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EVALUATION OF *IN-VITRO* ANTIARTHRITIC ACTIVITY OF WHOLE PLANT OF *PILEA MICROPHYLLA* (L.) LIEBM

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ABSTRACT
Medicinal plants provide major source of molecules with medicinal properties due to the presence of natural compounds. Arthritis is the swelling, pain and tenderness of one or major joints. Researchers show great interest in those medicinal agents that are derived from plants because the currently available drugs for alleviating pain & symptoms of Osteoarthritis & Rheumatoid arthritis either have certain side effects or are highly expensive. *Pilea microphylla* (Urticaceae), commonly known as artillery weed, rockweed or gunpowder plant, Mathil pacha has been reported to possess antioxidant, antidiabetic, radioprotective, antimicrobial, cytoprotective, antigenotoxic, antidepressant properties along with many ethnobotanic uses. The present study evaluates the pharmacological potential of Ethanolic Extract of *P.microphylla* [EEPM] & Aqueous Extract of *P.microphylla* [AEPM] of whole plant as an antiarthritic agent. The preliminary phytochemical screening of ethanol and aqueous extract showed presence of secondary metabolites like glycosides, saponins, phenolic compound, flavonoids, and alkaloids. In-vitro anti-arthritic activity of P. microphylla extract was evaluated by inhibition of protein denaturation by Egg Albumin [EA] and Bovine Serum Albumin [BSA] Method. The significant results in evaluation of anti-arthritic activity may be due to the combined effect of chemical constituents in the extract or individual effect. Hence ethanol and aqueous extract of P.microphylla offers to be an important source of bioactive constituents for the management of arthritis.

KEYWORDS: *Pilea microphylla*; Arthritis; *in-vitro*; Anti-arthritic; BSA; EA.

INTRODUCTION
A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for the synthesis of useful drugs.[1] The knowledge associated with traditional medicine has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals.[2]

RA is a chronic, inflammatory, and systemic autoimmune disease. Arthritis is the swelling and tenderness of one or more joints. The most common types of arthritis are osteoarthritis and rheumatoid arthritis. The primary symptoms of RA include pain, swelling, and destruction of cartilage and bone because of which permanent disability occur. Although the exact aetiology is unknown, but several hypotheses said that it is triggered by the combination of genetic predisposition and exposure to environmental factors like viruses. The exact pathophysiology is still unknown.[3] There are four main groups of drugs used to treat arthritis: Pain killers (analgesics), non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and corticosteroids (steroids).[4]

Plant Profile
Pilea microphylla [PM] is the largest genus of the Urticaceae family and one of the largest genera in the Urticales. It is native to Mexico and tropical South America & mainly utilized in gardens and landscapes as foliage or groundcover ornamental plant, but also for many ethnobotanical uses.[5] Pharmacologically, *Pilea* have been reported to possess antioxidant, antidiabetic, radioprotective, antimicrobial, cytoprotective, antigenotoxic, antidepressant properties. Folkoric uses of *Pilea microphylla* are:- Used for diarrhea and asthma, Entire plant infusion is used as a diuretic, Crushed leaves applied to sores and bruises, In the Antilles, sweetened decoction of roots used as diuretic, In Jamaica entire plant used for women in labor; used for infertility and inflammation. In Guatemala used for urinary problems. In Jamaican and Chinese medicine used for diabetes. In Trinidad and Tobago, leaves are used for inflammation and as womb cleanser.[6]

Fig. No. 1: *Pilea microphylla.*

Rationale of the Study

Cultivation, collection and availability of *P.microphylla* is very easy because it is a small, perennial herb which can be easily grown as a ground cover in a moist area. The appropriateness and strength of screening *P.microphylla* for its anti-arthritic activity was taken for the study, as they possess antibacterial activity and antimicrobial activity. The antimicrobial activity of *P.microphylla* was tested *invitro* by disc diffusion method and Minimum Inhibitory Concentration [MIC], and there is not enough report about its anti-arthritic activity. Thus, the present study is aimed to study the *in-vitro* antiarthritic activity of *P. microphylla.*

The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. Concern regarding the safety and cost of conventional arthritis management and therapies have sparked interest in natural remedies. Thus, the present study is aimed to study the *in-vitro* antiarthritic activity of *Pilea microphylla.*

MATERIAL AND METHODS

Materials used

Table No 1 : List of Chemicals and Reagents.

Reagents and Chemicals	Suppliers/ Manufactures
Ethanol	Medilise chemicals, Kannur
Distilled water	Nice chemicals Pvt Ltd, Cochin
Hydrochloric acid	Nice chemicals Pvt Ltd, Cochin
Potassium chloride	Spectrum Reagents & Chemicals Pvt Ltd
Sodium chloride	Medilise chemicals, Kannur
Disodium hydrogen phosphate	Nice chemicals Pvt Ltd, Cochin
Potassium dihydrogen phosphate	Medilise chemicals, Kannur
Bovine serum albumin	Kanton laboratories

Table No 2 : List of Equipment's.

Instruments	Manufactures
Hot air oven	Remi lab world, Mumbai
Incubator	Remi lab world, Mumbai
Compound microscope	Olympus , Japan
Digital microscope	Menzel vision, Mumbai
Electrical balance	Aczet Pvt Ltd
Micropipette	Eppendorf, Germany
Desiccator	Sorbead, Gujarat
UV Visible spectrometer	Hitachi- hitech science corporation, Japan
Distillation apparatus	Borosil India Company

Plant Collection and Authentication

The whole plant of *Pilea microphylla* were collected locally from Aluva region of Kerala in July 2022. *Pilea microphylla (L)* were authenticated by Prof. Dr. P. Sreeja, Dept. of Botany and Research Centre, Sir Syed College, Taliparamba, Kannur and a voucher specimen No of 9942 was deposited for future reference.

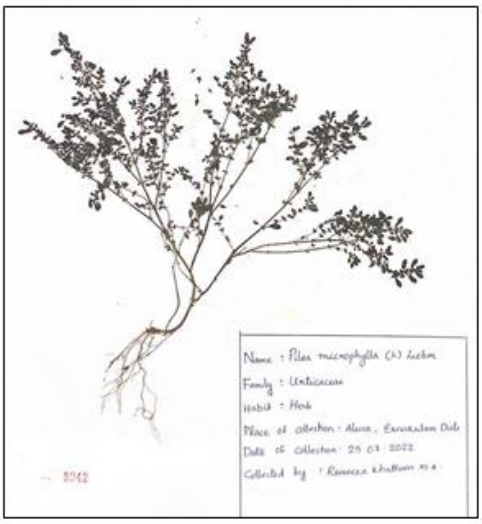

Fig. No. 2: Herbarium of *Pilea microphylla* (L) Liebm.

Pharmacognostical studies[7,8]

Macroscopic Evaluation

Macroscopic studies were carried out using morphological and organoleptic evaluation method. The size, shape & appearance, colour, odour of *P.microphylla* were observed.

Microscopical evaluation

a) Transverse section

Stem of *Pilea microphylla* were taken and clear sections were selected and mounted on a clean glass slide and covered with cover slip using glycerine. The sections were the viewed under low and high-power microscope attached with digital camera connected to the computer and application software.

b) Powder analysis

The powder was boiled with chloral hydrate to remove coloring matters, covered with a cover slip and viewed under microscope after mounting it on a glass slide. Then the powder was stained with phloroglucinol in presence of hydrochloric acid and the lignified structurewere viewed under microscope.

Fig. No. 3: *P.microphylla* Powder.

Physicochemical evaluation

Physiochemical constants like ash value, extractive value, moisture content, swelling index and mucilage content were carried out according to the standard procedure.

Phytochemical evaluation

Preparation of Extract

The powdered plant material (100g) was extracted by maceration method. In this process, the coarsely powdered crude drug is placed in a stoppered container with the solvent (Ethanol & water), allowed to stand at room temperature for a period of at least 7 days with frequent agitation until the soluble matter has dissolved. 2-3 drops of chloroform water is added to aqueous extract to prevent bacterial contamination. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquidare clarified by filtration or decanting after standing.

Fig. No. 4: A-Maceration; B-extract; C-distillation unit, D-dried extract.

In vitro Pharmacological Study

Anti- arthritic Activity

The invitro anti-arthritic activity was studied using bovine serum protein denaturation method[9,10,11] & egg Albumin method.

Principle

In arthritic diseases production of autoantigens may be due to denaturation of proteins. Denaturation of the protein involves the disruption of secondary, tertiary and quaternary structure of the molecules and finally leads to cell death, it occurs due to stress like a high level of salt, high temperature and high level of acidity. The mechanism of denaturation probably involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding. Denaturation of protein is well documented as contributing to inflammatory conditions like RA, diabetes, and cancer. Agents that can prevent protein denaturation, therefore, would be worthwhile for antiarthritic drug development. [12]

Inhibition of Protein Denaturation can be calculated using the equation:

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Test}) \times 100}{\text{Control}}$$

Bovine Serum Protein Denaturation Method

Preparation of Reagents

0.5% Bovine Serum Albumin (BSA):

Dissolved 500mg of BSA in 100 ml of water.

Phosphate Buffer Saline PH 6.3

According to IP 2007, Prepare PBS PH 6.4 by dissolving 1.70g of disodium hydrogen phosphate, 1.36g of potassium dihydrogen phosphate & 7.02g of sodium chloride in sufficient water to produce 1000ml. The pH was adjusted to 6.3 using 1N HCl.[13]

Method: Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of test solution of various concentrations.

Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of distilled water.

Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.[14]

Procedure

0.05 ml various concentrations (200, 400, 600, 800, 1000 µg/ml) of test dugs and standard drug diclofenac sodium (200, 400, 600, 800, 1000 µg/ml) were taken respectively, and 0.45 ml (0.5% w/V BSA) mixed. The samples were incubated at 37°c for 20 minutes and the temperature was increased to keep the samples at 60°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 660 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium.[15,16]

The percentage inhibition of protein denaturation can be calculated as.
% Inhibition of protein denaturation = 100 x [A 1 –A 2 /A 1]
Where: A 1 = Absorbance of control
A 2 = Absorbance of test /standard sample with albumin solution.[17]

Egg Albumin Denaturation Method
The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations (200, 400, 600, 800, 1000 µg/mL) of drug. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37 ± 2°C in a BOD incubator for 15minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in the concentrations of 200, 400, 600, 800, 1000 µg/mL was used as the reference drug and treated similarly for the determination of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula.
Percentage inhibition = [Abs control–Abs test sample /Abs Test Control] x 100
Each experiment was done in triplicate and the average was taken.[18]

RESULTS AND DISCUSSION

Macroscopic evaluation

Table No 3: Macroscopy.

Characters	Leaves	Stem	Root
Size	2-5mm	8-12 inches	4-6 inches
Shape	obovate	slender	irregular
Colour	Green	Green with purple tint	Pale brown
Odour	Characteristic	Characteristic	Characteristic
Taste	Astringent	Astringent	Astringent

Microscopic evaluation

Transverse section of *Pilea microphylla* Stem

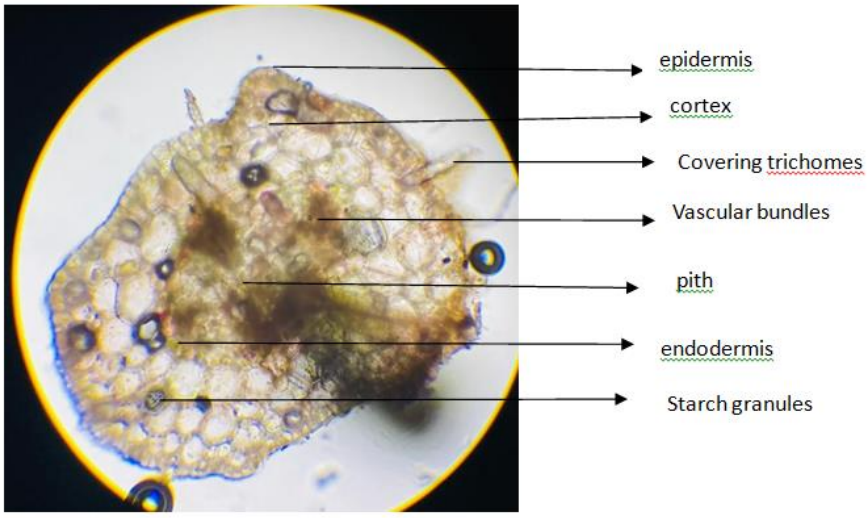

Fig No 5:(10x) TS of entire PM stem: A well-marked outer epidermis is present. Inner to epidermis 2-3 layered polygonl or hexagonal parenchyma cells are present. Epidermis is extended to form protective covering trichomes. Radially arranged vascular bundles with pith are visible. Single layered endodermis & Starch granules are also present.

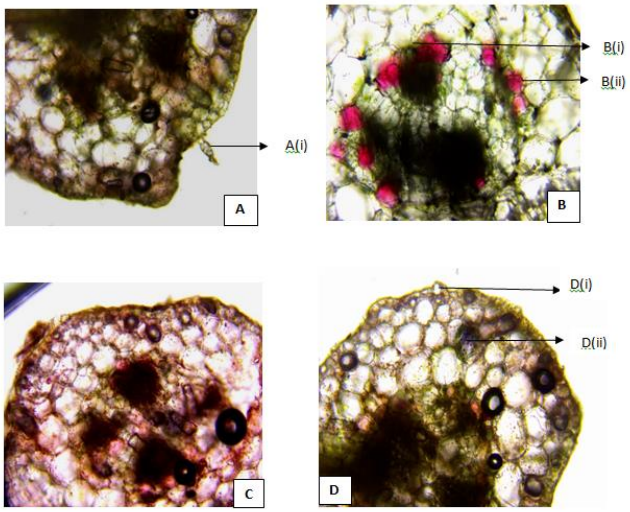

Fig No 6: A:(40x) covering trichomes **B:(40x) B(i)** radially arranged nonlignified phloem **B(ii)** lignified xylem bundles, **C:(40x)** TS of stem, **D:(40x) D(i)** with ridges, **D(ii)** starch granules.

Powder analysis

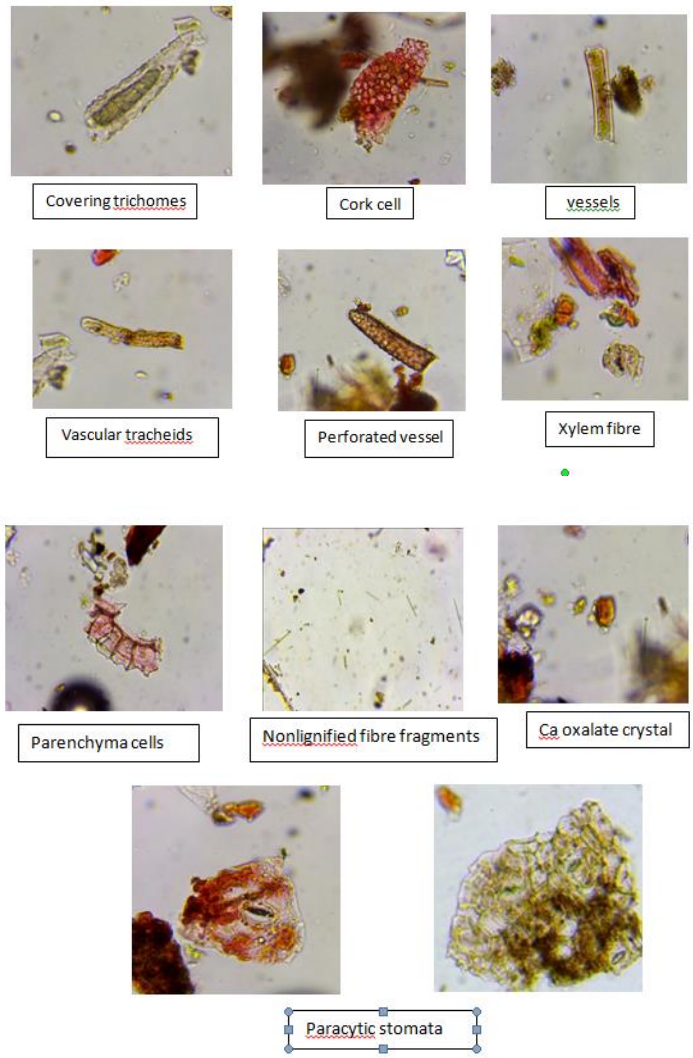

Fig No:7 Powder Analysis: Showed the presence of covering trichomes, parenchyma cells, cork cell, xylem fibers, vessels, tracheids, Ca oxalate crystals and characteristic paracytic stomata(stomata with 2 or 3 subsidary cells parallel to guard cells)

Physiochemical characters

Table No 4: Physicochemical parameters of *Pilea microphylla (L) Liebm.*

Physicochemical Parameters	Results
Ash value	**(Percentage w/w)**
Total ash	3.8
Acid insoluble ash	0.3
Water soluble ash	1.1
Extractive values	**(Percentage w/w)**
Water soluble extractive value	1.2
Ethanol soluble extractive value	0.8
Loss on drying	0.4
Swelling index	0.5ml
Foaming index	Nil
Mucilage content	0.1g

Phytochemical studies

Table 5: Qualitative chemical tests

SL.NO	Phytochemicals	Ethanolic extract	Aqueous extract
1	Carbohydrates	+	+
2	Proteins	_	_
3	Fats & Oils	_	_
4	Volatile oils	_	_
5	Steroids	_	_
6	Glycosides	+	+
7	Saponins	+	+
8	Phenolic compounds	+	+
9	Flavonoids	+	+
10	Alkaloids	+	+
11	Tannins	+	_

(+) = Presence
(-) = Absence

The phytochemical screening of alcoholic and aqueous extracts of whole plant of *Pilea microphylla* revealed the presence of flavonoid, glycoside, phenolic compound, saponins, alkaloids & carbohydrate in ethanolic and aqueous extract. Whereas tannins were present only in ethanolic extract.

In-***vitro*** **Anti-arthritic Activity**

A) BOVINE SERUM ALBUMIN METHOD [BSAM]

Table No 6: Percentage of inhibition of protein denaturation of Ethanolic & Aqueous extract of *Pilea microphylla* & Diclofenac.

Sample	Concentration (µg/ml)	Absorbance of test	% of inhibition
CONTROL	-	**1.088**	-
Standard Diclofenac **[SD]**	200	0.653	39.981
	400	0.548	49.632
	600	0.410	62.311
	800	0.314	71.138
	1000	0.251	76.930
Ethanolic extract of *Pilea microphylla* **[EEPM]**	200	0.277	74.547
	400	0.222	79.590
	600	0.158	85.474
	800	0.108	90.073
	1000	0.092	91.544
Aqueous extract of *Pilea microphylla* **[AEPM]**	200	0.064	94.115
	400	0.054	95.04
	600	0.045	95.86
	850	0.037	96.59
	1000	0.027	97.51

B) EGG ALBUMIN METHOD [EAM]:

Table No 7: Percentage of inhibition of protein denaturation of Ethanolic & Aqueous extract of *Pilea microphylla* & Diclofenac.

Sample	Concentration (µg/ml)	Absorbance of test	% of inhibition
CONTROL	-	**3.949**	-
Standard Diclofenac **[SD]**	200	1.438	63.585
	400	1.278	67.637
	600	1.015	72.119
	800	0.626	84.147
	1000	0.314	92.048
Ethanolic extract of *Pilea microphylla* **[EEPM]**	200	3.652	7.512
	400	3.542	10.300
	600	3.102	21.449
	800	2.548	35.472
	1000	1.500	62.017
Aqueous extract of *Pilea microphylla* **[AEPM]**	200	3.226	18.340
	400	3.027	23.341
	600	2.730	30.864
	850	2.162	45.253
	1000	1.328	66.371

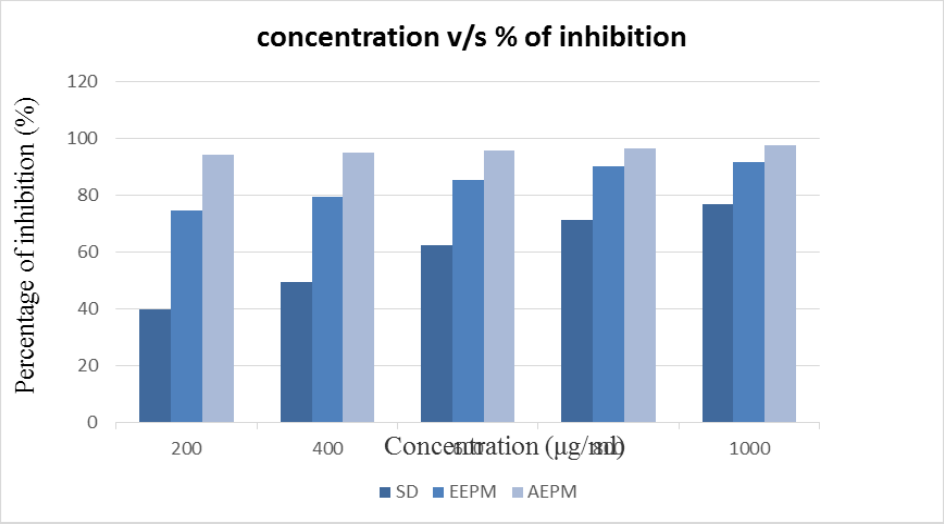

Fig. No 8: Graphical representation of SD, EEPM & AEPM using BSA Method.

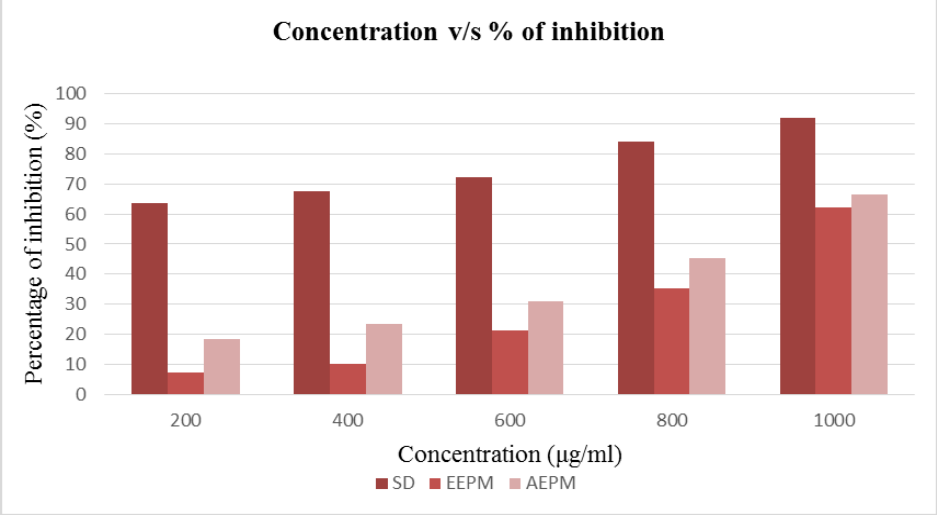

Fig. No 9: Graphical representation of SD, EED & EEPM using Egg albumin method.

DISCUSSION

Investigation of *in-vitro* antiarthritic activity of both ethanolic and aqueous extract of *Pilea microphylla* was done in 5 different concentrations (200, 400, 600, 800, & 1000µg/ml). Various *in-vitro* methods carried out include Bovine serum Albumin and egg albumin method.

The study of *in-vitro* antiarthritic activity on *Pilea microphylla* depend on the type of solvent used during Extraction. Ethanol is an organic polar solvent suitable for the extraction of phenolic compounds as it is effective, efficient & safe for human consumption whereas polar inorganic solvent such as water is usually used for the extraction of various bioactive phytochemicals in different experiment. This study showed that the highest anti-arthritic can be obtained from the aqueous extract of *Pilea microphylla* when compared to the ethanolic extract of the same.

% inhibition of protein denaturation of standard Diclofenac [SD] was found to be, 39.98, 49.63, 62.31, 71.13, 76.93 & EEPM was found to be 74.54, 79.59, 85.47, 90.07, 91.54 & AEPM was found to be 94.11, 95.04, 95.86, 96.59, 97.51 in 200, 400, 600, 800, & 1000µg/ml concentration respectively using Bovine serum albumin method [BSAM].

% inhibition of protein denaturation of standard Diclofenac [SD] was found to be, 63.58, 67.63,72.11, 84.14, 92.04 & EEPM was found to 7.5, 10.30, 21.44, 35.47, 62.01 & AEPM was found to be 18.3, 23.34, 30.86, 45.25,66.37 in 200, 400, 600, 800, & 1000µg/ml concentration respectively using Egg albumin Method [EAM].

In BSA and EA Method significant difference were noted, when ethanolic and aqueous extract were compared to standard Diclofenac. In BSA method ethanolic and aqueous extract showed more activity than SD. More activity of test than standard may be due to the crude extract form of the test. Whereas in EA method, the activity of EEPM & AEPM were less than the SD.

At the end of the study, when compared, in both BSA and EA method aqueous extract showed more pronouncing effect than ethanolic extract, it may be due to the presence of more class of chemical in aqueous extract than ethanolic extract and the action produced may be due to synergistic effect of the phytochemicals like phenolic compounds, flavonoids & glycosides. The protein denaturation inhibition action produced by the phytochemical may be due to its antioxidant, radical scavenging & ability to suppress the inflammation. Thus, act as natural anti-arthritic agents. Aqueous extracts are also able to form H bond with the hydroxyl group, so they are simple, economical & eco-friendly alternative.

From the result it was shown that the ethanolic and aqueous extract of *Pilea microphylla* effectively inhibit protein denaturation in a dose dependent manner. The percentage inhibition (%) against various concentration of different extract and standard were compared & graphically represented. Finally, it can be concluded that *Pilea microphylla* shows significant antiarthritic activity.

CONCLUSION

Inhibition of protein denaturation was studied to establish the mechanism of anti-arthritic effect of *Pilea microphylla*. *In-vitro* anti-arthritic method used for the study are Bovine Serum Albumin Method and Egg Albumin Method. From the results it may be concluded that the plant extract may control the production of auto antigens by preventing protein denaturation. Therefore, our present *in-vitro* studies on *P. microphylla* extracts demonstrated the significant anti-arthritic activity. Presence of active principles such as flavonoids, alkaloids, glycoside, and related polyphenols may be responsible for this activity. Hence, *P. microphylla* can be used as a potent anti-arthritic agent. Finally, it can be concluded from this study that the aqueous extract of *Pilea microphylla* can produce higher activity in inhibition of protein denaturation than ethanolic extract by using both Bovine serum albumin and Egg albumin method.

By further extensive research, we can explore the medicinal value of *P. microphylla* and make reasons why this is used traditionally for various diseases. The future scope of study involves the isolation and identification of individual bioactive phytoconstituent in *Pilea microphylla* underlying this high anti-arthritic potential.

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[Alabong, Pilea microphylla, artillery plant, Xiao ye leng shui hua: Philippine...]
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