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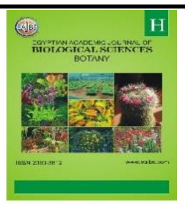

Antimicrobial Activity Evaluation of Different Plants Extracts against *Staphylococcus aureus* Bacteria

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ABSTRACT

Worldwide, Staphylococcus aureus (*S. aureus*) infection recurrences are still occurring, and the sharp rise in antibiotic resistance makes treating these infections more challenging. Epidemics of *S. aureus* pose risks to public health and place a financial strain on medical expenses globally. Laboratory experiment conducted assessed and contrasted five of botanical extracts as antibacterial that have been proposed in the past as having antibacterial qualities against S. aureus to respond to the current demand for efficient treatments against this pathogen. In short, the minimum inhibitory concentration (MIC) was established after *S. aureus* cultures were exposed to specific botanical extracts. That study was conducted to assess antimicrobial activity of five plant extracts i.e., *Salvia officinalis, Nigella sativa, Syzygium aromaticum, Myristica fragrans, Salvia rosmarinus* were investigated against *Staphylococcus aureus* bacteria activity, using agar disc diffusion technique. The results showed that there was a large variation in the rates of inhibiting the activity of *S. aureus* bacteria by using these various plant extracts. The largest zone of inhibition of *S. aureus* bacteria activity (9.14 mm) was recorded by using *Salvia rosmarinus* extract, while the lowest inhibition rate was recorded (4.67 mm) by *Myristica aromatic* extract.

INTRODUCTION

Bacteria are one of the most important microbial pathogens, becoming a major cause of food spoilage and numerous foodborne illnesses. This can be mitigated and food spoilage preserved using various chemicals in the food industry. However, the use of these preservatives has numerous adverse effects on human health, as these chemicals enter many food chains, leading to toxicity that accumulates and causes long-term complications. Given these negative effects, there has been a growing and urgent demand for natural preservatives and safer, more effective, and less complex solutions (Imran *et al*., 2021).

Despite notable progress in the safety and health of food production and processing methods, food safety remains one of the most crucial public health concerns. According to estimates, food-borne illnesses affect 30% of the population in affluent nations. About 2 million people perished in 2000 because of illnesses that caused diarrhea. As a result, we must create new strategies to eliminate or reduce food pathogens by fusing them with existing techniques. The use of natural preservatives is becoming more popular as industrial chemicals have a lot of negative effects and are mostly carcinogenic (Rahnama *et al.,* 2012).

One of the main causes of disease and death in developing countries is bacteria, where bacterial contaminants are the biggest threat to public health is posed by, which are mostly Gram-negative bacteria like Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, and Staphylococcus aureus. Numerous studies have shown that plant essential oils have antibacterial activity by disrupting the phospholipid bilayer of the cell membrane, increasing permeability and resulting in the loss of cellular components. They also degrade or inactivate genetic material and damage many enzyme systems (Solomakos *et al*., 2008).

Over the past few decades, there has been an increasing interest in the hunt for antimicrobial compounds from plants. However, because the specific antimicrobial techniques used in many of these investigations are not standardized, the results obtained from them cannot be directly compared. Numerous researchers have suggested that the assessment of antimicrobial activity from plant extracts requires well-established techniques that yield reliable results. However, several research detailing various approaches are still published in the literature (Othman *et al*., 2011).

Many studies plant extracts from medicinal and aromatic plants have demonstrated remarkable antibacterial activity comparable to currently tested and used modern drugs. So further research on efficacy, safety, and toxicity for these extracts (Kebede *et al*., 2021). Throughout human history, medicinal plants have been traditionally used to treat diseases. Ancient Egypt should be regarded as one of the first nations in the world that used that medicinal herb (Mousavi, 2004). Some plants were believed to be useful cures for specific illnesses even before people realized that germs existed (Mobaiyen *et al.,* 2015). As a result, these herbs' oils were utilized rather than their extracts, which are often used to treat infections (Rios and Recio, 2005). One may argue that plants are an underutilized source of potentially beneficial compounds. There are instances where herbal extracts can be applied against a lot of various bacteria (CHITSAZ *et al*., 2007; Jafari *et al*., 2020).

Substantial antibacterial activity has been demonstrated by the plant extracts, which are on par with the tested modern medications that are currently administered (Kebede *et al*., 2021).

The usage of herbal remedies has been revived as a traditional means of combating diseases due to advancements in medical science and new scientific breakthroughs in the field of drug manufacturing. 326 anti-infective (antibacterial, antifungal, antiparasitic, and antiviral) medications that are directly derived from nature or contain a component that mimics the action of a natural compound that have been found and used as treatments were listed by Newman and Craag 2016. The essential natural producers of phytochemicals that give medicinal plants their biological properties, including flavonoids, tannins, phenols, steroids, alkaloids, and terpenoids, have long been these plants (Yessuf, 2015). Phytomedicines are plant products made from fruits, flowers, seeds, roots, leaves, and barks. Several conventional analytical techniques have been used to identify and further define the bioactive components of plants (Thilakarathne *et al.,* 2018;).

Salvia officinalis is one of those medicinal plants that belongs to the *Lamiaceae* family. It is a perennial low shrub with traditional medicinal uses that comes from the Mediterranean region. There are around 900 species in its family. Inflammations of the mouth and throat, cough, bronchitis, angina, asthma, depression, excessive perspiration, and skin conditions have all been treated with it (Moreira *et al.,* 2013). More precisely, *S. officinalis* contains monoterpenes, diterpenes, triterpenes, and phenolic components that are known to have a variety of uses, including choleretic, hypoglycemic, stimulant, astringent, antimicrobial, antihypertensive, emmenagogue, antiperspirant, toning, and the treatment of dental abscess, stomach pain, and other buccopharyngeal disorders. Major terpenes including viridiflorol, manool, borneol, eucalyptol, and thujone are found in the essential oil of *S. officinalis*. The leaves of *S. officinalis* have already been found to contain rosmarinic acid, carnosol, flavonoids, ursonic acid, carnosic acid, oleic acid, tannic acid, fumaric acid, chlorogenic acid, polysaccharides, ursolic acid, caffeic acid, and estrogenic compounds (Mendes *et al*., 2020).

Nigella sativa, also referred to as black seeds, is a significant medicinal plant species that has become well-known for a variety of therapeutic uses because of its phytoconstituent-rich seeds. The primary component of *N. sativa* seeds, thymoquinone, is responsible for most of the pharmacological characteristics of the seeds (Franco-Ramos *et al*., 2020). Numerous other components, including proteins, carbohydrates, vitamins, dietary minerals (including Fe and Zn), crude fiber, alkaloids, saponins, steroids, terpenoids, p-cymene, limonene, and fatty acids, are also found in *N. sativa* seed extracts and/or oil, according to published research (Srinivasan, 2018). Various *N. sativa* seed extracts demonstrated antibacterial activity with varying levels of effectiveness against the harmful bacterial strains (*E. coli, P. aeruginosa, S. aureus,* and *B. subtilis*) because they include a range of chemical components and functional groups associated with their antibacterial capabilities (Shafodino *et al.,* 2022). On the other hand, Hadi *et al*., 2016 reported that the *N. sativa* seeds have pharmacological properties such analgesic, appetizer, antidiabetic, antioxidant, anti-inflammatory, radical scavenger, and antimicrobial properties due to their chemical composition.

Syzygium aromaticum commonly known as clove is one of aromatic plants which were traditionally used in ancient times for health care by the peoples of the world. Recently, it continued to be used as a cure for various diseases despite advances in modern medicine; this is due to the perpetual phenomena of resistance of microbial agents to conventional antibiotics and their side effects (Atchou *et al*., 2013). Clove is an aromatic, evergreen plant that grows 10 to 20 meters tall and is native to the Maluku Islands in Eastern Indonesia. It belongs to the *Myrtaceae* family (Alharbi, 2017). Traditional medicine uses it to treat gastrointestinal spasms, vomiting and nausea, stomach distention, coughing, diarrhea, dyspepsia, flatulence, uterine contraction, and nerve stimulation (Sulieman *et al*., 2007). According to scientific studies Clove contains antibacterial qualities, antiparasitic, antiviral, antimutagenic, anti-inflammatory, anti-ulcerative, antioxidant, anticoagulant, and antiseptic (Afanyibo *et al*., 2018).

The tree *Myristica fragrans* is a member of the *Myristicaceae* family, which has its origins in Indonesia. Thirteen percent of the tree's fruit is seed. The nutmeg spice is made by grinding the kernel of this seed. Generally, its seeds have around 60% oil. The essential oil that is derived from *M. fragrans* is frequently utilized in the culinary sector as a flavoring agent and in cosmetics as a fragrance-incorporating agent. Monoterpene hydrocarbons make up 80–90% of the essential oil produced by steam distillation, which ranges from 4% to 16%. It possesses strong antitumor, antibacterial, antifungal, anticancer, and hepatoprotective properties. Myristicin, eugenol, elemicin, and safrole are among the main bioactive chemicals that provide *M. fragrans* with their therapeutic qualities (Kuete, 2017; Al-Qahtani *et al*., 2022).

A perennial evergreen, rosemary was once known as *Rosmarinus officinalis* and is a member of the *Lamiaceae* family. Salvia *rosmarinus* was recently created by combining the genera Rosmarinus with the genus *Salvia* in a phylogenetic analysis (de Macedo *et al*., 2020). This fragrant shrub can reach a height of two meters, and its leaves are its primary component, serving a variety of functions. Recently, it was now recognized as one of the world's most important decorative and therapeutic plants. Rosemary has been cultivated for many years and utilized in phytocosmetics, cosmetics, and traditional medicine because of its many health benefits such as antibacterial, anti-inflammatory, antioxidant, anti-apoptotic, and anti-tumorigenic herbs. Traditional medicine uses rosemary extract to treat hair loss, urinary tract infections, chronic fatigue, peripheral vascular problems, and neurological diseases. In addition, rosemary has long been used as a diuretic, choleretic, tonic, rubefacient, emmenagogue, expectorant, choleretic, diaphoretic, antispasmodic, and anti-inflammatory. Because of its antioxidant and health-promoting properties, rosemary plants are growing in popularity (Anadón *et al*., 2021; Aamer *et al*., 2023).

Major components of rosemary oil, such as α-pinene, myrcene, 1,8-cineole, camphor, camphene, α-terpineol, and borneol, have been proven in recent research to have antibacterial action. *Bacillus subtilis*, *Proteus vulgaris, Pseudomonas aeruginosa, E. coli, Staphylococcus epidermidis*, and *Staphylococcus aureus* are all effectively combatted by rosemary oil (Chávez-González *et al*., 2016). Gram-positive Staphylococcus aureus is the most common cause of bacteremia-related mortality globally, accounting for an estimated 300,000 deaths annually and a case fatality rate of 15% to 30% (Tong *et al*., 2025).

The aim of this study is the *Staphylococcus aureus* bacteria susceptibility test for various three concentrations each plant extract and bacterial concentration were measured using disc diffusion as per the CLSI protocols.

MATERIALS AND METHODS

Experimental Site:

The experiment was conducted in the laboratories of the Department of Botany and Microbiology, Faculty of Science, Tobruk University, Libya, during July 2023.

Plant Material:

In vitro experimental study was carried out to evaluate antimicrobial effects and phytochemical screening of five different medicine plants (Table 1) i.e. (*Salvia officinalis, Nigella sativa, Syzygium aromaticum, Myristica fragrans, Salvia rosmarinus*) by three different concentrations (25, 50, 100 gr. dry plant/l) against *Staphylococcus aureus* bacteria growing in Mueller-Hinton Agar.

Table 1: The botanical data for the various plant species used.

Species	Family	Common name	Local Name	Plant part used
Salvia officinalis	*Lamiaceae*	Sage	Marameya	Leaves
Nigella sativa	*Ranunculaceae*	Black caraway, Black cumin	Habet Elbaraka	Leaves
Syzygium aromaticum	*Myrtaceae*	Clove	Koronfil	Leaves
Myristica fragrans	*Myristicaceae*	Nutmeg tree	Gozet Alteeb	Leaves
Salvia rosmarinus	*Lamiaceae*	Rosemary	Eklil Algabal	Leaves

Plant Extracts' Preparation:

For our experiment, only healthy plant samples devoid of morphological anomalies were used. To get rid of the surface contaminants, the samples were first thoroughly cleaned under running water. They were then left to cure at room temperature for an additional ten days. Before the samples were used in the trials, they were first finely ground into a powder to prepare extracts. The plan extracts were prepared by Aqueous extraction methods.

Media Used and Bacteria Strain:

Mueller-Hinton agar has been used in the laboratory as a bacterial growth medium for *Staphylococcus aureus*. It is a non-selective, non-differentiating agar medium used primarily for antibiotic susceptibility testing, particularly the Kirby-Bauer disk diffusion method.

The Agar Well Diffusion Method was used to assess the plant extracts' antibacterial properties according to Perez *et al*., 1990.

Statical Analysis:

The statistical analysis of the effect of different extract concentrations using a one-way analysis of variance, comparison of means, and a post hoc analysis using the least significant difference test was conducted at a significance level of 0.05 by the SPSS program.

RESULTS AND DISCUSSION

The Impact of Plant Extracts Sort on Bacterial Activity:

The data in the following Figure 1 illustrates the effect of plant extracts from five different plant species on the activity of *Staphylococcus aureus* (*S. aureus*). The results demonstrated that all plant extracts had the potential to be useful in inhibiting microbial development. against *S. aureus* with varying degrees of effectiveness, which may serve as synergistic agents or as natural substitutes for conventional antibiotics. Water extracts of *Salvia rosmarinus* leaf showed the largest zone of inhibition (9.14 mm) against *S. aureus*. While *Myristica aromatic* leaf extract recorded the lowest inhibition rate against bacteria *S. aureus* which reached (4.67 mm). On the other hand, the inhibition rates of *S. aureus* for the aqueous leaf extracts of *Salvia officinalis, Nigella sativa*, and *Syzygium aromaticum* ranged from 5.83 mm, 6.86 mm, and 7.17 mm for the three extracts, respectively.

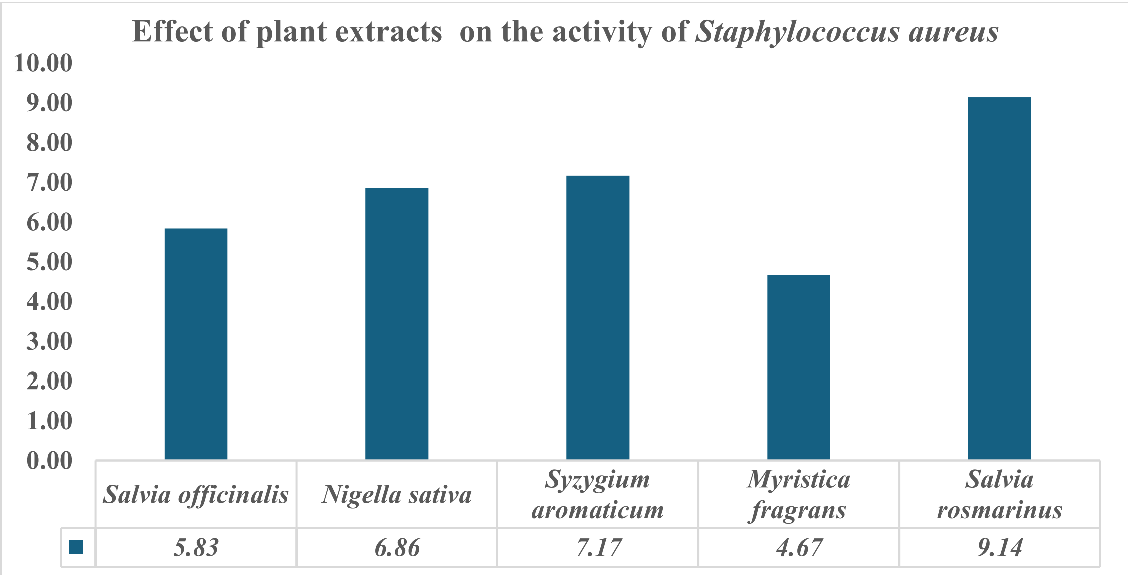

Fig. 1 Effect of plant extracts on activity of *Staphylococcus aureus* bacteria.

These plant extracts' antibacterial activity was assessed and documented in Table 2 and Figure 2. The findings showed that all plant extracts had the capacity to inhibit the microbiological growth of *S. aureus* bacteria with varying degrees of efficacy. *Nigella sativa* was the most effective extract at retarding the microbial growth of *S. aureus* bacteria at a concentration of 2.5% reaching 11.25 mm. However, the effect diminished at 5% concentration, and at 10% concentration, the extract had no inhibitory effect on bacterial growth.

The other plant extracts also showed variable antimicrobial activity and demonstrated inhibitory effects against *S. aureus* strains. The antibacterial activity varied with the extract concentration, with low concentrations (2.5%) of *Salvia officinalis* and *Myristica fragrans* plant extracts showing no inhibitory effect on *S. aureus*. The inhibitory effect increased with increasing extract concentration, reaching 8.5 mm and 3.5 mm at 5%

for both plants, respectively. It further increased with increasing concentration to 10%, reaching 9 mm and 10.5 mm for both extracts, respectively.

On the other hand, the different concentrations of *Salvia rosmarinus* leaf extract showed a great variation in the inhibitory effect and antibacterial activity against *Staphylococcus aureus* strains, where the inhibitory effect was recorded when using the extract at a concentration of (2.5 %), the highest rate of inhibition of bacterial growth was recorded, reaching 9.75 mm, which decreased with increasing the extract concentration to 5%, 10% to record 9.75 mm, and 8 mm for both concentrations, respectively. On the contrary, the antibacterial activity of the *Syzygium aromaticum* leaf extract increased with increasing concentration, ranging between 6.33 mm, 7.5 mm, and 7.67 mm for the three different concentrations, respectively (2%, 5%, and 10%).

The data in the table also shows that there are significant differences and effects between the antibacterial activity of the different plant species for which the aqueous leaf extracts were used, as well as significant differences between the three different concentrations used for all the different plant extracts.

These results agree with what was shown by Adegbodu *et al*., 2020 when evaluated the inhibitor effect of Rosemary, Nutmeg, and Cloves plant extracts on S. aureus bacteria.

Also, these results agree with Oo *et al*. (2021); they demonstrated the inhibitory effect of nutmeg seed extract on *Staphylococcus aureus* strains. They attributed the effect to the main components contained in nutmeg seed extracts, including myristicin, α-pinene, β-pinene, sabinene, 4-terpineol, methoxyeugenol, β-myrcene, elemicin, β-phellandrene, tetradecanoic acid, and γ-terpene. This also agrees with Ansory *et al*., 2018, and Nurjanah *et al*., 2017, on the pharmacological importance of nutmeg seed extracts, as they contain components that act as antimicrobial and inhibitory agents against Gram-positive and Gram-negative bacteria as well as fungi. Additionally, it has been discovered to possess antibacterial and antioxidant properties (Lima *et al*., 2012).

The results also agreed with what was explained by Abd Karim *et al*., 2020, that extracts of black seed (*Nigella sativa*) and *Syzygium aromaticum* showed antibacterial activity against *Staphylococcus aureus*, as it is a natural, safe and effective source of antibacterial and antioxidant compounds due to their containing biologically active compounds, such as their high content of phenol.

Generalie *et al.*, 2012 attributed the antibacterial and inhibitory effect of sage extract (*Salvia officinalis*) on *S. aureus* to the high content of compounds belonging to diverse chemical groups, including a high phenolic compound, saponins, tannins and flavonoids (Garcia *et al*., 2012).

Table 2: Effect of plant extracts of various plants and its concentration on activity of Staphylococcus aureus bacteria

Treatments	**Concentration (B)**			**Mean (A)**	**LSD $_{0.05}$ (A)**
Plant (A)	**C_1 (2.5 %)**	**C_2 (5 %)**	**C_3 (10 %)**		
Salvia officinalis	**0.00**	**8.50**	**9.00**	**5.83**	**0.29**
Nigella sativa	**11.25**	**9.33**	**0.00**	**6.86**	**1.03**
Syzygium aromaticum	**6.33**	**7.50**	**7.67**	**7.17**	**0.11**
Myristica fragrans	**0.00**	**3.50**	**10.50**	**4.67**	**2.18**
Salvia rosmarinus	**9.75**	**9.67**	**8.00**	**9.14**	**0.27**
LSD $_{0.5}$ (B)	**1.14**	**0.31**	**0.29**		

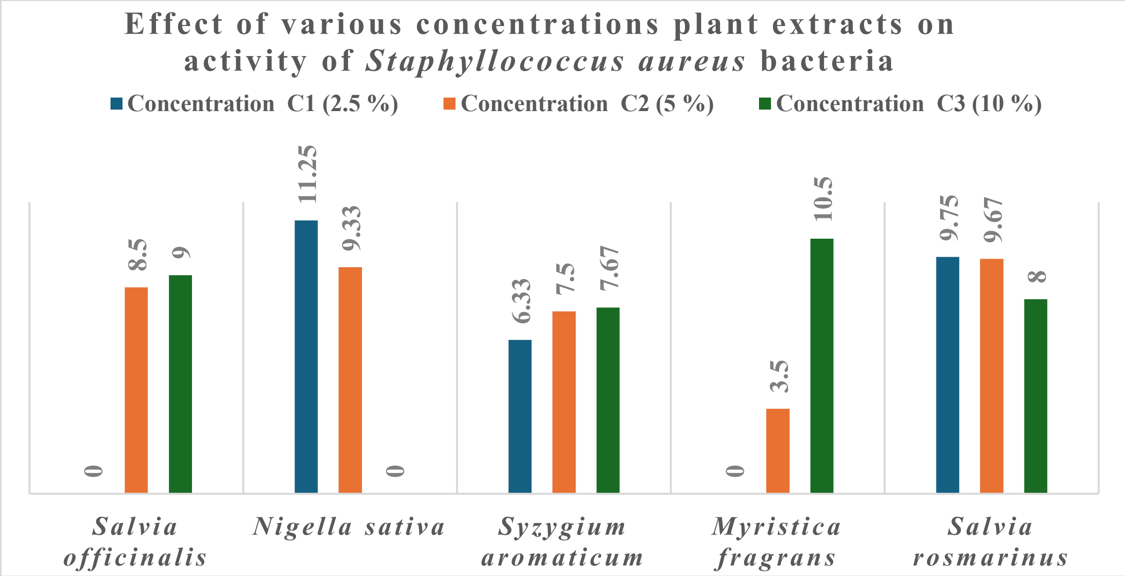

Fig. 2: Effect of various concentrations plant extracts on activity of *Staphylococcus aureus* bacteria.

CONCLUSION

Numerous health issues and food degradation can be caused by different strains of dangerous bacteria. Chemical preservatives can be used to avoid this, but doing so has negative health effects and allows chemicals to infiltrate various food chains, where they can cause toxicity and long-term issues. It is necessary to create natural preservatives that are less complicated, safer, and more effective considering these negative effects. For the prevention of food poisoning, existing plant extracts with demonstrated potential advantages (such as nutmeg, clove, rosemary, black cumin, and sage) can be utilized as natural substitutes for synthetic antibacterial agents.

Although plant extracts have demonstrated potent antibacterial activity comparable to that of currently studied and used chemical drugs, the production of effective antimicrobials from these plants should begin as soon as possible, and further research should be conducted on their clinical efficacy, safety, and toxicity which are safer and less expensive.

Declarations:

Ethical Approval: This study did not involve any live animals. It was based solely on experimental laboratory and analysis of plant extracts

Conflict of interest: The authors declare no conflict of interest.

Author's Contributions: I hereby verify that the authors mentioned on the title page has Contributed significantly to the idea and planning of the research, has carefully read the work, attested to the veracity and correctness of the data and its interpretation, and has given their approval for submission.

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Availability of Data and Materials: All data generated or analyzed during this study are included in this article.

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