

The Effect of Thyme and Peppermint Extracts on Some Species of *Candida* Yeast

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Abstract

This study was conducted at College of Education (Pure Sciences), University of Diyala for the period from July/2010 until February/2011 to evaluate the inhibitory effects of extracts from thyme and peppermint against some species of *Candida* yeast. Alcoholic extract from Thyme at concentrations 100 and 20 mg/ml showed the highest inhibitory effects against *Candida albicans*, *C. tropicalis*, *C. glabrata* and *C. krusie* 100%, and the lowest inhibitory 75.53% for *C. albicans*. Alcoholic extract from Peppermint at 100 and 20 mg / mL in percentages 100% for yeast *C. tropicalis*, *C. glabrata* and the lowest 70.78% for *C. albicans*. where acetic extract of thyme at concentrations 100 and 20 mg / ml in percentages 100% and 70.23% for *C. krusie*, then acetic extract from peppermint at concentrations 100 and 20 mg / ml in percentage 100% for *C. glabrata* and 59.30% for *C. albicans*. acetic extract from Thyme and peppermint at concentrations 100 mg / ml and antifungal Nystatin 50µ/ml showed the same effective inhibition against yeast *C. krusie*, *C. albicans* and *C. glabrata*, hot water extract from thyme at concentrations 100 and 20 mg / mL in percentage 97.91% and 67.50% for *C. glabrata* and *C. tropicalis*, Followed by hot water extract from Peppermint at concentrations 100 and 20 mg / mL in percentage 96.59%, and 62.19% for *C. krusie* and *C. tropicalis*. At last the cold water extract from thyme at concentrations 100 and 20 mg / ml in percentage 94.31% and 59.55% for *C. albicans* and *C. krusie*, Followed by cold water extract from Peppermint at concentrations 100 and 20 mg / ml in percentages 92.22% and 55.95% respectively, compared to the control treatment 0.0.

Keywords: Plants extracts, Yeasts and Antifungal

INTRODUCTION

Increased in recent years the importance of research on the frequency of fungal infections, attributable to the increasing number of patients in AIDS (Acquired Immunodeficiency), Syndrome, diabetes, pathogenesis of leukemia and tuberculosis (30), The Candidiasis diseases are considered an common opportunistic diseases in the world and it was resulting from infection from certain species of *Candida* genus, Including the oral infections and skin and systemic infections and genital urinary tract (42.53), *Candida albicans* Considered the president species of infection then other species such as *C. krusie*, *C. tropicalis*, *C. dubliniensis* and *C. glabrata* and other (54). The diagnosis of *Candida* species considered first step in treatment, it was observed that the yeasts *C. krusie*, *C. glabrata*, *C. tropicalis* and *C. dubliniensis* possesses high sensitivity to antifungal (49). At the present time many of the therapeutic lotions available to used to treat fungal diseases that develop slowly despite the lack of numbers compared to the lotions used to treat bacterial infections with the observation that most of the active substances included in these formulations are toxic when used at high concentrations, So limited use in the form of ointments skin surface (40) and for being possessed qualities of high toxicity to the liver, pancreas, so it requires to use antibiotics with natural sources (26,42) therefore, the possibility of extracting natural and anti materials was increased attention through the study of drugs for his close relationship the science botany and plant chemistry (15). The reasons for the trend towards medicinal plants is also free from industrial chemicals that cause side effects affecting the health of the patient, as well the scientific progress and industrial that provides methods and techniques are effective in keeping medicinal plants, with easy handling in different forms in the form of extracts sticky or tablets and pills dry led to increased orientation to the use of these plants (5). The plants are a repository for many of the active compounds, secondary metabolism compounds are simple installation and complex That's where some of them characteristics of anti-microorganisms in general, such as volatile oils and alkaloids and tannins and other (43) such as *Nigella* (*Nigella sativa*), thyme, garlic and other plants, what are containing the inhibitory effective materials of the fungi growth (4,16). The study aimed to:

- Preparation of alcoholic and acetone and water extracts of thyme and Peppermint plants.
- Qualitative assessment of the active compounds in the plant extracts.
- Determine the effectiveness of inhibitory plant extracts against some types of yeasts.
- Comparison of inhibitory efficacy of plant extracts with antifungal.

MATERIALS AND METHODS

Plants used

The thyme (*Thymus vulgaris*) leaves and peppermint (*Mentha piperita*) leaves and flowers of Labiatae (Lamiaceae). The leaves and flowers were dried at room temperature under air stream and then grinding samples were used in this study to get the powder required for Plant extracts.

Plant extracts

Four types of extracts of thyme and peppermint, a hot and cold water and alcohol and acetone, were prepared and used in this study.

Cold water extracts

Following the method of Parekh and Chanda (47) 10 g of the powdered samples in glass beaker with 100 ml of distilled water, put the flask in an incubator vibrators for 24 hours and at 37 m, then was nominated mix mediated medical gauze in glass tubes, have renounced quickly 5000 cycle / min for 10 minutes, was nominated was nominated output mediated filter paper, the filtrate is evaporated in an oven at a temperature 40 ° C for dry powder extract and save that in a dark glass bottle and sealed in frozen (- 20 m °) until use.

Hot water extract

According to the method of El-fallal and El-kattan (27) 10 g of the powder plant material were mixed with 100 ml of boiled distilled water, put glass beaker in the incubator vibrators in temperature of 28 C° for 30 minutes, was nominated mix the use of medical gauze, distributed filtrate in glass tubes and have renounced at 3000 r / min for 10 minutes, collecting the filtrate in glass dishes (diameter 20 cm) of water and dry it in the oven at a temperature 40 ° C until the water evaporates completely, to get a hot water extract powder, which saved the same way above.

Alcoholic extracts

Following the method of Shtayeh and Abo-Shdeib (56) 10 g of the powder plant materials were mixed with 100 ml of 70% ethanol, put the mixture in the incubator vibrating at a temperature of 35 C° for 24 hours, then was nominated mix using the medical gauze, distributed filtrate in glass pipes and quickly have renounced 3000 rev / min for 10 minutes, collecting the filtrate in glass dishes (diameter 20 cm), dry alcohol in the oven at a temperature 40 C°, to get the alcoholic extract powder which saved the same way.

Acetone extracts

Following the method above replacement with ethanol, acetone.

Concentrations used in the tests vital

The microbial concentrations 20, 40 and 60, 80 and 100 mg / ml were prepared in melting 10 g of powder plant extract in 10 ml of distilled water, and using the law of general dilution $C1V1 = C2V2$ and was sterilized by using filters minute (0.22 Micro Metter).

Antifungal effect on some species of genus Candida

The effects of antifungal Nystatin, Ketocanazole and Flucanazole were studied in some species of Candida, To find the Minimal Inhibitory Concentration (MIC) and the Minimal Fungicidal Concentration (MFC) by using the Sabouraud Broth Medium in the dilution method, according to the method of Shadomy and others (51), as follows: -

Preparation of inoculums

The inoculums were prepared at a rate of 10^5 cells/ml from the implanted four species of Candida developing on the Sabouraud Dextrose Ager (SDA) medium, at the age of 24 hours 24 hours.

Preparation of solutions antifungal

Antifungal were prepared In the melting 0.01 g of the antifungal in 100 ml from organic solvent Dimethyl Sulphoxide, Then prepared the dilutions required

Preparation of the Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MFC)

The original concentration of the anti-fungal 100 mcg / ml were prepared , And after that prepared a series of concentrations of multiplexed antifungal 0.05-50 µg / ml, In preparation a 12 tube each containing 2 ml of Sabouraud Sucrose Broth(SSB), 2 ml of anti original was added to the first tube to get concentration of 50 µg / ml, and the other concentration were prepared by multiplexed dilution method, with a tube of positive control (without antifungal) and a tube of negative control (without inoculums). Tubes were Inoculated with 0.05 ml from the suspended isolation , and incubated at 30 C° for 48 hours, tubes are mixed, It was observed the growth compared with the control tube, MIC values are represent the lowest concentration, who does not appear growth . MFC value were determined by implanting 0.01 ml of each tubes that did not appear growth and control tubes, And planted on the SDA medium free antifungal, then incubated at 30 C° for 48 hours, and examined after the emergence of growth in the control tube. The MFC Values represent the lowest concentration anti-fungal, which gives a negative result after secondary implantation.

Cytotoxicity of extracts of thyme and peppermint

The toxic cellular of thyme and peppermint extracts were estimated by the method of Xin-Guo and Ursella (59), 0.8 ml of extract was putting in a sterilized test tube with 0.2 ml of human red blood cells, to become the final

volume 1 ml, the tube was shaking for 30 minutes and incubated at 37 C°, and the tube Renouncing centrally in a Universal Centrifuge for 5 minutes at a rate of 1000 r / min, it the blood decomposition was observed, the treatment of control (test tube containing blood only) was used to note the differences in the decomposition of the blood.

The percentage of extracts of thyme and peppermint

The percentages of plant extracts were estimating by the method of Al-Balany (6)

% Extract = weight / weight of the powder × 100

Qualitative detection extracts of thyme and peppermint

The qualitative detection was conducted to identify the essential chemical components or active compounds in the extracts, including:

Detection of alkaloids

Alkaloids were detected by using the detector Marx and the detector Mayer, by the the method of Harborn (32).

Detection of tannins

Detecting lead acetate

The solution were preparing by melting 1g of lead acetate in 100 ml of distilled water, and added a few drops of the solution into a test tube containing 0.5 ml of extract, the emergence of white sediment gel textures were indicating to the presence of tannins (3).

Detect ferric chloride

Several drops of 1% ferric chloride were added to a test tube containing 0.5 ml of the plant extract. The appearance of a bluish green color were indicating to the presence of tannins (2).

Detection of saponins

1 - The aqueous solution of the powder plants was prepared in the test tube, the test tube were shaking strongly, The appearance of dense foam for a long time indicating to the presence of saponins (32)

2 - 3 ml of plant extract were added to 2 ml of 1% mercuric chloride (HgCl₂), the appearance of white sediment indicating to the presence of saponins (10).

Detection Glycosides

1 ml of the plant extract were putting in a test tube and added a 2 mL of reagent Benedict, and then transferred to a boiling water bath for 5 minutes, the appearance of red color indicates to a positive test (32).

Detection of resins

1 g of dry vegetable powdered were mixing with 10 ml of 95% ethanol, the solution were leaving for one minute in a water bath (100 C°), the solution was nominated and added a 10 ml of an aqueous solution of 4% hydrochloric acid, the appearance of turbidity indicates to the presence of resins (55).

Detection of flavonoids

1 - detection of potassium hydroxide alcohol

2 ml of the plant extract were mixing with 1 ml of alcoholic potassium hydroxide, the appearance of yellow color indicates to the presence of flavonoids) 36).

2 - Detection of flavonoids and flavonols

1 ml of plant extract were melting in 1 ml of concentrated sulfuric acid, the appearance of a dark yellow color was indicating to positive detection (10).

Detection of phenols

The detector of ferric chloride were preparing by melting 1 g of ferric chloride in 100 ml of distilled water. the filter paper was moisten in the plant extract, added drops of reagent Wohlen or ferric chloride to the filter paper, and the paper was exposing to ammonia vapor. The appearance of blue evidence indicates to phenols (2).

Detection of coumarins

1 g of dry plant powder were mixing with 10 ml of 95% ethanol in a test tube and then the tube is covered in nomination paper moistened with a solution of 5% dilute sodium hydroxide and the tube were putting in a boiling water bath for a few minutes. The appearance of greenish-yellow color when exposed the filter paper to the ultra violet (UV) indicates to the presence of coumarin (32).

Detection Fuocoumarins

1 ml of 10% alcoholic potassium hydroxide were adding to 1 ml of the plant extract, the appearance of yellow or greenish-yellow indicates to the Fuocoumarins (32).

Detection Triterpenoids

1 ml of concentrated sulfuric acid were adding to 1 ml of chloroform, and then the resulting solution was added to 2 ml of the plant extract. The appearance of red or purple indicates to the existence of triterpenoids (32).

Detection of volatile oils

Following the method of Harborn (32) The filter paper were satiated in 10 ml of filtrate extracts of thyme and peppermint and exposing to the source of ultra violet light, the appearance of a bright pink color indicates to the volatile oils.

PH measurement

PH was measured to the plant extracts after drying and solvent.

The effectiveness of extracts of thyme and peppermint in inhibitory growth of *Candida* spp

It was ascertained that non-contamination of the plant extract by planting 0.01 ml of plant extracts in the SDA medium and incubated for 3-7 days, according to the method of El-Kady et al (28), the plant extracts were mixing with the SDA medium and coolant to 50 C° in concentrations 20, 40, 60, 80 and 100 mg / ml and with two replicates each concentration, planting a disk (diameter 6 mm) from yeast colony developing in SDA medium for 7 days at the dish center, and two types of comparison were using, positive control which added to it anti fungal Nystatine at concentration 2 mg / ml at the SDA medium, and negative control without adding, all the dishes were planting in the same fungus, and incubated at the temperature of 28-30 C° for a week, diameter of growing colonized was measured (orthogonal diameter rate), and the percentage of inhibition was calculated by using the following equation:

$$\text{Inhibition percentage} = \frac{\text{Diameter of fungi in comparing plates} - \text{diameter of fungi in treatment plates}}{\text{Diameter of fungi in comparing plates}} \times 100$$

Statistical Analysis

The data were analyzed by practical experiment using CRD and the significant differences LSD with levels 0.01 and the SPSS program was use to analyze data (12).

Results and Discussion

Antifungal effect on some species of genus *Candida*

Table 1 showed the inhibitory effects of different group of antifungals against yeasts *C. albicans*, *C. glabrata*, *C. krusie* and *C. tropicalis*. The minimum inhibitory concentration (MIC) for a Flucanazole was between 6.25 - 12.5 µg / ml, followed by Ketacanazole 12.5-25 µg / ml, and then Nystatin 12.5-25 µg / ml, These results are consistent with that of Van den Bossche et al (58) where the group azoles two effects first Saitokrom p-450, which leads to the inhibition of Arxterol resulting from the removal 14.α methystrol. The second effect which results from the direct anti-fungal interaction with lipid membrane that lead to shattering membrane. The results also consist with that of Ingroff et al (34) where the group polinat such as Nystatin the influential when its union with sterol in the cell membrane, which leads to leakage of cell components and death, and the results also consistent with that of Al-Sadik (14), who found that the MIC for the Nystatin 32 µg / ml while it was 25 µg / ml for yeast *C. albicans*, Al-Hujami (8) mentioned that the value of MIC for Nystatin was between 12.5-25 µg / ml against *C. albicans*, and Arikan et al (19) found that the value of MIC for Nystatin was between 1-2 µg / ml. And The results also consist with that of Odds (45) where the value of MIC for Nystatin up to 50 µg / ml, and attributable the difficulty in determining the value of MIC to the widespread use and indiscriminate antifungal, which led to the emergence of differences in sensitivity between species within the same-genus dates back to the emergence of strains of different genotype strains wild (26.29), the effectiveness of antifungal and the time needed to kill the yeast dependent concentration, and vary the value of MIC in vitro antifungal for the different of medium and incubating temperature and duration of incubation, where increasing efficiency increase duration of incubation (9).

Table 1. Values MIC and MFC antifungal against *Candida* spp.

Yeasts	Antifungal	MIC µg/ml	MFC µg/ml
<i>C. albicans</i>	Flucanazole	12.5	50
	Ketacanazole	25	50
	Nystatin	25	50
<i>C. tropicalis</i>	Flucanazole	6.25	50
	Ketacanazole	12.5	50
	Nystatin	25	50
<i>C. glabrata</i>	Flucanazole	12.5	50
	Ketacanazole	12.5	50
	Nystatin	12.5	50
<i>C. krusie</i>	Flucanazole	12.5	50
	Ketacanazole	25	50
	Nystatin	25	50

Percentages and toxicity of extracts of thyme and peppermint

Alcoholic extracts gave the best percentages of 65.05% and 76.85% Table 2, followed by acetone extracts 64.00% and 68.65%, and the hot water extracts 61.45% and 56.45%, then the cold water extracts 38.60% and 44.60% for the peppermint and thyme sequentially. Variation of percentages attributable to difference in the

polarity of the solvent used distilled water and ethanol and acetone, These results are consistent with that of Bernard (22) where the variation is due to the different dielectric constant of the solvent (dielectric constant for the water 78.4, alcohol ethyl 24.5 and acetone 20.7), and the results also consistent with that of Al-Zuhairy (18) reduce the differences in the proportions active substances in the presence cold and hot water extracts and alcoholic extracts by 41.4% for the fruits of the Cokhrn (*Tribulus terrestris*) and 46% for Cleavers (*Echium vulgare*). And the results also consistent with that of Maogda Kutub (39) that the presence of compounds can be extracted with water to melt in the water more than organic solvents, and attributable to the chemical composition of these compounds and the nature of the materials associated with the portion of plant. The results in Table 2 showed cytotoxicity of alcoholic extracts from thyme, peppermint and acetone extracts from thyme effective toxicity to red blood cells of sheep's and human through decomposition bloody appeared in the glass (In vitro), and the results also showed the absence of toxic cellular for the extracts water extracts from thyme and peppermint, and acetone extracts from peppermint, the reason for this attributable to the low concentrations of compounds saponins in the plant, and these results are consistent with Mills et al (43) that the emergence of cellular toxicity due to the familiarity of the saponins to sterol entering the plasma membrane of the cell and remove it and the emancipation of hemoglobin, so the treatment of diseases interior materials rich compounds saponins oral and not intravenously, because the intestine do not absorbs saponins (50).

Table 2. Acid Function and percentage and Toxic Function

Extracts	Acid Function	%	Toxic Function
Cold water extract of thyme	6.04	44.60	No
Cold water extract of peppermint	8.60	38.60	No
Hot water extract of thyme	5.99	61.45	No
Hot water extract of peppermint	9.11	56.45	No
Acetone extract of thyme	6.30	68.65	Yes
Acetone extract of peppermint	4.22	64.00	No
Alcoholic extract of thyme	4.36	76.85	Yes
Alcoholic extract of peppermint	5.31	65.05	Yes

Qualitative Detection extracts of thyme and peppermint

the active compounds in the plant extract were investigating through using various chemical reagents. Qualitative detection showed that thyme and peppermint plant contain a number of active pharmaceutical components, glycosides, tannins, saponins, phenols and other relevant inhibitory effectiveness against *Candida* spp. And the results in Table 3 showed that thyme was contain the alkaloids in cold and hot water and alcoholic and acetone extracts, These results are consistent with that of Ateeq et al (20) where the wild thyme plant contain active substances including alkaloids, Glycosides, carbohydrates, tannins and volatile oils, etc., and the results vary with that of Al-Sadik (14) which is attributed to the difference in the environmental conditions of the plant food, alcoholic and acetone extracts from peppermint are containing alkaloids, And the results consistent with that of Jaber (35) that the alkaloids do not melt in water or melts partially and totally in acetone and alcohols, and exist tannins, Glycosides, carbohydrates, coumarins, Fuocoumarins, terpenoids, triterpenes and sterols in thyme and peppermint plants and all extraction methods, This results consistent with that of Al-Sadik (14), who found resins, tannins, saponins, Glycosides and flavonols in thyme. And saponins appeared in cold and hot water extracts from thyme and peppermint because they melt in the water and give the foam like soap, and do not melt in alcohol and acetone, and this results also consistent with that of found Jaber (35) who found that the main part of the saponins consists of sugar and often have sugar glucose. Phenols and flavonoids were existing in the thyme and peppermint plant and all extraction methods, This results are consistent with that of Al-Saeed et al (15) who considered phenols are active substance which is attributed to effective inhibitory of thyme, and appeared resins in extracts of alcohol and acetone because it is insoluble in water because they contain a portion oily, This results also consistent with that of Jabber (35) who consideration the resins was outputs to oxidize different types of essential oils, volatile oils were present in alcoholic and acetone extracts of thyme and peppermint, and did not appear in the water extract, because they melt in water at rates is very small.

Table 3. Qualitative Detection extracts of thyme and peppermint

Chemical Detection	Thyme Extract				Peppermint Extract			
	Cold water	Hot water	Alcoholic	Acetone	Cold water	Hot water	Alcoholic	Acetone
Alkaloids								
Markes reagent	-	-	+	+	-	-	+	+
Mayer reagent	-	-	-	-	-	-	+	+
Tannins								
Lead acetate 1% Detection	+	+	+	+	+	+	+	+
Ferric Chloride 1% Detection	+	+	+	+	+	+	+	+
Glycosides	-	+	+	-	-	+	+	-
Saponins								
Mercuric chloride 1% Detection	+	+	-	-	+	+	-	-
Dense foam Detection	+	+	+	+	+	+	+	+
Resins	-	-	+	+	-	-	+	+
Flavonoides								
Alcoholic potassium hydroxide Detection	+	+	+	+	+	+	+	+
Flavonoides and Flavonol Detection	+	+	+	+	+	+	+	+
Carbohydrate								
H2SO4 and Phenol Detection	+	+	+	+	+	+	+	+
Phenols								
Wholfin Detection	-	-	+	-	-	-	+	-

The effectiveness of extracts of thyme and peppermint in inhibiting the growth of yeast *Candida* spp.

The results in Table 4 showed that the antagonistic effect of thyme and peppermint extracts against *Candida* spp which depends on the type of extract and concentration and species of candida, as well as the type of plant, alcoholic extract showed high inhibitory effectiveness followed by acetone and cold water and hot water extracts, the rates of growth diameters of fungal colonies inversely proportionality with the concentration of extract, the rates of growth diameters of fungal colonies were less with increased concentration on the contrary, the percentage of inhibition where increases with the increased of extracts concentration. The results showed also that the lowest rates of growth yeasts *C.krusic*, *C.glabrata*, *C.tropicalis* and *C.albicans* in the concentration of alcoholic extract of thyme at 20 mg / ml 8.0, 8.5, 9.0 and 11.5 mm percentages inhibition of 82.97, 80.85%, 81.91% and 75.53%, and the highest percentages of inhibition at concentration 100 mg / ml, with diameters growth 0.0 mm, and a percentage of inhibition 100% for all species of yeasts, and the alcoholic extract of peppermint was gave at concentration 20 mg / ml with diameters growth 8.0, 8.5, 10.0 and 13.0 mm and a percentages of inhibition 83.15% 80.68 and 76.15% and 70.78%, and the highest percentages of inhibition at concentration 100 mg / ml with diameters growth

Table 4. Effectiveness of the alcoholic extract of thyme and peppermint in The inhibition of the growth of the yeast *Candida* spp

Plant	Yeast	Growth diameter (mm)/Extract conc.(mg/ml)						
		Cont.+	0.0	20	40	60	80	100
Thyme	<i>C.albicans</i>	0.0	47.0	11.5	4.0	2.0	0.5	0.0
	<i>C.tropicalis</i>	0.0	41.5	9.0	6.5	5.0	2.5	0.0
	<i>C.glabrata</i>	0.0	45.5	8.5	2.5	1.5	0.0	0.0
	<i>C.krusic</i>	0.0	44.5	8.0	2.5	1.0	0.0	0.0
Peppermint	<i>C.albicans</i>	0.0	44.5	13.0	9.5	5.0	2.0	0.5
	<i>C.tropicalis</i>	0.0	42.0	10.0	9.0	8.0	2.0	0.0
	<i>C.glabrata</i>	0.0	44.0	8.5	4.0	2.5	1.0	0.0
	<i>C.krusic</i>	0.0	47.5	8.0	2.5	1.5	0.5	0.5
Plant(0.35)	Fungi(0.50)	Conc.(0.66)		LSD P< 0.01				

Cont. + = positive control (anti-fungal Nystatin (2 mg / ml).

0.5, 0.0, 0.0 and 0.5 mm, with the percentages of inhibition 98.94%, 100%, 100% and 98.87% sequentially. Showed significant differences ($P < 0.01$) Appeared between the types of yeasts. *C.krusic* yeast showed high sensitivity against alcoholic extract of thyme, yeasts *C.glabrata* and *C.krusic* showed high sensitivity against the alcoholic extract of peppermint. These results consistent with Al-Jenabi (9), which mentioned that the inhibitory effectiveness of the alcoholic extract better than in water extract against *C.albicans*. Alcoholic extract of thyme at concentration 100 mg / ml showed inhibitory effectiveness equal to the effectiveness of the antifungal Nystatin for all types of yeasts, and at concentrating 80 mg / ml for yeasts *C.albicans*, *C.krusic* and *C.glabrata*, and the alcoholic extract of peppermint at concentration 100 mg / ml was gave inhibition effectiveness equal to the effectiveness of Nystatin for all types of yeasts, and at concentration 80 mg / ml was gave inhibition effectiveness equal to the effectiveness of Nystatin for yeast *C.krusic*, and the results also consistent with Al-Rjbo(11), who mention that the alcoholic extract was gave the highest percentage of inhibition 25.38% at concentration 4.5 mg / ml, higher than the percentage of inhibition of Nystatin at concentration 0.06 mg / disk, the effectiveness of the alcoholic extract may be attributed to the materials effective such us Flavonols that possesses the highest toxicity characteristics towards fungi by inhibiting the action of enzymes for metabolic reactions responsible basic non-specialist to interfere with the proteins, whereof leading to their inability to continue (43), and the results consistent with that of Al-Dhab (7) where the high portability for the ethyl alcohol to withdraw active compounds of the plant sample because of the high Polarity, However the results consistent with that of Carpinella et al (24) who mention to the effectiveness of ethanol extract of the plant Chinaberry (*Melia azedarach* L.) fruits in the inhibition of the growth of three types of fungi *Aspergillus flavus*, *Fusarium Moniliforme* and yeast *C. albicans*, because it contains active substances such as alkaloids and tannins and oils which working to weaken metabolic efficiency such us Succinate dehydrogenase enzyme effectiveness and its association with NADH, In addition to stopping the oxidative phosphorylation and the transmission of electrons chain that obtain during the process of respiration, where the alcohol groups effective overlapped with the protease enzyme composition who finally leads to stop the enzyme working (38). The acetone extracts came in second place in the inhibitory effectiveness, the reason for this was attributable to alcohols susceptibility to melts the active substances compared to other organic solvent (25). The results in Table 5 indicated that the highest rates of yeasts growth of *C.krusic*, *C.glabrata*, *C.tropicalis* and *C.albicans* in the acetone extract of thyme at concentration 20 mg / ml 9.0, 8.5, 9.0 and 12.5 mm, with the percentages of inhibition 79.54%, 81.11%, 78.31% and 70.23%, and the lowest rates of yeasts growth at concentration 100 mg / ml 0.0, 1.0, 1.5 and 0.5 mm with the percentages of inhibition 100%, 97.77%, 96.38% and 98.80%, respectively, Following by acetone extracts of peppermint, which gave the highest rates of growth at concentration 20 mg / ml of 9.5, 10.5, 11.5, and 17.5 mm with the percentages of inhibition 78.40%, 75.86%, 72.50% and 59.30% and the lowest rates of growth at concentration 100 mg / ml 1.0, 0.0, 1.5, and 2 mm with the percentages of inhibition 97.78% 0.100%, 96.25% and 95.34%. *C.krusic* yeast gave the highest degree of sensitivity against acetone extracts of thyme, and The yeasts *C.krusic* and *C. albicans* showed the same sensitivity to acetone extracts at concentration 100 mg / ml and antifungal Nystatin, the yeasts *C.glabrata* and *C.krusic* showed high sensitivity towards acetone extract of peppermint, the yeast *C.glabrata* showed the same sensitivity towards the acetone extract of peppermint at concentration 100 mg / ml and antifungal Nystatin, and these results are consistent with Sakharkar and Pati (52) who mention to the high inhibitory effectiveness of acetone and ethanol extracts of the leaves Senna (*Cassia alata*) against several isolates of filamentous fungi such as *Aspergillus niger*, *C.albicans* and *C.tropicales*, And there is an inverse relationship between the concentrations of extract and the number of cells being of increasing the percentage of inhibition (the small number of cells) with increase in the concentration of extract, this effect has attributed to the extracts effectiveness and their impact on the permeability of the cell membrane and permase enzyme action where the materials accumulate outside the cells (57).

Table 5. Effectiveness of the acetone extract of thyme and peppermint in The inhibition of the growth of the yeast *Candida* spp

Plant	Yeast	Growth diameter (mm)/Extract conc.(mg/ml)						
		Cont.+	0.0	20	40	60	80	100
Thyme	<i>C.albicans</i>	0.0	42.0	12.5	7.5	2.5	1.0	0.5
	<i>C.tropicalis</i>	0.0	41.5	9.0	6.5	5.0	2.5	1.5
	<i>C.glabrata</i>	0.0	45.0	8.5	6.5	3.0	2.5	1.0
	<i>C.krusic</i>	0.0	44.0	9.0	5.0	2.0	1.5	0.0
Peppermint	<i>C.albicans</i>	0.0	43.0	17.5	10.5	3.5	3.0	2.0
	<i>C.tropicalis</i>	0.0	40.0	11.0	9.0	5.0	3.0	1.5
	<i>C.glabrata</i>	0.0	43.5	10.5	6.0	2.5	1.5	0.0
	<i>C.krusic</i>	0.0	44.0	9.5	5.0	2.5	2.0	1.0
Plant(0.35)	Fungi(0.50)	Conc.(0.66)	LSD $P < 0.01$					

Cont. + = positive control (anti-fungal Nystatin (2 mg / ml)).

The hot water extract for thyme and peppermint ranked third in the inhibitory effectiveness Table 6, These results are consistent with that of Al-Thwehry (17) where the Inhibition of high effectiveness for the alcoholic extract and came in first, following by the acetone extract then water extract when studying the effect of extracts of *Zyzygium* (*Zyzygium aromaticum*) in the treatment of some infections of skin bacteria and fungi, The reason is attributable to the active compounds do not melt in water well and better melting material with heat factor which plays an important role in the melting, and The reason of the difference in effectiveness is attributable to the difference in polar solvents, Majeed et al (41) who mentioned that the few inhibitory effectiveness of the extracts from crude medicinal plants attributed to the little amount of effective materials in the extracts or the weakness of their effectiveness or was needing to separate the Effective components, Al-Rawi and Farty (13) also mention to the presence of effective components with each other in crude extracts sometimes cause a negative effects. The results in Table 6 also showed that the highest rates of yeasts growth of *C.krusie*, *C.tropicalis*, *C.glabrata* and *C.albicans* in hot water extracts of thyme at concentration 20 mg / ml of 8.5, 9.0, 13.0 and 13.0 mm with the percentages of inhibition 79.76%, 81.25%, 67.50% and 70.45%, and the lowest rates of yeasts growth at concentrating 100 mg / ml 1.0, 1.0, 4.5 and 1.5 mm, with the percentages of inhibition 97.61%, 97.91%, 88.75% and 96.59%, respectively. Hot water extracts of peppermint showed the highest rates of yeasts growth at concentration 20 mg / ml 9.0, 10.5, 15.5 and 13.0 mm, with the percentages of inhibition of 79.54%, 75.58%, 62.19% and 70.45%, and the lowest rates of yeasts growth at concentration 100 mg / ml of 1.5, 1.5, 3.5 and 3.0 mm, with the percentages of inhibition 96.59%, 98.80%, 91.46% and 93.18% sequentially. *C.krusie* yeast showed the highest sensitivity to hot water extracts of thyme, and *C. glabrata* and *C. Krusie* yeasts showed higher sensitivity to hot water extracts of peppermint. Inhibitory effectiveness of thyme and peppermint can be explained by Containing Some active compounds, such as terpenes, tannins, Flavonols, resins, saponins and volatile oils, This results are consistent with that of Kang et al (37) where the alkaloids inhibitory effectiveness against *C. albicans* through the inhibition of building chitin and Sterols which was important to construction of the fungal cell wall through the inhibition of important enzymes in their construction.

Table 6. Effectiveness of hot water extract of thyme and peppermint in The inhibition of the growth of the yeast *Candida* spp

Plant	Yeast	Growth diameter (mm)/Extract conc.(mg/ml)						
		Cont.+	0.0	20	40	60	80	100
Thyme	<i>C.albicans</i>	0.0	44.0	13.0	9.5	4.5	3.5	1.5
	<i>C.tropicalis</i>	0.0	40.0	13.0	10.5	8.5	5.0	4.5
	<i>C.glabrata</i>	0.0	48.0	9.0	4.0	4.0	2.5	1.0
	<i>C.krusie</i>	0.0	42.0	8.5	3.0	2.5	1.0	1.0
Peppermint	<i>C.albicans</i>	0.0	44.0	13.0	8.5	5.5	3.5	3.0
	<i>C.tropicalis</i>	0.0	41.0	15.5	14.5	9.0	5.0	3.5
	<i>C.glabrata</i>	0.0	43.0	10.5	5.0	3.0	3.0	1.5
	<i>C.krusie</i>	0.0	44.0	9.0	4.5	4.5	3.5	1.5
Plant(0.35)	Fungi(0.50)	Conc.(0.66)	LSD P< 0.01					

Cont. + = positive control (anti-fungal Nystatin (2 mg / ml)).

The cold water extract of thyme and peppermint ranked fourth in the inhibitory effectiveness Table 7. The reason for this is attributable to the active substances for the extracts do not melt in water, but well melting in organic solvents such as ethanol and methanol(1).

The results in Table 7 also showed the highest rates of yeasts growth of *C.krusie*, *C.tropicalis*, *C.glabrata* and *C.albicans* in cold water extracts of thyme at concentration 20 mg/ ml 18.0, 9.0, 11.0 and 14.5 mm with the inhibition of percentages 59.55%, 80.00%, 74.41% and 67.04%, and the lowest rates of yeasts growth at concentration 100 mg /ml 3.5, 3.5, 5.5 and 2.5 mm, with the inhibition of percentages 92.13%, 92.22%, 87.20% and 94.31%, respectively. The cold water extracts of peppermint gave highest rates of yeasts growth of *C. krusie*, *C. tropicalis*, *C. glabrata* and *C. albicans* at concentration 20 mg / ml 18.5, 13.5, 15.0 and 13.0 mm, with the inhibition of percentages 55.95%, 70.00%, 65.90% and 66.66%, and the lowest rates of yeasts growth at concentration 100 mg / ml 4.0, 3.5, 6.0 and 3.0 mm, with the inhibition of percentages 90.47%, 92.22%, 86.36% and 92.30% sequentially. The yeasts *C. krusie*, *C. glabrata* and *C. tropicalis* gave highest sensitivity toward cold water extracts of thyme, and yeast *C.glabrata* showed the highest sensitivity to cold water extracts of peppermint, and these results are consistent with that of Hamdan (31) who mention to the inhibitory effectiveness of the water extracts of mustarda (*Brassica alba*) seeds against bacteria and yeast *C.albicans*, and do not agree with that of Majeed (44) who mention to the lack of the effectiveness of

the water extracts of peppermint (*Mentha longifolia*) And not consistent well with that of Al-Jenabi (9), who stated that the hot and cold water extract of purslane (Verdolagas) plant did not show any effect in yeast *C.albicans* compared to the alcoholic extract, Bongoh and Hwang (23) in their study mention that the purslane extracts is antifungal against fungi *Aspergillus niger* and *Candida albicans*, and the reason for variation inhibitory effectiveness in extracts is attributable to the variation in the extraction method and solvent type and nature of the membranes of microorganisms (48) , The variation in inhibitory effectiveness of water extracts was attributable for chemical composition of the plant, and its effective compounds and concentration, as well as solubility in water or organic solvents during the extraction process, duration, and timing of plant harvesting and other factors.

Table 7. Effectiveness of cold water extract of thyme and peppermint in the inhibition of the growth of the yeast *Candida* spp

Plant	Yeast	Growth diameter (mm)/Extract conc.(mg/ml)						
		Cont.+	0.0	20	40	60	80	100
Thyme	<i>C.albicans</i>	0.0	44.0	14.5	12.5	10.5	5.0	2.5
	<i>C.tropicalis</i>	0.0	43.0	11.0	10.5	7.0	6.0	5.5
	<i>C.glabrata</i>	0.0	45.0	9.0	8.5	6.0	6.0	5.5
	<i>C.krusie</i>	0.0	44.5	18.0	5.5	5.5	5.5	3.5
Peppermint	<i>C.albicans</i>	0.0	39.0	13.0	8.5	5.5	5.5	3.5
	<i>C.tropicalis</i>	0.0	44.0	15.0	13.0	12.0	7.0	6.0
	<i>C.glabrata</i>	0.0	45.0	13.5	9.5	7.0	4.5	3.5
	<i>C.krusie</i>	0.0	42.0	18.5	8.5	7.5	5.0	4.0
Plant(0.35)	Fungi(0.50)	Conc.(0.66)	LSD P< 0.01					

Cont. + = positive control (anti-fungal Nystatin (2 mg / ml).

The results in table 8 indicate that the yeast *C. glabrata* showed the highest degree of sensitivity to extract from thyme and peppermint at concentration 100 mg / ml with no significant differences (P <0.01) with yeasts *C.albicans* and *C.krusie*, and these results are consistent with that of Omran and Esmailzadeh (46), who mention that the yeasts *C. krusie* and *C.glabrata* showed a higher sensitivity of yeast *C.albicans* toward oily extracts of plants thyme, pennyroyal and Lemon. The results also showed that the inhibitory effectiveness of thyme extracts against some types of yeast *Candida* spp. higher than in extracts of peppermint, and this is consistent with that of Ateeq et al (20) who mention that the extracted crude thyme possesses a higher inhibitory effectiveness against *Candida*, The results also consistent with that of Avijgan et al (21) who mention that the thyme Contains active substances such as essential oil and its excellent efficacy against *Candida*.

Table 8. Effectiveness of thyme and peppermint in concentration 100 mg/ml against *Candida* sp.

Yeast	Diameter(mm)	
	Thyme (100 mg/ml)	Peppermint (100mg/ml)
<i>C.albicans</i>	1.125	2.125
<i>C.tropicalis</i>	2.875	2.875
<i>C.glabrata</i>	1.375	1.250
<i>C.krusie</i>	1.250	2.250
Fungus (0.50)	LSD P<0.01	

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