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PHARMACOGNOSTIC PRESPECTIVE OF *ARTOCARPUS HETEROPHYLLUS LAM* LEAF AND ITS *IN-VITRO* ANTIRHEUMATIC ACTIVITY

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Keywords:

Artocarpus heterophyllus Lam, Rheumatoid arthritis, Inflammation, Moraceae

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ABSTRACT: *Artocarpus heterophyllus Lam*, popularly known as Ceylon jack tree or jackfruit, evergreen tree reaching 8-25 m high, belongs to the family Moraceae. The trunk is straight, rarely buttressed, with a circumference of 50-100 cm. The stems are straight, forming a 30° angle with the trunk. The leaves are alternate, simple, pinnately veined, coriaceous, glossy, 4-25 cm long x 2-12 cm, dark green on the upper side and pale green beneath. The leaf has many therapeutic properties especially its diabetes control, antioxidant and anti-aging properties. Traditionally leaves are useful in fever, boils, wounds and skin diseases. In the present study, we discuss and highlight the recent findings concerning the different classes of RA therapies including the conventional and modern drug therapies. Approximately, one per cent of the world population lives with rheumatoid arthritis (RA), with at least twice as many women affected as men. Selection of leaves are important as compared to the other parts because it includes more constituents and also can be cheaply available.

INTRODUCTION: Medicinal plants provide major source of molecules with medicinal properties due to presence of natural compounds. Medicinal plants are useful for curing human diseases and play an important role in healing due to presence of phytochemical constituents. Plant is an important source of medicine and plays a key role in world health. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world.

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This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions. Chemically prepared drugs may act quickly, but they have side effects which affect human body negatively in the long run, whereas, medicinal plants work in an integrated or pro-biotic with little or no adverse effects on the body.

Chronic diseases-including, cancer, diabetes, hypertension, stroke, heart disease, respiratory diseases, arthritis, obesity and oral diseases can lead to hospitalization, long-term disability, reduced quality of life, and death. Chronic diseases are characterized by high prevalence among populations, rising complication rates and increased incidence of people with multiple chronic conditions. Approximately, one per cent of the world population lives with rheumatoid arthritis (RA), with at least twice as many women affected

as men. The World Health Organization estimates more than 23 million people live with RA. Rheumatoid arthritis (RA) is one of more than 100 types of arthritis. This chronic autoimmune disorder affects the lining of synovial joints in about 0.5% of people and may induce severe joints deformity and disability. Rheumatoid arthritis (RA) is one of more than 100 types of arthritis. This chronic autoimmune disorder affects the lining of synovial joints in about 0.5% of people and may induce severe joints deformity and disability.

Rheumatoid arthritis impacts health life of people from all sexes and ages with more prevalence in elderly and women people. Non-steroidal anti inflammatory drugs (NSAIDs) (e.g.; Naproxen, Aspirin, Ibuprofen, oxaprozin, salsalate, diclofenac, mefenamic acid, meclofenamate, ketorolac *etc*.), corticosteroids (e.g.; Prednisone, Methylprednisolone, dexamethasone *etc*.), and disease-modifying antirheumatic drugs (DMARDs) (e.g.; Methotrexate, Hydroxychloroquine, sulfasalazine *etc.*) remain the most known treatments used against rheumatoid arthritis. Side effects such as Gastric irritation, Peptic ulcer, Gastrointestinal bleeding, Nausea, Abdominal discomfort, Kidney impairment, Cardiovascular effects, Rash, Diabetes, Osteoporosis, Metabolic syndrome, Hepatic dysregulations, Pneumonitis, Hematologic disorders, Infections, Nephrotoxicity, Hypercholesterolemia, Creatininemia, cellulitis, diarrhea, headache, hypertension, liver failure *etc*. (Reda Ben Mrid *et al* 2022).

However, not all patients respond well to these drugs and therefore, new solutions are of immense need to improve the disease outcomes. In the present review, we discuss and highlight the recent findings concerning the different classes of RA therapies including the conventional and modern drug therapies. Traditional medicinal plants are practiced worldwide for treatment of arthritis especially in developing countries where resources are meager. 485 plant species belonging to 100 families, traditionally used in arthritis. Several herbal remedies are promoted today for treating arthritis, including turmeric, ginger, Boswellia serrata, devil's claw, willow bark extract and feverfew *etc*. (Manjusha Choudhary 2015). Artocarpus genus (Family: Moraceae-mulberry family) received a great level of scientific interest as they consist of therapeutically active secondary metabolites and is economic source of food and widely used in traditional medicine. Artocarpus species are used as food and for traditional folk medicine in South-East Asia, Indonesia, Western part of Java and India. Artocarpus plants offer advantages as a profitable multipurpose crop for producing fruits and timber. Artocarpus has long been recognized and economically is of appreciable importance as a source of edible aggregate fruit; such as *Artocarpus heterophyllus* (Jack fruit), *Artocarpus altili*s (bread fruit) and *Artocarpus chempeden* (Chempedak) and yielding fairly good timber. *Artocarpus heterophyllus* popularly known as jack fruit is one of the important and commonly found trees in the home gardens of India and Bangladesh. The term jackfruit is derived from the Portuguese word Jaca which in turn is adopted from the word “Chakka” of Malayalam - A regional Indian language. Extracts of its plant parts have been applied in traditional medicine for the treatment of diarrhea, diabetes, malarial fever, tapeworm infestation, and as wound healing, antisyphilic, expectorant and also to treat anaemia, asthma and dermatitis.

Taxonomy of *Artocarpus heterophyllus lam:*

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperrms
Clade	Eudicots
Clade	Rosids
Family	Moraceae
Genus	Artocarpus
Species	*A. heterophyllus*

Leaves are thin-leathery and obovate-elliptic to elliptic. The leaves are smooth on the surface and rough on the undersides. Stipules are ovate-acute, deciduous and leave ring-like form on the twigs. The leaf has many therapeutic properties especially its diabetes control, antioxidant and anti-aging properties. It has distinctive flavour and fragrance and it is known be rich in vitamin. Jackfruit leaves has several therapeutic effect and work wonders when taken (especially on an empty stomach). Wide variations in leaf blade shape *i.e.,* elliptic, obovate, oblong, broadly elliptic and narrowly elliptic were recorded in the selected jackfruits. Different fruit shapes like ellipsoid, spheroid, oblong, clavate, oblong and irregular were recorded in the selected accessions.

Flake texture and flake flesh colour also showed wide variation. In seeds, different seed shapes such as ellipsoid, irregular, reniform, spheroid and oblong were recorded.

A. heterophyllus Lam. (AH), popularly known as Ceylon jack tree or jackfruit, is considered to be "poor man's food", as it is widely available in summer at an economical price when there is a shortage of agricultural produce in India. In India, it is also called "Panasa", "Atibruhatphala", "Kantaphal", and "Kanthal" due to its physical characteristics. The *A. heterophyllus* tree is also considered to be a source of flavonoids, sterols, volatile acids, carotenoids, and tannins, which vary depending on the stage of maturity. Moreover, it also has a unique combination of iron, vitamin C, vitamin B complex (especially vitamin B6, niacin, folic acid, and riboflavin) and minerals (mainly calcium and potassium). The fruit of *A. heterophyllus* has been employed in value-added products such as fruit juice, dried chips, jam, candies, jelly, marmalades, leather, and ice cream, depending on the sensorial characteristics at different stages. *A. heterophyllus* bears flowers after 6–8 years of planting, and flowers start to bloom from November to March. It is reported that, being a monoecious tree, the flowers are covered by two spathes of 5-10 cm in length. The central and peripheral regions bear male flowers, while footstalks from the main trunk bear female flowers (generally round in shape). The distinction between the flowers can be made based on surface characteristics: female flowers are generally large with a rough surface, whereas male flowers are small with a smooth surface. The leaves and stem show the presence of sapogenins, cycloartenone, cycloartenol, β sitosterol and tannins, they show estrogenic activity. A root contains β-sitosterol, ursolic acid, Betulinic acid and cycloartenone.

Traditionally leaves are useful in fever, boils, wounds and skin diseases. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic and constipating. The wood is nervine, antidiabetic, sedative and is useful in convulsions. The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent. The ash of Jackfruit leaves is used in case of ulcers. The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property and its pith is said to be abortifacient. Latex is used as an anti-inflammatory agent. Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world as a source of therapeutic agents for the prevention and cure of various diseases In India, there are more than 2500 plants species which are currently used as herbal medicaments. For than 3000 years, the herbal medicines are used either directly as folk medication or indirectly in the preparation of recent pharmaceuticals. Selection of leaves are important as compared to the other parts because it includes more constituents and also can be cheaply available. In India we don't give more importance to the *A. heterophyllus* leaf. Usually, they are burning along with other waste products. So, we will select to make an advantage from that.

MATERIAL AND METHOD:

Collection of Samples and Authentication: Fresh Leaf part of *Artocarpus heterophyllus* belonging to the family Moraceae was collected from kazhakkotam city at Trivandrum district of kerala. The identification and authentication of the medicinal plant were carried out by Dr. E. A. Siril Proffesor and head, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. The drug was shade dried, stored in airtight container and labelled. The drug was powdered and used for extraction, pharmacognostical, physico-chemical parameters, phytochemical, and pharmacological study. The RBC cell was collected from a laboratory in Trivandrum.

Pharmacognostical Study: The Leaf sample was subjected to morphological and microscopical examination

Morphology Study: The leaf part of plant *A. heterophyllus*. Lam was collected and subjected to morphological studys such as shape, size, colour, odour, taste.

Microscopy Study: The sample was subjected to transverse section. After collecting the plant

material, cut the material with razor blade to a thickness of 10- 20 µm and examined the sample under a microscope after phloroglucinol and HCL.

Powder Miroscopy: Spread a small quantity of the powder on the slide, treated with acetic acid and observed under microscope.

Quantitative Leaf Microscopy:

Vein Islet Number: Vein - islet number is the minute area of photosynthesis tissue encircled by the ultimate division of the conducting strands. A piece of the leaf was cleared by boiling in chloral hydrate solution and camera lucida and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field. The slide was placed on the stage. The veins included within the square were traced off, completing the outline of those islets which overlap two adjustment side of the square. The average number of vein islet from our adjoining square, to get the value for one square mm was calculated.

Vein Terminal Number: Vein termination number is the number of veinlet terminations per mm of leaf surface. A piece of the leaf was cleared by boiling in chloral hydrate solution and *Camera lucida* and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field.

The slide was placed on the stage. The veins included within the square were traced off, completing the outline of those islets which overlap two adjustment side of the square. The number of veinlet termination present within the square was counted and the average number of veinlet termination number from the four adjoining square to get the value for 1 square mm was found known as vein termination number.

Stomatal Number: Stomatal number is the average number of stomata per square millimeter of epidermis. A piece of leaf was cleaned and the upper and lower epidermis was peeled out separately by means of forceps. It was kept on slide and mounted in glycerin water. *Camera lucida* was attached and drawing board was placed for drawing the cells. A square of 1 mm by means of stage micrometer was drawn on it. The slide with cleared leaf was placed on the stage and the epidermal cells and stomata were traced. The number of stomata and the number of epidermal cells in each field were counted.

Stomatal Index: The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomatal index. A piece of leaf was cleaned and the upper and lower epidermis was peeled out separately by means of forceps. It was kept on slide and mounted in glycerin water. *Camera lucida* was attached and drawing board was placed for drawing the cells. A square of 1 mm by means of stage micrometer was drawn on it. The slide with cleared leaf was placed on the stage and the epidermal cells and stomata were traced. The number of stomata and the number of epidermal cells in each field were counted. The numbers of stomata were counted as stomatal number and the stomatal index using the above formula was calculated separately for upper and lower surface. Stomatal index can be calculated by using following equation:

$$I = S / E + S \times 100$$

Where,

I = stomatal index.

S = number of stomata per mm2.

E = number of ordinary epidermal cells per mm2.

Physical Study:

Ash Value: Ash content represents the inorganic residue remaining after ignition and complete oxidation of organic material. Ash values are helpful in determining the quality and purity of crude drug especially in powdered form.

Procedure:

Total Ash: Weighed and ignited flat, thin teared silica crucible and weighed about 2gm of powdered plant material into the crucible. Heated with a burner using a flame about 2cm height and supporting the dish about 7cm above the flame.

Heated till vapour almost ceace to be evolved, then lower the dish and the heat moved strongly until all the carbon is burned off, cooled in a desicator. Weighed the ash and calculate the total ash.

Acid – Insoluble Ash: Proceed as per the step mentioned in the procedure for total ash value of crude drug. Further using 25 ml of dil. HCL washed the total ash from the dish used for the total ash into 100 ml beaker. Placed wire gauze over a burner and boiled for 5 min. Filtered through as ashless filter paper, washed the residue twice with hot water. Ignited a crucible in a flame cooled and weighed. Put the filter paper and residue together into the crucible, heated gently until vapour cease to the evolved and then moved strongly until carbon has been released. Cooled in a desicator and weighed the residue and calculated acid – insoluble ash value of the drug.

Water – Soluble Ash

Using 25 ml of water, washed the total ash from the dish used for the total ash into 100ml beaker. Placed wire gauze over the burner and boiled for 5 min. Filtered through as ashless filter paper, washed the residue twice with hot water. Ignited the crucible in a flame cooled and weighed. Put the filter paper and residue together in the crucible, heated gently until vapour ceases to the evolved and then moved strongly until carbon has been released. Cooled in a desicator, weighed the residue and calculate acid – soluble ash of the drug.

Loss on Drying: Moisture content was determined by loss of water in terms of percent W/W by using the formula: Moisture content = Fresh weight – Dry weight / Fresh weight x 100. Procedure: Weighed an empty Petri dish and taken 5gm of sample into it. Put this sample in an oven and adjusted 105°C temperature and this sample in an oven for 3 hrs. Cooled the petri dish in a desicator and weighed the loss in weight is usually recorded as moisture.

Extractive Value: Extractive value helps to determine the number of active constituents present in the plant materials. Procedure:

Alcohol Soluble Extractive Value: Weighed accurately about 5 gm of powdered plant material into 250 ml conical flask with stopper and added 100 ml of alcohol. Shaken the flask frequently during first 6 hrs. Kept it aside without disturbing for 18 hrs and then filtered. Pippeted out 25 ml of filterate and evaporated to dryness in a weighed shallow flat-bottomed dish on a water bath. Then dried the residue at 105 °C to a constant weigh and calculated the percentage of alcohol soluble extractive value.

% of alcohol soluble extractive value = weight if residue / weight of drug x 100

Water Soluble Extractive Value: Weighed accurately about 5 gm of powdered plant material into 250 ml conical flask with stopper and added 100 ml of chloroform water. Shaken the flask frequently during first 6 hrs. Kept it aside without disturbing for 18 hrs and then filtered. Pippeted out 25 ml of filterate and evaporated to dryness in a weighed shallow flat-bottomed dish on a water bath. Then dried the residue at 105 °C to a constant weigh and calculated the percentage of water-soluble extractive value.

% of water-soluble extractive value = weight of residue / weight of drug x 100

Phyto Chemical Study: Extracts obtained were subjected to various chemical test to the chemical constituents presents in them. Results were tabulated.

Test for Alkaloids: The test solution was prepared by dissolving the extract in dilute HCL, the solution was filtered. The filterate was then subjected to the following test for the detection of the presence of alkaloids.

Dragendroff's test: To 2ml of the filterate was added to 1 ml of dragendroff's reagent (solution of potassium bismuth iodide). Formation of orange or reddish – brown precipitate indicates the test as positive.

Mayer's test: To 1 ml of test solution or filterate was added a drop or two of the Mayer's reagent (Potassium mercuric iodide.) Formation of yellow cream precipitate indicates the presence of alkaloids.

Hager's test: To 1 ml of the filterate or solution, added one drop or two drop of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate indicated the presence of alkaloids.

Wagner's test: Two drops of Wagner's reagent (Iodine in potassium iodide). Formation of yellow

or brown precipitate indicate the presence of alkaloids.

Test for Carbohydrates: The test solution was prepared by dissolving the extract individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test: Filterates were treated with 2 drops of alcoholic ᾰ-naphthol in a test tube and 2ml of con. sulphuric acid was added carefully along the sided of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Benedict's test: Filterates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of carbohydrates.

Fehling's test: Filtrates were hydrolyzed with diluted hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Test for Glycosides: Extracts were hydrolyzed with diluted hydrochloride acid and then subjected to test for glycosides.

Killer -killani test: Filterate were treated with glacial acetic acid containing a drop of ferric chloride. Formation of brown coloured ring indicates the presence of glycosides.

Test for Proteins: Extracts were prepared in water with phosphate buffer, soluble once are easily extracted by distilled water and insoluble once ae dissolved by alkaline treatment, then subjected to test for protein.

Biuret test: Filterate were added with 1ml of 4 % NaOH solution and a few drops of 1% copper sulphate solution. Formation of violet or pink color indicates the presence of protein.

Million's test: Filterate were treated with million's reagent. Formation of white precipitate, on warming the precipitate of brick red or it may be dissolved indicates the presence of protein.

Test for Amino Acid: Extracts was dissolved in water and subjected to test for amino acid.

Ninhydrin test: Filterate were treated with few drops of Ninhydrin solution and solution is treated in water bath for 10 minutes. Formation of purple or bluish colour indicates the presence of amino acid.

Test for Saponins: Extracts were dissolved in distilled water and subjected to test for saponins.

Foam test: Filterate were placed into a test tube and shaken vigorously for 2 minutes. Formation of persistent appearance of foam lasting for an at least 15 minutes indicates the presence of saponins.

Test for Flavonoids: Extracts were treated with 2M HCL and heated for about 30-40 min at 100°C. The extract was cooled and again extracted with ethyl acetate which was further concentrated to dryness and subjected to test for flavonoids.

Shinoda's test: Filterate were treated with 95% v/v methanol and few drops of Con. Hcl and 0.5 gm magnesium turnings. Formation of pink color indicates the presence of flavonoids.

Lead Acetate test: Filterate were treated with equal amount of lead acetate solution. Formation of yellow-coloured precipitates indicates the presence of flavonoids.

Sodium Hydroxide test: Filterate were treated with large amount of NaOH. Formation of yellow colour which decolorized after the addition of acid indicator.

Test for Tannins: Extract was dissolved in water and alcohol which is subjected to test for tannins.

Ferric Chloride test: Filterate were treated with 1ml of ferric chloride. Formation of dark blue or greenish black indicates the presence of tannins.

Gelatin test: Filterate were treated with few ml of 1% gelatin solution containing 10% NaCl. Formation of white principles indicates the presence of tannins.

Lead acetate test: Filterate were treated with few ml of 10% lead acetate solution. Formation of white precipitate indicates the presence of tannins.

Test for Steroids and Triterpenoids: Extract was dissolved in chloroform and subjected to test

Salkowski test: Filtrate were treated with 2ml chloroform and 3ml concentrated sulphuric acid carefully added to form a layer. Formation of reddish-brown coloration of the interface indicates the presence of terpenoids.

Liebermann-Burchard test: Filterate were filled with chloroform, acetic anhydride and few drops of Con. sulphuric acid. Formation of dark green color indicates the presence of terpenoids.

Test for Fats and Oils:

Filter Paper test: Filter paper gets permanently stained with oils.

Volatile Oil Extraction: Laboratories make use of the Clevenger apparatus for this purpose. The name of this apparatus was derived from its inventor, Joseph Franklin Clevenger in 1928. The Clevenger apparatus has three main parts- a round bottom flask in which the organic material is placed, a separator for automatically separating the distilled solution and a condenser. The apparatus is available in varied sizes to facilitate organic material. It looks similar to a glass double boiler having several valves to adjust the temperature. The process of extracting oil from an organic source involves the use of steam. The steam helps in avoiding the degradation of the essential oils.

At laboratory scale, we bring a few liters of water to a boil, and steam rises in a column containing the more or less finely ground plant. The vapor phase is then directed to a condenser, and the liquid is collected in a graduated burette. Thanks to a bended connection at the base of the burette, the hydrosol flows to the left in the beaker, while the essential oil remains in the burette. After typically 2 h of extraction (calculated from the first condensed drop), we can measure the volume of oil recovered and calculate the yield from the mass of plant introduced. This technique has the advantage of allowing us to recover the hydrosol. It also allows us to perform extractions on larger quantities of plants.

Toxicity Studies:

Preliminary Toxicological Screening Using Daphnia Magna: Select a range of concentrations that span those causing zero mortality to those causing complete mortality. Rinse all exposure chambers, except the chamber containing 100% test media, in dilution water. Mix concentrations and pour into each exposure chamber and measure 0.5 mL of the test media into a beaker and dilute to 500 ml. Using a graduated cylinder, pour out 50 mL into each exposure chamber and pour the rest into a beaker for chemical measurements. Continue these steps for all concentrations. Always work form the lowest concentration to the highest in order to minimize the risk of cross contamination. Using a wide bore pipette, randomly select and carefully place daphnia into each exposure chamber. Place the pipette tip below the surface and gently expel each daphnia individually into the chamber. The test begins when half of the organisms are in the exposure chambers. Measure and record mortality and survival at one hour and then at 24 and 48 hr. Measure and record temperature, dissolved oxygen, pH, conductivity, alkalinity, and hardness after the test begins and at the completion of the test. The test is complete at the end of 48 hours.

Pharmacological Study:

***In-vitro* HRBC Membrane Stabilization:** HRBC method was used for the estimation of anti-inflammatory activity *in-vitro*. The RBC cells was collected and washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 3 000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

$$\text{Percentage protection} = 100 - (\text{OD sample}/\text{OD control}) \times 100$$

Diclofenac sodium (5mg/ml) was choosen as the standard drug. The hemoglobin content in the supernatant solution and standard drug solution was estimated by using spectrophotometer at 560 nm.

RESULT AND DISCUSSION:

Morphological Study: Shape: Alternate, entire, simple, glossy, leathery, stiff, large and elliptic to oval in form with an entire margin, blunt apex,

tapers to base, glabrous surface, Netted pinnate venation. Leaves are often deeply lobed when juvenile and on young shoots. Size: 8 -10 cm in width, 15-17cm length. Colour: Green colour. Odour: Characteristic odour. Taste: Bitter taste.

Microscopic Study: TS of the leaf shows a dorsiventral structure with slightly wavy upper and much wavier lower epidermis. Mesophyll shows the presence of a single layer of compacted elongated palisade cells which is followed by spongy parenchyma. It consists of cambium, phloem, ground tissue, pith, xylem, vascular bundles.

Powder microscopy showed xylem vessels, epidermal cells withanomocytic stomata, covering trichomes, rosette form of calcium oxalate crystals and starch grains.

Quantitative Microscopy:

Quantitative Microscopy of Leaf Constants:

TABLE 1: QUANTITY MICROSCOPY OF LEAF OF *A. HETEROPHYLLUS. LAM* LEAF

Sl. no.	Quantitative microscopy	Observation
1	Stomatal Number	15.6
2	Stomata index	25.7
3	Vein islet number	15.4
4	Vein termination number	30.7

The leaf constants of *A. heterophyllus. Lam* was examined and found to be the following observations.

Stomatal number: 15.6

Stomatal index: 25.7

Vein-islet number: 15.4

Vein termination number: 30.7

Physical Study:

Parameters	Leaf Values % W/W
Water-soluble extractive value (%W/W)	5.4
Alcohol-soluble extractive value (%W/W)	6.0
Loss on drying at 105°C (%W/W)	0.168
Total ash value (%W/W)	27.0
Acid-insoluble ash value (%W/W)	14.07
Water-soluble ash value (%W/W)	24.80

Physical Study of *A. heterophyllus. Lam* leaf: The physical study of *A. heterophyllus. Lam* leaf was carried out and found the values of water extractive values, alcohol extractive values, total ash, water soluble ash, acid insoluble ash and loss on drying.

Phytochemical Study:

TABLE 2: PHYTOCHEMICAL STUDY OF *A. HETEROPHYLLUS. LAM* LEAF

Sl. no	Name of test	Alcohol extract	Water extract
1	Carbohydrate	+ve	+ve
2	Alkaloid	+ve	-ve
3	Glycoside	+ve	-ve
4	Flavonoids	+ve	+ve
5	Tannins	-ve	+ve
6	Protein	+ve	+ve
7	Amino Acid	+ve	+ve
8	Saponin	+ve	+ve
9	Steroids and Triterpenoids	+ve	+ve
10	Fats and Oils	-ve	-ve

The phytochemical study was carried out and the ethanolic extract of *A. heterophyllus. Lam* contain the presence of carbohydrate, alkaloid, glycoside, flavanoids, protein, saponin, steroids and

triterpenoids. The water extract contains the presence of tannins, protein, amino acid. Saponin, steroid and triterpenoids. Volatile oil extraction method was carried out using Clavancher apparatus and found the absence of oil in *A. heterophyllus. Lam* leaf part.

Toxicity Studies:

Preliminary Toxicological Screening using Daphnia Magna: The methods used to determine the LC_{50} differ depending on the results of the test. If there is no partial mortality in any replicate (i.e., all alive or all dead), then the Moving-Average Method may be used to determine the LC_{50}. If there is partial mortality with in a replicate, then the Probit Method should be used to calculate the LC_{50}. Also, the Lowest Observable Effect Concentration (LOEC) is recorded and the No Observable Effects Concentration (NOEC) is recorded. Since this is a simple acute test, only mortality is recorded up to 2000µg/ml and no mortality was observed.

TABLE 3: TOXICITY STUDY OF DAPHNIAMAGNA

Test Concentrations (µg/ml)	Mortality
100	Nil
500	Nil
1000	Nil
1500	Nil
2000	Nil

Pharmacological Study:

***In-vitro* HRBC Membrane Stabilization:** HRBC membrane stabilization was carried out after collecting RBC cells from the laboratory. Then alcohol extract of *A. heterophyllus. Lam* was added to the HRBC at different dilution (50,100 & 150 µg/ml) and then added phosphate buffer and hyposaline solution and it was again centrifugated at 3000 rpm and the solution was incubated at 37°C for 30 minutes, then analysed at 560 nm. The percentage haemolysis was estimated by assuming the hemolysis produced in the control as 100%. Diclofenac sodium was used as the standard drug (5mg/ml).

TABLE 3: PHARMACOLOGICAL STUDY OF *A. HETEROPHYLLUS. LAM*

	Absorbance (nm)
Control	0.854
Diclofenac sodium (Standard)	0.686
Ethanolic leaf extract concentration	
50 µg/ml	0.45
100 µg/ml	0.52
150 µg/ml	0.60

Antirheumatic Activity in HRBC Using Ethanol Leaf Extract: *A. heterophyllus. Lam* ethanolic extracts at different concentrations (50, 100, 150µg/mL) showed significant stabilization towards HRBC membranes. The percentage protection of ethanolic extract at concentration 150 µg/mL was higher than that of other concentrations. However, the percentage protection was found to be increased at higher concentration.

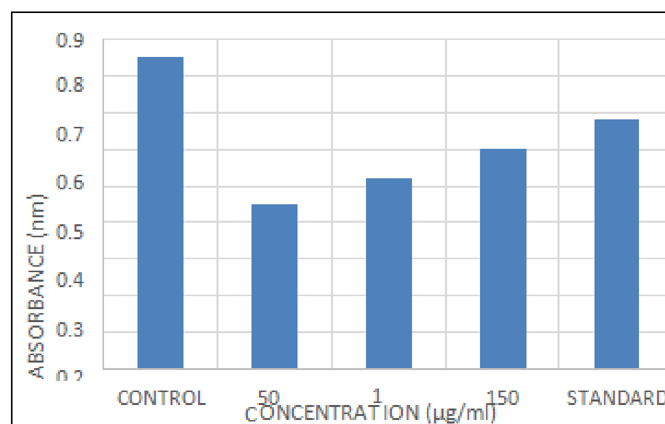

FIG. 1: GRAPHICAL REPRESENTATION OF ANTIRHEUMATIC ACTIVITY IN HRBC USING ETHANOLIC LEAF EXTRACT

Concentrations	Percentage (%)
Standard	39.7
50 µg/ml	10.5
100 µg/ml	15.12
150 µg/ml	25.74

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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