Original Article

Insilico Studies of Cardiospermum canescens derived compounds towards Anti inflammatory activity

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ABSTRACT

The objective was to predict the binding affinity and energy of phytochemicals of Cardiospermum canescens to synthesis a drug against Inflammation. The medicinal plant derived phytocompounds and the human 15-lipoxygenase-2 were undergone Molecular docking and interaction along with druggability assessment. The software, GOLD for the structure-based virtual screening to know receptor-ligand binding affinity and energy. The human 15-lipoxygenase-2 as receptor was obtained (PDB ID: 4NRE) from the Protein Data Bank (PDB). The prediction of pharmacokinetics, bioavailability and druglikeness for these small molecules was done by using SwissADME online tool. Present computational prediction (molecular docking) indicates that favourable fitness score was observed in Calycosin (40.53), Quercetin (36.80), Hentriacontanol (36.46), followed by Apigenin (33.41), rutin (28.19), Protocatechuic acid (28.12), Pentacatechualdehyde (27.88) and Pentadecanoic acid (27.29) of Cardiospermum canescens. The pharmacokinetics, bioavailability and druglikeness predictions showed may be Calycosin can be suitable drug candidate. Present in silico study by using software, the phytoligand Calycosin of Cardiospermum canescens may be considered as lead molecules to inhibit the inflammatory activity. In future, further functional (in vivo and in vitro) assay is suggested to validate the present predictive results.

Keywords: *In silico, Inflammation, virtual screening, Phytochemicals, Medicinal plant.*

1. INTRODUCTION:

Most of the Sapindaceaeous plants are rich in flavonoids, terpenoids, glycosides and cyanolipids. Several studies on these plants and their constituents have shown to display wide range of biological actions such as gastro protective, anti-inflammatory, antibacterial, antiviral, anticancer and anti-allergic activities (Takada et al., 2004; Ahmed et al. 1993; Umadevi and Daniel 1991; Ferrara et al. 1996). The herbs Cardiospermum helicacabum, Cardiospermum grandiflorum,

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Cardiospermum canescens, are herbaceous climbing vines, grown in various parts of India. Cardiospermum canescens is grown widely in tropical forests and wastelands in most parts of south India (Kirithiakr and Basu, 1999; Hooker, 1978). The whole plant of Cardiospermum canescens is used traditionally for rheumatism as a poultice (Rutomjee et al. 1984; Chopra et al., 1986). Many herbs used in traditional medicine for centuries, in most parts of the world have shown to have potent anti-inflammatory activity along with immuno modulatory effects with fewer or no side effects. The plant kingdom, therefore, is the most fruitful area for the discovery of new drugs for many such ailments including rheumatoid arthritis and related inflammatory disorders. Such avenues in the herbal drug research are constantly encouraging the researchers for the development of novel anti-inflammatory drugs.

Among several phytocompounds in crude extracts of this medicinal plant are well-known for experimental study. In experimental study, the flavonoids from Cardiospermum

canescens are suitable phytochemicals for the prevention of pain and inflammation. The researchers observed that synthetic drugs have potent side effects when used for the inhibition of above-mentioned target receptors for pain and inflammation. In present research scenario, researchers are showing interest for medicines from plant origin or phytomedicines to target inflammatory mediators without any adverse effects (Dragos D et al., 2017). According to Dragos et al., there is several plant species used to relief pain and prevent inflammation, oxidative stress, etc. during joint disorders.

Generally, in silico screening, protein or receptor is the main target to detect allosteric or inhibitory activity for drug action. Several compounds or ligands are derived from synthetic compounds or phytocompounds, which show favourable binding affinity and energy for the target. This may help in new and efficient drug development as a lead molecule(s). The virtual screening helps to detect large numbers of drug-like compounds, which are commercially available, computationally screened against targets to recognize the structure and function that are predicted to bind properly in an experiment (Reddy A.S. et al., 2007, Lavecchia A et al., 2013, Lionta E et al., 2014). Therefore, in recent trend, development of phytomedicines, in silico predictions play a vital role in the drug design and discovery process for pharmaceutical research. Moreover, the prediction of pharmacokinetics, bioavailability and druglikeness for small molecules has already been established by using SwissADME online tool (Daina A et al., 2017a, Daina A et al., 2016, Daina A et al., 2014, Daina A et al., 2017b, Adamu RM et al., 2018). The objective of the present study is to know the binding affinity and energy through molecular docking and interaction along with pharmacokinetics, bioavailability and druglikeness of these molecules.

2.   MATERIALS AND METHODS

The present in silico approach is based on molecular docking and interaction to detect the efficacy of selected compounds, along with pharmacokinetics, bioavailability and druglikeness of these molecules.

Selection of receptors

To prepare the protein, the crystal structures of lipoxygenase-2(PDB ID: 4NRE) from Homo sapiens were obtained from PDB database.

Selection of Ligands

For all the selected compounds, canonical SMILES (simplified molecular-input line-entry system) strings were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem).
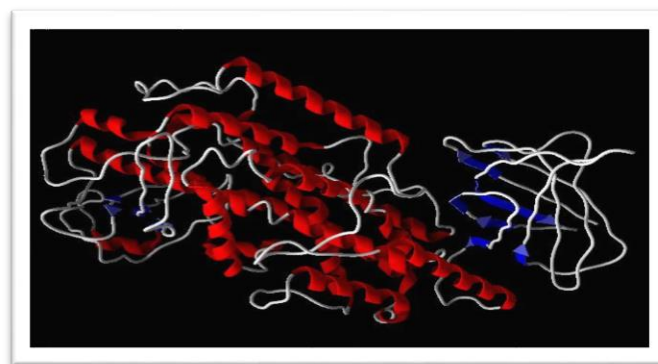
Fig 1: Ribbon structure of lipoxygenase-2(PDB ID: 4NRE)

Molecular docking and interaction for receptor-ligand binding:

After collecting the crystal structures, the possible binding sites of the protein from Homo sapiens was searched with CASTP server. From the binding site analysis of the protein we identified that, the binding pockets are identical in all chains and the largest binding pocket was taken for further docking studies. The crystal structure of these proteins was similar hence we have taken as representative structure for docking studies. The docking of drugs into the active site of this protein was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. We preferred Gold fitness score than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function.

Docking studies

The Eight compounds selected were pentadecanoic acid, apigenin, protocatechuic acid, protocatechualdehyde, hentriacontanol, calycosin, rutin, quercetin from Cardiospermum canescens. These compounds were fit in the active site of the target, considering both steric aspects (i.e., geometric shape) and functional group interactions, such as hydrogen bonding and hydrophobic interactions. The selected docked conformations of compounds into the proteins binding sites were shown.

Pharmacokinetics, bioavailability and druglikeness prediction of ligands

The predictive study of pharmacokinetics especially ADME, bioavailability and druglikeness of ligands were done through SwissADME online tool developed by Daina et al.(Daina A et al., 2017a) The tool predicts bioavailability radar as per six physicochemical properties such as lipophilicity, size, polarity, insolubility, flexibility and insaturation 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, (Daina A et al., 2016) and Daina et al. (Daina A et al., 2017a). The lipophilicity estimation (Log p/w) parameters such as iLOGP on free energies of solvation in n-octanol and water calculated by the generalized-born and solvent accessible surface area (GB/SA) model developed by Daina et al. (Daina A et al., 2014), XLOGP3 is an atomistic method including corrective factors and knowledge-based library developed by Cheng et al. (Cheng T et al., 2007), WLOGP is an implementation of a purely atomistic method based on the fragmental system of Wildman and Crippen (Wildman SA et al., 1999), MLOGP is an archetype of topological method relying on a linear relationship with 13 molecular descriptors implemented as per researchers (Moriguchi I et al., 1992, Moriguchi I et al., 1994) and SILICOS-IT is an hybrid method relying on 27 fragments and 7 topological descriptors as per earlier study (http://silicos-it.be.s3-website-eu-west-1.amazonaws.com/software/filter-it/1.0.2/filter-it.html, accessed June 2016). The Lipinski (Pfizer) filter is the pioneer rule-of-five was implemented in the tool from Lipinski et al. (Lipinski CA et al., 2001) and incorporated in this tool for the prediction of druglikeness [16]. The bioavailability radar for oral bioavailability prediction as per different physico-chemical parameters was developed by SwissADME tool (Daina A et al., 2007a). 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 20Å^2 < TPSA (topological polar surface area) < 130Å^2; 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 (Daina et al., 2017a).

3.   RESULTS AND DISCUSSION

In the present study the docking was done to detect active binding site for the targets. Computational prediction indicates that favourable fitness score was observed in Calycosin (40.53), Quercetin (36.80), Hentriacontanol (36.46), followed by Apigenin (33.41), rutin (28.19), Protocatechuic acid (28.12), Pentacatechualdehyde (27.88) and Pentadecanoic acid (27.29) of Cardiospermum canescens. Apigen informed interactions with lipooxygenase by hydrogen bondings and amino acids Leu57, Glu59 are involved in bonding as well as in Calycosin Arg63, Val64, in Hentriacontanol Gln119, Lys124, in pentadecanoic Arg63, Val 64, in Protocatechualdehyde Asp60, Gly62, in protocatechuic acid Val61, Gly62, in Quercetin Glu59, and in Rutin Asp60 are involved in bonding.

Table1: Interaction profiles of selected phytocompounds from Cardiospermum canescens with docking against lipoxygenase-2

S.No.	Ligand Name	Fitness	S(hb_ext)	S(vdw_ext)	S(hb-int)	S(int)
1.	Apigenin	33.41	1.98	27.28	0.00	-6.07
2.	Calycosin	40.53	3.66	31.38	0.00	-6.29
3.	Hentriacontanol	36.46	0.00	24.19	0.00	-9.73
4.	Pentadecanoic Acid	27.29	0.00	29.42	0.00	-13.17
5.	Protocatechu aldehyde	27.88	3.06	19.22	0.00	-1.61
6.	Protocatechuic Acid	28.12	3.98	19.68	0.00	-2.92
7.	Quercetin	36.80	2.98	30.30	0.00	-7.84
8.	Rutin	28.19	10.04	28.65	0.00	-21.24

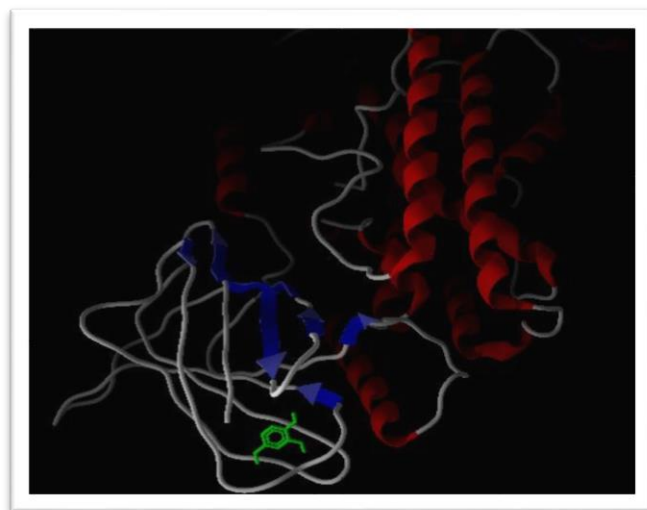*The fitness score is broken down into its constituent parts, specifically S(hb_ext), S(vdw_ext), S(hb_int), S(int).
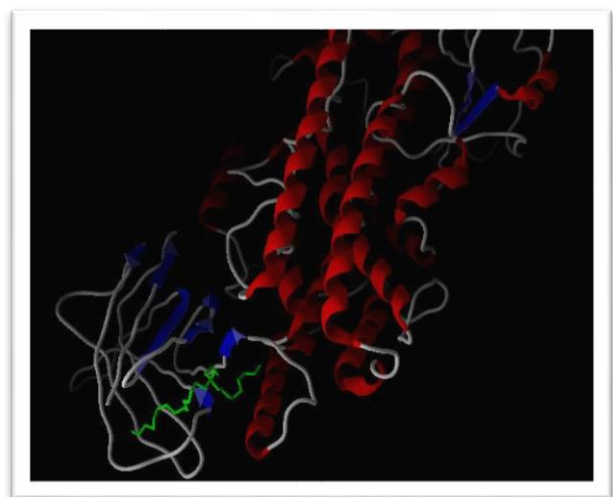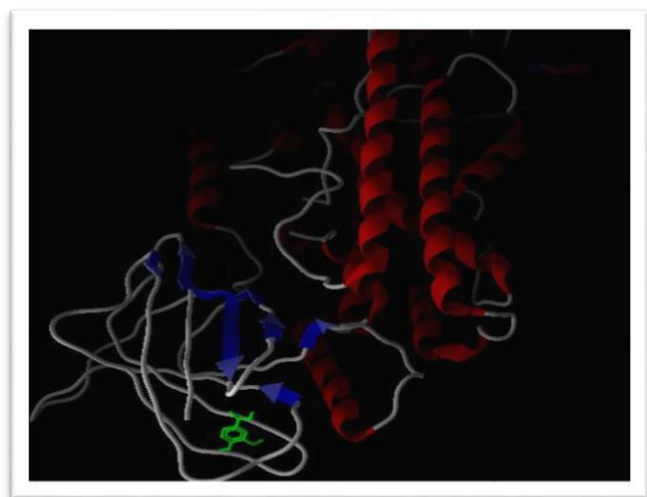
(A)
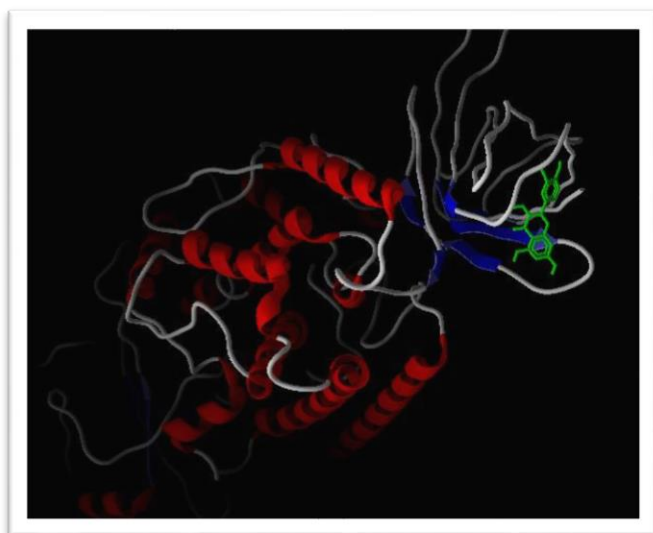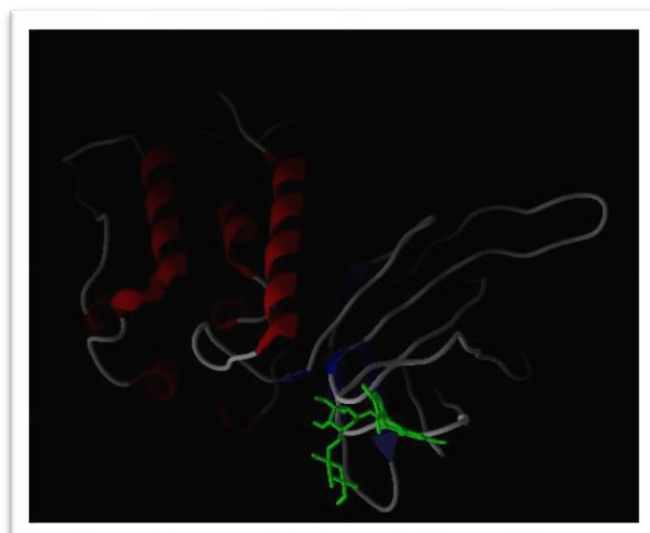

(B)

(C)

(D)

(E)
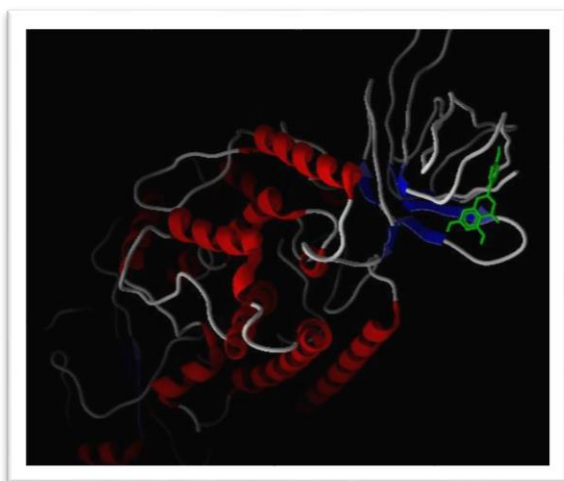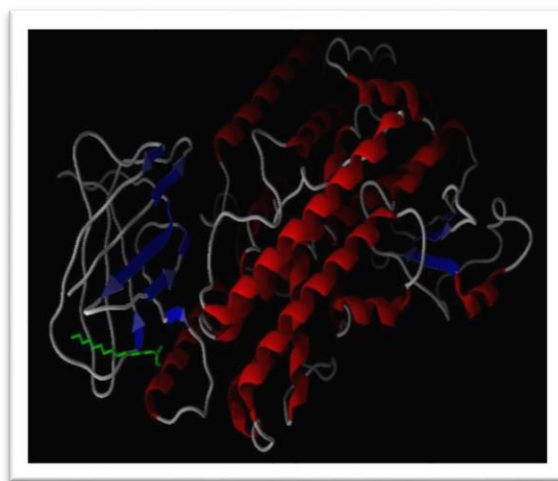
(F)

(G)

(H)

Fig 2: Docking Interactions of lipoxygenase-2 with A) Apigenin B) Calycosin C) Hentriacontanol D) pentadecanoie acid E) Protocatechualdehyde F) protocatechuic acid G) Quercetin H) Rutin

The following tables describe the predictive values for pharmacokinetics, bioavailability and druglikeness data on studied compounds. The molecules Calycosin, Apigenin, Quercetin, Pentadcanoic acid, Protocatechualdehyde and Protocatechuic acid showed high absorption rate while Rutin and Hentriacontanol obtained low absorption rate for GI absorption. No blood-brain permeability was obtained for Calycosin, Apigenin, Quercetin, Rutin, Protocatechuic acid and Hentriacontanol, but Pentadecanoic acid and Protocatechualdehyde showed penetration. Higher negative value obtained lower skin permeation (log Kp, cm/s) as Rutin (-10.26) followed by Quercetin (-7.05) and Protocatechuic acid (-6.42), Protocatechualdehyde (-6.37),Calycosin (-6.30), Apigenin (-5.80), Pentadecanoic acid (-3.07) and Hentriacontanol (-1.76). In case of metabolism, these molecules did not observe p-glycoprotein substrate while for cytochrome p450 as CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitors, Calycosin, Apigenin, Quercetin and Pentadecanoic acid obtained as inhibitor and Rutin, Protocatechualdehyde, Protocatechuic acid and Hentriacontanol as non-inhibitors for CYP1A2, All 8 molecules showed non-inhibitor for CYP2C19 and CYP2C9, and Calycosin, Apigenin and Quercetin obtained inhibitor while Rutin, Pentadecanoic acid, Protocatechualdehyde, Protocatechuic

acid and Hentriacontanol showed non-inhibitor for CYP2D6. The prediction of bioavailability and druglikeness, it was observed that All molecules bioavailability score showed same as 0.55 except Rutin as 0.17 The water solubility was obtained higher in Protocatechualdehyde and Protocatechuic acid, moderate in Pentadecanoic acid and just soluble for Calycosin, Apigenin, Rutin and Quercetin and Insoluble for Hentriacontanol.
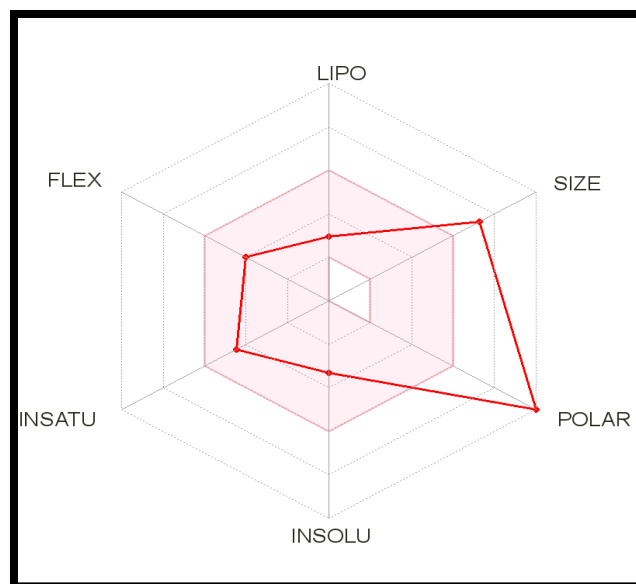
Table 2: Pharmacokinetics, bioavailability and drug likeness prediction of molecules

Bioavailability							
Sl. No.	Ligands	Skin permeation as log Kp (cm/s)	Bioavailability score	Water solubility as logS	iLOGP	XLOGP3	WLOGP
1.	Calycosin	-6.30	0.55	Soluble	2.40	2.44	2.88
2.	Apigenin	-5.80	0.55	Soluble	1.89	3.02	2.58
3.	Rutin	-10.26	0.17	Soluble	0.46	-0.33	-1.69
4.	Quercetin	-7.05	0.55	Soluble	1.63	1.54	1.99
5.	Pentadecanoic Acid	-3.07	0.55	Moderately Soluble	3.66	6.63	5.16
6.	Protocatechu aldehyde	-6.37	0.55	Very Soluble	0.79	1.09	0.91
7.	Protocatechuic Acid	-6.42	0.55	Very Soluble	0.66	1.15	0.80
8.	Hentriacontan ol	1.76	0.55	Insoluble	7.81	15.24	11.31

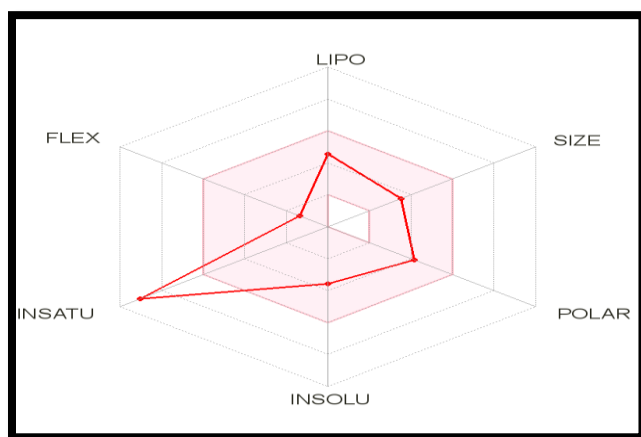| Pharmacokinetics								
Sl. No	Ligands	Gastro Intestinal absorption	Blood-Brain Permeant	P-glycoprotein substrate	CYP450 1A2 inhibitor	CYP450 2C19 inhibitor	CYP450 2C9 inhibitor	CYP450 2D6 inhibitor	CYP450 3A4 inhibitor
1.	Calycosin	High	No	No	Yes	No	No	Yes	Yes
2.	Apigenin	High	No	No	Yes	No	No	Yes	Yes
3.	Rutin	Low	No	Yes	No	No	No	No	No
4.	Quercetin	High	No	No	Yes	No	No	Yes	Yes
5.	Pentadecanoic Acid	High	Yes	No	Yes	No	Yes	No	No
6.	Protocatechualdehyde	High	Yes	No	No	No	No	No	Yes
7.	Protocatechuic Acid	High	No	No	No	No	No	No	Yes
8.	Hentriacontanol	Low	No	Yes	No	No	No	No	No

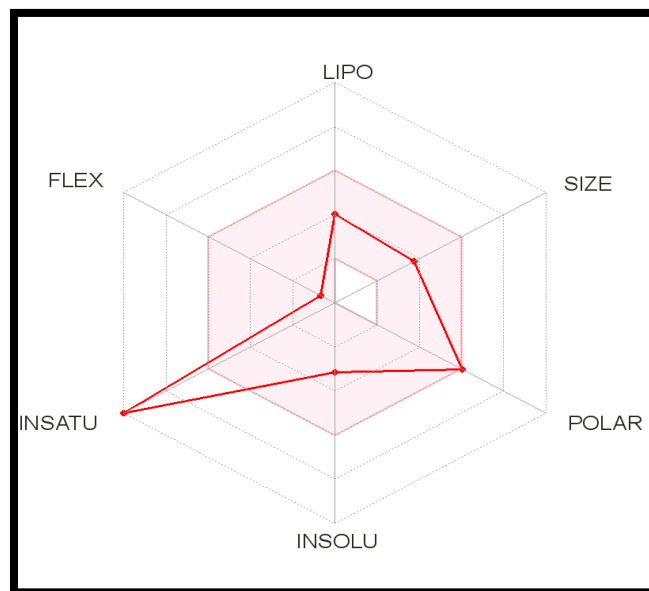| Drug likeness				
Sl. No.	Ligands	MLOGP	Lipinski rule	Lead-likeness
1.	Calycosin	0.77	Yes 0 violation	Yes
2.	Apigenin	0.52	Yes 0 violation	Yes
3.	Rutin	-3.89	No, 3 violations	No 1 violation
4.	Quercetin	-0.56	Yes 0 violation	Yes
5.	Pentadecanoic Acid	3.94	Yes 0 violation	No 3 violations
6.	Protocatechualdehyde	0.18	Yes 0 violation	No 1 violation
7.	Protocatechuic Acid	0.40	Yes 0 violation	No 1 violation
8.	Hentriacontanol	7.65	Yes 1 violation	No 3 violations

The study through molecular docking detects the exact ligand(s) for known target receptor. The molecular docking is widely used for new drug designing for therapeutic purposes. In other words, different ligands are screened to obtain Gibb's free energy bindings for affinity of drug towards the known target (Ganeshpurkar A et. al.,). The prediction of pharmacokinetics with special reference to ADME, bioavailability and drug like properties of small molecules are an important research interest by using SwissADME online tool for new drug design (Lionta E et. al.; Daina A et. al.; Daina A, Zoete V). 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 (Daina A et.al; Kwong E et.al,). But Calycosin showed suitable predictive data on physicochemical properties, pharmacokinetics, bioavailability and druglikeness. Interestingly, the 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 (Daina A et. al,).
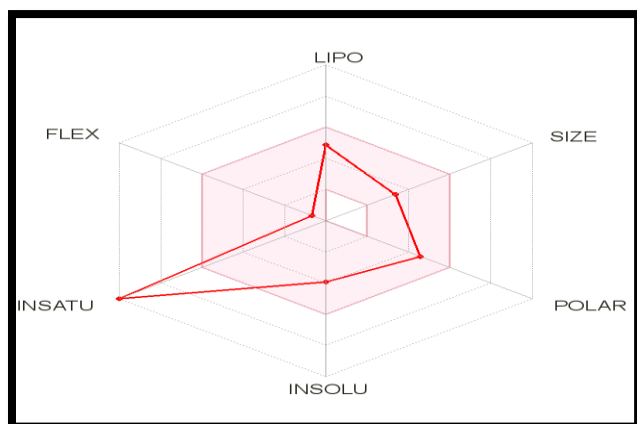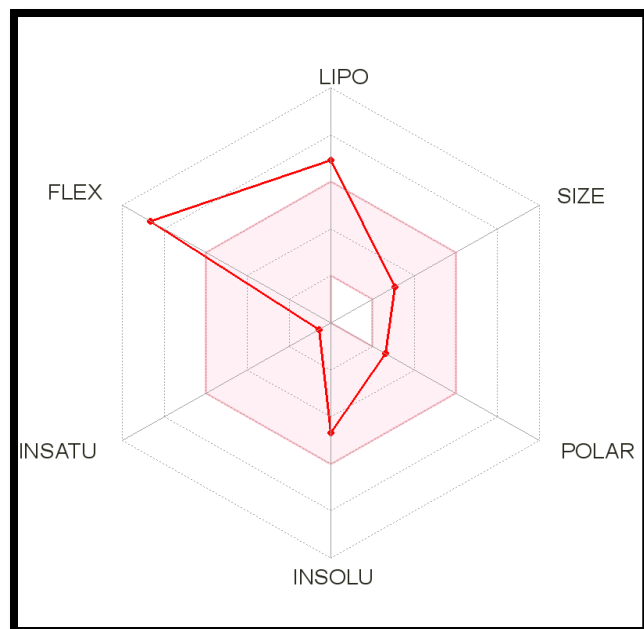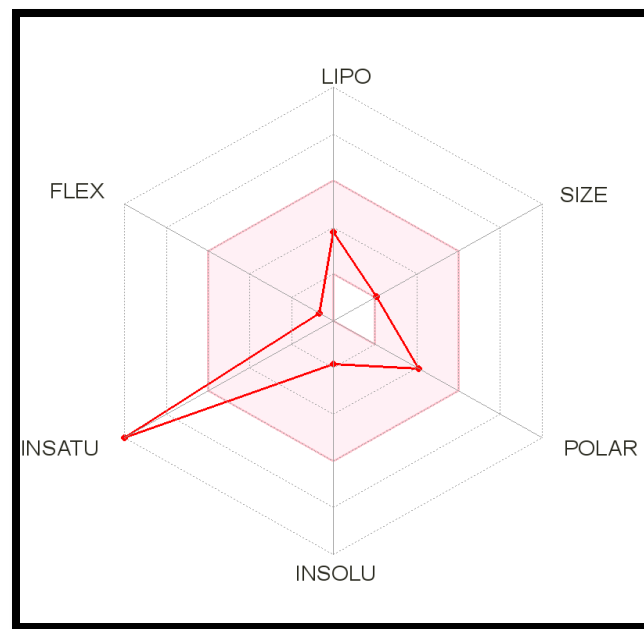

Rutin
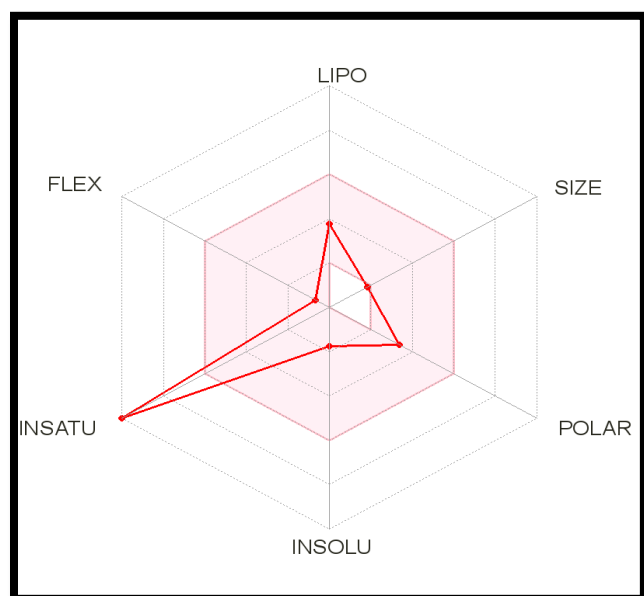
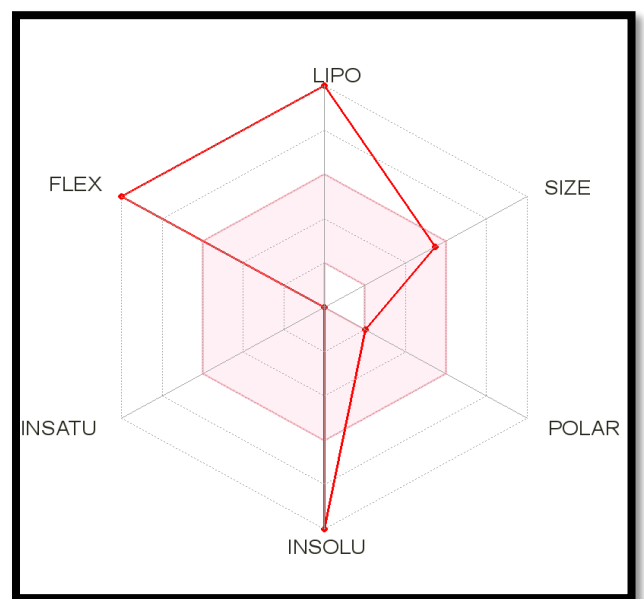

Calycosin

Quercetin

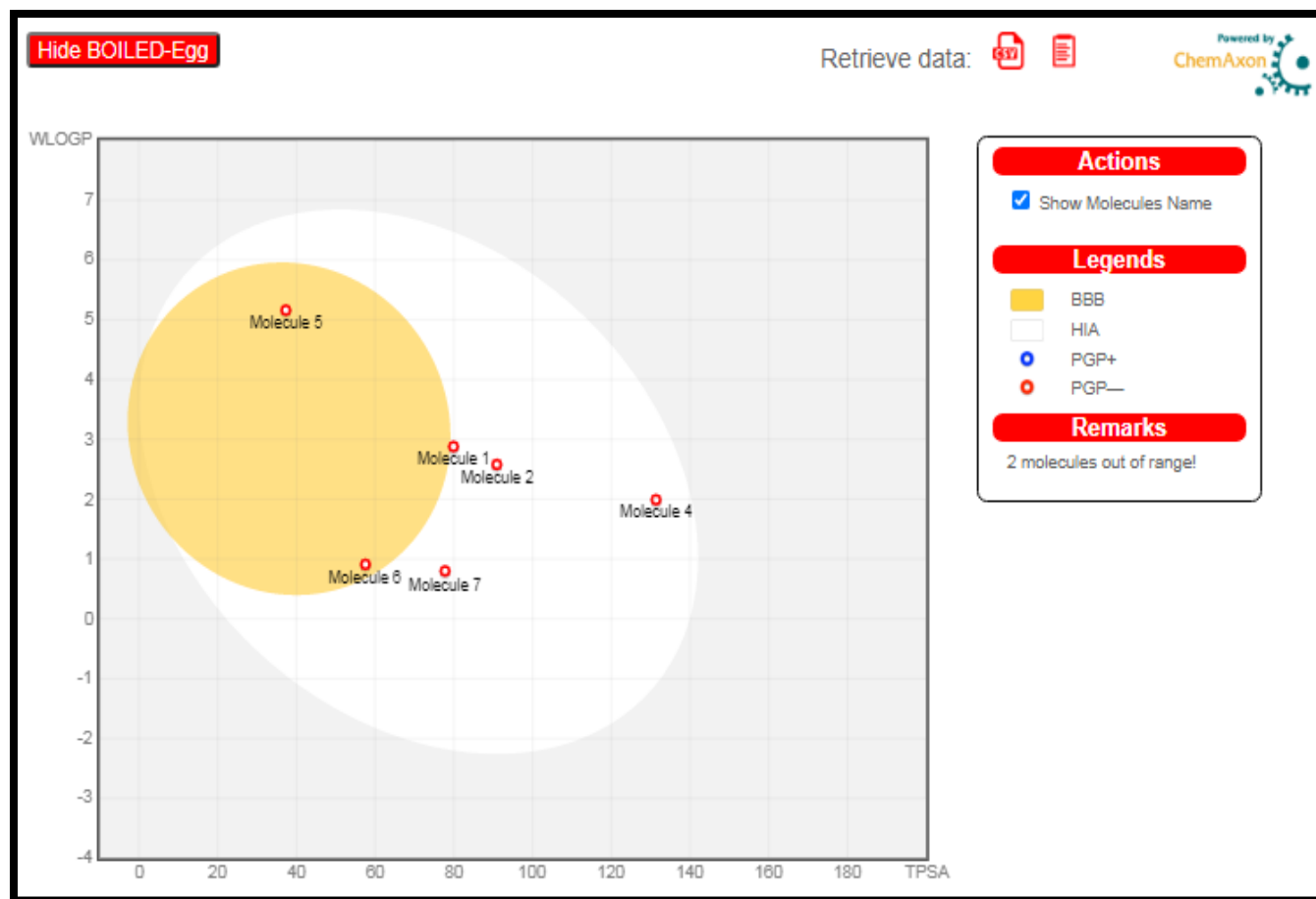Apigenin

Pentadecanoic Acid

Protocatechuic Acid

Protocatechualdehyde

Hentriacontanol

Fig: The Bioavailability Radar enables a first glance at the drug-likeness of a molecule.

Figure: The BOILED-Egg represents of selected compounds for intuitive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in function of the position of the molecules in the WLOGP-versus-TPSA graph

The study through molecular docking detects the exact ligand(s) for known target receptor. The molecular docking is widely used for new drug designing for therapeutic purposes. In other words, different ligands are screened to obtain Gibb's free energy bindings for affinity of drug towards the known target (Ganeshpurkar A et. al.,). The prediction of pharmacokinetics with special reference to ADME, bioavailability and drug like properties of small molecules are an important research interest by using SwissADME online tool for new drug design (Lionta E et. al.; Daina A et. al.; Daina A, Zoete V). 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 (Daina A et.al; Kwong E et.al,). But Calycosin showed suitable predictive data on physicochemical properties, pharmacokinetics, bioavailability and druglikeness. Interestingly, the 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 (Daina A et. al,). In overall predictive results, Calycosin can be suitable drug candidate as per bioavailability radar and BOILED-Egg representation. Furthermore, these predictive results should be validated by in vitro and in vivo functional and pharmacological assay.

4. CONCLUSION

Molecular docking indicates that favourable fitness score was observed in Calycosin (40.53), Quercetin (36.80), Hentriacontanol (36.46), followed by Apigenin (33.41), rutin (28.19), Protocatechuic acid (28.12), Pentacatechualdehyde (27.88) and Pentadecanoic acid (27.29) of Cardiospermum canescens. The prediction of pharmacokinetics with special reference to ADME, bioavailability and drug like properties of molecules, Calycosin can be lead compound for new drug candidate. However, it is suggesting further in vitro and in vivo assay to validate the present predictions.

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