Scientific Research Publishing

Journal of Biosciences and Medicines, 2020, 8, 96-103
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Disinfectant for Urinary Infection Caused by *Escherichia coli* by Using Natural Oils

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How to cite this paper: Harini, S., Abhipsapanigrahy and Suneetha, V. (2020) Disinfectant for Urinary Infection Caused by *Escherichia coli* by Using Natural Oils. *Journal of Biosciences and Medicines*, **8**, 96-103.
https://doi.org/10.4236/jbm.2020.85009

Received: March 26, 2020
Accepted: May 5, 2020
Published: May 8, 2020

Abstract

According to the WHO analysis, antibacterial treatment which is preferred for treating peptic ulcers and oral health problems are considered as a major improvement in the treatment but are responsible for several health problems that include vomiting, diarrhea and the increase in the bacterial resistance is a major concern. So, there is an urgent need for a good therapeutic alternative which is effective. Usage of essential oils to treat different ailments is found to be extremely effective with no side effects. Thus, essential oils are gaining importance in treatments due to their antibacterial, antifungal, antiprotozoal properties. Essential oils are also used as aroma therapeutic agents. They are complex mixture of low molecular weight compounds that include terpenoids, phenylpropanoids, oxygenated derivatives (aldehydes, ketones, alcohols, esters) which greatly differ in composition in different essential oils are the major components present. The bioactive properties and their medical potential are widely used in pharmaceutical industries. In this study, we use these essential oils to prepare a disinfectant and analyze the antimicrobial properties using the agar diffusion method. Our major concern in this study is to decrease the growth of *Escherichia coli* which causes urinary infection, by using disinfectant prepared using a mixture of essential oils of particular concentration.

Keywords

Essential Oil, Antibacterial Properties, Disc Diffusion Method, Pharmaceutical, Disinfectant, Urinary Infection

1. Introduction

Disinfectant is considered as the agents when applied to inanimate objects inhibit the growth of microorganisms. When studied about the traditional medicines, the usage of the essential oils was prominent in treating several health problems

and also in case of surgical conditions [1]. Thus the properties of these oils were studied and found to have antipathological activity. These are secondary metabolites of the plants and are prepared using steam distillation and solvents. About 43% of people find aromatherapy to be effective in relieving stress. Peppermint oil relieves people from migraine; Lavender oils helps to treat insomnia conditions in people with heart ailments. Thus, the idea of usage of these valuable essential oils in disinfectant preparation of ideal concentrations [2] and used to prevent the contamination by *Escherichia coli.* Urinary infection is more prevalent in the females compared to males; the risk factors include usage of poor hygienic washrooms, menopause and pregnancy (Wiley Online Library). The bacteria enter the urinary tract through urethra and multiply in bladder. The bacteria responsible for bladder related urinary infection is *Escherichia coli* [3]. By using essential oils such as clove oil, rose oil, cinnamon powder and peppermint oil of ideal concentration (500 μg/ml), using ethanol as a solvent [4], the disinfectant was prepared and agar diffusion method was performed to find the growth of *E. coli* to be decreased, thus maintaining hygienic conditions [5].

2. Materials and Methods

2.1. Preparation of Different Oils

Commercial oils were taken which have undergone FDA approved purity checks and a mixture of ideal concentration (500 μg/ml) by using ethanol as the solvent. **Figure 1** shows the commercial oils used [6].

2.2. Processing and Evaluation of Disinfectant

To find out which bacteria is present in major constituent in washrooms, the petri dishes are prepared under sterilized condition in laminar air flow chamber, the lid is opened to allow the contamination of the bacteria. **Figure 2** shows the colonies of the bacteria in the petri dishes after incubation. **Figure 3** shows that

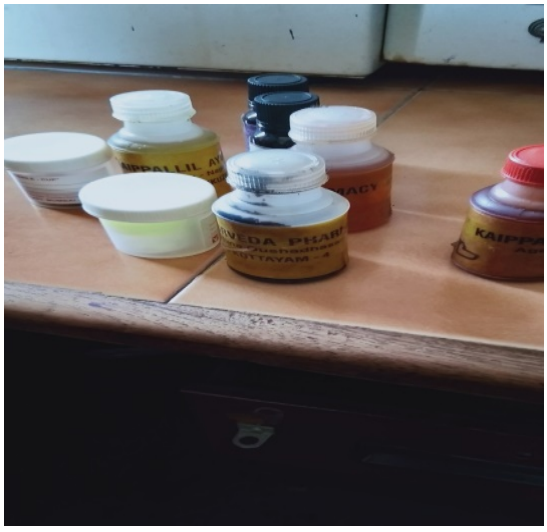

Figure 1. Commercial Essential oils.

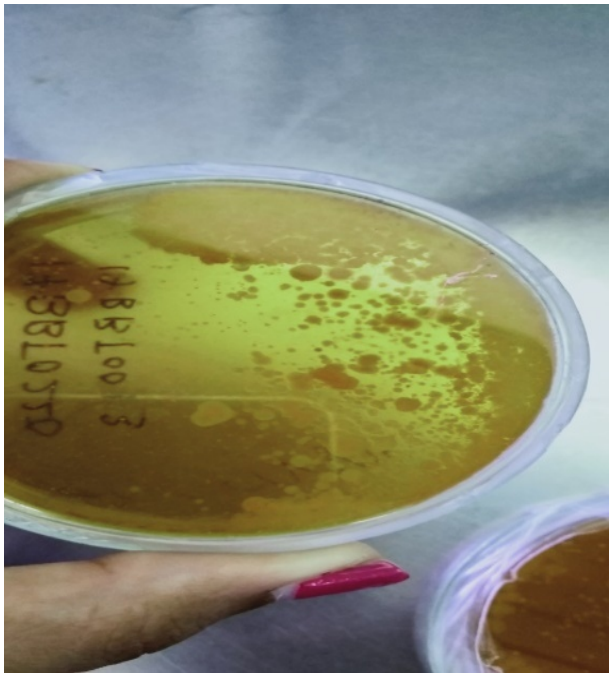

Figure 2. Contamination of petri dish in washrooms.

Figure 3. Incubator (37 degree Celsius).

the petri dishes are incubated (37 degree Celsius) for 24 hours. The bacteria is isolated and identified by biochemical test where a test kit named KI1001 AND KI1002 is used. The petri dish is swabbed aseptically by the isolated bacteria and by following the guidelines the agar diffusion method was performed by using the disinfectant made using essential oils [7]. The growth of the *Escherichia coli* was no more than 20%. The inhibition ranges from 6.4 mm to 11 mm. The lipopolysaccharides of the Gram negative bacteria might be responsible for quite good resistance of the bacteria.

2.3. Staining Techniques

The staining techniques are done to identify the type of the bacteria [8] present. Figure 4 shows the staining done using Gram staining and found out that it is Gram negative bacteria.

2.4. Staining for Examination

Different staining techniques like simple stain, Gram stain and Negative stain were performed.

2.5. Biochemical Test Kit

A kit that consists of different tests and by examining the color change the isolated bacteria is identified. The kits used are KI1001 and KI1002.

3. General Guidelines for Preparation of Oils

3.1. Clove Oil Preparation

Take 20 g of cloves in a mortar and pestle. Grind the cloves and put it in a white cloth. Tie it in one end of the cloth. Put it in the olive oil in small container. Place the small container in water containing big container. Boil for 2 hours. Allow the oil to cool and take the oil and crush the cloth to get the clove extract [9].

3.2. Preparation of Rose Oil

Fill the rose petals in the container with oil. Place the container in the big container containing water. Heat the container for 2 hours and cool it, then again heat it for 2 hours for 6 times. Cool down and extract the oil from the rose petals.

3.3. Preparation of Mint Oil

Crush the peppermint leaves with your hand or spoon. Put the leaves in the container. Fill the jar with olive oil covering the leaves. Let the oil steep for 2 days in sun and shake every 12 hours and keep it in the dark place.

3.4. Preparation of Cinnamon Powder

Take about 20 g of cinnamon in the mortar and pestle and grind it to get in powder form.

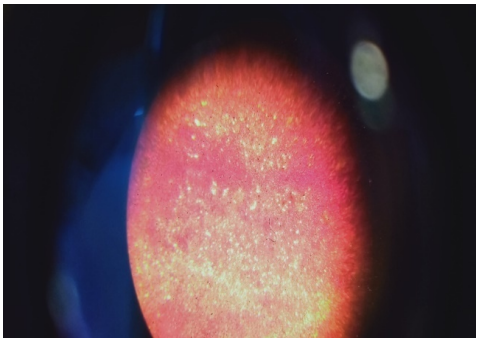

Figure 4. Gram staining (40×).

3.5. Preparation of Borneo Camphor Powder

Take about 10 g of Borneo camphor and smash it to make it into powder form.

Lavender oil, Tea tree oil, Glycerol, apple cider vinegar is also used.

The oils and the materials are taken in different proportions and mixed using the mixer for 10 - 15 minutes to make it into homogenous phase.

This disinfectant can be used in the floor of restrooms and also as aerosols.

Commercial oils obtained is FDA approved purity check was used for the preparation of the disinfectant and zone of inhibition was measured [10].

4. Biochemical Test

4.1. Procedure for the Biochemical Test Kit

Two kits KI1001 and KI1002 were taken. It consists of nearly 12 tests in each kit. KB0010 was gram negative rods and KB0020 was gram positive rods.

In KI1001—the tests that are performed to identify the bacterial colonies include Indole test, methyl red test, vogesproseawer's test, citrate utilization test, glucose test, adonitol test, arabinose test, lactose test, sorbitol test, mannitol test, rhamnose test, sucrose test.

In KI1002—the tests that are performed to identify the bacterial colonies include citrate utilization test, lysine test, ornithine test, urease test, phenyl alanine test, nitrate reaction test, hydrogen sulphide production test, glucose test, adonitol test, lactose test, arabinose test, sorbitol test.

4.2. Media Preparation

Suspend 3.8 g of Muller Hinton agar in 100 ml of distilled water in 150ml beaker. Autoclave it for 30 minutes and allows it to cool down in laminar air flow chamber. And pour it in the petri dish. About 5 petri dishes are prepared.

Suspend 4.1 g of Brain Infusion agar in 100 ml of distilled water in 150 ml beaker. Autoclave it for 30 minutes and allows to cool down in laminar air flow chamber. Pour it in petri dish; about 5 petri dishes are prepared.

4.3. Figures and Tables

Gas chromatography results that were reviewed from a few research journals, Table 1 and Table 2 provide the information about the major antibacterial compound present in the essential oils were studied.

Table 3 represents the Single factor ANOVA is performed using Excel and the F value is calculated.

Since F critical value is greater than F value, there is not much significant difference between the different concentrations of oils taken. The values taken were the number of bacterial cells [11] that was resistant to the application of different concentrations of oils. The bacterial cells were counted using colony counters [12].

This implies that when different concentrations of the disinfectants were pre-

pared (200 μg/ml, 300 μg/ml, 400 μg/ml, 500 μg/ml and 600 μg/ml) there was not much difference in the zone of inhibition layer [1]. It was the same when used different concentrations that is mentioned above.

Table 1. Percentage of antibacterial compound present in the natural oils.

NATURAL OILS	ANTIBACTERIAL COMPOUND	PERCENTAGE
CLOVE OIL	EUGENOL	83.13%
ROSE OIL	Beta-CITRONELLOL	30.24
MINT OIL	MENTHOL	29-48%
CINNAMON POWDER	PROANTHOCYANIDINS	21.99%

Table 2. Research reported on disinfectant from natural oils based on their properties.

Year of publication	Topic	Scientist	Name of the journal
2013	Observational study on preoperative surgical field disinfection: Povidone-iodine and Chlorhexidine-alcohol [1]	Magalini, S., Pepe, G., Panunzi, S.	European review for Medical and Pharmacological Sciences 17(24) 3367-3375.
1998	The testing of disinfectants [2]	Reybrouck, G.	Bio deterioration and Biodegradation 41(3-4)
2010	Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection [3]	Mulvey, M., Schilling, J., Hultgren, S.	Infection and Immunity 69(7) 4572-4579.
2015	Disinfectant choices in veterinary practices, shelters and households: ABCD guidelines on safe and effective disinfection for feline environments [4]	Addie, D., Boucraut-Baralon, C., Egberink, H., *et al.*	Journal of Feline Medicine and Surgery 17(7) 594-605.
2015	Antibacterial and antioxidant activities of Mentha piperita L. Arabian [5]	Rajinder, Singh., Muftah, A, M, Shushni., Asma, Belkheir	Arabian Journal of Chemistry
2005	Antibacterial and Antifungal Properties of Essential Oils [6]	Kalemba, D., Kunicka, A.	Current Medicinal Chemistry 10(10) 813-829
2005	Direct determination of per acetic acid and hydrogen peroxide and acetic acid in disinfectant solutions by far ultraviolet absorption spectroscopy [7]	Higashi, N., Yokota, H., Hiraki, S.	Analytical Chemistry 77(7) 2272-2277
2006	Addition polymers from Natural oils: A review [8]	Sharma, V., Kundu, P	Progress in Polymer Sciences (Oxford)
2011	Use of Essential Oils in Bioactive Edible Coatings: [9]	Sánchez-González, L., Vargas, M., González-Martínez, C., *et al.*	Food Engineering Reviews
2006	Fila mentation by *Escherichia coli* subverts innate defenses during urinary tract infection Justice [10]	Hunstad, D., Seed, P., *et al.*	Proceedings of the National Academy of Sciences 103(52) 19884-19889
2010	Food reservoir for *Escherichia coli* causing urinary tract infections [11]	Vincent, C., Boerlin, P., Daignault, D., *et al.*	Emerging Infectious Diseases 16(1) 88-95
	Molecular mechanisms of *Escherichia coli* pathogenicity [12]	Croxen, M., Finlay, B	Nature Reviews Microbiology
2010	Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection [1]	Mulvey, M., Schilling, J., Hultgren, S.	Infection and Immunity 69(7) 4572-4579

Table 3. Statistical Analysis to determine the significance between the treatments.

ANOVA: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	21	4.2	5.7		
Column 2	5	28	5.6	18.8		
Column 3	5	59	11.8	46.7		
Column 4	5	79	15.8	192.7		
Column 5	5	18	3.6	23.3		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F. crit*
Between Groups	573.2	4	143.3	2.494777	0.075591	2.866081
Within Groups	1148.8	20	57.44			
Total	1722	24				

5. Limitation

Although essential oils were found to have no side effects, when applied directly to skin in case of migraine, insomnia patients. It causes health problems that include rashes, asthma attacks (in case of aroma therapy), headaches and allergic reactions. Compound with high phenol content like cinnamon cause skin irritations. Swallowing of essential oils is not advisable.

6. Results

All the petri dishes that were taken in the wash rooms were contaminated by bacteria, and it was identified as *Escherichia coli*. Figure 2 shows the presence of the bacterial colonies after incubation in incubator at 37 degree Celsius. The odor of the disinfectant was pleasant with dominating peppermint and clove flavor and lasted for 5 hours and it was colorless. As mentioned above the zone of inhibition by agar diffusion method ranges 6.4 mm to 11 mm. This proves the antibacterial property of the essential oils. Based on the survey obtained from the students, 16 out of 20 students felt hygienic after usage of disinfectant in the washrooms. Thus, it consists of antimicrobial properties which prevent the outbreak of urinary and sexually transmitted diseases through contaminated washrooms. The different concentrations of different oils taken based on LSD method showed that there is no significant difference between the treatments, as all the concentration of the oils gives the same range zone of inhibition.

7. Discussion

Poor hygienic condition resulted in urinary infection problems in youngsters; to avoid the problem the disinfectant without the presence of ethanol is prepared. 80% of students find it hygienic after usage of the disinfectant. This is prepared by the usage of natural oils which consists of antimicrobial properties and odor properties.

Acknowledgements

Our sincere thanks to Dr.Viswanathan, Chancellor, VIT University for enabling the opportunity to create an innovative study and to fulfill our passions and special thanks to our beloved vice president G. V. Selvam for his constant encouragement.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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