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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. 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 (ΔG> -7 kcal/mol). At 100% (1 g/mL) 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. These activities cause 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

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 β-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 and 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 µg), gentamycin (10 µg), erythromycin (15 µg), vancomycin (10 µg), pefloxacin (10 µg), streptomycin (30µg), ampiclox (30 µg), amoxicillin (30 µg), ciprofloxacin (10 µg), rocephin (25 µg), zinnacef (20 µg), septrin (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), augmentin (30 µg) and tarivid (10 µg) 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 (Farag et al., 2017; Machová et al., 2019; Fredotovi'c 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, citronelly n-butyrate, 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-crystalized 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 (ΔG, 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	Size x = 30
		Center y = 19.108	size y = 2
		Center z = 43.258	size z=43.258
Dihydrofolate reductase	3SRW	Center x = 29.663	Size x = 26
		Center y = 43.494	Size y = 36
		Center z = 9.604	Size z = 38
D-alanine: D-alanine ligase (Ddl1)	2ZDQ	Center x = 48.35	Size x = 36
		Center y = 18.85	Size y = 30
		Center z = -1.47	Size z = 28
Isoleucyl_tRNA Synthetase (IARS)	1JZQ	Center x = -28.586	Size x = 24
		Center y = 3.282	Size y = 24
		Center z = -24.904	Size z = 42

Statistical analysis

The results obtained were recorded in Mean ± 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 raised colony	52	Cocci	*Staphylococcus* species
Pinkish round colony	9	Cocci	*Streptococcus* species
Whitish spread colony	105	Rod	*Pseudomonas* 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	*Strep. Mutans*	31.3
2	+	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staph. aureus*	5.4
3	-	-	+	-	+	+	-	-	-	+	-	A	A	-	*Pseudo. aeruginosa*	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- Vogesproskeur, 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) Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*
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
Septrin (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 (ΔG= -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 (ΔG=-7.6 kcal/mol). In the interaction with dihydrofolate reductase, vancomycin had the highest binding energy obtained from the standard antibiotics (ΔG = -7.4kcal/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-muurolene, 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 (ΔG= -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 ΔG= -4.2 kcal/mol and ΔG= -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 (ΔG= -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 (ΔG> -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-muurolene, trans-calamenene, and alpha-muurolene in nutmeg. Overall, luteolin 7-O- glucosides in ginger and Kaempferolin onion seem to have the highest affinity for the four proteins, as indicated by their free binding energy values (ΔG≥ -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 ΔG (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	Thiopropanal-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.

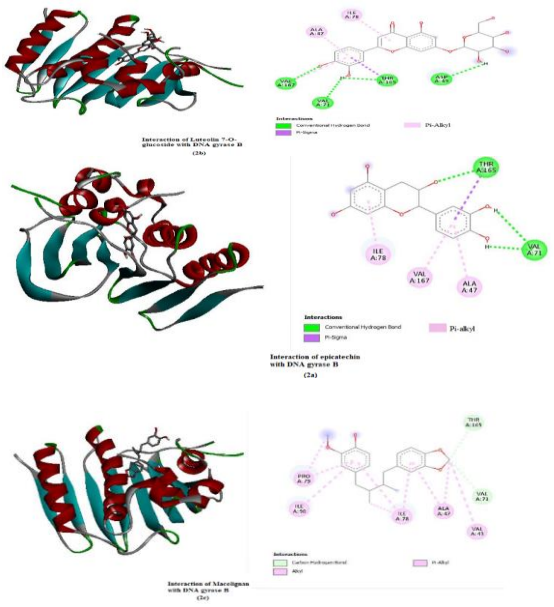

Figure 1. Structure of DNA Gyrase B in Complex with (a) Epicatechin,(b) Luteolin 7-O-glucoside, (c) Macelignan

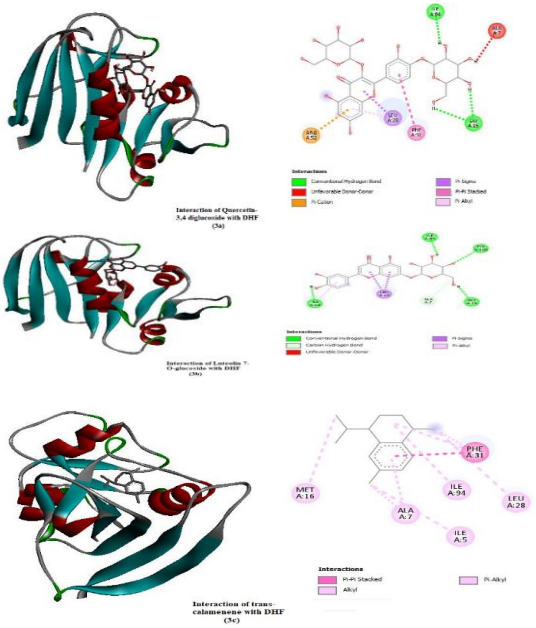

Figure 2. The Structure of Dihydrofolate Reductase (DHF) in Complex with (a) Quercetin- 3,4 diglucoside, (b) Luteolin 7-O-glucoside (c) Trans- calamenene

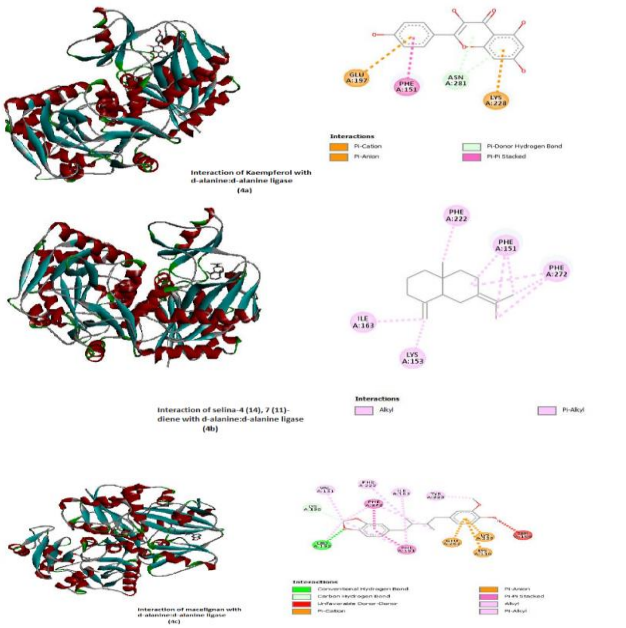

Figure 3. Structure of D-alanine: D-alanine Ligase in Complex with (a) Kaempferol, (b) Selina-4 (14), 7 (11)-diene (c) Macelignan

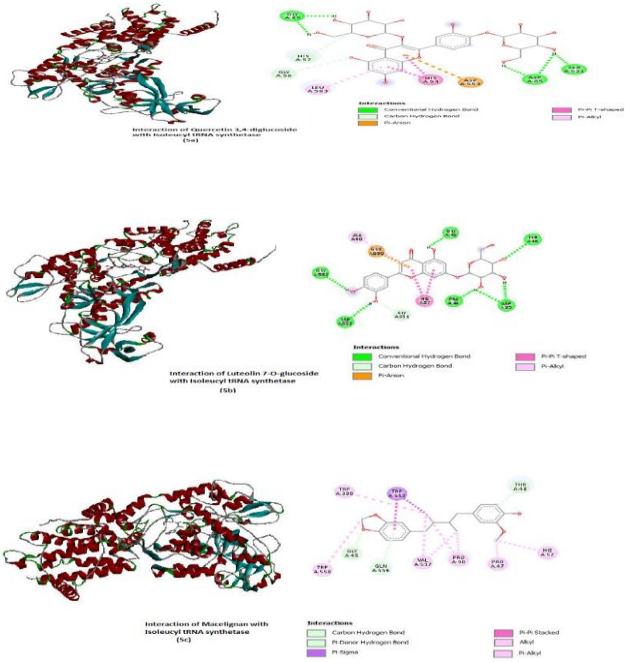

Figure 4. Structure of Isoleucyl tRNA Synthetase in Complex with (a) Quercetin 3,4-diglucoside, (b) Luteolin 7-O- glucoside, (c)Maceligna

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-votro antobacterial 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^{dc}	7.00 ± 1.73^{4}	7.00 ± 1.00^{4}
50	11.33 ± 1.12^{cd}	11.00 ± 2.65^{3}	11.67 ± 2.08^{32}
75	16.00 ± 2.00^{b}	$15.67 \pm 1.12ba^{2}$	14.00 ± 2.00^{cb23}
100	22.00 ± 1.00^{a}	20.33 ± 1.53^{ab1}	$20.67 \pm 1.53b^{1}$

Values with different superscripts (^{a,b,c}) across rows are significantly different at ($p<0.05$); values with different superscripts (^{1,2,3,4}) 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^{dc}	8.00 ± 0.00^{4}	7.33 ± 1.53^{4}
50	10.00 ± 1.00^{cd}	10.5 ± 0.71^{3}	14.00 ± 2.00^{3}
75	14.00 ± 1.73^{b}	$12.67 \pm 0.58c^{21}$	17.67 ± 1.53^{ab2}
100	20.00 ± 1.00^{a}	13.33 ± 1.12^{c12}	$24.33 \pm 0.58a^{1}$

Values with different superscripts (^{a,b,c}) across rows are significantly different at ($p<0.05$); values with different superscripts (^{1,2,3,4}) 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	$11.00 \pm 2.00_{dc}$	8.00 ± 1.00^{4}	8.67 ± 0.58^{4}
50	11.67 ± 1.53^{cd}	12.67 ± 1.16^{3}	12.67 ± 0.58^{3}
75	16.67 ± 1.12^{b}	$17.33 \pm 1.53ab^{21}$	15.67 ± 0.58^{bca21}
100	20.00 ± 1.00^{a}	$19.67 \pm 1.53ba^{12}$	$16.67 \pm 1.12c^{12}$

Values with different superscripts (^{a,b,c}) across rows are significantly different at ($p<0.05$); values with different superscripts (^{1,2,3,4}) 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 sparfloxacin). 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 sparfloxacin. *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 Poirel *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 ΔG, where the more negative the value of ΔG indicates, the higher the ligand affinity (Malmstrom and Watowich, 2011). This study obtained a negative ΔG 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 (Fredotovi´c *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 sucrase, 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 OTA, 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, OTA 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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REFERENCES

Abdel, F.M.A., Barghouth, M.H., Wassel, M.O., Deraz, O.H., Khalil, A.E., Sarsik, H.M., Ali-Mohsen, A.M., Qenawy, A.S. and ElFadl, R.K.(2022). Epidemiology of dental caries inpermanent dentition: evidence from a population-based survey in Egypt. BMC *Public Health,* 22: 2438. https://doi.org/10.1186/s12889-022-14844-9

Agabalogun, L. (2016). The spices of life: Testing the antimicrobial effects of garlic (Alliumsativum), Cinnamon (cinnamomum zeylanicum), and Clove (Syzygium aromaticum) against Streptococcus

mutans. [Last cited on 2016 Jul 12]. Available from:http:// www.google.com.

Ahmed, M.K., Hani, S.F., Ahmad, M.A. and Safaa, A.T. (2016). Antibacterial effect of onion. *Scholars Journal of Applied Medical Sciences,* 4(11D): 4128-4133.

Aldred, K.J., Kerns, R.J. and Osheroff, N. (2014). Mechanism of quinolone action and resistance. *Biochemistry*, 53: 1565-1574.

Al-Shahrani, M.A. (2019). Microbiology of dental caries: A Literature Review. *Annals of Medical and Health Science Research,* 9: 655-659.

Alves, M.J., Hugo, J.C., Froufe, A.F.T., Costa, A F.S., Liliana, G.O., Sara, R.M.O., Rui, M.V., Abreu, M.,P. and Isabel, C.F. (2014). Docking studies in target proteins involved in antibacterial action mechanisms: Extending the knowledge on standard antibiotics to antimicrobial mushroom compounds. *Molecules*,19: 1672-1684. doi:10.3390/molecules19021672

Amaral, S.M., Cortês A.Q. and Pires, F.R. (2009). Nosocomial pneumonia: importance of the oral environment. *Journal of Brazillians Pneumology*, 35:1116-1124.

Anwar, F., Muhammad, A., AbdullahIjaz, H., and Muhammad, S. (2019). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (foeniculum vulgare mill.) Seeds from pakistan. *Flavour and Fragrance Journal*, 24(4): 170-176. https://doi.org/10.1002/ffj.1929

Arif, H., Sohail, A., Farhan, M., Rehman, A.A., Ahmad, A. and Hadi, S.M. (2017). Flavonoids induced redox cycling of copper ions leads to generation of reactive oxygen species: A potential role in cancer chemoprevention. *International Journal of Biological Macromolecules*, 106: 569-578. https://doi.org/10.1016/j.ijbiomac.2017.08.049

Challaraj, E.S., Vinni, B. and Gayathri, N.K. (2020). A characteristic study on the effect of ginger and nutmeg extracts on pseudomonas and e. Coli biofilms. *International Journal of Research in Pharmaceutical Sciences*, 11(1): 386-396.

Cheng, Y., Liao, Y., Chen, D., Wang, Y. and Wu, Y. (2019). Prevalence of dental caries and its association with body mass index among school-age children in shenzhen, China. *BMC Oral Health*, 19: 270. https://doi.org/10.1186/s12903-019-0950-y

Chung, J. Y., Choo, J. H., Lee, M. H., and Hwang, J. K. (2006). Anticariogenic activity of macelignan isolated from myristica fragrans (nutmeg) against streptococcus mutans. *Phytomedicine*, 13(4): 261-266. doi:10.1016/j. phymed.2004.04.007

Clinical Laboratory Standards Institute (CLSI) (2006). Performance standards for antimicrobial disk susceptibility tests; Approved Standard 9th ed. *CLSI document* M2-A9. 26:1.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*,12(4): 564-582.

Cushnie, T.T. and Lamb A.J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38: 99-107. doi:10.1016/j.ijantimicag.2011.02.014

Devi, A., Singh, V. and Bhatt, A.B. (2012). Study of prevalence and sensitivity pattern of dental plaque against antibiotics and pomegranate. *International Journal of Pharmaceutical Sciences and Research*, 3(12):5062-5066.

Du, X., Li, Y., Xia, Y.L., Ai, S.M., Liang, J., Sang, P., Ji, X.L., and Liu, S.Q. (2016). Insights into protein-ligand interactions: mechanisms, models, and methods. *International Journal of Molecular Sciences*, 17(2): 1-34. doi:10.3390/ijms17020144.

Elamin, A., Garemo, M. and Mulder, A. (2021). Determinants of dental caries in children in the middle east and North Africa Region: A systematic review based on literature published from 2000 to 2019. *BMC Oral Health*, 21: 237. doi.org/10.1186/s12903-021- 01482-7

Fajriah, S., Megawati, S., Hudiyono, A, Kosela, S. and Hanaf M. (2016). Chemical constituents and potential cytotoxic activity of n-hexane fraction from myristica fatua houtt leaves. *International Symposium on Current Progress in Mathematics and Sciences*,1862: 0300871-0300876. doi: 10.1063/1.4991191

Farag, M. A., Sara, E.A., Rashad, H.H., Hesham, R.E., Haider, N.S., Annegret, L., Tarek, F.E., Fouad, O.F., and Ludger, A.W. (2017). Phytochemical profiles and antimicrobial activities of allium cepa red cv. And a. Sativum subjected to different drying methods: A comparative ms-based metabolomics. *Molecules,* 22: 761 779.doi:10.3390/molecules22050761

Fauzia, K.A., Miftahussurur, M., Syam, A.F., Waskito, L.A., Doohan, D. and Rezkitha, Y. (2020). Biofilm formation and antibiotic resistance phenotype of helicobacter pylori clinical isolates. *Toxin*, 12: 473. https://doi.org/10.3390/toxins12080473

Fredotovi´c, Ž., Puizina, J., Nazli´c, M., Maravi´c, A., Ljubenkov, I., Soldo, B., Vuko, E. and Baji´c, D. (2021). Phytochemical characterization and screening of antioxidant, antimicrobial and antiproliferative properties of allium cornutum clementi and two varieties of allium cepa l. Peel extracts. *Plants*, 10: 832. https://doi.org/10.3390/plants10050832

Griffith, A., Mateen, A., Markowitz, K., Singer, S.R., Cugini, C., Shimizu, E., Wiedman, G.R., and Kumar, V. (2022). Alternative antibiotics in dentistry: Antimicrobial peptides. *Pharmaceutics*, 14:1679. https://doi.org/10.3390/pharmaceutics14081679

Hemavathy, A., Shanthi, P., Sowndharya, C., Thiripura, S.S. and Priyadharshni, K. (2019). Extraction and isolation of bioactive compounds from a therapeutic medicinal plant- wrightia tinctoria (Roxb.). *International Journal of Pharmacognosy and Phytochemical Research* 11(3);199-204 DOI: 10.25258/phyto.11.3.15

Heo, S.M., Haase, E.M., Lesse, A.J., Steven, R.G. and Scannapieco, F.A. (2008). Genetic relationships between respiratory pathogens isolated from dental plaque and bronchoalveolar lavage fluid from patients in the intensive care unit undergoing mechanical ventilation. *Clinical Infectious Diseases*, 47: 1562-1570.

Ho, J.M., Bakkalbasi, E., Soll, D. and Miller, C.A. (2018). Drugging tRNA aminoacylation. RNA *Biology*, 15: 667 677. https:// doi. org/ 10. 1080/15476 286. 2018. 14298 79

Hong, W., Wang, Y. and Chang, Z. (2015). The identification of novel mycobacterium tuberculosis dhfr inhibitors and the investigation of their binding preferences by using molecular modelling. *Scientific Reports*,16: 15328.

Ifesan, B.O.T., Siripongvutikorn, S. and Voravuthikunchai, S.P. (2009). Application of eleutherine americana crude extract in homemade salad dressing. *Journal of Food Protection*, 72: 650-655.

Indu, M.N., Hatha, A.A.M., Abirosh, C. Harsha, U. and Vivekanandan, G. (2006). Antimicrobial activity of some of the south-indian spices against serotypes of escherichia coli, salmonella, listeria monocytogenes and aeromonas hydrophila. *Brazilian Journal of Microbiology*, 37(2): 1-9.

John Yun Niu, Iris Xiaoxue Yin, William Ka Kei Wu, Quan-Li Li, May Lei Mei, Chun Hung Chu (2021). Antimicrobial peptides for the prevention and treatment of dental caries: A concise review. *Archives of Oral Biology*, 122: 105022,https://doi.org/10.1016/j.archoralbio.2020.10502. ISSN 0003-9969,

Josef, Y., Beniam, G. and Kurt, R. (2015). Antibiotic resistance of pseudomonas aeruginosa at a single University Hospital Center in Germany Over a 10-year Period. *Public Library of Science Journal*, 10(10): 0139836.

Khushbu, Y. and Satyam, P. (2016). Dental caries: A review. Asian *Journal of Biomedical and Pharmaceutical Sciences,* 6(53): 1-7.

Kitamura, Y., Ebihara, A., Agari, Y., Shinkai, A., Hirotsu, K. and Kuramitsu, S. (2009). Structure of d-alanine-d-alanine ligase from thermus thermophilus hb8: cumulative conformational change and enzyme–ligand interactions. *Acta Crystallographica. Section D: Structurally*, 65: 1098-1106.

Kroemer, R.T. (2007). Structure-based drug design: docking and scoring. *Current Protein and Peptide Science*, 8: 312 328. http://dx.doi.org/10.2174/138920307781369382

Kumalo, H.M., Bhakat, S. andSoliman, M.E.S. (2015). Theory and applications of covalent docking in drug discovery: Merits and pitfalls. *Molecules*, 20: 1984-2000. http://dx.doi.org/10.3390/molecules20021984

Kumar, M., Barbhai, M.D., Hasan, M., Punia, S., Dhumal, S., Radha, R.N., Chandran, D., Pandiselvam, R., Kothakota, A., Tomar, M., Satankar, V., Senapathy, M., Anitha, T., De,y A, Sayed, A.A.S., Gadallah, F.M., Amarowicz, R. and Mekhemar, M. (2022). Onion (*Allium cepa L.*) peels: A review on bioactive compounds and biomedical activities. *Biomedicine and Pharmacotherapy*, 146: 112498. doi: 10.1016/j.biopha.2021.112498.

Babaeekhou, L. and Ghane, M. (2021). Antimicrobial activity of ginger on cariogenic bacteria: Molecular networking and molecular docking analyses. *Journal of Biomolecular Structure and Dynamics*, 9(6): 2164-2175. doi:10.1080/07391102.2020.1745283.

Machová, M., Tomáš, B., David, Š., Karel, V. and Petra, B. (2019). Release of volatile compounds from sliced onion analysed by gas chromatography coupled to mass spectrometry and its antimicrobial activity. *Journal of Food and Nutrition Research*, 58(4): 393-400.

Malmstrom, R.D. and Watowich, S.J. (2011). Using free energy of binding calculations to improve the accuracy of virtual screening predictions. *Journal of Chemical Information and Modeling*, 51(7): 1648-55. doi: 10.1021/ci200126v.

Marsh, P.D. (2003). Are dental diseases examples of ecological catastrophes? *Microbiology*, 149(2): 279-94.

Namwase, H., Najjuka, F. and Bbosa, G. (2021). Antibacterial activity of Corchorus olitorius L.and Acmella caulirhiza Del. on Streptococcus mutans, A Cariogenic Bacterium. *African Health Sciences*, 21(4): 1685-91. https://dx.doi.org/10.4314/ahs.v21i4.23.

Nelio, V., Daniela, A., Filipa, D., Margarida, P., Ana, V.,Liliana, R., Mariana, S., Vanessa, M., Fransisco, P., Beatriz, V., Joao, P. and Filipa, B. (2016). Dental caries: A review. *Journal of Dental and Oral Health*, 2(5): 1-3.

Nikolic, V., Ljubisa, N., Ana, D., Ivana, G., Maja, U., Ljijana, S., Jelena, S. and Bojana, D. (2021). Chemical composition, antioxidant and antimicrobial activity of nutmeg (*Myristica* fragrans houtt) seed essential oil. *Journal of Essential OilBearing Plants*, 24(2): 218-227 DOI: 10.1080/0972060X.2021.1907230

Nile, A., Nile, S.H., Kim, D.H., Keum, Y.S., Seok, P.G. and Sharma, K. (2018). Valorization of onion solid waste and their flavonols for assessment of cytotoxicity, enzyme inhibitory and antioxidant activities. *Food and Chemical Toxicology*, 119: 281-289.

Nishidonoa Yuto, Azis Saifudinb, Mikio Nishizawac, Takashi Fujitaa, Masatoshi Nakamotoa and Ken Tanakaa (2018). Identification of the chemical constituents in ginger (*Zingiber officinale*) responsible for thermogenesis. *Natural Product Communications,* 13(7): 1-5.

Oliver, A., Mulet, X., Lopez-Causape, C. and Juan, C. (2015). The increasing threat of pseudomonas aeruginosa high-risk clones. *Drug Resistance Updates*. 21(22): 41-59.

Panggabean, K.A., Rusmarilin, H. and Suryanto, D. (2019). The utilization of nutmeg seed (*Myristica* fragrans houtt) extract as an antimicrobial on tempeh sausage. IOP Conf. Series: *Earth and Environmental Science*, 260: 012087 doi: 10.1088/1755- 1315/260/1/012087

Poirel, L., Lambert T., Turkoglu, S., Ronco, E., Gaillard, J. and Nordmann, P. (2001). Characterizationof class 1 integrons from pseudomonas aeruginosa that contain the bla (vim-2) carbapenem-hydrolyzing beta-lactamase gene and of two novel aminoglycoside resistance gene cassettes. *Antimicrobial Agents and Chemotherapy*, 45: 546-552.

Popoola, O.D., Thomas, B.T., Agu, G.C. and Anyamene, C.O. (2017). Presence of efflux pump mediated antibiotic resistance in gram negative bacteria isolated from primary school pupils in ago-iwoye, ogun state, Nigeria. *African Journal of Science and Nature*, 5: 1-7.

Pranay, J. and Kumar, P. (2009). Antibiotic sensitivity pattern of streptococcus.mutans against commercially available drugs. *Journal of Pharmacy Research*, 2(7): 1250-1252.

Pratama, M.R.., Suratno, S. and Mulyani, E. (2018). Antibacterial activity of akar kuning (arcangelisia flava) secondary metabolites: molecular docking approach. *Asian Journal of Pharmaceutical and Clinical Research*, 11(11): 447-451. DOI 10.22159/ajpcr.2018.v11i11.29189

Raghavendran, K., Mylotte, J.M. and Scannapieco, F.A. (2007). Nursing home-associated pneumonia, hospital-acquired pneumonia and ventilator-associated pneumonia: The contribution of dental biofilms and periodontal inflammation. *Periodontology*, 44:164-177.

Rashid, N., Thapliyal C. and Chaudhuri P. (2016). Dihydrofolate reductase as a versatile drug target in healthcare. *Journal of Pharmacy and Pharmacology*, 7: 247-57.

Rathee M, and Sapra A. (2020). Dental Caries. In: StatPearls. Treasure Island (FL): StatPearls Publishing; Sayed, O.R, Farouk M. Gadallah O, Ryszard Amarowicz P, Mohamed Mekhemar Q, (2022).Onion (Allium cepa L.) Peels: A review on bioactive compounds and biomedical activities. *Biomedicine and Pharmacotherapy* 146: 12498-112514.

Schweitzer, B., Dicker, A.P. and Bertino, J.R. (1990). Dihydrofolate reductase as a therapeutic target. *Federation of American Societies for Experimental Biology Journal*, 4: 2441-52.

Sharma, K., Roscoe, P,, Vijender, Si,, Mohammed, A, (2016). Chemical composition and antimicrobial activity of fresh rhizome essential oil of zingiber officinale. *Pharmacognosy Journal*, 8(3) :1-6.

Singh, G., Kapoor I.P., Singh P., de Heluani C.S., de Lampasona M.P. and Catalan, C.A. (2008). Chemistry, Antioxidant and antimicrobial investigations on essential oil and oleoresins of zingiber officinale. *Food and Chemical Toxicology*, 46(10): 3295-302. doi: 10.1016/j.fct.2008.07.017.

Slayton, R. L., M. Fontana, D. Young, N. Tinanoff, B. Novy, R. D. Lipman, and L. Robinson. (2016). Dental caries management in children and adults. NAM Perspectives. Discussion Paper, *National Academy of Medicine*, Washington, DC. https://doi.org/10.31478/201609d

Trott, O. and Olson, A.J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry*, 31: 455-461. DOI 10.1002/jcc.21334

Warreth, A. (2023). Dental caries and its management. *International Journal of Dentistry*, 9365845. doi:10.1155/2023/9365845. PMID: 36636170; PMCID: PMC9831703.

Yadav, K. and Prakash, S. (2017). Dental caries: A microbiological approach. *Journal of Clinical and Infectious Disease Practice*, 22(1): 1-15.

Yousufi, K. (2012). To study antibacterial activity of allium sativum, zingiber officinale and allium cepa by kirby-bauer method. IOSR *Journal of Pharmacy and Biological Sciences,* 4(5):6-8.

Qiu, W., Zhou, Y., Li, Z., Huang, T., Xiao, Y., Cheng, L., Peng, X., Zhang, L.and Ren, B. (2020). Application of antibiotics/antimicrobial agents on dental caries. *BioMed Research International*, 5658212. doi: 10.1155/2020/5658212. PMID: 32076608; PMCID: PMC7013294.

Zhihao, Q., Liu, A., Li, P., Liu, C., Xiao, W., Huang, J., Liu, Z. and Zhang, S. (2020). Advances in physiological functions and mechanisms of (-) epicatechin. *Critical Reviews in Food Science and Nutrition,*1:23. doi:10.1080/10408398.2020.1723057.