

## Research Article

# *In-Vitro* and *In-Silico* Anti-Cariogenic Bacterial Activity of Selected Spices Commonly Consumed in South-West Nigeria

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## ABSTRACT

Dental caries is a preventable oral disease simply by practicing good oral hygiene; however, this disease remains a global health challenge due to the difficulties in management and treatment. Onion, ginger, and nutmeg extracts have been reported as potent antibiotics. Thus, the study investigated the efficacy of these three spices against cariogenic bacteria. Samples were collected from consented caried-patients at the Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun State, Nigeria. Disc and agar well diffusion methods were used to evaluate the antibiotic sensitivity of the spices against the isolates, respectively. The anti-cariogenic potential of significant compounds in each of the spices was further assessed against four bacterial target proteins (DNA gyrase B, dihydrofolate reductase (DHF), D-alanine: D-alanine ligase (Ddl1), and Isoleucyl-tRNA synthetase (IARS). Out of 100 samples collected, hundred and sixty-six (166) isolates were recovered: *Pseudomonas aeruginosa* (105), *Staphylococcus aureus* (52), and *Streptococcus mutans* (9). *In-silico* studies revealed that kaempferol in onion, luteolin 7-O-glucoside in ginger, and macelignan in nutmeg had high binding affinities for the four target proteins ( $\Delta G > -7$  kcal/mol). At 100% concentration, onions showed a higher zone of inhibition (20-22 mm) against the tested bacteria. In comparison, diameter zones of 20.33 mm and 20.67 mm for ginger, and 19.67 mm and 16.67 mm for nutmeg were observed against *Streptococcus mutans* and *Pseudomonas aeruginosa*, respectively. It was also observed that at higher concentrations of 100%, the tested spices had a higher inhibitory effect against the tested bacteria than the control antibiotics.

**Keywords:** Antibiotics, Cariogenic bacteria, Dental caries, Spices, Target proteins

## INTRODUCTION

Oral disease incidences in the last two decades have been alarming. Among this class of diseases, dental caries is one of the most common and has attained an endemic status, affecting about 4 billion people globally (Abdel *et al.*, 2022). Despite that, dental caries is a preventable oral disease by practicing good oral hygiene; however, this disease remains a global health challenge due to the

difficulties in management and treatment (Elamin *et al.*, 2019; Abdel *et al.*, 2022). High sugar consumption is considered to increase the risk of dental caries, causing oral dysbiosis and cavity acidity by biofilm bacteria activities. The activity causes crown demineralization, tooth decay, and root inflammation (Al-Shahrani, 2019; Rathee and Sapra, 2020). Dental caries has been reported as a risk factor for inflammatory bowel disease and gastric cancer. Hence, numerous preventive measures against this oral disease are well propagated by many international health organizations. Yet, the incidence remained on the

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rise, and several measures, including dental fillings, tooth extraction, and antibiotics administration, are being applied in managing and treating dental caries (Slayton *et al.*, 2016; Warreth, 2023). Different antibiotics have been used to treat dental caries, including penicillin, the first drug of choice; however, bacteria became resistant to the  $\beta$ -lactam antibiotics, and some other side effects caused a gradual reduction in uses.

The United States Food and Drug Administration warned against tetracycline due to teeth discoloration and other side effects. Clindamycin, metronidazole, and macrolides are also used; however, their use has gradually reduced over the decades due to their side effects and the resistance of cariogenic bacteria (Qiu *et al.*, 2020). Thus, other antimicrobials like fluoride, quaternary ammonium salts, chlorhexidine, calcium hydroxide, antimicrobial peptides (AMPs), and other remineralizing agents were developed for use (Qiu *et al.*, 2020; Griffith *et al.*, 2022). Moreover, fluctuations in the oral environment and biofilm formation, which promote antimicrobial resistance by preventing antimicrobial diffusion and enhancing plasmid exchange, also enable antimicrobial resistance (Cheng *et al.*, 2019; Fauzia *et al.*, 2020). Antibiotic resistance increases dental caries morbidity, leading to high treatment costs (Yadav and Prakash, 2017). Similarly, antibiotics have no remineralizing properties to treat dental caries, and this has led to seeking alternative antimicrobial agents from natural sources, predominantly plants, for treatment (John *et al.*, 2021). Thus, there is rising interest in searching for plant compounds with antimicrobial properties, especially from spices, to treat dental caries.

Plants are known to be a reservoir of bioactive compounds with medicinal properties, which makes them helpful in definite physiological action in treating various human diseases compared to the currently available antibiotics with high resistance rates and side effects (Hemavathy, 2019). Onion (*Allium cepa*), ginger (*Zingiber officinale*), and nutmeg (*Myristica fragrans*) are common spices consumed in foods and are used as flavor enhancers and preservatives due to their fragrance (Agabalogun, 2016). Furthermore, spices possess medicinal benefits such as antioxidant (Panggabean *et al.*, 2019), anti-inflammatory (Sharma *et al.*, 2016), and antimicrobial properties (Kumar *et al.*, 2022). There is a rising interest in the search for compounds in plants with antimicrobial properties, especially from spices.

A more recent method is the *in-silico* approach using molecular docking tools to identify antimicrobial compounds with high affinity for specific target proteins (receptors) (Kroemer, 2007). There is a sparse report concerning the antimicrobial reaction of these common spices against cariogenic bacteria. Hence, this study investigated the antibacterial potential of common spices against cariogenic bacteria using *in-silico* and *in-vitro* approaches. Moreover, there is a need for more attempts in the literature to explain the mechanisms of action of the established anticariogenic plant compounds. Hence, this

study seeks to explore *in-silico* studies and interactions with known antibiotic target proteins in bacteria to provide more insight into the anticariogenic mechanism of these spices.

## MATERIALS AND METHODS

### Study/area/subjects

The study was carried out in the Department of Medical Microbiology laboratory, Olabisi Onabanjo University, Ogun State, Southwestern Nigeria. The study subjects were patients who visited the Dental Clinic unit of Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu.

### Collection of specimens

Approval was obtained by the unit Head of the dental clinic, and 100 samples were collected from patients (female 54 and male 46) within the age range of 1 year and above who also gave their consent or whose guardians gave approval. With the assistance of a medical practitioner, one hundred (100) tooth specimens were aseptically collected from patients with apparent holes in their teeth. The specimens obtained via sterile swab sticks moistened in sterile distilled water were labeled, preserved in peptone water broth, and transported to the medical microbiology laboratory, where they were processed immediately.

### Culturing/subculturing of specimens

Each specimen was inoculated onto four different selective media: Cetrimide agar, *Streptococcus* agar, Mannitol salt agar, and MacConkey agar, using the spread plate method. Both primary plates and subcultured plates were incubated overnight at 37°C. The pure cultures were classified and identified based on their Gram staining reactions and biochemical tests. The isolates were stored on nutrient agar slants at 4°C for further use.

### Antibiotic susceptibility test for cariogenic bacteria

The antibacterial sensitivity of the isolates to 16 antibiotic discs (Oxoids, England) was determined using the Kirby-Bauer disc diffusion method using Mueller-Hinton agar plates. The pour plate method was used, and inoculated agar plates were allowed to dry before the antibiotic discs were aseptically inserted. All the plates (in triplicate), including those with antibiotics without organism (positive control), were incubated at 37°C for 24 hours, after which the diameter zones were taken using a ruler and then compared with the interpretation chart standard (CLSI, 2006). The following antibiotics: ampicillin (10  $\mu$ g), gentamycin (10  $\mu$ g), erythromycin (15  $\mu$ g), vancomycin (10  $\mu$ g), pefloxacin (10  $\mu$ g), streptomycin (30 $\mu$ g), ampiclox (30  $\mu$ g), amoxicillin (30  $\mu$ g), ciprofloxacin (10  $\mu$ g), rocephin (25  $\mu$ g), zinnacef (20  $\mu$ g), septrin (30  $\mu$ g), chloramphenicol (30  $\mu$ g), sparfloxacin (10  $\mu$ g), augmentin (30  $\mu$ g) and tarivid (10  $\mu$ g) were used.

### Preparation of the plant extracts

All the spices (onion, ginger, and nutmeg) were bought in the Ago-Iwoye community market, Ogun State. The plant materials were sun-dried for seven days, blended into a fine powder using a high-powered electric grinder (VTCL Electric machine grinder), and then stored in a clean, sterile bottle for further use. The extraction was done according to the method of Ifesan (2009) by soaking 25 g of each of the ground spices in 50 ml of hot water (80°C) and 50 ml of ethanol (95%) separately for one hour and three days respectively. Afterward, the plant solutions were filtered with a muslin cloth, and the filtrates were concentrated at reduced pressure (45°C) using a steam release valve on the water bath.

### Determination of the *in-vitro* antibiotic activity

The concentrated extracts were reconstituted using sterile distilled water (10 g in 10 ml), after which four concentrations were prepared using serial dilution. The agar diffusion method was employed, while the pour plate method was used in inoculating an overnight old culture of the isolates: *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Staphylococcus aureus* for sensitivity profile (CLSI, 2006).

### *In-silico* molecular docking

To ascertain the potential antimicrobial activity of the major chemical compounds in onion, ginger, and nutmeg, an *in-silico* docking study was done using four major bacterial two proteins that are antibiotic targets. A library of phytochemical compounds in each spice was formed using text mining analysis based on previous reports on the chemical compounds in each spice. Eight active compounds were reported in onion (Frag et al., 2017; Machová et al., 2019; Fredotović et al., 2021; Kumar et al., 2022), which include: kaempferol, allyl methyl trisulphide, quercetin-3,4 diglucoside, coumaric acid, epicatechin, ferulic acid, thiopropanal-S-oxide, vanillic acid; thirteen compounds was reported in ginger (Sharma et al., 2016; Nishidonoa, 2018; Babaeekhou and Ghane, 2021) which are camphene, gingerol, valencene, gingerdiol, citronellyl n-butylate, shogaol, zingiberene, gingerdiol 3,5 diacetate, selina -4(14), 7(11)-diene, alpha-pinene, beta-funebrene, beta phellandrene, and luteolin 7-O- glucoside; and six active compounds was reported in nutmeg (Chung et al., 2006; Fajriah et al., 2016; Panggabean et al., 2019; Nikolic et al., 2021) which are hexadecanoic acid, caryophyllene, gamma muurolene, trans-calamenene, alpha-muurolene, macelignan. These compounds were subjected to molecular docking in this study. As an addendum, five selected standard antibiotics- augmentin, erythromycin, ciprofloxacin, vancomycin, and septrin were also subjected to molecular docking as positive controls. All these compounds were acquired in SDF format from the Pub Chem database (<http://pubchem.ncbi.nlm.nih.gov>) and subjected to molecular docking to inhibit antibiotic target proteins in bacteria. The target proteins include DNA gyrase subunit B, dihydrofolate reductase (DHF), D-

alanine: D-alanine ligase (Ddl1), and Isoleucyl-tRNA synthetase (IARS) (Table 1) (Jianu *et al.*, 2021). The 3D structures of the mentioned target proteins were acquired from RCSB Protein Data Bank in PDB format (Berman *et al.*, 2000). The protein structures were prepared as suitable targets using the Autodock Tools (version 1.5.6, The Scripps Research Institute, La Jolla, CA, USA) (Trott and Olson, 2010). Co-crystallized ligands, metal atoms, undesired protein chains, and water molecules were removed from the protein structure using Pymol. After that, polar hydrogen atoms and Gesteiger charges were added, and docking grid maps were generated using the Autodock M.G.L. Tool 1.5.4. The grid box restriction of the search space was named coordinates and size (Table 1), considering the amino acids at the enzyme's active site. The target was thereby saved as the required file format (pdbqt). The dockings were done using AutoDock Vina 1.1.2 (Trott and Olson, 2010), a modern-day docking software produced by the Molecular Graphics Laboratory. Pymol and Discovery Studio were used for the docking analyses. The binding energy and intermolecular interaction were compared and analyzed using BIOVIA Discovery Studio visualizer version 19 (<https://discover.3ds.com/discovery-studio-visualizer-download>). The results obtained from the docking analysis were represented as ( $\Delta G$ , kcal/mol), which is the value of the free energy generated from the binding of the respective ligands to the individual proteins.

**Table 1.** Target Proteins and Their Molecular Docking Parameters

Protein	PDB ID	Grid Box Centre Coordinates	Grid box size
DNA gyrase B	1KZN	Center x = 19.577 Center y = 19.108 Center z = 43.258	Size x = 30 size y = 2 size z = 43.258
Dihydrofolate reductase	3SRW	Center x = 29.663 Center y = 43.494 Center z = 9.604	Size x = 26 Size y = 36 Size z = 38
D-alanine: D-alanine ligase (Ddl1)	2ZDQ	Center x = 48.35 Center y = 18.85 Center z = -1.47	Size x = 36 Size y = 30 Size z = 28
Isoleucyl tRNA Synthetase (IARS)	1JZQ	Center x = -28.586 Center y = 3.282 Center z = -24.904	Size x = 24 Size y = 24 Size z = 42

### Statistical analysis

The results obtained were recorded in Mean  $\pm$  Standard deviation (SD) of triplicate determinations. One-way ANOVA was used to statistically compare means' differences at a significance level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Isolation of cariogenic bacteria from the study population

A total of hundred (100) study subjects examined were 46 male and 54 female patients in different age groups (Table 2). Individuals between the ages of 11-20 years had the highest occurrence of dental caries, while the lowest occurrence was found in individuals between the ages of 41-50 (Table 2). Out of the hundred (100) samples -

**Table 2.** Distribution of Age and Sex in Study Population

Age (years)	Females	Males	Total
1-10	5	7	12
11-20	21	20	41
21-30	21	15	36
31-40	2	2	4
41-50	-	-	-
51 and above	5	2	7
TOTAL	54	46	100

**Table 3.** Cultural and Microscopic Characteristics of the Isolates

Appearance	Number of isolates	Shape	Probable organisms
Pink colony	raised 52	Cocci	<i>Staphylococcus</i> species
Pinkish colony	round 9	Cocci	<i>Streptococcus</i> species
Whitish colony	spread 105	Rod	<i>Pseudomonas</i> species
Total	166		

**Table 4.** Classification of Isolates Based on the Outcome of the Gram Reaction and Biochemical Tests

S/N	GR	CS	CT	CO	MO	IN	OX	CI	UR	MR	VP	G	L	M	Organism	% occurrence
1	+	-	-	-	-	-	-	-	-	-	-	A	A	A	<i>Strep. Mutans</i>	31.3
2	+	-	+	+	-	-	-	-	-	-	+	A	A	A	<i>Staph. aureus</i>	5.4
3	-	-	+	-	+	+	-	-	-	+	-	A	A	-	<i>Pseudo. aeruginosa</i>	63.3

**Keys:** GR- Gram stain, CS- Capsule stain, CT- Catalase, CO- Coagulase, MO- Motility, IN- Indole, OX- Oxidase, CI- Citrate, UR- Urease, MR- Methyl-red, VP- Vogesproskur, G- Glucose, L-Lactose, M-Mannitol, A-Acid production, (+)- positive, (-) - Negative.

collected, hundred and sixty-six (166) isolates within three genera: *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Staphylococcus aureus*, were recovered and identified based on their colony morphology, Gram stain reaction, and biochemical tests, hence indicating mixed infections (poly bacteria). *Pseudomonas aeruginosa* (63.3%) had the highest occurrence, followed by *Streptococcus mutans* (31.3%), while *Staphylococcus aureus* (5.4 %) was the least predominance) (Table 3 and Table 4).

#### Antibiotics profile of cariogenic bacteria

The three bacteria subjected to antibiotics testing showed varied susceptibility to the employed antibiotics (Table 5). They displayed moderate sensitivity to gentamycin,

pefloxacin, Rocephin, and vancomycin compared with the standard. *Staphylococcus aureus*, however, had the highest and lowest diameter zones of 17.67 mm and 14.00 mm against ciprofloxacin and, gentamycin respectively. *Streptococcus mutans*, on the other hand, had the highest and lowest zones of inhibition of > 17 mm and 11.33 mm against vancomycin and gentamycin, respectively. Augmentin, chloramphenicol, tarivid, and sparfloxacin had no inhibitory effects against *Staphylococcus aureus* and *Streptococcus mutans*. In contrast, *Pseudomonas aeruginosa* was resistant to more than half 10/16 of all the antibiotics tested but had the highest diameter zone of 18.67 mm against augmentin (Table 5).

**Table 5.** Calculated Average Inhibitory Zones (mm) of the Antibiotics on the Growth of the Isolates

Antibiotics	Inhibitory zone (mm) <i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>
Gentamycin (CN)	14.67 ± 1.53	14.00 ± 1.90	10.33 ± 1.03
Pefloxacin (PEF)	15.67 ± 1.15	14.67 ± 1.53	15.00 ± 0.00
Erythromycin (E)	-	17.00 ± 1.73	14.00 ± 1.00
Septin (SXT.)	-	17.33 ± 1.53	11.67 ± 0.58
Streptomycin (S)	-	14.67 ± 0.58	12.00 ± 2.00
Ciprofloxacin (C.P.X.)	-	17.67 ± 1.53	14.33 ± 0.58
Rocephin (R)	16.67 ± 1.53	15.67 ± 0.58	15.00 ± 0.00
Ampicillin (AM)	-	15.50 ± 0.71	-
Zinnacef (Z)	-	15.00 ± 0.00	-
Ampliclox (APX.)	-	15.33 ± 0.58	15.00 ± 1.73
Ampicillin (AMP.)	-	15.00 ± 0.00	-
Vancomycin (VA.)	11.33 ± 1.15	14.67 ± 1.15	17.67 ± 2.51
Augmentin (AU)	18.67 ± 2.31	-	-
Chloramphenicol (CH.)	-	-	-
Tarivid (OFX)	12.00 ± 2.00	-	-
Sparfloxacin (SP.)	-	-	-

The diameters of the inhibition zones are presented as the Mean (n = 3) ± standard deviation

#### In-silico docking

The molecular docking of some of the chemical compounds identified from the three spices tested against the four

antibiotic target proteins found in the tested bacteria is presented in heat map format (Table 6). The heat map table was constructed using a red-yellow design with the

lowest negative energy values highlighted in yellow. In contrast, the highest negative energy values are highlighted in red. This helps to identify potential compounds that would inhibit target proteins with more negative values than the standard antibiotics (control).

The interaction of the chemical compounds in the plant extract used with DNA gyrase B revealed that luteolin 7-O- glucoside in ginger had the highest negative free energy upon binding ( $\Delta G = -8.3$  kcal/mol), followed by kaempferol and epicatechin in onion. Macelignan in nutmeg also had relatively high binding energy values more significant than that of ciprofloxacin, a standard antibiotic ( $\Delta G = -7.6$  kcal/mol). In the interaction with dihydrofolate reductase, vancomycin had the highest binding energy obtained from the standard antibiotics ( $\Delta G = -7.4$  kcal/mol). Compounds in onion that showed a binding affinity more significant than that of vancomycin against dihydrofolate reductase (DHF) are kaempferol, quercetin 3,4 diglucoside, and epicatechin, while for ginger, the compounds are selina-4 (14), 7 (11)-diene, and luteolin 7-O-glucoside. However, only trans-calamenene in nutmeg had a higher binding affinity for DHF when compared to vancomycin.

The following compounds in onion: Kaempferol, coumaric acid, and ferulic acid; in ginger: gingerol, valencene, gingerdiol, shogaol, zingiberene, selina-4, 7 (11)-diene, beta-phellandrene, and luteolin 7-O-glucoside; and in nutmeg: gamma-murolene, trans-calamenene, and macelignan interacted more strongly with the target protein D alanine-d alanine ligase as observed from their higher negative free binding energy in comparison to the standard antibiotic-ciprofloxacin ( $\Delta G = -7.1$  kcal/mol) that gave the lowest free binding energy with the protein among all other antibiotics. Docking with D-alanine and ATP, the known substrates of the enzyme, gave  $\Delta G = -4.2$  kcal/mol and  $\Delta G = -7.6$  kcal/mol, respectively. Almost all compounds in all the three spices, except for allyl methyl trisulphide and thiopropanal-S-oxide, had higher affinities for the enzyme as revealed by their free binding energy values that were greater than that of D-alanine, the endogenous ligand of the enzyme. Septrin had the lowest binding free energy affinity for isoleucyl tRNA synthetase ( $\Delta G = -7.8$  kcal/mol) among the standard antibiotics used as

a positive control. Only quercetin 3, 4 diglucoside in onion, luteolin 7-O- glucoside in ginger, and macelignan in nutmeg had free binding energy values greater than

septrin. Results obtained from docking ATP, a known protein substrate, gave a binding free energy value ( $\Delta G > -6.7$  kcal/mol). Other compounds that showed a higher binding affinity for the protein greater than that of ATP include epicatechin in onion, valencene and selina-4 (14), 7 (11)-diene in ginger, gamma-murolene, trans-calamenene, and alpha-murolene in nutmeg. Overall, luteolin 7-O- glucosides in ginger and Kaempferol in onion seem to have the highest affinity for the four proteins, as indicated by their free binding energy values ( $\Delta G \geq -7.5$  kcal/mol). Vancomycin is the antibiotic with the highest affinity for all four target proteins used for the in-silico analysis in this study, as seen from its high negative free energy. At the same time, ciprofloxacin seems to have the lowest affinity for three of the antibiotic target proteins.

The key residues of the four different proteins that interacted majorly with the ligands in each space as revealed by PYMOL and Discovery Studio visualize (Table 7). The specific interaction of the ligand in each space with the highest negative binding free energy for DNA gyrase B are depicted in (Figure 1a-c). As seen from the interactions, the specific amino acid residues at the protein active site that interact with these ligands are ALA 47, ASN 46, ILE 78, THR 165, and VAL 71. The major interactions are through hydrogen bonds and pi-alkyl bonds. The amino acid residues in dihydrofolate reductase that interact with the ligands in each spice with the highest affinity for the protein are ASP 27, LEU 28, PHE 31, ALA 7, and ILE 94 (Figure 2a-c). The interactions are mainly pi-sigma, pi-pi, stacked hydrogen, and carbon-hydrogen bonds.

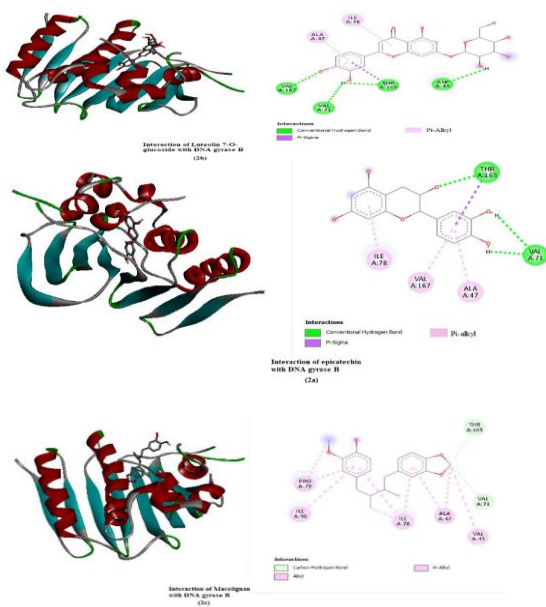
As seen from the interactions of the ligand with each spice that has the highest affinity for d-alanine:d-alanine ligase (Figure 3a-c), only two amino acid residues at its active site appear to interact commonly with the three ligands –PHE 151 and TYR 225. However, GLY 158, SER 159, VAL 161, GLU 197, PHE 222, TYR 223, TYR 225, LYS 228, ASN 284, GLU 282, and ASN 281 are the residues of the protein that interact with both kaempferol and macelignan. The interactions are mainly alkyl, pi-alkyl, pi-anion, and pi-cation. The interaction of isoleucyl tRNA synthetase with the ligand in each spice with its highest affinity is shown (Figure 4a-c). Amino acid residues in the protein interacting with the three ligands shown are ASP 553, TRP 518, GLY 45, HIS 57, and ASP 85. The interactions are mainly conventional hydrogen bonds, pi-anion, and carbon-hydrogen bonds.

**Table 6.** Heat Map of Recorded Docking Scores (Binding Free Energy Kcal/Mol) of the Compounds Present in Onion, Ginger, and Nutmeg and the Standard Antibiotics

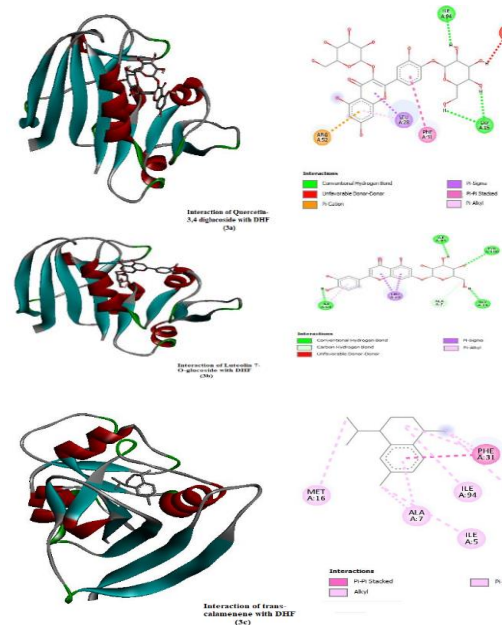
		Binding free energy $\Delta G$ (kcal/mol)				
		DNA Gyrase B	Dihydro Folate reductase	D ala:d ala ligase	Ile tRNA syn	
S/N	LIGANDS	1KZN	3SRW	2ZDQ	1JZQ	
1	Kaempferol	-7.7	-7.8	-8.6	-7.8	
2	Allyl methyl trisulphide	-3.3	-3.2	-3.8	-3.2	
3	Quercetin-3,4 diglucoside	-6.3	-9.0	-6.9	-8.4	
4	Coumaric acid	-6.0	-5.6	-7.7	-5.6	

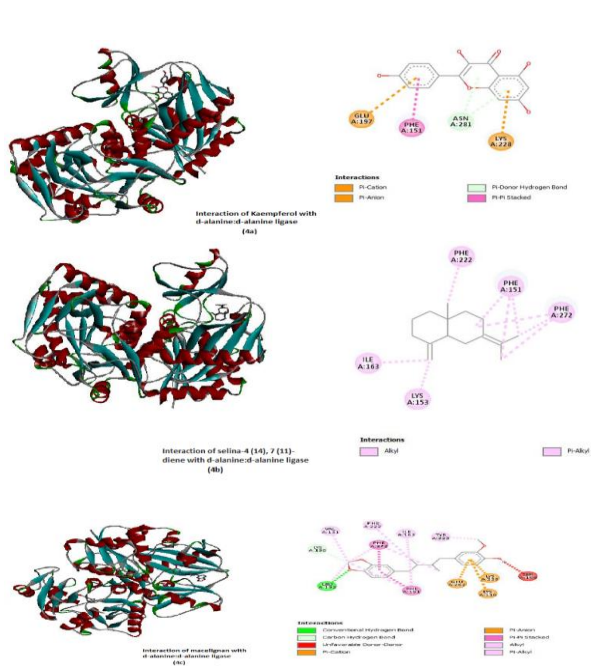
5	Epicatechin	-7.8	-8.0	-6.4	-7.6
6	Ferulic acid	-6.2	-6.1	-7.4	-5.9
7	Thiopropional-S-oxide	-3.0	-2.7	-3.3	-2.9
8	Vanillic acid	-5.9	-5.6	-6.6	-5.6
9	Camphene	-4.7	-5.3	-5.7	-5.5
10	Gingerol	-5.8	-6.2	-7.7	-5.8
11	Valencene	-7.3	-6.9	-8.0	-6.9
12	Gingerdiol	-5.8	-6.1	-7.8	-6.2
13	Citronellyl n-butyrate	-5.5	-5.3	-6.9	-5.8
14	Shogaol	-6.1	-5.9	-8.2	-6.1
15	Zingiberene	-7.1	-6.6	-8.5	-5.7
16	Gingerdiol 3,5 diacetate	-5.5	-6.6	-7.0	-6.2
17	Selina-4,(14), 7(11)-diene	-7.1	-7.9	-8.6	-7.1
18	Alpha -pinene	-4.7	-5.3	-6.3	-5.6
19	Beta-funebrene	-6.2	-7.0	-6.2	-6.5
20	Beta-phellandrene	-5.7	-5.7	-7.4	-5.5
21	Luteolin 7-O- glucoside	-8.3	-8.8	-7.5	-8.7
22	Hexadecanoic acid	-5.2	-5.1	-6.7	-5.4
23	Caryophyllene	-5.6	-7.1	-5.7	-6.6
24	Gamma muurolene	-6.4	-7.3	-7.6	-6.9
25	Trans-calamenene	-6.8	-7.5	-8.3	-6.8
26	Alpha-muurolene	-6.0	-7.4	-5.5	-6.8
27	Macelignan	-7.7	-7.4	-8.6	-8.0
28	Ciprofloxacin	-7.6	-4.1	-7.1	-8.1
29	Septrin	-8.0	-5.1	-9.2	-7.8
30	Erythromycin	-10.1	-6.3	-8.4	-11.4
31	Augmentin	-9.2	-5.7	-10.2	-9.1
32	Vancomycin	-13.6	-7.4	-14.0	-14.5
33	D-alanine			-4.2	
34	ATP			-7.6	-6.7

**Note:** The color scale varies from yellow to red (lowest recorded negative binding free energy to the highest negative binding free energy)  
 Key: Ligands numbered 1-8 are constituents of onion; 9-21 are constituents of ginger, 22-27 are constituents of nutmeg, 28-32 are standard antibiotics, 33 and 34 are known ligands of d-ala:d-ala ligase and isoleucyltRNA synthetase.

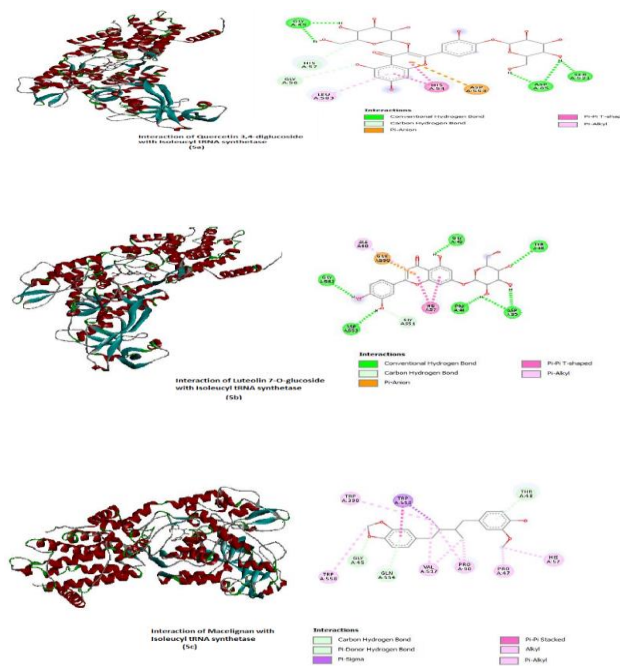


**Figure 1.** Structure of DNA Gyrase B in Complex with (a) Epicatechin, (b) Luteolin 7-O-glucoside, (c) Macelignan





**Figure 3.** Structure of D-alanine: D-alanine Ligase in Complex with (a) Kaempferol, (b) Selina-4 (14), 7 (11)-diene (c) Macelignan



**Figure 4.** Structure of Isoleucyl tRNA Synthetase in Complex with (a) Quercetin 3,4-diglucoside, (b) Luteolin 7-O- glucoside, (c)Maceligna

**Table 7.** Key Residues in Each Protein Interact with the Molecules

Antibiotic target protein	Key residues interacting with the ligands
DNA gyrase B	ILE 78, GLU. 50, A.S.N. 46, THR 165, V.A.L. 71, A.R.G. 76, A.L.A. 47, VAL 167, VAL 167
Dihydrofolate reductase	ASP 27, LEU 28, PHE 31, ALA 7, ILE 94, GLY 15
D-alanine: D alanine ligase	PHE 151, TYR 225, GLY 158, SER 159, VAL 161, GLU 197, PHE 222, TYR 223, TYR 225, LYS 228, ASN 284, ASN 281, GLU 282
Isoleucyl tRNA synthetase	ASP 553, TRP 518, GLY 45, HIS 57, ASP 85, PRO 46, PRO 47, GLU 550, GLN 554

**In-vitro antibacterial activity of the extracts against the tested bacteria**

The average inhibitory zones of the spices (onion, ginger, and nutmeg) against *Streptococcus mutans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are presented in Tables 8a, 8b, and 8c, respectively. There was a statistically significant difference in the zone of inhibitions exhibited by the extracts at 100% concentrations against the three tested organisms. The inhibitory effects of the extracts against the tested bacteria were organism, extract, and concentration-dependent. There were significant differences (p<0.05) observed in the inhibitory zones onion, ginger, and nutmeg at 25%, 50%, 75%, and 100% extract concentrations for each of the three organisms tested. For each of the three organisms, significant differences (p<0.05) were observed in all the concentrations tested except in onion extract, which was effective only at 50% and 75% concentrations. However, there appeared to be a statistically significant higher zone of inhibition at concentrations of 100% for all three

organisms. At 100% onion extract concentration, the highest zone of inhibition was obtained against *Streptococcus mutans*, although not statistically different from the other two organisms (Table 8a). The inhibitory effects of ginger extract against the tested organisms revealed no significant difference (p<0.05) in the inhibitory zones obtained for each of the three respective microorganisms. However, at a concentration of 75% ginger extract, there was a statistically significant (p<0.05) higher zone of inhibition against *Pseudomonas aeruginosa* than *Staphylococcus aureus* with no significant difference compared to that of *Streptococcus mutans*. However, it was observed that at a concentration of 100%, ginger extract was more active against *Streptococcus mutans* than *Staphylococcus aureus* at (p<0.05), but with no statistically significant difference in the inhibition zone obtained when compared to *Pseudomonas aeruginosa*.

For *Streptococcus mutans*, there was a significant (p<0.05) difference in the inhibition zones at all tested concentrations. Nonetheless, in *Staphylococcus*

*aureus* and *Pseudomonas aeruginosa*, there were significant differences ( $p < 0.05$ ) at the tested concentrations except for 25% and 50%. There was no statistically significant difference ( $p < 0.05$ ) in each inhibitory zone obtained at 25% and 50% nutmeg extract, respectively, for all three organisms. However, at 75% nutmeg concentration, there was a significantly ( $p < 0.05$ ) higher zone of inhibition against *Staphylococcus aureus* compared to *Streptococcus mutans*, even though there was no significant difference when compared to that of *Pseudomonas aeruginosa* at the same concentration. However, there is a significant difference ( $p < 0.05$ ) in the inhibitory zones obtained at 100 % nutmeg extract concentration against each of the three organisms, with

the highest zone of inhibition obtained against *Staphylococcus aureus*. For *Streptococcus mutans*, there was a significantly ( $p < 0.05$ ) higher zone of inhibition obtained at 100% compared to other concentrations, even though no significant difference ( $p < 0.05$ ) in the zone of inhibition obtained at both 25 and 50% extract concentrations. For *Staphylococcus aureus*, there was a significant difference ( $p < 0.05$ ) in the zones of inhibition obtained in the tested concentrations, with the highest zone recorded at 100%. For *Pseudomonas aeruginosa*, there was a significant difference ( $p < 0.05$ ) in the zones of inhibition obtained at all concentrations tested, although no significant difference ( $p < 0.05$ ) in the zones of inhibition obtained at both 75% and 100% (Tables 8a, 8b, and 8c).

**Table 8a.** Calculated Average Inhibitory Zones (mm) of the Spices on the Growth of *Streptococcus mutans*

Concentration (%)	Onion	Ginger	Nutmeg
25	10.33 ± 1.53 <sup>dc</sup>	7.00 ± 1.73 <sup>4</sup>	7.00 ± 1.00 <sup>4</sup>
50	11.33 ± 1.12 <sup>cd</sup>	11.00 ± 2.65 <sup>3</sup>	11.67 ± 2.08 <sup>32</sup>
75	16.00 ± 2.00 <sup>b</sup>	15.67 ± 1.12 <sup>ba</sup> <sup>2</sup>	14.00 ± 2.00 <sup>cb</sup> <sup>23</sup>
100	22.00 ± 1.00 <sup>a</sup>	20.33 ± 1.53 <sup>ab</sup> <sup>1</sup>	20.67 ± 1.53 <sup>b</sup> <sup>1</sup>

Values with different superscripts (<sup>a,b,c</sup>) across rows are significantly different at ( $p < 0.05$ ); values with different superscripts (<sup>1,2,3,4</sup>) across columns are significantly different at ( $p < 0.05$ )

**Table 8b.** Calculated Average Inhibitory Zones (mm) of Spices on the Growth of *Staphylococcus aureus*

Concentration (%)	Onion	Ginger	Nutmeg
25	8.33 ± 0.58 <sup>dc</sup>	8.00 ± 0.00 <sup>4</sup>	7.33 ± 1.53 <sup>4</sup>
50	10.00 ± 1.00 <sup>cd</sup>	10.5 ± 0.71 <sup>3</sup>	14.00 ± 2.00 <sup>3</sup>
75	14.00 ± 1.73 <sup>b</sup>	12.67 ± 0.58 <sup>c</sup> <sup>21</sup>	17.67 ± 1.53 <sup>ab</sup> <sup>2</sup>
100	20.00 ± 1.00 <sup>a</sup>	13.33 ± 1.12 <sup>c</sup> <sup>12</sup>	24.33 ± 0.58 <sup>a</sup> <sup>1</sup>

Values with different superscripts (<sup>a,b,c</sup>) across rows are significantly different at ( $p < 0.05$ ); values with different superscripts (<sup>1,2,3,4</sup>) across columns are significantly different at ( $p < 0.05$ )

**Table 8c.** Calculated Average Inhibitory Zones (mm) of Spices on the Growth of *Pseudomonas aeruginosa*

Concentration (%)	Onion	Ginger	Nutmeg
25	11.00 ± 2.00 <sup>dc</sup>	8.00 ± 1.00 <sup>4</sup>	8.67 ± 0.58 <sup>4</sup>
50	11.67 ± 1.53 <sup>cd</sup>	12.67 ± 1.16 <sup>3</sup>	12.67 ± 0.58 <sup>3</sup>
75	16.67 ± 1.12 <sup>b</sup>	17.33 ± 1.53 <sup>ab</sup> <sup>21</sup>	15.67 ± 0.58 <sup>bca</sup> <sup>21</sup>
100	20.00 ± 1.00 <sup>a</sup>	19.67 ± 1.53 <sup>ba</sup> <sup>12</sup>	16.67 ± 1.12 <sup>c</sup> <sup>12</sup>

Values with different superscripts (<sup>a,b,c</sup>) across rows are significantly different at ( $p < 0.05$ ); values with different superscripts (<sup>1,2,3,4</sup>) across columns are significantly different at ( $p < 0.05$ )

## Discussion

Antibiotic resistance increases dental caries-related morbidity with high treatment costs (Yadav and Prakash, 2017). Furthermore, antibiotics have no remineralizing properties to assist in managing dental caries. Thus, dental caries is a significant oral disease of public health importance and the primary cause of pain and tooth loss, presenting a high morbidity rate in the general population (Khushbu and Satyam, 2016; Nelio *et al.*, 2016). One hundred and five (105) isolates were identified with *Pseudomonas aeruginosa*, a Gram-negative bacterium being the most predominant. *Staphylococcus aureus* (109) and *Streptococcus mutans* (9) were the two Gram-positive bacteria isolated. It has been reported that the oral cavity is

an important reservoir of some Enterobacteriaceae (Raghavendran, 2007; Heo *et al.*, 2008; Amaral *et al.*, 2009) and potentially pathogenic bacteria such as *Pseudomonas aeruginosa*. Marsh (2003) reported that *Streptococcus mutans* and *Streptococcus sobrinus* are associated with humans and have a major role in the cause of dental caries. In this study, the response of the tested bacteria to the antibiotics differed. Vancomycin, augmentin, erythromycin, and ciprofloxacin were most active against *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively. Pranay and Kumar (2009) reported that *Streptococcus mutans* was moderately sensitive to vancomycin. In contrast, Devi *et al.* (2012) reported resistance of *Staphylococcus aureus* isolated from dental plaques to erythromycin and ciprofloxacin. *S. aureus* and *S.*



*mutans* used in this study could be considered multidrug resistant since they were resistant to more than three antibiotics (augmentin, chloramphenicol, tarivid, and sparfloracin). In addition to these four antibiotics, *S. mutans* was also resistant to amoxicillin. This is contrary to the study of Pranay and Kumar (2009), who observed the sensitivity of three strains of *Streptococcus mutans* against amoxicillin, Penicillin G, and chloramphenicol with maximum inhibitory zones. The discrepancy could be attributed to usage; according to Devi *et al.* (2012), antibiotics have proven more effective against dental plaques, but repeated use has side effects. Ciprofloxacin was not effective at all because there was no zone of inhibition. All bacteria isolated were resistant to chloramphenicol and sparfloracin. *Pseudomonas aeruginosa's* resistance to ten antibiotics out of the sixteen antibiotics tested is of great concern. The resistance of *Pseudomonas aeruginosa* to antibiotics is increasing globally due to its excessive use (Josef *et al.*, 2015). Oliver *et al.* (2015) also stated that *Pseudomonas aeruginosa* poses a growing threat because of its resistance ability to nearly all available antibiotics, which could be due to the selection of mutations in its chromosomal genes and its increasing ability to transfer resistance. This is attributed to the phytochemicals' interactions with bacterial target proteins. According to Poirrel *et al.* (2001), *P. aeruginosa* from clinical specimens have shown multidrug-resistant characteristics through different mechanisms. Also, a study by Popoola *et al.* (2017) reported antibiotic resistance in Gram-negative bacteria (of which *P. aeruginosa* is included) due to the presence of efflux pump transport protein. The multidrug resistance observed in *P. aeruginosa* is not surprising because there are evidences from researchers that there is an alarming resistance to antibiotics by Gram-negative bacteria than their Gram-positive counterpart. *Staphylococcus aureus*, *Streptococcus mutans*, and *Pseudomonas aeruginosa* tested in this study were susceptible to the extracts of onion, ginger, and nutmeg at varied concentrations used, with the higher the concentration, the higher the inhibition zone. Indu *et al.* (2006) reported that nutmeg and ginger at all concentrations tested showed antibacterial activity against some pathogenic microorganisms. Moreover, the result obtained by Challaraj *et al.* (2020) revealed that ginger and nutmeg extracts have antimicrobial and biofilm inhibition activity. Other plants, such as *C. olitorius* L. and *A. caulirhiza* Del., have also been reported to possess antibacterial activity against cariogenic bacteria (Namwase *et al.*, 2021).

At 100% onion concentration, high inhibitory zones were obtained among the tested bacteria than for some of the standard antibiotics that were sensitive to the organisms. This could be attributed to the fact that they contain higher bioactive compounds at a higher level. However, at a 10% extract concentration for ginger, a higher zone of inhibition against *Streptococcus mutans* and *Pseudomonas aeruginosa* was comparable to the zones of inhibition obtained for the standard antibiotics. Ahmed *et al.* (2016) reported the inhibiting effects of onion extract

against all the strains of *S. aureus* tested. Yousufi (2012) stated that the chloroform extract of onion exhibited a strong zone of inhibition (24 mm) against *S. aureus*, whereas the aqueous extract had a 19 mm zone of inhibition against *P. aeruginosa*. The bacterial effect of onion is not surprising as the spice has a natural preservative/food additive. In the case of nutmeg, a higher zone of inhibition was obtained at a concentration of 100% against *Streptococcus mutans* and *Staphylococcus aureus* compared to the zones of inhibition obtained for the standard antibiotics susceptible to the organisms. These results suggest that onion seemed more effective against the three cariogenic bacteria than ginger and nutmeg. However, both nutmeg and ginger also demonstrated significant antibacterial activity against two of the three cariogenic bacteria. This might be attributed to the synergistic effect of all chemicals in each plant compound working by various mechanisms to ensure antibacterial action. Generally, four different mechanisms are linked to antimicrobial action. These are cell wall/membrane synthesis inhibition, protein synthesis, nucleic acid synthesis inhibition, and anti-metabolite production (Alves *et al.*, 2014; Pratama *et al.*, 2018). Vancomycin seemed to be the antibiotic with the highest affinity for the bacterial target proteins, as seen from the results of the in-silico studies. Conversely, in the *in vitro* studies, the extracts of the three spices at a concentration of 100% were more effective against the three cariogenic bacteria, as seen from the higher zones of inhibition obtained when compared to the zones of inhibition obtained with vancomycin. The three spices used in this study all contain compounds with a high binding affinity for the target proteins. Their observed anticariogenic activity might be due to the high affinity of some of these compounds for the antibiotic target proteins working synergistically to inhibit the proteins responsible for bacterial DNA replication and protein synthesis, thereby impeding their growth. The stronger anticariogenic activity of onion, among other spices, might be due to its richer content of phenolic compounds. Phenolic compounds have been known to impede the growth of microbes by inhibiting their enzymes and indirectly interacting with microbial proteins (Cowan, 1999).

Molecular docking is a dynamic tool mainly employed to predict the affinities of compounds to target proteins and, as such, could be utilized in the search for new antimicrobial agents (Kumalo *et al.*, 2000). In this study, a total of 25 compounds known to be present in onion, ginger, and nutmeg were docked with target proteins known to be the target of antibiotics to predict the antibacterial effect of the plant compounds. The target proteins include dihydrofolate reductase, D-alanine:d-alanine ligase, DNA gyrase B, and isoleucyl-tRNA synthetase. DNA gyrase subunit B is a major antibiotic target protein due to its important role in bacteria's replication processes (Aldred *et al.*, 2014). Of major importance as an antibiotic target protein is also dihydrofolate reductase. This enzyme reduces dihydrofolate to tetrahydrofolate in a reaction that requires nicotinamide adenine dinucleotide phosphate (NADPH) as a co-factor. This enzyme is involved in biosynthetic pathways such as

purine and pyrimidines. Inactivation of this enzyme can eventually lead to cellular death due to aberrations in the biosynthesis of nitrogenous bases required for replication in bacteria (Hong *et al.*, 2015; Rashid *et al.*, 2016; Schweitzer *et al.*, 1990). D-alanine:d-alanine ligase is an enzyme responsible for producing the d-Ala-d-Ala peptide using energy derived from ATP. This d-Ala-d-Ala is the last peptide of the bacterial cell wall peptidoglycan layer from which the polymer is formed from the crosslinking of the peptidoglycan monomers (Kitamura *et al.*, 2009). Isoleucyl t-RNA synthetase is actively involved in synthesizing proteins in bacteria in a reaction that requires aminoacylation of the tRNA. Inhibition of this enzyme is a suitable mechanism of antimicrobial action (Ho *et al.*, 2018). The ligand affinity of the test is indicated by the value of  $\Delta G$ , where the more negative the value of  $\Delta G$  indicates, the higher the ligand affinity (Malmstrom and Watowich, 2011). This study obtained a negative  $\Delta G$  value for all tested compounds in each plant. This indicates that binding the ligands to the respective protein receptors is a spontaneous reaction favored thermodynamically (Du *et al.*, 2016). Among all compounds tested for onion, epicatechin had the highest binding free energy to DNA gyrase B. In contrast, quercetin 3,4 diglucoside had the highest free binding energy with dihydrofolate reductase and isoleucyl tRNA synthetase, while kaempferol has the highest binding affinity for d-alanine:d-alanine ligase. Quercetin 3,4 diglucoside has demonstrated antibacterial activity against gram-positive bacteria in previous studies (Fredotović *et al.*, 2021). Three major compounds in onion had a powerful binding affinity for all four antibiotic target proteins used in this study, as seen from their relatively high negative free energy values. The compounds are kaempferol, quercetin 3,4 diglucoside, and epicatechin. Kaempferol, quercetin 3,4 diglucoside, and epicatechin have all been reported to possess antioxidant activities (Arif *et al.*, 2017; Nile *et al.*, 2018; Zhihao *et al.*, 2020). Phenolic compounds have been reported to demonstrate observable antibacterial activity in addition to their antioxidant properties. Their antibacterial activity has alluded to their ability to modify cell membrane/cell wall permeability and rigidity, leading to the loss of integrity of the membrane when these compounds form hydrogen bonds with enzymes (Cushnie and Lamb, 2011). In ginger, luteolin 7-O-glucoside showed the highest binding free energy for DNA gyrase B, dihydrofolate reductase, and isoleucyl tRNA synthetase. However, selina-4 (14), 7 (11)-diene had the highest binding affinity for d-alanine:d-alanine ligase. Previous *in-silico* also showed that luteolin 7-O- glucoside demonstrated appreciable binding affinities for proteins of *Streptococcus mutans* such as glucan sucrose, glucan binding protein C, *S. mutans* antigen I/II carboxy-terminus and Apo form of D-alanine:D-alanine ligase thereby confirming its antimicrobial activity (Babaeekhou and Ghane, 2021). Selina-4 (14), 7(11)-diene is a sesquiterpene, and sesquiterpenes are quite abundant in ginger and have been reported to demonstrate antimicrobial activities against different microbes (Anwar *et al.*, 2019; Singh *et al.*, 2008). In nutmeg, macelignan showed the highest binding

affinity for DNA gyrase, protein, d-alanine:d-alanine ligase, and isoleucyl tRNA synthetase. At the same time, trans-calamenene had the most significant binding affinity for dihydrofolate reductase. Macelignan has been reported to show anti-cariogenic activity against cariogenic bacteria (Chung *et al.*, 2004). Trans-calamenene has been said to possess antibacterial activity (Fajriah *et al.*, 2016).

## CONCLUSION

Molecular docking studies show that some of the compounds in onion, ginger, and nutmeg had good binding affinities to the four antibiotic bacterial target proteins and, as such, were able to inhibit these proteins. This might be responsible for these extracts' in vitro anticariogenic activities against common cariogenic bacteria at varying levels. Hence, these spices, especially onions, represent new potential sources of anticariogenic compounds that can be utilized as dentrifices and in therapy against dental caries. More research on the isolation and characterization of the major lead compounds in each plant extract, i.e., kaempferol and quercetin 3,4 diglucoside in onion, luteolin 7-O-glucoside in ginger, and macelignan in nutmeg that will be useful for elucidation of their antibacterial mechanism is hereby recommended.

## AUTHORS' CONTRIBUTIONS

Conceptualization; GCA, Data curation; GCA, Formal analysis; GCA and ITS, Funding acquisition; GCA and ITS; Investigation; GCA, ITS, ASS and BTA, Methodology; GCA, ITS, TDA and AKA, Project administration; GCA and ITS, Resources; GCA and ITS, Software; GCA, ITS, TDA and AKA, Supervision; GCA, Validation; GCA, Visualization; GCA and ITS, Roles/Writing – original draft; Writing – review & editing; GCA, ITS, TDA, ASS, BTA and AKA. All authors have read and agreed to the published version of the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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