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RESEARCH ARTICLE

STUDY EFFECT OF PEPPERMINT OIL IN ENHANCING IRRITABLE BOWEL SYNDROME

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ABSTRACT

The goal of the study was to test the biological activities of Peppermint oil (*Mentha spicata* L.) extracted from leaves of Peppermint plant in growth of yeast *Candida albicans* isolated from the feces of 100 patients suffering from symptoms of Irritable bowel syndrome (IBS), and find out the relationship with demographic characteristics. The data and specimen have been collected for the period from 1st November 2011 to the end of February 2012, from Teaching Baghdad Hospital "Laboratory Central", chosen randomly according to the sample specifications from 11 years to 50 years. The study revealed potential inhibitory activity of peppermint oil against yeast, *Candida albicans* in vitro, and showed that the mean of age was (33.5) years, and most of them are women, living in urban. Gas chromatographic analysis revealed the presence of 31 volatile compounds in shoot, two of these compounds, Menthol and Terpinene were identified as terpenoids.

INTRODUCTION

Peppermint (*Mentha spicata* Linn.) is a herbaceous rhizomatous perennial plant belongs to the family Labiatae, is a large, cosmopolitan family of 180 genera and 3500 species, primarily annual or perennial herbs but with some shrubs or climbers, a few are small trees. The mint family is noted for the accumulation of an assortment of mono-, sesqui-, di- and triterpenes, caffeic acid and flavones are also present. Peppermint oil contains over 30 distinct chemical compounds and has been proven safe for consumption both in its plant and essential oil stages in toxicological investigations (WHO 2002, Raudenbush, 2004). Peppermint oil has a long tradition of medicinal use, with archaeological evidence placing its use at least as far back as ten thousand years ago as, the essential oils of peppermint, have been touted as an analgesic, anti-inflammatory, antiseptic, anti-infectious, antimicrobial, antispasmodic, astringent, carminative, digestive, expectorant, febrifuge, fungicidal, vasoconstrictor, decongestant, stimulant, cognitive enhancer, and stomachic (Nelson, 2001). Enteric coated capsules of peppermint oil have been shown to alleviate symptoms of Irritable bowel syndrome (IBS), including cramping, chronic abdominal pain, discomfort, constipation, bloating gas and diarrhea, according to the University of Maryland Medical Center (University of Maryland Medical Center 2014, Robert *et al*., 2001). Irritable bowel syndrome is a chronic functional disorder of the gastrointestinal tract, the causes of IBS are multi factorial; Stress, food allergies and intolerances medication, intestinal dyspepsia, parasites, hormone changes and overgrowth of Yeast and fungal within the colon are the predominant triggers for IBS. Various studies have shown that people with IBS have undiagnosed lactose 68% intolerance, when dairy products and lactose containing foods were eliminated 43.6% had total remission of symptoms and an additional another 40% had moderate improvements. (Lipski, 2010). This study was carried out to test the biological activity of peppermint oil against Monilia in vitro, and found out the relationship between demographic characteristic and irritable bowel syndrome.

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MATERIALS AND METHODS

Extraction of Peppermint oil

Peppermint leaves were collected from Hula river beach before flowering stage, washed in colander with water and remove brown leaves, the oil is extracted through a process called steam distillation , where water in the first chamber is heated using a controlled heat source to about 100 degrees Celsius until it boils and turns to steam and passes into plant chamber containing the fresh Peppermint leaves, "that more plant material we add, the more oil we can produce", then it's releases oil that evaporates with the steam and travels through a tube. As the steam and oil cool back down to liquid in the condensing chamber, the oil separates from the water and forms a thin layer at the top of the water that can be harvested (Johnson, 2014). An experimental study was carried out from 1st November 2011 to the end of February 2012, in order to study the biological activity test of Peppermint oil against Candida.

Yeast samples collection

Candida albicans collected from feces of 100 patients suffer from irritable bowel syndrome symptoms from Teaching Baghdad Hospital "Laboratory Central", chosen randomly according to the sample specifications from 11 years to 50 years, and both sex, then data taken according to questionnaire for this purpose.

Preparation of Monilia culture

Sabouroud Dextrose Agar (SDA), 65g dissolved in 1L, then boiling and sterilized in Autoclave at $115C^0$, 1.51bs, 15 minutes, after it cooled dispense 20 ml in Petri dish (Marwin, 2014).

Antimicrobial bioassay

After culturing Candida samples in blood agar to 24 hours then transport 1-2 colony from isolation colony by swab to the surface of Sabouroud pleat spared the sample in all pleat by carpet culturing method, then pitting the agar as hole in 6 mm in diameter then put 100 µl Peppermint oil in this hole, leaved to setting and then transport to incubator at 37 ^{0}C for 48 hours (Marwin, 2014).

Gas chromatographic analysis of peppermint oil

The experiment was done to diagnose and determine the volatile compounds in peppermint oil by (GC) Gas chromatograph connected with computer type, Shimadzu and record system type, "Pye Unicom AR-SS linear record", then injected (1) µl of peppermint oil in the system under separate condition illustrative in Table 1.

Table 1. Conditions of GC used for separate volatile compounds in peppermint oil

Primary temperature of column t_1	40 ^{0}C
Final temperature of column t_2	200 ^{0}C
Average of rising temperature	5 ^{0}C /mint
Injection temperature	300 ^{0}C
Detector temperature	325 ^{0}C
Carrie Gas	Helium, 25 cm/second
column dimensions	30 m × 0.25 mm
Diatomite	Supelco wax 10
Attenuation	0.01 ppm
Speed of record paper	10 mm/mint
Type of detector	(FID)Flame ionization detector
Size of material injection (sample)	1 µl

RESULTS

Biological activities

Peppermint oil significantly reduced growth of yeast *Candida albicans* isolated from the feces of 100 patients suffering from symptoms of Irritable bowel syndrome (Table 1). However the degree of reduction was different among the test yeast. The big inhibition zoon ranged (40-46)mm., (Figure 2) which have 47% of control while the small was 6mm, and the distribution of the matched symptoms indicates that the majority of the groups suffer from diarrhea 40%. Therefore there was significant association between inhibition zoon, symptoms and irritable bowel syndrome.

Table 1. Effect of Peppermint oil on *Candida albicans* by inhibition zoon and symptoms of Irritable bowel syndrome

No.	Inhibition zoon		Frequency	Percentage
1-	40-46		47	47
2-	47-52		23	23
3-	53-58		24	24
4-	59-64		6	6
	Total		100	100.0
X^2 obs. = 34.480	df = 3	X^2 crit. = 9.487	P < 0.050	
No.	Symptoms		F	%
1-	Diarrhea		40	40
2-	Constipation		25	25
3-	Dyspepsia		21	21
4-	Fatigue & weakness		14	14
	Total		100	100.0
X^2 obs. = 14.480	df = 3	X^2 crit. = 9.487	P < 0.050	

Demographic characteristic

The study shows that there was significant association between age, gender, residential area, and irritable bowel syndrome (Table 2). The ages that more affected ranged (31-40) years old who are 35% of the groups, and indicated the most of them are female were 65%, living at urban areas who were 88%. That revealed there was significant association between demographic data include (age, gender, residential area) and irritable bowel syndrome.

Table 2. Distribution of the study samples by their demographic characteristics age, gender, and residential area

No.	Age		Frequency	Percentage
1-	11-20		15	15
2-	21-30		20	20
3-	31-40		35	35
4-	41-50		30	30
	Total		100	100.0
X^2 obs. = 10.000	df = 3	X^2 crit. = 9.487	P < 0.050	

No.	Gender		F	%
1-	male		35	35
2-	Female		65	65
	Total		100	100.0
X^2 obs. = 9.000	df = 1	X^2 crit. = 3.841	P < 0.050	
No.	Residential area		F	%
1-	Urban		88	88
2-	Rural		12	12
	Total		100	100.0
X^2 obs. = 57.760	df = 1	X^2 crit. = 3.841	P < 0.050	

* The mean of age is (33.5).

Pearson correlation

The experement shwos that there was a stronge significant between age, gender, Residential area, Symptoms, Inhibition zoon and irritable bowel syndrome, 0.75, 0.43, 0.86 and 0.84 respectively, (Table 3) and gender have stronge significant with Residential area, Symptoms and Inhibition zoon, 0.50, 0.89 and 0.86 respectively, while Residential area have stronge significant with Symptoms and Inhibition zoon, 0.65 and 0.61 respectively, but Inhibition zoon have very stronge significant with Symptoms only, 0.92.

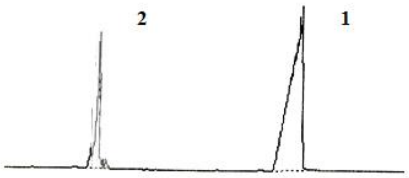
Table 3. Pearson correlation between age, gender, residential area, symptoms, inhibition zoon and irritable bowel syndrome

Variable		Gender	Residential area	Symptoms	Inhibition zoon
Age	Co.	.753**	430**	.863**	.840**
	Sig.	C1 .001	C2 .000	C3 .000	C4 .001
	N.	100	100	100	100
gender	Co.	_____	.503**	.892**	.863**
	Sig.		C5 .000	C6 .000	C7 .347
	N.		100	100	100
Residential area	Co.			.654**	.614**
	Sig.			C8 .000	C9 .000
	N.			100	100
Symptoms	Co.				.929**
	Sig.				C10 .000
	N.				100

C = Cell Co. = Correlation coefficient. Sig. = Significant (2- tailed). N. = Number of sample.
This table shows that the relationship in the all of the cells.
(C1 It means relationship between age and gender, C6 It means relationship between gender and symptoms …etc.).
[**. Correlation is significant at the 0.01 level (2-tailed)]

GC analyses

The results of Gas chromatographic analyses revealed the presence of 31 volatile compound in shoot of peppermint *Mentha spicata* two of these compounds were identified as terpenoids (Figure 1) but the others don't identified because we can't found the standard compounds.



1. Menthol and 2. Terpinene

Figure 1. GC analyses for volatile compounds of peppermint oil

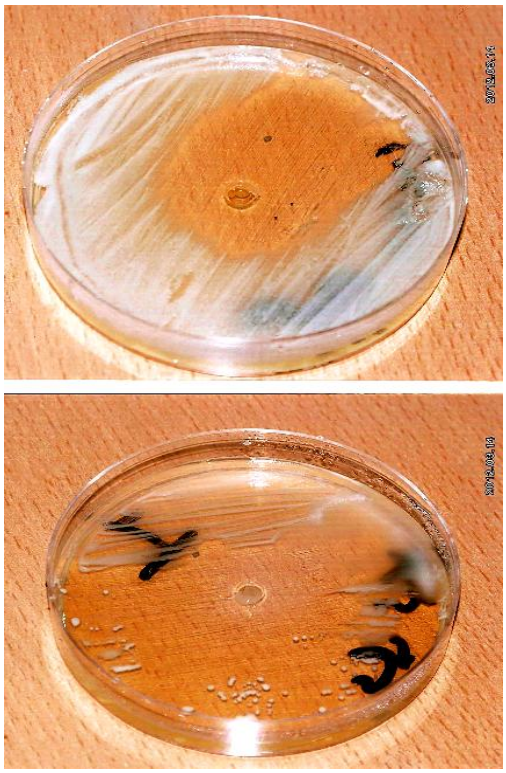


Figure 2. Effect of Peppermint oil on *Candida albicans* (Inhibition zone)

DISCUSSION

The inhibitory effects of chemical compounds released from peppermint oil by volatilization in growth of test yeast suggest that the volatile compounds contain about half of the oil is menthol, these include a variety of the compounds known as monoterpenes and the class of chemicals called sesquiterpenes (University of Maryland Medical Center, 2014, Agarwal *et al*, 2010). Menthol found is attributed to giving peppermint oil their medicinal properties of reducing growth of yeast (Ashley, 2011). The growth of yeast and fungal where found naturally within the colon in tolerable amounts like, *Candida albicans* come from ate pastry, undigested food and within the intestinal tract can provide nourishment and a home for yeast. Furthermore, the fermentation process, warm, moist colon walls and unbalanced pH environment can increase their growth. This overgrowth can lead to IBS (Candida, 2014). Other studies indicate to that Candidiasis, normal organisms which found in the human gut has proliferated because ongoing use of antibiotics. Use of this antibiotics predisposes to overgrowth Candida and destroy many of the beneficial bacteria in the gut, consequently, Candida able to proliferate since it is not kept in check by normal gut flora. Overgrowth of opportunistic bacterial pathogens also occurs in gut disease, like, proteus, streptococcus and staphylococcus are thought to contribute to IBS symptoms (Sarao, 2013).

Research studies have also been suggested that the compounds in the peppermint oil able to relax intestinal wall and sphincter smooth muscles through blocking calcium channel at cell receptor levels. This property of peppermint has been applied as an anti-spasmodic agent in the treatment of irritable bowel syndrome and other colic pain disorders (Nicole, 2013). Enteric coated capsules of peppermint oil have been shown to alleviate symptoms of Irritable bowel syndrome, including cramping, chronic abdominal pain, discomfort, constipation, bloating gas and diarrhea, according to the University of Maryland (Drugs.com 2014). As many as 20 percent of the adult population, have symptoms of IBS, making it one of the most common disorders diagnosed by doctors. It occurs more often in women than in men, that may be Candidiasis of the gut is commonly found in women who have a history of vaginal which make it easy to transition to anus, and it begins

before the age of 35 in about 50 percent of people (WHO 2010).

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