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Therapeutic potential of Marrubium vulgare (L.)against HCV NS5B polymerase and NS3 helicase: In silico approach

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ABSTRACT:
Hepatitis C virus (HCV) infection causessevere liver diseases such as chronic liver cirrhosis and hepatocellular carcinoma. Chronic HCV leads to morbidity and mortality globally because of its rapid mutation feature andcomplex influence onthe immunology system.Currently,the FDA approved boceprevir and sofosbuvir as hepatitis C virus (HCV) NS3 and NS5B inhibitors. The sustained virological response (SVR) rate for treatment with this approved drug is considerably low.This study focused on alternative remedies for the phytoconstituents of Marrubium vulgare (L.) synonym white horehound, which comes under the Lamiaceaefamily.The main aim is to screen the phytoconstituents for the novel anti-HCV based on the low binding energy anticipated ligand's favoured region against the receptor to make a stable complex via molecular docking analysis using Maestro 12.7 software (Schrodinger, LLC, NY, USA, 2021-1). We used the Qikprop 6.7 tool to determine the ligand's drug-likeness properties.We selected thirty-four phytochemicals from the M. vulgare for their pharmacokinetic properties, and docking evaluated the anti-HCV NS5B and NS3 activity based on the binding energy compared to the reference drugs sofosbuvir (-7.541 kcal/mol) and boceprevir (-3.774 kcal/mol). The result shows Luteolin (-7.932 kcal/mol) against NS5B and Sacranoside A (-5.445 kcal/mol) against NS3 helicase.These two top-hit phytoconstituents lead to significant activity based on the lowest binding energy. So, this work produces a promising drug candidate for antiviral activity against HCV.

KEYWORDS:HCV, NS5B polymerase, NS3 helicase, Marrubium vulgare (L.),ADME/T and MM/GBSA.

I.INTRODUCTION

Folk medicine of annual or perennial herb ofMarrubium vulgare (L.), commonly known as "horehound".It is used for many diseasesunder the Lamiaceae family [1]. It is distributed in Africa, Asia, and Southern Europe [2]. M. vulgare combine with other herbs to treat bronchitis. Moreover, it is commonly used for its antioxidant and antibacterial [3], analgesic and hypoglycemic effects [4].Tonic,antihypertensive recently inhibiting cyclooxygenase1 and acetylcholinesterase activities. [5].Aromatic, stimulant,expectorant, diaphoretic and diuretic properties, uterine, visceral and hepatic affections, and phthisis [6]. Anti-inflammatory [7], antiedematogenic activity [8]. The nature of M. vulgare istough and woody, with fibrous lateral roots and numerous stems. It grows from 20 to 100 cm high. Leaves are roundish and hoary on the surface. The white flowers in crowded axillary. The calyx and Corolla are tubular. Corolla is white to pale lavender. The seeds lie at the downof the calyx.M. vulgare has a musky odour that changesby drying into a pungent yet pleasant odour and bitter,aromatic taste. The different bioactive compounds include flavonoids, diterpenoids, and phenylethanoid glycosides[9]. Ithas trace amounts of essential oil, Non-volatile monoterpene, tannins,Sesquiterpene and lactone[10]. In identifying new therapies, plant-based compounds were found to be an alternative that can either be used directly or with structural modifications [11]. This plant has antiviral activity, so its phytoconstituents are utilized forthe anti-hepatitis c virus. Medicinal plants have been considered an alternative source of medicines for treating various human diseases, including liver diseases.The genome nature of HCV has already been reported. This studymainly focused on NS5B polymerase and NS3 helicase because these are essential for virus replication [12].Bioactive natural compounds offer diverse pharmacologicalactivities, low cost in production and are inherently safer than synthetic drugs [13].The drawback ofsynthetic medicines is high-cost and side effects. So, we target the anti-HCV drug without side effects and

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low cost. It is achieved by molecular docking studies, which are most frequently used nowadays.

II. MATERIALS AND METHODS

The literature study shows that the following proteins are closely associatedwith 3CJ5-NS5B polymeraseand 2ZJO – NS3 helicase.The proteins' 3D structurewas retrieved fromthe protein data bank (PDB). It was carried out in maestro 12.7, Schrodinger 2021-1[14].

Prediction Of "Drug-Likeness" Properties

We used the Qikprop 6.7 tool of a maestro for determining the drug-likeness properties. The selectedtotal of 34 phytoconstituents first determines the drug-likeness properties. Here, only fourteen phytocompounds passed all the parameters [15].

Preparation Of Proteins For Docking

The protein preparation wizard prepared two proteins, 3CJ5 - polymerase and 2ZJO - helicase used for docking. The primary steps were done, such as pre-processing, optimization, minimization, and deletion of water molecules. The protein receptor grid was generated. Finally, the docking of all ligandswasperformed by glide. The glide score (G score) determined Extra-precision (XP-module) [16].

PREPARATION OF LIGANDS

The 2D ("SDF") structures of all 34 ligands are prepared using the LigPrep tool of Schrödinger Maestro software. All ligands were minimized using the OPLS-3e force field module [17].

MOLECULAR DOCKING

The docking of all the phytochemicals toward the active site of the 3CJ5 (NS5B) and 2ZJO (NS3) targets was conducted using the Extra Precision (XP) set at the glide 9.0. we established the OPLS-3e force field throughout the study. All the ligands were docked with the receptors 3CJ5 (NS5B) and 2ZJO (NS3)and then ranked based on their docking scores representing binding energies [18].

Prime Mm-Gbsa Free Energy Calculation

The prime MMGBSA method exhibited each ligand molecule's relative binding-free energy (ΔG bind).Prime MM-GBSA calculates the power of optimized free receptors, free ligands, and a ligand complex with a receptor[19,20].

III. RESULTS AND DISCUSSION

The molecular docking studies of the phytoconstituents to active protein sites were carried out by an advanced docking module Schrodinger 2021-1; Maestro-12.7 version tofind the binding affinities of the phytocompounds. The ligands are docked towards the 3CJ5 and 2ZJO to establish their polymerase and helicase inhibition activity. The thirty-four selected phytoconstituents [10 and 21] PubChem ID structure is listed in [Table-1].

ADME/T STUDY RESULTS OF M. valugare PHYTOCHEMICALS

The ADME/T parameters for all the ligands can be evaluated in silico by using the Qikprop tool.The number of rotatable bonds (#rotor) of the molecule is 1-11.The percentage of oral absorption predicted compounds have 37.198 - 100 %. The compound's likely metabolic reactions range from 1 to 7. All the phytochemicals show in ranges. QPlogBB-compounds have within the limit. It computed the prediction of CNS activity −2 reported compounds are inactive, and +2 containing compounds are active. Log Kp, the compounds have a -5.544 to -1.79. QPlogKhsa compounds range from -1.085 to 0.793. QPlogHERG - above -5 means it causes toxicity. Here, no violation occurs. All the combinations are predicted HERG K+ values passed below -5. The determined Polar surface area of all the ligands ranges from 20.415 to 158.925. RO5 and RO3 both are within the recommended ranges. The ADME/T study report revealed that all the properties of the fourteen compounds are within the recommended ranges. The drug-likeness parameters for the phytoconstituents list are in [Table-2].

Docking Study Of M.Valugare Phytochemicals Against Ns5 Polymerase And Ns3 Helicase

HCV is the most harmful disease to human beings and may cause life-threading. Still, we do not have a vaccine; synthetic drugs also cause side effects. In this study, we have takenperfectly obeyed drug-likeness properties containing 14phytoconstituents fromM. valugaredocked againsttwo proteins in HCV. The binding energy, glide energy, glide$_{evdw,}$ and MM/GBSA were analyzed. The present work considered the most excellent G score exhibiting receptors of the respective protein.Among the two

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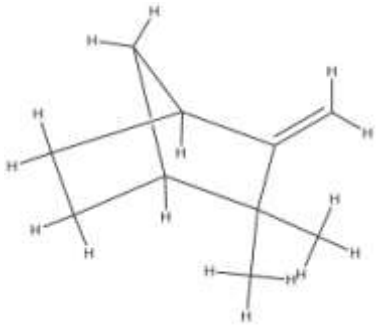
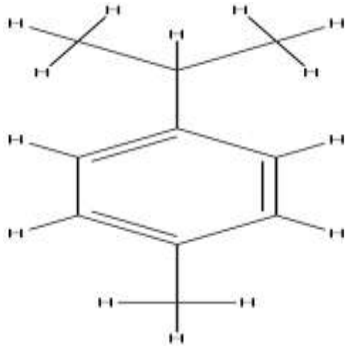
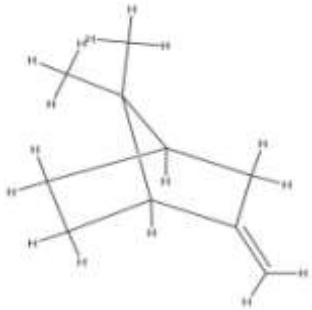
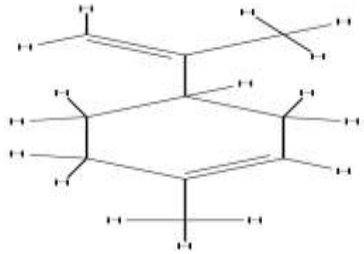
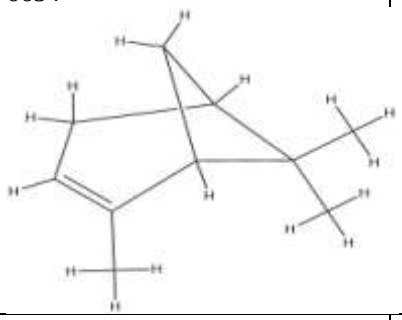
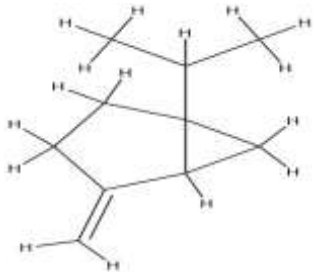
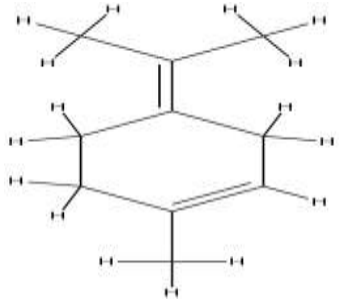
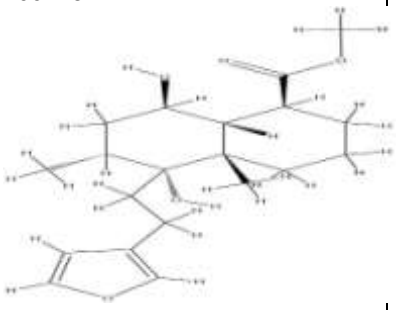
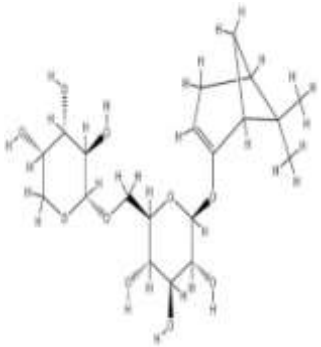
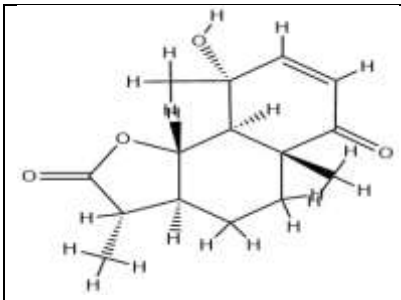
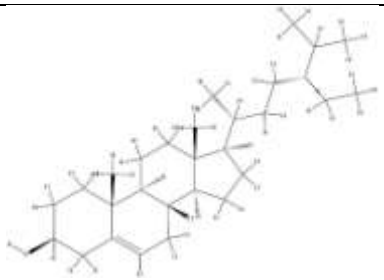
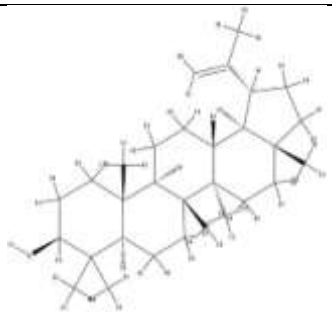
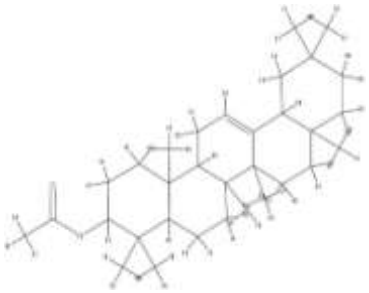
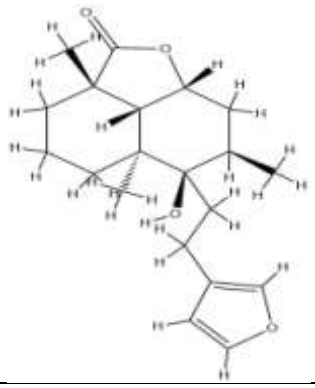
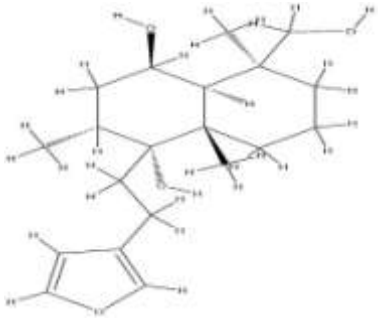
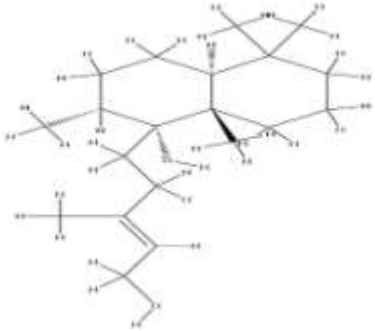
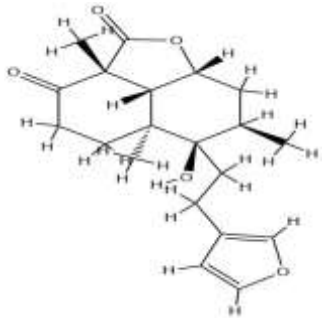
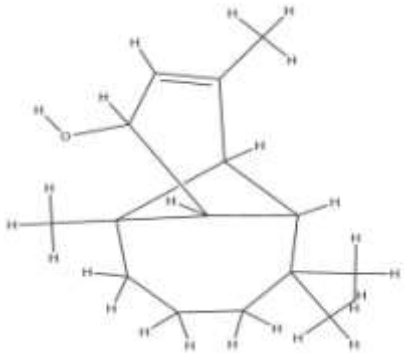
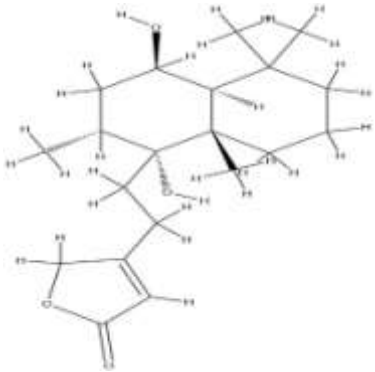
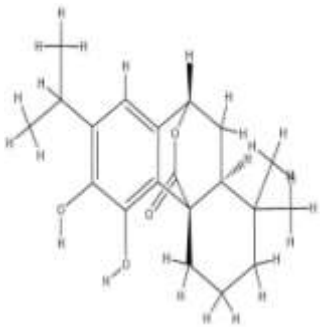
proteins, 3CJ5 has shown the highest G score withLuteolin phytocompound, glide$_{evdw}$ ranges from -26.733 to -1.841, the glide energy has maximum in -7.239 and minimum -35.355. the top hit compound contains free energy dG bind -28.27 kcal/mol.

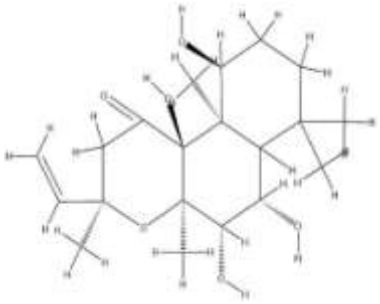
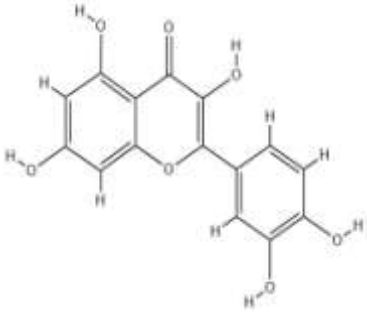
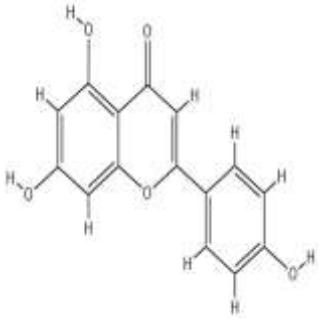
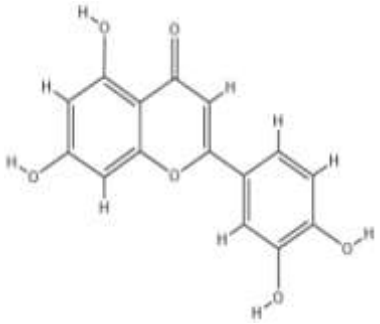
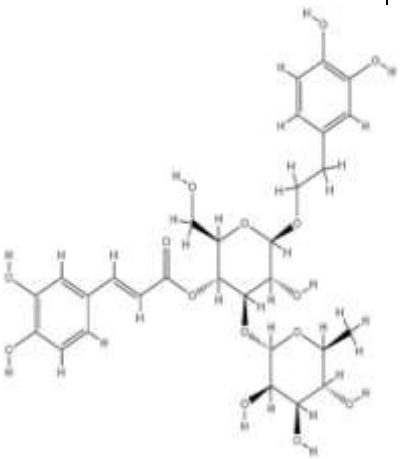
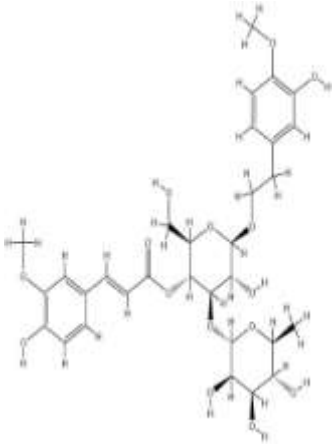
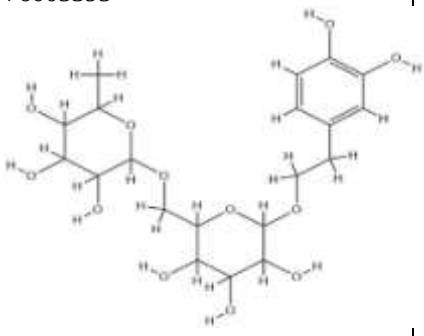
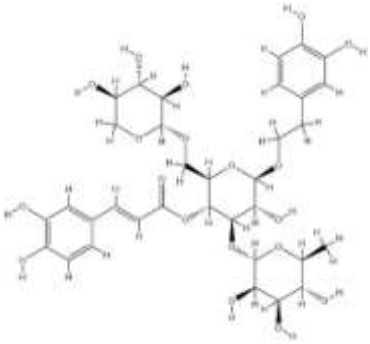
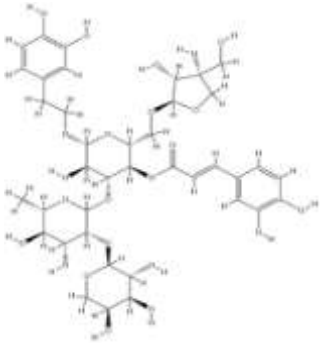
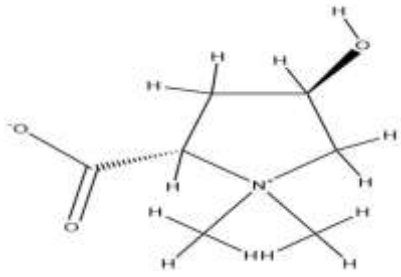
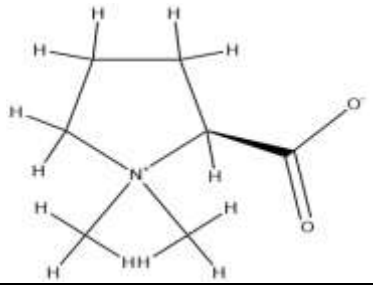
Table1: List of M. valugare phytochemicals with PubChem ID and chemical structure

Camphene 6616 	p-cymol 7463 	Fenchene 28930 
Limonene 22311 	α-pinene 6654 	Sabinene 18818 
α-terpinolene 11463 	Marrubic acid 200146 	Sacranoside A 102094959 
Vulgarin 94253	β-sitosterole 222284	Lupeol 259846

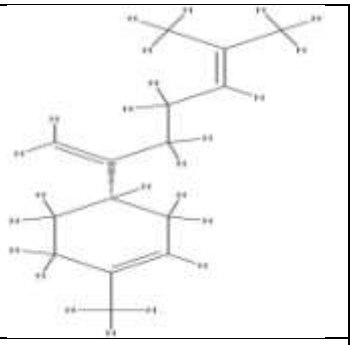
International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 314-324 www.ijprajournal.com ISSN: 2456-4494

		
β-amyrin 345510 	Oleanolic acid 10494 	Marrubiin 73401 
Marrubenol 10449689 	Peregrinol 7092583 	Peregrinin 7091745 
Vulgarol 85910272 	Deacetylvitexilactone 46883141 	Carnosol 442009 

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 314-324 www.ijprajournal.com ISSN: 2456-4494

Deacetylforskolin 10893929 	Quercetin 5280343 	Apigenin 5280443 
Luteolin 5280445 	Verbascoside 5281800 	Martynoside 5319292 
Forsythoside 76005395 	Arenarioside 6442994 	Ballotetroside 44584314 
Betonicine 164642 	Stachydrine 115244 	β-bisabolene 10104370

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 314-324 www.ijprajournal.com ISSN: 2456-4494



β-caryophyllene
5281515

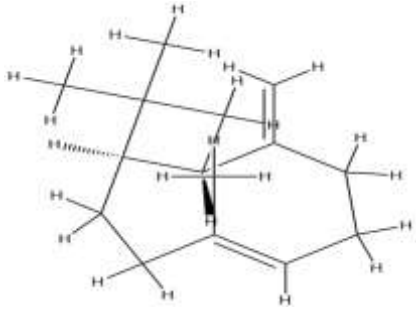


Table 2: ADME/T study results of M. valugare phytochemicals

Phytoconstituents name	#rotor	CNS	QPlogHERG	QPlogBB	QPlogKp	QPlogKhsa	HOA	%HOA	PSA	RO5	RO3
Vulgarol	1	1	-2.996	0.18	-2.084	0.455	3	100	20.415	0	0
Vulgarin	1	0	-3.387	-0.606	-3.707	-0.171	3	83.449	84.652	0	0
sacranoside A	11	-2	-4.782	-2.623	-4.912	-1.085	2	37.198	158.925	1	1
Quercetin	5	-2	-5.109	-2.419	-5.544	-0.343	2	51.649	143.331	0	1
Peregrinol	6	0	-3.807	-0.456	-2.258	0.793	3	100	37.524	0	0
Peregrinin	4	0	-3.819	-0.482	-2.446	0.1	3	100	83.683	0	0
Marrubiin	4	0	-3.772	-0.185	-1.79	0.454	3	100	58.563	0	0
Marrubic acid	6	0	-4.27	-0.548	-2.031	0.428	3	100	72.505	0	0
Marrubenol	7	-1	-3.872	-0.57	-1.939	0.235	3	100	61.29	0	0
Luteolin	4	-2	-	-1.955	-4.888	-0.198	3	61.20	121.44	0	0

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 314-324 www.ijprajournal.com ISSN: 2456-4494

			5.023					5	2		
Deacetyl vitexilact one	5	-1	-3.514	-0.737	-3.092	0.292	3	95.383	73.827	0	0
Deacetylf orskolin	5	-1	-2.786	-0.645	-3.099	-0.114	3	87.887	92.039	0	0
Carnosol	3	0	-3.437	-0.505	-3.079	0.389	3	100	70.488	0	0
Apigenin	3	-2	-5.114	-1.446	-3.989	-0.039	3	73.192	99.755	0	0
Recomm ender value	0 – 15	–2 (inacti ve), +2 (activ e)	< –5	–3.0 – 1.2	–8.0 to –1.0	–1.5 to – 1.5		>80 <25	7.0 – 200.0	Max 4	Max 3

HOA- human oral absorption: 1- Low; 2- Medium; 3- high.

Similarly, 2ZJO displayed the highest docking score withSacranoside A. It also glide$_{evdw}$-30.335, glide energy -44.107, and binding free energy was -40.43. NS5B polymerase and NS3 helicase vital role in the replication and assembly of the life cycle of HCV. Hence, both are attractive targets for many therapeutic goals.The known drugs are sofosbuvir and boceprevir used for HCV patients. Therefore, in this study, we used 14 phytochemicals for docking study valuesgiven in [Table-3].

Table 3: Docking study report of M. valugare phytochemicals with 3CJ5 and 2ZJO

NS5 polymerase (3CJ5)					NS3 helicase (2ZJO)				
Phytocons tituents name	docki ng score	glid$_{evd w}$	glide energ y	MM/G BSA dG bind	Phytocons tituents name	dockin g score	glide $_{evdw}$	glide energy	MM/G BSA dG bind
Luteolin	-7.932	-24.722	-32.955	-28.27	Sacranosi de A	-5.445	-30.335	-44.107	-40.43
Apigenin	-6.595	-25.636	-34.682	-31.96	Apigenin	-5.373	-24.048	-25.55	-22.17
Sacranosid e A	-5.765	-26.733	-35.355	-37.66	Luteolin	-5.176	-24.635	-31.473	-25.95
Peregrinin	-5.355	-19.239	-26.776	-44.23	Quercetin	-4.858	-29.024	-32.202	-33.26
Deacetylfo rskolin	-4.882	-16.68	-21.824	-25.34	Carnosol	-4.839	-17.078	-24.628	-35.36
Marrubiin	-4.583	-17.777	-23.21	-35.97	Deacetylf orskolin	-4.276	-28.024	-29.89	-28.58
Marrubic Acid	-4.486	-19.789	-25.586	-35.08	Vulgarin	-4.201	-21.735	-26.195	-33.18

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 314-324 www.ijprajournal.com ISSN: 2456-4494

Carnosol	-4.347	-21.611	-26.57	-43.2	Peregrinin	-3.772	-27.091	-25.661	-38.64
Vulgarin	-4.208	-17.55	-21.915	-18.59	Marrubic Acid	-3.144	-22.708	-22.082	-30.32
Peregrinol	-3.862	-15.68	-18.238	-31.55	Marrubenol	-3.14	-18.494	-21.247	-34.43
Vulgarol	-2.106	-13.449	-16.151	-14.68	Marrubiin	-2.847	-26.178	-25.672	-41.62
Marrubenol	-0.827	-1.915	-7.239	-20.2	Vulgarol	-2.8	-11.942	-13.362	-24.72
Deacetylvitexilactone	-0.700	-1.841	-8.542	-21.2	Deacetylvitexilactone	-2.566	-21.169	-25.955	-38.48
Sofosbuvir	-7.541	-31.764	-45.485	-30.48	Boceprevir	-3.774	-28.561	-38.651	-29.25

Energy unit in kcal/mol

Here, we illustrate only top hit phytocompounds from each protein. The Luteolin binding relationship towardsprotein NS5B binding energy was -7.932 kcal/mol. It makes two hydrogen bonds with the amino acids via water molecule bridge ARG 498 and LEU 474 H-bond distances 1.93 and 1.94Å within the binding pocket of the NS5B active site. Two pi-cation formed with the same residue of ARG 501 and pi-pi stacking interaction with TYR 477 are shown in [Figure1].The sacranoside A began five H-bonds with the residues of NS3, including GLY 237, GLY207, GLY 207, TYR 241 and TYR 241, and H-bond distances are 2.65, 2.40, 1.83, 2.62 and 2.35Å, respectively. The docking score was -5.445 kcal/mol, as illustrated in [Figure 2]. The reference drugs sofosbuvir and boceprevir also displayed a docking score was -7.541kcal/mol and bound to the residue of ARG 501; SER 476; ARG 498, and TYR 477 at the NS5B binding site via four H-bonds. Boceprevir showed a docking score was -3.774 kcal/mol and bound to the residues GLY 237, ALA233, and ALA233of the NS3 helicase binding site via three H-bonds shown[Figure 3].

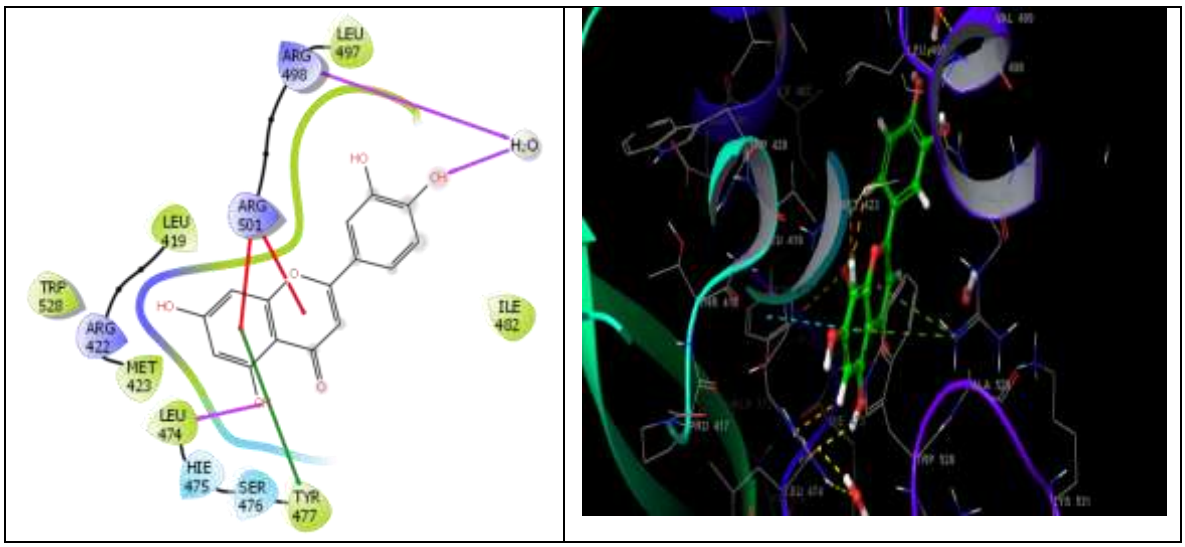


Figure 1: 2D and 3D structures of Luteolin complex withNS5B Polymerase protein.

International Journal of Pharmaceutical Research and Applications
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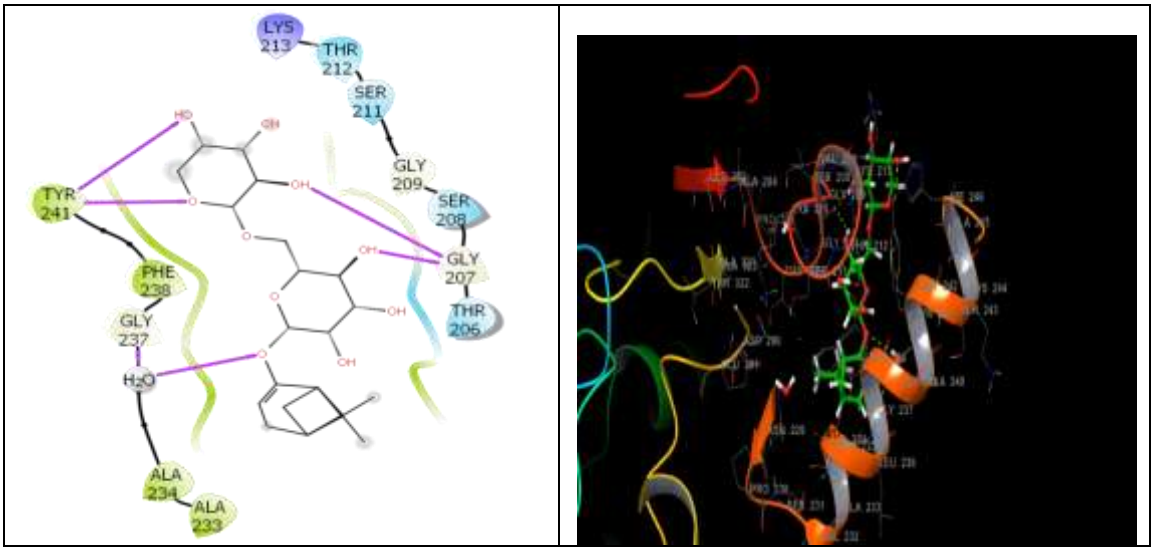

Figure 2: 2D and 3D structure of Sacranoside A complex with NS3 helicase protein.

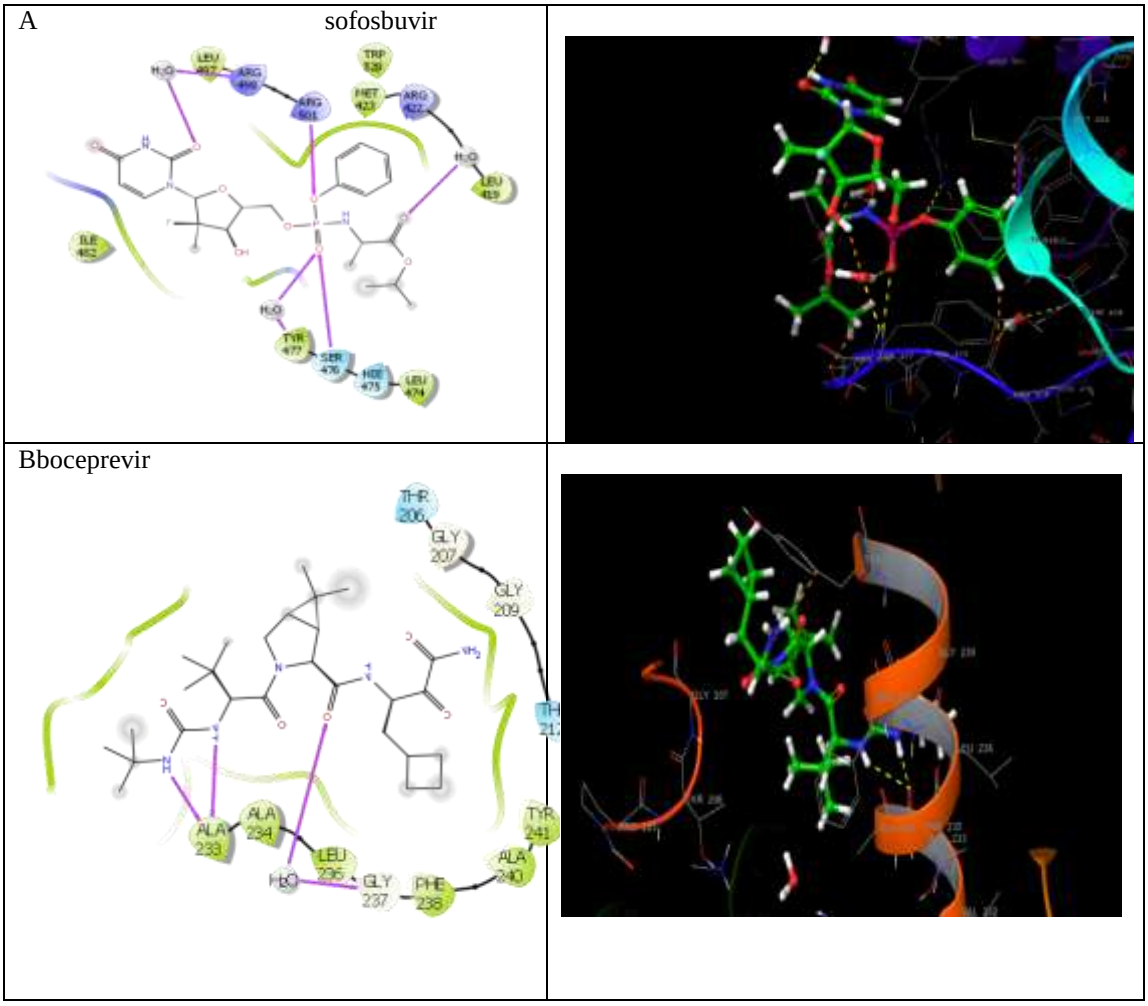

Figure 3: 2D and 3D structure reference drug A- sofosbuvir and B- boceprevir with polymerase and helicase protein.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 314-324 www.ijprajournal.com ISSN: 2456-4494

The M. valugare phytoconstituents have more medicinal value.Thus, the report incorporated to understand their capability as anti-HCV agents through in silico study.Luteolin has the best crucial score (-7.932 kcal/mol) compared to other docked phytochemicals. With this good docking scoremay possess against HCV. Luteolin docking score is higher compared to docked sofosbuvir. Sacranoside A has an excellent binding score compared to other docked phytochemicals.This satisfactory docking score of Sacranoside A compound against NS3 HCV protein. The docking score of Sacranoside A (-5.445 kcal/mol) is more than the reference drug boceprevir (-3.774kcal/mol). Henceforth, the Luteolin and Sacranoside A against NS5B and NS3 HCV.

IV. CONCLUSION

The present work has screened thirty-four phytocompounds from M. valugare via molecular docking and was docked against receptor proteins NS5B polymerase and NS3 helicase.All the "drug-likeness" parameters passed phytoconstituents were carried out docking study,that Luteolin and Sacranoside Arevealed more excellent G score at the active site by interacting with all crucial amino acid residues. These are exerted to inhibit enzyme activity. MM-GBSA free energy analysis reveals the stronger binding of the ligands to the receptors. So, Luteolin and Sacranoside A could be promising anti-HCV drugs that can be significant potential inhibitors for NS5B polymerase and NS3 helicase to develop a future antiviral drug.

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There are no conflicts of interest.

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It is not applicable.

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