Orignal Article

Phytocompound of pure thymol inhibit COVID-19 by binding to ACE2 receptor: *In silico* approach

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ABSTRACT

Due to the possible significant risk of COVID 19, various research have been done in recent years to discover and introduce COVID 19 antiviral medicines. Numerous studies have demonstrated that protease inhibitors, which are produced in high quantities in plant derivatives which can be particularly efficient in preventing virus-induced infection. The designed molecules (thymol derivatives) were effectively synthesized. Biological properties were used to confirm the purity and characterization of the synthesized substances. Also, acute toxicity study for the new product (thymol derivative) was determined . In this report, thymol derivative was analyzed by HPLC techniques to determine the new Thymol derivatives value, the theoretical and computer aided design studies showed that in docking and molinspiration score. As a result the Synthesised compound considered very good comparing with Favipiravir the well-known antiviral drug used against COVID-19. The analyzing data by using molinspiration was gave Thymol derivatives with more potential by Protease Inhibitor Receptors, and docking studies revealed that the synthesized molecule had a greater ligand binding affinity to the host receptor ACE2. According to various studies, the LD 50 of thymol taken orally is 640 mg/kg, while The LD50 value of the new thymol derivative was confirmed to be 1000 mg/kg.

Keywords: Thymol, thymol derivative, Corona virus, molinspiration and molecular docking

INTRODUCTION

A novel coronavirus (SARS-CoV-2) outbreak was discovered in Wuhan, China, before the end of 2019 and has spread around the world.[1] The World Health Organization reports that the number of confirmed cases of coronavirus infection has increased reached more than 37 million on October 10, 2020, with 1 million deaths (WHO). Coronaviruses are a major group of viruses that get their name from the crown-like spikes on their surfaces. People can be infected by a variety of viruses. Coronaviruses cause a variety of infections, from common colds to rare and dangerous respiratory infections including Severe Acute Respiratory Syndrome (SARSCoV) and Middle East Respiratory Syndrome (MERS-CoV).[2] The virus has since been termed SARS-CoV-2, and the disease it causes has been labeled COVID-19 by the World Health Organization.[3]

The virus is assumed to spread mostly from person to person, according to the US Centers for Disease Control and Prevention (CDC): respiratory droplets are produced when an infected person coughs or sneezes. These droplets may fall into your mouth or nose, or you may inhale them into your lungs. They believe that a person can contract COVID-19 by touching a virus-infected surface or object and then touching their mouth, nose, or eyes, but this is not thought to be the primary mode of transmission.[4]

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Thymol ($C_{10}H_{140}$) is a highly powerful component in several disinfectants intended to protect people from bacteria. Thymol is found in thyme essential oil, which is a naturally occurring blend of compounds obtained from the thyme plant.[5] It's commonly found in pesticides such animal repellents, fungicides, medical disinfectants, tuberculocidal drugs, and virucides. Perfumes, mouthwashes, pharmaceutical preparations, food flavorings, and cosmetics are all examples of where it can be used.[6] The major monoterpene phenol identified in thyme essential oil is thymol (2-isopropyl- 5-methylphenol). Antibacterial, anti-inflammatory, and antioxidant activities have been discovered in this molecule. Thymol could physically bind COVID-19 target proteins like spike protein and preventing COVID-19.[7]

In *Silico* research using of Molinspiration, which is a program that can evaluate a molecule and provide numerous metrics, including the capacity to estimate the potential of the compound acting on specific pharmacological targets.[8] Docking is a computer technique that predicts, When two molecules are joined together to form a stable complex, one molecule prefers one orientation over the other.[9] Using scoring functions, the strength of the link or binding affinity between two molecules can be predicted using the preferred orientation.. Many prominent docking approaches treat the ligand as flexible while keeping the protein conformation stiff.[10]

The goal of this study was to look for an alternative medicine that can block the function of the angiotensin converting enzyme (ACE2) which is worked as a SARS-CoV-2 receptor in a computer simulation. The formation of ester bonds between the two carboxyl groups of glutamic acid and the hydroxyl group of Thymol provides the basis for the overall synthesis procedure.

Materials and methods

Crystallization of Thymol

Pure Thymol was obtained: from the drug testing laboratories of the Iraqi Ministry of Health, national center for drug control and research. Thymol is often obtained from natural varieties, mainly Thymus species. Steam distillation or hydro distillation are used to extract the essential oils. After extraction, the aqueous and organic layers are separated in this process. To obtain pure thymol, distillation column chromatography or crystallization procedures might be applied.[11]

Synthesis, Characterization and Preliminary Pharmacological Evaluation of New Thymol derivative

Step one: Boc-glutamic acid 11.41 mmol was diluted in 20 mL of tetrahydrofuran (THF) that containing Triethylamine (TEA) 11.41 mmol and chilled in an ice bath at (-10°C). Ethyl chloroformate (ECF) 11.41 mmol was added drop by drop over a 10-minute period and the mixture was agitated constantly for another 30 minutes. Thymol (22.82 mmol) in distilled water 10 ml containing (TEA) 11.41 mmol was chilled to 0°C and immediately added to the aforementioned solution, stirring for 4 hours at -10°C and 2 hours at room temperature. The solvent was evaporated, and the precipitate was washed and filtered with diluted (HCl) 0.1N. The precipitate was collected and washed several times with water while stirring, then recrystallized from ethanol/toluene after being washed with ether (1:9).

Step two: To obtain the final chemical, the amino group of the collected precipitate was deprotected to yield a new derivative of Thymol, which was suspended in dichloromethane (DCM) (10 mL) and chilled to 0°C in an ice bath, then TFA (15 mL) was added with continuous stirring for 1 hour at 0°C. TLC was used to monitor the reaction's completion using the mobile phase methanol: chloroform (1:1). The precipitate was collected, suspended in 30 mL of methanol, and the pH was adjusted to 7 with a 5% methanolic NaOH solution. After adding ether a precipitate was produced, which was filtered and acetone washed before being recrystallized from ethyl acetate: petroleum ether (9:1). The precipitate was collected and dried at 50 degrees Celsius in an oven.[12]

HPLC

The New Thymol derivative was measured in an LC-20AD instrument using a reversed-phase HPLC-UV technique (Shimadzu, Kyoto). In a 40°C oven, UV detection was performed at a wavelength of 278 nm. Two columns were examined without the usage of safe guards: Sulfuric acid (0.5 mL of 2.5M) in 500 mL of acetonitrile and Sulfuric acid (0.5 mL of 2.5M) in water (A:B) Symmetry C18 (250 × 4.6 mm i.d., total) (500ml). To achieve the highest possible peak resolution, the flow rates were evaluated according to (Al-Mothafar. & Al-Shahwany).[13]

Experimental Design

In acute toxicity study, the new product (thymol derivative) was determined, albino mice male weighing between (20 – 25 g) were kept in animal house of Biotechnology Research Center / Al-Nahrain University in 25-28 °C laboratory conditions. To acclimate the mice to laboratory settings, all of them were given full access to water and food; the control animals (group I) were given distilled water orally, while the other groups II, III, and IV were given 1500, 1000, and 500 mg/kg orally, respectively, all animals were observed after treatment for 24 hours and the number of death recorded, The LD50 was calculated according to the literature.[21]

Theoretical and Computer Aided Studies

An in *Silico* research was conducted to gain a complete image of the produced molecules 3a–t. Molinspiration.[14] was used to compute the physicochemical, pharmacokinetic, and toxicological aspects of compounds, molinspiration cheminformatics software was used to predict drug-likeness and bioactivity analyses of leads, and Mcule software was

used to do docking experiments. This software includes a drug discovery platform as well as compound sourcing and hit detection. Docking experiments were conducted on the five most significant receptors in terms of activity, distribution, and metabolism. Molecular Docking study done by using 1-Click Docking program[15] on Angiotensin converting enzyme receptor (1R4L) and Protease-inhibitor receptor (1q91) .

The reference drug applied in these dockings is

Favipiravir (prodrug) is a purine base analog that is phosphoribosylated intracellularly to form active favipiravir ribofuranosyl-5B-triphosphate (favipiravir-RTP). It is a powerful and selective inhibitor of RNA viruses' RNA-dependent RNA polymerase (RdRp). The error-prone viral RdRp integrates favipiravir into the developing viral RNA, resulting in viral mutagenesis and chain termination.[16] Favipiravir works by blocking the viral RdRp enzyme, allowing it to be easily inserted into viral RNA while leaving human DNA alone. They came to the conclusion that nucleoside analogs (like favipiravir) have potential for COVID-19 treatments.[17]

Result and Discussion

Analysis of New Thymol derivative by HPLC

The result of HPLC method showed different peak of thymol's derivatete comparing with pure thymol, HPLC peaks of these compounds for each plant showed in the Figures (1 & 2). The full time of the new compound is about 14 min, while the pure thymol is about 1 min.

Theoretical Studies

The new designed compound have the following structure, which is a derivative of Thymol shown in Table 1.

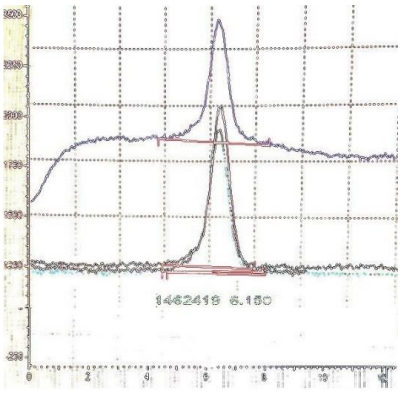

Figure 1: HPLC of derivative thymol

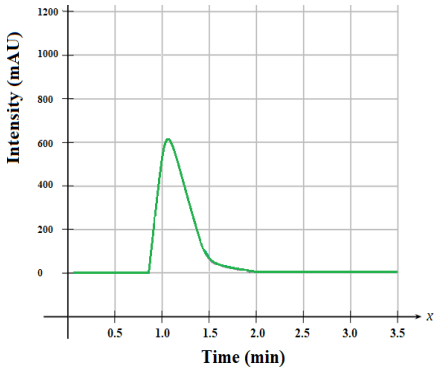

Figure 2: HPLC of pure thymol

Table 1: The theoretical properties of thymol and new thymol derivative :

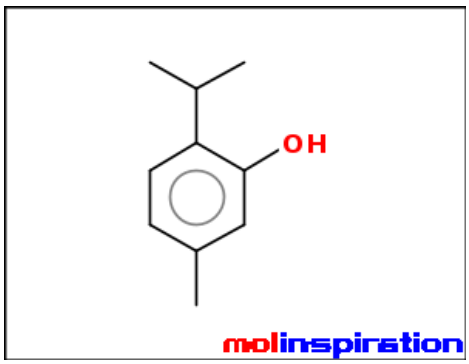	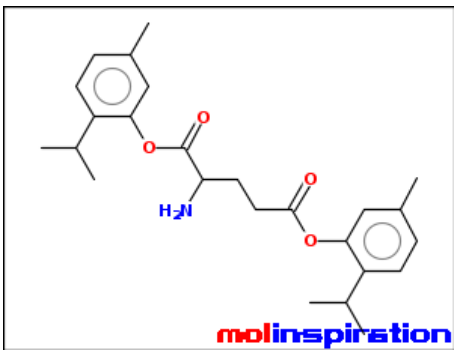
Thymol (5-Methyl-2-(propan-2-yl)phenol)[18] Properties Chemical formula $C_{10}H_{14}O$ Molar mass 150.221 g·mol^{-1} Melting point 49 to 51 °C Boiling point 232°C Solubility in water 0.9 g/L (20 °C)[19]	Thymol derivative Predicted Properties Chemical formula $C_{25}H_{33}NO_4$ Molar mass 411.53 Melting point 323.7°C Boiling point 759.38°C Log P: 5.9 (20°C)

Table 2: The theoretical Biological Activities of thymol, Synthesised compound and Favipiravir by using Molinspiration

Receptors	*Thymol*	*Synthesised compound*	*Favipiravir*
GPCR ligand	-1.05	-0.03	-0.43
Ion channel modulator	-0.53	0.02	0.42
Kinase inhibitor	-1.29	-0.15	-0.35
Nuclear receptor ligand	-0.78	-0.07	-1.14
Protease inhibitor	-1.34	0.07	-0.58
Enzyme inhibitor	-0.57	0.06	-0.18

Table 3: The theoretical study of thymol, New synthesised compound and Favipiravir as reference standard by using Docking program

	1R4L	*1q91*
Thymol	-6.0	-7.4
Synthesised compound	-9.0	-10.7
Favipiravir	-6.2	-6.9

1. Theoretical Biological Activities depending on (Molinspiration bioactivity score)[14]. The results shown in Ttable 2 must range from the worst (-2) to the best (2).

Molecular Docking study done by using 1-Click Docking program[15] on Angiotensin converting enzyme receptor (1R4L) and Protease-inhibitor receptor (1q91) on Thymol, New synthesised compound and Favipiravir as reference standard. The results shown in Table 3 above.

The Synthesised compound (thymol derivative) results considered very good comparing with Favipiravir the well-known antiviral drug used against COVID-19 and Thymol itself. The more negative values indicate higher binding affinity of ligand to the receptor

Each enzyme's binding pocket was successfully docked with the new Thymol derivative and the reference medication. The binding energies were measured in kilocalories per mol. The docked structure or conformation found at the end of each run, as well as the energies of these docked structures and their similarities to one another, are the most important results in a docking log file (DLG). The binding energies of the selected ligands were illustrated in Figures 3.

The thymol-ACE2 docked complex performed well in a Molecular Dynamics Simulation investigation. Because lower binding energy equates to better binding affinity, the molecules with the minimum binding energy of docking score were considered the best molecule for blocking the target receptor.[20]

Essential oil components can block the ACE2 receptor, making it more difficult for coronavirus to enter cells, potentially slowing the pandemic until the virus is eradicated. Despite the fact that these features are apparent *in silico*, more in vitro and clinical investigations involving SARS-CoV-2 should be explored for future research.[7]

Toxicity Studies

Thymol is toxic when consumed in large amounts, yet it is nearly non-toxic when applied topically. Even at lethal doses, oral treatment of thymol did not cause micronuclei in mice in vivo. According to various studies, the LD 50 of thymol taken orally is 640 mg/kg, which may have an effect on the peripheral nerve and sensation: lungs, thorax, or respiration: respiratory stimulation; lungs, spastic paralysis with or without sensory change; thorax, or respiration: respiratory stimulation0[22] Oral administration of new thymol derivative at dose 1500, 1000 and 500 mg/kg, the new synthesis compound produces different clinical symptoms and death in mice start at dose of 1500 mg/kg. The LD50 value of the new synthesis compound was confirmed to be 1000 mg/kg.

Conclusion

The thymol derivatives were successfully created by incorporating the aminomethyl group into the phytochemical thymol structure, according to the findings of this study. The analyzing data by using Molinspiration was gave Thymol derivatives were more potent with Protease Inhibitor Receptors, and docking studies revealed that the synthesized molecule had a greater ligand binding affinity to the host receptor ACE2 receptor. If administered to patients early in infection, an effective anti-viral for SARS-CoV-2 could eventually help to reduce viral load, avoid severe illness progression, and minimize person-to-person transmission. As soon as possible, benchmarking testing of this natural compounds versus additional prospective antivirals for SARS-CoV-2 with alternate modes of action is needed.

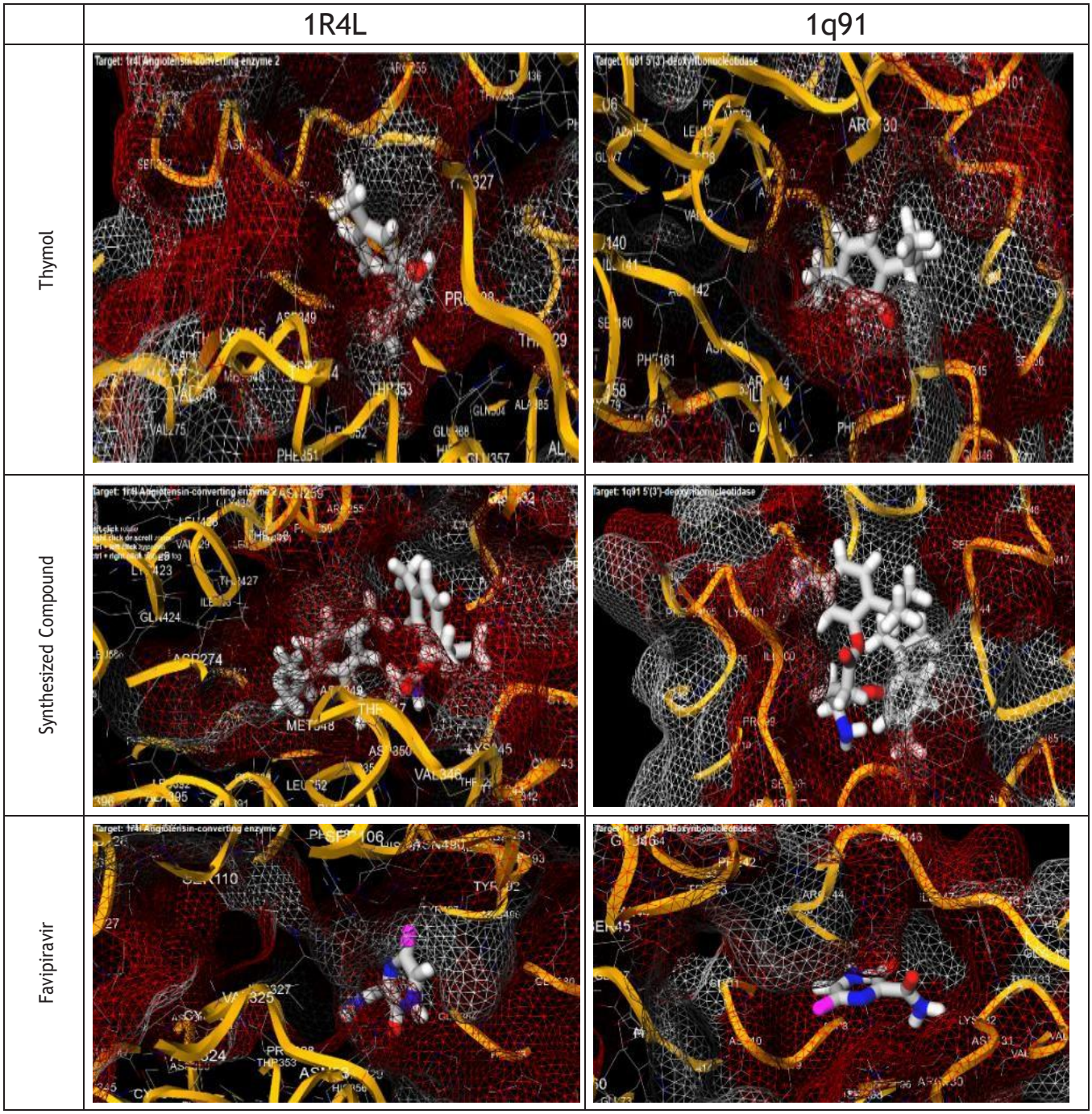

Figure 3: Docking binding of thymol, New synthesised compound and Favipiravir on 1R4L and 1q91

References

2. Chan, J. F. W., Yuan, S., Kok, K. H., To, K. K. W., Chu, H., Yang, J., ... & Yuen, K. Y. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The lancet*, *395*(10223), 514-523.
3. Mitchell, E. P. (2020). Corona virus: global pandemic causing world-wide shutdown. *Journal of the National Medical Association*, *112*(2), 113.
4. Gralinski, L. E., & Menachery, V. D. (2020). Return of the Coronavirus: 2019-nCoV. *Viruses*, *12*(2), 135.
5. Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., ... & Feng, Z. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England journal of medicine*.
6. Moghtader, M. (2012). Antifungal effects of the essential oil from Thymus vulgaris L. and comparison with synthetic thymol on Aspergillus niger. *Journal of Yeast and Fungal Research*, *3*(6), 83-88.
7. Singletary, K. (2016). Thyme: history, applications, and overview of potential health benefits. *Nutrition Today*, *51*(1), 40-49.

8. Rolta, R., Salaria, D., Kumar, V., SOURIRAJAN, A., & DEV, K. (2020). Phytocompounds of Rheum emodi, Thymus serpyllum and Artemisia annua inhibit COVID-19 binding to ACE2 receptor: In silico approach.
9. Gupta, A., Aniyery, R. B., & Pathak, A. (2017). In silico pharmacological and in vitro biological study of novel organotin-sorbate. *Int. J. Pharm. Sci. Res*, *8*(10), 4201-4212.
10. Lengauer, T., & Rarey, M. (1996). Computational methods for biomolecular docking. *Current opinion in structural biology*, *6*(3), 402-406.
11. Yuriev, E., Agostino, M., & Ramsland, P. A. (2011). Challenges and advances in computational docking: 2009 in review. *Journal of Molecular Recognition*, *24*(2), 149-164.
12. Dheer, D., Singh, D., Kumar, G., Karnatak, M., Chandra, S., Prakash Verma, V., & Shankar, R. (2019). Thymol chemistry: a medicinal toolbox. Current Bioactive Compounds, 15(5), 454-474.
13. Alwan, S. M., & Jabbar, S. S. (2015). Design, Synthesis and Preliminary Antimicrobial Evaluation of New Derivatives of Cephalexin. *Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683-3597, E-ISSN: 2521-3512)*, *24*(1), 85-91.
14. Al-Mothafar, N. A. & Al-Shahwany, A. W. Phenolic compounds from *Thymus vulgaris*, *Artemisia annua* extracts and pure Thymol were tested against twenty Pseudomonas spp. strains for antibacterial and anti-biofilm activities. IOP Journal of Physics. Under review.
15. Molinspiration Home Page. http://www.molinspiration.com/ (Accessed January 2017).
16. https://mcule.com/apps/1-click-docking/
17. Baranovich, T., Wong, S. S., Armstrong, J., Marjuki, H., Webby, R. J., Webster, R. G., & Govorkova, E. A. (2013). T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. *Journal of virology*, *87*(7), 3741-3751.
18. Agrawal, U., Raju, R., & Udwadia, Z. F. (2020). Favipiravir: A new and emerging antiviral option in COVID-19. *Medical Journal Armed Forces India*.
19. Favre, H. A., & Powell, W. H. (2013). *Nomenclature of organic chemistry: IUPAC recommendations and preferred names 2013*. Royal Society of Chemistry.
20. Sun, C., Cao, J., Wang, Y., Chen, J., Huang, L., Zhang, H., ... & Sun, C. (2021). Ultrasound-mediated molecular self-assemble of thymol with 2-hydroxypropyl-β-cyclodextrin for fruit preservation. *Food Chemistry*, *363*, 130327.
21. Simon, L., Imane, A., Srinivasan, K. K., Pathak, L., & Daoud, I. (2017). In silico drug-designing studies on flavanoids as anticolon cancer agents: Pharmacophore mapping, molecular docking, and Monte Carlo method-based QSAR modeling. Interdisciplinary Sciences: Computational Life Sciences, 9(3), 445-458.
22. Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of toxicology, 54(4), 275-287.
23. Rosado-Aguilar, J. A., Arjona-Cambranes, K., Torres-Acosta, J. F. J., Rodríguez-Vivas, R. I., Bolio-González, M. E., Ortega-Pacheco, A., ... & Aguilar-Caballero, A. J. (2017). Plant products and secondary metabolites with acaricide activity against ticks. Veterinary parasitology, 238, 66-76.