



## Bioactive compounds of *Poondu then* as potential inhibitors of main protease of SARS-Co-V2: A computational approach

P Saravana Siva<sup>1\*</sup>, N Mohaideen Jameela<sup>2</sup>, R Kaniyamuthan<sup>3</sup>, R Subhashri<sup>4</sup>, SM Vahitha BI<sup>5</sup>, K Sivasankari<sup>6</sup>

<sup>1</sup> Siddha Consultant, Agara Super speciality Siddha Clinic, Thirumullaivoiyil, Chennai, Tamil Nadu, India

<sup>2</sup> Siddha Consultant, Chennai Tamil Nadu, India

<sup>3</sup> PG Scholar, Department of Puramaruthuvam, Government Siddha medical College, Chennai, Tamil Nadu, India

<sup>4</sup> Siddha Medical Practitioner, TNSMC, Vaikakaran Street, Vepperry, Chennai, Tamil Nadu, India

<sup>5</sup> Siddha Medical Practitioner, Bismillah Nagar, Vettuvankeni, Chennai, Tamil Nadu, India

<sup>6</sup> Freelancer, San Jose, California, United states of America

### Abstract

The reported high virulence of COVID-19 and its increasing mortality worldwide as of late has necessitated the researchers to discover and introduce antiviral drugs against COVID-19. Since ancient times, plants and bioactive compounds have played an important role in the development of several clinically useful therapeutic agents. The results of various researches have suggested that protease inhibitors can be very effective in controlling virus-induced infection. The aim of this molecular docking study of the phytochemicals of *Poondu then* (PT) to identify the potential inhibitors of main protease of SARS-Co-V2. In the present study, phytochemicals were retrieved and then Molecular Docking was performed using Autodock 4.0.1. The results showed that the compounds Rosmarinic acid, Rutin, S-Allyl-L-cysteine, Pyrogallol served as inhibitors of protease of SARS-Co-V2. Among them the phyto compound of honey (*Then*) Rosmarinic acid had the highest binding affinity of -7.9 cal/mol when compared to the other 11 compound used in the study. The phyto compound had a good lipinski rule of five and had a better bioavailability of 0.56 and other properties. Hence it is noteworthy to conclude that Rosmarinic acid along with Rutin, S-Allyl-L-cysteine, Pyrogallol that are Phytochemicals of Garlic (*Poondu*) can and can be considered as potential candidates for further *in vitro* and *in vivo* studies.

**Keywords:** coronavirus, COVID-19, siddha, *Poondu then*, bioinformatic, garlic honey, docking, potential molecules

### Introduction

The 2019-novel coronavirus (nCoV) has been a significant source of global disaster in the 21<sup>st</sup> century. These viruses are either pleomorphic or spherical in shape and it is characterized by club-shaped projections of glycoproteins on its surface. The most important structural proteins of CoV are spike (S) protein (trimeric), membrane (M) protein, envelop (E) protein, and the nucleocapsid (N) protein. It is covered by an envelope like structure and has a single-stranded RNA genome [1]. CoV has one among the largest RNA genome among all RNA Viruses [2]. Three among the seven different strains of CoV, (SARS-CoV, MERS-CoV, and 2019-nCoV) have proved to be highly pathogenic. The 2019-novel coronavirus (nCoV), has been a cause of both lower and upper respiratory tract respiratory tract infections resulting in common cold, bronchiolitis, rhinitis, pharyngitis to pneumonia, and other system symptoms such as occasional watery and diarrhea [3].

The entry of virus into the host through droplet infection and close person to person contact is followed by establishing a cycle of infection and replication by binding to the primary target cells with the help of spike protein–host cell protein interaction (angiotensin converting enzyme-2 [ACE-2] [4]. The target cells of infection and replication are enterocytes, pneumocytes, epithelial renal tubules, tubular epithelial cells of kidney, immune cells, and cerebral neuronal cells [3]. The SARS-CoV genome encodes

a number of proteins. The replicase gene, which is a major component of the CoV genome encoded for 16 non-structural proteins NSPs in the form of two large PPs (PP1a and PP1ab). Two types of cysteine proteases act on these PPs to release the non-structural proteins (NSPs). The C-terminal end of these PPs is cleaved by chymotrypsin-like cysteine protease (main protease [M<sup>pro</sup>] or 3C-like protease [3CL<sup>pro</sup>] and the N-terminal end is processed by the M<sup>pro</sup> (also known as papain-like protease [PL<sup>pro</sup>] [5]. Currently, there is no specific antiviral drug for the treatment of CoV-associated pathologies. These proteins represent as important targets from drug discovery side [6]. As there is no well-defined therapy available, which specifically targets CoV, in this study, we have docked the possible protein structures which could be potential targets for the development of a therapeutic approach for the treatment of CoV with phytochemicals of *Poondu then* (Garlic Honey) a safe *Siddha* formulation for all ages. The selection of study drug for molecular docking was based on previous research works on the antiviral activity of its ingredients Garlic and Honey.

### Study drug Preparation and Indication

*Poondu Then* (PT) is a simple Siddha herbal formulation prepared by crushed Garlic (*Poondu*) taken in a clean cloth and the extract can be obtained through manual pressure by exposing to heat. The garlic extracted mixed with equal

amount of honey (*Then*) is known as *Poondu Then* (PT) [7]. This herbal remedy indicated in Siddha literature is a simple and cost effective home remedy that can be easily prepared and stored by common people. Both the ingredients have been scientifically explored individually by various researchers and have been perceived to be beneficial medicinally. Besides this, the folklore of South Indian culture has been using garlic and honey over centuries from pediatric to geriatric upper respiratory infections like sore throat, tonsillitis etc.

#### Garlic (*Allium sativum* L.)

Garlic (*Allium sativum* L.) has played a significant role in diet and medicine throughout the history. It has acquired a reputation in as a prophylactic as well as therapeutic medicinal plant in traditional medicines. Studies have reported that garlic extract has various phytochemicals like Allicin, diallyl trisulfide and ajoene and possess *in vitro* Antiviral activity against influenza A and B, cytomegalovirus, rhinovirus, HIV, herpes simplex virus 1, herpes simplex virus 2, viral pneumonia, and rotavirus [8]. Fresh garlic is estimated to contain approximately 4.38 to 4.65 mg of allicin per gram of garlic; thus for one fresh clove of garlic, weighing approximately 4 g, there is approximately 17.52 to 18.60 mg of allicin which may be responsible to play a central role in the biological activity of garlic [9, 10].

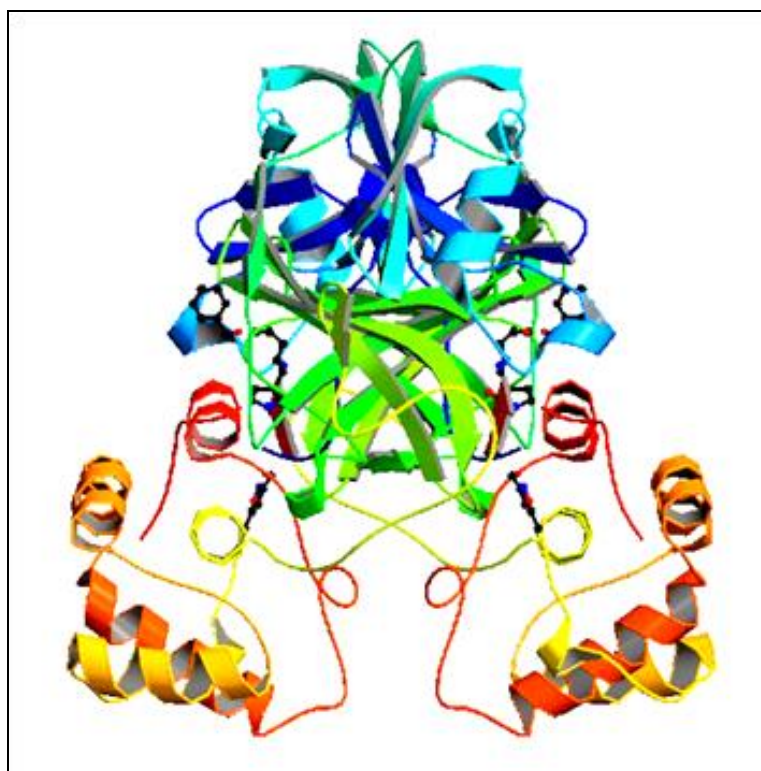
#### Honey

Honey is a natural substance appreciated for its therapeutic abilities. It is not only considered a food or a sweetener, but also used as a medicine for various pathologies. since ancient times. Its content in flavonoids and phenolic acids plays a key role on human health [11]. It has abundant flavones, flavanols, and flavonols through which it exerts antioxidant, anti-inflammatory, antimicrobial effects [12]. Honey is also able to reduce the number of inflammatory cells that are present in the fluid deriving from bronchoalveolar lavage, also inhibiting the bronchial hyperplasia of the goblet cells [13]. Honey can also be safely administered to infants and children with gastroenteritis [14]. A Research study as shown that honey has potent inhibitory activity against the influenza virus [15].

#### Materials and methods

##### Target preparation

The three dimensional structure of main protease of SARS-Co-V2 complexed with an inhibitor N3 was adapted for this study (fig. 1). The selected structure was optimized by adding the polar hydrogen atom. The macromolecule was converted into PDBQT format before performing docking.



**Fig 1:** The crystal structure of COVID-19 main protease in complex with an inhibitor N3

**Table 1:** List of phytochemicals of major components of poondu then

S.No	Major components	Key Phytochemicals
1	<i>Allium sativum</i>	Allicin, S-Allyl-L-cysteine, Pyrogallol, Rutin, Alliin, Ajoene
2	<i>Honey</i>	Pinocembrin, Pinobanksin, Chrysin, Galangin, Rosmarinic acid, Abscisic acid

#### Ligand preparation

The major component of the siddha medicine the *Poondu then* namely Honey and *Allium sativum* were taken for the study. Major phytochemicals detailed in (Table 1) were retrieved from Pubchem and all the phytochemicals were three dimensionally optimized before docking and all the phytochemicals were converted into PDBQT format for initiating docking.

### Molecular docking

Docking was performed using Auto Dock 4.0.1 and universal force field charges were added to all the atoms in the ligands. Docking calculations were performed for phytochemicals of honey and *Allium sativum* against the main protease of SARS-Co-V2. Essential hydrogen atoms and solvation parameters were added and the target protein and ligands were prepared in AutoDock. The X Y and Z coordinates were set at -25, 12 and 59 respectively. The grid was placed at 25 for X, Y and Z and autogrid program was generated. A total of 10 different docking pores were obtained for each docked molecules and the best pore was

selected based on the binding energy and the docked interaction profile were visualized using latest version of Pymol.

### Physiochemical and ADMET analysis

The physiochemical properties of all the phytochemicals used in the study was evaluated. The ADMET properties of the screened phytochemicals were obtained using the tool SwissADMET<sup>[16]</sup>. The toxicity and carcinogenicity profile of the phytochemicals were also evaluated using the tool preADMET.

**Table 2:** Physiochemical properties of phytochemicals

Phytochemicals	Formula	MW KDa	Heavy atoms	Aromatic heavy atoms	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	Lipinski violations
Alliin	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub> S	162.27	9	0	5	1	0	61.58	0
S-Allyl-L-cysteine	C <sub>62</sub> H <sub>104</sub> O <sub>33</sub>	1377.47	95	0	21	33	20	524.59	3
Pyrogallol	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	9	6	0	3	3	60.69	0
RUTIN	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	610.52	43	16	6	16	10	269.43	3
Alliin	C <sub>6</sub> H <sub>11</sub> NO <sub>3</sub> S	177.22	11	0	5	4	2	99.6	0
Ajoene	C <sub>9</sub> H <sub>14</sub> O <sub>3</sub> S	234.4	13	0	8	1	0	86.88	0
Pinocembrin	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	256.25	19	12	1	4	2	66.76	0
Pinobanksin	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	272.25	20	12	1	5	3	86.99	0
Chrysin	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	254.24	19	16	1	4	2	70.67	0
Galangin	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	270.24	20	16	1	5	3	90.9	0
Rosmarinic acid	C <sub>18</sub> H <sub>16</sub> O <sub>8</sub>	360.31	26	12	7	8	5	144.52	0
Abscisic acid	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	264.32	19	0	3	4	2	74.6	0

**Table 3:** Binding energy profile of phytochemicals against main protease

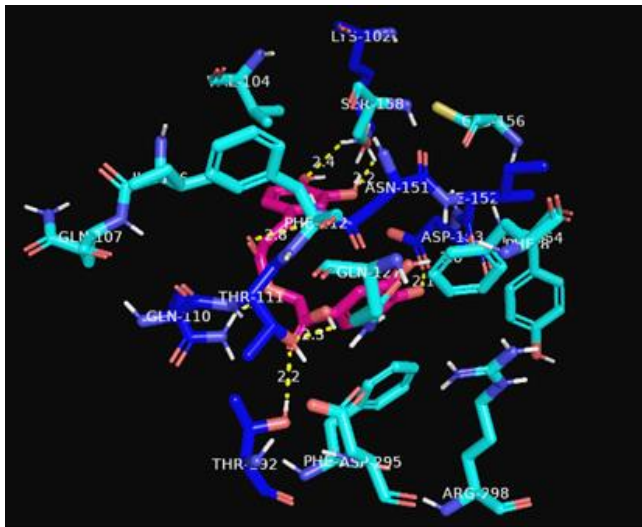
Pubchem ID	Phytochemicals	Binding energy Kcal/mol	No of HB bonds
65036	Alliin	-3.4	N
5386591	Ajoene	-4	1
87310	Alliin	-4.5	3
5281616	Galangin	-5	2
5281607	Chrysin	-5.1	2
5280896	Abscisic acid	-5.4	3
68071	Pinocembrin	-5.6	2
73202	Pinobanksin	-5.7	3
1057	Pyrogallol	-6.5	4
9793905	S-Allyl-L-cysteine	-6.8	6
5280805	Rutin	-7	7
5281792	Rosmarinic acid	-7.9	9

**Table 4:** Best ranked ligands against main protease of sars-CO-V2

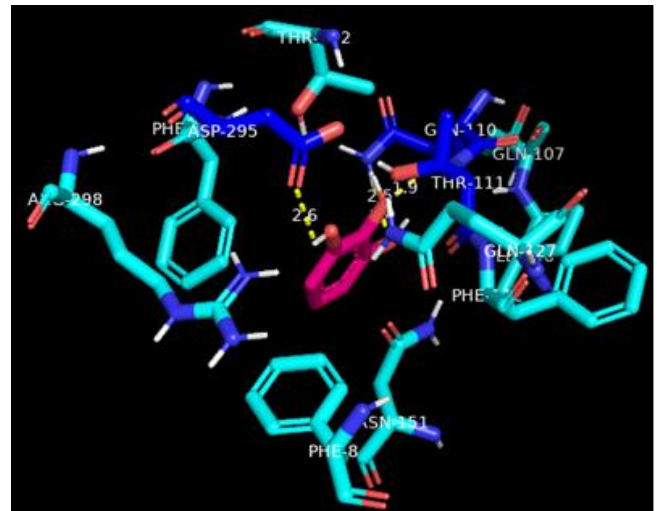
Phytochemicals	No of HB Interactions	Binding energy Kcal/mol	Amino acid Involved
Rosmarinic acid	9	-7.9	Lys102, Asp153, Ile152, Thr111, Asn151, Thr292, Gln110
Rutin	7	-7	Gly138, Glu288, Lys137, Gln127, Glu290
S-Allyl-L-cysteine	6	-6.8	Thr111, Gln110, Asn151, Thr292
Pyrogallol	4	-6.5	Gln110, Thr111, Asp295

**Table 5:** Admet analysis of the best ranked ligands

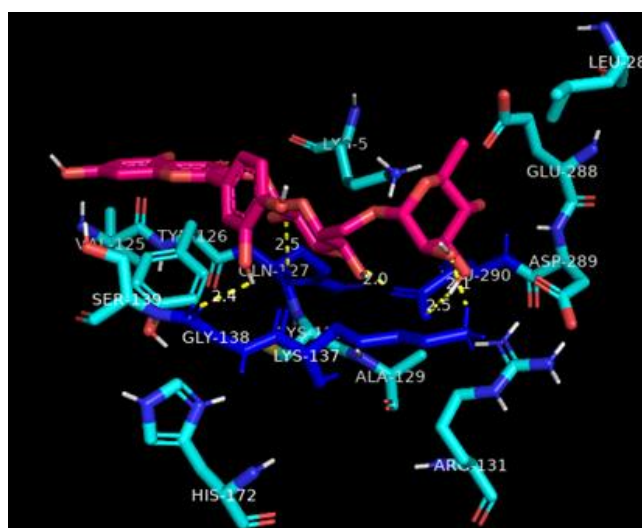
Phytochemicals	ESOL Class	GI absorption	BBB penetration	Pgp substrate	Bioavailability Score	Synthetic Accessibility
S-Allyl-L-cysteine	Moderately soluble	Low	No	Yes	0.17	10
Pyrogallol	Very soluble	High	Yes	No	0.55	1
RUTIN	Soluble	Low	No	Yes	0.17	6.52
Rosmarinic acid	Soluble	Low	No	No	0.56	3.38



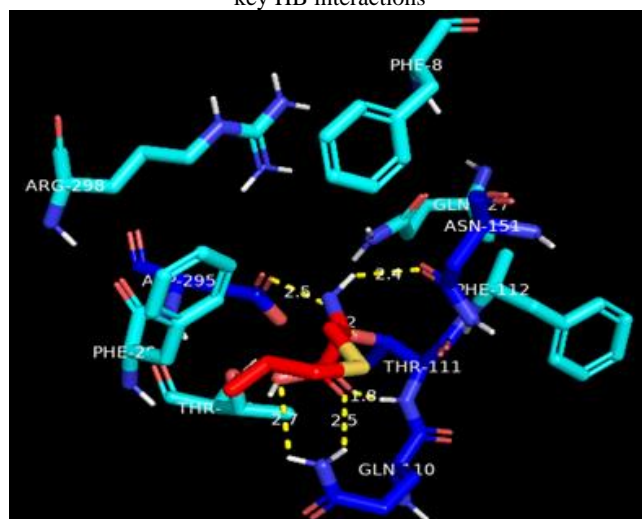
**Fig 2:** Docked Confirmation of Rosmarinic acid with the Main protease and key HB interactions



**Fig 5:** Docked Confirmation of Pyrogallol with the Main protease and key HB interactions



**Fig 3:** Docked Confirmation of Rutin with the Main protease and key HB interactions



**Fig 4:** Docked Confirmation of S-Allyl-L-cysteine with the Main protease and key HB interactions

### Result and discussion

The COVID-19 infection, a sudden outbreak in china has rapidly spread worldwide causing a massive infection and a global threat to the mankind. It has not only created panic situation but also lead to a large economic crisis. As of June 16, 2020 the number of global COVID-19 confirmed cases as reported by national authorities is 7,924,527 with the mortality of 434,367 and India has 332,424 cases and 9,520 deaths [17]. Many researchers are working on the various aspects of SARS-Co-V2 and in a war to create an effective drug. Despite of the massive infection still no appropriate drug is available. This study is a similar attempt to shed light into the use of phytochemicals as a drug of choice. In this study phytochemicals from the two medically important ingredients namely honey and *Allium sativum* has been analysed. Twelve major phytochemicals such as Allicin, S-Allyl-L-cysteine, Pyrogallol, Rutin, Alliin, Ajoene from *Allium sativum* and Pinocembrin, Pinobanksin, Chrysin, Galangin, Rosmarinic acid, Abscisic acid from honey were used detailed in Table 1. It was observed that out of the 12 compounds 10 compounds had no Lipinski rule violations expect for S-Allyl-L-cysteine and Rutin. The physicochemical properties of the phytochemicals used in the study is given in the Table 2. The potential effect of these phytochemicals to inhibit the Main protease were evaluated and tabulated in Table 3.

The best four compounds were screened based on the binding energy. The compounds namely Pyrogallol, S-Allyl-L-cysteine, Rutin and Rosmarinic acid showed the highest binding energy of -6.5 kcal/mol, -6.8 kcal/mol, -7 kcal/mol and -7.9 kcal/mol respectively. The compound Rosmarinic acid had the highest binding energy of -7.9 kcal/mol was the phyto compound isolated from honey. Rosmarinic acid is reported to have various biological activities including antioxidant [18], anti-inflammatory [19] and antimicrobial activities [20]. It is one of the major polyphenols present in herbal plants such as rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum*), oregano (*Origanum vulgare*), sage (*Salvia officinalis*), *Melissa officinalis* and many others [21].

Similar study was performed by S.K. Enmozhi *et al* in which Andragapholide had a binding energy of -3.094357 kcal/mol which is much higher than the Rosmarinic acid that showed the binding energy of -7.9 kcal/mol forming 9

significant HB interaction namely Lys102, Asp153, Ile152, Thr111, Asn151, Thr292, Gln110 that is also higher than Andragapholide with 4 HB interactions. The other three phytocompounds namely Rutin, S-Allyl-L-cysteine and Pyrogallol screened in this study has reported to have binding energy higher than the Andragapholide [22]. In a study by Aanouz *et al* the phyto compound Crocin was reported to have the highest binding energy of -8.2 kcal/mol formed various interaction but the conventional HB interactions were observed with Asn133, Thr135, Thr199, Arg131, Lys137, Lys5, Arg4, Phe3 and formed 7HB interaction [23]. In the present study though the binding energy was low compared to Crocin, the screened compound formed 9 potential HB interactions which is highly significant. Other compound Digitoxigenin and  $\beta$ -Eudesmol formed HB interactions with Gln110, Asp153 and Thr111 and similar interaction profile was observed with the phytocompounds of the study. The detailed interaction profile and the docking confirmation can be seen in Table 4 and fig (2-5).

The toxicity and ADME properties of the screened compounds reveal that the compounds are soluble in water followed by the compound Pyrogallol having a good gastro intestinal adsorption. Almost all the compounds are poor blood brain barrier penetrator except for the compound Pyrogallol. While looking into the bioavailability the compound Rosmarinic acid a good bioavailability and it is a known fact that the compound is a polyphenol and it is believed to have low toxicity and high beneficiary effect to human health [24]. The detailed ADME analysis for the screened phytocompounds is given in Table 5.

### Conclusion

It is interesting to note that the phytocompounds of both *Allium sativum* and Honey showed a significant binding energy and hydrogen bond interactions with the main protease of SARC-CoV2. Rosmarinic acid that exhibited the highest binding energy of -7.9 kcal/mol is one of the major phyto compound of Honey and a polyphenol. Similarly other potential features of Rosmarinic acid was that it exhibited 9 hydrogen bond interactions with no blood brain barrier penetration and is not a substrate of P- glycoprotein and had a bioavailability of 0.56. It is an obvious fact that phytocompounds of Siddha formulation *Poondu then* has potential phytocompound such as Rosmarinic acid. The other phytocompounds namely Rutin, S-Allyl-L-cysteine and Pyrogallol such as are not only effective but also safe with least or no side effects and can also serve as good inhibitors of Main protease of SARS-Co-V2. This computational study requires further *in vitro* and *in vivo* studies to prove its efficacy.

### Acknowledgement

The author wishes to acknowledge Mrs.Indu Purushothamman, Msc (Bioinformatics), Ph D., Research scholar, Presidency college, Chennai and Dr. R. Jeeva Gladys MD(s), Lecturer, Velumailu Siddha medical college, Sriperumbudur for their kind contribution and support towards publishing this study.

### References

1. Yang H, Bartlam M, Rao Z. Drug design targeting the main protease, the Achilles' heel of coronaviruses. *Curr. Pharm. Des.*,2006;12:4573–90.

2. Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses*,2012;4:1011–3.
3. Prajapat M, Sarma P, Shekhar N *et al*. Drug targets for corona virus: A systematic review. *Indian J Pharmacol*, 2020;52:56-65.
4. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA *et al*. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*, 2003;426:450–4.
5. Lindner HA, Fotouhi-Ardakani N, Lytvyn V, Lachance P, Sulea T, Ménard R. The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. *J Virol*,2005;79:15199–208.
6. Li F. Structure, function, and evolution of coronavirus spike proteins. *Annu. Rev. Virol*,2016;3:237–61.
7. Dr. Deva Asirvatham Samuel. Marunthu sei Iyalum Kalaiyum. 2nd edition.Chennai; Directorate of Indian Medicine and Homeopathy publishers, 2009, 243.
8. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. *Avicenna. J Phytomed*,2014;4:1-14.
9. Lissiman E, Bhasale AL, Cohen M. Garlic for the common cold. *Cochrane Database Syst. Rev.* 2014; 11.
10. Nakamoto M, Kunimura K, Suzuki J and Kodera Y. Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinyl dithiin, ajoene and diallyl polysulfides (Review). *Exp. Ther. Med*,2020;19:1550-53.
11. Noori S, Faiza S, Mohammed A, Amjed A, Khelod YS, Ahmad G. Effects of natural honey on polymicrobial culture of various human pathogens. *Arch. Med. Sci*,2014;10:246–250.
12. Moniruzzaman M, Yung An C, Rao PV, Hawlader MN, Azlan SA, Sulaiman SA, Gan SH. Identification of phenolic acids and flavonoids in monofloral honey from Bangladesh by high performance liquid chromatography: Determination of antioxidant capacity. *BioMed. Res. Int.* 2014, 737490.
13. Kamaruzzaman NA, Sulaiman SA, Kaur G, Yahaya B. Inhalation of honey reduces airway inflammation and histopathological changes in a rabbit model of ovalbumin-induced chronic asthma. *BMC. Complement. Altern. Med.* 2014;14:176.
14. Haffejee IE, Moosa A. Honey in the treatment of infantile gastroenteritis. *Br. Med. J. Clin. Res*, 1985;290:1866-1867.
15. Watanabe K, Rahmasari R, Matsunaga A, Haruyama T, Kobayashi N. Anti-influenza viral effects of honey *in vitro*: potent high activity of manuka honey. *Arch. Med. Res*,2014;45:359-65.
16. Daina A, Michielin O, Zoete V. Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. rep*,2017;3:42717.
17. World Health Organization, Sout East Asia. India Situation report. [https://www.who.int/india/emergencies/coronavirus-disease-\(covid-19\)/indiasituation-report](https://www.who.int/india/emergencies/coronavirus-disease-(covid-19)/indiasituation-report)
18. Ramanauskienė K, Raudonis R, Majiene D. Rosmarinic acid and melissa officinalis extracts differently affect glioblastoma cells. *Oxid. Med. Cell. Longev*, 2016; 1564257.

19. Sanbongi C, Takano H, Osakabe N. Rosmarinic acid in perilla extract inhibits allergic inflammation induced by mite allergen, in a mouse model. *Clin. Exp. Allergy*, 2000;34:971–977
20. Moreno S, Scheyer T, Romano CS, Vojnov AA. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free. Radic. Res*, 2006;40:223–231.
21. Corral-Lugo A, Daddaoua A, Ortega A, Espinosa-Urgel M, Krell T. Rosmarinic acid is a homoserine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator. *Sci. Signal*, 2016, 9.
22. Enmozhi SK, Raja K, Sebastine I, Joseph J. Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an in silico approach. *J Biomol. Struct. Dyn*, 2020;4:1-7.
23. Aanouz I, Belhassan A, El-Khatabi K, Lakhlifi T, El-Ldrissi M, Bouachrine M. Moroccan Medicinal plants as inhibitors against SARS-CoV-2 main protease: Computational investigations. *J. Biomol. Struct. Dyn*. 2020;5:1-9.
24. Cory H, Passarelli S, Szeto J, Tamez M, Mattei J. The role of polyphenols in human health and food systems: A mini-review. *Front. Nutr*, 2018, 5.