International Journal of Veterinary Sciences and Animal Husbandry

**ISSN:** 2456-2912
NAAS Rating: 4.61
VET 2025; 10(6): 07-12

www.veterinarypaper.com
Received: 05-03-2025
Accepted: 09-04-2025

**Akshay Durga Prasad M**
Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

**CM Jaikanth**
Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

**Ramesh Srinivasan**
Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

**Harsitha VM**
Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

**Ramasamy Thangamalai**
Assistant Professor, Veterinary Clinical Complex, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animals Sciences University, Salem, Tamil Nadu, India

**Corresponding Author:**
**Ramasamy Thangamalai**
Assistant Professor, Veterinary Clinical Complex, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animals Sciences University, Salem, Tamil Nadu, India

# Antimicrobial resistance pattern of staphylococcus species in mastitis affected dairy Cows

**Akshay Durga Prasad M, CM Jaikanth, Ramesh Srinivasan, Harsitha VM and Ramasamy Thangamalai**

**DOI:** https://www.doi.org/10.22271/veterinary.2025.v10.i6a.2297

**Abstract**
Inflammation is a crucial event for initiating tissue repair and regeneration following injury, can become detrimental when dysregulated, often progressing into chronic inflammation. Recent studies have emphasized that the resolution of inflammation is an active, regulated process mediated by Specialized Pro-resolving Mediators (SPMs) derived from omega-3 and omega -6 polyunsaturated fatty acids (PUFAs). This study focusses on the potential of Walnut (*Juglans regia* L.) seed phytoconstituents to modulate key enzymes involved in inflammation resolution such as, 5-lipoxygenase (5-LOX), 12-LOX, 15-LOX, and cyclooxygenase-2 (COX-2), through *in-silico* molecular docking analysis. Nine phytoconstituents from walnut seed, including retinol, ascorbic acid, linolenic acid, and linoleic acid, were evaluated for their physicochemical and pharmacokinetic properties using SwissADME and docked with inflammation-resolving enzymes using AutoDock Vina and results were visualized using Biovia Discovery studio. Retinol demonstrated the strongest binding affinity, particularly with 5-LOX and 12-LOX, while linolenic and linoleic acids showed consistent interactions across all targets. Most compounds exhibited favourable drug-likeness and high gastrointestinal absorption, with some capable of crossing the blood-brain barrier, highlighting their therapeutic potential against neural inflammation. From this study, it can be hypothesised that, walnut phytochemicals by serving as precursors to SPMs and exhibiting favourable interaction profiles with key enzymatic targets, may represent promising agents for the dietary management and therapeutic modulation of inflammation, ultimately aiding in the prevention of chronic inflammatory diseases.

**Keywords:** Inflammation resolution, SPMs, walnut, linolenic acid, lipoxygenases

## Introduction
Inflammation is the key process for initiating tissue repair and regeneration following injury, it becomes highly detrimental when dysregulated, often progressing into chronic inflammation and is a key contributor to the pathogenesis of several major diseases, including atherosclerosis, cardiovascular disorders, hepatic dysfunction, and chronic kidney disease [1]. In recent years, a paradigm shift in understanding the inflammatory cascade has emerged, largely driven by the pioneering work of Dr. Charles Serhan and colleagues. Their groundbreaking research revealed that the resolution of inflammation is not a passive cessation of immune activity but rather an active, tightly orchestrated biological process amenable to therapeutic modulation. This revelation led to the identification of a novel class of endogenous lipid mediators known as Specialized Pro-resolving Mediators (SPMs). These include Resolvins, Protectins, Maresins and Lipoxins, which are biosynthesized from dietary polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [2]. Through enzymatic pathways involving cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450, these omega-3 fatty acids are converted into bioactive mediators that actively facilitate the resolution of inflammation by clearing apoptotic cells, reducing leukocyte infiltration, promoting tissue repair, and restoring immune homeostasis [3]. Amidst this backdrop, Walnuts (*Juglans regia* L.) emerge as a promising candidate for nutritional and pharmacological exploration. They are naturally a nutrient-dense food and are rich in plant-derived bioactive peptides, essential amino acids, and polyunsaturated fatty acids, notably linoleic acid and α-linolenic acid. Walnut oil, derived from

the seed kernel, is especially valued for its multifaceted health benefits, including antioxidant antihypertensive, hypoglycaemic and neuroprotective activities [4, 5]. They are also reported to be enriched with bioactive constituents such as vitamin E, melatonin, phospholipids, squalene and flavonoids, which boasts their anti-inflammatory, lipid-lowering, anticarcinogenic and antioxidant properties [6].

Taking account of this rich phytochemical profile, the current study is designed through *in-silico* molecular docking analysis to investigate the potential of walnut-derived phytoconstituents to interact and modulate key enzymes involved in inflammation resolution, namely 12-lipoxygenase (12-LOX), 15-lipoxygenase (15-LOX), 5-lipoxygenase (5-LOX), and cyclooxygenase-2 (COX-2). This computational approach aims to elucidate the binding affinities and mechanistic interactions of walnut bioactives with these enzymatic targets, thereby exploring their prospective role in promoting the resolution of inflammation and preventing pathological prolongations.

## Materials and Methods

The lipoxygenase and cyclooxygenase enzymes play an important role in inflammation resolution pathways. Four vital enzymes involved in inflammation resolution were chosen for this study. The 3D structures of chosen enzymes 12- LOX, 15- LOX, 5-LoX and CoX-2 were obtained from RCSB-PDB and were used as protein targets.

Initially, the plants with high amount of PUFA contents were searched from literatures. The plant with high PUFA content selected for this study was Walnut seed. The PDB structures of the phytoconstituents of Walnut seeds were obtained from a curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) and the IMPPAT identifier and SMILES expression of phytoconstituents were given in the Table 1.

**Table 1:** Walnut seed phytoconstituents and their smiles

| S. No. | Name | IMPPAT ID | SMILES |
|---|---|---|---|
| 1 | Retinol | IMPHY001308 | OC/C=C(/C=C/C=C(/C=C/C1=C(C)CCCC1(C)C)C)C |
| 2 | Stearic acid | IMPHY004631 | CCCCCCCCCCCCCCCCCC(=O)O |
| 3 | Ascorbic acid | IMPHY006362 | OC[C@@H]([C@H]1OC(=O)C(=C1O)O)O |
| 4 | Palmitic acid | IMPHY007327 | CCCCCCCCCCCCCCCC(=O)O |
| 5 | Nicotinamide | IMPHY007385 | NC(=O)c1cccnc1 |
| 6 | [3-[Hydroxy-(2,3,4,5,6-pentahydroxycyclohexyl)oxyphosphoryl]oxy-2-octadecanoyloxypropyl] octadecenoate | IMPHY008728 | CCCCCCCCCCCCCCCCCC(=O)OCC(OC(=O)CCCCCCCCCCCCCCC)COP(=O)(OC1C(O)C(O)C(C(C1O)O)O)O |
| 7 | Oleic acid | IMPHY011797 | CCCCCCCC/C=CCCCCCCCC(=O)O |
| 8 | Linolenic acid | IMPHY012723 | CC/C=CC/C=CC/C=CCCCCCCCC(=O)O |
| 9 | Linoleic acid | IMPHY014990 | CCCCC/C=CC/C=CCCCCCCCC(=O)O |

The physicochemical characteristics and drug-likeness profiles of walnut seed phytoconstituents were meticulously evaluated using the SwissADME online platform. This assessment was conducted in accordance with established drug-likeness criteria, including Lipinski's Rule of Five, Veber's Rule, and Egan's Rule, among others, to predict their potential as orally bioavailable therapeutic agents. Additionally, pharmacokinetic parameters encompassing absorption, distribution, metabolism, excretion, and toxicity (ADMET) were analyzed to gain comprehensive insights into the systemic behavior of these bioactive compounds.

Furthermore, the three-dimensional structures of the phytoconstituents and selected protein targets involved in inflammation resolution namely, 5-LOX, 12-LOX, 15-LOX and COX-2 were retrieved in PDB format and subsequently converted into the PDBQT format to facilitate molecular docking simulations.

## Docking

The PDB structures of each walnut seed phytoconstituent were meticulously prepared for molecular docking by converting them into PDBQT format using AutoDock Vina. This process involved the removal of water molecules, the addition of polar hydrogens and the assignment of Kollman charges to ensure accurate representation of molecular interactions. Likewise, the target protein structures, the key enzymes implicated in the inflammation resolution pathways were similarly processed and optimized for docking. Each phytoconstituent was then individually docked with the selected protein targets using AutoDock Vina, employing the AutoGrid engine to define a grid box encompassing the active binding sites of the enzymes. The binding affinity for each ligand-protein complex was quantified based on the lowest docking score and most favourable binding energy (expressed in kcal/mol), which indicated the strength and stability of the interaction [7]. To further interpret and illustrate the molecular interactions, the resulting 3D conformations were visualized and analyzed using BIOVIA Discovery Studio Visualizer, enabling detailed observation of binding orientations, hydrogen bonding, and hydrophobic interactions between the walnut seed bioactives and the enzymatic targets.

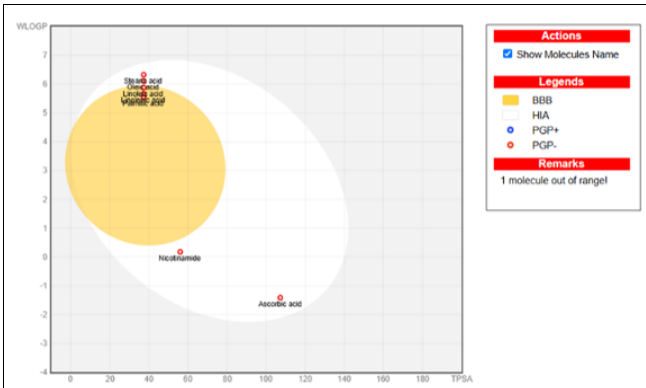
## Results

The physicochemical properties of the walnut phytoconstituents predicted using the SwissADME tool, are presented in Table 2. Out of the nine docked compounds, only one phytoconstituent octadecenoate has high molecular weight and has a greater number of hydrogen donors in comparison to all other compounds. Its topological polar surface area (TPSA) and iLogP value which are critical indicators of molecular polarity and membrane permeability are also significantly higher than those of the other phytoconstituents making octadecenoate the least favourable candidate in terms of oral bioavailability. In contrast, the remaining eight phytoconstituents demonstrated favourable physicochemical profiles, with optimal ranges of molecular descriptors, resulting in high predicted oral absorption and better overall drug-likeness potential.

**Table 2:** Physicochemical properties of walnut seed phytoconstituents

| S. No. | Name | MW | No. H-bond acceptors | No. H-bond donors | TPSA | iLOGP | GI absorption | BBB permeant | CYP1A2 inhibitor | Bioavailability Score |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Retinol | 286.45 | 1 | 1 | 20.23 | Not predicted | Not predicted | Not predicted | Not predicted | Not predicted |
| 2 | Stearic acid | 284.48 | 2 | 1 | 37.3 | 4.3 | High | No | Yes | 0.85 |
| 3 | Ascorbic acid | 176.12 | 6 | 4 | 107.22 | -0.31 | High | No | No | 0.56 |
| 4 | Palmitic acid | 256.42 | 2 | 1 | 37.3 | 3.85 | High | Yes | Yes | 0.85 |
| 5 | Nicotinamide | 122.12 | 2 | 1 | 55.98 | 0.7 | High | No | No | 0.55 |
| 6 | Octadecanoate | 867.14 | 13 | 6 | 219.32 | 7.55 | Low | No | No | 0.11 |
| 7 | Oleic acid | 282.46 | 2 | 1 | 37.3 | 4.27 | High | No | Yes | 0.85 |
| 8 | Linolenic acid | 278.43 | 2 | 1 | 37.3 | 3.36 | High | Yes | Yes | 0.85 |
| 9 | Linoleic acid | 280.45 | 2 | 1 | 37.3 | 4.14 | High | Yes | Yes | 0.85 |

The phytoconstituents of walnut seed with the ability to cross blood brain barrier (BBB) and high intestinal absorption (HIA) were also shown as boiled egg appearance derived from SwissADME (Fig-1). It is evident from the SwissADME results that palmitic acid, oleic acid, linolenic acid and linoleic acid crosses blood brain barrier whereas stearic acid, ascorbic acid and nicotinamide has high intestinal absorption.




| | |
|---|---|
| Retinol | Not predicted |
| Stearic acid | HIA |
| Ascorbic acid | HIA |
| Palmitic acid | BBB |
| Nicotinamide | HIA |
| octadecenoate | Out of range |
| Oleic acid | BBB |
| Linolenic acid | BBB |
| Linoleic acid | BBB |

**Fig 1:** Boiled egg of appearance of walnut phytoconstituents from SwissADME

The docking scores of the phytoconstituents of walnut seed against four enzymes of inflammation resolution are presented in the table 3. The 2D and 3D output structures of ligands showing higher binding affinities against the selected proteins are depicted in figures 2, 3 and 4. From the docking scores, it is evident that retinol, ascorbic acid and octadecenoate has good binding affinities against 12-LOX. Retinol showed highest binding affinity against 12-LOX (-7.8) (fig-2). Against the second enzyme 15-LOX, none of the phytoconstituents showed higher binding affinity of less than -6, however retinol, nicotinamide and ascorbic acid showed considerable binding efficacies of -5.8, -5.7 and -5.5 respectively. The docking of these phytoconstituents against the third enzyme 5- LOX revealed better binding affinity for retinol (-8.6), octadecenoate (-7.4), oleic acid (-6.4), linolenic acid (-6.4) and linoleic acid (-6.3) (fig 3). Similarly, against Aspirin Acetylated COX-2 enzyme the phytoconstituent retinol was found to exhibit the best binding score of -6.3 (fig 4) followed by Linolenic acid (-5.9) and Linoleic acid (-5.6).
The docking results showcased that retinol was the promising phytoconstituent from walnut seed which showed strong binding affinity against 12- LOX, 5- LOX and COX-2. It is also noticed from the docking results that retinol, ascorbic acid, linolenic acid and linoleic acid showed better binding interactions across all the inflammation resolution mediating enzymes in this study.

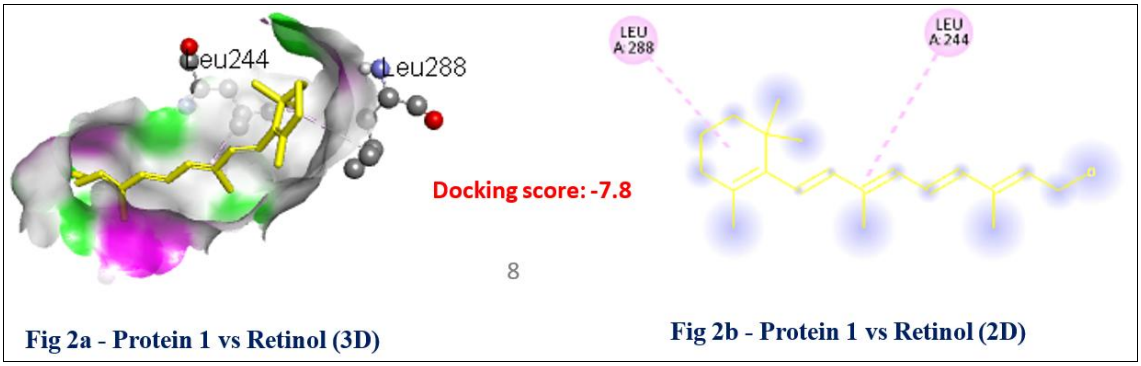



**Fig 2a - Protein 1 vs Retinol (3D)**

**Fig 2b - Protein 1 vs Retinol (2D)**

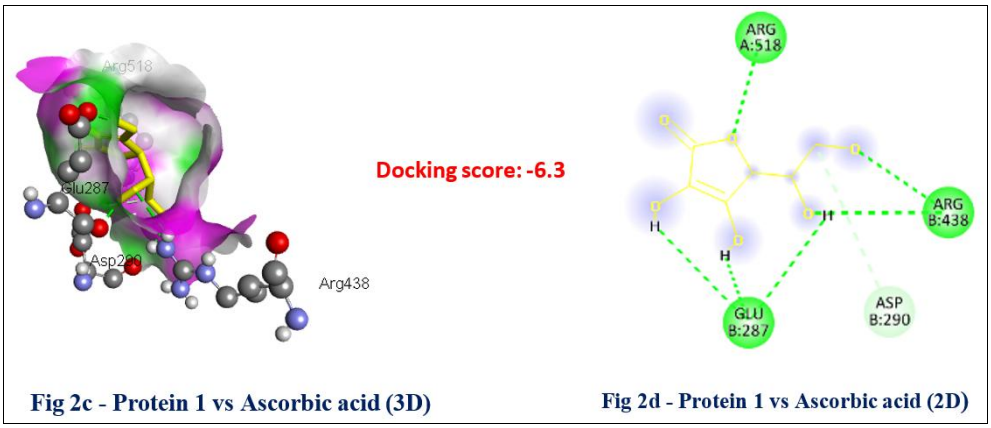



Fig 2c - Protein 1 vs Ascorbic acid (3D) Fig 2d - Protein 1 vs Ascorbic acid (2D)

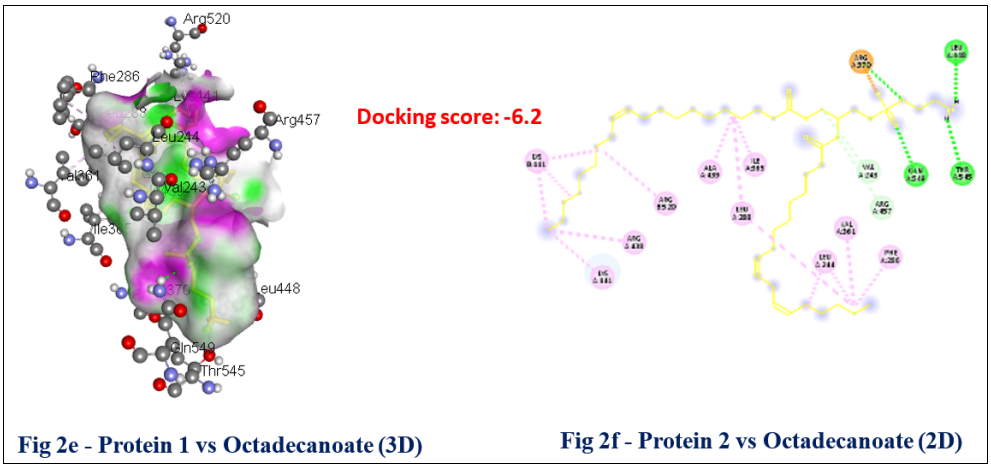



Fig 2e - Protein 1 vs Octadecanoate (3D) Fig 2f - Protein 2 vs Octadecanoate (2D)

**Fig 2:** 3D and 2D structures of ligands with highest binding affinities against Protein 1

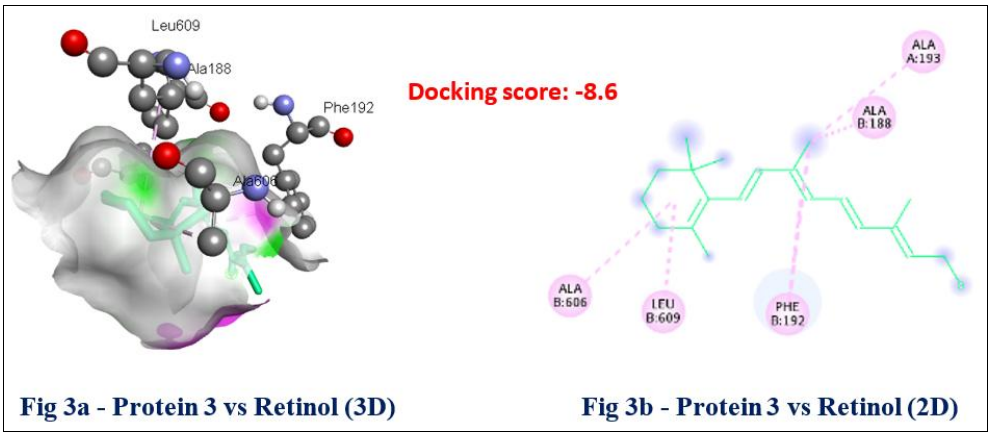



Fig 3a - Protein 3 vs Retinol (3D) Fig 3b - Protein 3 vs Retinol (2D)

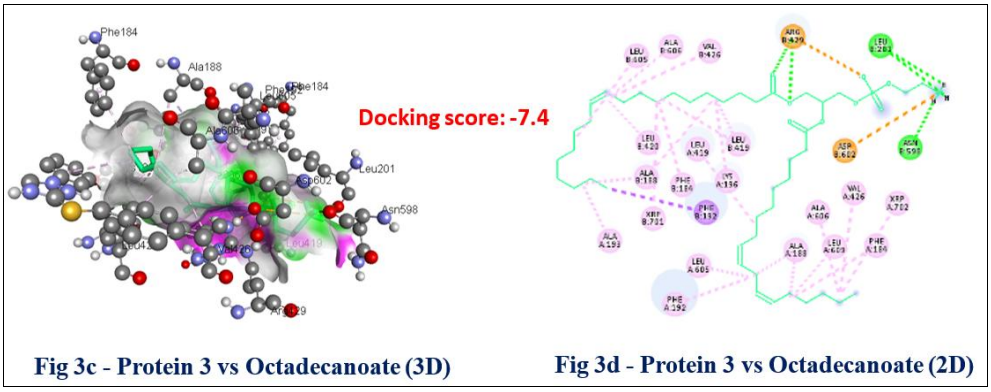
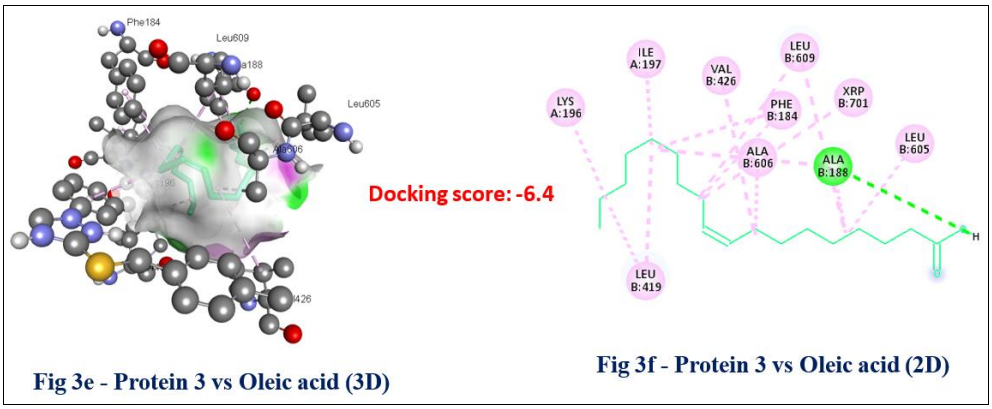



Fig 3c - Protein 3 vs Octadecanoate (3D) Fig 3d - Protein 3 vs Octadecanoate (2D)



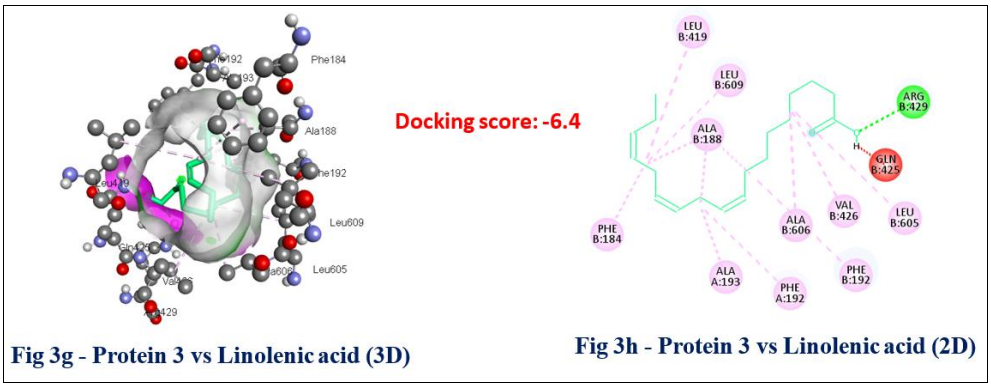




**Fig 3:** 3D and 2D structures of ligands with highest binding affinities against Protein 3

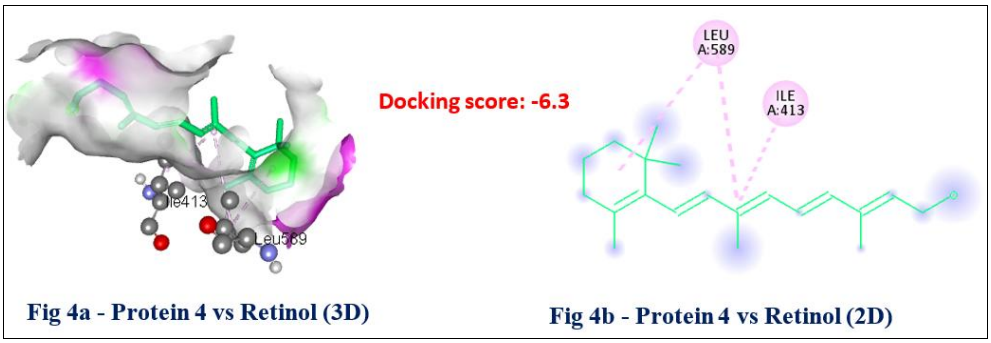



**Fig 4:** 3D and 2D structures of ligands with highest binding affinity against Protein 4

**Table 3:** Docking results of walnut seed phytoconstituents against inflammation resolution mediating enzymes

| S. No. | Ligands | Protein 1 - 12-lipoxygenase (8GHC) | Protein 2 - 15-lipoxygenase (7LAF) | Protein 3 - 5-Lipoxygenase (3O8Y) | Protein 4 - Aspirin Acetylated Cyclooxygenase-2 (5F19) |
|---|---|---|---|---|---|
| 1 | Retinol | -7.8±0.37 | -5.8±0.30 | -8.6±0.37 | -6.3±0.21 |
| 2 | Stearic acid | -5±0.26 | -4.5±0.29 | -5.8±0.16 | -5.4±0.33 |
| 3 | Ascorbic acid | -6.3±0.28 | -5.5±0.20 | -5.7±0.26 | -5.4±0.31 |
| 4 | Palmitic acid | -5.3±0.19 | -4.7±0.19 | -5.8±0.11 | -4.6±0.12 |
| 5 | Nicotinamide | -5.4±0.25 | -5.7±0.33 | -5.3±0.37 | -4.8±0.19 |
| 6 | Octadecanoate | -6.2±0.13 | -3.1±0.04 | -7.4±0.11 | -5.3±0.20 |
| 7 | Oleic acid | -5.7±0.11 | -4.7±0.31 | -6.4±0.18 | -4.6±0.23 |
| 8 | Linolenic acid | -5.3±0.15 | -5.1±0.24 | -6.4±0.15 | -5.9±0.28 |
| 9 | Linoleic acid | -5.7±0.22 | -5.2±0.40 | -6.3±0.11 | -5.6±0.28 |

## Discussion

The SPMs play a critical role in the resolution of inflammation, mainly by the inhibition of chemotaxis, enhancement of efferocytosis and mitigation of the influence of proinflammatory cytokines and chemokines [8, 9]. The synthesis of SPMs largely relies on the availability of omega-3 and omega-6 fatty acids at the site of injury which are mainly derived from PUFA enriched oils from plant and animal origin especially of fish origin. The EPA and DHA originating from fish oil were reported to have multiple health benefits and several studies have now indicated the potential of plant-derived omega-3 PUFAs to resolve inflammation and to protect against inflammatory diseases [10]. The consumption of alpha-linolenic acid of plant origin were reported to increase the proportion of EPA and DHA in membranes of inflammatory cells [11], thereby promotes resolution of inflammation. Weaver *et al*. (2009) [12] reported that high levels of omega-3 PUFA circulating concentrations might modulate the expression of genes known to be critical during inflammatory processes thereby reduces the pro-inflammation and promotes resolution of inflammation.

Walnuts are notable for their omega-3 fatty acid and

phytochemical content, which modulates the gut microbiota and promotes intestinal health [13]. Jimenez-Gomez *et al.*, (2009) [14] reported that walnut oil has been shown to suppress pro-inflammatory markers like TNF-α in peripheral blood mononuclear cells, enhancing systemic anti-inflammatory capacity. In this study, the phytoconstituents of walnut showed high gastrointestinal absorption and better bioavailability score for all constituents except Octadecanoate, this indicates that the walnut seed oil can replenish high amount of bioavailable omega-3 fatty acids at the inflammatory site making themselves available as precursor for the synthesis of SPMs. Further, the boiled egg representation of the phytoconstituents of walnut showed that some of its phytoconstituents to cross BBB which underlines the potential that walnut can be a potential candidate available to reduce neural inflammation.

Hersberger (2010) [15] reported that the anti-inflammatory effects of alpha-linolenic acid is due to its metabolic conversion to EPA and DHA which further acts as substrates for the synthesis of SPMs by the action of LOX and COX enzymes. It is highly evident in this study that linolenic and linoleic acids have good binding interaction with all the resolution mediating enzymes used in this study which might well prepose them to be futuristic candidate to bring resolution of inflammation. Further, being a nut with high nutritional value, walnuts when taken regularly can boast a rich reserve of PUFA in the circulating blood which at the time of crisis can help to curtail acute inflammation and bring resolution of inflammation at the earliest to protect from deadly chronic inflammation disorders.

## Conclusion

This study highlights the therapeutic potential of walnut (*Juglans regia* L.) phytoconstituents in promoting inflammation resolution through *in-silico* molecular docking analysis. Retinol, linolenic acid, and linoleic acid demonstrated strong binding affinities to key resolution enzymes *viz*., 12-LOX, 15-LOX, 5-LOX and COX-2 indicating their capability to modulate inflammatory pathways. Most phytoconstituents exhibited favourable pharmacokinetic properties such as drug-likeness, higher bioavailability and systemic efficacy. These findings suggest that walnut-derived bioactives, particularly omega-3-rich compounds, may serve as promising precursors for SPMs, offering a nutraceutical approach to curtail acute inflammation and further to prevent and manage chronic inflammation-related disorders.

**Acknowledgement:** Authors are highly thankful to Tamil Nadu Veterinary and Animal Sciences University, Chennai for providing technical and financial support.

**Author's Contribution:** Not available.

**Conflict of Interest:** Not available.

**Financial Support:** Not available.

## References

1. Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP. The resolution of inflammation. Nat Rev Immunol. 2013 Jan;13(1):59-66.
2. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2001 Jul;2(7):612-619.
3. Basil MC, Levy BD. Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. Nat Rev Immunol. 2016 Jan;16(1):51-67.
4. Liu D, Guo Y, Ma H. Production, bioactivities and bioavailability of bioactive peptides derived from walnut origin by-products: A review. Crit Rev Food Sci Nutr. 2023;63(26):8032-8047.
5. Ni ZJ, Zhang YG, Chen SX, Thakur K, Wang S, Zhang JG, *et al*. Exploration of walnut components and their association with health effects. Crit Rev Food Sci Nutr. 2022;62(19):5113-5129.
6. Hayes D, Angove MJ, Tucci J, Dennis C. Walnuts (*Juglans regia*) chemical composition and research in human health. Crit Rev Food Sci Nutr. 2016;56(8):1231-1241.
7. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010 Feb;31(2):455-461.
8. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. Identification and signature profiles for proresolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol. 2014 Jul 15;307(2):C39-54.
9. Duvall MG, Levy BD. DHA- and EPA-derived resolvins, protectins, and maresins in airway inflammation. Eur J Pharmacol. 2016 Aug 15;785:144-155.
10. Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM, *et al*. Dietary α-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. Am J Clin Nutr. 2007 Feb;85(2):385-391.
11. Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder PC. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. Prog Lipid Res. 2016 Oct;64:30-56.
12. Weaver KL, Ivester P, Seeds M, Case LD, Arm JP, Chilton FH, *et al*. Effect of dietary fatty acids on inflammatory gene expression in healthy humans. J Biol Chem. 2009 Jun 5;284(23):15400-15407.
13. Byerley LO, Samuelson D, Blanchard IVE, Luo M, Lorenzen BN, Banks S, *et al*. Changes in the gut microbial communities following addition of walnuts to the diet. J Nutr Biochem. 2017 Oct;48:94-102.
14. Jimenez-Gomez Y, Lopez-Miranda J, Blanco-Colio LM, Marín C, Perez-Martínez P, Ruano J, *et al*. Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. Atherosclerosis. 2009 Jun;204(2):e70-76.
15. Hersberger M. Potential role of the lipoxygenase derived lipid mediators in atherosclerosis: leukotrienes, lipoxins and resolvins. Clin Chem Lab Med. 2010 Aug;48(8):1063-1073.

**How to Cite This Article**
Durga Prasad AM, Jaikanth CM, Srinivasan R, Harsitha VM, Thangamalai R. Antimicrobial resistance pattern of staphylococcus species in mastitis affected dairy Cows. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(6):07-12.