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EXAMINING THE PHYTOCHEMICAL ANALYSIS AND ANTI-ARTHRITIC EFFECTS OF MELIA AZEDARACH LEAF EXTRACT: A HERBAL APPROACH OF ARTHRITIS MANAGEMENT

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Abstract

Introduction: Arthritis is the leading cause of disablement, affecting around 350 million people worldwide. Arthritis describes a condition that affects bone joints, joint tissues & connective tissues causing inflammation, pain, stiffness, limited movement and making it difficult to perform daily tasks affecting the quality of life. As of now, a definitive cure for arthritis has not been identified, several therapies are available to control symptoms and elevate the overall standard of life, including medications, physical therapy, lifestyle changes, and surgery etc. Objectives: Herbal treatments have been employed for centuries to manage a number of health disorders, and they continue to be an important form of treatment today due to their natural, safer, easily accessible and eco-friendly aspects. In continue with this herbal approach, we have targeted *Melia azedarach* (chinaberry) for its antiarthritic effect. *Melia azedarach* is a deciduous tree in the *Meliaceae* family, previous researches shown the anti-inflammatory, antifertility, astringent, antiseptic, diuretic, and deobstruent etc. But antiarthritic effect of *Melia azedarach* has not been studied. Methods: In this research, ethanolic extract of *Melia azedarach* leaf has been tested for antiarthritic activities using protein denaturation utilizing egg albumin, bovine serum protein denaturation, HRBC membrane stabilization and proteinase inhibitory assay in in-vitro models. The *Melia azedarach* extract also examined for phytochemical assay which shows the presence Flavonoids, Saponins, Glycosides, Phenols, Tannins, Alkaloids, Terpenes, Anthraquinone, Reducing sugar. Result: The result of the in-vitro analyses showed the significant inhibition in protein denaturation, proteinase and moderate inhibition in HRBC membrane stabilization with potential antiarthritic effect.

Keywords: Arthritis, Melia Azedarach, *In-vitro* Analysis, Phytochemical.

INTRODUCTION

Arthritis is the major source of disability in the world, affecting an estimated 350 million people.[1] The term "arthritis" refers to a disorder that affects bone joints, joint tissues,

and connective tissues, producing inflammation, discomfort, stiffness, and limited mobility, making daily tasks and physical activity difficult.[2,3,4] It can lead in persistent joint architectural damage and ligament degradation.[5]Arthritis may affect people of all ages, genders, and races, as well as infections and environmental factors like smoking and obesity, although it is more frequent in elderly persons and women.[6,7,8] Arthritis is a prevalent and typically chronic disease that can severely impair a person's quality of life.[9] Arthritis could be caused by a variety of causes such as injury, genetics, infection, age and environmental factors such as smoking and obesity.[6] Psoriasis and lupus are two medical diseases that may increase the chance of getting arthritis.[10,11] Arthritis symptoms differ based on the kind of arthritis. Some common symptoms are as follows: Stiffness and pain in the joints, Inflammation and tenderness in joints, Limited freedom of motion, Fatigue, Warmth and redness in the impacted area, Fever (in few types of arthritis).[3, 4, 12] The Symptoms may sometimes appear and go or worsen over time. Physical check-up, patient's medical history, and diagnostic tests such as X-rays, MRI, Uric acid test, Ultrasound or CT scans can all be used to diagnose arthritis. Blood tests may also be performed to search for inflammatory markers.[13, 14]

There are several forms of arthritis; however, the following are the most common:

1. *Osteoarthritis:* It is a form of arthritis that is prevalent in older adults and is caused by the gradual degeneration of joints due to wear and tear over time. While it can impact any joint in the body, it typically affects the hips, knees, hands, and spine.[15]
2. *Rheumatoid arthritis:* The described medical condition is autoimmune disease where the immune system targets the joint lining. Resulting in swelling, pain, and stiffness. Women are more prone to this condition, and it can affect individuals of any age.[8,16]
3. *Psoriatic arthritis:* A form of arthritis is linked to psoriasis, a skin disease marked by scaly, red spots. Symptoms of this arthritis include joint stiffness, swelling, and pain, and it may also lead to changes in the nails or skin.[11] There are various uncommon kinds of arthritis including ankylosing spondylitis, gout, and reactive arthritis.[17]

Epidemiology: A CDC assessment based on data from 2013 to 2015 found that 54.4 million (22.7%) persons in the United States experienced self-reported physician - determined arthritis, and 23.7 million of them had arthritis - related activity restriction. This figure is likely to rise as the population ages. Adults having co-morbid diseases such as cardiovascular disease, diabetes, and overweight had a greater frequency of physician-diagnosed arthritis than the general population (49.3%, 47.1%, and 30.6%, respectively).[18] From 1990 to 2010, disability caused by musculoskeletal problems increased by 45%. Osteoarthritis is the fastest growing significant health problem among them.[19]

Pathophysiology of Arthritis: While the pathophysiology of arthritis differs across the various types of the condition, the common denominator among them is that inflammation and damage to the affected joints can result in restricted mobility, stiffness, and discomfort.[2,3,20] Furthermore, the inflammatory response can cause alterations to the tissues surrounding the joints, leading to additional joint damage and functional impairment. Arthritis is a result of various mechanisms, which include: [21, 22]

Inflammation: Inflammation is a significant characteristic of most forms of arthritis, which involves the secretion of cytokines like TNF-alpha, IL-1, and IL-6 and from immune cells. These cytokines contribute to inflammation and damage in the joints. [23]

Immune system dysfunction: The immune system plays an important function in the onset and advancement of several types of arthritis. In cases of autoimmune arthritis, like rheumatoid arthritis, The immune system incorrectly assaults the body's own tissues, causing inflammation and joint damage.[24]

Synovial inflammation: Arthritis involves the inflammation of the synovial membrane, a delicate layer of tissue that envelopes the joints and produces synovial fluid. This fluid serves to lubricate and provide nourishment to the joints. However, in cases of arthritis, the synovial membrane becomes inflamed, producing an overabundance of synovial fluid, resulting in joint stiffness and swelling.[25]

Cartilage degradation: Cartilage is a sleek tissue that covers the edges of bones, facilitating their smooth movement over each other. Arthritis can cause damage and deterioration to this cartilage, resulting in limited mobility, stiffness, and joint discomfort. [26]

Bone remodeling: Arthritis can result in bone erosion and deformation, which may be influenced by alterations in bone remodeling. This process may be influenced by inflammatory cytokines and other factors.[27]

Neuromuscular dysfunction: Arthritis can cause changes in the neuromuscular system, leading to various symptoms such as joint stiffness, muscle weakness, and other related symptoms.[28]

Treatment of Arthritis: Despite there is currently no known cure for arthritis, numerous therapies are available to assist control symptoms and improve overall quality of life. Medication, physiotherapy, lifestyle modifications, and surgeries are among the therapies available. The kind and severity of arthritis, as well as the patient's specific demands and preferences, affect the method of treatment. Here are some common approaches that are used to manage arthritis:[29, 30]

Medications: Arthritis symptoms can be managed and disease progression slowed through the use of medications. NSAIDs, such as ibuprofen and naproxen, can aid in inflammation reduction and provide pain relief.[31,32]

1. *Physical therapy:* Physical therapy is a treatment option that can enhance joint mobility, strengthen muscles adjacent to the affected joint, and alleviate pain and stiffness.[33-35]
2. *Lifestyle modifications*: Apart from medication and physical therapy, changes in lifestyle can also be effective in treating arthritis. A balanced diet, frequent exercise, and adequate relaxation and sleep will help decrease arthritic symptoms and promote improved health.[36]
3. *Weight management:* Keeping a healthy body weight can be beneficial in reducing the pressure on joints that bear weight, such as the hips and knees, and could also aid in slowing down the progression of osteoarthritis.[37]

4. *Assistive devices*: Devices that provide assistance, such as braces, splints, and canes, can offer support to the affected joint, resulting in pain and stiffness reduction.[38]
5. *Surgery:* If arthritis becomes very severe, surgery may be required to fix or replace the damaged joint.[39]
6. *Complementary and alternative therapies*: Alternative and complementary therapies such as acupuncture, massage, or the use of dietary and herbal supplements, may provide relief for some individuals experiencing arthritis symptoms.[40]
7. *Education and support:* Getting educated about arthritis, its symptoms, and available treatments can be beneficial for individuals with arthritis to better manage their condition. Additionally, emotional and social support, such as from support groups, family, and friends, can help individuals cope with the challenges of living with arthritis.[41]

Herbal Treatment of Arthritis

Importance of Herbal Treatment

Herbal remedies have been utilized for treating various health issues for many years and they are still a crucial part of treatment in present times. Several scientific studies support the effectiveness of numerous herbal treatments, and ongoing research is expected to further reveal their importance in modern healthcare. Below are some reasons that emphasize the significance of herbal treatments.

1. *Natural and safe for long-term use:* Many herbal remedies are made from natural ingredients and have fewer adverse effects compared to pharmaceutical drugs. Additionally, they are often considered safe for extended periods of use.
2. *Accessible:* Herbal remedies are often more accessible and affordable compared to prescription drugs because they can be grown in one's backyard or found in the wild.
3. *Nutritional support:* Herbs and plants that are used in herbal treatments often contain high amounts of nutrients that can provide nutritional benefits to the body. For instance, nettle and dandelion are rich in vitamins and minerals, and can be used to supplement one's diet.
4. *Sustainable and eco-friendly:* Herbal treatments can be a sustainable and environmentally friendly practice since many herbs can be grown at home or in community gardens. This can reduce the need for transportation and packaging, making it a more eco-friendly option.

In summary, herbal treatments have significant value in promoting health and well-being due to their natural, comprehensive, and customized nature. As more people become interested in natural and alternative forms of medicine, the role of herbal treatments is expected to become more significant in modern healthcare. [42,43,44]

Plants used to Treat Arthritis

The various plants used in the treatment of arthritis are given below in table no. 1 along with their family, active ingredients, model used for arthritis activity, and result.

Table 1: The Plants used in Anti-Arthritis Activity

Plant Name	Family	Part of the plant used	Extraction method	Model used	Active ingredients	Parameters	Result	References
Boswellia Serrata	Burseraceae	Gum resin	HPLC	Collagen induced arthritis(CIA) rat model	▪ Keto beta bowswellic acid ▪ α-Boswellic acid ▪ Acetyle keto beta bowswellic acid	▪ TNF-α ▪ IFN ▪ IL-1 ▪ IL-6 ▪ IL-10 ▪ PGE2	Significant reduction in biochemical parameters when compared with standard	45
Eremostachys Laciniata	Lamiaceae	The rhizomes of Eremostachys Laciniata (L) Bunge,	Soxhlet extraction method	Clinical trials	▪ Iridoid glycosides ▪ Phenyl ethanoids ▪ Sterols	▪ Pain Score(1-10)	Significant reduction in pain score when compared with standard piroxicam drug.	46
Eucommia Ulmoides Oliv	Eucommiaceae	Cortex of Eucommia Ulmoides Oliv.	Solvent extraction	Collagen-induced arthritis (CIA) rat model		▪ Th17 ▪ IL-17 ▪ IL-10, ▪ TNF-α	Significant reduction in the symptoms of RA and other biochemical levels.	47
Matricaria Chamomilla L	Asteraceae	Whole plant	Solvent extraction		▪ Essential oil ▪ α-bisabolol ▪ Bisabolol oxide ▪ Guaiazulene	▪ *in silico* studies	α-bisabolol had the highest affinity for COX-2 of among all the substances tested.	48
Paeonia Lactiflora Pall	Ranunculaceae	Root	Ethanol reflux extraction	Collagen induced arthritis (CIA) rat model	▪ Paeoniflorin ▪ Albiflorin ▪ Oxypaeoniflorin	▪ TNF-α ▪ IL-1 ▪ PGE_2 ▪ cAMP	TGP significantly decreased production of IL-1, PGE_2, TNF-α and cAMP	49
Tripterygium Wilfordii Hook F	Celastraceae	Root	Solvent extraction	Collagen-induced arthritis (CIA) rat model	▪ Alkaloids ▪ Diterpenes ▪ Triterpenes ▪ Wilforine ▪ Wilfordine ▪ Wilforgine	▪ TNF-α ▪ IL-6 ▪ IL-8	Significant reduction in the TNF-α, IL-6, and IL-8.	50
Zingiber Officinale Rosc	Zingiberaceae	Rhizomes	Soxhlet extraction	▪ In vitro ▪ *in silico*	▪ 8-Gingerol ▪ 6-Gingerol	▪ Protein denaturation assay ▪ Protease inhibitory assay	The most effective anti-arthritic chemicals include 8-gingerol, 6-	51

					▪ 6-shogaol	▪ Membrane stabilization assay	gingerol, and Zingerone.	
					▪ Phenolics			
					▪ Flavanoids			
Aloe vera	Liliaceae	Leaf	Homogenization	Freund's complete adjuvant (FCA)-induced inflammatory arthritic		▪ Membrane lysis ▪ Protein denaturation ▪ TNF-α ▪ Cox-2	A. vera gel homogenate prevents tissue injury and decreases TNF-α and Cox-2 gene expression.	52
Cinnamomum cassia	Lauraceae	Twig	Ethanol reflux using rotary evaporators	Freund's Adjuvant-induced Arthritis	▪ Cinnamomulactone ▪ Cinnamaldehyde ▪ Coumarin ▪ Syringaresinol	▪ IL6 ▪ TNFα ▪ Body temperature	C. cassia twigs may be effective as a therapeutic or preventative agent for both acute and chronic rheumatoid arthritis or osteoarthritis.	53

Melia azedarach L

Traditional medicine is becoming most widely used and popular in both developed and developing nations. Chinaberry (*Melia azedarach L.*), one of the most significant medicinal plants in the Meliaceae family. The objective of this investigation was to discover anti-arthritic activity. And phytochemical contents of *Melia azedarach* leaves extracted using ethanol as the solvent.

Melia azedarach L. is a Meliaceae perennial deciduous tree that grows small to medium in size. *Melia azedarach L.* is said to get its name term Melia derived from the Greek word, which means' "manna or blossoming ash", referring to the leaves of tree's ' resemblance to Melia tree, and azedarach, which comes from the name of a toxic plant called "azadaracht" [54]. The tree is a native of South Asia (South of India, China, and Iran) that was brought to the New World, farmed, and naturalised. The plant is now found in Australia, India, Indonesia, and Pakistan, as well as Argentina, the Philippines, Brazil, and several Arab and African nations [55]. In many places, this plant is widely accessible and cultivated as a street tree. *Melia azedarach L.* leaves have been used to relieve joint discomfort for centuries. However, it has not been pharmacologically studied for rheumatoid arthritis. The current research was started to investigate the anti-arthritic efficacy of *Melia azedarach L* using protein denaturation utilizing egg albumin, bovine serum protein denaturation, HRBC membrane stabilization and proteinase inhibitory assay in in-vitro models. The antiarthritic efficacy of medicinal plants *Melia azedarach L.* (extract of leaf) was investigated using water and ethanol as solvents and checked against human arthritis to see whether it might

reduce inflammation at the location of the arthritis. A variety of phytochemical assays were performed on this extract to determine the presence of various components.

MATERIAL AND METHODS

Chemicals and Apparatus used

Bovine serum albumin or egg albumin, Methotrexate (standard drug), Alpha naphthol, Iodine, Pyridine, Picric acid, Alc. Hydroxide and other supportive chemicals have been procured from chemical store of Institute of pharmaceuticals sciences, Kurukshetra University, Kurukshetra India. All the instruments such as Plethysmometer, Capillaries, Heating mantle, Soxhlet apparatus, UV – Spectrometer, Incubator , Beakers 100ml, Test tubes, Glass rod, Volumetric flasks, Funnel, Round bottom flask, Centrifuge tubes, Pipette, Measuring cylinder, Weighing balance, Refrigerator used during the experimental work were available in Institute of pharmaceuticals sciences, Kurukshetra University, Kurukshetra India.

Extraction

Plant Material Collection

The M. azedarach leaves were collected from random Field, Assam down town University, Sankar Madhab Path,Gandhi Nagar, Panikhaiti,Guwahati, Assam, India. Following the selection of the plant, the following stage is to gather it and identify it botanically. Plant collection should be done by a professional botanist who meticulously records the collection location, season, plant state, and other details with proper voucher specimens, pictures, and written notes. *Melia azedarach* Linn aerial portions were gathered, cleaned with water from the tap, and air dried. For examination, the dried plant components were pulverised and stored. The plant material is normally stabilised by drying it at room temperature in a shaded location. The powdered plant material should next be treated to an appropriate extraction method once it has been dried or stabilised.

Preparation of Leave Extract

Fresh green leaves of M. azedarach were cleaned, separated, and washed with distilled water before drying in the shade for 30 days at a temperature of 21-30°C. In a beaker with a capacity of 5 L, 1,000 g of powdered M. azedarach leaves was added, along with 2 L of 70% methanol, and steeped for 72 hours with intermittent shaking and stirring. The residues were extracted three times, each time using the same fresh solvent and extract. For coarse filtration, the soaked plant material was filtered through numerous layers of muslin fabric one by one. The filtrate was filtered using Whatman no. 1 filter paper. In a rotary evaporator, the filtered extracts were concentrated under decreased pressure at 40°C. The semi-solid mass was collected and weighed to determine the yield, which was 29.4 percent (w/w), before being kept in the refrigerated (-8° C) until analysis.

Preparation of Ethanolic and Aqueous Extract of Melia azedarach (MAE)

Two procedures were employed –

Soxhlet Method: *M. azedarach* leaves (1kg) were shade-desiccated then crushed to produce a powder. At 60°C in a Soxhlet apparatus, equal amounts of the coarse powder were given across a 40-mesh sieve and thoroughly obtained with 90 percent (v/v) ethanol. To get an extract sample, the solvent was vaporized below pressure until

all the solvent was already eliminated, and the remaining water was eliminated by freeze drying. The extracts were kept in the refrigerator and a calculated portion of them was employed in the current study. In the shade, the leaves were then dried and pulverized in a mechanical grinder. The powdered material (200gms) was extracted in distilled water using a cold percolation process utilizing a Soxhlet apparatus around 55°C for 18 hours. The extract was concentrated in vacuo and weighed after being stored in a vacuum desiccator to remove all of the solvent.

Maceration Method: 100 gram of powder (stored) was soaked in 1.5 litres of 95% ethanol to make the ethanolic extract. The mixture was regularly stirred to aid the extraction of the active components. After 72 hours, this solution was sieved and filtered using a cotton layer and filter paper with pore sizes of 2.5 mm. The filtrate was evaporated for 8 hours at 82 ºC in a rota vapour. The resultant extract was put on a large Petri plate and allowed to dry at room temperature for two days, providing a completely dried ethanolic extract.

Using different fraction solvents, the considerable difference in yields of *Melia azedarach* leaf extracts was shown. In *Melia azedarach*, extracts using water and ethanol yielded 25.45gm, and 26.19gm, respectively.

A similar process was followed for aqueous extracts, with the exception that hot (100 ºC) and cold distilled water were utilized as solvents for infusion and maceration, respectively. To prevent fungal development, the infusion took three hours, and the maceration took 48 hours. After 7 days of filtrates in a vented oven heated to 50ºC, dried aqueous extracts were also produced. To make a stock solution each dried extract was used, after it stock solution was diluted with distilled water to make five distinct concentrations of 1.25, 2.5, 5, 7.5, and 10 mg/ml. 0.625, 1.25, 2.5, 3.75, and 5 mg/ml were the final concentrations examined.

Phytochemical Analyses of MAE

For phytochemical characterisation of significant bioactive compounds, MAE was exposed to a variety of qualitative assays. Himedia and Merck in India provided the chemicals utilised in the biochemical study. Qualitative tests were conducted to determine the existence of flavonoids, polysterols, tannins, saponins, alkaloids, phenolics, and carbohydrates [56-58].

1. *Benedict's test for Carbohydrates* - The extract was filtered after being dissolved in 5ml of distilled water. Benedict's reagents were used to treat the filtrate, which was then gently heated. An orange red precipitate indicates that carbohydrates are present in the sample.
2. *Terpenoids test* - The crude extract was diluted in 2 mL chloroform and evaporated until it was completely dry. 2ml concentrated H2SO4 was added to this, and it was heated for roughly 2 minutes. Grey colour indicating the presence of terpenoids.
3. *Salkowski's test for polysterols* - Separately, a little amount of the extract was suspended in 5ml of $CHCl_3$. The Salkowski test was next performed on the chloroform solution. Test of Salkowski: only some drops of concentrated H_2SO_4 were add up to 1ml of the above-prepared chloroform solution. The presence of phytosterols is shown by the brown colour generated.

4. *Foam test for Saponins* – A little amount of extract was mixed with 2ml of H_2O and shaken. If the foam formed lasts longer than 10 minutes, then the presence of saponins is confirmed.
5. *Ninhydrin test* - When heated with 2 millilitres of 0.2 percent Ninhydrin solution, the crude extract became violet, confirms the presence of amino acids and proteins.
6. *Test of Ammonium for flavonoid*s – some drops of 1% solution of ammonia were combined in a test tube containing a hydro methanolic extract of each plant sample. Flavonoid presence in the sample was indicated by a yellow hue.
7. *Test for tannins using Lead acetate* - A little amount of the extract was separately extracted in water, as well as the occurrence of tannins was determined by introducing a solution of lead acetate 10%. The occurrence of tannins is shown by the production of white precipitate.
8. *Perform a carbohydrate Fehling's tes*t – The two reagents used in this test are Fehling A and B. 2ml from each reagent was taken and added to crude extract and slightly heated. Formation of brick red precipitate confirm the presence of reducing sugars.
9. *Dragendorff's test for Alkaloids* - A little amount of extract was reacted with a few drops of weak HCl and filtered separately. The Dragendroffs reagent was employed to test the filtrates. The appearance of an orange-brown precipitate indicates the presence of alkaloids.
10. *Iodine test* – 2ml of iodine solution was mixed with crude extract. Appearance of dark blue or purple colour confirm the presence of carbohydrate.
11. *Test for Phenolics* - The extract (500 mg) was dissolved in 5 mL of pure water then 5% neutral FeCl3 were added to the mixture. Appearance of dark green colour confirm the presence of phenolic compounds.
12. *Molisch's test* - Molisch's reagent (2ml) was combined with Crude extract and well mixed. Then concentrated sulphuric acid (2ml) was gently added from the test tube's side. Appearance of a violet ring in the interphase confirm the presence of carbohydrate.
13. *Perform a protein Millon's test* – In this test crude extract is combined with 2ml Millon's reagent, it forms a white precipitate, which becomes red when heated gently, indicating the presence of protein.
14. *Liebermann's test for glycosides* - 2mL of acetic acid and chloroform were added to the crude extract. Ice was used to chill the concoction. H2SO4 was added in a concentrated form. The change in colour from violet to blue to green confirm that steroidal nucleus is present, i.e., glycone part of glycoside.
15. *Steroid test* - The crude extract was mixed with 2mL of concentrated H2SO4 then chloroform is added from sideways. A red color in the bottom chloroform layer indicated the presence of steroids. Another experiment involved mixing crude extract with 2mL chloroform. After that, 2ml of concentrated H2SO4 and CH3COOH were added to the mixture. The development of a greenish colour was detected the presence of steroids.

Evaluation of anti-arthritic Activity by using in-vitro Methods

Using egg albumin as a protein denaturation inhibitor: *M. azedarach* anti-arthritic efficacy in vitro was tested using a denaturation protein technique utilizing refreshing egg albumin of hen. The 5 mL reaction mixture included 02.8 mL phosphate buffered saline (pH 6.4), 2 mL fresh hen's egg albumin, 2 mL hydroalcoholic extract, butanoic as well as aqueous fractions of *M. azedarach*, as well as diclofenac sodium at different doses (12.5, 25, 50, 100, 200, 400, and 800 g/mL). For, n-butanol fraction, diclofenac sodium as well as aqueous fraction, a same amount of doubly distilled water was used, whereas DMSO was used as a control for aqueous methanolic extract. After 15 minutes of incubation at 37°C, the mixtures were heated for 5 minutes at 70°C. Its optical density was calculated at 660 nm after cooling, utilizing vehicle as a blank [59]. The following formula were used, the proportion inhibition of denatured protein was determined:

Inhibition %= 100 x [O.D of sample test /O. D of control -1]

Protein denaturation Inhibition method by serum albumin: Bovine serum albumin 0.45 ml (aqueous solution 5%) and *M. azedarach* extract 0.05 ml (at final volume 100 and 250 µg/ml) made up the reaction mixture (0.5 ml). A small amount of 1 N hydrochloride was used to alter the pH to 6.3. After being incubated at 37°C for 20 minutes, the mixture was heated at 57°C for 30 minutes. After chilling the samples, each tube was filled with 2.5 mL of phosphate buffer saline (pH 6.3). For the control test, turbidity was measured spectrophotometrically at 660 nm. Instead of extracts, 0.05 mL pure water was utilised, and the product control test lacked bovine serum albumin.

The following formula was used to compute the inhibition % of protein denaturation:

Inhibition % = (Optical density of test – Optical density of product control)/ O.D. of control X 100

The control represents complete protein denaturation. The results were compared to samples that had been treated with acetyl salicylic acid (250 mcg/ml).[60]

Procedure for stabilising the membrane of human red blood cells (HRBC): Using the HRBC membrane stabilisation technique, the activity of anti-arthritic of *M. azedarach* was also assessed. Blood was collected from a normal healthy individual who had not taken any NSAIDs for 15 days before to the trial and was then mixed with the equal quantity of sterile Alsevers solution. Centrifuged the blood mixture at 3k rpm for 15 minutes in a centrifugation machine, with the top layer gently removed using a sterile pipette or syringe. The remained packed cells at the bottom were detached as well as rinsed with solution isosaline before being suspended in isosaline at 10% v/v. The research employed a suspension of human RBCs.

Hypotonic saline (2 mL), phosphate buffer (1 mL), 0.5 mL of various doses of extract, and 0.5 mL suspension of RBC, reference sample, as well as control were all combined separately in the test mixes. The test solution was made up of 2 mL of hypotonic saline, 1 mL of phosphate buffer, 0.5 mL of diclofenac sodium as well as aqueous portions of *M. azedarach*, as well as 0.5 mL of 10% w/v human RBC in various concentrations (12.5, 25, 50, 100, 200, 400, and 800 g) in isotonic saline served as test controls. DMSO was used instead of water in the hydroalcoholic extract test control solutions. After 30 minutes of incubation at 37°C, the test solutions were centrifuged at 3000 rpm. The haemoglobin concentration was defined using a UV spectrophotometer at 560 nm after the supernatant liquid was emptied off. Using the

following formula, the proportion of stability of human RBC membrane or protection against hypotonicity-induced haemolysis was measured [61]:

"**Percentage protection = 100- [(optical density sample/optical density control) × 100]"**

Proteinase inhibitory assay

Take 1 mL of *M. azedarach* extract and 0.06 mg trypsin, 1 mL 25 mM tris-HCl buffers (pH 7.4), were included in the 2 mL reaction solutions. 1 mL of 0.8 percent casein solution was added after a 10-minute incubation time at 37 °C, followed by a 20-minute incubation period. To stop the reaction from continuing, 2 mL of 70% $HClO_4$ was added. Centrifuged the turbid suspension, and the supernatant solution was measured against a buffer as a blank at 280 nm [62].

Statistical Analysis

All data from the anti-arthritic research is presented as mean±SEM. One-way ANOVA was used for statistical analysis. Using the SPSS statistic computer software, we performed a post hoc test and a one-way ANOVA. A $p<0.05$ difference in mean values was deemed statistically significant.

RESULT

Phytochemical Analysis:

The result of the quantitative investigation of phytochemical components in several *Melia azedarach* leaf extracts is shown in table no. 2.

The presence of phytochemical constituents = +

The absence of phytochemical constituents = -

Table 2: The Quantitative Investigation of Phytochemical Components in Various Extracts of *Melia Azedarach Linn.* Leaves Yielded the Following Result

S.No.	Constituents	Aqueous extract	Ethanol extract
1	Steroids	+	+
2	Flavonoids	+	+
3	Saponins	+	+
4	Glycosides	+	+
5	Phenols	+	+
6	Tannins	+	+
7	Alkaloids	+	+
8	Terpenes	+	+
9	Anthraquinone	+	+
10	Reducing sugar	+	-

Antiarthritic Activity

Inflammatory arthritis is a synovial illness characterized by persistent inflammation of the joints, leading to disability due to joint damage. The % inhibition was determined with 25 to 100 µg/ml concentrations.

Antiarthritic activity by Bovine serum protein denaturation method

The plant extract *M. azedarach* showed 56, 66, 80, and 87 percent aqueous inhibition in the antiarthritic activity in in-vitro by Bovine serum protein denaturation method, whereas the ethanol extract *M. azedarach* showed 60, 70, 85, and 91 percent of

inhibitory activity at the same concentration (Table 3). The comparison of the antiarthritic activity of ethanolic and aqueous extract of *M. azedarach* of Bovine serum protein denaturation method is shown in figure 1.

Table 3: The Antiarthritic Activity of Plant Extract

S.No.	Concentration	%Inhibition	
		Aqueous extraction	ethanol extraction
1	25	56	60
2	50	66	70
3	75	80	85
4	100	87	91

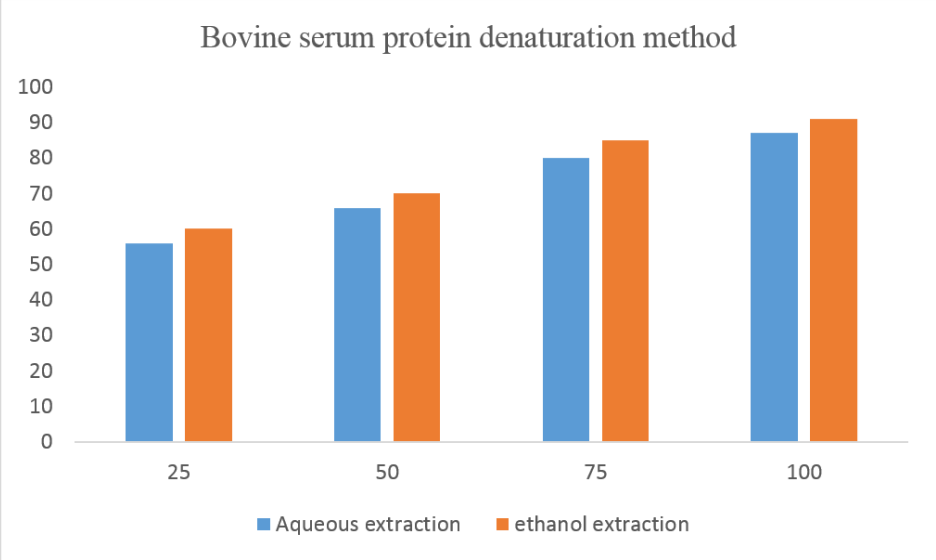

Figure 1: The Antiarthritic Activity of Plant Extract by Bovine Serum Protein Denaturation Method

Effect of Melia azedarach Linn on protein denaturation using egg albumin

Melia azedarach Linn. exhibited considerable protection against egg albumin denaturation at a variety of doses (12.5-800 g/ml). At 25-100 concentrations, the aqueous extract inhibited protein denaturation by 63, 65, 72, and 78 percent, while ethanol extract inhibited protein denaturation by 66, 72, 80, and 86 percent (Table 4). The comparison of the antiarthritic activity of ethanolic and aqueous extract of *M. azedarach* in protein denaturation using egg albumin method is shown in figure 2.

Table 4: *Melia Azedarach Linn* Effect on Protein Denaturation (Egg Albumin)

S.No.	Concentration	%Inhibition	
		Aqueous extraction	ethanol extraction
1	25	63	66
2	50	65	72
3	75	72	80
4	100	78	86

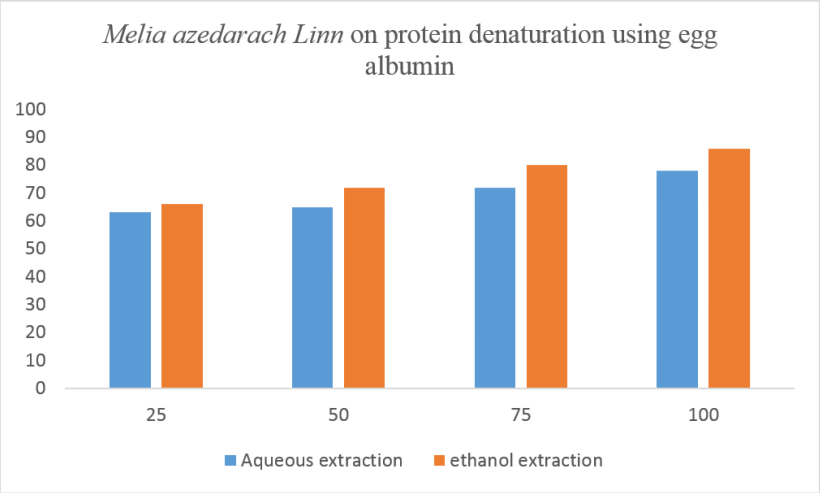

Figure 2: Effect of *M. azedarach Linn.* aqueous-ethanolic Extract and Fraction on Protein Denaturation Using Egg Albumin

Effect of Melia azedarach Linn on membrane stabilization

In the membrane stabilization model, there was a dose-dependent increase in percentage protection for all ethanolic and aqueous extract dosages. The maximum inhibition in % on membrane stabilization was seen in ethanol extraction 28, 35, 40 and 48 at (25–100 µg/ml) concentration, whereas in aqueous extraction, 20, 25, 32 and 40% inhibition were found (Table 5). The comparison of the antiarthritic activity of ethanolic and aqueous extract of *M. azedarach* in membrane stabilization method is shown in figure 3.

Table 5: Melia Azedarach Linn Effect on Membrane Stabilization

S.No.	Concentration	%Inhibition	
		Aqueous extraction	ethanol extraction
1	25	20	28
2	50	25	35
3	75	32	40
4	100	40	48

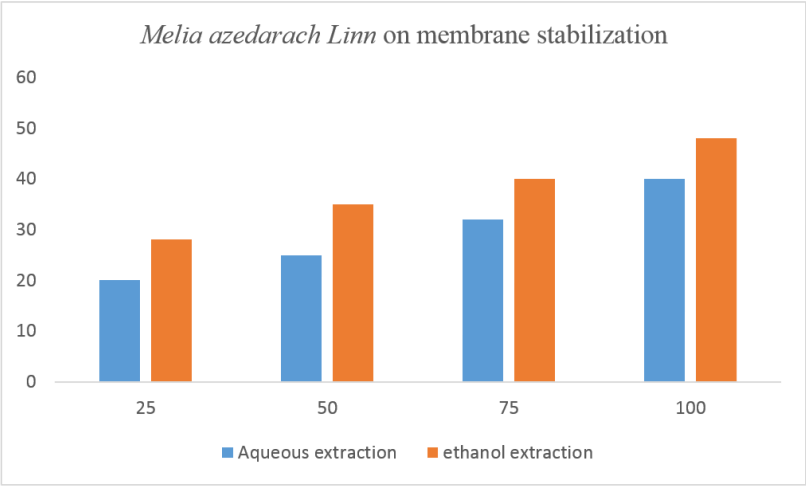

Figure 3: Effect of *M. azedarach Linn.* aqueous-ethanolic Extract and Fraction on Membrane Stabilization

Proteinase Inhibitory Assay

Proteinase, which destroys matrix tissues during inflammation, has been linked to the arthritic process. MMPs, in particular, are endopeptidases enzymes that are primarily engaged in cartilage matrix breakdown and bone joint deterioration. The antiproteinase activity was supplied by the two different extracts of *M. azedarach*, and the ethanol extract demonstrated a greater proteinase inhibitory effect (95% at 100 concentration) than aqueous extract (79%). The ethanol extract had a stronger inhibitory impact in this experiment (Table 6). The comparison of the antiarthritic activity of ethanolic and aqueous extract of *M. azedarach* in proteinase inhibitory assay method is shown in figure 4.

Table 6: Proteinase Inhibitory Assay

S.No.	Concentration	%Inhibition	
		Aqueous extraction	Ethanol extraction
1	25	46	60
2	50	58	70
3	75	66	80
4	100	79	95

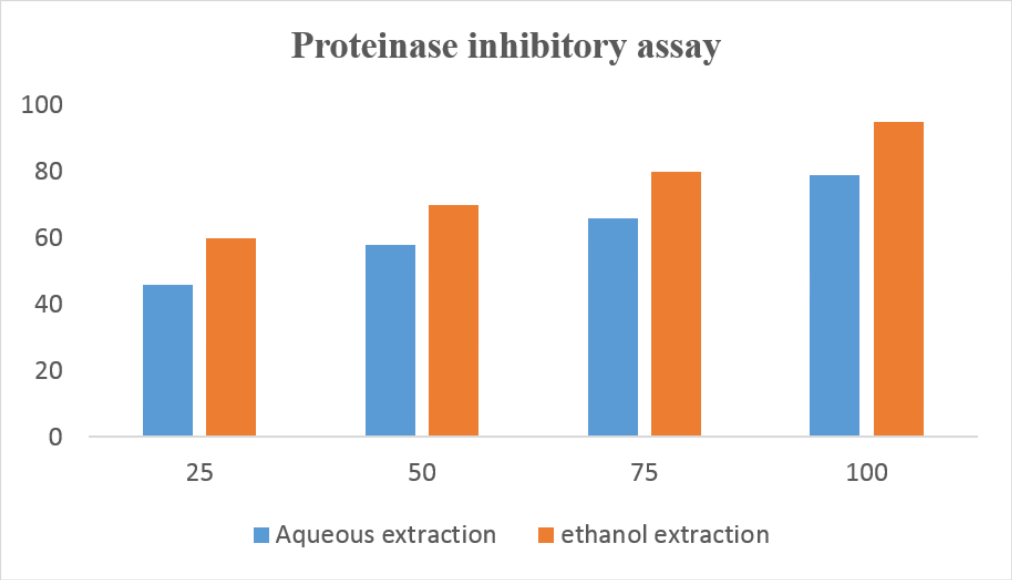

Figure 4: Effect of *M. azedarach Linn.* aqueous-ethanolic Extract on Proteinase Inhibitory Assay

Statistical Results of all 4 in-vitro tests

The t-test was used for paired samples to determine the significance difference in anti-arthritic activity of both aqueous and ethanol extract. Table 7 determines the descriptive statistics of all 4 groups. Table 8 denotes the correlation between both aqueous and ethanolic group of all 4 tests. A positive correlation which is almost equal to one was seen in all the 4 test groups. Table 9 determines the outcomes of paired t-test and the in 3 groups there is a significant difference in anti-arthritic activity of ethanol and aqueous extract. In three procedures, the ethanolic extract was shown to be more efficacious ($p<0.05$) than the aqueous extract. (Bovine serum albumin, membrane stabilization method and proteinase inhibitory assay method) while in protein denaturation by egg albumin method, the aqueous extract was seen to be more effective as compared to ethanolic extract ($p>0.05$).

Table 7: Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Aqueous_bovineserum	72.2500	4	13.91342	6.95671
	Ethanol_bovineserum	76.5000	4	14.10674	7.05337
Pair 2	Aqueous_eggalbumin	69.5000	4	6.85565	3.42783
	Ethanol_eggalbumin	76.0000	4	8.79394	4.39697
Pair 3	Aqueous_membranestabilization	29.2500	4	8.69387	4.34693
	Ethanol_membranestabilization	37.7500	4	8.42120	4.21060
Pair 4	Aqueous_proteinaseinhibitory	62.2500	4	13.86542	6.93271
	Ethanol_proteinaseinhibitory	76.2500	4	14.93039	7.46520

Table 8: Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Aqueous_bovineserum & ethanol_bovineserum	4	.999	.001
Pair 2	Aqueous_eggalbumin & ethanol_eggalbumin	4	.984	.016
Pair 3	Aqueous_membranestabilization & ethanol_membranestabilization	4	.994	.006
Pair 4	Aqueous_proteinaseinhibitory & ethanol_proteinaseinhibitory	4	.996	.004

Table 9: Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Aqueous_bovineserum Ethanol_bovineserum	-4.25000	.50000	.25000	-5.04561	-3.45439	-17.000	3	.000
Pair 2	Aqueous_eggalbumin Ethanol_eggalbumin	-6.50000	2.38048	1.19024	-10.28787	-2.71213	-5.461	3	.012
Pair 3	Aqueous_membranestabilization Ethanol_membranestabilization	-8.50000	1.00000	.50000	-10.09122	-6.90878	-17.000	3	.000
Pair 4	Aqueous_proteinaseinhibitory Ethanol_proteinaseinhibitory	-14.00000	1.63299	.81650	-16.59846	-11.40154	-17.146	3	.000

DISCUSSION

The antiarthritic activityof *M. azedarach* aqueous-ethanolic extract and fractions (ethyl acetate and aqueous) were examined in vitro. Furthermore, phytochemical analysis was carried out utilizing a variety of methods. *M. azedarach* inhibited protein (albumin) denaturation, stabilized HRBC membranes, inhibited paw/joint edema, and suppressed specific arthritic parameters, according to the results. Denaturation is caused by hydrogen, electrostatic, hydrophobic, and disulfide bonding changes. When compared to other fractions, plant extract had a larger inhibitory percentage in the bovine serum protein denaturation technique, and the protein inhibitory assay had the most suppressing impact (Fig. 1 and 5).

The increased absorbance of test samples compared to water suggested that *M. azedarach* can reduce protein denaturation thermally (egg albumin). Because the RBC membrane is like the lysosomal membrane, any agent's impact on RBC stability may be extrapolated to lysosomal membrane stabilization. Therefore, inhibition of RBC

haemolysis in a hypotonic medium provides an additional antiarthritic action. In the current research, plant extracts and fractions demonstrated appropriate dose-dependent stability of the RBC membrane. *M. azedarach's* ability to stabilize membranes might be linked to its ability to interfere with neutrophil lysosomal content release. The protective impact on erythrocyte lysis might be considered an explicit sign of *M. azedarach's* antiarthritic action.

Plant samples were subjected to phytochemical examination, which confirmed the existence of components with physiological and medicinal properties. Phytochemicals like saponins, tannins, phenols, steroids, terpenoids, flavonoids, alkaloids, and glycosides were found in the extraction of plants. The most common and biggest family of plant metabolites is phenolic chemicals. They have biological features like antiaging, antiapoptosis, antiinflammation, anticarcinogen, cardiovascular protection, antiatherosclerosis, the enhancement of endothelial function, and the prevention of cell proliferation and angiogenesis [8].

In this study we examine the effect of *M. azedarach* aqueous and ethanolic extract against antiarthritic activity. The result showed that the ethanolic extract of *M. azedarach* possess higher inhibition against arthritic in comparison to aqueous extract at the concentration 25 to 100 µg/ml.

CONCLUSION

The findings demonstrated that the plant investigated had medicinally essential components. There is a lot of evidence from previous research that the discovered phytochemicals are bioactive. Several studies have proved that these phytochemicals provide physiological and pharmacological qualities to the plants examined in the medication of many diseases. As a result, extracts from these plants might be considered a promising source of therapeutics. In light of the above, it's possible that *M. azedarach* has been shown to have a strong antiarthritic impact in laboratory experiments. Additional effort should be done to separate, purify, and characterize the active ingredients responsible for the antiarthritic activity of these plants, as well as further work to isolate, purify, and describe the active compounds responsible for the activity of these plants. In addition, further research into the likely mechanism of action of these extracts is urged. According to our research, more study is needed to determine the exact mechanism of action of *M. azedarach*, as well as the levels of pro-inflammatory cytokines, isolate active constituents, and characterize cells, to definitively establish *M. azedarach* as a potential safer modifying agent in the management of rheumatoid arthritis.

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