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Molecular docking studies, in vitro-brine shrimp lethal assay and antibacterial assessment of embelin, vilangin and phenyl vilangin against endodontic pathogen, *Enterococcus faecalis*

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Abstract

Complete debridement and disinfection of the root canal system are the primary requirement for successful endodontic treatment. *Enterococcus faecalis* is the most commonly found organism in a high percentage of root canal failures as a single organism or as a major component of the flora. Primary goal of this study is to assess the antibacterial effect of phytochemical agents namely embelin, vilangin and phenyl vilangin, respectively, against *E. faecalis*. And this is the first time that these materials are reported for antibacterial activity against the pathogen, *E. faecalis*. Our second goal is to predict the interaction between the active structure and the protein in the binding mode, and the third goal is to screen for cytotoxicity by brine shrimp lethality bioassay. All the compounds showed low to elevated level of antibacterial activity. Particularly, compound embelin, as well as its combination with NaOCl in the ratio 1:1, exhibits zone of inhibition value of 29 ± 0.89 and 32 ± 0.98 mm at a concentration of 80 µL and the MIC value of embelin was found to be 78.10 µg/mL. All the compounds exhibit low toxicity to high toxicity. The LC_{50} value of tested

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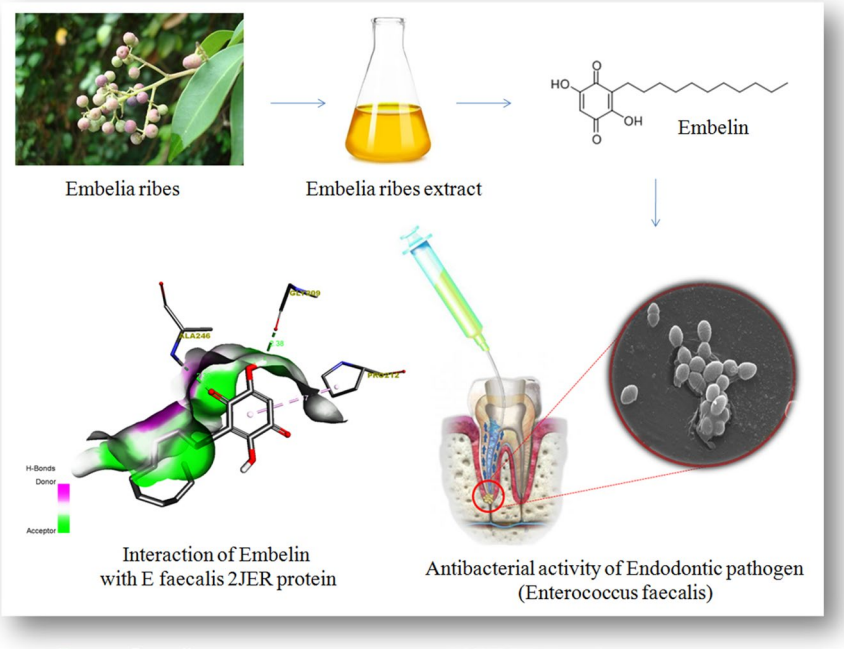
compounds was in the range of 17.5–80.0 µL with respect to positive control (vincristine sulphate). The result of lethality reveals the phenyl vilangin exhibits very minimal toxicity even under higher concentration, and in future, it may be investigated further for biocompatibility studies. The results of molecular docking reveal that embelin showed good interaction against 2JER protein with a higher binding energy value of − 7.2. Hence, these finding reveals that embelin and phenyl vilangin may be used as root canal irrigants in future.

Graphic abstract



Keywords Embelin · Vilangin · phenyl vilangin · Lethal assay · Cytotoxic · Docking and disc diffusion

Introduction

Enterococci are gram-positive pathogen, possessing the ability to grow in the presence or absence of oxygen. *E. faecalis* is found in 4–40% of primary endodontic infections. In fact, failed root canal treatment cases are nine times more likely to contain E. faecalis than primary endodontic infections. *Enterococcus faecalis* is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24 to 77%. Unlike primary endodontic infections, which are polymicrobial in nature and dominated by

gram-negative anaerobic rods, the microorganisms involved in secondary infections are composed of one or a few bacterial species. Among that, *E. faecalis* is the major and most commonly found organism in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora [1]. Enterococci survive very harsh environments including extreme alkaline pH (9.6) and salt concentrations.

Sodium hypochlorite is an effective irrigant for all presentations of *E. faecalis* including its existence as a biofilm [2]. The major drawback of sodium hypochlorite is that at high concentration of chloride ions, it possesses excellent antibacterial effect but at the same time, more cytotoxic towards normal tissues [3]. In order to resolve this issue, we are in constant search of an alternative irrigant over hypochlorite. By considering the biological structure and protein structure of *E. faecalis,* we preferred to try embelin, vilangin and phenyl vilangin as alternate phytochemical irrigants for endodontic treatment.

The aim of the present investigation is to assess the antibacterial activity of embelin, vilangin and phenyl vilangin against *E. faecalis* by disc diffusion method, and also, the MIC value was assessed for antibacterial investigation [4, 5]. Brine shrimp lethality assay was performed to study about the cytotoxicity of phytochemical irrigants based on the mortality percentage of nauplii and LC_{50} value. Finally, molecular docking method which is a recent technique to get insight about the exact binding location of the ligand and protein was also done. Embelin, vilangin, and phenyl vilangin ligands are selected to dock at the active areas of *2JER* of *E. faecalis* protein, and the binding energy value was calculated based on the docking efficiency.

Materials and methods

Chemicals and reagents

Embelin, formaldehyde, benzaldehyde, sodium hypochlorite, DMSO and Vincristine sulphate were sourced from Sigma Aldrich. Enterococcus Faecalis, Mueller-Hinton agar (MHA) and Sabouraud Dextrose agar were sourced from Sisco research laboratory chemicals, Mumbai, The optimized ligand molecules were docked into refined Enterococcus Faecalis protein (PDB ID: 2JER) protein using "LigandFit" in the AutoDock 4.2.6

Synthesis of vilangin and phenyl vilangin

Compound embelin was isolated from the berries of *Embelia ribes* Burm.f. (Family: Primulaceae). Vilangin and phenyl vilangin were synthesised by simple condensation of embelin with formaldehyde and benzaldehyde in acetic acid medium as reported by Bheema sankara Rao, and the synthetic scheme is shown in Fig. 1 [6]. The complete synthetic procedure and characterisation of phenyl vilangin involving Mass and NMR were reported in our previous work [7]

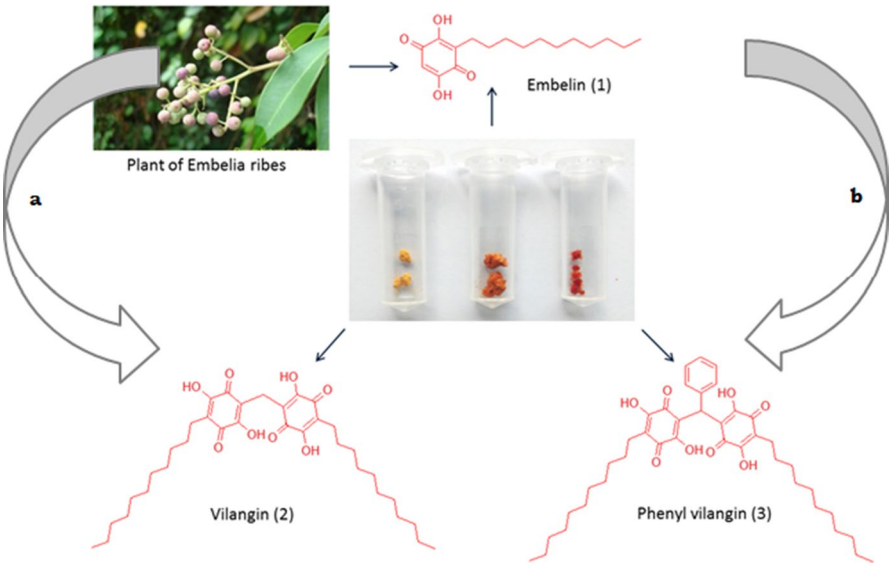


Fig. 1 Synthesis of vilangin (2) and phenyl vilangin (3) from embelin (1) by reacting with formaldehyde (a) and benzaldehyde (b)

Antimicrobial activity

Disc diffusion method

The antimicrobial activity of embelin and its derivatives were investigated through agar disc diffusion method. Mueller-Hinton agar (MHA) medium and Sabouraud Dextrose agar (SDA) medium are employed to study the antibacterial activity of samples against E. faecalis. Nutrient agar slant loaded with stock cultures was maintained at 4 °C. Active cultures for the study were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth that were incubated at 37 °C for 24 h. Sabouraud Dextrose agar and Mueller-Hinton agar media were poured and uniformly dispersed into the petri plates. Once the medium solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. 5 mm diameter of sterile discs was charged with various concentration (5 µL, 10 µL, 20 µL, 40 µL and 80 µL) of DMSO solution of sample under investigation and placed on the agar plates. The plates were incubated for 24 h, at 37 °C. All determinations were studied in triplicates. The diameters of zone of inhibition were correlated with standard hypochlorite. Determination of zone of inhibition by disc diffusion method was performed in two categories as shown in Table 1 (Determination of zone of inhibition of sample against standard hypochlorite) and Table 2 (Determination of zone of inhibition of sample having 1:1 ratio of hypochlorite and embelin derivatives.

Table 1 Zone of inhibition (mm) of samples and standard hypochlorite against E. Faecalis pathogen

Sample	Zone of inhibition (mm) at various concentration				
	5 µL	10 µL	20 µL	40 µL	80 µL
Embelin	10 ± 1.01	14 ± 0.65	20 ± 0.55	23 ± 0.65	29 ± 0.89
Vilangin	9 ± 0.95	12 ± 0.98	14 ± 0.50	16 ± 0.87	17 ± 0.75
Phenyl vilangin	10 ± 0.82	13 ± 1.01	15 ± 0.85	19 ± 0.54	23 ± 0.88
Sodium hypochlorite	20 ± 1.1	25 ± 0.82	29 ± 46	32 ± 0.78	40 ± 0.65

Table 2 Zone of inhibition (mm) of sample (Compound:HOCl, 1:1 ratio) and minimum inhibitory concentration of compound and standard hypochlorite against *E. Faecalis* pathogen

Sample	Zone of inhibition (mm) at various concentration					MIC[a] (µg/mL)
	5 µL	10 µL	20 µL	40 µL	80 µL	
Embelin:HOCl	13 ± 0.5	21 ± 0.97	24 ± 0.89	29 ± 0.23	32 ± 0.98	78.10
Vilangin:HOCl	9 ± 0.87	13 ± 0.78	15 ± 0.56	18 ± 0.65	19 ± 0.56	312.39
Phenyl vilangin:HOCl	12 ± 0.23	15 ± 0.97	17 ± 0.21	21 ± 0.98	26 ± 0.75	156.20
Sodium hypochlorite	–	–	–	–	–	39.05

[a]Minimum Inhibitory Concentration values are highlighted for embelin, vilangin and phenyl vilangin and sodium hypochlorite

Minimal inhibitory concentration (MIC)

Serial dilution of twofold was preferred to attain the concentration range of 1250–9.762 µg/mL. All the compounds including standard hypochlorite of different concentration range as mentioned above were prepared in sterile microwells to determine minimum inhibitory concentration. According to Sarker et al. [8], minimal inhibitory concentration was determined in nutrient broth accompanied with resazurin dye. All the samples and standard hypochlorite were dissolved in 1% DMSO and added to nutrient broth to a maximum dose of 1250 µg/mL. The microtitre plates were enclosed and the substances were agitated by plate shaker at the incubation temperature of 38 °C for 24 h. The microbial growth was observed by colour change in the wells.

Brine shrimp lethality assay

Hatching of the brine shrimp

In the present investigation, we employed brine shrimp lethality to determine the cytotoxicity of embelin, vilangin and phenyl vilangin [9, 10]. The brine shrimp eggs were procured from Philadelphia (USA). A rectangular jar containing 3 L of water was mixed with 28g ms of NaCl, and the jar was provided with proper aeration using air pump. About 15 gms of brine shrimp was sprinkled into the jar and the

compartment was illuminated by using 60–100 Watt bulb, placed few distance away from the jar. After incubation for 24 h, the hatched nauplii were separated from the empty egg and utilised for the experiment.

Bioassay

The suspension of brine shrimp nauplii (10 nos) was charged into test tube containing different concentrations (5 µL, 10 µL, 20 µL, 40 µL, 80 µL) of embelin, vilangin, phenyl vilangin and Vincristine sulphate (Control). And the tubes were left at room temperature for 24 h. After 24 h, the number of non-motile nauplii in each tube was counted. Again after 48 h, the number of survivors was observed and the mortality was recorded. The mortality endpoint of this bioassay is absence of controlled forward motion of nauplii for 30 s of observation. The mortality percentage of nauplii in each test tube was calculated using the formula given as follows:

$$\% \text{ Mortality } = \left[\text{Number of dead nauplii/Total number of nauplii under investigation}\right] * 100$$

Molecular docking

Protein preparation for docking

The 3D structure of Agmatine Catabolism of Enterococcus Faecalis (PDB ID: 2JER) was downloaded from Protein Data Bank (PDB), and before initiating the docking simulations, all non-protein molecules were removed from 2JER; for any alternative atom locations, only the first location was retained. All the docking calculations were performed by using AutoDock 4.2.6. E. faecalis protein was modified by adding polar hydrogens and then kept rigid in the docking process, whereas all the torsional bonds of ligands were set free by Ligand module in AutoDock Tools.

Ligand preparation for docking

The phenyl vilangin ligand was built using Chemsketch and optimized using "Prepare Ligands" in the AutoDock 4.2.6 for docking studies. The optimized ligand molecules were docked into refined *E. faecalis* protein (PDB ID: 2JER) using "Ligand Fit" in the AutoDock 4.2.6.

Computational details

All theoretical computations (quantum chemical calculations) were performed in Becke3-Lee-Yang-parr (B3LYP) with 6–311 + + G (d,p) functional by Gaussian 09 W program package on a computing system [11]. The electronic properties like HOMO and LUMO energies were confirmed using the DFT method. For achieving chemical reactivity of the molecule, the molecular electrostatic

potential (MEP) surface and the frontier molecular orbitals (FMOs) were visualized using the Gauss View 05 software. In order to understand the electronic properties, the theoretical UV–Visible spectra have been studied using TD-DFT method with 6e311 + + G (d,p) basis set for the gas phase.

Results and discussion

Chemistry

The chemistry of these compounds (1–3) has undergone a surge of interest because of the active pharmacore unit, 2,5-dihydroxy-[1, 4]-benzoquinone [12, 13] and the hydrophobic nature of alkyl chain from embelin, vilangin and benzene from phenyl vilangin. In our present investigation, carbon labelled C5 and C6 of benzoquinone derivative plays a vital role in reactivity (condensation) with formaldehyde and benzaldehyde because of the presence of hydroxyl unit (–OH) at C5 and active methylene unit at C6. The resonance stabilised dianion structure of embelin and phenyl vilangin shown in Fig. 2 exhibits more electron density at C6 and C3 position, which is also reflected from the molecular electrostatic potential diagram of phenyl vilangin. From the colour scheme of MEPs, red represents electron rich, that is the region where the hydroxyl hydrogen involve in resonance with quinonoid carbonyl oxygen and the partially positive charge represented by blue colour and that is due to the + I effect of alkyl chain at C3 position. The reactivity of embelin with formaldehyde and benzaldehyde was also reflected from the yield of the vilangin and phenyl vilangin. The reactivity of anion at C6 of embelin towards the carbonyl carbon of formaldehyde and benzaldehyde differs due to steric reason, because benzaldehyde is more sterically hindered by benzene ring than formaldehyde. Also from the MEPs, the green colour cloud is a neutral region, which comprises of hydrophobic alkyl as well phenyl unit. Hence, this green region only is responsible for the efficient interaction of bacterial cell wall, since it is made up of hydrophobic lipoproteins.

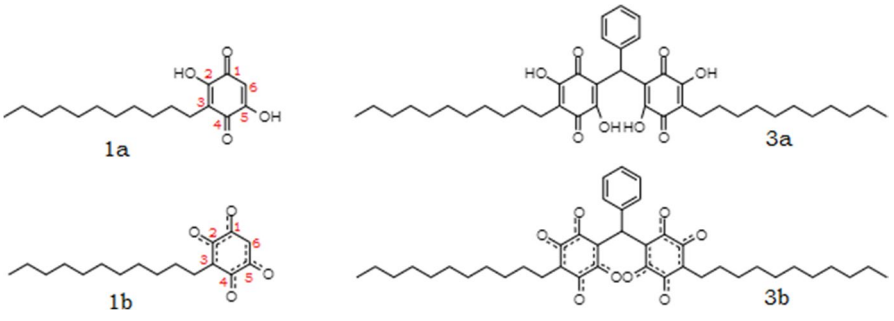


Fig. 2 Structure of embelin (1), phenyl vilangin (3) and its resonance stabilized dianion (1a and 1b)

Investigation of antibacterial activity

In the present study, benzoquinone derivatives were tested for their antibacterial activity against *E. Faecalis* bacteria, (gram-positive). The antibacterial activities of benzoquinone derivatives are being increasingly documented. Depending upon the nature of bacterial structure, some chemical structural modification is required to enhance the antibacterial activity. In the present work, the benzoquinone derivatives viz., embelin, vilangin and phenyl vilangin and the standard hypochlorite were tested for their antibacterial activity against *E. Faecalis* pathogen. The diameter of zone of inhibition and the MIC values are highlighted in Tables 1 and 2. It is explicit from the result that embelin and its combination with NaOCl (1:1) exhibit very good antibacterial activity. Particularly 1:1 ratio of embelin and NaOCl exhibits zone of inhibition value of 32 ± 0.98 mm at a concentration of 80 µL. The trend of zone of inhibition is found to be embelin > phenyl vilangin > vilangin. In addition to antibacterial assay minimum inhibitory concentration against E. faecalis was also investigated at different concentrations. From Table 2, it is clear that *E. Faecalis* is sensitive towards embelin next to standard NaOCl at a concentration of 78.10 µg/mL. Again the trend of MIC is similar to zone of inhibition. According to Overtone's concept of cell permeability, the lipid membrane surrounded by the cell favours the entry of only lipid soluble materials due to which liposolubility is an vital criterion controlling the antibacterial activity.

The hydrophobic/hydrophilic balance of chemical structure is usually regarded as the key factor to achieve pronounced activity against microbes and low toxicity to mammalian cells. In general, the gram-positive bacterial cell walls are usually thicker than gram-negative bacteria and the wall is usually made up of peptidoglycan layers and lipopolysaccharide. In our present study, the hydroxyl group in all the compounds exhibits hydrophilic interactions as electrostatic and hydrogen bonding with that of peptidoglycan layers and the same hydrogen bonding interaction of embelin with that of Enterococcus Faecalis protein is also reflected on docking studies shown in Figs. 5, 6, 7. On the other view, the undecyl unit ($C_{11}H_{23}$) at the third carbon of embelin is hydrophobic and is responsible for the adsorption and penetration of lipopolysaccharides. These two types of interaction can significantly impact the antimicrobial activity of surfaces. Based on the above discussion, the trend of hydrophobic character is phenyl vilangin > vilangin > embelin. Thus, the presence of low hydrophobic and the presence of carbonyl carbon and hydroxyl group have a significant influence on antibacterial activity.

Chemistry in antimicrobial activity

E. faecalis is the major bacteria which is responsible for endodontic infections and commonly found in high contents in root canal failure. Enterococcus and few Streptococcus species are the limited gram-positive bacteria shows significant level of glutathione (GSH). One of the significant functions of glutathione is to protect the cells against toxic substances by regulation of intracellular level of K^+, whose

transport and accumulation plays a vital role in maintenance of cell turgor and homeostasis of intracellular pH. Presence of glutathione in bacterial cell was found to intensify their resistance towards selective antibiotics. A probe through the literature reveals [14–18] that exposure of bacterial cell towards foreign bodies leads to decrease in the level of thiol content, thus the potential of bacterial cells to maintain a high level of glutathione largely determines their resistance to toxic substances. A survey through the literature reveals Streptococcus agalactiae, Streptococcus thermophilus, and Enterococcus faecalis grown in Todd-Hewitt medium must have the ability to synthesis GSH. The synthesis of GSH by these bacteria is the first reported case of GSH production by prokaryotes. Hence, it is more significant to focus on the mechanism behind chemical interaction with bacterial cell. In our present antimicrobial investigation, the trend in antimicrobial activity follows the order embelin > phenyl vilangin > vilangin. The possible mechanism of embelin with bacterial cell is shown in Fig. 3. E. faecalis being a gram-positive bacterium is usually made up of thicker cell wall than gram-negative bacteria, and the chemical constituent of cell wall consists of usually peptidoglycan layers and lipopolysaccharide. Adsorption and penetration are the two important factors which decide the first step of interaction between embelin and bacterial cell. The presence of hydrophobic undecyl unit ($C_{11}H_{23}$) at third carbon of embelin may be responsible for the adsorption and penetration of hydrophobic lipopolysaccharides. Complete exploration through the literature reveals, α, β-unsaturated carbonyl compounds *viz*., conjugated diones enable Michael addition of endogenous peptide glutathione to the olefinic bond. In

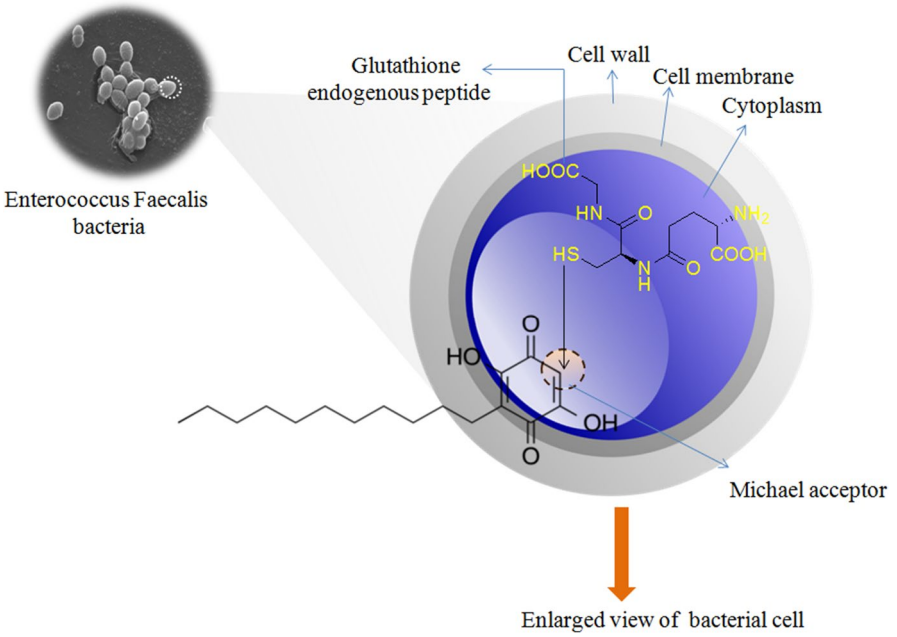


Fig. 3 Plausible mechanism of embelin with bacterial cell through Michael addition of endogenous peptide glutathione to the olefinic bond

our present investigation, there exists same Michael addition at carbon C6 of embelin with nucleophilic thiol unit of glutathione that leads to the formation of oxo anion at C4 carbon as shown in Figs. 3 and 4. The next step involves intra-molecular rearrangement with simultaneous elimination of hydroxyl group in the form of H_2O at C5 carbon. The possible outcome of this mechanism is found to be in good agreement with the work reported by Hubert et al. [19]. The net result from the mechanism indicates the loss of cells natural identity by decrease in the concentration of glutathione content. Thus, the effective reaction of embelin with glutathione is responsible for the antimicrobial activity. In general, most of the antimicrobial studies are concluded based on the zone of inhibition, culture and colony count. When we go for imaging tools, confocal microscopy can be used to study the volume of live and dead bacteria. Even though all these tools are valuable, they are technique sensitive, are prone for contamination during processing leading to altered results. But all these studies usually do not discuss the chemistry or mechanism behind the antimicrobial activity. This makes our study very unique where we have discussed in detail the mechanism behind the antimicrobial activity of embelin concluding that it could be a better alternative as an irrigant in the disciplines of endodontic.

Brine shrimp cytotoxicity studies

The brine shrimp lethality assay represents a rapid, inexpensive and simple it also proved to be a convenient method for monitoring biological activities of synthesised compound under investigation. The present study shows that hatched nauplii are unique for the preliminary cytotoxicity study. The mortality percentage and LC_{50} values of the brine shrimp lethality bioassay obtained for the synthesised compound extracts and that of the positive control, Vincristine sulphate, are presented in

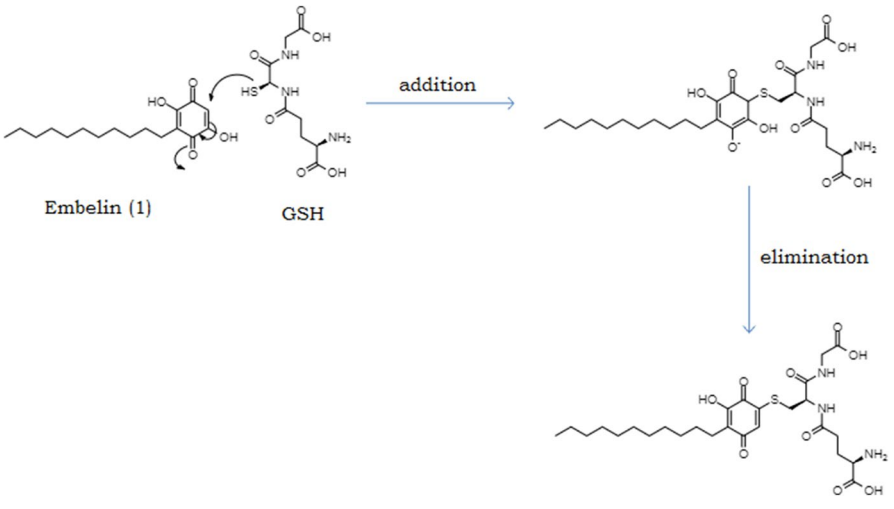


Fig. 4 Suggested pathway for the nucleophilic substitution at embelin

Table 3 Brine shrimp Lethality assay of compounds (1–3)

Compound name	Concentration level (µL)	Number of nauplii (Initial)	Number of nauplii dead after 24 h	Number of nauplii dead after 48 h	% mortality after 24 h	% mortality after 48 h	Brine lethality (LC_{50}, 48 h) µL
Embelin	5	10	0	0	0	0	17.5
	10	10	0	2	0	20	
	20	10	0	6	0	60	
	40	10	0	8	0	80	
	80	10	0	10	0	100	
Vilangin	5	10	0	0	0	0	40
	10	10	0	0	0	0	
	20	10	0	2	0	20	
	40	10	0	5	0	50	
	80	10	0	9	0	90	
Phenyl vilangin	5	10	0	0	0	0	80
	10	10	0	0	0	0	
	20	10	0	0	0	0	
	40	10	0	0	0	0	
	80	10	0	5	0	50	
Control	5–80	10	0	0	0	0	0

Table 3, respectively. The present study determined that the extent of lethality was directly proportional to the concentration of the sample under study.

There was no effect of lethality on any of the concentrations of the extract for 24 h. The brine shrimp were still actively moving in the test materials. All the synthesised compounds were non-toxic towards brine shrimp for 24 h of exposure but there is increase in toxicity at 48 h. Of the three synthesised benzoquinone derivatives (1–3), the mortality rate of embelin and vilangin after 48 h was increasing with increase in concentration from 5 to 80 µL with LC_{50} value of 17.5 and 40 µL. In particular, phenyl vilangin does not exhibit any mortality up to 4 µL and exhibited 50% mortality at concentration of 80 µL and it exhibited LC50 value under the same concentration. Overall, the maximum mortality took place at a concentration range of 40–80 µL. The result of lethality reveals that phenyl vilangin exhibits very minimal toxicity even under higher concentration, and in future, it may be investigated further for biocompatibility studies. The overall trend in lethality follows the order **1 > 2 > 3**. This may be attributed to the increase in hydrophobicity from embelin to phenyl vilangin. The present findings suggest that these compounds when used as root canal irrigants will be more compatible with the harmony of periapical tissues.

Molecular docking studies

Molecular docking studies were performed on embelin, vilangin and phenyl vilangin with Agmatine Catabolism of Enterococcus Faecalis (PDB ID: 2JER) target protein. Enterococcus faecalis makes ATP from Agmatine in three steps catalysed by Agmatine deiminase (AgDI), Putrescine transcarbamylase (PTC), and Carbamate kinase (CK). An antiporter exchanges Putrescine for Agmatine. We have cloned the *E. faecalis* ef0732 and ef0734 genes of the reported gene cluster for Agmatine catabolism, over expressed them in Escherichia coli, purified the products, characterized them functionally as PTC and AgDI, and crystallized and X-ray diffracted them. The *1.65-Angstroms-resolution* structure of AgDI forming a covalent adducts with an Agmatine-derived amidine reaction intermediate is described. We provide definitive identification of the gene cluster for Agmatine catabolism and confirm that Ornithine is a genuine but poor PTC substrate, suggesting that PTC (found here to be trimeric) evolved from Ornithine transcarbamylase. N-(Phosphonoacetyl)-putrescine was prepared and shown to strongly *(K (i) = 10 nM)* and selectively inhibit PTC and to improve PTC crystallization. We find that E. faecalis AgDI, which is committed to ATP generation, closely resembles the AgDIs involved in making polyamines, suggesting the recruitment of a polyamine-synthesizing AgDI into the AgDI pathway. The arginine deiminase (ADI) pathway of arginine catabolism probably supplied the genes for PTC and CK but not those for the Agmatine/Putrescine antiporter, and thus the AgDI and ADI pathways are not related by a single "en bloc" duplication event. The AgDI crystal structure reveals a tetramer with a five-blade propeller subunit fold, proves that AgDI closely resembles ADI despite a lack of sequence identity, and explains substrate affinity, selectivity, and Cys357-mediated-covalent catalysis. A three-tongued agmatine-triggered gating opens or blocks access to the active centre [20].

In drug discovery to reduce the time and cost and increase the efficiency of the medicine, molecular docking method has been used. It is a recent technique to get insight study of the exact binding location of the ligand and protein [21]. In this study, we can understand the transport of small molecule called ligand in biological systems. Auto Dock suite 4.2.6 is an excellent tool to get insights into the molecular mechanism of ligand–protein interactions bind to a receptor of the well-known three-dimensional structure. Embelin, vilangin, and phenyl vilangin ligands optimized structures obtained from Gaussian 09 were docked using Auto Dock Tools. These ligands were selected to be docked at the active areas of three receptors *2JER* of E. faecalis protein which were downloaded from the protein data bank (RCSB). In this protein, Chain-A was selected for docking studies. Ligand structures were made for docking by incorporating non-polar hydrogen (H) atoms, adding Gasteiger partial charges and defining rotatable bonds. A maximum grid box size of $112 \times 126 \times 126$ Å was created using Auto Grid. The grid box was assigned at the centre of the protein using x, y, and z coordinates of $21.936, -0.33$, and 62.649 were created with a grid spacing of 0.492 Å at the binding site for 2JER, respectively. Hundred docking runs were used to obtain the best-fit proteins and it had very high binding energy. Docked structures were visualized and a 2D (dimensional) graphic representation of protein–ligand interaction was created using Discovery studio visualize 4.0.

The ligand- receptor residue interaction, hydrogen bonds, distance of hydrogen bonds and molecular docking binding energies (kcal/mol) were obtained and shown in Table 4. Among these three compounds, embelin exhibited the highest binding energy value of − 7.2 against 2JER protein which may be the reason for its high biological activity. The different hydrogen bond interaction, hydrogen bond receptor-side surface interaction, and 2D-hydrogen bonds interaction representations are shown in Figs. 5, 6, 7.

Table 4 The ligand- receptor residue interaction between embelin, vilangin and phenyl vilangin with *2JER* protein

S.No	Pub chem. Id	Compound name	Binding energy (kcal/mol)	Residue interaction	Type of bond	Distance(Å)
1	**2JER**	Embelin	**− 7.2**	[ALA265]NH–O	Hydrogen	2.29
				H–OH[GLY209]	Hydrogen	2.38
2	**2JER**	Vilangin	− 5.8	[ASN117]NH–O	Hydrogen	2.11
				OH–O[GLY180]	Hydrogen	3.02
3	**2JER**	Phenyl Vilangin	− 6.9	C–O[GLU295]	Hydrogen	3.39
				C–O[GLU213]	Hydrogen	3.08
				C–O[GLU213]	Hydrogen	3.30
				[ILE299]N–O	Hydrogen	2.45

Bold indicates a pub chem id of protein code for *E. faecalis* bacteria subjected for docking studies

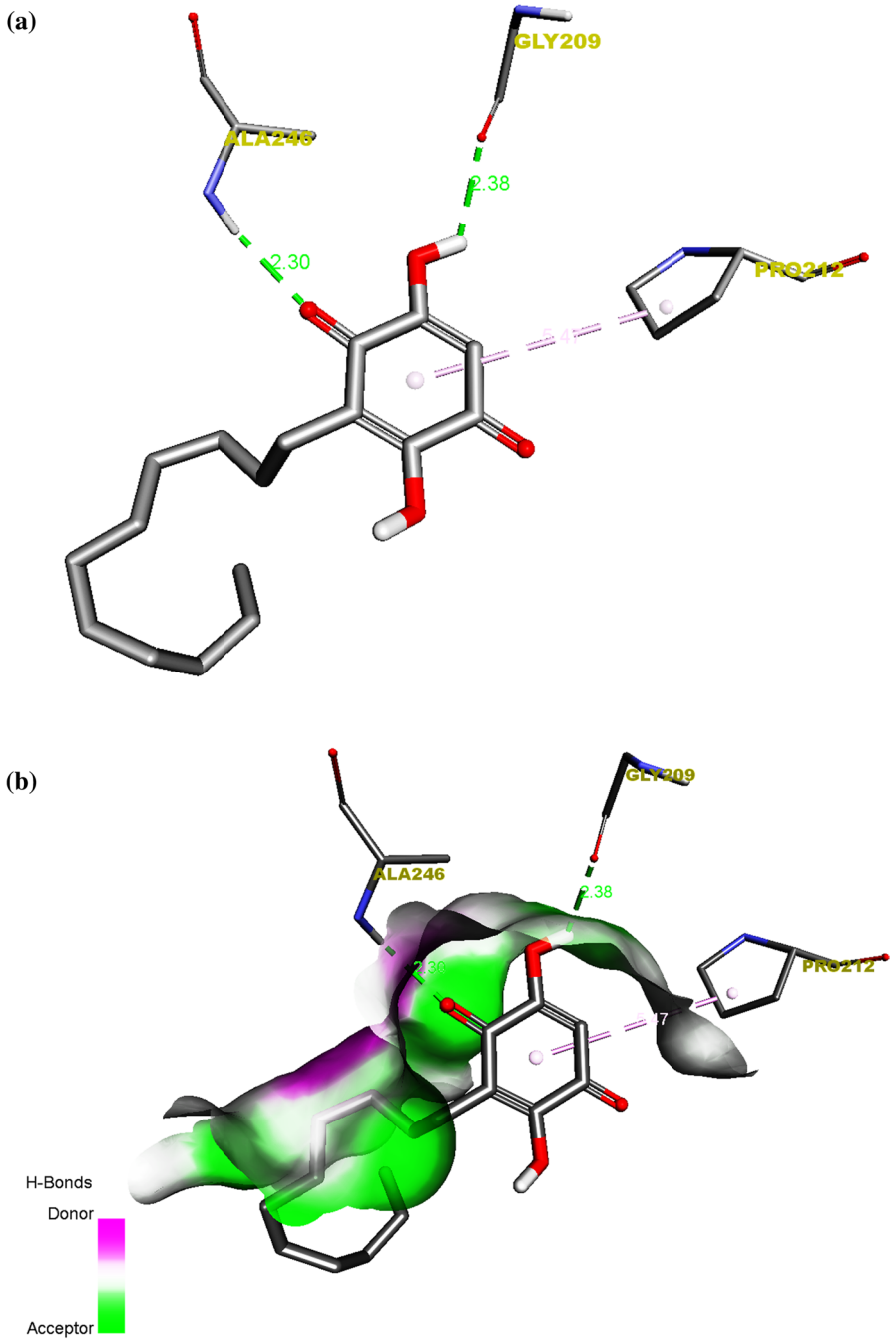


Fig. 5 a Hydrogen bond interaction of *embelin* with *2JER* protein. **b** Hydrogen bond receptor-side surface interaction of *embelin* with *2JER* protein. **c** 2D-Hydrogen bond interaction representation of *embelin* with *2JER* protein

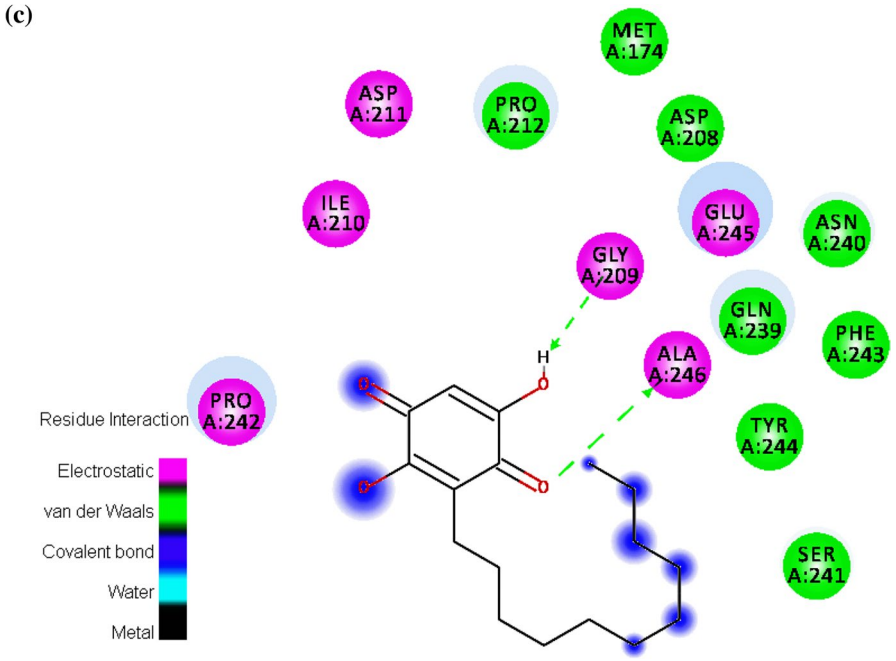


Fig. 5 (continued)

HOMO–LUMO analysis (Phenyl Vilangin)

In the present work, phenyl vilangin is subject for Gaussian studies, since the molecule exhibits appreciable results on antibacterial and brine shrimp lethal assay. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are known as frontier molecular orbitals (FMOs). Chemical stability is mostly influenced using the frontier orbitals (HOMO and LUMO). The FMOs plays a significant role in the optical and electrical properties and quantum chemistry [22]. The HOMO represents electron-donating ability, while the LUMO represents electron accepting ability [23]. The HOMO and LUMO energies are very helpful for physicists and chemists and are very important terms in quantum chemistry. The graphic representation of energies of molecular orbitals with molecular formula of phenyl vilangin ($C_{41}H_{56}O_8$) is shown in Fig. 8 (positive phase is represented by the red colour and negative phase represented in green colour). The HOMO lying at − 6.2929 eV and spreads around over the entire molecule whereas the LUMO located at − 4.0835 eV with large anti-bonding character, which reveals that the eventual charge transfer occurs within the molecule and the frontier orbital energy gap is 2.2095 eV.

In general both the HOMO and LUMO orbitals are the main orbital that helps to explain the chemical reactivity and kinetic stability of molecule. Dipole moments (Debye) of the title compound *phenyl vilangin* are shown in Table 5, representing that the dipole moment of this title compound can support more interaction with high dipole

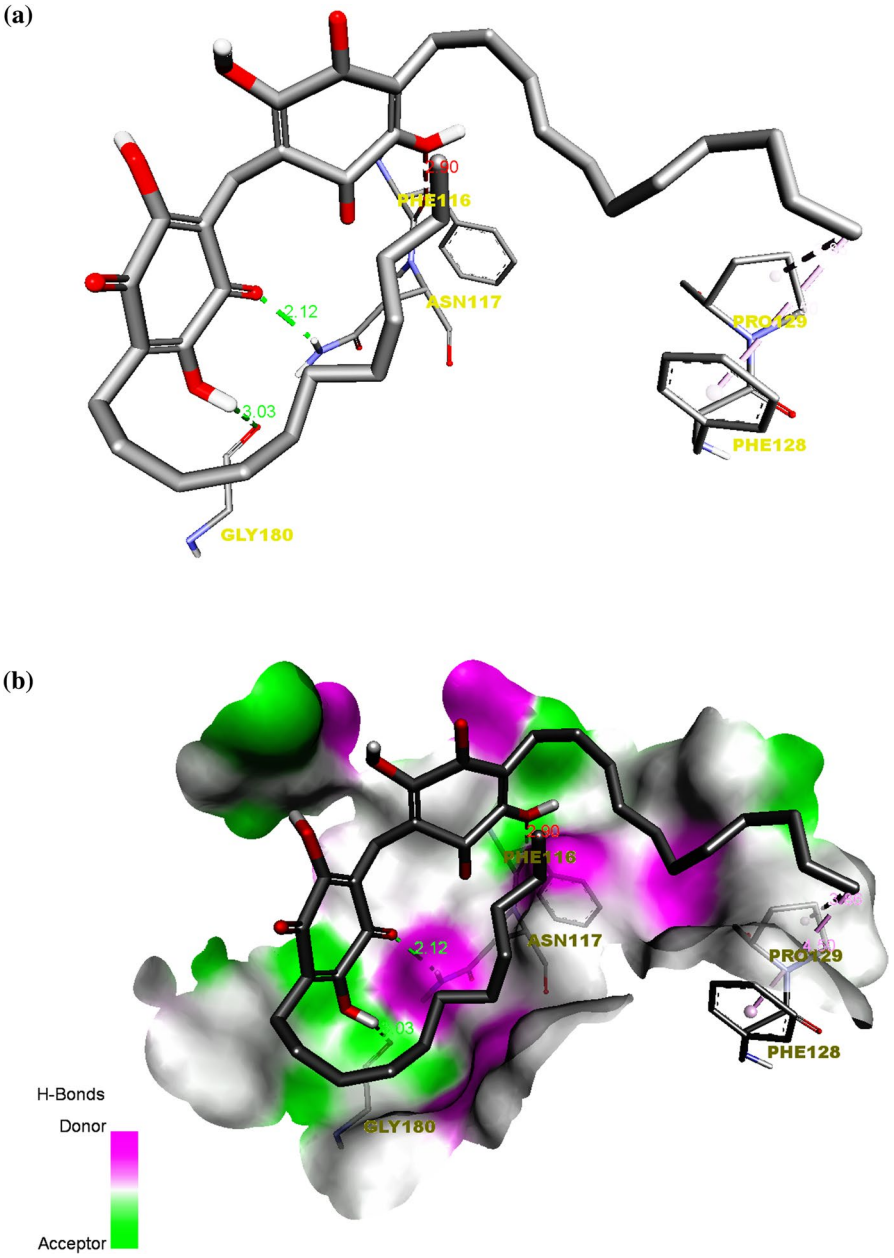


Fig. 6 a Hydrogen bond interaction of *vilangin* with *2JER* protein. **b** Hydrogen bond receptor-side surface interaction of *vilangin* with *2JER* protein. **c** 2D-Hydrogen bond interaction representation of *vilangin* with *2JER* protein

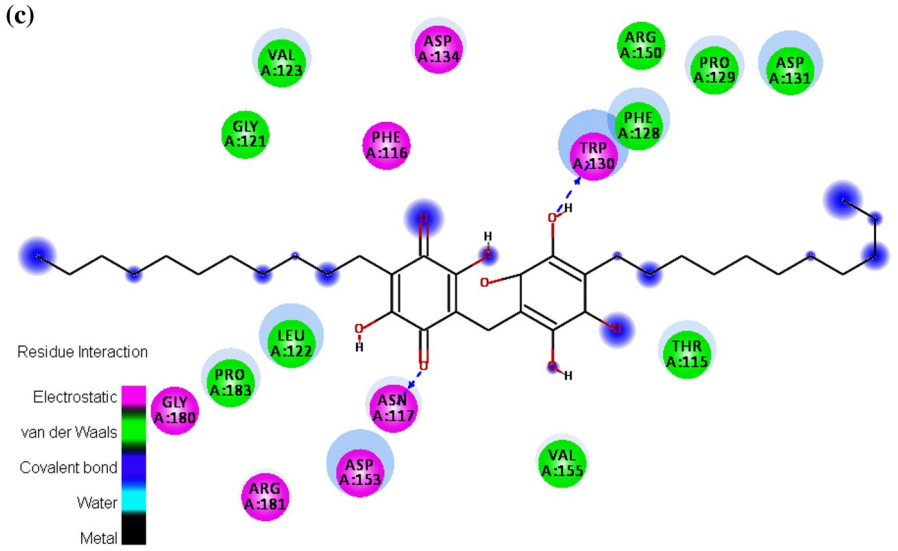


Fig. 6 (continued)

moment species especially in the biological systems**.** From Table 5, it is observed that title compound of *phenyl vilangin* has been exhibited a low value of chemical hardness η (eV) value of 1.1047 and high value of global softness S (eV) value of 0.2601; therefore, this compound has chemically reactive. Furthermore, the lowering in the HOMO and LUMO energy gap describes the ultimate charge transfer interactions that happen within the molecule and also possessed very good chemical and biological activities.

The lower value of electron affinity $A=4.0835$ (eV) for the phenyl vilangin molecule has accepted electrons for this reaction. As a result of having LUMO and HOMO energy gap (ΔE), the ionization potential (I), the electron affinity (A), global hardness (η) chemical potential (μ), global electrophilicity (ω) values can be estimated by the following formula.

$$\chi = I + A/2 \text{(Electronegativity)} \tag{1}$$

$$\mu = -(\mathrm{I} + \mathrm{A})/2 \text{(Chemical potential)} \tag{2}$$

$$\eta = I - A/2 \text{(Chemical hardness)} \tag{3}$$

$$\mathrm{S} = 1/2\eta \text{ (chemical softness)} \tag{4}$$

$$\omega = \mu^2/2\eta \text{ (Electrophilicity index)} \tag{5}$$

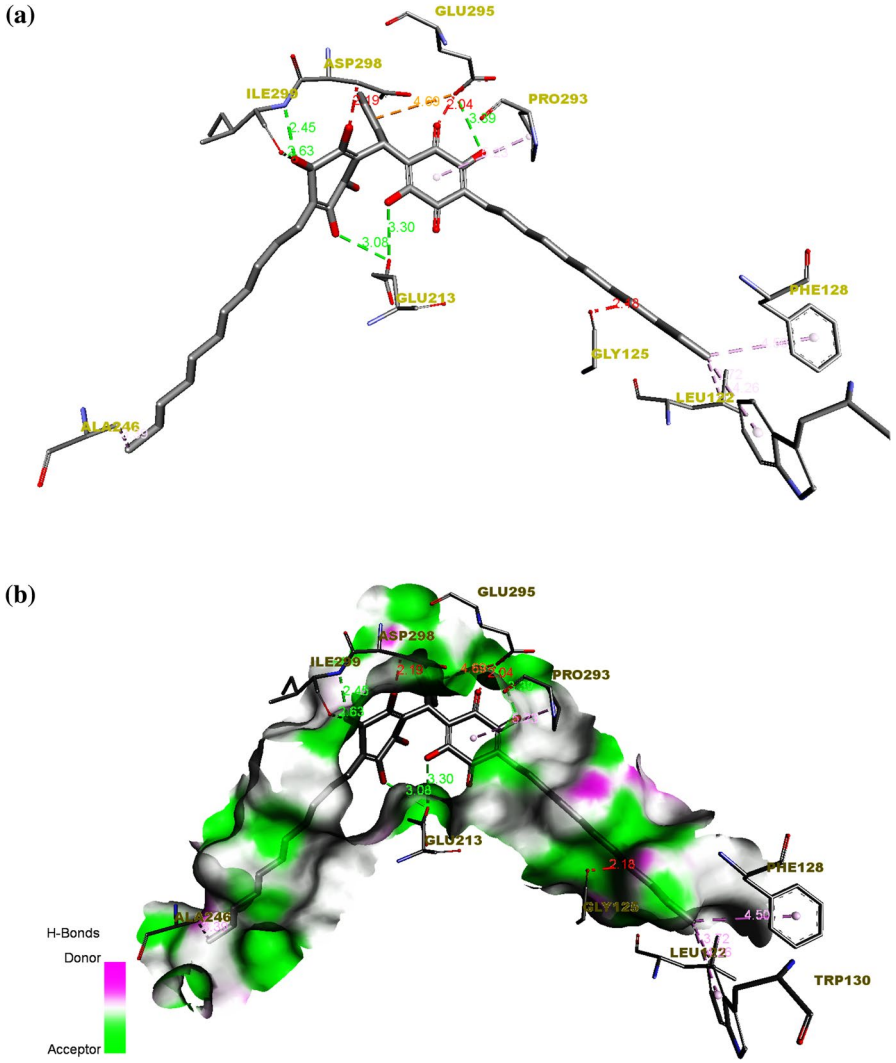


Fig. 7 a Hydrogen bond interaction of *phenyl vilangin* with *2JER* protein. **b** Hydrogen bond Receptor-side surface interaction of *phenyl vilangin* with *2JER* protein. **c** 2D-Hydrogen bond interaction representation of *phenyl vilangin* with *2JER* protein

Molecular electrostatic potential

Molecular electrostatic potential is a significant tool to better understand the relative polarity and reactivity of a molecule. Also, MEP is a valuable tool for expecting and examining the molecular interaction like hydrogen bonding, drug-receptor and enzyme–substrate interactions. The optimized structures at DFT/B3LYP/6–311 + + G (d, p) level of theory are used to generate MEPs of the isolated compound and *phenyl vilangin* is displayed in Fig. 9a and b. The MEP is a 3D-plot of

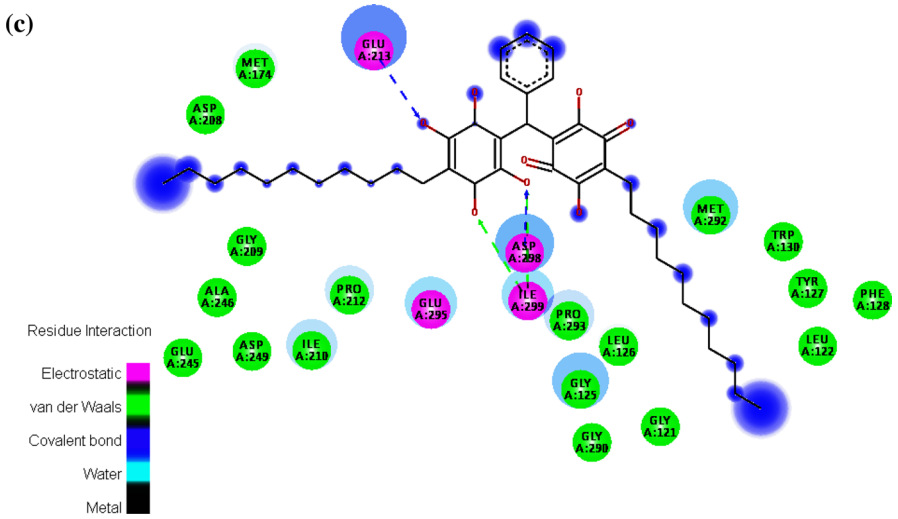


Fig. 7 (continued)

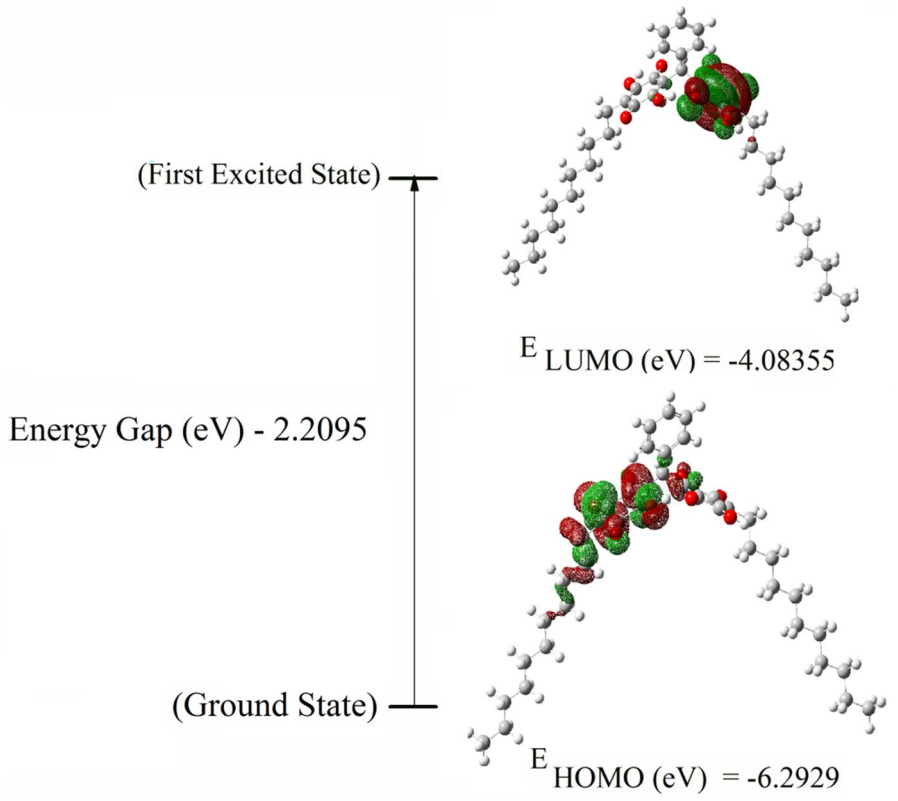


Fig. 8 Molecular orbital energy level diagram with HOMO and LUMO orbital of phenyl vilangin

Table 5 Calculated quantum chemical parameters HOMO–LUMO results *phenyl vilangin*

Quantum Parameters (eV)	Phenyl vilangin (HOMO–LUMO)
SCF energy (a.u)	−2197.221595
Dipole moment (Debye)	7.8207
E_{HOMO}	−6.2929
E_{LUMO}	−4.0835
ΔE_{gap}	2.2095
$I = -E_{HOMO}$	6.2929
$A = -E_{LUMO}$	4.0835
$\chi = \frac{I+A}{2}$	5.1882
$\mu = -(I+A)/2$	−5.1882
$\eta = (I-A)/2$	1.1047
$S = \frac{1}{2\eta}$	0.2601
$\omega = \frac{\mu^2}{2\eta}$	12.1832

electrostatic potential mapped onto electron density surface which depicts the size, shape, charge density and site of chemical reactivity of the molecules. In the colour scheme of MEPs, red represents electron rich, partially negative charge which is the favoured site for electrophilic attack, blue corresponds to electron deficient, partially positive charge which is the favoured site for nucleophilic attack, yellow coloured as slightly electron rich region; green coloured as neutral, respectively. Molecular electrostatic potential mapping is extremely helpful in the study of the molecular structure with its physiochemical property relationships and also biological studies [24]. The colour code of this map is in the range between -8.799×10^{-2}(deepest red) to 8.799×10^{-2} (deepest blue). It can be seen that the negative regions are mainly over the oxygen atom and the positive potential sites are around the hydrogen atoms.

UV–vis spectral analysis

The frontier molecular orbital acting a main role in discovering bioactive, electrical, in addition, optical properties. The test UV–Visible spectrum of *phenyl vilangin* title molecule has been recorded with methanol as a solvent and compared with a simulated spectrum with the 6–311 + + G(d,p) basis set in Fig. 10a, calculated using the TDDFT/B3LYP method, Fig. 10b.

Absorption spectroscopy of most of the organic molecules is based on transitions π–π*and σ–σ* in the UV–Vis region [25]. The UV–Vis absorption spectrum of phenyl vilangin is shown in Fig. 10 a and b and its exhibits a spectral range 300–800 nm. The theoretical UV–Visible spectra image was obtained from Gauss-Sum 3.0 program [10]. The significant absorption peaks observed are at 788, 664, and 617 nm. The most intense UV bands are observed due to absorption from highest occupied molecular orbital and lowest unoccupied molecular orbital

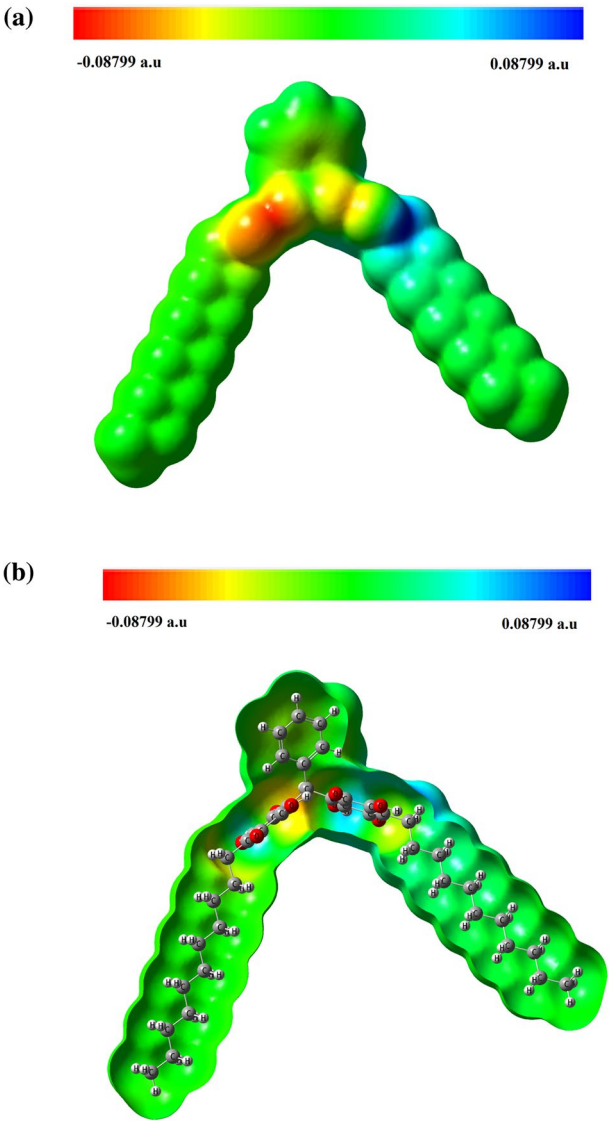


Fig. 9 a Molecular electrostatic potential (Overall view) of the compound phenyl vilangin. **b** Molecular electrostatic potential (Inner view) of phenyl vilangin

(HOMO to LUMO). In order to understand electronic transitions of compound, TD-DFT calculations on electronic absorption spectra were performed. Experimentally observed peaks are in good deal with the theoretical values calculated by TD-DFT method. The values of wavelength, electronic excitation energies and oscillator strength calculated by TD-DFT method are tabulated in Table 6.

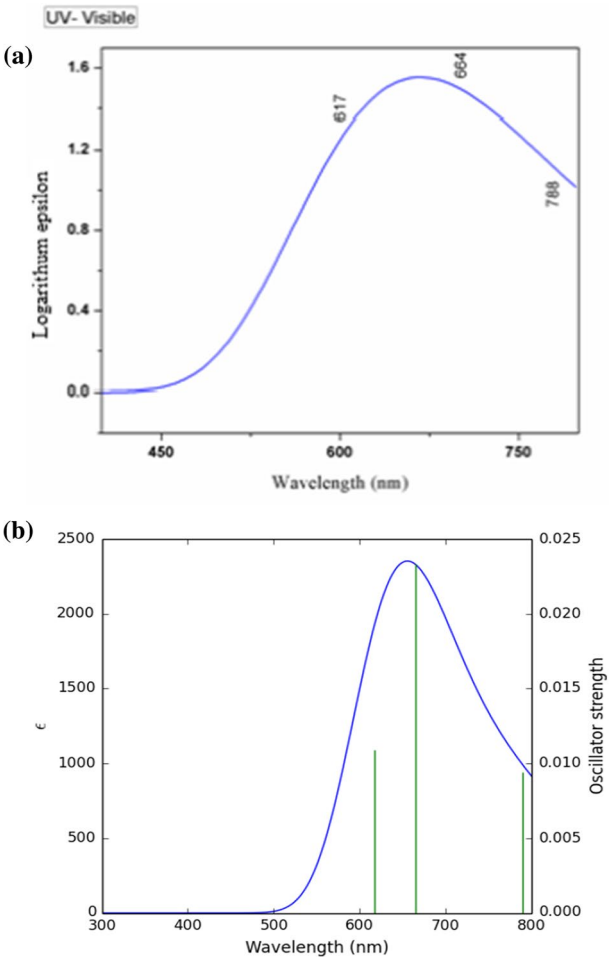


Fig. 10 **a** and **b** UV–Visible Spectrum of phenyl vilangin **a** Experimental **b** Theoretical

Table 6 The UV–visible wavelength (λ), band gap energy (ΔE) and oscillator strength (f) for phenyl vilangin calculated by TD-DFT/B3LYP method

Experimental	Calculated by TD-DFT/B3LYP/6–311 + + G(d,p)			
λ_{max} (nm)	Log (ε)	λ_{max} (nm)	E(eV)	(f)
788	1.0	788 HOMO → LUMO (95%)	1.5716	0.0094
664	1.5	664 HOMO-1 → LUMO (61%)	1.8659	0.0233
617	1.3	617 HOMO-3 → LUMO (55%)	2.0092	0.0109

Conclusion

Thus, the major conclusion drawn from this work is that the selection of suitable chemical structure with balanced hydrophobic and hydrophilic ratio decides its use as a root canal irrigant in the discipline of endodontics.

The prime outcome of this work reveals embelin as a very good antimicrobial agent and phenyl vilangin as a good cytocompatible agent. The resonance stabilised dianion structure of embelin and phenyl vilangin exhibits more electron density at carbon position C6 and C3, which is also reflected from the molecular electrostatic potential diagram of phenyl vilangin. The plausible mechanism proposed for antibacterial activity was found to be in good agreement with MIC value of embelin. It is certain from this work that by fine tuning the balance between hydrophobic and hydrophilic ratio, both antimicrobial property and biocompatible property can be achieved. This work may be extended further by synthesizing few more derivatives of embelin by incorporating various hydrophilic groups, and their impact on antimicrobial and biocompatibility properties can be investigated.

Declarations

Conflict of interest The authors have no conflict of interest.

References

1. A. Molander, C. Reit, G. Dahlen, T. Kvist, Int. Endod. J. **31**, 1 (1998)
2. A. Bystrom, G. Sundqvist, Int. Endod. J. **18**, 35 (1985)
3. Z. Mohammadi, S. Shalavi, A. Moeintaghavi, H. Jafarzadeh, TODENT J. **11**, 661 (2017)
4. N. Kandaswamy, N. Raveendiran, Res. Chem. Intermed. **41**, 7189 (2014)
5. N. Kandaswamy, Macromol. Res. **27**, 593 (2019)
6. C.H. BheemasankaraRao, V. Venkateswarlu, J. Org. Chem. **26**, 4529 (1961)
7. K. Narendran, S. Jayalakshmi, M. Nivedhitha, A. Saravanan, A. Ganesan, E. Sukumar, Indian J. Pharm. Sci. **82**, 909 (2020)
8. S.D. Sarker, L. Nahar, Y. Kumarasamy, Methods **42**, 321 (2007)
9. N. Meyer, N.R. Ferrigni, J.E. Putnam, Planta Med. **45**, 31 (1982)
10. P.N. Solis, C.W. Wright, M. Anderson, M.P. Gupta, D. Phillipson, Planta Med. **59**, 250 (1992)
11. M. J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, Gaussian, Inc, Wallingford CT. (2009)
12. I. Abraham, R. Joshi, P. Pardasani, R.T. Pardasani, J. Braz. Chem. Soc. **22**, 385 (2011)
13. S. Patai, Z. Rappaport, Wiley, New York. (1988)
14. R.C. Fahey, W.C. Brown, W.B. Adams, M.B. Worsham, J. Bacteriol. **133**, 1126 (1978)
15. A. Meister, M.E. Anderson, Annu. Rev. Biochem. **52**, 711 (1983)
16. L. Masip, K. Veeravalli, G. Georgiou, Antioxid. Redox Signal. **8**, 753 (2006)
17. D. Morris, M. Khurasany, T. Nguyen, J. Kim, F. Guilford, R. Mehta, D. Gray, B. Saviola, V. Venketaraman, Biochem. Biophys. Acta. **1830**, 3329 (2013)
18. G.V. Smirnova, O.N. Oktyabrsky, Biochemistry (Mosc.). **70,** 1199 (2005)
19. H. Hubert, K. Steinkellner, S. Z. Nele, A. Potthast, K. J. Edgar, R. Thomas, Chemcomm, **56**, 12845 (2020)

20. J.L. Llácer, L.M. Polo, S. Tavárez, B. Alarcón, R. Hilario, V. Rubio, J. Bacteriol. **189**, 1254 (2007)
21. U. Kragh-Hansen, Pharmacol. Rev. **33**, 17 (1981)
22. A. Frisch, A.B. Nielson, A.J. Holder, GaussView User's Manual (Gaussian, Inc., Pittsburgh, PA, 2000)
23. J.S. Muray, K. Sen, Theor. Chem. Acc. **103**, 343 (2000)
24. A. Savin, in *Recent Developments and Applications of Modern Density Functional Theory*, ed. J.M. Seminario (Elsevier, Amsterdam, 1996), p. 327
25. R.M. Silverstein, G.C. Bassler, T.C. Morrill, *Spectrometric Identification of Organic Compounds* (Wiley, Chichester, 1991)