



## Testing of Volatile Oils Activity Extracted from Different Medicinal Plants Against Some Fungi Isolated from Different Sources

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### Abstract

Volatile oil extracted from *Salvia officinalis* showed significantly more effective against growth of *T. rubrum* isolated from ulceration otitis externa with inhibition significant percentage 96.42% in concentration 1/25 then the concentration 1/50 with inhibition percentage 95.83%, two concentrations 1/75 and 1/100 showed low effects which inhibited fungal growth in percentage 89.28% and 81.54% respectively, While the volatile oil extracted from *Zingiber officinale* showed inhibited effect in percentage 92.26% in concentration 1/25 and 82.73% in concentration 1/50; The inhibition percentage was 79.76% and 77.97% in concentration 1/75 and 1/100 respectively. Volatile oil extracted from leaves of *Apium graveolens* was significantly more effective in concentration 1/25 then the volatile oil extracted from *Zingiber officinale* in the same concentration with a significant inhibition percentage 85.11% so volatile oil of leaves of *Mentha piperita* in concentration 1/25 with a significant inhibition percentage 81.54% and the concentration 1/100 from volatile oil of leaves of *Mentha piperita* Lower of all concentration inhibition of fungal growth above with inhibition percentage 70.23%.

Volatile oils extracted from *Salvia officinalis* was more significant effect against growth of yeast *C. albicans* isolated from ulceration otitis externa so the inhibition percentage was 85.11% followed with volatile oil of leaves of *Apium graveolens* with percentage 80.35% in concentration 1/50 then volatile oil of leaves of *Mentha piperita* with inhibition percentage 79.16% in the concentration 1/25 while inhibition percentage of volatile oil extracted from *Salvia officinalis* ranged between 79.76% and 69.64%, While volatile oil of leaves of *Apium graveolens* inhibition percentage between 78.57% and 70.23% respectively in concentrations 1/25, 1/100; Volatile oil of *Zingiber officinale* was lower effect which inhibited fungal growth with percentage 78.57% and 61.30% in higher and lower concentrations respectively, While different volatile oils extracts shows a different inhibition effects against *T. Mantagrophytes* isolated from skin scraping, So volatile oil extracted from leaves of *Mentha piperita* showed more effective in concentration 1/25 with inhibition percentage 97.16% then volatile oil extracted from *Salvia officinalis* with percentage 77.38% in same concentration, There were no significant difference in concentration 1/25 and 1/50 of volatile oil extracted from *Zingiber officinale* with inhibition percentage 73.80% for both concentrations and volatile oil of leaves of *Apium graveolens* was less effective which inhibited *T. Mantagrophytes* in inhibition percentage 69.04% and 64.88% at the concentrations of 1/25 and 1/50 respectively. There was no significant difference between concentrations 1/75 and 1/100 with a percentage of 59.52% for both of them.

**Keywords:** *Salvia officinalis*, *Zingiber officinale*, *Mentha piperita*, *T. Mantagrophytes*, *C. albicans*, *T. rubrum*

## اختبار فعالية الزيوت الطيارة المستخلصة من نباتات طبية مختلفة على بعض الفطريات المرضية

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### الخلاصة

اظهر الزيت الطيار المستخلص من أوراق نبات الميرامية *Salvia officinalis* تأثيراً تثبيطياً متوقفاً معنوياً عن باقي الزيوت المستخلصة من النباتات الطبية المختلفة المستخدمة في البحث على نمو الفطر *T. rubrum* المعزول من تقرحات الإذن الوسطى ونسبة تثبيط معنوية %96.42 عند التركيز 25/1 يليه التركيز 50/1 بنسبة تثبيط %95.83 وكان التركيزين 75/1 و 100/1 اقلها تأثيراً إذ ثبت نمو الفطر بنسبة %89.28 و %81.54 على التوالي، أما الزيت الطيار المستخلص من ثمار نبات الزنجبيل *Zingiber officinale* فأظهر تأثيراً تثبيطياً بنسبة %92.26 عند التركيز 25/1 و %82.73 عند التركيز 50/1 وكانت نسبة التثبيط المئوية %79.76 و %77.79 عند التركيزين 75/1 و 100/1 على التوالي؛ وتفوق الزيت الطيار المستخلص من أوراق نبات الكرفس *Apium graveolens* معنوياً عند التركيز 25/1 على الزيت الطيار لثمار نبات الزنجبيل عند نفس التركيز على نمو الفطر إذ بلغت نسبة التثبيط المئوية %85.11 كذلك الزيت الطيار لأوراق نبات النعناع عند نفس التركيز بنسبة تثبيط مئوية بلغت %81.54 وكان التركيز 100/1 من الزيت الطيار لاوراق نبات النعناع اقل التراكيز تثبيطاً لنمو الفطر أعلاه بنسبة تثبيط مئوية %70.23.

تفوق الزيت الطيار المستخلص من أوراق نبات الميرامية معنوياً في تثبيط نمو الخميرة *C. albicans* المعزول من تقرحات الاذن الوسطى حيث كانت نسبة التثبيط المئوية %85.11 يليه الزيت الطيار لاوراق نبات الكرفس بنسبة تثبيط %80.35 عند التركيز 25/1 ثم الزيت الطيار لاوراق نبات النعناع بنسبة تثبيط %79.76 عند نفس التركيز اعلاه في حين تراوحت نسبة التثبيط المئوية للزيت الطيار المستخلص من اوراق نبات الميرامية بين %79.76 و %69.64 لاعلى التراكيز واقلها على التوالي، اما الزيت الطيار لاوراق نبات الكرفس فتراوحت نسبة التثبيط المئوية بين %78.57 و %70.23 على التوالي عند التركيزين 50/1 و 100/1 وكان الزيت الطيار المستخلص من ثمار نبات الزنجبيل اقلها تأثيراً إذ ثبت نمو الفطر *T. Mantagrophytes* بنسبة %78.57 و %61.30 عند اعلى واقل التراكيز على التوالي.

في حين اظهرت الزيوت الطيارة المستخلصة من النباتات الطبية المختلفة تأثيراً تثبيطياً مغايراً على نمو الفطر *T. mentagrophytes* المعزول من القشطات الجلدية وتفوق الزيت الطيار المستخلص من اوراق نبات النعناع عند التركيز 25/1 ونسبة تثبيط مئوية بلغت %97.61 يليه الزيت الطيار المستخلص من اوراق نبات الميرامية بنسبة %77.38 عند نفس التركيز، ولم يكن هناك فرقاً معنوياً عند التركيزين 25/1 و 50/1 للزيت الطيار المستخلص من ثمار نبات الزنجبيل إذ بلغت نسبة التثبيط المئوية %73.80 لكلا التركيزين وكان الزيت الطيار لاوراق نبات الكرفس اقلها تأثيراً إذ ثبت نمو الفطر *T. mentagrophytes* بنسبة تثبيط %69.04 و %64.88 عند التركيزين 20/1 و 50/1 على التوالي ولم يكن هناك فرقاً معنوياً للتركيزين 57/1 و 100/1 إذ بلغت نسبة التثبيط المئوية %59.52 لكل منهما.

الكلمات المفتاحية : *Zingiber officinale, Mentha piperita, T. Mantagrophytes, C. albicans, T. rubrum*

## 1. Introduction

Skin infections are a high percentage of skin diseases in humans, especially in warm areas where the environment is suitable for growth of fungi as well as the availability of moisture, heat and keratin materials [1], Skin fungi infect the surface layer of the body so it is Keratinophilic tissue [2] Which includes hair, skin and nails as keratin is the main source of nutrition [3], Filamentous dermatophytes produces many enzymes such as pyrotechnics, keratinase, elastase, lipase, phospholipids, amylase and DNA ase [4], and carbon and nitrogen [5]. Dermatophytes differ from one species to another in their composition to these enzymes [6] [7]. The term Tinea is refers to infections caused by dermatophytes which it's mean Ring worm [1].

*Candida* is an opportunistic pathogenic yeast , the species *Candida albicans* is a natural human microbiota fungus. Although candidiasis is commonly regarded as a harmless commensal in healthy people, it may become overgrown and cause a variety of complications in the host, ranging from localized superficial infections to systemic life-threatening disseminated candidiasis like Oral Candidiasis, Vaginal Candidiasis, and Ears Candidiasis [8]. The ability of *C. albicans* to form biofilms, a densely packed population of cells that can develop on both abiotic and biotic substrates such as implanted medical devices and mucosal surfaces, is a significant virulence factor [9].

*C. albicans* infections associated with high morbidity and mortality rates, making them a significant clinical challenge [10] [11]. These infections have recently increased dramatically due to the increase in the incidence of immunodeficiency caused by Irradiation, Chemotherapy, Cancer, Diabetes and taking immunosuppressive treatments and over-taking treatments without medical advice and Acquired Immuno Deficiency Syndrome [12].

Due to the increase in resistance to the antifungal agents used to treat various fungi infections [13]. and the possession of the antifungal side effects on because of their toxicity to the mammalian cells [14], it was necessary to find alternatives to the antifungal agents that have high effectiveness against pathogenic fungi and are safe to use to eliminate the characteristic of antibiotic resistance in pathogenic fungi such as volatile oil extracted from some medicinal plants, this is the aim of this study.

## 2. Research Methods

### Medicinal plants used in research and extraction of volatile oil from its parts and sterilized

Four medical plants were selected to extract the volatile oil from their parts and study their affectivity, Which are the leaves of *Apium graveolens*, *Salvia officinalis* and *Zingiber officinale* fruits displayed in local markets of Mosul city, While *Mentha piperita* leaves was obtained from the Gardens of Mosul university, the plant samples were classified in the herbivores of college of Science/Biology department /University of Mosul, Volatile oil attended from the parts of plants was studied by steam distillation.

dried plant samples amount 500 g. of were crushed and placed in the drip of the distillation device. The distillation process was carried out, the oil was collected and dried using anhydrous calcium chloride. The samples were preserved at 4 ° C until Procedure the inhibition tests. Then sterilize the extracted oil with 1 cm<sup>3</sup> of it in 9 cm<sup>3</sup> of Ethylen glycol and sterilization using membrane filters with diameter 0.22 Micron was used to prepare concentrations used in research.

### Source of isolates and method of sampling

*Trichophyton mentagrophytes* and *T. rubrum* isolated from Scraps for skin by sterilizing the affected area superficially in ethanol 70% The edges of the infection were scraped using a sterile scalpel and the peels were taken and cultured on media Sabouraud's Glucose Agar (SGA) For three weeks, the fungi identification according to approved taxonomic keys. Yeast *Candida albicans* isolated from External ear ulcers with Ear Candidiasis By sterilization of external ear with cotton swab saturated with ethanol 70% it left to dry and then took a swab of the ear with a sterile cotton swab and cultured on SGA The samples were incubated at 27<sup>o</sup>c for three weeks, the fungi identification according to approved taxonomic keys [15].

### Inhibition test

Test the inhibitory effect of volatile oils extracted from medicinal plants studied on the growth of fungi *T. mentagrophytes* and *T. rubrum* by adding specific sizes of all extracted and sterilized oil to specific sizes of SGA media before hardening after blending well the concentrations (1/25, 1/50, 1/75, 1/100)cm<sup>3</sup>/cm<sup>3</sup> were obtained and then poured into sterile Petri dishes with a diameter of 9 cm, After hardening the media take a tablet from the edge of the fungal colony of the fungi studied by the cork

borer 0.5 cm diameter and put it in the center of the Petri dish in sterile conditions, Incubate the dishes in an inverted position at 27 ° C until the control dish is filled, The results were calculated by averaging two orthogonal diameter of each fungal colony by three replicates per concentration and each replicate one dish [16].

In *C. albicans*, inhibitory effect of volatile oils extracted from medicinal plants was tested using Agar Wells Diffusion method [17] [18]. *C. albicans* was cultured on the medium of the SGA (Thickness of medium 0.5 cm) by Streak plating by sterile loop (streaking), and the Wells was made in the plate with a diameter of 0.5 cm using the cork borer and care should be taken to leave adequate spaces between the Wells to avoid the overlap of inhibition zones, then put 0.5 cm<sup>3</sup> from different concentrations of each of the medicinal plants used in the study on the wells by syringe, control wells was filled with distilled water, Incubate the dishes at 27 ° C for 48 h. The results were taken by measuring the diameter of the inhibition zones around the wells. All results were statistically analyzed using the Duncan test to determine the significant differences at a probability level of 0.05 [19].

### 3. Results And Discussion

Table (1) and picture (1) appears that the volatile oil extracted from *Mentha piperita* leaves significantly exceeded another volatile oils in inhibiting the growth of the fungus *T. mentagrophytes* The percentage of inhibition was 97.19% at concentration 1/25 followed by the volatile oil extracted from the leaves of the *Salvia officinalis* with a percentage 77.38% of inhibition at the same concentration and then the volatile oil of *Zingiber officinale* fruit with a percentage of the inhibition of 73.80% at concentrations 1/25 and 1/50, While the percentage of inhibition was 72.61% at the concentration of 1/75 for the volatile oil of the *Salvia officinalis* leaves, while the concentration of 1/50 of the volatile oil for *Mentha piperita* leaves was affected by a percentage of 69.64%, followed by the concentration 1/25 of the volatile oil for the leaves of the *Apium graveolens* as the percentage of inhibition was 69.04% and did not differ significantly from the volatile oil of *Zingiber officinale* fruit by the inhibition percentage of 67.61% and 66.66% at concentrations 1/75 and 1/100 respectively.

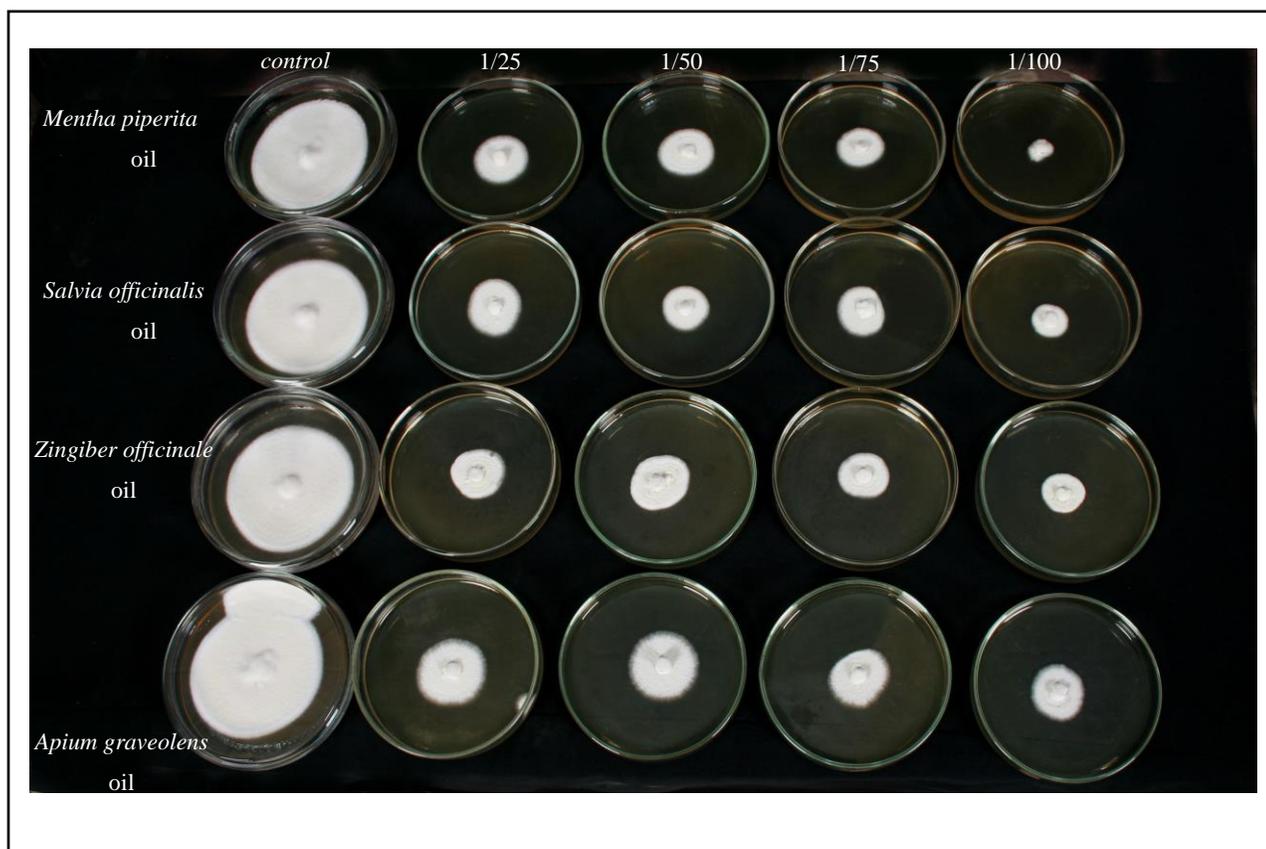
**Table 1.** Effect of different volatile oils on growth of fungi *T. mentagrophytes*

Type of plant oil	concentration cm <sup>3</sup> /cm <sup>3</sup>	Mean of colony diameter/cm*	percentage of inhibition %**
<i>Apium graveolens</i> oil	1/25	2.6	69.04 G
	1/50	2.95	64.88 J
	1/75	3.4	59.52 N
	1/100	3.4	59.52 N
<i>Mentha piperita</i> oil	1/25	0.2	97.19 A
	1/50	2.55	69.64 E
	1/75	3.0	64.28 K
	1/100	3.1	63.09 L
<i>Salvia officinalis</i> oil	1/25	1.9	77.38 B
	1/50	3.2	61.90 M
	1/75	2.3	72.61 D
	1/100	2.65	68.45 F
<i>Zingiber officinale</i> oil	1/25	2.2	73.80 C
	1/50	2.2	73.80 C
	1/75	2.72	67.61 H
	1/100	2.8	66.66I
Control	0	8.4	0.0 L

\* : Average values represent an average of three readings.

\*\* : The values that share a single alphabetical letter have no significant difference according to the Duncan test at a probability level of 0.05.

While the concentration 1/50 of the volatile oil of the leaves of the *Apium graveolens* showed a disincentive effect of 64.88% and the concentrations 1/75 and 1/100 for the volatile oil of *Mentha piperita* leaves with inhibition percentage 64.28% and 63.09% respectively, Finally, concentrations 1/75 and 1/100 for the volatile oil extracted from the leaves of the *Apium graveolens* were the least effective on the growth of *T. mentagrophytes* with percentage 59.52% for both of them.



**Figure 1.** Effect of volatile oil extracted from different medicinal plants with concentrations (1/25, 1/50, 1/75 and 1/100)  $\text{cm}^3 / \text{cm}^3$  against *T. mentagrophytes*.

The volatile oil extracted from the leaves of the *Salvia officinalis* plant showed a significant inhibitory effect on the growth of *C. albicans*, with inhibition percentage 85.11% at 1/25 concentration followed by the volatile oil extracted from the leaves of the *Apium graveolens* and *Salvia officinalis* leaves by inhibiting 80.35% and 79.76%, respectively, at 1/50 concentration, volatile oil from *Mentha piperita* and volatile oil from the leaves of the *Apium graveolens* inhibiting with percentage 79.16% and 78.57%, respectively, at 1/25 concentration, *Apium graveolens* oil did not differ significantly from *Zingiber officinale* oil at concentration 1/25 and *Mentha piperita* leaves oil at concentration 1/50 with 77.97%, Followed by *Salvia officinalis* oil with inhibition percentage 77.38% at concentration 1/75 and *Zingiber officinale* oil at concentration 1/50 with inhibition percentage of 75.59%, While *Mentha piperita* oil and *Zingiber officinale* oil showed an inhibition percentage 74.04% and 73.21% respectively at concentration 1/75, followed by volatile oil of *Mentha piperita* leaves in concentration 1/100 with inhibition percentage 72.61% and *Apium graveolens* oil with inhibition percentage 71.42% at 1/75

concentration and inhibition percentage 70.23% at 1/100. Finally the volatile *Salvia officinalis* oil and volatile oil of *Zingiber officinale* inhibited fungal growth with inhibition percentage 69.64% and 61.30%, respectively, at 1/100 concentration (Table 2).

**Table 2.** Effect of different volatile oils on growth of fungi *C. albicans*

Type of plant oil	concentration cm <sup>3</sup> / cm <sup>3</sup>	Mean of colony diameter/cm <sup>*</sup>	percentage of inhibition/% <sup>**</sup>
<i>Apium graveolens</i> oil	1/25	1.8	78.57 E
	1/50	1.65	80.35 B
	1/75	2.4	71.42 L
	1/100	2.5	70.23 M
<i>Mentha piperita</i> oil	1/25	1.75	79.16 D
	1/50	1.85	77.97 F
	1/75	2.15	74.04 I
	1/100	2.3	72.61 K
<i>Salvia officinalis</i> oil	1/25	1.25	85.11 A
	1/50	1.7	79.76 C
	1/75	1.9	77.38 G
	1/100	2.55	69.64 N
<i>Zingiber officinale</i> oil	1/25	1.8	78.57 E
	1/50	2.05	75.59 H
	1/75	2.25	73.21 J
	1/100	3.25	61.30 N
Control	0	8.4	0.0 O

\* : Average values represent an average of three readings.

\*\* : The values that share a single alphabetical letter have no significant difference according to the Duncan test at a probability level of 0.05.

Table (3) indicates the inhibitory effect of volatile oils extracted from different medicinal plants on the growth of *T. rubrum*, In which the volatile oil extracted from the leaves of *Salvia officinalis* significantly exceeded the concentrations of 1/25 and 1/50, with the percentage of inhibition 96.42% and 95.83% respectively, Followed by *Zingiber officinale* oil with 92.26% at concentration 1/25 and *Salvia officinalis* oil at 1/75 with 89.28%. The oil of the leaves of the *Apium graveolens* and *Zingiber officinale* showed a different inhibitory effect of 85.11% and 82.73% at concentration 1/25 and 1/50, The percentage of *Mentha piperita* oil 81.54% did not differ at 1/25 concentration with *Salvia officinalis* effect at concentration 1/100, The effect of *Apium graveolens* volatile oil, *Mentha piperita* volatile oil and volatile oil of *Zingiber officinale* was similar to the inhibition percentage 79.76% respectively at concentrations 1/50, 1/50 and 1/75 while the concentration 1/75 and 1/100 of the volatile oil of the *Apium graveolens* were affected by 79.16% and 78.57%, respectively, The percentage of inhibition of fungal growth was 77.97% for the volatile oil of *Zingiber officinale* at concentration 1/100. The

concentration of 1/75 and 1/100 of the volatile oil for *Mentha piperita* leaves was the lowest of the different concentrations, as the growth of *T. rubrum* was increased by 77.38% and 70.23% respectively.

Plants have been an important source of medicines since the dawn of human civilization. Despite the tremendous development in the field of morbidity during the 20th century, plants remain one of the major sources of medicine in the modern and traditional system of medicine throughout the world [20] [21]. Aromatic oils and secondary plant metabolism products have been widely applied in folk medicine, perfumery industries, food flavor and preservation, but only in recent years have begun to recognize their role as antimicrobial potential especially filamentous fungus *Trichophyton mentagrophytes* and *Aspergillus fumigatus* [22]. As well as determine the effects of essential herbal oils on human pathogenic fungi, especially *Trichophyton* spp. [23]. *Mentha longifolia* and *Salvia fruticosa* showed an inhibitory effect against all pathogens *Trichophyton mentagrophytes*

**Table 3.** Effect of different volatile oils on growth of fungi *T. rubrum*

Type of plant oil	concentration cm <sup>3</sup> /cm <sup>3</sup>	Mean of colony diameter/cm*	percentage of inhibition/%**
<i>Apium graveolens</i> oil	1/25	1.25	85.11 E
	1/50	1.7	79.76 H
	1/75	1.75	79.16 I
	1/100	1.8	78.57 J
<i>Mentha piperita</i> oil	1/25	1.55	81.54 G
	1/50	1.7	79.76 H
	1/75	1.9	77.38 L
	1/100	2.5	70.23 M
<i>Salvia officinalis</i> oil	1/25	0.3	96.42 A
	1/50	0.35	95.83 B
	1/75	0.9	89.28 D
	1/100	1.55	81.54 G
<i>Zingiber officinale</i> oil	1/25	0.65	92.26 C
	1/50	1.45	82.73 F
	1/75	1.7	79.76 H
	1/100	1.85	77.97 K
Control	0	8.4	0.0 N

\* : Average values represent an average of three readings.

\*\* : The values that share a single alphabetical letter have no significant difference according to the Duncan test at a probability level of 0.05.

and *Aspergillus fumigatus* [24]. *Salvia* species have long been described in traditional medicine for various indicators. Because of the widespread prevalence of this type of plant by the ethnic population, especially for the various infections ranging from skin diseases and gastrointestinal disorders, *Salvia*

*officinalis* oil showed an adverse effect on reverse dermatophyte and *Aspergillus* strains [25], *S. rhytidea* can also be effective against fungal infections. In view of the increased occurrence of candidiasis in the past decade, limitations on the use of antifungal drugs, the appearance of oxidative-resistant candidiasis and increased treatment failures, it is necessary to identify novel agents with antifungal properties. The results of phytochemical analysis showed that *S. rhytidea* extract was used. It was rich in flavonoids and tannins. The minimum inhibitory concentration (MIC) and the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of *S. Rhytidea* ranged from 3.125 to > 100 µg / ml and 6.25 to > 100 µg / ml, respectively. The value of inhibition of growth was shown as *C. tropicalis*, *C. krusei* and *C. albicans* [26]. The composition and antifungal activity of *Salvia officinalis* was also studied. On dermatophytes and *Aspergillus* [27]. Essential oils of red thyme, fennel, cloves, pine, sage, lemon and lavender balm is an inhibitory effect for the growth of clinical and environmental fungal strains [22]. *Zingiber officinale* is a medicinal plant that has been widely used in Chinese herbal medicines [28]. Plant extracts of *Zinigiber officinale* showed inhibitory growth of *Aspergillus niger* and *Aspergillus flavus* [29] [30], *Zingiber officinale* also showed antioxidant and antimicrobial activities against *Aspergillus niger* and *Candida albicans* [31].

#### 4. Conclusion

The results indicate that the volatile oil extracted from the different medicinal plants used in this study has different inhibitory effects on the three fungi studied *T. Mantagrophytes*, *T. rubrum* and *Candida albicans*.

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