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Full Length Article

In silico and *in vitro* analysis of bioactive compounds extracted from *Ocimum basilicum* against vancomycin-resistant enterococci

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A R T I C L E I N F O

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A B S T R A C T

Ocimum basilicum is an important herbal medicinal plant that has not been previously investigated for its biological potential against multi-drug resistant (MDR) clinical pathogens. This study explored the efficiency of *O. basilicum* phytocompounds as potent inhibitors of vancomycin-resistant enterococci (VRE) via *in vitro* and *in silico* analysis. An ethanolic extract of *O. basilicum* showed antimicrobial activity against 12 strains of vancomycin-resistant *Enterococcus faecalis* at varying concentrations. A total of 19 phytochemicals were analysed for ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) using the Swiss ADME server (http://www.swissadme.ch) to assess the pharmacological characteristics, including lipophilicity, water solubility, drug-likeness, pharmacokinetics and medicinal chemistry.

Among 19 compounds, 8 compounds (adipic acid, ethyl citrate, glutamic acid 5-ethyl ester, imidazole, palmitic acid, phthalic anhydride, 2-Propenoic acid 3-phenyl-methyl ester, & stearic acid) were selected as they fulfilled the Lipinski's rule of five. Autodock Vina was used to dock the selected phytocompounds into the target proteins (5ZHW, 4FUO, 1E4E, 4ECL, 6GED, 6ORI) of *E. faecalis*. Phthalic anhydride and the positive control antibiotic, linezolid showed stronger binding energy with all 6 target proteins revealing their therapeutic potential to treat VRE infections. These findings could be the baseline for the pharmaceutical sector to evaluate a chemical's safety profile and the in silico approaches to provide considerable advantages for both regulatory requirements and risk assessment criteria.

1. Introduction

In the last few decades, the incidence of multidrug resistance (MDR) has been reported among bacterial pathogens. Their continuous increase at a terrifying rate causes a public health threat worldwide. According to the Center for Disease Control and Prevention (CDC), antibiotic-resistant bacterial pathogens infect nearly 2.8 million people per year in the United States and kill more than 35, 000 people [1]. The majority of MDR bacteria cause nosocomial infection and some cause community-acquired infections. The excessive and inappropriate usage of antibiotics as therapy for human beings [2], animal husbandry, and aquaculture farms, besides the application of broad-spectrum antibiotics, can be one of the risk-associated factors most responsible for the increased spread of multidrug resistance [3].

Nosocomial infection cases are rapidly increasing, specifically in developing countries [4]. Recently, enterococci have gained more attention due to their ability to resist most antimicrobials, especially glycopeptide antibiotics [5]. Drug resistance, especially vancomycin resistance, is a serious threat to treating nosocomial infections [6]. Their intrinsic resistance to common antibiotics (penicillin, nalidixic acid, clindamycin, cephalosporin and aminoglycoside) is the major reason for their survivability in a hospital environment. Feasibly, their antibiotic resistance is acquired either *via* mutation or horizontal transfer of genetic material. The glycopeptides, vancomycin, and teicoplanin are commonly used for treating Gram-positive bacterial infections, especially staphylococcal and enterococcal infections. The widespread and

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frequent use of these glycopeptides in hospitals led to the development of VRE, which leads to serious health and economic impacts on healthcare professionals. Although the VRE case was first reported in 1986, from the UK, recently, they have been disseminated globally [7]. In the last two decades, VRE cases have increased by 20-fold [8] and turned out to be one of the leading causes of nosocomial infection worldwide among healthcare people. Although there has been a steady increase in enterococcal infection in India [9], very few outbreaks of VRE cases have been reported [7,10,11]. Among the nosocomial VRE, *Enterococcus faecium* accounts for the majority of infections and *Enterococcus faecalis* accounts for 2-20 % of infections. Other species, such as *Enterococcus durans* and *Enterococcus hirae* and *Enterococcus avium* are rarely reported [12].

Drug resistance among nosocomial pathogens is continuously increasing, their treatment has also become limited. Although synthetic antimicrobial metabolites have been already available in many countries, the usage of natural bioactive compounds derived from various sources like microbial, animals, or plants has attracted attention recently. Among all the natural sources, plant-derived bioactive compounds have exhibited more therapeutic applications in combating multi-drug resistant pathogens. *O. basilicum* is one of the well-known plants commonly used by the public due to its application in Ayurvedic and folk medicine [13,14]. The extract of various parts including leaves and essential oil are used as spices and flavors for various food products and as well as effective drugs for many infectious diseases in folk medicine due to their unique aroma, flavor and other biological activities [15].

Traditional research on medicinal plants is a time-consuming and highly expensive process due to the extraction of compounds and their qualitative and quantitative identification. Thus, most of the chemical compounds have not been completed for their studies to determine their biological activities. In recent years, with increasing knowledge of computer-based technologies, a number of successful new drugs from natural products have become more frequent, such as FDA (Food and Drug Administration)-approved drugs, Sinecatechins, Exelon, Rezadyne, etc. *In silico* analysis, it is more efficient to find efficient lead compounds from medicinal plants. This study aims to assess the docking efficiency of bioactive compounds extracted from *O. basilicum* against vancomycin-resistant enterococcal receptors targeting nosocomial infections.

2. Materials and Methods

2.1. Determination of the antimicrobial activity of Ocimum basilicum against VRE

According to our previous study [16], ethanol extract of *O. basilicum* was selected to assess their potential to inhibit the growth of 12 different strains of vancomycin-resistant (VR) *E. faecalis* (VRE056, VRE071, VRE123, VRE128, VRE134, VRE139, VRE145, VRE151, VRE162, VRE165, VRE170 & VRE177) The VR *E. faecalis* pathogens characterized elsewhere [6] were employed in this study. Agar well diffusion assay was used to determine the antimicrobial activity of ethanol extract of *O. basilicum* by following Backiam et al. [16]. Commercially available antibiotics being used to treat VRE infections namely, linezolid (10 μg/mL; Hi-Media, India) [17] were used as a positive control.

2.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of O. basilicum extract

The MIC and MBC of sweet basil leaf extract were determined to kill VR *E. faecalis* strains (n=12) by following the tube dilution method [16] with minor changes. Briefly, all the indicator pathogens were prepared freshly in Muller Hinton broth (Hi-Media, India) by adjusting their optical density (OD) to 0.5 (equivalent to the McFarland turbidity scale) with approximately 10^6- 10^7 CFU/mL. The ethanolic extract of the *O. basilicum* was diluted in a 96-well microtiter plate to reach a concentration ranging from 50-0.09 mg/mL. Each well was added with 50 μL of bacterial culture suspension and the OD was recorded after incubating at 37°C for 24 h to find out the lowest concentration that inhibited the bacterial growth. Finally, 50 μL of the treated bacterial culture from each well was separately swabbed on the surface of the nutrient agar (Hi-Media, India) plate and incubated for 12 h. The complete absence of growth on the nutrient agar plate was defined as MBC.

2.3. In silico analysis of phytocompounds of O. basilicum

2.3.1. Phytochemicals from O. basilicum

A total of 19 phytochemicals (Table 1) from ethanolic extract of *O. basilicum* were previously profiled using GC-MS (Gas chromatography-mass spectrometry) analysis [16] and enrolled in this study for *in silico* analysis against vancomycin-resistant enterococcal receptors.

2.3.2. Drug-likeness and toxicity prediction

Initially, all 19 compounds were predicted for ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) properties based on the well-established concept of Lipinski et al. [18]. This is an assessment of the pharmacokinetic properties of a compound and performed using the Swiss ADME server (http://www.swissadme.ch). This server evaluated all the compounds for their pharmacological features such as lipophilicity, water solubility, drug-likeness, pharmacokinetics and medicinal chemistry.

The structures of the identified compounds were converted to their canonical simplified molecular-input line-entry (SMILE) system and submitted to the Swiss ADME tool to find out the *in silico* pharmacokinetic properties such as the number of hydrogen donors, hydrogen acceptors, rotatable bonds, total polar surface area of the compound, lipophilicity, water solubility, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (P-gp) substrate, skin permeation, drug-likeness and medicinal chemistry.

Table 1
List of phytocompounds identified from *Ocimum basilicum* using GC-MS analysis (Backiam et al., 2023)

Name of the compound	Retention time	Formula	Molecular weight (g/mol)
Proline, 3,4-didehydro-	5.364	$C_5H_7NO_2$	113.11
1H-Imidazole	6.542	$C_3H_4N_2$	68.08
1, 5-dimethyl-1-vinyl-4-hexenyl butyrate	7.431	$C_{14}H_{24}O_2$	224.34
Estragole	8.753	$C_{10}H_{12}O$	148.20
2-Thiophenemethanamine	9.020	C_5H_7NS	113.18
Phthalic anhydride	10.164	$C_8H_4O_3$	148.11
1,5-Dioxaspiro[5.5]undec-3-en-2-one 7-isopropyl-10-methyl-4-(4-pent en-2-yl)-	10.653	C_6H_5NOS	139.18
2-Propenoic acid, 3-phenyl-, methyl ester	10.975	$C_{10}H_{10}O_2$	162.18
L-Glutamic acid 5-ethyl ester	11.586	$C_7H_{13}NO_4$	175.18
Ethyl citrate	13.675, 14.086	$C_8H_{12}O_7$	220.18
Bicyclo (3.1.1) heptane, 2,6,6-tri-methyl-, (1.alpha.,2.beta.,5.alpha.	15.319	$C_{10}H_{18}$	138.25
n-Hexadecanoic acid (Palmitic acid)	16.374	$C_{16}H_{32}O_2$	256.42
Phytol	17.563	$C_{20}H_{40}O$	296.53
9,12-Octadecadienoic acid (Z,Z)-	17.730	$C_{18}H_{32}O$	280.4
Octadecanoic acid (Steric acid)	17.963	$C_{18}H_{36}O$	284.5
2-Propenoic acid, 3-phenyl-, methyl ester	19.018	$C_{10}H_{10}O_2$	162.18
2-Propenoic acid, 3-phenyl-, methyl ester 1,3-Benzodioxole, 5-(1-propenyl)-	19.152	$C_{10}H_{10}O_2$	162.18
Triphenyl phosphate	19.740	$C_{18}H_{15}O_4P$	326.3
2,6,10,14,18,22-Tetracosahexaene (Squalene)	22.307	$C_{30}H_{50}$	410.7

The oral toxicity of the selected 8 compounds was assessed *via* ProTox II, a tool for the prediction of chemical compounds. ProTox II offers a free web server for *in silico* prediction for research people working in toxicology, pharmacology, and medicinal chemistry (http://tox.charite.de/protox_II). The chemical name as per Pubchem and the smile of the compound can be given in the tool to predict the toxicity.

2.3.3. Molecular docking: Proteins and ligands Pre-Preparation

The protonated low-energy 3D phytocompounds (ligands) were prepared using Autodocktools Version 1.5.7. A blind docking study was performed using AutoDock Vina version 10.0.22000.1219 to evaluate the binding efficiency of ligands to the receptor proteins of VRE pathogens. The target receptor proteins were prepared by removing ligands, water molecules, and heteroatoms and adding polar-charged hydrogen atoms. Further, a grid map was generated with a specific dimension for each target protein. The Lamarckian genetic algorithm was used to analyse the docking probability. The configuration files created for all the proteins generated the ten best poses for each of the ligands and scored using Autodock vina. The ligands were ranked based on the energy docked. The results of the docking were observed using BIOVIA Discovery Studio Visualizer 2021.

3. Results

3.1. Antimicrobial activity of O. basilicum extract

The antimicrobial activity of ethanolic extract of *O. basilicum* against 12 strains of vancomycin resistant (VR) *Enterococcus faecalis* have been assessed in this study. The ethanolic extract of *O. basilicum* exhibited significantly stronger antimicrobial activity against VR *E. faecalis* ($P<0.05$), as it inhibited the growth of all the 12 strains of VR *E. faecalis*. The ethanolic extract of *O. basilicum* showed the maximum zone of inhibition (24±0.617 mm) against VRE056, VRE071, VRE145 and VRE177, while linezolid exhibited significantly less activity against VRE056 ($P<0.05$), VRE071 ($P<0.001$), VRE145 ($P<0.001$), VRE177 ($P<0.01$) (Fig. 1).

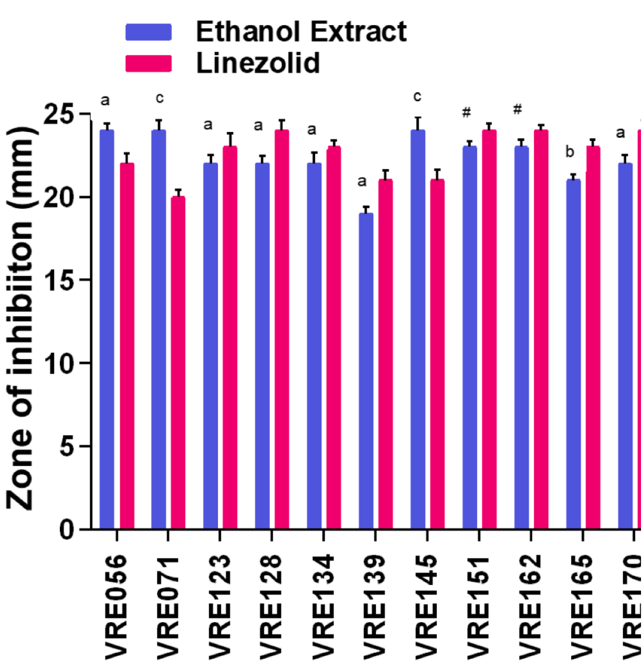

Fig. 1. Antimicrobial activity of ethanolic extract of *O. basilicum* leaves against 12 different vancomycin-resistant *Enterococcus faecalis* strains. The graph is plotted using ± SD of three independent experiments. Different letters of asterisks indicate significant differences from positive control and ethanolic extract. a: $P < 0.05$, b: $P < 0.01$, c: $P < 0.001$, #:nonsignificant

Significant antimicrobial activity, expressed as minimum inhibitory concentration of *O. basilicum* extract against VR *E. faecalis* strains is summarised in Table 2. The MIC of the ethanolic extract against 12 VR *E. faecalis* is ranged from 0.39 to 3.125 mg/mL. The strains, VRE056, VRE071, VRE145, and VRE177 were highly susceptible to sweet basil extract as these strains' growth was completely inhibited at the MIC of 0.39 mg/mL. Similarly, their MBC (3.125 mg/mL) was also lesser than the MBC required for other VRE strains (6.125- 25 mg/mL). This result was supported by agar well diffusion, which showed that the zone of inhibition against these four stains (VRE056, VRE 071, VRE145, VRE177) was significantly lesser compared to MBC for other 8 VRE strains

3.2. In silico drug-likeness and toxicity analysis

The present study aimed to assess the drug-likeness of the phytocompounds identified from *O. basilicum* leaf extract by analysing ADMET features including adsorption, distribution, metabolism, excretion and toxicity by using SwissADME tool. Among 19 compounds, 8 compounds such as adipic acid ethyl 2-octyl ester, 1-ethyl citrate, glutamic acid 5-ethyl ester, imidazole, palmitic acid, phthalic anhydride, 2-propenoic acid 3-phenyl-methyl ester, stearic acid were fulfilled the Lipinski's rule of five. Lipinski's five parameter rule states that the hydrogen bond donor should be less than 5, hydrogen bond acceptors should be less than 10, molecular weight should be less than 500 Da, log P should not be less than 5 and the total polar surface area should not be higher than 140 Å. The current *in silico* study using the SwissADME tool showed that the compounds (no=8) that obey Lipinski's rule of five are likely to be orally active. The total polar surface area (TPSA) value of the 8 compounds was in the range of 26.80 to 89.62 A° and all these values are less than the cut-off value, 140 A°. Similarly, the calculated rotatable bond value for 1-ethyl citrate, glutamic acid, imidazole, phthalic anhydride, 2-propenoic acid 3-phenyl-methyl ester was less than 10 indicating their structural stability (Table 3).

The skin permeation (Kp) values of the 8 compounds were in the range from -2.19 to -9.50 cm/s. revealing their low skin permeability. Except that of 1- ethyl citrate and L-glutamic acid 5-ethyl ester all the compounds showed lower skin permeability than the antibiotic (linezolid) tested in this study (-7.87 cm/s).

Among the 8 compounds, adipic acid, phthalic anhydride, palmitic acid, and propenoic acid showed potential for blood-brain barrier (BBB) permeation, while the remaining compounds and the tested antibiotic (linezolid) were negative for BBB permeation ability. Similarly, the *in silico* prediction *via* SwissADME also determined the cytochrome inhibitory potential of the compounds. Palmitic acid inhibited only 2 cytochromes, CYP1A2 and CYP2C9. And no more compounds inhibited any of the cytochromes. Likewise, no more compounds inhibited the substrate of permeability glycoprotein (P-gp substrate). The boiled egg

Table 2
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extract of *O. basilicum* against various vancomycin-resistant *Enterococcus faecalis* strains determined by tube dilution method

Indicator Bacteria	Ethanol extract (mg/mL)		Linezolid (mg/mL)	
	MIC	MBC	MIC	MBC
VRE056	0.39[d]	3.125 [d]	0.05	0.195
VRE071	0.39 [d]	3.125 [d]	0.05	0.195
VRE123	1.56 [d]	6.25 [d]	0.097	0.195
VRE128	1.56 [d]	3.125 [d]	0.097	0.195
VRE134	0.78 [d]	3.125 [d]	0.097	0.195
VRE139	1.56 [d]	6.25 [d]	0.097	0.195
VRE145	0.39 [c]	3.125 [d]	0.05	0.195
VRE151	3.125 [d]	25 [d]	0.195	0.39
VRE162	0.78[c]	6.125 [d]	0.39	1.56
VRE165	0.78 [c]	3.125 [b]	0.39	1.56
VRE170	1.56 [d]	12.5 [d]	0.39	1.56
VRE177	0.39 [d]	3.125 [d]	0.05	0.097

Table 3
In silico analysis of ADME/T properties of *O. basilicum* phytocompounds determined using Swiss ADME software

Characteristic properties	Phytocompounds								
	Adipic acid ethyl 2-octyl ester	1-Ethyl citrate	L-Glutamic acid 5-ethyl ester	Imidazole	Palmitic acid	Phthalic anhydride	2-Propenoic acid 3-phenyl-methyl ester	Stearic acid	Linezolid
Physicochemical properties									
SMILES	CCCCCCC(OC(=O)CCCCC(=O)OCC)C	CCOC(=O)CC(C(=O)O)(CC(=O)O)O	C(CC(=O)O)C(C(=O)O)N	c1ncc[nH]1	CCCCCCCCCCCCCCCC(=O)O	O=C1OC(=O)c2c1cccc2	COC(=O)C=Cc1ccccc1	CCCCCCCCCCCCCCCCCC(=O)O	CC(=O)NCC1CN(C(=O)O1)C2=CC(=C(C=C2)N3CCOCC3)F
No. heavy atoms	20	15	12	5	18	11	12	20	24
No. arom. heavy atoms	0	0	0	5	0	6	6	0	6
Fraction Csp3	0.88	0.62	0.71	0.00	0.94	0.00	0.10	0.94	0.5
No. rotatable bonds	14	7	6	0	14	0	3	16	5
No. H-bond acceptors	4	7	5	1	2	3	2	2	5
No. H-bond donors	0	3	2	1	1	0	0	1	1
Molar Refractivity	81.60	46.60	41.53	18.59	80.80	36.19	47.43	90.41	91.06
Topological polar surface area ($A^{\circ 2}$)	52.60	121.13	89.62	28.68	37.30	43.37	26.30	37.30	71.11
Lipophilicity									
Log $P_{o/w}$ (iLOGP)	3.63	0.43	1.26	0.00	3.85	1.16	2.30	4.30	2.58
Log $P_{o/w}$ (XLOGP3)	4.26	-1.03	-3.00	0.06	7.17	1.60	2.62	8.23	0.69
Log $P_{o/w}$ (WLOGP)	4.01	-0.77	-0.26	0.41	5.55	1.00	1.76	6.33	0.78
Log $P_{o/w}$ (MLOGP)	3.17	-.079	-2.45	-0.92	4.19	1.65	2.20	4.67	0.99
Log $P_{o/w}$ (SILICOS-IT)	4.41	-0.72	-0.30	1.36	5.25	1.87	2.20	6.13	1.25
Consensus Log $P_{o/w}$	3.90	-0.58	-0.95	0.18	5.20	1.45	2.22	45.93	1.26
Water solubility									
Log *S* (ESOL)	-3.38	-0.009	1.36	-1.04	-5.02	-2.17	-2.67	-5.73	-2.22
Solubility	1.21e-01 mg/ml; 4.21e-04 mol/l	1.77e+02 mg/ml; 8.05e-01 mol/l	4.01e+03 mg/ml; 2.29e+01 mol/l	6.21e+00 mg/ml; 9.12e-02 mol/l	2.43e-03 mg/ml; 9.49e-06 mol/l	1.00e+00 mg/ml; 6.76e-03 mol/l	3.48e-01 mg/ml; 2.15e-03 mol/l	5.26e-04 mg/ml; 1.85e-06 mol/l	2.03e+00 mg/ml; 6.01e-03 mol/l
Class	Soluble	Very soluble	Highly soluble	Very soluble	Moderately soluble	Soluble	Soluble	Moderately soluble	Soluble
Log *S* (Ali)	-5.08	-1.03	1.68	-0.22	-7.77	-2.12	-2.82	-8.87	-1.76
Solubility	2.40e-03 mg/ml; 8.39e-06 mol/l	2.07e+01 mg/ml; 9.42e-02 mol/l	8.38e+03 mg/ml; 4.78e+01 mol/l	4.14e+01 mg/ml; 6.08e-01 mol/l	4.31e-06 mg/ml; 1.68e-08 mol/l	1.12e+00 mg/ml; 7.55e-03 mol/l	3.48e-01 mg/ml; 2.15e-03 mol/l	3.80e-07 mg/ml; 1.33e-09 mol/l	5.86e+00 mg/ml; 1.74e-02 mol/l
Class	Moderately soluble	Very soluble	Highly soluble	Very soluble	Poorly soluble	Soluble	Soluble	Poorly soluble	Very soluble
Log S (SILICOS-IT)	-4.51	0.60	-.023	-1.08	-5.31	-2.45	-2.55	-6.11	-3.19
Solubility	8.81e-03 mg/ml; 3.08e-05 mol/l	8.77e+02 mg/ml; 3.98e+00 mol/l	8.38e+03 mg/ml; 4.78e+01 mol/l	5.61e+00 mg/ml; 8.24e-02 mol/l	1.25e-03 mg/ml; 4.88e-06 mol/l	5.30e-01 mg/ml; 3.58e-03 mol/l	4.54e-01 mg/ml; 2.80e-03 mol/l	2.19e-04 mg/ml; 7.71e-07 mol/l	2.18e-01 mg/ml; 6.47e-04 mol/l
Class	Moderately soluble	Soluble	Soluble	Soluble	Moderately soluble	Soluble	Soluble	Poorly soluble	Soluble
Pharmocokinetics									
GI absorption	High	High	High	High	High	High	High	High	High
BBB permeation	Yes	No	No	No	Yes	Yes	Yes	No	No
P-gp substrate	No	No	No	No	No	No	No	No	Yes

(*continued on next page*)

Table 3 (*continued*)

Characteristic properties	Phytocompounds								
	Adipic acid ethyl 2-octyl ester	1-Ethyl citrate	L-Glutamic acid 5-ethyl ester	Imidazole	Palmitic acid	Phthalic anhydride	2-Propenoic acid 3-phenyl-methyl ester	Stearic acid	Linezolid
CYP1A2 inhibitor	No	No	No	No	Yes	No	No	Yes	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	Yes	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	Yes	No	No	No	No	No	No	No	No
Log K_p (skin permeation) (cm/s)	-5.02	-8.37	-9.50	-6.67	-2.77	-6.07	-5.43	-2.19	-7.87
Drug likeness									
Lipinski	Yes; 0 violation	Yes, 0 violation	Yes, 0 violation	Yes; 0 violation	Yes; 1 violation: MLOGP>4.15	Yes; 0 violation	Yes; 0 violation	Yes; 1 violation: MLOGP>4.15	Yes; 0 violation
Ghose	Yes	No; 1 violation: WLOGP<-0.4	Yes	No; 3 violations: MW<160, MR<40, #atoms<20	Yes	No; 3 violations: MW<160, MR<40, #atoms<20	Yes	No; 1 violation: WLOGP>5.6	Yes
Veber	No; 1 violation	Yes	Yes	Yes	No; 1 violation: Rotors>10	Yes	Yes	No; 1 violation: Rotors>10	Yes
Egan	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No; 1 violation: WLOGP>5.88	Yes
Muegge	Yes	Yes	No; 2 violations: MW<200, XLOGP3<-2	No; 2 violations: MW<200, #C<5	No; 1 violation: XLOGP3>5	No; 1 violation: MW<200	No; 1 violation: MW<200	No; 2 violations: XLOGP3>5, Rotors>15	Yes
Bioavailability score	0.55	0.56	0.55	0.55	0.85	0.55	0.55	0.85	0.55
Medicinal chemistry									
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	1 alert
Brenk	1 alert: more_than _ 2_esters	0 alert	0 alert	0 alert	0 alert	2 alerts: beta_keto_anhydride, more_than_2_esters	1 alert: michael_acceptor_1	0 alert	0 alert
Lead likeness	No; 2 violations : 2 violations: Rotors>7, XLOGP3>3.5	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 2 violations: Rotors>7, XLOGP3>3.5	2 alerts: beta_keto_anhydride, more_than_2_esters	No; 1 violation: MW<250	No; 2 violations: Rotors>7, XLOGP3>3.5	Yes
Synthetic accessibility	3.04	2.94	2.23	1.0	2.31	1.49	1.91	2.54	3.32

diagram (Fig. S1) also revealed the overall fitness of the ADMET properties of the 8 compounds and antibiotic linezolid revealing their fitness to the ADMET features.

The *in silico* toxicity of the 8 compounds was predicted using the ProTox II tool and this analysis provided the LD_{50} value and the toxicity class of compounds tested. The LD_{50} value of ethyl citrate was predicted as 5900 and it belongs to toxicity class VI. Adipic acid, ethyl citrate, glutamic acid, and propenoic acid LD_{50} value was predicted as greater than 2000 and found to be toxicity class V. Palmitic acid, phthalic anhydride, stearic acid and the tested antibiotic (linezolid) LD_{50} value forecasted as $300 < LD_{50} \leq 2000$. Thus, these compounds belonged to toxicity class IV. The least LD_{50} value (220 mg/kg) was predicted for imidazole with toxicity class III. Moreover, all eight compounds were predicted to be inactive for hepatotoxicity.

3.3. Molecular docking

The target proteins such as 5ZHW, 4FUO, 1E4E, 4ECL, 6GED, 6ORI of *E. faecalis* and selected 8 phytocompounds such as adipic acid, ethyl citrate, glutamic acid 5-ethyl ester, imidazole, palmitic acid, phthalic anhydride, 2-Propenoic acid 3-phenyl-methyl ester, stearic acid was used to assess protein-ligand interaction. Autodock Vina results were created as a text file and the results of the docking study and the value of root mean square deviation (RMSD) were stored in the specific folder in a text file containing ten active torsion angles of the ligand docking with receptors from *E. faecalis*. Among the ten binding affinity score values, the first value in the RMSD table was found to be better with maximum docking affinity with the receptor protein and was expressed in kcal/mol. Among the 8 phytocompounds, phthalic anhydride had a good binding affinity with all the receptors of *E. faecalis* (Fig. 2). The binding affinity value of phthalic anhydride was found to be above -6.0 kcal/mol for the receptors such as 5ZHW, 4FU0, 1E4E, 4ECL, 6GED, 6ORI and the remaining phytocompounds showed less than -6.0 kcal/mol. Similarly, linezolid showed a binding affinity of -6.0 kcal/mol for the receptors, 5ZHW, 4FUO, 4ECL, 6GED and 6ORI (Table 4).

3.4. Visualization of docking results

The prepared protein and individual ligands from complex results were converted into pdbqt format, the rigid format of a molecule to picture *via* Biovia Discovery studio visualizer 2021 client. The binding affinity between the Enterococcal receptors and ligands (phytocompounds) was posturized and the interacted amino acid in the receptor protein was determined.

The interaction between *E. faecalis* receptor 5ZHW and phthalic anhydride was involved with 4 hydrogen bonds and 5 hydrophobic bonds. The amino acids SER216, TYR242, ILE222, ARG223, and TYR254 were involved in 9 reactions. A: SER216: HG-: UNL1:O showed closest interaction with 2.65788 Å. The interaction with the ligand linezolid involved 3 hydrogen bonds, 1 halogen bond, 2 electrostatic and 3 hydrophobic interactions. ARG223, ASP213, ASP228, ILE222, TRY254 were the amino acid residues involved in the interactions. The closest distance (2.62644 Å) was observed in A: ARG223:HH11-: UNL1: O (Fig. 3a).

The FUO and phthalic anhydride interaction formed 2 conventional type hydrogen bonds with the amino acids LYS195, ADP401 and 3

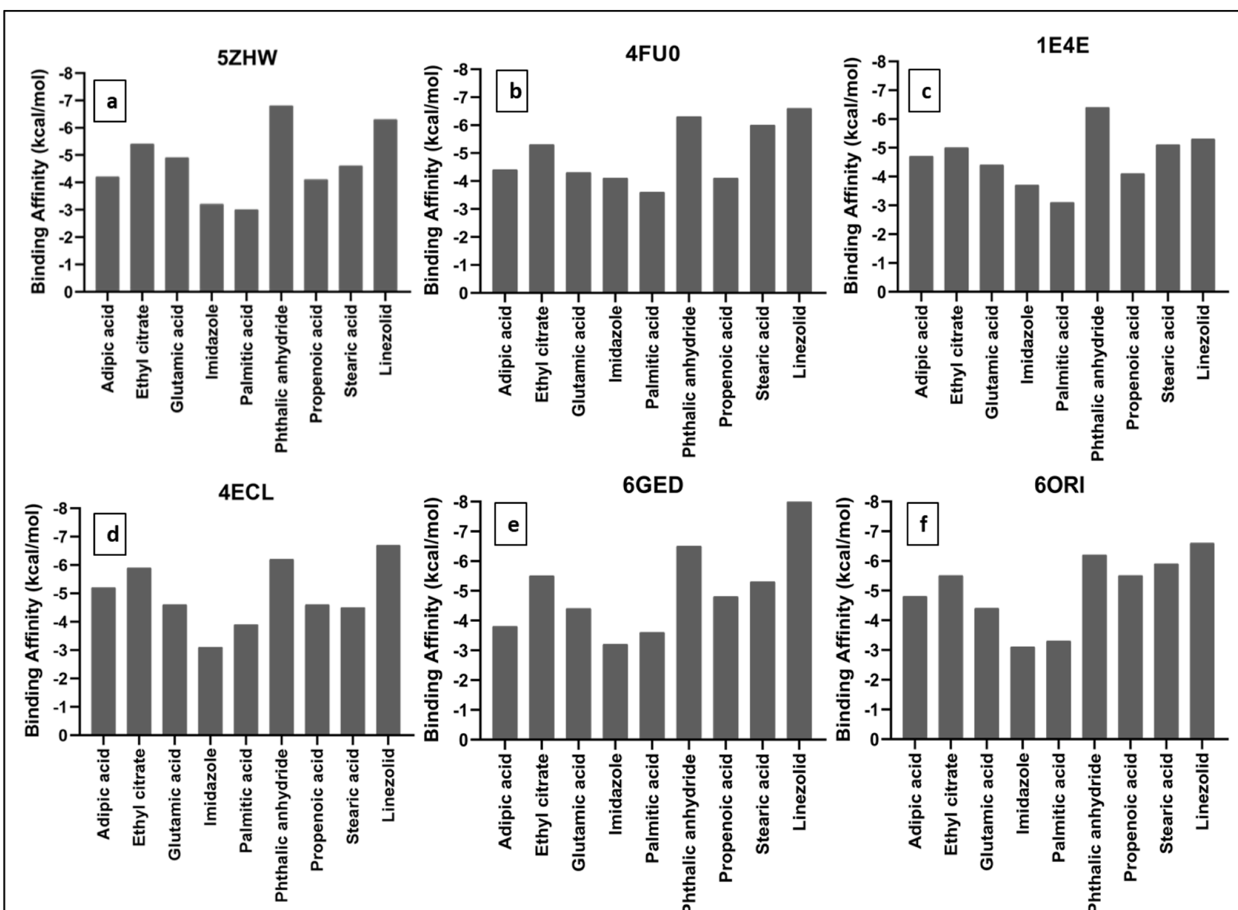

Fig. 2. The bar chart showing the RMSD values of binding affinity between *Enterococcus faecalis* bacterial cell receptors (5ZHW, 4FUO, 1E4E, 4ECL, 6GED & 6ORI) and phytochemicals (adipic acid, ethyl citrate, glutamic acid, imidazole, palmitic acid, phthalic anhydride, propenoic acid & stearic acid) and the positive control, linezolid.

Table 4
RMSD values of binding affinity between Enterococcus faecalis cell receptors and phytochemicals (A-Adipic acid; B-Ethyl citrate; C-Glutamic acid; D-Imidazole; E-Palmitic acid; F-Phthalic anhydride; G-Propenoic acid; H- Stearic acid; I-Linezolid [positive control]; The binding affinity values higher than -6.0 were shown in red colour)

E. faecalis receptors	Phytochemical compounds (Ligands) Binding affinity (kcal/mol)								
	A	B	C	D	E	F	G	H	I
5ZHW	-4.2	-5.4	-4.9	-3.2	-3.0	-6.8	-4.1	-4.6	-6.3
4FU0	-4.4	-5.3	-4.3	-4.1	-3.6	-6.3	-4.1	-6.0	-6.6
1E4E	-4.7	-5.0	-4.4	-3.7	-3.1	-6.4	-4.1	-5.1	-5.3
4ECL	-5.2	-5.9	-4.6	-3.1	-3.9	-6.2	-4.6	-4.5	-6.7
6GED	-3.8	-5.5	-4.4	-3.2	-3.6	-6.5	-4.8	-5.3	-8.2
6ORI	-4.8	-5.5	-4.4	-3.1	-3.3	-6.2	-5.5	-5.9	-6.6

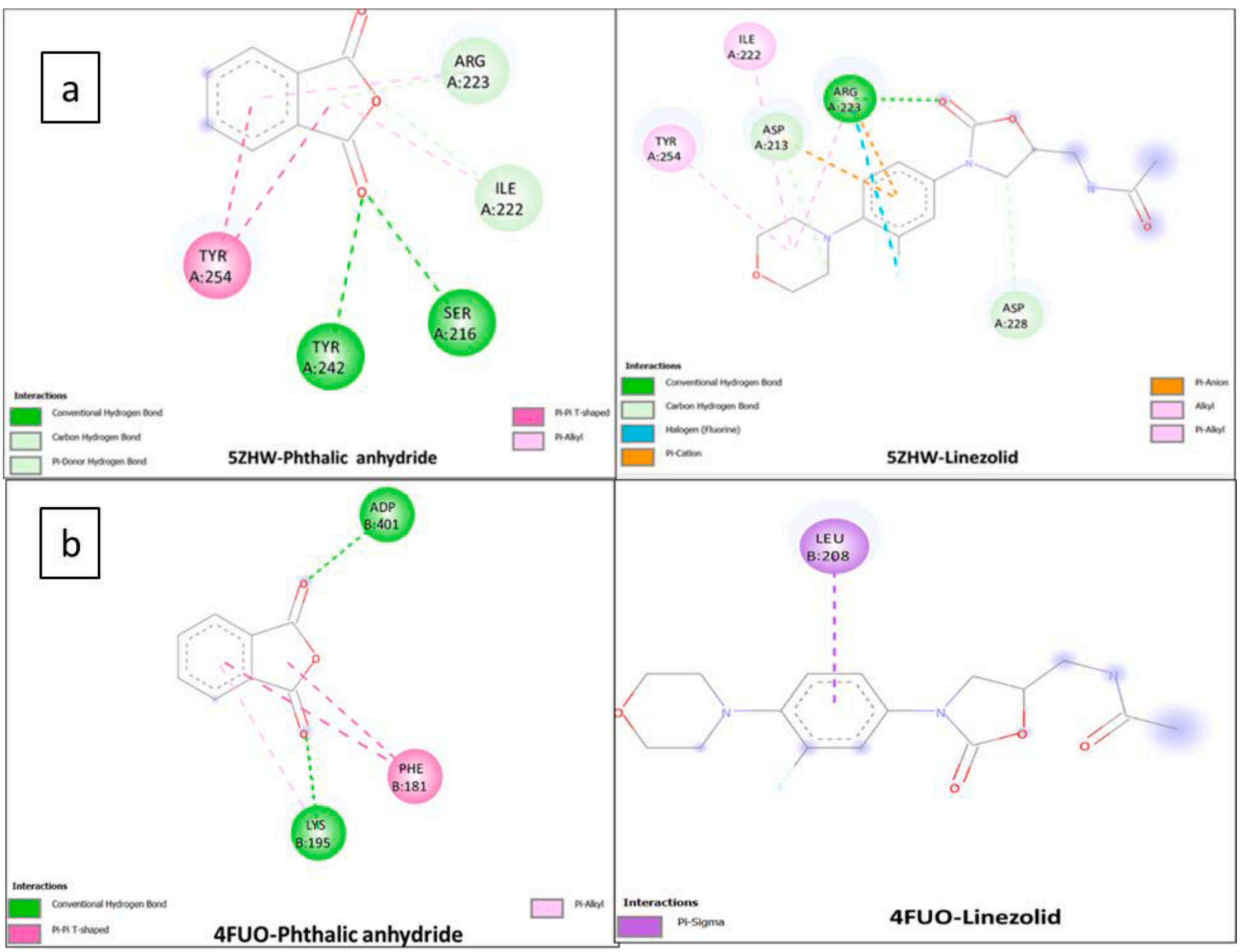

Fig. 3. Binding affinity between *E. faecalis* receptor (a) 5ZHW, (b) 4FUO and ligand phthalic anhydride (left) commercially available antibiotic linezolid (right)

hydrophobic (2 with T- shaped Pi-Pi and 1 with Pi-Alkyl) interactions with amino acid residues PHE181, LYS195. The shortest distance (2.05287 Å) was observed between B:ADP401:H2- UNL1-O. The interaction between FUO and linezolid formed with 1 hydrophobic force with LEU 208 with a distance of 3.90418 Å (Fig. 3b).

The interaction between the receptor 1E4E and phthalic anhydride was due to 2 hydrogen bonds and 4 hydrophobic. LYS171, PHE241, PHE169, VAL181, ILE240 were the amino acids involved in 6 interactions and ALYS171:HZ2-: UNL1:O showed strong interaction as it showed the least distance value of 2.62456 Å. Similarly, the interaction with linezolid was involved with 3 hydrogen bonds and ALA149, ARG300 were involved in 3 interactions. A: ALA149:HN-: UNL1:O showed the closest distance 1.88282 Å (Fig. 4a).

The phytocompound phthalic anhydride formed 6 hydrogen bonds with the active site amino acid residues ALA164, ASP165, SER203, TYR2-5, GLY206, SER203 of 4ECL receptor of *E. faecalis*. Further, 2 hydrophobic interactions of the types Pi-sigma, Pi-PI T shaped interactions with TYR205. The ligand linezolid formed 7 hydrogen bonds with ARG132, SER229, ASN358, CYS313, GLN316, GLY264 amino acid residues of 4ECL receptor. It also formed 1 halogen bond with CYS162 amino acid residue, 1 attractive electrostatic force with LYS311 and 1 hydrophobic interaction with HIS160. The closest binding was observed in A: SER229: HG -: UNL1:O with a distance of 1.8612 Å (Fig. 4b).

Phthalic anhydride formed one conventional hydrogen bond with DGC:3 with a distance of 2.35918 Å. Similarly, one conventional type hydrogen bond and one carbon type hydrogen bond are involved between 6GED and linezolid. In the 6GED receptor, DGC, DGD and DCC present in chains C and D were involved in the interaction (Fig. 5a).

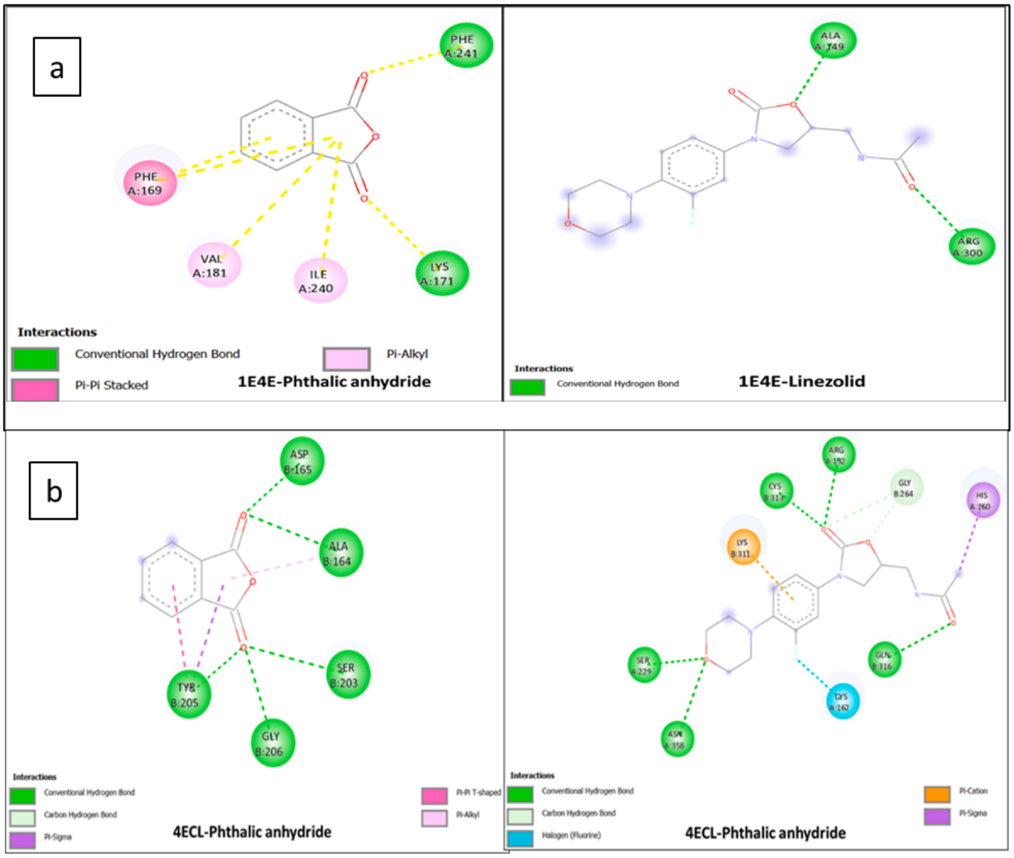

Fig. 4. Binding affinity between *E. faecalis* receptor (a)1E4E, (b) 4ECL and ligand phthalic anhydride (left) commercially available antibiotic linezolid (right)

The phthalic anhydride formed one conventional hydrogen bond with the amino acid ARG124 and one Pi-Alkyl bond with MET 213 amino acid of *E. faecalis* receptor. Similarly, linezolid formed two conventional hydrogen bonds and two carbon-hydrogen bonds with the amino acids (LYS 200, LYS200, I204, ASP196) of *E. faecalis* receptors. The strongest interaction was observed between A:LYS200:HZ3:B - : UNL1:O with a distance of 2.91436 Å (Fig. 5b).

4. Discussion

Increasing emergence of multi-drug resistant pathogens among the health care sector and unsolicited side effects of certain commercially available antibiotics has fashioned the awareness towards new bioactive compounds of plant origin. The present antimicrobial study results reveal that ethanolic extract of *O. basilicum* is effectively suppressing the growth of all the 12 strains of VR *E. faecalis*. This result was not amazing as one of our previous studies [16] reported that the plant extract inhibited Gram-negative MTCC strains. While the activity of *O. basilicum* against Gram-negative MTCC strains (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii*) was lesser than VR *E. faecalis* assessed in this study. The higher susceptibility of Gram-positive pathogens than Gram-negative bacteria may be due to differences in cell wall composition, in terms of the hydrophilic surface of the outer membrane and efflux pump that exclude a broad spectrum of substances including antibiotics and phytocompounds [19].

According to the tube dilution assay, the best antibacterial activity was observed for the ethanolic extract of sweet basil leaves, whose MIC ranged from 0.39 to 3.125 to inhibit the VR *E. faecalis* strains. According to Silva et al. [20], we considered MIC less than or equal to 1.56 mg/mL as considerable as effective inhibition. The MIC to completely inhibit the growth of VR *E. faecalis* is varying from each strain revealing that each strain requires different concentration of plant extract. Overall, the MIC of the ethanolic extract of sweet basil leaves is lesser than the effective MIC according to Silva et al. [20]. This difference in MIC of plant extracts is due to the presence of distinctive chemical constituents and volatile nature of the bioactive compounds [21].

The MIC and MBC of linezolid, a commercially available antibiotic, was approximately 8-16 fold higher than the ethanolic extract of *O. basilicum.* This may be due to the purity of commercially available antibiotics. However, further purification and drug formulation of the sweet basil extract could increase the antimicrobial activity. The present study is also in the same line as Kyaw et al. [22] who stated that commercial antibiotics are more potent than phytochemicals. Application of these phytochemicals as a single agent would always require high concentration for sufficient bioavailability. Due to the great antimicrobial potential of *O. basilicum* against VR *E. faecalis*, we planned to investigate the binding energy between the receptors of VR *E. faecalis* and the phytocompounds of sweet basil leaves identified using GC-MS analysis.

Drug discovery is a very complicated, expensive and time-consuming process as each step such as identification, characterization, production and validation need a couple of years with a well-trained scientist team [23]. Nowadays, the designing and validation of a drug molecule can be easy within a short time with the help of advancements in bioinformatics and reverse engineering technology [24]. Determination of drug likeness is a qualitative concept nowadays used in drug design with respect to some factors such as bioavailability, which can be estimated based on the molecular structure of the compound. In general, pharmacologists are more interested in the properties of drugs such as structural characteristics (Hydrogen bonding, lipophilicity, molecular weight, polar surface area), physicochemical properties (pH value, chemical stability, solubility, permeability) biochemical properties (protein binding affinity, metabolism) and pharmacokinetics and toxicity (half-life, half lethal dose, bioavailability). According to Lipinski's rule, a molecule to be used as a drug should fulfil Lipinski's rule of five. Based on ADMET analysis, 8

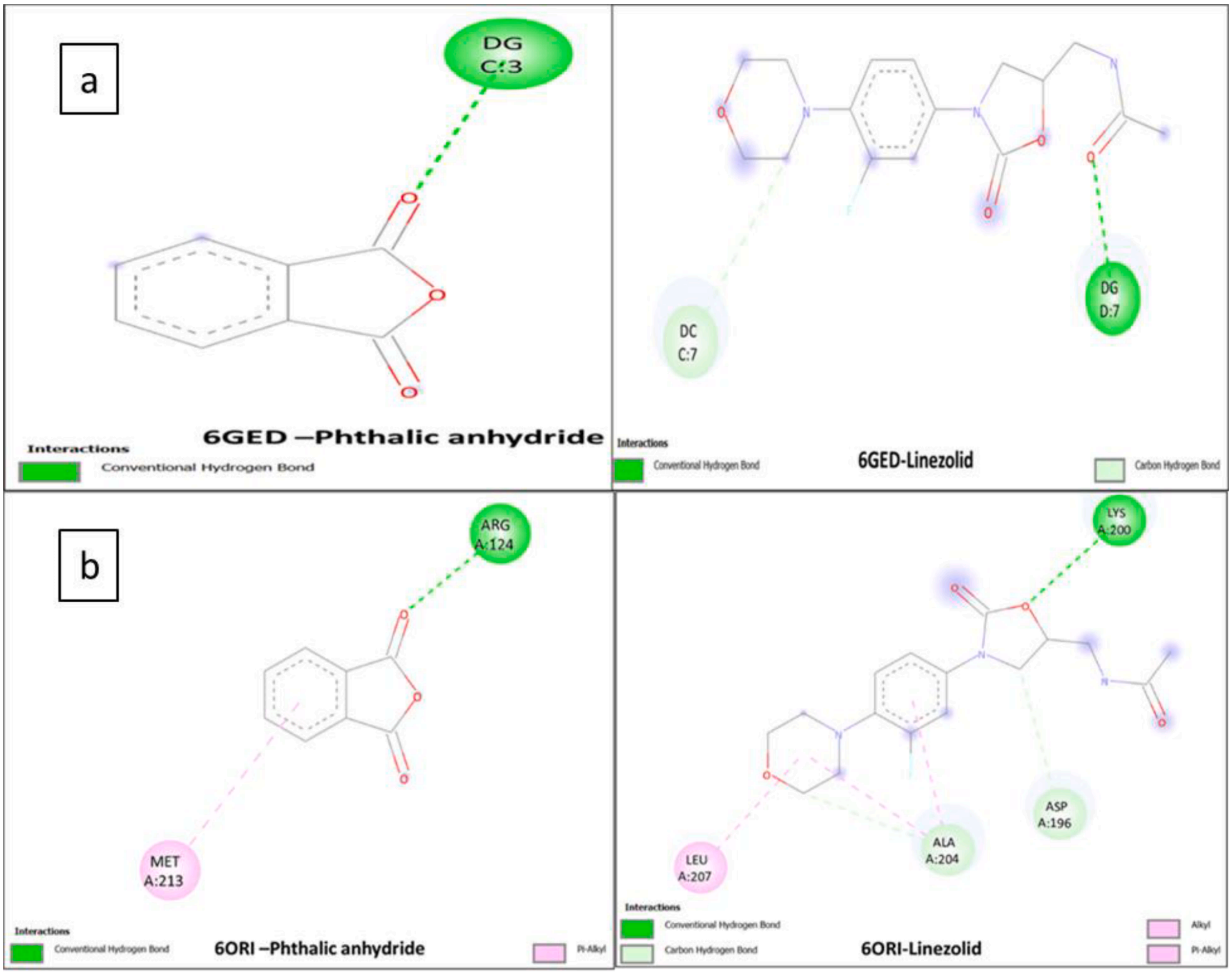

Fig. 5. Binding affinity between *E. faecalis* receptor (a) 6GED, (b) 6ORI and ligand phthalic anhydride (left), commercially available antibiotic linezolid (right)

compounds namely, adipic acid ethyl 2-octyl ester, 1-ethyl citrate, glutamic acid 5-ethyl ester, imidazole, palmitic acid, phthalic anhydride, 2-propenoic acid 3-phenyl-methyl ester, stearic acid are selected as they fulfilled Lipinski's rule of five. In addition, all these 8 compounds are found to be orally active and structurally stable and showed high gastrointestinal absorption. Among all compounds assessed, adipic acid, phthalic anhydride, palmitic acid, and propenoic acid have potential for BBB permeation revealing pharmacokinetics features.

In the case of toxicity endpoints, all 8 compounds were forecasted as inactive for immunogenicity, mutagenicity and toxicity. Similarly, these compounds except adipic acid and imidazole were forecasted to be noncarcinogenic.

Ligand-receptor interaction is a more crucial signalling phenomenon between the cells and other external molecules. This interaction is generally carried out through hydrophobic, non-covalent and van der Waals interaction and these are more significant for signal transduction, immune reaction and gene regulations. Molecular docking is the most advanced method to determine the protein-protein, protein-peptide, ligand-protein, and protein-nucleic acid interactions using various computer tools. Even though so many online open software tools are nowadays available for various docking studies, Autodock Vina is one of the most effective and reliable tools in bioinformatics [25]

Thus, in this study, we used Autodock for protein and ligand preparation and Autodock Vina for docking ligands and protein. The current study result reveals that the phytocompound phthalic anhydride showed stronger binding energy than other phytocompounds tested. Among the various receptors, 5ZHW showed stronger binding with phthalic anhydride and the docking interaction is favoured by H bond with Ser216, Tyr242, Ile222, Arg223 and hydrophobic interactions with amino acids such as Tyr254, Ile222, Arg223. This result reveals that the conserved amino acids serine, tyrosine, isoleucine, and arginine are crucially involved in binding with these receptors.

The higher binding energy values are noted for phthalic anhydride and receptors such as 5ZHW (-6.8 kcal/mol) and 1E4E (-6.4 kcal/mol). Interestingly it is noted that these binding energy values are lesser than the binding energy values of the antibiotic linezolid, which shows -6.3 and -5.3 kcal/mol against 5ZHW and 1E4E respectively. The positive control used in this study namely, linezolid is a common antibiotic used to treat infection caused by VRE. The results obtained in this study suggest that among the 8 compounds of ethanolic extract of *O. basilicum*, phthalic anhydride showed better and stronger binding energy towards the VRE target receptors and might be considered as a good inhibitor of VRE pathogens.

5. Conclusion

Natural bioactive compounds have been replacing synthetic drugs and antibiotics in treating infections and other disorders including stress-related illnesses. The current study results reveal an authentic solution for treating VR enterococcal infection by using effective phytocompounds. The ethanolic extract of *O. basilicum* demonstrated greater potential against 12 different VR *E. faecalis* strains at justifiable concentrations. In addition, the *in silico* study proved that the 8 phytocompounds identified from ethanolic extract of *O. basilicum* were found to be safe, non-toxic and non-carcinogenic. Further, phthalic anhydride showed a strong affinity towards the VR *E. faecalis* receptors revealing their antipathogenic potential. In the future, their toxicity potential will be assessed via both *in vitro* and *in vivo* approaches in order to consider the phytocompound (phthalic anhydride) of *O. basilicum* as a better therapeutic compound to treat VR enterococcal infections, as antibiotic resistance may be overwhelming.

CRediT authorship contribution statement

Senbagam Duraisamy: Conceptualization, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Arockia Doss Susai Backiam:** Investigation, Methodology. **Amutha Raju:** Writing – review & editing, Formal analysis. **Sukumar Ranjith:** Writing – review & editing. **Anbarasu Kumarasamy:** Writing – review & editing. **Senthilkumar Balakrishnan:** Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chphi.2024.100499.

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