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Effect of *Mentha piperita* essential oil against *Vibrio* spp. isolated from local cheeses

Shayma Thyab Gddoa Al-Sahlany*

Department of Food Science, Agriculture College, Basrah University, Basra City, Iraq

***Corresponding Author:** alsahlany.shayma@gmail.com

ABSTRACT

The aim of this study, *Vibrio* spp. was detecting in cheese manufacture and effect of essential oil of *Mentha piperita* on this bacteria. A 126 isolates of *Vibrio* spp. were isolated from 30 samples of two types of local cheeses. The samples were collected from 14 markets in Basrah city. 8 species from *Vibrio* genes was obtained and defined by microscopic and biochemical tests. *Vibrio parahaemclyticus* and *Vibrio cholera* were the highest percentage among other isolates. It was 33% and 25 % respectively. Essential oil of *Mentha piperita* was extracted from leaves. It was 2% (v:w) which used for *Vibrio* spp. isolates inhibition. *Vibrio logei* was most sensitive against 15 µl of Mentha *piperita* essential oil. The MIC of *Vibrio* spp. was.0035 ml excepted *V. cholera* was 0.0041 ml and V. *harveyi*, *V. logei* were 0.0027 mL.

Key word: *Vibrio* spp., *Mentha piperita*, essential oil, local cheese.

INTRODUCTION

Vibrio genus is belongs to Vibrionaceae family. *Vibrio* are a genus of Gram-negative bacteria, possessing a curved-rod shape (comma shape), facultative anaerobes that test positive for oxidase and do not form spores. Several species of which can cause foodborne infection, usually associated with eating undercooked seafood. Typically found in salt water (Machado & Gram, 2015).

The *Vibrio* spp. isolation from Egyptian soft Domiati cheese which content 5.4- 9.5% NaCl and this bacteria was identified by molecular methods (El-Baradei *et al*., 2007). Fourteen different species included *Vibrio* spp. were isolated from surface four cheeses (Mounier *et al*., 2005). Forty types of food samples from markets of Dhaka city including meat, fish, vegetables, fruits, street food, bakery shop food, fast food, sweets and dairy products. They were used *Vibrio* spp. isolation (Mrityunjoy *et al*., 2013).

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing (Baser & Buchbauer, 2010).

Essential oils have antimicrobial and antioxidant activity which used in medical, pharmacy and food keeping (Pirbalouti *et al*., 2013; Zengin & Baysal, 2014; Niamah & Alali, 2016). The Essential oil extract of *Mentha* species used as antimicrobial, antioxidant and antimutagenic (Mimica-Dukić *et al*., 2010; Mickiene *et al*., 2011). This essential oil content more bioactive compounds. The Linalool is major compound found in essential oil of Mentha (Silva *et al*., 2015). The aim of this study was to isolate *Vibrio* species from local cheeses found in market of Basrah city/ Iraq and study the effect of these essential oils from Iraqi *Mentha piperita* plant on this bacteria

MATERIAL AND METHODS

Cheese sampling

Two types' local cheeses were collected from 13 markets of Basrah city. The samples divided into two groups. One group was included 17 samples of white soft Iraqi cheese, another group was included 13 samples of braids cheese. 50 grams of cheese samples were transferred into biotechnology Lab./ Agriculture college / Basrah university, under sterile conditions.

Bacterial isolation

Eleven grams of cheese sample transferred to conical flask contents 99 ml of alkaline peptone water at pH 8.6 and incubated at 37℃ for 6 hours (Lesmana *et al*., 1985), thereafter 1ml of last dilations transferred to petri dish, poured Thiosulfate citrate bile salt agar (TCBS) media (LAB company, UK.) and incubated at 37℃ for 24-48 hours (Barrow & Feltham, 2003).

Vibrio spp. identification

All isolates were identified to be *Vibrio* spp. depending on microscopic examinations and

biochemical tests which included gram staining, spore forming, motility, oxidase test, Voges-Proskauer test, growth without NaCl, growth with (1, 3, 6, 12)% NaCl, myo-inositol, D-mannitol, L-arabinose, cellabiose and sucrose fermentation, ammonia production from arginine, acid and gas production from glucose, nitrate reduction, indole and citrate utilization (Holt, 1994).

Extraction of *Mentha piperita* essential oil

Essential oil was extracted from *Mentha piperita* leaves using Clevenger apparatus. 250 g of leaves mixed with 500 ml of distilled water was transferred into oil distillation for 1-3 hours at 95℃. The essential oil was then collected and determined by calibrated tube. It was kept in the freezer (Niamah & Alali, 2016).

Antibacterial activity essay

The antibacterial activity of essential oil extract from *Mentha piperita* leaves was determined by Agar diffusion method. 1 ml of *Vibiro* spp. was streaked by L- shape on Mueller-Hinton agar (Hi-media, India) and worked on 3 wells (6 mm) in agar. 5, 10 and 15 µl of essential oil extract were transferred to wells and Petri dishes kept in the refrigerator for 2 hours and incubated at 37℃ for 24-48 hours, effective inhibitory was estimated by measuring diameters of clear zones (Valgas *et al*., 2007).

Determination of minimal inhibitory concentration (MIC)

The MIC of essential oil extract from *Mentha piperita* leaves was determined by (Mann & Markham, 1998). The essential oil of *Mentha piperita* was added into molten Iso- sensitest agar (Oxoid, UK) with 0·25% (v/v) Tween 20 at 45-50℃. The range of essential oil concentrations was from 0.001 ml to 0.005 ml (v/v) %. 0.1 ml (10^6-10^8 cfu/ mL) of Vibrio spp. transferred plates and incubated at 37°C for 18-24 hours. The MIC was determined as the lowest concentration of oil to result in no growth of *Vibrio* spp. bacteria.

RESULTS AND DISCUSSION

Bacteria isolation

Vibrio spp. were found in all samples except four samples from braids cheese. The numbers of *Vibrio* spp. were high in white cheese than with braids cheese because of the braids cheese was produced by acidic method (Abd El Razig *et al*., 2002) and *Vibrio* spp. growth was weak in acidic media and the starter cheese do on anther bacteria inhibition (Widyastuti *et al*., 2014). The starter no add into with soft Iraqi cheese (Hanna & Nader, 1996). *Vibrio* spp. transferred to cheeses by way washing water, which is used after the industry and during the sales process.

Identification of *Vibrio spp.*

A 126 isolates from 152 isolates were selective after microscopic tests. Green colonies and yellow colonies were selected from TCBS cultures. The isolates were curved or straight form, Gram staining, non-spore forming and motile. The biochemical tests shown in table 2. 33(21.71%) isolates as *V. parahaemclyticus*, 25 (16.44%) isolates as *V. cholera*, 15 (09.86%) isolates as *V. vulnificus*, 12 (07.89%) isolates as *V. alginolyticus*, 12 (07.89%) isolates as *V. mimicus*, 11(07.23%) isolates as *V. damsela*,8 (05.26%) isolates as *V. campbellii*, 6 (03.94%) isolates as *V. harveyi*, 4 (02.63%) isolates as *V. logei* and 26 (17.10%) non *Vibrio* isolates (Farmer & Hickman-Brenner, 2006). The colony appearance on selective media was followed by conventional biochemical tests, for detection of *Vibrio* spp isolates. The phenotypic similarities of the eight species observed in the results of biochemical tests (Noguerola & Blanch, 2007). Carbohydrates fermentation and growth with NaCl were important tests to differentiate of Vibrio spp (Paydar, 2013). Asserts that the tests which have been applied in this study were able to efficiently differentiate these species. Thus, for detection of the species of the isolates, the conventional biochemical tests showed good method. However, the overall findings of these tests indicated that they are able to be used for detection of *Vibrio* spp.

Bacteria inhibition

The yield of *Mentha piperita* essential oil was 2 % (v:w). The table 3 show effect of essential oil extract from *Mentha piperita* leaves against *Vibrio* spp. isolates from cheese samples. All isolates were inhibited by essential oil and inhibition zones were different between *Vibrio* spp. isolates when increased concentration essential oil of *Mentha piperita* led increase diameters of inhibition. *V. harveyi* and *V. logei* were larger inhibition among anther isolates. The inhibition zones of this bacteria were (19.29 and 20.33) mm at 15 µl of *Mentha piperita* essential oils. The MIC was 0.0035 ml of isolates excepted *V. cholera* was 0.0041 ml and V. *harveyi*, *V. logei* were 0.0027 mL. The essential oil of *Mentha piperita* was contented more compounds as inhibitors of G^+ and G^- bacteria (Soković *et al*., 2010; Mahboubi & Kazempour, 2014). It don't have selective antimicrobial activity. The antimicrobial activity of

Table 1. Numbers of *Vibrio* spp. isolated from local cheese samples

Sample	Type of cheese	Name of the sampling site	Count (CFU/ g) of *Vibrio* spp. on TCBS
1	white soft	Old Basra	3×10^4
2	braids	Old Basra	33×10^2
3	white soft	Ashar	96×10^4
4	white soft	Ashar	42×10^5
5	white soft	Ashar	1×10^5
6	braids	Ashar	22×10^2
7	braids	Al-Qibla	77×10^3
8	white soft	Abil Khaseeb	95×10^4
9	white soft	Abil Khaseeb	44×10^4
10	white soft	Al Jumhouriya	52×10^4
11	white soft	Al Jumhouriya	99×10^3
12	white soft	Hay Alhussain	72×10^4
13	white soft	Hay Alhussain	66×10^4
14	braids	Al Hartha	45×10^2
15	braids	Al Hartha	56×10^2
16	white soft	Al Hartha	33×10^5
17	white soft	Hitteen	31×10^5
18	braids	Hitteen	67×10^2
19	white soft	Al Madeena	93×10^4
20	white soft	Al Madeena	25×10^5
21	braids	Al Madeena	Nail
22	braids	Al Madeena	Nail
23	braids	Al Nashwa	Nail
24	white soft	Al Zubair	26×10^5
25	braids	Al Zubair	1×10^2
26	braids	Al Zubair	53×10^2
27	white soft	Al Zubair	1×10^5
28	white soft	Um Qasr	55×10^4
29	braids	Al Meethag	32×10^3
30	braids	Al Ez	Nail

Table 2. Microscopic and biochemical tests of *Vibrio* spp. isolates

	Test	*V. parahaemclyticus* (n=33)	*V. cholera* (n=25)	*V. vulnificus* (n=15)	*V. alginolyticus* (n=12)	*V. mimicus* (n=12)	*V. damsela* (n=11)	*V. campbellii* (n=8)	*V. harveyi* (n=6)	*V. logei* (n=4)
	TCBS agar	G	Y	G	Y	G	G	G	Y	G
	Gram staining	-	-	-	-	-	-	-	-	-
	Spore forming	-	-	-	-	-	-	-	-	-
	Motility	+	+	+	+	+	+	+	+	+
	Oxidase	+	+	+	+	+	+	+	+	+
	Voges-Proskauer	-	±	-	+	-	+	-	-	-
Growth in	0% NaCl	-	-	-	-	+	-	-	-	-
Growth in	1% NaCl	+	+	+	+	+	+	+	+	-
Growth in	3% NaCl	+	+	+	+	+	+	+	+	+
Growth in	6% NaCl	+	-	+	+	-	+	+	+	-
Growth in	12% NaCl	-	-	-	+	-	-	-	-	-
Fermentation	Myo-inositol	-	-	-	-	-	-	-	-	-
Fermentation	D-mannitol	+	+	+	+	+	-	+	+	+
Fermentation	L-arabinose	+	-	-	-	-	-	-	-	-
Fermentation	Cellabiose	+	-	+	+	+	+	+	+	+
Fermentation	Sucrose	-	+	-	+	-	-	-	+	-
	Arginine dehydratase	-	-	-	-	-	+	-	-	-
	Gas from glucose	-	-	-	-	-	+	-	-	-
	Acid from glucose	+	+	+	+	+	+	+	+	+
	Nitrate reduction	+	+	+	+	+	+	+	+	+
	Indole	+	+	+	+	+	-	+	+	-
	Citrate utilization	-	+	±	-	+	-	-	-	-

*Symbols: n= N0. of isolates

G green**, Y** yellow**, +** positive**, -** negative**, ±** 50-70% positive

Table 3. Inhibition zones (mm) by concentrations and MIC of *Mentha piperita* essential oils

Vibrio spp. isolates	Concentrations of essential oils *Mentha piperita*			MIC (mL)
	5 µL	10 µL	15 µL	
V. parahaemclyticus (n=33)	11.55±0.30	15.13±0.43	18.20±0.36	0.0035
V. cholera (n=25)	12.18±0.25	14.77±0.48	17.08±0.22	0.0041
V. vulnificus (n=15)	11.85±0.66	16.19±0.79	18.20±0.15	0.0035
V. alginolyticus (n=12)	12.00±0.55	14.11±0.33	17.86±0.75	0.0035
V. mimicus (n=12)	12.56±0.90	15.88±0.44	18.26±0.56	0.0035
V. damsela (n=11)	11.11±0.35	14.63±0.22	17.20±0.44	0.0035
V. campbellii (n=8)	12.08±0.40	14.77±0.66	18.75±0.33	0.0035
V. harveyi (n=6)	13.54±0.21	18.13±0.49	19.29±0.56	0.0027
V. logei (n=4)	13.95±0.11	16.00±0.63	20.33±0.61	0.0027

*__Symbols: n=__numbers of isolates; each value is expressed as mean ± SD (n = 3)

essential oil of *Mentha piperita* came back to found monoterpene hydrocarbons compounds. Although these compounds are not abundant in the essential oil, and it was important activity. It is necessary to indicate that the other compounds can contribute to the improvement of this activity (Mkaddem *et al*., 2009). Many researches were reported sensitive of *Vibrio* spp. against essential oil of *Mentha piperita* (Yano *et al*., 2006; Snoussi *et al*., 2015).

CONCLUSION

All species of *Vibrio* isolation are pathogenic bacteria except *V. campbellii*, *V. harveyi* and *V. logei*. This bacteria found in two type local chesses. It transfers into cheese by water washing during the industry, storage and sales process. The cheeses sour by starters cultures bacteria content low numbers of *Vibrio* spp. The essential oil extract from *Mentha piperita* leaves have antibacterial activity against all *Vibrio* spp. isolation from chesses samples. Add essential oil of *Mentha piperita* to cheeses production is reducing the viability cells of microbes and inhibiting some species.

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