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Bioactive phenolic compounds isolated in *morus alba* leaves as potential inhibitor of Hepatitis C Virus NS3 Protease: A molecular docking approach

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Abstract *The devastating impact of hepatitis C virus on liver demand the design and development of highly effective therapeutic agents. Here, we employed computational tools to investigate the inhibitory activity of bioactive phenolic compounds isolated from Morus alba leaves against ~~the~~ hepatitis C virus NS3 protease. Results obtained from docking study ~~showed~~ indicated that 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside had a favourable docking score of -6.6 kcal/mol and was selected as the lead molecule. The ligand-receptor molecular interaction revealed that 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside interacted with HIS55, SER136, SER137, GLY135, LYS134, LEU133, VAL130, ALA155, ALA154, ARG153 and PHE152 at the active site of the target. The pharmacokinetics and drug-likeness of the lead molecule reveal that 3,4,5-trimethoxyphenol-1-O-β-D-rhamnopyranoside was soluble in all the class solvent employed for the assay. Meanwhile, CYP1A2, CYP2C19, CYP2D6, CYP3A4 and CYP2C9 Isoenzymes were not inhibited by 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside. The lead molecule was also noticed to obey Lipinski's, Egan, Veber and Muegge rules with 0.55 bioavailability score. Hence, 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside have demonstrated drug-like characteristic and may have the capacity to inhibit hepatitis C virus NS3 Protease if subjected to in vitro and in vivo assay.*

Keywords: Morus alba, molecular docking, ADMET, bioactive compound

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1.0 Introduction

The mulberry tree is cultivated in different part of the world. However, Mulberry tree plant is vastly grown in India, North America, South America, Europe, Pakistan and China (Huang, Ou, & Wang, 2013; Memon *et al.,* 2010). This tree plant belongs to the Moraceae (Family) and Morus (genus). The plant has about 24 Morus Species, one subspecies and over 100 known varieties (Akkol *et al*., 2015). Morus plant species have demonstrated great benefit in medicinal, economical, industrial, clinical, and domestic fields (Andreoni, 2005; Sánchez, 2000). In folk medicine, different species of this plant are used to treat several kinds of diseases, including fever, hypertension, arthritis, liver disorders and urinary system problems. tumours of fauces, aptha, asthma, cold, cough, diarrhoea, dyspepsia, oedema, fever, headache, hypertension, and wounds (Datta, 2002; Sánchez, 2000). Reported studies on mulberry revealed that the leaves of mulberry has several health

benefits (Arabshahi-Delouee & Urooj, 2007; Ercisli & Orhan, 2007; Eyduran *et al*., 2015; Koyuncu *et al*., 2014; Natić *et al*., 2015; Sánchez-Salcedo *et al*., 2015; Sánchez *et al*., 2014). Also, investigation carried out on different parts of this plant revealed potent biological activities including antioxidant (Wang *et al*., 2013; Wattanapitayakul *et al*., 2005; Yang *et al.,* 2010), antimicrobial (Choi & Hwang, 2005), anticancer (Chen *et al*., 2005; Huang *et al*., 2008), hypolipidemic (Kim *et al*., 2013; Liu *et al*., 2009), macrophage activating (Kang *et al*., 2006), neuroprotective (Niidome *et al*., 2007), antidiabetic (Lee *et al*., 2011), antihypertensive (Islam *et al*., 2008), antiviral activity, anxiolytic among others. Also, bioactive compounds such as 1-deoxynojirimycin (Enkhmaa *et al*., 2005; Park *et al*., 2003), quercetin 3-(6-Malonylglucoside) (Priya, 2012), deoxynojirimycin, Albano A (Asano *et al*., 2001), leachianone G (Gupta *et al*., 2013), and moralbosteroid (Mahmoud, 2013) have been isolated from different species of the plant.

Since the outbreak of hepatitis C virus (HCV) in 1982, about 170, million persons have been reportedly infected. Hepatitis C virus is a major cause of liver disease worldwide and contributing significantly to increasing mortality index. The transmission of this virus (HCV) has been reported to include blood transfusions from unscreened donors, injection drug use, unsafe therapeutic injections, and other health-care-related procedures. However, the treatment of HCV patients has been via combination regimens of pegylated interferons and (Shepard, 2005). The search for an effective drug with enhanced capacity to cure both early infection and patient with chronic HCV is receiving research interest. Therefore, the present study is aimed at investigating bioactive phenolic compounds in *morus alba* leaves as potential inhibitors for hepatitis C virus using the computer-aided approach via molecular docking analysis.

2.0 Materials and Methods

2.1 Receptor and ligand preparation

The 2D conformation of the bioactive compounds was build using ACD/ChemSketch 2018.2.5 Freeware version. The chemical structure was optimized using the Merck molecular force field (MMFF94)) on Avogadro interface (Hanwell *et al.*, 2012). The dock-prep tools on the UCSF Chimera interface were used to prepare the optimized chemical structures before the molecular docking step. The crystallized structure of the Hepatitis C Virus NS3 Protease in complex with a peptidomimetic inhibitor (ID: 1w3c) with a 2.3 Å resolution was retrieved from the Protein Data Bank (https://www.rcsb.org). The structure of the Hepatitis C Virus NS3 Protease consists of four distinct chains bounded to small chemical residues (DN1 and DN2). The preparation of the biological target (1w3c) was performed on the UCSF Chimera interface(Pettersen et al., 2004).

2.2 Molecular docking

AutoDockVina software was used to perform the molecular docking simulation (Morris *et al*., 1998). Specific docking of the ligands to the active site of the target protein was achieved by generating a grid box file of the ligand on the receptor. The grid box that defines the pocket of 1w3c protein was obtained from the AutoDock Vina functionality on UCSF Chimera interface (Pettersen et al., 2004). The grid box size and centre coordinates for the 1w3c were x(67.0874, 7.83168)Å, y (0.52812, 12.0096)Å and z (9.07564, 16.4572)Å respectively. The bioactive compound with the highest binding affinity for the receptor was selected for further *in silico* ADME analysis.

2.3 Validation and ADME analysis of lead molecule

Molecular Target studies are important in finding the phenotypical side effects or potential cross-reactivity caused by the action of small biomolecules (Gfeller *et al.*, 2014; Keiser *et al*., 2007). The online web server, SWISS-ADME (Adsorption, distribution, metabolism and excretion) was used to predict the drug-likeness, solubility uniqueness and pharmacokinetics characteristics of the lead molecule (https://www.swissadme.ch).

3.0 Results and Discussion

3.1 Molecular docking

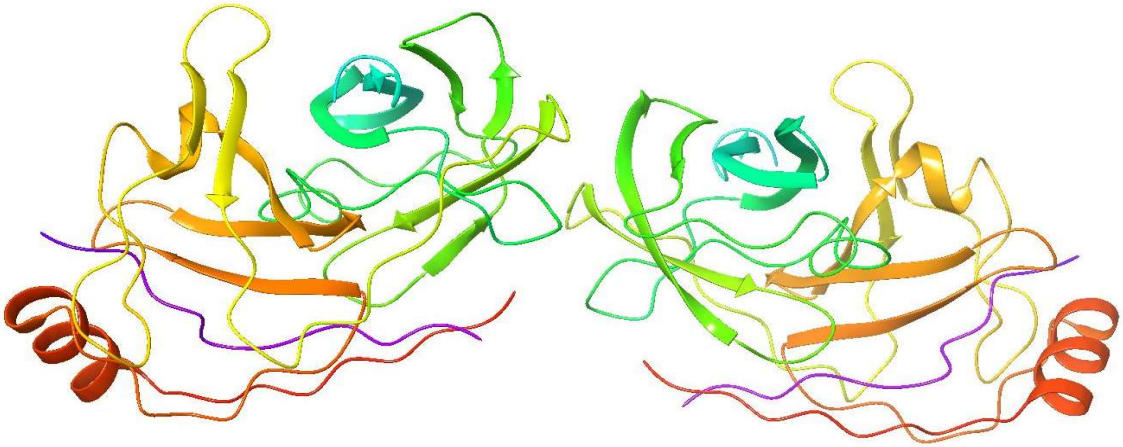
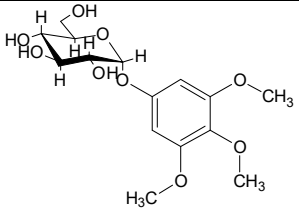
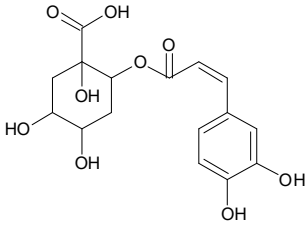
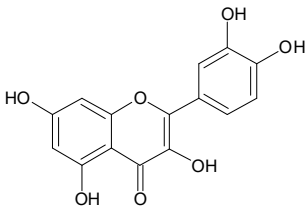
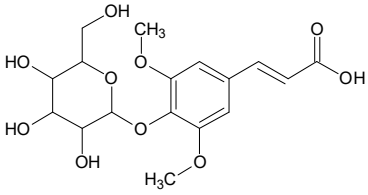
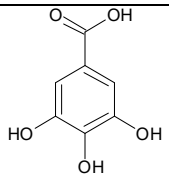


Fig 1: The crystalized structure of Hepatitis C Virus NS3 Protease (PDB ID: 1w3c).

Table 1: 2D chemical structure of bioactive compound isolated from the leaf extract of *morus alba* and their corresponding Glide score (G-Score) value calculated for the related query.

Code	Name	Score *ΔG (Kcal/mol)	2D Structure
MAL1	3,4,5-trimethyoxyphe-nol-1-O-β-D-rhamnopyranoside	-6.6	
MAL2	5'-O-E-caffeoyl-quinic acid (chlorogenic acid)	-60	
MAL3	3, 5, 7, 3' 4' Pen-tahydroxyflavone (quercetin)	-6.0	
MAL4	sinapic acid-O-glucoside	-5.0	

MAL5	3,4,5-trihy-droxybenzoic (gallic acid)	-4.7	

The docking study showed that MAL1 (3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside), MAL2 (5'-O-E-caffeoyl- quinic acid), MAL3 (3, 5, 7, 3' 4' Pentahydroxy flavone), MAL4 (sinapic acid-O-glucoside), MAL5 (3, 5, 7, 3' 4' Pentahydroxy flavone) had a favourable predicted docking score of -6.6 kcal/mol, -60 kcal/mol, -6.0 kcal/mol, -5.0 kcal/mol and -4.7 kcal/mol respectively. Hence, 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside was selected as the lead molecule (the compound with the best minimum energy). Fig 2 showed that 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside interacted with HIS55, SER136, SER137, GLY135, LYS134, LEU133, VAL130, ALA155, ALA154, ARG153 and PHE152 at the active site of the target. On the other hand, 5'-O-E-caffeoyl- quinic acid was observed to interact with 12 amino acids including ASP79, HIS55, VAL150, LEU133, LYS134, GLY 135, SER136, SER137, PHE152, ARG153, ALA154 and ALA155. Meanwhile, hydrogen bonds formation was observed for LEU133 and LYS134 residues (see Fig 3). Interaction between the ligand, 3, 5, 7, 3' 4' pentahydroxyflavone-1w3c and ASP79, HIS55, SER136, SER137, GLY135, LYS134, LEU133, VAL130, ALA155, ALA154, ARG153 and PHE152 (see Fig 4) was observed indicating possible reaction impact and consequences. Fig 5 shows the nature of sinapic acid-O-glucoside-1w3c interaction involving ALA155, ALA154, ARG153, PHE152, HIS55, VAL130, LEU133, LYS134, SER136, SER137 and GLY 135 in which a H-bond was formed with ALA155 residue within the active site of the target. Finally, 3, 5, 7, 3' 4' pentahydroxyflavone was noticed to interact with 10 amino acids ARG153, PHE152, HIS55, SER136, SER137, GLY135, LYS134, LEU133, ALA155 and ALA154 (see Fig 6).

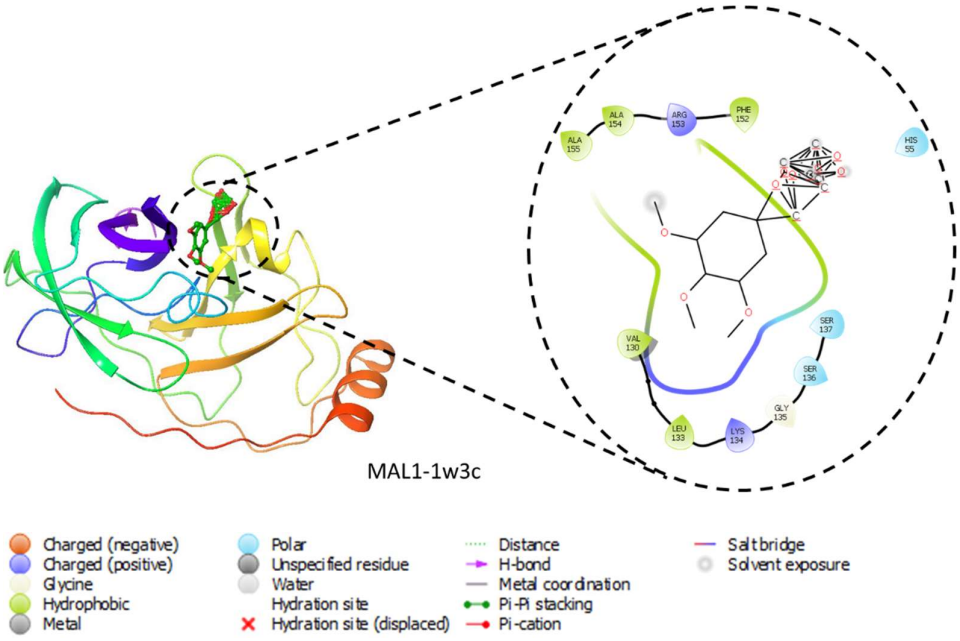


Fig. 2. Crystal structure of 1w3c complex with MAL1 showing also the binding site region and the residues that constitute this binding site region.

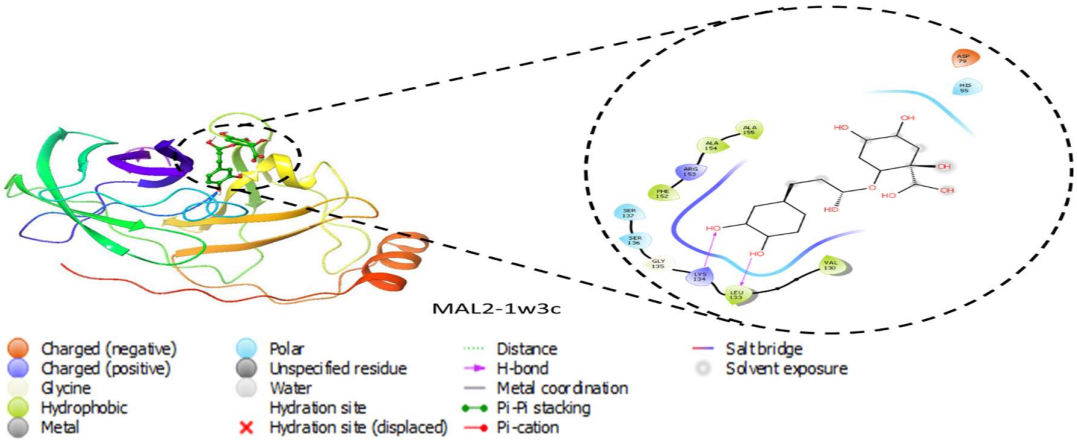


Fig. 3. Crystal structure of 1w3c complex with MAL2 showing also the binding site region and the residues that constitute this binding site region.

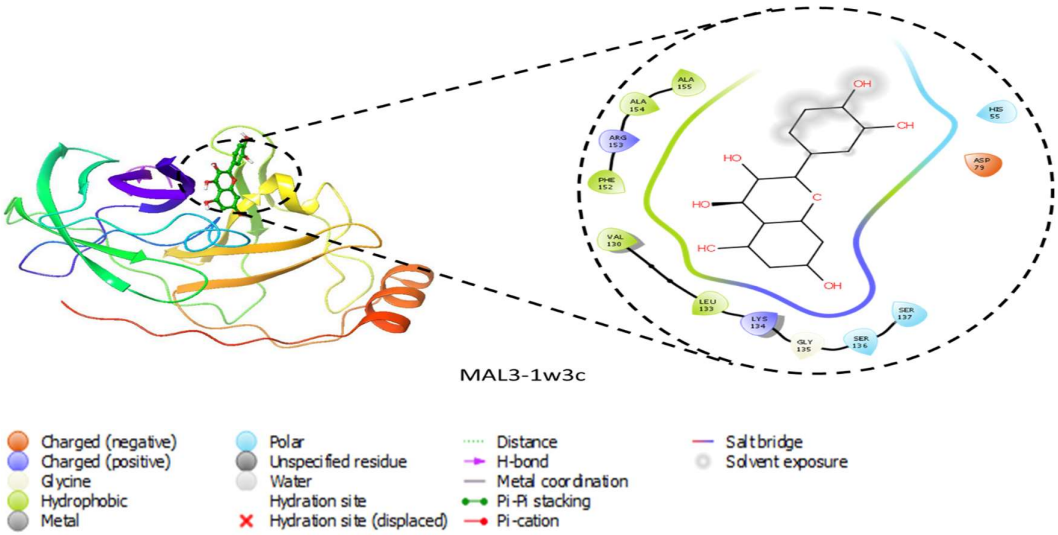


Fig. 4. Crystal structure of 1w3c complex with MAL3 showing also the binding site region and the residues that constitute this binding site region.

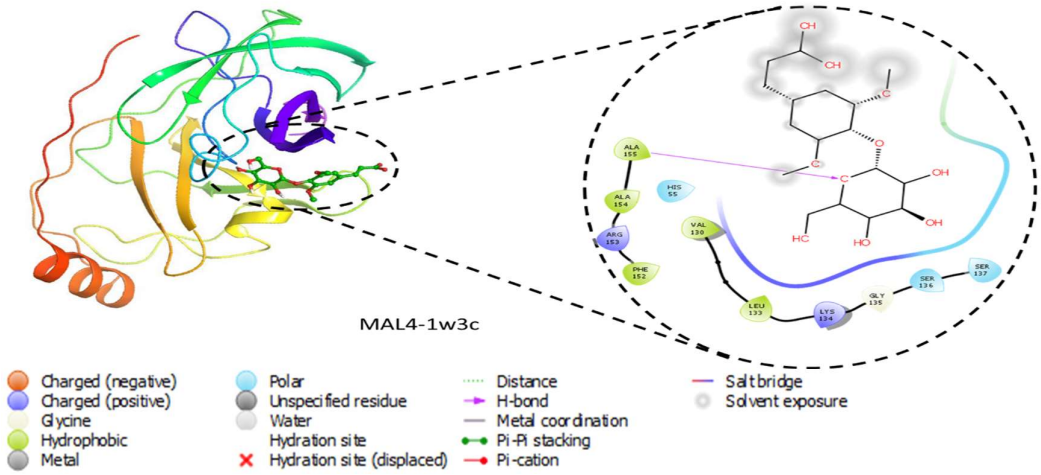


Fig. 5. Crystal structure of 1w3c complex with MAL4 showing also the binding site region and the residues that constitute this binding site region.

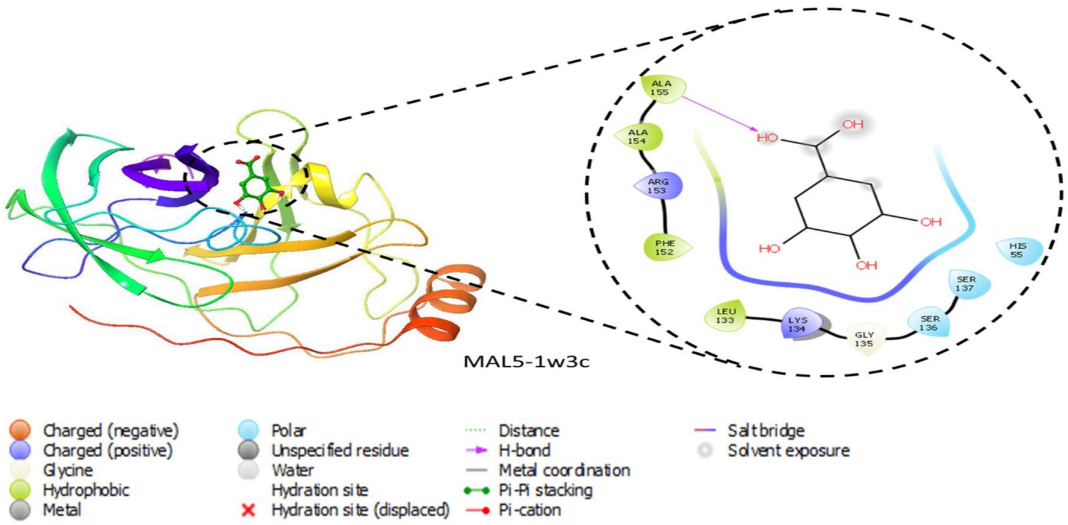


Fig. 6. Crystal structure of 1w3c complex with MAL5 showing also the binding site region and the residues that constitute this binding site region

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3.2 ADME assessment of potential hepatitis C virus NS3 protease inhibitors

The physicochemical space employed for the ADME prediction of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside is displayed in the coloured zone of the bioavailability radar shown in Fig. 8.

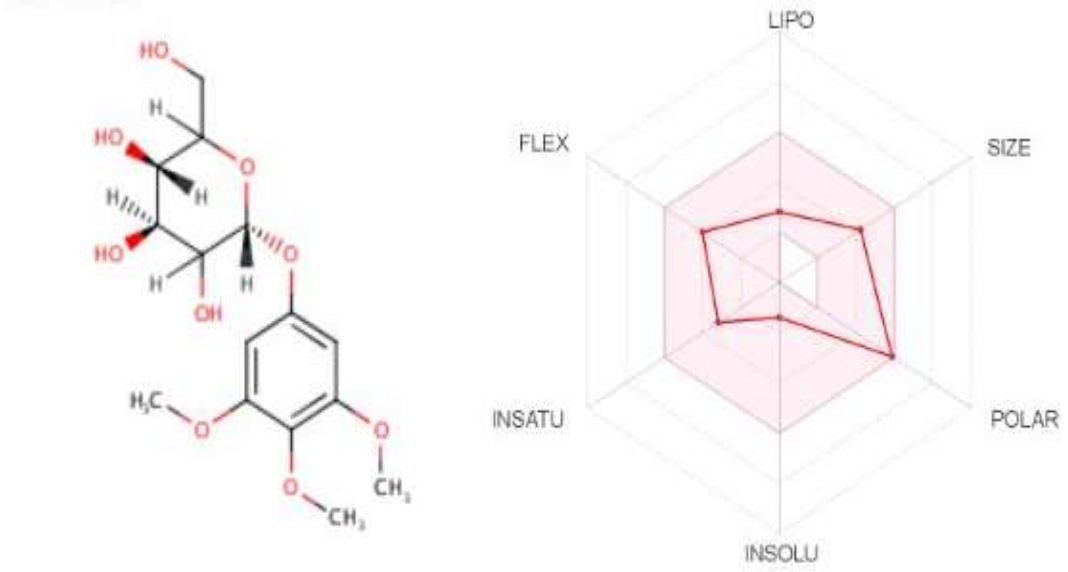

Fig. 7. The bioavailability radar of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside using Swiss ADME predictor.

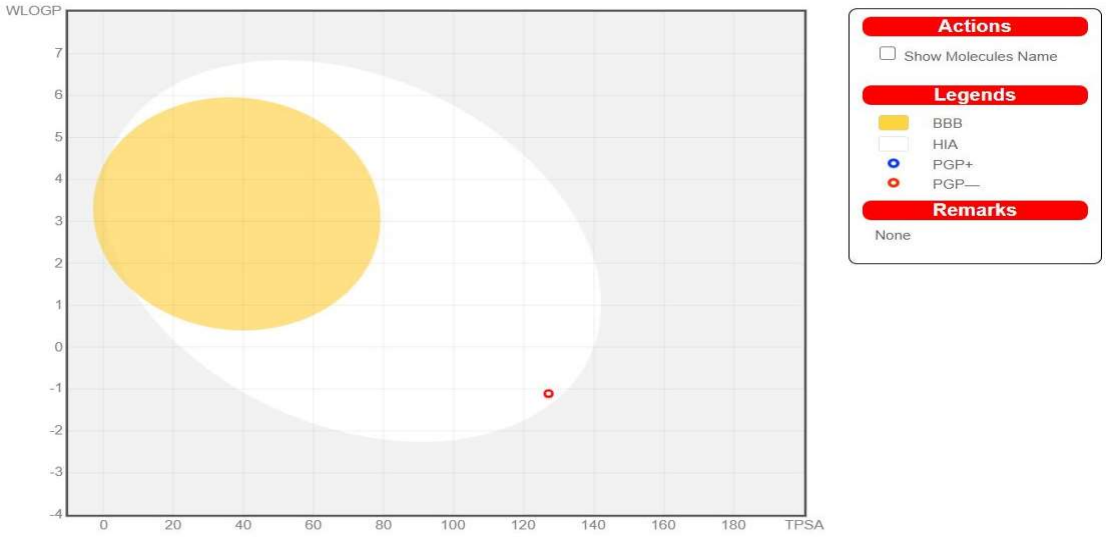

Fig. 8. Molecule falling in egg's yolk prediction 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside.

The pharmacokinetics, drug-likeness and medicinal chemistry friendliness of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside were obtained using SWISSADME online webserver. The physiochemical properties of the 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside: 24 heavy atoms, 9 hydrogen bond acceptors, 4 hydrogen bond donors, molar refractivity of 80.06 and topological polar surface area (TPSA) of the molecule is found to be 127.07 $Å^2$. On the other hand, the lipophilicity of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside was estimated and the parameters obtained included iLOGP (1.29), XLOGP3 (-0.58), WLOGP ~~is~~ (-1.11), MLOGP (-1.73), SILICOS-IT (-0.58) and Consensus P0/W (-0.54).

Table 2: Water solubility of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside.

Log *S* (ESOL)		-1.41
	Solubility	$1.34e^{+01}$ mg ml^{-1}; $3.88e^{-02}$ mol ml^{-1}
	Class	Very soluble
Log *S* (Ali)		-1.62
	Solubility	$8.35e^{+00}$ mg ml^{-1}; $2.41e^{-02}$ mol ml^{-1}
	Class	Very soluble
Log *S* (SILICOS-IT)		-0.56
	Solubility	$9.43e^{+01}$ mgml^{-1}; $2.27e^{-01}$ mol ml^{-1}
	Class	soluble

It is significant to state that 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside obeys Lipinski's, Egan, Veber and Muegge rules with 0.55 Bioavailability score (see Table 4) but–3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside did not obey Ghose score function. However, the blood-brain barrier (BBB) permeant and gastrointestinal absorption (GI) were hindered and relatively high, respectively. For further clarification, the BBB and GI were better demonstrated by using the molecule falling in egg's yolk model (Fig 8). As listed in Table 2, 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside was soluble in all the class solvent employed for the assay. Isoenzymes such as CYP1A2, CYP2C19, CYP2D6, CYP3A4 and CYP2C9 were not inhibited by 3,4,5-

trimethyoxyphenol-1-O-β-D-rhamnopyranoside. Therefore, the molecule will not result in a complicated biochemical process when administered as a therapeutic agent. Hence, we recommend further evaluation of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside as it could be the answer to our quest or may Clare the path to finding an effective drug for Hepatitis C Virus.

Table 3. Pharmacokinetics of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside.

GI adsorption	High
BBB permeant	No
P-GP substrate	No
CYP 1A2	No
CYP2C19	No
CYP2C9	No
CYP2D6	No
CYP3A4	No
Log Kp (skin permeation)	-8.82 cm s^{-1}

Table 4. Druglikeness of 3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside.

Lipinski	Yes, 0 violation
Ghose	No;1 violation
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55

4.0 Conclusion

The specific molecular docking study of natural products isolated from the leaf extract of *morus albaI* against Hepatitis C virus NS3 protease was performed. We observe that the lead molecule (3,4,5-trimethyoxyphenol-1-O-β-D-rhamnopyranoside) interacted with HIS55, SER136, SER137, GLY135, LYS134, LEU133, VAL130, ALA155, ALA154, ARG153 and PHE152 within the pocket of the receptor (1w3c). The ADME prediction revealed approving pharmacokinetics and druggable characteristics of the lead molecule. This result showed that this compound could have potential to be used as a therapeutic agent against Hepatitis C Virus. Hence, the lead molecule will require further wet-lab assessment under *in vivo* as well as *in vitro* conditions.

5.0 Acknowledgement

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6.0 References

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Declaration of interest

The authors declare no conflict of interest.