COVID-19: An In-Silico Analysis on Potential Therapeutic Uses of Trikadu as Immune System Boosters

Rukaiya Hasani Khasamwala

BS Abdur Rahman Crescent Engineering College: B S Abdur Rahman Crescent Institute of Science & Technology

Ranjani S

BS Abdul Rahman Institute of Science and Technology: B S Abdur Rahman Crescent Institute of Science & Technology

S Hemalatha (✉ hemalatha.sls@bsauniv.ac.in)

B S Abdur Rahman Crescent Institute of Science & Technology <https://orcid.org/0000-0002-8150-7721>

Research Article

Keywords: SARS-coV-2, Immune response, Trikadu, Ginger, Pepper, Long pepper, Protein-Ligand Interactions

Posted Date: August 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-773689/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Applied Biochemistry and Biotechnology on January 6th, 2022. See the published version at <https://doi.org/10.1007/s12010-021-03793-5>.

Abstract

Corona virus pandemic outbreak also known as COVID-19 has created an imbalance in this World. With the vaccines still in the trials, Scientists have adopted the use of natural or alternative medicines which are consumed mostly as dietary supplements to boost the immune system using herbal remedies. India is famous for traditional medicinal formulations which includes, 'Trikadu' – A combination of three acrids namely *Zingiber officinale*, *Piper nigrum* and *Piper longum* which has antioxidant properties that boost our immune system hence acting as a strong preventive measure. In this study AutoDock 4.0 was used to study interaction between the phytochemicals of Trikadu with RNA Dependent polymerase protein and Enveloped protein of the virus. Analysis of the results showed that Coumarin, Coumapherine and Bisdemethoxycurcumin showed strong bonding interactions with both the proteins. We can conclude that Trikadu are the potential molecules which can be incorporated in the diet to boost the immune system as a preventive measure against the virus.

1. Introduction

The Coronavirus (COVID-19) is an infectious disease-causing respiratory ailment affecting the lungs caused by a novel strain of virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Corona viruses are a group of viruses found in many species including cattle, camel and bats which is responsible for the outbreak and respiratory illness (1). The Virus has a main presenting symptom which is pneumonia and is suspected of having originated from a local sea-food market whose source is an unknown animal most likely assumed to be bat for being responsible for the emergence and spread of the novel strain of the Coronavirus (2).

The World Health Organisation (WHO) along with the Scientists have adopted using alternative traditional medicine which has benefits and working with institutions in selecting the traditional medicines which are showing high level of efficacy in clinical trials and using it as a treatment against covid-19 (3, 4, 5).

The immune system is a strong protection against foreign pathogens and by boosting it, we can strengthen the line of defense. We can avoid the immune weakening food and instead add immune boosting food to our diet. This will avoid our body to be in a state of inflammation which further weakens our immune system. Avoid processed food, dairy, trans fat and excessive carbohydrates. Increase daily consumption of plant-based food to increase antioxidants in the body and combat oxidative stress which will boost mitochondrial and cellular functions. Spices are highly antioxidant and immune protective (6).

We have chosen 'Trikadu', for this study is a mixture of three acrids Ginger (*Zingiber officinal Roscoe*), Pepper (*Piper nigrum*) and Long Pepper (*Piper longum*) due to their extensive anti-oxidant properties as well as anti-inflammation. The active components of these phytochemicals help in boosting the immune system by minimizing the inflammation and the combination of these effective phytochemicals can be used as potential agents in combating the virus and aiding in faster recovery. Herbs are still found in 40% of the prescribed medicines from doctors and the interest in using herbal

remedies is preferred over chemical drugs due to less side effects in humans as it is in accordance with nature in its raw composition (7).

Ginger belongs to the family *Zingiberaceae* is famous for its anti-inflammatory properties and is widely used as a medicinal product that is herbal in nature and which also shares similarities with anti-inflammatory drugs that are non-steroidal in nature. Extracts of Ginger consists of a complex multicomponent mixture of biologically active components including compounds which are structurally similar like gingerols, paradols and shogaols accounts majorly for the anti-inflammatory properties(8).

Black pepper (*Piper nigrum*) is a native plant to the tropical regions of India and is also known as the "King of Spices". It is used in Ayurveda, Unani and siddha for thousands of years. According to Ayurveda, the pungency and the heating properties of the pepper help in metabolizing food as it helps in digesting it. The heat acts as a stimulant which clears congestion in the Respiratory System (9).

Long Pepper is used in the traditional system of Ayurvedic medicine. It is most commonly used to treat diarrhoea, cholera, stomachache and respiratory infections. Long pepper is closely related to *P.nigrum* and comparatively has a hot flavour. The odour is peculiar and produces a very strong pungent taste which leaves the tongue numb. The piperine content is around 3-5% in *P. longum* (10).

Coumarin (2H-1-Benzopyran-2-one) comprises phenolic substances which are found in plants. They are used in essential oils, green tea and chicory. It is a constituent of Pepper plant and is used in anti-inflammatory activity, anti-coagulant, anti-bacterial, anti-fungal, antiviral and anticancerous. They have wide pharmacological uses and can be used as a novel therapeutic target for ailments (11).

.Demethoxycurcumin and Bisdemthoxycurcumin are the ingredients of long pepper. It has potential for curing many diseases affecting the liver, Alzheimer's disease and Parkinson's. It possesses anti-inflammatory, anti-viral, antibacterial and antioxidant properties. Demethoxycurcumin and Bismethoxycurcumin give the yellow colour to the plant (12).

The Spike protein mediates the fusion of the viral and host membrane which contains a Receptor Binding Domain (RBD) that attaches itself to the cell during the entry of the Virus (13). The receptor of SARS-CoV-2 is an Angiotensin converting enzyme (ACE-2). The Spike proteins involved in the cycle of virus include 6lxt which is an enveloped protein that plays a role in budding, fusion of envelope and pathogenesis that are involved in the life cycle of virus (14). 6M71 protein is a SARS-CoV-2 is a RNA-Dependant RNA Polymerase in complex with cofactors. The RNA Dependant RNA polymerase is the central component of the viral replication and transcription machinery, it is also appeared to be the primary target of the antiviral drug remdesivir (15, 16)

Hence these two proteins are selected to perform In-silico analysis using Molecular docking with the 11 Bio-Active components (Ligands) of the three spices to study their potential therapeutic effectiveness and the scope of using it as an immune-booster in combating the virus and an effective alternative remedy with no side-effects in case of immuno-suppressed individuals too.

2. Materials And Methods

The two proteins 6LXT and 6M71 are docked with the compounds of Ginger, Pepper and Long Pepper using the softwares Marvin view, AutoDock 4.0, BIOVIA Discovery studio and Pymol for simulation and visualization (17). The Best conformational pose is decided based on the RMSD table which gives the binding energy from which the interaction with the least binding energy are selected and their conformations are observed using AutoDock which shows how many Hydrogen bonds are formed and the pose with more number of hydrogen bonds formed is the more stable one (18).

2.1. Molecular Docking

Protein and Ligand Preparation is done using AutoDock after downloading the structures from Protein Data Bank (PDB) and PubChem. Free-energy scoring function based on linear regression analysis and AMBER force field to give an RMSD table which gives different interaction sites based on their binding energies. It is used widely in blind docking and is very useful where the Binding site location is unknown. This also provides good quality of conformations of ligands and a strong correlation between the constant of inhibition and the experimental ones. The water molecules are removed from the proteins, so it does not interfere during the binding using BIOVIA Discovery Studio. Polar Hydrogen is added using AutoDock while preparing the protein. The Grid is prepared using Blind Docking and AutoGrid is run which gives the electrostatic potential maps and desolvation maps which are used by AutoDock to guide the docking process using the active site as the site of binding. AutoDock is then run which gives the RMSD Table that consists of the interactions and binding energies of the proteins and ligands based on the runs. The runs with the highest ranking and minimum binding energies are selecteAd. Visualize the interactions between the protein and ligand formed by Hydrogen bonds using BIOVIA Discovery studio.

3. Results And Discussion

The Protein structures were obtained from PDB and docked with 11 compounds of Ginger, Pepper and Long Pepper of the Trikadu mixture. The AutoDock gave the possible conformations of the ligand as well as the RMSD table which gave the binding affinities of the protein and the ligand. The conformations of each ligand were further analysed by analysing the number of hydrogen bonds formed to check the interactions with the target proteins and find out the amino acids involved in the interactions. PubChem id, Binding energy, root mean square division values and residues of target ligand molecule which interact with the proteins are given in the tables (1 and 2) below. These interactions are observed using BIOVIA Discovery studio to visualize the hydrogen bonds and the amino acids involved.

3.1 Molecular Docking Studies

Both the proteins (6lxt and 6m71) are docked with 11 Phytocompounds of the Trikadu extract which acts as an immune booster that enables the first line of defense to boost the immune system (Fig1, Fig2) .

From the Comparative analysis (Fig3) , we can observe that the binding energy of the "Coumarin"(323) compound was the lowest with the 6lxt (Enveloped protein) that is -6.44 Kcal/mol and the highest binding energy was of the interaction between (10)-Gingerol (168115-6lxt) and 6lxt (Enveloped protein) which was -3.10 Kcal/mol. Hence, Coumarin has the most stable interaction followed by Coumapherine (10131321), Demethoxycurcumin (5469424) and Bisdemethoxycurcumin (5315472) showing that they are more effective in blocking the active site of the protein by interacting with them, indirectly leading to boosting the immune system and making it immune towards the virus.

These spices have been placed in the category of functional foods which are known for their health benefits in boosting the immune system (19, 20). These phytochemicals have antioxidant properties needed and also prevent food rancidity hence making the consumption safe (21). Previous research also concludes that Ginger is a good source as an anti-oxidant and can be used as a supplement to boost the immune system (19).

From the comparative analysis done above in the Fig.4, We can see that the Binding energy of the Coumapherine (10131321) and Bisdemethoxycurcumin (5315472) are the lowest being -7.50Kcal/mol and -7.48Kcal/mol. This shows that the two ligands are highly stable and require the least binding energy to interact with the 6m71 (RNA - Dependant RNA Polymerase protein). Following them are Demethoxycurcumin (5469424) and Coumarin (323). This data tells us as to which compounds are more effective in blocking the protein and can be effective as a preventive measure against Covid-19 and can be consumed on regular levels to boost the immune system.

From the Analysis of results, we can see that the compounds Coumarin, Coumapherine and Bisdemethoxycurcumin show good binding activity and are an active component of Pepper and Ginger respectively. The Highest Binding energy is again of (10)-gingerol which means it has the least number of Hydrogen bonds formed with both the proteins of 6lxt and 6m71 and is unstable.

The phytochemicals present in pepper is an excellent source for harnessing therapeutic effects using bio-availability of the drug metabolism. The oxidative damage is protected by quenching of free radicals which in turn increases the anti-oxidants and prevents oxidative stress. It also activates the delay of procarcinogens growth by using drug metabolizing systems hence being a potential antitumor agent (22). The phytochemicals of Long Pepper (*Piper longum*) are used extensively in Ayurveda, Unani and siddha for disease related to respiratory tracts (23).

This study is performed to reduce the severity of the symptoms of Covid-19 virus by using Trikadu which acts as a natural immune booster using natural spices including Ginger (*Zingiber officinale*), Pepper (*Piper nigrum*) and Long Pepper (*Piper longum*). These spices are responsible and mainly involved in boosting the immune system by possessing strong anti-inflammatory, anti-oxidant properties which increases the immune response and acts as a second line of defense by keeping the body enriched with the required anti-oxidants. Covid-19 is linked to the diet and maintaining a healthy diet during a pandemic increases the survival of the host and keeps the body healthy and not in an inflamed state. The future scope of the study includes formulating an antioxidant drink which consists of the spices Ginger, Pepper

and Long pepper and the combination of all the three spices. This formulated powerful drink can be used in adequate dosage and consumed on a regular basis to boost the immune system and keep the body enriched with antioxidants and in an anti-inflammatory which will promote healing faster and increase the production of antibodies in blood. The future work also includes Using different spices and performing an *In-silico* analysis to find the scope of the Natural spices whose Bioavailability is high and put it to good use as a second line of defense in avoiding many health conditions and increasing the lifespan simultaneously. Hence, This In-silico analysis showed the scope of therapeutic effectiveness of Trikadu combination of three acrids Ginger, Pepper and Long Pepper in battling Covid-19 and controlling the Pandemic.

4. Conclusion

This docking study using the phytochemicals of Trikadu shows that they are more effective in blocking the protein of COVID19. Thus, Trikadu can be an effective preventive measure against COVID-19 and can be consumed on regular levels to boost the immune system by possessing strong anti-inflammatory, antioxidant and also acts as a second line of defense by keeping the body enriched with the required antioxidants. COVID-19 is linked to the diet and maintaining a healthy diet during pandemic keeps the body healthy.

Declarations

Funding

All authors are thankful to B.S. Abdur Rahman Institute of Science & Technology, Chennai for providing research facilities in school of life sciences. The authors also gratefully acknowledge the Ministry of Science and Technology, Department of Science and Technology (KIRAN Division) (GoI), New Delhi. (Ref No. DST/WOS-B/2018/1583-HFN (G)) and DST/SATYAM/COVID-19/2020/213 (G).

Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest

Availability of data and material

Data will be available on request

Code availability

Not Applicable

Ethics approval

Not Applicable

Consent to participate

Not Applicable

Consent for publication

All authors read and approved the manuscript for publication

Authors' contributions

SH conceived and designed research. SR, SRHK, SNS conducted computational experiments. All authors wrote the manuscript. All authors read and approved the manuscript.

References

1. Pal M, Berhanu G, Desalegn C, Kandi V. (2020) Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*. 2020;12(3):e7423.
2. Singhal, T. A Review of Coronavirus Disease-2019 (COVID-19). (2020) *Indian J Pediatr*. 2020;87(4):281-286.
3. Ang L, Song E, Lee HW, Lee MS. (2020) Herbal Medicine for the Treatment of Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J Clin Med*. 2020;9(5):1583.
4. Lin LT, Hsu WC, Lin CC.(2014) Antiviral natural products and herbal medicines. *J Tradit Complement Med*. 2014;4(1):24-35.
5. Kiran G, Karthik L, Shree Devi MS, Sathiyarajeswaran P, Kanakavalli K, Kumar KM, Ramesh Kumar D. (2020) In Silico computational screening of KabasuraKudineer - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein. *J Ayurveda Integr Med*. 2020;S0975-9476(20)30024-3.
6. Yashin A, Yashin Y, Xia X, Nemzer B. (2017) Antioxidant Activity of Spices and Their Impact on Human Health: A Review. *Antioxidants (Basel)*. 2017;6(3):70.
7. Ekor M. (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014;4:177.
8. Grzanna R, Lindmark L, Frondoza CG.(2005) Ginger—an herbal medicinal product with broad anti-inflammatory actions. *J Med Food*. 2005;8(2):125-132.
9. Salehi B, Zakaria ZA, Gyawali R, et al. Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications. *Molecules*. 2019;24(7):1364. *Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications. Molecules* **2019**, 24, 1364.
10. Kumar S, Kamboj J, Suman, Sharma S. (2011) Overview for various aspects of the health benefits of Piper longum linn. fruit. *J Acupunct Meridian Stud*. 2011;4(2):134-140.

11. Leyva-Jiménez FJ, Lozano-Sánchez J, Cádiz-Gurrea ML, Arráez-Román D, Segura-Carretero A. (2019) Functional Ingredients based on Nutritional Phenolics. A Case Study against Inflammation: Lippia Genus. *Nutrients*. 2019;11(7):1646.
12. Sandur SK, Pandey MK, Sung B, et al. (2007) Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. *Carcinogenesis*. 2007;28(8):1765-1773.
13. Belouzard S, Millet JK, Licitra BN, Whittaker GR. (2012) Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses*. 2012;4(6):1011-1033.
14. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. (2009) The spike protein of SARS-CoV—a target for vaccine and therapeutic development. *Nat Rev Microbiol*. 2009;7(3):226-236.
15. Gao Y, Yan L, Huang Y, et al. (2020) Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*. 2020;368(6492):779-782.
16. Chaw, S., Tai, J., Chen, S. et al. (2020) The origin and underlying driving forces of the SARS-CoV-2 outbreak. *J Biomed Sci* 27, 73 (2020).
17. Elfiky AA.(2020) Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study]. *Life Sci*. 2020;253:117592.
18. Pantsar T, Poso A. (2018) Binding Affinity via Docking: Fact and Fiction. *Molecules*. 2018;23(8):1899.
19. Mao QQ, Xu XY, Cao SY, et al. (2019) Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). *Foods*. 2019;8(6):185.
20. Serafini M, Peluso I. (2016) Functional Foods for Health: The Interrelated Antioxidant and Anti-Inflammatory Role of Fruits, Vegetables, Herbs, Spices and Cocoa in Humans. *Curr Pharm Des*. 2016;22(44):6701-6715.
21. Lee MT, Lin WC, Yu B, Lee TT. (2017) Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals - A review. *Asian-Australas J Anim Sci*. 2017;30(3):299-308.
22. Takooree H, Aumeeruddy MZ, Rengasamy KRR, Venugopala KN, Jeewon R, Zengin G, Mahomoodally MF. (2019) A systematic review on black pepper (*Piper nigrum* L.): from folk uses to pharmacological applications. *Crit Rev Food Sci Nutr*. 2019;59(sup1):S210-S243.
23. M. M. Pandey, Subha Rastogi, A. K. S. Rawat. (2013) "Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation", *Evidence-Based Complementary and Alternative Medicine*, vol. 2013,.

Tables

Table 1
Results 6lxt protein docked with 11 phytocompounds of Trikadu

S.No	Phytocompound	PubChem_ID	Binding energy	No. Of Hydrogen Bonds formed	RMSD Value	Interacting Residues
1	Gallic acid	370	-5.02	4	0.00	LYS947, SER943, ER940, LU1182, ARG1185, LYS1181, ASP1184, GLN1180
2	Kaempferol	5280863	-5.25	4	0.00	ARG1185, LYS1181, GLU1182, SER940, SER943, LYS947, ASP1184, GLN1180
3	Demethoxycurcumin	5469424	-5.89	3	0.00	ARG1185, LYS1181, GLU1182, SER940, SER939, SER943, ALA942, ASP1184, GLN1180, GLY946, ILE1183, YS947, GLY946
4	Coumarin	323	-6.44	1	0.00	ASN955
5	Coumaperine	10131321	-5.98	3	0.00	SER940, GLU1102, ARG1185, SER943, LYS947, GLN1180, ASP1184, ILE1183, GLN949, ASP950

S.No	Phytocompound	PubChem_ID	Binding energy	No. Of Hydrogen Bonds formed	RMSD Value	Interacting Residues
6	Bisdemethoxycurcumin	5315472	-5.60	5	0.00	GLN949, ASP950, GLN954, ASN953, VAL1177, VAL1176, LYS947, SER1175, ASN1178
7	Gingerol	442793	-3.79	3	0.00	ASP950, ASN953, VAL1177, GLN949, SER1175, LYS947, VAL951, GLN954, ASN1178, VAL1176
8	(10)-Gingerol	168115	-3.10	2	0.00	ARG1185, LYS1181, SER940, ASP1184, GLU1180, LYS947, VAL951, GLU1182, SER943
9	(8)-Gingerol	168114	-3.44	2	0.00	ALA942, SER943, GLY946, ASP950, VAL1177, VAL1176, ASN1178, VAL951
10	Shogaol	5281794	-5.24	3	0.00	ARG1185, GLU1182, LYS1181, SER940, SER943, ASP1184, GLN1180, LYS947, ALA942, ASN1187

S.No	Phytocompound	PubChem_ID	Binding energy	No. Of Hydrogen Bonds formed	RMSD Value	Interacting Residues
11	Paradol	94378	-5.00	3	0.00	ARG1185, GLU1182, LYS1181, SER940, SER943, ASP1184, GLN1180, LYS947, ALA942

Table 2
Results 6m71 protein docked with 11 phytochemicals of Trikadu

S.No	Phytochemicals	PubChem_ID	Binding Energy	No.of Hydrogen Bonds formed	RMSD Value	Interacting Residues
1	Gallic acid	370	-5.92	3	0.00	MET629, ASN628, PRO627, PRO677, THR462, ASN459, GLU350, ARG349, VAL315
2	Kaempferol	5280863	-6.11	4	0.00	LYS621, CYS622, PRO620, TYR619, ASP760, ARG553, ASP618, ARG624
3	Demethoxycurcumin	5469424	-6.87	3	0.00	PHE396, ASN628, PRO677, ARG457, CYS395, THR394, PRO461, THR462
4	Coumarin	323	-6.48	3	0	PRO627, ASN628, ARG349, PRO461, PRO677, ASN459, MET629, GLU350, THR462, VAL315
5	Coumapherine	10131321	-7.50	2	0.00	ASN459, THR462, ASN628, MET463, MET629, GLU350, VAL315, PRO461, PRO677

S.No	Phytocompounds	PubChem_ID	Binding Energy	No.of Hydrogen Bonds formed	RMSD Value	Interacting Residues
6	Bisdemethoxycurcumin	5315472	-7.48	5	0.00	SER682, THR556, ARG555, THR687, ASN691, SER681, ASP623, THR680, ARG624, ALA558, TYR456, LYS676, ARG553, MET452
7	Gingerol	442793	-4.52	4	0.00	ASP623, LYS621, PRO620, TYR619, ASP618, PHE793, ASP164, SER795
8	(10)-Gingerol	168115	-4.28	1	0	ASN628, THR319, PRO677, ASN459, LEU460, THR462, PRO461, TYR456, ARG457, VAL315
9	(8)-Gingerol	168114	-5.64	3	0	ASN628, SER664, GLY678, CYS395, THR394, PHE396, THR319, SER664, VAL315, GLU350, SER318
10	Shogaol	5281794	-5.14	2	0.00	THR556, TYR456

Figure 2

Docking images of SARS CoV-2 6m71 (RNA - Dependant RNA Polymerase protein) against Trikadu compounds: 2a. Gallic acid, 2b. Kaempferol, 2c. Demethoxycurcumin, 2d. Coumarin, 2e. Coumapherine, 2f. Bisdemethoxycurcumin, 2g. Gingerol, 2h. (10)-Gingerol, 2j. (8)-Gingerol, 2j. Shogaol, 2k. Paradol

Figure 3

Binding energies of 6lxt protein interaction with the 11 compounds are compared using a graphical visualization

Figure 4

Binding energies of 6m71 protein interaction with the 11 compounds are compared using a graphical visualization