

In vitro Evaluation of the Anti-inflammatory and Antioxidant Potential of the Siddha Formulation *Serankotai Nei*

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Abstract

Serankotai Nei is made of purified *Semecarpus anacardium* nuts mixed with milk and ghee has long been credited to be effective in the treatment of inflammatory diseases. The pharmacology of this formulation is unproven. The current research gives the confirmation of its dual antioxidant and anti-inflammatory functions using the standardized in vitro experiments. Three complementary models such as Protein denaturation inhibition, Human Red Blood cell (HRBC) membrane stabilization, and Lipoxigenase (LOX) activity inhibition were used to measure the anti-inflammatory activity. The antioxidant potential was determined with DPPH, hydrogen peroxide, ABTS, and nitric oxide radical scavenging assay at 10–100 mg/mL. Serankotai Nei was found to have a high level of protection at 100–500 mg/mL with up to 89% inhibition of protein denaturation, 84% stabilization of HRBC membranes, and 78% LOX inhibition, similar to the standards of diclofenac sodium and NDGA. The formulation had high dose dependent activity with IC₅₀ value of 2248 mg/mL, which corresponds to standard antioxidants. The results of the study support that Serankotai Nei has a strong dual effect. This bifunctional effect has therapeutic benefits in complex disorders like rheumatoid arthritis, in which oxidative stress and inflammation go hand in hand.

Keywords: Protein Denaturation, Erythrocyte, drug interactions, Lipoxigenase inhibitors, Free Radical Scavengers

1. INTRODUCTION

Oxidative stress and inflammation determine many chronic diseases, such as diabetes, cardiovascular diseases, neurodegenerative diseases, cancer, and aging Nguyen, T. H., Le, H. D., Kim, T. N. T., The, H. P., Nguyen, T. M., Cornet, V., Lambert, J., & Kestemont, P. (2020). In case of inflammation, reactive species are released by activated neutrophils and macrophages. A variety of mechanisms can be used to describe the anti-inflammatory effects of a drug, and they may include Protein Denaturation Inhibition, Cyclooxygenase (COX) pathway inhibition, Free Radical Scavenging, neutralizing ROS to prevent cellular damage, Cytokine Modulation, NF-κB Pathway inhibition, Lipoxigenase (LOX) interference (Ramos-González, E. J., Bitzer-Quintero, O. K., Ortiz, G., Hernández-Cruz, J. J., & Ramírez-Jirano, L. J. (2021)., Bhol, N. K., Bhanjadeo, M. M., Singh, A. K., Dash, U. C., Ojha, R. R., Majhi, S., Duttaroy, A. K., & Jena, A. B. (2024)., Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M.,

et al. (2024). Oxidative stress is exacerbated by excess reactive oxygen / nitrogen species which increase the expression of pro-inflammatory genes. Agents that have anti-inflammatory and antioxidant properties are therefore useful. *Semecarpus anacardium* Linn. (popularly referred to as Serankotai or Bhallataka) has been a well known Siddha and Ayurvedic medicine due to its wide range of medicinal uses. Serankotai nei, is cooked using purest Serankotai (Vallathy), which is mentioned in Agathiyar Vaithiya Vallathy (Mohan, 2001). It has been found that Serankotai has antifungal, anti-inflammatory, antioxidant, antibacterial, anticarcinogenic, antiaatherogenic properties, and improves memory (Sharma, R. A., & Yadav, N 2022). It is reported to be skin irritant due to its nuts and oil because they have the oxirane $C_{13}H_{18}O_3$ functional group (Gopinath & Arunadevi, 2024). Therefore, the purification of semecarpus should be performed cautiously *Semecarpus anacardium* Linn. fruits were reported to have an important anticancer effect by inducing apoptosis, preventing tumor growth, and changing the immune reactions, thus justifying their use in cancer treatment in the past Mishra, A. K., Neha, S. L., Jain, A., Jagtap, C. Y., Dane, G., Paroha, S., & Sahoo, P. K. (2024). Although there are these reports, pharma-logical validation of the classical Siddha formulation Serankotai Nei which is prepared using milk and ghee as its vehicles is limited. Its scientific foundation is the key to the reconciliation of traditional assertions with modern pharmacology as well as to investigate its role as a complementary therapy agent.

As such, the current research was taken to critically analyze the anti-inflammatory and antioxidant capacity of Serankotai Nei with the help of standardized in vitro tests. The objective of this work is to present experimental data that will substantiate the conventional assertions, as well as to establish a basis on which future mechanistic/clinical studies can be conducted.

2. METHODOLOGY:

2.1 Plant authentication :

Nuts of Serankottai (*Semecarpus anacardium* Linn.) were harvested in the forest areas of Coimbatore districts in Tamil Nadu. The vegetable matter received adequate taxonomic identification and authentication and a specimen numbered PARC/2023/3985 was allocated to it. Authentication was done by DR.P. Murugan , Botanist, Sri Sairam Siddha Medical College and Research Centre .

2.2. Purification of Serankotai nuts:

Different nuts are then taken and cleaned by washing them carefully using a soft cotton cloth to get off all the surface impurities. Since the seeds are corrosive in nature, they are therefore purified and used in medicines. The traditional method involves a three day period of moistening of the nuts in cow milk with the milk being renewed after 24 hours or they can be moistened in the milk by boiling Llanchezian, R., Joseph, C. R., & Rabinarayan, A(2012). The proteins in the milk serve to counter the corrosive phenolic elements, such as urushiol such as Bhilawanol and anacardic acid and minimise the irritant activity. The cotyledons are then removed carefully by soaking and the nuts dried thoroughly by being exposed to the sun till hard. The completely dried and de-toxicated nuts are then extracted or added to ghee (Nei) to formulate therapeutic preparations. This purification phase is important to note, as the nut oil which is not purified can give rise to free radicals and cause an undesirable reaction like factitious dermatitis Neema, S., Dabbas, D., Pathania, V., & Vasudevan, B. (2023), Tripathi, Y. B., Pandey, N., & Tripathi, P. (2008).

2.3 Preparation of Serankotai nei :

Pure nuts of *Semecarpus anacardium*, cow milk and ghee are the main ingredients used to make the formulation called Siddha formulation Serankottai Nei. First, 200 g of decoction purified nuts is boiled in 500 ml of cow milk until the active principles are released into the decoction. A decantation is followed by again repeating the procedure with a fresh 500 ml of milk to allow complete removal of irritant compounds and as many therapeutic constituents as possible to be obtained. These milk decoctions are then combined and filtered and clarified butter (ghee) is added which would be a stabilizing and cooling medium. This mixture is then heated to low flame till all the milk is evaporated

and the medicated ghee is left behind rich in the nut extract. The end product is cooled down, filtered to eliminate coarse particles and kept in airtight containers. This method of preparation enables the neutralization of the corrosive nature of the nut besides increasing bioavailability, decreasing irritancy, and reproducibility (Vijayalakshmi, T., Muthulakshmi, V., & Sachdanandam, P., 2000). Serankotai Nei administered in 5-10 ml volumes with warm water, twice or thrice a day, on an empty stomach, in combination with severe dieting, is safe and does not cause side effects to treat chronic diseases such as arthritis, neurological and skin diseases.

2.4 Anti-Inflammatory Activity

2.4.1 Egg Albumin Denaturation Assay

The anti-inflammatory effect of unidentified crude extracts can be determined in vitro by avoiding the degradation of egg albumin. To prepare a 5mL reaction mixture, 0.2 mL of 1-2% egg albumin solution (either fresh hen eggs or commercially available egg albumin powder) were mixed with 2 mL of sample extract or standard (Diclofenac sodium) at various concentrations and 2.8mL of phosphate-buffered saline at a room temperature of 37°C. The absorbance at 280 nm was measured in an appropriate UV/Vis spectrophotometer after cooling using triple-distilled water as a blank (Madhuranga and Samarakoon, 2023).

2.4.2 Heat-Induced Haemolysis Of The Rbc Membrane

1ml of the test sample (acetyl salicylic acid as standard) at concentrations of 100, 200, 300, 400, and 500 µg/mL in isotonic phosphate buffered saline (PBS) and 1ml of a 10% RBC solution made up the assay combination. After centrifuging the reaction mixtures for five minutes at 2800 g and incubating them for a total of twenty minutes at 54 °C in a water bath, the absorbance of the supernatant was measured at 540 nm using a spectrophotometer (Hasan et al., 2024). The following equation was used to determine the percent inhibition of haemolysis:

$$\text{Inhibition of haemolysis (\%)} = 100 \times \left[1 - \frac{OD_2 - OD_1}{OD_3 - OD_1} \right]$$

where OD₁ = unheated test sample, OD₂ = heated test sample, and OD₃ = heated control sample.

2.4.3 Lipoxygenase Inhibitory Assay

Lipoxygenase inhibitory tests were conducted in line with one of the published procedures, using the Lipoxygenase Inhibitor Screening Assay (LISA) Kit (Cayman Chemical Co.). This kit included 0.1 M of Tris-HCl buffer (pH 7.4), 1 and 2 of developing agents (chromogen), soybean 15-lipoxygenase (15-LOX), arachidonic acid and KOH. The assay buffer was then diluted by ten folds using HPLC-grade water. Chromogen was prepared newly by combining the two agents 1 and 2 in equal parts. In the case of blanks 100 mL of buffer was added in the wells and in positive controls, 990 mL of buffer and 10 mL of 15-LOX. A solution with substrate was prepared by adding 25 mL KOH and 25 mL arachidonic acid in ethanol and diluted with 950 mL water and utilized after 30 minutes. Sample, buffer (980 mL), and 15-LOX (10 mL) were added to test wells after which 10 mL of substrate was added to initiate reactions. It was stirred by shaking plates 5-10 min after which 100 mL of chromogen was added to end catalysis. The amount of hydroperoxides formed was recorded at 490 nm (Sharanya et al., 2023). NDGA was used as standard, and five concentrations (100-500mg/mL) of Serankotai nei were conducted in duplicates. The following equation was used to determine the percent inhibition:

$$\text{Percentage inhibition} = \left[\frac{\text{Amount of HP produced in the blank sample} - \text{Amount of HP produced in the Test Sample}}{\text{Amount of HP produced in the blank sample}} \right] \times 100$$

2.5 Antioxidant Assay:

2.5.1 DPPH (2, 2-Diphenyl 1-2 Picrylhydrazyl) Assay

The antioxidant activity of the test drug sample Serankotai nei was measured using a 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) assay, which is a free radical scavenging assay. DPPH scavenging assay was

detected by the use of standard ascorbic acid and Serankotai nei 10-100 mg/ ml concentration of the sample solution was combined with 1 ml of 0.3 mM DPPH methanol solution to form the final mixture which was allowed to react at room temperature. After incubation at 37°C (USER) absorbance was determined with Serankotai nei at different concentrations (10 ug, 20 ug, 40 ug, 60 ug, 80 ug, and 100 ug/ml). The absorbing sample was prepared with methanol as a blank and the absorbance was measured at 517 nm in a double-beam UV spectrophotometer (Sivaraman and Perumal, 2015). The DPPH radical scavenging activity of the Serankotai nei and Ascorbic acid as a percentage was determined by the following formula:

$$\text{Percentage DPPH Radical scavenging} = \frac{A(C)-A(S)}{A(C)} \times 100$$

Where,

A -Absorbance , C- Control , S – Sample

The effective concentration of *Serankotai nei* required to scavenge DPPH radical by 50% (IC₅₀ value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations.

2.5.2 Nitric Oxide Radical Scavenging Assay

Lipoxygenase inhibitory tests were conducted according to a published procedure by using Lipoxygenase Inhibitor Screening Assay (LISA) Kit (Cayman Chemical Co.). The kit was made up of the following products: Tris-HCl buffer (0.1 M, pH 7.4), chromogen (agent 1 and 2), soybean 15-lipoxygenase (15-LOX), arachidonic acid, and KOH. The Assay buffer was diluted 10 times with water of HPLC grade. The agents 1 and 2 were mixed in equal proportions to prepare chromogen. In the case of blanks, 100 mL buffer was incubated in wells, positive controls had 990 mL buffer and 10 mL 15-LOX. The substrate solution (25 mL of KOH + 25 mL arachidonic acid in ethanol, dissolved by 950 mL of water) was utilized in 30 minutes. Sample, 15-LOX, and buffer were added to test wells (10 mL sample, 980 mL buffer, and 10 mL 15-LOX), then reactions were initiated by the addition of 10 mL substrate. Plates were shaken 5 min, followed by the addition of 100 mL of chromogen to stop catalysis. The measurement of hydroperoxides was done at 490 nm. NDGA became a standard, and five (100-500 mg/mL) concentrations of *Serankotai nei* were run in a duo (Siripongvutikorn et al., 2024). The percentage nitrite radical scavenging activity of the *Serankotai nei* and gallic acid was determined with the help of the following formula:

$$\text{Percentage NO Radical scavenging} = \frac{A(C)-A(S)}{A(C)} \times 100$$

Where,

A -Absorbance , C- Control , S – Sample

2.6 Statistical Analysis :

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test.

3. RESULTS

4. DISCUSSION

The current study confirms the protective effect of the Siddha formulation *Serankotai Nei* in inflammation and oxidative stress, two mutually enhancing processes of pathogenesis of chronic diseases like rheumatoid arthritis, diabetes, cancer, and neuro degeneration.

The use of herbal preparations as a possible alternative to conventional medications is gaining momentum because of the limitations of existing treatments of acute and chronic inflammatory conditions (Gupta, M., Singh, N., Gulati, M., Gupta, R., Sudhakar, K., & Kapoor, B ., 2021). *Serankotai nei* was used in this study at five concentrations (100-500 µg/mL) on three assays of anti-inflammatory tests.

One of the most common pathological processes in rheumatoid arthritis, cancer, and diabetes is protein denaturation, as autoantigens cause an inflammatory response (Silvestrini & Silvestrini, 2023; Dharmadeva, S., Galgamuwa, L. S., Prasadine, C., & Kumarasinghe, N., 2018). In the egg albumin assay, Serankotai nei gave a dose-dependent inhibition of 76.33% at 500 µg/mL, which is lesser than diclofenac sodium (95.83%)(Figure 1).

The anti-inflammatory activity was also confirmed by the HRBC membrane stabilization to 78.8% and 97.54% inhibition of hemolysis, respectively, in the presence of 500 µg/mL of the compound and diclofenac (Figure 2). It is essential because the release of lysosomal enzymes due to the instability of cells aggravates tissue damage (Yesmin et al., 2020, Bi, J., Sun, Y., Guo, M., Sun, X., Sun, J., Jiang, R., Wang, N., & Huang, G., 2025).

Therefore, Serankotai nei exhibits protection effects similar to NSAIDs and natural bioactive compounds. Clinical significance of stabilization of erythrocyte and lysosomal membranes is that, attack of these membranes causes release of proteolytic enzymes that increase tissue injury. Serankotai Nei has the potential to reduce secondary injury in the process of inflammation by maintaining membrane integrity, which augments its protein stabilizing activity.

Another significant inhibition was lipoxygenase (LOX) inhibition with Serankotai nei performing with an 82.1% inhibition at 500 µg/mL which is similar to that of NDGA (91.5%) (Figure 3). As LOX enzymes are linked to the synthesis of leukotriens using arachidonic acid, the result suggests the high potential of Serankotai nei in the inhibition of pro-inflammatory pathways(Wang et al., 2021). Lipoxygenase inhibition showed the highest inhibitory process with the highest inhibitor concentration of 82.1% in Serankotai Nei at 500 µg/mL similar to the standard NDGA, indicating the potent inhibitory capacity of the compound with inflammatory leukotriene pathways (Table 1). This makes the formulation a candidate in conditions where leukotriene signalling is the central role.

The antioxidant assay showed moderate and significant free radical scavenging. In DPPH assay, the inhibition was between 9.64% and 44.19%, and the IC₅₀ was 115.2 µg/mL versus 35.71 µg/mL in ascorbic acid (Sadowska Bartosz & Bartosz, 2022). The level of nitric oxide scavenging was lower (IC₅₀ 139.2 µg/mL compared with 35.41 µg/mL of gallic acid) (Table 2), whereas the level of ABTS inhibition was rather higher (IC₅₀ 93.85 µg/mL compared with 19.47 µg/mL of gallic acid). Scavenging of hydrogen peroxide was mediocre (IC₅₀ 136.8 µg/mL vs. 33.4 µg/mL of BHA)(Table 3). Among them, the greatest inhibition was demonstrated with ABTS, which implies that it has moderate but steady antioxidant activity.

Oxidative stress is an interdependent process with inflammation (Manful, C. F., Fordjour, E., Ikumoinin, E., Abbey, L., & Thomas, R., 2025 Sharifi Rad et al., 2020). During inflammation, reactive oxygen and nitrogen species include superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite that are produced by activated neutrophils and macrophages (Canton, M., Sánchez-Rodríguez, R., Spera, I., Venegas, F. C., Favia, M., Viola, A., & Castegna, A. (2021). Agents which can regulate both oxidative stress and inflammatory processes can therefore have holistic therapeutic effects. Such a candidate is serankotai nei, which is dual-active.

The antioxidant and anti-inflammatory activity of Serankotai Nei is(provides) a therapeutic benefit over single target therapies, especially in multifactorial pathologies such as rheumatoid arthritis in which oxidative stress and inflammation are inseparable.

The future directions involve the use of molecular docking experiments to determine active components, animal experiments to determine in vivo applicability, and clinical trials to determine effectiveness in chronic inflammatory diseases. The involvement of cytokine modulation and NF-κB in pathology should be further mechanistically clarified, which will enhance the pharmacological foundation of this Siddha formulation.

5. CONCLUSION

This study provides the first pharmacological evidence that *Serankotai Nei* exerts strong anti-inflammatory and antioxidant effects, validating its traditional use. The dual activity highlights its

translational potential for disorders such as Dermatitis and **rheumatoid arthritis**, where oxidative stress and inflammation are tightly interlinked. Unlike single-target therapies, *Serankotai Nei* offers the advantage of simultaneously modulating inflammatory pathways and neutralizing free radicals, thereby broadening its therapeutic relevance. Future work should advance this formulation through **in vivo validation, mechanistic pathway studies, and clinical trials**, paving the way for its integration as a complementary therapy in modern medicine.

6. CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this article.

7. ACKNOWLEDGEMENTS

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9. ABBREVIATIONS :

HRBC – Human Red Blood Cell, **LOX** – Lipoxygenase, **COX** – Cyclooxygenase, **NF-κB** – Nuclear Factor kappa-light-chain-enhancer of activated B cells, **ROS** – Reactive Oxygen Species, **DPPH** – 2,2-Diphenyl-1-picrylhydrazyl (radical scavenging assay), **ABTS** – 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (radical scavenging assay), **NO** – Nitric Oxide (radical scavenging assay), **PBS** – Phosphate Buffered Saline, **UV/Vis** – Ultraviolet/Visible (spectrophotometry), **HPLC** – High Performance Liquid Chromatography, **DS** – Diclofenac Sodium, **NDGA** – Nordihydroguaiaretic Acid, **AA** – Ascorbic Acid, **GA** – Gallic Acid, **BHA** – Butylated Hydroxyanisole, **IC₅₀** – Inhibitory Concentration at 50% (measure of potency), **ANOVA** – Analysis of Variance, **SD** – Standard Deviation, **OD** – Optical Density, **HP** – Hydroperoxides, **RBC** – Red Blood Cell

10. AUTHORS CONTRIBUTION

P. Chakravarthi – Software, Data curation, Conceptualization, Investigation S. Kanimozhi - Writing - Original draft, Validation, Resources, Supervision E. Nandhini - Methodology, Reviewing and Editing, R. Sathish Adithya - Reviewing and Editing, Validation

10. REFERENCES

- Bi, J., Sun, Y., Guo, M., Sun, X., Sun, J., Jiang, R., Wang, N., & Huang, G. (2025). Lysosomes: guardians and healers within cells- multifaceted perspective and outlook from injury repair to disease treatment. *Cancer cell international*, 25(1), 136. <https://doi.org/10.1186/s12935-025-03771-5>
- Bhol, N. K., Bhanjadeo, M. M., Singh, A. K., Dash, U. C., Ojha, R. R., Majhi, S., Duttaroy, A. K., & Jena, A. B. (2024). The interplay between cytokines, inflammation, and antioxidants: mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine compounds. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 178, 117177. <https://doi.org/10.1016/j.biopha.2024.117177>
- Canton, M., Sánchez-Rodríguez, R., Spera, I., Venegas, F. C., Favia, M., Viola, A., & Castegna, A. (2021). Reactive oxygen species in macrophages: Sources and targets. *Frontiers in Immunology*, 12, 734229. <https://doi.org/10.3389/fimmu.2021.734229>
- Dharmadeva, S., Galgamuwa, L. S., Prasadanie, C., & Kumarasinghe, N. (2018). In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *Ayu*, 39(4), 239–242. https://doi.org/10.4103/ayu.AYU_27_18
- Gopinath, P., & Arunadevi, R. (2024). Acute toxicity study of hexane extract of *Sodhita Semecarpus anacardium* L. drupe in Wistar albino rats and its prediction using in-silico tool. *TI*, 31(1), 1–8. <https://doi.org/10.18311/ti/2024/v31i1/35349>
- Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., et al. (2024). NF-κB in biology and targeted therapy: New insights and translational implications. *Signal Transduction and Targeted Therapy*, 9(1), 53. <https://doi.org/10.1038/s41392-024-01757-9>

- Gupta, M., Singh, N., Gulati, M., Gupta, R., Sudhakar, K., & Kapoor, B. (2021). Herbal bioactives in treatment of inflammation: An overview. *South African Journal of Botany*, 143, 205–225. <https://doi.org/10.1016/j.sajb.2021.07.027>
- Hasan, M. M., Islam, M. E., Hossain, M. S., Akter, M., Rahman, M. A. A., Kazi, M., Khan, S., & Parvin, M. S. (2023). Unveiling the therapeutic potential: Evaluation of anti-inflammatory and antineoplastic activity of *Magnolia champaca* Linn's stem bark isolate through molecular docking insights. *Heliyon*, 10(1), e22972. <https://doi.org/10.1016/j.heliyon.2023.e22972>
- Kim, M. E., & Lee, J. S. (2025). Advances in the regulation of inflammatory mediators in nitric oxide synthase: Implications for disease modulation and therapeutic approaches. *International Journal of Molecular Sciences*, 26(3), 1204. <https://doi.org/10.3390/ijms26031204>
- Llanchezian, R., Joseph, C. R., & Rabinarayan, A. (2012). Urushiol-induced contact dermatitis caused during *Shodhana* (purificatory measures) of *Bhallataka* (*Semecarpus anacardium* Linn.) fruit. *Ayu*, 33(2), 270–273. <https://doi.org/10.4103/0974-8520.105250>
- Madhuranga, H. D. T., & Samarakoon, D. N. A. W. (2023). In vitro anti-inflammatory egg albumin denaturation assay: An enhanced approach. *Natural Ayurvedic Medicine*, 7(3), 000411.
- Manful, C. F., Fordjour, E., Ikumoinein, E., Abbey, L., & Thomas, R. (2025). Therapeutic strategies targeting oxidative stress and inflammation: A narrative review. *BioChem*, 5(4), 35. <https://doi.org/10.3390/biochem5040035>
- Mishra, A. K., Neha, S. L., Jain, A., Jagtap, C. Y., Dane, G., Paroha, S., & Sahoo, P. K. (2024). Effectiveness of *Semecarpus anacardium* Linn. fruits in cancer and inflammatory diseases: A mini review. *Fitoterapia*, 175, 105978. <https://doi.org/10.1016/j.fitote.2024.105978>
- Mohan, R. C. (2001). *Agathiyar Vaidhiya Vallathy-600*. Thamarai Noolagam.
- Neema, S., Dabbas, D., Pathania, V., & Vasudevan, B. (2023). Marking nut dermatitis: A case series on factitious dermatitis. *Medical Journal Armed Forces India*, 79(4), 470–473. <https://doi.org/10.1016/j.mjafi.2020.11.022>
- Nguyen, T. H., Le, H. D., Kim, T. N. T., The, H. P., Nguyen, T. M., Cornet, V., Lambert, J., & Kestemont, P. (2020). Anti-Inflammatory and Antioxidant Properties of the Ethanol Extract of *Clerodendrum Cyrtophyllum* Turcz in Copper Sulfate-Induced Inflammation in Zebrafish. *Antioxidants (Basel, Switzerland)*, 9(3), 192. <https://doi.org/10.3390/antiox9030192>
- Ramos-González, E. J., Bitzer-Quintero, O. K., Ortiz, G., Hernández-Cruz, J. J., & Ramírez-Jirano, L. J. (2021). Relationship between inflammation and oxidative stress and its effect on multiple sclerosis. *Neurología*, 39(3). <https://doi.org/10.1016/j.nrleng.2021.10.010>
- Sadowska-Bartosz, I., & Bartosz, G. (2022). Evaluation of the antioxidant capacity of food products: Methods, applications and limitations. *Processes*, 10(10), 2031. <https://doi.org/10.3390/pr10102031>
- Sharanya, C., Abhithaj, J., Arun, K., Eeda, Koti Reddy, Bhat, Vignesh, Variyar, E.J. Sabu, A , Haridas, M. (2023). Lipoxigenase inhibitory synthetic derivatives of methyl gallate regulate gene expressions of COX-2 and cytokines to reduce animal model arthritis. *Scientific Reports*, 13, 10644. <https://doi.org/10.1038/s41598-023-37613-z>
- Sharifi-Rad, M., Kumar, N. V. A., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Mishra, A. P., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N., Calina, D., Cho, W. C., & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694. <https://doi.org/10.3389/fphys.2020.00694>
- Sharma, A., Sharma, R. A., & Yadav, N. (2022). In vitro antimicrobial activity of *Semecarpus anacardium* nuts against human pathogenic bacteria. *International Journal of Life Science and Pharmaceutical Research*, 12(1), L83–L91. Available from: <https://ijlpr.com/index.php/journal/article/view/1035>
- Silvestrini, B., & Silvestrini, M. (2023). Medical implications of the relationships among protein denaturation, necrosis and inflammation: An intriguing story. In *Tendons—Trauma, inflammation, degeneration, and treatment*. London: IntechOpen. <https://doi.org/10.5772/intechopen.108018>

Siripongvutikorn, S., Pumethakul, K., Yupanqui, C. T., Seechamnaturakit, V., Detarun, P., Utaipan, T., Sirinupong, N., Chansuwan, W., Wittaya, T., Samakradhamrongthai, R. S. (2024). Antioxidant and nitric oxide inhibitory activity of the six most popular instant Thai curries. *Foods*, 13(2), 178. <https://doi.org/10.3390/foods13020178>

Sivaraman, D., & Perumal, P. (2015). In-vitro screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of *Ipomoea aquatica* Forsk: Therapeutic lead for Alzheimer's disease. *Journal of Applied Pharmaceutical Science*, 5(2), 12–16.

Tripathi, Y. B., Pandey, N., & Tripathi, P. (2008). Purification of nuts of *Semecarpus anacardium* Linn., a herbal drug for arthritis. *Indian Journal of Experimental Biology*, 46(6), 447–452.

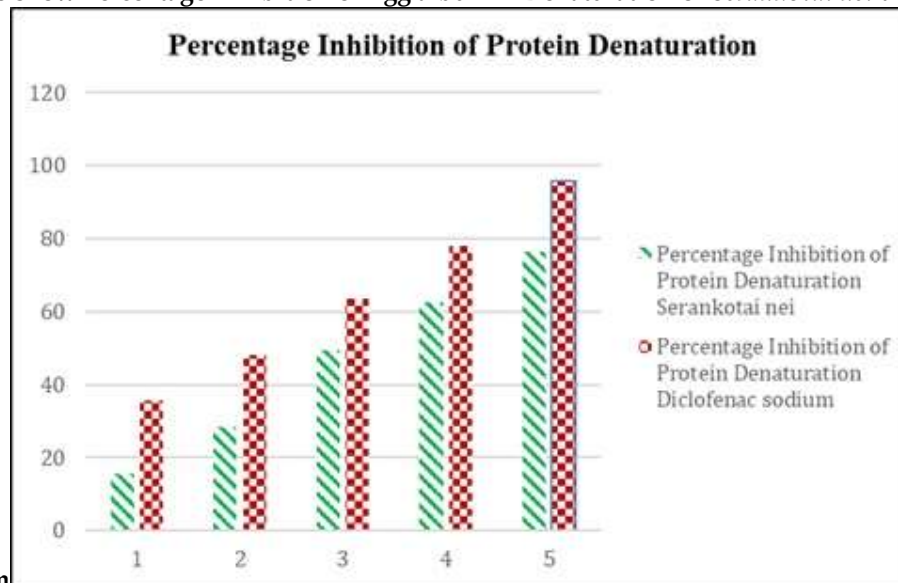
Vijayalakshmi, T., Muthulakshmi, V., & Sachdanandam, P. (2000). Toxic studies on biochemical parameters carried out in rats with *Serankottai nei*, a Siddha drug—Milk extract of *Semecarpus anacardium* nut. *Journal of Ethnopharmacology*, 69(1), 9–15. [https://doi.org/10.1016/S0378-8741\(99\)00020-3](https://doi.org/10.1016/S0378-8741(99)00020-3)

Wang, Bei, Wu, Lujin, Chen, Jing, Dong, Lingli, Chen, Chen, Wen, Zheng, Hu, Jiong, Fleming, Ingrid, Wang, Dao Wen (2021). Metabolism pathways of arachidonic acids: Mechanisms and potential therapeutic targets. *Signal Transduction and Targeted Therapy*, 6(1).

Yesmin, S., Paul, A., Naz, T., Rahman, A. A., Akhter, S. F., Wahed, M. I., Emran, T. B., & Siddiqui, S. A. (2020). Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of *Choi* (*Piper chaba*). *Clinical Phytoscience*, 6(1), 59.

3.1 Egg Albumin Denaturation Assay :

Figure 1 : Graph to show Percentage Inhibition of Egg albumin Denaturation of *Serankottai nei* and

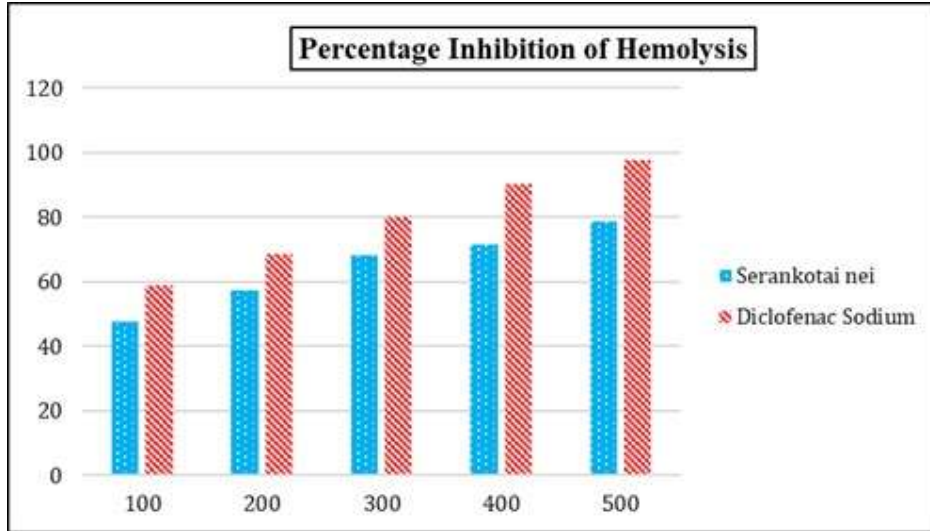


Diclofenac sodium

Data are given as Mean \pm SD (n=3)

3.2 Heat-Induced Haemolysis Of The Hrbc Membrane

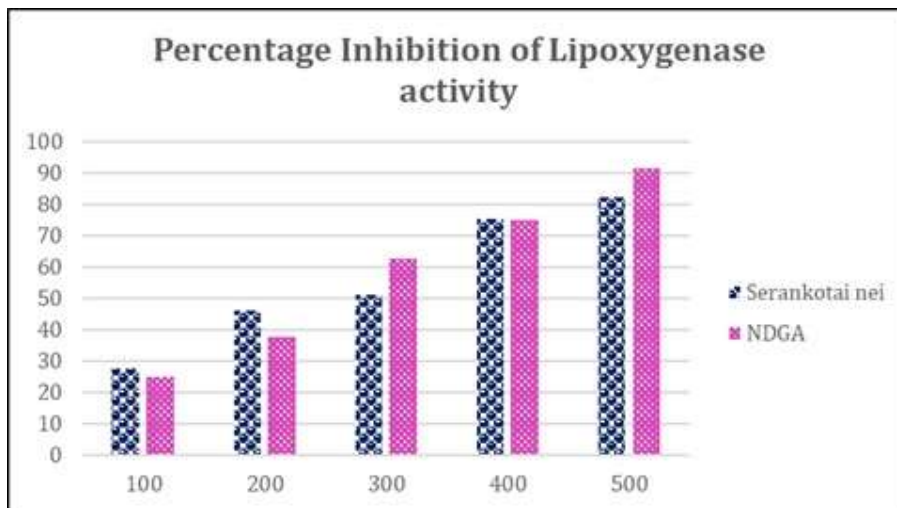
Figure 2 : Graph to show Percentage Inhibition of Haemolysis of *Serankottai nei* and Diclofenac sodium



Data are given as Mean \pm SD (n=3)

3.3 Lipoxygenase Inhibitory Assay

Figure 3 : Graph to show Percentage Inhibition of Haemolysis of *Serankotai nei* and NDGA



Data are given as Mean \pm SD (n=3)

Table 1 Percentage Inhibition seen in inhibition using Egg Albumin Denaturation , Hemolysis , Lipoxygenase Activity for *Serankotai nei* and standard Data are given as Mean \pm SD (n=3) (Sn = Serankotai Nei, DS= Diclofenac sodium , GA = Gallic Acid, NDGA = Nordihydroguaiaretic Acid)

Table 2 : Percentage inhibition of Serankotai Nei compared with standard antioxidants (Ascorbic acid, Gallic acid, BHA) in four radical scavenging assays (DPPH, Nitric Oxide, Hydrogen Peroxide, ABTS)

at concentrations 10–100 µg/mL. Values are Mean ± SD (n = 3). Higher % inhibition indicates stronger scavenging activity. (Sn = Serankotai Nei, AA = Ascorbic Acid, GA = Gallic Acid, BHA = Butylated Hydroxyanisole)

Concentration (µg/ml)	DPPH Radical Scavenging Assay	Nitric Oxide Radical Scavenging Assay	Hydrogen Peroxide Radical Scavenging Assay	ABTS Radical Scavenging Assay
Concentration (µg/mL)	% Inhibition of Albumin Denaturation	% Inhibition of Egg Inhibition	% Inhibition of Assay	% Inhibition of Lipoxygenase Activity
100	Sn:15.55 ± 1.88	Sn:47.51 ± 0.54		Sn:27.63 ± 1.22
10 µg/ml	Sn: 9.642 ± 5.385; AA: 30 ± 12.57	Sn : 4.612 ± 3.781 GA:24.64 ± 5.808	Sn : 8.62 ± 5.533 BHA: 35.07 ± 3.859	Sn : 8.351 ± 4.671 GA:34.54 ± 1.81
20 µg/ml	Sn: 15.22 ± 0.174; AA: 40.53 ± 1.062	Sn : 12.63 ± 3.185 ± 0.21 GA:39.24 ± 6.765 ± 1.23	Sn : 13.88 ± 5.314 BHA: 43.22 ± 3.698	Sn: 19.09 ± 0.3204 GA:15.92 ± 0.366
40 µg/ml	Sn: 22.32 ± 2.015; AA: 52.93 ± 3.604	Sn : 20.29 ± 3.21 GA:50.6 ± 4.376	Sn : 20.87 ± 6.263 BHA: 56.49 ± 3.396	Sn : 30.05 ± 7.516 GA:65.46 ± 1.146
60 µg/ml	Sn: 29.15 ± 1.245; AA: 76.37 ± 1.2699	Sn : 26.05 ± 2.18 ± 0.23 GA:59.35 ± 7.887 ± 1.591	Sn : 26.86 ± 4.384 BHA: 64.16 ± 10.88	Sn: 37.48 ± 10.67 GA:81.34 ± 1.012
80 µg/ml	Sn: 39.32 ± 7.676; AA: 75.77 ± 2.641	Sn : 31.67 ± 4.917 GA:82.36 ± 4.907	Sn : 32.36 ± 5.291 BHA: 79.15 ± 8.317	Sn : 44.73 ± 8.511 GA:88.85 ± 0.7407
100 µg/ml	Sn: 44.19 ± 6.359; AA: 90.71 ± 3.358	Sn : 35.06 ± 5.13 GA:93.84 ± 4.548	Sn : 37.22 ± 4.684 BHA: 86.85 ± 7.889	Sn : 52.91 ± 10.07 GA:98.84 ± 0.2587

Table 3 : IC₅₀ values (µg/mL ± SD) of *Serankotai Nei* compared with standard antioxidants in four radical scavenging assays (DPPH, Nitric Oxide, Hydrogen Peroxide, and ABTS). Data are expressed as mean ± SD (n = 3). Lower IC₅₀ values indicate stronger radical scavenging activity

Assay	Test Drug / Standard	IC ₅₀ Value (µg/mL) ± SD
DPPH Radical Scavenging	<i>Serankotai Nei</i>	115.2 ± 22.73
	Ascorbic Acid	35.71 ± 8.778
Nitric Oxide Scavenging	<i>Serankotai Nei</i>	139.2 ± 16.58
	Gallic Acid	35.41 ± 2.198
Hydrogen Peroxide Scavenging	<i>Serankotai Nei</i>	136.8 ± 13.28
	BHA	33.4 ± 8.017
ABTS Radical Scavenging	<i>Serankotai Nei</i>	93.85 ± 24.16
	Gallic Acid	19.47 ± 1.423