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In Silico Prediction of selected major Phytochemical constituents of Chandraprabha Vati as CYP 2C9 Inhibitor

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ABSTRACT

Background: Computational docking is a crucial technique for developing better novel drugs. The behaviour of a test molecule in the coupling site of the desired receptor target can be described using a docking method. In recent years world's population relies mainly on natural and herbal based marketed formulation for health care. In this study components of the main ingredients Shilajit and Shudha guggulu in the preparation of Chandraprabha Vati is evaluated for the binding affinity towards CYP2C9. It is done by using a comparator drug Glimepiride since it is metabolises using CYP2C9.

Purpose: There is an increase in usage of ayurvedic drugs as an add-on or supplement along with the therapeutic drug plan.purpose of this study is to know the interaction potential of the components of the Herbal formulation.

Study Design: In Silico Study.

Materials and methods: Online tools and Database such as Protein data bank, IMPPAT, Pubchem, pkCSM, CB-Doc were used for choosing of protein, Docking, ADMET prediction, Drug-likeness of the components.

Results and Discussion: The vina score of the Phytochemical constituents of Chandraprabha Vati was observed, many exhibit good binding effect. It demonstrates the Binding affinity of the phytochemicals with the target.

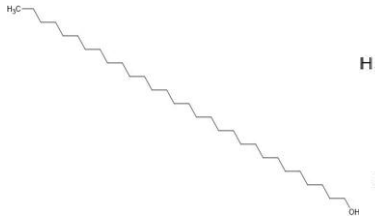
Conclusion: This current study concludes that Episesamin, Stigmasterol, Thunbergene, E- Guggulusterone, Cholesterol, beta-sitosterol, Mukulol, Isorhamnetin, 16alpha-Hydroxyprogesterone have Good Binding affinity to the Target as well as predicted to have CYP2C9 inhibition property.

Keywords: Computational tool,CYP2C9, Docking, Drug-likeness, Binding Affinity, Binding score

1.INTRODUCTION

Computational docking is a crucial technique for developing better novel drugs. The procedures of drug development and discovery need a significant investment of time and resources. To accelerate the process different online tools and software are available to predict the effect of the drug in particular disease1.The behaviour of a test molecule in the coupling site of the desired receptor target can be described using a docking method. The capacity to forecast the useful binding strength between the ligand and the receptor complex is a prerequisite for a docking technique. New active medications with fewer side effects have been found in computational chemistry. Various bioactive compound's binding attractions have been predicted, and particular sites of interaction between the bioactive compounds and target proteins have been explained in molecular docking analysis. In recent years world's population relies mainly on natural and herbal based marketed formulation for health care2,3. Many studies focus on Natural compounds to use it as a source for the manufacturing of the medicines. Research studies on herbal medicinal products is important for the safety of human health4,5. Identification and knowing the pharmacological effects of herbal medicinal products is essential for the uplifting to modernizing its use. Because of the presence of multiple components in a single herbal formulation, the side effects and Drug interaction potential need to be studied do achieve safe use of the Herbal marketed drug6,7. Determining the precise chemical components of medicinal plants and their primary biological roles is challenging due to the diversity and complexity of these compound's chemical makeup.

Worldwide, herbal therapy is widely used to treat a range of medical conditions. It has been observed that inhibiting CYP450 enzymes decreases the metabolism of medications that are CYP450 substrates, altering its pharmacokinetics8,9. Chandraprabha vati is an Ayurvedic classical formulation used to reduce the intensity of a set of complex clinical disorders which correlates in many ways with obesity, metabolic syndrome and Diabetes mellitus. In recent days Chandraprabha Vati is widely used in the treatment of Diabetes Mellitus10. In this study components of the main ingredients Shilajit and Shudha guggulu in the preparation of Chandraprabha Vati is evaluated for the binding affinity towards CYP2C9. It is done by using a comparator drug Glimepiride since it is metabolises using CYP2C9. This study was done to predict the binding effect of selected major phytochemical constituents of Chandraprabha Vati. The major constituents of the Ayurvedic drug includes Shilajit(Asphaltum Punjabianum), Shudha guggulu(Commiphora weightii). Shilajit and Shudha guggulu is present upto 80% in the Chandraprabha vati formulation, hence these two components were selected. Shilajit contains Fulvic acid, Benzoic acid, Hippuric acid, Resin. Shudha guggulu contains Quercetin, Ellagic acid, quercetin 3-O-glucuronide, Avicularin, Vanillic acid , Isorhamnetin, 4-Hydroxybenzoic acid, 3,4- Dihydroxybenzoic acid, alpha-Camphorene, Myrcene, E- Guggulsterone, Mukulol, (2E,6Z,10E) - 3,7,11-trimethyl-14- propan-2-ylcyclotetradeca-2,6,10-trien-1-ol, 20 Hydroxypregn-4-en- 3-one, Cholesterol, 1-Triacontanol, Pluviatilol, Thunbergene, Ferulic acid, beta Caryophyllene, Stigmasterol, Episesamin, Stearic acid, Palmitic acid, Neocembrene, beta- Sitosterol, Oleic acid, Campesterol, Linoleic acid, 2 ,2-Dimethyl-3-(3- methylenepent-4-enyl)oxirane,16alpha-Hydroxyprogesterone, Pelargonidin. There have been no previous study to check the binding effect of constituents of Chandraprabha Vati with the particular protein used in the present Study11. Apart from treating Diabetes, Chandrprabha Vati has a wide range of uses in the treatment of Urinary tract infection, Bloating, Constipation, Liver cirrhosis, Hernia, Haemorrhoids, to regulate thyroid glands, joint and bone disorders, to promote strength and immunity. Chandraprabha Vati is available in doses like 250 mg and 500 mg.



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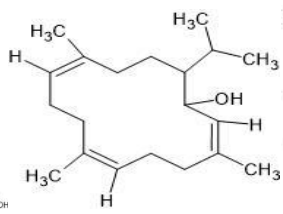

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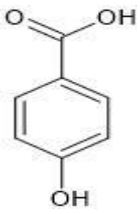

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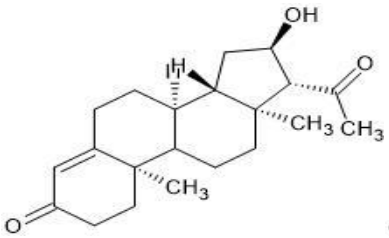

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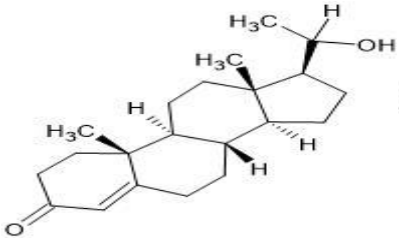

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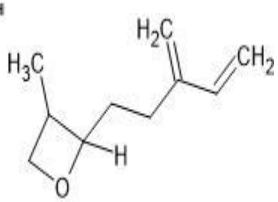

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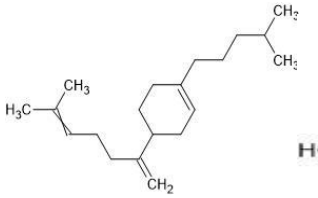

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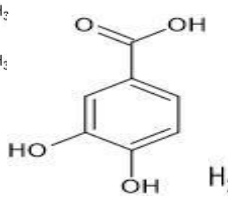

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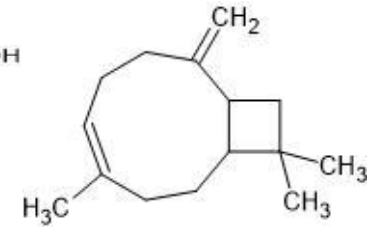

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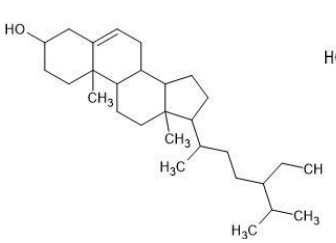

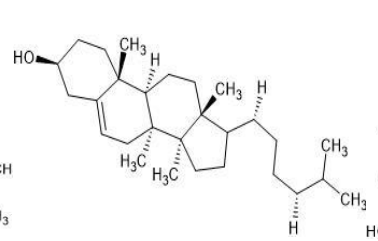

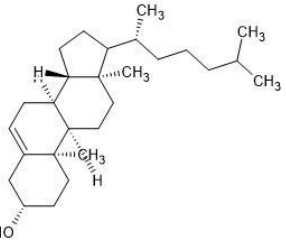

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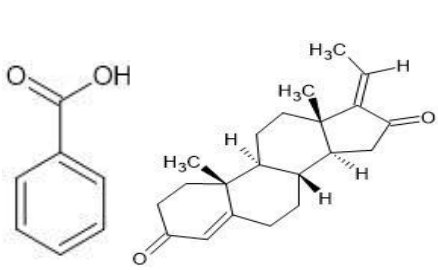

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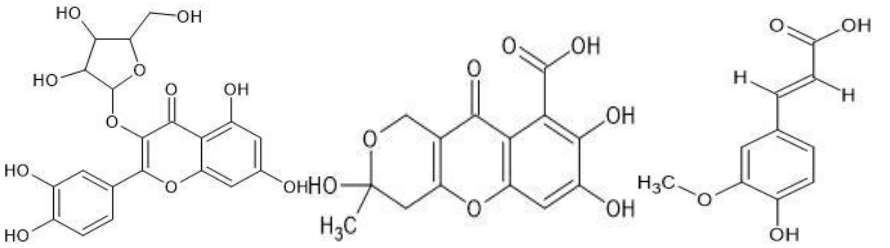

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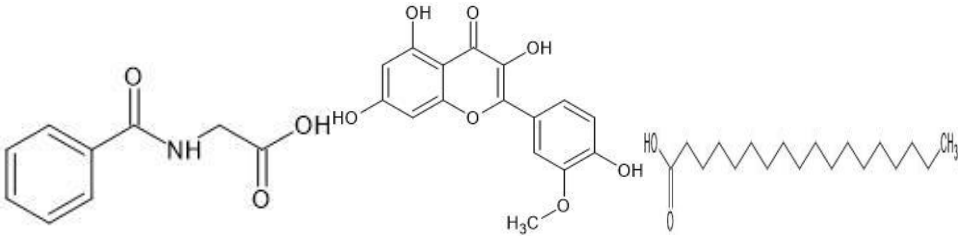

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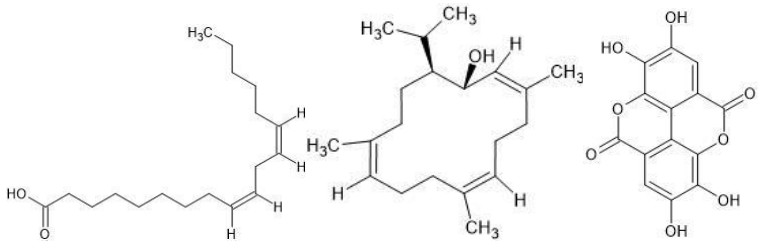

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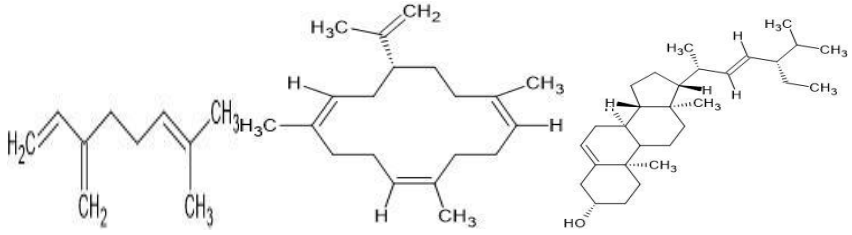

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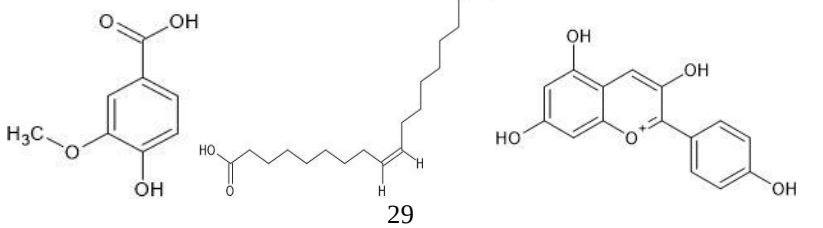

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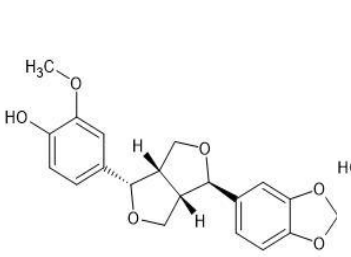
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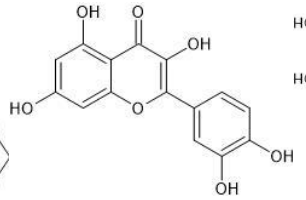
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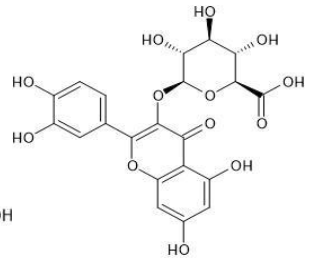
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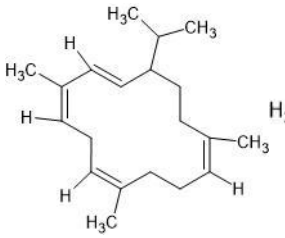
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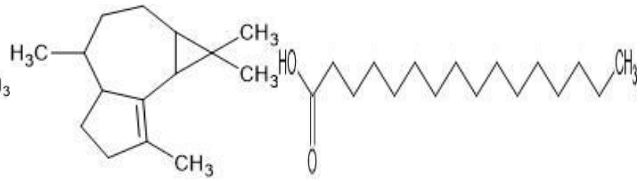
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S.no	Phytochemicals	S.no	Phytochemicals
1.	1-triacontanol	19.	Hippuric acid
2.	(2E,6Z,10E)-3,7,11-trimethyl-14-propan-2-ylcyclotetradeca-2,6,10-trien-1- ol	20.	Isorhamnetin
3.	4-Hydroxybenzoic acid	21.	Palmitic acid
4.	16alpha-Hydroxyprogestrone	22.	Linoleic acid
5.	20-Hydroxypregn-4-en-3-one	23.	Mukulol
6.	2,2-Dimethyl-3-(3- methylenepent-4-enyl)oxirane	24.	Ellagic acid
7.	alpha-Camphorene	25.	Myrcene
8.	3,4-Dihydroxybenzoic acid	26.	Neocembrene
9.	beta-Caryophyllene	27.	Stigmasterol
10.	beta-sitosterol	28.	Vanillic acid
11.	Campesterol	29.	Oleic acid
12.	Cholesterol	30.	Pelargonidin
13.	Benzoic acid	31.	Pluviatilol
14.	E-Guggulsterone	32.	Quercetin
15.	Episesamin	33.	Quercetin 3-O-glucuronide
16.	Avicularin	34.	Thunbergene
17.	Fulvic acid	35.	Resin
18.	Ferulic acid	36.	Stearic acid

2.MATERIALS AND METHODS

The phytochemical constitutents of Shilajit and Shuddha guggulu were gathered from IMPPAT - online database for Phytochemicals of Indian medical Plants. The structure, molecular formula and canonical smiles of the Phytochemicals were attained from PUBCHEM Database. The Pharmacokinetic parameters ADMET prediction was done using pkCSM online tool. Docking was done using Cytochrome P450 (CYP) 2C9 TCA007 Inhibitor Complex as Protein which is sourced from Protein data bank. CB DOC2 online Docking software was used to Dock the Protein and the ligand.

3.RESULTS AND DISCUSSION

3.1.Binding Activity Prediction

The vina score of the Phytochemical constituents of Chandraprabha Vati was observed, many of them exhibit good binding effect. The vina score is a measure of Binding affinity prediction between the ligand and the protein, in which a lower indicates a stronger binding affinity. Table 1

Table 1: Binding affinity of the phytochemicals

S.no	Phytochemical Constituents	Pubchem ID	Chemical Formula	Vina Score
1.	Episesamin	72307	$C_{20}H_{18}O_6$	-10.7
2.	Stigmasterol	5280794	$C_{29}H_{48}O$	-9.9
3.	Thunbergene	11747713	$C_{20}H_{32}$	-9.7
4.	Pluviatilol	70695727	$C_{20}H_{20}O_6$	-9.5
5.	E-Guggulsterone	6439929	$C_{21}H_{28}O_2$	-9.5
6.	Campesterol	173183	$C_{28}H_{48}O$	-9.4
7.	Cholesterol	5997	$C_{27}H_{46}O$	-9.2
8.	Neocembrene	5281384	$C_{20}H_{32}$	-9.0
9.	beta-sitosterol	222284	$C_{29}H_{50}O$	-9.0
10.	Mukulol	13255923	$C_{20}H_{34}O$	-8.9
11.	Quercetin	5280343	$C_5H_{10}O_7$	-8.8
12.	20-Hydroxypregn-4-en-3-one	161109	$C_{21}H_{32}O_2$	-8.7
13.	Isorhamnetin	5281654	$C_{16}H_{12}O_7$	-8.6
14.	Pelargonidin	440832	$C_{15}H_{11}O_5$	-8.4
15.	16alpha-Hydroxyprogestrone	243761	$C_{21}H_{30}O_3$	-8.2
16.	(2E,6Z,10E)-3,7,11-trimethyl-14-propan-2-ylcyclotetradeca-2,6,10-trien-1- ol	5368823	$C_{20}H_{34}O$	-8.1
17.	Quercetin3-O-glucuronide	5274585	$C_{21}H_{18}O_{13}$	-8.0
18.	Ellagicacid	5281855	$C_{14}H_6O_8$	-8.0
19.	Resin	133110026	$C_{15}H_{24}$	-7.6
20.	Avicularin	5490064	$C_{20}H_{18}O_{11}$	-7.6
21.	alpha-Camphorene	101750	$C_{20}H_{32}$	-7.5
22.	beta-Caryophyllene	5281515	$C_{15}H_{24}$	-7.4
23.	Fulvicacid	5359407	$C_{14}H_{12}O_8$	-7.4
24.	Ferulicacid	445858	$C_{10}H_{10}O_4$	-6.4
25.	Hippuricacid	464	$C_9H_9NO_3$	-6.4
26.	Linoleicacid	5280450	$C_{18}H_{32}O_2$	-6.2
27.	Oleicacid	445639	$C_{18}H_{34}O_2$	-5.8
28.	Vanillicacid	8468	$C_8H_8O_4$	-5.7
29.	Stearicacid	5281	$C_{18}H_{36}O_2$	-5.7
30.	Palmiticacid	985	$C_{16}H_{32}O_2$	-5.7
31.	Benzoicacid	243	$C_7H_6O_2$	-5.5
32.	3,4-Dihydroxybenzoicacid	72	$C_7H_6O_4$	-5.5
33.	1-triacontanol	68972	$C_{30}H_{62}O$	-5.4
34.	Myrcene	31253	$C_{10}H_{16}$	-5.4
35.	4-Hydroxybenzoicacid	135	$C_7H_6O_3$	-5.3
36.	2,2-Dimethyl-3-(3-methylenepent-4-enyl) oxirane	122371	$C_{10}H_{16}O$	-5.3

It demonstrates the Binding affinity of the phytochemicals with the target protein [Cytochrome P450 (CYP) 2C9 TCA007 Inhibitor Complex], providing molecular-level evidence to support their potential therapeutic effects12,13,14. This compares the binding effect of glimepiride to the Phytochemicals of Chandraprabha Vati, since both drug and herbal formulation is used in the treatment of Diabetes mellitus. Episesamin, stigmasterol, Thunbergene, Pluviatilol E-Guggulsterone, Campesterol Cholesterol, Neocembrene Beta-sitosterol Mukulol, Quercetin, 20-Hydroxypregn-4-en-3-One, Isorhamnectin, Pelargonidin 16alpha-Hydroxyprogesterone exhibit similar or more binding affinity towards the protien compared to Glimepiride.The Image of Vina score and Image of the binding of Protein and Ligand was added from Figure 1 to Figure 3.

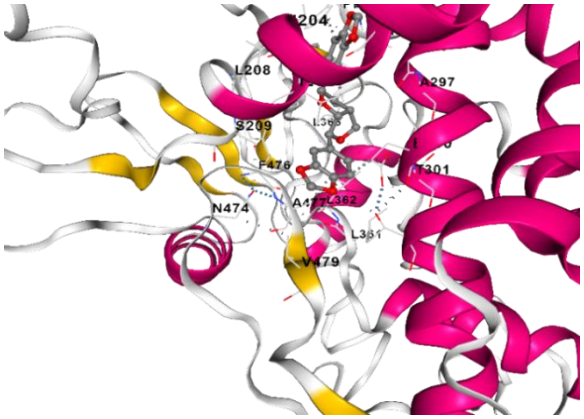

Figure 1: Binding of Episesamin with Protein 5w0c

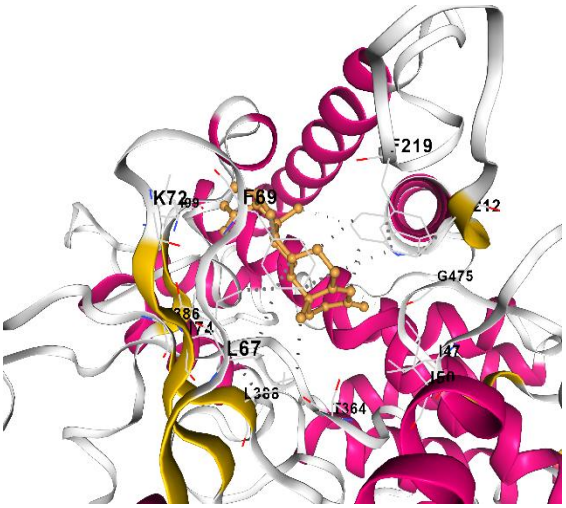

Figure 2: Binding of Stigmasterol

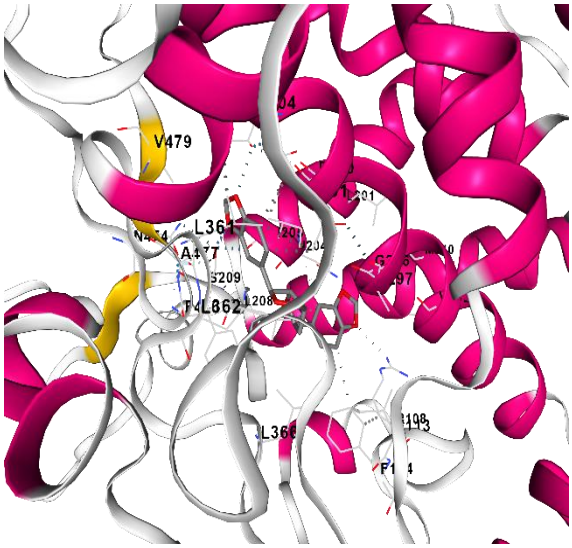

Figure 3: Binding Score of the Thunbergene

3.2.Pharmacokinetic Properties

Among 36 phytochemicals of Chandraprabha Vati, 19 constituents was predicted to have CYP2C9 inhibitory effect. Table 2.

Table 2: CYP2C9 Inhibition effect of Phytochemicals

S.no	Phytochemical constituents	CYP2C9 inhibition
1.	Episesamin	Yes
2.	stigmasterol	Yes
3.	Thunbergene	Yes
4.	Pluviatilol	No
5.	E-Guggulsterone	Yes
6.	Campesterol	No
7.	Cholesterol	Yes
8.	Neocembrene	No
9.	beta-sitosterol	Yes
10.	Mukulol	Yes
11.	Quercetin	No
12.	20-Hydroxypregn-4-en-3-one	No
13.	Isorhamnetin	Yes
14.	Pelargonidin	No
15.	16alpha-Hydroxyprogesterone	Yes
16.	(2E,6Z,10E)-3,7,11-trimethyl-14-propan-2-ylcyclotetradeca-2,6,10-trien-1- ol	No
17.	quercetin3-O-glucuronide	Yes
18.	Ellagicacid	Yes
19.	Resin	No
20.	Avicularin	Yes
21.	alpha-Camphorene	Yes
22.	beta-Caryophyllene	No
23.	Fulvicacid	Yes
24.	Ferulicacid	Yes
25.	Hipuricacid	No
26.	Linoleicacid	No
27.	Oleicacid	No
28.	Vanillicacid	No
29.	Stearicacid	No
30.	Palmiticacid	Yes
31.	Benzoicacid	Yes
32.	3,4-Dihydroxybenzoicacid	No
33.	1-triacontanol	No
34.	Myrcene	Yes
35.	4-Hydroxybenzoicacid	No
36.	2,2-Dimethyl-3-(3-methylenepent-4-enyl) oxirane	Yes

pkCSM online tool predicted Episesamin, Stigmasterol, Thunbergene, E- Guggulusterone, Cholesterol, beta sitosterol, Mukulol, Isorhamnetin, 16alpha-Hydroxyprogesterone, quercetin 3-O-glucuronide, Ellagic acid, Avivularin, alpha-Camphorene, Fulvic acid, Ferulic acid, Palmitic acid, Benzoic acid, Myrcene, 2,2-Dimethyl-3 (3-methylenepent-4-enyl) oxirane have CYP2C9 inhibitory effect.

Among all the components 9 phytochemicals have inhibitory effect for CYP2C9 as well as have good binding affinity compared to Glimrpiride. Table 3

Table 3: Phytochemicals shows Positive CYP2C9 Inhibition and exhibit similar or more binding effect compared to Glimepiride

S. no	Phytochemicalconstituents	CYP2C9 inhibition	Vina Score
1.	Episesamin	Yes	-10.7
2.	Stigmasterol	Yes	-9.9
3.	Thunbergene	Yes	-9.7

4.	E-Guggulusterone	Yes	-9.5
5.	Cholesterol	Yes	-9.2
6.	beta-sitosterol	Yes	-9.0
7.	Mukulol	Yes	-8.9
8.	Isorhamnetin	Yes	-8.6
9.	16alpha-Hydroxyprogesterone	Yes	-8.2

3.3.Molecular characteristics

pkCSM software was applied to check the Drug-likeness and ADME parameters for the selected constituents[15,16]. In a study done by Pradeepa et al states all the phytochemicals follows the Lipinski rule which is similar to this current study.

The physicochemical properties of drugs meant for oral administration have been effectively predicted using the rule-of-fives. ADME of the compounds was checked to predict the CYP inhibitory effect[17,18]. According to Lipinski's rule, an orally active drug-like molecules cannot have more than one violation of the following requirements.

- Not morethan5hydrogen bonddonars.
- Not morethan10hydrogen bondacceptors.
- Molecularmass lessthanorequalto500.
- Anoctanolwaterpartitioncoefficientlessthan orequaltofive[19,20.]

This study focuses only on the major components of Chandraprabha Vati. In the future study remaining compounds will be analysed along with Molecular dynamic to prove the Binding site of the Ligands

4.CONCLUSION

Based on the Results of the conducted study, among 36 selected Phytochemicals 15 compound's vina score indicates they have similar or more binding affinity compared to Glimepiride. 19 compounds shows CYP2C9 Inhibitory effect in the Prediction tool. This current study concludes that Episesamin, Stigmasterol, Thunbergene, E- Guggulusterone, Cholesterol, beta-sitosterol, Mukulol, Isorhamnetin, 16alpha-Hydroxyprogesterone have Good Binding affinity to the Target as well as predicted to have inhibitory effect for CYP2C9. Further Studies on Molecular dynamics will help to prove the accurate Binding site of these ligands with the protein.

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6.Credit author statement

Ahalya S.P- Performing the experiments and Writing - original draft.
Vijayakumar T.M- Supervision, review.
Satish Kumar R.C- Editing the original draft.

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