Antimicrobial Effects of Essential Oils on *Staphylococcus aureus*

MacKenzie M. Bryant, Katelynn M. Andera, and Bryan D. Monesson-Olson

Alfred State – SUNY College of Technology

Introduction

The use of essential oils has become more popular as researchers explore new treatments for antibiotic resistant bacteria. Beyond antibacterial properties, essential oils have antifungal and antiviral effects (Hyldgaard et. al, 2012). Tea tree oil has been shown to be useful in dental applications, acne treatments, tinea pedis (athlete's foot), and bacterial vaginosis (Carson et. al, 2006). Another essential oil, cinnamon bark oil, has shown synergistic properties with tea tree and other essential oils (Nuryastuti et. Al, 2009).

There are many essential oils and much information has yet to be discovered about them. Two essential oils that are said to exhibit antibacterial properties are saro (*Cinnamosma fragrans*) and patchouli (*Pogostemom cablin*). We believe that the use of essential oils can play a large role in the future in regards to seeking alternative ways to treat skin infections, including MRSA.

The goal of our research was to investigate the antibacterial properties of patchouli and saro *in vitro* against a common skin pathogen, *Staphylococcus aureus.* We set up a time kill assay in order to evaluate how quickly the oils inhibited growth of the bacteria. Our data suggests that there is an additive or synergistic effect when combining these two oils.

Methods

Stock organism

- *Staphylococcus aureus* stock culture was obtained by microbead inoculation (Presque Isle)

Time Kill Assay

- *Staphylococcus aureus* was inoculated into solutions containing different concentrations of essential oils, Tween 80, and distilled water
  - Concentrations of each oil(s): 1%, 2%, 3% and control (no oil)
  - Patchouli and saro combination 3% = 1.5% patchouli and 1.5% saro
- After 10, 30, and 60 minutes, 100-µL of the solutions were placed into an inactivation broth containing lecithin, yeast extract, and peptone

Serial Dilutions & Plating

- Serial dilutions of each inactivation broth were performed: 1:10, 1:100, and 1:1000 containing 0.9-g/L NaCl
- The original inactivation broth and serial dilutions were plated onto TSA agar plates and incubated, colonies were counted
- Plates that contained over 200 colonies were reported as "200+"

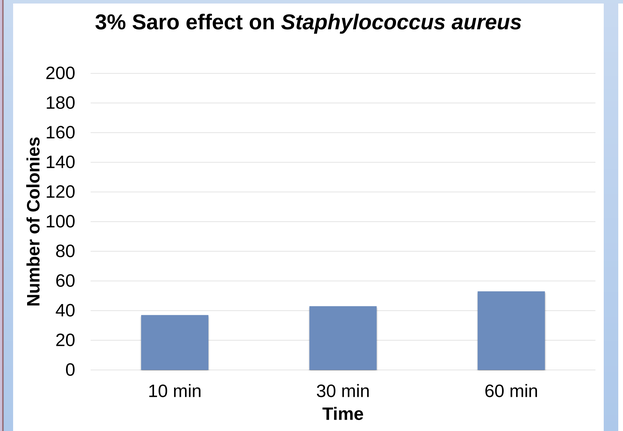
Results

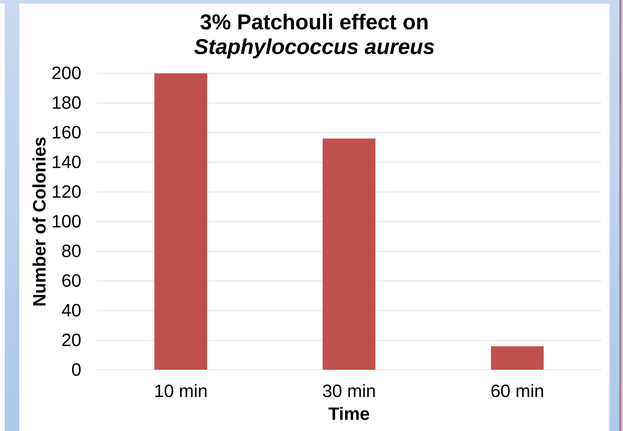
Saro 1%	10 min	30 min	60 min
UD	200 +	200 +	200 +
1:10	200 +	200 +	200 +
1:100	200 +	200 +	200 +
1:1000	28	198	183
Saro 2%	**10 min**	**30 min**	**60 min**
UD	200 +	200 +	200 +
1:10	200 +	200 +	200 +
1:100	200 +	200 +	200 +
1:1000	200 +	188	200 +
Saro 3%	**10 min**	**30 min**	**60 min**
UD	200 +	200 +	200 +
1:10	200 +	200 +	200 +
1:100	200 +	200 +	200 +
1:1000	37	43	53
Control	**10 min**	**30 min**	**60 min**
UD	200 +	200 +	200 +
1:10	200 +	200 +	200 +
1:100	200 +	200 +	200 +
1:1000	199	200 +	200 +

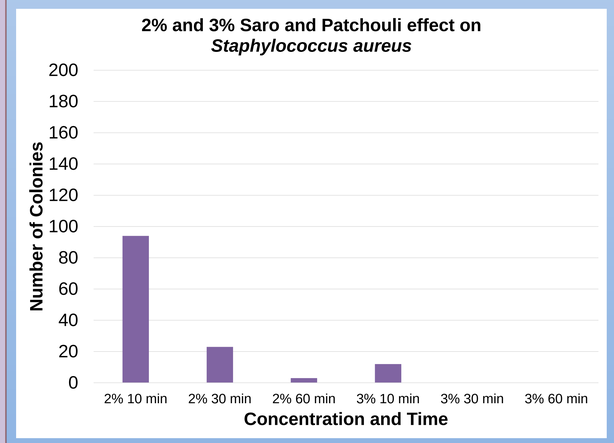
Patchouli 1%	10 min	30 min	60 min
UD	200 +	200 +	0
1:10	200 +	200 +	200 +
1:100	200 +	200 +	40
1:1000	200 +	195	2
Patchouli 2%	**10 min**	**30 min**	**60 min**
UD	199	200 +	71
1:10	200 +	182	27
1:100	40	41	5
1:1000	4	3	0
Patchouli 3%	**10 min**	**30 min**	**60 min**
UD	200 +	200 +	200 +
1:10	200 +	200 +	200 +
1:100	200 +	200 +	200 +
1:1000	200 +	156	16
Control	**10 min**	**30 min**	**60 min**
UD	0	0	0
1:10	0	0	8
1:100	0	0	0
1:1000	0	0	0

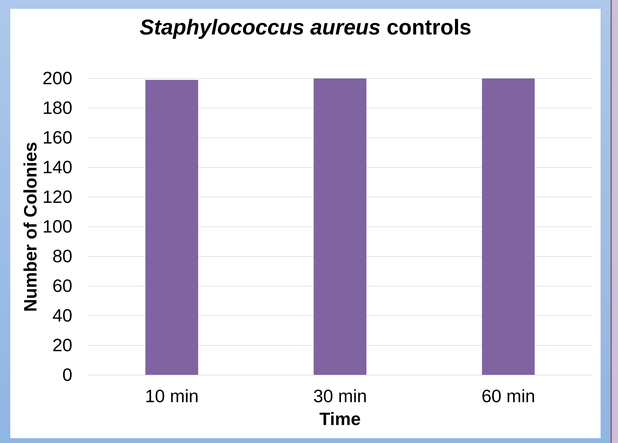
Saro + Patchouli 1%	10 min	30 min	60 min
UD	200 +	200 +	0
1:10	200 +	65	0
1:100	127	16	0
1:1000	20	2	0
Saro and Patchouli 2%	**10 min**	**30 min**	**60 min**
UD	200 +	200 +	200 +
1:10	200 +	200 +	88
1:100	200 +	95	21
1:1000	94	23	3
Saro and Patchouli 3%	**10 min**	**30 min**	**60 min**
UD	200 +	30	0
1:10	200 +	3	0
1:100	65	1	0
1:1000	12	0	0
Control	**10 min**	**30 min**	**60 min**
UD	200 +	200 +	4
1:10	200 +	200 +	200 +
1:100	200 +	200 +	200 +
1:1000	54	35	71

UD = Undiluted









Conclusions

Our preliminary data has shown that the essential oils patchouli and saro have antimicrobial properties. The addition of the 3% concentration of patchouli to the media caused a steep decline in the number of *Staphylococcus aureus* colonies over time. After one hour, nearly all bacterial growth was inhibited. The addition of 3% saro to the media inhibited *Staphylococcus aureus* growth, although the inhibition was greatest at 10 minutes. The antimicrobial properties of the saro seemed to have "worn off" as time passed after the initial inoculation, allowing more bacteria to grow.

When patchouli and saro were combined, bacterial growth was severely inhibited as time went on. After 60 minutes almost no colonies remained. Even at the 2% concentration, (1% patchouli, 1% saro) all growth was inhibited by 30 minutes when the the oils were put together in a blend at 3% concentration (1.5% patchouli and 1.5% saro). This could suggest additive or synergistic properties between the two oils.

Several issues make it difficult to interpret our data. The controls during the patchouli trial had no growth. We believe this error was due to inoculation of the bacteria too early after the test tubes and water were taken out of the autoclave. During the saro and patchouli combination trial, there was a high amount of growth in the 2% concentration. This issue could possibly be from oil inoculation errors or pipette errors. Lastly, some inactivation broth plates (S+P, control, 60 min) had little to no growth on them, their following dilutions had more growth. We believe this could have been caused by plating errors. We plan on repeating these experiments and eliminating these errors. As we continue our research, we will further investigate the potential synergistic properties of these two oils, testing more specific concentrations of oils (ex. 2.0%, 2.25%, 2.50%, etc.) will help up determine a minimum inhibitory concentration (MIC) value that can help us mathematically evaluate the level of synergism.

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Acknowledgements

We would like to thank Amy Werner and Michael Putnam for their help and knowledge with this project.