Indian Journal of Chemistry
Vol. 63, April 2024, pp. 390-393
DOI: 10.56042/ijc.v63i4.7084

# Embelin as an alternative reference standard for Delta-9-Tetrahydrocannibinol

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*Received 11 December 2023; accepted (revised) 22 March 2024*

Delta-9-Tetrahydrocannibinol (Δ9-THC), has shown neuroprotective effects on animal models, but cannot be used as a drug for treatment as it is controversial to use an addiction drug for treatment. Further, there have been toxic and addictive effects on humans. Embelin (isolated from *Embelia ribes*) falls under herbal medicine category. In this study it has been computationally shown that the pharmacokinetic and drug-likeliness effects of Embelin are similar to Δ9-THC, and thus, it can be used as an alternative reference standard as supported by docking studies.

**Keywords**: Reference standard, Delta-9-Tetrahydrocannibinol, Embelin, Docking studies, Molecular mechanics

Cannabis is fast growing, annual flowering plant and believed to have beneficial effects when smoked or ingested. Cannabis may be used to treat a wide range of conditions including anxiety and epilepsy, as well as for nausea suppression, appetite enhancement *etc.* and many medicinal and recreational products are developed[1]. Addiction of drug of abuse is a big challenge for society and policy makers[2]. Delta-9-Tetrahydrocannibinol (Δ9-THC) (Fig. 1), that is primarily responsible for producing the well-documented effects on perception, mood, emotion, and cognition that together constitute the psychotropic effects of cannabis[3]. There are few reports where endocannabinoids are used as a potential drug target for neurodegeneration[4]. Δ9-THC, which is constituent of Cannabis has shown neuroprotective action but cannot be used as a drug for treatment as it is controversial to use an addiction drug for treatment and also it has toxic effects on humans when given as doses of any concentration. Cannabis (Marijuana, Δ9-THC content more than 0.3%) is a schedule 1 controlled substance in India.

Δ9-THC is monitored by the various authorities in many countries with instrumental analytical methods, which require calibration with standards. The US Pharmacopeia (USP) general chapter <11> defines reference-standard materials as "highly characterized specimens of drug substances, excipients, reportable impurities, degradation products, compendial reagents, and performance calibrators". Due to the controlled nature of Δ9-THC, it is difficult to procure reference standard of Δ9-THC in India, unlike the countries where cannabis are allowed for recreational purposes[2]. To facilitate analyses we tried to study feasibility of using embelin, a structurally similar compound with the similar physicochemical properties as Δ9-THC, as a reference standard. Naturally occurring bioactive molecules are known for their diverse biological applications such as antimicrobial, anticancer, anti-inflammatory, and analgesic activities. Embelin (Fig. 1) is a naturally occurring benzoquinone that inhibits the growth of cancer cells, making it a potent anticancer drug. However, the low water solubility of embelin restricts its clinical applicability[5,6].

The parameters required to consider application of embelin as an alternative standard of Δ9-THC are (i) crossing the Blood Brain Barrier (BBB) (ii) other factors include that it should be in unionized form (iii) molecular weight should be approximately 400 to 500 Da (iv) log *P*-value approximately 2 and (v) should have the ability to form 7 to 10 hydrogen bonds[5-8]. Embelin fulfil all these required parameters. Our objective to demonstrate that the embelin can be utilized as alternative reference materials of Δ9-THC will support in the routine monitoring program by the local authorities and consequently can lead to reduced dependence of Δ9-THC.

## Experimental Details

### Chemicals, materials and reagents

Ethyl acetate (AR grade), Chloroform (AR Grade), n-Hexane (95% purity), Silica gel were procured from SRL Chemicals, Mumbai, India. *Embelia ribes* fruit and

leaves were obtained from local market in Kolkata, India. Δ9-THC standard was procured from Dr. Ehrenstorfer (Product Code: DRE-A17405100ME-100). The following chemo informatics tools were used for Docking and ADME analysis: Auto Dock Vina (1_1_2_win32.msi), UCSF Chimera (version 1.15), ANTECHAMBER algorithm, Swiss PDB viewer (SPDBV)(version 4.1), Discovery studio (2021 Client), Swiss ADME (web server).

**Molecular docking**

The docking (that is protein-ligand binding energy (ΔG) analysis) of the compounds Δ9-THC and Embelin was performed using Auto Dock Vina as an extension in UCSF Chimera. Standard operative protocol of docking was conducted. The protein structure of single domain camelid antibody fragment in a complex with C-terminal of alpha synuclein was retrieved from RCSB Protein Data Bank (PDB)[9,10]. PDB-ID 2X6M in PDB format, the protein has good ligand structure and goodness of fit to experimental data. As per docking protocol, removal of all water and solvent molecules, co-crystallized residues (if any) and mirror chain (if any) was ensured using UCSF Chimera software. Next part was the protein structure preparation and performed by Chimera. The protein structures were prepared by assigning the hydrogen atoms, charges and energy minimization using Dock Prep tool. The charges were assigned as per the Austin Model-1 bond-charge-increment (AM1-BCC) method which quickly and efficiently generates high-quality atomic charges for protein and the charges were computed using ANTECHAMBER algorithm. The energy minimization was performed using Swiss PDB viewer (SPDBV). The target proteins after minimization of energy was then saved in PDB format for future docking purpose.

Embelin and Δ9-THC used for the *in silico* interaction assays is the medicinal plant's secondary metabolites which is present in PubChem (Pub Chem ID – 3218) for Embelin, retrieved from there in SMILES format, and SMILES format for Δ9-THC was retrieved from PubChem (PubChem ID – 16078) and pasted in ChemDraw and cleaned up, after which it was copied to Chem3D pro where their energy minimization was carried using MM2 calculations, after that it was saved in SDF (3D) format.

Prior to molecular docking of ligand and protein, the ligands were optimized by addition of hydrogen and addition of charge using Gasteiger algorithm, and energy minimization was performed using one thousand steepest descent steps with 0.02 Å step size with an update interval of ten and then again saved in PDB format in structure editing wizard of Chimera software, which is driven by the Chemoinformatic principle of electronegativity equilibration and then the files were saved in PDB format. A grid box which assigns the binding region was chosen in such a way that it would cover the protein's active site for the hydrophobic surface of the concave region of protein to fit in properly the hydrophobic surface of the ligand giving the best binding score. For visualising in different formats, we used the software Discovery studio and UCSF Chimera.

**Isolation of Embelin**

The leaves and fruits of *Embelia ribes* (500g), which are the only part that people consume, grow throughout the plains and hilly regions of India and collected from local vendors. The leaves and fruits were air dried and extracted with 1% ethylacetate in n-hexane in a Soxhlet apparatus for 72 h. The total extract was concentrated using rotary evaporator and kept at room temperature for some time, then weighed and found to have 29 g of yellowish semi-solid clay. This was dissolved in chloroform and purified using a silica gel column chromatography with (1:99) ethylacetate in n-hexane.

**Results and Discussion**

**Pharmacokinetic similarity of Embelin and Δ9-THC**

*In silico* ADME analysis was conducted to investigate physio-chemical properties of the potent hits, such as water solubility, lipophilicity, bio-radar (for orally acceptable molecules), drug likeness and pharmacokinetics by using the website http://www.swissadme.ch, but the toxicity of these molecules cannot be investigated by using Swiss ADME with their SMILES profile. From this server we compare the pharmacokinetic similarity between Embelin and Δ9-THC.

**Isolation and structural description of Embelin**

The chemical structure of Embelin extract isolated from *Embelia ribes* (500g) was confirmed using $^{1}H$ and $^{13}C$ NMR (given in supplementary file).

**Pharmacokinetic similarity of Embelin and Δ9-THC**

The result from SwissADME server is very comforting as Embelin and Δ9-THC shows nearly 100% similarities in pharmacokinetic parameters (Table 1) which makes embelin a good substitute of Δ9-THC. Embelin and Δ9-THC both passes through BBB and has high gastrointestinal absorption, but

Table 1 — Pharmacokinetics similarity of Embelin and Δ9-THC

| Parameter | Δ9-THC | Embelin |
|---|---|---|
| Gastrointestinal absorption | High | High |
| BBB permeant | Yes | Yes |
| *P*-glycoprotein substrate | No | No |
| Cytochrome P450 1A2 (CYP1A2) inhibitor | No | No |
| Cytochrome P450 2C19 (CYP2C19) inhibitor | Yes | Yes |
| Cytochrome P450 family 2 subfamily C member 9 (CYP2C9) inhibitor | Yes | Yes |
| Cytochrome P450 2D6 (CYP2D6) inhibitor | Yes | Yes |
| Cytochrome P450 3A4 (CYP3A4) inhibitor | No | No |
| Skin permeation coefficient (log Kp) | –3.27 cm/s | –4.25 cm/s |

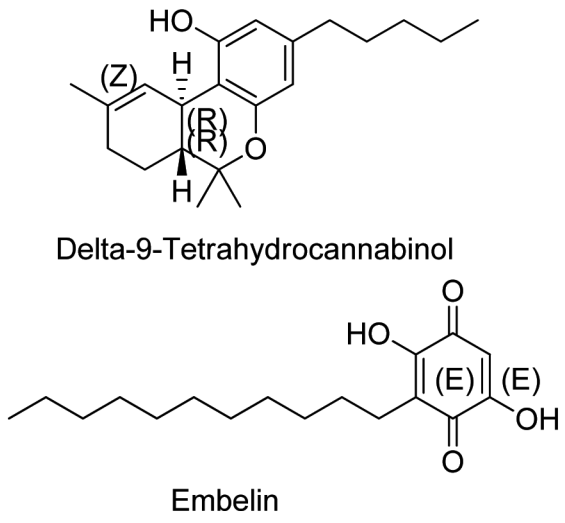


Fig. 1 — Chemical Structures of Δ9-THC and Embelin

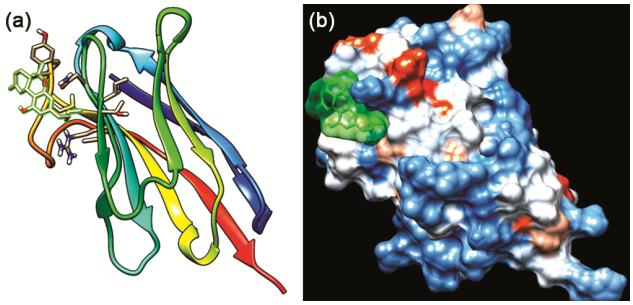


Fig. 2 — (a) Ribbon pose of Δ9-THC (b) Hydrophobic pose of Δ9-THC; with 2X6M protein

Embelin does not show any cytotoxic effect or addiction of any kind.

## Docking studies

The protein ligand binding conformational energies between the chosen protein PDB-ID 2X6M and the ligands Embelin and Δ9-THC which is the main phyto-compound of the leaves of *Embelia ribes* and cannabinoid plant are obtained out using *in-silico* docking. The calculations reveal the highest free energy change for these interactions and ΔG = –5.4 kcal/mol for Δ9-THC inside a grid box of –34.57 × 15.19 × 3.58 Å with size 30 × 30 × 30 Å (Fig. 2 and 3) and ΔG = –5.2 kcal/mol for Embelin (Fig 4 and 5) inside a grid box of –34.57 × 15.19 × 3.58 Å with size 30 × 30 × 30 Å along x- , y- and z- axes.

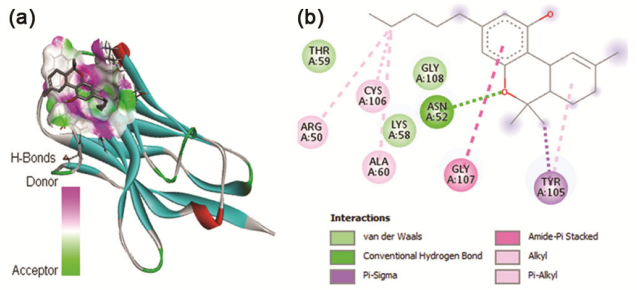


Fig. 3 — (a) H-Bond pose of Δ9-THC (b) 2D pose of Δ9-THC; with 2X6M protein

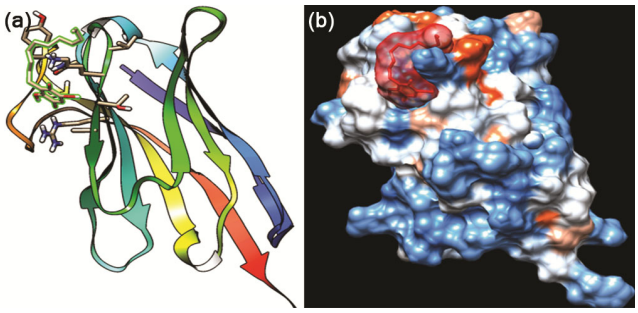


Fig. 4 — (a) Ribbon pose of Embelin (b) Hydrophobic pose of Embelin; with 2X6M protein.

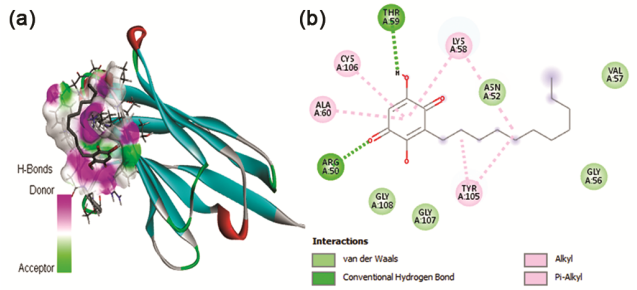


Fig. 5 — (a) H-Bond pose of Embelin (b) 2D pose of Embelin; with 2X6M protein.

## Conclusion

All these data demonstrated that Embelin and Δ9-THC showed similar activity. Embelin is non-addictive and non-toxic and may be used as an alternative reference standard for Δ9-THC. These

findings could be used in the routine drug of abuse monitoring program by local authorities.

## Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

## Acknowledgements

This work was supported by the Department of Health research (DHR), Grant-in-aid scheme, Grant/ Award No. R.11013/10/2021-GIA/HR.

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