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Full Length Research Paper

Assessment of antibacterial virtues of *Teucrium capitatum* (Ja'da) on pathogenic bacteria (From Folk to Complementary Medicine)

Bilal Ahmad Ghareeb* and Doha Hisham Weld-Ali

Department of Biology and Biotechnology, Arab American University-Jenin (AAUJ)
P. O. Box 240 Jenin, Palestine.

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There are more than 200 species of *Teucrium* including *Teucrium capitatum* which is used by Palestinians and Jordanians as a folk medicine. *Teucrium capitatum* is extracted here by original procedures inspired uniquely from the old Palestinian and Jordanian traditions (juice and infusion). These techniques combine the virtues of the plant with an appropriate osmotic pressure. The aim of this study is to assess for the first time the antibacterial capacity of *T. capitatum* on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermis*. Of special interest, bacteria caused colic and gastric infection like *E. coli* and *P. aeruginosa*. This application copes up with the traditional use of *T. capitatum* against colic and intestinal infections. Results show antibacterial virtue in *T. capitatum*. The juice method is more efficient against pathogens than the infusion method. The bacterial species mostly inhibited by *T. capitatum* is *P. aeruginosa* (inhibition of 58% and 19% of growth at 16.5 mg of fresh Ja'da/ml of bacterial medium extracted by juice and infusion methods respectively). *S. epidermis* is inhibited by 50% and 42% at the same dose for both methods of extraction respectively. Similar results are obtained when applying the same dose on *E. coli* (growth inhibition is 43% and 36% for both methods respectively). *T. capitatum* also inhibited 23% and 17% of the growth of *S. aureus* when applied at the same dose for the two methods respectively. Findings here are consistent with the traditional use of *T. capitatum* as a treatment for inflammatory bowel disease caused by bacterial infections. This plant can, therefore, be recommended for further medical assessment for the appropriate dose(s) and appropriate target pathogenic bacterial strains in addition to the potential side effects notably on liver.

Key words: *Teucrium capitatum*, pathogenic bacteria, colic, gastroenteritis, medicinal plants, Arab and Palestinian folk medicine.

INTRODUCTION

Palestinian and Jordanian historic heritage as a part of the Arabic culture harbor a reservoir of traditions of use of medicinal plants as folk medicines. It is estimated that 80% of humans depend on traditional medicine for their primary health care (Gurib-Fakim and Mahomoodally, 2013).

The whole above ground parts of *Teucrium capitatum* (Ja'da) are used in traditional Palestinian folk medicine especially in Palestinian bank areas and Al-Nakab desert, as well as Jordanian and Persian medicine to fight against colic and diarrhea. It is used as herbal tea also in some Mediterranean cultures (Shebaro, 1997). These traditions should not be underestimated as they emerge

*Corresponding author. E-mail: bilal.ghareeb@aauj.edu, ghareebbilal@gmail.com. Tel: + 972 4 241 8888 Ext. 1144, + 972 4 2520 030, + 972 569 727 717; Fax: + 972 4 2510 810.

from thousands of years of empirical experience. However, the traditional and popular reports should be assessed in a scientific context and this is the goal of this research. *T. capitatum* is assayed in this research for its antibacterial virtues against bacterial infections causing colic and gastric problems as well as urinary and skin infections.

T. capitatum is known popularly as Cat Thyme or (Ja'da). Its flowers are small and range from pink to white (Figure 1). Leaves grow in September, October, and November; the flowers appear in April, May, June, July, and August (Ishtayeh, 2008). Leaves appear opposite, entire, dentate, and color of the flower is cream and white. They are captured from the mountain of Akkaba – Jenin area (Palestine). *T. capitatum* is a perennial, pubescent, aromatic plant, 20-50 cm high, with green to greyish leaves and white flowers. It grows wild in southern Europe, central and south-west Asia and North Africa. This plant is scarcely found in continental France. In the world (as mentioned above), there are more than 200 types of *Teucrium*, for example *Teucrium ajugaceum*, *Teucrium botrys*, *Teucrium chamaedrys*, *Teucrium polium*, *Teucrium capitatum* (under study in this research) and others (Mostefa-Kara, 1992; Dao, 1993).

T. capitatum is reported to contain many important alkaloids known as cetkaderan as well as saastron, carbohydrate (glucose, fructose, sucrose, ramnoz, raffinose), sterolat and turbines, flavone, gelokozydat, flavonoids, and volatile oil. More than 45 compounds are described including the compound responsible for the well known bitter taste of Ja'da (pkrobolin) (Al-Qahtani, 2005).

Studies report that the oil isolated from *T. capitatum* grown in Portugal was characterized by a high content of oxygenated monoterpenes (33.0%), isomenthone (7.7%) being the major constituent. Another oil from a population collected from the same region was dominated by monoterpenes and sesquiterpene hydrocarbons (43.9% and 23.2%, respectively), α-pinene (7.7%), sabinene (11.2%) and β-pinene (10.3%) being the main compounds. The oils from other three populations were characterized by a high content of both sesquiterpene hydrocarbons (23.0%, 32.2% and 33.2%) and oxygenated sesquiterpene (39.7%, 23.4% and 20.4%). T-cadinol (24.1%) and α-cadinol (9.8%) were the major compounds in the oil (José et al., 2004; Calzada et al, 2006; Mo Tutin and Wood, 1972; Maranta, 1981).

This study assesses the antibacterial virtues of *Teucrium capitatum* (Ja'da) on selected bacterial species of relevant importance (*E. coli*, *Staphylococcus epidermis, Staphylococcus aureus* and *Pseudomonas aeruginosa).* This study accompanies assaying *Teucrium capitatum* on both G +ve (e.g. *S. Aureus*) and G–ve bacteria (e.g. *E. coli*) (Madigan and Martinko, 2005). Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibiotics especially those related to pensillin, because of their impenetrable wall (Gladwin, 2007). The pathogenic capability of Gram-negative bacteria is often associated with certain components of Gram-ve cell envelope, in particular, the lipopolysaccharide layer (also known as LPS or endotoxin layer) (Desvaux et al., 2009).

MATERIALS AND METHODS

Bacteria

All bacteria species were obtained from Government Hospital – Jenin. They include *E. coli* pathogenic, *S. epidermis*, *S. aureus and P. aeruinosa*. They are grown on appropriate media prepared kindly by Mr. Saied Al-Khaseeb (Biology and Biotechnology Department, Arab Amarecan University, Jenin).

Teucrium capitatum was kindly provided by friends from Akkaba village on the 13th of November, 2012, and kept in a freezer at -20°C after weighting.

Plant extracts

T. capitatum was extracted in NaCl solution (0.9%) using two methods:

(1) Juice was made by crushing or maceration with gloved hands for 3 min (in Arabic mars) and applied on the above ground parts (leaves and stems). This was accomplished after washing of the plant and letting it to dry for 1 h. The extract was collected and filtered using sterile micro filter (0.2 µm) and kept in freezer till application on bacteria.

(2) Infusion method was done by putting the above-ground parts of the plant in boiled 0.9% NaCl solution and set for infusion till the extract attains room temerature (5 h), and then the infusion extract was filtered using 0.2 µm and freezed till application on cells.

Bacterial medium

“Nutrient Agar” is the most popular agar media in the market today. It is used to culture a wide range of bacteria, molds, and yeasts, though it is not too good for fungus. This medium contains agar, casein and tryptone. The nutrient broth plates are by far the most convenient way to culture bacteria and molds (Feng et al., 2002; Fisher et al., 2007). It contains 9 g NaCl/L which is added to preserve the osmotic pressure of the medium so that any antibacterial effect will be attributed to the plant rather than to a disturbed osmocity. The medium was autoclaved for 45 min and cooled to 50°C, after which it was poured on plates (Petri dishes) (Dorland, 2012).

A series of dilutions and volumes were applied on bacteria as demonstarted in Table 1 and Figures 2 to 5. The used doses of *T. capitatum* extract were 3.5, 8, 12

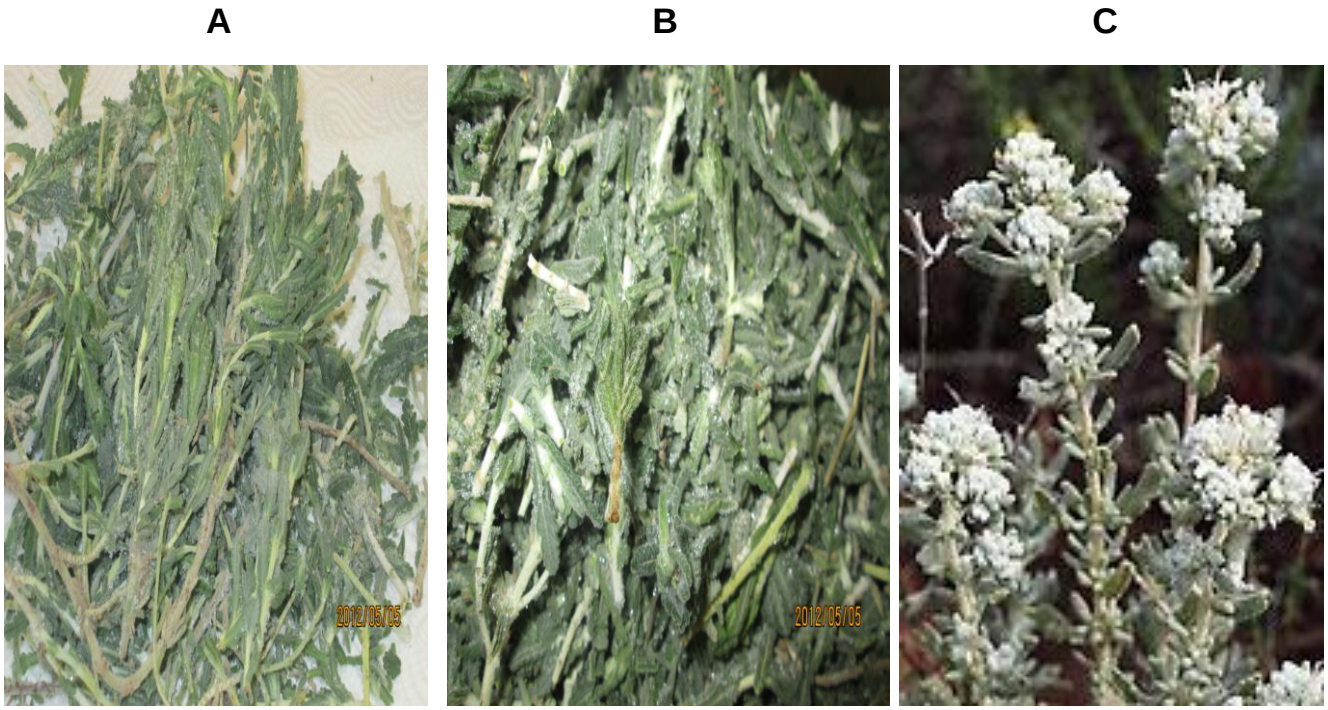


Figure 1. Leaves and flowers of *Teucrium capitatum*.

and 16.5 mg of fresh Ja'da/ml of bacterial medium. About 80 µl of an overnight bacterial culture were added to agar medium plates and let on 37°C for an overnight culturing then bacteria were counted to assess the effect.

We used 3 controls, 2 negative controls (bacteria in the medium without any addition of *Teucrium capitatum*), and bacteria (80 µl of an overnight culture) in the medium with lettuce, but without *T. capitatum*. The positive control consists of Ampicillin Antibiotic in bacterial medium (1%) and bacteria (80 µl), without *T. capitatum.* We used 3 replicates for each volume, and 80 µl of an overnight culture. *S. epidermis* showed a slow growth pattern and was therefore allowed to grow for 2-overnight period at 37°C. Colony-forming unit (CFU), introduced early in microbiology (Breed et al., 1916). (estimate of viable bacterial or fungal numbers) was blotted against a range of doses of *Teucrium capiatatum*.

RESULTS AND DISCUSSION

Table 1 depicts the number of colonies of the four pathogenic species of bacteria upon applying different doses of *T. capitatum* compared to appropriate controls (bacteria without *T. capitatum*, bacteria with ampicillin and lettuce). In addition, the two extraction procedures are contrasted here (the maceration or crushing of juice method versus the soaking or infusion method). Assays were done thrice and the average is shown.

Effect of *T. capitatum* on *P. aeruginosa*

Pseudomonas aeruginosa: It has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia (Klaus-Dieter, 2012).

Pseudomonas aeruginosa is a gram-negative that belongs to the family Pseudomonadaceae. It produces the blue green pigment pyocyanin. The effect of *T. capitatum* on *P. aeruginosa* is demonstrated in Figure 2.

Clearly at the dose of 16.5 mg of fresh Ja'da/ml of bacterial medium, *T. capitatum* shows an antibacterial effect as compared with the controls. Lettuce did not reduce the growth of *P. aeruginosa*. When added at 3.5 and 8 mg of fresh Ja'da/ml of bacterial medium, the growth of *P. aeruginosa* was inhibited by 10-23%. At 16.5 mg of fresh Ja'da/ml of bacterial medium, the growth of *P. aeruginosa* was inhibited by 58%. However, surprisingly, the penicillin shows an unexpected profile.

Effect of *T. capitatum* on *E .coli*

UPEC (uropathogenic *E. coli*) is one of the main causes of urinary tract infections. It is part of the normal flora in the gut. Gastroenteritis is an inflammation or infection of the gastrointestinal tract, particularly the stomach and

Table 1. The number of bacterial colonies counted after applying different concentrations and volumes of *T. capitatum* (extracted by the two methods: juice and infusion) on 80 µl of an overnight bacterial culture on nutrient agar plates.

Variable	Plate #	Bacteria control without *Teucrium capitatum*	Ampicillin Antibiotic 1%	3.5 mg of fresh Ja'da/ml of bacterial medium	8 mg of fresh Ja'da/ml of bacterial medium	12 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh lettuce/ml of bacterial medium
P. aeruginosa juice	1	91	111	86	76	67	38	85
	2	94	110	83	69	68	41	88
	3	92	113	89	66	70	37	81
	Ave =	92.33	111.33	86	70.33	68.33	38.66	84.66
P. aeruginosa infusion	1	91	111	65	93	78	74	85
	2	94	110	111	63	80	72	88
	3	92	113	104	76	89	78	81
	Ave=	92.33	111.33	93.33	77.33	82.33	74.66	84.66
E. coli juice	1	23	3	26	21	15	10	29
	2	20	0	25	16	17	12	38
	3	18	0	18	22	12	13	22
	Ave=	20.33	1	23	19.66	14.66	11.66	29.66
E. coli infusion	1	23	3	20	12	19	12	29
	2	20	0	22	15	12	15	38
	3	18	0	21	20	17	13	22
	Ave=	20.33	1	21	15.66	16	13.33	29.66
S. epidermis juice	1	105	0	124	75	64	54	98
	2	111	0	111	64	67	59	105
	3	130	0	124	77	61	60	108
	Ave=	115.33	0	119.66	72	64	57.66	103.66
S. epidermis infusion	1	105	0	94	82	75	63	98
	2	111	0	93	81	72	68	105
	3	130	0	88	78	69	69	108
	Ave=	115.33	0	91.66	80.33	72	66.66	103.66
S. aureus juice	1	47	0	56	44	34	36	26
	2	46	0	53	47	37	35	31
	3	42	0	49	39	36	32	30
	Ave=	45	0	52.66	43.33	35.66	34.33	29
S. aureus infusion	1	47	0	54	37	29	35	26
	2	46	0	50	41	38	33	31
	3	42	0	53	42	35	29	30
	Ave=	45	0	52.33	40	34	32.33	29

intestines, and can be caused by a bacterial infection but also by other causes like viruses and parasites. The virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis (Eves

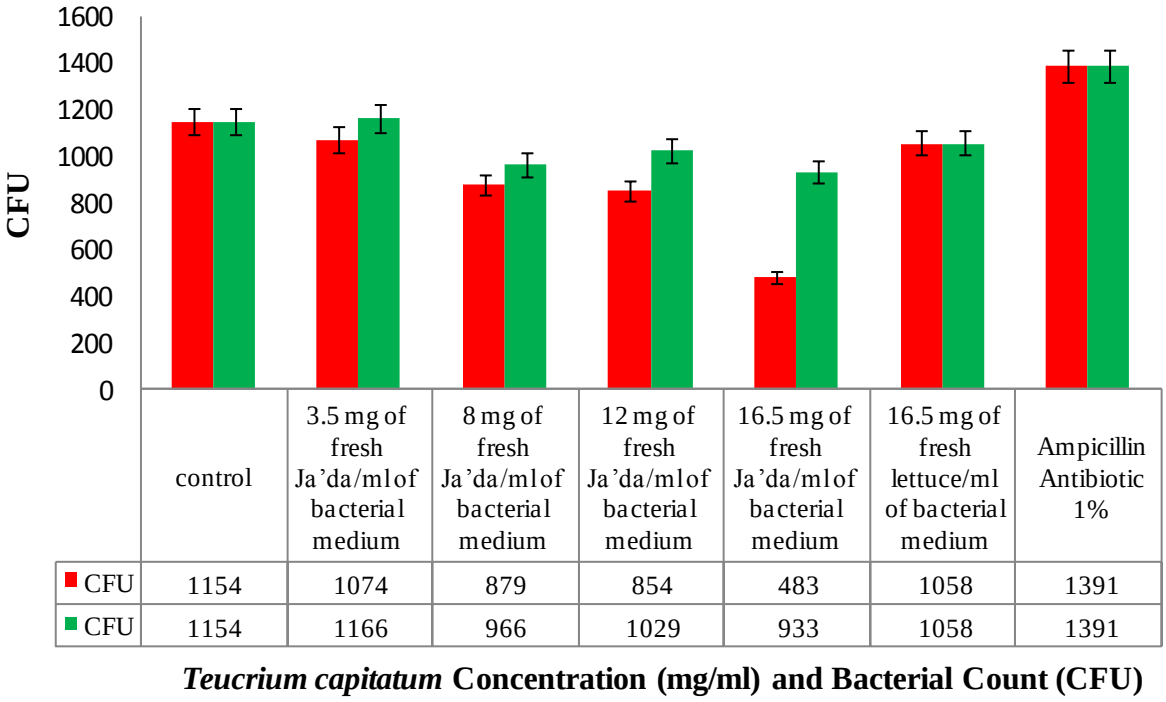


Figure 2. Effect of *Teucrium capitatum* on *Pseudomonas aeruginosa*. Red columns depict the effect of *Teucrium capitatum* plant extracted by juice method or maceration on *P. aeruginosa*. Green columns describe the effect of *Teucrium capitatum* plant extracted by infusion. Values are means ± SD; N = 3; P-value = 5%.

and Rivera, 2010).

E. coli and related bacteria constitute about 0.1% of gut flora and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Feng et al., 2002).

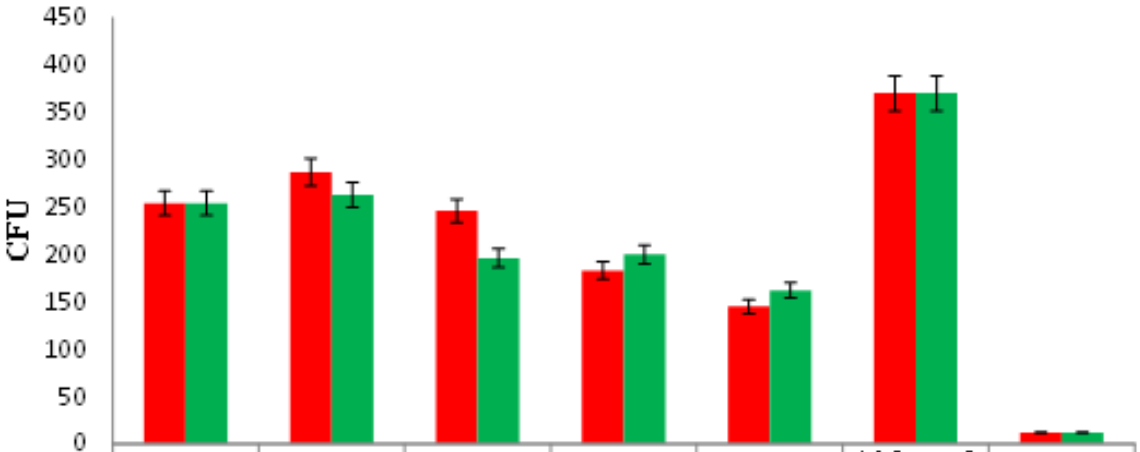
Strains of *E. coli* produce potentially lethal toxins. Food poisoning caused by *E. coli* can result from eating unwashed vegetables or poorly butchered and undercooked meat. O157:H7 is also notorious for causing serious and even life-threatening complications such as hemolytic-uremic syndrome. This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach (Reid et al., 2001).

E. coli can harbor both heat-stable and heat-labile enterotoxins. The latter, termed LT, contain one A subunit and five B subunits arranged into one holotoxin, and are highly similar in structure and function to cholera toxins. The B subunits assist in adherence and entry of the toxin into host intestinal cells, while the A subunit is cleaved and prevents cells from absorbing water, causing diarrhea (Tauschek et al., 2002). As shown in Figure 3, the effect of *T. capitatum* is independent of the extraction method whether extracted by juice or infusion methods. Lettuce did not reduce the growth of the tested uropathogenic *E. coli* bacteria. When added at 3.5 or 8 mg of fresh Ja'da/ml of bacterial medium, *T. capitatum* has no antibacterial effect on the growth of *E. coli*. It is only at 16.5 mg of fresh Ja'da/ml of bacterial medium, that *T. capitatum* inhibits the growth of *E. coli* by 43 and 36% (when juice extract and infusion of *T. capitatum* are used respectively). The effect of *T. capitatum* on *E .coli* is shown in Figure 3.

Effect of *T. capitatum* on *S. epidermis*

S. epidermis lives on the skin. But sometimes it can convert into pathogenic bacteria and cause skin diseases. This bacterium is a gram positive bacterium and is just one of 33 known species belonging to the genus *Staphylococcus*. It is part of the human skin flora (commensal), and consequently a part of the human flora. It can also be found in the mucous membranes and in animals due to contamination (Queck and Otto, 2008).

Although *S. epidermis* is not usually pathogenic, patients with compromised immune systems are often at risk for developing an infection. These infections can be both nosocomial and community acquired, but they pose a greater threat to hospital patients. *S. epidermis* is also a major concern for people with catheters or other surgical



	control	3.5 mg of fresh Ja'da/ml of bacterial medium	8 mg of fresh Ja'da/ml of bacterial medium	12 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh lettuce/ml of bacterial medium	Ampicillin Antibiotic 1%
CFU	254	287	246	183	145	370	12
CFU	254	263	196	200	162	370	12

***Teucrium capitatum* Concentration (mg/ml) and Bacterial Count (in CFU)**

Figure 3. Effect of *Teucrium capitatum* on *E. coli*. Red columns describe the effect of *Teucrium capitatum* extracted by juice method. Green columns describe the effect of *Teucrium capitatum* extracted by infusion method. Values are means ± SD; N = 3; P-value = 5%.

implants because it is known to cause biofilms that grow on these devices (Salyers et al., 2002). The effect of *T. capitatum* on *S. epidermis* is shown in Figure 4.

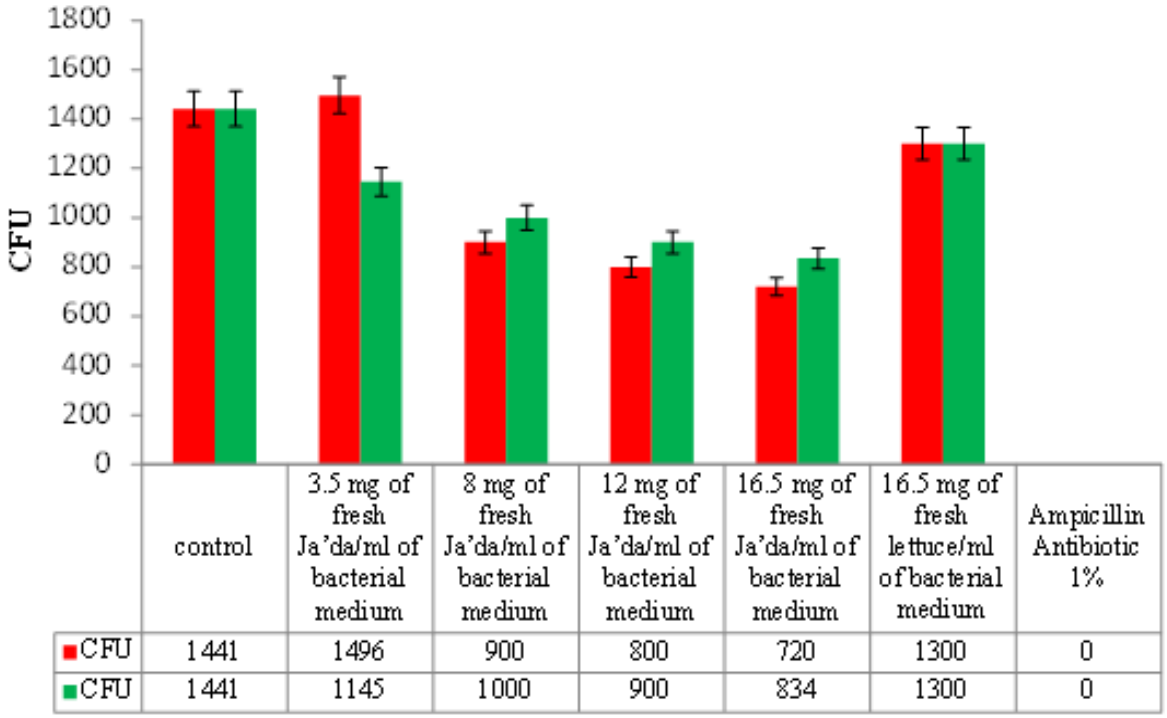
The effect of *T. capitatum* on *S. epidermis* is demonstarted in Figure 4. *T. capitatum* exerts a stronger antibacterial effect on *S. epidermis* compared with *E. coli*. Lettuce did not reduce the growth of bacteria and exhibited a classical control profile. When *T. capitatum* extracted by juice method was added at 3.5, 8 mg of fresh Ja'da/ml of bacterial medium, the growth of *E. coli* was inhibited by 20%. At 16.5 mg of fresh Ja'da/ml of bacterial medium, inhibition is of 50% and 42% using juice and infusion methods respectively. However, ampicillin also exhibited a classical control profile.

Effect of *T. capitatum* on *S. aureus*

S. aureus is frequently found in the human respiratory tract. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (for example, boils), respiratory disease (for example, sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. Antibiotic-resistant forms of pathogenic *S. aureus* have emerged like MRSA (Kluytmans et al., 1997).

S. aureus is gram positive pathogenic bacteria which is widespread in damaged or spoiled food. Depending on the strain, *S. aureus* is capable of secreting several exotoxins. Symptoms include fever, erythematous rash, hypotension, shock, multiple organ failure, and skin desquamation. Lack of antibody to some strains (for example, TSST-1) plays a part in the pathogenesis of toxic shock syndrome. Other strains of *S. aureus* can produce an enterotoxin that is the causative agent of *S. aureus* gastroenteritis (Cenci-Goga et al., 2003). This gastroenteritis is self-limiting, characterized by vomiting and diarrhea one to six hours after ingestion of the toxin with recovery in 8 to 24 h. Symptoms include nausea, vomiting, diarrhea, and major abdominal pain (Patel et al., 1987). The effect of *T. capitatum* on *S. aureus* is shown in Figure 5.

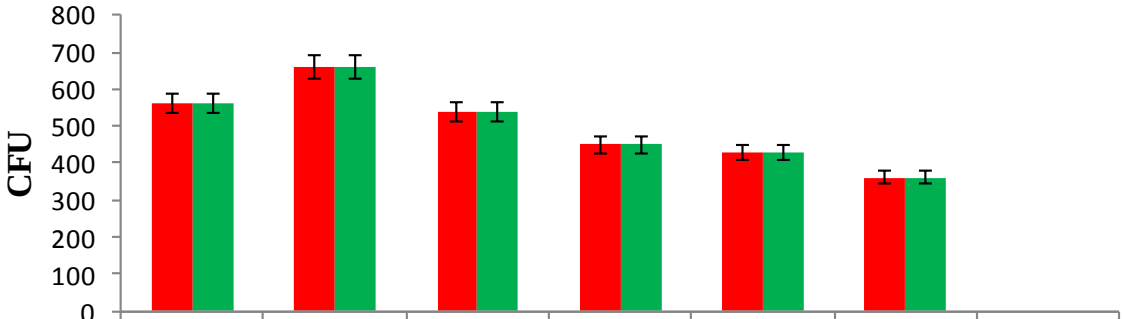
S. aureus is less affected by *T. capitatum* extract when compared with the other species of bacteria used so far in this study. However, when added at a dose of 16.5 mg of fresh Ja'da/ml of bacterial medium, bacterial growth was inhibited by 23% and 17% for the juice and soaking methods respectively. Interestingly, lettuce shows a kind of inhibition of *S. aureus*, whereas ampicillin shows the



	control	3.5 mg of fresh Ja'da/ml of bacterial medium	8 mg of fresh Ja'da/ml of bacterial medium	12 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh lettuce/ml of bacterial medium	Ampicillin Antibiotic 1%
CFU	1441	1496	900	800	720	1300	0
CFU	1441	1145	1000	900	834	1300	0

***Teucrium capitatum* Concentration (mg/ml) and Bacterial Count (in CFU)**

Figure 4. Effect of *Teucrium capitatum* on *S. epidermis*. Red columns describe the effect of *Teucrium capitatum* extracted by juice method on *S. epidermis*. Green columns describe the effect of *Teucrium capitatum* extracted by infusion. Values are means ± SD; N = 3; P-value = 5%.



	control	3.5 mg of fresh Ja'da/ml of bacterial medium	8 mg of fresh Ja'da/ml of bacterial medium	12 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh Ja'da/ml of bacterial medium	16.5 mg of fresh lettuce/ml of bacterial medium	Ampicillin Antibiotic 1%
CFU	562	661	538	450	429	362	0
CFU	562	661	538	450	429	362	0

***Teucrium capitatum Concentration* (mg/ml) and Bacterial Count (in CFU)**

Figure 5. Effect of *Teucrium capitatum* on *Staphylococcus aureus*. Red columns describe the effect of *Teucrium capitatum* extracted by juice method. Green columns describe the effect of *Teucrium capitatum* extracted by infusion. Values are means ± SD; N = 3; P-value = 5%.

expected pattern of a control.

PERSPECTIVES

Further investigations are needed to determine the active antimicrobial agents in *T. capiatum* as well as to determine their mode(s) of action. As previously mentioned, *T. capitatum* contains monoterpenes and sesquiterpene hydrocarbons (José et al., 2004; Calzada et al, 2006; Mo Tutin and Wood, 1972; Maranta, 1981). Such components were demonstrated to act as antimicrobial agents though in another plant species (oregano) (Lambert et al., 2001).

The primary mode of antibacterial action of thymol, sesquiterpene and monoterpenes is not fully known, but is believed to involve outer and inner membrane disruption, and interaction with membrane proteins and intracellular targets. In addition to interacting with membrane phospholipids, evidence has accumulated thymols and sesquiterpenes hydrocarbons interaction with membrane proteins and intracellular targets, which hinder cell recovery after temporary exposure. The ability of sesquiterpene and monoterpenes to interact with proteins was examined using the protein bovine serum albumin (BSA) and the organic compound deferoxamine, which is also rich in amine groups but otherwise known for its Fe^{3+} chelating properties. These compounds react similarly to that of amine groups in bacterial membrane proteins (Juven et al., 1994). It is hypothesized that thymol and monoterpenes form a complex protein with membrane-bound or periplasmic proteins by means of hydrogen bonds and hydrophobic interactions. Interaction with membrane proteins was further supported by other studies. *Salmonella enterica* was exposed to sub-lethal concentrations of thymol, and observed accumulation of misfolded outer membrane proteins and up-regulation of genes involved in synthesis of outer membrane proteins (Di Pasqua et al., 2010). Due to the reported negative effect on liver (Dourakis et al., 2002), further assays should be conducted on hepatic cell lines.

Conclusions

This study is the first to assess the antimicrobial virtues of *T. capitatum* on pathogenic bacteria. This herb is being used for a very long time by Palestinians and Jordanians to combat against stomach and intestinal colic. This study aimed to validate this folk traditional knowledge. *T. capitatum* was found to have a greater antimicrobial effect when extracted using the originally employed juice method (in Arabic mars) as compared with the infusion method.

In a decreasing order of magnitude, *T. capitatum* inhibited the growth of *P. aeruginosa*, *S. epidermis*, *E. coli* and *S. aureus,* notably when added at 16.5 mg of fresh Ja'da/ml of bacterial medium. The plant juice by crushing or maceration method accompanies maceration with squeezing under pressure, and it is expected, therefore, to extract lipids and lipid soluble as well as water soluble compounds. It is an original method, the frequent method in the literature is just the infusion method (described above). Another method found in the literature but not used here is extraction by boiling in alcoholic solution 50%. This method accompanies the disadvantage of inactivation of active substances by boiling with the traces of alcohol that could bias the results through killing of bacteria.

RECOMMENDATIONS

The results presented in this study need further assays in cells to fine tune the appropriate antimicrobial doses of *T. capitatum* on cells. Experiments on animals are essential to pass through humans where validation should be conducted with strict medical control. Hopefully, it would be possible to take advantage of this herb with its compounds and oils to manufacture drugs against colic and stomach infections.

Importantly, it should be taken into consideration that *T. capitatum* may have side effects on the liver and kidney (Dourakis et al., 2002).

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