

## Invigorating *Clitoria ternatea* L. an Ayurvedic traditional herb as a competent brain drug on par with *Hypericum perforatum* L. to contest P-glycoprotein across the blood brain barrier

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The vital phytochemicals from the leaves of *Clitoria ternatea* L. were extricated using GC/MS assay is pursued by a molecular docking study, considering the phytochemicals as ligands against the efflux protein (P-Glycoprotein) which determines the active compound that surpasses the blood brain barrier. Five potential compounds were considered competent to the optimized phytochemicals of *Hypericum perforatum* L. among which Hexanoic acid, 4-hexadecyl ester furnished a minimum binding affinity energy value of -8.734kcal/mol and interacted with the amino acid residues GLN721 of the active site. Whereas, Pseudohypericin is the foremost phytochemical of *Hypericum perforatum* L. exhibited a docking interaction of -8.096 kcal/mol with a distinct amino acid residue MET982. This work endorses *Clitoria ternatea* L. as a competent brain drug across the blood brain barrier on par with an established European folk medicinal plant *Hypericum perforatum* L. (St.John's Wort).

**Keywords:** Ayurveda, *Clitoria ternatea* L., *Hypericum perforatum* L., Blood brain barrier, P-Glycoprotein

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Innumerable curable modes such as *Ayurveda*, *Siddha* and *Unani* are distinguished with a deep rooted ancient horarti, has been practiced in India<sup>1</sup>. *Shankpushpi* is a presumed drug in *Ayurveda* that has been used for centuries as a brain and nervine tonic<sup>2</sup>. The term *Shankpushpi* is derived from Sanskrit language that instances a twining herb whose flowers resembles the shape of conch shell or *shanka* which is a sacred instrument found in the hands of lord Shiva. Pharmacognostically this has four ethanobotanical taxonomy named to *Evolvulus alsinoides* L. (Convolvulaceae), *Convolvulus prostratus* Forssk syn. *Convolvulus pluricaulis* Choisy (Convolvulaceae), *Canscora alata* (Roth) Wall. syn. *Canscora decussata* (Roxb.) Schult. & Schult.f. (Gentianaceae), and *Clitoria ternatea* L. Though all the four plants are catalogued under the same class Magnoliopsida, *Clitoria ternatea* L. separates itself at the level of sub-class belonging to Rosidae, while the other three belong to Asteridae<sup>3</sup>. *Medhya rasayana* a rejuvenating recipe containing *Shankpushpi* is used in many *Ayurvedic* formulations, either singly or in

combination with other herbs, meant for sleeplessness, epilepsy, hallucinations and anxiety<sup>4</sup>. Pharmacognostic studies suggest that *C. pluricaulis* can be considered as the actual source which is used in the formulation to treat hypertension<sup>5</sup>, while *E. alsinoides* and *C. ternatea* L. as the alternative sources of *shankpushpi*<sup>6</sup>. *Clitoria ternatea* L., is distinct among other herbs and some ancient practitioners used it as brain drug in several *Ayurvedic* assortments<sup>7,8</sup>. Indian traditional medicine has devised rich medical expertise knowledge of treatise which was handwritten in Sanskrit language as pre-European information but, after the establishment of British rule western medicine was looked upon as the leading system of medication. This might be one of the reasons due to which *Ayurveda* could not stand parallel to the western medicine. An ambiguity is reflected in the interpretation and mode of action in the formulations and drugs prescribed by this traditional medicine. However current scenario has realised the significance of *Ayurveda*, where biomedical and *Ayurvedic* scientists are making attempts to combine the best of different healing traditions to meet the challenge faced in healthcare

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requirements of modern era<sup>9</sup>. Therefore there is a robust desire to evaluate the phytoconstituents of *Clitoria ternatea* L. on par with the European folk medicinal plant *Hypericum perforatum* L. (St. John's wort) as an efficient brain drug which can facilitate itself through the blood brain barrier. *Hypericum perforatum* L. belongs to the family *Hypericaceae*, with this species in particular being called as *St. John's wort* or *Perforate St. John's wort* to distinguish it from other species in the plant. Primarily, an extensive amount of clinical research shows that St. John's wort extracts are effective in treating major depressive disorder. In the United States, *St. John's wort* products are described as a "wound healer" for nervous individuals and are available over-the-counter as top selling herb in the form of dietary supplements<sup>10</sup>. The chief phytoconstitutions Hypericin and Hyperforin are well established phytochemicals against depression. Though *Clitoria ternatea* L. lacks reputable compounds, it also has a magnificent compilation of phytochemicals<sup>11</sup>. Which are on par with *St. John's wort*. This study asserts a molecular docking of the phytochemicals against an efflux protein P-Glycoprotein that is layered in the blood-brain barrier (BBB) which is a dynamic and highly selective permeable interface between central nervous system (CNS) and periphery that regulates the brain homeostasis<sup>12</sup>. Increasing evidences of neurological disorders and restricted drug delivery process in brain make BBB as distinct drug target. Recently P-gp is being used as therapeutic target for optimizing CNS drug delivery that is based on pharmacogenomics data analysis<sup>13</sup>. The result of the over-expression of membrane proteins like P-gp, exhibits power to exclude many kinds of drugs out of cell but the pathway of drugs to the binding site and the detailed drug-P-gp interaction process are not clear. A former study has reported that a putative aromatic gating may be present in P-gp<sup>14</sup>, but there is no direct evidence showing which residue plays the role of gating in the way to the binding site. The substrate promiscuity of P-gp, as its enigmatic property, is also not well elucidated. Molecular simulation may be an alternative to discover the pathway of drugs to the binding site and the detailed drug-P-gp interaction process. The conventional molecular dynamics has been used to explore the binding energy of P-gp and ligands<sup>15-17</sup>. Regardless of synthetic neurological drugs herbal medicine improves a better

sustainability and phytochemicals can endorse as perfect ligands in combating blood brain barrier. This study uplifts *Clitoria ternatea* L. as a proficient *Ayurvedic* drug that fetches solution against neurological problems.

## Methodology

### Materials and Chemicals

All chemicals used were of analytical grade purchased from, sigma Aldrich Ltd.

### Plant collection and identification

The healthy Plant samples were collected from the premises of Bishop Heber College, Tiruchirappalli, Tamil Nadu, India. The leaves from the whole plant was segregated, cleaned and allowed to dry under shade. The identification and voucher specimen number AAM 001 of the plant was sorted out and deposited at the Rapinat Herbarium and Centre for Molecular Systematic, St. Josephs College, Tiruchirappalli, Tamil Nadu. The plant was identified as *Clitoria ternatea* L. by Dr S John Britto (Director). The plant specimen was confirmed by comparison with reference herbarium specimen.

### Preparation of aqueous extract

The leaf samples were cleaned, shade dried and powdered. 100 gm of powdered leaf sample were weighed and boiled with 1 L of distilled water for 10 min at 70 °C. The plant extract yielded was filtered and evaporated to dryness and further used for analysis.

### Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis of the plant was performed using a Perkin-Elmer Clarus 500 system comprising an AOC-20i auto-sampler. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

### Identification of phytochemicals

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

### Docking analysis

Molecular docking studies were carried to identify the binding affinities and interaction between the inhibitors and the target protein (Permeability glycoprotein) P-Gp using Glide software (Schrodinger Inc. U S A- Maestro version 10.2). Glide (Grid-based Ligand Docking with Energetic) is one of the most accurate docking tool available for ligand-protein, protein-protein binding studies. Glide was found to produce least number of inaccurate poses and 85% of Glides binding models had an RMSD of 1.4 Å or less from native co-crystallized structures. The study also incorporated the pharmacological descriptions of the compounds by ADMET analysis.

### Preparation of Ligands

The 3-dimensional structures of the phytochemicals considered as ligands were retrieved and downloaded as .mol files from the site of Pub Chem. The molecules were processed using the LigPrep tool from Schrodinger to obtain the perfect conformation by the addition or removal of hydrogen atoms with respect to the OPLS\_2005 force field.

### Preparation of Protein Target

The target protein P-Glycoprotein (Fig. 1) was retrieved from Protein Data Bank (PDB ID 4M1M). Water molecules were removed and a single chain was selected between two chains, but water that connects between the ligand and the protein are sometimes retained. The structure forming bonds from the ligand or a cofactor to a protein metal were deleted by adjusting the ligand bond orders and formal charges. The minimization was done to restrain the input protein coordinates by a selected RMSD tolerance.

### GLIDE / Ligand Docking

Grid generated output file was uploaded as an input for Ligand docking against protein prepared targets in GLIDE.XP mode was adopted by selecting Flexible docking mode.

### Results and discussion

The phytoconstituents from the aqueous leaf extract of the plant *Clitoria ternatea* L. belonging to the family Papilionaceae, was extricated to persuade its medicinal significance as a brain drug. In the traditional (Asian) Indian systems of medicine particularly in *Ayurveda*, the roots, seeds and leaves of *Clitoria ternatea* L. have widely used as a brain

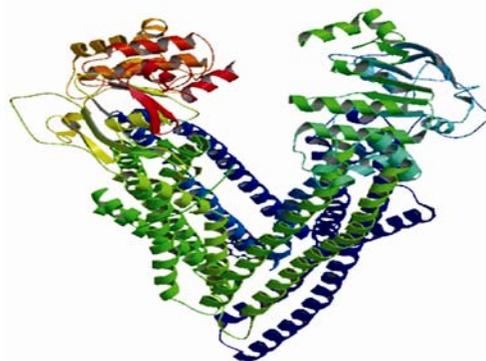


Fig. 1—Crystal structures of Target protein P-Glycoprotein

tonic and is believed to promote memory and intelligence<sup>21</sup>. Research works have illustrated that the roots of the plant have an extended antiquity to promote mental power memory retention and alleviate psychotic stress<sup>18</sup>. Moreover the leaves of the plant comprise of affluent phytochemicals which require a profound study<sup>19,20</sup>. Hence leaf was desired as the vital part that reveals the utmost essential phytochemicals.

### Extraction of the volatile phytochemicals by GC-MS assay

The chromatogram of GC-MS analysis of aqueous leaf extract of *Clitoria ternatea* L. is exemplified in the Fig. 2, which conferred 17 different compounds that were detected and catalogued in parallel to the NIST library. Relatively the essential phytoconstituents such as sugar, flavonoid, volatile terpene, fatty acid methyl ester, fatty acid ethyl ester, aldehyde and fatty alcohol were extricated among which, (Triterpene) Squalene (10.58%), (Fatty alcohol) Hexanoic acid,4-hexadecyl ester (0.92%), (Fatty alcohol) 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-(4.0%), (Aldehyde) 9,17-Octadecadienal, (Z)-( 0.6 %) and (Diterpene alcohol) Phytol (11.9629%) were the foremost exudates with a retention time of 43.81, 36.37, 33.15, 32.99 and 32.13 mins. Interestingly, four compounds (4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-Dihydro actinidiolide, Phytol and 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-) of *Clitoria ternatea* correlated as efficient compounds targeting neurological problems. 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl,2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl Dihydroactinidiolide and Phytol are secondary metabolites grouped as Terpenes. It is reported that terpenes exhibits a wide range of neurological effects within the central

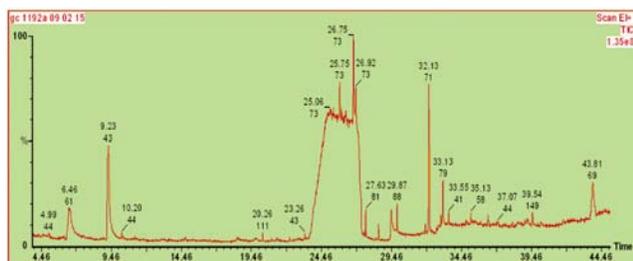


Fig. 2—GC/MS profile extricating phytochemicals from Aqueous extract of *Clitoria ternatea*

nervous system of insects. The neurotoxic deterrent properties of many monoterpenes in insects have been shown to include interactions with the octopaminergic system (analogous to the noradrenergic system in vertebrates), cholinesterase inhibition, and multiple direct interactions with the GABA system, including blockade of GABA-gated chloride channels and allosteric binding to GABA<sub>A</sub> receptors<sup>21</sup>. The antidepressant and anti-inflammatory effects of St John's wort extract were initially attributed to the naphthodianthrones<sup>20</sup> and more recently to hyperforin<sup>22</sup> and the range of flavonoid constituents. High concentrations of hypericin and flavonoid compounds inhibit Monoamine oxidase (MAO - A and MAO - B) activity thereby instigating as an antidepressant<sup>23</sup>. 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl is a fragment of flavanoid which is extricated in the aqueous extract negligibly in a high level with a retention time of 0.66 of 10.20%. A vast number of natural, plant-based extracts and chemicals are purported to have beneficial effects on human brain function<sup>24</sup>. This GC-MS study exhorts the necessitate to assay the essential phytochemicals that fortifies *Clitoria ternatea* L. as a prominent neurological drug.

### Molecular docking studies

An *in silico* assay was executed to determine the best compound by docking against the efflux protein P-Gp (Permeability Glycoprotein) that interacts with a drug strongly leading to a reduced absorption in the gut and very limited entry into the brain. The active site associated with the QZ59-RRR ligand that is considered to be positive binder composing of amino acid residues, such as MET88, PHE332, ILE338, PHE338, GLN721, TYR949, PHE724, PHE974, VAL978, TYR303, PHE728, SER975 and LEU335<sup>21</sup>. In the present study, four phytochemicals extracted from the aqueous leaf extracts of *Clitoria ternatea* L. (hexanoic acid-4-hexadecylester, 9,12,15-Octadecatrien-

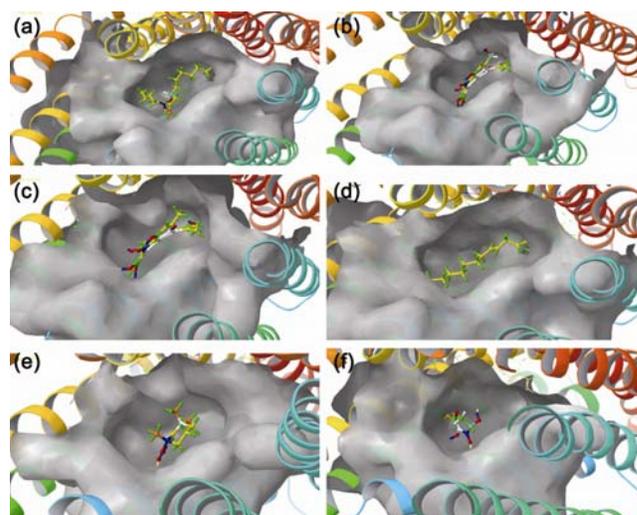


Fig. 3—docking pose of the phytochemicals a. hexanoic acid, 4-hexadecyl ester b. Pseudohypericin c. Hypericin d. 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- e. 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- f. 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl into the active sites

1-ol,(Z,Z,Z)-,2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl- and 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl) were docked computationally into the active site (Fig. 3) of the P-Gp (Permeability Glycoprotein). To endorse their inhibitory potency efficiently, standardized phytochemicals of *Hypericum perforatum* L. (Hyperforin, Adhyperforin, Pseudohypericin and Hypericin) were also docked with the target protein as reference compounds. The phytochemicals had the potential to dock with the target proteins and their interaction details are listed in Table 1. The target protein was counteracted by all the compounds of *Clitoria ternatea* L. where, hexanoic acid-4-hexadecyl ester provided a least docking score of -8.734kcal/mol pursued by 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) - (-5.987kcal/mol). Hexanoic acid-4-hexadecyl ester is a major compound extricated from the aqueous extract of *Clitoria ternatea* L. which impeded competently when compared to the test compound of *Hypericum perforatum* L. while, the two reference compounds Pseudohypericin and Hypericin (Figs. 3 b & c) counteracted with the target protein with a least score of -8.096 kcal/mol and -8.021kcal/mol. The study investigated diversity in the amino acid residues such as TYR 306, GLN 986, MET 982 which are distinct from the amino acids of the active site. Most of the residues in proteins active site are hydrophobic and are involved in a sturdy hydrophobic interaction. The ligands in the study exhibited a good interaction

Table 1—Hit list of the ligands with the target protein P-Gp

S.No	Name of the Compound	Binding Score Energy Value (Kcal/mol)	No of Hydrogen Bonds	H <sub>2</sub> Bond length	Interacting AminoAcid residue
1.	hexanoic acid, 4-hexadecyl ester -	8.734	1	2.00	GLN 721
2.	Pseudohypericin	-8.096	1	2.06	MET982
3.	Hypericin	-8.021	1	2.12	GLN 986
4.	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	-5.830	1	2.07	GLN986
5.	2(4H)-Benzofuranone, 5,6,7,7 a-tetrahydro-4,4,7a-trimethyl-	-5.695	1	2.19	GLN 721
6.	4H-Pyran-4-one,2,3-dihy 3,5-dihydroxy-6-methyl dro-	-5.196	2	2.03	GLN 721
				1.88	TYR 306
7.	Hyperforin	-	-	-	-
8.	Adhyperforin	-	-	-	-

Table 2—Qik Prop properties of the phytocompounds

S.No	Compounds	MW*	SASA*	logPo/w*	logPw*	logBB*	% Human Oral Absorption	PSA*
1	hexanoic acid, 4-hexadecyl ester	340.588	875.895	7.799	0.124	-1.078	100	34.695
2	Pseudohypericin	277.406	560.664	3.533	5.504	0.414	100	28.898
3	Hypericin	180.246	385.861	1.56	4.234	0.048	95.951	41.316
4	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	144.127	324.154	-0.578	9.146	-0.644	71.073	79.396
5	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	504.452	665.364	3.214	11.059	-2.721	38.181	167.511
6	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	520.451	675.696	2.134	14.09	-3.357	23.039	189.734
7	Venlafaxin	264.45	729.307	5.826	2.219	-0.944	100	23.116

with the target protein's active site (GLN 721) attesting a strong hydrophobic interaction. *In silico* descriptions (Table 2) of the compounds was performed through ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) which is an imperative technique used to unveil the pharmaceutical significance of the phytocompounds. Many *in silico* technologies in drug discovery have increased the possibility of finding the new lead compounds at much shorter time period. However, unfavorable ADMET properties have been identified as a major cause of failure even for very promising drug candidate molecules<sup>25-29</sup>.

Consequently, earlier prediction of ADMET properties through *in silico* methods can increase the success rate of candidate molecules prior to reaching development. Ecopharmacognosy, enables the sustainability of traditional medicine and supports the

*in silico* evaluation of known compounds as enzyme inhibitors prior to *in vitro* testing to conserve the natural resources<sup>30</sup>. Network ethnopharmacology is an advanced technique that scientifically explores traditional knowledge and improves the existing knowledge on drug discovery<sup>31</sup>. Traditional ADME analyses relied heavily on whole animal assays and the more labor intensive biochemical studies. High throughput screening methods, fast ADMET profiling assays, and computational approaches have allowed the pharmaceutical industry to identify quickly the less promising drug candidates in the very early development stage so that time and valuable resources are not spent pursuing compounds that have little probability of reaching the general population. Of the fore-mentioned properties, the drug's aqueous solubility will likely be one of the first properties measured. Aqueous solubility is a major indicator of

the drug's solubility in physiological gastrointestinal fluids and is a major indicator of the drug's oral bioavailability. Hexanoic acid, 4-hexadecyl ester and Pseudohypericin exhibit 100% human intestinal absorption, with high *in vitro* plasma protein binding hence, indicates that both phytochemicals can be used as drug molecules.

Lipophilicity is another of the physical properties that is measured in the early stages of drug testing to predict the transport of molecules from the gastrointestinal tract into the epithelial cells that line the inner and outer surfaces of the body. Most common drugs cross cellular barriers by transcellular pathways (across epithelial cells) that require the drug to enter the outer portion of the lipid bilayer of the cell membrane. The drug then diffuses to the inner lipid layer and travels across the cell before crossing the cell membrane once again to exit. Lipophilicity was introduced to describe a compound's affinity to be in lipid-like environment. Several solvent systems have been suggested as a surrogate to represent the lipid membrane against water. For convenience and economical reasons, the partition coefficient of the drug candidate between 1-octanol and a series of aqueous buffers has become the standard measure of lipophilicity. The intrinsic lipophilicity (logarithm of the water-to-octanol partition coefficient,  $\log P_o/w$ ) describes the equilibrium distribution of molecular drug candidate (unionized form of the molecule) between water and the aqueous buffer, and is independent of *pH*. The effective lipophilicity (logarithm of the water-to-octanol distribution coefficient) reflects the concentration ratio of the neutral drug molecule plus all ionized forms that may be present in the aqueous buffered solution at the given *pH*. The effective lipophilicity is highest in hexanoic acid, 4-hexadecyl ester thereby facilitates and confers a better pharmacokinetic property with a high BBB permeation, normal polar surface area (0.0 to 150.0) values and restricting into the optimistic molecular weight. The *in silico* study characterises hexanoic acid, 4-hexadecyl ester as a promising ligand that passed through the pre-screening phase on pharmacological properties such as, ADMET and docking studies endorses the phytochemical to be a more effective brain drug that can pass through the blood brain barrier.

### Conclusion

The current study aggravates the importance of the plant *Clitoria ternatea* L. an ancient *Ayurvedic*

medicinal herb on par with the western folk drug *Hypericum perforatum* L. (*St John's wort*). The phytochemicals hexanoic acid-4-hexadecyl ester and 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-furnished a least binding energy affinity with the efflux protein P-Gp and can establish as potential lead molecules in developing an efficient brain drug that can surpass the blood brain barrier. The effectiveness of these potent phytochemicals as a synergistic effect can be further endorsed by performing clinical studies which would elevate the incredible competence of *Ayurvedic* medicine on parity with western medicine.

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