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Coronavirus Spike (S) Glycoprotein (2019-Ncov) Targeted Siddha Medicines Kabasura Kudineer and Thonthasura Kudineer –*In silico* Evidence for Corona Viral Drug

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Abstract

COVID19 is the prime threat to the human race now. Currently the world faces Covid-19 out-break across all continents and it is characterized by its membrane proteins due to mutations. Siddha Medicine is one of the oldest medical system in the world which is believed to be originated more than 10,000 years ago which is prevalent in the ancient Tamil land. Siddha medicine classifies disease and disorders into 4448 types and has remedy for more than 64 types of fever. Among these Kabasura Kudineer and Thonthasura Kudineer are two siddha formulations used against fevers due to respiratory infections. The present study was carried out to evaluate these two formulations against COVID 19 using *in silico* docking methods. For that the active principles/phytocompounds from the ingredients of the formulations were docked against coronavirus spike glycoprotein trimmer (PDB ID: 3JCL) using iGEMDOCK software. 10 phytocompounds showed promising activity against COVID spike glycoprotein. This study showed that 10 phytocompounds which act as ligands to bind with viral proteins to prevent the binding of host receptors. Of these Cucurbitacin B (-112.09), Cardiofoliolide (-111.5), Apigenin (-98.84) and Pyrethrin (-92.98) were observed as more effective with less bind energies required for binding with spike proteins to prevent the fusion lead viral replication. Since Kabasura Kudineer contains more active phyto constituents, the higher activity was observed than Thontha sura Kudineer. The study demonstrated that Kabasura Kudineer could be a potential siddha medicine for COVID 19 provided further preclinical and clinical confirmatory studies.

Keywords: COVID19, Kabasura Kudineer, Siddha Medicines, Thonthasura Kudineer

1. Introduction

Novel coronavirus (2019-nCoV, COVID-19) was first reported from Wuhan, China, on 31 December 2019. Though, Coronaviruses (CoV) are a large family of viruses that familiar in humans, this new type is said to be novel due to its complex envelop protein raised from multiple mutations[1]. These mutations also alter the tissue tropism and eventually harboring in to a variety of hosts by damaging host's immune response[2]. Thus they make a wide alarming threat to health concerns globally.

Corona viruses (CoV) are positive-strand RNA viruses enveloped by the specific proteins (S), called, spikes. These proteins mediate the viral entry and host specification. They have two parts, namely membrane proteins (M) and envelop proteins (E) and both of them involved in

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pathogenesis. Some of them have envelope-associated Hemagglutinin-Esterase protein (HE)[3]. The spikes give them as crown appearance and the name cause ('crown'). CoV families cause various range of illness differing from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV)[4]. They are zoonotic viruses that are transmitted between human and other animals. SARS-CoV was transmitted from civet cats to humans; MERS-CoV from dromedary camels to humans, but, 2019-nCoV firstly discovered in sea food market[5]. The symptoms include respiratory problems, fever, cough, shortness of breath and breathing difficulties are few of the common symptoms of 2019-nCoV infection. In more severe cases, they can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death (37%). Due to its rapid spread, World Health Organization (WHO) announced medical emergency and researches are in full swing against 2019-nCoV worldwide[6].

The research analysis revealed that COVID19 exist in two forms, S and L form. " S" said to be less virulence and lesser adoptable than "L" to human receptor, angiotensin-converting enzyme (ACE2). Mutations cause the variation in fitting, i.e., L form is mutated.

The drug development is a consuming process by means of time, cost, humans and molecular techniques. The *in vitro* drug screening methods are helping the researchers for opting out the novel and optimal drug candidates against various diseases in close proximity[7]. Nilavembu Kudineer was prominently used against different viral infections on their out bursting during past several years. In this present study Kabasura Kudineer chooranam and Thonthasura Kudineer are the polyherbal siddha formulations that are used to treat different types of fevers irrespective of their seasons. This pilot *insilico* study aims to evaluate their activity against infection caused by COVID19.

2. Materials and Methods

2.1 Siddha Formulations

The Siddha classical formulations Kabasura kudineer chooranam (15 ingredients of herbs *Zingiber officinale, Piper longum, Syzygium aromaticum, Tragia involucrate, Anacyclus pyrethrum, Hygrophilla auriculata, Terminalia chebula, Adathoda vasica, Coleus amboinicus, Saussurea lappa, Tinospora cordifolia, Clerodendrum serratum, Andrographis paniculata, Sida acuta, Cyperus rotundus)* and Thonthasura Kudineer Chooranam (10 ingredients of herbs *Zingiber officinale, Adathoda vasica, Andrographis paniculata, Tinospora cordifolia, Ellettaria cardamomum, Solanum xanthocarpum, Trichosanthes cucumerina, Tephrosia purpuria, Mollugo cerviana, Vitis vinifera)* were selected for *in silico* docking analysis (Table 1&2). The active phytomarkers zingiberene, Vasicine, Cordifolioside B, Cucurbitacin B, Andrographolide, Apigenin, Carvacol, Costunolide, Pyrethrin, Eugenol were subjected to evaluate the interaction with Corona virus spike glycoprotein trimer 3JCL using iGEMDOCK software (version 2.0).

Table 1. Ingredients of Kabasura kudineer

Sl. No.	Tamil Name	Botanical Name	Major active Phytocompound
1.	Chukku	*Zingiber officinale*	Zingiberene
2.	Thippili	*Piper longum*	Piperine
3.	Kirambu	*Syzygium aromaticum*	Eugenol
4.	Sirukanchori	*Tragia involucrate*	Costunolide
5.	Akkirakaram	*Anacyclus pyrethrum*	Pyrethrin
6.	Mulliver	*Hygrophilla auriculata*	Apigenin
7.	Kadukkaithol	*Terminalia chebula*	Chebulic acid
8.	Adathodai	*Adathoda vasica*	Vasicine

9.	Karpuravalli	*Coleus amboinicus*	Myrtenol
10.	Kostam	*Saussurea lappa*	Costunolide
11.	Seendhil	*Tinospora cordifolia*	Cordifolioside B
12.	Siruthekku	*Clerodendrum serratum*	Carvacol
13.	Nilavmebu	*Andrographis paniculata*	Andrographalide
14.	Vattathiruppi	*Sida acuta*	Carvacol
15.	Korai kizhangu	*Cyperus rotundus*	Amentoflavone

Table 2. Ingredients of Thonthasura kudineer

Sl. No.	Tamil Name	Botanical Name	Major active Phyto compound
1.	Chukku	*Zingiber officinale*	Zingiberene
2.	Adathoda	*Adathoda vasica*	Vasicine
3.	Nilavembu	*Andrographis paniculata*	Andrographalide
4.	Seenthil	*Tinospora cordifolia*	Cordifolioside A
5.	Elam	*Ellettaria cardamomum*	Eugenol
6.	Kandangathri	*Solanum xanthocarpum*	Stigmasterol
7.	Peipudal	*Trichosanthes cucumerina*	Cucurbitacin-B
8.	Mutkavelai	*Tephrosia purpuria*	Stigmasterol
9.	Parpadgam	*Mollugo cerviana*	Vitexin
10.	Thrakshai	*Vitis vinifera*	Vitexin

2.2 Target Protein

CoV spike protein, coronavirus spike glycoprotein trimer (PDB ID: 3JCL) was obtained from PDB and used for the study.

2.3 Preparation of Ligand

The major compounds present in the ingredients of Kabasura Kudineer Chooranam (Table 1&2) and Thonthasura Kudineer Chooranam was selected and their SDF obtained from Pubchem database. The SDF files were converted into PDB file format using OPEN BABEL software.

2.4 Protein-Ligand Docking

The initial rough docking was performed in iGEMDOCK software (version 2.0) with a population size of 150 and 70 generations, set as default.

3. Results and Discussion

The core theme of the Structure based Drug Discovery (SBDD) from bioactive small molecules is based on the efficient binding energy of the docked complex. In the present study, two active compounds of siddha formulations were evaluated against corona viral membrane protein for their inhibiting ability of the active protein. CoV spike protein contained 1324 residues and principally determines the host range. It contains chain A, B and C (Figure 1). The subunit S1, recognize the host cell receptor and S2 mediate the membrane fusion. Inhibiting the spike protein is an efficient drug targeting method in corona viral therapy[8].

The results of the protein ligand docking were shown in (Table 1&2). The active phyto compounds Cordiofoliside B, Andrographolide, Vasicine were found in both Kabasura Kudineer and Thonthasura Kudineer whereas Apigenin and Pyrethrin present only in Kabasura

Kudineer. At the same time Cucurbitacin B found in *Trichosanthes cucumerina* of Thonthasura Kudineer. Both of them have four plants in common (*Z. officinalius, J. adathoda, A. paniculata* and *T. cordifolia*. The similarity in formulations had showed the significances of those plants against viral infections. The curious analysis of the chemical structures of those active molecules from different plants had showed that those compounds are

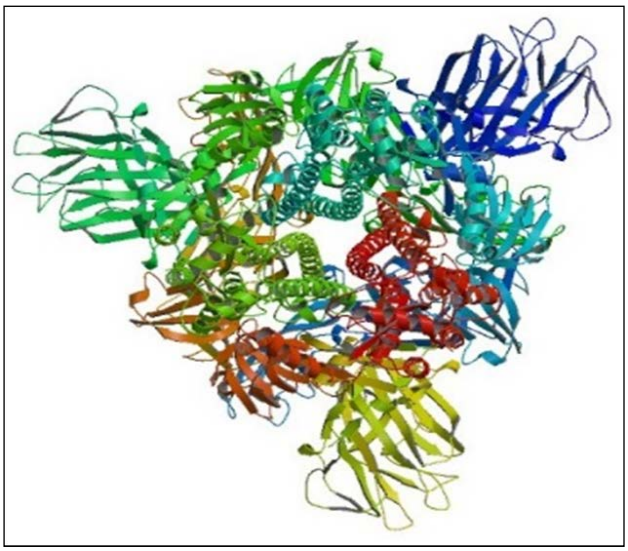

Figure 1. Coronavirus spike glycoprotein trimer 3JCL.

of with different derivatives of same mother compounds (Figure 1).

The prominent bioactive molecules from those plants were tabulated and their physiochemical properties were retrieved from public databases (https://pubchem.ncbi.nlm.nih.gov). On virtual screening, it was known that those compounds bound at different binding pockets of the viral proteins. The individual binding energy and the amino acids that contributed the docking were given in (Table 1).

In our study, the Cucurbitacin Bbonded with GLY794, SER795 and Cardiofoliolide B with PRO695, CYS697, GLN824 Table 4 on glycoprotein region which may inhibit the actual conformational change and inhibit the viral population bursting by exhibiting more efficient and stronger hydrogen bonds with active sites followed by other ligands Apiginin, Pyrethrin, Andrographolide, Vasicine, Carvacol, Eugenol and zingiberene.

The lesser energy shows the greater possibility of the drug candidates. iGEMDOCk was used to find out the docking score in this study. The energy values obtained by iGEMDOCK of the drug targets showed that Cucurbitacin B (-112.9), Cardiofoliolide B (-111.5), Apigenin (-98.84), Pyrethrin (-92.98) and Vasicine (-82.98) were showed higher docking energy against the viral protein. These results could be attributed to previous studies[9,10].

The present study confirmed that the active molecules of the respective medicinal plants *Trichosanthes cucumerina*, *Tinospora cordifolia*, *Hygrophilla auriculata*, *Anacyclus pyrethrum Andrographis paniculata*, *Adathoda vasica*, *Saussurea lappa*, *Clerodendrum serratum*, *Syzygium aromaticum*, *Zingiber officinale*, might inhibit the viral

Table 3. Docking study between Drug targets with Ligands (Phytocompounds of Kabasura kudineer and Thonthasura Kudineer using iGEMDOCK software

Sl. No.	Drug targets or protein with ligand	Rough docking energy values with iGEMDOCK				
		Total energy (Kcal/ mol)	V.D.W. (Kcal/ mol)	H.Bond (Kcal/ mol)	Electro-static (Kcal/ mol)	Aver Con pair (Kcal/ mol)
1	Zingiberene	-63.03	-63.03	0	0	26.33
2	Vasicine	-82.98	-76.32	-5.66	0	0
3	Cordifolioside B	-111.5	-84.34	-27.15	0	0
4	Cucurbitacin B	-112.9	-93.12	-31.34	0	0
5	Andrographolide	-89.34	-68.45	-21.23	0	0
6	Apigenin	-98.84	-81.45	-17.39	0	0
7	Carvacol	-72.15	-60.22	-11.93	0	0
8	Costunolide	-74.95	-66.97	-7.98	0	0
9	Pyrethrin	-92.98	-86.32	-6.66	0	0
10	Eugenol	-69.78	-565.78	-13	0	0

Table 4. Results of rough docking and accurate docking performed with a software iGEMDOCK between the drug targets with ligands (major compounds) present in the Kabasura Kudineer & Thonthasura Kudineer

Sl. No.	Plant	Common Name	Compound	Binding domain	Name of the Amino acid
1	*Andrographis paniculata*	Nilavembu	Andrographolide	H-S	ASN 783, ASN 910
				V-M	PHE 909
				V-S	PHE 909, ASN 910,ASN 615
				H-M	GLN 1028, SER 632
2	*Hygrophila auriculata*	Mulliver	Apigenin	H-S	VAL 57, ASN 60, LYS 881, ASP 902
				V-M	TYP 58, ASP 902, LEU 903,
				V-S	TYR 58, TYR 59, ASN 60, ARG 901, LEU 903
3	*Syngium aromaticum*	Kirambu	Carvacol	H-S	ARG 637
				V-M	TYR 311, SER 635, ILE 666
				V-S	TYR 311, SER 635, ARG 637
				H-M	SER 309, TYR 311
4	*Tragia involucrate*	Sirukanchori	Costunolide	H-S	TYR 583,
				V-M	ASP 782,
				V-S	GLN 1028, GLY 590, ASN 615
				H-M	LEU 616,
5	*Syngium aromaticum*	Kirambu	Eugenol	H-S	ARG 637
				V-M	TYR 311
				V-S	TYR 634, ARG 787
				H-M	SER 309
6	*Anacyclus pyrethrum*	Akkirakaram	Pyrethrin	H-S	GLN 404, HIS 428
				V-M	GLN 404, LEU 405
				V-S	GLN 404, LEU 405, HIS 405, ASN 407
7	*Adathoda vasica*	Adhathodai	Vasicine	H-S	GLN 404, LEU 405,
				V-M	GLN 404, HIS 428
				V-S	TYR 311
				H-M	GLY 590, ASN 615
8	*Tinospora cordifolia*	Seendhil	Cardiofoliolide B	H-M	PRO695, CYS697, GLN824
				H-S	ARG700, ASN1091,
				V-M	PRO695, ILE1090, ASN1091, GLN824
				V-S	HIS716, ILE1090, GLN824
9	*Trichosanthes cucumerina*	Peipudal	Cucurbitacin B	H-M	GLY794, SER795
				V-S	LEU64, GLN 267, GLU 307
				H-S	ASN801, GLN 267
10	*Zingiber officialis*	Chukku	Zingiberene	V-S	ASN 783, GLN 1028, ARG 1050, ASN 615
				V-M	SER 1026, 1027

pathogenesis at various levels spanning from prevention to cure. Apigenin 12 amino acids in spike protein Eugenol showed lowest number of interaction (5 amino acids) among the compounds followed by costunolide. Most of the compounds (pyrethrin and apigenin etc.) present in the formulations showed interaction with the different regions of the same amino acids (V-M, VS, H-S and H-M types).

Zingiberene bind to six different amino acids present in various regions of the spike protein. Out of the all of the compounds in Kabasura Kudineer, Zingeberene, Andrographalide, Vasicine, Cardiofoliate B and Cordiofolioside A showed interactions with CH regions. Zingiberene and andrographalide are present in both of the formulations, the present *in silico* analysis revealed that Zingiberene binds to four different amino acids in central helix (CH) region of the S2 subunit whereas Andrographalide bind to GLN 1028 on CH region. The CH region is an essential component in membrane fusion during the viral entry[11]. Their cooperative binding might interact with five amino acids in CH region. These results showed that Kabasura Kudineer chooranam could be a potential candidate in prevention of viral infection. Except, Zingiberene and Andrographalide, other compounds present in Thonthasura Kudineer showed no interactions with amino acids present in CH regions.

The other compounds mainly interacted with S2 subunit (amino acid 755 – 1000). Apigenin, interacted with Lys881, Arg901, Asp902 and Leu903 present in the fusion peptide region. These four interactions could be a potential drug candidature for future studies. All other compounds interacted with S1 subunit of the spike protein (Figure 2).

The mechanism of action of the phytocompounds present in the Kabasura Kudineer siddha formulation attracting/binding multiple aminoacids at different sites of viral proteins which corroborated with the well-known malarial drug, artimisinin[12]. This showed the synergistic activity of phytocompounds not only against the viral proteins which also modulate the immune system for fighting against the viral replication. The present study confirmed that the active molecules of the respective medicinal plants *Trichosanthes cucumerina*, *Tinospora cordifolia*, *Hygrophilla auriculata*, *Anacyclus pyrethrum Andrographis paniculata*, *Adathoda vasica*, *Saussurea lappa*, *Clerodendrum serratum*, *Syzygium aromaticum Zingiber officinale*, might inhibit the viral pathogenesis at various levels spanning from prevention to cure. The present study revealed that Kabasura Kudineer Chooranam and Thonthasura Kudineer Chooranam would be the functionally significant formulations against corona viral protein it was demonstrated that Kabasura

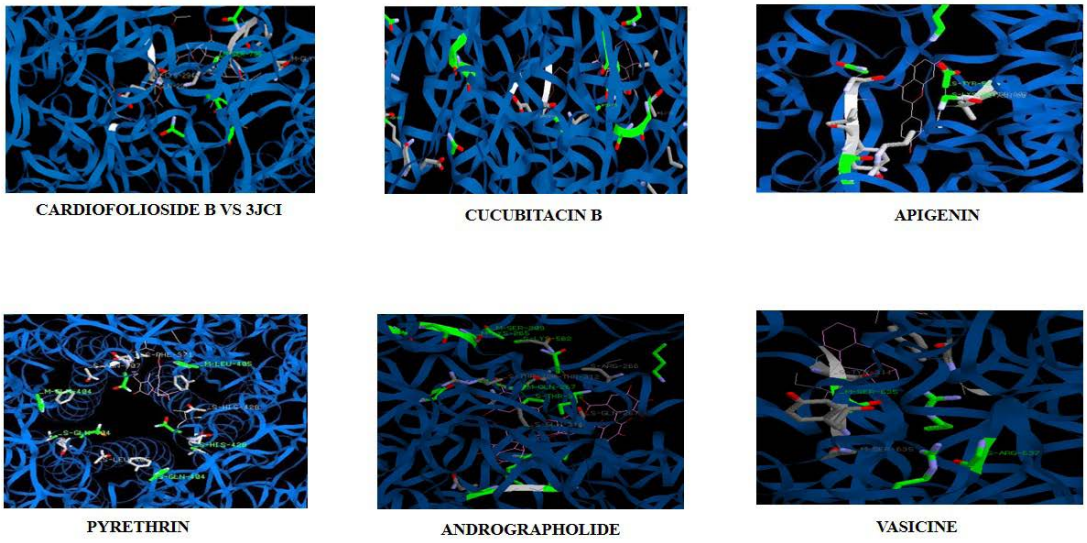

Figure 2. Binding of effective phyto active marker compounds from KSK & TSK on coronavirus spike glycoprotein trimer 3JCL.

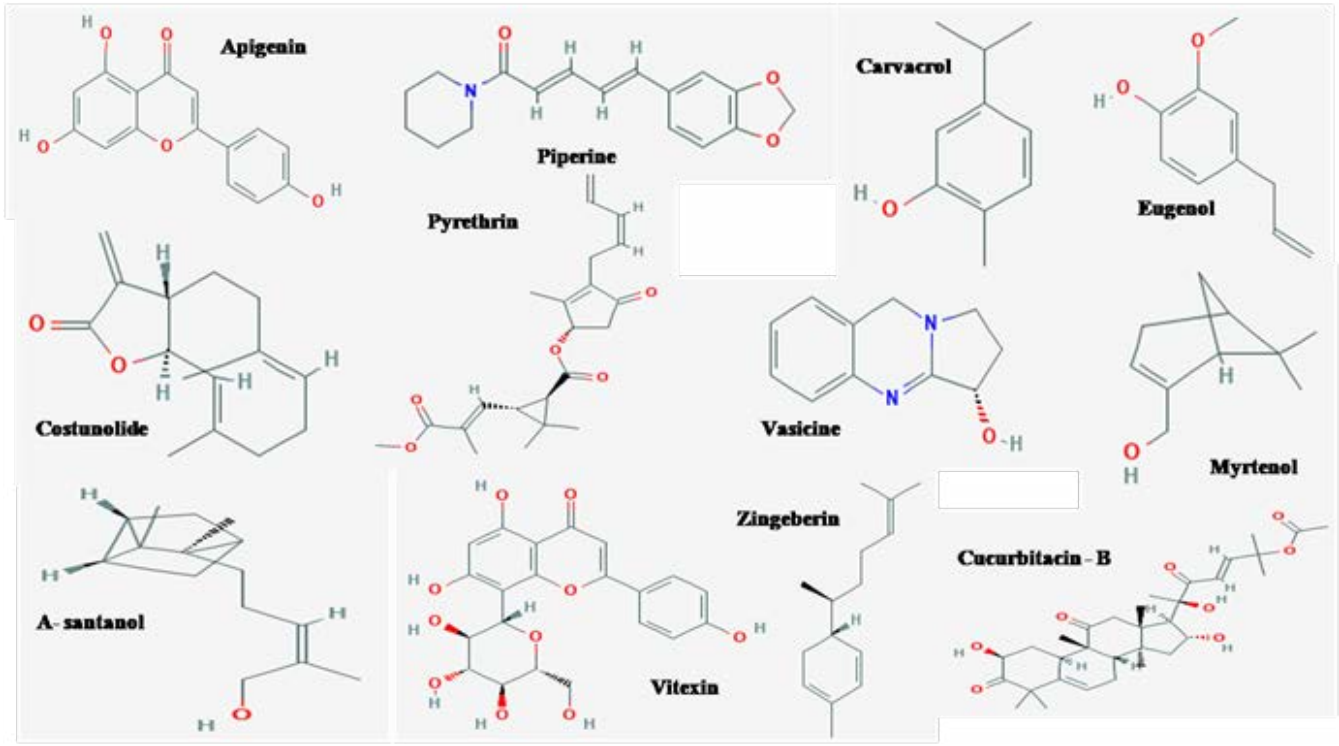

Figure 3. Structures of major bioactive principles present in the Kabasura kudineer and Thonthasura kudineer chooranan (https://pubchem.ncbi.nlm.nih.gov/)

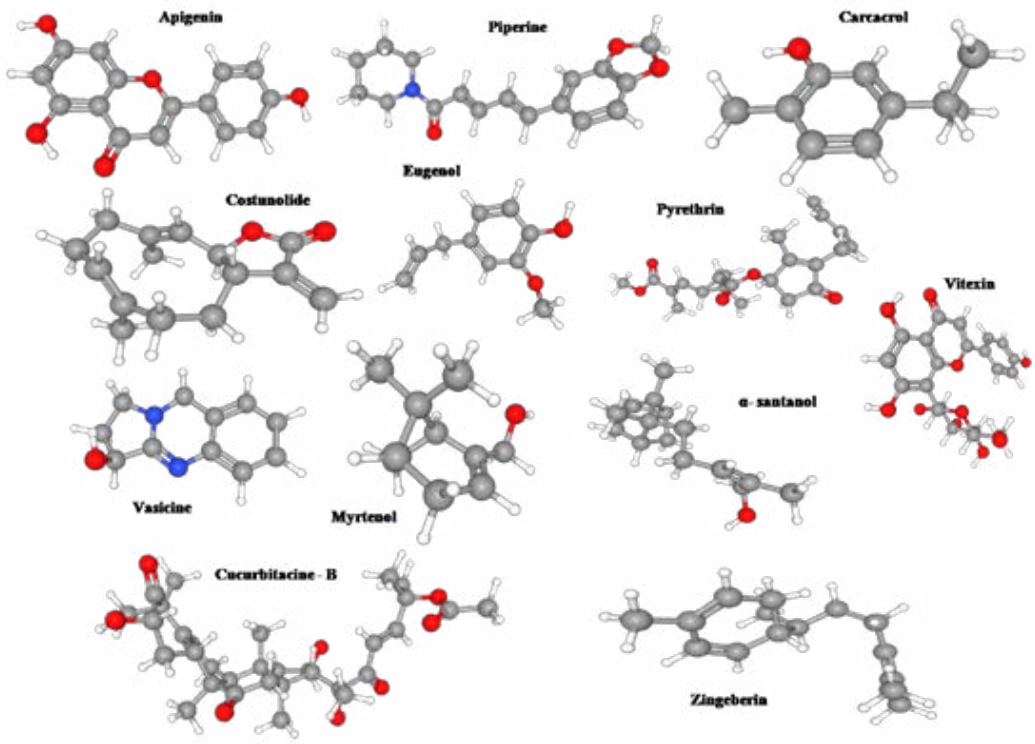

Figure 4. Three dimensional Structures of major bioactive principles present in the Kabasura kudineer and Thonthasura kudineer chooranan (https://pubchem.ncbi.nlm.nih.gov/)

Kudineer Chooranam showed more efficient inhibitory effect against viral replication.

4. Conclusion

The present study demonstrated the efficacy of siddha medicines Kabasura Kudineer and Thonthasura Kudineeer against COVID 19 infection. The phytocompounds showed promising activity against the viral spike glycoprotein. Since more effective phyto compounds present in Kabasura Kudineer which prevent the spike proteins to bind with host cell receptor, the activity was higher than Thonthasura Kudineer. Further preclinical and clinical pharmacology studies are required to develop Kabasura Kudineer as a potential siddha drug against COVID 19.

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