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ANTIBACTERIAL PHYTOCHEMICALS IN *MACROTYLOMA UNIFLORUM* (LAM.) VERDC. ON DNA-GYRASE B: AN *IN SILICO* STUDY

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ABSTRACT: The medicinal plant, *Macrotyloma uniflorum* (Lam.) Verdc. is a common pulse and the seed of this plant containing several phytochemicals that inhibit the multiplication of bacteria. The objective of the present predictive study was to detect the receptor-ligand binding energy and interaction through molecular docking for phytocompounds established in *M. uniflorum* seed on bacterial DNA gyrase B receptor (PDB ID: 3G7B). *In silico* study especially molecular docking and pharmacokinetics, bioavailability, drug-likeness and medicinal chemistry prediction was performed by using PyRx tool (Version 0.8) and Swiss ADME online tool to know binding affinity and energy along with druggability. The molecular interaction was visualized in the molecular graphics laboratory (MGL) tool (Version 1.5.6). The common phytoligands of 16 numbers and antibiotic of 1 number were selected for present prediction. The result of molecular docking revealed that favourable binding energy was observed in daidzein (-7.5 Kcal/mol) followed by stigmasterol (-7.3 Kcal/mol) of *M. uniflorum* when compared to ciprofloxacin (-6.4 Kcal/mol) on DNA gyrase B receptor. In the pharmacokinetics, bioavailability and drug-likeness prediction, Daidzein can be suitable drug candidate, which may be potent lead antibacterial compound. In conclusion, the binding was obtained opposite to the active site, which may be due to non-competitive inhibition. Moreover, in future research this predictive data should be validated with further toxicological, pharmacological and bacterial inhibition study for confirmation of natural antibacterial compound.

KEYWORDS: *Macrotyloma uniflorum*; Phytoligands; Bacterial DNA gyrase B; Molecular docking; Pharmacokinetics; *In silico* study

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1. INTRODUCTION

The leguminous medicinal plant, *Macrotyloma uniflorum* (Lam.) Verdc. is commonly known as horse gram and found in different places of India. Several researchers have been reported that the phytoconstituents present in the plant parts used as antibacterial, antiurolithiatic against calcium oxide crystals, calcium phosphate crystals and uric acid crystals, etc. [1, 2, 3, 4, 5, 6, 7, 8]. The aqueous extract of this plant is used to prevent urinary tract infection (UTI) mainly by bacterial infection through traditional knowledge of phytomedicines [3]. The recurrence UTI may cause nephrolithiatic (kidney calculi or stones) or urolithiatic (urinary tract calculi or stones) effect [9, 10, 11], which is prevented by the extract of this plant [6, 12]. On the other hand, Srinivas et al. [13] studied potential antinephrolithiatic efficacy by aqueous seed extract of *M. uniflorum* on albino rat model. Moreover, it was observed that aqueous extract of *M. uniflorum* seed had the capacity of antibacterial activity [14]. Antibacterial agents are important medicines to prevent various bacterial infection but there is possibilities of side effect or resistance to pathogenic bacteria [15, 16]. To prevent bacterial resistance, researchers are showing interest to inhibit the growth of bacteria by using plant derived medicines or phytomedicines. Among several enzymes in bacteria, DNA gyrase enzyme is most effective for metabolic regulation in bacteria and help in the process of DNA replication, and known as type II topoisomerase [17, 18, 19]. Although, plant-based phytomedicine is suitable as antibacterial natural products and prevent bacterial resistance. Interestingly, in experimental study the crude extracts of plant parts of *M. uniflorum*, and also seeds show the antibacterial and/or antiurolithiatic or antinephrolithiatic activity [11, 14] and separation of each phytochemical to know their efficacy in wet lab can be suitable for the above-mentioned therapy that may need long duration, huge laboratory expanses, animal test, etc. But *in silico* study through computational prediction especially molecular docking and pharmacokinetics especially ADME (absorption, distribution, metabolism and excretion) supports the identification of lead compound(s) within a short duration. In this context, receptor (macromolecule)-ligand (small molecule) binding affinity and energy value estimation is a suitable technique for structure-based drug designing and exact phytocompound or combination of few phytochemicals can easily be predicted [20, 21, 22]. Present *in silico* study was to detect suitable receptor-ligand binding energy and interaction through molecular docking and druggability potential for common phytocompounds of *M. uniflorum* on bacterial DNA gyrase B receptor (PDB ID: 3G7B).

2. MATERIALS AND METHODS

Selection of protein (receptor)

The crystal three-dimensional structure of protein of bacterial DNA gyrase B (PDB ID: 3G7B) was downloaded from the website of protein data bank (www.rcsb.org/). Ronkin et al. [18] have investigated the X-ray diffraction crystallographic structures of the bacterial DNA gyrase B. They obtained receptor with inhibitory molecule (*Staphylococcus aureus* gyrase B co-complex with

methyl({5-[4-(4-hydroxypiperidin-1-Yl)-2-phenyl-1,3-thiazol-5-Yl]-1H-pyrazol-3-Yl}methyl)carbamate inhibitor) at 2.30Å resolution. The 3-D ribbon structure is exhibited in Figure 1 after visualizing in MGL tool developed by The Scripps Research Institute [23].

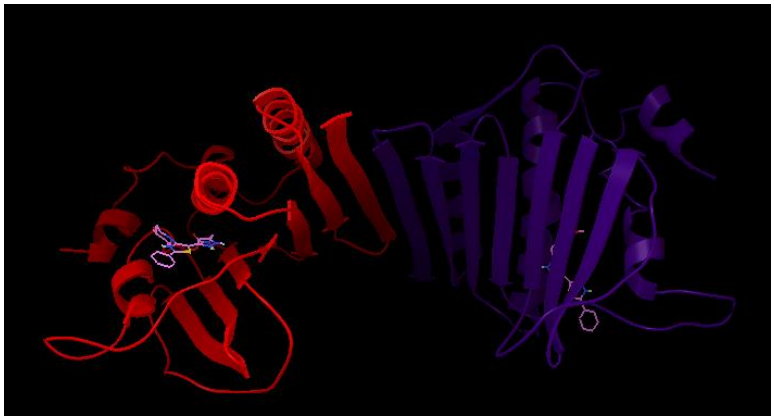

Figure 1: Three-dimensional (3D) ribbon structure of DNA gyrase B (PDB ID: 3G7B) [Chain A = red colour and Chain B = blue colour, both the chains are attached with inhibitory molecules (line structure in CPK at 471 and 472 position)]

Selection of phytochemicals and synthetic compound (ligands)

The common phytochemicals such as phenolic compounds (3,4-dihydroxy benzoic acid, vanillic acid, caffeic acid, p-cumaric acid, ferulic acid, chlorogenic acid, syringic acid and sinapic acid), flavonoids (daidzein, quercetin, kaempferol and myricetin), phytosterols (stigmasterol and β-sitosterol), and anthocyanidins (cyanidin and malvidin) present in the seed of *M. uniflorum* were selected as per previous reports [3, 4, 8, 24, 25, 26]. In the present study, common 16 phytoligands of *M. uniflorum* and 1 synthetic ligand (ciprofloxacin antibiotic) were taken**.** The Canonical SMILES (simplified molecular-input line-entry system) string of these ligands were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) and .pdb file of each phytochemical was obtained from CORINA online server (www.mol-net.de) after inserting SMILES string in particular place. The photograph of seeds is exhibited in Figure 2.

Figure 2: Seeds of *Macrotyloma uniflorum* Lam.

Study of molecular docking and interaction

The docking was carried out by a virtual screening tool, PyRx (Version 0.8) developed by Trott and Olson [27]. The molecular docking was visualized the output .pdbqt file by using MGL tool [23] and the results of three-dimensional structure of pose and interaction were rendered by using MGL tools [23]. Docking was done for 16 phytoligands and 1 ciprofloxacin (synthetic ligand) with bacterial DNA gyrase B (PDB ID: 3G7B) receptor to detect suitable binding energy value. The phytoconstituents and synthetic drug (ligands) with the DNA gyrase B receptor to identify the residues involved in each case of receptor-ligand interaction for the detection of antibacterial property. Table 1 describes the 3-D grid box size values and central position values for docking site on the studied target receptor with a grid spacing of 0.375 Å. Finally, all the 17 ligands were studied to detect binding pose, energy value and interactions. The resultant structural complexes of the individual ligand/receptor binding were finally observed in MGL tool [23], to know some specific contacts between the atoms of the ligands and amino acids of the DNA gyrase B receptor.

Table 1: Grid size for studied receptor (in Å)

Receptor	Size			Position from centre		
	X	Y	Z	X	Y	Z
DNA gyrase B	69.0284	46.5718	71.4694	27.0389	0.0933	8.8102

Pharmacokinetics, bioavailability and drug-likeness prediction of ligands

The predictive study of pharmacokinetics especially ADME, bioavailability and drug-likeness of ligands were done through SwissADME online tool developed by Daina et al. [28; 29]. The tool predicts bioavailability radar as per six physicochemical properties such as lipophilicity, size, polarity, solubility, flexibility and saturation to detect druglikeness. The ADME properties viz. passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeation as well as substrate or non-substrate of the permeability glycoprotein (P-gp) as detected positive or negative in the BOILED-Egg model within the tool developed by Daina, and Zoete, [30] and Daina et al. [28]. The estimation of lipophilicity (Log p/w) parameters such as iLOGP was calculated for n-octanol and water on free energies of solvation as per the generalized-born and solvent accessible surface area (GB/SA) model developed by Daina et al. [31], XLOGP3 is an atomistic method including corrective factors and knowledge-based library developed by Cheng et al. [32], WLOGP is an implementation of a purely atomistic method based on the fragmental system of Wildman and Crippen [33], MLOGP is an archetype of topological method relying on a linear relationship with 13 molecular descriptors implemented as per researchers [34, 35] and SILICOS-IT is an hybrid method relying on 27 fragments and 7 topological descriptors (http://silicos-it.be.s3-website-eu-west-1.amazonaws.com/software/filter-it/1.0.2/filter-it.html,accessed June 2016) [28]. The Lipinski

(Pfizer) filter is the pioneer rule-of-five was implemented in the tool from Lipinski et al. [36] and incorporated in this tool for the prediction of druglikeness [28]. The bioavailability radar for oral bioavailability prediction as per different physico-chemical parameters was developed by SwissADME tool [28, 29]. The ranges of each parameter was mentioned as LIPO = lipophilicity as -0.7 < XLOGP3 < +5.0; SIZE = size as molecular weight 150gm/mol < MV < 500gm/mol; POLAR = polarity as 20Å² < TPSA (topological polar surface area) < 130Å²; INSOLU = insoluble in water by log S scale 0 < Logs (ESOL) < 6; INSATU = insaturation or saturation as per fraction of carbons in the sp3 hybridization 0.3 < Fraction Csp3 < 1 and FLEX = flexibility as per rotatable bonds 0 < No. rotable bonds < 9 [28, 29].

3. RESULTS AND DISCUSSION

The results of binding energy values (Table 2) indicated that phytoligands such as daidzein and stigmasterol showed -7.5 and -7.3 Kcal/mol respectively while β-sitosterol, quercetin, cyanidin, myricetin, malvidin, chlorogenic acid, kaempferol, sinapic acid, caffeic acid, p-cumaric acid, ferulic acid, 3,4-dihydroxy benzoic acid, syringic acid and vanillic acid were obtained -6.9, -6.9, -6.9, -6.8, -6.7, -6.6, -6.3, -5.3, -5.3, -5.2, -5.2, -5.0, -5.0 and -4.9 Kcal/mol respectively when compared to synthetic ligand ciprofloxacin (-6.4 Kcal/mol). In case of daidzein, contact residues were obtained LYS170, ASN82, GLN66, GLN210, SER226, GLU224, GLU68 and THR80 in chain B having two hydrogen bonding THR212 and unknown in chain B while for stigmasterol, contact residues THR212, THR80, GLU68, ASP81, THR171, ASN82, GLN66 and LYS170 in chain B were obtained without hydrogen bonding in comparison with ciprofloxacin the contact residues GLU41, HIS45, TRP49, ARG42 and ARG198 in chain B having one hydrogen bond contact THR194 in chain B were obtained (Figure 3, 4 and 5). According to Gross et al. [37] and Jagadeesan et al. [38], it was known that active site residues such as GLU50, ASN54, GLU58 and THR173 are found in DNA gyrase B, which located in the ATP binding pocket and functions mainly involved in ATPase activity. The present *in silico* study revealed that the phytoligand (daidzein), and synthetic ligand (ciprofloxacin) both were showed binding pose opposite to the active site in chain B of bacterial DNA gyrase B (PDB ID: 3G7B) having two hydrogen bonding and one hydrogen bonding, which may be due to non-competitive inhibition. In several experimental studies, it was observed that crude extract of *M. uniflorum* in different solvents are potential for the inhibition of pathogenic bacterial growth. These bacteria are *Bacillus subtilis*, *Bacillus careus*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysentriae*, *Escherichia coli*, etc. [2, 3, 4]. In another experiment on antibacterial potential of natural compounds by Abreu et al. [39], observed isoflavonoids such as luteolin, apigenin, chrysin, genistein and daidzein from *Cytisus striatus* as antibiotic adjuvants against methicillin-resistant *Staphylococcus aureus* (MRSA), which support the present prediction on DNA gyrase B receptor for phytoligand daidzein obtained favourable binding energy followed by stigmasterol (Table 2).

Table 2: Energy values for receptor-ligand binding

Sl. No.	Ligands	Binding energy (Kcal/mol)
Phytocompounds		
1.	Daidzein	-7.5
2.	Stigmasterol	-7.3
3.	β-sitosterol	-6.9
4.	Quercetin	-6.9
5.	Cyanidin	-6.9
6.	Myricetin	-6.8
7.	Malvidin	-6.7
8.	Chlorogenic acid	-6.6
9.	Kaempferol	-6.3
10.	Sinapic acid	-5.3
11.	Caffeic acid	-5.3
12.	p-Cumaric acid	-5.2
13.	Ferulic acid	-5.2
14.	3,4-dihydroxy benzoic acid	-5.0
15.	Syringic acid	-5.0
16.	Vanillic acid	-4.9
Synthetic antibiotic		
1.	Ciprofloxacin	-6.4

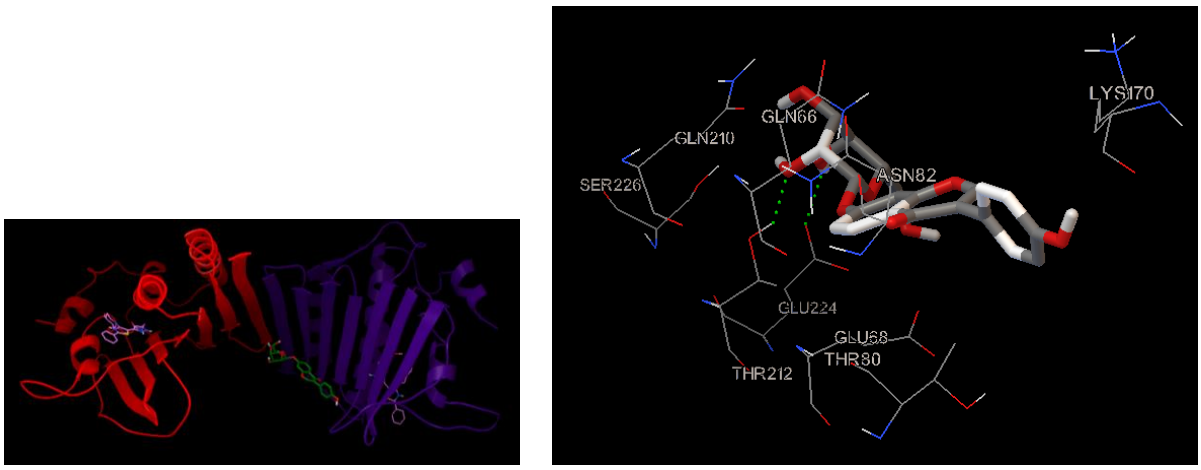

Figure 3: Daidzein binding pose and interaction

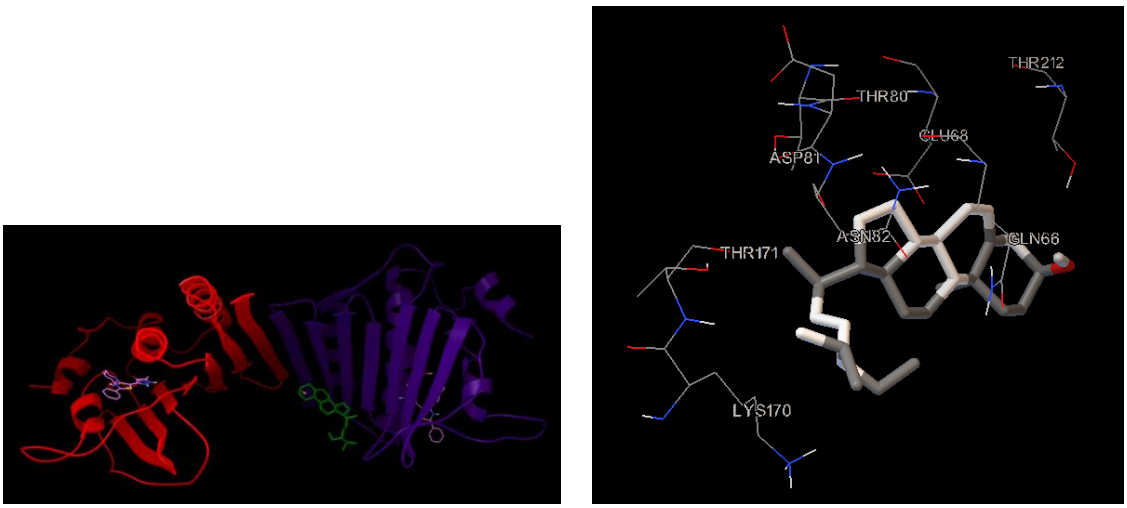

Figure 4: Stigmasterol binding pose and interaction

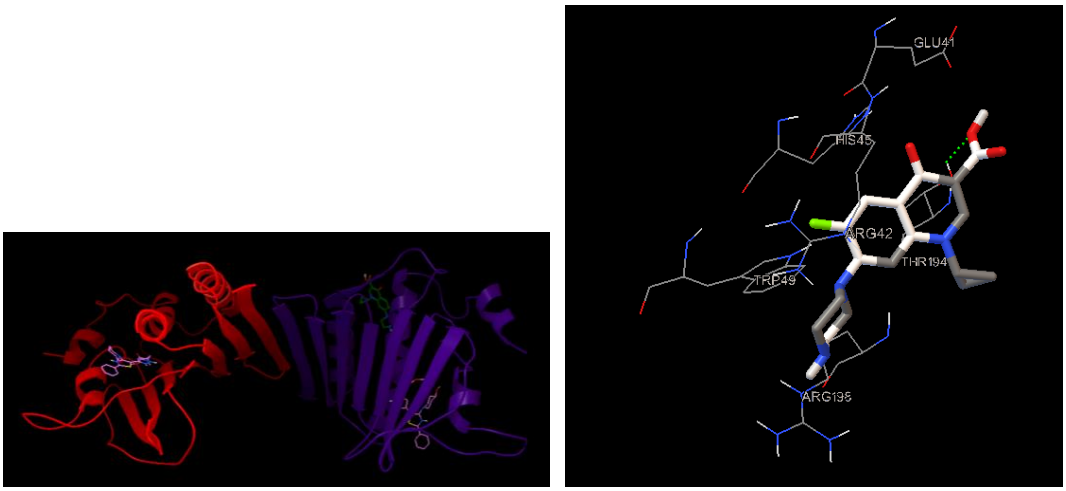

Figure 5: Ciprofloxacin binding pose and interaction

In recent research, it was reported that antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *S. aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas fluorescens* and *Klebsiella pneumoniae* by stigmasterol isolated from the stem bark of *Neocarya macrophylla*. Moreover, Yusuf et al. [40] have investigated antibacterial properties of stigmasterol isolated from the stem bark of *Neocarya macrophylla* plant. They tested on several species viz. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *S. aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae* and no inhibitory effect was observed on few bacterial species such as VRE, *S. typhi* and *K. pneumoniae*. In the present prediction, the favourable energy value of daidzein and stigmasterol was found close proximity, which may support after confirming wet lab experimentation. The results on predictive values for pharmacokinetics, bioavailability, drug-likeness and medicinal chemistry data on studied phyto and synthetic ligands (Table 3). For

pharmacokinetics prediction, the GI absorption rate was obtained higher for daidzein and ciprofloxacin while lower for stigmasterol. No blood-brain permeability was observed for stigmasterol and ciprofloxacin while daidzein observed penetration positive. In case of skin permeation (log Kp, cm/s), higher negative value was obtained for ciprofloxacin followed by daidzein and lower for stigmasterol. Both phytoligands did not show p-glycoprotein substrate activity except ciprofloxacin. To detect inhibitory activity for cytochrome p450 as CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitors, daidzein showed inhibition except others two for CYP1A2, CYP2D6 and CYP3A4. The prediction of bioavailability data revealed that same bioavailability scores were obtained for all studied small molecules. The water solubility data obtained moderately soluble both phytoligands while soluble for synthetic ligand (Table 3). In case of bioavailability, other parameters such as iLOGP, XLOGP3, WLOGP, MLOGP and SILCOS-ST data were also predicted. For iLOGP, stigmasterol observed higher value followed by ciprofloxacin while daidzein showed lower value. For XLOGP3, stigmasterol obtained higher value and daidzein showed lower value while ciprofloxacin obtained negative value. For WLOGP, stigmasterol observed higher value followed by daidzein and ciprofloxacin and for MLOGP, former was same followed by ciprofloxacin and daidzein. For, SILCOS-IT, stigmasterol observed higher value followed by daidzein and ciprofloxacin.

Table 3: Prediction of Pharmacokinetics, bioavailability, drug-likeness and medicinal chemistry of phyto and synthetic ligands

Pharmacokinetics								
Sl. No.	**Ligands**	**GI absorption**	**BB permeant**	**PGP substrate**	**CYP450 1A2 inhibitor**	**CYP450 2C19 inhibitor**	**CYP450 2C9 inhibitor**	**CYP450 2D6 inhibitor**
1.	Daidzein	High	Yes	No	Yes	No	No	Yes
2.	Stigmasterol	Low	No	No	No	No	Yes	No
3.	Ciprofloxacin	High	No	Yes	No	No	No	No
Pharmacokinetics				**Bioavailability**				
Sl. No.	**Ligands**	**CYP450 3A4 inhibitor**	**Skin permeation as log Kp (cm/s)**	**Bioavailability score**	**Water solubility as logS & SILICOS-IT**	**iLOGP**	**XLOGP3**	**WLOGP**
1.	Daidzein	Yes	-6.10	0.55	Moderately soluble & -4.98	1.77	2.47	2.87

2.	Stigmasterol	No	-2.74	0.55	Moderately soluble & -5.47	4.96	8.56	7.80
3.	Ciprofloxacin	No	-9.09	0.55	Soluble & -3.50	2.24	-1.08	1.18

Bioavailability				**Drug-likeness**				
Sl. No.	**Ligands**	**MLOGP**	**SILICOS-IT**	**Lipinski rule**	**Ghose filter**	**Veber filter**	**Egan filter**	**Muegge filter**
1.	Daidzein	1.08	3.02	Yes; 0 violation	Yes	Yes	Yes	Yes
2.	Stigmasterol	6.62	6.86	Yes; 1 violation	No; 3 violations	Yes	No; 1 violation	No; 2 violations
3.	Ciprofloxacin	1.28	1.90	Yes; 0 violation	Yes	Yes	Yes	Yes

Medicinal chemistry					
Sl. No.	**Ligands**	**Lead-likeness**	**Pan assay interface structure**	**Brenk structural alert**	**Synthetic accessibility score**
1.	Daidzein	Yes	0 alert	0 alert	2.79
2.	Stigmasterol	No; 2 violations	0 alert	1 alert	6.21
3.	Ciprofloxacin	Yes	0 alert	0 alert	2.51

GI = Gastro-intestinal; BB = Blood-brain; PGP = p-Glycoprotein

The bioavailability radar (Figure 6) for oral bioavailability prediction for each ligand showed daidzein and ciprofloxacin are with the range of >-0.7 and <+5 for LIPO as XLOGP3. The SIZE as molecular weight (gm/mol) was showed 254.24 for daidzein, 331.34 for ciprofloxacin and 412.69 for stigmasterol. The POLAR as TPSA (Å²) 70.67 and 74.57 for daidzein and ciprofloxacin while 20.23 for stigmasterol. The INSOLU Logs (ESOL) values were showed negative for all ligands. The INSATU (insaturation) as per Csp3 data, 0.00 for daidzein, 0.86 for stigmasterol and for 0.41 ciprofloxacin. The data for FLEX as per no. of rotable bonds 1 no., 5 and 3 nos. for daidzein, stigmasterol and ciprofloxacin respectively.

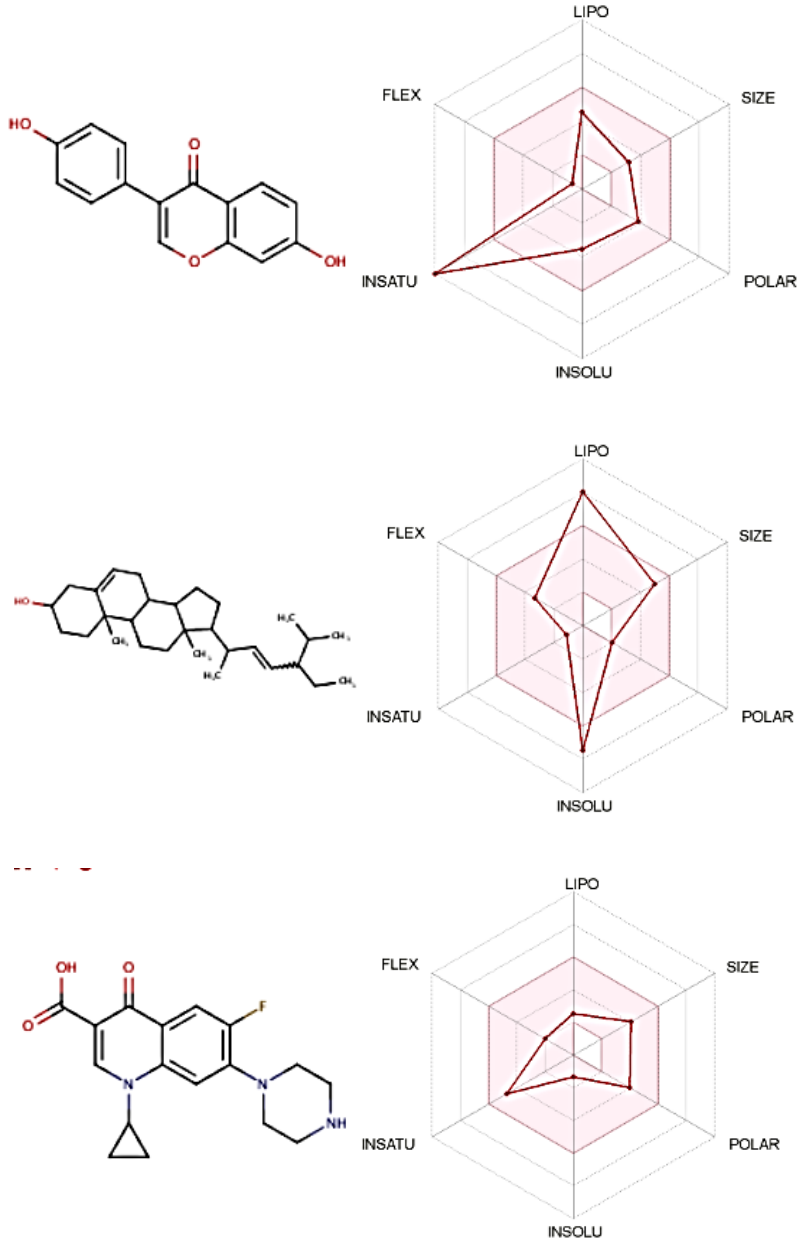

Figure 6: Molecular structure and bioavailability radar (pink area exhibits optimal range of particular property) for studied small molecules such as diadzein, stigmasterol and ciprofloxacin (LIPO = lipophilicity as XLOGP3; SIZE = size as molecular weight; POLAR = polarity as TPSA (topological polar surface area); INSOLU = insolubility in water by log S scale; INSATU = insaturation as per fraction of carbons in the sp3 hybridization and FLEX = flexibility as per rotatable bonds

The inbuilt BOILED-Egg model represented that daidzein and ciprofloxacin showed the capability of GI absorption while blood-brain barrier penetration was found only for daidzein among other two small molecules. All small molecules were found PGP negative as non-substrate in predictive model except ciprofloxacin (Figure 7).

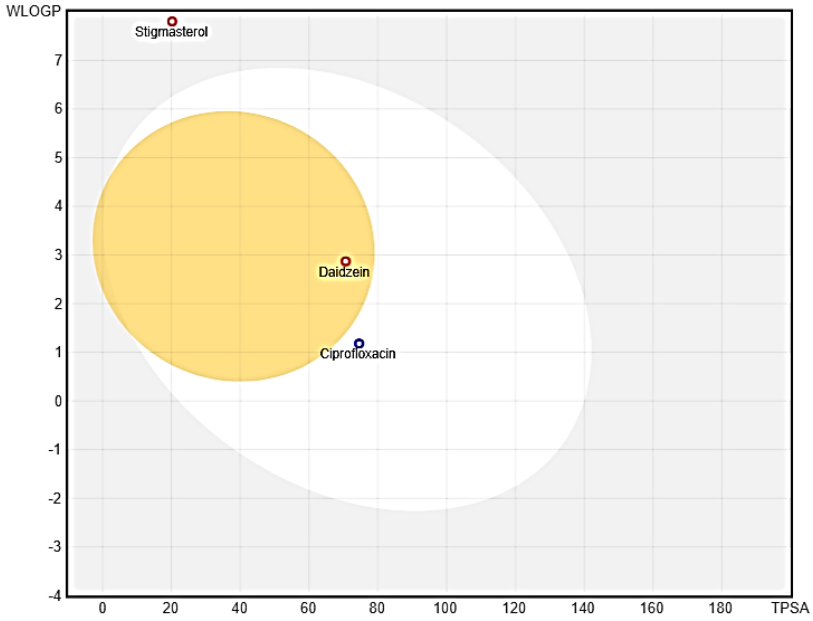

Figure 7: The BOILED-Egg represents for intuitive evaluation of passive gastrointestinal absorption (HIA) white part and brain penetration (BBB) yellow part as well as blue and red points PGP positive and negative in function of the position of the small molecules in the WLOGP-versus-TPSA graph

Besides molecular docking and interaction for receptor-ligand binding, the prediction of pharmacokinetics, bioavailability, drug-likeness and medicinal chemistry of small molecules are the present research interest by researchers for new drug design and an easy screening can easily be done by using SwissADME online tool [21, 22, 28, 29, 30, 31]. It was well-known that the physicochemical properties such as solubility and lipophilicity prediction are also detected the small molecule whether progressing a successful drug candidate [21, 22, 28, 29, 30, 31]. The graphical representation of Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) has already been proposed as an accurate predictive model, which helps by computational prediction of the lipophilicity and polarity of small molecules [28, 29]. In overall predictive results, flavonoid diadzein can be suitable drug candidate after isolation from the seeds of *M. uniflorum* as per bioavailability radar and BOILED-Egg representation. Furthermore, these predictive results should be validated by *in vitro* and *in vivo* toxicological and pharmacological assay for the prevention of bacterial pathogenicity with special reference to UTI.

4. CONCLUSION

In *in silico* study, the docking binding energy value prediction revealed that the among 16 natural compounds selected from *M. uniflorum*, diadzein showed favourable binding energy value followed by stigmasterol on bacterial DNA gyrase B receptor compared to synthetic antibiotic (ciprofloxacin). All these small molecules showed binding opposite to the active site, which may be due to non-competitive inhibition against studied receptor when compared to established synthetic antibiotic.

Besides docking, the prediction of pharmacokinetics, bioavailability, drug-likeness and medicinal chemistry, the small molecule, daidzein can be a lead compound for new drug candidate for antibacterial phytomedicine. However, it is suggested further *in vitro* and *in vivo* assay for toxicology, pharmacology and bacterial inhibition study for antibacterial compound to validate the present predictions.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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