Pooja R Karker et al /J. Pharm. Sci. & Res. Vol. 11(9), 2019, 3195-3200

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Exploring Flavonoids for the Inhibition of Hepatitis C Virus by Targeting NS3 Protease

Pooja R Karker[1,2] and Ravikumar Y S[2]*

[1] *Department of Biochemistry, REVA University, Bangalore-560 064, Karnataka, India*

[2] *Department of Biotechnology, M.S. Ramaiah Institute of Technology, Bangalore-560 054, Karnataka, India*

Abstract:

Hepatitis C Virus (HCV) Infection causes deadly complications of the liver by attacking its hepatocytes. The NS3 protein of the virus is of prime importance as it is responsible for the processing of the polyprotein. Hence, it's wise to choose this protein as the target to come up with HCV inhibitors. In this study, 25 plants were chosen based on their significance in inhibiting liver disorders. A library of 108 flavonoids was constructed and docked against the NS3 protein of the virus. 16 flavonoids were obtained as the lead molecules having a binding affinity of lesser than -8.0 kcal/mol. Further, we performed simulation studies for 3 of the flavonoids and obtained 5,7-Dihydroxy-3-methoxy-8-methylflavone as the lead. 1016 analogs of this flavonoid were searched and they were individually docked against HCV NS3 protease to obtain 2-(2,6-dihydroxyphenyl)-2,5,7-trihydroxy-3H-chromen-4-one as the lead molecule with a binding affinity of -6.6 kcal/mol. However, in-vitro studies have to be performed in order to confirm the inhibitory effect of these flavonoids.

1. INTRODUCTION

Hepatitis C virus (HCV) infection is one of the leading diseases damaging the liver at a chronic level [1] and the estimation reveals that around 180 million people are infected with the virus [2]. Long-term HCV infection often causes severe liver diseases, including advanced fibrosis, cirrhosis and hepatocellular carcinoma, making HCV the most common reason for liver transplantation in developed countries [3]. Having this evident medical requirement, extensive efforts have been disbursed to develop effective therapeutic strategies for hepatitis C infection [4]. Combined therapy using pegylated interferon (PegIFN) - α and ribavirin for 24 or 48 weeks was the only promising treatment for HCV infection until 2011 [5]. However, this therapy is very expensive and the success rate is very low; Thereby increasing the need to implement efficacious therapy for HCV treatment without the presence of these analogs.

HCV consists of a single-stranded genomic RNA which in turn contains a single open reading frame (ORF) that is necessary for the translation and replication of the viral genome. It undergoes internal splicing to encode structural proteins including the Core protein C, envelope glycoproteins (E1 & E2) and p7 transmembranal protein; Non-structural proteins include NS3, NS4A, NS4B, NS5A and NS5B that is vital for the RNA replication [6]. The protease NS3/4A and the RNA polymerase NS5B are the two pivotal druggable viral targets involved in HCV replication and hence they have been widely used in anti-viral screening. NS3 is a protein with multifunction consisting a serine protease in the N-terminal and an RNA helicase in the C-terminal [7]. The NS3 serine protease has emerged as a prime target for the design of inhibitors as antiviral agents as helicase is responsible for cleaving the polyprotein into 10 mature proteins; if this is targeted then there virus replication will not take place.

Plants have always been a primary option for treatment of diseases. Many traditional medicinal plants have been reported to have strong antiviral activity and some of them have already been used to treat viral infections. Likewise, many herbs and isolated phyto constituents have been investigated for inhibitory effect against HCV [8]. Unlike bacteria, viral genome is subjected to rapid mutations and a new drug has to be developed very frequently. The cost that is involved in the research and development of a new anti-viral drug is very high to be afforded by the developing countries. Therefore plant based products such as flavonoids have been selected based on their known effects and its specificity and then it is screened to check the efficacy. The flavonoids interact with the different stages in the replication cycle of viruses. For example, some flavonoids work on the intracellular replication of viruses, whereas others inhibit the infectious properties of the viruses [9].

2. MATERIALS AND METHODS

2.1 Criteria for the Selection of Plants

25 plants were selected based on the following criteria: Plant extracts reported antiviral effect, Plants used by Traditional medical practitioners like Ayurveda and Chinese medicine to treat liver disorder and Plants reported to inhibit viral protease enzyme [ex HCV protease].

2.2 Selection of compounds

From the list of selected plants using the criteria mentioned above, the flavonoids that were reported in these plants were searched in online tools using search engines such as NCBI, BMC, Science Direct, Google patent search, Herbalgram.org etc.

2.3 Docking Studies

Molecular docking of the flavonoids with NS3 protease is done by the following steps:

2.3.1 Preparation of Ligands

The 2D structure of each flavonoid selected was drawn using Chem Draw software and was saved as a MOL file. These 2D structures were converted into 3D structures and optimized by minimizing their energy using Avogadro software and saved as a pdb file and were used as input file for opening in Auto Dock. The molecule was opened in Auto Dock software and the torsion was set in each molecule by making the number of rotatable bonds nil in them and was saved as a pdbqt file.

2.3.2 Preparation of Receptor Proteins

The structure of the protein (NS3 protease) was downloaded from Protein Data Bank using PDB ID 1dy8A in pdb format. This was opened in Auto Dock and optimized by deleting the water molecule, removing the heteroatoms etc. and was saved as a pdbqt file. The grid box (having confirmation: center_x = 62.1, center y = -12.092, center z = 8.457) was chosen for the protein on their active site (His57, Ser139, Arg155, Ala157, Ala156).

2.3.3 Molecular Docking

Both the compound and the protein were docked using Autodock vina. The best ranked model with lowest binding energy was analyzed further and visualized using Ligplot software [10].

2.4 Molecular Dynamic Simulation

To validate our docking procedure we did molecular dynamics simulation of the NS3 HCV protein using GROMACs software.

2.5 ADMET Study

Absorption, distribution, metabolism, excretion, toxicology (ADMET) study was performed *In silico* for all the 108 flavonoids by using the OSIRIS software. The drug-likeliness and toxicity of the compounds irrespective of the protein was determined.

3. Results & Discussions

3.1 Selection of plants

Based on the biological significance, 25 plants were selected which have already been used to treat several liver diseases, treat viral infections and inhibit viral proteases:

Table 1: List of plants

Sl. No	Plant Name	References
1.	*Plantago major*	[11]
2.	*Phyllanthus urinaria*	[12]
3.	*Terminalis chebula*	[13]
4.	*Phyllanthus amarus*	[14]
5.	*Cichorium intybus*	[14]
6.	*Solanum nigrum*	[11]
7.	*Cassia occidentalis*	[11]
8.	*Foeniculum vulgare*	[11]
9.	*Careya arborea Roxb*	[11]
10.	*Phyllanthus niruri*	[11]
11.	*Silybum marianum*	[14]
12.	*Picrorhiza kurroa*	[14]
13.	*Panax pseudoginseng*	[14]
14.	*Hypericum perforatum*	[14]
15.	*Trigonella foenum graecum*	[14]
16.	*Polygonum cuspidatum*	[15]
17.	*Glycyrrhiza glabra*	[16]
18.	*Ocimum basilicum*	[17]
19.	*Pistacia lentiscus*	[18]
20.	*Trachyspermum ammi*	[18]
21.	*Zingiber officinale*	[14]
22.	*Cochlospermum tinctorium*	[19]
23.	*Ecballium elaterium*	[20]
24.	*Glycyrrhizae Radix*	[21]
25.	*Boerhaavia diffusa*	[22]

Out of these 25 plants, 108 flavonoids with anti- microbial and anti- HCV activity were found.

3.2 Results of the docking study

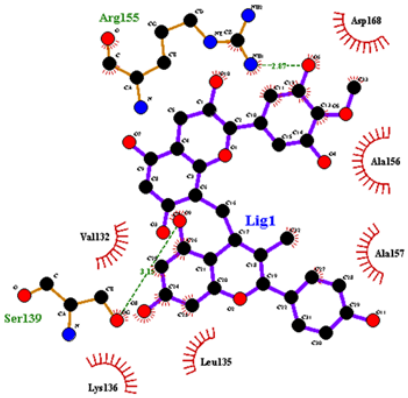
From the docking study that was performed, we obtained 16 molecules that should binding affinity of <-8 kcal/mol with the HCV NS3 protease. About 50 flavonoids showed the affinity of <-7 kcal/mol

Table2: Binding energy of top 10 Flavonoids against NS3 protease

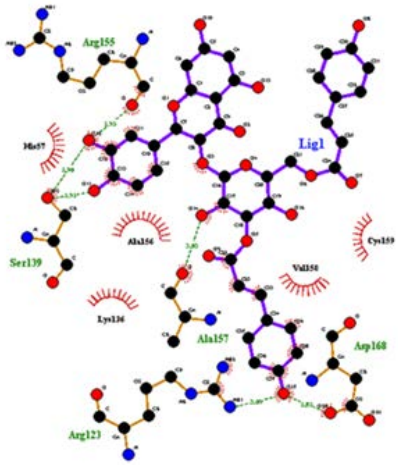
Sl No.	Flavonoids	Binding affinity(-kcal/mol)
1	Oligomeric proanthocyanadins	9.3
2	Quercitroside	9.3
3	3-O-a-l-rhamnosyl quercetin	8.8
4	Eriodictyol-7-O-rutinoside	8.8
5	Rutin	8.8
6	Kaempferol-3-O-rutinoside	8.6
7	Nirurin	8.6
8	Nepetin-7-glucoside	8.5
9	Quercetin-3-glucoside galactoside	8.4
10	Liquiritin apioside	8.3

3.3 Ligplot Analysis of the Lead Molecules

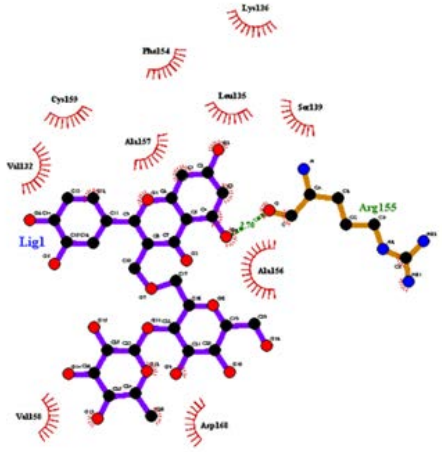
These lead molecules were viewed on ligplot software to study about the interactions of these molecules with the protease.



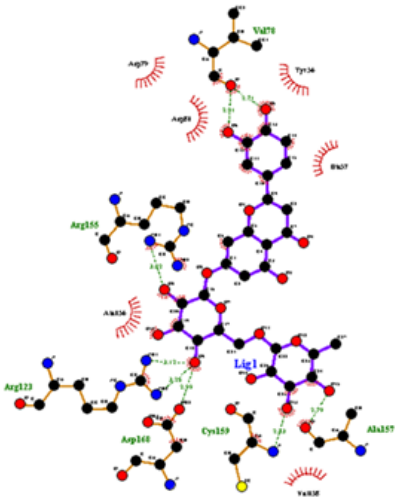
1) Oligomeric proanthocyanadins



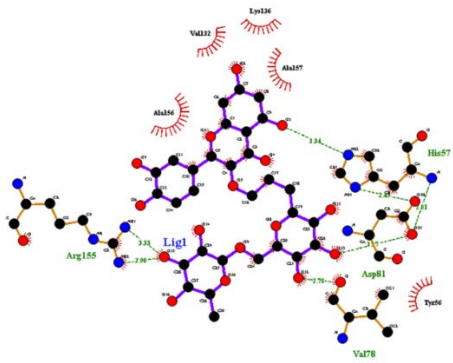
2) Quercitroside



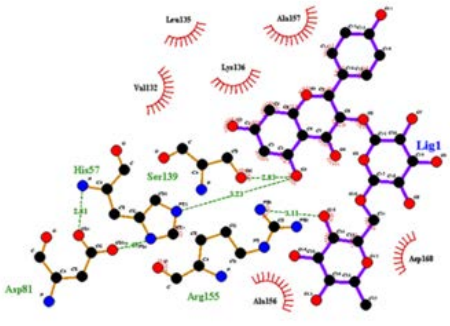
3) 3-O-a-l-rhamnosyl quercetin



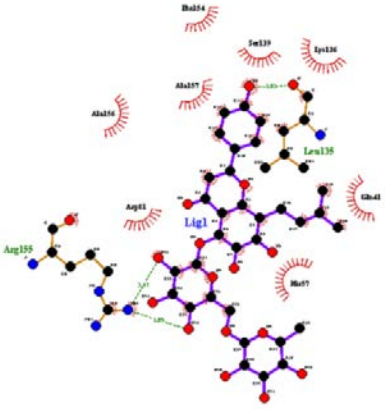
4) Eriodictyol-7-O-rutinoside



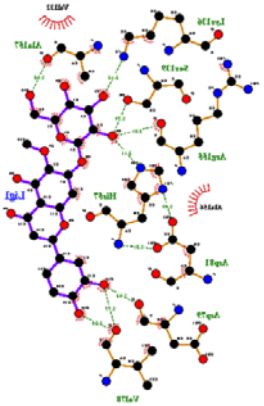
5) Rutin



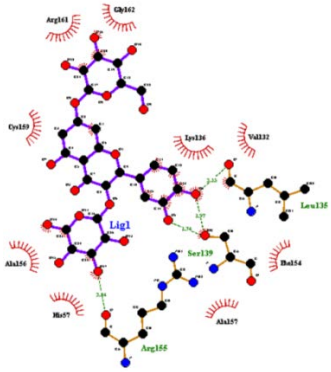
6) Kaempferol-3-O-rutinoside



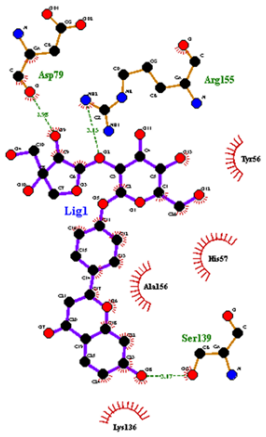
7) Nirurin



8) Nepetin-7-glucoside



9) Quercetin-3-glucoside galactoside



10) Liquiritin apioside

3.4 MD Simulation Results

Oligomeric Proanthocyanadins and Quercitroside were chosen as the positive control with binding affinity of -9.3 kcal/mol and 5,7-Dihydroxy-3-methoxy-8-methylflavone with binding affinity of -5.8 kcal/mol as negative control was chosen for MD simulation studies. 5,7-Dihydroxy-3-methoxy-8-methylflavone showed better interactions than Oligomeric Proanthocyanadins and Quercitroside. 1016 analogs of 5,7-Dihydroxy-3-methoxy-8-methylflavone was searched in PubChem and again docked individually against NS3 protease. Among them 2-(2,6-dihydroxyphenyl)-2,5,7-trihydroxy-3H-chromen-4-one should the least binding affinity of -6.6 kcal/mol with NS3 protease and was chosen as the lead molecule.

Table 3: Lead Molecules of the Analogs

SL NO.	COMPOUND NAME	BINDING AFFINITY (-Kcal/mol)
1	2-(2,6-dihydroxyphenyl)-2,5,7-trihydroxy-3H-chromen-4-one	6.6
2	3-methoxy-2-(4-methylphenyl)-4-oxochromene-6-carboxylic acid	6.5
3	Cedeodarin	6.5
4	Scutevulin	6.5
5	2-(3,4-dihydroxyphenyl)-7-hydroperoxy-5-hydroxy-3-methoxychromen-4-one	6.5
6	(3R)-2alpha-(4-Hydroxyphenyl)-6,8-dimethyl-3,5,7-trihydroxy-2,3-dihydro-4H-1-benzopyran-4-one	6.5
7	6-chloro-2-(3-hydroxyphenyl)-5,7-dimethyl-2,3-dihydrochromen-4-one	6.4
8	2-(4-fluorophenyl)-3-methoxy-4-oxochromene-6-carboxylic acid	6.4
9	8-C-Methylkaempferol	6.4
10	5,7,2',3'-Tetrahydroxyflavone	6.4

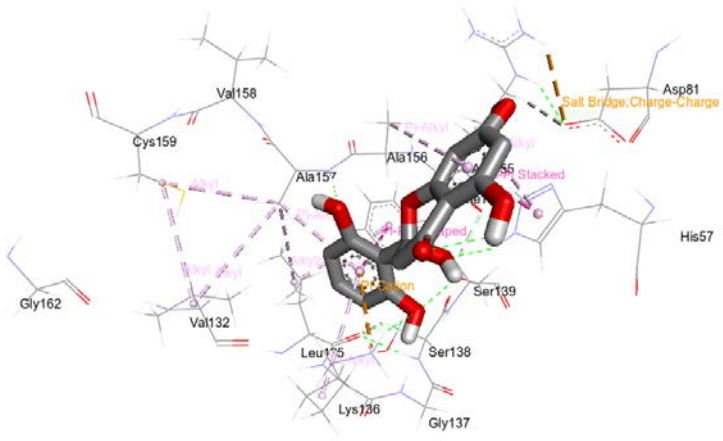


Fig 1: Interaction of 2-(2,6-dihydroxyphenyl)-2,5,7-trihydroxy-3H-chromen-4-one with the active site of NS protease

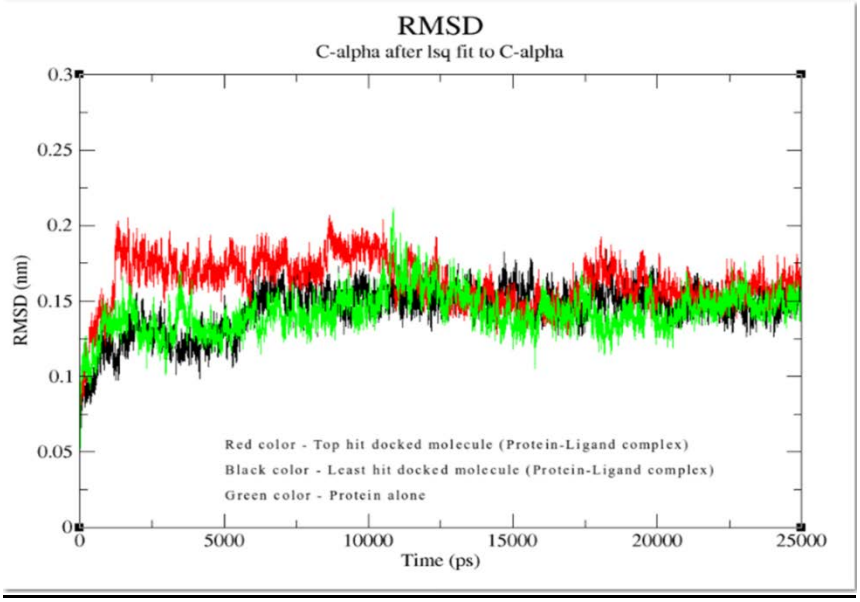


Fig2: RMSD plot of flavonoids

DISCUSSIONS

Molecular docking studies were performed with all these 108 flavonoids against the active site of NS3 protease. When the ligands and the protein is docked together, Autodock Vina searches for the best confirmation of the complex with least binding energy and hence the highest stability. In this way, it gives an output file of 9 best ranked models with different binding affinities in kcal/mol. The confirmation which is ranked 1st has the least binding affinity and is considered to be the best possible orientation that can be obtained in docking. For example Nirurin: 1 to 9 models have the binding affinity of -8.6, -8.1, -8.0, -7.8, -7.2, -7.2, -7.0, -6.9, -6.8 kcal/mol respectively. The model with the affinity -8.6 kcal/ mol will be selected for the further analysis. About 50 flavonoids exhibited a binding affinity of lesser than -7.0 kcal/ mol and 16 molecules showed a binding affinity of lesser than -8.0 kcal/mol. Lesser the value of the binding affinity better is the result obtained. Oligomeric Proanthocyanadins and Quercitroside show the best interaction with NS3 protease having a binding affinity of -9.3 kcal/ mol. This is followed by 3-O-A-L-Rhamnosyl Quercetin, Eriodictyol-7-O-Rutinoside, Rutin having a binding affinity of -8.8 kcal/ mol. The ligplot analysis of Oligomeric proanthocyanadins which has the least binding affinity shows hydrophilic interactions with Arg155 and ser139 and hydrophobic interactions with Asp168, ala156, ala157, leu135, val132 and lys136. On the other hand, Quercitroside shows hydrophilic interactions with Arg155, ser139, ala157, asp168 and hydrophobic interactions with arg123, His57, ala156, lys136, val158, cys159. Eriodictyol-7-O-rutinoside though it has lesser binding affinity than the above mentioned compounds, it has interactions with most number of residues (12) among the lead molecules; hydrophilic interactions withVal78, arg155, arg123, asp168, cys159 and Hydrophobic interactions with ala157, Asp79, asp81, tyr56, his57, val158, ala156. Rutin having a binding affinity of -8.8 kcal/mol interact with His57, asp81, val78, arg155 hydrophilically and with Tyr56, ala156, val132, lys132, ala157 hydrophobically. Strangely, 3-O-A-L-Rhamnosyl Quercetin interacts with a single amino acid Arg155 whereas it interacts with 10 amino acids Lys136, phe154, leu135, ser139, ala157, cys159, val132, ala156, asp168, val158 hydrophobically. Kaempferol-3-O-rutinoside and Nirurin have the same binding affinity of -8.6 kcal/mol whereas they interact with His57, ser139, asp81, arg155 (hydrophilic), Val132, lys136, leu135, ala157, asp168, ala156 (hydrophobic) and Leu135, arg155 (hydrophilic), Phe154, ser139, lys136, ala157, ala156, asp81, his57, gln41(hydrophobic) respectively. Nepetin-7-glucoside have hydrophilic interactions with Lys136, ser139, arg155, ala157, his57, asp81, asp79, val78 and hydrophobic interactions with Val132 and ala156. On the other hand, Quercetin-3-glucoside galactoside have hydrophilic interactions with Leu135, ser139, arg155 and hydrophobic interactions with Gly162, arg161, cys159, ala156, his57, ala157, phe154, val132, lys136. Liquiritin apioside interact with Arg155, asp79, ser139 amino acids in a hydrophilic manner and Tyr56, his57, ala156, lys136 in a hydrophobic manner.

ADMET (Absorption, distribution, metabolism, excretion and toxicity) studies were carried out by In silico studies to determine the drug likeliness of the compound irrespective of the protein that it interacts with. The druglikeliness and the toxicity of the compounds are listed under the table 4. Cassiaoccidentalin B showed the maximum drug likeliness of 3.037 and with no toxicity issues followed by nirurin which showed drug likeliness of 2.075; In spite of good drug likeliness it showed low reproductive effecting ability of the molecule. Any value that is positive is a suitable druggable molecule. Oligomeric Proanthocyanadins though it showed very good interactions with the protein, it exhibited high reproductive effecting issues.

Further molecular dynamic simulation studies were performed for three of the flavonoids: 5, 7-Dihydroxy-3-methoxy-8-methylflavone, Oligomeric Proanthocyanadins and Quercitroside to understand the interaction in real life conditions. In molecular docking these compounds had shown a binding affinity of -5.8, -9.3 and -9.3 kcal/ mol respectively. This proves that 5, 7-Dihydroxy-3-methoxy-8-methylflavone has not exhibited very good results in molecular docking. Under simulation studies Dihydroxy-3-methoxy-8-methylflavone showed better results than Oligomeric Proanthocyanadins and Quercitroside when RMSD was calculated as a function of time. We can see that this is contradictory when compared to the results obtained from docking studies (Fig2). By docking 1016 analogs of Dihydroxy-3-methoxy-8-methylflavone against NS3 protease 10 lead molecules were obtained (Table 3); 2-(2,6-dihydroxyphenyl)-2,5,7-trihydroxy-3H-chromen-4-one showed the best interaction with a binding affinity of 6.6 kcal/ mol (Fig1).

REFERENCES

1. Dubuisson J, Cosset F (2014) Virology and cell biology of the hepatitis C virus life cycle. *J Hepatol*. 61: 3-13.
2. Geddawy A, Ibrahim Y, Elabahie N, Ibrahim M (2017) Direct Acting Anti-Hepatitis C Virus Drugs: Clinical Pharmacology and Future Direction. J Transl Int Med. 5(1):8-17.
3. Scheel T, Rice C (2013) Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. *Nat Med*. 19: 837–849.
4. Bartenschlager R, Lohmann V, Penin F (2013) The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection. *Microbiology*. 11: 482-496.
5. Imran M, Manzoor S, Khattak NM, Khalid M, Ahmed QL, Parvaiz F, Tariq M, Ashraf J, Ashraf W, Azam S, Ashraf M (2013) Current and future therapies for hepatitis C virus infection: from viral proteins to host targets. *Arch Virol*. 10: 510-516.
6. Calland N, Dubuisson J, Rouillé Y, Seron K (2012) Hepatitis C Virus and Natural Compounds: New Antiviral Approach. *Viruses*. 4: 2197-2217.
7. Moradpour D, Penin F, Rice C (2007) Replication of hepatitis C virus. *Microbiol*. 5: 76-94.
8. Kumar S, Pandey A (2013) Chemistry and Biological Activities of Flavonoids: An Overview. *Scientific World J*. 1: 1-13.
9. Nijveldt R, Nood V, Hoorn V, Boelens P, Norren K, Leeuwen P (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr. 74: 418–425.
10. Karkera P, Telkar S, Usha B, Swamy H and Ravikumar Y (2016). Computational Screening of Flavonoids for HCV Inhibition. IJRET. 5: 31-37.

11. Najmi A, Pillai K, Pal S and Aqil M (2005) Free radical scavenging and hepato protective activity of jigrine against galactosamine induced hepatopathy in rats. Journal of Ethnopharmacology. 97 (3): 521-525.
12. Yang C, Cheng H, Ling T, Chiang L and Ling C (2005) Acetone, ethanol and methanol extracts of Phyllanthus urinaria inhibit HSV-2 infection in vitro. Antiviral Research 67: 24-30.
13. Kim T, Kang S, Jung K, Kang J, Lee E, Han H and Kim S (2001). Antiviral activities of extracts isolated from Terminalis chebula Retz., Sanguisorba officinalis L., Rubus coreanus Miq. and Rheum palmatum L. against hepatitis B virus. Phytotherapy Research 15:718-720.
14. Coon J and Ernst E (2004) Complementary and alternative therapies in the treatment of chronic hepatitis C: a systematic review. Journal of Hepatology. 40: 491-500.
15. Wang R, Kong J, Wang D, Lien L and Lien E (2007) A survey of Chinese herbal ingredients with liver protection activities. Chin Med. 2:5.
16. Gajula D, Verghese M, Boateng J, Walker B, Shackelford L, Mentreddy S, Cedric S (2009) Determination of Total Phenolics, Flavonoids and Antioxidant and Chemopreventive Potential of Basil (Ocimum basilicum L. and Ocimum tenuiflorum L.). Int J Cancer Res. 5: 130-143.
17. Jagtap S, Deokule S and Bhosle s (2006) Some unique ethnomedicinal uses of plants used by the Korku tribe of Amravati district of Maharashtra, India. Journal of Ethnopharmacology 107: 463–469.
18. Ljubuncic P, Azaizeh H, Portnaya I, Cogan U, Said O, Saleh K and Bomzon A (2005) Antioxidant activity and cytotoxicity of eight plats used in traditional Arab medicine in Israel. Journal of Ethnopharmacology. 99:43-47.
19. Nergard C, Diallo D , Inngjerdingen K , Michaelsen T, Matsumoto T, Kiyohara H, Yamada H and Paulsen B (2005) Medicinal use of Cochlospermum tinctorium in Mali Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. Journal of Ethnopharmacology. 96: 255-269.
20. Gerges H, Khalil R, Mansour E, Magdalou J, Chahined R and Ouainia N (2007) Cucurbitacins from Ecballium elaterium juice increase the binding of bilirubin and ibuprofen to albumin in human plasma. Chemico-Biological Interactions. 169: 53-62.
21. Nakayama T, Suzuki S, Kudo H, Sassa S, Nomura M and Sakamoto S (2006). Effects of three Chinese herbal medicines on plasma and liver lipids in mice fed a high-fat diet. Journal of Ethnopharmacology. 109(2):236-240.
22. Samvatsar S and Diwanji VB (2000). Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. Journal of Ethnopharmacology. 73: 313-316.