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# Molecular docking studies of *delonix elata* heat shock protein (HSP70) ATPASE: An enzyme target for brain attack

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**Abstract**
Heat Shock Protein (HSP 70) Atpase enzyme is neurological disorder enzyme present human brain cells. Quercetin (QU), Rutin (RU) and Hesperitin (HE) was taken as ligand for molecular docking with brain targets. Heat Shock Protein (HSP 70) Atpase whose crystallographic structures are available on the PDB database 2E1Q was used for the docking analysis using the Schrödinger Suite tool. The docking studies of the enzyme Heat Shock Protein (HSP 70) Atpase with two ligand quercetin, rutin and hesperitin reveals that these lead molecules can be used in drug development for brain attack. Phytochemical screening of alkaloid, steroid, triterpenoid, flavonoid, protein, amino acid, tannins, phenolics, glycosides, saponins, carbohydrates, volatile oils, fatty acids, emodins of phytoconstituents have been carried out.

**Keywords:** heat shock protein, *Delonix elata*, quercetin, rutin, hesperitin and brain attack

## 1. Introduction
Heat shock protein 70 (Hsp70a) is a molecular chaperone that is expressed in response to stress. In this role, Hsp70 binds to its protein substrates and stabilize them against denaturation or aggregation until conditions improve [1]. In addition to its functions during a stress response, Hsp70 has multiple responsibilities during normal growth; it assists in the folding of newly synthesized proteins [2, 3] the sub cellular transport of proteins and vesicles [4] the formation and dissociation of complexes [5] and the degradation of unwanted proteins [6, 7]. Thus, this chaperone broadly shapes protein homeostasis by controlling protein quality control and turnover during both normal and stress conditions [8]. Consistent with these diverse activities, genetic and biochemical studies have implicated it in a range of diseases, including cancer, neurodegeneration, allograft rejection, and infection. This review provides a brief review of Hsp70 structure and function and then explores some of the emerging opportunities (and challenges) for drug discovery. The 70 kDa group of heat shock proteins or HSP70 is a highly conserved family of proteins, being present from bacteria to man. In most species, there are multiple genes for [9]. Members of this protein family include constitutive or cognate (HSC70) and the stress inducible forms (HSP70).

The term HSP70 is used here, unless otherwise specified, to refer to inducible as well as the cognate forms. These proteins are believed to function mainly as molecular chaperones helping in protein transport and translocation [10, 11]. Among the very diverse and wide-ranging roles of the HSP70 family proteins, an emerging field of considerable significance concerns their expression in neurons. Several isoforms of HSP70, including the constitutively present cognate forms (HSC70), are known to exist in neurons [12, 13]. In fact, one of the first glimpses of the function of HSP70 came from studies on bovine brain showing an interaction of HSP70 family protein with folded proteins like clathrin [14]. Clathrin uncoating of the synaptic vesicles (SV) is an important step in SV recycling pathway [15, 16]. Before the vesicle is recycled and can fuse to early endosomes, its clathrin cover has to be removed. *β*-internexin, a cytoskeletal-associated protein, with clathrin-uncoating ATPase activity in rat brain is a member of the HSP70 family [12]. This HSP70 family protein interacts with conformationally flexible regions of clathrin light chains and allows the release of bound clathrin while ATP is hydrolyzed [17].

## 2. Materials and methods
### Plant Material
Plant material of *Delonix elata* (L.) was collected from Narthamalai, Pudukottai, Tiruchirappalli, Tamil Nadu, during the month of December 2008. The plant specimen was identified with Gamble, Flora of the Presidency of Madras and the identity is confirmed with the herbarium specimen deposited in Department of Botany, Periyar EVR College (Autonomous) Tiruchirappalli, Tamil Nadu.

### Preparation of the Extract
Plant materials leaf and root was washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The plant materials was identified and authenticated by Botanical Survey of India (Southern Circle, Coimbatore Tamil Nadu, India). A voucher specimen of both has been deposited for future reference in the Department of Botany, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam – 637 205, Tiruchengode - 637 205, Tamil Nadu. *Delonix elata*

(L.) leaves and root were chopped into small pieces, shade dried. Dried samples were powdered in a Wiley mill. Powdered samples were stored in polythene containers at room temperature. The leaves and root samples were taken for analysis to detect the presence of certain biologically active compound(s). The extract contains polar components of the material and 2µl sample of the solution was employed in HPL and GC-MS for analysis of different compounds.

**Identification of certain biologically active compounds**

*Delonix elata* leaf and root were chemically screened to find out the presence alkaloids (AL), flavonoids (FL), protein (PR), amino acids (AA), carbohydrates (CR), tannins (TA), phenolics (PH), glycosides (GL), saponins (SA), or absence of bio active compounds like steroid (ST), triterpenoid (TT), volatile oils (VO), fatty acids (FA), emodins (EM).

**3. Extraction**

*Delonix elata* (Leaves - 50g) and (Root - 50g) coarse sample using Soxhlet method, extraction 24 hrs and using Methanol (MT) solvent.

**Preliminary phytochemical screening**

The condensed extracts of different solvent used for preliminary phytochemical screening were carried out using standard procedures to test the presence of bioactive compounds [22, 23 & 24].

**4. Qualitative Analysis of phtyochemicals**

The analysis of phytochemicls from the solvent free extract of *Delonix elata* leaves and root was individually carried out using various qualitative test for alkaloids, flavonoids, protein, amino acid, tannins, phenolics, glycosides, saponins and carbohydrates compounds.

Extraction of phytochemicals

The individual phytochemical was extracted in the appropriate solvent and stored in air -tight containers at 4ºC till further use.

Phytochemical screening

The test was performed on methanol extract of *Delonix elata* (L.) Gamble leaves (Table 1).

**Table 1:** Phytochemical screening of *Delonix elata* (L.) Gamble (leaves and root)

| Phytoconstituent | Test | Methanol |
|---|---|---|
| Alkaloids | Mayer's test | + |
| Tests for steroids and sterols | Salkowski's test | - |
| Test for triterpenoids | Hirshorn test | - |
| Flavonoids | Shinoda test | + |
| Protein and Amino acids | Ninhydrin test | + |
| Tannins and Phenolics | Gelatin test | + |
| Glycosides | Legal test | + |
| Saponins | Alcoholic vanillin test | + |
| Carbohydrates | Benedict's test | + |
| Volatile oils | Aliquot test | - |
| Fatty acids | Filter paper test | - |
| Emodins | Ammonium hydroxide test | - |

*+ Present; - Absent*

Phytochemical analysis revealed that Methanol extract of *Delonix elata* leaves and root contains alkaloids, flavonoids, protein, amino acid, tannins, phenolics, glycosides, saponins and carbohydrates compounds (Table -1).

**Maceration**

Powdered dried leaves (1g) and root (1 g) were macerated with methanol: water (1:1; v/v, 10 mL) and left at rest (7 days, room temperature). The material was filtered and the crude extract obtained was analyzed directly by GCMS and HPLC-UV. This procedure was repeated in triplicate.

**5. Gas Chromatography (GC)**

A common form of mass spectrometry is Gas Chromatography - Mass Spectromertry (GC/MS or GC - MS). In this technique, a gas chromatography is sued to separate compounds. The stream of separated compounds is fed on line into the ion source, a metallic filament emits electrons, which ionize the compounds. The ions can then further fragment, yielding predictable patterns. Itact ions and fragments pass into the mass spectrometer's analyzer and are eventually detected. The ample were analysed by GC - Mas supplied by FISONS instruments the GC - Mas model is GC 8000 series and MS is MD 800. GC column dimension : 30 mm × 0.25 mm × 0.5 mm. AB-35 MS fused silica column. Helium gas is engaged as a carrier gas at the rate of 1 ml/min. The GC-MS oven temperature was programmed as follows

1. 50 °C (hold 2.5 minutes) to 150 °C at the rate of 15 °C/min
2. ii. increased to 200 °Cat a rate of 3 °C/min
3. increased to 300 °C at a rate of 8 °C/min
4. kept for another 8 minutes at 300 °C

**GC conditions:** Injector temperature 250ºC at 6ºC/mts and be held at this temperature for 10mts. The ion source temperature is 200ºC and the interface temperature is 250ºC.

**Gas chromatography: mass spectrometry (GC - MS)**

The spectras was obtained in the EI mode with 70 eV ionization energy. The compounds were identified by comparison with the standards, if not available, the mass spectra was matched with the in built library. The methanolic extract of the plant drug powder, viz., leaf and root were injected by hypodermic syringe into the inlet port of GC. The full scan MS of the compounds were measured from m/z 80 - 750. MS data were acquired in the negative ionization mode. The results can be expressed in terms of retention time (Rt), which is the time required for elution of sample or RV - the volume of carrier gas required to elute a component from the column. These parameters are nearly always expressed in terms relative to a standard compound ($RR_v$ or $RR_T$) which may added to the sample extract or which could take the form of the solvent used for dissolving the sample. GC provides both quantitative and qualitative data on plant substances, since measurements of the area under the peaks shown on the GC trace are directly related to the concentrations of the different components in the original mixture. GC is automatically linked to mass

spectrometry and the value of the technique is that it requires only microgram amounts of material, that it may yield a complex fragmentation pattern, which is often characteristic of that particular compound. MS, in essence, consits of degrading trace amounts of an organic compound and recording the fragmentation pattern according to mass.
The sample vapor diffuses in to the low-pressure system of the mass spectrometer where it is ionized with sufficient energy to cause fragmentation of chemical bonds. The resulting positively charged ions are accelerated in a magnetic field, which disperses and permits relative abundance measurements of ions of give mass - to - charge ratio. The resulting record of ion abundance versus mass constitutes the mass spectral graph, which thus consits of a series of lines of varying intensity at different mass units. In many cases, some of the parental compound will survive the vaporization process and will be recorded as a molecular ion peak. Very accurate mass measurement (0. 0001 mass units) can then be performed on this and particular ion. The accuracy is such as to indicate the exact molecular formula of the substance, so that conventional lethal analysis no longer necessary. MS is frequently used in conjunctions with GLC, and the combined operation provides both qualitative and quanitative identification of the many structurally complex components that my be present together in a particular plant extract (Zaeba *et al*., 2005). Some of the many applications of MS data to plant biochemical research are covered in two Treatises (Waller, 1972), (Waller and Dermer, 1980).

**6. Qualitative and quantitative analysis**
The quantitative determination of the chemical compounds was based on the comparison of peak areas of samples with those in GCMS library. Some of the identified compounds are quercetin, rutin, beta amyrin and hesperitin as described below: Quercetin- The quercetin was identified in *Delonix elata* L. (leaves) in GCMS analysis. Figure 2 shows that the comparison of abundance of quercetin in leaf parts of the plant extracted by the methanol solvents. The leaves sample contain the least quercetin compounds.

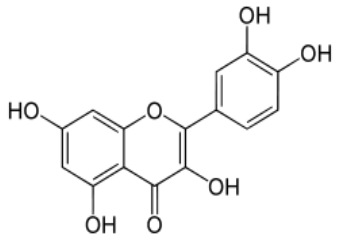


**Fig 2:** Quercetin chemical composition ($C_{15}H_{10}O_{7}$)

As a result, the best extraction conditions for quercetin compound in *Delonix elata* L. were 24 hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves.

**Rutin**
Besides quercein, rutin was also one of the chemical compounds that can be determined from *Delonix elata* L. leaves in the GCMS analysis which was next to the peak of rutin. The rutin was identified in *Delonix elata* L. (leaves) in GCMS analysis. Figure 3 shows that the comparison of abundance of rutin in leaf parts of the plant extracted by the methanol solvents. The leaves sample contain the least rutin compounds.

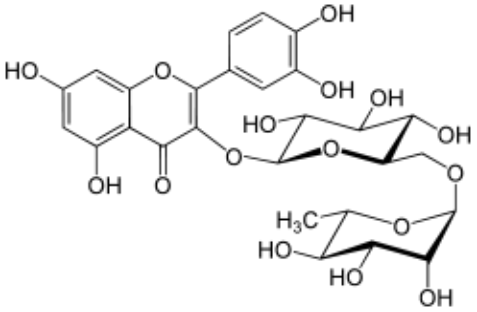


**Fig 3:** Rutin chemical composition ($C_{27}H_{30}O_{16}$)

As a result, the best extraction conditions for rutin compound in *Delonix elata* L. were 24 hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves. *Delonix elata* (L.) leaves show the presence of quercetin and rutin. In addition, methanol has been identified as the better extraction solvent. This was because almost all the parts of plant which were extracted with methanol solvent proposed the higher percentage area in the result of gas chromatography as shown in Figure 3.

**Hesperitin**
The hesperitin was identified in *Delonix elata* L. (root) in GCMS analysis. Figure 4 shows that the comparison of abundance of hesperitin in root parts of the plant extracted by the methanol solvents. The root sample contains the least hesperitin compounds.

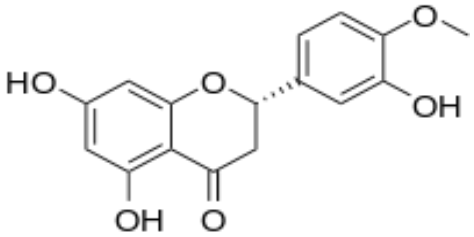


**Fig 4:** Hesperitin chemical composition ($C_{16}H_{14}O_{6}$)

As a result, the best extraction conditions for hesperitin compound in *Delonix elata* L. were 24 hours extraction period with methanol as solvent and the most favourable parts of the plant for Soxhlet extraction process was leaves.

**7. Acute Oral Toxicity Studies**
The method used defined doses (2000 µg/kg body weight) and results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for classification of chemical which cause acute toxicity. Three male rats 250-300 gm were used for the study, since the herbal extracts are relatively nontoxic, the starting dose level of methanolic leaf (Quercetin and Rutin) and root extract of *Delonix elata* (Hesperitin) was selected 2000 µg/kg/bw/p.o. the drug was administered orally to rats which were fasted over night with water *ad libitum* before administration of the drug. Body weights of the rats before and after treatment were noted.

**Structure of the proteins molecules**

Structure of the Hsp 70 ATPase protein molecule 3 (PDB 4108 - Fig. 8).

**Molecular Models Binding Proteins**

**A. Hsp 70 ATPpase - ligand interaction**

The interaction profile of Hsp 70 ATpase with leaf extract of Rutin molecule showed that the ligand interacted ARG 19 (O), GLY 22 (O), ASN 90 (H, O), TYR (O, H) and ARG 19 (H, O), receptor via H, O atom types (Fig 5.6. )forming hydrogen bonds with the bond distance of 1.87 Å, 2.15 Å, 2.42 Å, 2.5 Å, 1.95 Å, 1.96 Å and 1.91Å, total number of bonds 7 respectively (Table 7). The higher interaction of Hsp 70 ATPpase with Hesperitin can be noticed from the Glide score; -10.48 as higher Interaction profile (Table 2). The interaction profile of Hsp 70 ATpase with leaf extract of Quercetin molecule showed that the ligand interacted ARG 90 (O, H), GLY 22(H, O), MET 192 (H,O), THR 190 (H, O), GLY 186 O, H) receptor via H, O atom types (Fig 9,10,11, 12, 13 and 14 )forming hydrogen bonds with the bond distance of 2.33Å, 2.11 Å, 2.47 Å, 2.21 Å and 1.89 Å, and total number of bonds 5 respectively (Table 3).

The higher interaction of Hsp 70 ATPpase with Quercetin can be noticed from the Glide score; -9.13 as higher Interaction profile (Table 3). The interaction profile of Hsp 70 ATpase with root extract of Hesperitin molecule showed that the ligand interacted ASN 90 (H, O and O, H), and GLY 186 (O, H) receptor via H, O atom types (Fig 9, 10, 11, 12 and 13) forming hydrogen bonds with the bond distance of 2.22Å, 2.36 Å and 1.88 Å, total number of bonds 3 respectively (Table 2). The higher interaction of Hsp 70 ATPpase with Hesperitin can be noticed from the Glide score; -7.76 as higher Interaction profile (Table 2), Fig. 12 graph showing rutin molecule high, quercetin medium and hesperidin low interaction profile. Fig. 16 graph showing glide score and docking score rutin (-12.84), (-12.84) and Quercetin (-7.65), (-7.64), (Table 3).

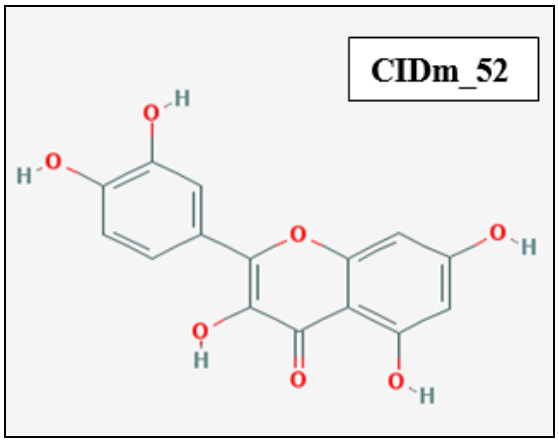


**Fig 5:** Structure of Quercetin compounds

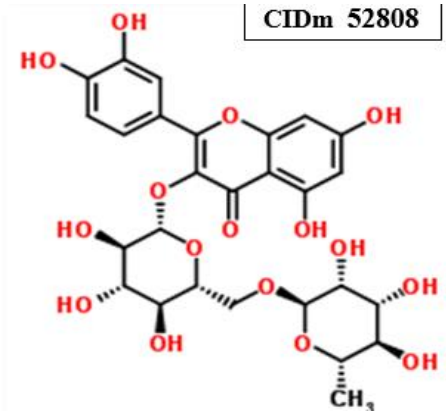


**Fig 6:** Structure of Rutin compounds

(2D structure) (2 D structure)

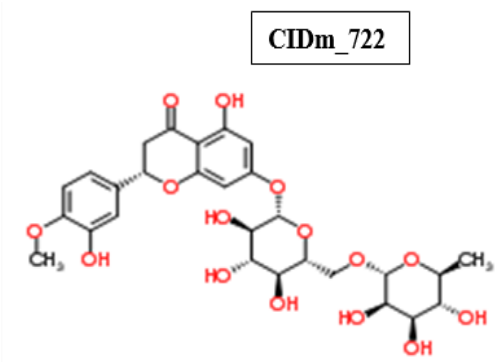


**Fig 7:** Structure of Hesperitin compounds (2D structure)

**Table 2:** Structure of Desired Compounds

| Sr. No. | Name of the compound | Molecule code (2D) |
|---|---|---|
| 1. | Hesperitin | 72281.1 |
| 2. | Quercetin | 5280343.1 |
| 3. | Rutin | 5280805.1 |

**Protein Structure**

**Fig 8:** HSP 70 Protein Molecular Models Binding Proteins HSP 70 ATPASE 41O8 - Rutin (5280805)

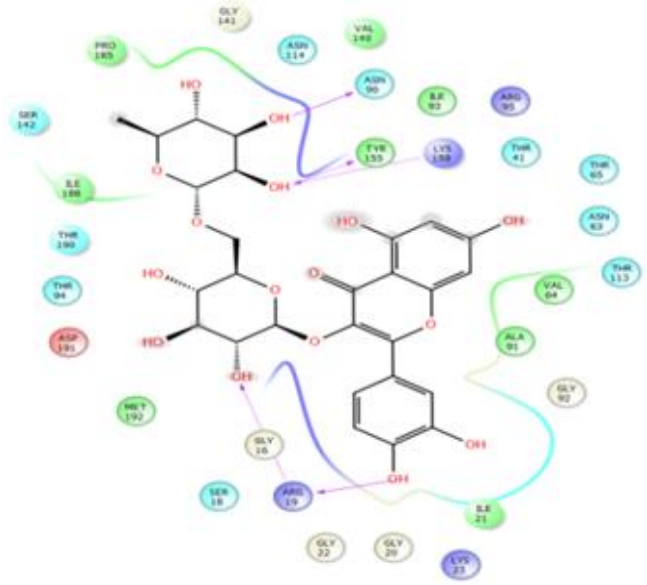

**Fig 9:** 2D – Representation

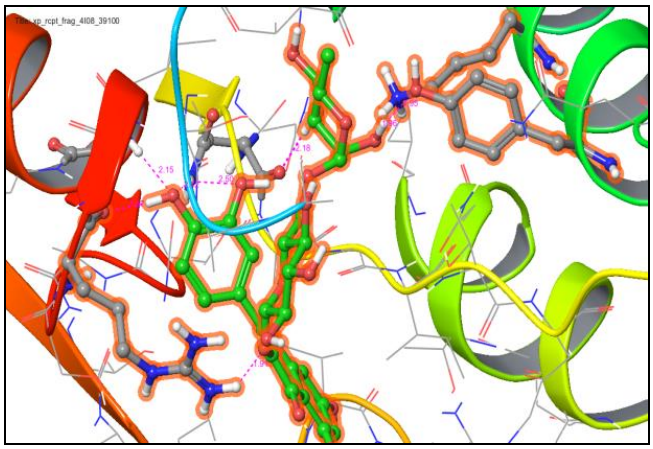

**Fig 10:** 3D- Dimensional Representation

HSP 70 ATPASE 41O8 - Quercetin (5280343)

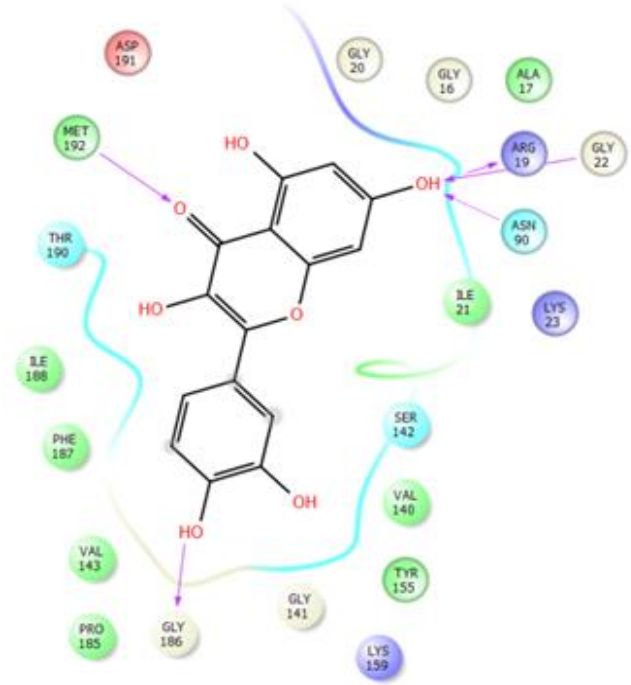

**Fig 11:** 2D Representation

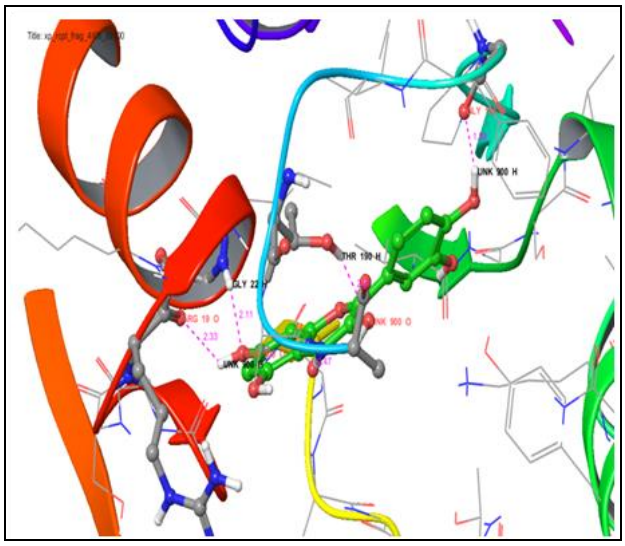

**Fig 12:** 3D Representation

HSP 70 ATPASE 41O8 - Hesperitin (72281)

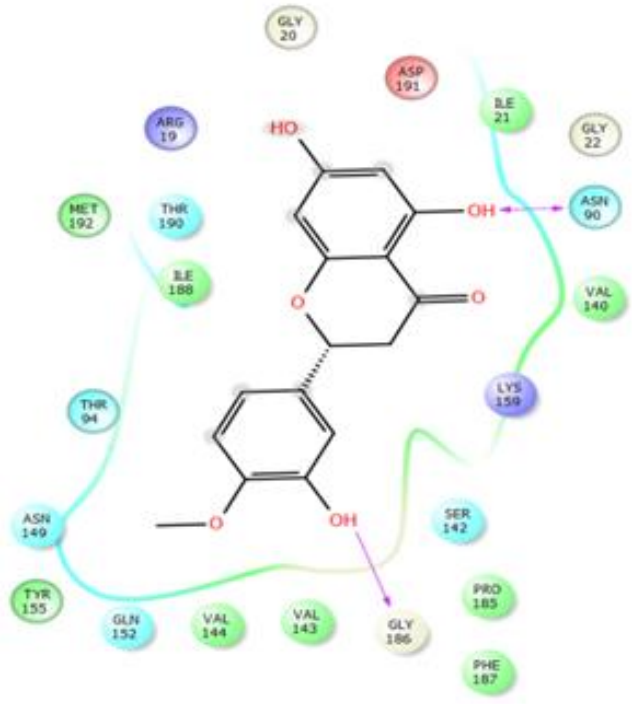

**Fig 13:** 2D Representation

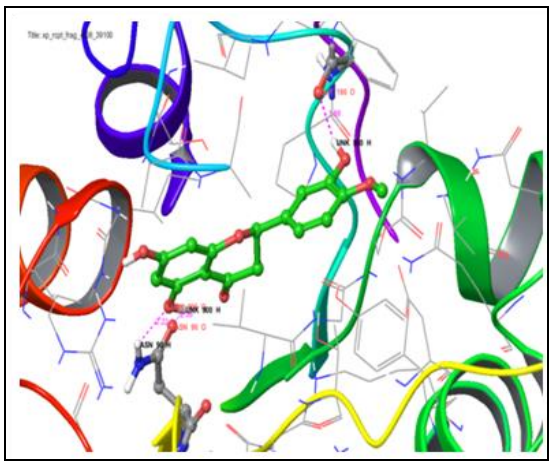

**Fig 14:** 3D Representation

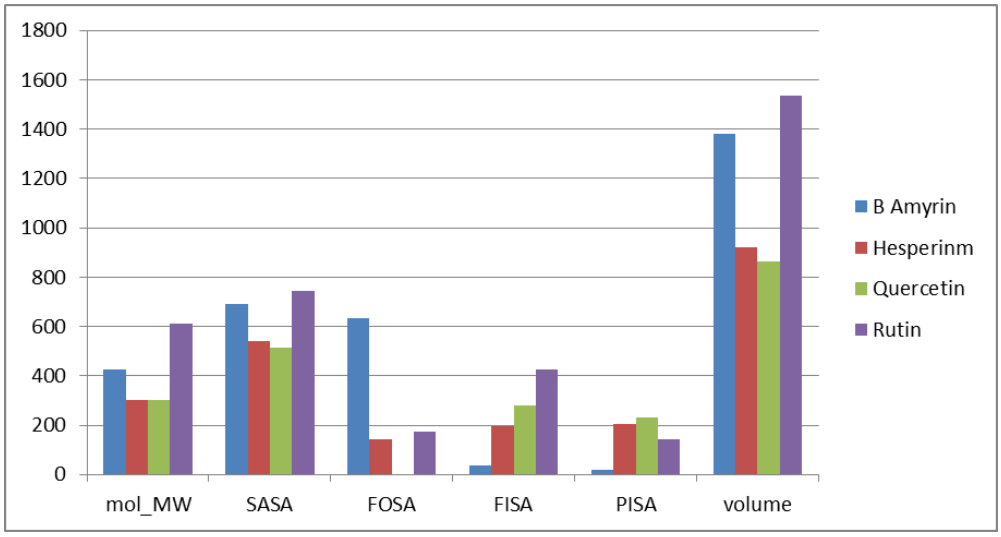


Fig 15: Toxicity Prediction

**Table 3:** Toxicity Qikprop Results

| zLigand | mol.MW | SASA | FOSA | FISA | PISA | Volume |
|---|---|---|---|---|---|---|
| B - amyrin | 426.724 | 690.833 | 634.767 | 36.751 | 19.315 | 1381.075 |
| Hesperitin | 302.283 | 541.774 | 141.243 | 197.701 | 202.839 | 19.955 |
| Quercetin | 302.24 | 512.193 | 0 | 281.814 | 230.38 | 861.42 |
| Rutin | 610.524 | 742.66 | 173.321 | 425.554 | 143.786 | 1535.939 |

Toxicity Prediction graph (Fig. 15) β- amyrin, hesperitin (Middle), quercetin(low) and rutin (High) were recorded. Toxicity qikprop (Table 3) result β- amyrin (sasa 690), hesperitin (sasa 541.774), Quercetin (sasa 512), Rutin (sasa 742).

**Table 4:** Interaction Profiled of Interacted Complex

| Molecule | Residues | Protein Atom | Ligand Atom | Bond Length | Total No of Bond | Extract | Remarks |
|---|---|---|---|---|---|---|---|
| Quercetin | AGR90 | 0 | H | 2.33 | 5 | Leaf | Interacted |
| | GLY 22 | H | 0 | 2.11 | | | |
| | MET 192 | H | 0 | 2.47 | | | |
| | THR 190 | H | 0 | 2.21 | | | |
| | GLY 186 | 0 | H | 1.89 | | | |
| Rutin | AGR19 | 0 | H | 1.87 | 7 | Leaf | Interacted |
| | GLY 22 | 0 | H | 2.15 | | | |
| | ASN90 | H | 0 | 2.42 | | | |
| | | 0 | 0 | 2.5 | | | |
| | TYR155 | 0 | H | 1.95 | | | |
| | | H | 0 | 1.96 | | | |
| | AGR 19 | H | 0 | 1.91 | | | |
| Hesperitin | ASN 90 | H | O | 2.22 | 3 | root | Interacted |
| | | O | H | 2.36 | | | |
| | GLY 186 | O | H | 1.88 | | | |

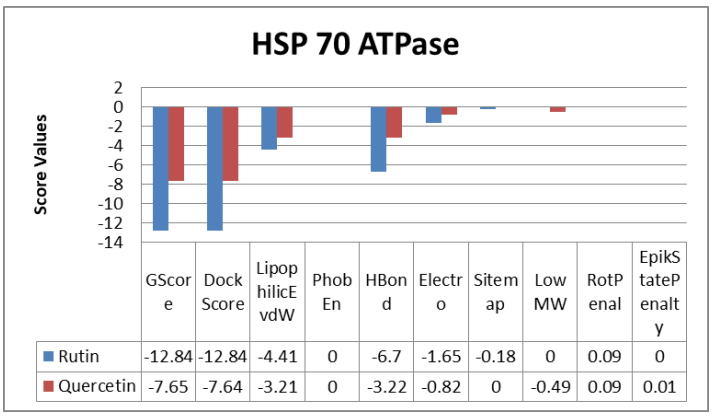


**Fig 16:** HSP 70 ATPASE

ADMET Property

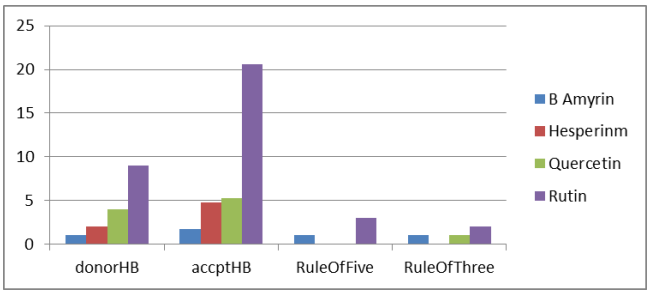


**Fig 17:** ADMET Property

ADMEt Property of the donor hydrogen bond and accept hydrogen bond (Fig.17) graph showing β- amyrin, hesperitin, quercetin and rutin molecule peak showing high, middle and low interaction value recorded the Fig 17.

## Conclusion

The development of novel compounds with biological activity is an urgent need. In the present study the HSP 70 ATPASE and Acetyl choline esterase (Ach) protein was successfully docked hesperitin, quercetin and rutin molecule. Drug interaction study to have a track in the ongoing race between drug development and new drugs especially new compounds which are more important for the discovery of new hits using molecular methods. The Fitness scores of prodigiosin and cycloprodigiosin were calculated using the Schrödinger Suite software. Hence, it is concluded that could be a potent brain attack target molecule against HSP 70 ATPASE which may be worth for further clinical trials.

## Acknowledgments

I, hereby acknowledge Dr. P. Shanmugavel, Ph. D, D.Sc., Associate Professor and Murugesh Easwaran, Senior Research Fellow(SRF), DBT Bioinformatics Centre, Bharathiar University, Coimbatore - 641 046 for providing me knowledgeable insight regarding Bioinformatics tools which were of much relevance for my research work.

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