

## EVALUATION OF ANTIBACTERIAL EFFICACY OF LEMON GRASS OIL, TEA TREE OIL, EUCALYPTUS OIL AND LEMON JUICE, AS A HAND SANITIZER

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### ABSTRACT

**Background-**Many studies have shown that understaffing and increased workload is risk factor for health care-associated epidemics and the goal of hand hygiene is to obtain sufficient reduction of microbial counts on the skin to prevent cross-transmission of pathogens among patients. There are also studies showing herbal hand sanitizers having significant bacteriostatic effect on hands.

The study will be undertaken to assess the antibacterial efficacy of lemon juice and three different natural oils.

**Materials and Method:** Three groups consisting of health workers (group I, group II, group III and Group IV) were included in the study. Sterile cotton swab sticks were used to take swabs from both hands and swabs were inoculated on the part of the agar plates marked pre application on to Cystein lactose electrolyte deficient agar plates. Lemon juice and natural oils

(Eucalyptus, Tea tree oil and Lemon grass) was asked to rub the thoroughly on the palms. Later after 1 min, swabs were collected again from the palms and inoculation was done on the respective agar plates, in the part marked post. This procedure was repeated for six consecutive days.

**Results:** Lemon juice was slightly better than lemon grass oil, tea tree oil and eucalyptus oil in eradicating microbes from the hands of health care workers and also lemon grass oil showed better antibacterial efficacy than eucalyptus oil and teatree oil. **Conclusion:** The household herbal oils and fresh extracts can be used in preventing spreading of infections, namely by its use as an antiseptic for topical and for hand cleaning.

**Key words:** Hand sanitizer; Antibacterial; Plant extracts; Lemon juice; Eucalyptus oil, Lemon grass oil, tea tree oil.

### INTRODUCTION

Nosocomial infections are the result of three factors occurring in tandem: high prevalence of pathogens, high prevalence of compromised hosts, efficient mechanisms of transmission from patient to patient. The hands of health care workers are the primary mode of transmission of these multidrug-resistant pathogens and infections to patients.<sup>1</sup> Normal human skin harbours bacteria (between 10<sup>2</sup> and 10<sup>6</sup> CFU/cm<sup>2</sup> ) and during daily activity, health care workers progressively accumulate microorganisms on their hands from direct patient contact or contact with contaminated environmental surfaces and devices.<sup>1</sup> Though proper hand hygiene

is the single most important, simplest, and least expensive means of preventing health care-associated infections and the spread of antimicrobial resistance; but, unfortunately poor hand-hygiene practices are observed due to lack of scientific knowledge, unawareness of risks, misconceptions (eg, glove use obviates the need for hand hygiene), unavailability of hand hygiene facilities, understaffing and patient overcrowding.<sup>2</sup> Hand washing refers to the application of a nonantimicrobial or antimicrobial soap; and mechanical friction is generated by rubbing the hands together for 1 minute, followed by rinsing with water, and then drying thoroughly with a disposable towel. But in the current scenario of mechanized life style a consumer will always prefer readymade formulation of alcohol hand rub rather than hand washing.<sup>1</sup> Traditionally, microorganisms residing on the hands are divided into resident and transient flora. Transient flora (e.g. *Staphylococcus aureus*, Gram-negative bacilli, *Candida* species) colonizes the superficial skin layers for short periods, is usually acquired by contact with a patient or contaminated environment and these microorganisms are easily removed by mechanical means such as hand washing and are responsible for most health care associated infections and the spread of antimicrobial resistance.<sup>3</sup> In contrast to hand washing, the objective of alcohol hand-rub is a more effective and rapid reduction of skin flora by killing all transient flora and most resident flora.<sup>3</sup> According to World Health Organization (WHO) definition a medicinal plant, is a plant that can be used for therapeutic purposes and or its compounds be used as a pioneer in the synthesis of semi-synthetic chemical drugs. Plants are still considered to be the potential source of medical compounds. Many countries to treat oral health and many disease especially infectious diseases including diarrhea, fever and cold are using plants traditionally and in addition to this, many recreational compounds used in traditional medicine have plant root. To overcome the increasing number of bacterial strains resistant to various antibiotics, many attempts have been done to use the antimicrobial potential of plants. Many resistant strains such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Staphylococcus* and *Enterococcus* has caused problems in treating infections caused by these bacteria. Studies on extracts prepared from collected plants with antimicrobial compounds having different mechanisms of action against resistant microbial strains are of clinical importance.<sup>5</sup> Hence this study was performed to assess the antimicrobial efficacy of four concentrated natural plant extracts such as eucalyptus oil (*Eucalyptus globules*), lemon grass oil (*Cymbopogon flexuosus*), tea tree oil and fresh juice of lemon (*Citrus Limon*) as hand sanitizers. A tall, evergreen tree, Eucalyptus is one of the world's important and most widely planted genera native to Australia and Tasmania, successfully introduced worldwide. Eucalyptus species are well known as medicinal plants because of their biological and pharmacological properties. In the international pharmacopeia, the most important and represented species, however, is *Eucalyptus globulus* (*E. globulus*) which is the main furnisher of essential oils. These essential oils are in great demand in the market, for its various applications such as, as anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge, fumigant, hemostat, inhalant, insect repellent, preventitive, etc. The antibacterial activity of Eucalyptus extracts has been due to the components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool,  $\beta$ - pinene,  $\gamma$ -terpinene,  $\alpha$ - terpinol, alloocimene and aromadendrene. The essential oils from the leaf of *E. globulus* showed varying degrees of antibacterial activity against two clinical isolates. From the above experiment it can be inferred that extract suggest significant growth inhibiting effects on Gram-positive (*E. coli*) and Gram-negative bacteria (*S. aureus*). The efficacy of leaf oil of *E.*

*globulus* against these microorganisms may provide a scientific ground for the application of the herb in the prevention and treatment of bacterial infections The results of this study

present the herb as a good candidate to explore new alternative antibacterial agents to combat pathogenic microorganisms.<sup>6</sup>

*Cymbopogon citrates* (DC.) stapf, commonly known as lemongrass and other *Cymbopogon* species is a tall, coarse grass with a strong lemon taste. It is a perennial herb widely cultivated in the tropics and sub-tropics, designates two different species, East Indian *Cymbopogon flexuosus* (DC.) stapf and West Indian, *Cymbopogon citratus* (DC.) stapf. The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties. Further, many workers had reported about the antibacterial activity of lemongrass oil against a diverse range of organisms comprising gram positive and gram negative organism, yeast and fungi. The chemical composition of the oil analyzed by gas chromatography (GC)/mass spectrometry (MS) identified fifteen components where neral (39.0%), geranial (33.3%), limonene (5.8%) and geranyl acetate (4.2) were the most abundant constituents.<sup>7</sup> Lemon is an important medicinal plant of the family *Rutaceae*. Different parts (viz., leaves, stem, root and flower) of Lemon is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of against clinically significant bacterial strains. Biological activities including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities are due to the presence of Citrus flavonoids. They function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation. In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses.<sup>8</sup>

### Materials and Method

The present study was an open, noncomparative prospective study. Twelve healthy volunteers each without any clinical signs of dermal abrasion, trauma and infection were included in the study. Sterile cotton swab sticks were used to take swabs from both hands, including fingers. Sterile CLED (Cystein lactose electrolyte deficient) agar plates were serially numbered and were divided into two halves and one part was marked as pre application and other part were marked as post application.

The swabs were inoculated on CLED agar on the part marked pre application. Later approximately 0.5 ml of three oils (eucalyptus oil, lemon grass oil, tea tree oil) and lemon juice were squeezed out on the palms of the subjects and they were asked to rub the gel thoroughly on the palms, back of the hands, fingers until the hands became dry. After one minute of application the swabs were taken by the same method and inoculation was done on the respective agar plates, in the part marked post. The agar plates were incubated in an incubator at 37°C for 24 to 48 hours. The smear prepared from the plates was stained by Gram's stain and were examined for bacterial presence. The same procedure was repeated for six consecutive days on all subjects. The microorganisms were classified into either GPC (gram positive cocci), GPB (gram positive bacillus), GNC (gram negative cocci), GNB (gram negative bacillus) and these isolated organisms were identified by biochemical reactions. The predefined primary efficacy parameter was the microbiological count percent reduction for following microorganisms after one minute hand rub: GPC (Gram positive cocci), GNB (Gram negative bacilli), GPB (gram positive bacilli), MSSA (Methicillin sensitive staphylococcus aureus), E. Faecalis (figure 2), CONS (Coagulase Negative Staphylococci), NFGNB (Non fermentative gram negative bacilli), micrococci, K. Pneumonia (figure 3) and Candida species were isolated.

## Results

On assessing the effect of eucalyptus oil as hand sanitizer on the microbes present on hands of three health care workers, it was observed that there was considerable reduction in the growth of micrococci. Certain microorganism such as, GPC, GNB, GPB, MSSA, E. faecalis were completely eliminated after 6 days whereas, CONS and NFGNB were unaffected after application of Eucalyptus oil as hand sanitizer (Table 1).

EUCALYPTUS OIL						
No of days	Health care worker 1		Health care worker 2		Health care worker 3	
	Pre application	Post application	Pre application	Post application	Pre application	Post application
1.	Micrococci	Reduced growth	Micrococci	Reduced growth	MSSA	MSSA No growth
2.	GPC	No growth	CONS	CONS	E. Faecalis	No growth
3.	GNB	Reduced growth	CONS	CONS	MSSA	no growth
4.	Acinetobacter	Acinetobacter	GPC	Reduced growth	GPC	Reduced growth
5.	GNB	No growth	Micrococci	Reduced growth	GPC	Reduced growth
6.	Micrococci	Reduced growth	NFGNB	NFGNB	GPC	Reduced growth

**Table 1: Shows efficacy of eucalyptus oil on microorganism isolated from study subjects.**

LEMON GRASS OIL						
No of days	Health care worker 1		Health care worker 2		Health care worker 3	
	Pre application	Post application	Pre application	Post application	Pre application	Post application
1.	Micrococci	Reduced growth	Micrococci	No growth	MSSA	Reduced growth
2.	K.pneumonia	No growth	GPC	Reduced	NFGNB	No

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	e			growth		growth
3.	K.pneumonia e	No growth	NFGNB	No growth	NFGNB	No growth
4.	NFGNB	No growth	CONS	No growth	NFGNB	No growth
5.	CONS	No growth	GNB	No growth	Micrococci	No growth
6.	Micrococci	Reduced growth	GNB	No growth	Spores	Spores

**Table 2: Shows efficacy of lemon grass oil on microorganism isolated from study subjects.**

Spores, NFGNB and candida species were unaffected with the use of lemon juice when used as a hand sanitizer, but there was considerable reduction of micrococci and certain microorganism such as micrococci, GPC, GNB, GPB were completely eliminated after 6 days (Table 3).

FRESH LEMON JUICE						
No of days	Health care worker 1		Health care worker 2		Health care worker 3	
	Pre application	Post application	Pre application	Post application	Pre application	Post application
1.	Budding yeast cell (Candida)	Budding yeast cell (Candida)	Micrococci	Reduced growth	MSSA	No growth
2.	Budding yeast cell, (Candida)	Budding yeast cell, (Candida)	Micrococci	No growth	GNB	Reduced growth
3.	GPC	No growth	Spores	Spores	No growth	No growth
4.	GPC	No growth	GPB	Reduced growth	No growth	No growth
5.	Micrococci	No growth	Micrococci	No growth	NFGNB	NFGNB
6.	GPC	No growth	Spores	Spores	Spores	Spores

**Table 3: Shows efficacy of fresh lemon juice on microorganism isolated from study subjects.**

TEA TREE OIL						
No of days	Health care worker 1		Health care worker 2		Health care worker 3	
	Pre application	Post application	Pre application	Post application	Pre application	Post application
1.	Micrococci	Reduced growth	Micrococci	Reduced growth	MSSA	Reduced growth
2.	K.pneumoniae	Reduced growth	GPC	Reduced growth	NFGNB	No growth
3.	K.pneumoniae	Reduced growth	NFGNB	Reduced growth	NFGNB	No growth
4.	NFGNB	Reduced growth	CONS	Reduced growth	NFGNB	No growth
5.	CONS	Reduced growth	GNB	Reduced growth	Micrococci	No growth
6.	Micrococci	Reduced growth	GNB	No growth	Spores	Spores

**Table 4: Shows efficacy of tea tree oil on microorganism isolated from study subjects.**

When overall efficacy of three plant extracts were compared it was found that lemon juice was slightly better than lemon grass oil eucalyptus oil and tea tree oil in eradicating microbes from the hands of health care works and also lemon grass oil showed better antibacterial efficacy than eucalyptus oil and tea tree oil (Table 5).

Natural plant extracts	NFGNB	GPC	CONS	SPORES	CANDIDA species
<b>Eucalyptus oil</b>	No effect	Eliminated	No effect	-----	-----
<b>Lemon grass oil</b>	Eliminated	Reduced growth	Eliminated	No effect	-----
<b>Lemon juice</b>	Eliminated	Eliminated	Eliminated	No effect	No effect
<b>Tea Tree Oil</b>	Reduced growth	Reduced growth	Reduced growth	No effect	-----

**Table 5: Shows comparison of overall effect of three natural plant extracts on microorganisms isolated from hands of health care workers.**

## DISCUSSION

The goal of hand hygiene is a sufficient reduction of microbial counts on the skin to prevent cross-transmission of pathogens among patients. According to recently revised hand-hygiene guidelines, the use of an alcohol-based hand rub (ABHRs) is the preferred method of hand hygiene. Given the health effects of alcohol ingestion, it can be assumed that alcohol absorption throughout inhalation and in a lesser extent via dermal contact might induce the negative health effects in the long term. Some healthcare workers have complained of an unpleasant smell associated with the use of alcohol rubs (ABHRs).<sup>9</sup> According to a study by Bessonneau and Thomas (2012)<sup>9</sup> the use of ABHRs leads to the absorption of very low doses of alcohol, but sudden and repeated inhalation of high alcohol concentrations raises the question of possible adverse health effects. Latest trend being the use of Herbal Hand Sanitizers have shown reliable efficacy in eliminating all common bacteria's from the hands and on the surface of the inanimate objects and is negative for any adverse reaction like skin drying or irritation.<sup>9</sup> Mondal et al (2004)<sup>1</sup> tested the efficacy and safety of pure Hands herbal hand sanitizer in healthy volunteers and inanimate objects before and after application for 7 consecutive days. Their results revealed that (*Escherichia coli*, *Proteus mirabilis*, *Shigella sonnennei*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) were eliminated, from the hands of all volunteers over the period of 7 days. The authors concluded that PureHands Herbal Hand Sanitizer has a significant bacteriostatic effect on the hands surface and on the surface of inanimate objects.<sup>1</sup> Hence the present study was undertaken to assess the efficacy of common household plant extracts as a hand sanitizer in health care workers for 6 consecutive days. Warnke et al (2013)<sup>2</sup> tested the antimicrobial efficacy of Eucalyptus, tea tree and lemongrass oils on Vancomycin resistant *Enterococcus* (VRE) species, Methicillin resistant *Staphylococcus aureus* (MRSA), *E. coli*, *K. Pneumonia*. Eucalyptus oil was in no case the most potent inhibitor, however it did perform relatively moderately against all pathogens; its greatest zone of inhibition was against MRSA (8-14 rnm) and the smallest was against *P. aeruginosa* (0-3 rnm). Lemongrass oil demonstrated the largest zones of inhibition out of all the substances against VRE(13-18 rnm) and MRSA(20-29 mm), yet showed relatively moderate inhibition against *E. coli* ESBLstrains (2-13 rnm) and poor inhibition against MR *P. Aeruginosa* (0- 2 mm) and *Klebsiella* ESBL strains (2- 4 mm).<sup>2</sup> The authors concluded that such oils may be a promising force as supportive topical treatment in the case of stubborn and multi-resistant hospital-acquired infections in the clinical setting.<sup>2</sup> In a study done by Mulyaningsih (2011) showed that *Eucalyptus globus* fruit extract exerted the most pronounced activity against MRSA.<sup>10</sup> Trivedi & Hotchandani (2004)<sup>11</sup> tested different concentrations of eucalyptus oils on agar plates containing *Klebsiella*, *Proteus*, *Pseudomonas*. Different doses of oil of eucalyptus, i.e., 5, 10, 25, 50, 100, 150 and 200 micro liters were poured in the wells with the help of a sterile micropipette. The results of this study showed that, *E. Coli* and *Klebsiella* spp. were sensitive to 5 µl; *S. aureus* to 25 µl while *Pseudomonas* and *Proteus* spp. required 50 ml of eucalyptus oil. It was further observed that with an increasing dose of oil of eucalyptus, the resulting diameter of the zone of inhibition increased for all the organisms. The results of the study revealed that oil of eucalyptus has antibacterial activity against Grampositive as well as Gram negative bacteria resistant to commonly used antimicrobial agents.<sup>11</sup> The chemical compositions of the leaf oils of various *Eucalyptus* species had been reported. The major component is 1,8- cineole, but a main contributor for the bioactivity was assumed to be -terpineol, which showed eight-fold higher activity than 1,8- cineole against *S. aureus*. 1,8- Cineole had not been reported as an active principle in other eucalyptus oils.<sup>6</sup> The present study showed considerable reduction in the growth of micrococci and certain microorganism such as, GPC, GNB, GPB, MSSA, *E. Faecalis* were completely eliminated after 6 days. Plant et al (2015)<sup>12</sup> tested antimicrobial activity of 31

types of essential oils by disk diffusion method on four diverse strains of bacteria *E. coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Serratia marcescens* and found that lemon grass oil showed better bacterostatic activity against the four selected strains of microbes compared to Eucalyptus oil and lemon extract.<sup>12</sup> In a study by Naik et al. (2010)<sup>7</sup> lemongrass oil was found effective against many test organisms except *P. aeruginosa*. Gram positive organisms (*S. aureus*, *B. cereus* and *B. subtilis*) were found more susceptible than gram negative organisms (*E. coli*, *K. pneumoniae*, *P. aeruginosa*). The antibacterial activity was found progressively increasing with the increase in concentration of oil. The maximum effect was found at 30% concentration and minimum effect was observed at 5% concentration of oil.<sup>7</sup> Results with methanol extract of lemon grass plant prevented bacterial growth of *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* which with increasing concentration, their antibacterial effect also increased. This showed that most plant extracts have inhibition effect on Gram positive bacteria and little effect on Gram negative bacteria.<sup>5</sup> This inhibition effect can be related to its active compounds that include: steroids and terpenoids, alkaloids, citral, geraniol, flavonoids, eugenol, citronolal, geranyl acetate, beta cariofilin, tannins, phenolic compounds, saponin and farnesul.<sup>5</sup> In another study by Soares et al.,<sup>13</sup> *C. citratus* essential oil exhibited high antifungal and antibacterial properties. Although inhibition was higher in Grampositive bacteria and effect in Gramnegative bacteria was also shown. At the tested concentrations of the oils an extensive *Candida* spp. inhibition was noted. This effect of inhibition was particularly pronounced in *Non Candida Albicans* (NCA) species. The activity of Lemon grass oil over Gram-positive multidrug- resistant bacterial strains and over *C. albicans* and NCA species indicated a potential role in preventing spreading of hospital infections, namely by its use as an antiseptic for topical and for hand cleaning.<sup>13</sup> Microorganism in the present study such as *K. Pneumonia*, NFGNB, CONS, GPC, GNB, GPB were completely eliminated after 6days whereas spores were unaffected with use of lemon grass oil as hand sanitizer. Scientific name of lemon is *Citrus limon*, its plant family is Rutaceae and the main component present in lemon is Limonene. The most important active ingredients of lemon are monoterpenes such as limonene,  $\gamma$ -terpinene,  $\beta$ -pinene and the aldehydes geranial and neral.<sup>14</sup> According to a study done by Maruti et al, (2011)<sup>8</sup> shows that the peel of lemon is not only an astringent but also is a good antimicrobial agent. the authors state that since certain skin flora like *Pseudomonas* and *Micrococcus* can grow in presence of sebum, especially when it is secreted in excess (in certain person), and cause purulent skin infections and serve as a predisposing factor for other types of skin infections like acne, simple use of lemon juice can prevent such types of infections and could help in keeping a good and healthy skin.<sup>8</sup> Nada et al (2013)<sup>3</sup> tested pure culture of 6 types of gram positive and 8 types of gram negative and one yeast isolates by different types of lemon extracts, extracts of peel juice of citrus, and sweet lemon. The juice of citrus lemon had antimicrobial activities more than other types of extracts. The juice of lemon showed inhibition against Gram positive strains such as *S. Aureus*, *S. Agalactiae*, *Enterococcus faecalis*, *S. Pneumonia*, *S. Pyogens*. Gram negative bacerias that were inhibited by Lemon juice were *E. Coli*, *Enterbacter aerogenes*, *K. Pneumonia*, *Proteus Species S. Typhi* *Acinetobacter Species* and *M. Catarrhalis*.<sup>3</sup> To our surprise the lemon juice extract was effective against *Candida albicans* and in the present study there was no action against the *Candida*. This emphasizes on further evaluation of antifungal efficacy of lemon juice. They found the reduction in gram positive as well as gram negative bacteria. They concluded that lemon extracts have an important role as antimicrobial effect.<sup>3</sup> Olsen et al (1994)<sup>15</sup> tested the antibacterial efficacy of vinegar, lemon juice and baking soda on *serratia marcescens* present on bathroom soil. They found that vinegar and to a lesser extent lemon juice was effective



against this bacterium presumably because of its very low pH. The authors stated that vinegar outperformed lemon juice because acetic acid is known to be a more effective bactericidal than citric acid.<sup>15</sup> With the use of lemon juice in the present study, spores, NFGNB and *Candida* species were unaffected, but certain microorganism such as micrococci, GPC, GNB, GPB were completely eliminated after 6 days. When overall efficacy of three plant extracts were compared in the present study, it was found that lemon juice was slightly better than lemon grass oil and eucalyptus oil in eradicating microbes from the hands of health care workers and also lemon grass oil showed better antibacterial efficacy than eucalyptus oil. In recent years, considerable progress has been made in understanding more fully the responses of different types of bacteria (mycobacteria, nonsporulating bacteria, and bacterial spores) to antibacterial agents. Resistance to bactericidal agents can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids (self replicating, extrachromosomal DNA) or transposons (chromosomal or plasmid integrating, transmissible DNA cassettes). Intrinsic resistance is demonstrated by GNB, bacterial spores, Mycobacteria, and, under certain conditions, Staphylococci.<sup>16</sup> With growing concerns about the development of biocide resistance and cross-resistance with antibiotics, it is clear that clinical isolates should be under continual surveillance and possible mechanisms should be investigated.<sup>16</sup>

## CONCLUSION

Although the oils used in our study is effective against the microorganism such as NFGNB, CONS and GPC. Lemon juice and lemon grass oil had almost same effects on these microorganisms as compared to eucalyptus oil and tree tea oil. But these oils were ineffective against spores and *Candida*. Lemon juice can be used as efficient hand sanitizer but since the sample size was very small, further research and development of these oils in consumer products is warranted.

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