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In silico-modelling of phytochemicals in septic arthritis

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Abstract
Septic arthritis is a serious medical condition associated with severe morbidity and mortality. However, the clinical manifestations can be broad with conditions that resemble this synovial infection and require different assessment and care. Septic arthritis generally manifests with monoarticular joint pain with urticaria, swelling with pain on lesion and movement. Fever is present in many cases, but most are usually mild. Diagnostic tests can assist with the treatment, but the standard procedure to cure this infection is Arthrocentesis or joint aspiration. The management of septic arthritis includes intravenous antibiotics by intermittent infusion and orthopedic incisions. This descriptive research work discusses the in silico docking studies by using phytochemicals like Andrographolide, Capsaicin, Curcumin, Epigallocatechin gallate, Eugenol, Gingerol, Thymoquinone, Piperine with Proteins with PDB id's 2MZW, 1TVF, 1VQQ, and 3VSL respectively and could display anti-arthritic activity against the host-pathogen like *Staphylococcus aureus* which is the primary pathogen that causes in the pathogenesis of septic arthritis. Clearly, these phytochemicals should be further examined in preclinical and clinical trials to validate their effective efficacy against the disease.

Keywords: Septic arthritis, Staphylococcus aureus, docking, phytochemicals, active sites, receptor

Introduction
Septic arthritis is also known as bacterial acute arthritis, is a chronic inflammatory disease condition caused by an opportunistic infection. Septic arthritis affects mainly in large joints, such as the knees, but it might significantly impact any joint. In developed countries, the estimated prevalence of this infection is estimated to be about 6 cases per 100,000 populations, with the highest concentrations among commonly viewed under the age of 15 and over 55 years of age. The causes of septic arthritis are complex, ranging from benign to death. Septic arthritis is one of the most troubling causes in a patient with monoarticular arthritis. Septic arthritis is caused by a bacterial infection that is *S. aureus* in the joint, which can result in massive joint damage if not managed effectively. The fatality rate can be huge, varying from 3 to 25%. Septic arthritis may be vague, considering the seriousness of the disease, with many patients missing the typical symptoms, symptoms, or clinical features.
There are also a series of disorders that may be mistaken for septic arthritis, making the diagnosis much more complicated. During SA, both host and bacterial factors are thought to be pathogenic. The cartilage-synovium conjunction is typically the first site of joint destruction, followed by pannus formation and cartilage and bone destruction. A dramatic induction of polymorphonuclear granulocytes to activated macrophages, followed by T cells, depicts the inflammatory process. This event caused permanent joint damage and is related to the development of a series of cytokines. The outcome of SA is determined by the treatment's efficiency and agility. Even a few days of treatment delay can lead to permanent joint damage and a higher risk of death.
Therefore, there is a pressing need to create an intense enemy of this particular disease, specialists for the avoidance of the flare-up and stop bacterial infections causing septic arthritis. Repurposing of realized little particles is by all accounts an exceptionally productive path so as to create strong medications to battle diseases in this brief timeframe. As of late, various endeavors were made to plan novel inhibitors or utilize drug repurposing ways to deal with recognition hostile to medications.

Procedure
1. ligand Screening
For the initial Ligand screening purposes, a web-based tool named Swiss ADME (https ://www.swiss adme.ch/) was used to eliminate a few compounds according to Lipinski's rule of five parameters. For a compound to qualify as ligand it should Have < 500 Da molecular

weight, a high lipophilicity i.e. value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less than 5. Any compound with more than 2 violations was ruled out for further study (Lipinski2004).

2. Protein Preparation and Active site Determination

Required protein in pdb format was downloaded from the website **rcsb.org**, commonly known as the **Protein Data Bank**. 3D conformers of the ligand were downloaded from PubChem.

Using **PyMOL (Version 2.4.1)** software water molecules as well as native ligands from the protein were removed, defined as cleaning/purification of the protein for further application. **Using a web server called Deep Site** Active Pockets of the proteins were calculated. The results calculated by the web server were in the form of different ids, centers and scores. Scoring In deep site was using neural networking based on following instructions using DCNN architecture. Center values for the grid were selected keeping score greater than 0.98.

UCSF Chimera (Version 1.14) was used to prepare the receptor using DockPrep function. **Dock Prep** prepared structures for Docking using these functions:

- deleting water molecules
- repairing truncated sidechains
- adding hydrogens
- assigning partial charges
- writing files in Mol2 format

1. In silico Docking Using Auto dock Vina

Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular **Docking Studies**. Center values and size of the grid of different scores were used from **DEEPSITE** calculations done above.

Following Parameters were set in auto dock vina.

Receptor options

- **Add hydrogens in Chimera** (**true**/false) – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar (potentially H-bonding) hydrogens to identify atom types for scoring purposes.
- **Merge charges and remove non-polar hydrogens** (**true**/false) – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the processed receptor (and there may not have been any lone pairs to start with)
- **Ignore waters** (**true**/false)
- **Ignore chains of non-standard residues** (**true**/false) – ignore chains composed entirely of residues other than the 20 standard amino acids.
- **Ignore all non-standard residues** (true/**false**) – ignore all residues other than the 20 standard amino acids.

For Ligands

- **Merge charges and remove non-polar hydrogens** (**true**/false) – note Auto Dock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the ligand output files
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with)

Docking parameters

- **Number of binding modes** (1-10, **10**) – maximum number of binding modes to generate
- **Exhaustiveness of search** (1-8, **8**) – thoroughness of search, roughly proportional to time
- **Maximum energy difference (kcal/mol)** (1-3,**3**) – maximum score range; binding modes with scores not within this range of the best score will be discarded.

The docking results were calculated by Auto dock vina using it's Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative sign and least RMSD values were chosen for further studies.

4. Residue Analysis

PyMOL was used for visualization of interactions of the docked structure at the ligand sites. Discovery Studio 2020 was used to study the ligand interactions and total number of residues. It was also used to plot the 2D structure of the interactions and residues.

Statistical Analysis: Descriptive, estimation and Hypothesis testing with confidence interval of 95% was applied to data using formula 1 given below.

$$CI = \bar{x} \pm z\frac{s}{\sqrt{n}}$$

CI = confidence interval

$\bar{x}$ = sample mean

z = confidence level value

s = sample standard deviation

n = sample size

Formula 1 used for calculation of confidence interval

Results and Discussion

The docking result was obtained from Autodock vina in the form of Dock score for all the four proteins docked with above mentioned ligands.

Docking Results of Staphylococcus aureus proteins

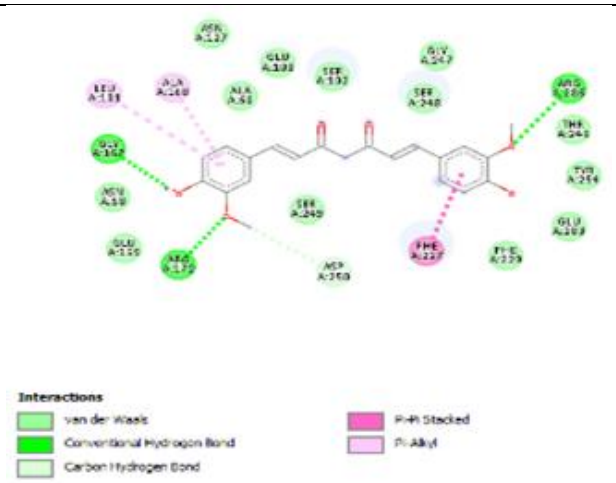
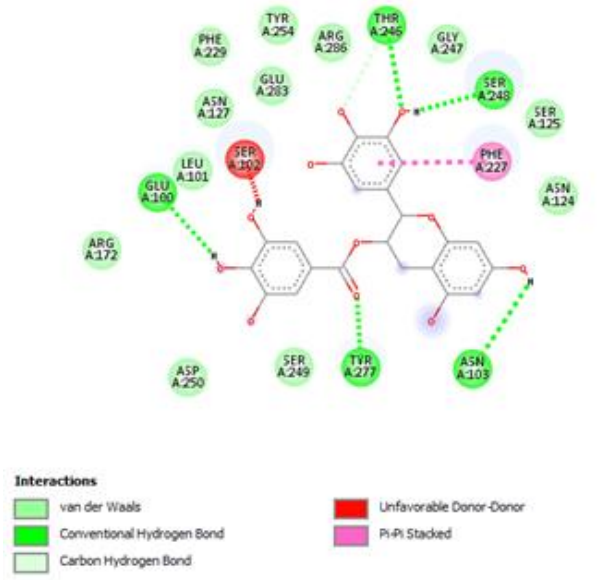
PDB-ID 1TVF

For ITVF, 3 active sites for chain A and 2 active sites for chain B were selected out of which the first active site of chain A was selected with a Deepsite score of 0.998066127. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 1 and Table 2 shows the post statistical docking scores with Ligand Protein interactions.

Table 1: Docking results of phytochemicals with PDB-ID 1TVP

Ligands	Dock score
Andrographolide	-7.3
Capsaicin	-7.2
Curcumin	-7.7
Epigallocatechin gallate	-8.6
Eugenol	-5.5
Gingerol	-6.4
Thymoquinone	-5.7
Piperine	-7.1

Table 2: 2D amino acid interactions of curcumin and epigallocatechin gallate with PDB-ID 1TVF

Ligands	Dock score	Interactions
Curcumin	-7.7	
Epigallocatechin gallate	-8.6	

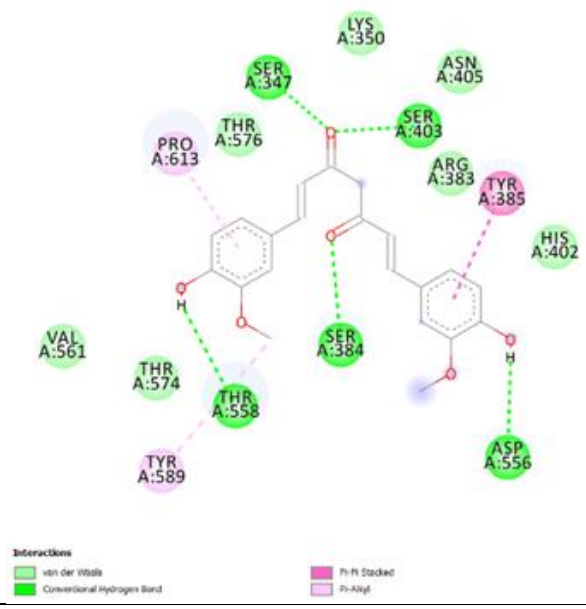
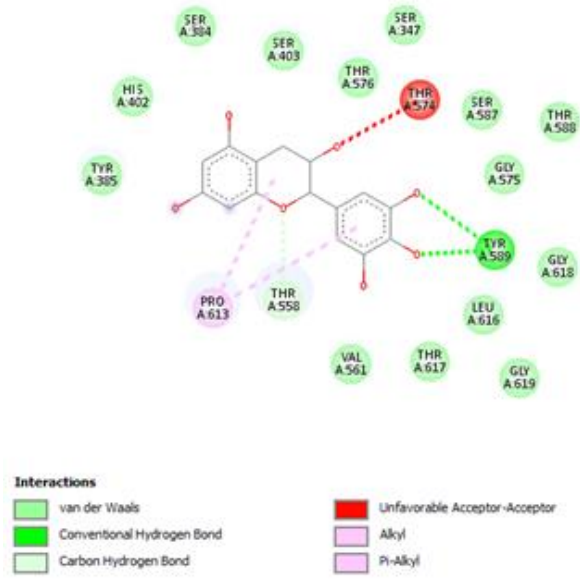
PDB-ID 3VSL

For 3VSL, 3 active sites for chain A and 4 active sites for chain B were selected out of which the second active site of chain A was selected with a Deepsite score of 0.999304295. The selection was made on the basis of the highest binding energy of the ligand receptor. The docking results before statistics are shown in Table 3 and Table 4 shows the post statistical docking scores with Ligand Protein interactions.

Table 3: Docking results of phytochemicals with PDB-ID 3VSL

Ligands	Dock score
Andrographolide	-7.3
Capsaicin	-6.2
Curcumin	-7.7
Epigallocatechin Gallate	-9.1
Eugenol	-5.3
Gingerol	-6.5
Thymoquinone	-5.2
Piperine	-7.1

Table 4: 2D amino acid interactions of curcumin and epigallocatechin gallate with PDB-ID 3VSL

Ligands	Dock score	Interactions
Curcumin	-7.7	
Epigallocatechin gallate	-9.1	

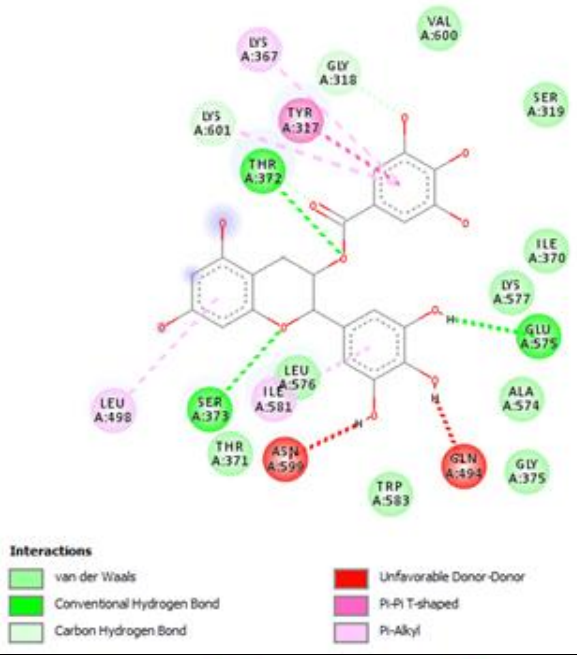
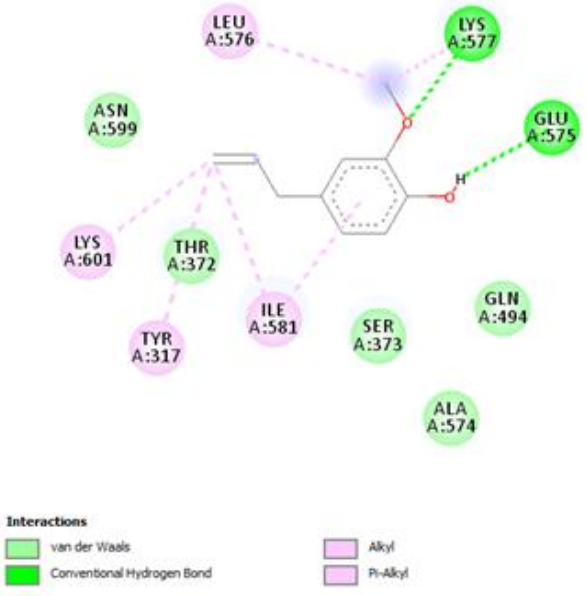
PDB-ID 1VQQ

For 1VQQ, 2 active sites for chain A and 2 active sites for chain B were selected out of which the first active site of chain A was selected with a Deepsite score of 0.998640954. The selection was made on the basis of the highest binding energy of the ligand receptor. The docking results before statistics are shown in Table 5 and Table 6 shows the post statistical docking scores with Ligand Protein interactions.

Table 5: Docking results of phytochemicals with PDB-ID 1VQQ

Ligands	Dock score
Andrographolide	-7
Capsaicin	-6.2
Curcumin	-6.9
Epigallocatechin Gallate	-9.2
Eugenol	-9.3
Gingerol	-6.2
Piperine	-6.8
Thymoquinone	-5.6

Table 6: 2D amino acid interactions of epigallocatechin gallate and eugenol with PDB-ID 1VQQ

Ligands	Dock score	Interactions
Epigallocatechin gallate	-9.2	
Eugenol	-9.3	

PDB-ID 2MZW

For 2MZW, 3 active sites for chain A and 4 active sites for chain B were selected out of which the first active site of chain A was selected with a Deepsite score of 0.999576986. The selection was made on the basis of the highest binding energy of the ligand receptor. The docking results before statistics are shown in Table 7 and Table 8 shows the post statistical docking score with Ligand Protein interactions.

Table 7: Docking results of phytochemicals with PDB-ID 2MZW

Ligands	Dock score
Andrographolide	-8.8
Capsaicin	-7.5
Curcumin	-9.8
Epigallocatechin Gallate	-9.2
Eugenol	-6.1
Gingerol	-7.2
Thymoquinone	-7.1
Piperine	-8.8

Table 8: 2D amino acid interactions of curcumin and epigallocatechin gallate with PDB-ID 2MZW

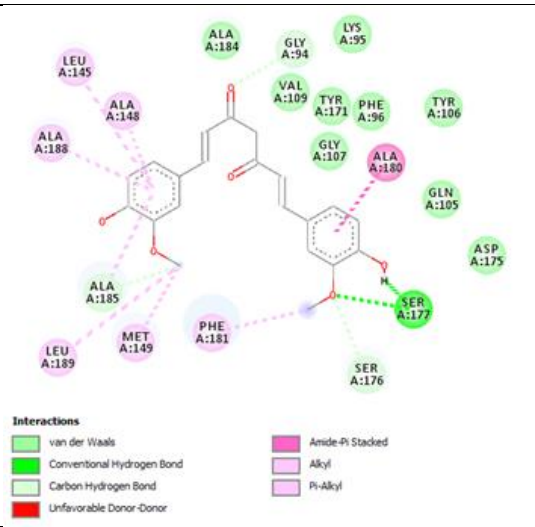
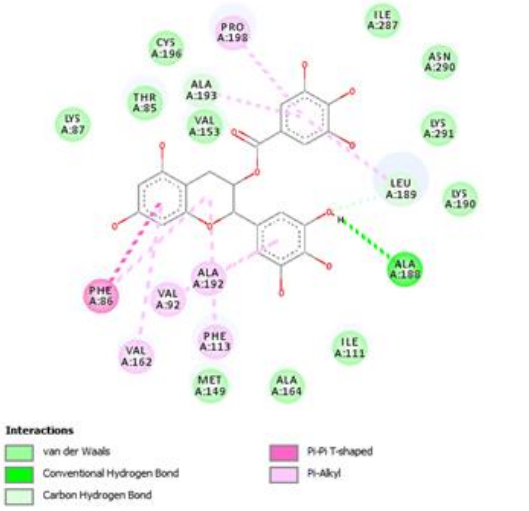
Ligands	Dock score	Interactions
Curcumin	-9.8	
Epigallocatechin gallate	-9.2	

Table 9: Summarizes the results showing ligands and their interacted proteins that were considered in the study for the targeted disease

Ligands	Proteins Interacted	Target Disease
Andrographolide	1VQQ 2MZW 3VSL 1TVF	Septic Arthritis caused due to Staphylococcus aureus
Capsaicin		
Curcumin		
Epigallocatechin gallate		
Eugenol		
Gingerol		
Thymoquinone		
Piperine		

Conclusion

All eight ligands were studied using bioavailability radar. Our results proposed that epigallocatechin gallate showed best docking results with all PDB-IDs 1TVF, 2MZW, 3VSL as well as 1VQQ. Besides this curcumin showed its best docking results with 1VQQ, 2MZW, and 3VSL. Apart from these two, eugenol showed its best docking result with PDB-ID 1VQQ. To find the effectiveness and to propose the exact mechanism in-vitro studies can be encouraged on Curcumin, Epigallocatechin gallate as well as Eugenol by targeting proteins of organism responsible for septic arthritis that are discussed above to understand the mechanism and a potential cure for septic arthritis.

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References

1. Bang JS, Oh DH, Choi HM, Sur BJ, Lim SJ, Yeon JY *et al*. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1β-stimulated fibroblast-like synoviocytes and in rat arthritis models. Arthritis Res Ther 2009, 11(2).
2. El-Ghazaly MA, Fadel NA, Abdel-Naby DH, Abd El-Rehim HA, Zaki HF, Kenawy SA. Potential anti-inflammatory action of resveratrol and piperine in adjuvant-induced arthritis: Effect on pro-inflammatory

cytokines and oxidative stress biomarkers. Egypt Rheumatol 2020, 42(1).
3. Umar S, Golam Sarwar AHM, Umar K, Ahmad N, Sajad M, Ahmad S *et al*. Piperine ameliorates oxidative stress, inflammation and histological outcome in collagen induced arthritis. Cell Immunol 2013;284(1-2).
4. Umar S, Zargan J, Umar K, Ahmad S, Katiyar CK, Khan HA. Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. Chem Biol Interact 2012, 197(1).
5. Faisal R, Shinwari L, Jehangir T. Comparison of the therapeutic effects of thymoquinone and methotrexate on renal injury in pristane induced arthritis in rats. J Coll Physicians Surg Pakistan 2015, 25(8).
6. Vaillancourt F, Silva P, Shi Q, Fahmi H, Fernandes JC, Benderdour M. Elucidation of molecular mechanisms underlying the protective effects of thymoquinone against rheumatoid arthritis. J Cell Biochem 2011, 112(1).
7. Tekeoglu I, Dogan A, Demiralp L. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phyther Res 2006, 20(10).
8. Tekeoğlu I, Dogan A, Ediz L, Budancamanak M, Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phyther Res 2007, 21(9).
9. Arjumand S, Shahzad M, Shabbir A, Yousaf MZ. Thymoquinone attenuates rheumatoid arthritis by downregulating TLR2, TLR4, TNF-α IL-1, and NFκB expression levels. Biomed Pharmacother 2019, 111.
10. Mushtaq Z, Tahir Nadeem M, Arshad MU, Saeed F, Ahmed MH, Bader Ul Ain H *et al*. Exploring the biochemical and antioxidant potential of ginger (Adric) and turmeric (Haldi). Int J Food Prop 2019, 22(1).
11. Shahrajabian MH, Sun W, Cheng Q. Pharmacological Uses and Health Benefits of Ginger (*Zingiber officinale*) in Traditional Asian and Ancient Chinese Medicine, and Modern Practice. Not Sci Biol 2019, 11(3).
12. Funk JL, Frye JB, Oyarzo JN, Timmermann BN. Comparative effects of two gingerol-containing zingiber officinale extracts on experimental Rheumatoid arthritis. J Nat Prod 2009, 72(3).
13. Padher SV, Madgulkar A. Evaluation of topical formulations containing gingerol and piperine in rheumatoid arthritis. Int J Res Pharm Pharm Sci 2017, 2(6).
14. Mateen S, Shahzad S, Ahmad S, Naeem SS, Khalid S, Akhtar K *et al*. Cinnamaldehyde and eugenol attenuates collagen induced arthritis via reduction of free radicals and pro-inflammatory cytokines. Phytomedicine 2019, 53.
15. Grespan R, Paludo M, De Paula Lemos H, Barbosa CP, Bersani-Amado CA, De Oliveira Dalalio MM *et al*. Anti-arthritic effect of eugenol on collagen-induced arthritis experimental model. Biol Pharm Bull 2012, 35(10).
16. Min SY, Yan M, Kim SB, Ravikumar S, Kwon SR, Vanarsa K *et al*. Green tea epigallocatechin-3-gallate suppresses autoimmune arthritis through indoleamine-2,3-dioxygenase expressing dendritic cells and the nuclear factor, erythroid 2-like 2 antioxidant pathway. J Inflamm (United Kingdom) 2015, 12(1).
17. Ahmed S. Green tea polyphenol epigallocatechin 3-gallate in arthritis: Progress and promise Arthritis Research and Therapy 2010, 12.
18. Lee SY, Jung YO, Ryu JG, Oh HJ, Son HJ, Lee SH *et al*. Epigallocatechin-3-gallate ameliorates autoimmune arthritis by reciprocal regulation of T helper-17 regulatory T cells and inhibition of osteoclastogenesis by inhibiting STAT3 signaling. J Leukoc Biol 2016, 100(3).
19. Karatas A, Dagli AF, Orhan C, Gencoglu H, Ozgen M, Sahin N *et al*. Epigallocatechin 3-gallate attenuates arthritis by regulating Nrf2, HO-1, and cytokine levels in an experimental arthritis model. Biotechnol Appl Biochem 2020, 67(3).
20. Zhang N, Weber A, Li B, Lyons R, Contag PR, Purchio AF *et al*. An Inducible Nitric Oxide Synthase-Luciferase Reporter System for *in vivo* Testing of Anti-inflammatory Compounds in Transgenic Mice. J Immunol 2003, 170(12).
21. Chen L, Lu Y, Zhao L, Hu L, Qiu Q, Zhang Z *et al*. Curcumin attenuates sepsis-induced acute organ dysfunction by preventing inflammation and enhancing the suppressive function of Tregs. Int Immunopharmacol 2018, 61.
22. Daily JW, Yang M, Park S. Efficacy of Turmeric Extracts and Curcumin for Alleviating the Symptoms of Joint Arthritis: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Vol. 19, Journal of Medicinal Food 2016.
23. Borbély É, Kiss T, Szabadfi K, Pintér E, Szolcsányi J, Helyes Z *et al*. Complex Role of Capsaicin-Sensitive Afferents in the Collagen Antibody-Induced Autoimmune Arthritis of the Mouse. Sci Rep 2018, 8(1).
24. Aggarwal BB, Shishodia S. Suppression of the nuclear factor-κB activation pathway by spice-derived phytochemicals: Reasoning for seasoning. In: Annals of the New York Academy of Sciences 2004.
25. JCW L, TK C, DS N, SR S, JS, WF W. Andrographolide and its analogues: Versatile bioactive molecules for combating inflammation and cancer. Vol. 39, Clinical and Experimental Pharmacology and Physiology 2012.
26. Li X, Yuan K, Zhu Q, Lu Q, Jiang H, Zhu M *et al*. Andrographolide ameliorates rheumatoid arthritis by regulating the apoptosis–NETosis balance of neutrophils. Int J Mol Sci 2019, 20(20).
27. Gupta S, Mishra KP, Kumar B, Singh SB, Ganju L. Andrographolide attenuates complete freund's adjuvant induced arthritis via suppression of inflammatory mediators and pro-inflammatory cytokines. J Ethnopharmacol 2020, 261.
28. Wang DA, Tambyah PA. Septic arthritis in immunocompetent and immunosuppressed hosts. Vol. 29, Best Practice and Research: Clinical Rheumatology 2015.
29. Ross JJ. Septic Arthritis of Native Joints. Vol. 31, Infectious Disease Clinics of North America 2017.
30. Morris GM, Ruth H, Lindstrom W, Sanner MF, Belew RK, Goodsell DS *et al*. Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009, 30(16).
31. Dassault S. BIOVIA. http://www3ds.com/products-services/biovia/. 2014.
32. Accelrys Inc. BIOVIA Materials Studio. Http://AccelrysCom/. 2017.
33. DeLano WL. The PyMOL Molecular Graphics System, Version 2.3. Schrödinger LLC 2020.

34. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC *et al*. UCSF Chimera: A visualization system for exploratory research and analysis. J Comput Chem 2004, 25(13).
35. Cletus A Ukwubile, Jude A Odugu, Troy S Malgwi, Mathias Simon Bingari. Evaluation of in vitro anthelminthic, antiproliferative and antihypertensive potentials of Rauwolfia vomitoria Afzel. (Apocynaceae) leaf extract. Int J Adv Biochem Res 2019;3(1):64-74. DOI: 10.33545/26174693.2022.v6.i2a.107.
36. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S *et al*. PubChem 2019 update: Improved access to chemical data. Nucleic Acids Res 2019, 47(D1).
37. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A *et al*. PubChem substance and compound databases. Nucleic Acids Res 2016, 44(D1).
38. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D *et al*. Drug Bank: A knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res 2008, 36(Suppl. 1).
39. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR *et al*. DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic Acids Res 2018, 46(D1).
40. 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, 7.
41. Trott O, Olson AJ. Auto Dock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2009.
42. Jiménez J, Doerr S, Martínez-Rosell G, Rose AS, De Fabritiis G. Deep Site: Protein-binding site predictor using 3D-convolutional neural networks. Bioinformatics 2017, 33(19).
43. Lim D, Strynadka NCJ. Structural basis for the β-lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus. Nat Struct Biol 2002, 9(11).
44. Tomlinson JH, Thompson GS, Kalverda AP, Zhuravleva A, O'Neill AJ. A target-protection mechanism of antibiotic resistance at atomic resolution: Insights into Fus B-type fusidic acid resistance. Sci Rep 2016, 6.
45. Yoshida H, Kawai F, Obayashi E, Akashi S, Roper DI, Tame JRH *et al*. Crystal structures of penicillin-binding protein 3 (PBP3) from methicillin-resistant staphylococcus aureus in the Apo and cefotaxime-bound forms. J Mol Biol 2012, 423(3).
46. Kwatra B, Roy R, Bhowmik R, Sengupta S. DRUG REPURPOSING : IN SILICO MODELING OF COVID-19. Res J Life Sci Bioinformatics, Pharm Chem Sci. 2021;7(2):19-40.
47. Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K *et al*. The protein data bank. Acta Crystallogr Sect D Biol Crystallogr 2002, 58(6 I).