Studies on the Anti-Arthritic Properties of NYCTANTHES ARBOR-TRISTIS and Cinnamon Bark in vitro

1. Aradhana Singh, 2. Dr. Dhiraj Kumar
1,2 Institute of technology and management, GIDA

Abstract:

Rheumatoid arthritis (RA) is an autoimmune disease characterized by bone and joint degeneration. The anti-inflammatory chemotherapy drugs available today have adverse effects but only offer short-term respite. With little adverse effects, herbal therapies have shown to be effective in reducing the symptoms of RA. In this study, we investigated the potential of cinnamon bark and Nyctanthes arbor-tristis using in vitro techniques. A qualitative analysis of the contents in a methanolic extract of plants included triterpenes, alkaloids, polysaccharides, glycosides, phenolic compounds, peptides, and free amino acids. TLC and HPTLC are used to assess extract's active ingredients. At 100 μg/mL, the extract exhibits the strongest scavenging characteristics with anti-arthritic effect. Protein denaturation, membrane destabilization, and protease inhibitor method are some of the techniques used to test anti-inflammatory effects.

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

Introduction:

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that causes inflammation of the joint and synovial membranes. It also causes discomfort, degeneration of cartilage, and deformation of bone cartilage. Specifically, it is known that inflammatory mediators play a critical role in the initial development of inflammation, stiffness, and disability in RA. Infections and inflammatory mediators constantly cause this autoimmune illness of the synovial joints [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].

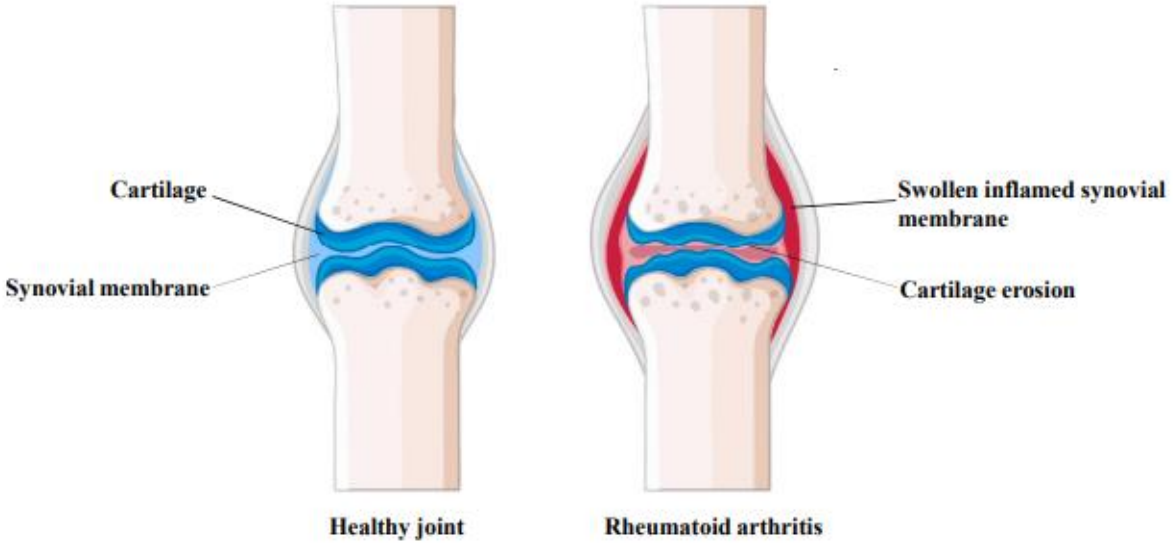

Fig.1 Rheumatoid arthritis

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:

Using silica gel HPTLC plates, four different mobile phases that have been previously described for the separation of flavonoids were assessed: The following are the ratios of acetic acid and formic acid (100:11:11:26, v/v), methyl ethyl ketone to formic acid and water (50:30), and ethyl acetate to formic acid and water (6:1:1, v/v) [15].

2.4 Protein denaturation:

Mizushima and Kobayashi's (1968) denaturation of proteins approach was used to conduct the in vitro anti-arthritic experiment. The final mixture (5 ml) contained 2 ml of plant extracts at different concentrations (10, 20, 50, and 100 µg/L), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 0.2 ml of egg albumin. The control group uses an identical volume of double-distilled water. After fifteen minutes of incubation at 37 °C in a BOD incubator, the mixture was heated for five minutes at 70 °C. Upon cooling, the vehicle functioned as a point of reference for 660 nm absorbance measurements. [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:

To create a 2.0 mL reaction mix, add 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. Incubation of the mixture lasted five minutes at 37°C. Each extract received a 1.0 mL addition of 0.8% (w/v) casein. An additional twenty minutes were spent incubating the combination. To stop the process, 2.0 mL of 70% (v/v) perchloric acid was added to each extract. After centrifuging the unclear suspension, the hydrolyzed peptide supernatant's absorption capacity was measured at 280 nm using a buffer solution as a blank.

3. Result:

3.1 Extraction of plant compound:

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

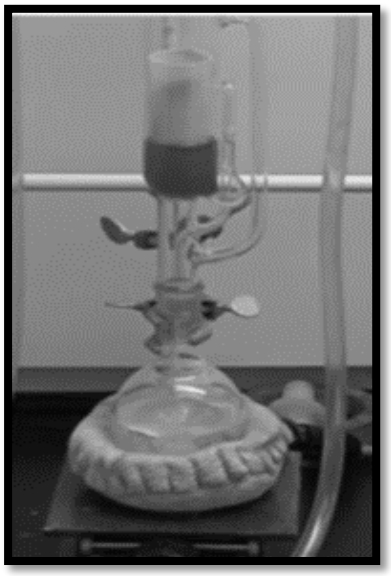

Fig.3 Soxhelt apparatus

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.

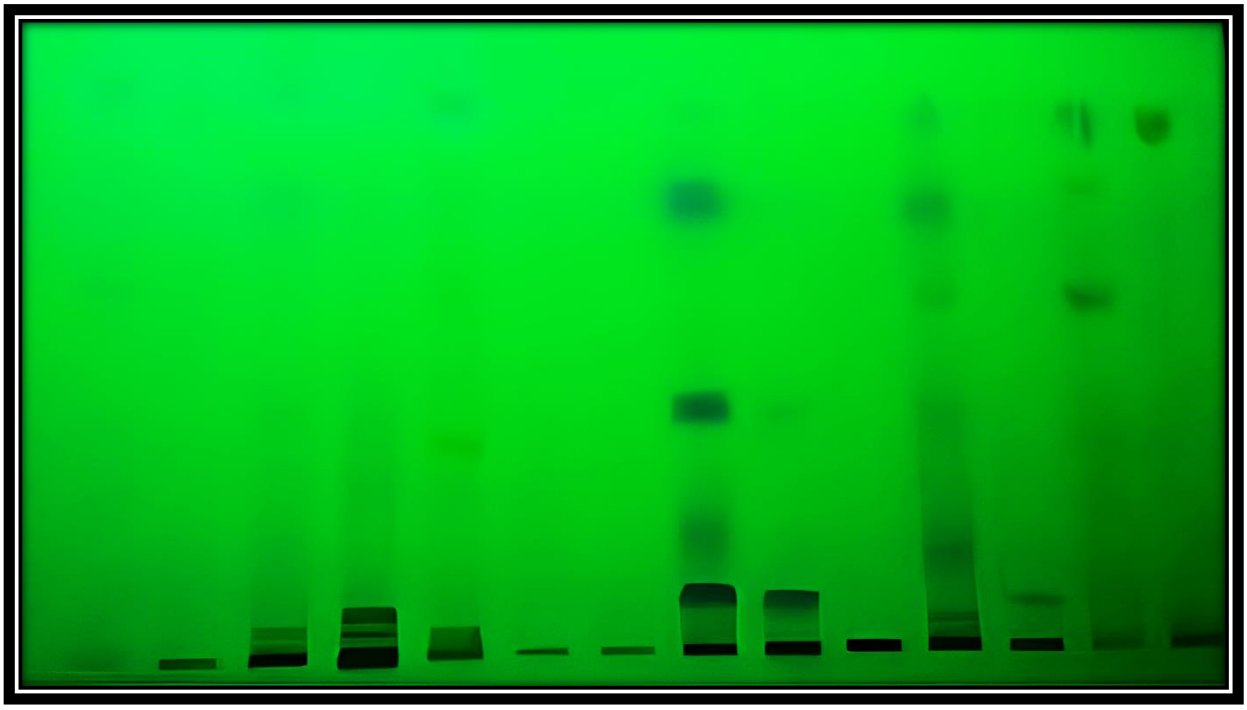

Fig.4 fingerprint using HPTLC

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

Discussion:

Increased muscle permeability, granulocyte and mononucleate cell movement, and the development of granulomatous tissue are the hallmarks of inflammation, a multifaceted process. Usually, it hurts. 63 percent. Everyone feels pain, yet pain is an unpleasant emotion that is hard to measure. Both centralised processes, which are triggered by a range of pain perception input, and peripheral sensory afferent neurons, which are activated in disease, can result in peripheral or neurological pain. A number of benefits, including as sensitivity to potent antinociceptives and little tissue injury, make the hot-plate paradigm an ideal tool for studying the peripheral antinociceptive impact. The functions that bradykinins and prostaglandins play in pain have been the subject of hypotheses. It is believed that phenolic compounds decrease prostaglandin synthesis [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].

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