In vitro Investigations on the Anti-Arthritic Characteristics of Cinnamon Bark and NYCTANTHES ARBOR-TRISTIS

Aradhana Singh[1], Dr. Dhiraj Kumar[2]

[1,2]Institute of technology and management, GIDA

Abstract: An autoimmune condition known as rheumatoid arthritis (RA) is characterized by joint and bone degradation. Current anti-inflammatory chemotherapeutic medications have negative side effects but provide momentary relief. Herbal remedies have demonstrated beneficial impact on RA symptoms with little side effects. In this work, we used in vitro methods to examine potential of Nyctanthes arbor-tristis and Cinamon bark. Alkaloids, polysaccharides, glycosides, phenolic compounds, peptides, free amino acids, and triterpenes were among the constituents that were qualitatively examined in methanolic extract of plants. HPTLC and TLC analyze substances that are active in extract. Extract show greatest scavenging properties in anti-arthritic action at 100 µg/mL. Anti-inflammatory activity investigate by following methods, such as protein denaturation, membrane destabilization and protease inhibitor method.

Keywords: rheumatoid arthritis, anti-inflammatory, protein denaturation, methanolic extract

Introduction:

An autoimmune, inflammatory, chronic condition associated with inflammation of the synovial and joint membranes is rheumatoid arthritis (RA). In addition, it results in bone cartilage distortion, pain, and cartilage degradation. Particularly, it has been established that inflammatory mediators are essential for the development of inflammation, stiffness, and disability at the outset of RA. It is an autoimmune disease of the synovial joints that is incessantly triggered by infections and inflammatory mediators [1]. Rheumatoid arthritis affects 0.5% of people worldwide each year, with women being afflicted more often than males by a ratio of three [2]. Degradation of bone and cartilage (Figure 1) and a worse quality of life owing to pain and limited mobility are caused by RA [13]. Strong ties exist between RA and an auto-immune response brought on by a range of environmental variables (epigenetics, genetics, and the diversity of the microbiome, among others), as well as the virus rubella. The three stages of RA development include inflammatory synovium, pain and edema, and eventually degeneration of bone and cartilage that results in joint damage [3]. A multitude of proinflammatory cytokines play a role in RA, including TNF-α (tumor necrosis factor), interleukin-1b, cyclooxygenase II (COX-II), enzymes of lysyl oxidase (LOX), prostaglandin-endoperoxide synthase (PTGS), prostaglandins, H2O2, TGF (transforming growth factor), and MCSF (macrophage colony-stimulating factor) [4]. Through years, allopathic medications have improved and been demonstrated to help halt the advancement of illnesses by treating symptoms and enhancing the quality of life for individuals who have been harmed by them [5]. However, medication has harmful side effects that raise risk of respiratory and urinary tract infections [6]. Long-term use of various pharmaceuticals has also been connected to major adverse effects in human embryos, just as prolonged use of RA treatments may result in issues including pneumonia and tuberculosis [7]. Herbal remedies are becoming more popular as secure substitutes for these dangerous illnesses. Compared to synthetic medications, they do not have as many negative effects on the human body. In India, around 2500 plant species are now utilized to make herbal remedies [8].

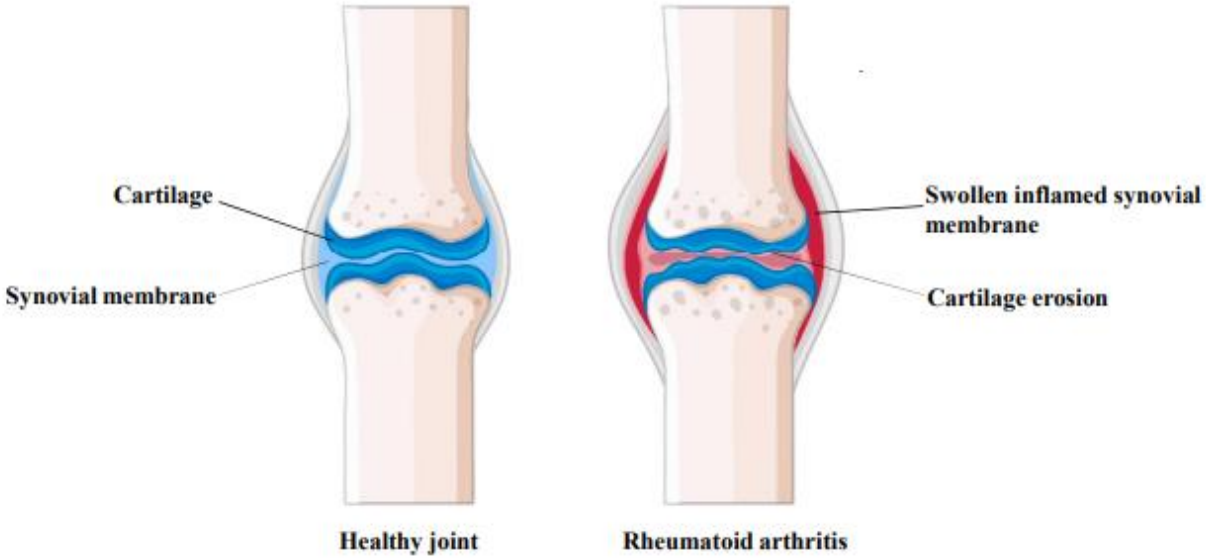

Fig.1 Rheumatoid arthritis

Moreover, a lot of primitive herbal medicines and the ingredients in them have strong antioxidant properties and scavenge free radicals, which both encourage cartilage degradation and inflammatory reactions [9]. Natural chemical components taken from therapeutic plants have ability to influence and modify pro-inflammatory signaling expression on the inflammatory pathway, which lessens the arthritic impact [10]. Night jasmine, or Nyctanthes arbor-tristis Linn, is a plant that belongs to the Oleaceae family. This particular Nyctanthes species is indigenous to South and Southeast Asia. The plant is native to India and may be found growing in the Himalayas, parts of Nepal to the east of Assam, Tripura, Bengal, Jammu, and Kashmir, and as far south as central area of the Godavari river [11]. According to Ayurveda, plant has great therapeutic potential. Nyctanthes have been shown to be a source of advantageous chemicals that may be utilized as pharmaceuticals, research intermediates for novel compounds, and most recent leads for medication synthesis in contemporary day [12]. Because the blossoms of this plant have antibilious, carminative, stomachic, and astringent properties, they can be used to treat piles and many skin conditions. Historically, RAs utilized powdered stem bark to treat rheumatic joint discomfort [14].

2. Material and methds:

2.1 Sample Collection, Authentication, and Extraction:

Fresh harsingar (Nyctanthes arbor-tristis) leaves and cinnamon bark were collected from the campus of ITM ,GIDA, Gorakhpur and authenticated by NBRI, Lucknow. Shadow-dried leaves were ground into a powder for extraction. Using Soxhlet device, extraction of secondary metabolites from plants was accomplished effectively.

2.2 Plant extracts preparation:

The amount to which the necessary phytoconstituents were dissolved determines which solvent should be used. Ethanol was continuously heated throughout the extraction process of certain plant components. The fine powder was securely sealed in a Soxhlet apparatus and recovered for 72 hours at 60°C with the solvent ethanol, shaking periodically. Evaporation lowered the volume of the extraction to a very tiny degree. For phytochemical research, the resultant ethanol extract of the herbal plant was utilized.

2.3 Preliminary Phytochemical Analysis:

The ethanolic extracts of cinnamon bark from Nyctanthes were used for a preliminary phytochemical analysis. This plant has a significant number of flavonoids, alkaloids, tannins, phenolic compounds, sterols, saponins, protein, and amino acids [15}.explained in figure .2.

Tests	Methods
Terpanoid	1gm extract was mixed with 2ml chloroform and concentrated H_2SO_4.
Flavanoid	1ml extract was mixed with NaOH and HCl.
Phlobatanins	1 millilitre of extraction was mixed in purified water & processed. In a 2 percent HCl, the filtrate was diluted.
Tannins-	1% lead acetate was added in 1ml extract.
Coumarine	In 2ml of extraction, 3ml of 10% NaOH was applied
Steroid	1 mL extraction was mixed in 10 mL chloroform, and an appropriate amount of sulphuric Acid was applied to the test tube's sides.
Saponin	One millilitre of extraction was combined with three millilitres of dw and allowed for shaken vigorously.

Fig.2. Preliminary phytochemical test

2.3 HPTLC and TLC analysis of extracts:

Four distinct mobile phases that have been previously characterized for the separation of flavonoids were evaluated using silica gel HPTLC plates: The ratios of ethyl acetate to formic acid and water (6:1:1, v/v), acetic acid and formic acid (100:11:11:26, v/v), and methyl ethyl ketone to formic acid and water (50:30) are shown [15].

2.4 Protein denaturation:

The in vitro anti-arthritic experiment was carried out using the denaturation of proteins technique developed by Mizushima and Kobayashi (1968). The final combination (5 ml) included 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 2 ml of extracts from plants at various concentrations (10, 20, 50, and 100 µg/L). The same amount of double-distilled water is used as a control. The mixture was then incubated for fifteen minutes at 37 °C in a BOD incubator, followed by five minutes of heating at 70 °C. After cooling, the car served as a reference to measure the absorbance at 660 nm [18].

2.5 Membrane stabilization:

10 milliliters of recently collected human blood were extracted and placed in centrifuge tubes that had been heparinized. Then, it is mixed with an equal amount of Alsever's solution (0.5 percent isosaline, which is prepared by dissolving 8.5 g of NaCl in water, autoclaving it for 15 minutes at 121 °C, and then allowing it to cool to room temperature) and centrifuging the mixture using 100 milliliters of purified water. One milliliter of HRBC solution was mixed with equal parts of botanical extracts in a range of concentrations (10, 20, 50, and 100µg/mL). Upon incubation for 30 minutes at 37 °C, each test mixture underwent centrifugation.

2.6 Protease inhibitor:

250 µL of trypsin, 1.0 mL of 25 mM Tris-HCl buffer (pH 7.4), and 1.0 mL of an aqueous solution containing 100–1000 µg/mL of each extract were added to generate a 2.0 mL reaction mix. The mixture was incubated at 37°C for five minutes. A 1.0 mL addition of 0.8% (w/v) casein was added to each extract. The mixture was incubated for a further twenty minutes. Each extract has received 2.0 mL of 70% (v/v) perchloric acid to halt the process. The hydrolyzed peptide supernatant's absorption ability was evaluated at 280 nm using a buffer solution as a blank, following the centrifugation of the unclear suspension.

3. Result:

3.1 Extraction of plant compound:

As seen in figure 3, the Soxhlet device was effectively used to extract plant secondary metabolites.

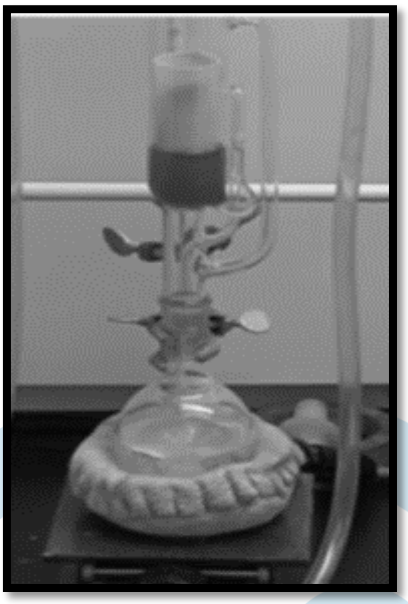

Fig.3 Soxhelt apparatus

3.3. Qualitative phytochemical Evaluation of Polyherbal extract:

Table 1: Phytochemical screening of Polyherbal extract.

Test	**Nyctanthes**	**Cinnamon bark.**
Phenols	+	+
Glycosides	+	+
Terpenoids	+	+
Flavonoids	+	-
Alkaloids	+	-
Saponin	+	-
Tannins	+	+
Carbohydrate	+	+
Steroids	-	+

3.4. HPTLC and TLC analysis of extracts.

The following is arrangement of flavonoids found in these plants: Quercetin > Vitexin > Luteolin > Rutin. Most flavonoids have bands associated with their florescence that are visible at 366 nm but not at 254 nm. Figure 4 explain HPTLC fingerprints.

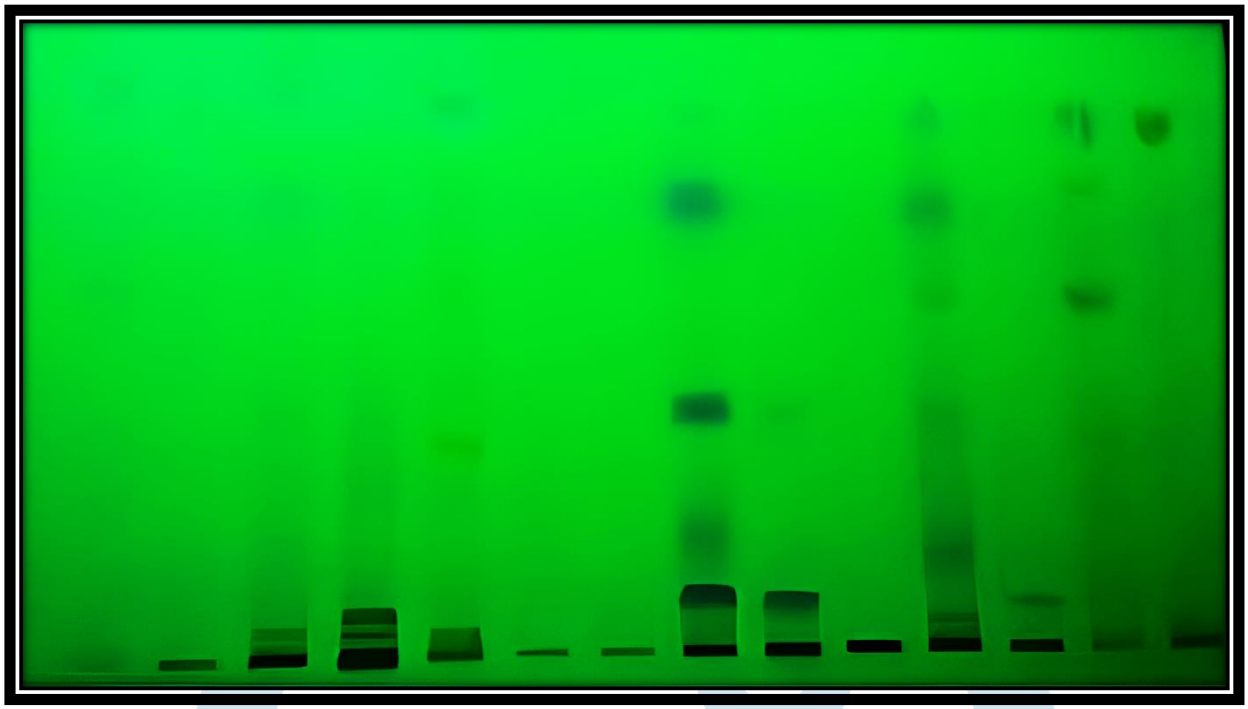

Fig.4 fingerprint using HPTLC

3.5. Protein denaturation:

µg/ml	**Cold water**	**Hot water**
0.01	19.29±1.34	13.26±0.96
0.1	19.58±0.62	16.30±0.93
1	20.71±0.66	22.43±1.49
10	22.43±1.32	24.74±0.75
100	23.73±3.36	25.09±2.27
1000	27.65±0.73	27.71±0.72

3.6. Membrane stabilization:

S. no.	µg/ml	%
1	10	44.05 ± 1.02
2	20	51.04 ± 0.59
3	50	57.34 ± 1.74
4	100	63.46 ± 1.23

3.7. Protease inhibitor:

S. no.	µg/ml	**%**
1	10	43.35
2	20	56.12
3	50	61.69
4	100	63.79

Discusion:

A complex process, inflammation is characterized by increased muscle permeability, granulocyte and mononucleate cell migration, and the formation of granulomatous tissue. It usually causes discomfort. (63%). Even though everyone experiences pain, it is an unpleasant feeling that is difficult to quantify. Peripheral or neurological pain may be caused by both centralised processes, which are triggered by a variety of pain perception input, and peripheral sensory afferent neurons, which are activated in illness. The hot-plate paradigm was used to study the peripheral antinociceptive effect because it offers several advantages, including sensitivity to strong antinociceptives and little tissue damage. There have been theories about the roles played by prostaglandins and bradykinins in pain. Phenolic substances are assumed to reduce prostaglandin production [19]. Acetic acid exposure is known to cause the peritoneum to produce unpleasant chemicals, which causes the wiggling reflex [20].

Conclusion:

The denaturation of protein molecules has been well documented in the literature and is linked to inflammatory processes in conditions like arthritis [20]. The suppression of protein denaturation may play a key role in the antirheumatic impact of NSAIDs [21]. Many scientists have already evaluated the effects of different plant components on denaturation of proteins. The effects of Semecarpus anacardium bark on bovine albumin are a few examples, Wedeliatrilobataon cow albumin, Albucasetosaon egg albumen [22,23].

References:

1) Prasad, P.; Verma, S.; Ganguly, N.K.; Chaturvedi, V.; Mittal, S.A. Rheumatoid arthritis: Advances in treatment strategies. Mol. Cell. Biochem. 2023, 478, 69–88
2) Garg, R.; Garg, A. The Research Trends and Scientometric Assessment of Rheumatoid Arthritis in India During 2016–2021. Curr. Rheumatol. Rev. 2023, 19, 26–35.
3) Guo, Q.; Wang, Y.; Xu, D.; Nossent, J.; Pavlos, N.J.; Xu, J. Rheumatoid arthritis: Pathological mechanisms and modern pharmacologic therapies. Bone Res. 2018, 6, 15.
4) Siouti, E.; Andreakos, E. The many facets of macrophages in rheumatoid arthritis. Biochem. Pharmacol. 2019, 165, 152–169.
5) Wei, Z.F.; Jiao, X.L.; Wang, T.; Lu, Q.; Xia, Y.F.; Wang, Z.T.; Guo, Q.L.; Chou, G.X.; Dai, Y. Norisoboldine alleviates joint destruction in rats with adjuvant-induced arthritis by reducing RANKL, IL-6, PGE2, and MMP-13 expression. Acta Pharmacol. 2013, 34, 403–413.
6) Choudhary, M.; Kumar, V.; Gupta, P.; Singh, S. Investigation of antiarthritic potential of Plumeria alba L. leaves in acute and chronic models of arthritis. Biomed. Res. Int. 2014, 2014, 474616
7) Choudhary, M.; Kumar, V.; Gupta, P.K.; Singh, S. Anti-arthritic activity of Barleria prionitis Linn. leaves in acute and chronic models in Sprague Dawley rats. Bull. Fac. Pharm. 2014, 52, 199–209.
8) Rengasamy, K.R.; Khan, H.; Gowrishankar, S.; Lagoa, R.J.; Mahomoodally, F.M.; Khan, Z.; Suroowan, S.; Tewari, D.; Zengin, G.; Hassan, S.T.; et al. The role of flavonoids in autoimmune diseases: Therapeutic updates. Pharmacol. Ther. 2019, 194, 107–131.
9) Suroowan, S.; Mahomoodally, F. Herbal products for common auto-inflammatory disorders—Novel approaches. Comb. Chem. High Throughput Screen. 2018, 21, 161–174.
10) Tasneem, S.; Liu, B.; Li, B.; Choudhary, M.I.; Wang, W. Molecular pharmacology of inflammation: Medicinal plants as antiinflammatory agents. Pharmacol. Res. 2019, 139, 126–140.
11) Suroowan, S.; Mahomoodally, F. Herbal products for common auto-inflammatory disorders—Novel approaches. Comb. Chem. High Throughput Screen. 2018, 21, 161–174.
12) Shrivastava, R.; Bharadwaj, A.K. Nyctanthes arbortristis an Important Medicinal Plant of Madhya Pradesh State—A Review. Pharm. Biosci. J. 2018, 6, 10–15.
13) Scott DL, Steer S. The course of established rheumatoid arthritis. Best practice & research Clinical rheumatology. 2007;21(5):943-967.
14) Singh, J.; Singh, A.P.; Singh, A.P. Nyctanthes arbor-tristis: A comprehensive review. World J. Curr. Med. Pharm. Res. 2021, 3, 74–78.
15) Prashant, T.; Bimlesh, K.; Mandeep, K.; Gurpreet, K.; Harleen, K. Phytochemical screening and extraction: A review. Int. Pharm.Sci. **2011**, 1, 98–106.
16) Kokate, C.K. Practical Pharmacognosy; Vallabh Prakashan: New Delhi, India, 2005; pp. 107–111.
17) Saba AB, Oguntoke PC and Oridupa OA. Anti-inflammatory and analgesic activities of ethanolic leaf extract of Calotropis procera. African Journal of Biomedical Research. 2011: 14(3):203-8.
18) Ravi, R., Ritesh, J., Narendra, M.P.A. and Khadabi, S.S. 2009. Anti – inflammatory action of Abuliton indicum sweet leaves by HRBC Membrane stabilization. Research Journal of Pharmacy and Technology., 2(2): 415 – 416.

19) Alcarez MJ And Ferrandiz ML. Journal of Ethanopharmacology, 1987: 209: 21.
20) Andrade SF, LGV Cardoso, JCT Carvalho and JK. Bastos. Anti- inflammatory nad anti nociceptive activities of extract, fractions and populnoic acid from bark wood of Austroplenckiapopulenea, Journal of Ethanopharmacology 2007:109:464-471.
21) Olesen J, Thomsen LL and Iversen H. Nitric oxide is a key molecule in migraine and other vascular headaches. Trends in pharmacological sciences. 1994: 15(5):149-53.
22) www.WebMD medical reference/causes of pain.
23) Shikha Chauhan. Opioid Analgesics, Amity University
24) MJ Christie, CW Vaughan and SL Ingram.Opioids, NASIDs and 5- lipoxygenase inhibitions act synergistically in brain via archidonic acid metabolism, inflammation research.1999: 48: 1-4.