Asian Journal of Chemistry; Vol. 34, No. 7 (2022), 1688-1692

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23653

Preservative Action of Ginger and Garlic Ratio Mixture on Fresh Leaves of *Moringa oleifera* with its Computational Evidences

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Received: 3 December 2021; *Accepted*: 7 February 2022; *Published online*: 15 June 2022; AJC-20841

In food industries worldwide, food processing methods are tremendously needful to meet the present consumer's demands. There are continuously increasing efforts to develop food preservatives by avoiding chemicals for which natural sources are the only alternative. *Morninga oleifera* (Moringaceae) is highly nutrious and medicinaly valued plant which will be detoriate with the microbial and environmental factors. The preservation of food materials is really challenged without using the chemical preservatives. The use of spice property of ginger and garlic has a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. They both have the radical scavenging activity otherwise considered ideal antioxidants beneficial in many reported diseases including the antifungal, antibacterial and antiviral and antiparasitic agents. Therefore, the present study has been undertaken to investigate the popularly used ginger and garlic in the management of antimicrobial activity of leaves of *Morninga oleifera* by *in vitro* as well as *in silico* approaches.

Keywords: Food processing, Preservatives, Radical scavenging activity, Antioxidants, *In silico* approach.

INTRODUCTION

Preservative additives are subdivided into antimicrobials, anti-darkening and antioxidants. Food processing methods are tremendously needful to meet the present consumer's demands in the food industries worldwide [1]. There are continuously increasing efforts to develop food preservatives by avoiding chemicals for which the natural phyto compounds are the only alternative. Consequently, consumers look for products that are labeled which guarantee the absence of chemical additives. Such cases have led to the want of natural additives and hence the search begins, those natural additives are claimed as antioxidant preservatives by the food industries [2]. All the raw materials of food have some biochemical process and are susceptible to attack from a microorganism that affects their sensory properties and resulting toxicity formation. When foods are exposed to air then fats, oils, flavouring substances, vitamins and colours, it spontaneously oxidize. The presence of oxygen, heat, light and moisture and transition metals are major factors responsible for food deteriorations for which compounds having antioxidants property inhibit the oxidative process and reduce a change in taste, colour and nutritional value of food [3]. Ascorbic acid, carotenoids, phenolic compounds and tocopherols are some natural antioxidants used in food [4]. Ginger and garlic used as a spice have a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. They both have the radical scavenging activity otherwise considered ideal antioxidants beneficial in many reported diseases including the antifungal, antibacterial, antiviral and antiparasitic agents [5-11].

Therefore, the present study has been undertaken to investigate the popularly used ginger and garlic in the management of selected antimicrobial activity for the preservation of *Morninga oleifera*. Further, molecular docking results in between the selected constituents of ginger and garlic of alliin, paradol, ezingerone, gingerol, shogaols and E-ajoene, respectively with the selected receptor of *Aspergillus niger* has been carried out.

EXPERIMENTAL

All plant materials ginger, garlic and *Moringa oleifera* were collected in February 2021 at the rural district of Jajpur, India. DPPH, Folin-Ciocalteu reagent and gallic acid were obtained from Sigma-Aldrich, USA. Ferrous chloride and

sodium carbonate were obtained from Sisco Research Laboratories Pvt. Ltd., India. The other chemicals and solvents used were of analytical grade available commercially. The fresh forms of garlic, ginger and moringa leaves were made into pieces, air-dried and made into powdered forms.

Extraction of plant material: The powdered plant samples (20 g) were percolated at room temperature with 99.9% ethanol for garlic, ginger and *Moringa oleifera* leaves separately in 400 mL beakers. The beakers were covered with foil paper, shaken and left to stand for 2 weeks with regular shaking. After 2 weeks the suspensions were filtered and the filtrates were concentrated using a rotary-evaporating machine at 40 ºC. The extracts were marked accordingly and stored in the refrigerator for further analysis [12].

Preparation of stock solution: Stock solutions of these garlic and ginger extracts were prepared by dissolving 2 mg extract of each plant in 10 mL of DMSO. Then made a combination of ginger and garlic extract in different ratios *viz*. 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1.

Determination of minimum inhibitory concentration (MIC): Bacterial species of *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter asburiae* and *Bacillus subtillis* species were obtained from the Biotechnology Centre, Siksha 'O' Anusandhan University. Both dilution methods were used to determine the minimum inhibitory concentration (mic). Cultured the bacteria in plate inoculated with each stock solution from range 2, 4, 8, 16, 32 to 1024 µL. Standard antibiotic ciprofloxacin was used in the assay for comparison. All the tests carried out in triplicates of the plate were incubated aerobically at 37 ºC for 48 h [13]. Zero growth of dilution was taken as MIC value.

Preparation of preservative combination of ginger and garlic extract: Moringa leaves washed with distilled water thoroughly. Then 200 g of fresh leaves were vacuumed packed in a plastic bag (20 cm × 30 cm) and fresh leaves coadministrated with ginger and garlic powder in a specific ratio (4:6) and vacuumed packed in a plastic silo bag. Kept at room temperature (25-30 ºC for unsealed to sample on the day of 2, 5, 10, 15, 20) [14].

Antioxidant activity: Antioxidant activity was determined by the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) method with some modifications [15]. Ethanol extracts of *Moringa oleifera* leaves were adjusted to various concentrations (10, 20, 30, 40, 50 µg/mL) to a test tube with 1 mL of extract an aliquot of 1 mL of 0.1 mL DPPH radical in methanol was added. As a control, pure ethanol was used and left to stand in dark for 60 min at room temperature. The absorbance was measured at 517 mm. The radical scavenging activity (RSA) was calculated using the following equation:

$$\text{RSA}\ (\%) = 1 - \frac{A}{B} \times 100$$

where A is the absorbance of sample and B is the absorbance of control. To scavenge 50% of radical (IC_{50}), the concentration in mg/mL was obtained from the regression equation.

Determination of total phenolic content: The amount of total phenolic was determined using Folin-Ciocalteu reagent with little modifications [16]. Moringa extract (0.5 mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent and left at room temperature for 5 min, 2 mL Na_2CO_3 was added to the mixture and again kept at room temperature for 1 h and finally the measured the absorbance at 760 nm at UV-visible spectrophotometer (V-630, Jasco, UK). The phenolic content was determined from a standard curve of gallic acid ($Y = 0.0158x + 0.0133$, $R^2 = 0.9943$) and expressed as milligram Gallic acid equivalents (GAE)/100 g dry weight.

Receptor selection and identification: The receptor selection with the 3D protein structure of amino acid sequence and other water molecules and hetero atoms retrieved from PDB and optimized by using Swiss-PDB viewer (SPDBV software) [17-19]. *Aspergillus niger* phytase receptor protein data bank (PDB) was retrieved in FASTA from NCBI with their characteristics of Ident = 98.42%, query cover 100%. Chain A. PDB id3k4p structure was retrieved from the web address http://www.rcsb.org. SPDBV software was used to remove the water molecules as well as heteroatoms also present with retrieved protein, to avoid drug docking interference and saved in PDB format. The fasta sequence of the receptor was as follows:

TCDTVDQGYQCFSETSHLWGQYAPFFSLANESVISPE
VPAGCRVTFAQVLSRHGARYPTDSKGKKYSALIEEIQQ
NATTFDGKYAFLKTYNYSLGADDLTPFGEQELVNSGIK
FYQRYESLTRNIVPFIRSSGSSRVIASGKKFIEGFQS
TKLKDPRAQPGQSSPKIDVVISEASSSNNTLDPGTC
TVFEDSELADTVEANFTATFVPSIRQRLENDLSGVSLT
DTEVTYLMDMCSFDTISTNTVDTKLSPFCDLFTHEEW
INYDYLQSLKKYYGHGAGNPLGPTQGVGYANELIARL
THSPVHDDTSSNHTLDSNPTTFPLNSTLYADFSHD
NGIISILFALGLYNGTKPLSTTTVENITQTDGFSSAWTV
PFASRLY

3D protein structure and amino acid sequence and other data retrieved from PDB and binding studies were done using the CASPT server. Computed Atlas of Surface Topography of proteins (CASTp) is a web server that provides online services for locating, delineating and measuring surface pockets, interior cavities and cross channels geometric and topological properties of protein structures to make the protein functional or active. It is found that 158 pockets were present in *Aspergillus niger* phytase. As per the pocket, information is concerned the above picture shows Red mark pocket consisting of area (SA) = 3208.75, volume (SA) = 6827.679

Ligand design and optimization: From the PubChem database, all the 3D structures of the selected constituents of ginger and garlic in structural data format (SDF) were retrieved. Further, the conversion of SDF format into PDB was done through OPEN BABEL graphical user interface (GUI) software for all selected phyto ligands [18].

Receptor ligand docking: Docking is a process to get assume regarding the orientation of a molecule using drug docking parameters through AutoDockTool-1.5.6, in which the grid box was set to X = 26.128, Y = 30.4 and Z = 23.785 with spacing = 0.497 Å covering all the reported active residues like GLU 78, ASN 82 and THR 228. These tools also help to

TABLE-1
MINIMUM INHIBITORY CONCENTRATION (MIC) OF GARLIC AND GINGER EXTRACT

Bacteria	Ginger (µg/mL)	Garlic (µg/mL)	Ginger and garlic in the different ratios (µg/mL)								
			01:09	02:08	03:07	04:06	05:05	06:04	07:03	08:02	09:01
E. coli	51.6 ± 2.95	52.3 ± 2.90	51 ± 2.51	50.6 ± 2.61	51.4 ± 2.74	25.2 ± 2.52	52.4 ± 2.84	51.2 ± 1.99	51.8 ± 3.08	52.5 ± 3.11	50.7 ± 2.95
Klebsiella sp	207.4 ± 2.85	102.6 ± 2.88	103.5 ± 2.63	103.3 ± 3.24	51.4 ± 2.97	51.5 ± 3.52	102.8 ± 2.55	102.9 ± 3.27	103.4 ± 3.27	204.6 ± 3.55	204.5 ± 2.86
S. aureus	102.6 ± 2.88	51.2 ± 3.75	51.5 ± 2.83	51.8 ± 3.93	50.6 ± 3.41	25.4 ± 2.71	51.1 ± 3.66	102.2 ± 3.03	102.8 ± 3.23	102.5 ± 4.01	102.7 ± 2.91
Enterobacter. sp.	102.3 ± 2.85	102.7 ± 3.49	103.1 ± 3.50	102.2 ± 3.09	102.1 ± 3.72	103.2 ± 3.48	101.8 ± 3.42	102.4 ± 3.55	102.1 ± 4.33	101.8 ± 3.39	102.6 ± 3.40
Bacillus sp.	102.6 ± 2.88	51.7 ± 3.32	51.6 ± 3.47	51.5 ± 3.78	51.2 ± 3.72	52 ± 3.82	102.5 ± 3.43	102 ± 3.53	102.5 ± 3.58	101.5 ± 3.32	101.5 ± 3.66

Values are expressed mean ± SEM

predict the binding stability between the two molecules using the scoring function [19-21]. In each docking process, the program was set to under 10 times known as RUN to obtain the binding energy for each pose of interaction and generate a root mean square deviation (RMSD) table. Each of the protein-ligand was observed through Discovery Studio Visualizer 3.5 and the interaction was saved as an image file.

RESULTS AND DISCUSSION

The MIC values of ginger extracts were 51.2, 204.8, 102.4, 102.4, 102.4 µg/mL against *E. coli*, *K. pneumonia*, *S. aureus*, *E. asburiae*, *B. subtillis*, respectively (Table-1). The MIC were as low as 51.2 µg/L of extracts against *E. coli* bacteria is suggestive of the best antibacterial potential of the bioactive principles of ginger extracts. In the case of MIC values of garlic extracts were 51.2 µg/mL against *E. coli*, *S. aureus* and *B. subtillis* (Table-1). In the case of mixing of ginger and garlic in a different ratio, the MIC value obtained that ratio (4:6) of ginger and garlic had low MIC value than other ratios against all microbes due to having some synergistic effect of ginger and garlic. It is found that *Moringa oleifera* leaves preserved for 20 days and containing phenolic compound **31-35** (mg GAE/100 g) and DPPH value didn't decrease as like fresh leaves where it degrades in short time period or end of the day (Table-2).

The current experimental finding so far has helped to understand ligand-receptor interaction. The retrieved protein *Aspergillus niger* phytase (PDB 3k4p) was solved by X-ray diffraction technique at 1.43 Å resolution, with R-value free,

TABLE-2
DETERMINATION OF ANTIOXIDANT AND PHENOLIC CONTENT OF *Moringa oleifera* LEAVES EXTRACT

Day	DPPH (µg/mL)	Total phenolic content (mg GAE/100 g)
Day 0	51.7 ± 3.52	34.7 ± 3.40
Day 2	50.7 ± 3.52	34.3 ± 3.90
Day 5	50.5 ± 4.07	34.6 ± 4.17
Day 10	49.3 ± 4.01	33.6 ± 4.21
Day 15	47.8 ± 4.01	32.0 ± 4.38
Day 20	47.4 ± 3.35	33.1 ± 4.68

Values are expressed Mean ± SEM

work and observed was 0.0.275, 0.0.215 and 0.218, respectively as depicted in the experimental findings of the protein data bank. The selection of phyto ligands was done on the basis of active constituents of ginger and garlic which was reported in the literature. These ligands mostly exerted their property of antifungal activity due to their inherent antioxidant nature. The present finding of the molecular docking studies showed out of six numbers of Phyto ligands, alliins present in garlic produced the highest binding energy -5.75 kcal/mol with binding interaction residue of GLU 78, ILE 79, ASN 82, VAL 227, THR 228, LEU 229, GLU 233 which is depicted in Fig. 1. Further, H-bond interaction of the same was also found with GLU 78, ASN 82, THR 228, GLU 233. This energy value agrees excellently with the previously experimented pathway represents best to moderate interactions covering conventional hydrogen, carbon-hydrogen, pi-anion, pi-lone pair and pi-alkyl bond (Table-3) [21]. The best blocking of amino acid active sites resulting good inhibition antifungal activity.

TABLE-3
MINIMUM BINDING ENERGY OF SELECTED CONSTITUENTS OF GARLIC AND GINGER AGAINST *Aspergillus niger*

Compounds	Pubchem CID	AUTO DOCK		Interacting amino acid residue	H-bond interaction
		Minimum binding energy (Kcal/mol)	Run		
Zingerone	31211	-4.13	9	LEU 229, GLU 78, THR 228, VAL 227	THR 228, GLU 78
Paradol	94378	-4.38	10	GLU 223, GLU 78, VAL 227, LEU 229, ASN 82	GLU 233, GLU 78, ASN 82
Gingerol	3016110	-2.76	7	Not found	Not found
Shogaols	5281794	-4.06	9	ASN 82, THR 228, LEU 229, VAL 227, ILE 79, GLU 78, GLU 233	ASN 82, GLU 78, GLU 233
Alliin	87310	-5.75	9	GLU 78, ILE 79, ASN 82, VAL 227, THR 228, LEU 229, GLU 233	GLU 78, ASN 82, THR 228, GLU 233
E-Ajoene	538659	-3.98	5	LEU 229, LEU 75, VAL 227, ASN 82	ASN 82, VAL 227

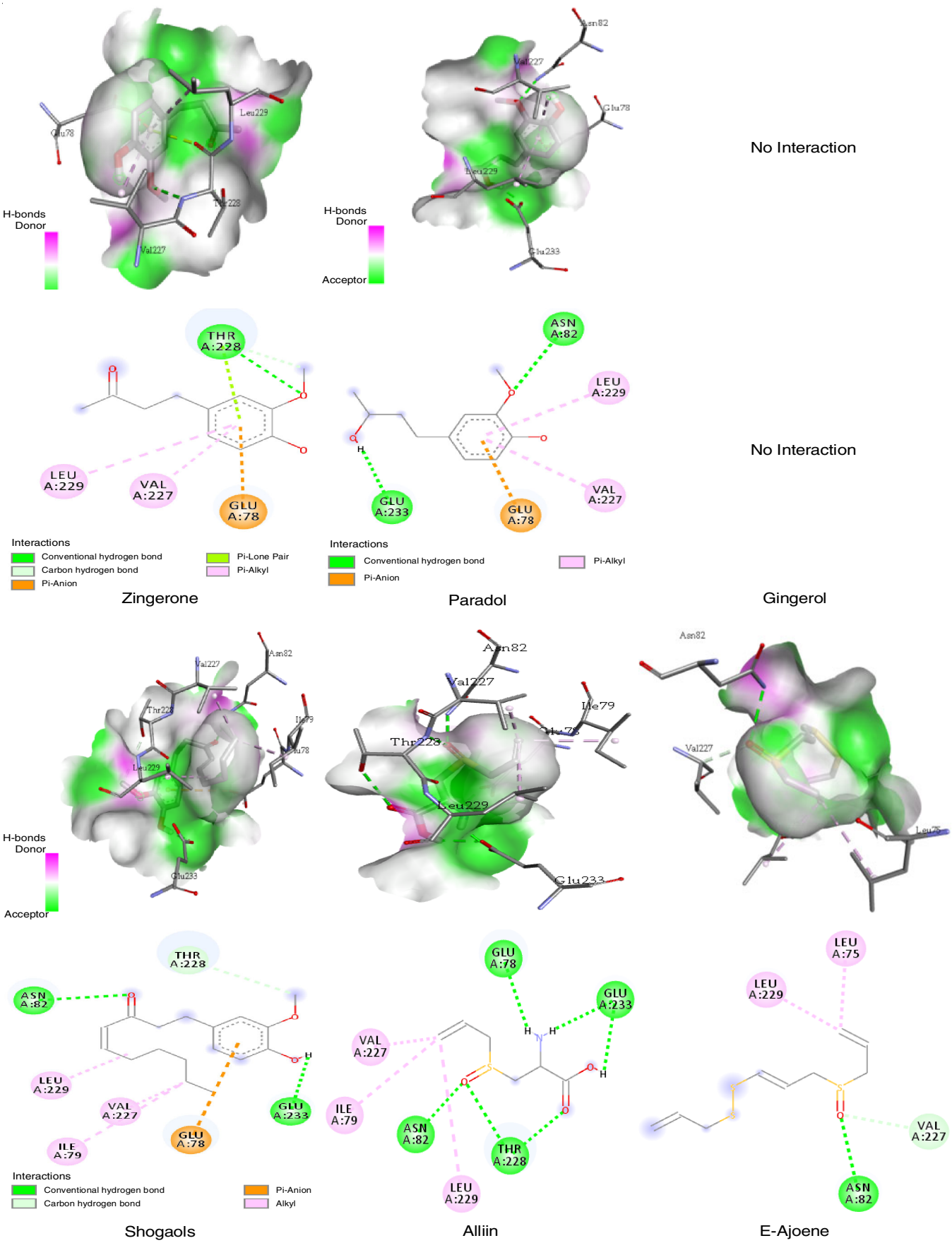

Fig. 1. Visualization of 3D models of docked complexes depicted by Discovery Studio Visualizer 3.5, depicting interactions of *Aspergillus niger* phytase receptor with selected constituents of ginger and garlic

Conclusion

A developed food preservatives by avoiding chemicals for which the mixture ration of ratio (4:6) of ginger and garlic producing highest presevativative effect against selected species of the microorganism. Selected constituents of ginger and garlic showed binding affinity of the receptor could be the reason for the preservation.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. B.B. Subudhi, Drug Development Lab of SPS and staffs of CBT, SOA University for their co-operation and help.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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