Anti-bacterial and anti-depressant properties of docosahexaenoic acid metabolites produced by gut bacteria

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Abstract

The potential of Docosahexaenoic acid (DHA) and its derivatives produced by the gut bacteria is not well studied. The present work focuses on the role of DHA metabolites on human physiology. Fermentation of DHA was performed on MS basal medium by bacteria such as Bacillus sp., Lactobacillus sp., Clostridium sp., Escherichia coli, Staphylococcus sp. and Enterococcus sp. The metabolites like 2-Nonadecanone, Z,Z-6,27-Hexatriactontadien-2-one, cis-9-Hexadecenal, Eicosanal, Stigmast-5-En-3-ol and Oleate were found to be predominant and showed strongest antimicrobial activity against Bacillus subtilis and Staphylococcus epidermidis.

In silico docking analysis revealed that Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester (Gscore: -10.232), Hexatriacontane (Gscore: -8.884), (Z)-3-(Heptadec-10-en-1-yl) phenol (Gscore: -8.047), Dotriacontane (Gscore: -7.582), Decanedioic acid, bis(2-ethylhexyl) ester (Gscore: -7.074), 10(E),12(Z)-Conjugated linoleic acid (Gscore: -6.986), Benzene,1,3-Bis(1,1-Dimethylethyl)- (Gscore: -6.68), 2-Pentacosanone (Gscore: -6.653), Furan, tetrahydro-2,5-dimethyl- (Gscore: -6.124), 8-Octadecanone (Gscore: -6.007), 9,17-Octadecadienal, (Z)- (Gscore: -5.859), 2 Nonadecanone (Gscore: -5.751) and 2-Heptodecanone (Gscore: -5.743) have an ability to bind with Monoamine oxidases (PDB ID: 2Z5X). Thus, these compounds are appropriate drug candidates for use as anti-depressant molecules.

Keywords: Docosahexaenoic acid, metabolites, anti-depressant, anti-bacterial, gut bacteria.

Introduction

Dietary supplements containing omega-3 polyunsaturated fatty acids (PUFAs) are attributed with an array of health benefits. The levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in human blood and tissue are strongly dependent on the intake of foods and supplements enriched with these fatty acids. Proper supply of Arachidonic acid (AA) and DHA is required for infant brain growth and functional development and breast feeding provides AA and DHA to infants[7,21]. DHA is involved in cell signaling; a deficiency of some omega-3 fatty acids can lead to a lack of learning capacity.

DHA is the major structural fatty acid found in the gray matter of the brain and retinal tissues of human and other mammals. Human can only synthesize very less quantities of DHA and the rest of their needs are met only from food sources[17]. DHA in brain tissue is high in young children which help in improving their mental ability later in life[15].

DHA supplements are also known to improve the gut microbiota by increasing an abundance of short-chain fatty acid producers[13]. The microbicidal activity of DHA and their derivatives has been reported in some earlier studies. Different enveloped viruses, parasites and pathogenic bacteria such as *Pseudomonas aeruginosa, Bacillus subtilis, Listeria monocytogenes, Helicobacter pylori, Staphylococcus aureus* and *Neisseria gonorrhea* were found susceptible to the metabolites of DHA[4,24].

Antibacterial activity of DHA derivatives has been proven in some previous studies which indicated that it disturbed the bacterial cell membrane functions[4,6,20,24].

In addition to their antimicrobial and antiviral properties, these molecules also possess anti-inflammatory and anti-tumor properties. However, the relationship between dietary intakes of DHA with the gut microbiota is still unknown. To know more about the metabolomics between the gut microbiota and dietary DHA, the bio-converted DHA metabolites using gut microbiome were investigated in this work and were further evaluated for their antibacterial properties.

The antibacterial properties of long chain fatty acids are well known. Polyunsaturated fatty acids (PUFA) are known to have an inhibitory effect because they are easily incorporated in the outer cell membranes of bacteria. It is possible that by opening the permeability channels, the required concentration gradients between an organism and its environment can be dissipated, resulting in the death of the organism[11]. There are fewer studies on the DHA metabolism by the gut bacteria and the role of metabolites in preventing pathogen growth. Consequently, the present study focused on the microbial degradation of DHA and the evaluating medicinal properties of microbial metabolites.

Material and Methods

Growth Media: The SCS (single source of carbon) media were prepared to cultivate the normal intestinal flora of human. The medium composed of the following ingredients (in grams per liter): 5 g $(NH_4)_2SO_4$, 3 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 15 mg EDTA, 4.5 mg $ZnSO_4.7H_2O$, 4.5 mg $CaCl_2.2H_2O$, 3 mg $FeSO_4.7H_2O$, 1 mg $MnCl_2.4H2O$, 1 mg H_3BO_3, 0.4 mg $Na_2MoO_4.2H_2O$, 0.3 mg $CuSO_4.5H_2O$, 0.3 mg $CoCl_2.6H_20$ and 0.1 mg KI. The pH of the medium was adjusted to 5.5 using 0.1N HCl before sterilization using 0.22 µm filter. Solid medium was prepared by adding 15 g agar per liter of liquid SCS media followed by autoclaving at 121 °C[28].

Fecal Sample Collection: The stool samples were obtained from four healthy volunteers ranging from 20 to 75 years old living in the same neighborhood. The samples were transported to the laboratory for the further process within 15 hours of collection.

Isolation and Characterization of Gut Microbial Flora from Human Fecal Sample: Approximately 125 mg of fecal material was dissolved in 5 mL of SCS media. The diluted sample was then transferred (2.5 µL) to a cool SCS medium (5 mL) and incubated at 37 °C for 24 to 48 h. Pure cultures were obtained by plating cultures on an SCS agar medium plate and incubated at 37 ℃ for 24-48 h. Single colonies were picked and re-treated on SCS plates and other isolates were identified by morphological and biochemical characteristics. The most prominent isolates were characterized by standard bacteriological methods and by amplification of the 16S rRNA gene. MEGA 5.0 software was used for sequential alignment and phylogenetic tree generation.

Bioconversion of DHA: Bioconversion of DHA was carried on 50 mL SM broth supplemented with 200 mg of DHA. Five different probiotics like *Bacillus* sp., *Lactobacillus* sp., *Clostridium* sp., *Escherichia coli*, *Staphylococcus* sp. and *Enterococcus* sp., were inoculated at levels of 500 µL followed by 24 h incubation at 37 ℃ and bioconversion was allowed to continue.

Extraction of Fatty Acids from Bio-converted Broth: Bio-converted broth were suspended in 3 mL of 4 mol L^{-1} sodium hydroxide and incubated at 90 °C for 90 min. After cooling, the pH of the sample was adjusted to 2.0 with 0.1N hydrochloric acid. Fatty acids were then extracted by adding 2 mL anhydrous diethyl ether and separated by centrifugation at 5500×g for 10 min. The upper phase was removed and dehydrated by adding anhydrous sodium sulfate. The dehydrated fatty acids were collected and dried under a stream of nitrogen.

Next, 50 µL bistrimethyl silyltrifluoroacetamide (BSTFA) was added and the mixture was incubated at 70 ℃ for 30 min and dried under a stream of nitrogen. The fatty acids were dissolved in 100 µL hexane for GC-MS analysis[27].

GC-MS Analysis: Fatty acid composition analysis was performed on the Shimadzu GCMS QP 2020 that employed a fused silica column packed with SH-Rxi-%Sil MS (30 m × 0.25 mm ID × 250 µm df) and the components were separated using helium as carrier gas at a constant flow of 1 mL/min. The injector temperature was set at 280 ℃ during the chromatographic run. 1 µL of extract sample was injected into the instrument and the oven temperature was set as follows: 40°C (2 min) followed by 280°C at the rate of 10°C min^{-1} and 280°C, where it was held for 3 min. The mass detector conditions were: transfer line temperature 280 °C, ion source temperature 230 °C and ionization mode electron impact at 70 eV, a scan time 0.2 s and scan interval of 0.1 s. The fragments were from 40 to 550 Da. The spectra of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2017) library.

Standard antibiotics: Ampicillin was used as positive control and distilled water, Dimethyl sulfoxide (DMSO) solvent was used as negative control for anti-bacterial susceptibility test.

Bacterial strains: Standard clinical pathogens of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherischia coli*, *Klebsiella pneumonia* and *Staphylococcus epidermidis* were obtained from SKS Clinical Laboratory, Salem, Tamil Nadu, India. These bacterial strains were purified and their identity was confirmed (Data not shown).

Antimicrobial activity of the DHA metabolites: The antimicrobial activity of the compound was determined using agar-well diffusion method. The ampicillin discs (Nam KhoaBioTek Company, Vietnam) (10 µg/mL) were used as positive controls. Negative controls were distilled water and DMSO soaked in paper discs. 900 µg of the bio-converted DHA extract was weighed and dissolved in 10 mL DMSO to obtain a concentration of 90 µg/mL of the extract; this was the initial concentration of extract used to determine antimicrobial activity of the extract. Mueller Hinton agar was prepared, sterilized at 121 ºC for 15 min and the sterilized medium was seeded with 0.1 mL of the standard inoculum spread evenly over the surface of medium with a sterile swab.

Wells were bored into the solidified media and inoculated using a standard broth borer of 6 mm in diameter. 0.1 mL of the extract solution of 90 µg/mL concentration was then introduced into each well on the medium. The seeded medium was then incubated at 37 ºC for 24 h. The plates were observed for the growth inhibition zone and the results were recorded in millimetres (mm).

Minimal Inhibitory Concentration Assay: The minimum inhibitory concentration (MIC) of the crude extract were measured using the procedure described above[26]. Briefly, stock solutions were prepared by adding 3.4 mL of DMSO to 4 mg of crude extract in a vial. Stock solutions were then

diluted in series (6 times) in 100 µL of nutrient broth in 96 well plates to attain the desired concentrations (588, 294, 147, 74, 37 and 18.5 µg/mL). Thereafter, 100 mL (in duplicate) of each of these solutions was seeded with 100 mL of an overnight bacterial culture brought to 0.5 Mc Farland in nutrient broth. Ampicillin served as positive control while the negative control was made up of 50 % nutrient broth in DMSO. Viable bacteria were confirmed in the presence of resazurin dye after 4 h of incubation and MICs were recorded for every concentration of extract.

Molecular Docking

Ligand Preparation: Based on the GC-MS analysis, 82 compounds were uploaded in two-dimensional (2D) SDF (Spatial Data File) format using the PubChem database (https:/pubchem.ncbi.nlm.nih.gov/, accessed in March 2021). Ligand was prepared using LigPrep tool of Maestro v 11.1. The pH 7.0 ± 2.0 was used to generate ionizing states of compounds with Epik 2.2 (force field: OPLS4) in Schrödinger ver.11.1. A maximum of 32 stereo-isomers per ligand was chosen.

Protein Preparation: The three-dimensional (3D) structures of the Human Monoamine Oxidases-A (PDB ID: 2Z5X) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/, accessed on 12 March 2021) in PDB format. The Protein Preparation Wizard (Schrödinger ver.11.1) was used to prepare the 2Z5X receptor using the following processes: optimization, removal of water molecules and minimization (Force field: OPLS4).

Receptor Grid Generation and Glide Molecular Docking: Grid generation (Schrödinger Maestro ver.11.1) for the selected receptor was achieved using the default settings (Force field: OPLS4). Receptor grids were calculated for proteins prepared for observation of the placement of various bound ligands in the predicated active site during the docking procedure. The Van Der Waals radius scale factor and partial atomic load were 1.00 and 0.25 respectively. A cubic box of specific dimensions centered on the centroid of the active site residues was obtained for the receptor. The bounding box was adjusted to 14 × 14 × 14 Å for docking experiments. Ligand docking was followed by flexible standard accuracy (Schrödinger ver.11.1) and docking score and ligand docking interactions were registered.

Results and Discussion

About 65 bacterial colonies were isolated from the human fecal samples. Based on colony morphology, 16 bacteria were selected for further biochemical characterization [Table 1] as per the description of Bergey's Manual of Systematic Bacteriology. Among these 16 bacterial isolates, *Lactobacillus* sp., *Clostridium* sp., *Escherichia coli*, *Bacillus* sp., *Enterococcus* sp. are the gut bacteria which were found to be commonly present in both normal human intestine and the diseased human intestine[2]. These five gut bacteria were further used for bioconversion of DHA and the predominant bacteria was analyzed for molecular characterization based based on 16s rRNA and NCBI blast analysis. The sequence result showed 100% similarity with *Bacillus cereus* (Accession No. MK571682.1) [Fig. 2].

Lactobacillus sp., *Clostridium* sp., *Escherichia coli*, *Bacillus* sp. *and Enterococcus* sp., have multiple roles in the human body which stabilize the gastric acid, hepatic, bile and digestive enzymes of gastrointestinal tract and it can modulate gut microbiota and microbiota associated metabolic pathways[16]. These gut bacteria are predominantly present in both the healthy and diseased individuals[2]. Therefore, these microbes were used for *in vivo* study to evaluate the metabolomics between the gut bacteria and DHA. After 24 h, bio-converted DHA broth was subjected to fatty acid extraction. The crude extract was further analyzed using GC-MS and tested for antibacterial activity. The normal gut flora isolated from human fecal matter converted DHA into various therapeutic metabolites as analyzed by GC-MS and was found to possess various medicinal properties [Fig. 1].

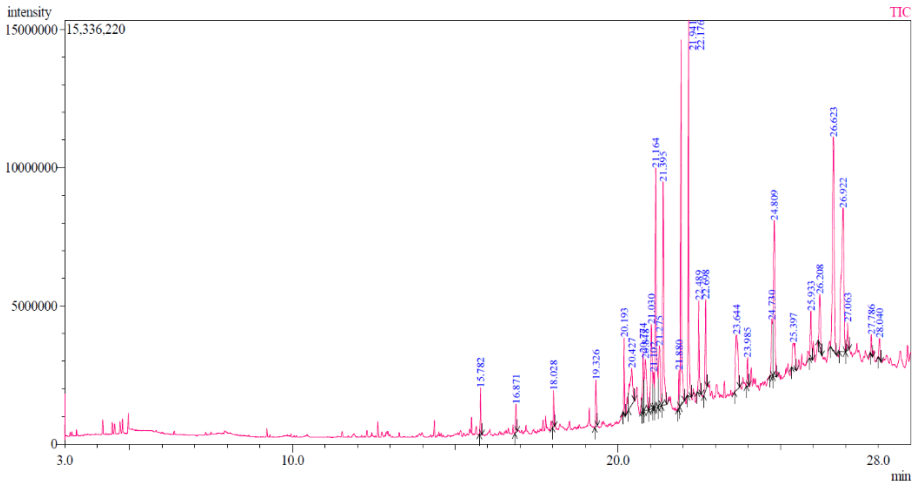


Fig. 1: GC-MS analysis of bioconverted DHA metabolites

Table 1
Morphological and biochemical characteristics of gut bacteria from fecal sample

Bacterial Isolate	Bacterial Name	Gram Staining	Shape	Motility	Spore	Catalase	Oxidase	MR	VP	Indole	Nitrate Reduction	Fermentation of Fructose	Fermentation of Glucose	Fermentation of Lactose	Fermentation of Maltose	Fermentation of Mannitol	Fermentation of Ribose	Fermentation of Sucrose
H1	*Enterococcus sp.*	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
H2	*Lactobacillus sp.*	+	Rod	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
H3	*Clostridium sp.*	+	Bacilli	+	+	-	-	-	-	-	-	+	+	-	-	+	-	-
H4	*Eschericia coli*	-	Bacilli	+	-	+	-	+	-	+	+	-	+	+	+	-	-	+
H5	*Citrobacter sp.*	-	Bacilli	+	-	+	-	+	-	-	+	-	+	+	-	+	-	+
H6	*Enterobacter sp.*	-	Rod	+		+	-	-	+	-	+	-	+	+	-	+	-	+
H7	*Proteus sp.*	-	Rod	+		+	-	+	-	+	+	-	+	-	+	-	-	+
H8	*Streptococci sp.*	+	cocci	-		-		+	+	-	-	-	+	-	+	+	-	-
H9	*Bacillus sp.*	+	Bacilli	+	+	+	-	+	+	-	-	+	+	-	+	+	-	+
H10	*Staphylococcus sp.*	+	cocci			+	-	+	-	-		-	+	+	+	+	-	+
H11	*Bifidobacterium sp.*	+	Rod	-	-	-	-			-	-	+	+	+	+	-	-	+
H12	*Clostridiumsp.,*	+	Coccobacilli	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+
H13	*Leuconostoc sp.*	+	Cocci	-		-	-					-	+	+	-	-	-	+
H14	*Enterococcus sp.*	+	Cocci	-	-	-	-					-	-	-	-	+	-	+
H15	*Fusobacterium sp.*	-	Rod	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-
H16	*Bacteroides sp.*	-	Rod	-	-	+	-			-		-	+	+	+	-	-	+

These gut bacteria converted DHA into 82 metabolites [Table 4] **a**mong which some metabolites were reported to possess antibacterial properties. 2-Nonadecanone is a ketone which showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* extracted from *Chromolaena odorata* Linn.[26]. Cis-9-Hexadecenal is C_{16} mono-unsaturated fatty-aldehyde group which was reported as an anti-melanogenic compound and possesses antimicrobial properties[9,25]. Stigmast-5-En-3-Ol, Oleat is reported to be a bacteriostatic compound[18] and oleic acid was used in the wound dressing as an antibacterial compound[8]. Octadecanoic acid also named as stearic acid, an unsaturated fatty acid reported antibacterial activity against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*[19]. n-hexadecanoic acid was reported to have anti-bacterial effect against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Escherichia coli, Escherichia coli O157:H7, Salmonella typhimurium, Proteus mirabilis* and *Klebsiella pneumoniae*[23]. Phenol, 2,4-Bis(1,1-Dimethylethyl)- was reported to be anti-pathogenic against uropathogen *S. marcescens*[1].

Commonly, *Lactobacillus* sp., *Clostridium* sp., *Escherichia coli, Staphylococcus* sp. and *Enterococcus* sp. are present in healthy ones[12] but tend to decrease in the intestinal microbiome if the individual is said to be in a diseased state[10]. The most common activity among all metabolites obtained from bioconverted DHA extract showed their anti-bacterial activity. In an earlier study, DHA was bioconverted using *P. aeruginosa PR3* and the crude extract showed effective antibacterial activity against four gram-positive bacteria *Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus* (ATCC 6538) and *S. aureus* (KCTC 1916) and seven gram-negative bacteria, *Enterobacter aerogenes, Escherichia coli, E. coli* O157:H7, *Pseudomonas aeruginosa, Salmonella enteritidis and S. typhimurium*[11].

In this study, analysis of the bioconverted Docosahexaenoic acid produced by *Lactobacillus* spp., *Clostridium* spp., *Escherichia coli, Bacillus cereus, Enterococcus* spp. crude extract showed an effective antibacterial activity against clinical pathogens *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Staphylococcus epidermidis and Staphylococcus aureus* when compared with the standard antibiotic Ampicillin. From the GC-MS analysis, compounds from bioconverted DHA having various medicinal properties were identified and most of the compounds were observed to possess antimicrobial activity. To ensure further, the antibacterial activity for the bioconverted DHA extract was evaluated [Table 2, Fig. 3]. Among 5 tested microorganisms, the extract presented the strongest antimicrobial effect against *Lactobacillus* spp., *Escherichia coli and Bacillus cereus.*

Bioconverted DHA extract was against *Bacillus subtilis* with the diameter of inhibition zone of about 25 mm, 26 mm and 24 mm. *Clostridium* spp., *Lactobacillus* spp., *Escherichia coli, Enterobacter* spp. and *Bacillus cereus* bioconverted DHA extract showed activity against *Staphylococcus aureus* (20 mm, 19 mm, 18 mm, 20 mm), *Escherichia coli* (15 mm, 16 mm, 14 mm, 15 mm, 17 mm), *Klebsiella pneumonia* (19 mm, 18 mm, 20 mm, 19 mm, 20 mm) and *Staphylococcus epidermidis* (16 mm, 15 mm, 14 mm, 15 mm, 13 mm). There was no activity found against the DMSO and distilled water which were used as controls.

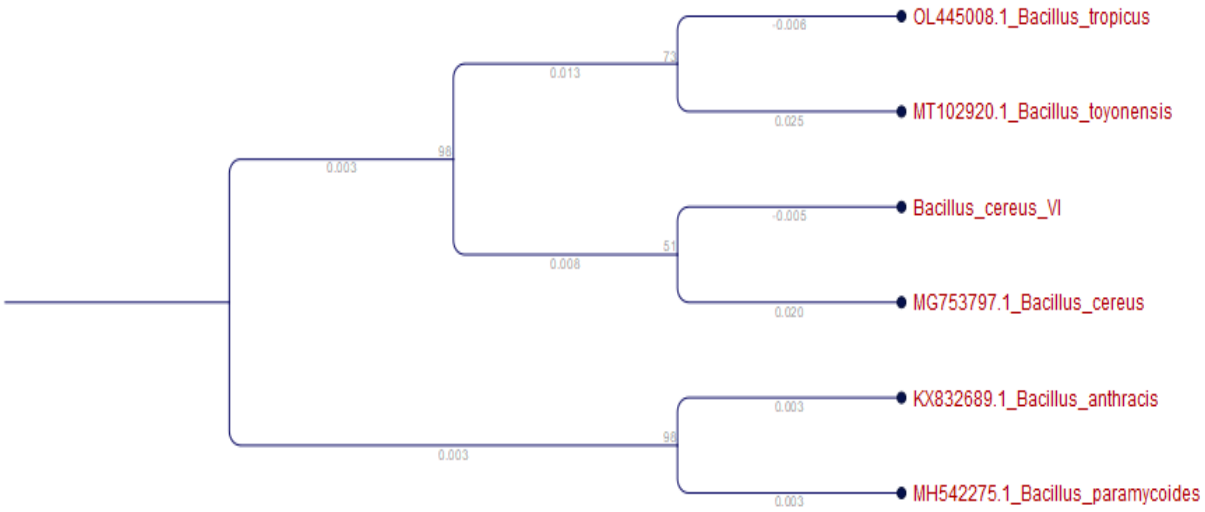


Fig. 2: Phylogenetic tree for 16s rRNA sequence of *Bacillus cereus*

Table 2
Antibacterial activity of bioconverted DHA extract

S. N.	Clinical Pathogens	Zone of Inhibition (mm)							
		Bio converted DHA extract of *Lactobacillus* sp. (90 µg/mL)	Bio converted DHA extract of *Clostridium* sp. (90 µg/mL)	Bio converted DHA extract of *Bacillus cereus* (90 µg/mL)	Bio converted DHA extract of *Escherichia coli* (90 µg/mL)	Bio converted DHA extract of *Enterobacter* sp. (90 µg/mL)	Ampicillin (10 µg/mL) (Positive Control)	DMSO	Sterile Distilled water (Negative Control)
1.	*Bacillus subtilis*	25	24	26	25	24	28	0	0
2.	*Escherichia coli*	15	16	14	15	17	18	0	0
3.	*Klebsiella pneumonia*	19	18	20	19	20	21	0	0
4.	*Staphylococcus epidermidis*	16	15	14	15	13	17	0	0
5.	*Staphylococcus aureus*	20	19	18	20	19	21	0	0

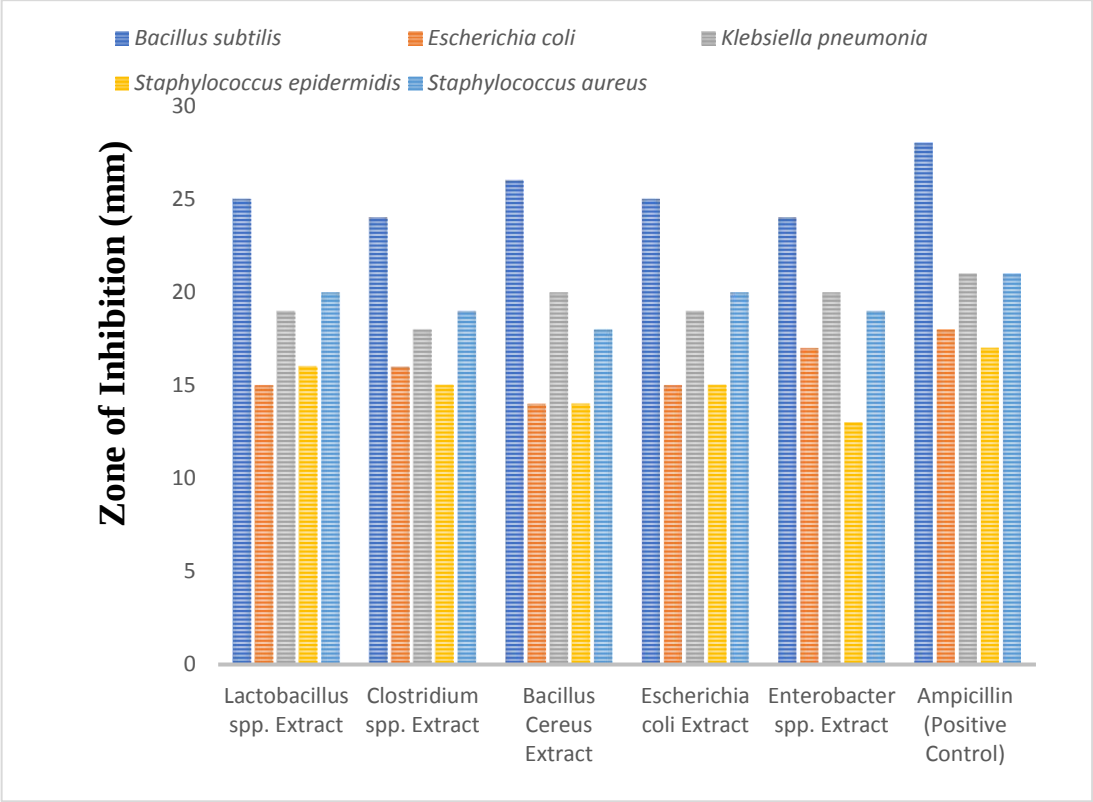


Fig. 3: Antibacterial activity of bioconverted crude extracts

Table 3
Minimum inhibitory concentration of different crude extract compared with standard drugs

S.N.	Clinical Pathogens	Minimum inhibitory concentration (MIC, µg/mL)					
		Bio converted DHA extract of *Lactobacillus* spp.	Bio converted DHA extract of *Clostridium* spp.	Bio converted DHA extract of *Bacillus cereus*	Bio converted DHA extract of *Escherichia coli*	Bio converted DHA extract of *Enterobacter* spp.	Ampicillin (Positive Control)
1.	*Bacillus subtilis*	18.5	18.5	18.5	18.5	18.5	26
2	*Escherichia coli*	294	147	294	294	294	26
3.	*Klebsiella pneumonia*	147	74	294	147	182	26
4.	*Staphylococcus epidermidis*	294	294	74	294	74	26
5.	*Staphylococcus aureus*	74	74	147	147	124	26

The antibacterial activity of the crude extract was tested against five different clinical bacterial strains using microdilution method. The MICs of the different crude extract of bioconverted docosahexaenoic acid against five different clinical bacterial strains were depicted in table 3. Tested compounds exhibited broad spectrum of antibacterial activity and their efficacy was concentration dependent. For instance, amongst the five different strains, *Bacillus subtilis* was the most susceptible strain to all the crude extract with an MIC 18.5 µg/mL. Therefore, these compounds appear to be 1.4 time more potent than ampicillin (MIC 26 mg/mL). Furthermore, the crude extract of *Lactobacillus* spp. showed MICs of 74 µg/mL against *Staphylococcus aureus*, 74 µg/mL MIC of *Clostridium* spp., crude extract against *Klebsiella pneumonia*, 74 µg/mL MIC of *Bacillus cereus* crude extract against *Staphylococcus epidermidis* and 74 µg/mL MIC of *Enterobacter* spp. crude extract against *Staphylococcus epidermidis*.

Moreover, it was discovered that some compounds were more active than crude extracts for other bacterial strains. There was a significant concentration of metabolites in crude

extract such as 2-Nonadecanone (13.27 %), cis-9-Hexadecenal (11.48 %), Stigmast-5-En-3-Ol (7.54 %), Oleic acid (13.93 %), Octadecanoic acid (32.53 %), n-hexadecanoic acid (34.68 %), phenol, 2,4-Bis(1,1-Dimethylethyl) (31.59 %) which have been previously reported as antibacterial compounds and may be responsible for the antibacterial effect of crude extract of DHA metabolites.

These results could be related to the synergistic and/or antagonistic effects of various metabolites in the crude extract. Due to numerous compounds in the crude extract with varying potency against individual bacterial strains, an antagonistic action of these compounds will diminish the crude extract's toxicity towards those strains.

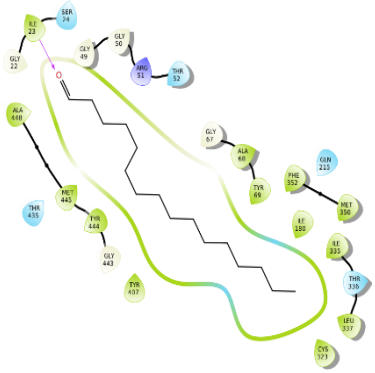
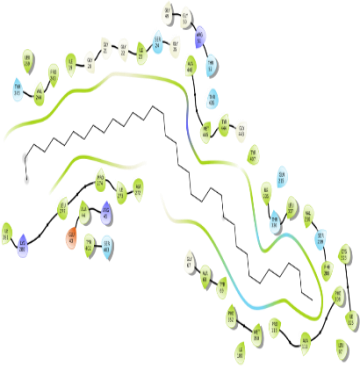
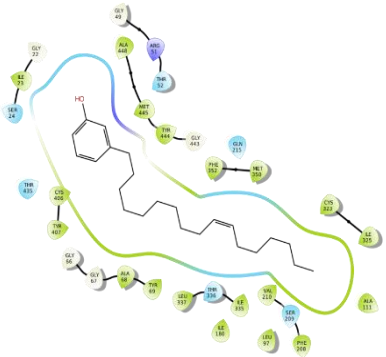
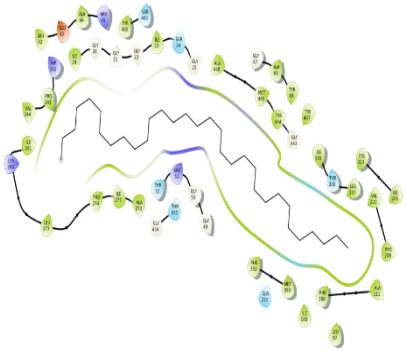
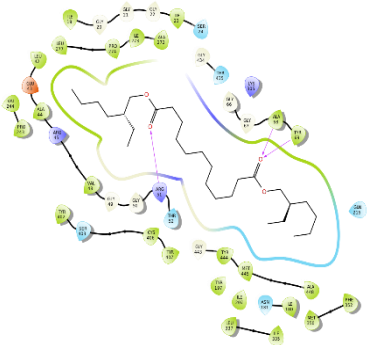
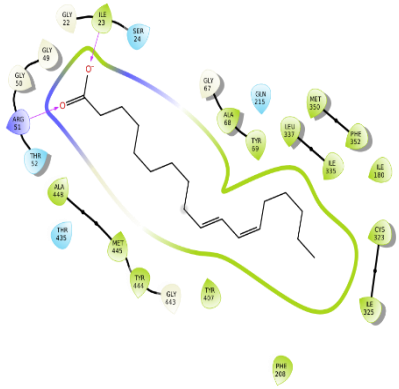
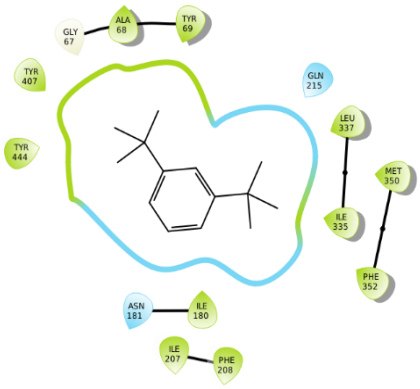
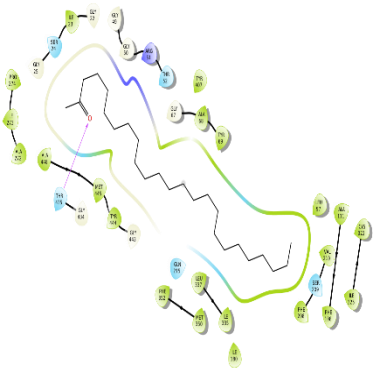
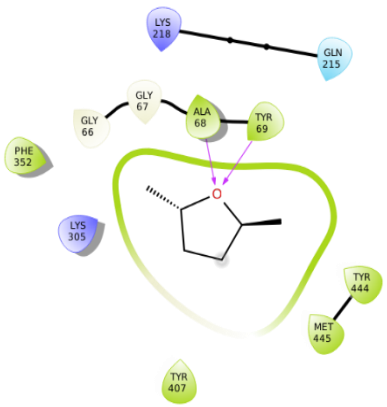
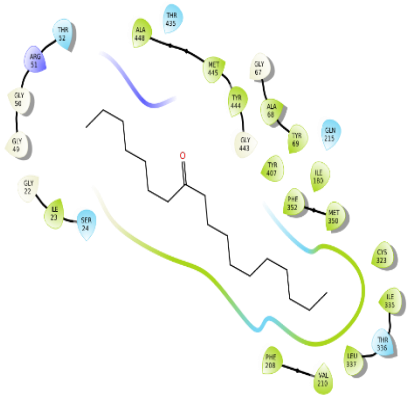
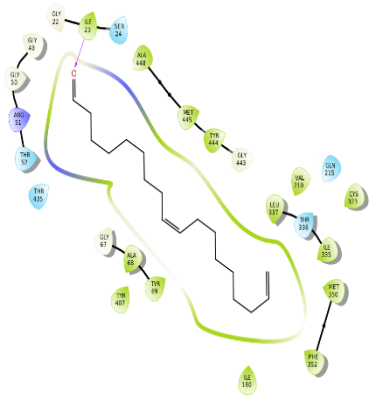
Monoamine oxidases-A is primarily responsible for destroying serotonin and norepinephrine which may lead to depression. The inhibitors of Monoamine oxidases-A are therefore used to treat the depression. In the present study, structure-based computational modeling of ligand-receptor interactions was performed focusing on ligand molecules derived from the bioconversion of DHA. A total of 82 compounds were identified from the bioconverted DHA using GC-MS which are listed in fig. 1 and table 4. The crystal structure of human monoamine oxidases (PDB ID 2Z5X) was considered as the receptor for the present study. Imipramine, a protease inhibitor (PI) has been used as the control for molecular docking throughout our experiment. It is widely used in the treatment of depression. Imipramine selectively binds to monoamine oxidases, inhibits it by reuptake of serotonin and norepinephrine, thus elevating the levels of these neurotransmitters in the brain[3].

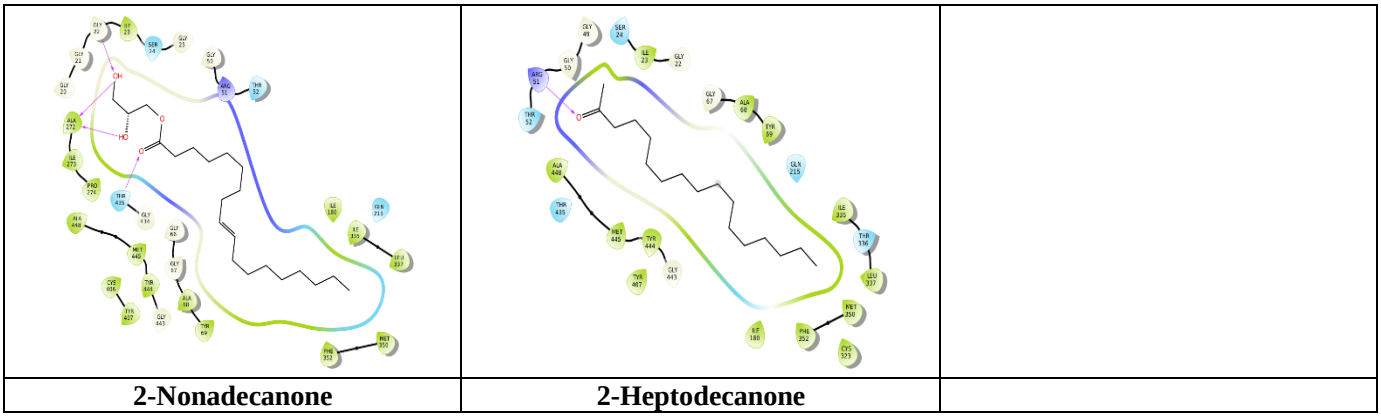
Both experimental and computational evidences suggest the ability of imipramine in inhibiting the depressant effect caused by monoamine oxidases[3]. Molecular docking was performed using bioconverted DHA metabolites against monoamine oxidases enzyme receptors, 2Z5X with imipramine as the control. Docking was done using Glide module (Maestro software) of Schrodinger ver.11.1. The strength of the receptor-ligand interactions was determined based on the Glide score. The higher is the negative Glide score, the better is the interaction between the protein and the ligand[22]. Potential ligands were shortlisted based on the ligand efficiency, Glide score and docking score. The receptor, 2Z5X was docked with 82 ligands which were derived from the bioconversion of DHA. In the docking experiment, fourteen ligands were shown to possess considerable docking scores against 2Z5X [Table 5]. Among them, Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester displayed the highest predicted binding affinity with a Glide score of -10.232.

Table 4
Metabolites from bioconverted Docosahexaenoic acid using gut bacteria

S.N.	Retention Time (min)	Percentage %	Name of the Compound
1	20.774	34.68	n-Hexadecanoic acid
2	22.698	32.53	Octadecanoic acid
3	15.782	31.59	Phenol, 2,4-Bis(1,1-Dimethylethyl)-
4	4.165	22.59	Benzene, Methyl-
5	27.000	17.11	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
6	22.489	13.93	Oleic Acid
7	22.176	13.27	2-Nonadecanone
8	21.941	12.91	Z,Z-6,27-Hexatriactontadien-2-one
9	43.881	11.48	cis-9-Hexadecenal
10	27.952	10.69	Butyl 4,7,10,13,16,19-docosahexaenoate
11	23.462	10.31	Palmitoyl chloride
12	26.922	10.27	Tetrapentacontane
13	25.389	9.83	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
14	27.214	8.7	Octadecanoic acid, 2,3-dihydroxypropyl ester
15	16.871	8.53	Hexadecane
16	18.019	8.29	Eicosane
17	21.395	7.89	Eicosanal-
18	23.874	7.70	Glycidyl palmitate
19	24.730	7.63	Heneicosane
20	26.623	7.54	Stigmast-5-En-3-Ol, Oleat
21	28.162	7.18	Decanedioic acid, bis(2-ethylhexyl) ester
22	6.560	6.18	Oxime-, methoxy-phenyl-
23	18.671	5.89	Tetradecanoic acid
24	8.781	5.49	1-Hexanol, 2-Ethyl-
25	12.619	5.18	Dodecane, 2,6,11-trimethyl-
26	3.172	5.02	Heptane

27	14.351	5.0	Tetradecane
28	25.490	4.9	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethy
29	23.653	4.90	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-
30	9.401	4.43	Cyclotrisiloxane, hexamethyl-
31	26.680	4.30	n-Propyl 9-octadecenoate
32	4.460	4.01	3-Hexanone
33	24.162	3.75	Hexatriacontane
34	4.538	3.59	2-Hexanone
35	25.582	3.50	Glycidyl palmitate
36	15.499	3.05	Hexadecane, 2,6,10,14-Tetramethyl-
37	27.861	2.73	Methyl 4,7,10,13,16-docosapentaenoate
38	20.193	2.69	2-Heptadecanone
39	22.308	2.62	Octadecane
40	28.368	2.56	Squalene
41	24.218	2.32	1H,5H-Cyclopropa[G][1,2,4]Triazolo[1,2-A]
42	26.786	2.31	1-Cyclohexyldimethylsilyloxybutane
43	26.880	2.26	Octadecanoic acid, propyl ester
44	11.521	2.19	Dodecane
45	21.275	2.15	(Z)-3-(Heptadec-10-en-1-yl)phenol
46	26.220	1.90	Silikonfett Se30 (Grevels)
47	20.757	1.90	1-Butyl 2-(8-Methylnonyl) Phthalate #
48	26.959	1.87	cis-10-Pentadecenoic acid, isobutyl ester
49	9.209	1.86	Octane, 6-Ethyl-2-Methyl-
50	3.365	1.82	Furan, tetrahydro-2,5-dimethyl-
51	24.491	1.75	Eicosanoic acid
52	24.115	1.67	Cyclodecasiloxane, eicosamethyl-
53	19.326	1.64	Hexadecanal
54	24.112	1.50	Cyclodecasiloxane, eicosamethyl-
55	25.824	1.48	Bis(2-ethylhexyl) phthalate
56	6.999	1.48	1-Propene, 3,3-Dichloro-
57	22.429	1.46	9,12-Octadecadienoic Acid (Z,Z)-
58	22.421	1.44	10(E),12(Z)-Conjugated linoleic acid
59	28.188	1.41	Dotriacontane
60	12.282	1.41	Benzene, 1,3-Bis(1,1-Dimethylethyl)-
61	21.880	1.34	8,11-Heptadecadienal, (8Z,11Z)-
62	20.574	1.29	4-Pentyl-Cyclohexanecarboxylic Acid
63	25.561	1.28	Myristic acid glycidyl ester
64	21.102	1.28	9,17-Octadecadienal, (Z)-
65	4.716	1.18	Hexanal
66	20.411	1.15	Hexadecanoic acid, methyl ester
67	24.814	1.13	1,16-Dibromohexadecane
68	23.985	0.99	2-Pentacosanone
69	20.709	0.98	2,6,10,15,19,23-Hexamethyltetracosane
70	27.063	0.97	8-Octadecanone
71	9.212	0.95	Octane, 6-Ethyl-2-Methyl-
72	20.581	0.94	4-Pentyl-Cyclohexanecarboxylic Acid
73	4.766	0.90	2-Hexanol
74	25.397	0.89	Stigmasta-5,22-Dien-3-Ol, Acetat, (3-Beta,2
75	12.730	0.85	4-Propylbenzaldehyde
76	16.778	0.81	1-Hexadecanol
77	4.691	0.76	3-Hexanol
78	13.282	0.75	Dodecane, 4,6-dimethyl-
79	28.040	0.72	9-Heptadecanone
80	27.786	0.70	Z,Z-6,28-Heptatriactontadien-2-one
81	24.915	0.61	3-({[2-(4-Fluorophenyl)Ethyl]Amino}Methyl
82	12.623	0.48	Dodecane, 4,6-dimethyl-

		
Imipramine	**Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester**	**Hexatriacontane**
		
(Z)-3-(Heptadec-10-en-1-yl) phenol	**Dotriacontane**	**Decanedioic acid, bis(2-ethylhexyl) ester**
		
10(E),12(Z)-Conjugated linoleic acid	**Benzene,1,3-Bis(1,1-Dimethylethyl)-**	**2-Pentacosanone**
		
Furan, tetrahydro-2,5-dimethyl-	**8-Octadecanone**	**9,17-Octadecadienal,(Z)-**



2-Nonadecanone	2-Heptodecanone	

Fig. 4: Interaction diagram with H-bonds and other interactions of a 2Z5X with (A) Imipramine (B) Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester (C) Hexatriacontane (D) (Z)-3-(Heptadec-10-en-1-yl) phenol (E) Dotriacontane (F) Decanedioic acid, bis(2-ethylhexyl) ester (G) 10(E),12(Z)-Conjugated linoleic acid (H) Benzene,1,3-Bis(1,1-Dimethylethyl)- (I) 2-Pentacosanone (J) Furan, tetrahydro-2,5-dimethyl- (K) 8-Octadecanone (L) 9,17-Octadecadienal, (Z)- (M) 2 Nonadecanone and (N) 2-Heptodecanone showing different polar and non-polar interactions and bonds

The next in line were Hexatriacontane (Gscore: -8.884), (Z)-3-(Heptadec-10-en-1-yl) phenol (Gscore: -8.047), Dotriacontane (Gscore: -7.582), Decanedioic acid, bis(2-ethylhexyl) ester (Gscore: -7.074), 10(E),12(Z)-Conjugated linoleic acid (Gscore: -6.986), Benzene,1,3-Bis(1,1-Dimethylethyl)- (Gscore: -6.68), 2-Pentacosanone (Gscore: -6.653), Furan, tetrahydro-2,5-dimethyl- (Gscore: -6.124), 8-Octadecanone (Gscore: -6.007), 9,17-Octadecadienal,(Z)- (Gscore: -5.859), 2 Nonadecanone (Gscore: -5.751) and 2-Heptodecanone (Gscore: -5.743) .

The docking interaction between the control and protein 2Z5X was appreciably high with a Glide score of -5.394. Molecular docking revealed that one ligand atom of the control interacted with various amino acid residues of the protein at position 23(Isoleucine) respectively. Isoleucine made a hydrophobic interaction with the 'O' atom of imipramine [Fig. 4A]. 82 metabolites derived from the bioconversion of DHA were docked against 2Z5X. Among them, Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester displayed the highest binding efficiency with a Glide score of -10.232. Docking results revealed that 3 ligand atoms of Hexadecanoic acid and 2-hydroxy-1 (hydroxymethyl) ethyl interacted with amino acid residues of the protein at positions 22, 51 and 272 with glycine, arginine and alanine respectively.

Glycine makes a hydrogen bond interaction with the 'OH' atom of the ligand, arginine makes a positively charged hydrogen bond interaction with the 'O" whereas alanine makes a hydrophobic hydrogen bond interaction with OH [Fig. 4B]. The next molecule interacting with 2Z5X with a high Glide score was Decanedioic acid, bis(2-ethylhexyl) ester (Gscore: -7.074). Decanedioic acid, bis(2-ethylhexyl) ester made a positively charged 'O' bond hydrogen interaction with threonine, 'O' bond hydrophobic hydrogen bond interaction with alanine and 'O' bond hydrophobic hydrogen bond interaction with tyrosine at positions 51, 68 and 69 respectively, similar to that of hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester [Fig. 4F]. 10(E),12(Z)-Conjugated linoleic acid came in the next position with a Glide score of -6.986. It made an 'O' bond hydrophobic hydrogen bond interaction with isoleucine and 'O' bond positively charged hydrogen bond interaction with arginine at 23 and 51stposition [Fig. 4G]. 2-Pentacosanone (G score: -6.653) interacted with threonine amino acid residue of 2Z5X at 435th position. It made a polar hydrogen bond interaction with the 'O' atom of the protein [Fig. 4I]. 9,17-Octadecadienal, (Z) (Gscore: -5.859) interacted with isoleucine amino acid residue of 2Z5X at 23rd position and it made a hydrophobic hydrogen bond with the 'O' atom of the protein [Fig.4L].

2 Nonadecanone (Gscore: -5.751) glycine makes a glycine hydrogen bond interaction with 'OH' at 22 position, alanine makes hydrophobic hydrogen bond interaction with 'OH' at 272 position and theronine makes polar hydrogen bond with 'O' at 435 position [Fig. 4M]. 2-Heptodecanone (Gscore: -5.743) makes "O" bond positively charged hydrogen bond with arginine at 51st position [Fig.4N].

Thus, in this study, the monoamine oxidases protein namely, 2Z5X was considered as target to screen the bioconverted metabolites of DHA and identify potential drugs against depression that have appreciable binding affinity towards this receptor protein playing an essential role as an antidepressant. In the docking experiments, four ligands showed considerable docking score against 2Z5X [Table 5].

Among them, Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester (Gscore -10.232), Hexatriacontane (Gscore: -8.884), (Z)-3-(Heptadec-10-en-1-yl) phenol (Gscore: -8.047), Dotriacontane (Gscore: -7.582), Decanedioic acid, bis(2-ethylhexyl) ester (Gscore: -7.074), 10(E),12(Z)-Conjugated linoleic acid (Gscore: -6.986), Benzene,1,3-Bis(1,1-Dimethylethyl)- (Gscore: -

6.68), 2-Pentacosanone (Gscore: -6.653), Furan, tetrahydro-2,5-dimethyl- (Gscore: -6.124), 8-Octadecanone (Gscore: -6.007), 9,17-Octadecadienal, (Z)- (Gscore: -5.859), 2 Nonadecanone (Gscore: -5.751) and 2-Heptodecanone (G score: -5.743) were found to possess G score greater than even that of the control (imipramine) (G score: -5.394).

Table 5
Docking scores for receptor (2 Z5X) and ligands (Compounds)

S.N.	Ligands	Glide Ligand Efficiency	Docking Score	Glide Score	Amino acid Residue position	Type of Interactions
1.	Imipramine (Control)	-0.257	-5.394	-5.394	Gly 22	Glycine
					Ile 23	Hydrophobic Hydrogen bond with "O"
					Ser 24	Polar
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Tyr 407	Hydrophobic
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
2.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-0.445	-10.232	-10.232	Gly 20	Glycine
					Gly 22	Hydrogen Bond with "OH"
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 25	Glycine
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged Hydrogen Bond with "O"
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Gln 215	Polar

					Ala 272	Hydrophobic interaction Hydrogen bond with "OH"
					Ile 273	Hydrophobic
					Pro 274	Hydrophobic
					Ile 335	Hydrophobic
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Tyr 407	Hydrophobic
					Gly 434	Glycine
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
3.	Hexatriacontane	-0.247	-8.884	-8.884	Ile 19	Hydrophobic
					Gly 20	Glycine
					Gly 21	Glycine
					Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 25	Glycine
					Glu 43	Negatively charged
					Ala 44	Hydrophobic
					Arg 45	Positively charged
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Leu 97	Hydrophobic
					Phe 108	Hydrophobic
					Ala 111	Hydrophobic
					Pro 113	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Ser 209	Polar
					Val 210	Hydrophobic
					Gln 215	Polar
					Pro 243	Hydrophobic
					Val 244	Hydrophobic
					Thr 245	Polar
					Leu 259	Hydrophobic
					Ala 272	Hydrophobic
					Ile 273	Hydrophobic
					Pro 274	Hydrophobic
					Leu 277	Hydrophobic
					Lys 280	Positively charged
					Ile 281	Hydrophobic
					Cys 323	Hydrophobic

					Ile 325	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Tyr 402	Hydrophobic
					Ser 403	Polar
					Tyr 407	Hydrophobic
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
4.	(Z)-3-(Heptadec-10-en-1-yl)phenol	-0.335	-8.047	-8.047	Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 49	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 66	Glycine
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Leu 97	Hydrophobic
					Ala 111	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Ser 209	Polar
					Val 210	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 325	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Cys 406	Hydrophobic
					Tyr 407	Hydrophobic
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
5.	Dotriacontane	-0.237	-7.582	-7.582	Ile 19	Hydrophobic
					Gly 20	Glycine
					Gly 21	Glycine
					Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 25	Glycine
					Leu 42	Hydrophobic
					Glu 43	Negatively charged
					Ala 44	Hydrophobic
					Arg 45	Positively charged
					Gly 49	Glycine

					Gly 50	Glycine
					Arg 51	Positively charged
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Leu 97	Hydrophobic
					Phe 108	Hydrophobic
					Ala 111	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Val 210	Hydrophobic
					Gln 215	Polar
					Hip 242	Positively charged
					Pro 243	Hydrophobic
					Val 244	Hydrophobic
					Ala 272	Hydrophobic
					Ile 273	Hydrophobic
					Pro 274	Hydrophobic
					Leu 277	Hydrophobic
					Lys 280	Positively charged
					Ile 281	Hydrophobic
					Cys 323	Hydrophobic
					Ile 325	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Ser 403	Polar
					Tyr 407	Hydrophobic
					Gly 434	Glycine
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrohobic
6.	Decanedioic acid, bis(2-ethylhexyl) ester	-0.221	-7.074	-7.074	Ile 19	Hydrophobic
					Gly 20	Glycine
					Gly 21	Glycine
					Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Ile 42	Hydrophobic
					Glu 43	Negatively charged
					Ala 44	Hydrophobic
					Arg 45	Positively charged
					Val 48	Hydrophobic
					Gly 49	Glycine
					Gly 50	Glycine

					Arg 51	Positively charged Hydrogen Bond with "O"
					Thr 52	Polar
					Gly 66	Glycine
					Gly 67	Glycine
					Ala 68	Hydrophobic Hydrogen Bond with "O"
					Tyr 69	Hydrophobic Hydrogen bond with "O"
					Ile 180	Hydrophobic
					Asn 181	Polar
					Tyr 197	Hydrophobic
					Ile 207	Hydrophobic
					Gln 215	Polar
					Pro 243	Hydrophobic
					Val 244	Hydrophobic
					Ala 272	Hydrophobic
					Ile 273	Hydrophobic
					Pro 274	Hydrophobic
					Leu 277	Hydrophobic
					Lys 305	Positively charged
					Ile 335	Hydrophobic
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Tyr 402	Hydrophobic
					Ser 403	Polar
					Cys 406	Hydrophobic
					Tyr 407	Hydrophobic
					Gly 434	Glycine
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
7.	10(E),12(Z)-Conjugated linoleic acid	-0.349	-6.982	-6.986	Gly 22	Glycine
					Ile 23	Hydrophobic

						Hydrogen bond with "O-"
					Ser 24	Polar
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged Hydrogen bond with "O"
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 325	Hydrophobic
					Ile 335	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Leu 337	Hydrophobic
					Tyr 407	Hydrophobic
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
8.	Benzene,1,3-Bis(1,1-Dimethylethyl)-	-0.477	-6.68	-6.68	Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Asn 181	Polar
					Ile 207	Hydrophobic
					Phe 208	Hydrophobic
					Gln 215	Polar
					Ile 335	Hydrophobic
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic

					Tyr 407	Hydrophobic
					Tyr 444	Hydrophobic
9.	2-Pentacosanone	-0.256	-6.653	-6.653	Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 25	Glycine
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Leu 97	Hydrophobic
					Phe 108	Hydrophobic
					Ala 111	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Ser 209	Polar
					Val 210	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 325	Hydrophobic
					Ile 335	Hydrophobic
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Ala 272	Hydrophobic
					Ile 273	Hydrophobic
					Pro 274	Hydrophobic
					Gly 434	Glycine
					Thr 435	Polar Hydrogen bond with “O”
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
10.		-0.875	-6.124	-6.124	Gly 66	Glycine

	Furan, tetrahydro-2,5-dimethyl-				Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Gln 215	Polar
					Lys 218	Positively charged
					Lys 305	Positively charged
					Phe 352	Hydrophobic
					Tyr 407	Hydrophobic
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
11.	8-Octadecanone	-0.316	-6.007	-6.007	Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Phe 208	Hydrophobic
					Val 210	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Tyr 407	Hydrophobic
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
12.	9,17-Octadecadienal, (Z)-	-0.308	-5.859	-5.859	Gly 22	Glycine
					Ile 23	Hydrophobic Hydrogen bond with “O”

					Ser 24	Polar
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Val 210	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Tyr 407	Hydrophobic
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
13.	2 Nonadecanone	-0.288	-5.751	-5.751	Gly 20	Glycine
					Gly 21	Glycine
					Gly 22	Glycine Hydrogen bond with “OH”
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 25	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged
					Thr 52	Polar
					Gly 66	Glycine
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic

					Gln 215	Polar
					Ala 272	Hydrophobic Hydrogen bond with "OH"
					Ile 273	Hydrophobic
					Pro 274	Hydrophobic
					Ile 335	Hydrophobic
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Cys 406	Hydrophobic
					Tyr 407	Hydrophobic
					Gly 434	Glycine
					Thr 435	Polar Hydrogen Bond with "O"
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic
14.	2-Heptodecanone	-0.319	-5.743	-5.743	Gly 22	Glycine
					Ile 23	Hydrophobic
					Ser 24	Polar
					Gly 49	Glycine
					Gly 50	Glycine
					Arg 51	Positively charged Hydrogen bond with "O"
					Thr 52	Polar
					Gly 67	Glycine
					Ala 68	Hydrophobic
					Tyr 69	Hydrophobic
					Ile 180	Hydrophobic
					Gln 215	Polar
					Cys 323	Hydrophobic
					Ile 335	Hydrophobic
					Thr 336	Polar
					Leu 337	Hydrophobic
					Met 350	Hydrophobic
					Phe 352	Hydrophobic
					Thr 435	Polar
					Gly 443	Glycine
					Tyr 444	Hydrophobic
					Met 445	Hydrophobic
					Ala 448	Hydrophobic

Hence, Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester, Hexatriacontane, (Z)-3-(Heptadec-10-en-1-yl) phenol, Dotriacontane, 10(E),12(Z)-Conjugated linoleic acid, Benzene,1,3-Bis(1,1-Dimethylethyl)-, 2-Pentacosanone, Furan, tetrahydro-2,5-dimethyl-, 8-Octadecanone, 9,17-Octadecadienal, (Z)-, 2 Nonadecanone, 2-Heptodecanone were proposed to possess better inhibitory property towards 2Z5X than that of the control. Thus, these compounds are strongly recommended as potential drug candidates for treating depression.

Conclusion

Various metabolites were extracted by fermentation of DHA using the microorganisms generally found in our gut mictobiota, namely, *Lactobacillus* spp., *Clostridium* spp., *Escherichia coli, Bacillus cereus* and *Enterococcus* spp. *In silico* studies of docking confirmed the binding of metabolites with the target protein. These metabolites were found to exhibit anti-bacterial properties and anti-depressant effect. Hence, it was concluded that DHA metabolites produced by gut microbiome could possess various therapeutic roles in the human system.

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