines

*Secophthalideisoquinolines

*Benzophenathiridines

*Spirobenzylisoquinolines

*Protopines

* Morphinadienones

2.7 Isoquinolin Alkaloid

Alkaloids that possess an isoquinoline skeleton are among the most common of all alkaloids. *Fumaria* genus of these are derived from a 3,4-dihydroxytyramine (dopamine) precursor that undergoes a Schiffbase addition with aldehydes of different origin. At least 4000 compounds of this structural type are known.

The simplest type of isoquinoline alkaloids is based on carbonyl compounds such as glyoxylic acid, pyruvic acid, and an aldehyde derived from leucine. The second major structural type, benzylisoquinoline alkaloids and tetrahydrobenzylisoquinoline alkaloids are based on 3,4-dihydroxytyramine (dopamine) precursor and an aldehyde derived from tyrosine, 4-hydroxyphenylacetaldehyde. A third group, including emetine and a series of structurally related alkaloids, involves the same amine precursor condensing with secologanin, an iridoid monoterpene. Isoquinoline alkaloids are mostly found in the Cactaceae, but in some other families as well. About 130

isoquinoline alkaloids are known. They are derived from a 3,4-dihydroxytyramine (dopamine) precursor and have an isoquinoline skeleton.The skeleton is based on a 3,4-dihydroxytyramine (dopamine) precursor and carbonyl compounds such as glyoxylic acid, pyruvic acid, and an aldehyde derived from leucine (Internet).

2.8 Previous Phytochemical Studies

Although many literatures have been published about *Fumaria,* Platonova *et al*., (1956) and Hakim *et al*., (1961) reported the isolation of fumaramine, coptisine and sanguinarine from *Fumaria micrantha* which later on was designated as synonym for *Fumaria* .

Smolenski *et al.,* (1972) mentioned that *Fumaria officinalis* as a plant containing alkaloids in his screening article of plant containing alkaloids. Popova *et al.,* (1983) reported the isolation of alkaloid from this species namely isoquinolin alkaloid, new alkaloid named as *officinalis*.

2.9 Herbal Medicine

Records from ancient Egypt, Assyria, China and India show that the use of plants for medicinal purposes extends back to earliest recorded history, Indeed, their use is probably a natural progression from the distinctiveness for fumaria of animals to utilize such plants and constitutes a recognition of the complete spectrum of consumable plants, commencing with those that are poorly dietary; passing through those foods known to be necessary for health (fresh vegetables, carrots, fruit, which are now known to provide essential vitamins); on to products consumed in reasonable quantity as foods but known to have medicinal effects (e.g. figs, prunes, mucilage's acting as mild laxatives).

Finally the recognition of purely medicinal plants, some apparently quite 'safe' and others more potent (e.g. containing cardio active glycosides), which can be

consumed only in small quantities but which at such dosage are suitable for the treatment of certain diseases.

2.10 Functions of Alkaloid in Plants

The characteristic nature of alkaloids have and their often very marked pharmacological effects when administered to animals naturally led scientists to speculate on their biological role in the plants in which they occurred .In spite of many suggestions over the years , however , little convincing evidence for their function has been forthcoming .The following points are noteworthy:

* Being of such diverse nature, alkaloids as a group could not be expected to have a common role (if any) in the plant, except possibly in situations requiring a nonspecific basic compound.
* Alkaloids often occur in plants in association with characteristic acids- for example, the tropane alkaloids of the *Solanaceae* and *Erythroxylaceae* are esters, In some cases the alkaloids could provide either a means of storing or transporting in soluble form the particular acids.
* As the majority of alkaloids are biosynthesized from readily available units by a series of ubiquitous reactions, their presence in the plant may be purely chance, depending on the enzymes present and the availability of precursors. Being apparently harmless to the plant, they are not eliminated through necessity by natural selection.
* By the use of grafts, plants which normally accumulate alkaloids in the aerial parts (e.g. *Nicotine*, *Datura*) are produced free of alkaloids. The lack of alkaloid in the scion appears in no way to impair its development, which suggests the non – essential nature of the alkaloid.
* Plants which do not normally contain alkaloids appear usually to suffer no adverse reaction when administered alkaloids (colchicines is an exception). Some ‘foreign’ alkaloids may be metabolized.

* Current research constantly demonstrates not only those alkaloids participate in plant metabolism over the long term, but also that daily variation in alkaloid content (qualitative and quantitative) is very common in some species. This implies that even if the presence of alkaloids is not vital to the plant, they do participate in metabolic sequences and are not solely the waste, end products of metabolism (Larson and Marly, 1984).

2.11 Allergy

Allergy is an abnormal immune reaction of the body to allergens such as pollen, dust, certain foods, drugs, animal fur, animal pets, animal excretions, feathers, microorganisms, cosmetics, textiles, dyes, smoke, chemical pollutants and insect stings. Certain conditions such as cold, heat, or light may also cause allergic symptoms in some susceptible people. Some allergens are just specific to some individuals but not to others. Allergens may act via inhalation, ingestion, injection or by contact with the skin. The resulting allergy may cause the victim to have a medical problem such as hay fever (allergic rhinitis), or atopic dermatitis (eczema), or allergic asthma, with symptoms ranging from sneezing, rhinorrhea, nasal itch, obstruction to nasal air-flow, loss of sense of smell, watery and itching eyes as in allergic rhinitis; and skin itching, skin redness and skin lesion as in atopic dermatitis. Allergic patients suffer not only irritating symptoms but also an impairment of the quality of life. In 1989 allergy diseases affected 10% of the world's population (Vercelli and Geha1989). Today, allergy is even more common. It is now the most wide spread immunological disorder in humans, and the most prevalent and rapidly increasing chronic health problem, particularly in the industrialized countries where allergy affects one in four individuals. Furthermore allergy also causes a socioeconomic problem resulting in huge economic losses. In the U.S. an estimated 20% or more of its population is allergic to something (Lichtenstein, 1993), In Central Europe, pollinosis alone affects 10 percent of its population

(Puls and Bock, 1993). The incidence of allergic asthma in the U.S. rose by 60% between 1979 and 1989 (Bousquet, 1994), In the USA alone it costs billions of dollars per year (HayGlass, 1995). Allergic rhinitis alone affects 10-15% of the US population (Broide, 1995).

2.12 The Treatment of Allergy

The treatment of allergic diseases has benefited from the gradual understanding of allergy mechanisms. Allergic reaction is due to a change in the immunoreactivity of an individual. From current knowledge, its mechanism (Kay and Lichtenstein, 1993), when the antigen is presented to T-helper cells, B-lymphocytes will overproduce allergen-specific IgE antibodies. In the case of antigen contact, IgE bound to mast cells leads to mast cell activation and degranulation. As a result, mast cells release abnormal amounts of mediators such as histamine, PAF (platelet-activating factor), leukotrienes and prostaglandin D. These mediators will dilate blood vessels, increase permeability of small blood vessels, stimulate nerve endings, stimulate secretion of mucus in airways, or constrict bronchial airways, so as to induce local inflammation and cause various immediate symptoms or chronic symptoms.

Immunotherapy is a specific form of controlled allergen admission that changes immunoactivity into allergen tolerance. It has been used for more than 80 years but still represents a controversial treatment of allergic diseases (Mailing, 1994). It is appreciated that the efficacy of allergen immunotherapy is currently very low. Severe symptomatic reactions occasionally may occur, especially in asthmatic patients. In certain countries (UK and the Scandinavian countries) the use of immunotherapy has been greatly curtailed due to adverse reactions (International Rhinitis Management Working Group, 1994b; HayGlass, 1995).

2.13 Traditional Chinese Medicine (TCM)

With thousands of years of experience in treating diseases with natural materials, TCM still plays an important role in the health-care system of modern China and is officially recognized not only in China, but also in Japan and in some other eastern and south-eastern Asian countries which have the same cultural tradition (Zhu and Woerdenbag, 1995). People their still prefer to use traditional herbs for the treatment of allergy. There are several TCM prescriptions effective for allergic symptoms (Chen, 1996). Some of them are, the Minor-Bupleurum-Combination with *Pinellia magnolia* Combination (Saibokuto), consisting often herbs including Perilla and the Ephedra-Magnolia-Combination containing *Perilla*, have been traditionally used for the treatment of bronchial asthma. Studies showed that Saibokuto, and Minor-Blue-Dragon-Combination.

(Syoseiryuto) clinically exhibit inhibitory effects on type I allergic reaction (Umesato, 1984) and Saibokuto was found to inhibit histamine release and mast cell degranulation (Toda, 1988). Furthermore, there has been increasing interest in western countries for TCM. British scientists achieved impressive results with a TCM prescription (Zemaphyte) containing 10 Chinese herbs for the treatment of severe atopic asthma (Sheehan, 1992; Cooper, 1994; Sheehan and Atherton, 1994; Latchman 1995). In recent years, many researchers have been interested in elucidating the function of TCM as biological response modifiers or immune regulators. It has also been confirmed that some common vegetables or herbs contain nonnutritive components that may provide protection against chronic diseases including allergy and even some forms of cancer (Haranaka, 1985; Chen, 1989; Kawakita, 1990; Yamazaki, 1992).

2.14 The Effect of Some Herbs on Pathogenic Bacteria

Herbal drugs such as the leaves of tea plant or perilla have also been used as food for a long time. The biological (antimicrobial) activities of these materials are usually not as those antibiotics. However, when the active components are accumulated, significant effects to the living organism can be produced, even though activity is relatively weak. Bacteriostatic compounds in the plants may also be useful for the development of food additives to preserve the food for a longer time without harmful side effects (Terauchi *et al*., 1997).

2.14.1 *Staphylococcus aureus*

Staphylococcus aureus is a major pathogen for humans. Almost every person will have some types of *S. aureus* infection during poisoning or minor skin infections to sever life-threatening infections. *Staphylococcus aureus* infection can also result from direct contamination and a wound like the post operative Staphylococcal wound infection or infection following trauma (chronic osteomylitis subsequent to an open fracture, meningitis following skull fracture) (Baily and Scott, 1974). The natural history of *Staphylococcus aureus* can be summarized as follows: many ceonates and mist children and adults become intermediately colonized by *S. aureus*, harboring the organism either in their nasopharynx, or in their vagina and exceptionally in the rectum or perinea area. From these sites, *S. aureus* can contaminate any site on skin or mucous membrane, or other subjects by interpersonal transfer by air or direct contact (Lecture and Macfadden, 1999).

2.14.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa are Gram negative rods and has assumed an important role as a causative organism especially in hospital-acquired infections and an increasingly frequent cause of death due to a bacterial species. Also *P. aeruginosa* plays a role in the urinary tract infections and skin burns and

similarly patient with lowered resistance and children. In addition the wide spread use of antibiotic in hospitals leads to an increase in the incidence of resistance strain of *Pseudomonas aeruginosa* (Ronald, 1995).

Further more, *Pseudomonas aeruginosa* play an important role in spoilage of food, dairy products, meat, poultry and eggs, so that it causes different intestinal infection.

2.14.3 *Escherichia coli*

Escherichia coli is a Gram-negative bacterium, rods related to Enterobacteriaceae family. *E. coli* is a member of the normal intestinal flora and also found as normal flora in the upper respiratory and genital tracts. The pathogenesis of *E. coli* includes:-

* Urinary Tract Infection
* *E. coli* associated Diarrhea Disease**.**
* Sepsis**:** When normal host defenses are inadequate, *E. coli* may reach the blood stream and causes sepsis.
* Newborns may by highly susceptible to *E. coli* sepsis (Jawetz, 1998).

2.15 The Effect of Some Herbs on Pathogenic Fungi

During the past five decades, there has been a significant increase in studies concerning fungal infections in both human and lower animals. Historically, a dermatophyte *Trichophyton schoenleinii* was the first microorganism that was proved to cause an infectious disease of humans. Although fungal infections of the skin were (and still are) regarded as the most common and relatively beginning of disease, their profile has taken a new and direct aspect reflected in new pathological condition (John *et al*., 1996).

Fungi cause direct harm to man and animal by means of toxins or by inducing allergic reaction, or by progressive mycosis. About one –fifth of the world population suffers from mycosis. In man most infections involve the skin,

hair and nail (superficial mycosis). These mycotic infection are unpleasant and be difficult to cure, but will not normally be lethal. Infections inside the body (deep-seated mycosis) are much more dangerous. They may become generalized and fatal unless treated. The agents of most superficial mycosis are a group known as dermatophytes, belonging to the genera microsporium, Epidermatophyton and trichophyton. The various dermatophytes usually cause virtually identical lesions of the infections involving the same part of the body (McFadden *et al.*, 1999). Dermatophytosis refers to infections of the skin, nails, or hairs that are caused by fungi classified as deratophyts.

Normally, it is not considered that dermatophytic fungi can cause systemic disease, in fact with only one miner exception, non-of these fungi can grow at 37°C. the dermatophytic fungi includes numerous species of fungi, which consist of three major genera. These organisms occur world wide mainly in the soil and on certain animals.

2.15.1 *Trichophyton rubrum*

Trichophyton rubrum is a cosmopolitan, anthropophilic species. It has recently become the most frequent etiologic agent of human ringworm especially tinea cruris through out the word. *Trichophyton rubmrum* is reported to cause tinea pedis in children as young as age two years; it does not invade the nail plate.

Trichophyton rubmrum is chronic and some individuals become lifetime carriers once infected by this fungus, (John, 1996). It may also cause tinea corporis. Skin lesions caused by *Trichophyton rubmrum* have red margin; the central portion may appear to be relatively clear, although scaling may be apparent. Once rare occasion, this organism may cause tinea capotis. Infected hair does not fluoresce and hair invasion is endothrix. Because culture of *Trichophyton rubmrum* is quite variable, identification can be frustrating.

However most mycologists believe that identification is important because rigorous and extensive therapy is often required.

The other form of *Trichophyton rubmrum* is called the granular form, this white colony is very flat, lacks, aerial hyphae and has pronounced granular appearance; undersurface of the colonies of *Trichophyton rubmrum* are usually red. Sometimes this red color is very bright, but often it has such a deep shade that it appears to have brown mixed with it (Bulmer, 1979). *Trichophyton rubmrum* is the most common isolate from all regions of the body except the scalp.

2.15.2 *Trichophyton mentagrophytes*

This fungus is a worldwide cause of athlete's foot. It also can cause tinea capitis, tinea barbae and tinea cruris. In many instance these infections are sporadic. According to many authorities infections by *Trichophyton metagrophytes* are not difficult to cure. There are several different variants of this organism; some are anthropophilic, while others are zoophilic. If hair is infected, the infection is endothrix (Bulmer, 1979). *Trichophyton metagrophytes* is cosmopolitan species and is the most commonly isolated dermatophyte from man and animal. Typical colonies of *Trichophyton mentagrophytes* are flat with a cream to buff or tan, powdery surface. *Trichophyton mentagrophytes* tend to produce deeply pigmented colonies (Kwon Chung and Bennett, 1992). It has several different colonial forms. One major form is called "downy" because the culture is white. Very fluffy and grossly has downy appearance. Usually this form is anthropophilic. The other major form of *Trichophyton metagrophytes* is "granular"; usually this colonial type is zoophilic in origin and when it infected man, may induce considerable inflammation (Bulmer, 1979). The several side of the colony is rose brown, occasionally orange to deep red in color (Bailey and Scott, 1974). *Trichophyton mentagrophytes* is most commonly isolated in

clinical laboratories and it produces a positive urease reaction within two or three days (Koneman *et al.*, 1978).

2.15.3 *Trichophyton tonsurance*

This organism is anthropophilic and is worldwide, although major endemic areas are North and Central America, Northern Europe, Southeast Asia and many of South Pacific Islands.

The organisms cause endothrix hair infections in which the inside of the entire shaft seems to be filled with spores. The type of hair infection causes the hair to burst open or to grow in a coil in the stratum corneum. These coils of hair are grossly visible and are referred to as "black dot" tinea pedis. *Trichophyton tonsurance* may cause tinea corporis, tinea pedis and occasionally onychomycosis.

Some people refer to this organism as the etiological agent of adult ringworm of the scalp; this disease is considered to be contagious. Infected hairs do not fluoresce. Colonies of *Trichophyton tonsurance* look powdery and are cream yellow to reddish brown color. The underside of the colonies is a rich red to brown color. Colonies often have suede appearance, i.e., short, aerial hyphae with many wrinkles and folds. The identification of *Trichophyton tonsurance* can be improved by using a series of *Trichophyton* agars, Skin and nail lesion caused by *Trichophyton tonsurance* are indistinguishable from other ringworms (Kwon- Chung and *et al*., 1992).

2.16 Cytotoxiciy

Several experiments carried out for the sole the purpose of determining the potential cytotoxiciy of compounds under study, either because they are being used as pharmaceutical or cosmetic and must be shown non toxic, or because they are designed as anticancer agents, when Cytotoxiciy may be critical to their action.

Cytotoxiciy is a complex event *in vivo*, where its expression may be manifest in a wide spectrum of effects, from simple cell death as in the toxic effects of anticancer drugs on both cells of tumor and normal cell of the bone marrow, skin, or gut, to complex metabolic aberrations such as neuro – or nephrotoxicity, where no cell death my occur, only function change. Definition of cytotoxicity will tend to very depending on the nature of the study, whether cell are killed or simply have their metabolism altered. While anticancer agent may be required to kill cells (through it need not), the proof of the absence of toxicity of another pharmaceutical may require more subtle analysis of minor metabolic change or an alternation in cell – cell signaling such as might give rise to an inflammatory or allergic response (Ian Freshney *et al*., 1994).

It is important that any measurement can be interpreted in terms of the *in vivo* response, or at least with the understanding that clear differences exist between *in vitro* and *in vivo* measurements. Measurements of toxicity *in vitro* are a purely cellular event as presently carried out. It would be very difficult to re-create the complex pharmacokinetics of drug exposure, for example *in vitro*, and there will usually be significant difference in drug exposure time and concentration, rate of change of concentration, metabolism, tissue penetration, clearance, and excretion. Although it may be possible to stimulate these parameters, e.g., using multicultural tumor spheroids for drug penetration, most studies concentrated on a direct cellular response.

Many nontoxic substances become toxic after metabolism by the liver; in addition, many substances that are toxic *in vitro* may be detoxified by liver enzymes. For testing *in vitro* to be accept as an alternative to animal testing, it must be demonstrated that potential toxic reach the cells *in vitro* this may require additional processing by purified liver microsomal enzyme preparations or co-culture with activated hepatocytes (Ian Freshney *et al*., 1994).

The nature of the response must also be considered carefully. A toxic response must also consider carefully. A toxic response *in vitro* may be

measured by changes in cell survival or metabolism, while the major problem *in vivo* may be a tissue response, an inflammatory reaction or fibrosis. For *in vitro* testing to be more effective, constriction of models of these responses will to be required, perhaps utilizing cultures reassembled from several different cell types and maintained in the appropriate hormonal milieu.

It should not be assumed that complex tissue and even systemic reactions cannot be simulated *in vitro.* Assays for the inflammatory response, teratogenic disorders, or neurological dysfunctions may be feasible *in vitro,* given a proper understanding of cell- cell interaction and the interplay of endocrine hormones with local paracrine and autocrine factors. Normal cell are specialized, they have a specific form and function that suit them to the role they play in the body. Normal cell are growing under controlled mechanism, contact inhibition, one organized layer, and differentiated cells (Comess, 1990). For example, mouse embryo fibroblast cell line and chick embryo cell line. Normal cell line can enter the cell cycle for about 50 times, and then they die, while cancer cell can enter the cycle repeatedly. In the body, a cancer cell divides to form an abnormal mass of cell called a (tumor), which invaded and destroys neighboring tissue (Silivia, 1998).

Materials and Methods

3.1 Equipment and Apparatus

The following equipments and apparatus were used throughout the study:

No.	Equipment	Company (Origin)
1	Autoclave	Gallenkamp
2	Balance	Mettler (Switzerland)
3	Centrifuge	M. SE (U.K.)
4	Distillator	Kenet (England)
5	ELISA	Organo Teknika (Microwell System)
6	Incubator	Gallenkamp
7	Milipore filter	Millipore corp (U.S.A.)
8	Oven	Gallenkamp
9	pH meter	Metter-Toledo (U.K.)
10	Reflex	Locally Made
11	Rotary Evaporator	Buchi (Switzerland)
12	Water Bath	Gallenkamp

3.2 Chemicals

The following chemicals were used throughout the study:

No.	Name	Company (Origin)
1	Ammonia	BDH (England)
2	Chloroform	BDH
3	Diethyl Ether	BDH
4	Ethanol	BDH
5	Ethyl Acetate	BDH
6	H_2SO_4	BDH
7	HCl	BDH
8	Hexane	BDH
9	KI	BDH
10	Mercuric chloride	BDH
11	Methanol	BDH
12	$MgCl_2$	BDH
13	Petroleum ether	BDH
14	petroleum ether	BDH
15	Potassium iodide	BDH

3.3 Plant Collection and Preparation

Fumaria officinalis plant was collected by cutting it from its root, from the west region of Iraq, and then identified by the Iraqi national herbarium (Baghdad Abu-Graib).

After cleaning the plant it was left in a dark ventilated place for drying, the dried plant cutted into small pieces for grinding to make it in powdery form, and then kept until used.

3.4 Plants Extract

3.4.1 Water Extract

Plant powdered material macerated with distilled water in a ratio of (1:7g/ml) (50 g of the powdered plant mixed with 350 ml. of sterilized distilled water). During extraction the mixture was shacked continuously in a shaker incubator for 5-7 hours at room temperature; the suspension was filtered through a filter of fine gauze to get rid of the large particles then filtered through a filter paper (Whatman No.1) the filtrate was concentrated using vacuum rotary evaporator at 40°C.

3.4.2 Ethanolic Extract

Fifty grams of *Fumaria officinalis* plant powder was extracted with 500 ml of 70% ethanol under continuous stirring for few hours at room temperature, the suspension was filtered using vacuum rotary evaporator at 40°C. pH was adjusted to 3 and then chloroform was added (3 times), the upper layer was discarded and the lower layer was collected (Figure 3-1) (Montanari *et al*., 1998).

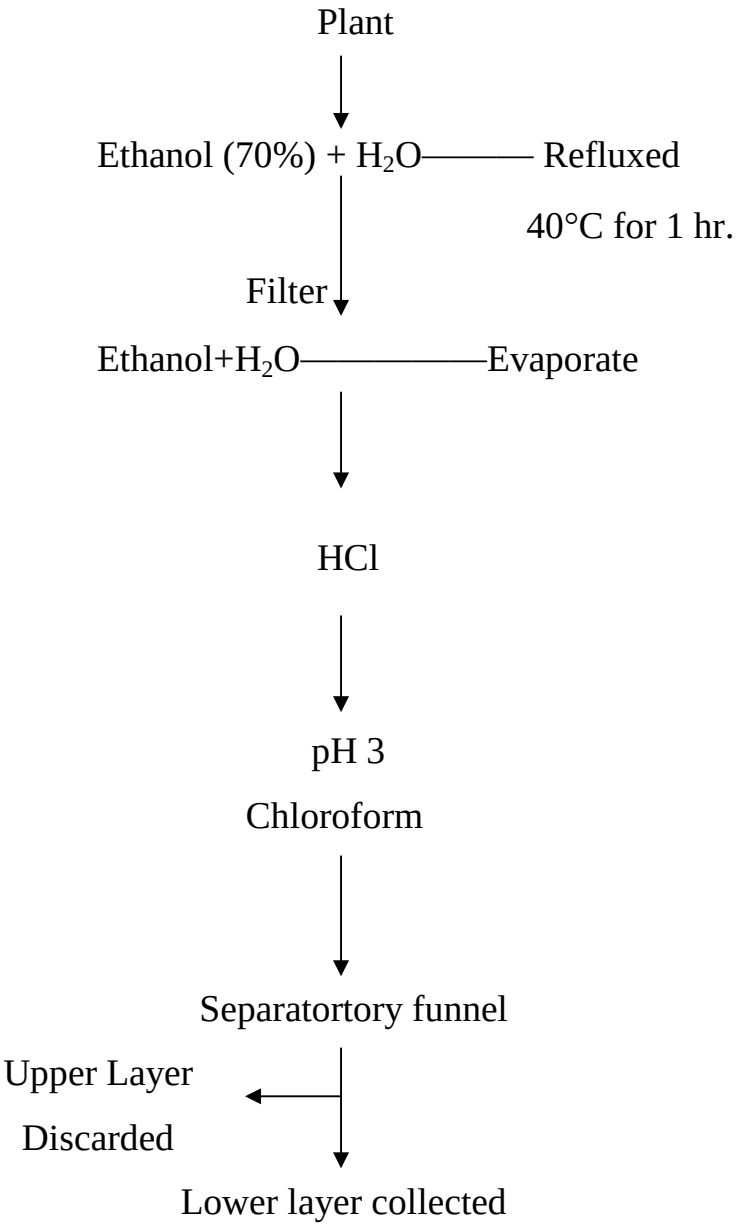

Figure (3-1) Ethanolic extraction of *Fumaria officinalis* plant (Montanri, 1998)

3.5 Preparation of Reagent and Solution

The following reagents are used for detection of active compound (alkaloid) in the plant extract:-

3.5.1 Mayer's Reagent

Solution A: 385g of mercuric chloride was dissolved in 600 ml of distilled water.

Solution B: 5g of potassium iodide was dissolved in 10ml of distilled water.

A and B solution were mixed, the volume was completed to 100 ml by adding distilled water (Smolenski *et al*., 1972). This reagent is used for detecting of alkaloid.

3.6 Detection of Some Active Compounds

Different chemical reagents and solutions which were used for detection of active compounds found in *Fumaria officinalis*, and as follows

3.6.1 Detection of Alkaloids

Fifty milliliter of aqueous extract of plant was boiled with 4% (vol/vol) of HCl, then 0.5 ml from aqueous extract of the plant was taken and put in watch glass with Mayer's reagent, the appearance of white precipitate is an indication for the presence of alkaloid, Hussein, (1981).

3.6.2 Detection of Glycosides

Two equal parts of Fehling reagent (Biotechnology Research Center / Al-Nahrain University) and plant extraction were mixed in boiling water bath for 10 minutes; the appearance of red color is an evidence for the presence of glycosides (Shihata, 1951).

3.6.3 Detection of Flavonids

Ethanolic extract of the plant were partitioned with petroleum ether, the aqueous layer was mixed with ammonia solution, and the appearance of dark color is an evidence for the presence of flavonoid (Harborne, 1974).

3.6.4 Detection of Resins

Ten ml of acidify distilled water with 4ml hydrochloric acid added to the plants extract, detection of positive test is by appearance of turbidity Shihata, (1951).

3.7 The Microorganisms Used for Antimicrobial Activity

No.	Microorganisms	Origin
1	*Staphylococcus aureus*	Biotechnology Department / College of Science / AL-Nahrain University
2	*Pseudomonas aeruginosa*	
3	*Escherichia coli*	
4	*Trichophyton rubrum*	
5	*Trichophyton tonsurns*	
6	*Trichophyton mentagrophytes.*	

3.8 Preparation of Culture Media

3.8.1 Sterilization Methods (Cappuecino and Sheman, 1987)

*Culture media were sterilized by autoclaving at 121°C and 15 Ib/in2 for 15 min.

*Glassware were sterilized in oven at 180-200 C for 2 hours.

3.8.2 Preparation of Culture Media

The following culture media were used routinely in our study:

***Nutrient agar:** Bacteria were cultured on nutrient agar prepared according to (Tilton *et al*., 1992) using 8g Nutrient broth 2g agar dissolved in distilled water and completed to 100 ml by D.W.

***Modified Sabouraud Dextrose Agar:** Fungi were cultured on modified sabouraud dextrose agar according to Finegold *et al*., 1982, by the following ingredients:

Peptose	10g	Glucose	20g
Agar	20g	Cycloheximide	500g
Cephalexin	500g	D.W.	1L

Then it was sterilized by autoclaving

3.8.3 Ethanolic Extract for Antibacterial Detection

One ml of chloroform was added to the ethanolic extract (which is insoluble in water), then 2 g of solution were dissolved in 20 ml sterile distilled water, from this different concentrations was prepared (250 Ppm, 500 Ppm, 750Ppm), according to equation**:-** .

$$Ppm = \frac{X * 1000}{\text{Media size}}$$

X=the amount of plant extract were used

Nutrient agar media were prepared for control plates. The medium was mixed well, poured in Petri-dishes and left to be solidified. 100µl overnight

culture was spotted on the agar medium and left without separation. The inoculated plates were placed at room temperature for 30 minutes to allow absorption of excess moisture. The plates were incubated for 24 hours at 37°C (NCCLs, 1993).

3.8.4 Ethanolic Extract for Antifungal Detection

To ethanolic extract were used 6 g of plant extract were dissolved in 60 ml sterile D.W., were as plant dissolved in 1 ml of chloroform concentration were prepared started with (5, 10, 20, 25 and 30 mg/ml) as the following equation:

$$C_1V_1 = C_2V_2$$

Plant extract was added to modified Sabouraud dextrose agar containing cephalaxin and cycloheximide at ratio 1.5:1.5 (vol/vol), all petridishes were inoculated with fungal spore and incubated at 30°C for 7-14 days (Al-Samaraei *et al*., 2001).

The diameter of fungal colonies was determined after the period of incubation, and then the inhibition percent was calculated according the following equation:

$$\text{Percent} = \frac{\text{Inhibition average of colony diameter in treated plate} - \text{Average of colony diameter in control plate}}{\text{Average of colony diameter in control plate}} x100$$

3.9 Management of Laboratory Animal

Healthy mature albino mice 8-12 week old and 25-35 gm of weight were obtained from the animal house of biotechnology research center / AL-Nahrain University. They were kept in an air conditioned room (22-24°C) with an automatically controlled photoperiod of 13 hrs in animal cage of opaque plastic measuring (29×15×12) cm. Five mice were kept in each cage, containing wooden shave. Tap water and diet were freely available for the animals. The mice were kept two week for adaptation. During this period, abnormal and sick mice were excluded from the experiments. The cages were cleaned and sterilized with 70% ethanol every one week regularly.

Twenty male mice were used in this study, these mice are divided into four groups (A, B, C and D), consisting of five mice in each cage.

3.10 *In Vivo* Treatment

In this experiment four (five in each cage) mice were used per each of the following treatment:-

1-Goup A (Ethanolic extract treated).

2-Group B (Cortisone treated).

3-Group C (Ethanolic and cortisone treated).

4-Group D (Untreated as control).

3.10.1 Ethanolic Extract Treatment

The ethanolic extract was suspended in ethanol and distributed in three test tubes, each one contains 1 ml of extract with different concentrations as the following :-

* 1mg / kg / body: - 1 ml of extract + 1 ml of ethanol.
* 3 mg / kg / body: - 1ml of extract + 2 ml of ethanol.
* 5 mg / kg / body: - 1ml of extract only.

All these different doses were given as 0.1 ml (80µg/ml) for each mouse orally.

3.10.2 Cortisone Treatment

A quantity of 0.1 ml of cortisone was given to group of mice in concentration of (80µg/ml) daily for two weeks, after the end of this period; the serum is collected by taking the blood by heart puncture.

3.10.3 Ethanol and Cortisone Treatment

As mentioned and explained in section (3.10.1) the ethanolic extract treated mice (3 group plus 1 control) for two weeks ,the same mice were treated with cortisone in 0.1 ml with concentration of (80µg/ml) for each group for also two weeks, after this treatment, the serum was collected from heart.

3.10.4 Control Group

In this group, the mice left without any treatment (control) and by the same way the blood sample are withdrawn by heart puncture.

3.11 Enzymatic Assay

3.11.1 GPT and GOT Tests

According to Reitman and Frankel (1957), blood was collected from the mice by heart puncture .The serum was separated by centrifuging at 5000 rpm for 10 min. then, the serum was taken and treated as follows:

Two test tubes were used for each sample, the 1st one contained the blank reagent and 2nd one contains the sample. These samples were treated as in the following:-

	GPT	GOT
Reagent 1 Reagent 2	1 ml —	— 1 ml
Incubate for 5 min at 37°C.		
Serum	0.2 ml	0.2 ml
Mix and incubate at 37°C	— 1 hour	— 30 min.
Reagent 3	1 ml	1 ml
Mix. Let stand for 20min at room temp		
NaOH (0.4 N)	10 ml	10 ml
Mixed wait 5 min. measure under condition identical to those used for the standard curve (Appendix 1).		

Wavelength: 505 nm

Activities of these two enzymes in the serum were estimated from the activity table attached with kit of each enzyme.

3.11.2 ALP Test

Sample used in this test was the same of serum sample used for GPT& GOT tests. To estimate the activity of the ALP enzymes, procedure of Kind and King (1945) was used: four test tubes for each sample were prepared, the 1st one contain the sample, the 2nd is the blank sample, the 3rd contained the standard sample and the 4th is the blank reagent, as shown below:-

	Serum sample	Serum bank	Standard	Reagent blank
Reagent	2 ml	2ml	2ml	2ml
Incubate for 5 minutes at 37°C.				
Serum Reagent 2	50µl —	— —	— 50µl	— —
Incubate for exactly 15 min at 37°C.				
Reagent 3	0.5ml	0.5ml	0.5ml	0.5ml
Mixed well or preferably vortex.				
Reagent 4 Serum Distilled water	0.5ml — —	0.5ml 50µl —	0.5ml — —	0.5ml — 50µl
Mix and let stand for 10 minutes in the dark. Measure.				

$$\text{Calculation} = \frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD standard}} \times n$$

3.11.3 Bill Test

Samples used in this test were the same as ones used in GPT and GOT, and ALP tests. To estimate the activity of the Bill enzymes, procedure of Kind and King, (1945) was used: four test tubes for each sample were prepared, the 1st one contains the sample, the 2nd is the blank sample, the 3rd contained the standard sample and the 4th is the blank reagent, as follows:

	Macro -method		Micro- method	
	Blank	sample	Blank	sample
Bilitrol sample Reagent1 Working solution	100µl 1ml —	100µl — 1 ml	40 1ml	
Mix. record absorbance after incubation at:				
20- 25°C 37°C	5 min 3 min		10 min 5 min	

Reagent 1: 20 volumes

Reagent 2: 1 volume

Wavelength: 550 nm (Hg 546nm)

$$\text{Sample concentration} = \frac{(A_{\text{sample}} - A_{\text{blank}})_{\text{sample}}}{(A_{\text{sample}} - A_{\text{blank}})_{\text{Bilitrol}}} \times n$$

n: Bilitrol titer.

3.12 Allergy Test

3.12.1 Animals

Healthy adult male rabbit was obtained from the animal house of Biotechnology research center / AL-Nahraine University.

Two male rabbit, weighed 2-3kg was used.

The animal kept in suitable environmental condition of 20-25°C and photoperiod of 12 hrs. daily.

3.12.2 Disc Preparation

A sterile filter paper has been cut into discs of 2mm in diameter and then socked in a chromate for 24hr., in a concentration 0.005mg in 1 ml of sterile distilled water, while other are socked in the *Fumaria officinalis* extract and other was left as positive and negative control.

3.12.3 Animals Treatment

- Both sides of the animal cleaned with sterile distilled water and then shaved with paste.
- After removal of the hair from both side and cleaned with distilled water and cotton.
- The discs that were prepared from (3.12.2) step were used as the following:-

3.12.3.1 With Chromate

The sides were treated with discs socked with chromate.

3.12.3.2 With Extract

The sides were treated with discs that were treated with *Fumaria officinalis* extract.

3.12.3.3: Without Treatment (Control)

The sides were left without any treatment as a negative control.

3.12.3.4: With Ointment

The side is treated with ointment as a positive control.

3.13 Cytotoxiciy Preparation

3.13.1 Cytotoxiciy Effect on Mouse Embryos Fibroblast Cells

3.13.1.1 Fibroblast Cell Preparation

This procedure was done according to the method which described by Freshney, (1994).

In this experiment a pregnant mouse 11-13 days was used for preparation of fibroblast primary. The mouse was killed by cervical dislocation and swab the ventral surface liberally with 70 % alcohol and the Uteri was removed which were filled with embryos by sterile technique and placed it in a sterile Petri dish containing 10-20 ml PBS, wash with PBS 2-3 times to remove the blood. The intact Uteri was transferred to another Petri dish, dissected out the embryos by treating the uterus with sterile forceps and scissors, the embryos freed from the membranes and placenta, placed one side of the dish to bleed, then transferred to another Petri dish, wash 2-3 times with PBS to remove the blood, chopped finely, placed the pieces in a universal with 10-20 ml PBS, allowed the pieces to be settled, removed the supernatant fluid, repeated the washing 2-3 times. transfer the pieces to trypsinization flask which contain magnetic bar and 50 ml of trypsin (0.25 % trypsin), stir at about 200 rpm for 15 min at 37°C, allow the pieces to be settle, collected supernatant centrifuged at 2000 rpm for 10 minutes, resuspend the pellet in to 10 ml tissue culture medium (RPMI 1640 supplemented with 10 % F.C.S), fresh trypsin was added to the pieces and continue to stir and the steps were repeated until no further disaggregating is apparent.

3.13.1.2 Culture

The cells suspension was collected which were centrifuged and kept in RPMI with 10 % F. C. S culture the cells on tissue culture flasks and incubated at 37°C for 4 days.

3.13.1.3 Harvesting

After 4 days of incubation the cells were grew as a monolayer. To remove monolayer detachment, the medium was discarded, and PBS was added (5ml / 25 cm^3) to the side of the flask opposite the cells, the cells were rinsed and PBS was discarded.

Trypsin versen (3ml / 25cm^3) was added to the side of the flask opposite the cells, turned the flask over to cover the monolayer completely, left for 15-30 seconds and be sure that the monolayer has not detected, cells round up and the monolayer should slide down the surface, then RPMI medium was added (0.1 - 0.2 ml / cm^3) supplemented with 10 % F. C. S dispersed cells by repeated pipetting over the surface bearing the monolayer. Finally, the cell suspension was pipette up and down for a few times and then the cell suspension was collected in sterile universals.

3.13.1.4 Cytotoxiciy Test

For this test the micro titer plates (96 wells flat bottom) were used for plant extract and positive control (normal saline) and their replicative lines started from column 2 to column 12 , 50 ml medium (RPMI 1640 supplemented with 10 % F.C.S) was added for negative control line and its replication 50 µl medium were added from row 1 to 12 . Fifty micro liter of plant extract was added to row 1 and 2, and to all its replicative lines, and then from rows 2 double fold dilutions we prepared, the same steps we prepared to make positive control lines but with the addition of 50 µl from normal saline instead of plant extract and prepared dilutions by the same way. Finally150 µl from fiberoblast cells suspension were added to all wells, incubated at 37°C for 4 days.

3.13.1.5 Reading the Result

After 4 days of incubation, 50 µl from neatural red stain was added to each well and reincubated at 37°C for 2 hours, the stain was discarded gently washed with PBS, then 20 ml from the eluting solution was added to each well, read by ELISA reader at OD 492 nm and the results were recorded.

3.13.2 Cytotoxiciy Effect of Extract on S.U.99 Plasmacytoma Cell Line

Plasmacytoma cell line S.U.99 used in this study was supplied by Al-Nahrain University, Department of Biotechnology. This tumor cell was induced, cultured and characterized in Al-Nahrain University, Department of Biotechnology, this procedure was done according to the method which described by Naradra and Henry, (2001) with some modifications.

The S.U.99 cells attached to the flask surface were removed by gently pipeting with sterile technique, then 12 µm (1mg/ml) was added from human halotransferrin and incubated at 37°C for 2 hrs. After incubation the S.U.99 cells were cultured with extract as in the fibroblast cells procedure which described earlier. The positive control was done by culturing the S.U.99 cells with halotransferrin and normal saline while the negative control was done by culturing the S.U.99 cells with halotransferrin only.

Results and Discussion

4.1 Plant Extracts

4.1.1 Aqueous Extract

Fifty grams of *Fumaria officinalis* plant powder was used for preparation of this extract; the weight of the residue resulted after evaporation of distilled water was 10g, this extract appeared brown in color.

4.1.2 Ethanolic Extract

Fifty grams of *Fumaria officinalis* plant powder was used for preparation of this extract; the pH value of the ethanolic extract was 6.7. The weight of the residue resulted after evaporation of ethanol was 18.5g; this extract appeared dark green in color.

4.2 Effect of Plant Extract on the Growth of Bacteria

Plant extract of *Fumaria officinalis* was obtained and minimum inhibitory concentration was used (250 Ppm, 500 Ppm, 750 Ppm), for the ethanolic extract. The antibacterial activity of any extract depends on the type of extract, concentration and the type of microorganism.

4.2.1 Ethanolic Extract

Ethanolic extract of *Fumaria officinalis* plant posse's minimum inhibitory activity against *Pseudomonas aeruginosa,* at a concentration of (250 Ppm and 500 Ppm) the maximum inhibitory concentration was at 750 Ppm while in *E. coli* and *Staphylococcus aureus* no inhibitory activity was found in all concentrations (Table 4-1) (Fig.4-1). Ethanolic extract of many medicinal plant have a several active compounds such as alkaloid which have Antimicrobial activities against many microorganisms (Beg, 2001).

Table (4-1) Effect of *Fumaria officinalis* ethanolic extract on the growth of bacteria

Concentration of the extract (Ppm)	*E. coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
250	—	+	—
500	—	+	—
750	—	++	—

– = no inhibition.

+ = Inhibition zone diameter between 0.5-2 mm.

++ = Inhibition zone diameter between 13-18 mm.

Ethanolic extract showed significant activity on certain pathogenic bacteria; *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa* (Emerawa and Olayiwola, 1991). According to these results we can indicate that medicinal plant with active compounds such as alkaloids have significant inhibition activities.

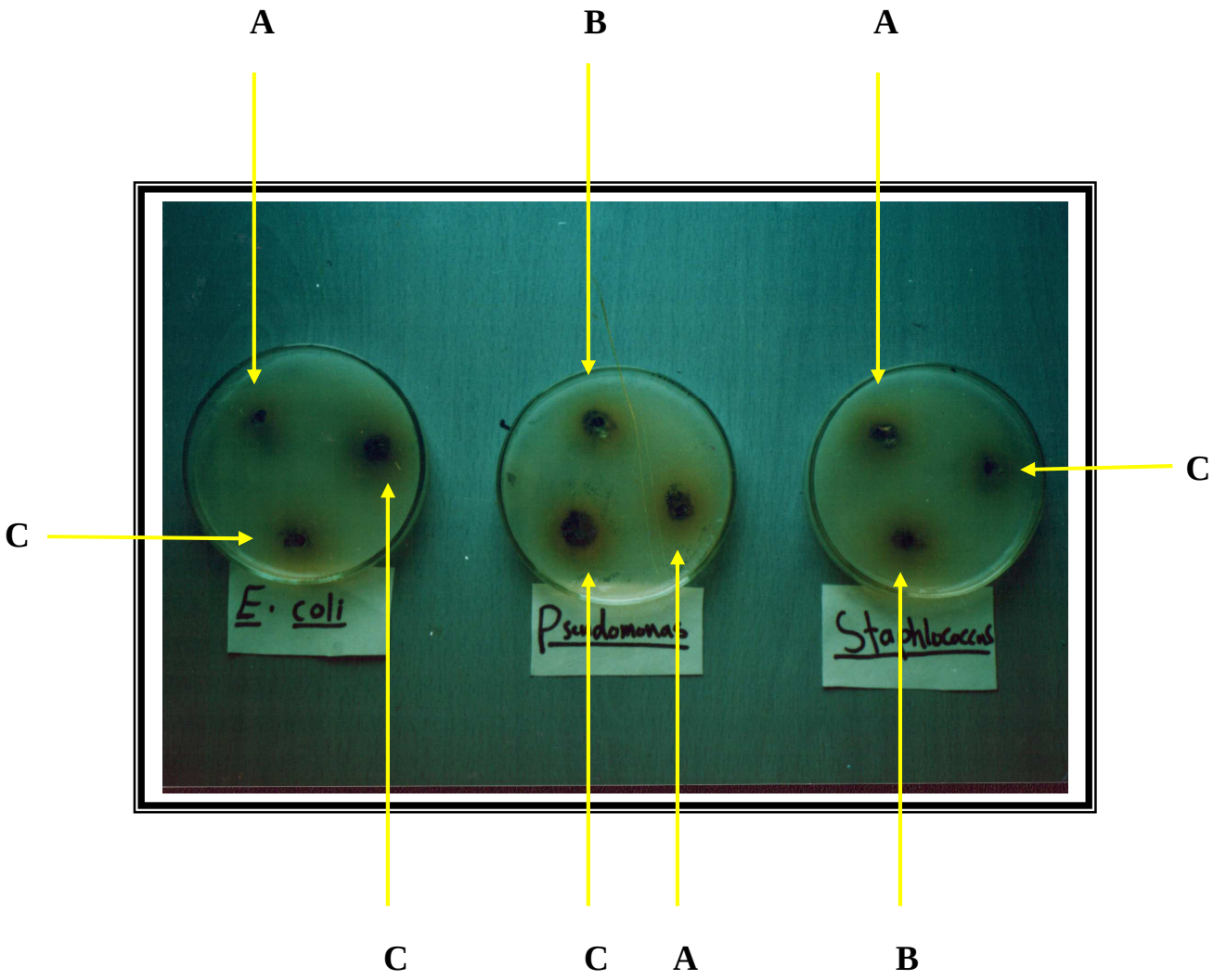

Figure (4-1) Effect of *Fumaria officinalis* ethanolic extract on some pathogenic bacterial growth on nutrient agar at 37°C for 24 hr.

A: 250 Ppm **B: 500 Ppm** **C: 750 Ppm**

4.3 Effect of Plant Extract on the Growth of Fungi

The effect of *Fumaria officinalis* extract varies according to method of extraction, the concentration used and the type of fungus.

4.3.1 Ethanolic Extract

The ethanolic extract of *Fumaria officinalis* showed different inhibitory effects. The ethanolic extract showed no inhibitory effect against *Trichophyton rubrum* at the concentrations: 5mg/ml, 10mg/ml, and 20mg/ml but has a little inhibitory effect in 25mg/ml and 30mg/ml, (Fig. 4-2), the percentage of inhibition at the mentioned concentrations were (32.2, 33.3, 42.2, 77.7, 83.3) % respectively, where as the growth of *Trichophyton mentegrophyte* was inhibited completely by 10mg/ml ethanolic extract (Fig.4-3), the percentage of inhibition at the same concentrations (22, 88.8, 100, 100, 100) % respectively. *Trichophyton tonsurans* slightly inhibited at the concentrations of 5mg/ml, 10mg/ml, 20mg/ml, but in the concentration of 25mg/ml, 30mg/ml the growth was inhibited completely (Fig.4-4). The percentage of inhibition at these concentrations (12.5, 41.25, 47.5, 100, and 100)% (Table 4-2) respectively From these results we conclude that *Trichophyton rubrum* and *Trichophyton tonsurans* were more resistant to ethanolic extract of *Fumatria officinalis* than *Trichophyton mentagrophytes.*

Table (4-2) Effects of *Fumaria officinalis* ethanolic extract on the growht of fungal colonies (mm)

Concentration (mg/ml)	*Trichophyton rubrum*		*Trichophyton mentagrophytes*		*Trichophyton tonsurans*	
	Average of Colonies Diameter (mm)	Percentage of Inhibition %	Average of Colonies Diameter (mm)	Percentage of Inhibition %	Average of Colonies Diameter (mm)	Percentage of Inhibition %
Control	90	0	90	0	80	0
5	61	32.2	70.2	22	70	12.5
10	60	33.3	10	88.8	47	41.25
20	52	42.2	0	100	42	47.5
25	20	77.7	0	100	0	100
30	15	83.3	0	100	0	100

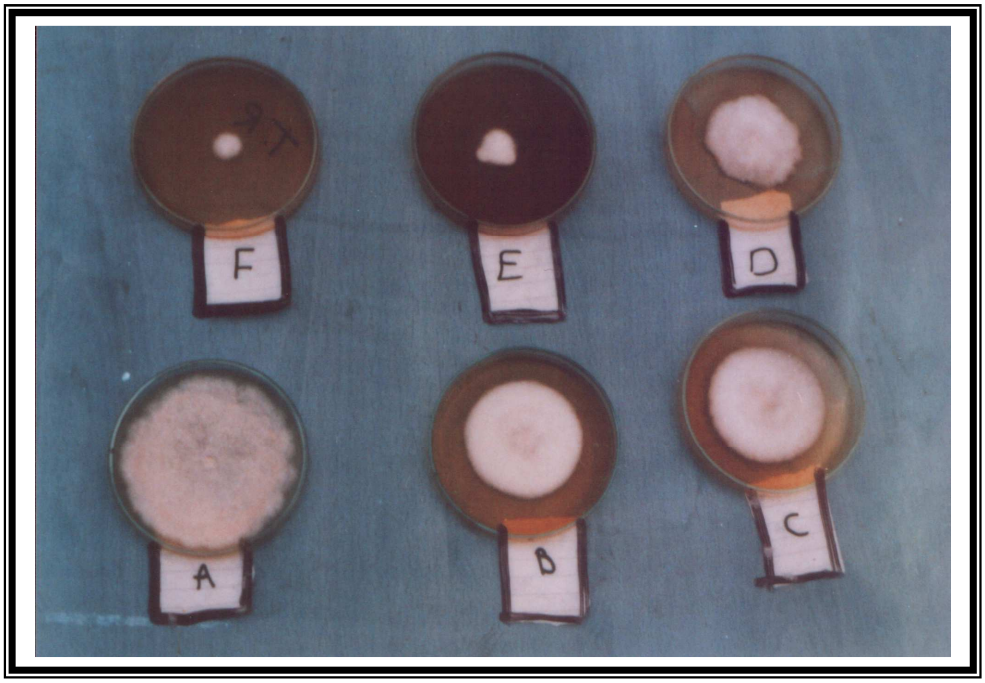

Figure (4-2) The effect of ethanolic extract on the Growth of *Trichophyton rubrum*

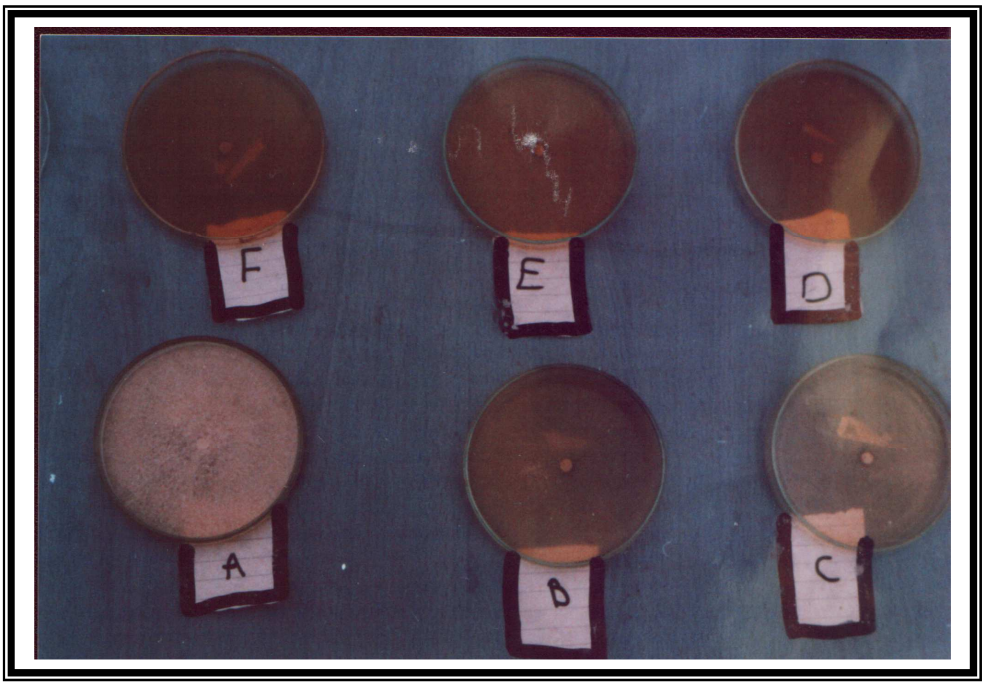

Figure (4-3) The effect of ethanolic extract on the Growth of *Tricophyton mentagrophytes*

A= Control plate B=5mg/ml C= 10mg/ml

D=20mg/ml E= 25mg/ml F= 30mg/ml

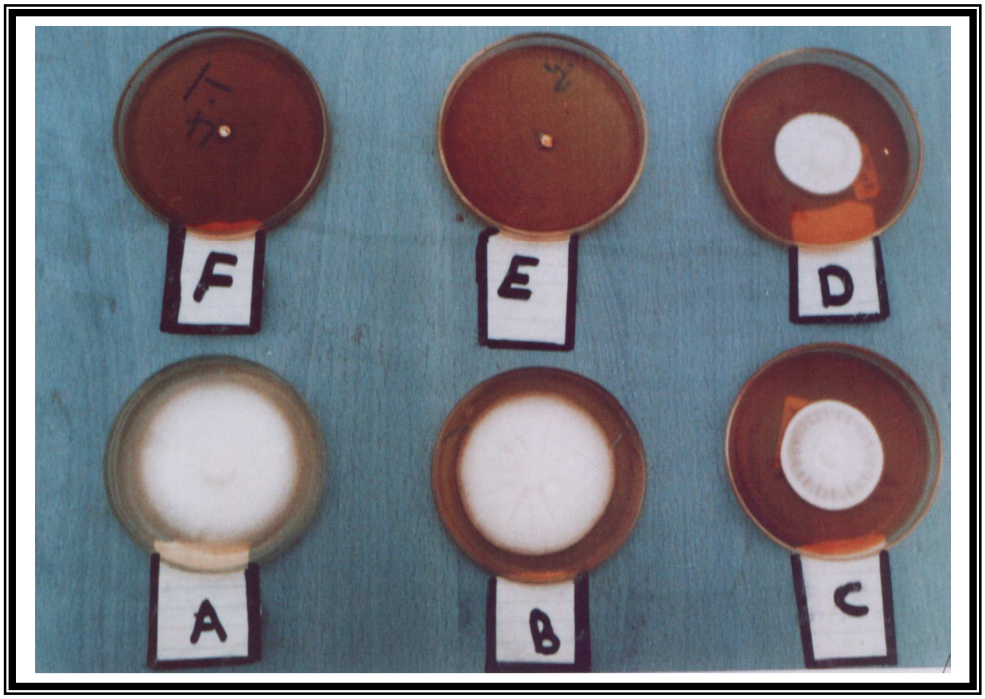

Figure (4-4) The effect of ethanolic extract on the Growth of *Tricophyton tonsurans*.

A= Control plate B=5mg/ml C= 10mg/ml

D=20mg/ml E= 25mg/ml F= 30mg/ml

In general pharmaceutical studies of antifungal agent on drug are classified into:-

1. Drug that disrupt the cell membrane.
2. Drug that inhibit mitosis.
3. Drug that inhibit deoxyribonucleic acid (DNA) synthesis (Laurence *et al*., 1997).

Three general modes of action of different plant extract were recognizing as follows:

1. Inhibition of microbial cell wall formation or biosynthesis of some essential protein.
2. Disruption of deoxyribonucleic acid (DNA) metabolism.
3. Alteration of normal function of cellular membrane (Tyler, 1988).

Some drug can be administrated alone or as poly- pharmaceutical in the form of decoctions, infusions, tablets, powders, confections, preserves, conserves, syrups, linctuses and calcined preparation.

In the field of preventive and primary health care, it is notable that most of Unani medicines are not directly curative and therefore are not liable to produce harmful side effects in contrast with some chemical or synthetic drugs (Chopra *et al*., 1978).

4.4 Effect of Plant Extract on Liver Enzymes GPT, GOT, ALP and BILL

The effect of ethanolic extract of *Fumaria officinalis* on the liver enzymes has been administrated by comparing the results that were obtained animal serum that was treated with ethanolic plant extract in three different concentrations (1mg/ml, 3mg/ml, 5mg/ml) compared with control result as follow:

4.4.1 Effect of Plant Extract on GPT

It was found that the values of GPT enzyme when treated with the extract only in concentrations of (1mg, 3mg/ml) is higher than the normal (control) values, while when treated with (5mg/ml) the value of GPT enzyme is lower than in control, and also all these values were higher than cortisone treated values, while treated with cortisone in concentration of 80µl/body weight shoe values less than the normal or control value.

Also, in comparing the values between that of animals treated with extract only and with that treated with cortisone and extract was found that these values in concentration of (1mg and 3mg/ml) is higher than values that obtained from those treated with extract and cortisone, while the value of enzyme in concentration of 5mg/ml is less than the value that obtained from treatment with extract and cortisone (Table 4-3).

All these values of GPT enzymes that coming from treated with cortisone and ethanolic plant extract in concentration of (1mg/ml, 3mg/ml, and 5mg/ml) were higher than control and cortisone values, and the value of control was higher than cortisone.

From the results that show in table (4-3), GPT enzyme levels varies in the three concentration, which mean that ethanolic extract of *Fumaria officinalis* are affected on this enzyme because as we know that the liver is especially rich in this enzyme than other organ, so this varies may refer either to circularity frailer with shock or hypoxia. Although these levels may become evaluated whenever disease processes affect liver cell condition.

Table (4-3) Effect of *Fumaria officinalis* plant ethanol extract on liver enzyme GPT

Treatment with ethanolic extract	GPT	Treatment with cortisone + ethanolic extract	GPT	Difference	t	Significance difference
1mg	84.5 ± 5.5*a	1mg	35.6±0.85 a	48.9	9.98	p≤0.05
3mg	74.0 ± 2.0* b	3mg	38.2±0.2 a	35.8	16.3	p≤0.05
5mg	20.5±0.5 c	5mg	36.4±0.4*** a	15.9	9	p≤0.001
control	24.2 ± 2.31 c		24.0±2.31			
cortisone	8.5± 0.5		8.5± 0.5			

The similar letters between vertical treatments means no significant difference (p≤0.01).

* The presence of significance difference (p≤0.1)

*** The presence of significance difference (p≤0.001).

4.4.2 Effect of Plant Extract on Enzyme GOT

It was found from the result obtained, that the values of GOT enzyme when treated with extract in concentration of (1mg, 3mg, and 5mg /ml) are less than normal or control value, while the enzyme values when treated by concentration of (1mg, 3mg, and 5mg /ml) were greater than cortisone treated value, while level of enzyme treated with cortisone was less than control value.

In comparing between the values that obtained from treated with extract only, and those treated with cortisone and extract, the values of the later treatment is greater than that obtained from treated with extract only.

The values of GOT enzyme treated with cortisone and then with ethanolic extract was less than control value and greater than enzyme treated with cortisone only in concentration of 80µl/body weight, (Table 4-4)

From the above mentioned result we can conclude that the ethanolic plant extract affected according to it's different concentrations on the level of GOT enzyme, as we know that GOT is present in high concentration in cardiac cell and skeletal muscle, kidney and erythrocyte, so any damage occur to any one of these tissue may increase plasma level, in addition this enzyme is related with heart disorder.

Table (4-4) Effect of *Fumaria officinalis* plant ethanolic extract on liver enzyme GOT

Treatment with ethanolic extract	GOT	Treatment with cortisone + ethanolic extract	GOT	Difference	t	Significance difference
1mg	29.15±1.85b	1mg	46.9±0.1±b**	17.7	9.1	p≤0.01
3mg	28.0±9.0b	3mg	41.8±0.2b*	13.8	3.56	p≤0.05
5mg	29.5±0.5b	5mg	44.5±0.5b*	15	3.5	p≤0.05
control	109.08±5.2a		109.08±5.2a			
cortisone	25.5±0.5b		25.5 ± 0.5c			

The similar letters between vertical treatments means no significant difference (p≤0.01).

* Significance difference at the level of (p≤0.1).

** Significance difference at the level of **(**p≤0.01).

Experimental work by Gorbunov *et al*., (1980) reported that *Fumaria officinalis* alkaloid have cardiovascular activity by decreasing or preventing myocardial activity by decreasing preventing myocardial ischemia caused by occlusion of coronary artery, in addition these differences in values is not related to heart disorder but also may he related with muscular diseases which include muscular dystrophy, myocardial infection and acute viral or toxic hepatitis.

4.4.3 Effect of Plant Extract on Enzyme ALP

It was found from the results that the value of ALP enzyme when the animal was traded with *Fumaria officinalis* ethanolic extract by concentrations of (1mg, 3mg, 5mg /ml) is higher than normal or control value, while the values of ALP that treatment occurred with plant ethanolic extract in concentrations of (1mg, 3mg, and 5mg /ml) was greater than cortisone treated value, whereas the value of enzyme treatment occurred with cortisone in concentration of 80µl/body weight was greater than control value, (Table 4-5)

While the value of ALP enzyme after treatment with ethanolic plant extract in concentration of (1mg, 3mg, 5mg /ml) was greater than cortisone treated value, and the value of enzyme after treatment with cortisone in concentration of 80µl/body was greater than control. The ALP enzyme values that obtained after treatment with cortisone then with ethanolic plant extract in three different concentrations (1mg/ml, 3mg/ml and 5mg/ml) was greater than value compeering with value that obtain from treated with plant extract only, in addition the value that obtained after treatment with cortisone and plant extract was greater than control and cortisone treated value, From the result obtained, the decreasing in values of enzyme that treated with plant extract and cortisone which mean that the enzyme has been affected by these treatments and one of the most reasons for the decreasing is the bone disease and also liver disease which associated with the animal treatment.

Table (4-5) Effect of *Fumaria officinalis* plant ethanolic extract on liver enzyme ALP

Treatment with ethanolic extract	ALP	Treatment with cortisone + ethanolic extract	ALP	Difference	t	Significance difference
1mg	53.8±2.5 b	1mg	68.18±2.96 c	14.37	2.624	N.S
3mg	78.08±1.01 a	3mg	551.4±2.97 a	473.32	241.5	p≤0.001
5mg	45.94±4.44 b	5mg	121.54±5.93 b	75.6	50.91	p≤0.01
control	25.5±1.15 c		25.5±1.15 d			
cortisone	34.09±4.44 c		34.09±4.44 d			

The similar letters between vertical treatments means no significant difference (p≤0.01).

4.4.4 Effect of Plant Extract on Enzyme BILL

It was found from the results that the value of BILL enzyme in concentration of (1mg/ml) that treated with extract is greater than when compaired with normal or control value.

While the values of enzyme in concentration of (3mg and 5mg/ml) were less than control value.

The value of Bill enzyme that treated with plant extract in concentration of (3mg/ml) was less than cortisone value, while the second value that obtains from treatment with (5mg/ml) was the same value of cortisone.

The value of Bill enzyme that obtained after treatment with cortisone and then with ethanolic extract in three different concentrations (1mg/ml, 3mg and

5mg/ml) was greater than values of Bill enzyme with plant extract only and the value of enzyme that treated with cortisone and then with plant extract was greater than control and cortisone values.

For the comparing between cortisone values that obtain from treated with 80µl/body weight, the cortisone value was greater than control value.

Table (4-6) Effect of *Fumaria officinalis* plant ethanolic extract on liver enzyme

Treatment with ethanolic extract	BILL	Treatment with cortisone + ethanolic extract	BILL	Difference	t	Significance difference
1mg	11.21±7.67a**	1mg	68.18±0.01	5.03	17.6	p≤0.01
3mg	2.07±0.3a	3mg	551.4±0.01	5.2	2.27	N.S
5mg	5.31±2.36a	5mg	121.54±0.03	5.28	2.27	N.S
control	7.67±2.40a		7.67±2.40a			
cortisone	5.31±1.18a		5.31±1.18a			

The similar letters between vertical treatments means no significant difference (p≤0.01).

** Significance difference at the level of (p≤0.01).

According to the resulting obtained, the Bill enzyme has been affected, and this effected has been shown by varies in values of enzyme when compared with control, these difference is related with abnormal of billirubion metabolism, experimental work by Reynier *et al*., (1977) found that *Fumaria* extract increase the volume of bill secretion when administered intraduodenaly to anesthetized rats with hypocholeresis, these abnormalities is characterized by increase of

bilirubin in the blood and brownish-yellow pigmentation of the skin, sclera and mucus membranes.

4.5 Cytotoxic Assay of Crude Alkaloid Plant Extract

Cytotoxic assay is an important and highly selective assay that allows determining the cytotoxic effect of many agents, with different dilutions, on different types of normal and tumor cell culture (Carmichal *et al*., 1987).The neutral red method, as originally developed by (Borenfreuned and Puener, 1985). Is simple, accurate and yields reproducible result. The key component is the vital dye, neutral red. Viable cells will take up the dye by active transport and incorporated dye into lysosomes, whereas non viable cells not take up the dye. After the cell has been allowed to incorporate the dye they are briefly washed and fixed. The incorporated dye is then liberated form the cell in an acidified ethanol solution. an increase or decrease in number of cell or their physiological state results in a concomitant change in the amount of dye incorporated by the cell in the culture, this indicate the degree of cytotoxicity caused by test material (Piala and Yale 1964).

Two kinds of cell culture were used in this study to determine the cytotoxic effect of wild type *Fumaria officinalis* crude alkaloid.

The first cell culture was mouse embryo fibroblast cells, which represent normal cell culture. The results (fig.4-5) revealed that the crude alkaloid in concentrations of (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.03, 0.01. 0.009, 0.004 and 0.002 mg/50µl) has show no cytotoxic or inhibition effect on the of mouse embryo fibroblast but in contrast it showed a stimulating effected on their growth in compared with the control, but when the dilution of crude alkaloid extract increased the stimulating effect has increased too, Fig (4-5).

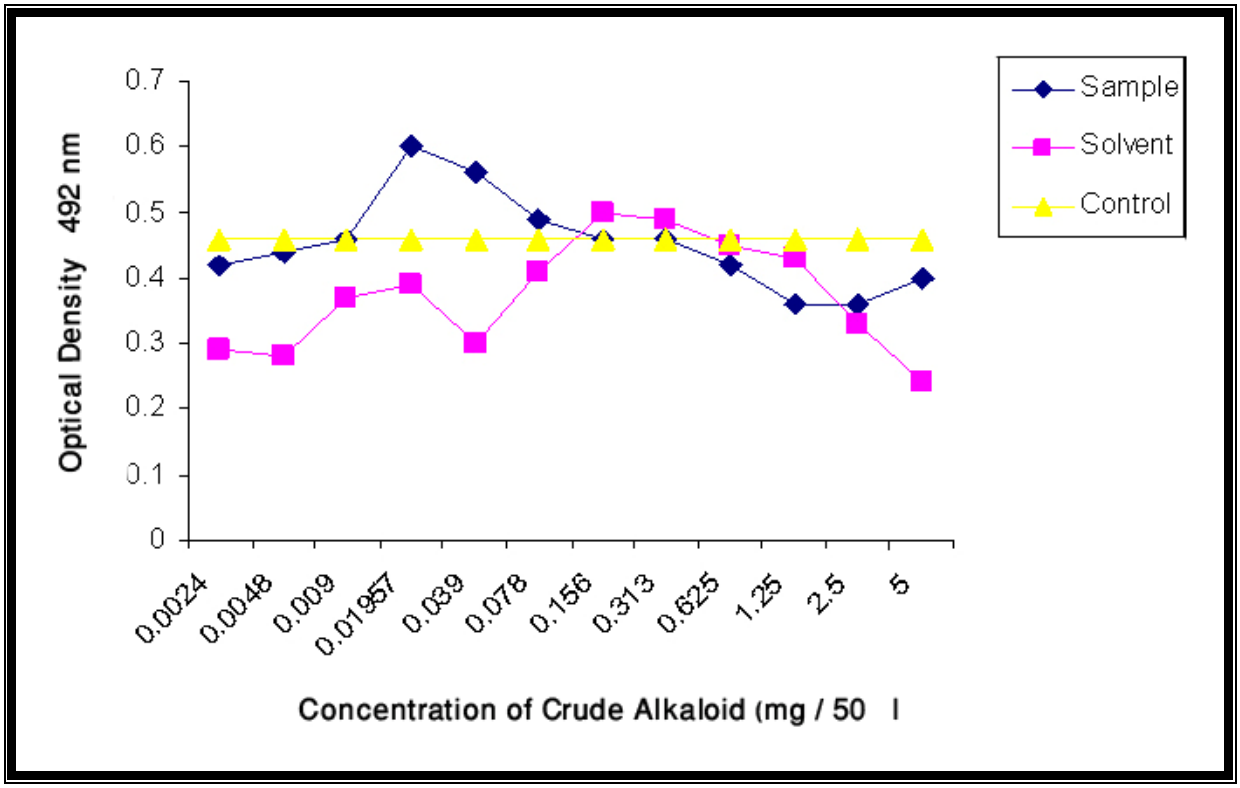

Figure (4-5) Cytotoxic assay of crude *Fumaria officinalis* ethanolic extract on mouse embryo fibroblast

Investigations showed that some of plant extract have no inhibition or cytotoxic effect on normal cell and specially extracts that contain alkaloid or polysaccharide (Eherkem *et al*., 1988).

Fig (4-5) showed that the ethanolic extract of *Fumaria officinalis* plant have stimulation properties specially in concentration (0.07, 0.03 and o.o1 mg/50µl).

The second cell line was myeloma cell used to determine the cytotoxic effect of wild type *Fumaria officinalis* crude extract. Result (Fig.4-6) showed that at the concentrations (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.03, 0.01. 0.009, 0.004 mg/50µl) have a great inhibition and cytotoxic effect against this tumor cell line when compared with control, and when the dilution of crude alkaloid extract increased the cytotoxic effect has increased too, whereas no significance effect is shown by low concentrations (Fig.4-5).

Results (Fig 4-5) show also a little effect of the solvent (alkaloid extract dissolved in saline) on myeloma cell line.

This affected could be attributed to the permeability of these cells since myeloma cell-line is tumor is a malignant cell-line, so their function and properties have many defects and one of these defects is their permeapolity.

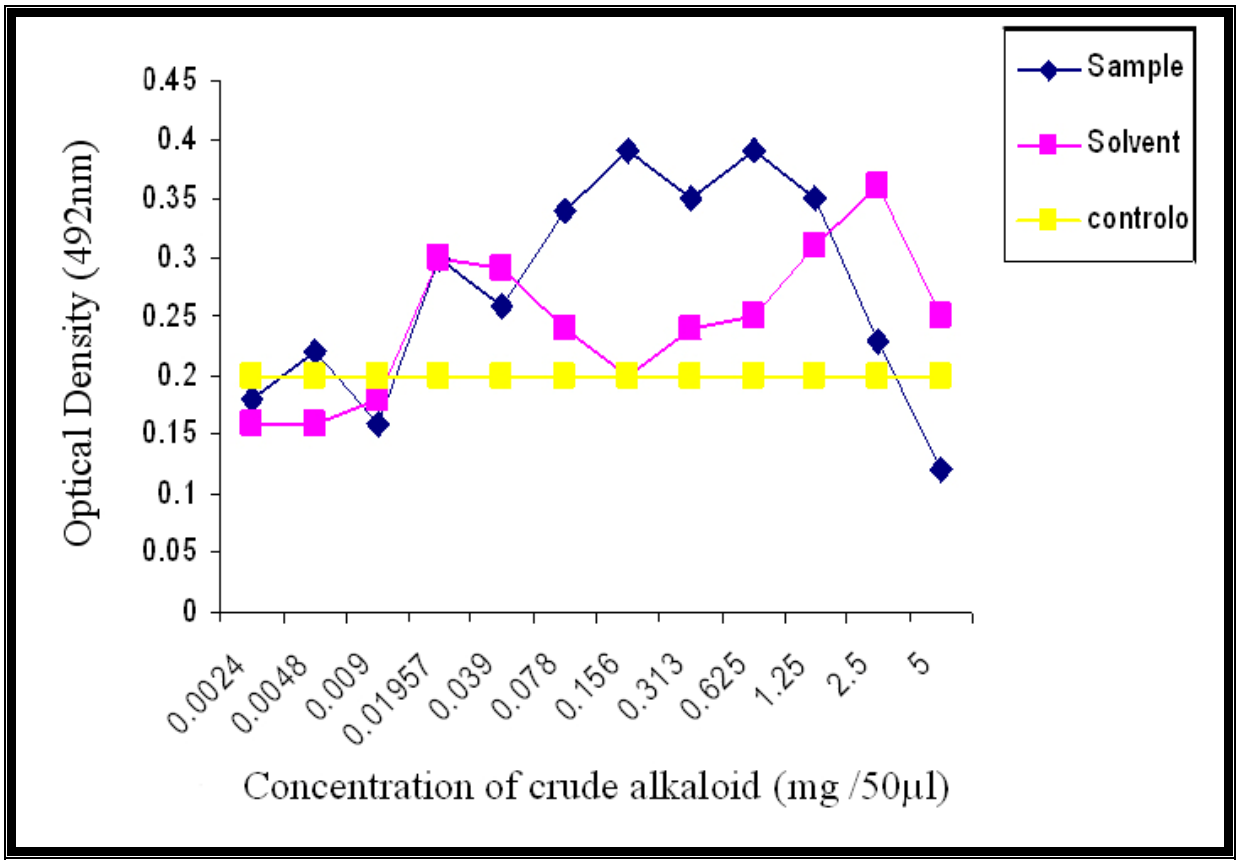

Figure (4-6) Cytotoxic assay of crude *Fumaria officinalis* ethanolic extract on myloma cell line

4.6 Allergy Assay

Allergy is an abnormal immune reaction of the body to allergens such as pollen, dust, certain foods, drugs, animal fur, animal pets, animal excretions, feathers, microorganisms, cosmetics, textiles, dyes, smoke, chemical pollutants and insect stings, cosmetics.

Healthy male rabbit was used in this study to reveal the inhibitory effect of crude *Fumaria officinalis* alkaloid extract to the immune response. The result shows that after treating the animal skin with chromate in concentration of 0.005mg/ml in a form of discs for 24 hrs. (figure 4-7), the immune response appears as a red zone, then, the animal was treated with both ethanolic extract of *Fumaria officinalis* and watery extract as a natural product and also with ointment as a synthetic product, the results obtained was as the following:

1) Animal treated with ethanolic extract for one week have a side effect which revealed as redness of the skin, and this redness is due to the presence of ethanol with the crude extract.
2) Animal treated with watery extract for the same period of the time (one week also) shows the ability of this crude extract to inhibit the erethema that caused by introducing the chromate, and this is due to the presence of alkaloid (isoquinolin alkaloid).
3) Animal treated with ointment also gave an indication that it also inhibits the redness that occurs on the skin but in a period faster than that treated with watery extract.

According to these results we can conclude that the crude water extract of *Fumaria officinalis* may have certain anti allergic activity (Figure 3-8).

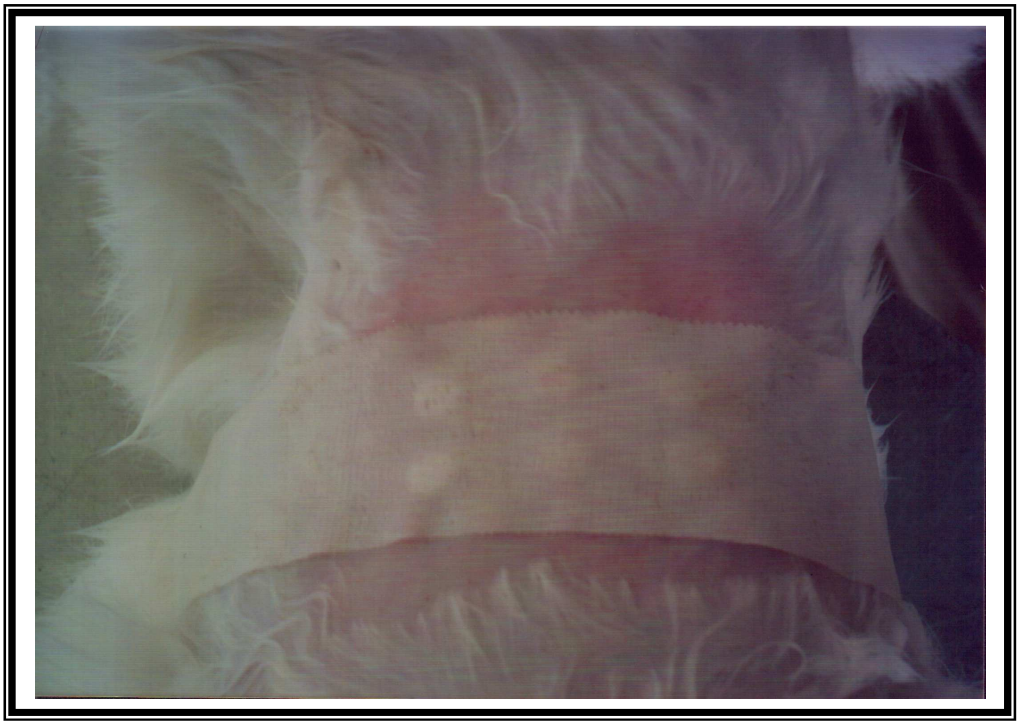

Figure (4-7) Allergy assay on rabbit skin using different discs containing ethanol, chromate with negative control.

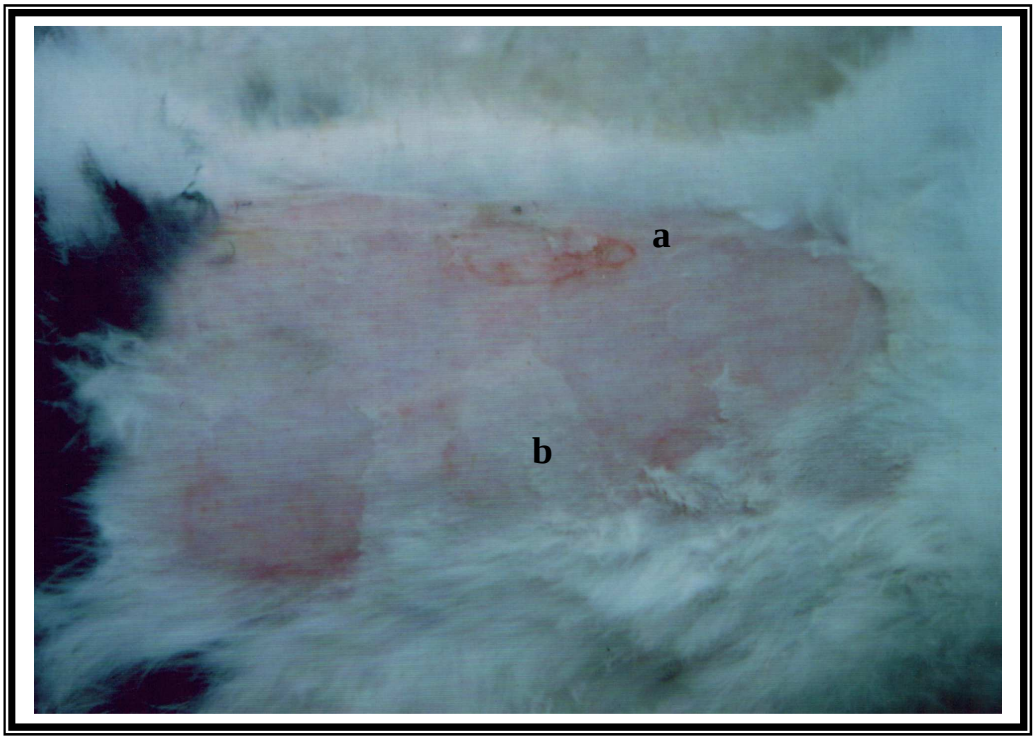

Figure (4-8) Treatment of chromate effecting using aqueous extract of *Fumaria officinalis*

a- Chromate effect, b- Aqueous extracted effect.

Conclusions and Recommendations

5.1 Conclusions

1) *Fumaria officinalis* is a good natural source for the production of antibacterial and antifungal agents.
2) The presence of secondary metabolite in *Fumaria officinalis* which represent alkaloid (isoquinolin alkaloid).
3) Ethanolic extract of *Fumaria officinalis* showing a greet activity of inhibition on the bacterial and fungal growth.
4) Ethanolic extract of Fumaria officinalis plant shown an effected on liver enzymes.
5) *Fumaria officinalis* alkaloid extract has a good stimulating effect for the growth for normal cell culture.
6) Crude alkaloid ethanolic extract of *Fumaria officinalis* may exhibit a good antitumor activity.
7) Aqueous extract of *Fumaria officinalis* plant exhibit antiallergic properties.

5.2 Recommendations

1) Perform further studies on the biological activities of *Fumaria officinalis.*

2) More advance methods are needed for isolation, purification and identification of different active components for the plant *Fumaria officinalis.*

3) More research is needed on the biological actives of *Fumaria officinalis*, including: cytotoxicological activity and allergic activity.

4) Experimental work on the application of *Fumaria officinalis* extract.

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Word Wide Web Reference

http://www.isoquinolin-alkaloid-of-Fumaria

Appendix 1

Absorbance	U/l	Absorbance	U/l
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

Absorbance	U/l	Absorbance	U/l
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

الخلاصة

هذا البحث قد اوصى به لدراسة الفعالية البايولوجية لمستخلص نبات الشاترك على الحيوانات المختبرية.

ان عشب الشاترك نبات واسع الانتشار وله سمعة عريضة بين خبراء العشبة في العراق وهو تقليد شعبي.

الكشوفات الكيمياوية كانت قد اجريت لتشخيص المواد الفعالة المحتواة فيه.

نوعان من المستخلصات كان قد احضر من مسحوق نباتي لنبات الشاترك وهما: المستخلص المائي والمستخلص الكحولي وهذان المستخلصان المختلفان من المسحوق النباتي لنبات الشاترك اختبر فعاليتهما خارج جسم الحي.

لقد اظهرت التحقيقات الكيمياوية وفحوصات البرنامج لنبات الشاترك العراقي الحاوي على شبه القلويدات احتواء على مواد فعالة مختلفة وقد وجد بان المادة الفعالة الاساسية في هذا النبات هو شبه القلوي.

الاحياء المجهرية المتسخدمة في هذه الدراسة كانت:

اظهرت الدراسات التي اجريت خارج الجسم الحي ان المستخلص الكحولي لنبات الشاترك يملك فعالية قوية ضد الاحياء المجهرية والاحياء الفطرية بتراكيز مختلفة.

على العكس من ذلك اثبت المستخلص المائي فعالية عالية كمثبط مناعي اكثر من المستخلص الكحولي.

كذلك الدراسات التي اجريت خارج الجسم الحي على انزيمات الكبد قد اظهرت قيم مختلفة حسب التراكيز المتخلفة التي استخدمت من المستخلص الكحولي لنبات الشاترك.

كما اظهرت الدراسة التي اجريت على الخلايا الليفية لاجنة الفئران ان المستخلص الكحولي له تاثير تحفيزي لنمو هذه الخلايا عكس الخلايا السرطانية التي كان لها التاثير في تثبيط نموها.

Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Sciences

Biological Study on the Activity of *Fumaria officinalis* Extracts

A thesis
Submitted to the College of Science of Al-Nahrain University
In partial fulfillment of the requirement for the Degree of M. Sc.
in Biotechnology

By

Mohanned Hasan Hussein AL- Azawi
B. SC. 2001
AL-Nahrain University

Thu Al-Quda 1426

December 2005

بسم الله الرحمن الرحيم

قُل لو كانَ البحرُ مِداداً لكلمتِ

ربي لَنفِدَ ٱلبحرُ قَبلَ أن تَنفَد كلمتُ

ربي ولو جئنا بمثله مددا

صدق الله العظيم

الاية (١٠٨) الكهف

الى مقام النبي الاعظم ...

محمد رسول الله (صلى الله عليه وسلم)

الى من اقتدي به .....

ابي

الشمعة التي نورت دربي.....

والدتي

الى من رافقني في خطواتي.....

إخوتي وأخواتي

اهدي ثمرة جهدي

مهند

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And to all staff of Iraqna Call Center.

Mohanned

Supervisor Certification

I certify that this thesis was prepared under my supervision in the College, Al-Nahrain University of Science as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:
Supervisor:
Dr. Khulood W. Al-Samarraei
Scientific Degree: Assist Professor
Date:

Signature:
Supervisor:
Dr. Alice K. Melconian
Scientific Degree: Professor
Date:

In review of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:
Name: **Dr. Nabeel Al-Ani**
Scientific Degree: Assistant professor.
Title: Head of Biotechnology Department.
Date:

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:
Name:
Scientific Degree:
Date:
(Chairman)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: **Dr. Laith Abdul Aziz Al-Ani**

Scientific Degree: Assistant Professor

Title: Dean of College of Science

Date:

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

دراسة الفعالية البيولوجية لمستخلص ألشاترك

رسالة
مقدمة إلى كلية العلوم في جامعة النهرين
وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الإحيائية

من قبل

مهند حسن حسين العزاوي

بكلوريوس تقانة احيائية 2001
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