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The antibacterial activity of essential oils from *Tagetes erecta* and *Thuja occidentalis*

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ABSTRACT

Plants produce phytochemicals that have potential medicinal benefits. Essential oils are phytochemicals that have been recognized to have antibacterial properties. Bacteria are becoming resistant to current therapeutic agents. New methods need to be developed to help protect livestock, crops, and people from harmful bacteria such as *Staphylococcus aureus* and *Escherichia coli*. This study utilized steam distillation to extract essential oils from the leaves, stems, and flowers of *Tagetes erecta* and the branches and needles of *Thuja occidentalis*. The essential oils collected were studied for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. These bacteria were utilized, because these are bacteria that are commonly associated with disease and spoilage in crops, livestock, and people. It was also important that a gram-negative and gram-positive bacterium was studied. Both *Tagetes erecta* and *Thuja occidentalis* essential oils showed antibacterial activity. An analysis of the compounds was done using GC-MS. The major components of *Tagetes erecta* essential oil were d-limonene, dihydrotagetone, piperitone, piperitenone, and caryophyllene oxide. *Thuja occidentalis* essential oil had alpha-pinene, 3-carene, and alpha-cedrol as its major components.

Keywords: *Antibacterial, essential oil, natural product, phytochemicals, therapeutic agent.*

INTRODUCTION

Natural products are compounds that are produced by living organisms such as microorganisms, animals, and plants. About 60 percent of cancer drugs contain natural products (Solowey et al., 2014). Phytochemicals, biologically active plant compounds, are natural products that are used in flavors, perfumes, and medicine. The importance of plant-based drugs is beginning to be understood, because they are less harmful to the body (Bakkali et al., 2008; Cavanagh and Wilkinson, 2002; Jirovetz et al., 2002). Essential oils are phytochemicals that are oily, volatile, natural liquids characterized by a strong aroma (Al-Bayati, 2009; Bakkali et al., 2008; Burt 2004). Essential oils are extracted from the leaves, stems, flowers, buds, twigs, fruits, roots, and seeds of a plant through steam distillation, solvent extraction, expression, and hydrodistillation (Bakkali et al., 2008; Burt 2004). Essential oils are compounds synthesized through the secondary metabolism of plants (Bakkali et al., 2008; Nascimento et al., 2000). A secondary metabolism is a process that does not correlate with the growth, development, and reproduction of an organism. Essential oils have been recognized to have antiparasitic, antiviral, and antibacterial properties (Bakkali et al., 2008; Burt, 2004; Essawi and Srour, 2000; Goni et al., 2009; Nascimento et al., 2000).

Many strains of bacteria are mutating to become resistant to therapeutic agents; therefore, there is an urgent need to discover new drugs and therapies (Essawi and Srour, 2000; Nascimento et al., 2000). Traditional methods of using plant compounds are being revitalized to protect crops and livestock from disease and spoilage (Dorman and Deans, 2000; Schillinger and Lücke, 1989).

Plants that contain essential oils repel insects and herbivores from eating it. (Bakkali et al., 2008). *Tagetes erecta* (marigold) and *Thuja occidentalis* (arborvitae) are rarely eaten. This study focuses on the antibacterial activity of their essential oils on *Staphylococcus aureus* and *Escherichia coli*. In this study, the essential oils from *Tagetes erecta* and *Thuja occidentalis* were extracted using steam distillation. The discoveries found could carve a path of future medical contributions along with broadening current scientific knowledge.

MATERIALS AND METHODS

i. Extraction of Essential Oils

The leaves, stems, and flowers obtained from *Tagetes erecta* were cut and put into the collection flask in the steam distillation apparatus (Figure 1). The hot water pushes steam through the collection flask, breaking the plant membranes so the essential oils in the plant material are collected (Cassel et al., 2009; Harborne, 1998). A steam distillation was also performed on the twigs and needles from *Thuja occidentalis*. Each successful extraction was collected and dried using anhydrous sodium sulfate and put into a labeled glass vial.

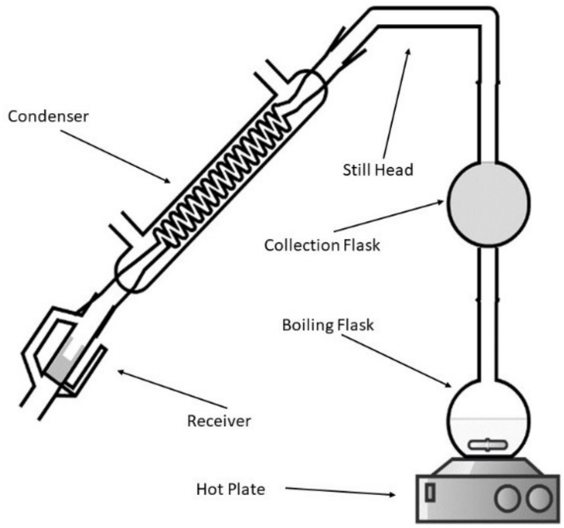

Figure 1. Steam distillation apparatus. The essential oils were collected in the receiver.

ii. Antibacterial Activity

The antibacterial activity of *Tagetes erecta* and *Thuja occidentalis* essential oils was tested using the disc diffusion method. The *Tagetes erecta* and *Thuja occidentalis* essential oils extracted using steam distillation were tested against *Staphylococcus aureus* and *Escherichia coli* with a 100% concentration. If the bacteria were resistant to the essential oil, no further tests were done. If the bacteria were susceptible to the essential oil, a 50% and 25% solution was made and tested. The essential oils were diluted with dimethyl sulfoxide (DMSO), which was used as the control.

Escherichia coli and *Staphylococcus aureus* were cultured in 10 milliliters of Mueller Hinton Broth and incubated at 37 ˚C. *Escherichia coli* and *Staphylococcus aureus* were uniformly spread on Mueller Hinton Agar plates. 17 microliters of a solution was added to a disc. Each concentration of *Tagetes erecta* and *Thuja occidentalis* essential oils were tested against *Escherichia coli and Staphylococcus aureus* three times.

After the bacteria and solutions were added to the plates, they were sealed and incubated at 37 ˚C for 24 hours. The distance between the edge of the disc and bacteria was measured using a centimeter ruler (Prabuseenivasan, 2006). Only a 100% solution of *Tagetes erecta* was done on *Escherichia coli*, because there was no evidence of the bacteria growth being inhibited.

iii. Essential Oil Analysis

20 microliters of the essential oil was dissolved in dichloromethane. An analysis of the *Tagetes erecta* and *Thuja occidentalis* essential oils solution was done using a gas chromatograph mass spectrometer (GC-MS).

RESULTS

i. Antibacterial Activity

The susceptibility of *Escherichia coli* and *Staphylococcus aureus* to *Tagetes erecta* and *Thuja occidentalis* was tested using the disc diffusion method. Only a 100% solution of *Tagetes erecta* essential oil was tested against *Escherichia coli*, because the bacteria were resistant to *Tagetes erecta* essential oil. Three concentrations of *Tagetes erecta* essential oil were tested against *Staphylococcus aureus*. *Tagetes erecta* essential oil was mixed with DMSO to create a 50% and 25% composition of *Tagetes erecta* essential oil. All three concentrations of *Tagetes erecta* essential oil inhibited the growth of *Staphylococcus aureus*. Table 1 summarizes the zone of inhibition of *Tagetes erecta* essential oil.

Table 1. Zone of inhibition of *Tagetes erecta* essential oil against *Escherichia coli* and *Staphylococcus aureus*. The solutions were made using *Tagetes erecta* essential oil and DMSO.

Tagetes erecta Composition	Bacteria Strain	
	Staphylococcus aureus	*Escherichia coli*
100%	3.9 mm	No Activity
50%	0.9 mm	No Activity
25%	0.1 mm	No Activity

Three concentrations of *Thuja occidentalis* essential oil were tested against *Staphylococcus aureus* and *Escherichia coli*. *Thuja occidentalis* essential oil was mixed with DMSO to create a 50% and 25% composition of *Thuja occidentalis* essential oil. All three concentrations of the *Thuja occidentalis* solutions inhibited the growth of both *Escherichia coli* and *Staphylococcus aureus*. Table 2 summarizes the zone of inhibition of *Thuja occidentalis* essential oil.

Table 2. Zone of inhibition of *Thuja occidentalis* essential oil against *Escherichia coli* and *Staphylococcus aureus*. The solutions were made using *Thuja occidentalis* essential oil and DMSO.

Thuja occidentalis Composition	Bacteria Strain	
	Staphylococcus aureus	*Escherichia coli*
100%	6.0 mm	4.8 mm
50 %	4.2 mm	4.7 mm
25%	3.2 mm	1.2 mm

ii. Essential Oil Analysis

A GC-MS was used to determine how many components were present in *Tagetes erecta* and *Thuja occidentalis* essential oils along with what compounds were present. *Tagetes erecta* essential oil was found to have over 100 components. Dihydrotagetone, piperitone, piperitenone, d-limonene, and caryophyllene oxide were the major compounds. Table 3 summarizes the components of *Tagetes erecta* essential oil.

Thuja occidentalis essential oil was found to have at least 100 components. 3-carene, alpha-cedrol, and alpha-pinene were the major compounds. Table 4 summarizes the components of *Thuja occidentalis* essential oil.

Table 3. 30 components with the highest concentration in *Tagetes erecta* essential oil. * denotes the major components in the essential oil. # denotes components that are uncertain in the *Tagetes erecta* essential oil.

Components of *Tagetes erecta* essential oil	Percentage of the Solution
Dihydrotagetone*	17.99%
Piperitone*	15.94%
Piperitenone*	12.37%
D-limonene*	11.55%
Caryophyllene oxide*	7.96%
P-cymen-8-ol	5.72%
Trans-verbenol	3.10%
Isoartemisia ketone	2.74%
Cedrene	2.53%
Trans-verbenol	2.07%
Cis-ocimenone	1.96%
5-(1-hydroxy-1-methylethyl)-2-methyl-2-cyclohexene-1,4-diol#	1.55%
Bornyl acetate	1.40%
2-methyl-2-bornanol	1.22%
Car-3-en-5-one#	1.04%
Spathulenol	1.02%
1-methyl-3-(1-methylethenyl)-benzene	1.01%
Caryophyllene oxide#	0.86%
N-(3-amino-2-hydroxy-phenyl)-acetamide	0.82%
Unknown	0.76%
5-(1-hydroxy-1-methylethyl)-2-methyl-2-cyclohexene-1,4-diol#	0.73%
P-cymene	0.72%
Cis-tagetone	0.68%
Alpha-terpineol	0.66%
Unknown	0.65%
3-methoxy-p-cymene	0.54%
Sabinene	0.49%
Tau-cadinol	0.44%

Table 4. 30 components with the highest concentration in *Thuja occidentalis* essential oil. *denotes the major components in the essential oil. # denotes components that are uncertain in the *Thuja occidentalis* essential oil.

Components of *Thuja occidentalis* essential oil	Percentage of the Solution
3-carene*	25.98%
Alpha-cedrol*	19.53%
Alpha-pinene*	17.42%
Alpha-terpinyl acetate	5.36%
Alpha-terpinyl acetate	2.41%
Caryophyllene Oxide	2.39%
Alpha-fenchene	1.91%
Car-3-en-5-one#	1.87%
Beta-myrcene	1.85%
Cryptone	1.78%
P-cymene	1.72%
M-cymen-8-ol	1.69%
D-limonene	1.68%
(3R, 3aR, 5S, 6R, 7aR)-3,6,7,7-tetramethyloctahydro-3a,6-ethanoinden-5-ol	1.59%
Sabinene	1.45%
Beta-phellandrene	1.18%
Alpha-phellandren-8-ol	1.06%
Cedrene	1.04%
Beta-pinene	0.94%
(3R,6R)-3-hydroperoxy-3-methyl-6-(prop-1-en-2-yl) cyclohex-1-ene	0.90%
Bornyl acetate	0.89%
Cedryl acetate	0.83%
Trans-verbenol	0.82%
Unknown	0.76%
Alpha-humulene	0.61%
Cis-verbenol	0.59%
Eucarvone	0.52%
(3R,6R)-3-hydroperoxy-3-methyl-6-(prop-1-en-2-yl) cyclohex-1-ene	0.47%
Car-3-en-5-one#	0.39%
O-cymene	0.37%

DISCUSSION

Essential oils are hydrophobic, which allows essential oils to penetrate the lipids in the cell membrane of bacteria. (Knobloch et al., 1986; Liolios et al., 2009). The cell contents will begin to leak, due to the lipids becoming permeable (Knobloch et al., 1986; Burt, 2004). An excessive loss of the cell contents will lead to cell death (Denyer and Hugo, 1991).

This study showed that *Tagetes erecta* essential oil shows antibacterial activity against *Staphylococcus aureus*, but it was not active against *Escherichia coli*. *Thuja occidentalis* essential oil showed antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. This study did not separate compounds from the essential oils and test for their antibacterial activity. Therefore, it cannot be concluded which compound has antibacterial properties or if the antibacterial activity was due to a mixture of compounds. This study can conclude that both *Tagetes erecta* and *Thuja occidentalis* essential oils have antibacterial properties. *Thuja occidentalis* showed more antibacterial activity than *Tagetes erecta* against *Escherichia coli*. Further study needs to be done to discover what individual or mixture of compounds of *Tagetes erecta* and *Thuja occidentalis* essential oils have antibacterial properties.

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