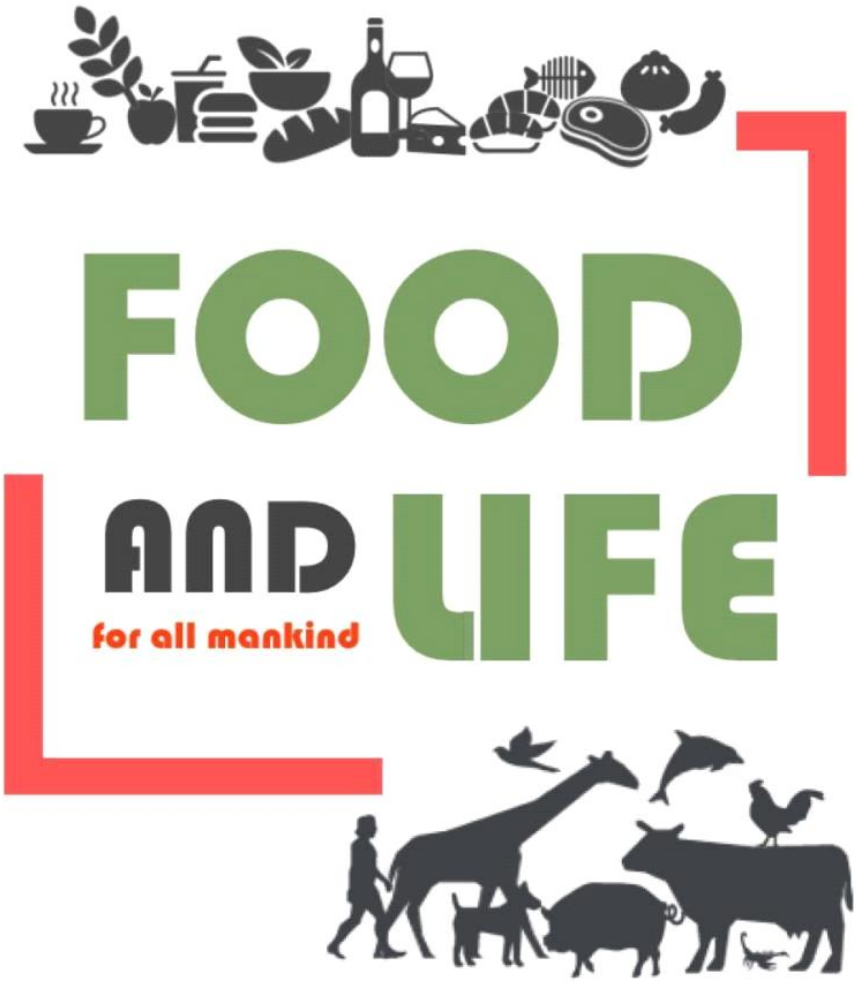

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Effectiveness of *Teucrium* herb as an antibacterial agent

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Abstract

The purpose of this study was to evaluate the antibacterial effect of *Teucrium* extracts prepared by using acetone, ethanol, and distilled water against *Escherichia coli* and *Bacillus subtilis* bacteria. In this experiment, three different forms of *Teucrium* plant were used, fresh leaves were collected from the villages of Irbid, It was divided into two parts, part is fresh leaves and the other part have been dried and grinded in the laboratory, and there is another form of *Teucrium* plant obtained from the medicinal herbs store. The extracts of the *Teucrium* were prepared. The antibacterial activity of these extracts was examined using *the well Diffusion Method*. The results showed that the extracts of the *Teucrium* plant that prepared by using acetone and ethanol obtained from the medicinal herbs store had the highest inhibitory capacity, while the same extracts for the other forms of this plant showed varying inhibition ratios. *Teucrium* extracts can be effective as antibacterial against *E. coli* and *B. subtilis* bacteria.

Keywords: antibacterial agent, antibacterial effect, the effectiveness of extracts

Introduction

Since prehistoric times, therapeutic plants, often known as medicinal herbs, have been identified and employed in traditional medical procedures (Vashvaei et. 2015). Numerous chemical compounds are produced by plants for a variety of purposes, including protection against herbivorous mammals, fungi, insects, and illnesses (Ben Othman et. 2017). Numerous phytochemicals have been shown to have potential or confirmed biological activity. Because they are more widely available and less expensive than contemporary medications, medicinal herbs are frequently employed in non-industrialized countries. *Teucrium* is a genus of Lamiaceae plants, most of which are perennial. Some of the New World species are annuals. *Teucrium* is primarily found in the Western Irano-Turanian and Mediterranean regions, known for its anti-diabetic, anti-inflammatory, hypotensive, anorexic, antispasmodic, antiulcer, antipyretic, and antinociceptive qualities, it is commonly available in Jordan and Palestine (Talib et. 2010). *Teucrium* is used as a tonic and digestive in the treatment of gastritis and enteritis. It is also recognized as a superior depurative and febrifuge (Nenad et. 2007). The essential oils and alcoholic extracts of *Teucrium*, however, were said to have antibacterial properties against both Gram positive and Gram-negative microorganisms. Although *Teucrium* is considered as one of the prominent plants used by domestic people, scientific data illustrate that this herb can lead to critical toxicity to body organs such as the stomach, liver and kidney. In addition to humans, also scholars have opinions on the reliability of the use of the herb. Some scientific studies have reported that the plant has a negative effect on the liver, whereas some others opine that the herb has poisonous effects due to the various phytochemical ingredients (Samira et. 2013). In the lower intestine of warm-blooded species, *Escherichia* (also known as *E. coli*) is a Gram-negative, facultatively anaerobic, rod-shaped coliform bacteria of the genus Escherichia. The majority of *E. coli* strains are not harmful, but some serotypes have the potential to seriously injure their hosts and occasionally result in food-related product recalls. By creating vitamin K2 and limiting the colonization of the intestine with pathogenic bacteria, the benign strains, which are a typical component of the gut flora, can benefit their hosts. A genus of rod-shaped, gram-positive bacteria belonging to the phylum Firmicutes is called *Bacillus subtilis* (Alizade et. 2011).

Obligate aerobes or facultative anaerobes are both possible

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for Bacillus species. Bacillus is a ubiquitous group of pathogenic organisms that comprises both free-living and parasitic species. The bacteria can form oval endospores, which are not genuine spores but to which the bacteria can reduce themselves and stay in a dormant state for very long periods of time, when the environment is harsh.

This work aims to study the antibacterial activities of extracts of *Teucrium* that prepared by using of ethanol, acetone and distilled water (DW).

Materials and Methods

Plant material

Fresh *Teucrium* plant were collected from *Tupnah* and *Dair Yousef* village (*Irbid, Jordan*), it was divided into two parts, the first part was used as fresh, the other part was dried at room temperature for five days and then grinded, and there is other dried sample of *Teucrium* plant collected from the medicinal herbs store in *Irbid* city.

Extracts preparation

Three extracts were made for each type of *Teucrium* plant [acetone, ethanol and DW extracts]. Three samples of *Teucrium* plant were weighed, each sample was 5 grams, the leaves were cut after weighing into small pieces, each five grams was placed in a bottle, 50 mL of DW was added to the first sample, 50 mL of ethanol was added to the second sample, and 50 mL of acetone was added to the third sample. After 24 hours, the samples were filtered and left at room temperature to evaporate. After that, the precipitate material was dissolved for use in the experiment. The same technique was used for other forms of the *Teucrium* plant (fresh *Teucrium* was dried at room temperature for five days and other dried sample of the *Teucrium* plant were collected from the medicinal herbs store).

Preparation of the agricultural medium

In this experiment, *nutrient agar* was used and prepared by mixing 15 g of *nutrient broth* with 17 g of *agar* in flask containing 1 L DW and putting it on Magnetic Stirrer until became homogeneous, then, it was placed in the autoclave for 3 hours, after that poured it in the petri dish and put it in fridge.

Bacteria strains

The bacterial strains that have been used in this experiment are:

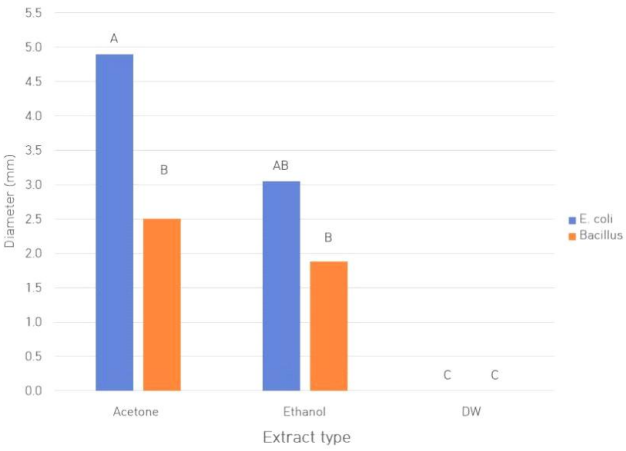

Fig. 1. The mean of the inhibition zone diameter of fresh *Teucrium* sample. DW, distilled water.

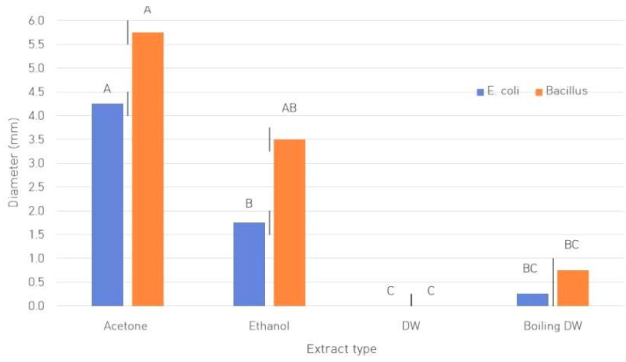

Fig. 2. The mean of the inhibition zone diameter of dried *Teucrium* sample. DW, distilled water.

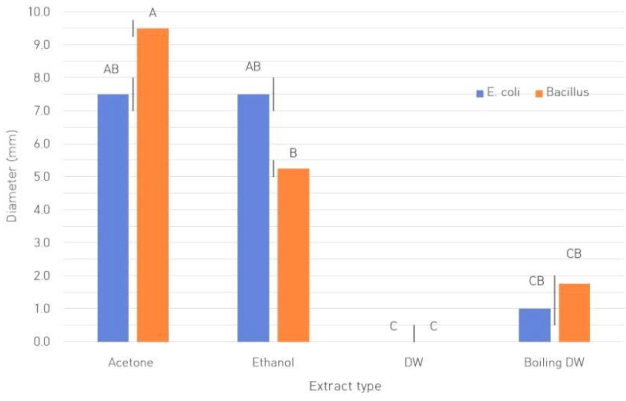

Fig. 3. The mean of the inhibition zone diameter of medicinal herbs store. DW, distilled water.

Gram negative bacteria are *E. coli (ATCC 25922).*

Gram positive bacteria are *Bacillus subtilis.*

Table 1. Result of fresh *Teucrium* sample

Extract	*Escherichia coli*				*Bacillus*			
	Diameter				Diameter			
	First hole	Sec hole	Mean	SD	First hole	Sec hole	Mean	SD
Acetone	5	4.8	4.9	0.01	2	3	2.5	0.5
Ethanol	3	3.1	3.05	0.0025	2.25	1.5	1.875	0.14
DW	0	0	0	0	0	0	0	0

These are the diameter values of the inhibition zone minus the diameter of the primary hole (5 mm).
The measurement unit used is mm.
DW, distilled water.

Table 2. Result of dried *Teucrium* sample (unit: mm)

Extract	*Escherichia coli*				*Bacillus*			
	Diameter				Diameter			
	First hole	Sec hole	Mean	SD	First hole	Sec hole	Mean	SD
Acetone	4.5	4	4.25	0.06	5.5	6	5.75	0.25
Ethanol	2	1.5	1.75	0.25	3	4	3.5	0.5
DW	0	0	0	0	0	0	0	0
Boiling DW	0	0.5	0.25	0.25	1	0.5	0.75	0.25

These are the diameter values of the inhibition zone minus the diameter of the primary hole (5 mm).
DW, distilled water.

Table 3. Result of *Teucrium* sample were collected from the medicinal herbs store (unit: mm)

Extract	*Escherichia coli*				*Bacillus*			
	Diameter				Diameter			
	First hole	Sec hole	Mean	SD	First hole	Sec hole	Mean	SD
Acetone	7	8	7.5	0.5	9	10	9.5	0.5
Ethanol	8	7	7.5	0.5	6	4.5	5.25	0.75
DW	0	0	0	0	0	0	0	0
Boiling DW	0.5	1.5	1	0.5	1.5	2	1.75	0.25

These are the diameter values of the inhibition zone minus the diameter of the primary hole (5 mm).
DW, distilled water.

Test the activity of the extracts:

Well diffusion method was used to test the activity of the extracts on bacteria. After the results showed that the dry plants had a high inhibitory effect compared with the fresh plant, the extract of boiled DW from the dry plant and dried plant was made, then these extracts were tested on bacteria by the same technique.

Results

The results show the extracts that prepared by using acetone and ethanol have a high inhibitor effect compared with the DW extracts Which did not show any inhibitor effect (Fig. 1, Fig. 2, Fig. 3).

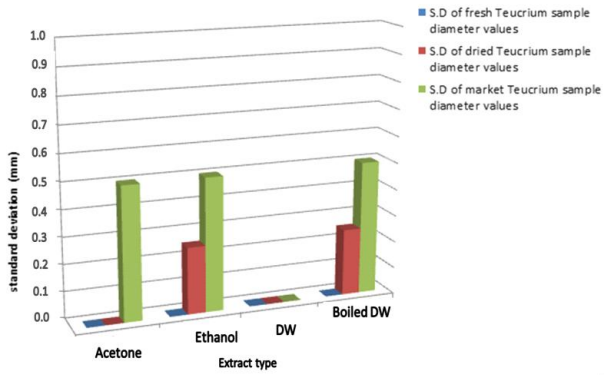

Fig. 4. The standard deviation of the diameter values of the inhibition zone for the three type of *Teuctium* (*Escherichia coli*). DW, distilled water.

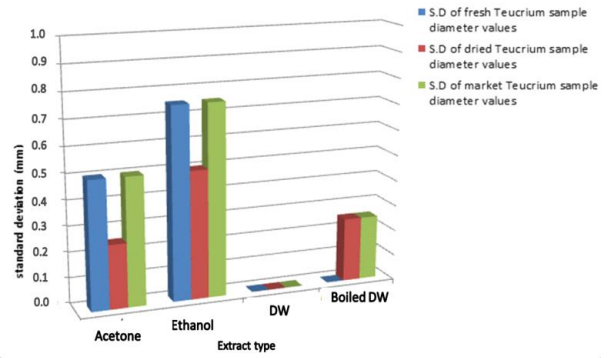

Fig. 5. The standard deviation of the diameter values of the inhibition zone for the three type of *Teuctium* (*Bacillus*). DW, distilled water.

Discussion

The results of this study showed different inhibitory ratios for different types of *Teucrium* according to the solution used. This proved that the *Teucrium* contains substances that have antibacterial effects, and it emerged that the extracts of the plant that were prepared by using acetone and ethanol have a high inhibitory effect. The extracts of the plant that were prepared by using acetone obtained from the medicinal herbs store and plant that has been dried in the laboratory has a high inhibitory ability on *B. subtilis* and *E. coli*, as well as for the extract that was prepared by using ethanol also had a high inhibitory rate (Table 1, Table 2, Table 3). May be due to the fact that the dry plant can easily penetrate by the fluids more than the fresh plant, the physical and chemical properties of acetone and ethanol have helped their interaction with the inhibitory substances in the plant and their deposition easily and at high concentrations because acetone and ethanol are volatile compounds. As for aqueous extracts that showed no inhibitory activity, it may be due to the fact that the plant absorbed water, which means that the concentration of the inhibitory substance is very low because it is mixed with water. In the fresh plant extracts, the inhibitory activity was lower than the two dry plants (Table 1, Table 2, Table 3). This may be due to the difficulty of penetrating the fluid into the plant leaves and interacting with the inhibitory material, resulting in a small amount of inhibitory material, resulting in reduced inhibitory activity, and this is what the results showed (Fig. 4, Fig. 5).

Conflicts of Interest

The authors declare no potential conflict of interest.

Acknowledgments

Not applicable.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

Author Contributions

The article is prepared by a single author.

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