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

Therapeutic Activity of Crud Alkaloidal Extract of *Fumaria officinalis* in Dermatophyte (*Trichophyton rubrum*). (Study by Light Microscop).

A thesis
Submitted to the College of Science of AL-Nahrain University
As partial fulfillment of the requirements for the degree of Master of Science in Biotechnology

By

RAFAL SHAKEB ABD AL-WAHAB AL-ANI

B. Sc. Biotechnology (2002)

Al-Nahrain University

Safar **1427**

March **2006**

بسم الله الرحمن الرحيم

فَبَدَاَ بِاوعيتهم قَبلَ وِعاءِ اخيهِ ثمَ استخرجها مِن وِعاءِ اخَيهِ كذلكَ كِدنا لِيُوسفَ ما كانَ لياخذَ اخاهُ في دين الملكِ الا ان يَشَاء اللهُ نَرفَعُ دَرجَاتِ مَن نشاءُ وَفَوقَ كُلِّ ذي عِلمٍ عَليمٌ

صَدق الله العَظيم

يوسف : ٧٦

الاهداء

الى من هو سبب وجودي...

والدي العزيز

الى من الجنة تحت اقدامها...

والدتي العزيزة

الى من اشددبهم ازري

واشركهم في امري ...اخوتي واخواتي

رفل

Acknowledgment

Praise is to almighty Allah, the most gracious, the most merciful, for enabling me to accomplish this work.

I wish to express my sincere thanks to my supervisors **Dr. Khulood W. Al-Sammarraie** and **Dr. Salim R. Al-Obaidy** for their valuable assistance helpful suggestions and penetrating remarks that without them, this study would probably never have taken their present shape.

Special thanks to the Deanery of the College of Science and to the head of department of Biotechnology / AL-Nahrain University for their help and facilities.

Sincere thanks to the staff of Biotechnology Department in College of Science, Al-Nahrain University for their cooperation.

I am also grateful to Dr. Hazim and Rose for their helping in the period of this research.

I am also grateful to Miss. Asma'a Obaid, Miss Ebtihal and my friends Hala, Shaymaa, Noor and Dunya for their help and support me during my work.

Finally, I thank my family, father, mother, brothers and sisters my God bless them all.

Rafal

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:

Title:

Chairman:

Signature:	**Signature:**
Name:	**Name:**
Title:	**Title:**
Member:	**Member:**
Signature:	**Signature:**
Name:	**Name:**
Title:	**Title:**
Member/Advisor:	**Member/Advisor:**

I hereby certify upon the decision of the examining committee

Signature:

Name: Dr. Laith A. Z. Al- Ani

Title: Assistant Professor

Address: Dean of the College of Science

Date:

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

Signature:	**Signature:**
Supervisor:	**Supervisor:**
Dr. Khulood W. Al-Samarai	**Dr. Salim Rasheed Al-Obaidy**
Title:	**Title:**
Date:	**Date:**

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

Signature:

Dr. Nabeel Al-Ani

Title: Chairman of Biotechnology Department

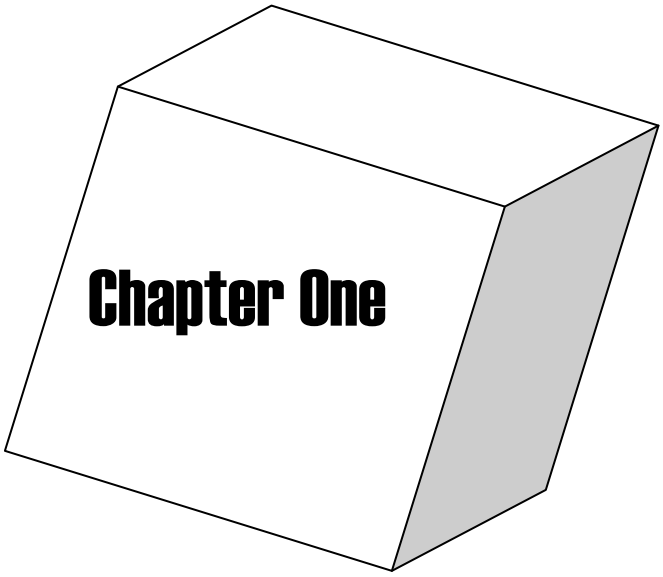
Date:

Summary

This investigation was planned to study the histopathological changes caused by *Trichophyton rubrum* and study the therapeutic activity of *Fumaria officinalis* (ethanolic extract) on the infected skin by using light microscope (*in vitro* and *in vivo*).

- Clinical features of animal skin infected with *Trichophyton rubrum* was represented by scaly area with alopecia, irregular margin with papule, boil formation, redness, swelling, ulceration and loss of hair.
- Light microscopic studies have shown certain degenerative changes in the infected area these changes were represented by oedema with heavy acute inflammatory cell in dermis area extended to hypodermis, abscess formation and congestion of blood vessels.
- Clinical features pf animals skin infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract represented that crust tend to drop-off spontaneously after the five day, leaving white scar, usually slightly depressed along it's entire length.
- Healing after eleventh day of the treatment with *Fumaria officinalis* extract the incision was completely covered with newly formed epithelium and the hair seen in comparism with fugnidin ointment with which healing was represented by slower regenerative changes, crust tend to drop-off spontaneously and the incision which covered the area were less than those covered the area treated with *Fumaria* extract, indicating the efficiency of *Fumaria* ethanolic extract as antifungal agent.

– Light microscopic studies revealed certain regenerative changes in the infected area after the treatment with *Fumaria officinalis* extract, these changes were represented by more moderate inflammatory reaction with accumulation of neotrophile and monocyte in the epidermis layer.



Introduction and Literature Review

Introduction and Literature Review

1-1 Dermatophytes:

Dermatophytes are a group of keratinophilic fungi, which invade the superficial area of the body like skin, hair and nails (Hawksworth *et al*., 1983; Zurita and Hay, 1987; Myrvic and Weiser, 1988; Midgley *et al*., 1997).

Dermatophytes are referred to as "tinea" infections, they are also named for the body site involved (Ajello, 1974).

Dermatophytes are also known as "ringworm" fungi, this name has been in use at least from the sixteenth century and was coined to describe the circular lesion produced by dermatophytes on the skin or scalp (Kwon *et al*., 1992).

Many plants can be used as crude drugs in different countries, medicinal plants are used as health care products in traditional medicine as raw materials for the pharmaceutical industry and for the extraction of different active compounds such as alkaloids, volatile oils, phenolic compounds, …etc (Arora, 1965).

According to asexual reproduction, dermatophytes are classified into three genera which are: *Trichophyton, Micrsporum* and *Epidermophyton* (Emmons *et al*., 1977; Vanbreusegham, 1977; Haward and Haward, 1983).

These genera include 41 species, *Trichophyton* has 22 species while *Microsporum* has 17 and two species only belong to *Epidermophyton* (Vanbrensegham, 1977; Haward and Haward, 1983; Rippon, 1988).

Some countries improved the cultivation and research of medicinal plants including chemical constituents, their therapeutic applications in addition to morphology and taxonomy.

In the last few years, research on the medicinal plants had increased and different plants have been screened for thier antimicrobial studies (Al-Shamma and Mitscher, 1979; Ikram and Inamul, 1980; Tomesi *et al*., 1986).

In folk medicine, the herb *Fumaria officinalis* has been used for skin disease, cystitis, atherosclerosis, rheumatism, arthritis, as blood purifier, hypoglycemia and for infections (Dute, 1985).

Fumaria is derived from Latin (Fumus terrae) "Smoke of the earth" refer to the smoke-like smell of some species or to smoke rising from the ground as the wispy foliage may look like (Hoffman, 1995).

There are over 55 species of fumaria of which over a dozen are known to be medicinal. Fumitory has been mentioned in all of the old herbals from Grieve to Culpepper and from everyone form Pliny and Dioscorides to Chaucer and Shakespeare (Grieve, 1996; Bown, 2001).

The Aims of This Study

1. Preparation of ethanolic extract from *Fumaria officinalis* aerial parts.
2. *In vitro* study on the antifungal activity of aerial parts of *Fumaria officinalis* against *Trichophyton rubrum.*
3. Histopathological study on the effect of fumaria ethanolic extract in the treatment of skin layer changes caused by *Trichophyton rubrum* in experimental animals.

1-2 Historical Review

Dermatophytes are a group of morphological and physiological related molds, some of which cause well defined infections: dermatophytoses (tinea or ringworm) (Devroey, 1985).

The first known on the dermatophytes started in 1839 by schoenlein when he observed (Arthrospore or conidia and hyphae in hair infected with favus and proved that these fungi transmitted by infections from man to another (Ajello, 1977).

In 1845 (Robert Remake) described the causative of favus which was *Trichophyton schoenleini* while Malmsten 1845 described the fungus *Trichophyton tonsurans* (Emmous *et al*., 1977).

While Charles Robin in 1847 described and named *Trichophyton mentagrophytes* (Haward and Haward, 1983).

The first systemic studies on the dermatophytes was established by Raymond Sabouraud 1892. This work included taxonomy, morphology and laboratory methods for identification of dermatophytes, according to this study dermatophytes are classified into 4 groups: *Achorion, Trichophyton, Microsporum and Epidermophyton,* depending on the basis of clinical aspect of the disease combined with the cultural and microscopic characteristics of the fungus by developing a media containing crude peptone with either crude maltose or honey (Haward and Haward, 1983).

Although Raymond Sabouraud established taxonomic criteria for dermatophytes, the classification of these fungi remained chaotic until 1934, mainly because many clinicians descried new species based not only on morphology of fungi but also on clinical manifestation of the disease. Emmons *et al*., (1977) established our current taxonomic system of

dermatophytes. They refined the genera *Trichophyton*, *Microsporum and Epidermophyton*. depending on spore morphology and accessory structures of fungi (Emmons *et al*., 1977; Vanbrenseghem, 1977; Haward and Haward, 1983).

The number of recognized dermatophytes reached about 41 species. These include 22 species of *Trichopyton*, 17 species of *Microsporum* and 2 species of *Epidermophyton* (Rippon, 1988). Devroey and Vanbreuseghem, 1977 classified dermatophytes into different species which are *Trichopyton*, *Microsporum*, *Epidermophyton*, *Keratinomyces* and *Microides* depended on a sexual phase (Vanbreuseghem, 1977). The dermatophytes have been divided into 3 ecological groups these groups are geophiles, zoophiles and anthropophilies (Georg, 1960; Takashio, 1979).

1-3 Sources of Infection and Way of Transmission

Several factors are known to influence the spread and development of the ringworm infection. Such as the virulence of the pathogens, host resistance, host preference and source of infection (Philot, 1977).

Individual dermatophytes differs considerably in their host rang and importance as agents of disease in man and animals. The differences in host specificity have been attributed to the differences in keratin of the hosts (Rippon, 1982).

The organisms are transmitted by either direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair, brushes, clothing, furniture, theater seats, caps, bed, lineus, towels, hotel rugs and locker room floors (Mackenzie, 1963; Philpot, 1977, Rippon, 1988; Alan and James, 2000).

1-3-1 Geophiles

Geophiles exist as saprophytes in the soil and have the ability to competitively colonize keratinous substrates successfully (Barlow, 1976; Matsumoto and Ajello, 1987). Chmel and Buchvald (1970) found that most of infections were from gardeners and farm workers and over 60% of the reported cases were form areas with high amounts of soil. Their distribution appears to be related to the distribution of available keratin (Marplas, 1965; Mantovani, 1978; Devroy, 1985).

These dermatophytes are generally contacted directly form soil containing a high number of spores and are only rarely transmitted from man to man or lower animals to man (Ajello, 1974; Devroy, 1985).

1-3-2 Zoophiles

Zoophiles species are basically animal pathogens. Often with a single preferred animal host or very limited host range, outside which they are found only in exceptional circumstances (English, 1972).

The usual route of infection is by direct contact. However, indirect contact and air borne infection may also account for the transmission of the disease (Jungerman and Schwartzman, 1972).

There are important differences between rural and urban areas in the principle pathogen encountered and in the way by which the ringworm is transmitted (George *et al*., 1956).

In urban area, it has been estimated that approximately 20% of human infections were of animal origin, while in rural areas the infection of human from 80% of animal origin (Jungerman and Schwartzman, 1972).

Six species of dermatophytes were found to cause ringworm to animal and transmitted to human. These include: *Microsporum canis*,

Microsporum distortum, Trichophyton verrucosum, Trichophyton mentegrophytes, Trichophyton equinum and *Trichophyton gallinae* (George, 1960).

Human infection due to *Microsporum distortum, Trichophyton gallinae* and *Trichophyton equinum* are rare. *Trichophyton gallinae and Trichohphyton equinum* are specific for the gallinaceous birds and horses respectively and rarely can produce infection in other animals or man (Torres and George, 1956).

Zoophilic dermatophytes rarely grow actively as saprophytes but survive in a dormant state on contaminated materials of animal origin (Devroy, 1988).

Microsporum canis, Trichophyton verrucosum and *Trichophyton mentagrophytes* are common agents of ringworm in animals but are also frequently associated with human infection (Takashio, 1979; Katoh *et al.*, 1990).

1-3-3 Anthrophiles

Infection is mainly associated with the community, because the transmission is from man to man. Close human contact is an important factor in the spread of infection.

Gentles and Holmes, (1957) showed a definite relationship between the incidence of tinea pedis and the use of communal bath. The incidence among bathers and non-bathers was 21% and 28% respectively.

George (1960) reported a high rate of infection in communal bathers, compared with those bathed only at home. This gave a good evidence for the occurrence of cross infection. Also she mentioned that the percentage

of infected individuals was correlated directly with the amount of washing and cleaning in swimming pools.

In a survey on *Trichophyton tonsuranse* infection is some schools, (Mackenzie, 1961) the fungus was recovered from the hair brushes, combs and some bedding materials. The existence of carriers is an important source of infection.

Esteves *et al*., (1959) stated that tinea cruris could be acquired from towels contaminated by scales of infected feet. Careless exchange of towels or clothing may also transmit the infection. In an outbreak of tinea cruris, Neves and Xavier (1964) were able to isolate the fungus from clothes and other objects touching the groins, bedpans and water closets. They mentioned that indirect transmission was more likely occurred in tinea cruris rather than directly transmission.

Anthrophiles are primarily adapted for parasitism of man (Carretta and Ajello, 1990; Tanaka *et al*., 1992). Some species occasionally cause ringworm in animals, for example *Trichophyton rubrum* has been reported to be causative of infection in a dog (Georg, 1960; Kaplan, 1985).

Since transmission in man to man, contracting the disease therefore require human contact (Mackenzie, 1988).

The spread of anthrophiles is more common in communities like schools, barracks, prisons and the family (Philpot, 1977; Rippon, 1982).

In concentrated communities, the use of facilities such as shower rooms, and common headgear leads to rapid spread of infections (Baxter, 1980).

1-4 Dermatophytoses

All the dermatophytes cause tinea and related diseases. The word tinea comes from the Latin root meaning a growing worm, the common name for some form of tinea is ringworm (Myrvic and Weiser, 1988).

Ringworm has different clinical manifestation in different areas of the body (Martin, 1999). Although many different body sites may be involved each focus of infection is due to local inoculation.

Inflammation is often greatest at the advancing margin, leaving a central area with some clearing.

The nomenclature ringworm follows from irregular inflammatory border of the skin lesion (Fried Lander, 2000). While the name of the clinical disease is done by appending a Latin term designating the body site to the word tinea (Rodgers, 2001).

Single species of dermatophytes can induce different types of clinical manifestation, depending on the site of body affected (Rebell and Taplin, 1974; Mackenzie *et al*., 1986; Rippon, 1988).

1-4-1 Tinea Capitis (Ringworm of the Scalp)

Tinea capitis, the most common dermatophytosis in children, is an infection of the scalp and hair shafts (Abdel-Rahman, 1997).

Transmission is fostered by poor hygiene and overcrowding and can occur through contaminated hats, brushes, pillow cases and other inanimate objects (Aly, 1999).

Tinea capitis is characterized by irregular or well demarcated alopecia and scaling, when swellen hairs fracture a few millimeters from the scalp, "Black dot" alopecia is produced (Elewski, 2000) it's caused by *Trichophyton* and *Microsporum.*

1-4-2 Tinea Corporis

Tinea corporis or ringworm typically appears as single or multiple annular, scaly lesions with central clearing, a slightly elevated, reddened edge, and sharp margination on the trunk, extremities, or face (Hubbard, 1999; Rosen, 1997). *Trichophyton rubrum* and *Microsporum canis* are the most common.

1-4-3 Tinea Barbae

Tinea barbae is involves the skin and coarse of the beard and mustache area (Shear *et al*., 1998). This dermatophytes infection occur in adult men and hirsute women, because the usual cause is a zoophilic organism, farm workers are most often affected (Noble, 1998) it's caused by *Trichophyton verrucosum.*

1-4-4 Tinea Faciei

Tinea faciei tends to occur in the non bearded area of the face.

The patient may complain of itching and burning, which become worse after sunlight exposure (Gupta, 1997).

Some round or annular red patches are present (Zuber, 2001).

1-4-5 Tinea Mannum

Tinea mannum is a fungal infection of one or occasionllay both hands (Goldstein, 2000). It often occurs in patient with tinea pedis. The palmer surface is diffusely dry and hyperkeratotic (Rodgers, 2001).

1-4-6 Tinea Cruris

Tinea cruris, frequent called "Jock itch", is a dermatophyte infection of the groin (Higgins, 2000). This dermatophytosis is more common in men than in women and is frequently associated with tinea pedis (Evans *et al*., 1993).

The most common species that cause infection is *Epidermophyton floccosum.*

1-4-7 Tinea Pedis

Tinea pedis, or athlete's foot, has three common percentation.

It is characterized by fissuring, maceration, scaling in the interdigital spaces of the fourth and fifth toes, usually caused by *Trichophyton rubrum* (Temple *et al*., 1999).

1-4-8 Tinea Unguium

Tinea unguium, a dermatophyte infection of the nail, is a subset of onchomycosis, which also may be caused by yeast and nondermatophyte molds (Noble, 1998).

Risk factors for this infection include aging diabetes, poorly fitting shoes and the presence of tinea pedis (Lawry *et al*., 2000).

1-5 Microscopic Features of Trichophyton

Species of this genus are widely distributed and are the most common cause of tinea corporis, capitis and barbae. Most of them are anthrophilic and few are zoophilic.

Microscopically: it's characterized by club shaped thin walled macro-conidia with 8-10 septa ranging in size from 8 by 4 μ – 15 by 8 μ.

The macro-conidia are born singly at the terminal end of the hyphae or in short branches. While the micro-conidia are usually spherical or clavate in shape ranging from 2-4 µ in size (George, 1960; Hashimoto, 1978; Kaman and Forslind, 1985).

The most common species are *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton verrucosum* and *Trichophyton mentagrophytes.*

Trichophyton rubrum

- Pathogenicity: - Infects the skin and nails, only rarely the beard, hair or scalp.
- Rate of growth: - Slow, mature within 14 days.
- Colony morphology: Surface granular or fluffy, white; while the reverse of dish cultured agar is deep red or purplish; occasionally brown, yellow orange or even colorless.
- Microscopic morphology: Septate hyphae with lateral, tear-shaped micro-conidia.

Macro-conidia may be abundant, rare or absent; when present they are long, narrow thin-walled, with parallel sides, and have two to eight cells.

1-6 Medicinal Plants

Fumaria officinalis

Arabic name: Shartarah

English name: Fumaria

Family name: Fumariaceae

Genus: *Fumaria*

Species: *officinalis*

Other name: fumitory, earth smoke.

1-6-1 Description of the Plant

Fumaria officinalis is an annual that reaches 10-50 cm with a hollow, greenish blue stem. The leaves are of the same hue, and alternate in 3-pinnate sections. The flowers are small and are arranged in clusters or spikes (Grieve, 1994; Fleming *et al*., 2000).

1-6-2 Distribution

Habital is native to Europe and northern Africa. Introduced to Asia, North America and Australia.

Fumariaceae, as a family, is composed of 17 genera (Meikle, 1977) and about 400 species chiefly distributed in the temperate and subtemperate regions of the northern hemisphere (Townsend *et al*., 1980).

In Iraq, fumariaceae family is represented by three genera namely Fumaria, corydalis and hypecoum.

The first one includes annual herbs and so as the third, while the second includes perennial herbs (Rechinger, 1964).

Fumaria is mostly found in the middle and northern parts of the country and in the north west sector of the desert.

Fumaria officinalis is an invasive, weedy plant that enjoys diches, fields and gardens (Gorbunov *et al*., 1980; Fetrow and Avila, 1999).

1-6-3 Biological Activities

Fumaria officinalis was used in folk medicine, the herb has been used for skin diseases, cystitis, atherosclerosis, rheumatism, arthritis, as blood purifier, hypoglycemia and for infections (Duke, 1985, Hahn, 1993).

Reynier *et al*., (1977) reported that fumaria extract increase the volume of the bile secretion when administrated intraduodonally to

anesthetized rats with hypocholeresis. Also he demonstrated that fumaria extract has both antispasmotic and spasmolytic activity.

A study by Gorbunov *et al*., (1980) demonstrated that *Fumaria officinalis* alkaloids have cardiovascular activity, by decreasing or preventing myocardial ischemia caused by occlusion of the coronary artery.

Fumaria decoction makes a curative lotion for milk-crust on the scalp of an infant (Khory and Katrak, 1981).

The Japanese make a tonic from it, cows and sheep eat it, and the later are said to derive great benefit from it (Fernandez, 1982).

Physicians and writers from Dioscorides to Chaucer, and from the fourteenth century to Cullen and modern times evaluate its purifying power (Lust, 1983).

Fumitory has been highly valued since at least Roman times for it's tonic and blood cleansing effect upon the body (Phillips and Foy, 1990).

The herb is antispasmodic, slightly diaphoretic, mildly diuretic, laxative and weakly tonic (Launert, 1981; Grieve, 1984; Chopra *et al* 1986).

French and German physicians still preferil to most other medicines as a purifier of the blood while sometimes the dried leaves are smoked in the manner of tobacco, for disorders of the head. Externally fades freckles, antiseptic, anti inflammatory lotion for spot and eczema (Mallard, 1998). Some caution should be exercised in the use of this herb since excess doses cause hypnotic and sedative effects, especially if it's taken for more than about 8 days (Bown, 2001).

It is particularly valuable in the treatment of all visceral obstructions, particularly those of the liver, in scorbutic affections and in troublesome

eruptive disease of the skin, especially eczema for which it can be taken internally and externally (Hoffman, 1995).

An intravenous administration of the alkaloids from fumitory resolved myocardial ischemia and arrhythmias in dogs with induced coronary blood flow disorders (Grieve, 1996).

Protopine has been shown in animal models to be antihistamine and sedative in low doses, yet stimulating and convulsant at high doses. It is considered lithotriptic because it prevents the build-up of biliary calculi (Fetrow, 1999). Fumitory may also used as an eye wash to ease conjunctivitis (Mitch, 1997).

Fumitory is contraindicated in glaucoma because there is a possibility of increasing intraocular pressure (Mills, 2000).

There is also of theory on what fumitory might interact with. If some one is taking it as antihypertensive there might be an increased hypotensive effect which could dangerously lower some one's blood pressure.

1-6-4 Active Compounds

The most prominent constituents of fumitory are the alkaloid fumarine, fumaric acid, and considerable amounts of inorganic matter, especially potassium salts (Fernandez, 1982).

Fumaric acid was early identified as present and its isomerism with malic acid which was established later (Hoffman, 1995).

Fumaric acid is obtained by heating malic acid ($C_4H_6O_5$) with water acidulated with sulphuric acid, 180°C (356°F), where by one molecules of water is split off.

It has been used for the treatment of psoriasis, but a too high dosage increase sugar level in blood (Zienty, 1962; Davis, 1964).

It has used as a food acidulant and as a row material in the manufacture of unsaturated polyester resins, quick-setting inks, furniture, paper sizing chemical and aspartic acid (Birt and Weyens, 1974; Mallard and Linstorn, 1998).

Fumitory occur in colorless prisms or stellate scales, somewhat soluble in cold, more soluble in hot water, to which it imparts an acid reaction; is also soluble in alcohol and ether, and forms salts with bases mostly soluble in water but all of which are insoluble in absolute alcohol (Grieve, 1994; Mitch, 1997).

The alkaloid fumaria has been believed to be identical with corydaline, but it differs both in formula and in its reaction to sulphuric and nitric acids. It occurs in colorless, tasteless crystals, freely soluble in chloroform, less soluble in benzene and still less soluble in alcohol and ether, sparingly soluble in water (Meikle, 1977).

Alkaloids (candine, protopine, fumarine, fumaricine fumarilline, estilopine, cryptonine, sanguinarin, coryladine, …… etc.) main of all is fumarine, (0.13%) which though it shows antihistaminic and antiamathic properties, on aprolonged dosage may lead to respiratory disorders (James and Williams, 1974).

The leaves yield by expression a juice which has medicinal properties. An extract, prepared by evaporating the expressed juice or a decoction of the leaves, throws out upon its surface a copious saline efflorescence (Preisner and Shamma, 1980).

In spite of all compounds mentioned before also it contain mucilage, amino aids, resin, phlobaphene, quercitin, benzophen, anthridines, chlorogenic acid, caffeic acids, etc. (Hoffman, 1995; Fetrow, 1999).

Fumaria officinalis also contain isoquinoline (Guinan and Shamma, 1983), one of them are Berberine which has general antimicrobial, trypanocidal, antifungal, anthelminthic and tuberculostatic propeties. It is widly used for treatment of malaria, amoebiasis and leshmaniasis (James and Williams, 1974).

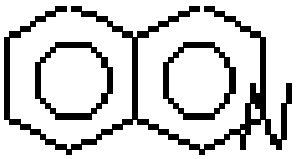
Also there are Protoberberine alkaloids which are one of the most widely distributed groups of benzyl isoquinoline alkaloids. They occure in at least 10 plant families one of them are Fumaraceae (Deans, 1992). It is widely used for treatment of malaria, amoebiasis and leshmaniasis (James and Williams, 1974).

Fumaria officinalis also contain protopine alkaloids berberine or protoberine alkaloids (Shimizu *et al*., 1994).

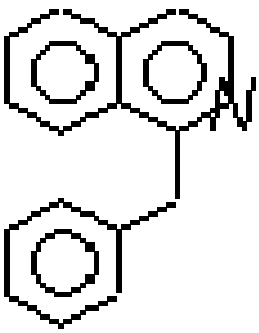
Protopine has smooth muscle relaxant, hypotensive and bacteria as well as ocytocis properties (Rienstra *et al*., 1998).

1-7 Alkaloids of *Fumaria officinalis*

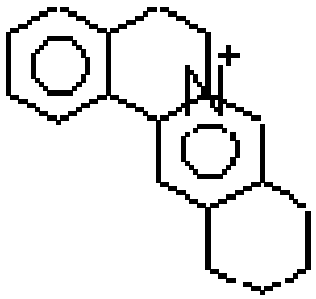
There are many types of *Fumaria officinalis* and these include the following groups of alkaloid and organic acid (Preisner and Shamma, 1980).



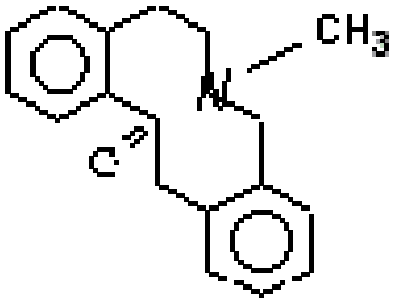
Isoquinolines



Benzylisoquinolins

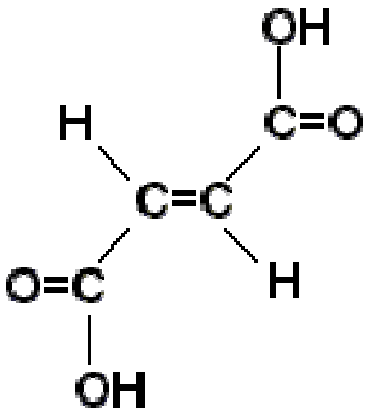


Protoberberines

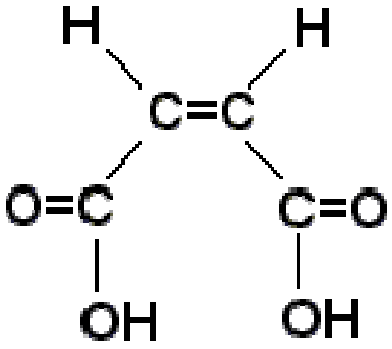


Protopines

while the organic acids are

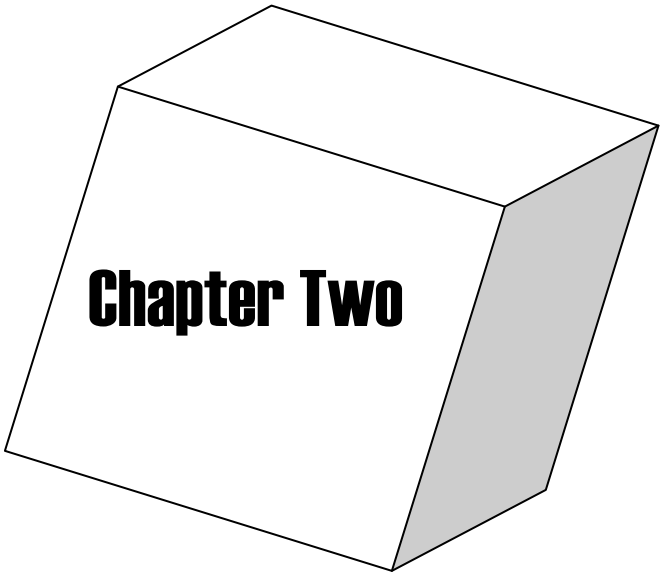


Fumaric acid



Maleic acid

Fumaria officinalis
http://www.ibiblio.org/herbmed



Materials and Methods

Materials and Methods

2-1 Materials

2-1-1 Equipment and Apparatus

The following equipments and apparatus were used in this study:

No.	Equipment	Company (Origin)
1.	Autoclave	Gallenkamp (England)
2.	Electric balance	Metter (Switzerland)
3.	Electric oven	Gallenkamp (England)
4.	Incubator	Gallenkamp (England)
5.	Light microscope	Olympus (Japan)
6.	pH meter	Radiometer
7.	Rotary-evaporator	Buchi (Germany)
8.	Haemocytometer	Assistent (Germany)
9.	Water bath	Gallenkamp (England)

2-1-2 Chemicals

No.	Materials	Company (Origin)
1.	Chloroform	BDH (England)
2.	Ethanol	Ajax (Australia)
3.	Methylene blue	Fluka
4.	Peptone	Oxoid (England)
5.	D-glucose	BDH (England)
6.	Agar	Oxoid (England)

-White Field Ointment

In this study, white field ointment was used to treat the infected area. This is considered as fungicidal drug. It's manufactured by the state company for drug industries and medical appliances Sammara, Iraq. The fungidin ointment contains:

1% clotrimazole.

2.2 Methods

2.2.1 Collection of the Plant:

The plant was collected from the local markets and identified by the Iraqi National herbarium (Baghdad, Abu-Graib).

The aerial parts of the plant were air dried at room temperature and grinded into a powder form.

2.2.2 Extraction of Alkaloidal Components (Ethanolic Extract)

Fifty grams of plant powder was extracted with 250 ml of ethanol 70% using reflex apparatus for 4 hrs then filtered it by Whitman No. 1 HCL was added to make the pH 3 then evaporated at 40°C under vacuum, put it in separatory funnel, 25 ml of chloroform was added to the residue in a separatory funnel.

The chloroform layer was collected. This step was repeated three times. The collected chloroform layers were mixed and evaporated by rotary evaporator representing the total alkaloids (Harborne, 1973).

2.2.3 Detection of Alkaloids

Ten ml of plant extract was acidified by adding HCL, test it by Mayer's and appearance of white precipitate indicates the presence of alkaloids (Smolensk *et al*., 1972).

2.2.4 Preparation of Culture Media:

2.2.4.1 Sterilization Methods (Cappuccino and Shermany, 1987):

a- Culture media was sterilized by autoclave at 121°C / 15 pounds / $inch^2$ for 15 min.

b- Glass wares were sterilized in the electric oven at (180-200)°C for 2 hr

2.2.4.2 Preparation of Modified Sabouraud Agar:

Fungi were cultured in modified Sabouraud dextrose agar prepared according to Finegold *et al*., 1982, by mixing the following ingredients:

Peptone	10 g
Glucose	20 g
Agar	20 g
Cycloheximide	0.5 g
Cephalexin	0.5 g
Distilled Water	1000 ml

The cycloheximide was added to this media to prevent the growth of saprophytic fungi, while the cephalixin was added to prevent the growth of bacteria (Beneke and Rogers, 1980).

2.2.5 Preparation of the Extract Ointment:

The ointment of *Fumaria* extract was prepared by mixing about 6 ml of glycerol with equal volume of the ethanolic extract.

2.2.6 Laboratory Animals:

In this study, three groups of mice were used.

These mice were received from the Biotechnology Research Center of Al-Nahrain University.

They were divided as the following:

1. The first group: three animals used as a control to study the normal structure of skin.
2. The second group: three animals were used, infected with *Trichophyton rubrum*. Animals of this group were used to examine the histopathological changes caused by *Trichophyton rubrum*.
3. The third group: nine animals used each one was infected with *Trichophyton rubrum*, after the appearance of lesions, the infected area was treated with plant extract and compared with that treated with the ointment.

Note: animals in second and third group were infected with 1 ml ($6.95x10^6$ spore / ml) of fungal suspension (Intraperitonial IP)

2.2.7 Preparation of Antifungal Samples:

The stock solution was prepared by dissolving 6 g of plant extracts residue with 60 ml sterile distilled water, where as extracts which were insoluble in water 2% of ethanol extract was added then the volume completed with

sterile distilled water. The plant extracts were prepared at different concentration started with 2 mg / ml, 4 mg / ml and 6 mg / ml.

Plant extracts were added to modified Sabouraud dextrose agar containing cephalexin and cycloheximide at the ratio 1.5 : 1.5 ml, all petridishes were inoculated with fungal spore and incubated at 37°C for 7-14 days (Al-Samarei *et al*., 2001).

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

$$\text{Inhibition percent\%} = \frac{\text{Average diameter of fungal growth in control plate} - \text{Average diameter of fungal growth in treated plate}}{\text{Average of fungal growth in control plate}} * 100$$

2.2.8 Spore Suspension

Spore suspensions were prepared according to Faraj method (Faraj, 1990), spores were harvested by adding 5 ml of sterilized distilled water containing 0.1% tween 80 to aid wetting and separation of the spores, then the fungal growth was separated by a loop. The suspension was filtered through sterile cotton wool, the filtrate was centrifuged tubes, further washed with distilled water. The supernatant was removed and the spores were washed twice by resuspending in sterile distilled water and further centrifuged. Then 5 ml of sterile distilled water was added to the supernatant and mixed vigorously by the vortex for 1 min. One drop of the

suspension was added to haemocytometer by Pasteur pipette, spores were calculated under large power X40 of light microscope using the following equation:

Concentration of spores = $(Z \times 4 \times 10^6)$ / n spores / ml

Where n: total No. of small squares

Z: total No. of spores.

2.2.9 Animal Treatment

After 11 days of infection, the lesions were exerted, and the animals were treated topically:

1. The first groups: three animals infected with *Trichophyton rubrum* were treated with *Fumaria* extract.
2. The second groups: animals infected with *Trichophyton rubrum* were treated with *Fumaria* extract and glycerol.
3. The third groups: animals infected with *Trichophyton rubrum* were treated with fungidin ointment.

2.2.10 Light Microscopic Study

Samples obtained from the skin, were prepared for histopathological studies.

1. Samples were fixed in 10% formalin for 24 hr. (Bancroft and Stevens, 1982)
2. Placed in 70% ethanol overnight.

3. Dehydration by 4 changes of (80%, 90%, 95% and 100%) ethanol for 2 hrs for each concentration.
4. Placed in xylene for 2 hrs to clear the tissue.
5. Then embedded in melting paraffin (melting point of paraffin in 58°C) for 2-3 hrs at 60-70°C in the oven.
6. Blocked in paraffin wax and section were cut by a microtome 4-5 μm in thickness.
7. Tissue section fixed on glass slides by Mayer's albumin.

a) Haematoxylin-eosin Staining:

i. Tissue section on slides was dewaxed by xylene.
ii. Washed shortly in 3 changes of absolute alcohol.
iii. Then 95% alcohol and 70% alcohol.
iv. Washed in water for 5 min.
v. Stained with haematoxylin for 5-10 min washed in water 5 min.
vi. The slides were then placed in eosin for 10-15 sec.
vii. Washed in water for 2-3 min, acid alcohol for 1% dipping.
viii. The sections were then dehydrated in (70, 89 and 95)% alcohol, few seconds for each and 2 changes of absolute alcohol.
ix. The slides were left, then to dry.
x. Put in xylene 15-30 min, and covered by cover slip with Canada balsam (Bancroft and Stevens, 1982).

b) Periodic Acid-Schiff (PAS) Staining Procedure:

i. Cut paraffin sections at 5 μm.
ii. Deparffinzed by xylene.
iii. Absolute alcohol.

iv. Add alcohol 95%.

v. Rinsed in distil water.

vi. Periodic acid solution for 5 min (Oxidizer).

vii. Rinsed in distil water.

viii. Placed in Schiff's leuco. Fuchsin for 15 min. for pink color to develop.

ix. Stained in Harries's Hematoxyline for 6 min, or light green counter stain for a few sec. Light green is recommended for counter staining sections in which fungi are to be demonstrated. Omit step 12 through 16 if light green is used.

x. Rinsed in tap water.

xi. Differentiated in acid alcohol-3 tolo quick dips.

xii. Washed in tap water.

xiii. Dip in ammonia water to blue sections.

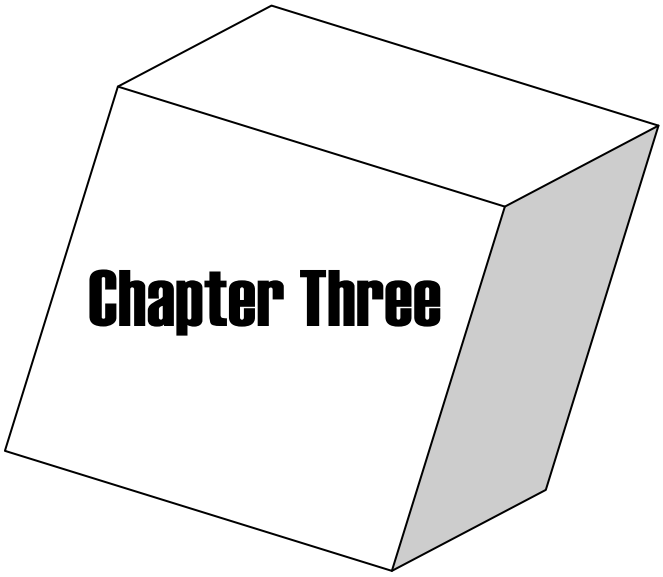
xiv. Wash in running tap water 10 min.

xv. Alcohol, 95%.

xvi. Absolute alcohol, 2 changes.

xvii. Xylene, 2 changes.

Mountin In permount (Ambrogi, 1960).



Results and Discussion

Results and Discussion

3-1 Ethanolic Extract of Fumaria

The resulted volume of the ethanolic extract is 6 ml after evaporation the weight of the residue equal to 1g it appeared with dark brown color.

3-2 Detection on Alkaloids

By using Mayer's reagent

A drop of Mayer's reagent was added to few drops of the plant extract, a white precipitate was observed indicating presence of alkaloids.

3-3 Effect of Plant Extract on growth of Dermatophytes

There are many researches about the effect of plant, and its active component to inhibit dermatophytes.

In our study, we used ethanolic extract of *Fumaria officinalis* against the Fungus *Trichophyton rubrum* and determined its inhibition effect at concentration 2, 4 and 6 mg / ml using sabouraud dextrose agar and incubate it for 7 days (Figure 3-1).

Depending on our results, it is clear that ethanolic extract of *Fumaria officinalis* with different concentration has an inhibitory effect (Table 3-1)

Table (3-1) Inhibition diameter of *Trichophyton rubrum* showing percentage of fungal growth by *Fumaria officinalis* extract.

Fumaria officinalis (mg / ml)	Diameter of fungal growth (cm) (m ± s)		Inhibition (%)
0	a	8 ± 0.21	0
2	b	4.75 ± 0.086	40.6
4	c	2.5 ± 0.013	68.7
6	d	0.5 ± 0.05	93.7

Numbers with different litters are significantly different at 0.05.

Even low concentration of the extract has an antifungal activity. Many plant active compounds are considered as antimicrobials including alkaloids, phenols, volatile oils (Al-Ani, 2002; Al-Zawabaiy, 2003). So this inhibitory effect is attributed to the alkaloid found and detected in *Fumaria officinalis* extract. *Fumaria* alkaloids are one the major active plant ingredient considered as antibacterial (Dornberger and Lich, 1987) and as antifungal (Guerin an Reveilleve, 1984; Rios *et al*., 1987).

There are many study ensure that this plant contains alkaloids (Reynier, 1977; Guinadeau and Shamma, 1983).

Depending on the site of action, pharmaceutical studies classified antimicrobials into:

1. Drug that inhibit cell wall synthesis.
2. Drug that inhibit protein synthesis.
3. Drug that inhibit nucleic acid synthesis.
4. Drug affecting cytoplasmic membrane

(Laurence *et al*., 1997).

3-4 Clinical Features of the Infected Animals

After 16 days of infection with *Trichophyton rubrum* Figure 3-2, 3-3, 3-4, 3-5 and 3-6 pointed certain degenerative changes due to the adherence of the fungal conidia into the keratinophilic layer of the skin producing skin infection.

The conidia enter the skin through an abrasion, germinate and the hyphae begin to grow in the stratum corneum. The hyphae invade the hair follicle and enter the cortex of the hair by dissolving the keratin. The hyphae and conidia were carried to the surface by the growing hair which often breaks off.

Invasion of the hair causes the shaft to be weak and break, resulting in circular, scaly areas with loss of hair as in Figure 3-2, 3-3, 3-4, 3-5 and 3-6. skin area infected with *Trichophyton rubrum* show irregular margin with papule, macule with boil formation, redness and swelling (2 x 2 cm) (figure 3-2). This indicates the invasion of the hyphae into stratum corneum inducing a hyperkeratosis with crust.

While other figures has shown swelling with ulceration, bullons like lesion, bullons like redness and loss of hair due to the effect of the pathogenic fungus figure (3-3, 3-4, 3-5 and 3-6) (1.2x1.2 cm, 1.25x1.25 cm, 1.6x1.6 cm, and 2x2 cm) respectively.

Irregular margin were seen, redness, slightly swelling with ulceration and deep seated margin figure (3-5) (1.6x1.6 cm). This represented by elevation of the skin due to accumulation of inflammatory cells.

This in turn leads to dilation of the blood vessels causing redness of the skin.

Dermatophytes have the ability to use keratine as food sources because of keratinase enzyme and the infected area have loss of hair, redness area and dilation of blood vessels (Koneman *et al*., 1992).

The resulting inflammatory response by the host is the most intense at the area of the recent invasion. Similar changes were observed by Lucky, (1985) on *Trichophyton* infection cause tinea capitis.

Some *Dermatophytes* produce only mild or no inflammation or immune reaction; in such cases, the organism may persist indefinitely, causing intermittent remissions and exacerbations of a gradually extending lesion with a scaling slightly raised border (Ohst, 2004)

The specific clinical features in general depend on the inflammatory response of the host. Lesions usually appears as erythematous area with fine scales, often in circular patterns, the scales represent increased epidermal turnover in response to the inflammation (Weitzman and Summerbell, 1995).

The disorders caused by the *Dermatophytes* are appropriately called dermatophytoses, because of the characteristic ring shaped lesion that produced, these dermatophytoses are popularly called ringworm; they are also referred to as tinea (Latin for "worm " infections.)

After the treatment with *Fumaria officinalis* extract regenerative changes were observed in all animal groups. The crust tends to drop-off spontaneously after the 5 day leaving a white scar, usually slightly depressed along its entire length.

This initial depression of the healing incision line disappeared by the 11 day of the treatment. The incision was completely covered with newly formed epithelium and the hair as seen in Figure (3-7, 3-8, 3-9, and 3-10)

which represent the skin infected and treated with the extract. The regeneration was gained after the treatment with this extract.

The incision was completely covered with newly formed epithelium and the hair as seen in figure (3-7, 3-8) which represents animal skin infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract and glycerol, representing normal appearance of previous infected area.

Figure (3-9) and (3-10) represent the skin of mice treated with *Fumaria officinalis* showing slight remaining of previous infected area, growth of hair and still showing mark between regenerative area and normal area.

Physical inspection involves observing the skin, hair, nails and mucous membranes. Noting colour, size of lesions, moistness/ dryness, texture and anatomic location and distribution of lesions (Gupta, 1999).

Comparing these results with that animal infected with *Trichophyton rubrum* and treated with fugidin ointment, slower regenerative changes were seen. The crust tends to drop off spontaneously and the incision which was completely covered the area were less then those that covered the area treated area with *Fumaria officinalis*. In group treated with fugidin redness still persist with ulceration, crusting and slightly loss of hair (figure 3-11) indicating that *Fumaria* extract is more active than fugidin in healing the infected area suspecting that time for healing by *Fumaria* extract is less than that in fugidin ointment which is also another fact for the activity of this plant extracts.

3-5 Histological Examination by Light Microscopic Examination

A- Control Animal

The light microscopic examination of the skin of the control animal, figure (3-12) has shown that the skin consists of two distinct but lightly attached layers.

1- The epidermis layer.

2- The dermis layer

Surface layer consist of many layers of dead plate keratin material, underneath these was keratinocytes with thick plasma membrane (stratum corneum), while stratum spinosum consists of several layers of large keratinocytes overlying the stratum basales. The dermis consists of connective tissue, blood vessels and nerves tissue.

Similar structure was found in several mammalian animals such as rats, guinea pigs and rabbits (Mawafy and Cassens, 1975; Shigeo and Youtako, 1986).

B- Experimental Animals:-

Animals infected with *Trichophyton rubrum* have shown certain histological changes with adherence of the fungal conidia to the Keratinocytes caused keratinolysis which promote conidial invasion and growth in the epidermal layer.

Pierard *et al*., (1997) found that fungal toxic activity of different species promote fungal growth on stratum corneum. The adjacent epidermis showed abnormalities in which it lost its normal structure of the

skin. The epidermis adjacent to the infected region showed certain degenerative changes in the nucleus and the cytoplasm.

Histological section of represented skin indicated the presence of edema with heavy acute inflammatory cell in dermis area extended to hypodermis with abscess formation, (figure 3-13).

Other section of infected skin shows edema and heavy inflammatory cell reaction in dermis area and underdermis (hypodermis area) this is seen in (figure 3-14). In addition to the appearance of polymorph neutorphils and necrotic debris (figure 3-15). The inflammatory reaction extended to the deep dermis layer forming an abscess (figure 3-16).

Those clinical signs result from inflammatory response and as a result of penetration and digestion of the non-viable outer skin layer and of hair shafts by infective fungal elements.

Arthrospores (the infective element) present on the broken hairs, collars and brushes from infected or carrier animals and contamination of the environment comes from these sources in which arthrospores invade hair shafts and stratum corneum (the outer – most layer of the epidermis) (Weinberg *et al.*, 2005)

An infected hair follicle may rupture and spread organisms to tissue beneath the skin (subcutaneous tissue) producing a firm. Sometimes painful nodule known as a "Pseudomycetoma" are found (Mukherjee, 2003).

The dermatophytes have the ability to invade keratinized tissue (skin, hair, and nails) but are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host (Serrano, 2004) chronic dermatophytosis is mostly caused by *Trichophyton rubrum*, and there is some evidence that mannun

produced by this fungus suppresses or diminishes the inflammatory response (Santos, 2005).

Most fungi can survive a wide range of temperatures (from 10^{o}C to 40^{o}C). the human body temperatures of 37^{o}C is quite high for the survival and growth of most fungi with exceptions like *Candida albicans*, which usually grows well in a laboratory temperature of 37^{o}C, and the dermatophytes (ringworm fungi), which grow well at 28.3 to 30^{o}C. The human body is capable of being their host (Elewski, 1997).

Other suggestions explained that the ability of dermatophytes to invade the skin and cause cutaneous inflammation is due to the presence of exogenous enzymes like elastase, alkaline phosphotase and leucine arylamidase enzyme which play a significant role in the infection (Brasch and Zaldue, 1994). Dermatophytes have the ability to invade skin because, this invasion to deratinophilic layer results in degeneration and transmission of fungus to hair follicle (Voisard *et al*., 1999) so that's why fungal spores are found in hair follicles in stratum dermis.

These results confirm with the findings of Droule *et al*., (1991), that the fungal conidia induce the hyperkeratin lysis of the skin layer with the infection of stratum corneum and padded into the underlying dermis layer.

Fungus can become pathogenic either when the hosts defense mechanism breaks down or when environmental conditions allow the fungus to grow in overabundance, overtaking the body's normal defenses. Among the approximately 50000 species of fungi, there are some 200 pathogens (agents capable of causing disease). About 20 of them are commonly isolated in cutaneous (relating to skin) infections (Kane, 1997).

It is well known that appearance and increase in number of inflammatory cells and conjestion of blood vessels are an inflammatory

response to infection and indicating that fungus has the ability to invade epidermis layer and extended to dermis layer (figure 3-16).

There are four defense mechanisms against dermatophytes infection includes:

1. The skin's natural barrier function.
2. Skin turnover.
3. Serum inhibitory factor.
4. Cellular immune system (type IV delayed hypersensitivity reaction) (Czaika, 1998).

C- Animal Treatment with *Fumaria* Extract

This extract was used since it contains alkaloidal compounds which are considered as a strong anti-dermatophytic agent (Damirdagh, 2000).

Figure (3-17 and 3-18) represented skin infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* and glycerol showing still more moderate inflammatory reactions.

These two figures appeared with certain regenerative changes because of migration of neutrophiles and monocyte from inner of blood vessels into the outer of blood vessels.

These regenerative changes referred to the action of alkaloidal compound of *Fumaria officinalis* extract which in figure (3-1) ensure the results that alkaloidal extraction has a strong antifungal activity during the *in vitro* study on *Trichophyton rubrum* in spite of low concentration of the extract.

Costo, (1985) referred to the migration of phagocytic cells into the wound and this is really resulted in dialation of blood vessels in the dermis

layer and infiltrates tissue with inflammatory cells like neutrophiles and monocyte.

The skin infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* showed after a time, the presence of abundant inflammatory reaction (figure 3-19 and 3-20).

Studies of drug efficacy, however, must make this distinction, since the complete cure that may precede environmental reacquisition of the organism represents successful therapy, where as recrudescent disease indicates in adequate therapy (Kim, 1999).

Infected skin treated by fugidin in (figure 3-21) shows still more abundant inflammatory cell reaction, while figure (3-22) of infected skin treated by fugidin in show still more abundant inflammatory reaction and abscess formation.

By comparing the results of the infected and treated skin we observed that the infected skin treated with *Fumaria officinalis* gave a better response than that of treated with fugidin after 11 day of treatment. This healing could be achieved from all the parameter or signs of regeneration used in our study. These results pointed the efficiency of *Fumaria* extract as an active antifungal agent.

In general pharmaceutical studies of antifungal agents 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 recognized 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 the cellular membrane (Tyler *et al*., 1988).

Some drugs can be administered alone or as poly-pharmaceutical (following the principle of therapeutics) in the form of decoctions, tablets infusions, powders, confections, electuaries, preserves, conserves, syrups, linctus, calcined preparations etc.

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

Figure (3-1) antifungal activity of *Fumaria officinalis* against *Trichophyton rubrum* at concentration 2, 4 and 6 mg / ml.

A: Control; B: 2 mg / ml; C: 4 mg / ml; D: 6 mg / ml

Figure (3-2) Morphological changes caused by *Trichophyton rubrum* (2x2 cm).

Note: Papule, macule with boil formation .

Figure (3-3) Morphological changes caused by *Trichophyton rubrum* (1.5x1.5 cm).
Note: The swelling with ulceration of skin.

Figure (3-4) Morphological changes caused by *Trichophyton rubrum* (1.25x1.25 cm).
Note: Swelling area (ballous like lesion) and slight ulcertin.

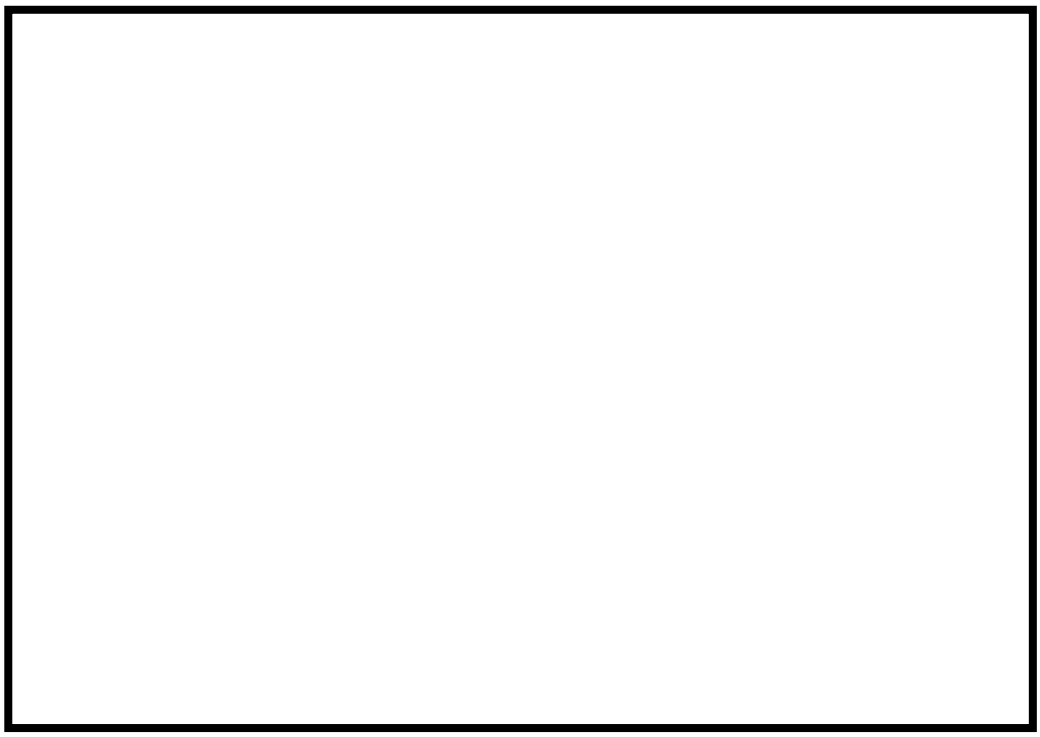
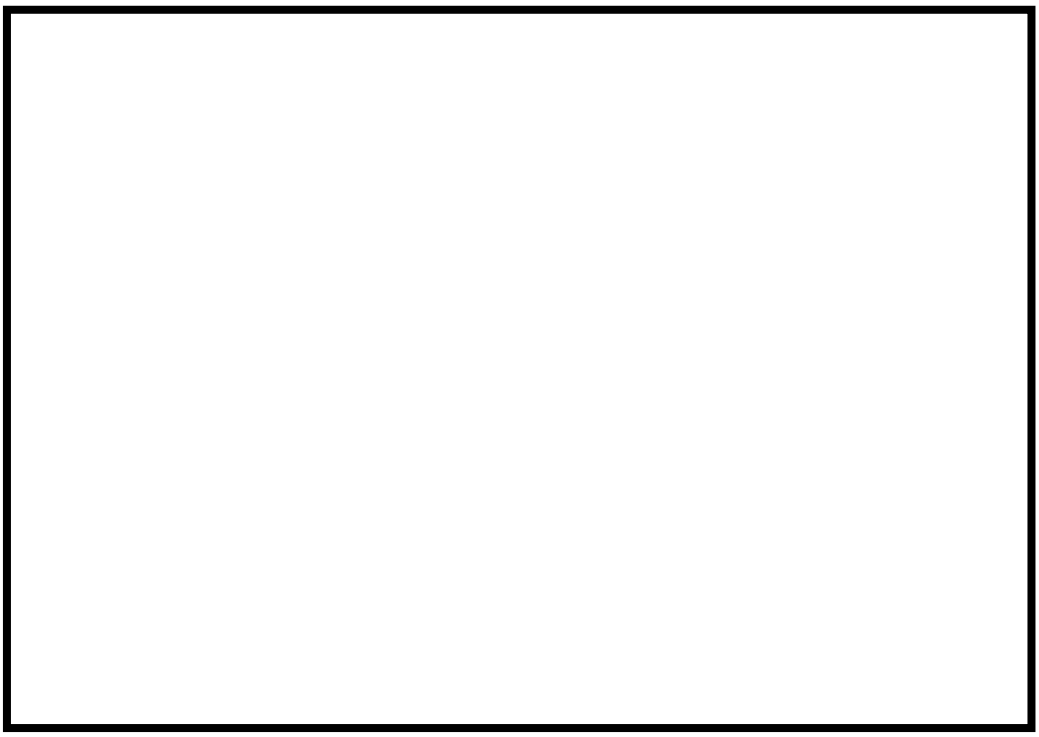


Figure (3-5) Morphological changes caused by *Trichophyton rubrum* (1.6x1.6 cm).
Note: Irregular margin with deep seated margin with loss of hair.

Figure (3-6) Morphological changes caused by *Trichophyton rubrum* (1.25x1.25 cm).
Note: Slightly swelling with ballons like redness with loss of hair.



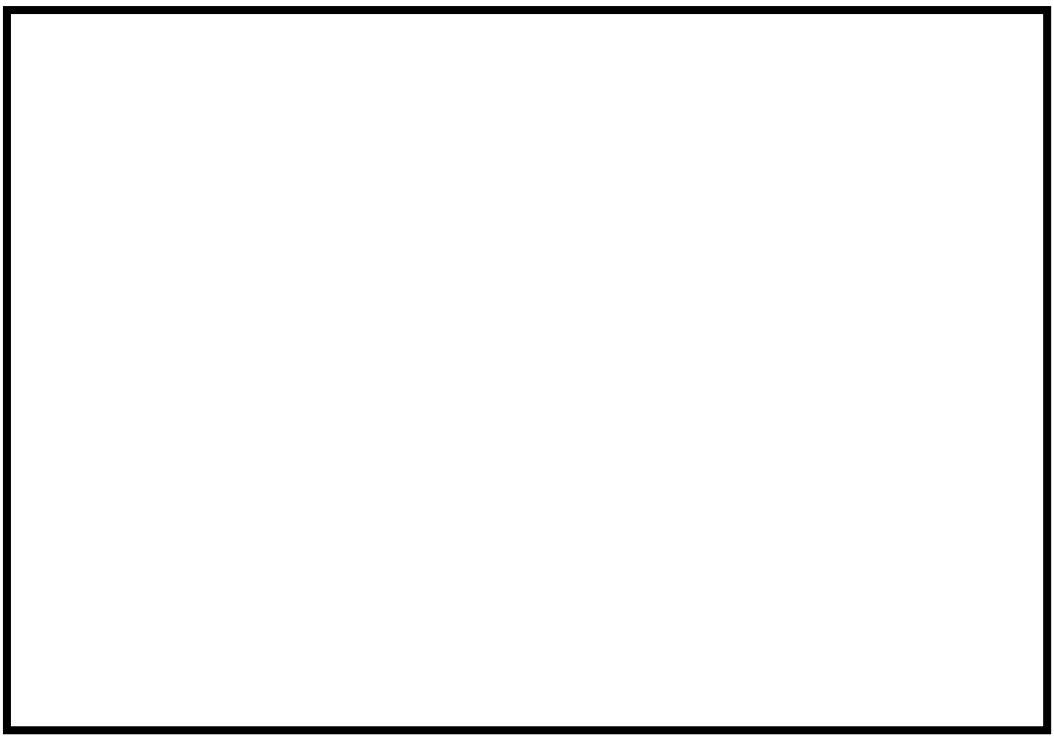


Figure (3-7) Morphological repair of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* + glycerol.

Figure (3-8) Morphological repair of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* + glycerol.

Figure (3-9) Morphological repair of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract.

Figure (3-10) External features of mice infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract.

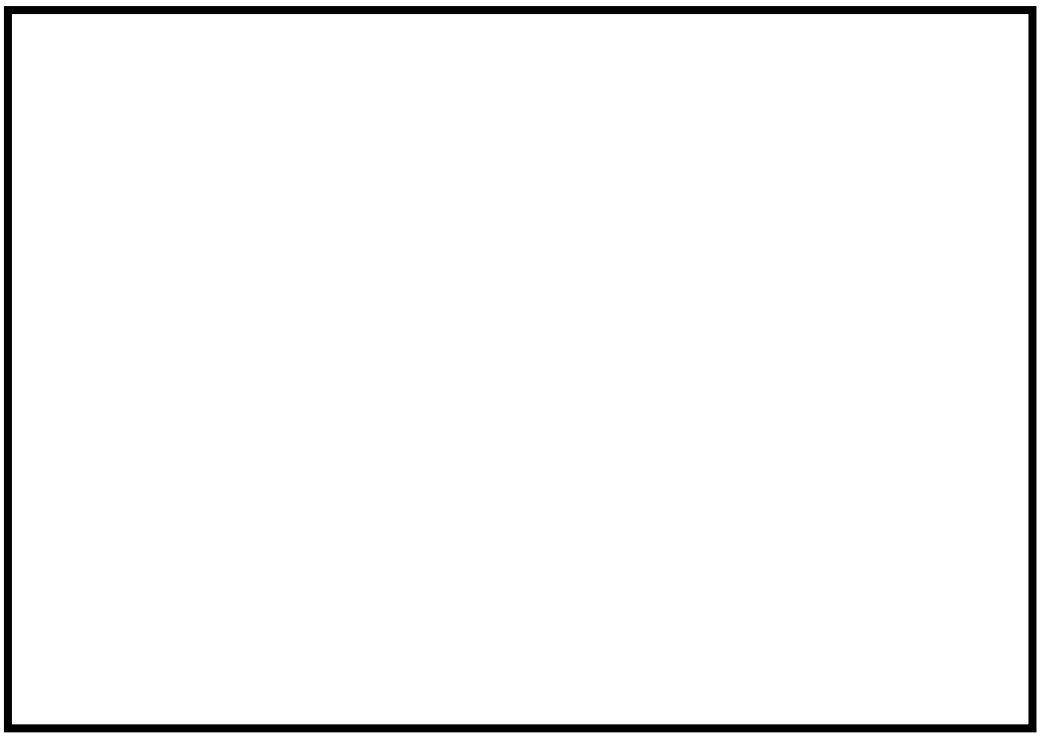


Figure (3-11) Morphological repair of an animal infected with *Trichophyotn rubrum* and treated with fugidin.

Figure (3-12) C. S of normal skin of mice.

Note: Colagen fiber (→) stratum corneum (↑↑) stratum basales (◄) stratum spinosum (▲)

Haematoxylin-Eosin stain (200x).

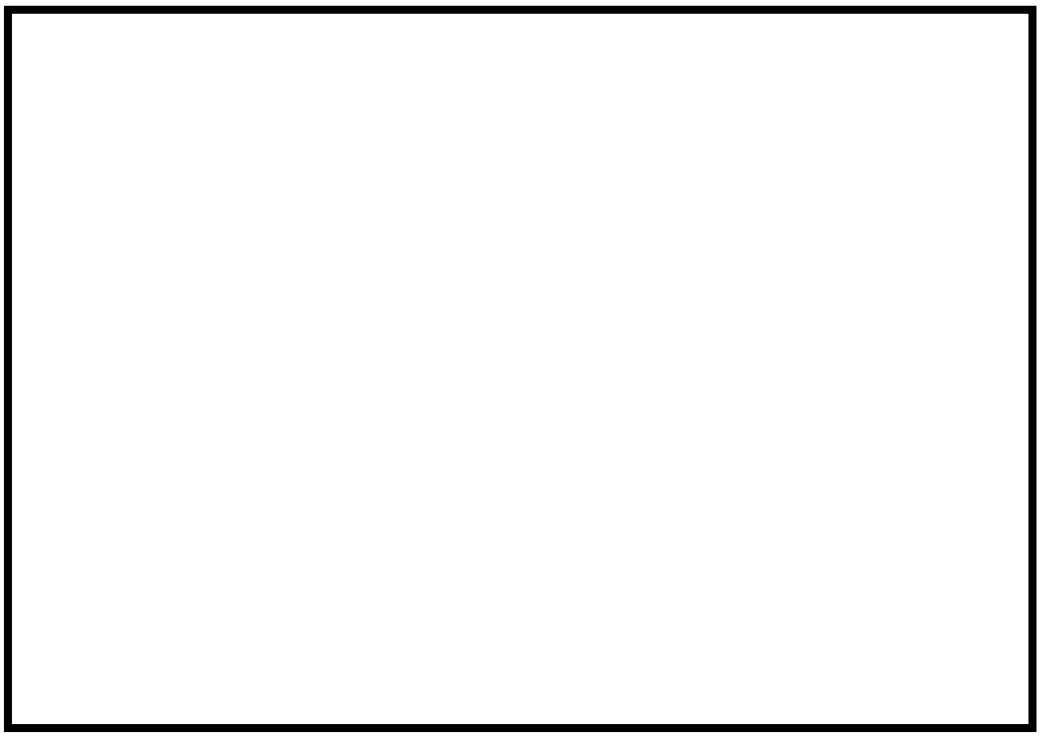


Figure (3-13) C. S in skin layer of an animal infected with *Trichophyton rubrum*.
Note: Oedema with heavy acute inflammatory cell (↑) in dermis area extended to hypodermis with absces formation (▲)
Haematoxylin-Eosin stain (400x).

Figure (3-14) C. S in skin layer of an animal infected with *Trichophyton rubrum*.
Note: Oedema (↑) and inflammatory cell reaction (▲)
Haematoxylin-Eosin stain (100x).

Figure (3-15) C. S in skin layer of an animal infected with *Trichophyton rubrum*.
Note: Polymorph neutrophiles (↑), necrotic debris (↕).
Haematoxylin-Eosin stain (200x).

Figure (3-16) C. S in skin layer of an animal infected with *Trichophyton rubrum*.
Note: Inflammatory reaction extend to the deep dermis layer forming an abscess (→).
Periodic Acid-schiff (PAs) stain (200x).

Figure (3-17) C. S in skin layer of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract with glycerol.
Note: Still more moderate inflammatory reactions (→).
Haematoxylin-Eosin stain (40x).

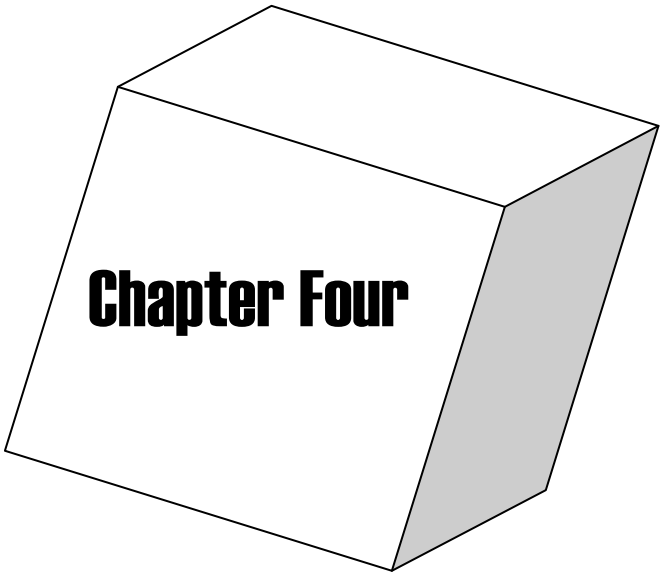
Figure (3-18) C. S in skin layer of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract with glycerol.
Note: Moderate inflammatory reactions (▲)
Haematoxylin-Eosin stain (400x).

Figure (3-19) C. S in skin layer of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract.
Note: Presence of inflammatory reactive changes (→).
Haematoxylin-Eosin stain (100x).

Figure (3-20) C. S in skin layer of an animal infected with *Trichophyton rubrum* and treated with *Fumaria officinalis* extract.
Note: Still presence abundant inflammatory reactions.
Haematoxylin-Eosin stain (200x).

Figure (3-21) C. S in skin layer of an animal infected with *Trichophyton rubrum* and treated with fugidine ointment.
Note: Still more abundant inflammatory reactive (→).
Haematoxylin-Eosin stain (100x).

Figure (3-22) C. S in skin layer of an animal infected with *Trichophyton rubrum* and treated with fugidin ointment.
Note: Still more abundant inflammatory reactions and abscess formation (→).
Haematoxylin-Eosin stain (40x).



Conclusions and Recommendations

Conclusions

From our study, we conclude the following points:

1. Skin infected with *Trichophyton rubrum* is accomplished by certain morphological changes like papule, macule, boil formation, ulceration, redness and loss of hair.

2. *Fumaria officinalis* extract with it's contents of active ingredient has the ability to heal the skin infected with *Trichophyton rubrum*. This extract is more efficient than the fugidin ointment, this could be concluded depending on the same period needed for healing in each one.

Recommendations

1. Further studies on separation, identification and purification of active compounds of *Fumaria officinalis*.
2. Further research on the pharmacological activity of this plant including cytotoxicity, immunoresponse and antitumor activity.
3. Propagation through plant tissue culture technique.

References

Abdel-Rahman, S. M. and Nahata, M. C. (1997). Treatment of tinea capitis. Ann. Pharmacolther. ; 31: 338-48.

Ajello, L. (1960). Geographic distribution and prevalence of the dermatohpytes. Annal of New York Acadeym of Science. 89: 30-38.

Ajello, L. (1974). Natural history of dermatophytes and related fungi. Mycopathol. Mycol. Appl. 53: 93-110.

Ajello, L. (1977). Mikstones in the history of medical mycology: the Dermatophytes. Recent advances in medical and veterinary Mycology, proceedings of the sixth congress of the international society of Tokyo, Tokyo. 3-11.

Alan, S. and James, L. (2000). Pathology, 2nd ed. Harcourt Publisher Limited. 23 (1): 489-492.

Al-Ani, A. O. (2002). Effect of *Tencrium polium* extract on the growth of some pathogenic microorganism. M.Sc. Thesis. Department of Biotechnology, College of Science, Saddam University.

AL-Sammarae, K. W.; Al-Rekabi, S. and Ahmed, B. R. (2001). Effect of leaves extract of *Withania samnifera* of the growth of some *Dermatophytes*. The second conference in medical and biological science. Zart privat University, P. 40.

Al-Shamma, A. and Mitscher, L. A. (1979). Comprehensive survey of indigenous Iraqi plants for potential economic value 1. Screening results of 327 species for alkaloids and anti-microbial agents. J. of Natural Products. 42: 633-642.

Aly, R. (1999). Ecology, Epidemiology and diagnosis of tinea capitis. Pediatr infect. Dis. J. 18: 180-5.

Al-Zobaiy, A. K. (2003). Therapeutic activity of *Trncrium polium* extract on selected dermatophytes. M.Sc. Thesis Department of Biotechnology, College of Science, Saddam University.

Ambrogi, L. P. (1960). Manual of histologic and special staining techniques, 2nd ed., McGraw-Hill Book Company. INC. New York.

Arora, R. B. (1965). Cardiovascular pharmaco-therapeutic of six medical plants indigenous to Pakistan and India. In: Hamdard pharmacopia of eastern medicine, Hamdrad foundation Pakistan, P. 422-448.

Bancroft, J. D. and Stevens, A. (1982). Theory and practice of Histological Techniques. 2nd ed. Churchill Livingstone: 483-516.

Barlow, A. J. E. (1976). Recent advances in fungus diseases. Int. J. Dermatol.; 15: 418-424.

Baxter, M. Rush-Murno, F. M. (1980). The superficial mycoses of man and animals in new Zealand and their diagnosis (3rd ed). Massey University.

Beneke, E. S. and Rogers, A. L. (1980). Medicinal Mycology Manual, with human mycosis monograph, 4th ed. Minneapolis-Burgess Publishing Comp. 20-75.

Birt, M. A. and Weyens. E. (1974). Hydrocarbon Process. 53 (11): 132.

Bown, Deni. (2001). New encyclopedia of herbs and their uses. Dorling Kindersley. London.

Brasch, J. and Zaldue, M. (1994). Enzyme pattern of dermatophytes. Department of dermatology, University of Kiel, Germany. 37 (2): 11016.

Chmel, L. and Buchvald, J. (1970). Ecology and transmission of *Microsporum gypseum* from soil to man. Sabouraudia, 8: 149-156.

Chopra, R. N.; Anand, K. K.; Sharma, M. L.; Bupinder Singh and Ghatak, B. J. (1978). Medicinal plant with strong potential. Indian Journal of experimental biology. (16) P: 1216-1217.

Chopra, R. N.; Nayar, S. L. and Chopra, I. C. (1986). Glossary of Indian Medicinal plants (Including the supplement). Council of scientific and industrial Research, New Delhi.

Czaika, V.; Tietz, H. J.; Schulze, P. and Sterry, W. (1998). Dermatomykose durch Trichophton verrucosum bei Mutter and Kind. Hantarzt. 48: 576-580.

Damirdagh Al-Wandawi, I. S.; Nokalan, A. M. and Al-Banaa, Y. M. A. (2000). Fungicidal effect of caffeine and harmala on *C. albicans*. J. of Diala Vol. 1 No. 2 Part. 2. Special issue third Sci. (April 2000) P. 65-73.

Damirdagh, I. S. and Al-Janabi, A. H. (2000). Inhibition of colony growth of some dermatophyte by some plant extracts. Al-Mustansiriya. J. Sci. 4 (1): 53-63.

Davis, R. H. (1964). Mechanism of inheritance. 2. Heterokaryosis. Pp. 567-588.l In G. C. Anisworth and A. S. Sussman (eds.), The Fungi. Vol. II. Academic Press, New York.

De Vroey, C. (1985). Epidemiology of ringworm (Dermatophytoses). Seminars in Dermatol. 4: 185-200.

De Vroey, C. (1988). *Dermatophytes*, A "nomen dubinm"? In: Tumbay E (ed). FEMS-symposium of dermatoses in man and animals. Izmir, Turkey. 1988: 16-23.

Deans, J. A. (1992). Lango's Handbook of Chemistry. 14th ed. McGraw. Hillbook. To Co. Inc. New York.

Dornberger, K. and Lich, H. (1987). Screening hach antimicrobial sowie potentiell concerostatisch wirksamen pflan-zeninhaltsstofen. Pharmazie. 37 (3): 215-221.

Droule, R.; Bickord, A. A.; Walker, R. L. and Channing, S. E. (1991). Favus in backyard flock of game chicken. California Veterinary Diagnostic Laboratory System. University of California. 35 (3): 625-630.

Dute. J. A. (1985). Die amphocholeretishe wirkung der *Fumaria officinalis*. Z. Alleg Med. 34: 1819.

Elewski, B. E. (2000). Tinea capitis: a current perspective. J. Am. Acad. Dermatol. 42 (1 pt 1): 1-20.

Elewski, B. E. and M. A. Charif. (1997). Prevalence of onychomycosis in patients attending a dermatology clinic in northeastern Ohio for other conditions. Arch. Dermatol. 133: 1172-1173.

English, M. P. (1972). Ecological aspects of *Dermatophytes* regarded essentially as anthrophilic. In: Preuser J. H. (ed). Medical Mycology, Zbl Bkt supple 8. Gustav Fisher Verlag; NY: 53-59.

English, M. P. (1980). Medical mycology: Studies in biology no. 119. Edward Arnold, London.

Ennons, C. W.; Pinford, C. S. and Utz, J. P. (1977). Medical Mycology. 3rd ed. Lea and Sebiger, Philadelphia: 117-167.

Estsves, J. Brandao, F. N.; Neves, H. and Custodio, J. S. (1959). Bol. Clin. Hosp. Lisbook. 23: 307. (Cited by Neves and Xayier, 1964, 76: 429-436.

Evans, E. G.; Dodman, B.; Williamson, D. M.; Brown, G. J. and Bowen, R. G. (1993). Comparison of terbinafine and clotrimazole in treating tinea pedis. BMJ. 307: 645-7.

Faraj, M. K. (1990). Regulation of mycotoxin formation in *Zea mays*. Ph. D. Thesis, Department of bioscience and biotechnology. University of Strathelyde, Glascow, U. K.

Fernandez, M; Grandson. (1982). To Planta Medicinal. Pamplone: Editions university of Navarro, P. 85.

Fetrow, Charles, W. and Avila, Juan, R. (1999). Professional's Handbook of Complementary and Alternative Medicines. Springhouse Corporation. Spring house, P. A.

Finegold, S. M.; Nartin, W. J. and Scott, E. G. (1982). Anti-microbial susceptibility test and assay in Baily and Scott Diagnostic Microbiology, 6th ed. Chap. 36, p. 404-585.

Fleming, Thomas, *et al.* (ed.) (2000). PDR for herbal medicines. Medical Economics Company Montvale, NJ.

Freg, D., Oldfield, R. J. and Bridjer, R. C. (1979). A color atlas of pathogenic fungi. Year Book Medical Publishers, Chicago.

Friedlander, S. F. (2000). The optimal therapy for tinea capitis. Pediatr Dermatol. 17: 325-6.

Garretta, G.; Ajello, L. and Padhye, A. A. (1990). Occurrence of *Arthroderma gloriae,* a geophile keratinophilic ascomycete, in Italy. J. Med. Vet. Mycol; 28: 99-102.

Gentles, J. C. and Holmes, J. G. (1957). Foot ring worm in coal miners. Brit. J. Induster. Med. 14: 22-29.

George, L. K. (1960). Epidemiology of dermatophytoses: sources of infection, mode of transmission and epidemiology. Ann. N. Y., Acad. Sci., 89: 69-77.

George, L. K.; Hand, E. A. and Meyges, R. A. (1956). Observation of rural and urban ringworm. J. Invet. Derm., 27: 335-353.

Goldstein, A. O.; Smith, K. M.; Lves. T. J. and Goldstein, B. (2000). Mycotic infections. Effective management of conditions

involving the skin, hair and nails. Geriatrics; 55: 40-2, 45-7, 51-2.

Gorbunov, N. P.; Sukhanov, A. A. and Bolotova, M. F. (1980). "Pharmacological correction of Myocardial ischemia and arrhythmias in reversible coronary blood flow disorders and experimental myocardial infraction in dogs" Kardiodogiya, 20 (5): 34-7. Through C. A. 93: 161224m.

Gorbunove, N. P. *et al*.(1980). "Pharmacological correction of myocardial ischemia and arrhythmias in reversible coronary blood flow disorders and experimental myocardial infarct in dogs". Kardiologia. May. 20 (5): 84-7.

Grieve Maude (1996). A modern Herbal. Barnes and Noble Books. New York (orig. 1931).

Grieve, M. (1984). A modern Herbal. Peuguin ISBN. 0-14; 046-440-9.

Grieve, M. (1994). A modern Herbal. Tiger Books International. London.

Guerin, J. C. and Reveilleve, H. P. (1984). Active antifonque dextraits vegetaux a usage therapeutique I. Etude de 41 extraits sur 9 souches Iongiques. Ann. Pharm. Fr. 42(6): 553-559.

Guinau deau, H. and Shamma, M. (1983). Izmiriue: a new protopine alkaloid. J. Nat. Prod. 46(6): 234-935.

Gupta, A. K. and N. H. Shear. (1999). The new oral antifungal agents onychomycosis of the townails. J. Eur. Acad. Dermatol. venerol. 13: 1-13.

Gupta, A. K.; Richard, K. S. and Piet, D. (1997). Current management of onychomycosis. Dermatoclinics, 15 (1): 121.

Hahn, R. Nahrstedt, A. (1993). High content of hydroxyl cinnamic acids esterified with (+)-D-Malic-acid in the upper parts of *Fumaria officinalis* . In: PM 59 (2): 189.

Harbone, J. B. (1973). Phytochemical methods sciences paper backs, Chapman and Hall.

Hashimoto, T. (1978). Carotenogenosis associated with arthrosporulation of *Trichophyton* species. J. Microbiol., 136: 1120-1126.

Haward, D. H. and Hawared, L. F. (1983). Fungi pathogenic for human and animals. Part. A. Vol. 3, Marcel Dekker.

Hawksworth, D. L.; Sutton, B. C. and Ainsworth, G. C. (1983). Ainsworth and Bisby's Dictionary of fungi common wealth, mycological institute, Kew. P 1-445.

Hellgren, L. and Vincent, J. (1980). Lipolytic activity of some dermatophytes. Department of Dermatology, University of Trondheim, Norway. J. of Medi. Microbiol. Vol. (13): 155-157.

Hoffman, David (1995). The new holistic herbal. Barnes and Noble Books. New York.

Hoffman, J. J.; Timmermann, B. N., Mclaughlin, S. P. and Punnapayat, K. (1995). Potential antimicrobial activity of plants from south western United States. Int. J. of Pharmacognosy, 31 (2): 101-115.

Hoffman, J. J.; Timmermann, B. N.; Mclaughlin, S. P. and Punnapayak, K. (1993). Potential antimicrobial activity of plants from the South Western United States. Int. J. of Pharmacognosy, 31 (2): 101-115.

Hubbard, T. W. (1999). The predictive value of symptoms in diagnosing childhood tinea capitis. Arch. Pediatr. Adolesc. Med. 153: 1150-3.

Ikram, M. and Inamul, H. (1980). Screening of medicinal plants for antimicrobial activity fitoterapia. 51: 231-235.

Itiggins, E. M.; Fuller, L. C. and Smith, C. H. (2000). Guidelines for the management of tinea capitis. British Association of dermatologists. Br. J. Dermatol. 143: 53-8.

James, M. N. G. and Williams, G. J. (1974). To Esso Research and Engineering Co. P: 30, 1249.

Jungerman, P. F. and Schwartzman, R. M. (1972). Veterinary Medica Mycology, Chapter 1. Lea and Febriger Philadelphia.

Kaaman, T. and Forslind, B. (1985). Ultra-structural studies on experimental hair infection caused by *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Acta. Derm. 65: 536-569.

Kane, J.; Summerbell, R. C.; Singler, L.; Krajden, S. and Land, G. (1997). Laboratory handbook of dermatophytes, a clinical guide and laboratory manual of dermatophytes and other filamentous fungi from skin, hair and nails-star publishing company, Belmont, California.

Kaplan, W. and Gump, R. H. (1985). Ring worm in the dog caused by *Trichophyton rubrum.* Vet. Med. 53: 139-142.

Katoh, T.; Sano, T. and Kagawa, S. (1990). Isolation of *Dermatophyte* from clinically normal scalps in *Microsporum canis* infections using the hairbrush method. Mycopathologia; 112: 23-25.

Khory, R. N. and N. N. Katrak. (1981). Materia Meidica of India and their Therapeutics, Neeraji Publishing House, New Delhi.

Kim, J. A; Takizawa, K.; Fukushima, K.; Nishimura, K. and Miyaji, M. (1999). Identification and genetic homogeneity of *Trichophyton tonsurans* isolated from several regions by random amplified polymorphic DNA. Mycopathologia 145: 1-6.

Koneman, E. W. (1985). Practical laboratory Mycology. 3rd edition. Williams and Wilkins. Baltimore.

Kwon chung, K. J. and J. E. Bennett, P. G. (1992). 105-150 Lea and Febiger. Philadelphia.

Laurence, D. R.; Benuett, P. N. and Brown, M. J. (1997). Clinical pharmacology. 8th ed. Churchill Livingstone p. 197.

Launert. G. (1981). Edible and Medicinal Plants. Itamlyn ISBN 0-600; 37216-2. Covers plant in Europe adrowing of each plant, quite a bit of interesting information.

Lawry, M. A.; Haueke, E.; Strobeck, K.; Martin, S.; Zimmer, B. and Romano, P. S. (2000). Methods for diagnosing onchomycosis: a comparative study and review of the literature. Arch. Dermatol. 136: 1112-6.

Lust. J. (1983). The Herb Books. ISBN 0-553-23827-2.

Mackenzie, D. W. R. (1961). The extra human occurrence of *Trichophyton tonsurans* in a residential school. Sabouraudia, 1: 58-64.

Mackenzie, D. W. R. (1988). Antigenic composition and serology of *Dermatophytes* and dermatophytoses in man and animals. Izmir, Turkey. 31-38.

Mallard, W. G. and Linstorn, P. J. (1998). Nist chemistry webbook, Nist. Standard Reference database number 69. National Institute os standard and Technology. Gaithersburg, MD 20899. (http://webbook.nist. gov).

Mantovani, A. (1978). The role of animal in the epidemiology of mycoses. Mycopathologia; 65: 61-66.

Marples, M. J. (1965). The ecology of *Microsporum canis* Bodin in New Zealand. J. Hygiene; 54: 378-387.

Martin, A. G. and Koboyashi, G. S. (1999). Superficial fungi infection: dermatophytosis, tinea nigra, piedra. In: freedberg 1M, *et al*.,

eds. Fitzpatrick's Dermatolgoy in general medicine. Vol. 2. 5th ed. New York: McGraw-littl, 2337-57.

Mastsumoto and Ajello, L. (1987). Current taxonomic concepts pertaining to the dermatophytes and related fungi. Int. J. Dermatol. 26: 491-9.

Meikle, R. P. (1977). "Flora of Cyprus" Vol. I; Benthammoxon Trust, Royal Botanic Gardens, Kew, U. K.

Midgley, G. and Clayton, Y. M. (1972). Distribution of *Dermatophytes* and *Candida* spores in the environment. Br. J. Derm. 86: 71-77.

Midgley, G.; Clayton, Y. M. and Hay, R. J. (1997). Medical Mycology. Mosby-Wolfe Publishing, London, P. 155.

Simon, M. and Kerry, B. (2000). Principles and practice of phytotherapy. Churchill Livingstone, Edinburgh, Scotland.

Mitch, L. W. (1997). Fumitory (Fumaria officinalis L) No. 59 of the series "Intriguing World of Weeds" Weed Technology. 11: 843-845.

Mowafy and Cassens (1975). Microscopic structure of pig skin. J. of Animal Science, Vol. 41 (5): 1281-1287.

Mukherjee, P. K.; Leidich, S. D.; Isham, N.; Leitner, I.; Ryder, N. S. and Ghannoum. M. A. (2003). Clinical *Trichophyton rubrum*. Microbiology 151: 145-155.

Myrvic, N. Q. and Weiser, R. S. (1988). Fundamental of medical bacteriology and mycology. Second ed., Lea and Febiger.

Neves, H. and Xavier, N. C. (1964). The transmission of tinea cruris, Brit. J. Derm. 76: 429-436.

Noble, S. L.; Forbes, R. C. and Stamm, P. L. (1988). Diagnosis and management of common tinea infection. Am. Fam Physican. 58: 163-74, 177-8.

Odd, F. C. (1979). Candida and Candidosis. Leicester University Press.

Ohst, T.; de Hoog, S.; Presber, W.; Stavrakieva, V. and Graser, Y. (2004). Origins of microsatellite diversity in the *Trichophyton rubrum-T. violaceumclade* (dermatophytes). J. Clin. Microbiol. 42: 4444-4448.

Philips, R. and Foy, N. (1990). Herbs pan Books Ltd. London ISBN 0-330-30725-8.

Philpot, C. M. (1977). Some aspects of the epidemiology of tinea, Mycopathologia, 62, 1: 3-13.

Philpot, C. M. (1977). The use of nutritional tests for the differentiation of dermatophytes. Sabouraudia. 15: 141-150.

Pierard, G. E.; Pierard Franshimot, C. and Arrese, J. E. (1997). Povidone-I doine wash solutions in the prevention of superficial mycoses.

Department of Dermatology. CHU Sart. Tilman, Liege, Belgium. Eur. J. Clin. Pharmacol, 53 (2): 101-104.

Preisner, R. M. and Shamma, M. (1980). "The Spirobenzyl. Isoquinoline" Lioydia, 43 (3), 305-318.

Rebell, G. and Taplin, D. (1974). *Dermatophytes*: their recognition and identification. 2nd ed. Coral Gables, Fla, U. of Miami. Press.

Rechinger, K. U. (1964). "Flora *Iranica fumaraceae*". No. 110/20.4.74 Akademische Druck-4. Verlagsanstalt, Graz-Austria.

Reynier, M.; Lagrange, E.; Haring, J. and Vigoronx, M. (1977). "Effect of *Fumaria* extract, protopine, and papaverine on the biliary secretion of the hypocholeretic rat". Trav. Soc. Pharm. Montpellier, 37 (2): 73-84. Through, C. A. 87: 111808k.

Rienstra, J. C.-Kiracofe; Graham, D. C. and Schaefer, H. F. (1998). Molecular Phys. 94, 767.

Rippon, J. W. (1982). Medical Mycology, the pathogenic fungi and the pathogenic actinomycetes, 3rd ed. W. B. Saunders Company. P196-275.

Rippon, J. W. (1988). Medical Mycology, the Pathogenic fungi and the pathogenic actinomycetes, 3rd ed. W. B Saunders Company. P. 196-275.

Rippon, J. W. (1988). Medical Mycology. The pathogenic fungi and the pathogenic actinomycetes, 3rd ed. W. B. Sannders Company. 196-275.

Rodgers, P. and Bassler, M. (2001). Treating onchomycosis. Am. Fam. Physiscain. 63: 663-72 677-8.

Rois, J. L.; Recio, M. C. and Villar, A. (1987). Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. J. Ethanopharmacology, (21) P: 139-152.

Rosen, T. (1997). Dermatophytosis: diagnostic pointers and therapeutic pitfalls. Consultant; 37: 1545-57.

Santos, D. A. and Hamdan, J. S. (2005). Evaluation of broth microdilution antifungal susceptibility testing conditions for *Trichophyton rubrum*. J. Clin. Microbiol. 43: 1917-1920.

Serrano-Gomez, D.; Dominguez-Soto, A.; Ancochea, J.; Jimenez-Heffernan, J. A. Leal, J. A. and Corb, A. L. (2004). Dendritic cell. Specific Intercellular Adhesion molecule 3-grabbing nonintegrin Mediates binding and internalization of *Aspergillus famigatus* conidia by dendritic cell and Macrophages. . Immunol. 173: 5635-5643.

Shear, N. H.; Einarson, T. R.; Arikian, S. R.; Doyle, J. J. and Van Assche, D. (1998). Pharmacoeconomic analysis of topical treatments for tinea infections. Inf. J. Dermatol. 37: 64-71.

Shigeo Kondo and Yutaka Hozumi (1986). Organ culture of skin of rabbits. J. Dermatol. Vol. 13 (2): 92-100.

Shimizu, F.; Nibu, Y.; Osaki, T. and Shimad, R. (1994). Chemistry SOC. Japan. 68, 16.

Smolenst, J. J.; Silnis, H. and Faranssnoth, N. R. (1972). Alkaloid screening coyadia, 35: 11-34.

Takashio, M. (1979). Taxonomy of *Dermatophytes* based on their sexual states. Mycologia, 71 (5): 968-976.

Tanaka, S.; Summerbell, R. C.; Tsuboi, R.; Kaaman, T.; Sohnle, P. G.; Mastumoto, T. and Ray, T. C. (1992). Advanced in dermatophytes and dematophytoses. J. Med. Vet. Mycol. 30: 29-39.

Temple, M. E.; Nahata, M. C.; Koranyi, K. L. (1999). Pharmacotherapy of tinea capitis. J. Am. Board Fam. Pract. 12: 236-42.

Tomesi, C. N.; Vial, A. A.; Buschi, C. A.; Rofi, R. D.; Schteingart, G. A.; Inigo, R. P. A.; Zallochi, C. M. and Pomoilio, A. B. (1986). Anti-microbial screening higher plants. Part II. Fitoterapia, 57: 46-50.

Tornes, G. and Georg, L. K. (1956). A human case of *Trichophyton gallinae* infection disease contracted from chicken, Arch. Derm. 74: 191-197.

Townsend, C. C.; Guest, E. and Omar, S. A. (1980). Flora of Iraq. V. 4 part 1. Univ. Press Glasgow.

Txler, V. E. (1988). Pharmacognosy. 9th edition. Lea and Febger. Philadelphia.

Vanbreuseghem, R. (1977). Modern classification of *Dermatophytes*, *Dermatologica*., 155: 1-6.

Vincent, J. (1980). Lipolytic activity of some dermatophytes. Department of Dermatology, University of Trandheim, Norway. J. of Med. Microbiol. Vol. (13): 155-157.

Voisard, J. J.; Weill, F. X.; Beylot, B. M.; Vergier, B. and Dromer, C. (1999). Dermatophilic granuloma caused by *Microsporum canis*. Service de Dermatologie Hospital, Hantleveque CHU, de Brodeaux, Pessac, France. 198 (3): 317-319.

Weinberg, J. M; Koestenblatt, E. K. and Jennings, M. B. (2005). Utility of histopathologic analysis in the evaluation of onychomycosis. J. Am. Podiatr Med Assoc. 95: 258-263.

Weitzaman, I. and Summerbell, R. C. (1995). The dermatophytes. Clinical Microbiology Service Colombia Presbyterian Center, New York, U.S.A, Clin. Microbiol. Res. 8 (2): 240-259.

Weitzman, I.; Summerbell, R. C. (1995). The dermatophytes clinical microbial. Rev. 240-259.

Wolters, B. (1976). Serial experiments on transformation and degradation of secondary plant substances by microorganisms. Planta Medica. 29: 41-53.

Zienty, F. B.; Vingard, B. D. and Schleppni, K. A. A. (1962). J. Organic Chemistry. 27, 3140.

Zuber, T. J.; Baddam, K. (2001). Superficial fungal infection of the skin. Where and how it appears help determine therapy. Post grad Med. 109 (1): 117-20, 123-6, 131-2.

Zurita, J. and Hay, R. J. (1987). Adherence of *Dermatophytes* microconidia and arthroconidia to human deratinocytes *IN vitro.* J. Inv. Dermat. 89 (5): 529-534.

World Wide Web Reference

http://www.ibiblio.org/herbmed

الخلاصة

استهدفت الدراسة الحالية التحري عن التغيرات النسيجية المرضية التي تسببها الفطريات الجلدية المرضية ودراسة الفعالية العلاجية للمستخلص الايثانولي لنبات الشاترك بواسطة المجهر الضوئي ويمكن تلخيص النتائج كما ياتي:-

- سبب الاصابة بالفطر *Trichophyton rubrum* تغيرات مظهرية في جلد الحيوان وتتضمن ظهور منطقة محرشفة وسقوط الشعر وظهور الندب وتكون خراجات واحمرار وانتفاخ مع تقرح المنطقة.
- بينت الدراسة النسيجية بالمجهر الضوئي ان الاصابة بالفطر *Trichophyton rubrum* ادت الى حدوث تغيرات تنكسية في المناطق المصابة تمثلت بوجود الادمة وتكوين الخراج مع ظهور الخلايا الالتهابية في البشرة ومنطقة تحت البشرة مضافا الى ذلك توسع الاوعية الدموية.
- ادت المعالجة بمستخلص الشاترك الايثانولي الى تغيرات بالجلد تمثلت بنقصان التقشر تدريجياً بعد اليوم الخامس من المعالجة تاركا ندب بيضاء.
- اتضح بعد احدى عشر يوما من العلاج بمستخلص نبات الشاترك ان المنطقة المصابة قد غطت بنسيج ظهاري جديد مع ظهور الشعر وعند مقارنتها بالعلاج مع مرهم الـ Fugidin المضاد للفطريات حيث كانت التغيرات البنائية بشكل ابطأ وذلك من خلال النسبة الاقل للتقشر ونمو الشعر خلال نفس الفترة الزمنية مما يؤكد كفاءة مستخلص نبات الشاترك كمادة مضادة للفطريات.
- بينت الدراسة النسيجية حدوث تغيرات بنائية محددة للطبقات المصابة بالفطر *Trichophyton rubrum* والمعالجة بمستخلص نبات الشاترك (مستخلص ايثانولي) تمثلت بتجمع الخلايا المناعية مثل العدلة واحادية النواة في طبقة البشرة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

الفعالية العلاجية لمستخلص القلويد الخام لنبات الشاترك في الفطريات الجلدية الممرضة (*Trichophyton rubrum*)

دراسة باستخدام المجهر الضوئي

رسالة

مقدمة الى كلية العلوم جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

رفل شكيب عبد الوهاب العاني

بكلوريوس تقانة احيائية جامعة النهرين ٢٠٠٢

صفر ١٤٢٧

اذار ٢٠٠٦

Information

Name: Rafal Shakeeb Abd Al-Wahab Al-Ani
Degree: M.Sc. in Biotechnology
Date:5-3-2006
Phone No.: 5554455
e-mail: rafalshakeeb@yahoo.com
Address: Baghdad-Iraq, Hay Al-Khadraa, Sec. 641, St. 76, Hous No. 18

الاسم: رفل شكيب عبد الوهاب العاني
الشهادة: ماجستير بايوتكنولوجي
تاريخ المناقشة: ٥-٣-٢٠٠٦
رقم الهاتف: ٥٥٥٤٤٥٥
العنوان: بغداد - حي الخضراء، محلة ٦٤١، زقاق ٧٦، دار ١٨