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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

Abstract: 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. Eight (8) species from *Vibrio* genes was defined by microscopic and biochemical tests. *Vibrio parahaemclyticus* and *Vibrio cholera* were the highest percentage of isolates. It was 33% and 25 %. Essential oil of *Mentha piperita* was extracted from leaves plant was 2% (v:w) which used for isolating inhibition. *Vibrio logei* was most sensitive against 15μl of *Mentha piperita* essential oil. 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 is belongs to Vibrionaceae family. *Vibrio* species 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 [1].

Vibrio spp. isolation from Egyptian soft Domiati cheese which content 5.4- 9.5% NaCl and these bacteria were identified by molecular methods [2]. Fourteen different species included *Vibrio* spp. were isolated from surface four cheeses [3].

Forty types offood 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 [4].

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 [5].

Essential oils have antimicrobial and antioxidant activity which used in medical, pharmacy and food keeping [6-8]. The Essential oil extract of *Mentha* species used as antimicrobial, antioxidant and antimutagenic [9, 10]. This essential oil content more bioactive compounds. The Linalool is major compound found in essential oil of *Mentha* [11]. The aim study, *Vibrio* spp. isolation from local cheeses which found in market of Basrah city/ Iraq and study effect of essential oil extraction from Iraqi *Mentha piperita* plant on this bacteria.

MATERIALS AND METHODS

Cheese Samples: Two types of local cheeses were collected from 13 markets of Basrah city. The samples were divided into two groups. One group was included 17 samples of white soft Iraqi cheese, another group was included 13 samples of braids cheese. Fifty (50) grams of cheese samples were transfer into Biotechnology Lab./ Agriculture College / Basrah University, under sterile conditions.

Bacteria Isolation: Eleven (11) grams of cheese sample transfer to conical flask contents 99 ml of alkaline peptone water at pH 8.6 and incubated at 37°C for 6 hours [12], thereafter 1ml of last dilations transfer to Petri dish, pour Thiosulfate citrate bile salt agar TCBS media (LAB company, UK.) and incubated at 37°C for 24-48 hours [13].

Corresponding Author: Shayma Thyab Gddoa Al-Sahlany, Department of Food Science, Agriculture College, Basrah University, Basra City , Iraq.

***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 [14].

Extraction of *Mentha Piperita* Essential Oil: Essential oil was extracted from *Mentha piperita* leaves using Clevenger apparatus. 250 g of leaves with 500 ml of distilled water was transferred into oil distillation for 1-3 hours at 95°C. The essential oil was then collected and determined by calibratedtube. It was kept in the freezer [8].

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 workedon 3 walls (6 mm) in agar. 5, 10 and 15µl of essential oil extract were transferred to walls and Petri dishes kept in the refrigerator for 2 hours and incubated at37 ?C for 24-48 hours, effective inhibitory was estimated by measuring diameters of clear zones [15].

Determination of Minimal Inhibitory Concentration (MIC): The minimal inhibitory concentration (MIC) of essential oil extract from *Mentha piperita* leaves was determined by Mann and Markham, [16].

RESULTS AND DISCUSSION

Bacteria Isolation: *Vibrio* spp. was 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 acidity method [17] and *Vibrio* spp. growth was week in acidity media and the starter cheese do on anther bacteria inhibition [18]. The starter no add into with soft Iraqi cheese [19]. *Vibrio* spp. transfer 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. The isolates were curved or straight form,G ?, 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 [20].

Table 1: The 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	AbilKhaseeb	95×10^4
9	White	AbilKhaseeb	44×10^4
10	White	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

Colony appearance on selective media was followed by conventional biochemical tests, for detection of *Vibrio* spp.The phenotypic similarities of the eight species observed in the results of biochemical tests [20].

Bacteria Inhibition: The yield of *Mentha piperita* essential oil was 2 % (v:w). Data presented in Table 3 show the 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 to increase diameters of inhibition. *V. harveyi* and *V. logei* were larger inhibitions among

Table 2: The 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	-	-	-	-	+	-	-	-	-
	1% NaCl	+	+	+	+	+	+	+	+	-
	3% NaCl	+	+	+	+	+	+	+	+	+
	6% NaCl	+	-	+	+	-	+	+	+	-
	12% NaCl	-	-	-	+	-	-	-	-	-
Fermentation	Myo-inositol	-	-	-	-	-	-	-	-	-
	D-mannitol	+	+	+	+	+	-	+	+	+
	L-arabinose	+	-	-	-	-	-	-	-	-
	Cellabiose	+	-	+	+	+	+	+	+	+
	Sucrose	-	+	-	+	-	-	-	+	-
	Arginine dehydratase	-	-	-	-	-	+	-	-	-
	Gas from glucose	-	-	-	-	-	+	-	-	-
	Acid from glucose	+	+	+	+	+	+	+	+	+
	Nitrate reduction	+	+	+	+	+	+	+	+	+
	Indole	+	+	+	+	+	-	+	+	-
	Citrate utilization	-	+	±	-	+	-	-	-	-

*Symbols: n numbers of isolates, G green, Y yellow, + positive, - negative, ± 50-70% positive

Table 3: Diameters of inhibition zones (mm) by concentrations and MIC of *Mentha piperita* essential oils

	Concentrations of essential oils *Mentha piperita*			
Vibrio spp. isolates	5 µl	10 µl	15 µl	MIC (mL)
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

anther isolates. The inhibition zones of these bacteria were (19.29 and 20.33) mm at 15 µl of *Mentha piperita* essential oils. The MIC was 0.0035 mL of All isolates excepted *V. cholera* was 0.0041 mL and *V. harveyi*, *V. logei* were 0.0027 ml.

The essential oil of *Mentha piperita* was more compounds as inhibitors of G+ and G ? bacteria [22, 23]. It does not have selective antimicrobial activity. The antimicrobial activityof 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 theother compounds can contribute to the improvement of this activity [24]. Many researches were reported sensitive of *Vibrio* spp. against essential oil of *Mentha piperita* [25, 26].

CONCLUSION

Some species of *Vibrio* are pathogenic bacteria and anther species are spoilage food. It found in water and many types of food such as fish, sea food, salad and dairy products. It transfer into cheese by water washing during the industry, storage and sales process. The cheeses added starterscultures bacteria content low numbers of *Vibrio* spp. The essential oil extract from *Mentha piperita* leaves have antibacterial activity against all *Vibrio* spp. isolation from two types of local cheese.

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