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# Antimicrobial Activity of Tea Tree oil against Pathogenic Bacteria and Comparison of Its Effectiveness with Eucalyptus Oil, Lemongrass Oil and Conventional Antibiotics

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**Abstract** Tea Tree oil (TTO) is known to have antibacterial effects and this study was aimed to determine the abilities to control pathogenic bacteria and also compared the antimicrobial effectiveness of Eucalyptus oil (ECO), Lemongrass oil (LGO) and antibiotics those are using for bacterial infection. This study of antimicrobial activity against ten pathogenic bacteria: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aeromonas hydrophila*, *Escherichia coli*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Streptococcus agalactiae* was done by Broth dilution method and Agar well diffusion method. The essential oils used in this study were commercially available. The inhibition of bacterial growth after 24 hours incubation exhibits greater results than 6 hours incubation in most of the cases. After 24 hours incubation, TTO showed minimum 96.94% against *E. coli* and maximum 100% inhibition against seven bacteria selected for this study whereas ECO showed minimum 37.02% against *E. coli* and maximum 100% inhibition against *S. aureus*, *P. vulgaris* and *A. hydrophila* and the another essential oil that is LGO exhibited minimum 69.08% against *E. coli* and maximum 100% inhibition against five bacteria chosen for this study. The inhibition zones from each extract were measured and an activity index was calculated from the mean zone sizes. All Essential oils showed some degree of antimicrobial properties with the highest activity index (1.6) being from TTO against *S. agalactiae*. At last, established a comparison between tea tree oil and some broad spectrum antibiotics using well diffusion method. Tea tree oil exhibited observable zone against all the bacteria contrariwise, among nine antibiotics only two of them showed noticeable zone of inhibition to all the bacteria tested. According to this study, TTO has demonstrated remarkable antibacterial activity which was more efficient than ECO and LGO and, moreover, it is expected that TTO will gradually take place of conventional antibiotics to treat bacterial infection.

***Keywords:*** *essential oils, activity index, inhibition percentage, broth dilution, well diffusion*

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## 1. Introduction

The world seems to be running out of effective antibiotics. While any antimicrobial resistance is concerning, the increasing incidence of antibiotic-resistant Gram-negative bacteria has become a particular problem as strains resistant to multiple antibiotics are becoming common and no new drugs to treat these infections will be available in the near future [1]. The problem of resistance is also due to an abuse of antibiotics. Many people will go to a doctor and demand an antibiotic when they have a cold or a flu, for which these antibacterial compounds are useless. In many countries it is possible to buy antibiotics over the counter. Often, if people are poor, they will not take the full dose all of that leads to resistance [2]. Nowadays, use of alternative therapies with mainstream medicine has gained the momentum. Aromatherapy came into existence after the scientists deciphered the antiseptic and skin permeability properties of essential oils [3]. Essential oils are a rich source of biologically active compounds [4]. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential [5]. Tea tree oil may have a clinical application, especially for clearance of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage or as a hand disinfectant to prevent cross-infection with Gram-positive and Gram-negative epidemic organisms [6]. Tea tree oil's antimicrobial terpenes content also makes it popular for combating acne. Not only does it kill MRSA

or staph infections, but it will also kill *Propionibacterium* acnes that live inside hair follicles and can lead to inflammation and acne. It has the ability to kill parasites and fungal infections, which is why it's so popular for use in fighting toenail fungus, ringworm and athlete's foot. Apply undiluted tea tree oil twice daily to affected areas like nails or feet to relieve symptoms, and possibly completely heal these unsightly ailments [7]. The antibacterial properties in eucalyptus essential oil are well established, and its antiseptic nature makes it appropriate for treating wounds like burns, sores, cuts, and abrasions [8]. A diluted lemongrass mixture may assist in facilitating nutrient assimilation and boosts the functioning of the digestive system, which is helpful in alleviating bowel problems and digestive disorders [9]. Lemongrass contains substances that are used to alleviate muscle pain, reduce fever, and to stimulate uterus and menstrual flow [10].

# 2. Materials and Methods

## 2.1. Strains of Bacteria

The following strains of bacteria were used: *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Aeromonas hydrophila, Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis, Klebsiella pneumonia and Streptococcus agalactiae*. All strains of bacteria were isolated from clinical sample and maintained in laboratory fridge through regular subcultures.

### 2.2.1. Broth Dilution Method

At first, two tubes were prepared where one of them contain 5 ml of Brain Heart Infusion Broth (BHIB) and another tube contained mixture of 4ml of BHIB with1ml of tested oil. Bacterial suspension matched with McFarland 0.5 and transferred 10 µl per tube prepared earlier. After mixing the broth and suspension well incubated the tube at 37°C for 24 hours. After 24 hours 900 µl of saline was taken separately in sets of 6 tubes for broth and 4 tubes for broth with oil. One hundred microliters of bacterial suspension from broth were added to the 1st tube and 100 µl solution was transferred to the 2nd tube and this procedure was repeated till 6th tube. Similarly, 100 µl of bacterial suspension from broth with oil was added to the 1st tube and 100 µl solution was transferred to the 2nd tube and this procedure was repeated till 4rd tube. Before transferring the solution every tube was subjected to vortex for uniform mixing. As the study planned to detect inhibition rate in two different time interval so the previous step needed to repeat at 6 hours and 24 hours incubation.

### 2.2.2. Detection of Inhibition Percentage

After specific incubation period 100 µl of the samples was spread on the agar plate containing nutrient agar from each diluted both broth and broth with oil tube. One hundred microliter from original tube containing oil and broth was spread on agar plate. All the plates were incubated at 37°C for 24 hours. CFU in Oil mixed with broth and CFU in broth of each spread plate was counted and compared. Rate of inhibition in case of every diluted tube was then calculated and averaged to detect actual inhibition Percentage.

## 2.3. Agar Well Diffusion

Fresh subculture plates, incubated for 24 hours, were placed under the laminar chamber and used to make standard bacterial suspensions in labeled test tubes. Next, an autoclaved cotton swab was used to perform lawn culture for the uniform growth of bacteria. After that, a cork borer was dipped into the agar to make three holes or wells in the media. Each well was labelled and accordingly filled with 50 microlitres of TTO, ECO, LGO and Cefepime disc as positive control. The plates are incubated for 24 hours at 37°C, after which clear zones were formed around the control disc and the extracts which gave positive results. These inhibition zones were measured in millimetres using a ruler and recorded. All antimicrobial tests were repeated twice and the average of the inhibition zones is noted. An activity index was calculated from the results to measure the relative efficacy of the fruit extracts. The following formula was used:

$$\text{Activity Index}(\text{AI}) = \frac{\text{Zone of Inhibition of Essential Oil}}{\text{Zone of Inhibition of Cefepime}}.$$

# 3. Results and Discussion

Essential oils have great medicinal benefits as they contain the essence of herbs and flowers in concentrated form. The aroma molecules are very potent organic plant chemicals that make the surroundings free from disease, bacteria, virus and fungus. Their versatile character of antibacterial, antiviral, anti-inflammatory nature along with immune booster body with hormonal, glandular, emotional, circulatory, calming effect, memory and alertness enhancer, is well documented by many scientists [11]. It's known to everyone that most antibiotics no longer work; infections are getting harder to cure. Hence, it is high time to find alternative to antibiotics from natural sources.

## 3.1. Inhibition percentage

$$\text{CFU} = \frac{\left(\begin{array}{l}\text{Number of colonies}\\ \times\text{reciprocal of the dilution factor}\end{array}\right)}{\text{Volume of plated suspension}}$$

$$\text{Inhibition percentage} = \{1-\left(\frac{\text{CFU in oil Dilution}}{\text{CFU in broth Dilution}}\right)\}\times 100.$$

The results of our investigation showed that in most of the cases the inhibition percentage of tested essential oils against selected pathogenic bacteria for this study exhibits greater results after 24 hours incubation than 6 hours incubation.

Tea Tree oil shows great promise as an antimicrobial agent than Eucalyptus oil and Lemongrass oil according to

the results of our investigation. J. May *et al*., (2000) researched based on the time-kill approach, determined the killing rate of tea tree oil against several multidrug-resistant organisms, including MRSA, glycopeptide-resistant *enterococci*, aminoglycoside-resistant *klebsiellae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, and also against sensitive microorganisms. A rapid killing time (less than 60 min) was achieved with both tea tree oils (standard and chemically cloned) with most isolates, but MRSA was killed more slowly than other organisms [6]. In this study, efforts were made to determine the effectiveness of incubation period on Inhibition percentage.

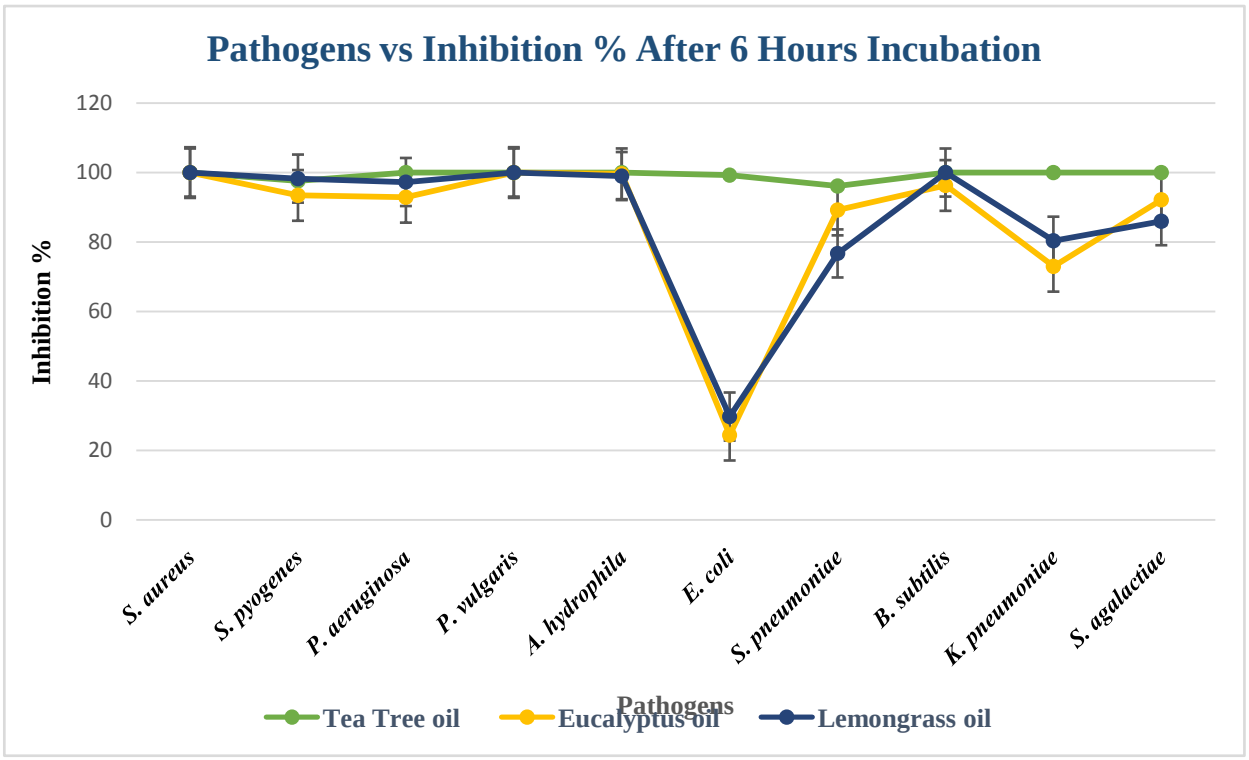


**Figure 1.** Inhibition percentage of Essential oils after 6 hours incubation against pathogenic bacteria. Vertical bars indicate Standard deviations from the mean

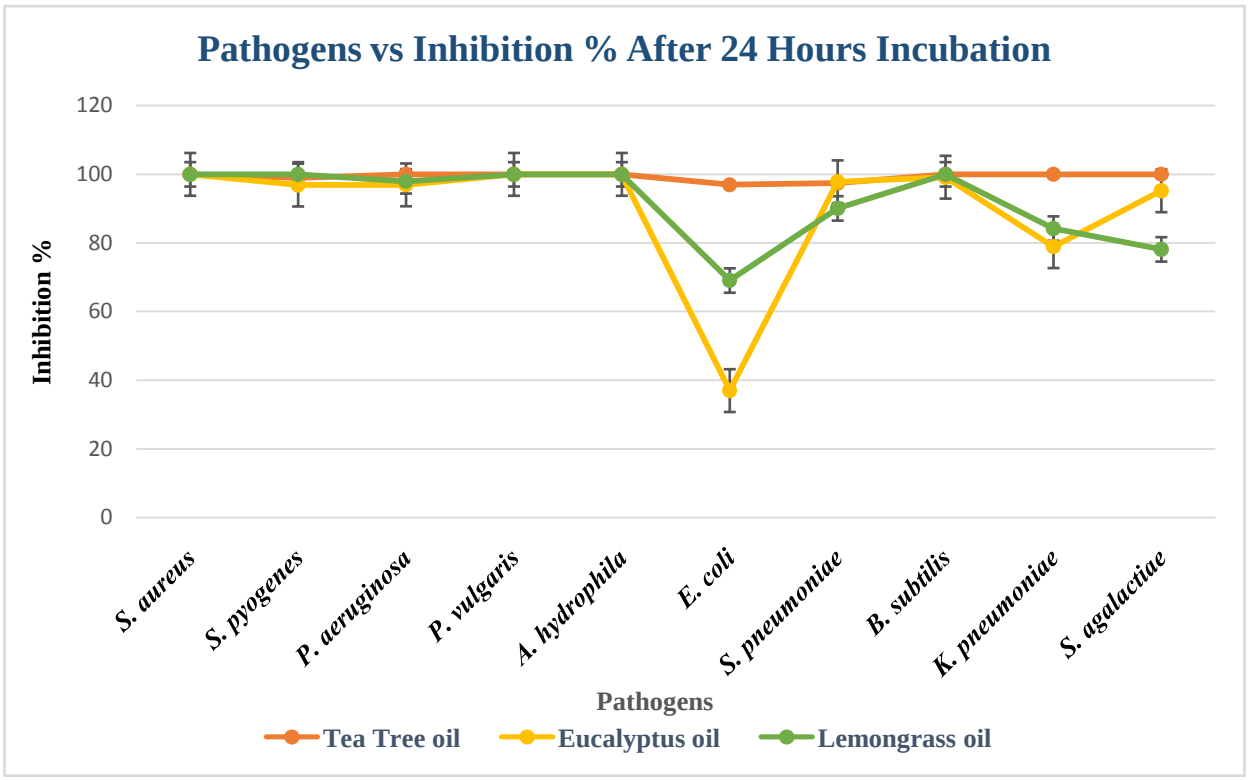


**Figure 2.** Inhibition percentage of Essential oils after 24 hours incubation against pathogenic bacteria. Vertical bars indicate Standard deviations from the mean

## 3.2. Antimicrobial Activity of Essential oils

Tea tree oil proved to be the strongest antimicrobial agent in this research. In fact, the highest inhibition zones in our entire investigation, 36.3 mm was that of TTO, against *A. hydrophila*, appearing 1.1 times stronger than the control antibiotic Cefepime. Besides that, TTO showed significant activity against all the pathogens selected for this study. Conversely, ECO and LGO didn't exhibit satisfactory results; most of the bacteria exhibited very low degree of sensitivity except against *B. subtilis* and *S. agalactiae*. Fitzpatrick (2010) investigated effectiveness of Tea tree oil, fresh garlic, an industrial cleaner and deodorizer Quad 10, and mouthwash Listerine against five bacteria: *B. subtilis*, *E. coli*, *M. roseus*, *S. luteus*, and *S. marcescens*. TTO showed the most consistent inhibitory action with all bacteria, except *Sarcina luteus*, controlled to at least one centimeter radius when measured from the edge of the disk solution. Fresh garlic and Listerine had no effect on controlling the bacteria as they showed no or little zone of inhibition [12]. In this study, antibacterial activity of TTO was determined by tested on ten bacteria which helped to establish a steady comparison with another essential oils and antibiotics.

Carson *et al.* (2006), Carson and Riley (1995), Cox *et al.* (2001) Tea tree oil has shown inhibitory effects on bacteria with *E. coli* [13,14,17]. Similarly, Lee *et al.*, 2013 found that TTO presented dose-dependent inhibitory effects against the growth of *P. acnes* and *S. aureus*, while the inhibitory effects against *P. acnes* were stronger than those against *S. aureus* [16]. .In this experiment antimicrobial action of TTO was tested on other bacteria and moreover, this experiment also determined how TTO compared to other essential oils and conventional antibiotics thought to control bacteria.

## 3.3. Tea Tree Oil vs. Conventional Antibiotic

Aggarwal, (2006) in his research stated that the use of herbal medicine is becoming popular due to toxicity and side effects of antibiotics. This has led to sudden increase in the number of herbal drug manufacturing [15]. Another important section of this study was to establish comparative analysis of antimicrobial efficacy between TTO and conventional antibiotics against selected pathogenic organism. The purpose of this section was to find out a replacement of antibiotics which has nearly similar ability to control bacterial growth as antibiotics has several side effects and it was done by agar diffusion assay. Nine antibiotics and TTO involved in this segment. TTO appeared 30.6 mm ZOI against *A. hydrophila* which is greater than eight antibiotics out of tested nine antibiotics. Undoubtedly TTO proved itself as a great substitution of conventional antibiotics as TTO showed remarkable activity against all the pathogenic bacteria selected for this study. On the other hand, all the bacteria used in this study were resistant to only two antibiotics (Cefepime and Cefuroxime Sodium) out of nine antibiotics. The claimed statement presented by constructed table of average zone of inhibition in response to TTO and conventional antibiotic discs against selected bacteria for this study (Table 2).

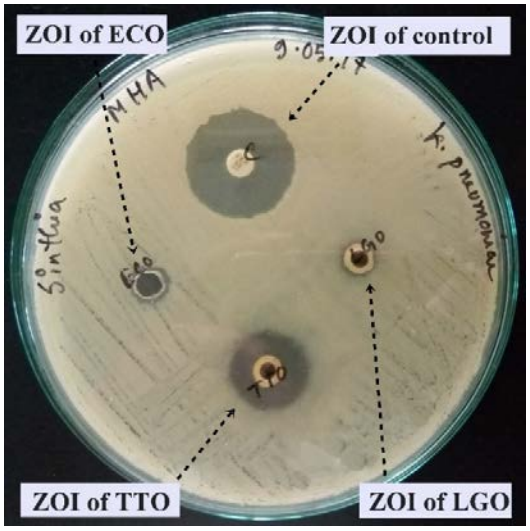


**Figure 3.** Antibacterial effect of Essential oils Against *K. pneumonia*

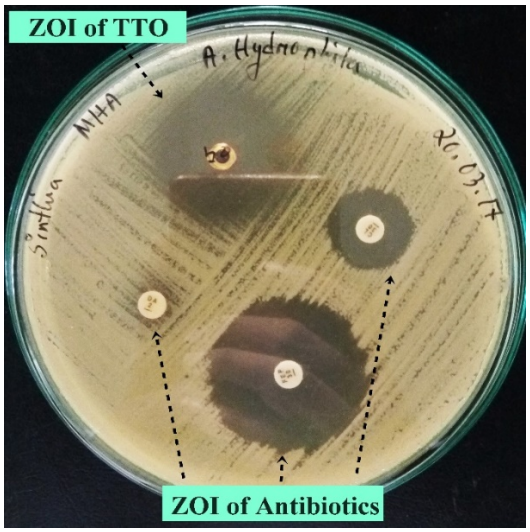


**Figure 4.** ZOI of Tea Tree Oils and Antibiotics against *A. hydrophila*

**Table 1. Antimicrobial Activity for Essential Oils**

| Bacteria | Mean ZOI* for Cef* (mm) | Mean ZOI for TTO (mm) | AI* for TTO (mm) | Mean ZOI for ECO (mm) | AI for ECO (mm) | Mean ZOI for LGO (mm) | AI for LGO (mm) |
|---|---|---|---|---|---|---|---|
| *S. aureus* | 27.3 | 16.7 | 0.6 | 12.3 | 0.4 | 11.7 | 0.4 |
| *S. pyogenes* | 34.3 | 20.7 | 0.6 | 13.3 | 0.3 | 15.0 | 0.4 |
| *P. aeruginosa* | 18.7 | 22.7 | 1.2 | 11.7 | 0.6 | 15.3 | 0.8 |
| *P. vulgaris* | 32.0 | 20.0 | 0.6 | 12.0 | 0.3 | 22.7 | 0.7 |
| *A. hydrophila* | 33.0 | 36.3 | 1.1 | 22.3 | 0.6 | 17.7 | 0.5 |
| *E. coli* | 32.3 | 24.3 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| *S. pneumoniae* | 34.7 | 19.7 | 0.5 | 13.7 | 0.3 | 14.7 | 0.4 |
| *B. subtilis* | 21.0 | 22.7 | 1.0 | 18.3 | 0.8 | 24.3 | 1.1 |
| *K. pneumoniae* | 25.0 | 19.3 | 0.7 | 9.3 | 0.3 | 0.0 | 0.0 |
| *S. agalactiae* | 10.3 | 17.3 | 1.6 | 14.0 | 1.3 | 16.3 | 1.5 |

*ZOI = Zone Of Inhibition, *Cef = Cefepime, *AI = Activity Index

**Table 2. Average zone of inhibition in response to TTO and conventional antibiotic**

| Bacteria | Name of antibiotics Zone of Inhibition (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rifampicin | Cefepime | Cephalexin | Erythromycine | Amoxycillin | Sulphamethoazole | Doxycycline | Cefuroxime Sodium | Clindamycin | Tea Tree Oil |
| *S. aureus* | 32.6 | 25.3 | 33.3 | 28.6 | 35.3 | 24.3 | 30.6 | 34.3 | 29.3 | 18.6 |
| *S. pyogenes* | 0 | 34.2 | 0 | 0 | 0 | 0 | 0 | 14.6 | 0 | 17.3 |
| *P. aeruginosa* | 0 | 31.6 | 0 | 0 | 0 | 0 | 0 | 16.3 | 0 | 18.6 |
| *P. vulgaris* | 17.6 | 35.3 | 19.3 | 14.3 | 0 | 21.6 | 27.6 | 28.3 | 0 | 21.3 |
| *A. hydrophila* | 8.6 | 31.3 | 19.6 | 0 | 0 | 24.3 | 21.3 | 23.6 | 0 | 30.6 |
| *E. Coli* | 37.6 | 30.3 | 37.6 | 0 | 38.6 | 22.3 | 34.3 | 35.3 | 24.6 | 15.6 |
| *S. pneumoniae* | 25.6 | 17.6 | 28.6 | 26.3 | 40.3 | 27.3 | 10.3 | 35.3 | 23.3 | 17.6 |
| *B. Subtilis* | 0 | 24.3 | 11.3 | 0 | 0 | 9.6 | 11.6 | 14.6 | 0 | 14.3 |
| *K. pneumoniae* | 26.6 | 10.3 | 35.6 | 30.3 | 36.6 | 38.3 | 35.3 | 16.6 | 28.3 | 16.6 |
| *S. agalactiae* | 20.3 | 18.3 | 24.3 | 24.3 | 32.3 | 29.6 | 16.6 | 31.3 | 24.3 | 16.3 |

### 3.4. Limitations

Wilkinson and Cavanagh (2005), and Carson *et al*., (2006) showed that TTO presented better antibacterial activity toward anaerobic bacteria than aerobic bacteria. They used mass spectrophotometry to separate two major components, terpinen-4-ol and 1,8-cineole, were used to evaluate skin toxicity by a single topical application [13,18]. On the other hand, in our study, the components were not separated through mass spectrophotometry as they did in their research and also they had applied TTO directly on erythema and edema instead of pathogenic bacteria which was not done in this study. Hammer *et al*., (2006) concluded that it may be used externally in its diluted form by the majority of individuals without adverse effect (provided oxidation is avoided) [19]. Topical application of high concentrated TTO can cause adverse reactions like skin irritation, allergic contact dermatitis, systemic contact dermatitis, erythema multiform like reactions, and systemic hypersensitivity reactions [19, 20]. We didn't provide any constructed information about appropriate dose which will treat infection without any kind of adverse effect. Therefore, this study could have been better if we could separate components through spectrophotometry and applied essential oils directly with ascertained dose on bacterial infection.

### 3.5. Further Scope

As the antimicrobial efficacy of the tested essential oil have been established, further research is required keeping the limitations in mind. In this study, we have used commercial essential oils since preparing fresh oil wasn't feasible in our country. This results could be compared with freshly prepared essential oils. Analysis by spectrophotometry could help isolate and identify the major components contributing to the antimicrobial properties of the essential oils. In our investigation we tested essential oils on selected pathogenic bacteria those are responsible for bacterial infection. These essential oils would be more acceptable for treatment if the tested oils were applied directly on infection area with safe doses instead of individual bacteria.

## 4. Conclusion

Slowly, science is catching up in explaining why tea tree oil is such an effective antimicrobial agent. In this study, it's been proven that Tea Tree oil has noticeable antimicrobial activity against bacteria which are responsible for bacterial infection in compared to Eucalyptus oil, Lemongrass oil and conventional antibiotics. In the final analysis, the potential of Tea Tree Oil to be used as natural antimicrobial agent is recommendable as antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aeromonas hydrophila*, *Escherichia coli*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Streptococcus agalactiae* were demonstrated. The use antibiotics would never be in the first place. Tea tree oil itself as fully effective against all the bacteria used in this study. The development of Tea Tree oil would be a great alternative to conventional antibiotics against bacterial infections.

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