Asian Journal of Chemistry; Vol. 29, No. 10 (2017), 2123-2128

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20622

Chemical Profiling and Antibacterial Efficacy of Different Ginger Accessions from Uttarakhand, India

JYOTSNA DHANIK, ANKITA VERMA, NEELAM ARYA, OM PRAKASH and VIVEKANAND*

Department of Chemistry, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar-263 145, India

*Corresponding author: E-mail: imvivekanand@gmail.com

Received: 3 March 2017; *Accepted*: 31 May 2017; *Published online*: 31 August 2017; AJC-18504

In recent years, a great significant attention has been drawn to natural compounds rather than synthetic compounds due to their less side effects or without side effects in therapeutic. Present work deals with chemical composition and *in vitro* antibacterial activity of methanolic extracts of five ginger accessions collected from different places of Uttarakhand, India. Molecular docking is performed between phytocompounds identified through GC-MS analysis and bacterial protein PDB ID: 1QFE and PDB ID: 2VF5. By comparing the scoring results of compositions in extracts of ginger rhizomes with bacterial protein, we can infer the antibacterial activity about selected phytocompounds. Molecular-docking analysis and antimicrobial test proved that methanolic extract of ginger accession from Dharchula had strong antibacterial effects.

Keywords: Ginger, Antibacterial, Molecular docking, Zingerone, Gingerol.

INTRODUCTION

Food borne illnesses by microorganisms causes threat to consumers, food industry and food safety authorities. Natural extracts from plants have been used for several years for different purposes and now a days uses as alternative remedies and food preservatives [1]. The antibacterial potential of plant extracts and oils can be useful for the preservation of raw and processed food, in the pharmaceutical industry and as alternative medicines and natural therapies [2]. Spices and herbs are well known for their antimicrobial and antioxidant properties and have the ability to produce multidimensional flavours in food [3].

Zingiberaceae family harbours many commercially important plants including *Zingiber officinale* (Ginger). Rhizome of the plant is used as spice, flavourant and herbal medicine for antiemetic, antipyretic and hypotensive effects, since ancient times [4]. Due to its universal appeal and adaptability, ginger is cultivated in most tropical and subtropical countries [5]. The crop is grown throughout India from temperate hilly regions to subtropical wet areas.

The structural diversity of plant-derived compounds is immense and, the impact of antimicrobial action they produce against microorganisms depends on their structural configuration. The discovery of novel drugs from natural sources is highly important because many isolated molecules are complex. Some of them are less potent, but can serve as pharmacophore, for chemical modification and for drug designing. In modern drug designing, molecular docking is routinely used for understanding drug-receptor interaction. Molecular docking provides useful information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. The recent expansion of antimicrobial drug research has occurred because there is a critical need for new antimicrobial agents to treat these life threatening invasive infections. The development of antimicrobial resistance has increased in this century and there is a need for developing new antimicrobial agents, which will be more selective, potent and less toxic compared to the existing drugs in clinical treatment. The main objective of this study was to determine the *in vitro* antibacterial activity of methanolic extracts of five ginger accessions collected from different places of Uttarakhand and molecular docking studies were performed, in order to provide insights into the mechanism of action of potential antibacterial drugs for resistant micro-organisms.

EXPERIMENTAL

Five accessions of ginger rhizomes were collected from different places of Uttarakhand State *viz.* of Kumaun region (29°36′N, 79°42′E). There are various methods employed for the preparation of extracts from the plant. Ginger rhizomes were washed, shade dried at room temperature for at least 15 days and pulverized into fine powder. The material was extracted

by successive soaking for a period of 72 h each in methanol and stored in sterilized amber coloured bottles in refrigerator for further study. The extracts were analyzed by gas chromatography-mass spectrometry.

Gas chromatography mass spectrometry (GC-MS): GC-MS analysis of extracts were performed using gas chromatograph HP 6890 with mass selective detector MS 5973 (Agilent technologies, USA) fitted with a DB-5 fused silica column (30 m × 0.25 mm; 0.25 μm film; thickness), with electronic pressure control and split-splitless injector. Helium flow rate through the column was 1 mL/min in a constant flow mode. The initial column temperature was 50 °C rising 250 °C at a rate 5 °C/min. The MS detector acquisition parameters were that the transfer line was held at 260 °C and the detector was held at 280 °C. The detection was performed in full scan mode from *m/z* 41 to 450. After integration, the fraction of each component in total ion current (TIC) was calculated with the aid of an automatic system of processing data of GC-MS supplied by NIST mass spectra library.

Identification of the components: The components were identified by comparing the spectrum of unknown component with the known component stored in NIST and Wiley libraries. The KI values of compounds were compared with those recorded in the literature [6]. The composition percentage and Kovats indices of the sample material was recorded and presented in Table-1.

Antibacterial screening

Sources of test organisms: The antibacterial screening of essential oil of ginger accessions was evaluated against five pathogenic bacterial strains *Escherichia coli, Salmonella typhi, Bacillus subtilis, Pseudomonas aeruginosa* and *Microbacterium* sp. The bacterial strains used for antibacterial study was isolated from different meat samples in Department of Veterinary and Public Health, Pantnagar, India. Antibacterial screening of the extracts against these bacteria was done by disc-diffusion method with slight modification and was measured by zone of inhibition [7].

Preparation of bacterial inoculums: For the preparation of bacterial inoculums, Luria Bertani broth (Hi-media) for *Escherichia coli*, buffered peptone water (Hi-media) for *Salmonella typhi*, nutrient broth for *Bacillus subtilis*, *Microbacterium* sp. and *Pseudomonas aeruginosa* were weighed and poured in distilled water as per manufactures instructions. The test tubes containing culture media was sterilized in an autoclave at 120 °C and 15-20 lbs for 0.5 h. Bacterial colonies were inoculated in test tubes in above prepared broths. The test tubes containing bacterial colonies were incubated for 24 h in incubator. Next day cultures showed a marked turbidity in the tubes and were used to conduct further experiment.

Preparation of agar plates: Difco Nutrient Agar (1.5 %) was used for the preparation of plate media. The media was prepared in distilled water, autoclaved and gently cooled. There after, the prepared media was poured in petriplates (dia. 9 cm) in laminar flow and kept undisturbed as such till it got solidified. After solidification, these Petri plates were incubated at 37 °C overnight for sterile testing.

Antibacterial screening of extracts by disc diffusion method: Antibacterial screening of methanolic extracts was performed by disc diffusion method, which is the most common method to evaluate the antimicrobial activity [8,9]. Bacterial inoculums 100 μL was added to the agar plates and uniformly spread over the surface using spreader. Sterilized disc of 5 mm diameter soaked in different methanolic concentrations of extracts (250, 500, 750, 1000 μg/mL) were placed on the inoculated plate. These plates were incubated at 37 °C overnight to observe the zone of inhibitions around the disc, which were compared with the zone of inhibitions formed by the standard antibiotic gentamicin (10 μg/disc). The sterile paper discs impregnated with methanol served as negative control. These inoculated plates were firmly closed with the maximum possible aseptic precautions. After incubation, relative susceptibility of each organism was determined by a clear zone of inhibition of growth around the disc impregnated with the extracts as well as the antibiotic. Zone of inhibition (mm) was measured with the help of scale.

Molecular docking: Molecular docking study of selected phyto compounds with protein was done by using molegro virtual docker (MVD) [10]. The structure of identified compounds was drawn by ChemDraw Ultra 8.0 and their corresponding MDL MolFile (.mol) was prepared using Chem3D Ultra v8.0 integral option (save as MDL MolFile (.mol)). The crystal structure of Type 1 3-dehydroquinate dehydratase from *Salmonella typhimurium* (PDB ID: 1QFE) and glucosamine 6-phosphate synthase in complex with glucosamine 6-phosphate from *E. coli* (PDB ID: 2VF5) was obtained from Protein Data Bank in PDB file. The protein structure of target enzyme in PDB file and ligand (synthesized compound) in Mol file were imported in MVD and bond orders, hybridization states and angles were assigned if missing. Electrostatic type surface of protein was created. Potential binding sites of target protein were obtained by detecting maximum of 5 cavities setting parameters as molecular surface (expanded van der Waals), maximum number of cavities (n = 5), minimum cavity volume (10), probe size (1.20), maximum number of ray checks (n = 16), minimum number of ray hits (n = 12) and grid resolution (0.80). All docking calculations were carried out in docking wizard using the grid-based MolDock score (GRID) function with a grid resolution of 0.30 Å. The binding site on the receptor was defined as extending in X, Y and Z directions around the dock molecule with a radius of approximately 30-35 Å. The MolDock optimization search algorithm with a maximum of ten runs was used through the calculations, with all other parameters kept as defaults. Multiple poses for each run were retained based on root mean square division clustering threshold set at 1.00 and ignoring similar poses with RDSM threshold 1.00. The best one pose with lowest MolDock score was selected manually. Using default parameters maximum 5 cavities were detected in the target protein for potential binding with selected best pose. Sphere center of protein with sphere radius 30-33 Å were selected for further docking studies. Best protein-ligand interaction was selected on the basis of MolDock score; Re Rank score and number of hydrogen bonds [11].

Statistical analysis: Results are presented as mean ± standard deviations. One way analysis of variance (ANOVA) was performed by Tukey's test ($p < 0.05$) was evaluated by using SPSS16 Statistical Package for Social Science.

TABLE-1
COMPARATIVE STUDY OF METHANOLIC EXTRACTS OF FIVE GINGER ACCESSIONS FROM UTTARAKHAND

Compounds	Dharchula	Champawat	Dwarahat	Didihat	Pithoragarh	KI
Isobutane	–	–	–	0.56	–	365
Allyl acetate	0.13	–	–	–	–	670
Cyclopropyl methyl ketone	–	–	0.10	–	–	730
Myrcene	1.03	–		–	–	991
3-Pentyl bromide	–	–	0.15	–	–	1009
α-Ocimene	–	0.11	–	-	–	1039
2-Methyl-4-ethyl-5-propyloxazole	–	–	–	0.17	–	1067
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	–	–	–	0.54	–	1134
β-Sesquiphellandrene	–	–	10.18	0.91	–	1149
Caprinaldehyde	–	–	–	0.65	–	1204
β-Geraniol	–	–	1.35	–	–	1255
Neryl acetate	–	–	2.92	–	–	1365
n-Undecanol	–	2.18	–	–	–	1371
β-Elemene	–	–	–	0.31	–	1389
β-Caryophyllene	–	–	8.72	-	–	1418
Disulpfide, dipropyl	–	–	–	0.13	–	1421
trans-α-Bergamotene	4.32	1.13	–	–	–	1436
β-*cis*-Farnesene	–	–	–	–	4.26	1443
Pelargonic acid, methyl ester	–	0.99	0.39	–	0.16	1476
α-Curcumene	–	–	1.85	–	1.33	1483
α-Zingiberene	–	–	–	2.57	–	1495
α-Farnesene	–	–	17.90	0.86	3.43	1508
β-Bisabolene	–	–	–	–	2.23	1509
γ-2-Cadinene	–	–	1.25	–	–	1513
β-Curcumene	–	–	37.48	–	14.02	1517
5-(T-butyl)-4-(2'methylpropyl)thiophen-2-one	0.63	–	–	–	–	–
1-Isopropyl-2-nonynlacetate	0.08					–
Zingerone	11.83	4.64	1.44	20.41	13.62	1653
1-Hexadecyne	–	-	-	2.33	-	1664
Myristic acid	–	-	-	-	1.10	1720
trans-Nuciferol	–	-	-	0.38	-	1758
α,β-Dihydroferulic acid, ethyl ester	–	-	-	-	2.34	1768
2,5-Dimethyl-5-nitrohexanal	–	-	-	0.34	-	-
Palmitic acid, methyl ester	–	-	-	1.54	-	1878
7-Hexadecenoic acid, methyl ester, (Z)	–	-	-	2.53	-	1886
Palmitic acid	10.85	12.15	-	3.76	-	1984
9,12-Hexadecadienoic acid, methyl ester	–	-	-	2.59	-	2093
Petroselinic acid	–	-	-	31.47	-	2175
Gingerol	34.06	20.06	4.18	13.37	19.43	2396
Dioctyl pthalate	–	–	2.38	-	4.05	2682
cis-8-Shogaol	5.91	–	1.13	1.21	4.39	–
Methyl-(3R)-(-)-5-oxo-3-propylpentanoate	0.16	–	–	–	–	–
7-Methyl-bicyclo[2,2,1]heptane	2.40	–	–	–	–	–
3,3,6-trimethyl-1,2-dioxane	0.36	–	–	–	–	–
(-)-Nortrachelogenin	13.51	9.61	–	5.56	5.21	3263
3-(2-Cyclohexen-1-yl)pentane-2,4-dione	0.45	0.98	–	–	–	–
6,8-Dodecadien-1-ol	–	4.62	–	–	–	–
Carinol	–	22.21	–	–	1.72	3296
2,4-Cyclohexadien-1-ol,2,6,6-trimethyl-1-(3-methyl-4-pentenyl)	–	4.94	–	–	–	–
(4E,6S)-6-[(*tert*-Butyldiphenylsilyl)oxy]-4-hepten-1-ol	–	–	0.04	–	–	–
3-Bromo-4-chloro-4,5-dihydro-3-hydroxymethyl-3H-furan-2-one	–	–	0.11	–	0.07	–
Tetradecanoic acid, 12-methyl-, methyl ester	–	–	–	0.23	–	–
Methyl (3A. α, 7A, β)-5-oxo-hexahydroindane-3A-oxoacetate	–	–	–	0.09	–	–
(Z)-3-(Fluoromethyl)-7-methylocta-2,6-dien-yl-acetate	–	–	–	–	0.08	–
(1R*,5S*,8R*)-8-Hydroxytricyclo[6.3.1.0(1,5)]dodecan-10-one	–	–	–	–	0.07	–
5,9-Undecadien-2-one, 10-methyl-6-(trifluoromethyl), (Z)	–	–	–	–	0.09	–
Ricinoleic acid	–	–	–	–	0.15	–
5,8,9,10-Tetrahydro-10-hydroxymethyl-2-methoxy-3,6-dimethylbenzocyclooctene	–	–	–	–	0.14	–
o-Methyl dehydrocassine	–	–	–	–	0.64	–
1,2,2-(2H(3)-4-Methoxyphenylethene	–	–	–	–	1.13	–
2H-Pyran-3,4-diol, tetrahydro-5-[[3-(2-hydroxy-1-methylpropyl)-oxiranyl]methyl]-2-[2-methyl-3-(3-methyl-5-isoxazolyl)-2-propenyl]-,[2S-[2.α(E), 3β,4β, 5α[2R*,3R*(1R*,2R*)]]]	–	–	–	–	1.39	–
Oxirane,2,3-dichloro-2-ethyl-3-methyl-, *trans*	–	–	–	–	0.30	–
Total	85.72 %	83.62 %	91.57 %	92.51 %	81.35 %	

RESULTS AND DISCUSSION

The chemical composition of methanolic extracts of five accessions of ginger rhizomes are shown in Table-1 accounting 85.72, 83.62, 91.57, 92.51 and 81.35 % from Dharchula, Champawat, Dwarahat, Didihat and Pithoragarh, respectively. From the above results it was concluded that the extracts of ginger rhizomes is a complex mixture of several compounds, dominated by zingerone, gingerol, β-sesphellandrene, α-zingiberene, α-farnesene, *cis*-8-shogaol, carinol, *trans*-α-bergamotene, β-caryophyllene, β-curcumene, β-bisabolene, β-farnesene, α-curcumene, palmitic acid and (-) nortrachelogenin and many of which are present in traces.

The *in vitro* antimicrobial activity of extracts of different ginger rhizomes, against five species of microorganisms, estimated by the zone of inhibition varied according to samples and bacteria strains (Table-2). Results revealed that the inhibition of bacterial growth was dose dependent and extract of ginger rhizomes collected from Dharchula showed strongest antibacterial activity in comparison to other. *E. coli, Salmonella typhi* and *Microbacterium sp.* are the most sensitive bacteria as they are inhibited by most of the extracts while *Bacillus subtilis* and *Pseudomonas aeruginosa* were the most resistant bacteria since the extracts showed weak or no inhibition towards it. It is well known that the antibacterial activity of extracts depends on their chemical composition. Major or trace compounds in the extracts might give rise to the antimicrobial activity exhibited. Results found are in accordance with Azu and Onyeagba, which confirmed that ginger extracts show potential inhibition against *E. coli* and *S. typhi* [12]. However, Kaushik and Goyal [13] reported significant inhibition of ginger rhizomes against *E. coli*.

The bacterial toxicity of methanolic extracts of ginger might be possibly due to the presence of mono and sesquiterpenoids or might also be due to the synergistic and antagonistic action of several compounds identified in GC-MS analysis.

Major compounds like zingerone, 6-gingerol, 8-shogaol, 6-shogaol, nortrachelogenin, isocaryophyllene, β-elemene, carinol, *trans*-nerolidol, α-curcumene, α-zingiberene, β-curcumene might be responsible for the antibacterial activity of this plant, as the antibacterial activity of these compounds have already been reported [14-21].

Bajpai *et al*. [22] also support our explanation that as a result of the presence of mono and sesquiterpenoids within plant extract, considered to be the main cause for their antibacterial mode of action. Since these compounds have different ways of effect since these compounds not only attack cell walls and cell membranes *i.e.*, affecting their permeability and release of intracellular constituents (*e.g.* ribose, sodium glutamate) but they also interfere with membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity).

Comparative docking of bacterial protein PDB ID: 1QFE and PDB ID: 2VF5 with selected phytocompounds from extracts of ginger rhizomes were done to support the *in vitro* antibacterial activity. Tables 3 and 4 show the binding energy, number of hydrogen bonds, hydrogen bond length and moldock score of all the selected phytocompounds with bacterial protein. Docking studies revealed all the phytocompounds showed good binding energy toward the target protein (PDB ID: 1QFE) ranging from -3.3 to 26.1 kJ mol^{-1} and moldock energy between -91.37 to 9057.28 kJ mol^{-1} and with target protein (PDB ID: 1VF5) binding energy ranging between-9.8 to-19.0 kJ mol^{-1} and mol dock energy between -94.00 to 9321.35 kJ mol^{-1}.

TABLE-2
EFFECT OF METHANOLIC EXTRACTS OF FIVE GINGER ACCESSIONS ON HUMAN PATHOGENIC BACTERIA BY DISC DIFFUSION METHOD

Ginger accessions	Conc. (ppm)	Zone of inhibition (mm)				
		E. coli	*Salmonella typhi*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Microbacterium sp.*
Dharchula	250	13 ± 0^{abc}	12.67 ± 0.577^{abcde}	11 ± 0^{bcd}	11.67 ± 0.577^{bcde}	10 ± 0^{a}
	500	13.67 ± 0.577^{abcde}	15.33 ± 0.577^{efghi}	11.67 ± 0.577^{cde}	12.33 ± 1.155^{cdef}	11.33 ± 0.577^{abcd}
	750	14.67 ± 0.577^{bcdef}	16 ± 1^{fghi}	12.67 ± 0.577^{ef}	13.33 ± 0.577^{efg}	12.67 ± 0.577^{de}
	1000	20 ± 0^{h}	18 ± 0^{i}	13.33 ± 0.577^{f}	15 ± 0^{g}	16 ± 0^{h}
Champawat	250	12.67 ± 0.577^{ab}	10.33 ± 0.577^{a}	0 ± 0^{a}	10.67 ± 0.577^{bc}	10.33 ± 0.577^{ab}
	500	13.67 ± 1.528^{abcde}	11.33 ± 0.577^{abcd}	0 ± 0^{a}	11.33 ± 1.155^{bcd}	10.33 ± 0.577^{ab}
	750	16 ± 0^{defg}	14 ± 1^{defg}	0 ± 0^{a}	12 ± 0^{cde}	11.67 ± 0.577^{bcde}
	1000	16.33 ± 0.577^{efg}	15.33 ± 0.577^{efghi}	0 ± 0^{a}	13 ± 0^{def}	12 ± 0^{cde}
Dwarahat	250	11.33 ± 1.155^{a}	10.67 ± 0.577^{ab}	0 ± 0^{a}	0 ± 0^{a}	10.33 ± 0.577^{ab}
	500	13.33 ± 1.155^{abcd}	11 ± 1^{abc}	0 ± 0^{a}	0 ± 0^{a}	10.67 ± 0.577^{abc}
	750	13.67 ± 2.309^{abcde}	14 ± 1^{defg}	0 ± 0^{b}	11.67 ± 0.577^{bcde}	10.67 ± 0.577^{abc}
	1000	16 ± 0^{defg}	15.33 ± 0.577^{efghi}	0 ± 0^{a}	13 ± 0^{def}	11 ± 0^{abc}
Didihat	250	14.33 ± 0.577^{bcde}	11.33 ± 0.577^{abcd}	10.67 ± 0.577^{bc}	10.67 ± 0.577^{bc}	10 ± 0^{a}
	500	15.33 ± 1.155^{bcdefg}	13.33 ± 0.577^{bcdef}	11 ± 0^{bcd}	11.67 ± 0.577^{bcde}	10.67 ± 0.577^{abc}
	750	17.33 ± 0.577^{fgh}	14.33 ± 1.155^{efgh}	12 ± 0^{de}	12 ± 0^{cde}	11.33 ± 0.577^{abcd}
	1000	17.67 ± 0.577^{gh}	17 ± 0^{hi}	12.67 ± 0.577^{ef}	13 ± 0^{def}	13 ± 0^{ef}
Pithoragarh	250	15.67 ± 0.577^{cdefg}	13.67 ± 1.528^{cdefg}	10 ± 0^{b}	10 ± 0^{b}	10.67 ± 0.577^{abc}
	500	16 ± 1^{defg}	14.33 ± 2.082^{efgh}	10.67 ± 0.577^{bc}	11.33 ± 1.155^{bcd}	11 ± 1^{abc}
	750	17.33 ± 0.577^{fgh}	16.33 ± 0.577^{ghi}	10.67 ± 0.577^{bc}	12.67 ± 0.577^{def}	14.33 ± 0.577^{fg}
	1000	17.67 ± 0.577^{gh}	17 ± 0^{hi}	12 ± 0^{de}	14 ± 0^{fg}	15 ± 0^{gh}

Values are means of three replicates ± Standard deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$).

TABLE-3
DOCKING RESULTS OF SELECTED PHYTOCOMPOUNDS FROM METHANOLIC EXTRACTS OF GINGER RHIZOME WITH CRYSTAL STRUCTURE OF TYPE 1 3-DEHYDROQUINATE DEHYDRATASE FROM *Salmonella typhimurium* (PDB: 1QFE)

Compound	Mol doc score (Kcal/mol)	Rerank score	Hydrogen bonds	Energy (kcal/mol)	Hydrogen bond length	Amino acid interacted
Zingerone	-94.004	-74.818	2	-1.87 -1.52	2.52 3.29	Met 15, Gly 14, Glu 13, Pro 16, Gly 12, Gly 220, Arg 198, Val 200, Pro 199, Met 1, Glu 192, Ala 222
Carinol	9321.356	1251.109	4	3.86, -2.5, 12.8, 12.03	1.85, 2.63, 0.82, 0.91	Arg 48, Ser 232, Ala 233, Pro 234, Ala 231, Glu 230
cis-8-Shogaol	-94.348	6.200	2	-2.5 -1.86	2.79 2.94	Glu 6, Arg 82, Arg 48, Gly 88, Gly 87, Phe 145, Ala 233, Ser 232, Glu 236, Arg213, Met 205
(-)-Nortrachelogenin	-121.935	-85.412	7	-1.4, -0.29, -1.01, -1.82, -2.41, -2.40, -1.5	3.34, 3.49, 3.39, 3.23, 3.11, 2.58, 3.28	Glu 13, Gly 14, Met1 5, Glu 217, Pro 16, Lys 17, Gly 12, Met1, Glu 192, Ala222

TABLE-4
DOCKING RESULTS OF SELECTED PHYTOCOMPOUNDS FROM METHANOLIC EXTRACTS OF GINGER RHIZOME WITH CRYSTAL STRUCTURE OF TYPE 1 3-DEHYDROQUINATE DEHYDRATASE FROM *E. coli* (PDB: 2VF5)

Compound	Mol doc score (Kcal/mol)	Rerank score	Hydrogen bonds	Energy (kcal/mol)	Hydrogen bond length	Amino acid interacted
Zingerone	-91.372	-78.858	7	-2.5, -1.69, -2.38, -1.49, -2.5, -0.41, -1.86	2.78, 2.50, 3.12, 2.47, 2.76, 3.06, 2.89	Leu 346, Ala 299, Ser 303, Cys 300, Ser 347, Thr 302, Thr 352, Ser 349, Gln 348, Ser 401, Ala 400, Gln 488
Carinol	9057.288	147.904	9	-0.59, -2.5, -1.94, -0.05, -2.5, -1.50, -2.5, -0.11, -2.5	3.18, 2.98, 3.21, 3.53, 3.05, 3.29, 3.09, 3.54, 3.06	Gln 408, Ser 303, Thr 302, Ala 404, Gln 348, Ser 401, Lys 603, Val 605, Glu 488, Leu 601, Ala 602, Val 399
cis-8-Shogaol	-103.288	-79.386	5	-2.5, -2.09, -2.5, -0.25, -2.5	2.66, 3.18, 3.06, 3.46, 2.74	Gln 348, Ser 303, Lys 603, Cys 300, Thr 352, Val 399, Thr 302, Val 399, Leu 484
(-)-Nortrachelogenin	-131.949	-90.349	9	-2.5, -1.81, -2.5, -1.69, -2.26, -0.95, -2.31, -2.5, -2.5	2.80, 3.23, 2.97, 2.90, 3.14, 3.18, 2.57, 3.01, 3.07	Gln 488, Ala 602, Val 399, Ala 400, Ser 604, Gly 301, Cys 300, Glu 488, Val 399, Ser 303, Thr 352, Ser 347, Ser 349, Gln 348

Results clearly reveals that (-) nortrachelogenin shows maximum (7) hydrogen bonds with protein (PDB ID: 1QFE) while for protein (PDB ID: 2VF5) carinol and (-) nortrachelogenin shows maximum (9) hydrogen bonds. Mol dock score and rerank score of (-) nortrachelogenin for protein (PDB ID: 1QFE) is-121.93 kJ mol^{-1} and -85.41 and for protein (PDB ID: 2VF5) it is -131.94 kJ mol^{-1} and -90.349, respectively. Mol dock score and rerank score of carinol for protein (PDB ID: 2VF5) is 9057.20 kJ mol^{-1} and 147.90. It means, if it had lower energy so it was more stable in binding. The primary aim of selecting these bacterial protein was to distinguish the major pathway through which *Zingiber officinale* exhibits its antibacterial potential. From our docking results we found that it was (-) nortrachelogenin on which active constituents from *Zingiber officinale* were found to be most active. The presence of –OH group in the phenolic compounds plays an important role in the antimicrobial activity, promotes the delocalization of electrons which then act as proton exchangers and reduce the gradient across the cytoplasmic membrane of bacterial cells. This will cause the collapse of the proton motive force and depletion of the ATP pool and ultimately leading to cell death [23]. Antimicrobial activity of plant antimicrobials could also vary depending on the type of microorganisms, extraction method, culture medium, size of inoculum and method of determination [24].

(-) Nortrachelogenin is a lignan belonging to group of polyphenolic compounds. Lee *et al*. [18] evaluated the antimicrobial activity of (-)-nortrachelogenin on various pathogenic bacterial strains. Studies on *E. coli* O157 confirmed that the antibacterial activity is exerted by causing damage to the bacterial membrane. Properties associated with high antibacterial activity demonstrated by this study show the high application potential of this compound and instigate new and further study of its application. Motivate studies to explore the consumer product formulation containing viability (-) nortrachelogenin and carinol or its derivatives.

Conclusion

Plants are rich source of drugs, used in primary health care for treating human ailments. The advantages of using plants as drugs are that they are safe, low-cost and more reliable than the synthetic products. Hence plants can be used as effective pharmacological agents. Since, there is a growing demand for food that is free of synthetic chemicals as preservatives, it is necessary to examine and identify alternative and safe approaches for controlling food borne pathogens. Even though many

natural products are currently being used for the preservation and extension of the shelf-life of foods, there are still many unexplored sources. The use of natural compounds from plants could open up the possibility of using them as novel antimicrobials. However, despite their potential, the use of natural antimicrobials in food systems remains limited mainly due to the side effects of undesirable flavour or aroma. Therefore, further research is needed to determine the optimum levels of antibacterials that can be safely applied in food systems without unduly altering any sensory characteristics.

ACKNOWLEDGEMENTS

One of the authors (Jyotsna Dhanik) is thankful to Department of Science and Technology, New Delhi, India for providing DST-INSPIRE Scholarship for research work.

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