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## A comparative Study in the Antibacterial effect of Eugenol as Hand Wash Material with two Types of Soap

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### Abstract

Hands have consistently been an important vehicle in the transmission of disease<sup>(1)</sup>. Thus, thorough hand-washing remains the single most important factor in preventing infection specially in hospitals and labs.

Twenty-nine non-clinical volunteers (do not work or come in contact with a clinical or hospital setting) that lacked visible skin injuries, eczema or apparent skin disease were used, those subjects were all tested by a material of each of the three used in the study weekly and laboratory tests were done pre- and post washing.

All three material were effective , Eugenol extract as effective as the bar and lotion soap. And this was confirmed statistically.

Eugenol has a great antibacterial action even in small concentration and this may be due to steroids and other constituents that are found in the composition of its volatile oil. High concentrations are more effective and this may give us a new revolution in prevention of bacterial infection.

**Key words: Eugenol, hand wash, lotion soap, antiseptic, antibacterial agent.**

### Introduction

Hands have consistently been an important vehicle in the transmission of disease<sup>(1)</sup>. Thus, thorough hand-washing remains the single most important factor in preventing infection<sup>(1)</sup>. While many studies have focused on the effects of repeated hand-washings in clinical settings, such as that performed before a surgical procedure<sup>(2,3)</sup>. In this study 3 materials were used in hand washing, these material are Eugenol oil, lotion soap and bar soap of known trade mark.

Botanical name for Eugenol is *syzygium aromaticum*. It is a tree of hot countries, grows at a height of 30 to 40

feet. It begins flowering in about seven years and continues to produce for another 80 or more years. It is a pyramidal ever green tree; bark smooth grey, leaves lanceolate; flower buds borne in small clusters at the end of branches , greenish, turning pink at the maturity, aromatic; then turn to brown with pin like appearance . In recent medicine, Eugenol prescribed as anti-pyretic, sterilizing agent, analgesic, curing skin ulcers, for headache, epilepsy, promote gastric function....etc.<sup>(4)</sup>. The antimicrobial activity of Eugenol was studied on 8 types of microorganisms, the results

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showed a well documented antimicrobial effect in addition to antifungal activity. Further studies showed the effect of dental materials that contains Eugenol in comparison with non Eugenol materials<sup>(5, 6, 7, 8)</sup>.

In this study we compared between the antibacterial effect of this plant extract with bar soap (lifebuoy®) and lotion soap (Palmolive®).

## Materials and Methods

Twenty-nine non-clinical volunteers (do not work or come in contact with a clinical or hospital setting to differentiate from those researches done on clinical and hospital settings) that lacked visible skin injuries, eczema or apparent skin disease were used. During washing and sampling, the wearing of rings and wristwatches was not allowed. The participants were also requested not to use an antibacterial soap eight hours prior to sampling and not to use any type of washing of one's hand one hour prior to sampling.

The study was conducted over a 3 week period in which three materials, Eugenol oil, lifebuoy bar soap (the antimicrobial agent is triclosan or chloroxylenol®) and the Palmolive® lotion soap that does not contain active antimicrobial agent.

Eugenol oil extraction is done by using another oil e.g. sunflower oil (because pure Eugenol oil is volatile); for this type of extraction there is the hot and cold methods, the hot method was chosen.

A 750 g of Eugenol flowers were collected for each 500 ml of sunflower oil. The oil and the flowers put all together in a container which is already placed over a pot contains boiling water, the system was heated kindly for 3 hours. The final mix is filtered the product is then kept in a dark, dry and clean containers until use. Each

week a different material was tested on all 29 volunteers. At seven day intervals, a pre-wash sample (to determine a baseline enumeration of bacteria for that day) and a post-wash sample were obtained.

To establish a baseline enumeration of bacteria, a pre-wash sample was collected using the "glove juice method" (3). A sterile non-powdered Nitrile glove (N-Dex, Best Manufacturing Company, Menlo, GA) was aseptically applied to the test subject's hand. Twenty-five ml of sterile Tryptic Soy broth, TSB, (Becton Dickinson, Sparks, MD) was then aseptically added to the gloved hand. The glove was secured to the wrist with self-adherent bandage (Medi-Rip, Conco Medical Company, Rock Hill, SC). The gloved hand was palpated on all surfaces for a period of 1 minute. During this process the subject also was asked to clench a fist twice and dislodged any material from underneath his/her fingernails. The glove juice was then aseptically transferred into a sterile microcentrifuge tube by means of a sterile disposable plastic pipette.

Sampling by the glove juice method assisted in the accurate representation of the natural flora on the subject's hands by collecting microorganisms from all surfaces of the hand. Along with the normal flora, this method also recovered potential pathogens residing on the skin.

The subject washed his/her hands with a test soap for a regulated two-minute period. With tap water running (35 to 37°C), the hands were rinsed and 2 ml of extract, soap or lotion soap was dispensed into the palm. The hands were thoroughly cleaned. Next, the right palm scrubbed the top of the left hand and vice versa. Next, the subject was asked to clean under the fingernails as much as possible. Finally, the hands were rinsed for 1

minute to ensure soap residue left on the hands was removed. The hands were then dried with paper toweling and the post-wash sample was immediately taken which is the same as pre-wash sampling.

One ml sample was taken from the recovered TSB "glove juice" (pre-wash and post-wash) and diluted in sterile TSB. Previous trials established optimal dilutions of 1:10, 1:100, and 1:1000. These dilutions were chosen to attain countable plates. Dilutions were made and 50  $\mu$ l of each solution was then spread plated onto TSA, (Becton Dickinson, Sparks, MD) plates. All processing of samples was executed within 10 minutes of collection. The plates were incubated at 35°C for 48 h before visual examination.

Bacterial counts were performed on countable plates that consisted of 30-300 bacterial colonies. Each colony was assumed to be one bacterium and thus bacterial numbers were converted to colony forming units (CFUs). To obtain CFUs/ml the number of colonies counted was divided by the dilution of the plate. The total number of bacteria was then enumerated and converted to log<sub>10</sub> values. Each unique colony type was also counted and re-struck for isolation on TSA plates. The isolated colonies were then characterized using a general classification scheme as illustrated in. Because previous studies indicated that catalase positive.

## Results

Gram positive cocci were the majority of bacteria found on human skin; these isolates were further differentiated into Staphylococcus and Micrococcus. Moreover, the intent of characterization was not to identify to the species level, but to estimate the prevalence of the different types of organisms found on normal skin.

## Statistical Analysis

The mean log<sub>10</sub> values of the pre-wash and post-wash samples for each washing material or soap were compared by paired t-tests to show whether the immediate reduction for each material was statistically significant. Using  $\alpha = 0.05$ , the samples from all three materials showed a significant difference between the pre and post-wash ( $p < 0.001$ ) as seen in Table 1.

A one-way ANOVA with repeated measures was then used to compare the log<sub>10</sub> bacterial reduction between all three of the materials used. The ANOVA analysis compared the reduction for each material in relation to the others. The analysis produced a P value of 0.67 that indicates that there is no statistically significant difference in the bacterial reduction between the three materials used.

## Discussion

No previous study was found that Eugenol oil or extract was used as hand wash material.

Each of the three materials provided a significant immediate reduction in the amount of bacteria on the hand following the hand-washing procedure. However, it was also found that there is no difference in the immediate effectiveness between the three materials. This supports the antiseptic theory of Eugenol<sup>(5, 10)</sup> and confirmed that there is no immediate difference between a regular lotion soap, a bar soap and this plant extract.

All materials tested significantly reduced the amount of bacteria on the hands following a hand-wash, all had similar reductions. This may be due to the mechanical action of vigorously rubbing soap and water together for at

least 10 seconds which is a major contributor to the cleansing process<sup>(9)</sup>.

In conclusion, the results of this study suggest the use of an antibacterial Eugenol extract in prevention of bacterial infection that is transmitted by hands. We must ask if there are another plant extracts that are efficient in the field of disinfection.

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Table 1. Pre-wash and post-wash bacterial log<sub>10</sub> counts [mean ± standard deviation (n)] as well as difference between pre-wash and post-wash [mean ±-standard deviation (n)], 95% confidence interval and P values for each material.

Mean ±SD (n)	Eugenol extract	Lifebuoy ® bar soap	Palmolive ® lotion soap
Pre- wash	4.87 ± 0.69 (27)	5.20 ± 0.99 (26)	4.97 ± 0.70 (25)
Post- wash	4.43 ± 0.67 (27)	4.78 ± 0.77 (26)	4.49 ± 0.73 (25)
Pre- wash- post- wash	0.44 ± 0.42 (27)	0.42 ± 0.44 (26)	0.48 ± 0.53 (25)
P value	<0.001	<0.001	<0.001