



Antibacterial Activity of *Mitragyna Speciosa* Korth. Extract on *Streptococcus mutans*

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Research Article

Abstract

Objective: The purpose of this study aimed to evaluate the antibacterial effect of *Mitragyna speciosa* Korth. (Kratom) extract on *Streptococcus mutans*.

Materials and Methods: Crude extracts of kratom leaves were prepared using four extraction methods: maceration with 95% ethanol, maceration with 50% ethanol, decoction, and squeezing. *S. mutans* cultured in Mueller Hinton Broth was plated on Mitis Salivarius Agar. Paper discs containing kratom extracts from the four different extraction methods, and 0.12% chlorhexidine (as a positive control) were placed on agar plates. The inhibition zones were measured in millimeters, with clear zones indicating bacterial growth inhibition. The experiments were performed in triplicate. Data were recorded numerically and supplemented with images for analysis.

Results: The experiments indicated a similar clear zone area in shape and size. A clear inhibition zone was seen around the filter disc soaked in 0.12% chlorhexidine, indicating its antibacterial activity against *S. mutans*, while discs soaked in other extracts allowed normal bacterial growth. Red and white kratom extracts were unable to inhibit bacterial growth regardless of the extraction method used (maceration with 95% or 50% ethanol, decoction, or squeezing). However, reduced clear zones were observed around paper discs soaked with extracts from white kratom (squeezing method) and red kratom (maceration with 95% ethanol), which exhibited a slight tendency to inhibit bacterial growth.

Conclusion: Despite previous reports of antibacterial properties, kratom extracts obtained by the extraction methods in this study probably had a slight or no effect on *S. mutans*. Further investigation will provide more information on the effect of kratom on *S. mutans*.

Keywords: *Mitragyna speciose*/ Anti-bacterial agents/ Kratom leaves/ Mitragynine/ *Streptococcus mutans*

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Introduction

Kratom or thom, scientifically known as *Mitragyna speciosa* Korth., is a tropical herbal plant native to Southeast Asia, especially in Malaysia and Thailand.^{1,2} It belongs to the Rubiaceae plant family (coffee). It has been used for at least a century as traditional medicine for pain and fatigue relief among agricultural workers and laborers.³ Thai folk healers use its pharmacologic properties to relieve

pain, alleviate diarrhea, regulate blood sugar levels, and treat coughing.^{2,4,5} It has been reported to be used as an opioid substitute for opioid withdrawal treatment.^{2,3}

Moreover, it also provides analgesic effects, opioid agonist activity, and CNS depressant from the primary active compound, mitragynine. Known as 9-methoxy-corynantheidine (Figure 1), which is a

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dominant indole alkaloid among over 25 alkaloids extracted from kratom leaves, constitutes approximately two-thirds of its alkaloid content.^{3,6,7} Kratom has distinct strains, characterized by green, red, or white leaf veins. The red vein strain, or red kratom, (Figure 2a) requires the shortest period for cultivation and has the most potent effect.^{7,8} Conversely, the green vein strain, or green kratom, needs an extended period for growing and exhibits lower potency than red kratom. The white vein strain, or white kratom (Figure 2b), falls between the two, requiring an intermediate growth duration.

Initially sorted as a Category 5 on the Table of controlled narcotic drugs according to Thailand's Narcotics Act B.E. 2522 (1979), it faced stringent regulations, including a prohibition on consumption, cultivation, trade, and distribution. However, the Narcotics Act No.8 B.E. 2564 (2021) led to its removal from Category 5 on the Table. Subsequently, the Narcotics Act B. E. 2565 (2022) legalized its

cultivation, possession, and sale with the required authorization from the Office of the Narcotics Control Board (ONCB) if it is used as a composition in foods, medicines, cosmetics, and herbs.

Fresh *M. speciosa* leaves can be directly chewed to extract the juice. The leftover fiber is indigestible and must be discarded. The leaves can also be consumed after being removed from the stem, midrib, and veins, or chewed with betel nut (*Areca catechu*).¹ Dried kratom leaves can be blended and used as cigarette filler for smoking or brewed as tea for consumption.^{1,6} Sweeteners and lime juice are added to reduce the bitterness of kratom tea and enhance its opioid effects by facilitating opioid extraction through acidity. Kratom cocktail (4x100), kratom mixed with Coca-Cola and cough syrup, is a popular alternative way for consumption.⁶ This recipe provides the active ingredients, including mitragynine, codeine, caffeine, chlorpheniramine, or phenylephrine.⁷

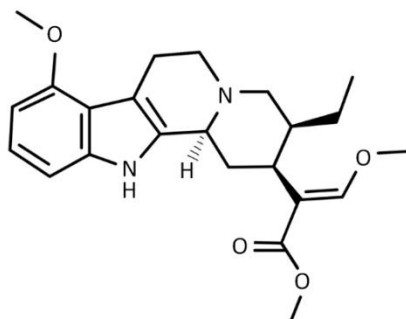


Figure 1 Chemical structure of mitragynine



Figure 2 (a) Red vein kratom leaf (b) White vein kratom leaf

Kratom has diverse pharmacological effects, including antinociceptive, psychostimulant, antidepressant, anti-inflammatory, antioxidant, and antibacterial properties.^{2,9,10} Mitragynine inhibits mechanical and thermal noxious stimuli by engaging supraspinal μ - and δ - opioid receptor subtypes to provide an antinociceptive effect.^{3,11} As an antidepressant property, mitragynine stimulates serotonin production and secretion and affects the neuroendocrine HPA axis system.¹² Mitragynine inhibits the inflammatory pathway by affecting the COX-2 mRNA expression and prostaglandin E₂ production.¹³ Methanolic and alkaloid extracts of *Mitragyna speciosa* Korth. leaf showed a zone of inhibition against *Salmonella typhi* and *Bacillus subtilis* in the preliminary antibacterial screening assay.¹⁴ The extracts were analyzed by high performance thin layer chromatography (HPTLC) method, and mitragynine bands were visually detected in all extracts with varying intensity. Mitragynine was believed to be an active compound against specific bacteria. Another study investigating the antimicrobial activity of kratom leaf 50% acetic acid extracts reported that mitragynine could inhibit *Staphylococcus aureus* and *Escherichia coli*.¹⁵ Quercetin, the secondary metabolite of *Mitragyna speciosa*, was found in the in silico study. The interaction between the ATP1 protein and quercetin was predicted to be an antibacterial mechanism of kratom leaf methanolic extract on *Streptococcus pneumoniae* and *E. coli*.¹⁶ Since *S. mutans* belongs to the same genus as *S. pneumoniae*, the expected antibacterial mechanism should be the same.

Streptococcus mutans has been known as a key pathogen of dental caries since it was isolated from carious lesions by J. Clarke in 1924. It was named under *Streptococcus mutans* due to the belief that the oval-shaped cells observed were mutant forms of Streptococci.¹⁷ The strong association between caries experience and *S. mutans* has been suggested and confirmed in a systematic review and

meta-analysis.¹⁸ *S. mutans*, a Gram-positive, facultatively anaerobic cocci bacteria, belonging to the *Streptococcus* genus, plays a crucial role in dental plaque formation as a primary colonizer, and is notably implicated in the pathogenesis of dental caries or cavities.¹⁹ This bacterium thrives in the oral cavity, particularly in biofilms on tooth surfaces, where it evades host defence mechanisms and utilizes nutrition, such as sucrose, as a substrate for fermentation. Through this process, *S. mutans* produces acids, mainly lactic, as metabolic by-products and lowers the pH of the surrounding environment, leading to the demineralization of tooth enamel, ultimately resulting in the development of cavities.¹⁹

S. mutans conversion to a harmful pathogen is enabled by several virulence factors. Attachment to tooth surfaces to initiate biofilm formation and generating acidity are important examples of its abilities to cause dental caries.²⁰ One such factor is the binding ability of bacterial surface proteins, such as glucosyltransferases and adhesins, to the host-derived molecules like salivary glycoproteins and extracellular matrix proteins. This adhesion allows *S. mutans* to colonize and form biofilms.¹⁹ Moreover, *S. mutans* can metabolize various carbohydrates, including sucrose, glucose, and fructose through enzymatic pathways that generate energy and acidic by-products. These acids damage tooth enamel and create an environment conducive to the growth of acidogenic and acid-tolerant microorganisms, further exacerbating dental caries.¹⁹ Research on *S. mutans* opens pathways to review its effect on dental health, oral disease, and its role in potential strategies for prevention and dental caries management. Understanding the mechanisms of *S. mutans* pathogenicity is crucial for developing effective interventions to maintain oral health and prevent tooth decay.

Due to its prior categorization as a Controlled Narcotic in Thailand, research on kratom's antibacterial properties has been limited. Kratom is usually consumed via chewing, the effect of kratom on the oral cavity is interesting, especially the effect on oral bacteria. *S. mutans* is a major cariogenic bacterium and is also in the same genus as *S. pneumoniae*, on which kratom presented antibacterial interaction. Therefore, this study aims to investigate whether kratom exhibits antibacterial effects on *S. mutans*.

In traditional Thai medicine, kratom is used to relieve many symptoms, including diarrhea, cough, and fatigue. Contemporarily, it is used as an energy booster and an oral wound healing promoter since its removal from Category 5 status.

As an antibacterial screening test, four different extraction methods for *Mitragyna speciosa* (kratom) had been selected, including maceration with 95% ethanol, maceration with 50% ethanol, decoction, and squeezing. The believed bioactive compound in antibacterial activity represented in the kratom leaf is mitragynine, which is a lipophilic alkaloid. The extract from maceration using the 95% ethanol method was expected to show the highest

antibacterial activity among all methods, as this is suitable for extracting lipophilic alkaloids.²¹ While the extract obtained by the decoction method was predicted to have no clear zone around the disc. Mitragynine should have been degraded during boiling in water in a decoction process, as the previous study reported that it is stable at the temperature range of 4-40 °C.²²

Materials and Methods

Kratom Extract Preparation

Samples of red kratom 1,765 grams were collected from the Chumphon province of Thailand, while white-veined samples 1,729 grams were collected from the Pattani province. All samples were processed to obtain the crude extract at the Department of Thai Traditional Medicine, Faculty of Medicine, Thammasat University. The kratom leaves were cleaned and extracted using four methods: maceration with 95% ethanol, maceration with 50% ethanol, decoction, and squeezing, as shown in Figure 3. The extraction methods were applied from basic plant extraction methods and the previous studies.^{21,23,24}

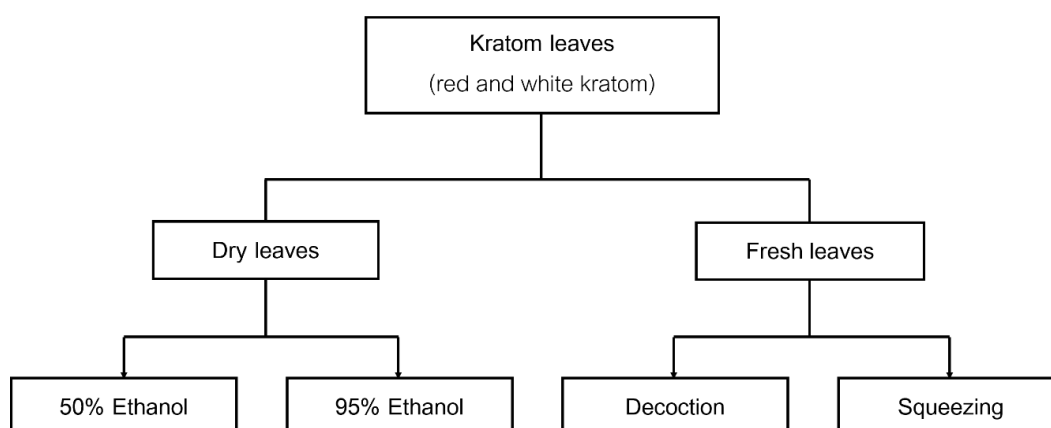


Figure 3 Methods for kratom extract preparation

Maceration: The dry red and white vein kratom leaves were macerated with a solid-solvent ratio (1:3) in either 95% (137 g and 198.81 g, respectively) or 50% ethanol (142 g and 181.53 g, respectively), each for three days, then filtered. These processes were repeated three times. The extracts were pooled and concentrated using a rotary evaporator under reduced pressure at 45 °C. The yields of red and white vein kratom extracts obtained using 95% ethanol were 14.7% and 12.5%, respectively. When using 50% ethanol, the yields were 22.4% for the red vein kratom extract and 18.6% for the white vein kratom extract.

Decoction: The fresh red and white vein kratom leaves (724 g and 324 g, respectively) were boiled in water with a solid-solvent ratio (1:3) for 15 minutes and then filtered to obtain the extract. The extracts were filtered, combined, and concentrated with a freeze-dryer (LyoFreeze, New York, US). The yields of red and white vein kratom extracts were 6.45% and 8.75% respectively.

Squeezing: Fresh red and white vein kratom leaves (42 g and 80.65 g, respectively) were mixed with water in a solid-solvent ratio (1:3), then blended. The extracts were filtered, combined, and concentrated with a freeze-dryer (LyoFreeze, New York, US). The yields of red and white vein kratom extracts were 10.68% and 15.48%, respectively.

Paper Disc Preparation

The crude extracts obtained from all extraction methods were prepared for paper disc soaking. The maceration with 95% and 50% ethanol extracts were prepared at 500 mg/mL concentration in absolute DMSO. In contrast, the decoction and squeezing extracts were dissolved at a concentration of 100 mg/mL in sterile water. 10 microliters of each extract solution and 0.12% chlorhexidine were placed at the center of 6 mm paper discs.

Bacterial Culture Preparation

S. mutans, a common dental caries pathogen, was chosen for testing the inhibitory growth effect of *M. speciosa* in this study. Initially, *S. mutans* Clake (ATCC 25175) was plated on Mitis Salivarius Agar (M-S agar) and then incubated, aerobically, at a temperature of 37 °C with 5% CO₂ for 24 hours. A single bacterial colony was inoculated into Mueller Hinton Broth (MHB) and incubated aerobically at a temperature of 37 °C for 4 hours. The culture density was measured and adjusted to 0.5 McFarland.

Antibacterial Testing

The bacterial culture in MHB was plated on M-S agar, and then 6 mm paper discs with 0.12% chlorhexidine as a positive control. Paper discs with kratom extracts from the four different extraction methods were put on the agar plate. After aerobically incubating at a temperature of 37 °C with 5% CO₂ for 48 hours, the clear zone diameter was measured from one edge to another and recorded in millimeters. The experiments were performed in triplicate. The result was interpreted from a clear zone around the paper disc.

Statistical Analysis

This research collected data on a numerical scale and recorded images.

Results

The results from triplicated experiments shown in Figure 4a-c indicate a similar clear zone area in shape and size. A clear zone of approximately 16.2 mm was observed around paper discs soaked in 0.12% chlorhexidine, as shown in Table 1. In contrast, paper discs soaked with all kratom extracts allowed bacterial growth. This indicates that only 0.12% chlorhexidine could inhibit the growth of *S. mutans*. Red and white kratom extracts were likely to inhibit bacterial growth as reduced clear zones (positions 2 and 3 in Figure 4a-c)

were observed around paper discs soaked with extracts from white kratom (squeezing method), and red kratom (maceration with 95% ethanol). The growth in these areas was less robust when compared to other areas on the culture media without the extract-soaked paper discs.

Apart from the 0.12% chlorhexidine, which inhibited the growth of *S. mutans*, and the extracts from white kratom by squeezing and red kratom by maceration with 95% ethanol, which showed potential to inhibit *S. mutans* growth. The extracts from both types of kratom obtained by other extraction methods could not inhibit the growth of *S. mutans* at all.

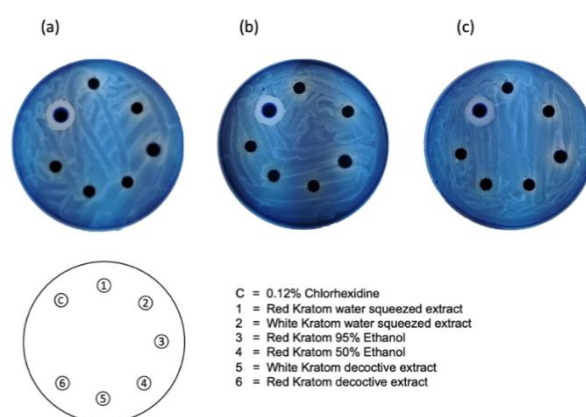


Figure 4 Zone of inhibition of kratom extract compared with 0.12% chlorhexidine on *S. mutans*

Table 1 Antibacterial activity (zone of inhibition) of kratom extract compared with 0.12% chlorhexidine on *S. mutans*

Microorganism	Zone of Inhibition (mm)						
	Control	Water extracts		EtOH Extracts		Decoctive extracts	
	0.12% CHX	Red veins	White veins	95%	50%	Red veins	White veins
<i>S. mutans</i>	16.2	N/A	N/A	N/A	N/A	N/A	N/A

EtOH: Ethanol.

Discussion

Mitragyna speciosa, commonly known as kratom, has garnered significant interest following its removal from Category 5 of the controlled narcotic drugs in the Narcotics Act No. 8 2564 (2021). This interest is due to its various active compounds, particularly the dominant alkaloid mitragynine, which provides various pharmacological effects, including antinociceptive, psychostimulant, antidepressant, anti-inflammatory, antioxidant, antibacterial, and anticancer properties.²⁵ Compounds in kratom may also cause adverse health effects, such as constipation. Consequently, other herbs, such as *Ficus carica*, have often been integrated to mitigate these effects.⁵

Although kratom has been used for at least a century as a pain alleviator and fatigue reliever among agricultural workers and laborers by chewing

the leaves or through processing methods that make it suitable for oral ingestion, there were fewer studies of kratom in Thailand due to the Narcotics Act B.E. 2522 (1979). The oral health benefits of using kratom, including its effects on the oral tissues, have not been studied extensively.

Salim et al. reported the antibacterial effects of kratom methanol extract on *S. pneumoniae* and *E. coli* bacteria in vitro.¹⁶ The results indicated that the extract exhibited antibacterial activity against both pathogens at concentrations ranging from 25% to 100%. Notably, the secondary metabolite of mitragynine, quercetin, was found to interact with the ATP1 protein of the bacteria, suggesting that this interaction may be a key mechanism underlying the observed effects.

Additionally, Niyomdech et al. investigated the effects of kratom leaf extracts on the inhibition

of *S. aureus* and *E. coli*.¹⁵ Various solvents were used for extraction. Acetic acid yielded the highest percentage, followed by ethanol. Both solvent extracts exhibited positive antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at a 6 mg/ml concentration of mitragynine.

Mitragynine is considered a significant active compound that may have various effects, including antibacterial activity. However, the levels of mitragynine in the different extracts were not analyzed in this study, as the objective aimed for the preliminary antibacterial screen assay for further study. The results may lack a specific measurement of the mitragynine level, leading to a limited ability to explain how the levels of mitragynine could influence the capacity to inhibit or eliminate *S. mutans*.

The four extraction methods, including maceration with 95% ethanol, maceration with 50% ethanol, decoction, and squeezing, for *Mitragyna speciosa* (kratom) use different techniques, temperatures, durations, and solvent polarities. These differences affect the extraction efficiency, quality, and quantity of bioactive compounds, which can impact the antibacterial properties of kratom. Maceration with 95% ethanol is a highly effective extraction method for lipophilic alkaloids, such as mitragynine in kratom.²¹ It is suitable for pharmaceutical or research purposes requiring purified extracts. Maceration with 50% ethanol yields a broader range of both polar and non-polar substances, particularly more water-soluble compounds, as the solvent is an ethanol-water mixture. Decoction is primarily used for extracting water-soluble compounds; however, it may not be suitable for thermolabile alkaloids.²³ Squeezing is a simple, traditional extraction method, but it yields low extraction efficacy.

Although previous studies have indicated that using acetic acid as a solvent showed the highest yield of kratom extraction, ethanol and water

were chosen to be the primary solvents in this study as these solvents are more biocompatible when used in the oral cavity. This decision was made to prioritize the safety and edibility of the extracts.

The antibacterial mechanism of kratom extract on *S. pneumoniae* and *E. coli* was predicted in an in silico study as the interaction between the ATP1 protein and quercetin, which is the secondary metabolite of *Mitragyna speciosa*.¹⁶ Since *S. mutans* belongs to the same genus as *S. pneumoniae*, it was anticipated that they would share a similar antibacterial mechanism. However, differences in extraction methods, solvents, and extract concentrations may have influenced the results of this study.

According to this study's results, most kratom extracts do not affect the *S. mutans* growth, as the clear zones only appear around a paper disc soaked with 0.12% chlorhexidine, which is currently recommended as the best available in reducing *S. mutans* and oral biofilm *in vivo*. Taste alteration, mouth and tongue numbness/pain, xerostomia, and oral tissue discoloration have been reported with 0.12% chlorhexidine usage. The extracts from white kratom by squeezing and red kratom by maceration with 95% ethanol may slow the growth of *S. mutans*. This suggests that if the concentration of these two extracts (white kratom by squeezing and red kratom by 95% ethanol maceration) showed slight effects, and suggested mechanisms or concentrations for future investigation.

This study is highly relevant as it represents the first investigation into the effects of kratom on oral bacteria growth, specifically *S. mutans*, as there was no prior research focusing on oral pathogens. While kratom did not exhibit antibacterial activity against cariogenic pathogens, it may stimulate saliva flow rate and enhance mechanical plaque removal, potentially benefiting dental treatments.

As there has been no confirmed report that kratom can increase saliva flow rate yet, it may indirectly stimulate salivation through its effects on the autonomic nervous system. Kratom impacts the parasympathetic nervous system,²⁶ which regulates salivary gland activity. In 2023, Dodds et al. concluded that chewing gum results in a higher unstimulated salivary flow rate in the elderly and those with xerostomia.²⁷ This is related to the duration of chewing gum history. It may imply that chewing kratom leaves may increase the salivary flow rate and mechanical plaque removal as well.

The belief that chewing kratom prevents oral diseases, such as dental caries, may be unfounded. This study indicates that kratom consumers should be encouraged to recognize the importance of regular oral health maintenance. Therefore, this research advocates for a shift in perspective in oral hygiene care.

Further investigation into the effects of kratom extract on oral tissues as well as other pathogenic microorganisms in the oral cavity, will provide a clearer understanding of kratom's effects, which aims to provide a comprehensive understanding of kratom as more people in all age groups, particularly among adolescents and working adults, are likely to access it easily.

Conclusion

Kratom extracts obtained by the extraction methods in this study demonstrated minimal or no effects on the growth of *S. mutans*, which plays an important role in dental caries. Although prior studies demonstrated its antibacterial properties against other bacteria, the kratom extracts cannot be recommended as caries-preventive agents based on the findings of this study. Further investigation will provide more information on the effect of kratom on *S. mutans*.

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ฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดไมทราไจนา สเปซิโอซา ต่อเชื้อสเตรปโตค็อกคัส มิวแทนส์

ทิพย์พรรณ สาธิตธรรมพร¹ มัทธน พูลเกษร¹ ภูริทัต กนกกังสดาล² นิขมน มุขสมบัติ²
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บทความวิจัย

บทคัดย่อ

วัตถุประสงค์: เพื่อประเมินฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดจากใบกระท่อมต่อเชื้อสเตรปโตค็อกคัส มิวแทนส์

วัสดุอุปกรณ์และวิธีการ: สารสกัดหยาบจากใบกระท่อมเตรียมด้วย 4 วิธี ได้แก่ การแช่ด้วยเอธานอลความเข้มข้นร้อยละ 95 การแช่ด้วยเอธานอลความเข้มข้นร้อยละ 50 การต้ม และการคั้น เชื้อสเตรปโตค็อกคัส มิวแทนส์ที่เพาะเลี้ยงในอาหารเหลวชนิดมูลเลอร์ อินตัน ถูกเพาะบนอาหารแข็งชนิดไมดิส ซาลิวาเรียส แล้ววางแผ่นกรองที่มีสารสกัดกระท่อมจากทั้ง 4 วิธี และคลอร์เฮกซิดีนความเข้มข้นร้อยละ 0.12 (เป็นตัวควบคุมเชิงบวก) บนอาหารแข็ง พื้นที่ที่ยับยั้งถูกวัดเป็นมิลลิเมตร โดยพื้นที่ใบบ่งชี้การยับยั้งการเติบโตของแบคทีเรีย การทดลองทำซ้ำสามครั้ง ข้อมูลถูกบันทึกเป็นตัวเลขและเสริมด้วยภาพถ่ายสำหรับการวิเคราะห์

ผล: การทดลองแสดงให้เห็นพื้นที่ใบบ่งชี้การยับยั้งการเติบโตของแบคทีเรียได้ ไม่ว่าจะใช้วิธีสกัดแบบใดก็ตาม (การแช่ด้วยเอธานอลความเข้มข้นร้อยละ 95 หรือ 50 การต้ม หรือการคั้น) อย่างไรก็ตามพบว่ามึบริเวณใสลดลงรอบๆ แผ่นกรองที่แช่ด้วยสารสกัดจากกระท่อมสีขาว (วิธีการคั้น) และกระท่อมสีแดง (การแช่ด้วยเอธานอลความเข้มข้นร้อยละ 95) มีแนวโน้มเล็กน้อยที่จะยับยั้งการเติบโตของแบคทีเรีย

บทสรุป: แม้ว่าจะมีรายงานก่อนหน้านี้เกี่ยวกับคุณสมบัติในการต่อต้านแบคทีเรีย แต่สารสกัดจากกระท่อมที่ได้จากวิธีสกัดในการศึกษานี้ อาจมีผลต่อเชื้อสเตรปโตค็อกคัส มิวแทนส์เพียงเล็กน้อย หรือไม่มีผลเลย การศึกษาเพิ่มเติมในอนาคตจะช่วยให้ข้อมูลเพิ่มเติมเกี่ยวกับผลของกระท่อมต่อเชื้อสเตรปโตค็อกคัส มิวแทนส์

คำไ้รหัส : ไมทราไจนา สเปซิโอซา/ สารต้านแบคทีเรีย/ ใบกระท่อม/ ไมทราจินิน/ สเตรปโตค็อกคัส มิวแทนส์

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