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Phyto-activity of *Syzygium aromaticum* extract against pathogenic bacteria isolated from chronic tonsilitis patients

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ABSTRACT

Because of its antibacterial and antioxidant characteristics, the naturally occurring spice *Syzygium aromaticum* (clove) is used as a medicine and a preservative. Although it is said to have originated in Indonesia, clove is now grown in numerous coastal regions at higher altitudes all over the world. Clove has been used for ages for its medicinal properties, which have been shown effective against a wide variety of illnesses. Clove has been found to be effective as an antibiotic, antiviral, anti-inflammatory, hepatoprotective, stress-reducing, interoceptive, and anesthetic. Clove's larvicidal efficacy against dengue is a novel use. Extraction of clove's volatile oil has found commercial usage in the creation of pharmaceutical, nutritional, and personal care products. Eugenol is a crucial phenolic molecule found in clove oil's various phytoconstituents. With regard to the issues raised, this study carried for detection activity of clove on pathogenic *S. aureus* and *S. pyogen* isolated from patients with chronic tonsillitis. The present study noted that the activity of methanolic extract was higher than that of aqueous extract in all concentrations except for the 1% concentration, that had no activity against both bacteria. Also, it was noted that the *S. aureus* had higher susceptibility than *S. pyogen* for both extracts.

Keywords: *Syzygium aromaticum,* EO, clove, *S. aureus, S. pyogen*

INTRODUCTION

As knowledge in the fields of nutrition and medicine expands, plants play an ever-increasing role in the lives of humans. Flavoring agents, culinary additives, coloring agents, preservatives, and medications are just a few of the many uses for the dried root, seed, bark, fruit, or flowers of plants known as spices (Sachan et al., 2018). The discovery of spices, which have been used for flavoring since prehistoric times, was a cause for celebration. Essential oils are described as an aromatic product of complex composition that is extracted from a botanically defined raw vegetable material using hydro-distillation, steam distillation, or a suitable mechanical technique, as stated in the European Pharmacopoeia (Machadora et al., 2020). Clove, or *Syzygium aromaticum,* is one of the world's most expensive spices and the second most traded spice overall. *Caryophyllus aromaticus*, *Caryophyllus silvestris*, *Eugenia caryophyllus*, *Jambosa caryophyllus*, and *Myrtus caryophyllus* are all names for the clove tree (Kaur and Kaushal, 2019). Cloves, which are native to North Maluku Islands in Indonesia but are harvested at various times around the world, are widely cultivated, and can be found in stores year-round. Some wild clove variants are located in Bacan, Ternate, Motir, Tidore, Makian, and western sections of Irian Jaya, whereas the majority of cultivated clove comes from Indonesia, Madagascar, and Pemba. Only the states of Karnataka, Tamil Nadu, and Kerala are able to support clove growing in India. After Indonesia, India is now the world's largest user of clove. To put it simply, clove is a fragrant tree (Ginigaddara, 2018; Kaur and Kaushal, 2019). The

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word clove comes from the French words for nail (clou) and clove (clove). The clove tree, also known as the conical myrtle, reaches a maximum height of 10-12 meters and has a straight trunk (Khan, 2022). The dense, grayish, semi-erect branches are a dark shade of green. The large, opposing leaves are oblong to elliptic in shape, simple obovate in outline, hairless, and covered in oil glands. Flowering on this tree starts at the 7-year mark and lasts for the next 80 years. Oils extracted from clove stems, clove buds, and clove leaves are all commercially accessible. The chemical make-up, aroma, and color of clove essential oils vary widely. The quantity of secondary metabolites present in clove essential oil is influenced by environmental, climatic, agricultural, and genetic variables (Nair, 2021).

Inflammation of the palatine tonsils, or tonsillitis, is most commonly (if not always) brought on by bacteria. Tonsillitis episodes are marked by high body temperature, a sore throat, odynophagia, swollen tonsils with or without exudate, and cervical lymphadenopathy (Klagisa et al., 2022). Clinical diagnosis of tonsillitis relies on anamnestic history and physical examination. (Sydorchuk, 2018). When more than two bouts of tonsillitis are experienced within a 12-month period, the possibility of recurrent tonsillitis should be explored (Wilson et al., 2023). Antibiotics are the standard treatment for tonsillitis episodes. An abscess in the peritonsillar space can develop if inflammation from the palatine tonsil spreads to the neighboring peritonsillar tissue (Klagisa et al., 2022). The most typical purulent consequence of acute tonsillitis is a peritonsillar abscess (PTA). Clinical history and physical examination are used to confirm a PTA diagnosis. Microbiological analysis results show little variation between those with tonsillitis and healthy controls in studies comparing the two groups (Sanmark et al., 2020). Without including comparisons to other patient groups, many studies just look at the microbiological outcomes of PTAs (Montero et al., 2017; Klagisa et al., 2022). The reasons for the failure of conservative therapeutic techniques in cases of tonsillitis are not well understood, and there are not many studies available on the topic. Research shows that S. aureus is highly prevalent in tonsillar samples from people who have tonsillitis (Smith and Heubi, 2018). The most common causes of tonsillitis are the bacteria *Staphylococcus aureus* and *Streptococcus pyogen.* It is unknown, however, what part these organisms play in the development of abscesses, in the development of resistance to antibacterial therapy, and in the etiology of tonsillitis aggravation. It is important to investigate alternative defensive mechanisms, such as biofilm formation, because S. aureus does not exhibit great resistance to antibiotics in tonsillitis, in contrast to *S. pyogen.* Antibiotic resistance has been linked to the development of biofilms (Katkowska et al., 2020; Michalik et al., 2020). Clove essential oil's antibacterial action in pasteurized milk has been documented against *S. aureus* and *L. monocytogenes,* among others (Radünz et al., 2019). Clove oil was found to be highly effective against *Penicillium sp., Aspergillus flavus,* and *S. aureus,* all of which were isolated from dried fish (Decapterus maruadsi) (Chouhan et al., 2018). Clove oil's antibacterial properties were tested on *L. innocua, C. diversiformis,* and *S. aureus,* three gram-positive bacteria, and four gram-negative bacteria. via the broth microdilution method: *S. typhimurium, E. coli, S. liquefaciens,* and *S. putrefaciens* (de Meneses et al., 2019).

MATERIALS AND METHOD

Plant collection

Two fresh plant parts of *Syzygium aromaticum* was collected from a Thi-Qar province market, evaluated for their antimicrobial activity against two pathogenic bacteria, were isolated from tonsillitis patients.

Phytochemical extraction drying

The newly gathered plant components were cleaned in running water and then in sterile distilled water before being used in the extraction process. The substance was dried in a 50° C oven for 48 hours before being ground into a powder (Köprü et al., 2020).

Preparation of bacterial suspension

The tests bacteria *S. aureus* and *S. pyogen* were isolated from Nasiriyah city in Thi-Qar province. By adding sterile distilled water, the concentration of two harmful bacteria was brought up to the 0.5 McFarland standard (1.5×10^8 CFU/ml). Adjusting the turbidity of a microbiological suspension against McFarland standards ensures that the total number of microorganisms remains within an acceptable range. After thoroughly mixing 9.95 ml of 0.18M H_2SO_4 (1.0% w/v) with 0.05 ml of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2 \cdot 2H_2O$), we had our 0.5 McFarland standard. The McFarland standard tube could be sealed snugly for up to 6 months to prevent evaporation loss. The test and the standard were shown on a white backdrop separated by a black line to facilitate comparison (Julianti et al., 2017).

Preparation of aqueous and methanolic extracts

The extraction of certain plant parts yields an extract, which is a combination of phytochemicals (Kazlauskaite et al., 2022). The phytochemicals in the plants were extracted using a solvent, specifically 95% ethanol and distilled water. In order to create 100 ml of ethanol extract (1%, 5%, 10%, 15%, 20%, and 25% w/v), 1, 5, 10, 15, 20, and 25 g of powdered plant material were dissolved in adequate sterile ethanol and distilled water, respectively. For 24 hours, the mixture was left undisturbed in a sterile flask covered with aluminum foil to prevent evaporation, before being filtered using sterilized Whatman no.1 filter paper. The filtered extract was then evaporated in a water bath until only 1, 5, 10, 15, 20, and 25 milliliters of the original extract remained. Extracts prepared in this way, both ethanolic and aqueous, were tested right away for antibacterial activity using the agar well diffusion technique.

Statistical analysis

The current data were statistically analyzed by statistical software program SPSS version 26, based in using One way ANOVA for mean comparison, least significant difference LSD for mean difference, independent sample t-test, and Scatter blot for drawing the activity diagrams.

RESULTS

Antimicrobial activity of aqueous extract on *S. aureus* and *S. pyogen* according to concentrations

The present study showed the activity of aqueous extracts was increasing with concentration increase against both *S. aureus* and *S. pyogen.* Also, it was noted that the 1% concentration did not exhibit activity against *S. aureus,* while both 1% and 5% concentrations did not exhibit activity against *S. pyogen,* the study also recorded the high activity against *S. aureus* was 13.3 ± 0.26, while against *S. pyogen* 11.2 ± 0.23. in the other hand the study noted the *S. aureus* bacteria effects more than *S. pyogen* in all concentrations with except 1% concentration at p. value <0.05, as in Table 1.

TABLE 1. Antimicrobial activity of aqueous extract on *S. aureus* and *S. pyogen* according to concentrations

Concentrations	*S. aureus*	*S. pyogen*	p. value
	Aqueous extract inhiation zone Mean ± SD		
1%	0.00 ± 0.00[f]	0.00 ± 0.00[e]	1.000
5%	0.76 ± 0.30[e]	0.00 ± 0.00[e]	<0.001
10%	3.66 ± 0.37[d]	1.70 ± 0.36[d]	<0.001
15%	10.1 ± 0.58[c]	4.86 ± 0.28[c]	<0.001
20%	11.9 ± 0.40[b]	9.46 ± 0.20[b]	<0.001
25%	13.3 ± 0.26[a]	11.2 ± 0.23[a]	<0.001
p. value	<0.001	<0.001	
LSD	0.65	0.40	

Antimicrobial activity of methanolic extract on *S. aureus* and *S. pyogen* according to concentrations

The present study showed the activity of aqueous extracts were increasing with concentration increase against both *S. aureus* and *S. pyogen.* Also, it was noted that the 1% concentration did not exhibit activity against *S. pyogen.* The study also recorded the high activity against *S. aureus* was 15.5 ± 0.40, while against *S. pyogen* it was 14.4 ± 0.75. On the other hand, the study noted the *S. aureus* bacteria significantly effects more than *S. pyogen* in 1%, 5%, 15% and 20% concentrations, while non-significantly in 10% and 25% concentrations at p. value < 0.05, as in Table 2.

TABLE 2. Antimicrobial activity of methanolic extract on *S. aureus* and *S. pyogen* according to concentrations

Concentrations	*S. aureus*	*S. pyogen*	p. value
	Methanolic extract inhiation zone Mean ± SD		
1%	1.06 ± 0.11[f]	0.00 ± 0.00[f]	1.000
5%	2.96 ± 0.32[e]	2.40 ± 0.34[e]	0.030
10%	5.96 ± 0.72[d]	4.63 ± 0.60[d]	0.072
15%	12.2 ± 0.58[c]	7.70 ± 0.36[c]	<0.001
20%	14.1 ± 0.56[b]	12.2 ± 0.25[b]	0.006
25%	15.5 ± 0.40[a]	14.4 ± 0.75[a]	0.088
p. value	<0.001	<0.001	
LSD	0.88	0.81	

A comparison between aqueous and methanolic extracts on *S. aureus* according to concentrations

The methanolic extract exhibited significant antimicrobial activity against *S. aureus* more than aqueous extract in all extract concentrations, at p. value <0.05, as in Table 3.

TABLE 3. A comparison between aqueous and methanolic extracts on *S. aureus* according to concentrations

Concentrations	Aqueous	Methanolic	p. value
	S. aureus Response Mean ± SD		
1%	0.00 ± 0.00	1.06 ± 0.11	<0.001
5%	0.76 ± 0.30	2.96 ± 0.32	0.001
10%	3.66 ± 0.37	5.96 ± 0.72	0.008
15%	10.1 ± 0.58	12.2 ± 0.58	0.012
20%	11.9 ± 0.40	14.1 ± 0.56	0.005
25%	13.3 ± 0.26	15.5 ± 0.40	0.001

A comparison between aqueous and methanolic extracts on *S. pyogen* according to concentrations

The methanolic extract exhibited a significant antimicrobial activity against *S. pyogen* more than aqueous extract in all extract concentrations, with the exception of 1%, which did not exhibit significant difference at p. value <0.05, as in Table 4.

TABLE 4. A comparison between aqueous and methanolic extracts on *S. aureus* according to concentrations

Concentrations	Aqueous	Methanolic	p. value
	S. pyogen Response Mean ± SD		
1%	0.00 ± 0.00	0.00 ± 0.00	1.00
5%	0.00 ± 0.00	2.40 ± 0.34	<0.001
10%	1.70 ± 0.36	4.63 ± 0.60	0.002
15%	4.86 ± 0.28	7.70 ± 0.36	0.001
20%	9.46 ± 0.20	12.2 ± 0.25	<0.001
25%	11.2 ± 0.23	14.4 ± 0.75	0.002

DISCUSSION

The current study noted the phyto activity of clove methanolic extract proved higher activity than clove aqueous extract in all concentration except for the 1% concentration which had no activity against both bacteria. Also, *S. aureus* was recorded having hight susceptibility for both extract activity than *S. pyogen.*

The emergence of antibiotic-resistant bacteria in poor countries was triggered by both the infections themselves and the greater availability of some over the counter medications. Incorrect dosing and the inappropriate use of antibiotics for viral infections may also play a role. Bacterial susceptibility to different antibiotics may exhibit geographical and temporal variations (Gebremariam et al., 2015). Clove essential oil offers antimicrobial activity against a wide variety of infections. The –OH groups in the meta and ortho locations of the primary chemical composition have been linked to the antibacterial action. The cytoplasmic membrane of microorganisms can interact with these functional groups (Rajkowska et al., 2016; Behbahani et al., 20119). The study of Ayushi et al., (2020), detected

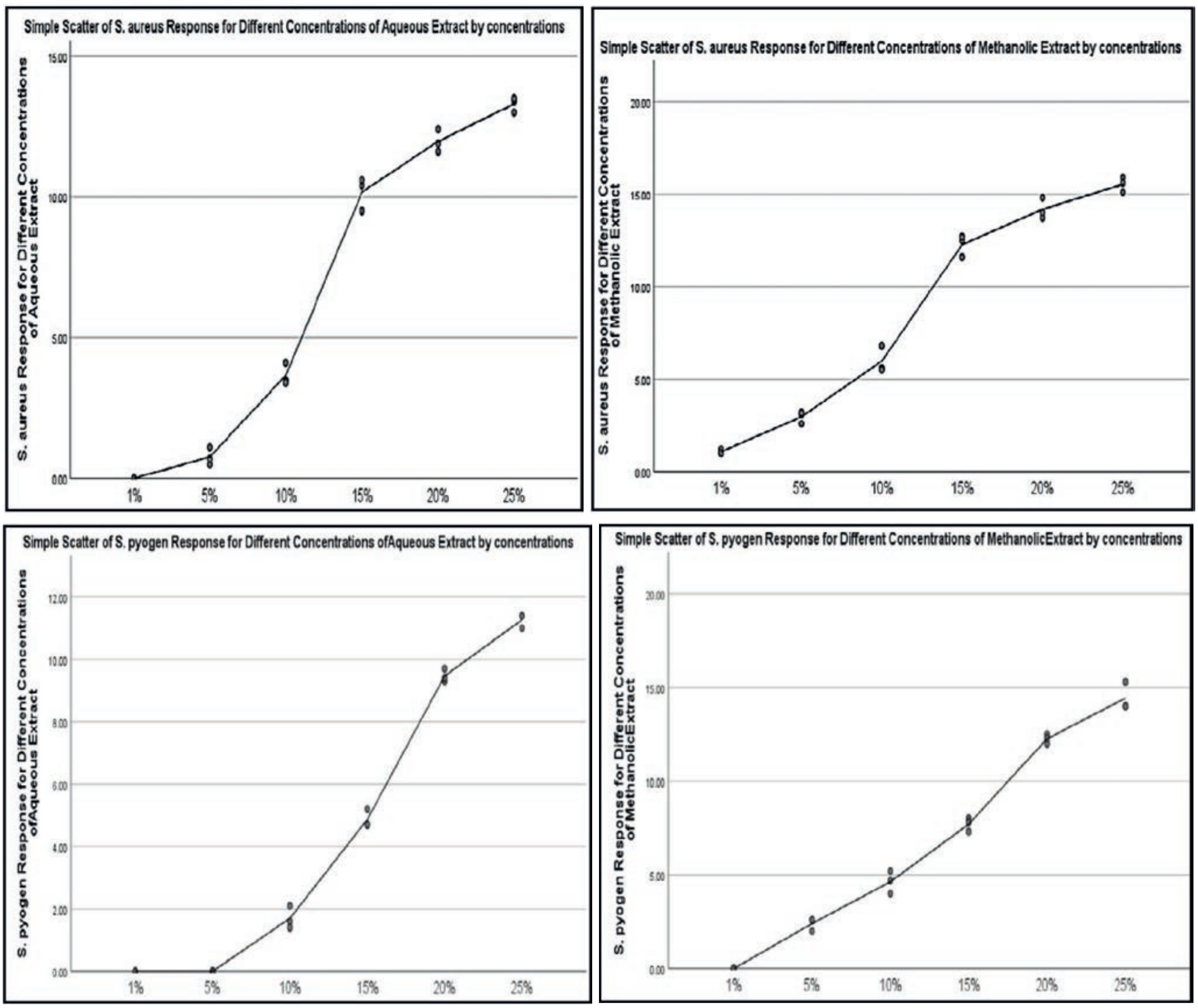

FIGURE 1. The scatter blot of aqueous and methanolic extracts show the activity of extract increased with concentrations increase, which also exhibited low and high activity against both bacteria

the clove oil extract has showed to be effective against *Staphylococcus SPP* and *A. niger,* and also noted the germicidal activities against *S. aureus, P. aeruginosa,* and *K. pneumonia,* which have been shown by dispensing clove oil in 0.4% v/v concentrated with sugar solution. A study of Mohamed et al., (2018), involved 200 patients with corneal ulcer; their study showed 81.3% activity of clove extract against pathogenic bacteria, and also noted 97.5% of *S. aureus* inhibited by clove extract, and 76% of *S. aromaticum.* The study of Ginting et al., (2021), involved extracts of clove and cinnamon against gram negative bacteria isolated from chicken meat and their results revealed the effect of clove and cinnamon essential oils on both *E. coli, K. pneumoniae,* and ESBL producing *E. coli* from chicken meat observed through SEM showed changes in bacterial cell structure. In the studies of Abdel-Moaty et al., (2016), and Qian et al., (2020), the antibacterial activity of clove and cinnamon essential oils were tested against ESBL-producing bacteria as a comparison to the previous studies, and reported the essential oil of both plants had higher activity than antibiotics.

There may be a number of factors contributing to the recent uptick in research and usage of medicinal plants as alternative medicines in the prevention of infections. To begin with, secondary metabolites from plants have enormous promise as antibacterial agents. Secondly, essential oils (EOs) can kill microorganisms without the harmful side effects of synthetic antibiotics. One more benefit of EOs resulting from the research is their ability to kill Gram-positive bacteria across the board. This detrimental effect on cellular permeability has been repeatedly described as the mechanism of action of *S. aromaticum* oil in studies of *S. aureus* and *S. pyogen* in recent years (Xu et al., 2016), which is in agreement with the negative impact of *S. aromaticum* oil on *S. aureus* cellular permeability found here.

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