

## Therapeutic efficacy of some extracts of hot pepper fruits (*Capsicum annum*) and Sumac seeds (*Rhus coriaria*) on the growth of some isolated dermatophytes species

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### Abstract

**Background:** Fungal infections are one of the common skin diseases with difficulty in their treatment approach. The present efficient drugs for fungal infection are limited.

**Aim:** To determine the therapeutic efficacy of plant extracts as alternative antifungal agents.

**Materials and methods:** 100 clinical samples [68 from female and 32 from male] were collected during the period from March to July 2017 from subjects attending Dermatology Clinic in Salah Uldean General Hospital. Fungal infection was diagnosed with using KOH wet preparation. Fungal species identified by using conventional approach. The active ingredients existing in the plant extracts were detected and analyzed through qualitative and quantitative detection technique of chemical compounds using a high performance liquid chromatographic device (HPLC). Agar diffusion method was used to determine antifungal activity of plant extracts.

**Results:** Direct microscopic examination showed that there were (75%) positive samples, while culture shows (67%) positive samples. The isolated dermatophytes belong to *Epidermophyton*, *Microsporum*, and *Trichophyton* genus. The predominant dermatophytes were *T. rubrum* (25%) species. The highest frequency of infection was in the age group of 11-20 years. The sensitivity of the tested fungi to the aqueous and alcoholic plant extracts varies. Alcoholic extract of the hot pepper plant was more effective as antifungal than the aqueous extract of the same plant. However, aqueous hot pepper extracts was more effective against *T. mentagrophyte* than that of alcoholic extract. Additionally, alcoholic Sumac extract shows higher efficacy than aqueous extract.

**Conclusion:** Hot pepper and Sumac extracts show antifungal activity against *Microsporum canis*, *Trichophyton rubrum* and *T. mentagrophyte*.

**Key words:** Antifungal, Dermatophytes, Hot pepper, Sumac, *Trichophyton*, *Epidermophyton*, and *Microsporum*.

### Introduction

The skin is exposed to many fungal infections, termed mycoses, which include superficial mycoses, coetaneous mycoses, and subcutaneous mycoses. Dermatophytes are a group of fungi that can invade the stratum corneum of skin, hair and nails for humans and animals, causing mycosis [1]. These fungi are known as keratinophilic and cause ringworm or Tinea infections. Dermatophytes belong to three genus, *Trichophyton*, *Epidermophyton*, and *Microsporum* and has the ability to produce status enzymes for keratin [2]. Dermatophytes cannot infect the internal tissues of the body because most types of dermatophytes are unable to grow under the degree of (37 °C) such as *T.Verrucosum* because of existence serum which

contains inhibitory factors for fungal growth. Dermal inhibitor factor, including Transferrin and lower concentration of iron in these tissues, so it is called cutaneous mycoses. In rare cases, however, the infection may extend to deep tissue, especially in patients with immune deficiency and those who use immunosuppressive drugs and individuals infected with Human Immunodeficiency Virus [3]. The main trait that made the fungus is important from the medical point of view is its ability to analyze the natural hard keratin through the secretion of many extracellular enzymes such as keratinase, collagenase and protease. Most of these fungi are present in the soil and involved in decomposition [4]. The fungus attack on keratinocytes tissues leads to the emergence of a number of clinical forms, which vary depending on the type and virulence of the fungus, the immune status of the host and the infection site. Skin fungal infections are termed Dermatophytosis or Tinea, a Latin word means Cutworm [5]. This term is used for fungal infections that begin as a small rash and then spread in an annular shape because the fungus grows evenly in all directions, forming a circular lesion on the skin or scalp that resembles the holes that the moth causes in clothing [6]. Due to the rapid increase in the infection level by dermatophytes diseases, there has been a tremendous development in the manufacture of antifungal drugs, but these antibiotics may be expensive, long duration of treatment course, antifungal agents side effects, and high toxicity on blood cells and kidney. Therefore, the recent trends are the use of medicinal plants extracts as the active substance. The human body is more compatible with treatment by herbal medicine than treatment by chemical medicine as digestive system has adapted to the response to these plant foods [7]. Due to the absence of adequate studies on the role of medicinal plants in treating skin diseases, this study was conducted.

### **Materials and methods**

The 100 clinical samples were collected during the period from March to July 2017 from subjects attending Dermatology Clinic in Salah Uldean General Hospital. The clinical examination and initial clinical diagnosis performed by dermatologist. The direct examination (wet preparation) of the samples was carried out after sampling from the affected site by scraping.

### **Direct microscopic examination and implantation of samples**

Direct microscopic diagnosis was done by taking a sterile glass slide and then placing the skin scale, hair or nail samples. A drop or two drops of 10% potassium hydroxide solution were then added. The slide left for 20 minutes and then examined under the microscope using force (40 x) to confirm the presence of spores and Hypha. The nail samples were placed in test tubes and a small amount of 10% potassium hydroxide solution was added and left in the incubator at 37 °C for 3- 4 hours. A small amount of this sample was then placed on a clean glass slide and covered with a slide cover and examined under the microscope at power of (40 x) to observe the Hypha fungal structures. Sabouraud Dextrose Agar media was used with cyclohexamide and chloramphenicol (SDACC) to culture the remaining skin, hair and nail scales. The inoculated dishes were then incubated for a period of (10-20) days and examined continuously every three days and if there is no growth in the dishes after 21 days of incubation then the results are recorded as negative.

### **Preparation and sterilization of extracts**

The plant material was grinded with an electric mill and 40 g of the grinded plant was added to 160 ml of sterilized distilled water. The mixture mixed using the electric mixer and left in the fridge for 24 hours for the purpose of soaking. It was then filtered through several layers of filter papers (Wattman No.1) to remove the non-grinded plant parts and the remaining fibers, then pour the extract in sterile glass dishes, placed in an oven at a temperature not exceeding 40° C until the entire

water evaporates and the dried extract placed in glass bottles with a tight lid and kept in the refrigerator until using. The alcoholic extract was prepared in the same manner as the aqueous extract, except for the replacement of the aqueous solvent with ethyl alcohol at a concentration of 95%. Then, 1 g of dried plant extract and solvent was taken in 10 ml of sterile distilled water. Thus the concentration of the stock extract solution was 100 mg/ml. This extract was sterilized using Milipore filters with a diameter of 0.22 Mm. This standard concentration was considered as a source for the preparation of the subsequent solutions used in the study [8].

### **General chemical detection of active compounds in plant extracts used in the study**

Chemical compounds were identified and analyzed through qualitative and quantitative detection of chemical compounds using high pressure liquid chromatography (HPLC). This analysis was conducted at the Ministry of Science and Technology / Baghdad. Stationary phase column with the phenomenex type and the minute size is 3 Mm was used. The mobile phase is acetic acid at a flow velocity of 1 ml.min<sup>-1</sup> at 30°C using ultraviolet radiation along a 245 nm wavelength Where 0.1 g of sample dissolved in 5 ml aqueous solution of methanol, the centrifugation was performed at a speed of 7500 rpm for 15 minutes and the pure suspension was treated with charcoal to remove the dyes and was dried and then re-suspended in 0.1 ml of methanol by the mixer, The mixture was passed through a single-use filter and 20 µL of the model was injected into the HPLC [9].

### **Test the inhibitory activity of plant extracts against some pathogenic fungi**

It was ascertained that the plant extract was not contaminated after filtration and filtration by micro-filters 0.22 micron. The Agar diffusion method was followed by three isolates of isolated dermatophytes: the 20 ml of Sabouraud Dextrose Agar media was poured in each dish and after the hardening of the culture media, (0.15 ml) of the used concentrations (10, 20, 30, 40 mg/ml) of aqueous and alcoholic extracts were put on the surface of the hardened media and made replicated for each concentration, a 7 mm tablet was placed from the edge of the fungal colony of the studied fungi at 7 days, The control dish was added to the fungus without the addition of the plant extract. The sterilized distilled water was added in the hole as a control agent and all dishes containing fungi were incubated at (25-28°C) for (7- 10) days and the diameter of the developing colony was measured (the mean of two orthogonal diameter) [10].

**Statistical analysis:** The results were statistically analyzed by applying the Minitab program using the ANOVA and the arithmetic average was measured using the multimodal Duncan test and the probability level of  $p \geq 0.05$ .

### **Results and discussion**

The isolated dermatophytes were belong to *Epidermophyton*, *Microsporum*, and *Trichophyton* genus and this result was consistent with others [11]. However, the present study finding was not agreed with others [12] who isolated *Microsporum*, and *Trichophyton* only. Additionally, in a recent study in Salah Uldean governorate, Iraq, only *Trichophyton* genus was isolated from clinical specimens [13]. Sixty-seven samples were culture positive (67%). The predominantly isolated fungal species were *T. rubrum*, which form 37.3% of the total isolates, Fig 1. This results agreed to that reported by Sumit et al [14]. The results showed that the percentage of infection among females (68%) was higher than that of males (32%). The present study shows that *T. capitis* was the predominant fungal infection (49.25%), followed by *T. corporis* (29.85%) and *T. manum* (25.37%), while the lowest one was *T. unguium* (5.97%), Fig 2. Fungal infection incidence was higher in 11-20 age group.

### General chemical detection of active compounds in plant extracts of hot pepper and sumac

The results of the chemical detection of some active substances of hot pepper and sumac plants are shown in Figs. 3-7. Terpene and Capsaicin were detected in hot pepper, while Terpene, Tannin and volatile oils were found in sumac.

### Effect of aqueous and alcoholic extract of sumac plant

The results of the present study showed that the inhibitory capacity of the extract increased with increasing concentration. The highest effect was at concentration of 40 mg/ml on the studied fungi which include *T. rubrum*, *T. mentagrophytes* and *M. canis*. The lowest effect was that of concentration (10 mg/ml) and this may be attributed that high extracts concentrations have the ability to penetrate through the plasma membrane mechanism, which has an excellent ability to selective permeability. The inhibitory effect of the aqueous extract for sumac plant on *T. rubrum* fungi was 67.7% (colony diameter =21 mm) while the fungal colony diameter in plates without treatment was (65 mm). The lowest effect of the aqueous extract on the same fungus was with the concentration of (10 mg/ml), with a mean colony diameter of 52 mm (20%). The diameter of *T. mentagrophytes* fungus was 22.5 mm at 40 mg/ml concentration with an inhibitory effect of 62.5%. The colony diameter of the fungus without treatment was 60 mm. The lowest effect of the aqueous extract on the same fungus was at the concentration (10 mg/ml), with mean colony diameter of 39 mm and inhibitory effect of 35%. *M. canis* fungus growth was inhibited at rate of 56.4% at 40 mg/ml sumac extract concentration and the colony mean diameter was (24 mm) compared to (55 mm) in plates without treatment. While extract of 10 mg/ml concentration, shows inhibitory effect of 21.8%, with a mean colony diameter of (43) mm, Figure(8).

The results of the statistical analysis showed significant differences at the level of 0.05 for alcoholic extract of sumac plant as shown in Figure (9). It was observed that there was a complete inhibition (100%) of this extract on *T. rubrum* at 40 mg/ml concentration compared to the diameter of the fungus without treatment (65 mm). The lowest effect of alcoholic extract on the same fungus was at 10 mg/ml concentration, with 48.5 mm diameter (25.4%). *T. mentagrophytes* fungus mean colony diameter without treatment was 60 mm, while in that with 40 mg/ml concentration treatment shows mean colony diameter of 34 mm, with inhibitory effect of 43.3%. Extract concentration of 10 mg/ml demonstrated the lowest inhibitory effect (8.3%) with a mean colony diameter of 55 mm. Also the concentration of 40 mg/ml of alcoholic extract shows highest inhibitory effect against *M. canis* fungus (42.7%), with mean colony diameter of (31.5) mm compared to the fungus mean diameter of 55 mm in plates without treatment. While the concentration of 10 mg/ml of sumac alcoholic extract did not show any inhibitory effect on the *McCain's* fungus.

### Effect of aqueous and alcoholic extract of hot pepper plant

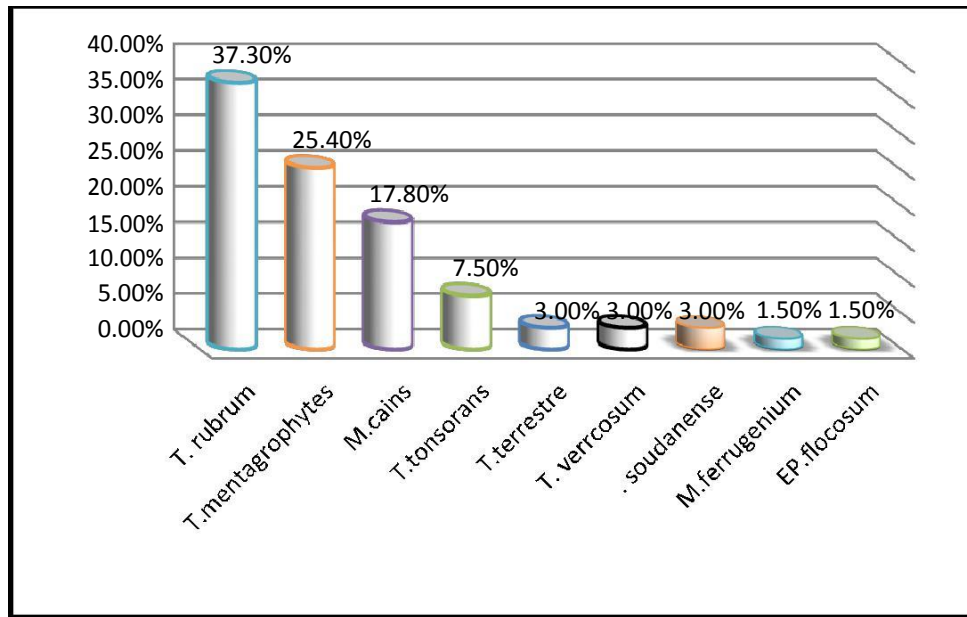
The results showed a complete inhibition of the aqueous extract of hot pepper on *T. rubrum*, *T. mentagrophytes* and *M.canis* at concentration of 40 mg/ml. For *T. mentagrophytes*, the lowest effect was 10% (mean colony diameter =54 mm) at the concentration of 10 mg/ml compared with the mean diameter of 60 mm without treatment. For *M. canis* fungus, the lowest effect was 5.5% at 10 mg/ml concentration (52 mm) compared to the fungus diameter without treatment (55 mm), Fig.10.

The alcoholic extracts of hot pepper show inhibitory effect of 51.5% at 40 mg/ml concentration with a mean colony diameter of 31.5 mm, while that plates without treatment was (65 mm), Figure(11). The lowest effect of the alcoholic extract on the same fungus was at 10 mg/ml concentration, with a mean colony

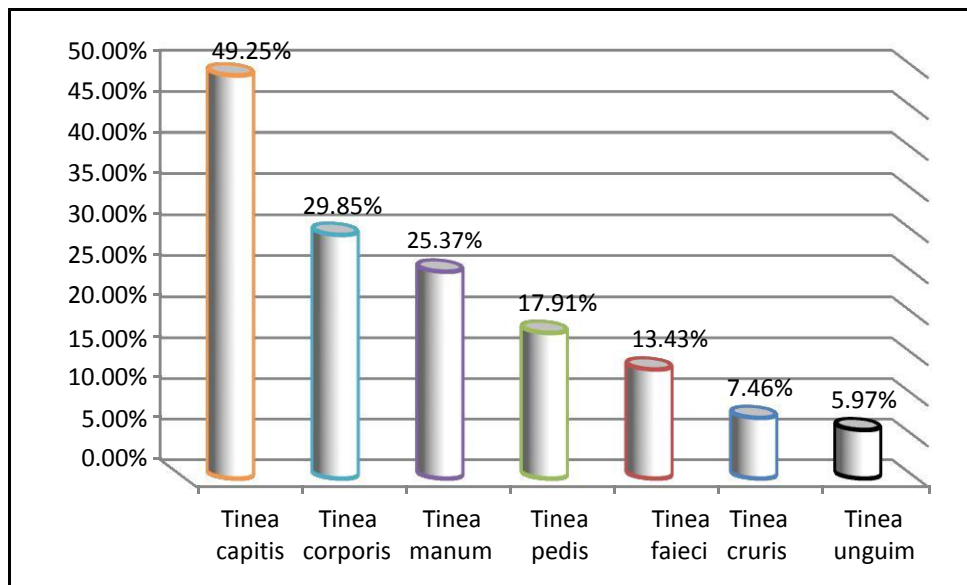
diameter of 62 mm. For *T. mentagrophytes* fungus, the mean colony diameter of the fungus without treatment was 60 mm. The highest effect was at 40 mg/ml concentration (62.5%), with a mean colony diameter of 22.5 mm. While the concentration of 10 mg/ml shows the lowest inhibition effect (8.3%) as the mean colony diameter was 55 mm. Unfortunately, the alcoholic extract concentration of 40 mg/ml shows low inhibitory effect against *M. canis* fungus. Additionally, 10 and 20 mg/ml concentration of alcoholic extracts did not show any inhibitory effect against *M. canis* fungus, Figure(11).

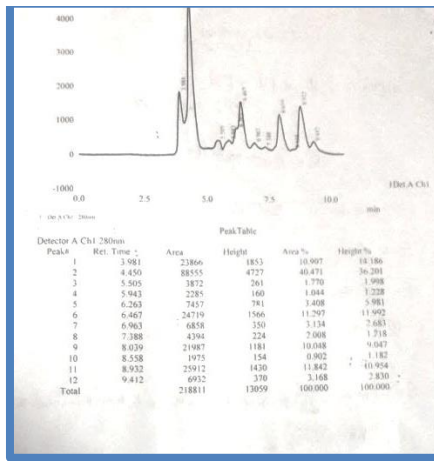
The inhibitory effect of studied plant extracts against tested fungi is due to their containment of tannins, terpene, resins, capsaicin and volatile oils, which are known as antibiotics, disinfectant materials, sterile materials and toxic oxidants material for microorganisms [15]. Glycosides inhibit the enzymes and transport proteins present in the cell membrane and affect living components such as mitochondria, and lead to cells poisoning and death and proved its ability of inhibition to some microbes, including fungi. While Tannins act to precipitate the proteins associated with the cellular membranes of fungi and thus affect the process of entry and exit of substances into and out of the fungal cell as well as their formation a complexities with polysaccharides and their effect on enzymes [16]. Turbinones are non-nitrogenous chemical compounds, which are a volatile oil derivative and it is characterized by being anti-fungal [17]. While the inhibitory activity of the turbinones occurred by its ability to tear the cellular membranes by lipophilicity compounds. Capsaicin is also an effective chemical anti-inflammatory agent for many bacterial, viral and fungal pathogens and a natural antioxidant [17]. This is due to the fact that fungi are not affected by the chemical compounds found in the aqueous and alcoholic extracts or by some substances that led to stimulate the fungus to growth. This may be due to the nature of the fungus itself in terms of cellular membranes, the relationship of this with the mechanism of action of the effective compound found in each plant or because of the difficulty of penetrating the extract to the cell wall and the sensitivity of the tested fungi against the extracts (aqueous and alcoholic) that were used in the experiment. . The superiority of the alcohol extract may be attributed to its effect on the lack of effective compounds that can be extracted by water, and may be due to the containment of alcoholic extracts on sulfur compounds effective against the germs that are dissolved in alcohol [18]. The active compounds found in medicinal plants are soluble in organic solvents such as ethanol. Their concentration is higher in the alcoholic extract than in the aqueous extract. This result agree with a previous study [19] who referred to the variation in the composition of cellular membranes from one fungus to another and affect the sensitivity of that fungus towards the extract or oil used. In conclusion, the extracts of hot pepper and sumac show an antifungal activity against the tested mentagrophytes. This finding warranted a confirmatory study in a large scale in vitro and in vivo research program.

**Fig. 1: Types of Isolated Dermatophytes**

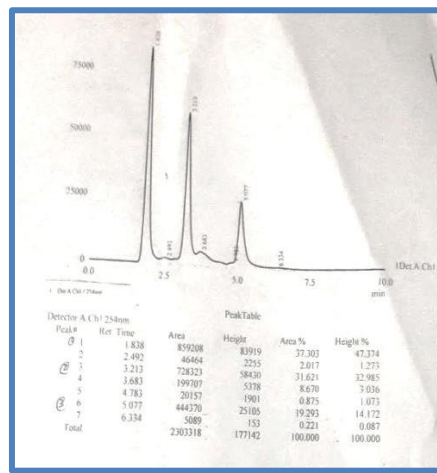


**Fig. 2: Percentage distribution of fungal infections by body area.**

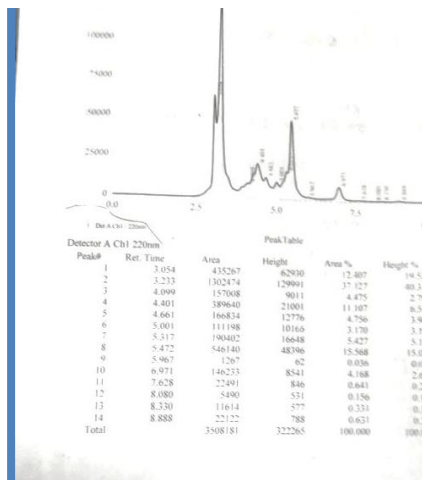




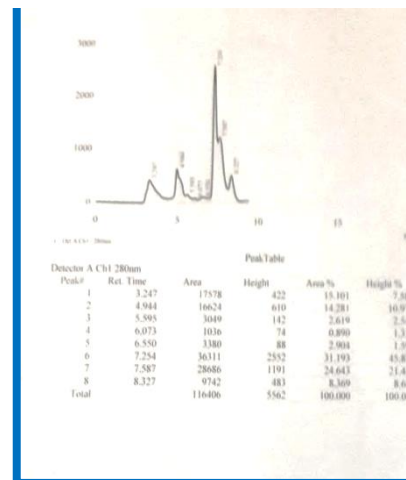
Figure(3) Tannin Sumac



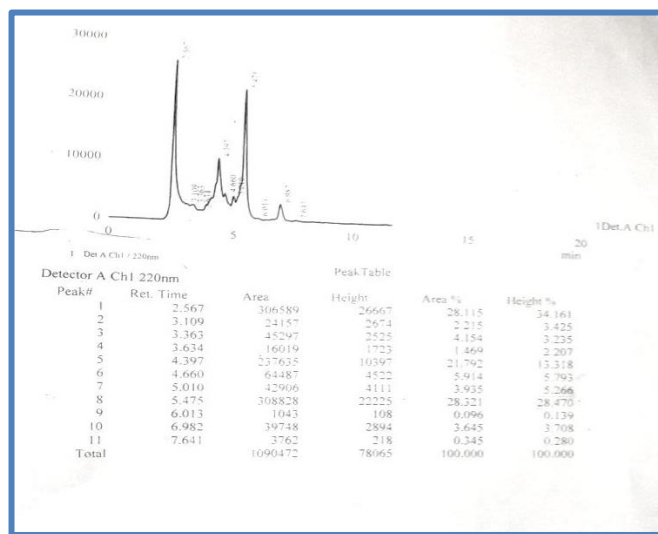
Figure(4) volatile oils in Sumac



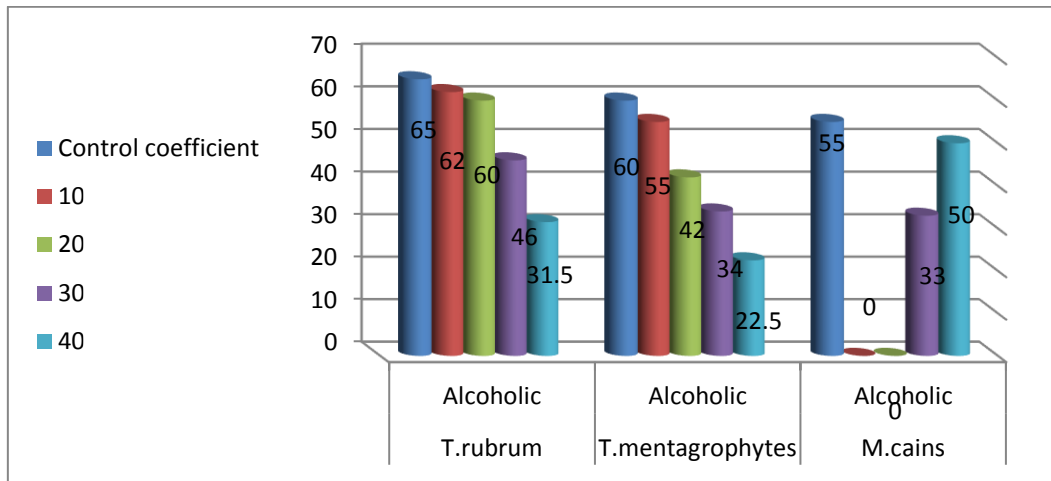
Figure(5) Terpene in hot pepper



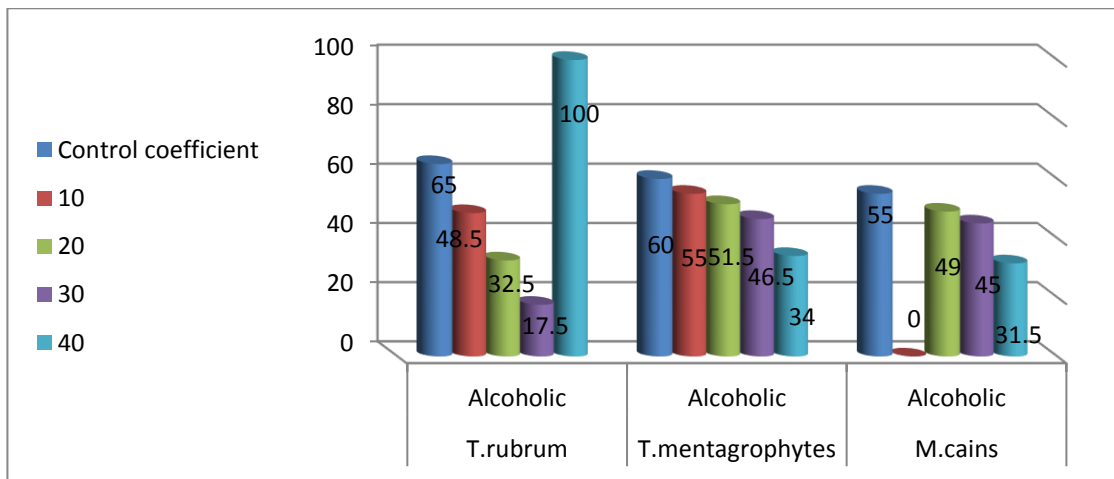
Figure(6) Capsaicin in hot pepper



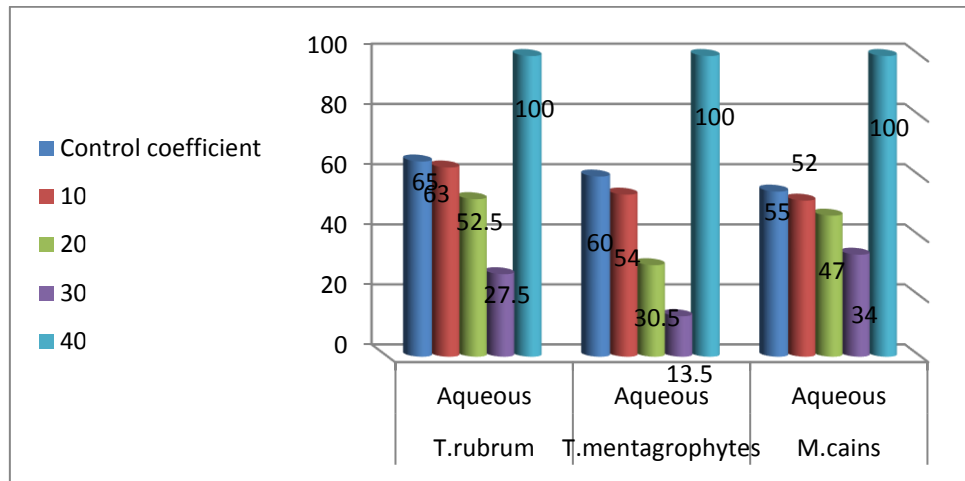
Figure(7) Terpene in Sumac



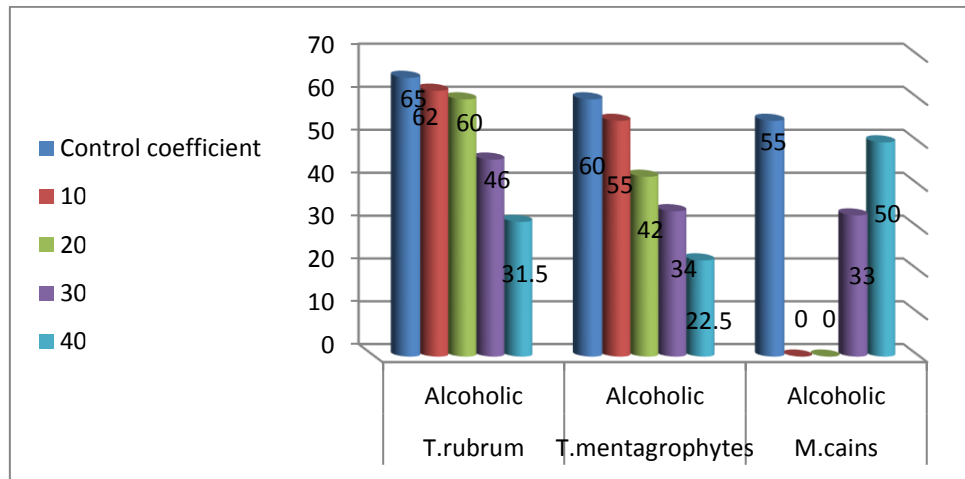
**Figure (8) Effect of aqueous extract of Sumac seeds (*Rhus coriaria*) on the fungi studied.**



**Figure (9) Effect of alcoholic extract of Sumac seeds (*Rhus coriaria*) on the fungi studied.**



**Figure (10) Effect of aqueous extract of hot pepper fruits (*Capsicum annuum*) on the fungi studied.**



**Figure(11) Effect of alcoholic extract of hot pepper fruits (*Capsicum annuum*) on the fungi studied.**

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