

## **The In vivo And In vitro Effects of Eugenia Caryophyllus, Quercus infectoria and Terminalia Extracts On Dermatophytes Infections**

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### **Abstract :**

A total of 340 specimens were collected from skin or hair of patients suffering from dermatophytic infections, during a period of one year (July/2005 to July/2006) at AL-Hussein Hospital / Karbala And Mirjan Teaching Hospital / Babylon . Four species of dermatophytes belong to the genus *Trichophyton* were isolated in this study;

- *Trichophyton mentagrophytes* var. *mentagrophytes* .
- *Trichophyton mentagrophytes* var. *interdigitale*.
- *Trichophyton verrucosum*.
- *Trichophyton rubrum* .

The prevalent clinical type of tinea (T.) infections was *Tinea corporis* which accounted for 154 cases (45.25%), then *Tinea pedis* 79 cases (23.23%), *Tinea cruris* 61 cases (17.94%), *Tinea capitis* 25 cases (7.35%), and *Tinea manuum* 21 cases (6.17%). The response of these isolates toward; aqueous, alcoholic and acetic extracts of three different medicinal plants; fruit of *Quercus infectoria* , fruit of *Terminalia citrina* and flower of *Eugenia caryophyllus* was investigated. The results of preliminary chemical analysis revealed that *Quercus infectoria* contained; Glycosides , Tannins , Saponins , Flavonoids , Carbohydrates , Phenols , Coumarins , Coumarins and Volatile oils , while *Terminalia citrina* contained; Glycosides , Tannins , Saponins , Resins , Flavonoids , Carbohydrates , Phenols , Coumarins and Volatile oils , whares, *Eugenia caryophyllus* contained ; Tannins , Saponins , Resins , Flavonoids , Carbohydrates , Phenols , Coumarins and Volatile oils. Phenols, Volatile oils of *Quercus infectoria*, *Eugenia caryophyllus* and Tannins of *Terminalia citrina* were extracted and studied their inhibition activity against the isolated fungi. The active components showed higher activity against fungal isolates than crude extracts. The results indicated that the effect of the active components (Phenols , Tannins and volatile oils) being extracted from the three plants in treatment of the laboratory infected skin of rat with *Trichophyton mentagrophytes* were highly as anti-inflammatory, antibacterial as well as antifungal. A prepared ointments from phenols extracted of *Quercus infectoria*, Tannins and volatile oils extracted of *Terminalia citrina* and *Eugenia caryophyllus* in the recovery period in mice infected by *T.mentagrophytes* were 13 days, 14 and 15 days respectively in compared with the recovery period of 16 days by using Clotrimazole.

### **المستخلص :**

جمعت 340 عينة من جلد و شعر مرضى مصابين بالخمج الفطري الجلدي، للمدة من تموز 2005 ولغاية تموز 2006 في مستشفى الحسين في كربلاء ومستشفى مرجان التعليمي في بابل. تم عزل وتشخيص 4 أنواع جلدية تعود إلى الجنس *Trichophyton* فطرية وهي:-

- *Trichophyton mentagrophytes* var. *mentagrophytes*.
- *Trichophyton mentagrophytes* var. *interdigitale*.
- *Trichophyton verrucosum*.
- *Trichophyton rubrum*.

كذلك حددت إن أكثر الأنواع السريرييه من الخمج الفطري أنتشارا هو سعفه الجسم ونسبتها 154 حاله (45.25%)، تليها سعفه القدم 79 حاله (23.23%)، وسعفه المغين 61 حاله (17.94%)، سعفه الرأس 25 حاله (7.35%)، وسعفه اليد 21 حاله (6.17%). استجابة هذه العزل تم اختبارها تجاه المحلول المائي، الكحولي والأسيتوني لمستخلص النباتات الطبية الثلاثة المختلفة: ثمار العفص، ثمار أهليلج، أزهار القرنفل النتيجة الأولية للتحليل الكيميائي أظهرت إن مستخلصات أزهار القرنفل احتوت على التانينات والصابونينات والراتنجيات والفلافونيدات والفينولات والفيوكيومارينات والكيومارينات والزيوت الطيارة ثم استخلصت المركبات الفينولية والزيوت الطيارة من نباتي العفص والقرنفل والتانينات من نبات الأهليلج واختبرت فاعليتها تجاه العزلات الفطرية. وقد أوضحت النتائج أن المركبات الفعالة أظهرت فاعلية تثبيطية عالية إزاء العزل الفطرية أعلى من المستخلصات الخام. تضمن اختبار كفاءة المركبات الفعالة (الفينولات والتانينات والزيوت الطيارة) المستخلصة من النباتات الثلاث في معالجة الأخمج الجلدية الفطرية المستحدثة في الحيوانات المختبرية وقد أظهرت فاعلية تثبيطية عالية باعتبارها مضادة للالتهابات والبكتريا إضافة لتضادها للفطريات. عند معالجة الفئران المصابه بالخمج الفطري مختبريا بالمرهم المحضر من الفينولات المستخلصة من ثمار العفص، والتانينات والزيوت الطيارة من نبات الأهليلج و القرنفل تم شفاؤها بعد مرور 13 يوم، 14 يوم، 15 يوم على التعاقب مقارنة بالشفاء للفترة 16 يوم باستعمال كلوتراميزول كريم

### **Introduction :**

Fungal infections occur worldwide and affect all ages and all races. However, prevalence of the organisms varies in different countries. There have been an important increase in the incidence of fungal infections during the last decade Millions of people throughout the world are affected by superficial fungal infections, which are the most common skin diseases seen in the out-patient dermatological clinic .These infections, which occur in both healthy and immunocompromised persons, are caused by dermatophytes, yeasts and other moulds. Approximately ninety percent of fungal Skin infections are caused by dermatophytes (1, 2). Dermatophytes are fungal organisms which are capable of colonizing or infecting keratinized tissue such as stratum corneum, hair and nails. Although dermatophytes infections are not life-threatening, they are one of the most common infections of man and may result in significant morbidity(3). Dermatophytes infections are caused by members of genera represented by; *Microsporum*, *Tricophyton*, and *Epidermophyton*s and can be classified according to whether they reside predominantly on humans ( Anthropophilic), on animals ( zoophilic) or in the soil ( geophilic) . In general, the zoophilic and geophilic organisms cause a dramatic inflammatory response in humans as compared with the Athrophilic organisms (3). Commonly the fungal skin infections caused by dermatophytes are called the Tinea infections. Tinea infections are contagious and can be passed through direct contact or by contact with clothing, from shower and pool surfaces, and even from pets (3). The estimated lifetime risk of acquiring a dermatophytic infection is between ten and twenty percent (4) .Recognition and appropriate treatment of these infections reduces both morbidity and discomfort and lessens the possibility of transmission. The present line of treatment involves use of anti-fungal, either topical such as tolnaftate, terbinafine hydrochloride, and imidazoles such as ketoconazole, miconazole nitrate and clotrimazole. Or systemic such as Griseofulvin, Terbinafine hydrochloride, flucanazole.. Recently herbal therapies have been used successfully in treating dermatological disorders including dermatophytosis in Europe and Asia for thousands of years, and now it is becoming increasingly popular among patients and physicians(5).As the treatment of Tinea infections is continued for a couple of weeks after clinical subsidence. Recurrences are common. Infections by fungi are often chronic and require prolonged treatment by antimycotic drugs that are expensive and sometimes uneffective (5 ). It has long been suspected that a major problem in clinical practice is the lack of patient compliance causing failure of drug therapy or the reason for poor control of the disease (6). One of the main reasons attributable to non compliance is the cost of medicines in the treatment of superficial mycoses. Fungal infections need long term therapy involving several weeks. But the patient discontinues the application, due to cost factor, when the clinical manifestations subside resulting in the recurrence of the disease. As a result superficial fungal infections become chronic, causing enormous physical and psychological distress to the sufferers (6). Since *Eugenia caryophyllus*, *Querus infectoria* and *Terminalia citrina* extracts are totally inexpensive even for repeated applications, the cost factor as a barrier is easily overcome and the patients can get rid of the fungal infections. More over there is no report of any composition comprising *Eugenia caryophyllus*, *Querus infectoria* and *Terminalia citrina* extracts for dermatophytes treatment. The present study prepared a novel herbal composition in an attempt to detect their chemical compositions and to demonstrate the antifungal activity of them.

### **Materials and methods**

Through a period of one year (July/2005 to July/2006) 340 samples were collected from patients aged between 5 and 55years suffering from dermatophytic infections (Tinea corporis ,Tinea pedis ,Tinea cruris, Tinea capitis and Tinea mannum,) at AL-Hussein Hospital/ Karbala and Mirjan Teaching Hospital / Babylon .From which scales or hair were taken and diagnosed microscopically (infected scales or hair of patients where placed on a slide in a drop of 20% KOH, a cover slip added and the specimen was examined immediately and again after 20 minutes. In skin branching hyphae or chains of arthrospores are seen. In hair, most species form dense sheaths of spores around the hair (ectothrix), or inside the hair shaft (endothrix). Culture: the identification of dermatophytes by culturing the specimens on Sabouraud's dextrose agar containing cyclohexamide and

chloramphenicol to suppress mould and bacterial growth, incubated for 2- 4 weeks at 25-28°C. Species are identified on the bases of colonial morphology {growth rate, surface, texture, and any pigmentation, micriscopical morphology (macroconidia, microconidia) and in some cases nutritional requirement was added} ( 7 ). We used three types of medical plants that were diagnosed and specified by specialist from Department Of Microbiology, College of Science ,University Of Babylon, as follows in table (1);

Table (1) the name, family & the type of the three used medical plants.

NUMBER	NAME	FAMILY	TYPE
1	<i>Terminalia citrine Roxb</i>	<i>Combretaceae</i>	Fruit
2	<i>Eugenia carryophyllus Spreng</i>	<i>Myrtaceae</i>	Flower
3	<i>Quercus infectoria Oliv</i>	<i>Cupressaceae</i>	Fruit

Preparations of aqueous extracts according to Al Mashadani;(1999) ; and Ahmed et al(1998) methods in which distilled water was added to powder plants in a ratio of 1:5 W:V and mixed for 24 hours by using shaker water patch, then filtered through a piece of cloth and filter paper to remove the residual . This method was repeated many times to get enough material of extracts. The same method was used to get alcohol extracts by using ethyl alcohol 95% and, acetone extracts by using acetone 70%. The stock solutions were prepared for every type of these plant extracts by dissolved 1000mg of these extracts in 10ml of distal water to get 100mg/ml concentration, and then from this concentration we prepared the following concentrations,(1,2,3,4.5,6,7,8,9,10)mg/ml,the concentration was calculated according to the following low;  $C_1V_1 = C_2V_2$

C=concentration V=volume

We used the method of EL-kady et al (1993) to know the effect of these plants extracts on the isolated dermatophytes by mixing dried plant extracts with Sabouraud`s dextrose agar in fluid form at 50°C in the concentrations ,(1,2,3,4.5,6,7,8,9,10) mg/ml and repeat every one of these extract three times ,then after solidification of the agar, we put a 6mm disc containing the isolated dermatophytes in the center of the dish. The result was compared with two dishes containing Sabouraud`s dextrose agar in solid form that contains the same isolated dermatophytes, in which ;one was left as such , and other contains clotrimazole 2mg/ml , all were inocubated at 25-28°C for 2- 4 weeks. Then measurement of the diameter of the dermatophyte growth, the mean of the two perpendicular diameters, the results were recorded, the percent of growth inhibition was known by using the following formula:

$$\frac{\text{Colony diameter of content} - \text{colony diameter of the test}}{\text{Colony diameter of content}} \times 100$$

Identification and separation of some active components by using different chemical reagents like ; Dragendroff reagent, Marqus reagent, Wagner reagent, Mayer reagent, Lead acetate test, Ferric chloride test, Molish test, Folin reagent, Ferric chloride reagent,and others and Thin Layer chromatography, using the solvents; butanol, acetic acid, and distal water in ratio of 40, 10,50, and measure the rate of flow of the isolates.

15 white rats aged 2-3 months, weighted 25-30gm were taken from College Of Pharmacy/Kufa university, distributed in special plastic box containing good environment with sufficient water and food. After shaving the skin and induced mild erosions on one area, inoculation of *Trichophyton metagrophytes* which was isolated from the patients by a loop from the sample kept in physiological saline was done in this area that later on developed dermatophyte infection. These rats were divided into five groups, each group consists of three rats as follows; first group was left with out treatment. Second group was treated with tannins extracts from *Terminalia citrine* .Third group was treated by volatile oils of *Eugenia carryophyllus*

Forth group was treated with phenolic compound of *Quercus infectoria*.

And Fifth group was treated with topical clotrimazole 1% cream.

We used the present statistical analysis system 4×9×3 by using (SAS,2001) complete randomized design (CRD). Ointment prepared by mixing 7mg of plants powder with 1gm of Vaseline.

**Results :**

The most prevalent clinical type of tinea (T.) infections was *T.corporis* which accounted for 154 cases (45.25%), then *T.pedis* 79 cases (23.23%), *T.cruris* 61 cases (17.94%), *T.capitis* 25 cases (7.35%), and *T.mannum* 21 cases (6.17%). Only 260(76.47%) samples taken from 340 patients suffering from dermatophytes infections gave positive results by direct microscopical examinations, and 123(36.1%) by culture. Four species of dermatophyte belong to the genus *Trichophyton* were diagnosed as follows:

- *Trichophyton mentagrophytes* var. *mentagrophytes* 45(17.3%).

- *Trichophyton mentagrophytes* var. *interdigitale*.34(13.07%)

-*Trichophyton rubrum*36 (13.84%).- *Trichophyton verrucosum* 8(3.07%). Table (2) As well As There were many isolates of non-dermatophyte filamentous fungi and Yeasts were Identified and neglected like; - *Aspergillus* spp. -*Rhizopus* spp. - *Penicillium* spp.and- *Candida albicans*.

Table (2) shows; types, and number (No.) of tinea (T) cases, the number and percentage of isolated dermatophytes species.

No.	Types of tinea	No. Of case	No.of positive results by microscopy	T.m.var m.	T.m.var i.	T.v.	T.r.
1	<i>T.corporis</i>	154	50	15	10	5	20
2	<i>T.pedis</i>	79	28	8	15	0	5
3	<i>T.cruris</i>	61	27	12	5	0	10
4	<i>T.capitis</i>	25	10	6	2	2	0
5	<i>T.mannum</i>	21	8	4	2	1	1
Total		340	123	45	34	8	36

**T.m.var m.**= *Trichophyton mentagrophytes* var. *mentagrophytes*

**T.m.var i.**= *Trichophyton mentagrophytes* var. *interdigitale*

**T.v.**= *Trichophyton verrucosum*

**T.r.**= *Trichophyton rubrum*

Preparation of aqueous, alcoholic and acetonic extracts from 3 different medicinal plants;, and investigation of their activity against the these dermatophyte selected isolates in the laboratory in which their effects were depended on ;the type of extract, and it's concentrations ,the type of species of dermatophyte colony. Alcoholic extract was gave the best results in inhibition of dermatophyte colonies ,followed by acetonic then aquagenic type. The diameter of colony growth was inversely related to the extract concentration, the mean diameter of colony growth is decreased with increase of concentration of extracts, however the percentage of growth inhibition was increased with increase of concentration of extracts table (3,4,5,)

**Table(3 effects of acetonic, alcoholic and aquagenic extracts of *Quercus infectoria* in the growth diameter(mm) of isolated *trichophytos***

Conc. mg/ml	Acetonic extract				Alcoholic extract				Aquagenic extract			
	T.v.	T.r.	T.m.i.	T.m.m	T.v.	T.r.	T.m.i.	T.m.m	T.v.	T.r.	T.m.i.	T.m.m
1	16 FG	18 EF	18 EF	20 E	14 GHI	16 FG	18 EF	18 EF	18 EF	20 E	20 E	20 E*
2	12 HIJ	15 FGH	15 FGH	16 FG	12 HIJ	12 HIJ	13 GHIJ	14 GHI	13 GHIJ	14 GHI	16 FG	18 EF
3	10 JKL	10 JKL	11 IJK	12 HIJ	8 KLM	8 KLM	10 JKL	10 JKL	10 JKL	10 JKL	13 GHIJ	15 FGH
4	6 MNO	8 KLM	8 KLM	10 JKL	4 NOP	6 MNO	7 LMN	8 KLM	6 MNO	8 KLM	10 JKL	10 JKL
5	4 NOP	6 MNO	6 MNO	8 KLM	2 PQ	4 NOP	4 NOP	6 MNO	4 NOP	6 MNO	6 MNO	6 MNO
6	2 PQ	2 PQ	3 OPQ	3 OPQ	0 Q	2 PQ	2 PQ	2 PQ	2 PQ	2 PQ	4 NOP	4 NOP
7	0 Q	0 Q	2 PQ	2 PQ	0 Q	0 Q	0 Q	0 Q	0 Q	2 PQ	2 PQ	2 PQ
8	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q
Control (-)	55 D	60 C	75 B	80 A	55 D	60 C	75 B	80 A	55 D	60 C	75 B	80 A
Clot. 2mg/ml	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q

There is no significant difference between concentration marks with same letters  $P \geq 0.01$ . The results in the table represented the mean of three times  $LSD=3.341$

**Table(4). effects of acetonc, alcoholic and aquagenic extracts of *Terminalia citrina* in the growth diameter(mm) of isolated *trichophytos*.**

Conc. mg/ml	Acetonc extract				Alcoholic extract				Aquagenic extract			
	T.v.	T.r.	T.m.i.	T.m.m	T.v.	T.r.	T.m.i.	T.m.m	T.v.	T.r.	T.m.i.	T.m.m
1	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
2	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
3	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
4	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
5	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
6	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
7	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
8	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
Control (-)	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
Clot. 2mg/ml	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E

There is no significant difference between concentration marks with same letters  $P \geq 0.01$ . the results in the table represented the mean of three times  $LSD=3.560$

**Table(5). effects of acetonc, alcoholic and aquagenic extracts of *Eugenia carryophyllus* in the growth diameter(mm) of isolated *trichophytos*.**

Conc. mg/ml	Acetonc extract				Alcoholic extract				Aquagenic extract			
	T.v.	T.r.	T.m.i.	T.m.m	T.v.	T.r.	T.m.i.	T.m.m	T.v.	T.r.	T.m.i.	T.m.m
1	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
2	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
3	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
4	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
5	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
6	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
7	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
8	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
Control (-)	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E
Clot. 2mg/ml	16 HI	20 FG	20 FG	23 EF	16 HI	18 GH	20 FG	20 FG	18 GH	20 FG	20 FG	25 E

There is no significant difference between concentration marks with same letters  $P \geq 0.01$ . the results in the table represented the mean of three times  $LSD=3.218$

Table (6) the mean time(by days)of improvements of infected lab. animals with dermatophyte.

No Group	ointment type	Duration of of improvement/ by days
1	phenolic extract of <i>Terminalia citrine</i>	13
2	volatile oils of <i>Eugenia carryophyllus</i>	15
3	tannis extract of <i>Quercus infectoria</i>	14
4	Clotrimazole 1%	16
5	Controlled group (with out treatment)	32

**Discussion :**

Ring worm is a common dermatophytes infection that constitutes an important public health problem among population World wide, as it is highly contagious and can be transmitted through body contact (person to person transmission),mainly refuge carrus, or schools, or through inanimate objects like; clothes and combs(11). The playing habit of children make them in contact with contaminated soils and animals like gout, sheep, cats and local dogs which are a known source of infections(11).The presence of a symptomatic carriers yield significant growth of dermatophytes may be equal to symptomatic suffers might increase the spread of infections in the community

(12,13-).So dermatophytosis is a common dermatological problem especially in children (higher prevalence of was found amongst children under the age of 10 years than older ones, suggesting that ring worm is mainly a prebatal disease (13)) that constituted 8.2% of dermatological diseases in Iraqi children in 1989 (14), and 2.7% in 2000(7).While in our study we found that dermatophytosis was constitute 23% of the out patient in the dermatological clinic in Hilla 2007. Tinea corporis was the most common type (45%),then tinea pedis ( 23%), tinea cruris (17%), tinea capitis (7.35%), tinea mammum (6.17%) Of the total number of the collected cases., incimparison to the previous study tinea capitis was (3%),tinea cicinata( 3%), tinea fasciei (1.2%) and tinea pedis (0.4%)(10). The existing medicines in the treatment of superficial fungal infections have limited therapeutic spectrum and cause several side effects besides the cost factor. Many medicinal plants have a potential role in the treatment of fungal infections like cassia alata leaf extract (15) and the used medical plants in our study has goode anti-inflammatory, antibacterial as well as antifungal effects.The mechanism of this effects were related to their contents of phenolic compounds that made a hydrogen binding with most active enzymes in the fungus to stop it's metabolic, growth, and protein synthesis (16). Tannis connected with keratin layer of skin to increase its barrier effects against fungal invasions as well as stimulates the effects of the inflammatory cells. The volatile oils inhibited the fungal growth by decrease the effects of Succinate dehydrogenase and bonded with NADH to prevent oxidation –phosphoration as well as electron transports(17,18). Regarding the effect of the type of extract ,the best was alcohol followed by acetone ,and the least was the water extract. So there is a clinical significant difference between the effect of these three extracts P.value< 0.01 however in comparison of these results with another drug like clotrimazole (2mg/ml) was the same effect. So we can use this plants extracts in the treatment of dermatophytosis in the future as a new line of treatment, available and to reduce the coast of therapy.

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