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PHYTOCHEMICAL INVESTIGATION AND *IN-VITRO* ANTI-ARTHRITIC EVALUATION OF VARIOUS EXTRACTS OF *FICUS AURICULATA* LOUR. LEAVES

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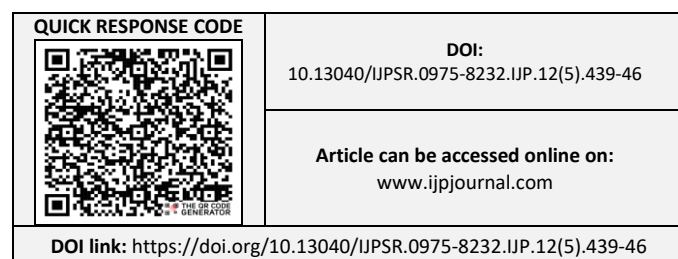
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ABSTRACT: Plants have been used for medical purposes since prehistoric periods. Approximately 60% of the world population and 80% of the population of developing countries depend on traditional medicinal plants due to their safety, accessibility, affordability and they are more economic. Inflammation of the joints is known as arthritis. More than 100 distinct forms of arthritis and associated disorders that affect the joints are referred to as arthritis, which is not a single illness. The connective tissue that envelops joints and other tissues. Anti-arthritic activity was mainly reducing the total leukocyte migration as well as lymphocyte and the monocytes / macrophage migration. The current study was designed to evaluate the *in-vitro* anti-arthritic activity of various extracts like Pet ether, Chloroform, Ethanol, Aqueous of *Ficus auriculata* Lour leaves. The extraction was done by using Soxhlet apparatus method (Pet ether, Chloroform, Ethanol) and maceration method was done for aqueous extract and the extract was subjected to phytochemical screening. The *in vitro* anti arthritic activity of various extracts was performed by using the bovine serum protein denaturation method. The result revealed that various extracts contain phytochemical constituents like alkaloids, flavonoids, glycosides, phenols and tannins and carbohydrates. The various extracts exhibited significant *in-vitro* anti arthritic activity when compared to the standard drug (Diclofenac) respectively.

INTRODUCTION: India ranks among the world's top producers of therapeutic plants. Drugs originating from plants are used as a prototype to create more medications that are less harmful and more effective. Medicinal plants may have therapeutic effects because of a variety of secondary metabolites, including phenols, alkaloids, flavonoids, saponins, and sterols.

The relationship between a medicinal plant's phytochemicals and pharmacological action is receiving more and more attention ¹. The Greek phrase "illness of the joints" is the source of the English term "arthritis." According to its definition, it is either acute or chronic joint inflammation that commonly coexists with pain, suffering, and structural damage from a primary injury.

Arthralgia is not the same as arthritis; the latter is defined as pain or discomfort localized to a joint, with little regard for the cause of the pain or discomfort (which may be brought on by inflammation in the joint). Despite its prevalence, not much is known about arthritis. Inflammation of the joints is referred to as arthritis.



Although there are over 100 distinct forms of arthritis and associated illnesses that impact joints, the tissues surrounding the joint, and other connective tissue, the term "arthritis" does not refer to a single disease. This illness is rheumatic ². Chronic autoimmune rheumatoid arthritis (RA) is characterized by cartilage degradation, joint edema, and synovial inflammation. RA frequently results in severe disability ³.

Rheumatoid arthritis (RA) and other autoimmune illnesses afflict millions of individuals. RA is distinguished by articular injuries accompanied by inflammatory synovial cell proliferation, leading in a nearly complete functional impairment. It affects approximately 1% of the overall population. According to epidemiological data, women are 2-3 times more likely than men to have this disease ⁴.

The frequency of it varies with age. While it may affect anyone at any age, the 25–50 age group is the most prevalent for arthritis ⁵. The precise cause of the condition is unknown, although a few theories suggest that a combination of genetic predisposition and vulnerability to outside influences, such as viruses.

Release of certain free radicals, such as superoxide and nitrous oxide, which are byproducts of cellular metabolism, although the precise pathophysiology is still understood. Such free radicals may cause T-cells to produce interleukins (IL) and tumour necrosis factor (TNF- α), which in turn affects the production of growth factors, cytokines, and adhesive molecules on immune cells, eventually leading to tissue destruction and inflammation. The inflammatory cells' infiltration, neovascularization, and hyperplasia of the synovial membrane cause RA's pathological alterations, which include articular damage and cartilage erosion ⁶.

The large, rounded leaves of the *Ficus auriculata* (Moraceae) or Roxburgh fig, are a common sight throughout Asia ⁷. *Ficus auriculata* Lour. is a dioecious tree that grows to a height of 4 to 10 meters. It is heavily composed of white latex across the whole plant. The bark has a rough texture and a grayish brown colour. The branchlets are rusty brown in colour. Pear-shaped fruits often grow on leafless branchlets at the base of the trunk and on the main branches ⁸.

The leaves are broadly ovate-cordate, alternating, and have an obtuse-mucronate apex with a shallowly dentate whole edge. Figs are pear-shaped fruits (Syconus), with 8–12 prominent longitudinal ridges. The tiny, sessile male flowers have an ovoid anther, lengthy filaments, two stamens, a translucent, spatulate, thinly membranous calyx, and three lobes. The tiny, sessile or pedicellate female flowers have an oval ovary and three lobes on the calyx ⁹.

F. auriculata leaves are crushed, and the resulting paste is administered to the wounds. Additionally, they are useful for dysentery and diarrhea. Its stem bark juice is useful for treating diarrhoea and cuts. For dysentery and diarrhoea, roasted figs are consumed. Root latex is used to treat cholera, the mumps, vomiting, and diarrhea ¹⁰.

The contents of flavanols (myricetin, quercetin, and kaempferol) were determined. Furthermore, from the petroleum ether, Chloroform, and Ethanol fractions of the alcoholic extracts of the leaves and fruits, botulinic acid, lupeol, stigma sterol, bergapten, scopoletin, β -sitosterol-3-O- β -D-glucopyranoside, myricetin, and quercetin-3-O- β -D-glucopyranoside were identified.

One flavonoid is quercetin, which has anti-inflammatory and antioxidant properties. By blocking the production of inflammatory cytokines, lowering lipopolysaccharide induced cyclooxygenase (COX-2) levels, and inhibiting nuclear factor-kappa β (NF- κ B) and AP-1 activity, quercetin reduces the clinical symptoms of arthritis. It inhibits the growth of synoviocytes and the recruitment of neutrophils and macrophages ^{11, 12, 13}.

Ficus auriculata leaves showed presence of phenols, flavonoids, glycosides, resin, tannins, triterpenes, polyphenol, alkaloid, sterol, coumarins according to phytochemical study.

These substances support the plant's medicinal properties, which include analgesic, antibacterial ¹⁴, antioxidant ¹⁵, and anti-inflammatory ¹⁶, anti-fungal ¹⁷, Hepatoprotective ¹⁸, anti microbial ¹⁹, anti-cancerous ¹⁴, anti hyperglycemic ²⁰, Anti-arthritic properties.

FIG. 1: *FICUS AURICULATA* PLANT SPECIES

Need for Study: The goal of currently used anti rheumatic drug to reduce pain, swelling, delay the progression of disease, minimize the disability and ultimately improving the patient life. Most of these objectives are achieved by the combination of NSAIDS, DMARDS, Corticosteroids being used till now, the potential side effect gives a limitation of side effects. Now's it's the growing concern all over the development of new safe, potent, less toxic, anti arthritic drug. Hence there is a need to explore the more naturally available alternatives, so that their therapeutic values can be assessed a expanded.

MATERIALS AND METHODS:

Chemical Used: Potassium dihydrogen Phosphate (KH_2PO_4), Sodium Hydroxide (NaOH), Bovine Serum Albumin, Diclofenac Sodium.

Collection of *Ficus auriculata* (Leaves): Fresh leaves were collected from Rudraprayag, Uttarakhand for the study. The leaves were gently washed under running tap water and then distilled water for a few seconds to reduce the accumulation

of impurities. After washing it was made into powder using the traditional pestle and mortar method and the powder was stored in an airtight container. The plant was authenticated from Botanical Survey of India, Northern Regional Centre 192, Kaulagarh Road, Dehradun, Uttarakhand, India which is a Government of India Organization run by the Ministry of Environment, Forest, and Climate Change, under Accession number 1481.

Preparation of Various Extracts of *Ficus auriculata* Leaf Samples: Fresh leaves were used for extraction. The leaves were collected and washed. To obtain a constant weight the leaves were crushed properly. *Ficus auriculata* was done using 25gm with 250ml of water for aqueous extract by Maceration method and Pet ether, Chloroform, and Ethanol by Soxhlet method. The extracts were concentrated in a water bath until it was completely dried. The dry extract was kept in a sealed beaker.



EXTRACTION OF LEAVES



VARIOUS EXTRACTS OF LEAVES

FIG. 2: EXTRACTION OF LEAVES

Phytochemical Investigation ²¹: Phytochemical Investigation was carried out to check for the presence of phenolic compounds, alkaloids, glycosides, tannins, and flavonoids in the various extracts such as Pet ether, Chloroform, Ethanol and Aqueous of *Ficus auriculata* Lour. Leaves.

Test for Alkaloids:

Drangendroff's Test: A few drops of Drangendroff's reagent (potassium bismuth iodide) were added 2-3 ml of extract, Orange Brown ppt was obtained.

Mayer's Test: A few drops of Mayer's reagent (potassium mercuric iodide solution) were added to 2-3 ml of extract, White ppt was obtained.

Wagner's Test: A few drops of Wagner's reagent (iodine potassium iodide solution) were added to 2-3 ml of extract, Reddish Brown ppt was obtained.

Hager's Test: A few drops of Hager's reagent (saturated solution of picric acid) were added to 2-3 ml of extract, Yellow ppt was obtained.

Tannic acid Test: To the extract, add tannic acid solution gives buff coloured ppt.

Test for Flavonoids:

Sulphuric Acid test: When sulfuric acid is added, the flavonoids dissolve and produce a bright yellow solution.

Zinc HCl Test: The test solution was heated using HCl and zinc. The tint changed from pink to red.

Shinoda Test: After adding a few pieces of magnesium ribbon to the test solution, the conc. HCl became pink to magenta red.

Lead Acetate Test: The residue was mixed with lead acetate solution. Precipitation became yellow.

Alkaline Reagent Test: A few drops of sodium hydroxide solution should be added to the test solution. When a few drops of diluted acid are added, the intense yellow hue that first appeared turns colourless, signifying the presence of flavonoids.

Test for Saponins:

Foam Test: Water was added to the extract and mixed briskly. The presence of foam.

Haemolytic Test: One drop of blood was put on a glass slide and extracted. The hemolytic zone was seen.

Test for Phenols & Tannins:

Acetic Acid Test: When acetic acid solution is added to the extract, tannins are confirmed by the production of a red colour.

Dilute Nitric Acid Test: The presence of tannins is confirmed when the extract is treated with a diluted solution of nitric acid; the production of a red to yellow colour.

5% FeCl₃ Test: Adding FeCl₃ solution to the extract indicates the presence of hydrolysable tannins as blue colour formation and condensed tannins as green colour creation.

Lead Acetate Solution: When lead acetate solution is added to the extract, produced white ppt.

Dilute Iodine Test: When dilute iodine solution is added to the extract, Transient Red color solution produced.

Test for Glycosides:

Killer-Killani Test: Add 1 drop of 5% FeCl₃ and conc. H₂SO₄ to the extract of glacial acetic acid. Where two came together, a reddish brown coloured developed. Blue-green-looking liquid layers and top layer were seen.

Legal's Test: After adding 1 ml of sodium nitroprusside and 1 ml of pyridine to the extract, the colour changed from pink to red.

Baljet's Test: The extract gained a yellow to orange colour when sodium picrate was added.

Bromine Water Test: The extract was mixed with bromine water, which produces a yellow precipitate.

Test for Carbohydrates:

Molisch's Test: Add a few drops of concentrated sulfuric acid through the test tube's walls to the 2 ml of test solution after adding alcoholic naphthol.

The presence of carbohydrates was verified at the junction by the formation of a purple to violet colour ring.

Barfoed's Test: When 1 ml of the test solution is heated over a water bath with 1 ml of Barfoed's reagent added, the creation of cupric acid produces a green colour that indicates the presence of monosaccharide.

Fehling's Test: When 1 ml of Fehling's A and B is added to 1 ml of test solution and heated on a water bath, the presence of carbohydrates is confirmed by the production of a brick-red precipitate.

Benedict's Test: The presence of the carbohydrates is confirmed by adding 1 ml of Benedict's reagent and heating on a water bath to 2 ml of test solution. A reddish-brown precipitate forms.

Iodine Test: To 3ml of test solution, add Dil. Iodine solution. Blue color appears which disappears on boiling and reappears on cooling.

Tannic Acid: With 20% of tannic acid test solution gives ppt.

***In-vitro* Anti- Arthritic Activity of Various Extracts of *Ficus auriculata* Leaves:**

Bovine Serum Albumin Protein Denaturation Method:

Principle: The process of denaturing proteins involves applying external stressors or compounds such as heat, strong acids, or bases, concentrated inorganic salts, organic solvents, or strong bases that cause the proteins to lose their secondary and tertiary structures. In diseases like rheumatoid arthritis, denaturation of proteins is a well-established source of inflammation. The primary mechanism of antiarthritic non-steroidal anti-inflammatory medications [NSAIDS] is to protect against denaturation.

Method:

Test Solution (0.5ml): Includes 0.05ml of test solution at different concentrations (10, 20, 30, 40, 50µg/ml) and 0.45 ml of bovine serum albumin (0.5% W/V aqueous solution).

Test Control Solution (0.5ml): Includes 0.05ml of distilled water and 0.45ml of bovine serum albumin (0.5% W/V aqueous solution).

Standard Solution (0.5ml): Includes 0.05ml of different doses (10, 20, 30, 40, 50µg/ml) of

Diclofenac sodium and 0.45ml of bovine serum albumin (0.5% W/V aqueous solution). Reagent preparation.

0.5% Bovine Serum Albumin (BSA): 50 mg of BSA should dissolve in 50 ml of water.

Phosphate Buffer Saline (PH 6.4): Dissolve 27.21gm of KH_2PO_4 in 1 litre of distilled water. And dissolve 8gm of NaOH in 1 litre of distilled water. Transfer 50ml of KH_2PO_4 and 11.6ml of NaOH in 250ml of volumetric flask. Shake the solution then adjust the PH to 6.4.

Procedure: A mixture of 0.45ml (0.5% W/V BSA) was prepared by mixing 0.05ml of different concentrations (10, 20, 30, 40 and 50µg/ml) of the reference medication Diclofenac sodium and the test drug respectively. For three minutes, the samples were incubated at 57° C. Once the solutions have cooled, mix with 2.5 ml of phosphate buffer. The absorbance was measured. measured at 660 nm using a UV-Visible Spectrophotometer. A 100% denaturation of proteins is represented by the control. A comparison was made between the outcomes and Diclofenac sodium. The following formula can be used to determine the percentage inhibition of protein denaturation.

$$\text{Percentage inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$



FIG. 3: BEAKER CONTAINING BOVINE SERUM PROTEIN DENATURATION

Evaluation of Physical Parameters of *Ficus auriculata* Lour Leaf:

Ash Values: Ash value is helpful in determining the quality and purity of crude drug, especially in the powdered form. Vegetable drugs are ashed to get rid of any organic material that would otherwise interfere with an analytical result.

When burned, crude pharmaceuticals typically leaves behind an ash that contains phosphates, carbonates, and silicates of sodium, potassium, calcium, and magnesium.

Total Ash Value: Weighed accurately about 2-3gm of powdered drug in a tared silica crucible, incinerated at a temperature not exceeding 450°C for 4hrs, until free from carbon, cooled and weighed. Total ash value was calculated using the following formula:

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drug taken}} \times 100$$

Water Soluble Ash Value: Boiled the ash with 25ml of water, filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450°C for 4hrs. Cooled in a desiccator and weighed. Calculated the percentage of water - soluble ash with reference to the air- dried drug using the following formula:

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash} - \text{Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Acid Insoluble Ash Value: Boiled the ash with 25ml of 2M HCl, filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450°C for 4 hrs. Cooled in a desiccator and weighed. The percentage of acid

insoluble ash with reference to the air- dried drug was calculated using the following formula:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Extractive Value:

Alcohol Soluble Extractive Value: 5gm accurately weighed coarse drug was macerated with 100ml of alcohol (90% v/v) in a stoppered flask for 24hrs, shaking frequently during first 6hrs. It was then filtered through a filter paper. 25ml of alcoholic extract was evaporated to dryness in a tared China dish and weighed. The percentage w/w of alcohol soluble extractive with reference to the air- dried drug was calculated using the following formula:

$$\% \text{ Alcohol soluble extractive value} = \frac{\text{Wt. of residue}}{\text{Wt. of crude drug taken}} \times 80$$

Water Soluble Extractive Value: 5gm accurately weighed coarse powdered drug was macerated with 100ml of chloroform water I.P in a stoppered flask for 24hrs, shaking frequently during first 6 hrs. It was then filtered through filter paper. 25ml of chloroform water was evaporated to dryness in a tared China dish and weighed, Calculated the percentage w/w of water- soluble extractive with reference to the air- dried drug using the following formula:

$$\% \text{ Water soluble extractive value} = \frac{\text{Wt. of residue}}{\text{Wt. of crude drug taken}} \times 80$$

RESULTS AND DISCUSSION:

Phytochemical Investigation:

TABLE 1: PHYTOCHEMICAL SCREENING OF *FICUS AURICULATA* LOUR

Contents	Ethanolic Extract	Pet Ether Extract	Chloroform Extract	Aqueous Extract
Alkaloids	Present	Present	Present	Present
Glycosides	Absent	Present	Present	Absent
Flavonoids	Present	Present	Present	Absent
Phenol & Tannins	Present	Absent	Present	Present
Carbohydrate	Present	Present	Present	Present



FIG. 4: PHYTOCHEMICAL INVESTIGATION

TABLE 2: ORGANOLEPTIC CHARACTERISTICS OF *FICUS AURICULATA* LOUR. LEAF

S. no.	Parameter	Observation
1.	Colour	Green
2.	Odour	faint
3.	Taste	bitter

TABLE 3: EVALUATION OF PHYSICAL PARAMETER OF *FICUS AURICULATA* LOUR. LEAF

S. no.	Parameter	Leaf
1.	Total ash value	29.2
2.	Acid insoluble ash value	14.7
3.	Water soluble extractive value	0.526

In-vitro Anti-Arthritic Activity of Various Extracts (Pet ether, Chloroform, Ethanol, and Aqueous) of *Ficus auriculata* Lour leaves:

TABLE 4: *IN-VITRO* ANTI-ARTHRITIC ACTIVITY OF VARIOUS EXTRACTS (PET ETHER, CHLOROFORM, ETHANOL, AND AQUEOUS) OF *FICUS AURICULATA* LOUR LEAVES

Concentration µg/ml	% inhibition of Pet ether Extract	% Inhibition of Chloroform Extract	% inhibition of Ethanol Extract	% inhibition of Aqueous Extract	% inhibition of Reference (Diclofenac)
10µg/ml	70.64	77.73	40.61	49.15	73.03
20µg/ml	69.53	60.44	71.38	44.06	70.78
30µg/ml	63.79	70.20	63.10	89.83	84.26
40µg/ml	62.69	60.10	56.66	76.27	68.53
50µg/ml	68.65	57.36	59.57	72.88	66.29
Mean	67.060	65.166	58.264	66.438	72.578
SD	3.57894	8.54588	11.30662	19.26787	6.99699
S.E.M	1.60055	3.82183	5.05647	8.61685	3.12915

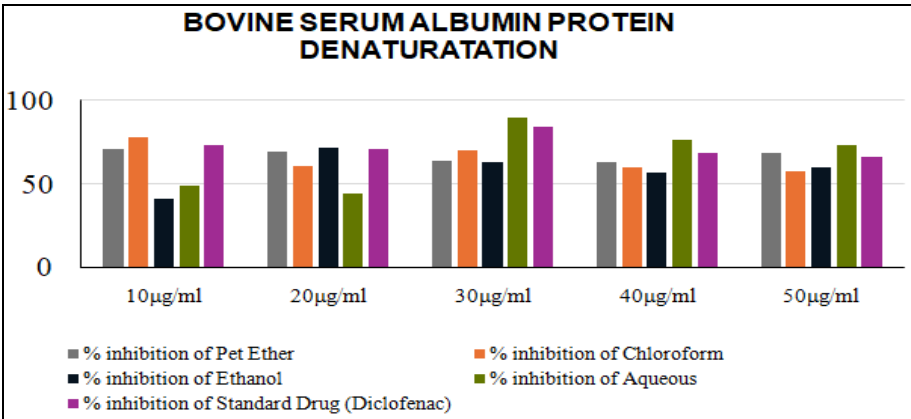


FIG. 5: GRAPH REPRESENTING % INHIBITION OF PROTEIN DENATURATION AS ANTI ARTHRITIC POTENTIAL OF VARIOUS EXTRACTS

CONCLUSION: Additionally, plants are appealing sources for the development of novel, highly effective, and safe therapeutic molecules for the management of rheumatoid arthritis. Scientific research into the potential benefits of herbal plants is crucial for optimal patient treatment and protection. Herbal medications are highly sought after in developed countries for basic healthcare due to their effectiveness, safety, and lack of negative side effects. The purpose of this study is to

Bovine Serum Albumin Protein Denaturation Method: The various extracts (Pet ether, Chloroform, Ethanol, and Aqueous) of *Ficus auriculata* Lour leaves was tested for their in vitro Anti-Arthritic activity by using Bovine serum albumin denaturation method, where the test substances is exposed to different concentrations 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml, in order to determine the percentage inhibition. The percentage inhibition of test substances is almost equal to standard (Diclofenac Sodium).

describe the anti-arthritic properties of a medicinal plant from the Moraceae family. *Ficus auriculata* leaves were extracted using the Soxhlet method with a variety of solvents, including pet ether, chloroform, and ethanol, as well as the maceration method with water. The anti-arthritic properties were also investigated *in-vitro* using the bovine serum protein denaturation method at different concentrations ranging from 10µg/ml to 50µg/ml. The phytochemical components of *Ficus auriculata*

leaves including alkaloids, glycosides, flavonoids, phenol, and tannins, provide strong anti-arthritic properties. We draw the conclusion from the study that, at the quantities tested, the different *Ficus auriculata* leaf extracts exhibit considerable Anti arthritic activity. To create novel products produced from plants that can cure arthritis, further study is still required.

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CONFLICTS OF INTEREST: This study was conducted in accordance with established ethical principles, and the authors declare that there are no conflicts of interest.

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