Research Article

Clove (*Syzygium aromaticum*) Extract has Antibacterial and Antifungal Effects against Human Scalp Microbes

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Submission Date: 13-06-2024; Revision Date: 14-07-2024; Accepted Date: 16-08-2024.

ABSTRACT

Background: Since ancient times, humans have treated themselves with herbs and found them effective against many diseases. Therefore, they transferred their experience to the next generations. However, utilizing these natural products was based on personal experience rather than scientific evidence. Thus, scientifically testing these assumptions is necessary. Clove (*Syzygium aromaticum*) is rich in phytochemicals, making it a good pain reliever and disinfection agent against many microbes, including those that might infect hair. **Materials and Methods:** This study screened microbes isolated from the human scalp and subjected them to different clove aqueous extraction concentrations. 6 volunteers provided samples from their scalps, and two microorganisms were found: *Staphylococcus aureus* and *Cryptococcus neoformans*. Clove extract was added at different concentrations-100%, 50%, 20%, and 0%-to Petri dishes treated with 1 mL of liquid cultured bacteria or fungi. Each Petri dish was divided equally into 4 quarters, and each quarter contained a well created with a sterile cork. Two wells were filled with plant extract, and two others with 1 mL of water (control). **Results:** After incubation, inhibition zones were evaluated, and the clove extract prevented the development of both microbes. However, only *C. neoformans* growth inhibition was dose-dependent. **Conclusion:** The results indicate that clove extract is effective against isolated scalp microbes (*S. aureus* and *C. neoformans*) and can be used as a treatment for scalp problems.

Keywords: Clove, Scalp microbes, *Staphylococcus aureus*, *Cryptococcus neoformans*.

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INTRODUCTION

The utilization of plants and herbs has been known as a source of medicine in old nations for a long time.[1] Thus, people strive to know more about them to treat themselves, and this knowledge gets transferred among generations. However, people always relied on their own experience and recommendations from others without understanding these plants' content and effective components. Thus, several studies have been conducted to reach this understanding and discovered that certain fragrant herbs, such as cinnamon, oregano, clove, thyme, etc., have antibacterial, antiviral, anticarcinogenic, and antifungal properties.[2] Among these herbs, clove (*Syzygium aromaticum*) has drawn high interest because of its potent antioxidant and antibacterial properties.[3]

S. aromaticum is a kind of dried flower bud of the Myrtaceae family endemic to the Indonesian Maluku islands; however, it has recently been planted in various countries globally.[4] The evergreen clove plant tree can reach a height of thirty or forty feet, and its leaves are leathery with multiple tiny depressions.[5] The flower buds are used as a spice, flavoring, or fragrance in items such as toothpaste, soaps, and makeup.[6] The commercial portion of the clove tree comprises its buds and leaves, and the production of flowering buds starts four years after the tree is planted.[7] When the buds

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DOI: 10.5530/ajbls.2024.13.61

are pre-flowering, they are gathered by hand or with an organic phytohormones.[8]

Clove is commercially used for medicinal purposes and in the perfume industry.[9] It may substitute chemical preservatives in many foods because of its antioxidant and antimicrobial qualities.[3] High concentrations of several chemical ingredients with an antioxidant action are thought to be responsible for clove's efficiency against various degenerative illnesses.[9] For example, clove has been utilized as an anti-inflammation because of its considerable flavonoid content.[10] It also promotes peristalsis, raises stomach hydrochloric acid, and acts as a carminative.[11] Clove is efficient against various intestinal issues; it has therapeutic properties that help relieve nausea, indigestion, loose movements, and flatulence and can help with vomiting, diarrhea, and gastric irritation symptoms.[12] Clove powder mixed with honey is used to cure acne; it can help heal cuts and bites when mixed with water.[6] Aromatherapists use pure clove oil to treat arthritis and rheumatic symptoms.[13] Moreover, clove's fragrant oils have an irritating and stimulating impact and can mildly elevate body temperature and improve blood circulation.[6] Traditionally, Clove Essential Oil (CEO) has been applied to treat injuries, burns, infections, and toothaches.[14]

CEO is rich in phenolic compounds, with eugenol as the main constituent, making up at least 50% of the identified thirty compounds. β-caryophyllene, α-humulene, and eugenyl acetate make up for 10%-40% of CEO. Minor or trace components, including α-copaene, 4-(2-propenyl)-phenol, chavicol, and α-cubebene account for less than 10%. These compounds have various biological actions, such as antibacterial, antifungal, insecticidal, and antioxidant. Therefore, eugenol is widely applied in dentistry due to its ability to permeate tooth pulp tissue and enter the bloodstream. Additionally, clove-derived sesquiterpenes exhibit anticarcinogenic properties.[9]

The FDA classifies CEOs as widely accepted as safe (GRAS), which explains why it is found in foods, medications, beauty products, sanitary products, and scents. Thus, cloves are used in perfumes and soaps as a cleaning agent in histology and other industrial applications. Traditional Chinese and Indian medicine employs cloves as a warming and stimulating ingredient. Clove treats a variety of pathogen-related disorders in tropical Asia, including scabies, infectious diseases such as malaria, cholera, and pneumonia. It was originally developed in America to treat numerous bacterial and protozoan infections, as well as viruses, worms, and other food-borne pathogens. In India, clove and its essential oils were found effective against dandruff,[15,16] making them good candidates to eliminate infections and scalp microbes. This study examined different clove extract concentrations on two microbes isolated from the human scalp: *Staphylococcus aureus* and *Cryptococcus neoformans*.

MATERIALS AND METHODS

Medium Preparation

The Potato Dextrose Agar (PDA) medium was produced following the supplier's instructions: 39 g of PDA was added to 1L of distilled water, followed by 15 min of autoclaving at 121°C. The hot liquid was spread evenly among 10 cm plates, each containing approximately 39.3 mL of PDA and reaching a depth of 0.5 cm. The Nutrient Agar (NA) medium was made in accordance with the manufacturer's guidelines. 28 g of NA powder was added to 1 L of distilled water, heated until dissolved, then autoclaved for 15 min at 121°C. The hot liquid was distributed among 10 cm plates, each holding roughly 39.3 mL of NA, down to a 0.5 cm depth.

Sample Isolation and Purification

Samples were taken from the scalps of six volunteers using a swap. They were cultured on PDA for fungi and NA media for bacteria. The PDA Petri dishes were then inculpated at 25°C for five days and the NA Petri dishes at 37°C for 24 hr. Next, pure colonies were isolated using a sterile inoculation loop and spread on fresh media. For fungus, isolated colonies were cultured for 5 days at 25°C and bacteria for 24 hr at 37°C.

Bacteria and Fungi Staining

Fungi Staining

One drop of lactophenol blue was placed on a clean slide, and a small tuff of fungus was added and mixed gently. Next, a cover glass was put over the mix, and the sample was examined under a light microscope.

Gram Staining

A small amount of tap water was poured on a glass slide, and bacteria dissolved with the water until an even and thin film of microbes was formed, after which the slide was air dried. Next, the slide was heated to fix the bacteria and allowed to cool. The slide was stained with Crystal Violet for 1 min by flooding and then rinsing with water. Next, an iodine solution was applied for 1 min by flooding the slide and rinsing it with water. The slide was decolorized for 3 sec with Gram Stain Decolorizer and rinsed with water. Next, the slide was drenched with Safranin about 30 sec before being cleaned with water. The bacteria were then observed under a light microscope's oil immersion objective.

Aqueous Clove Extraction

The extraction was prepared as described in[17] with modifications; Fresh clove buds have been chopped into a smooth powder with an electric grinder. The following amounts were used for the different final concentrations: 4 g of clove in 25 mL of distilled water for 20%; 10 g of clove in 20 mL of distilled water for 50%; and 10 g of clove in 10 mL of distilled water for 100%. Next, flasks were placed for 30 min in a 90°C water bath and incubated at 37°C overnight. Then, each flask was diluted in 16 mL of distilled water. The extractions were added as slurry of 1 mL/well, except the 100% concentration, which was added as 1 g/well.

Antimicrobial Screening

The extracts' antibacterial and antifungal characteristics were evaluated using the agar well dispersion method, as described by.[18] 1 mL of liquid cultured bacteria or fungi was transferred onto the middle of a Petri dish that was sterilized with PDA for fungi and NA for bacteria. After solidification, sterile cork borers with a diameter of 6 mm were used to make wells in the agar plates. Four plates were used: two for extracts and two for controls. Each extract was applied in 1 mL to the corresponding well. Plates were then left to incubate for 24 hr at 37°C for bacteria and 5 days at 25°C for fungus. Antimicrobial activity was assessed by determining the zone of inhibition that was the diameter of the well formed during the time of incubation. 1 mL of water was used as a negative control.

Statistical Analysis

The statistical analysis was carried out using IBM SPSS software. A one-way analysis (ANOVA) was performed to compare the major effects of various clove extract concentrations (100%, 50%, 20% and 0%) on the growth of *S. aureus* and *C. neoformans*. Tukey's HSD multiple comparison test was used to analyse differences among various treatments. Every difference was found to be significant ($p<0.05$).

RESULTS

Bacteria and Fungi Identification

Bacteria were identified by preparing a bacterial smear and performing a Gram staining. Based on their cellular morphology (shape) and staining characteristics (Gram-positive or Gram-negative), the bacteria were identified as Gram-positive *S. aureus* (Figure 1). Meanwhile, fungi were identified by lactophenol blue staining and observed under the microscope. Based on their cellular morphology (shape), the fungi were identified as *C. neoformans* (Figure 2).

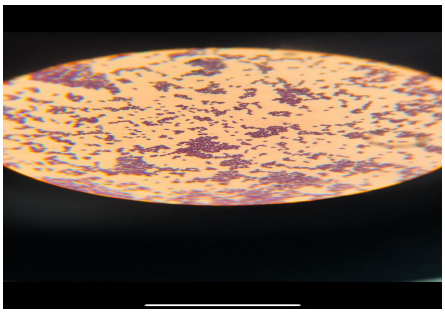

Figure 1: *Staphylococcus aureus* under microscope.

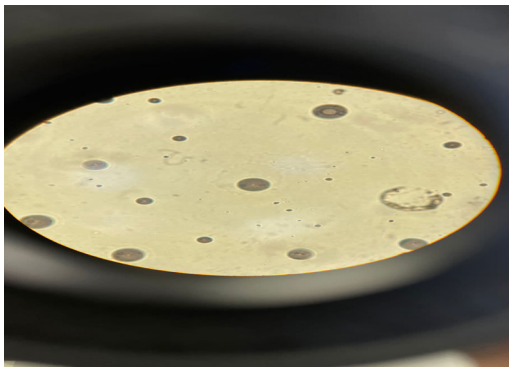

Figure 2: *Cryptococcus neoformans* under microscope.

Effect of Aqueous Extracts of Clove on Scalp Microorganisms

S. aureus and *C. neoformans* growth was inhibited by clove extracts (Figure 3). The *S. aureus* inhibition zone did not vary with the extract concentration but significantly differed from the control at all extract concentrations (Figure 4; $p<0.001$). The *C. neoformans* inhibition zone was concentration-dependent: 100% extract displayed the highest inhibition compared to 50% ($p=0.009$), 20% ($p<0.001$), and the control ($p<0.001$). Similarly, 50% extract had a higher inhibitory effect than 20% ($p=0.039$) and the control ($p<0.001$). And finally, 20% extract displayed higher inhibition than the control ($p<0.001$) (Figure 5).

DISCUSSION

This study explored the impact of clove (*S. aromaticum*) on human scalp microbes, revealing a robust effect of clove extracts leading to the inhibition of both bacteria and fungi. Clove composed of several phytochemicals with antimicrobial potential[9] that inhibited the growth of *S. aureus* and *C. neoformans*, with the *S. aureus* inhibition zone consistently and significantly different from the control at all concentrations. Whereas the *C. neoformans* inhibition zone was concentration dependent. Fungal cell walls comprise a more complex mixture of glucans, chitin, and glycoproteins.[19,20] On the other hand, the main component of bacterial cell walls is peptidoglycan,

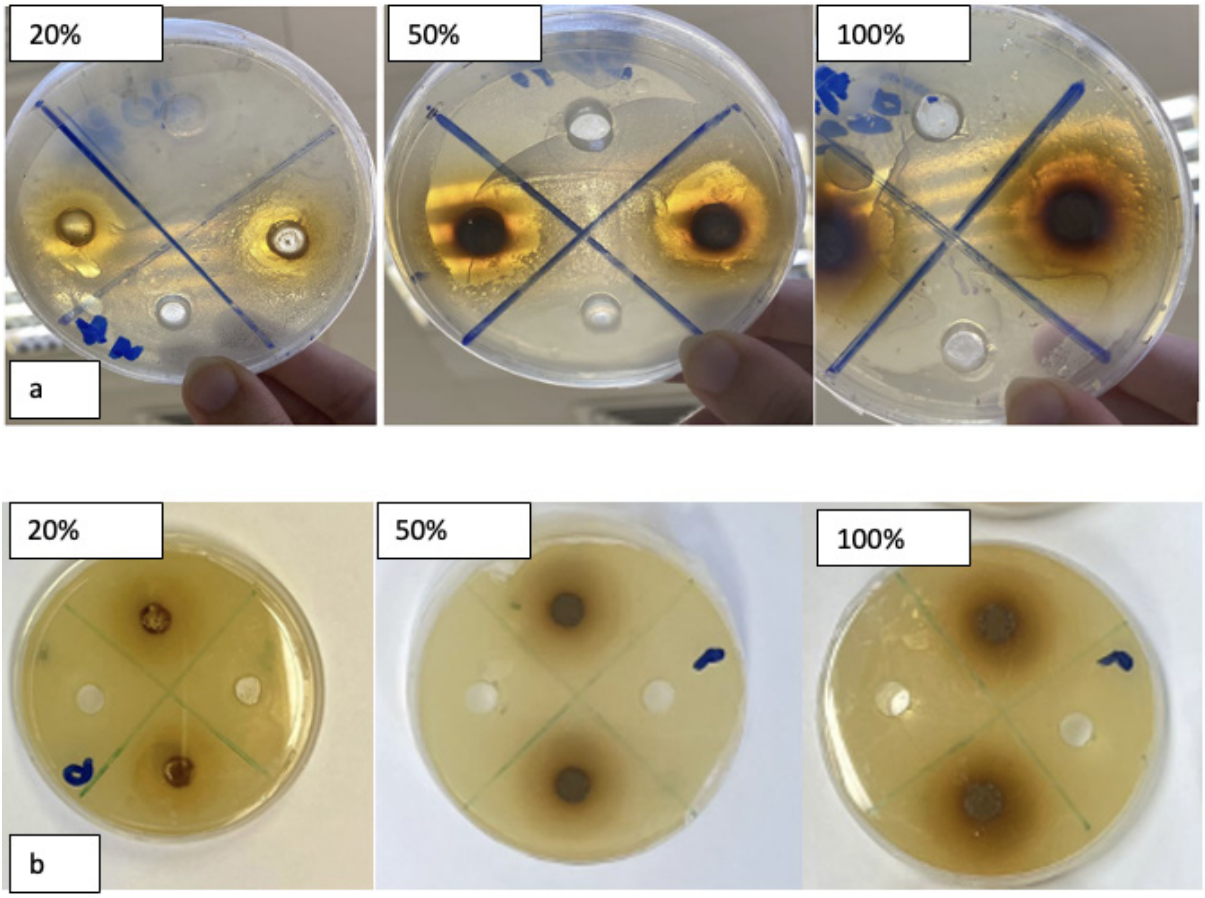

Figure 3: The inhibition zone (cm) of aqueous extracts of clove (0%, 20%, 50%, 100%) on (a) *Staphylococcus aureus* and (b) *Cryptococcus neoformans*.

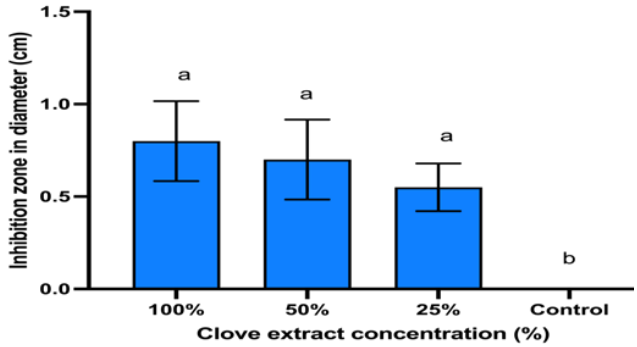

Figure 4: Effect of aqueous extracts of clove on the *Staphylococcus* aureus growth. Values represent the means of inhibition zone as affected by the clove extract concentration (0%, 20%, 50%, 100%). Panels show mean±SE; *n*=8. Panels with the same symbols show no difference (α=0.05).

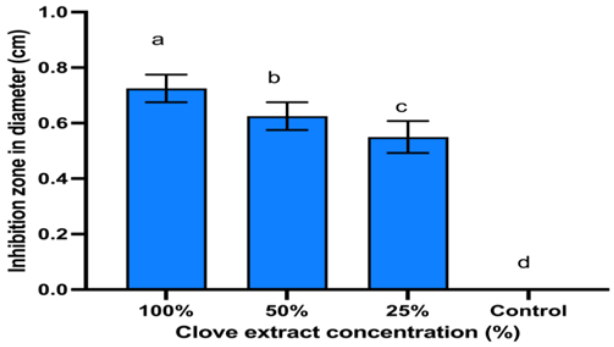

Figure 5: Effect of aqueous extracts of clove on the *Cryptococcus neoformans* growth. Values represent the means of inhibition zone as affected by the clove extract concentration (0%, 20%, 50%, 100%). Panels show mean±SE; *n*=8. Panels with the same symbols show no difference (α=0.05).

a special polymer absents from fungi.[21] Thus, these differences could be because of the different nature of the bacterial and fungi cell walls that control the clove extract cell penetration.

Clove is rich in phytochemicals and essential oils,[22] which strongly impact the isolated scalp microbes. These results are supported by several studies that utilized clove against diverse microbes in different body areas, including the skin. For example, a study examined the effect of clove oil extract on fungal skin infection caused the yeast Candida albicans, *Epidermophyton floccosum*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*.[23] CEO and its volatile vapor were found to strongly suppress the formation of spores and

fungal growth of the investigated dermatophytes.[23] Another study examined the effect of clove oil extract on bacterial growth and demonstrated that all the oil extracts possess antimicrobial activity against all types of bacteria and yeast tested.[24]

S. aureus is a dangerous Gram-positive bacterium that causes many infections affecting the skin and lungs,[25] and, more commonly, the subcutaneous tissues.[26] It is a biomarker of seborrheic dermatitis, a common scalp infection resulting in greasy hair, dandruff, erythema, itchy skin, and hair loss.[27,28] Therefore, its treatment is critically needed to improve people's health, appearance, and confidence. However, many treatments became less effective due to the developing resistant strains.[29] Thus, finding a natural treatment could be more affordable and practical. In this study, the clove extract application was effective against this microorganism, suggesting its practicality as a cheap human remedy to eliminate the infection.

C. neoformans is the highest-ranking fungus on the World Health Organization's fungal critical pathogen list.[30] It is an opportunistic, extensively dispersed fungus, primarily of natural origin, that can cause infections that are fatal.[30] It is present in the biotic community of a scalp[31] and considered pathogenic against humans,[32] causing skin issues, such as papules, maculopapular lesions, or violaceous nodular lesions.[33] Fungal disorders related with its diseases include dandruff, skin colonization, invasive bloodstream infections, and increased morbidity as well as mortality levels.[34] However, many treatments became ineffective due to its developed resistance.[30] Thus, finding alternatives that can be effective and harmless, such as herbs and other natural resources, has become critical. This study revealed a potential for clove to eliminate this fungus through significant fungal growth inhibition compared to a control treatment.

These results indicate that clove is an affordable and effective method to treat scalp bacterial and fungal diseases, providing a practical and cheap solution to improve scalp health and hair appearance. Hence, examining cloves on a larger spectrum of pathogenic microorganisms may help treat many diseases they cause. Therefore, it is of utmost importance for future studies to gather a large sample size to explore various types of microorganisms that can infect the scalp, to demonstrate and broaden the impact of such natural treatments on a larger scale.

CONCLUSION

Clove extracts have been demonstrated to be an efficient and effective way to prevent the growth of *S. aureus* and *C. neoformans* on the scalp. This is due to the impact of clove's abundant phytochemicals and essential oils on isolated scalp microbes. These extracts exhibit potent antimicrobial potential against these microorganisms, providing a natural and effective remedy to protect the scalp. Thus, the successful application of clove extract against *C. neoformans*, the top-ranked fungus on the fungal critical pathogen list, and *S. aureus*, a dangerous bacterium, further reinforces the effectiveness of this treatment. These findings not only enhance the health of the scalp and the appearance of hair but also reassure the audience about the potential of this natural remedy.

ACKNOWLEDGEMENT

The author thanks Ms. Amasi, Ms. Eshraq, Ms. Huda, Ms. Shorooq, and Ms.Taif for setting up the experiment.

ETHICAL APPROVAL

Consent and approval were obtained from the volunteers before samples were collected.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

CLO: Clove essential oil; **FDA:** Food and Drug Administration; **PDA:** Potato dextrose agar; **NA:** Nutrient agar; **GRAS:** Generally Recognized as Safe.

SUMMARY

In this study, *Staphylococcus aureus* and *Cryptococcus neoformans* have been isolated from human scalp then subjected to different concentrations of clove extracts. Both microorganisms exhibited limited growth responding to these extracts. These results suggest utilizing clove in eliminating hair microbes and improving its health and appearance.

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Cite this article: Almuziny M. Clove (*Syzygium aromaticum*) Extract has Antibacterial and Antifungal Effects against Human Scalp Microbes. Asian J Biol Life Sci. 2024;13(2):491-6.