International Journal of Secondary Metabolite
2024, Vol. 11, No. 4, 833–852
https://doi.org/10.21448/ijsm.1438721
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Chemical profile by GC-MS and protective effect of Algerian cloves (*Syzygium aromaticum*) against *Lactobacillus* spp. and *Streptococcus* spp. isolated from dental caries

Ouahiba Benhamada[1,2*], **Nabila Benhamada**[2,3], **Lilia Boussouf**[1,4], **Essaid Leghouchi**[2,3]

[1]Department of Applied Microbiology and Food Sciences, Faculty of Nature and Life Sciences, University of Jijel, 18000 Jijel, Algeria
[2]Laboratory of Biotechnology, Environment and Health, Faculty of Nature and Life Sciences, University of Jijel, 18000 Jijel, Algeria
[3]Department of Cell and Molecular Biology, Faculty of Nature and Life Sciences, University of Jijel, 18000 Jijel, Algeria
[4]Laboratory of Biomathematics, Biophysics, Biochemistry, and Scientometry, Faculty of Nature and Life Sciences, University of Bejaia, 06000 Bejaia, Algeria

ARTICLE HISTORY
Received: Feb. 18, 2024
Accepted: July 04, 2024

KEYWORDS
Tooth decay,
GC-MS,
Clove,
Antibacterial activity,
Polyphenols,
Flavonoids.

Abstract: The oral cavity is home to a large and diversified microbial flora, which plays an important role in the genesis of multiple diseases, including tooth decay. Indeed, tooth decay is the most common ailment in the world, with almost everyone having experienced it at least once in their lifetime. Examining plants used in traditional medicine is one of the research approaches used to discover novel, potent antibacterial chemicals with a broad spectrum of action, as present antibacterials have significant drawbacks. This study aims to examine the chemical composition of Algerian clove *Syzygium aromaticum* using GC-MS and to evaluate the antibacterial activity of the methanol extract against bacteria isolated from dental caries caused by *Streptococcus* spp. and *Lactobacillus* spp. The results show a strong extraction yield of 29.7%, with high amounts of polyphenols and flavonoids calculated at 178.82 mg GAEQ/g and 24.13 mg QEQ/g. The principal chemical elements of *S. aromaticum* peel methanol extract were identified as eugenol (61.23%) and eugenol acetate (26.45%) based on mass spectrum data and retention times. The methanol extract has a significant antibacterial effect against tested strains, with MICs ranging from 111.37 to 445.5 mg/mL. Higher concentrations of polyphenols resulted in a significant increase in inhibition zone diameter against S1 ($r^2 = 0.94$, $p < 0.001$), L3 ($r^2 = 0.94$, $p < 0.001$), L5 ($r^2 = 0.93$, $p < 0.001$), and L9 ($r^2 = 0.96$, $p < 0.001$).

1. INTRODUCTION

Dental caries, one of the most common chronic illnesses of the oral cavity worldwide, persists in the modern period despite access to the most advanced sciences and technologies in dental treatment. Bacterial fermentation of dietary carbohydrates, specifically sucrose, breaks down tooth-hard acellular tissue (Kabra *et al.*, 2012; Riaž *et al.*, 2023). One of the most pressing

*CONTACT: Ouahiba BENHAMADA wahibabenhamada@yahoo.fr City of 402 Log, Mazeghitane, Block 13 n 12, Jijel (18000), Algeria

e-ISSN: 2148-6905

issues of our time is the ineffectiveness of antibiotic treatments. Given the numerous barriers to the use of known antibacterials, it is critical to search for novel, effective antibacterial compounds with a broad spectrum of action.

One of the study methodologies is to investigate plants used in traditional medicine. Because medicinal plants contain a high concentration of antimicrobial compounds, they have been shown to be beneficial in treating a number of ailments, including bacterial disorders (Featherstone, 2000). Several studies have tested plant extracts for antibacterial action against pathogenic bacteria (Dogruoz *et al.*, 2008; Amadi *et al.*, 2016; Mostafa *et al.*, 2018; Srikacha & Ratananikom, 2020). Medicinal plants are widely used in the treatment of dental caries and dental care-associated infections because they contain phytochemicals such as flavonoids, polyphenols, terpenes, and alkaloids (de Oliveira Carvalho *et al.*, 2020; Sharaf *et al.*, 2021; Foda *et al.*, 2022; El-Sherbiny & Mahmoud, 2022). Clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) is one of the most expensive spices and has been used for centuries as a food preservative and for a number of medicinal purposes. Clove was originally grown in Indonesia, but it is now grown all over the world, mainly in Brazil's Bahia state. This plant has a high potential for use in food, cosmetics, medicine, and agriculture. It is a rich source of bioactive chemicals such as hydroxycinnamic acids, hydroxybenzoic acids, phenolic compounds, and flavonoids. Cloves' principal phenolic components include eugenol, eugenyl acetate, caryophyllene, and gallic acid, which account for their high antioxidant capabilities (Cortés-Rojas *et al.*, 2014; Gengatharan & Abd Rahim, 2023).

Clove plants are widely used as traditional remedies due to their anti-helminthic, anti-inflammatory, anti-spasmodic, anti-pyretic, anti-allergic, antifungal, anti-carcinogenic, anti-allergic, antiviral, antioxidant, anti-mutagenic, anti-arthritis, and anti-parasitic properties. Cloves are being employed for their antibacterial characteristics (Saikumari *et al.*, 2016; Ajobiewe et *al.*, 2022; Yakubu Bello *et al.*, 2022). This study aims to contribute to a better understanding of this plant and to promote its traditional use for therapeutic purposes. We investigated the chemical composition by GS-MS and in vitro the antibacterial activity of the methanol extract of *S. aromaticum* growing in northern Algeria. The agar-well diffusion method was used against *Lactobacillus* spp. and *Streptococcus* spp. isolated from dental caries.

2. MATERIAL and METHODS

2.1. Plant Material

The species used in this study is *S. aromaticum* (Table 1); however, it has also been known as *Eugenia caryophyllata*, *Eugenia caryophyllus*, and *Eugenia aromatica* (Penot, 2016; Kaur & Chandrul, 2017).

Table 1. Scientific classification of clove (Kaur & Chandrul, 2017).

Classification unit	Classification
Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Eudicots
(unranked)	Rosids
Order	Myrtales
Family	Myrtaceae
Genus	*Syzygium* Gaertn.
Species	*S. aromaticum* (L.) Merr. & L.M.Perry

The flower buds were employed; they are dark brown "nail" in color, 12 to 17 mm long, with a lower calyx (hypanthus) up to 4 mm thick, surmounted by four leathery and divergent lobes made up of the four fleshy sepals spreading in a cross. The four paler, non-spreading petals are yellow-brown and create a headpiece that conceals numerous bent stamens and a short upright style on a nectar-bearing disc at the base (Figure 1a). The bilocular inferior ovary's receptacle

tube is angular, wrinkled, and carries a large number of seeds. These buds have a distinct aroma and flavor that is fragrant, scorching, and pungent (Wichtl & Anton, 1999).

2.2. Methanol Extract Preparation

2.2.1. *Sampling and grinding*

The clove buds were procured at a local store in Jijel, Northeast Algeria, in April 2022. The samples were dried at room temperature for three days, then in an oven at 40°C for five days. Subsequently, the plants were ground with a coffee grinder until a fine dry powder was obtained (Figure 1b).

2.2.2. *Phenolic compounds extraction*

According to Owen *et al.* (1999), methanol is the ideal solvent for extracting phenolic chemicals because it boosts extraction efficacy and is easier to remove. It also works well when coupled with water (80%) (Qasim *et al.*, 2016; Nakilcioglu & Otles, 2021). The complete extract was made by macerating 30 g of clove powder with 300 mL of 80% methanol in a flask on a magnetic stirrer for 48 hours in the dark at room temperature. After maceration, the clove solution was filtered via filter paper and evaporated at 40°C in a Heidolph-type steam rota until the solvent was entirely evaporated (Figure 1c). The extract was collected and kept in the freezer at -20 °C until use.

Figure 1. *S. aromaticum* in forms: clove buds (a), crushed cloves (b), and clove methanol extract (c).

2.2.2.1. Extraction yield determination. The extraction yield is calculated as the weight of the clove methanol extract (Figure 1c) divided by the weight of the powdered plant (Figure 1b). This yield was computed as a percentage using Eq. (1):

$$Y(\%) = W_d/W_p \times 100 \quad (1)$$

where, Y is the extraction yield as a percentage, W_d is the weight of dry extract in grams, and W_p is the weight of the powdered plant in grams.

2.2.2.2. Phenolic compounds determination. The total polyphenols were determined using the Folin-Ciocalteu (FC) method (Slinkard & Singleton (1977), with gallic acid as a standard: 1 mL of clove extract was mixed with 1/10 mL of FC reagent and 2% Na_2CO_3. The mixture was stirred and incubated in the dark at room temperature for 2 hours. The absorbance was measured at 760 nm using a spectrophotometer. The results were expressed in mg gallic acid equivalent per gram dry weight (mg GAEQ/g) using the equation ($y = 6.574$ x, $r^2 = 0.99$) derived from the calibration curve established with gallic acid.

2.2.2.3. Flavonoids compounds determination. Determination was based on the principle of direct determination by aluminum trichloride using the method of Meda et al. (2005). Flavonoids have a free hydroxyl group in positron that, when combined with aluminum trichloride, forms a yellowish complex through ion chelation. The yellow color produced is proportional to the amount of flavonoids in the extract (Basli *et al.*, 2012). Then, 2 mL of crude methanol extract was combined with 2 mL of aluminum chloride methanol solution (2% $AlCl_3$). After 15 minutes, a spectrophotometer was used to read the wavelength at 415 nm against a blank. A calibration curve was generated in parallel under the same operating conditions, with

Quercetin serving as a positive control. The results are expressed in milligrams of Quercetin equivalent per gram dry weight (mg QEQ/g) using the equation obtained from the standard curve (y = 31.68 x, r^2 = 0.99).

2.3. GC-MS analysis of *S. aromaticum* methanol extract

The phytochemical compounds in the previously prepared crude methanolic extract of *S. aromaticum* were analyzed using the Shimadzu GC-MS QP2010 El 70 ev quadrupole model. A mass-selective detector was designed with a 200°C ion source and a 250°C interface. MS analysis was performed using an OV 1701 capillary column with a film thickness of 0.25 µm and a length of 25 meters. Helium was used as a carrier gas in a split-less injection (20:0 split ratio) mode at 250 °C, with a volume of 1µL and a flow rate of 1.00 mL/min. The injection was performed at a constant linear speed of 40.6 cm/sec, with a purge flow of 1.2 mL/min and a total flow of 22.2 mL/min. The temperature was initially set at 90.0 °C and gradually increased to 250 °C at a rate of 10 °C per minute. The sample's total run time was set at 52 minutes. The mass spectrum range was set from 40.00 to 350.00 m/z.

2.4. Phytochemical Compounds Identification

To identify the compounds, their names, structures, and molecular weights were determined by comparing their spectra to those found in the National Institute of Standards and Technology database library (Nist05.LIB). Components were identified using GC retention time (RT), and MS fragment interpretation was performed by comparing the results to the Nisto5.LIB database.

2.5. Evaluation of the Antibacterial Activity of Methanol Extract

2.5.1. *Bacteriological samples*

A sterile excavator was used to collect ten samples from dental surgeons in Jijel wilaya from carious lesions and dentin softenings of patients with tooth decay. These samples were placed in sterile tubes with nutrient broth, transported to the laboratory, and incubated at 37°C for 24 hours.

2.5.2. *Isolation and identification of bacteria*

After incubation, the samples were isolated on Columbia and MRS agar plates to detect *Streptococcus* spp. and *Lactobacillus* spp., which are the most involved in the formation of dental caries. Sterile swabs were soaked in the nutrient bowls for each sample and deposited on the surface of the agar, which had previously been poured into a Petri dish and cooled before being inoculated using the streak technique. The dishes were then incubated at 37°C for 24 h for *Streptococcus* spp. and 48 h for *Lactobacillus* spp. Following incubation, the bacteria were identified in three stages:

• *macroscopic examination* consists of studying colony morphology (shape, appearance, outline, surface, color) from cultures obtained on agar of Colombia and MRS media;

• *microscopic examination* is performed on a bacterial smear, prepared from the suspect colonies in pure cultures, then fixed and stained by the Gram method to determine their morphology and Gram type (positive or negative Gram);

• *biochemical identification of bacteria* was performed using API 20 campy, catalase, and oxidase assay (Delarra, 2007).

2.5.3. *Transplanting bacterial strains*

The various bacterial strains were transplanted onto appropriate agar media using the streak method 24-48 h before antibacterial activity testing and then incubated in an oven at 37°C for 24-48 h to obtain a fresh culture and isolated colonies (La *et al.*, 2008).

2.5.4. *Inoculum preparation*

Colonies well isolated from fresh cultures were transferred to tubes containing sterile physiological water to produce bacterial suspensions with turbidity close to 0.5 McFarland (10^6 CFU/mL) (Kablan *et al.*, 2008; Kuete *et al.*, 2010; Souad *et al.*, 2010).

2.5.5. *Aromatogram*

The antibacterial activity of the methanolic extract was assessed using the Mueller-Hinton agar diffusion method (Souad *et al.*, 2010). The culture medium is poured into 90-mm Petri dishes. After solidification, each dish is inoculated with a sterile swab soaked in bacterial inoculum. After inoculation, sterile 5 mm diameter Whatman No. 1 paper discs were placed on the seeded agar with sterile forceps and filled with 10 µL of different concentrations of clove methanol extract. The plates were kept in the fridge at 5 °C for 2 hours to allow the plant extract to diffuse and then incubated at 37 °C for 24 hours. The absence of microbial growth around the discs creates a translucent halo (inhibition zone). The inhibition zone diameter is measured and expressed in mm. The negative control contained methanol, whereas the positive contained penicillin.

2.5.6. *Determination of minimum inhibitory concentration (MIC)*

The disk diffusion method, as described by Aneja and Joshi (2010) and Mostafa *et al.* (2018), was used to determine the MIC values of *S. aromaticum* extract concentrations in Mueller-Hinton agar, which was then inoculated with pathogenic strain bacterial suspensions. Because of its coagulated appearance, the methanol extract of *S. aromaticum* was first diluted by a decimal dilution (1/10) to a concentration of 891 mg/mL. Different concentrations of clove methanol extract were prepared separately in a geometric progression of 1/2 ratio, with concentrations ranging from 891, 445.5, 222.75, 111.37, and 55.68 mg/mL. Filter paper discs (8 mm in diameter) were moistened with 10 µL of various clove methanol extract concentrations and placed on top of Mueller-Hilton agar plates. All Petri dishes containing the seeded medium were refrigerated at 5 °C for 2 h to allow clove extract diffusion before being incubated at 37 °C for 24 h. The MIC was determined to be the extract's lowest concentration, indicating a distinct zone of inhibition (Nkere & Iroegbu, 2005; Aneja *et al.*, 2009).

2.6. Statistical Analysis

Three repetitions were performed for each concentration to calculate the standard deviation (SD). The ORIGIN 6.0 system was used to conduct the statistical analysis, which involved testing univariate variance (one-way ANOVA). The results were expressed as mean ± SD. A distinction was considered insignificant when $p > 0.05$ (NS), significant when $0.01 < p < 0.05$, very significant when $0.001 < p < 0.01$, and highly significant when $p < 0.001$. The correlation matrices between polyphenols and antibacterial activity were examined using STATISTICA Version 10.

3. RESULTS

3.1. Extraction Yield and Contents of Polyphenols and Flavonoids

The methanol extract of *S. aromaticum* produced a high yield of 29.7%. 8.91 g of dry residue was recovered from 30 g of powdered plant, containing 178.82 mg GAEQ/g polyphenols and 24.13 mg QEQ/g flavonoids.

3.1. Macroscopic and Microscopic Identification of Isolated Strains

Figure 2 and Figure 3 show the macroscopic and microscopic appearance of the isolated strains, respectively. Figure 2 depicts the colonies of isolated bacteria from the Columbia medium as a cream-colored mat. On MRS medium, growth results in the formation of isolated cream-white colonies with a uniform outline.

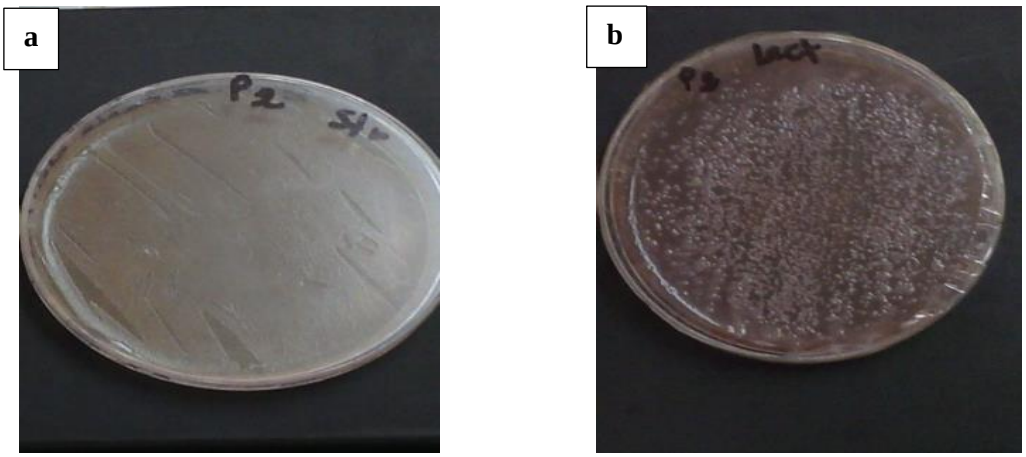

Figure 2. Macroscopic appearance of isolated bacterial colonies on Columbia Agar (a) and MRS Agar (b)

Gram staining revealed two types of cell arrangements: isolated and chain-shaped rounded cocci (Streptococci) from Columbia Agar (a) and isolated bacilli from MRS Agar (b).

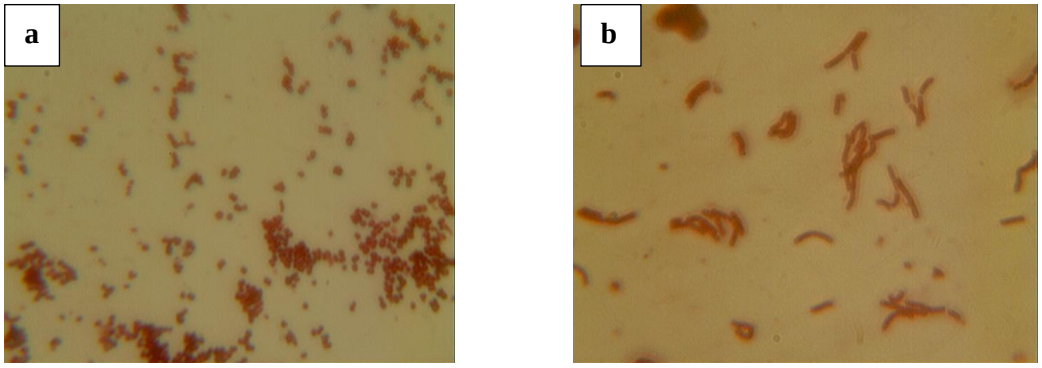

Figure 3. Microscopic appearance of isolated bacterial colonies on Columbia Agar (a) and MRS Agar (b) after Gram coloration

Streptococcus spp. were isolated from all ten samples, but *Lactobacillus* spp. could only be isolated from samples 1, 2, 3, 5, 9, and 10. Streptococci were found to be the most common etiological agents in the formation of dental caries, as opposed to lactobacilli. Table 2 lists the biochemical characteristics of the isolated bacteria. The findings clearly indicate that the samples belong to the genera *Streptococcus* spp. and *Lactobacillus* spp.

Table 2. Biochemical characteristics of isolated bacteria.

Biochemical test	ONPG	LDC	ODC	ADH	Citrate	H_2S	Urease	TDA	Indole	VP	Gelatinase	Glucose	Mannose	Inositol	Sorbitol	Raffinose	Saccharose	Melibiose	Amylase	Arabinose	Catalase	Oxidase
S	+	+	+	+	+	-	+	+	-	+	+	-	+	-	-	+	+	+	+	+	-	-
B	+	+	+	+	+	-	+	+	-	-	-	+	+	-	+	+	-	-	+	+	-	-

S. Streptococci, B. Bacilli, +. Positive reaction, -. Negative reaction

3.3. Estimation of Chemical Constituents of *S. aromaticum* by GC-MS

A GC-MS analysis of the chemical composition of *S. aromaticum* methanol extract revealed 31 peaks (Figure 4). Table 3 lists *S. aromaticum*'s phytochemical components, including their names and chemical formulas, concentrations (%), retention time (RT), and biological activities.

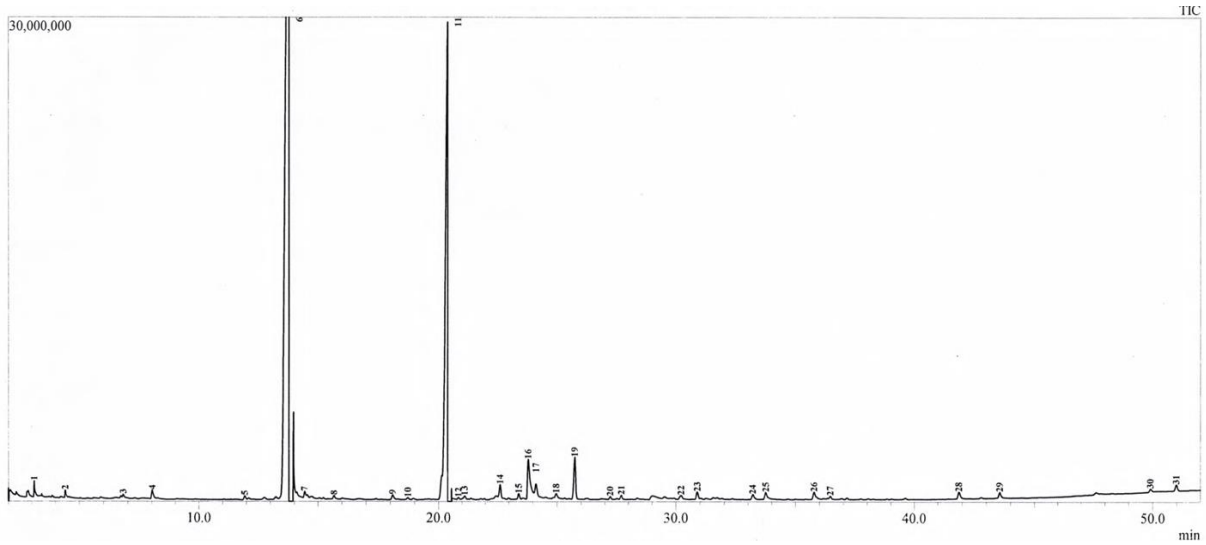

Figure 4. GC-MS chromatogram of methanol extract of *S. aromaticum*, where the x-axis represents retention time (mn) and the y-axis represents abundance.

By comparing mass spectral data and retention times, the main chemical constituent of *S. aromaticum* peel methanol extract was identified as eugenol (61.23%), which was detected at 13.177 mn. The other major constituent found in the methanol extract was eugenol acetate (26.45%) at 20.371 mn, followed by Pyrogallol (2.72%) and 2',3',4' Trimethoxyacetophenone (1.99%), while the other components had low values ranging from 0.09% to 0.74%.

The 20 chemical components of *S. aromaticum* are exhibited in biological activities including Pyrogallol, 2',3',4' Trimethoxyacetophenone, Benzyl Benzoate as antibacterials, 4H-Pyran-one, 2,3-dihydroxy-3,5-dihtdroxy-6-methyl-, Pentadecanoic acid, 14-methyl, methyl ester, 9-Octadecanoic acid (Z)-, methyl ester as antioxidants, caryophyllene, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, Caryophyllene oxide, estragole as anti-inflammatory, vanillin as anti-carcinogenic, and antioxidant, androsterone as neurosteroid and anticonvulsant, 4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl as antioxidant and anti-inflammatory, naphthazarin as antibacterial and antifungal, Di-n-octyl phthalate as antimicrobial and insecticidal (Table 3). The major chemical constituents of eugenol and eugenol acetate exhibit a variety of biological activities, including antibacterial, antiviral, and antifungal properties. Eugenol has anticancer, anti-inflammatory, and antioxidant properties.

Table 3. Lists of chemical components of *S. aromaticum* detected by GC-MS.

PN	Name of compounds	Molecular formula	RT	%	Biological activities	References
1	D-Alaninol	C_3H_9O	3.092	0.40	Undefined	
2	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	4.416	0.22	Undefined	
3	Cyclopentane, 1-acetyl-1,2-epoxy-	$C_7H_{10}O_2$	6.817	0.13	Undefined	
4	4H-Pyran-one, 2,3-dihydroxy-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	8.018	0.40	Antioxidant	Chen *et al.* (2021)
5	Caryophyllene	$C_{15}H_{24}$	11.936	0.15	Anti-inflammatory	Gyrdymova & Rubtsova (2021)
6	Eugenol	$C_{10}H_{12}O_2$	13.771	61.23	Antibacterial, antiviral, antifungal, anticancer, anti-inflammatory and antioxidant	Ulanowska & Olas (2021); Cheikhyoussef *et al.* (2022)
7	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	14.436	0.16	Anti-inflammatory	Brustugun *et al.* (2005); Lu *et al.*

						(2005); Xu *et al.* (2007)
8	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	15.666	0.18	Undefined	
9	Vanillin	$C_8H_8O_3$	18.096	0.25	Anti-carcinogenic, antioxidant	Arya *et al.* (2021)
10	Butanoic acid, 3-oxo-, 1-methylpropyl ester	$C_8H_{14}O_3$	18.726	0.09	Undefined	
11	Eugenol acetate	$C_{12}H_{14}O_3$	20.371	26.45	Antibacterial, antiviral, antifungal	Hemeda *et al.* (2022)
12	α-Cedrene	$C_{15}H_{24}$	20.843	0.17	Undefined	
13	Lingustral	$C_9H_{14}O$	21.111	0.25	Undefined	
14	Caryophyllene oxide	$C_{15}H_{24}O$	22.626	0.64	Anti-inflammatory	Gyrdymova & Rubtsova (2021)
15	Androsterone	$C_{19}H_{30}O_2$	23.417	0.24	Neurosteroid Anticonvulsant	Reddy & Rogawski (2012); Zolkowska *et al.* (2014)
16	Pyrogallol	$C_6H_6O_3$	23.803	2.72	Antibacterial	Tinh *et al.* (2016); Oliveira *et al.* (2022)
17	10-12-Pentacosadiynoic acid	$C_{25}H_{42}O_2$	24.130	0.74	Undefined	
18	Acetophenone, 4'-hydroxy-	$C_8H_8O_2$	24.968	0.21	Antifungal	Mohammadi Ziarani *et al.* (2020)
19	2',3',4' Trimethoxyacetophenone	$C_{11}H_{14}O_4$	25.739	1.99	Antibacterial	Freitas *et al.* (2020)
20	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	$C_{13}H_{16}O_3$	27.220	0.13	Undefined	
21	Benzyl Benzoate	$C_{14}H_{12}O_2$	27.692	0.19	Antibacterial	Diastuti *et al.* (2019)
22	Pentadecanoic acid, 14-methyl, methyl ester	$C_{19}H_{34}O_2$	30.217	0.23	Antioxidant	Vijisaral Elezabeth & Arumugam (2024)
23	Palustrol	$C_{15}H_{26}O$	30.888	0.32	Undefined	
24	Clovane diol	$C_{15}H_{26}O_2$	33.196	0.31	Undefined	
25	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl	$C_{17}H_{14}O_5$	35.743	0.43	Antioxidant, anti-inflammatory	Gupta *et al.* (2023)
26	9-Octadecanoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	35.777	0.43	Antioxidant	Mazumder *et al.* (2020)
27	Naphthazarin	$C_{10}H_6O_4$	36.455	0.12	Antibacterial, antifungal	Ryu *et al.* (1993) Duvauchelle *et al.* (2021)
28	3-[(Benzo[1,3]dioxol-5-ylmethylene)-amino]-2-methyl-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d]pyrimidin-4-one	$C_{19}H_{17}N_3O_3S$	41.873	0.41	Undefined	
29	Pyrrole-3-carboxylic acid, 5-(3-hydroxypropyl)-2-methyl-5-(1,2,4(4H)-triazol-3-yl)-,ethyl ester	$C_{16}H_{20}N_6O_4$	43.551	0.31	Undefined	
30	Di-n-octyl phtalate	$C_{24}H_{38}O_4$	49.911	0.15	Antimicrobial, insecticidal	Huang *et al.* (2021)
31	Estragole	$C_{10}H_{12}O$	50.984	0.35	Anti-inflammatory	Rodrigues *et al.* (2016)

3.4. Antibacterial Activity of *S. aromaticum* Methanol Extract

The results in Table 4 and Table 5 show the antibacterial activity of *S. aromaticum* methanol extract against *Streptococcus* spp. and *Lactobacillus* spp. isolated from various samples in the form of bacterial growth inhibition halos around the discs, respectively. Table 6 shows the antibacterial activity results for the negative control (filled with methanol) and positive control (penicillin).

Table 4. Antibacterial activity of clove methanol extract against *Streptococcus* spp. $p = 0.011$.

	Inhibition zone diameters (mm)				
	Clove methanol extract concentrations (mg/mL)				
Isolates	891	445.5	222.75	111.37	55.68
S1	23.33±1.52	12±2.64	0	0	0
S2	17.67±0.57	12±1	10±0	9±0	0
S3	9±0	8.5±2.12	0	0	0
S4	22.67±3.05	9.33±0.57	8.67±0.57	8.33±0.57	0
S5	17.33±0.57	9±1	7.5±0.70	7±0	0
S6	23.33±1.15	12.67±0.57	10.33±0.57	9±0	0
S7	11.33±1.52	9.5±0.70	9±1	8.33±1.54	0
S8	15±0	13.69±0.79	0	0	0
S9	15.33±0.57	11.33±1.15	9 .67±0.57	9.67±2.51	0
S10	10±1.73	9.33±0.57	9±0	9.33±1.15	0

S: Streptococcus

The data in the table are represented as the mean ± SD.

Table 5. Antibacterial activity of clove methanol extract against *Lactobacillus* spp. $p = 0.00368$.

	Inhibition zone diameters (mm)				
Isolates	Clove methanol extract concentrations (mg/mL)				
	891	445.5	222.75	111.37	55.68
L1	36.33±1.50	16.5±2.12	10.67±1.15	10±0	0
L2	24.67±2.08	12.5±0.70	12±0	11±0	0
L3	35±0	11.5±2.12	9±0	0	0
L5	40±0	28±2	19±1.41	9±0	0
L9	40±0	18.33±0.57	13	8.50	0
L10	19.67±4.72	9.67±0.57	9.67±0.57	9±0	0

L: Lactobacillus

The data in the table are represented as the mean ± SD.

According to Table 4, *Streptococcus* spp. exhibit remarkable sensitivity to methanol extract, with an increase in the diameters of the inhibition zones observed at various extract concentrations. Except for S1, S3, and S8, all other *Streptococcus* spp. strains were slightly more sensitive, with a zone of inhibition at 111.37 mg/mL. Table 5 shows that Lactobacilli strains exhibit hypersensitivity, as evidenced by the large diameters of the inhibition zones when compared to Streptococci strains. These findings indicate that Lactobacilli are more sensitive to *S. aromaticum*'s methanol extract than Streptococci.

Clove's antibacterial properties demonstrated effective inhibition of test bacterial strains at an extract concentration of 891 mg/mL. The maximum zone of inhibition was against *Lactobacillus* spp. isolates L5 and L9 (40 mm), followed by L1 (36.33 mm) and L3 (35 mm), 2 (24.67 mm), S1 and S6 (23.33 mm), and S4 (22.67 mm). Most of the other extract concentrations tested against the other isolates resulted in inhibition zone diameters ranging from 8.33 mm to 19.67 mm. Except for L3, the inhibitory effect of *S. aromaticum* extract on all tested *Lactobacillus* spp. began at 111.37 mg/mL, with inhibition zones ranging from 8.5 to 11 mm.

Table 6. Antibacterial activity of Penicillin and Methanol (80%) against *Streptococcus* spp. and *Lactobacillus* spp.

Streptococcus spp.	Inhibition zone diameter (mm)		*Lactobacillus* spp.	Inhibition zone diameter (mm)	
	Penicillin	Methanol (80%)		Penicillin	Methanol (80%)
S1	18	0			
S2	17	0			
S3	20	0	L1	22.5	0
S4	19.5	0	L2	23	0
S5	18.5	0	L3	23	0
S6	22	0	L5	24	0
S7	21	0	L9	24.5	0
S8	20	0	L10	22	0
S9	19.5	0			
S10	21.5	0			

Table 7 summarizes the MIC values for *S. aromaticum* methanol extract against *Streptococcus* and *Lactobacillus* spp. It can be noted that 60% of the tested *Streptococcus* spp. had the same MIC of 111.37 mg/mL (S2, S4, S6, S7, S9, and S10). However, except for L3, methanol extract has the same MIC of 111.37 against all tested *Lactobacillus* spp.

Table 7. MIC of *S. aromaticum* methanol extract against *Streptococcus* spp. and *Lactobacillus* spp.

Streptococcus spp.	Minimum inhibitory concentration of *S. aromaticum* extract (mg/mL)	*Lactobacillus* spp.	Minimum inhibitory concentration of *S. aromaticum* extract (mg/mL)
S1	445.5	L1	111.37
S2	111.37	L2	111.37
S3	445.5	L3	222.75
S4	111.37	L5	111.37
S5	445.5	L9	111.37
S6	111.37	L10	111.37
S7	111.37		
S8	445.5		
S9	111.37		
S10	111.37		

3.5. Correlation analysis

Figure 5 and Figure 6 show correlation matrices between polyphenols in mg gallic acid equivalent per g dry weight, corresponding in dilutions $(GAEQ/g)_{cd}$ of the methanolic extract and inhibition zone diameters against *Streptococcus* spp. and *Lactobacillus* spp. (Table 8). The findings reveal significant positive correlations between polyphenols and inhibition zone diameters for all tested strains.

Table 8. Corresponding polyphenols in methanol extract dilutions.

Dilution ratio	Concentrations (mg/mL)	Polyphenols $(GAEQ/g)_{cd}$
1/10	891	159.5
1/20	445.5	79.75
1/40	222.75	39.87
1/80	111.37	19.93
1/160	55.68	9.96

$(GAEQ/g)_{cd}$: mg gallic acid equivalent per g dry weight corresponding in dilutions

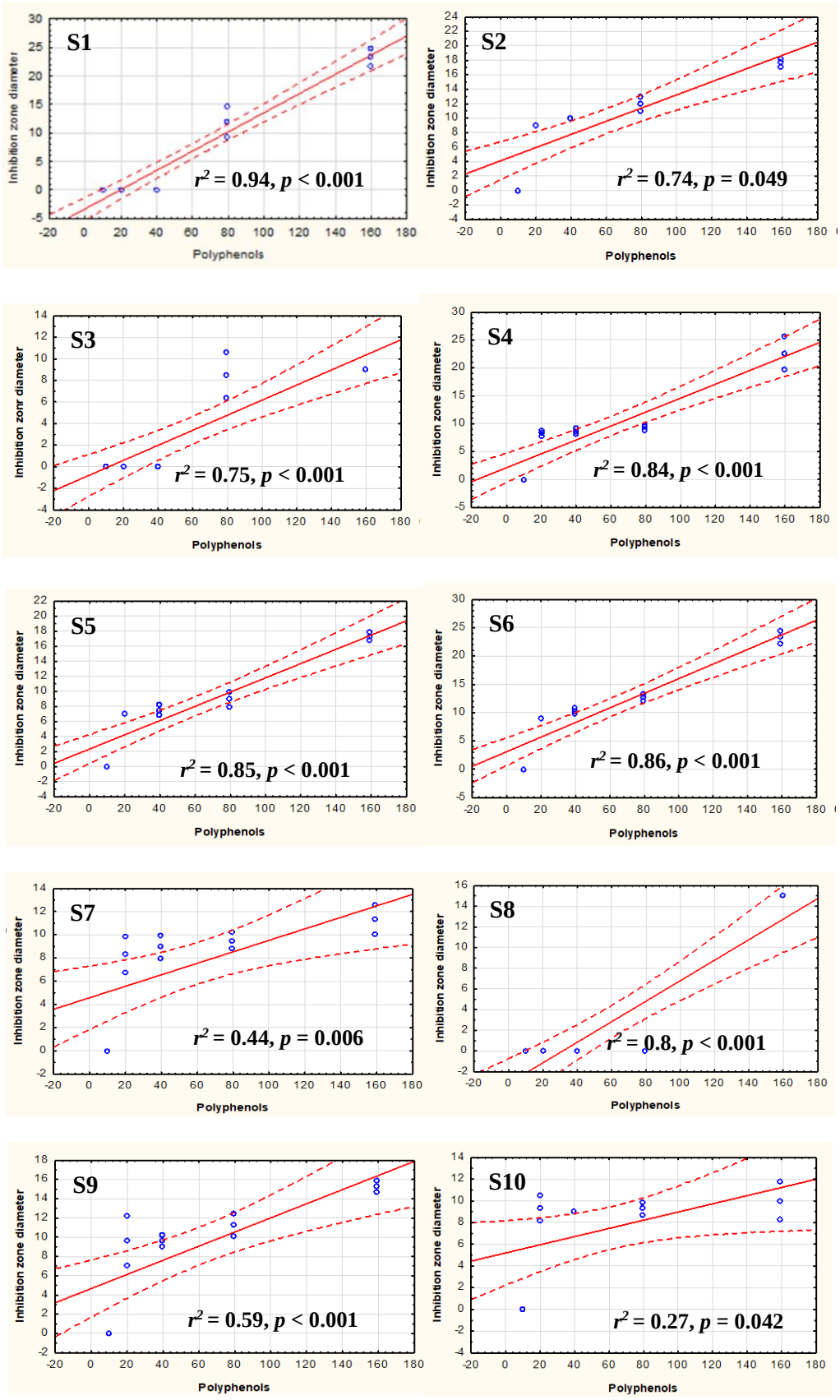

Figure 5. Correlation matrices between polyphenols and inhibition zone diameters against *Streptococcus* spp.

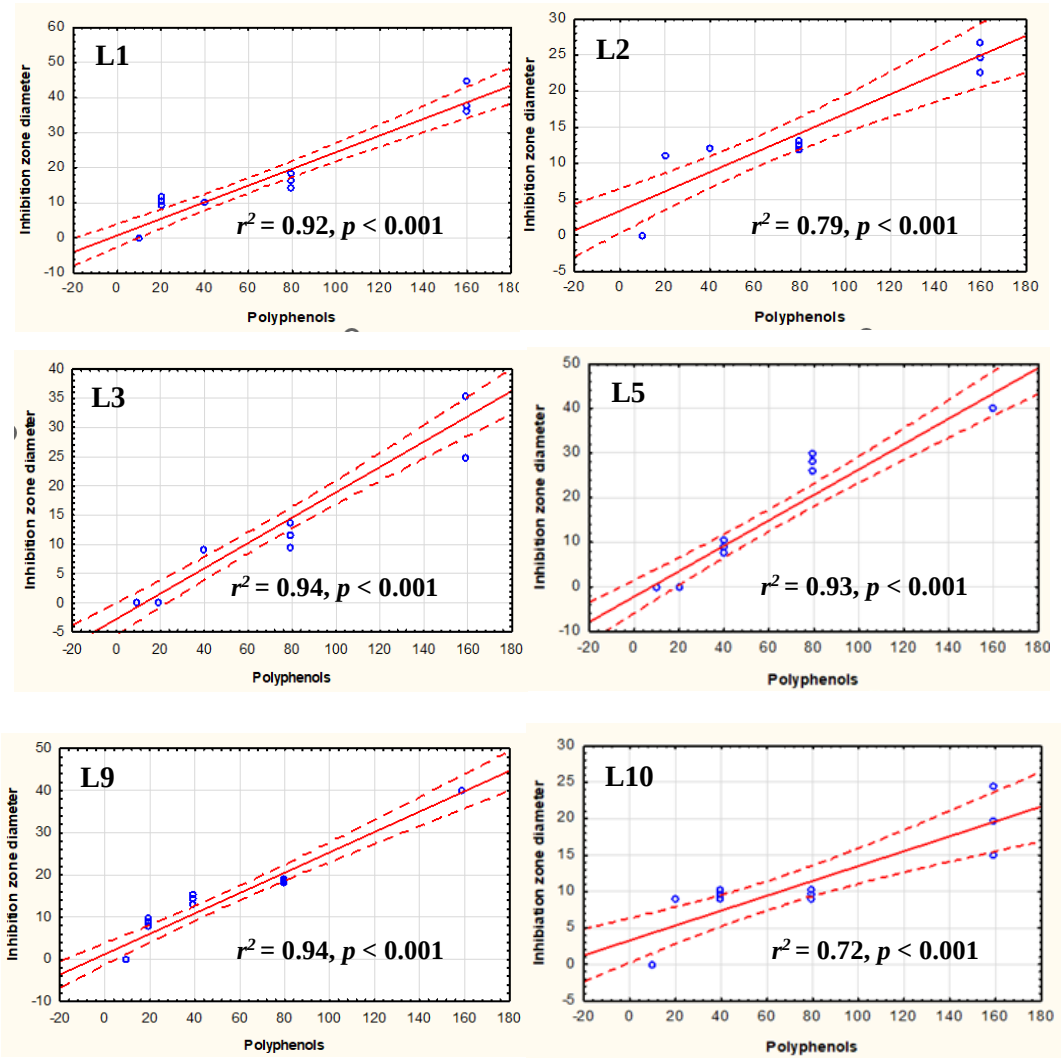

Figure 6. Correlation matrices between polyphenols and inhibition zone diameters against *Lactobacillus* spp.

4. DISCUSSION and CONCLUSION

According to a review of the studies, methanol is typically identified as the most effective solvent for phenolic compound extraction due to its polar property and ability to solubilize and recover optimal amounts of plant active components (Sultana *et al.*, 2014; Qasim *et al.*, 2016; Shafira *et al.*, 2020; Nakilcioglu & Otles, 2021; Benhamada *et al.*, 2022). In the case of our experiment, we were able to obtain a high extraction yield estimated at 27 %, which differs from the results found by Sabiu-Haxhijaha *et al.* (2021), who obtained yields of 5.67% and 1.07% using hydrodistillation and ultrasound extraction methods, respectively. Khan *et al.* (2022) indicated that the maximum yield of clove extract was 28.2%.

Using GC-MS for quantitative phytochemical screening, the *S. aromaticum* methanol extract was discovered to contain 31 compounds, with the largest peak area at 13.77 RT and 20.37 RT, indicating the presence of eugenol (61.23%) and eugenol acetate (26.45%), respectively, among its principal chemical constituents. Bhuiyan *et al.* (2010) identified 31 components in *Syzigium caryophyllatum* bud oil, the most important of which were eugenol (49.7%), caryophyllene (18.9%), benzene,1-ethyl-3-nitro (11.1%), and benzoic acid,3-(1-methylethyl) (8.9%). Eugenol was the most abundant oil constituent in *S. aromaticum* buds (72.08-82.36%), with eugenyl acetate essential oil (8.6 - 21.3%) coming in second (Kaur *et al.*, 2017).

Our findings are consistent with those of Ratri *et al.* (2020), who discovered that eugenol (85.01%) and eugenyl acetate (13.06%) are the main components detected by GC-MS in Island clove oil. Nonetheless, the petroleum ether extract of *S. aromaticum* contained the primary

chemical constituents eugenol (21.72%), phenol, 2 methoxy-4-(2-propenyl)-acetate (16.75%), espintanol (10.41%), and caryophyllene oxide (9.55%) (Alghazzaly *et al.*, 2022). Several other studies have also reported that eugenol is the primary component of cloves (Cortés-Rojas *et al.*, 2014; Cheikhyoussef *et al.*, 2022: Jadhav *et al.*, 2022; Khan *et al.*, 2022; Kiralan & Ketenoglu, 2022; Frohlich *et al.*, 2023; Gengatharan & Abd Rahim, 2023).

Bacteria are one of the leading causes of tooth decay. In this study, microbial analysis of tooth decay revealed the presence of two Gram-positive bacterial genera: *Lactobacillus* spp. and *Streptococcus* spp. Munson *et al.* (2004) found that Gram-positive bacteria dominate the oral bacterial flora, and Prajapati and Raol (2013) isolated *Streptococcus* spp. and *Lactobacillus spp.* from dental caries, confirming these findings. Our results are also consistent with those of Aneja and Joshi (2009) and Prajapati and Raol (2014), who indicated that *Streptococcus* spp. and *Lactobacillus* spp. are the primary, culturable agents responsible for caries lesions.

Another study conducted by Almaamori (2023) revealed that bacteria associated with tooth decay include *Staphylococcus* spp., *Streptococcus pyogenes*, *Bacillus cereus*, *Escherichia coli*, and *Proteus* spp. Mallya and Mallya (2020) reported the same conclusions. In fact, *Streptococcus mutans*, *Lactobacillus*, and *Actinomycetes* were the bacteria most frequently associated with tooth decay, despite the fact that *Lactobacillus* spp. is not the caries initiator but does play a role in the development and progression of dentin caries. *Streptococcus* spp. was isolated from 10 samples, leading us to conclude that *Streptococcus* spp. isolates are the most common etiological bacteria of dental caries. Cai and Kim (2023) and Riaz *et al.* (2023) found that *Streptococcus mutans* is the most commonly associated with dental caries.

To assess the efficacy of medicinal plant extracts, which can rival that of antibacterials, we evaluated the antibacterial activity of the methanol extract of *S. aromaticum* in this work. The findings show that methanol clove extract has significant activity against the tested strains, resulting in varying inhibition zone diameters depending on the concentration used ($p = 0.011$ and $p = 0.00368$ against *Streptococcus* spp. and *Lactobacillus* spp., respectively). Our findings are consistent with those of Alghazzaly *et al.* (2022), who found that *S. aromaticum* extract has antibacterial activity against strains of the viridans group Streptococci, indicating a potential natural treatment alternative.

Our results also indicate that methanol extract from cloves has significant antimicrobial activity in the bacteria tested. Hugar *et al.* (2017) reported that clove oil was highly effective against *E. faecalis* and could thus be used as a disinfectant. Yakubu Bello *et al.* (2022) concluded that clove oil could be used as a natural preservative because it inhibits the growth of Bacillus cereus. Ajobiewe *et al.* (2022) mentioned the same result, indicating that clove has antibacterial activity against antibiotic-resistant *Escherichia coli*. The observed variation in inhibition zone diameters can be attributed to either differences in bioactive molecule composition in the extract or their mechanism of action on Gram-positive bacteria.

Numerous studies have demonstrated the antibacterial effect of natural active ingredients. *S. aromaticum*'s antibacterial properties are thought to be due to its high polyphenol and flavonoid content (Shrivastava *et al.*, 2014; Oshomoh *et al.*, 2015). Increasing polyphenol concentrations resulted in a significant increase in inhibition zone diameter against tested strains, especially against S1 ($r^2 = 0.94$, $p < 0.001$), L3 ($r^2 = 0.94$, $p < 0.001$), L5 ($r^2 = 0.93$, $p < 0.001$), and L9 ($r^2 = 0.96$, $p < 0.001$). According to Alghazzaly *et al.* (2022), *S. aromaticum*'s antibacterial activity is due to polyphenolic constituents such as eugenol, phenol, 2-methoxy-4-(2-propenyl)-acetate, espintanol and Caryophyllene oxide.

Saini et *al.* (2019) and Bai *et al.* (2023) reported that eugenol is the main component of clove essential oil, and eugenol clearly has antibacterial effects on *S. aureus* and *E. coli* related to cell wall and membrane damage, inhibition of biofilm formation, oxidative stress-mediated apoptosis, and disruption of DNA synthesis. Joseph and Sugatha (2011) discovered that clove has antibacterial properties against a variety of foodborne pathogens, including *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus sp*.

According to the findings of Shehadi *et al.* (2014), these substances have been shown in vitro to have effective antimicrobial activity against a wide range of microorganisms. Their mode of action is most likely due to their ability to form a complex with soluble extracellular proteins from the bacterial wall, which destroys the cell membrane. In the same context, essential oils, flavonoids, and polyphenols may cause potassium ion leakage at the membrane level, resulting in irreversible membrane damage. This potassium permeability is a precursor to death (El-Haci *et al.*, 2012).

The MIC results of methanolic extracts indicated that *S. aromaticum* could be used to control and prevent pathogenic bacteria. These findings are consistent with those of Mostafa *et al. (*2018), who discovered that the most potent plant extracts with bacteriostatic and bactericidal properties against highly susceptible strains of foodborne pathogenic bacteria (*S. aureus* and *P. aeruginosa*) were ethanolic extracts of *S. aromaticum*. Furthermore, it has been demonstrated that flavonoids' toxicity to microorganisms occurs either through the deprivation of metal ions such as iron or through non-specific interactions such as the formation of hydrogen bonds with proteins in microorganism cell walls (adhesins) or enzymes (Basli *et al.*, 2012).

In conclusion, the obtained results highlight the antibacterial properties of the methanol extract of Algerian clove due to its high bioactive component content, which showed significant growth inhibitions for all *Lactobacillus* spp. and *Streptococcus* spp. isolated from dental caries. These findings led us to propose using essential oils, such as cloves, as a natural antibiotic for the treatment of dental caries, as well as in the preparation of toothpaste and mouthwashes. Future research should focus on extracting, separating and purifying the bioactive components of *S. aromaticum*, as well as studying their mechanisms of action on antibiotic resistance in vivo.

Acknowledgments

The authors would like to express their gratitude to all those who contributed to this study. The authors would also like to thank the Algerian Ministry of Higher Education and Scientific Research and the General Directorate of Research and Development Technologies (DGRSDT) of Algeria.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Ouahiba Benhamada: Investigation, Resources, Visualization, Software, Formal Analysis, Methodology and writing original draft. **Nabila Benhamada:** Analysis, Interpretation and Language revision. **Lilia Boussouf:** Interpretation and Language revision. **Essaid Leghouchi:** Supervision and Validation

Orcid

Ouahiba Benhamada https://orcid.org/0000-0003-2474-5739
Nabila Benhamada https://orcid.org/0000-0001-8088-8561
Lilia Boussouf https://orcid.org/0000-0002-6827-6340
Essaid Leghouchi https://orcid.org/0000-0001-9087-1050

REFERENCES

Ajobiewe, H.F., Elisha, E., Ibrahim, A.E., Ajobiewe, J.O., Salami, A.O., Umeji, L.C., … Alau, K.K. (2022). Antimicrobial activity of clove plant flower bud extract (*Syzygium aromaticum*) on *Escherichia coli. Scholars Journal of Applied Medical Sciences, 10*(5), 673-684. https://doi.org/10.36347/sjams.2022.v10i05.002

Alghazzaly, A., Elsherbiny, G., Moghannem, S., & Sharaf, M. (2022). Antibacterial, antibiofilm, antioxidants and phytochemical profiling of *Syzygium aromaticum* extract. *Egyptian Journal of Aquatic Biology and Fisheries*, *26*(5), 207-218.

Almaamori, A.M. (2023). Oral health and dental caries. *World bulletin of public health*, 14

Amadi, E.K., Kareru, P.G., Keriko, J.M., & Kiptoo, J. (2016). Antimicrobial activity of selected medicinal plants' extracts against *streptococcus pneumoniae*. *International Journal of Botany Studies*, *1*(1), 20-22.

Aneja, K.R., & Joshi, R. (2010). Antimicrobial activity of *Syzygium aromaticum* and its bud oil against dental cares causing microorganisms. *Ethnobotanical Leajlets*, *14*, 960-975.

Aneja, K.R., Joshi, R., & Sharma, C. (2009). Antimicrobial activity of Dalchini (*Cinnamomum zeylanicum* bark) extracts on some dental caries pathogens. *Journal of Pharmacy Research, 2*(9), 1387-1390.

Arya, S.S., Rookes, J.E., Cahill, D.M., & Lenka, S.K. (2021). Vanillin: a review on the therapeutic prospects of a popular flavouring molecule. *Advances in Traditional Medicine, 21*(3), 1–17. https://doi.org/10.1007/s13596-020-00531-w

Bai, J., Li, J., Chen, Z., Bai, X., Yang, Z., Wang, Z., & Yang, Y. (2023). Antibacterial activity and mechanism of clove essential oil against foodborne pathogens. LWT- *Food Science and Technology*, *173*, 114249. https://doi.org/10.1016/j.lwt.2022.114249

Basli, A., Chibane, M., Madani, K., & Oukil, N. (2012). Activité antibactérienne des polyphénols extraits d'une plante médicinale de la flore d'Algérie: *Origanum glandulosum* Desf. *Phytothérapie, 10*, 2-9. https://doi.org/10.1007/s10298-012-0683-9

Benhamada, O., Benhamada, N., & Leghouchi, E. (2022). Polyphenols and antibacterial activity of *Xanthoria parietina* (L.) Th. Fr. methanol extract under lead stress. *Journal of Applied Biological Sciences*, *16*(3), 537-552. https://doi.org/10.5281/zenodo.7114289

Bhuiyan, M.N.I., Begum, J, Nandi, N.C., & Akter, F. (2010). Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston). *African Journal of Plant Science*, *4*(11), 451-454.

Brustugun, J., Tonnesen, H.H., Edge, R., & Navaratnam, S. (2005). Formation and reactivity of free radicals in 5-hydroxymethyl-2-furaldehyde–the effect on isoprenaline photostability. *Journal of Photochemistry and Photobiology, 79*(2), 109-119. https://doi.org/10.1016/j.jphotobiol.2004.12.005

Cai, J-N., & Kim, D. (2023). Biofilm ecology associated with dental caries: understanding of microbial interactions in oral communities leads to development of therapeutic strategies targeting cariogenic biofilms. *Advances in Applied Microbiology*, *122*, 27-75. https://doi.org/10.1016/bs.aambs.2023.02.001

Cheikhyoussef, A., Cheikhyoussef, N., Rahman, A., & Hussein, A.A. (2022). Clove (*Syzygium aromaticum*): Chapter 14 - Clove (*Syzygium aromaticum*) phenolics: Extraction, compositions, and biological activities. *Chemistry, Functionality and Applications*, 215-233. https://doi.org/10.1016/B978-0-323-85177-0.00036-7

Chen, Z., Liu, Q., Zhao, Z., Bai, B., Sun, Z., Cai, L., … Xi, G. (2021). Effect of hydroxyl on antioxidant properties of 2,3 dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one to scavenge free radicals. *RSC Advances*, *11*(55), 34456–34461. https://doi.org/10.1039/d1ra06317k

Cortés-Rojas, D.F., de Souza, C.R.F., & Oliveira, W.P. (2014). Clove (*Syzygium aromaticum*): a precious spice. *Asian Pacific Journal of Tropical Biomedicine, 4*(2), 90-96. https://doi.org/10.1016/S2221-1691(14)60215-X

de Freitas, T.S., da Cunha Xavier, J., Pereira, R.L.S., Rocha, J.E., Muniz, D.F., da Silva, P.T., … Coutinho, H.D.M. (2020). Direct antibacterial and antibiotic resistance modulatory activity of chalcones synthesized from the natural product 2-hydroxy-3,4,6-trimethoxyacetophenone. *FEMS-Microbiology Letters*, *367*(15). https://doi.org/10.1093/femsle/fnaa124

Delarras, C. (2007). Microbiologie pratique pour le laboratoire d'analyse ou de contrôle sanitaire. *Ed. Lavoisier*. P, 128, 129, 269, 271.

de Oliveira Carvalho, I., Purgato, G.A., Píccolo, M.S., Pizziolo, V.R., Coelho, R.R., Diaz-Muñoz, G., & Alves Nogueira Diaz, M. (2020). In vitro anticariogenic and antibiofilm activities of toothpastes formulated with essential oils. *Archives of Oral Biology*, *117*, 104834. https://doi.org/10.1016/j.archoralbio.2020.104834

Devi, B., Ramasubramaniaraja, R., Carism, & Devi, B. (2009). Dental caries and medicinal plants –An overview. *Journal of Pharmacy Research*, *2*, 1669-1675.

Diastuti, H., Chasani, M., & Suwandri, S. (2019). Antibacterial Activity of Benzyl Benzoate and Crotepoxide from Kaempferia rotunda L. Rhizome. *Indonesian Journal of Chemistry*, *20*(1), 9. https://doi.org/10.22146/ijc.37526

Dogruoz, N., Zeybek, Z., & Karagoz, A. (2008). Antibacterial Activity of Some Plant Extracts. *IUFS Journal of Biology*, *67*(1), 17-21.

Duvauchelle, V., Majdi, C., Bénimélis, D., Dunyach-Remy, C., Meffre, P., & Benfodda, Z. (2021). Synthesis, structure elucidation, antibacterial activities, and synergistic effects of novel juglone and naphthazarin derivatives against clinical methicillin-resistant *Staphylococcus aureus* Strains. *Frontier in Chemistry*, *9*, 773981. https://doi.org/10.3389/fchem.2021.773981

El-Haci, I.A., Atik-Bekkara, F., Didi, A., Gherib, M., & Didi, M.A. (2012). Teneurs en polyphénols et pouvoir antioxydant d'une plante médicinale endémique du Sahara Algérien. *Phytothérapie*, *10*, 280-285. https://doi.org/10.1007/s10298-012-0726-x

El-Sherbiny, G.M., & Elbestawy, M.K.M. (2022). A review – plant essential oils active against *Helicobacter pylori*. *Journal of Essential Oil Research*, *34*(3), 203-215. https://doi.org/10.1080/10412905.2022.2025464

Featherstone, J.D.B. (2000). The science and the practice of caries prevention. *The Journal of the American Dental Association*, *131*(7), 887-899. https://doi.org/10.14219/jada.archive.2000.0307

Foda, A.M., Kalaba, M.H., El-Sherbiny, G.M., Moghannem, S.A., & El-Fakharany, E.M. (2022). Antibacterial activity of essential oils for combating colistin-resistant bacteria. *Expert Review of Anti-infective Therapy*, *20*(10), 1351-1364. https://doi.org/10.1080/14787210.2022.2101997

Freitas, T.S., da Cunha Xavier, J., Pereira, R.L.S., Rocha, J.E., Muniz, D.F., da Silva, P.T., … Coutinho, H.D.M. (2020). Direct antibacterial and antibiotic resistance modulatory activity of chalcones synthesized from the natural product 2-hydroxy-3,4,6-trimethoxyacetophenone. *FEMS-Microbiology Letters, 367*(15). https://doi.org/10.1093/femsle/fnaa124

Frohlich, P.C., Santos, K.A., Ascari, J., dos Santos Refati, J.R., Palú, F., Cardozo-Filho, L., & da Silva, E.A. (2023). Antioxidant compounds and eugenol quantification of clove (*Syzygium aromaticum*) leaves extracts obtained by pressurized liquid extraction and supercritical fluid extraction. *The Journal of Supercritical Fluids*, *196*, 105865. https://doi.org/10.1016/j.supflu.2023.105865

Gengatharan, A., & Abd Rahim, M.H. (2023). The application of clove extracts as a potential functional component in active food packaging materials and model food systems: A mini-review. *Applied Food Research*, *3*(1), 100283. https://doi.org/10.1016/j.afres.2023.100283

Gupta, S., Park, S.E., Mozaffari, S., El-Aarag, B., Parang, K., & Tiwari, R.K. (2023). Design, Synthesis, and Antiproliferative Activity of Benzopyran-4-One-Isoxazole Hybrid Compounds. *Molecules*, *28*(10), 4220. https://doi.org/10.3390/molecules28104220

Gyrdymova, Y., & Rubtsova, S. (2021). Caryophyllene and caryophyllene oxide: a variety of chemical transformations and biological activities. *Chemical Papers*. *76*(2). https://doi.org/10.1007/s11696-021-01865-8

Hemeda, N.A., Hegazy, G.E., Abdelgalil, S., Soliman, N.A., Abdel-Meguid, D.I., & El-Assar, S.A. (2022). Maximization of red pigment production from *Streptomyces* sp. LS1 structure elucidation and application as antimicrobial/antifouling against human pathogens and marine microbes. *Journal of Genetic Engineering and Biotechnology*, *20*, 168. https://doi.org/10.1186/s43141-022-00452-y

Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., & Shao, H. (2021). Phthalic Acid Esters: Natural Sources and Biological Activities. *Toxins (Basel)*, *13*(7), 495. https://doi.org/10.3390/toxins13070495

Hugar, S., M Patel, P., Nagmoti, J., Uppin, C., Mistry, L., & Dhariwal, N. (2017). An in vitro comparative evaluation of efficacy of disinfecting ability of garlic oil, neem oil, clove oil, and tulsi oil with autoclaving on ndodontic K Files tested against *Enterococcus faecalis*. *International Journal of Clinical Pediatric Dentistry*, *10*(3), 283-288. https://doi.org/10.5005/jp-journals-10005-1451

Jadhav, J.J., Jadeja, G.C., & Desai, M.A. (2022). Clove (*Syzygium aromaticum*): Chapter 28 - Effect of extraction techniques on the yield, composition, and quality of clove (*Syzygium aromaticum*) essential oil. *Chemistry, Functionality and Applications*, 485-500. https://doi.org/10.1016/B978-0-323-85177-0.00027-6

Joseph, B., & Sujatha, S. (2011). Bioactive Compounds and its autochthonous microbial activities of extract and clove oil (*Syzygium aromaticum* L.) on some food borne pathogens. *Asian Journal of Biological Sciences*, *4*(1), 35-43. https://doi.org/10.3923/ajbs.2011.35.43

Kablan, B.J., Adiko, M., & Abrogoua, D.P. (2008). Évaluation in vitro de l'activité antimicrobienne de *Kalanchoe crenata* et de *Manotes longiflora* utilisées dans les ophtalmies en Côte d'ivoire. *Phytothérapie*, *6*(5), 282-288. https://doi.org/10.1007/s10298-008-0332-0

Kabra, P., Loomba, K., Kabra, S.K., Majumdar, D.S.P., & Kuma, N. (2012). Medicinal plants in the treatment of dental caries. *Asian Journal of Oral Health & Allied Sciences*, *2*(1), 12-16.

Kaur, D., & Chandrul, K. (2017). *Syzygium aromaticum* L. (Clove): A vital herbal drug used in periodontal disease. *Indian Journal of Pharmaceutical and Biological Research*, *5*(2), 45-51. https://doi.org/10.30750/ijpbr.5.2.9

Khan, M. K., Hassan, S., Imran, M., & Ahmad, M.H. (2022). Clove (*Syzygium aromaticum*): Chapter 22 - Extraction of bioactive compounds from clove (*Syzygium aromaticum*). *Chemistry. Functionality and Applications*, 405-417. https://doi.org/10.1016/B978-0-323-85177-0.00010-0

Kiralan, M., & Ketenoglu, O. (2022). Clove (*Syzygium aromaticum*): Chapter 36 - Food applications of clove (*Syzygium aromaticum*) extracts. *Chemistry, Functionality and Applications*, 607-617. https://doi.org/10.1016/B978-0-323-85177-0.00030-6

Kuete, V., Dongfack, M.D., Mbaveng, A.T., Lallemand, M-C., Van-Dufat, H.T., Wansi, J-D., … Wandji, J. (2010). Antimicrobial activity of the methanolic extract and compounds from the stem bark of *Drypetes tessmanniana*. *Chinese Journal of Integrative Medicine*, *16*(4), 337-343. https://doi.org/10.1007/s11655-010-0527-8

Li, Y.H., & Lu, X.Y. (2005). Investigation on the origin of 5-HMF in *Shengmaiyin decoction* by RP-HPLC method. *Journal of Zhejiang University Science B*, *6*, 1015–1021. https://doi.org/10.1631/jzus.2005.B1015

Mallya, P.S., & Mallya, S. (2020). Microbiology and clinical implications of dental caries – a review. *Journal of Evolution of Medical and Dental Sciences*, *9*(48), 3670-3675. https://doi.org/10.14260/jemds/2020/805

Mazumder, K., Nabila, A., Aktar, A., & Farahnaky, A. (2020). Bioactive Variability and In Vitro and In Vivo Antioxidant Activity of Unprocessed and Processed Flour of Nine Cultivars of Australian *lupin* Species: A Comprehensive Substantiation. *Antioxidants (Basel)*, *9*(4), 282. https://doi.org/10.3390/antiox9040282

Meda, A., Lamien, C.E., Romito, M., Millogo, J., & Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan Honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571-577. https://doi.org/10.1016/j.foodchem.2004.10.006

Mohammadi Ziarani, G., Kheilkordi, Z., & Mohajer, F. (2020). Recent advances in the application of acetophenone in heterocyclic compounds synthesis. *Journal of the Iranian Chemical Society*, *17*, 247–282. https://doi.org/10.1007/s13738-019-01774-4

Moroh, J-L.A. (2008). Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) milne-redheat (rubiaceae) sur la croissance in-vitro des souches *d'Escherichia coli*. *Bulletin de la Societe Royale des Sciences de Liege*, *77*. 44 - 61.

Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N., & Bakri, M.M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 361-366. https://doi.org/10.1016/j.sjbs.2017.02.004

Munson, M.A., Banerjee, A., Watson, T.F., & Wade, W.G. (2004). Molecular analysis of the microflora associated with dental caries. *Journal of clinical microbiology*, *42*(7), 3023-3029. https://doi.org/10.1128/JCM.42.7.3023-3029.2004

Nakilcioglu, T.E., & Otles, S. (2021). Influence of extraction solvents on thepolyphenol contents, compositions, and antioxidant capacities of fig (*Ficus carica* L.) seeds. *Annals of the Brazilian Academy of Sciences Printed*, *93*(1), 1678-2690. https://doi.org/10.1590/0001-3765202120190526

Nkere, C.K., & Iroegbu, C.U. (2005). Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida*. *African Journal of Biotechnology*, *4*(6), 522-526.

Oliveira, L.C.C., Rodrigues, F.A.A., dos Santos Barbosa, C.R., dos Santos, J.F.S., Macêdo, N.S., de Sousa Silveira, Z., Coutinho, H.D.M., & da Cunha, F.A.B. (2022). Antibacterial Activity of the Pyrogallol against *Staphylococcus aureus* Evaluated by Optical Image. *Biologics*, *2*, 139-150. https://doi.org/10.3390/biologics2020011

Oshomoh, E.O., ldu, M., & Udinyiwe, O.C. (2015). Phytochemical screening and antimicrobial sensitivity of clove flower (*Syzygium aromaticum*, L. Merrill and Perry) bud on dental pathogens. *International Journal of Pharmacy and Pharmaceutical Research*, *3*(2), 1-12.

Owen, P.L., & Johns, T. (1999). Xanthine oxidase inhibitory activity of northeastern North American plant remedies used forgout. *Journal of Ethnopharmacology*, *64*,149-160. https://doi.org/10.1016/s0378-8741(98)00119-6

Penot, E. (2016). Analyse technico-économique de la filière amont de production d'essence de girofle à Fénérive- Est, Madagascar: de la feuille à l'alambic.

Prajapati, R.A., & Raol, B.V. (2014). Characterization of microbial flora isolated from human teeth carious lesions. *Life Sciences Leajlets*, *47*, 01-08.

Qasim, M., Aziz, I., Rasheed, M., Gul, B., & Khan, M. (2016). Effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes. *Pakistan Journal of Botany*, *48*(2), 621-627.

Ratri, P.J., Ayurini, M., Khumaini, K., & Rohbiya, A. (2020). Clove oil extraction by steam distillation and utilization of clove buds waste as potential candidate for Eco-Friendly Packaging. *Jurnal Bahan Alam Terbarukan*, *9*(1), 47-54. https://doi.org/10.15294/jbat.v9i1.24935

Reddy, D.S., & Rogawski, M.A. (2012). "Neurosteroids - Endogenous Regulators of Seizure Susceptibility and Role in the Treatment of Epilepsy". In Noebels JL, Avoli M, Rogawski MA, et al. (eds.). Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th Edition. Bethesda (MD): National Center for Biotechnology Information (US). National Center for Biotechnology Information (US). PMID 22787590

Riažˇ, A., Umar, M., & Farah, N. (2023). Prevalence, causes and management of dental caries. *Journal of Xi'an Shiyou University, Natural Science Edition*, *19*(5), 1033-1053.

Rodrigues, L.B., Martins, A.O.B.P.B., Cesário, F.R.A.S., Castro, F.F., de Albuquerque, T.R., Fernandes, M.N.M., … de Menezes, I.R.A. (2016). Anti-inflammatory and antiedematogenic activity of the *Ocimum basilicum* essential oil and its main compound estragole: In vivo mouse models. *Chemico-Biological Interactions*, *257*, 14-25. https://doi.org/10.1016/j.cbi.2016.07.026

Ryu, C.K., Kim, D.H., Kim, H.J., & Chung, S.Y. (1993). The antimicrobial activities of some 1,4-naphthalenediones (IV). *Archives of Pharmacal Research*, *16*, 327-330. https://doi.org/10.1007/BF02977525

Sabriu – Haxhijaha, A., Jordanovska, S., & Popovska, O. (2021). Analysis of clove (*Syzygium aromaticum* L.) oil obtained with hydrodistillation and ultrasound-assisted extraction. UDC582.776.2-113.551:542.61. https://doi.org/10.20544/HORIZONS.B.09.2.P07

Saikumari, D., Shiva Rani, S.K., & Saxena, N. (2016). Antibacterial Activity of *Syzigium aromaticum* L. (Clove). *International Journal of Current Microbiology and Applied Sciences*, *5* (11), 484-489. http://dx.doi.org/10.20546/ijcmas.2016.511.056

Saini, M., Alam, S., Prajapati, R., Kr, L., & Thakur (2019). Analysis and estimation of eugenol content in microemulsion formulation containing clove oil (*Syzigium aromaticum*). *Journal of Applicable Chemistry*, *8*(1), 195-202.

Shafira, K., Azad, A.K., Zubair, L., & Helal Uddin, A.B.M. (2020). Extraction and quantification of eugenol from clove buds using HPLC. *Current Chromatography*, *07*(1) https://doi.org/10.2174/2213240607999200818161356

Sharaf, M.H., El-Sherbiny, G.M., Moghannem, S.A., Abdelmonem, M., Islam, A., Elsehemy, I.A., … Kalaba, M.H. (2021). New combination approaches to combat methicillin-resistant *Staphylococcus aureus* (MRSA). *Scientific Reports*, *11*(1), 1-16. https://doi.org/10.1038/s41598-021-82550-4

Shehadi, M., Awada, F., Oleik, R., Chokr, A., Hamze, K., Hamdan, H.A., & Kobaissi, A. (2014). Comparative analysis of the anti-bacterial activity of four plant extracts. *International Journal of Current Research and Academic Review*, *2*(6), 83-94.

Shrivastava, K., Sahu, S., Mishra, S.K., & De, K. (2014). In vitro antimicrobial activity and phytochemical screening of *Syzygium aromaticum*. *Asian Journal of Research in Phannaceutical Science*, *4*(1), 12-15.

Slinkard, K., & Singleton, V.L. (1977). Total phenol analysis: automation and comparison with manual methods, *American Journal of Enology and Viticulture*, *28*(1),49-55.

Souad, A., Chalghem, I., Kassah-Laouar, A., Laroui, S., & Souad, K. (2010). Activité antioxydante et antimicrobienne d'extraits de *Cuminum cyminum* L. *Lebanese Science Journal*, *11*, 69-81.

Srikacha, N., & Ratananikom, K. (2020). Antibacterial activity of plant extracts in different solvents against pathogenic bacteria: An in vitro experiment. *Journal of Acute Disease*, *9*(5), 223-226. https://doi.org/10.4103/2221-6189.291288

Sultana, B., Anwar, F., Mushtaq, M., Aslam, M., & Ijaz, S. (2014). In vitro antimutagenic, antioxidant activities and total phenolics of clove (*Syzygium aromaticum* L.) seed extracts. *Pakistan Journal of Pharmaceutical Sciences*, *27*(4), 893-899.

Tinh, T.H. Nuidate, T., Vuddhakul, V., & Rodkhum, C. (2016). Antibacterial Activity of Pyrogallol, a Polyphenol Compound against *Vibrio parahaemolyticus* Isolated from The Central Region of Thailand. *Procedia Chemistry*, *18*, 162-168. https://doi.org/10.1016/j.proche.2016.01.025

Ulanowska, M., & Olas, B. (2021). Biological Properties and Prospects for the Application of Eugenol - A Review. *International Journal of Molecular Sciences*, *22*(7), 3671. https://doi.org/0.3390/ijms22073671

Vijisaral Elezabeth, D., & Arumugam, S. (2014). GC-MS analysis of bioactive constituents of Indigofera suffruticosa leaves. *Journal of Chemical and Pharmaceutical Research*, *6*(8), 294-300.

Wichtl, M., & Anton, R. (1999). Plantes thérapeutiques. 2nd Ed. *Lavoisier*, Paris, 106-108.

Xu, Q., Li, Y.H., & Lu, X.Y. (2007). Investigation on influencing factors of 5-HMF content in Schisandra. *Journal of Zhejiang University Science B.*, *8*(6), 439-345. https://doi.org/10.1631/jzus.2007.B0439

Yakubu Bello, R., Umar, S., Wakil, S., & Bello, A. (2022). Antibacterial activity of clove extracts (*Syzygium aromaticum*) against *Bacillus cereus*. *International Journal of Advanced Academic Research*, *8*(8), 33-46. https://doi.org/10.46654

Zolkowska, D., Dhir, A., Krishnan, K., Covey, D.F., & Rogawski, M.A. (2014). Anticonvulsant potencies of the enantiomers of the neurosteroids androsterone and etiocholanolone exceed those of the natural forms. *Psychopharmacology*, *231* (17), 3325-3332. https://doi.org/10.1007/s00213-014-3546-x