In Vitro Effect of *Terminalia Arjuna* (Roxb.) Wight & Arn Plant Extracts Against Anti-Arthritic and Anti- Glycation Activities in Egg Albumin

Alagusundaram. P[1]*, Asharaja A[1]

[1]*Research scholar (Reg No:21111152021002), Department of Zoology, PMT College, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli), Melaneelithanallur-627 953, Tenkasi District, Tamil Nadu, India
[1]Assistant Professor, Department of Zoology, PMT College, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli), Melaneelithanallur-627 953, Tenkasi District, Tamil Nadu, India

***Corresponding Author:** Alagusundaram. P
*Email: alagusundaram143@gmail.com

Abstract
Many natural plant ingredients have been utilized for centuries in siddha therapy to suppress inflammatory pathways and wound heal in diabetic patient with little side effects. Many parts of the *Terminalia arjuna* plant are utilized in siddha medicine to treat a range of illnesses. However, there is a dearth of literature evaluating the pharmacological characteristics with anti-inflammatory and anti-glycation activities. The aim of the study was to investigate anti-inflammatory and anti- glycation property of *T. arjuna* using egg albumin denaturation activity. *Terminalia arjuna* (bark and leaf) extraction was performed using heated at 70°C for 2 hour. The concentration gradient of extracts was prepared using water and 70% ethanol. The extract with egg albumin was incubated in a water bath at 57 oC for 3 minutes and One nonsteroidal anti-inflammatory drug acetyl salicylic acid was used as reference drugs. The percentage inhibition of protein denaturation was calculated by spectrophotometry. . A mixture of egg albumin (EA) and 500 mM glucose in 100 mM phosphate-buffered saline (pH 7.4) was incubated at 37°C for 20 days in the dark with a gentle shaking followed by the addition of plant extracts. The percentage inhibition of advanced glycation end products (AGEs) was calculated by spectrophotometry. Both aqueous and 70% ethanol extracts from leaves exhibited substantial inhibition percentages, particularly at higher concentrations (48% of 200,59% of 400 µg/ml, 61.22% 600 µg/ml), (52% of 200,64% of 400 µg/ml, 71% 600 µg/ml) respectively over bark extract. The inhibition rate of egg albumin denaturation in extraction increased gradually with concentration. Significantly higher inhibition was showed in leaf extract than bark extracts. In addition, Lowest browning of 78.5 %,64.22%,39% were seen in the ethanolic extract of leaf when 200 µg/mL, 400 µg/mL,600 µg/mL were treated to glycated egg albumin that had been compared without extract (100%) over other extract. the inhibition rate of Advanced glycation end products (AGEs) was also significantly higher (AGEs) in leaf plant extract. The means ± SD of the data ($n = 3$, $p < 0.05$) are significant value displayed. Anti-inflammatory and anti- Advanced glycation end products (AGEs) activity increases with the concentration of *Terminalia arjuna* plant extract.

Keywords: *Terminalia arjuna*, egg Albumin, anti-inflammatory activity, Advanced glycation end products

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive, debilitating condition marked by pain, swelling, and stiffness in the synovial joints. This terrible illness' precise etiology is uncertain. But there is a substantial correlation between it and an autoimmune reaction brought on by a variety of internal and environmental stimuli. When current medicines are used regularly, negative effects are always caused, which over time could offset beneficial effects. Inflammation, a physiological reaction to harm resulting from physical trauma, toxic chemicals, or microbiological agents, in addition to other illnesses such as rheumatism, is currently a significant issue impacting the morbidity of the labor force globally(Shams *et al*.,2021). Rheumatoid joint inflammation is estimated to impact approximately 1% of the global population, with the exact cause still to be determined. Rheumatoid arthritis can cause irreparable joint deterioration and an elevated risk of mortality when it abruptly advances into an inflammation that affects numerous body systems. It is associated with the degeneration of cartilage at joints, which causes discomfort, swelling, and stiffness when bones rub against one another(Vieira-Sousa *et al*.,2020; Ajithkumar *et al*.,2020).

Then, white blood cells swarm the joints, secreting chemicals like interleukins and tumor necrosis factor-alpha (TNF-alpha), which exacerbates pain, swelling, and damage to the joints. Extracellular activity of lysosomal enzymes, which are produced during inflammation, exacerbates the situation (Sheelarani *et al*.,2014; Vaijayanthimala *et al*.,2019).

Advanced glycation end products (AGEs) are created by the non-enzymatic condensation reaction known as glycation, which occurs when reducing sugars and protein amino groups reorganize to produce stable ketoamines. This spontaneous reaction is dependent on the degree and duration of hyperglycemia, the protein half-life, and the permeability of the tissue to free glucose. Increased glycation and the buildup of tissue AGEs have been connected to problems in diabetes because they can modify immunogenicity, diminish ligand binding, vary protein half-lives, and

disrupt enzymatic activity. Hyperglycemia is one of the clinical features of diabetes that causes the AGEs to be produced. Consequently, another crucial therapeutic target for the management of diabetes complications is glycation(Wong *et al*.,2022 ;Anwar *et al*.,2020).
Since, so far no scientific evaluation has been made to carry out in vitro anti-inflammatory, antiarthrhitic and anti-glycation end products (AGEs) activity in *Terminalia arjuna* plant extracts

MATERIALS METHODS

Plant Collection and Extraction

The *Terminalia arjuna* leaves and bark were obtained in Melaneelithanallur, Sankarankoil, Tenkasi District, Tamil Nadu. The bark and leaves that had been collected and cleaned, then shade dried for 2 week. Subsequently, it was ground into a fine powder and kept at 4°C in an airtight glass container shielded from the sun. Using a Soxhlet extractor at a ratio of 1:10 (w/v), the plant leaves and bark were extracted in Distilled water .
The Soxhlet extraction process heats the solvent up to temperature (>70°C). The evaporated solvent is contained within the apparatus by the condenser unit To get clear of the solvent, rotary evaporation was applied to the extracted liquid. The resulting semisolid extract was kept for future use in a freezer at 4º C in an airtight container. This solid extract dissolved in water and 70 % solvent based on the biochemical test (Karunanithi & Venkatachalam.,2019). The whole study was conducted at the PMT College's Department of Zoology in Melanelithanallur, Tenkasi District,TamilNadu , from 2023 to 2024.

In vitro studies *of* anti-arthritic activities by Inhibition of protein denaturation in *T. arjuna* extract

The inhibition of protein denaturation was carried out with little modification .The test solution was made with 0.05 ml of *T. arjuna* leaves and bark extract (100 and 250 µg/ml) in aqueous and 70% ethanol and 0.45 ml of Egg albumin (75 percent v/v phosphate-buffered saline). The test control (0.5 ml) consisted of 0.05 ml of distilled water and 0.45 ml of Egg albumin. On the other hand, the product control (0.5 ml) comprised 0.05 ml of extract and 0.45 ml of distilled water, whereas the standard contained 0.05 ml of acetyl salicylic acid and 0.45 ml of Egg albumin. The samples were incubated at 37 oC for 20 minutes, and then they were heated to 57 oC for 3 minutes. After cooling, 2.5 ml of phosphate buffer saline (pH 6.3) was added to each tube .At 660 nm, the absorbance was determined using spectrophotometry. 100% protein denaturation was represented by the control. The outcomes were contrasted with acetyl salicylic acid. The following formula was used to get the percentage inhibition of protein denaturation (Vaijayanthimala *et al*.,2019;Ajithkumar *et al*.,2020).
Percentage Inhibition = 100-[(Ac – As)/Ac×100]
Where Ac = Absorbance of control and As = Absorbance in presence of extract/ acetyl salicylic acid.

InVitro Glycation of Egg albumin in *T. arjuna* extract

The protein glycation inhibitory activity was done according to the method by anwar et al 2020 with minor modifications. A mixture of 500 μL of egg albumin (EA) at (75 % v/v aqueous solution) and 400 μL of 500 mM glucose in 100 mM phosphate-buffered saline (pH 7.4) was incubated at 37°C for 20 days in the dark with a gentle shaking followed by the addition of plant extracts. Some of the incubated samples contained various levels of *Terminalia arjuna* leaves and bark extract (200–600 µg/mL).Added 10 μL of 3 mM/L of sodium azide to prevent bacterial contamination. The reaction was ended by adding 10 µl of trichloroacetic acid (TCA), left at 4°C for 10 minutes followed by being centrifuged at 8,000 rpm for 5 minutes, and dissolved in alkaline phosphate buffer saline (pH 10). The production of browning Advanced glycation end products (AGE)s was measured wavelength of 460 nm.. Every experiment was conducted three times.
It's interesting to note that it was once believed that egg albumin with glucose that had not been exposed to extract could brown to a maximum of 100%. The sample absorbance, As, and the control absorbance, Ac, were entered into the formula to yield the relative percent browning of the other samples. As a result, the different samples' degree of browning decreased in a dose-dependent manner(Vieira-Sousa *et al*.,2020; Anwar *et al*.,2020).
% of browning = [(Ac − As)/Ac] × 100,
where Ac = Absorbance of egg albumin and glucose system and As = Absorbance of egg albumin and glucose system incubated with *Terminalia arjuna* extract.

Statistical analysis

All the results were expressed in mean ± standard deviation through tables and graphs. The statistical significance value of the results were analyzed by 2-way ANOVA followed by SPSS.

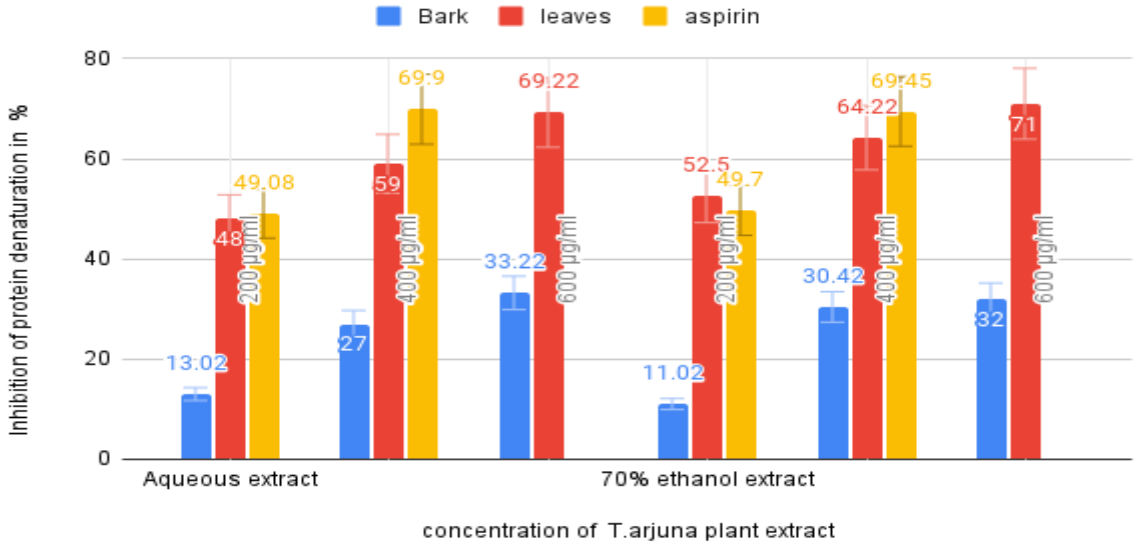
Result

Invitro antiarthiritc activity by Protein denaturation method

Bark Extracts: The aqueous extracts from bark showed inhibition rate of percentages across the tested concentrations (13.02% of 200 µg/ml,27% of 400 µg/ml, 33.22% of 600 µg/ml) The 70% ethanol extracts from bark demonstrated moderate to good inhibition percentages across the tested concentrations (11.02% of 200 µg/ml, 30.42% of400 µg/ml, 32 % of 600 µg/ml).

Leaf Extracts**:** Both aqueous and 70% ethanol extracts from leaves exhibited substantial inhibition percentages, particularly at higher concentrations (48% of 200,59% of 400 µg/ml, 61.22% 600 µg/ml), (52% of 200,64% of 400 µg/ml, 71% 600 µg/ml) respectively. The leaf extracts consistently showed higher inhibition than the corresponding bark extracts, suggesting that leaves may contain more potent bioactive compounds with stronger inhibitory effects.Aspirin, used as a reference compound, compare to plant extracts in varies concentration

Figure-1*: In vitro* studies *of* anti-arthritic activities by Inhibition of protein denaturation in extract *T.arjuna*



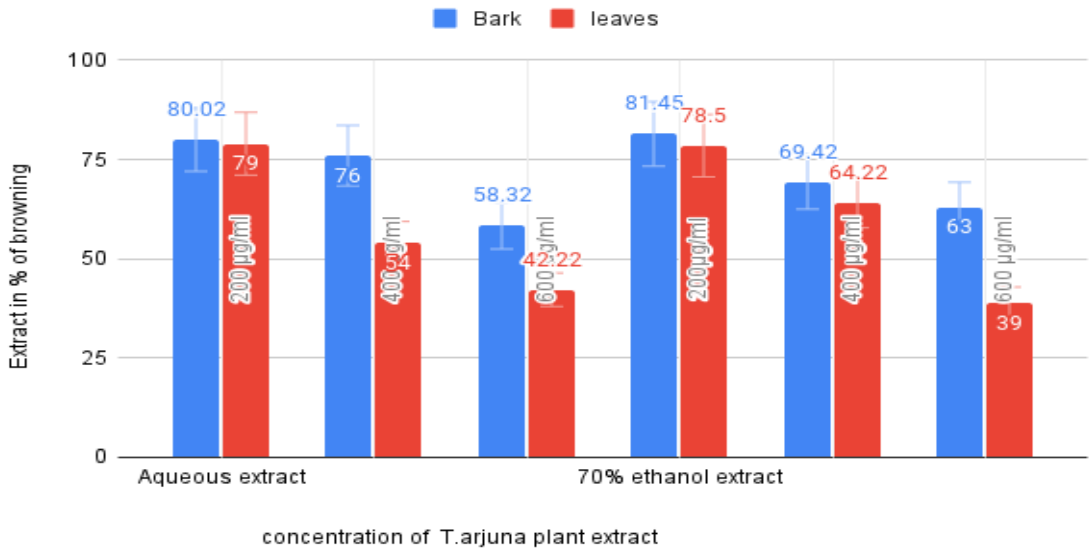
All the value were expressed in mean ± standard deviation (n=3); $p < 0.05$.

Effect of *T. arjuna* Extract on Browning

Egg albumin was incubated with glucose at 37 °C with or without plant extract for a duration of 20 days. The rate of browning is measured at 420 nm, and browning intensity is the first indication of glycation. The data shows that the ethanolic extract decreased browning in a dose-dependent manner. Browning of 80.02%,76 %,58.32% were seen in the aqueous extract of bark when 200 µg/mL, 400 µg/mL,600 µg/mL were treated to glycated egg albumin that had been compared without extract (100%) as well as ethanolic extract of bark showed browning 81.45 %,69.42%,63% respectvely. Browning of 79 %,54%,42.22% were seen in the aqueous extract of leaf when 200 µg/mL, 400 µg/mL,600 µg/mL were treated to glycated egg albumin that had been compared without extract (100%) as well as ethanolic extract of leaf showed browning 78.5 %,64.22%,39% respectvely

These results imply that when extract is present, browning is inversely correlated with decreased synthesis of glycated brown products. The least amount of browning was seen in the egg albumin that was incubated with glucose; this could be the consequence of internal structural alterations that occur over time.

Figure-2: Inhibition of AGE formation by *T.arjuna* plant extract



ss. The means ± SD of the data (n = 3, $p < 0.05$) are displayed

Discussion

Figure-1 explore that comparison of Protein denaturation to anti-arthritic activities The auto antigen production in certain arthritic disease may be due to denaturation of protein.

Aspirin, used as a reference compound, demonstrated notable inhibition percentages comparable to or sometimes higher than the plant extracts. This indicates that the tested plant extracts may possess similar or potentially novel bioactive compounds with anti-inflammatory or other inhibitory properties comparable to aspirin

The results exhibit that the ethanolic extract leaves was controlling the production of auto antigen and prevent the denaturation of protein

Loranthus micranthus from *H. brasiliensis* was reported to have rich polyphenol content and anti-inflammatory properties (Agbo *et al*., 2014). Various biological activities such as anti-analgesic, anti-inflammatory, anti-diabetic, and anti-arthritic were attributed to *Citrus maxima* (Vijaylakshmi and Radha, 2015,)

Terminalia arjuna plant leaf can be used as a potent natural anti-arthritic agent. The results show that the extracts of *Terminalia arjuna* leaves exhibiting anti-arthritic activities might be due to the presence of high concentration active compounds such as polyphenolic content, alkaloids,triterpenoids, and flavonoids than bark extract(Vaijayanthimala *et al*.,2019)

Figure-2 explore that When the plant extract is present, browning is reduced. The first sample, which is egg albumin treated with glucose for 20 days, is thought to exhibit 100% glycation, or browning. In first day showed least of browing in test sample due to natural color of plant .Highest browing will be seen in control sample over the test sample in last day of experiment . Samples 2–4 contained egg and glucose with varying concentrations (200–600 μg/mL) of plant extract. The browning intensity (the degree of glycation) is found to decrease with an increase in the concentration of the ethanolic extract. Sample 5 contained egg albumin incubated in the absence of glucose and plant extract as negative control. It showed the least glycation. It had the least amount of glycation. ethanolic extract leaves showed least glycation over aqueous extract in 39% of 600 μg/ml concentration.

The observed concentration-dependent activity suggests that the effectiveness of T. arjuna extracts in inhibiting AGE formation is influenced by the concentration of bioactive compounds present. Higher concentrations initially led to increased inhibition, although this effect plateaued or declined at the highest concentrations tested(Mohd Dom *et al*.,2020).

Bark extract may contain elevated amount of carbohydrates such as lignin,cellulose,whereas leaves extract contain lowest carbohydrate,highest phenolic and flovonoid content .leaves extract showed low degree of browing(Anwar *et al*.,2020;)

The significant inhibition of AGE formation by *T. arjuna* extracts, particularly from leaf, highlights their potential therapeutic value in managing conditions associated with AGE accumulation, such as diabetes and aging-related disorders(Kazeemet *et al*.,2012).

T. arjuna extracts contain bioactive compounds that possess anti-glycation properties. Further investigation into the specific compounds responsible for this activity could lead to the development of novel therapeutic agents or functional food ingredients.

Conclusion

Inhibition activity of the bark and leaf of *T. arjuna* extracts, along with aspirin, demonstrate promising inhibitory effects on protein denaturation and AGE formation,particularly leaf of *T. arjuna* extracts exhibit high inhibitory activity on AGE formation . These findings highlight the potential of *T. arjuna* plant-derived compounds as valuable sources of natural inhibitors of protein denaturation and anti-glycation agents with various applications in healthcare .

Acknowledgment

The author would like to thank Department of zoology,PMT college,Melaneelithanallur-627 953

Financial support and sponsorship

Nil

Conflicts of interest;

There are no conflicts of interest.

REFERENCES

1. Agbo, M.O., Nworu, C.S., Okoye, F.B.C. and Osadebe, P.O., 2014. Isolation and structure elucidaton of polyphenols from Loranthus micranthus Linn. parasitic on Hevea brasiliensis with antiinflammatory property. *EXCLI journal*, *13*, p.859.
2. Ajithkumar, T.G., Mathew, L., Sunilkumar, K.N., Rajagopal, R., Alfarhan, A., Kim, Y.O., Kim, H. and Kim, H.J., 2020. In vitro assessment of anti-inflammatory and anti-arthritic effects of Helicanthes elasticus (Desv.) Danser accessions collected from six different hosts. *Saudi Journal of Biological Sciences*, *27*(12), pp.3301-3306.
3. Anwar, S., Almatroudi, A., Allemailem, K.S., Jacob Joseph, R., Khan, A.A. and Rahmani, A.H., 2020. Protective effects of ginger extract against glycation and oxidative stress-induced health complications: An in vitro study. *Processes*, *8*(4), p.468.

4. Vieira-Sousa, E., Eusébio, M., Ávila-Ribeiro, P., Khmelinskii, N., Cruz-Machado, R., Rocha, T.M., Bernardes, M., Santos-Faria, D., Silva, J.L., Santos, H. and Miguel, C., 2020. Real-world longterm effectiveness of tumor necrosis factor inhibitors in psoriatic arthritis patients from the Rheumatic Diseases Portuguese Register. *The Journal of Rheumatology*, *47*(5), pp.690-700.
5. Wong, C.Y., Leong, K.H., He, X., Zheng, F., Sun, J., Wang, Z., Heh, C.H. and Kong, K.W., 2022. Phytochemicals of six selected herbal plants and their inhibitory activities towards free radicals and glycation. *Food Bioscience*, *46*, p.101557.
6. Kazeem, M.I., Akanji, M.A., Hafizur, R.M. and Choudhary, M.I., 2012. Antiglycation, antioxidant and toxicological potential of polyphenol extracts of alligator pepper, ginger and nutmeg from Nigeria. *Asian Pacific journal of tropical biomedicine*, *2*(9), pp.727-732.
7. Mohd Dom, N.S., Yahaya, N., Adam, Z. and Hamid, M., 2020. Antiglycation and antioxidant properties of Ficus deltoidea varieties. *Evidence-Based Complementary and Alternative Medicine*, *2020*.
8. Karunanithi, A. and Venkatachalam, S., 2019. Ultrasonic-assisted solvent extraction of phenolic compounds from Opuntia ficus-indica peel: Phytochemical identification and comparison with soxhlet extraction. *Journal of food process engineering*, *42*(5), p.e13126.
9. Shams, S., Martinez, J.M., Dawson, J.R., Flores, J., Gabriel, M., Garcia, G., Guevara, A., Murray, K., Pacifici, N., Vargas, M.V. and Voelker, T., 2021. The therapeutic landscape of rheumatoid arthritis: current state and future directions. *Frontiers in pharmacology*, *12*, p.680043.
10. Sheelarani, T., Gopal, V., Seethalakshmi, S. and Chitra, K., 2014. In vitro anti-inflammatory and anti-arthritic activity of selected medicinal plant. *Int J Pharm Sci Rev Res*, *28*(2), pp.162-163.
11. Vaijayanthimala, P., Sakthipriya, M. and Sangameswaran, B., 2019. In vitro anti-arthritic activity of Cissus quadrangularis stem extract. *IN VITRO*, *12*(1)
12. Vijaylakshmi, P. and Radha, R., 2015. An overview: Citrus maxima. *The Journal of Phytopharmacology*, *4*(5), pp.263-267.