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IN-VITRO ANTI-ARTHRITIC ACTIVITY OF WHOLE PLANT OF EPIPREMNUM AUREUM (LINDEN & ANDRE) G.S. BUNTING

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ABSTRACT

The plant *Epipremnum aureum* (Linden & Andre) G.S Bunting is a perennial, evergreen, climbing vine belongs to the family Araceae. In the present study, pharmacognostical, phytochemical constituents and in vitro anti-arthritic potential of whole plant of *Epipremnum aureum* were evaluated. Pharmacognostical evaluation included macroscopic, microscopic and physicochemical evaluation. Ethanol (EEEA) and water (AEEA) were used as solvent for extraction. Preliminary phytochemical screening was done by performing different chemical tests and glycosides, alkaloids, flavonoids, carbohydrate and phenolic compounds were present. The *in-vitro* anti-arthritic potential was evaluated using heat induced Bovine Serum protein denaturation method and Egg Albumin denaturation method. The activity of both of theplant extract was compared with standard anti-inflammatory drug Diclofenac. In the result it was found that the EEEA and AEEA of the plant showed significant reduction in protein denaturation. EEEA at concentration of 200, 400, 600, 800 and 1000 µg/ml showed 92.22%,93.95%, 95.29%, 96.52% and 97.02% inhibition respectively, whereas AEEA showed 96.52%,97.03%, 97.54%, 97.85% and 98.26% inhibition. In egg albumin denaturation method at 200, 400, 600, 800 and 1000 µg/ml EEEA showed 18.10%, 24.15%, 40.83%, 53.78% and 67.85respectively. Similarly, the AEEA showed % inhibition as 19.01%, 24.65%, 37.33%, 56.48% and 69.61%. The results reveal promising anti arthritic potential of the plant. However furtherpharmacological investigation using isolated active ingredients can be carried out to confirm its efficacy and mechanism of action and can result in an eco-friendly cost effective antiarthriticherbal drug with anti-inflammatory potential contributing towards the better healthcare of human society.

KEYWORDS: *Epipremnum aureum;* Anti-arthritic; Protein denaturation; Inflammation; Phytochemical; BSA; Egg albumin; *In-vitro.*

1. INTRODUCTION

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products, today, symbolize safety, in contrast to the synthetics that are regarded as unsafe to human and environment. Different chemical constituents contained in plant exhibit different activities for alleviating abnormal health of human/animals.[1] Throughout the world, herbal medicine has provided many of the potent medicines to the vast arsenal of drugs available to modern science, both in crude form and as a pure chemical, upon which modern medicines arestructured.[2] Therefore, traditional medical practitioners appreciate to use different parts of plant having several chemical constituents to treat various disorders.[3]

Epipremnum aureum is a perennial evergreen vine belonging to the family Araceae. It is a monocot that grows vigorously and rapidly covering a wide area. It is native to South East Asiaand Solomon Islands and mainly seen in temperate regions but later get introduced in tropical and sub-tropical forests. This plant possess antioxidant, antibacterial, antifungal, anti-termite, anti-tumor, and antiulcer activity.[4]

Fig. 1: *Epipremnum aureum.*

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh whole plants of *Epipremnum aureum* (Linden &Andre) G.S. Bunting was collected from Kozhikode district, Kerala on August 2022. The plant was dried under shade and powdered andthis powder was used for further pharmacognostic and phytochemical studies. Dried specimenof *Epipremnum aureum* was well pressed and mounted on herbarium sheet with the help of fevicol and it is labelled. The plant was authenticated by Dr. Sreeja P, M.Sc., Ph.D., PG Dept. of Botany & Research Centre, Sir Syed College, Taliparamba. Kannur district, Kerala, India. Also, a voucher specimen numbered 9944 was also kept in the respective college.

Fig. 2: Herbarium of Epipremnum aureum (Linden & Andre) G.S Bunting.

2.2 Pharmacognostical studies

2.2.1 Macroscopic studies

Macroscopical studies of Epipremnum aureum were examined and determined by the evaluation of external characters like colour, taste, odour, size and shape of the leaf, stem and root.

2.2.2 Microscopic characters

For transverse microscopy, free hand sections were taken. Thin sections were selected, stained with Saffranin, mounted in glycerin & observed through Trinocular 'Leica' microscope attached with 'Leica DFC 295' digital camera connected to the computer and Leica Application Software LAS Version 3.6.1.

For powder microscopy, sufficient amount of powder was taken on a microscopic slide, add 1-2 drops of saffranin. Spread the sample evenly over the slide & mount with glycerin. Observethrough Trinocular 'Leica' microscope attached with 'Leica DFC 295' digital camera connected to the computer and Leica Application Software LAS Version 3.6.1. And the procedure was repeated in 2-3 slides to get maximum characters.

2.2.3 Physicochemical evaluation

Physiochemical constants like ash value, extractive value, moisture content, swelling index and mucilage content were carried out according to the standard procedure.[5]

2.2.4 Phytochemical studies

Preliminary phytochemical screening was carried out by cold maceration method. Extraction was carried out by using ethanol and water. Then phytochemical screening of ethanolic and aqueous extracts of *Epipremnum aureum* was performed. The extracts obtained were subjected to various chemical tests for the detection of secondary metabolites.[6]

2.2.5 In-vitro pharmacologic study

Inhibition of protein denaturation using bovine serum albumin

0.05 ml various concentrations (200, 400, 600, 800 and 1000 µg/ml) of test drug and standard drug diclofenac sodium (200, 400, 600, 800 and 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-Visiblespectrophotometer at 660 nm. The control 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Inhibition of protein denaturation using egg albumin

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, and 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 and 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:[7]

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}^{[8]}}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Macroscopic evaluation

From macroscopic evaluation, colour, odour, taste, size and shape of the leaf, stem and root of the plant were determined.

Table 1: Morphological and Organoleptic character.

Characters	Leaf	Stem	Root
Size	Mature leaves upto100 cm long and 45 cm broad, but juvenile leaves are smaller	4 cm in diameter	1 cm in diameter
Shape	Heart shaped	Trailing, cylindrical	Cylindrical andslender
Colour	Green variegated with yellow, white, and cream colour	Green	Green or Browncolour
Odour	Characteristic	Characteristic	Characteristic
Taste	Slightly bitter	Characteristic	Characteristic

Fig 3: Juvenile Epipremnum aureum plant- A: Leaf, B: Stem, C: Root.

3.2 Microscopic evaluation

Transverse section of the leaf, stem and root were performed.

a) Transverse section of leaf

Transverse section of the leaf passing through midrib shows the following important tissues and as shown in the fig 4:

Upper epidermis: Covered with cuticle, cells are radially elongated and compactly arranged in monolayer. It is followed by 2-3 layers of chlorenchyma cells.

Mesophyll: Differentiated into a 2 layered palisade tissue, followed by 5-6 layers of spongy cells.

Vascular bundles: Present in mid-rib, conjoint, collateral, and closed. Each vascular bundle issurrounded by a sheath of sclerenchymatous cells. Each vascular bundle consists of xylem lying towards the upper epidermis and phloem towards the lower epidermis.

Lower epidermis: A single layer of parenchymatous cells covered with cuticle.

b) Transverse section of stem

T.S of stem is circular in outline with ridged margin. The following are the important tissues seen from periphery to the centre and are shown in the fig 5:

Epidermis: Ridged, made up of compactly arranged parenchymatous cells, followed by a few layers of hypodermal chlorenchyma.

Cortex: Many layered, parenchymatous, presence of vascular bundles in a ring fashion.

Endodermis: Single layered, followed by pericycle.

Pith: Large with scattered vascular bundles which are similar to those present in the cortex.

Vascular bundles: Conjoint, collateral, and closed i.e. both xylem and phloem are enclosed together, phloem is present towards the outer side, and cambium is absent. They resemble an oval shape that is surrounded by sclerenchymatous sheath. Phloem fibres are also present.

c) Transverse section of root

The transverse section of root is circular in shape with wavy margin. The following are the important tissues seen from the periphery to the centre and as shown in the fig 6:

Epidermis: Single celled, parenchymatous, single celled, and elongated trichomes are present.

Hypodermis: Multi-layered, collenchymatous.

Cortex: Multi-layered, parenchymatous, contain starch granules,

Endodermis: Made up of sclerenchymatous tissues. Inner to the epidermis, a single layered pericycle is present. On the endodermis, a specialised black coloured strip is present, called Casparian strips.

Vascular bundles: Well developed xylem and phloem are arranged in a ring fashion, surrounded by sclerenchymatous patches, comprising of 10 or more groups of xylem, alternating with patches of phloem. Xylem exarch i.e., metaxylem towards the centre, and protoxylem towards the periphery. Xylem and phloem are separated by parenchymatous strips.

Pith: Inner to the vascular bundles, a well cleared pith is present, parenchymatous, with small patches of sclerenchyma cells.

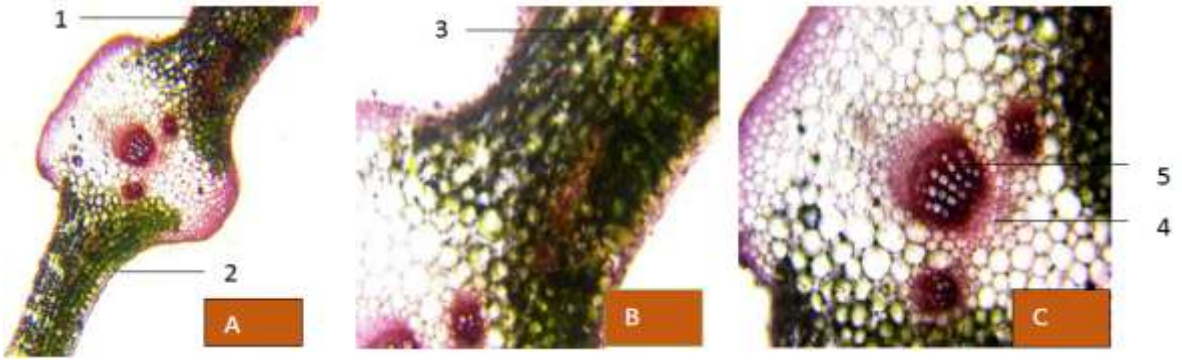

Fig. 4: T.S of leaf A- Leaf passing through the midrib (10X) 1: Upper epidermis, 2: Lowerepidermis, B- (40X) 3: Mesophyll, C-(40X) 4: Xylem, 5: Phloem.

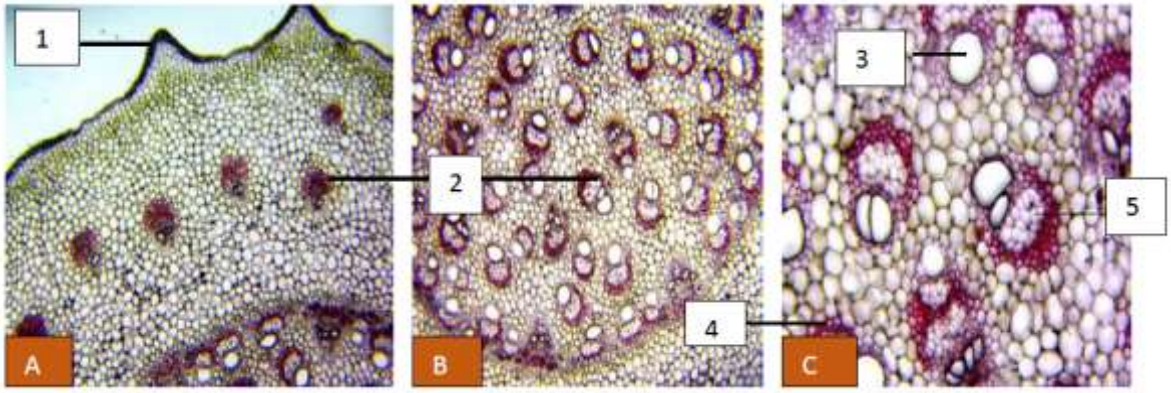

Fig. 5: T.S of stem A- (10X) 1: Epidermis, B-(10X) 2: Vascular Bundles, C-(40X) 3:Xylem, 4: Pericyclic fibres, 5: Phloem.

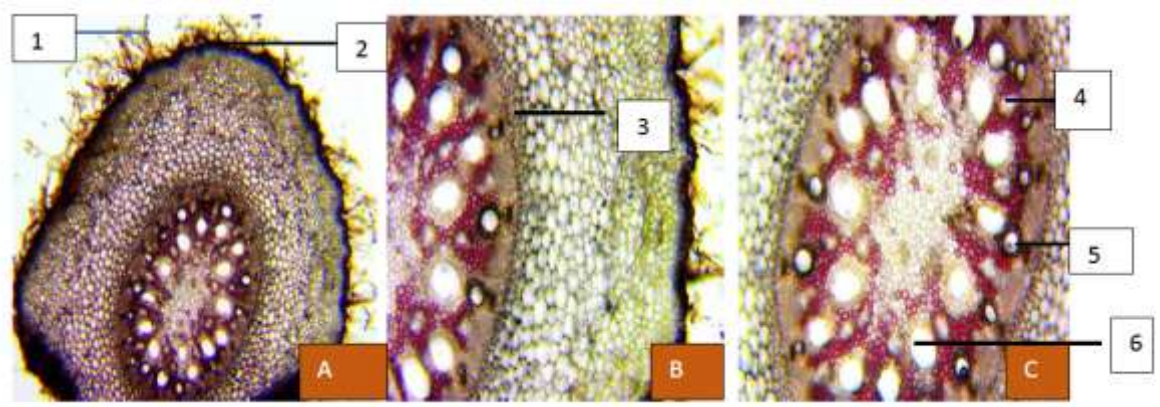

Fig. 6: T.S of root A- (10X) 1: Trichomes, 2: Epidermis, B- (40X) 3: Endodermis, C- (40X) 4: Phloem, 5: Protoxylem, 6: Metaxylem.

3.3 Powder microscopy of whole plant

The powder microscopy of the plant revealed the presence of trichomes originating from the epidermal cells and scattered cork cells as shown in the fig 7. Pitted xylem vessels are seen in purple colour which indicates that it is lignified. Cluster of parenchymatous cells are seen. Non-lignified fibres are clearly seen in an isolated manner. Paracytic stomata was seen in the epidermal cells. Prismatic calcium oxalate crystals are seen along with clusters of starch grains. Brown matters were also visible when viewed under the microscope.

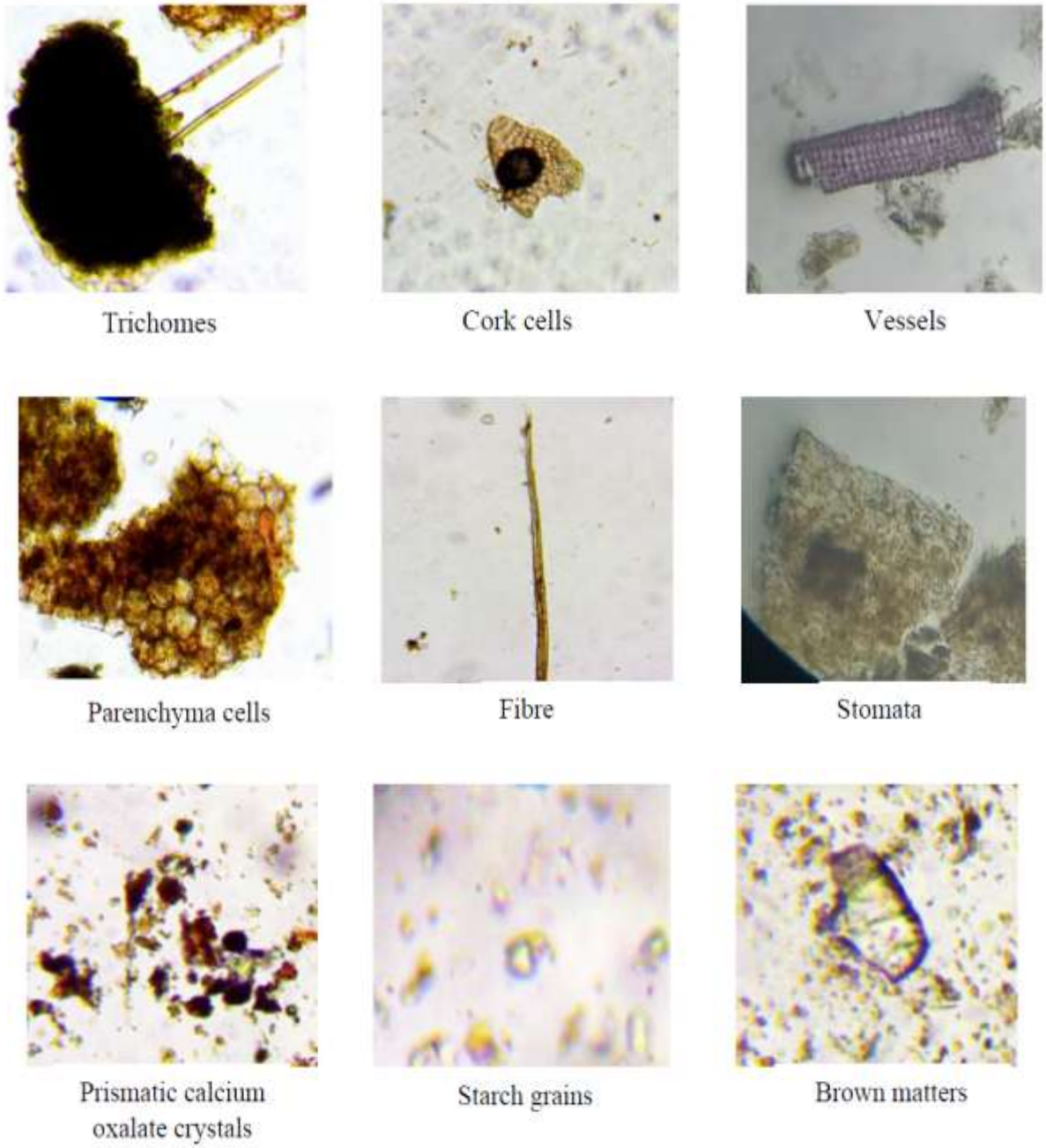

Fig. 7: Powder microscopy.

3.4 Physicochemical characters

Evaluation of identity, quality and purity is of prime importance to assure the safety of the drugs. Various parameters like solvent extractive values, moisture content, ash values etc can be used to establish the identity and quality of the plant drug. Determination of ash values is significant as it helps in detecting low grade products and exhausted drugs. Ash value mainly increases with adulteration and contamination. Insufficient drying may sometimes lead to enzymatic deterioration of active constituents therefore determination of moisture content is another important parameter to be assessed. Details of physicochemical properties were givenin table 2.

Table 2: Physicochemical parameters.

Parameters	**Results**
Ash value	**Percentage w/w**
a) Total ash	15.58
b) Acid insoluble ash	3.18
c) Water soluble ash	6.75
Extractive value	**Percentage w/w**

a) Ethanol soluble extractive	9.5
b) Water soluble extractive	8.48
Loss on drying	7.23
Swelling index	3 ml
Foaming index	Nil
Mucilage content	0.76% w/w

3.5 Phytochemical studies

Preliminary phytochemical screening was carried out by cold maceration method using ethanol and water. Phytochemical analysis showed the presence of carbohydrates, phenolic compound, alkaloids, flavonoids, phytosterols, steroids, tannins, and terpenoids.

Table 3: Qualitative chemical tests.

SL No.	Phytochemicals	Ethanolic extract	Aqueous extract
1.	Carbohydrate	+	+
2.	Proteins	-	-
3.	Fats and oils	-	-
4.	Steroids	+	-
5.	Phytosterols	+	-
6.	Volatile oils	-	-
7.	Tannins	+	-
8.	Glycosides	+	+
9.	Phenolic compounds	+	+
10.	Flavonoids	+	+
11.	Alkaloids	+	+
12.	Terpenoids	+	-

(+) – Presence(-) – Absence

The detection and presence of these bioactive secondary metabolites in the plant extracts is highly indicative of pharmacological importance of the plant in terms of its medicinal uses. The flavonoids, alkaloids, and phenolic compounds are widely distributed secondary metabolites in plants having anti-arthritic activity.

3.6 Pharmacological study

***In-vitro* anti-arthritic activity**

Inhibition of protein denaturation using bovine serum albumin

Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins. Denaturation of proteins 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.

The absorbance of each extract was recorded and the percentage of inhibition was calculated.

Table 4: Percentage inhibition of protein denaturation by EEEA, AEEA and SDusing BSA.

Sample	Concentration (µg/ml)	Absorbance test at 660 nm	% of inhibition
Control	-	0.978	-
Standard Diclofenac(SD)	200	0.653	33.23%
	400	0.548	43.96%
	600	0.410	58.07%
	800	0.314	68.20%
	1000	0.251	74.33%
Ethanolicextract of *E. aureum* (EEEA)	200	0.076	92.22 %
	400	0.059	93.96%
	600	0.046	95.29%
	800	0.034	96.52%
	1000	0.029	97.03%
Aqueousextract ofE.aureum (AEEA)	200	0.034	96.52%
	400	0.029	97.03%
	600	0.024	97.54%

	800	0.021	97.85%
	1000	0.017	98.26%

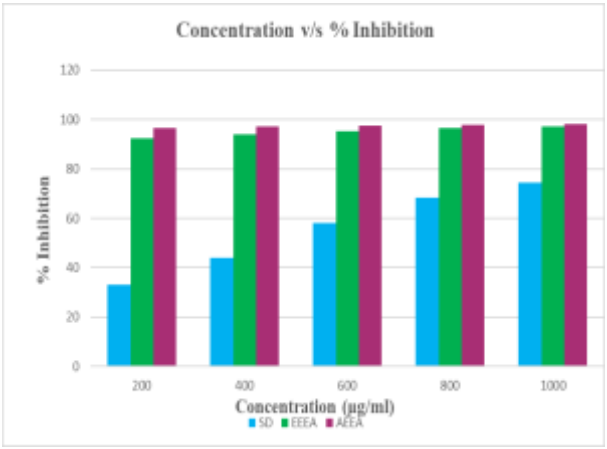

Fig. 8: Percentage inhibition of protein denaturation using BSA by EEEA, AEEA, SD.

Inhibition of protein denaturation using Egg Albumin

Table 5: % Inhibition of protein denaturation by EEEA, AEEA and SD using eggalbumin.

Sample	Concentration (µg/ml)	Absorbance of test at 660 nm	% of inhibition
control	-	3.739	-
SD	200	1.438	61.54%
	400	1.278	65.81%
	600	1.015	72.85%
	800	0.626	83.25%
	1000	0.314	91.60%
EEEA	200	3.062	18.10%
	400	2.836	24.15%
	600	2.212	40.83%
	800	1.728	53.78%
	1000	1.202	67.85%
AESD	200	3.028	19.01%
	400	2.817	24.65%
	600	2.343	37.33%
	800	1.627	56.48%
	1000	1.136	69.61%

The aqueous and ethanolic extract of *E.aureum* were evaluated for anti-arthritic activity over the standard Diclofenac sodium. In this study the AEEA showed significant anti-arthritic activity in both BSA denaturation method and Egg Albumin denaturation method, when compared to EEEA.

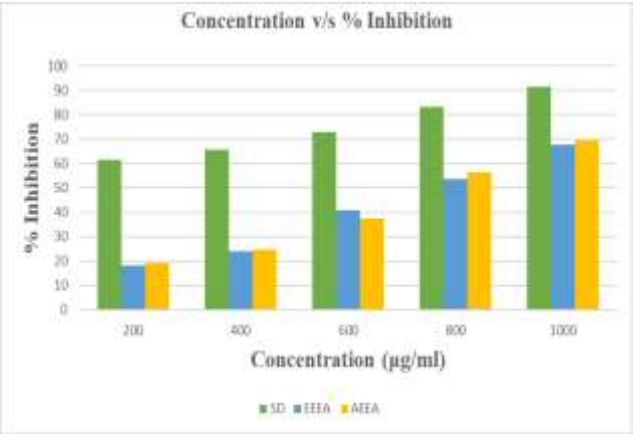

Fig. 9: Percentage inhibition of protein denaturation using egg albumin by EEEA, AEEAand SD.

In BSA method the maximum inhibition was observed at 1000 µg/ml. the aqueous extract showed maximum inhibition of 98.26%, while the ethanolic showed 97.02%. In case of standard drug Diclofenac maximum inhibition of 74.33% was observed at 1000 µg/ml. The % inhibition of protein denaturation by EEEA at the concentration of 200, 400, 600, 800 and 1000µg/ml was found to be 92.22%, 93.95%, 95.29%, 96.52% and 97.02% respectively. Similarly, the % inhibition of protein denaturation by AEEA was found to be 96.52%, 97.03%, 97.54%, 97.85% and 98.26% at concentration of 200, 400, 600, 800 and 1000 µg/ml respectively. The SD exhibited % inhibition of 33.23%, 43.96%, 58.07%, 68.20% and 74.33% at the respective concentrations. The plant extract exhibited more activity compared to the standard. This is may be due to the combined effects of phytoconstituents present in the plant extract. As SD is a single moiety, while the plant extract is in a non-characterised, crude form, the intact phytoconstituent responsible for the increased % inhibition is not crystal clear. Carrying out a successive solvent extraction, may help to find the distinct phytochemical that is actually responsible for the inhibition of protein denaturation.

In egg albumin denaturation method, both the extracts showed significant anti-arthritic activity. But, AEEA showed a slight increased percentage inhibition than the EEEA. The % inhibition of protein denaturation by EEEA was found to be 18.10%, 24.15%, 40.83%, 53.78% and 67.85% at the concentration of 200, 400, 600, 800 and 1000 µg/ml respectively. Similarly, the AEEA showed % inhibition as 19.01%, 24.65%, 37.33%, 56.48% and 69.61%. The % inhibition by SD was 61.54%, 65.81%, 72.85%, 83.25% and 91.60% respectively. The major constituents present in the aqueous extract were flavonoids, phenolic compounds, alkaloids and glycosides. So, the synergistic effect of these constituents may be responsible for the increased activity of aqueous extract.

There is a slight difference in the activity shown by the extract in BSA denaturation method and Egg Albumin denaturation method. This variation may be due to the difference in concentration and combined effects of phytoconstituents especially in case of total extract.

As per the literature survey denaturation of protein is one of the causes of rheumatoid arthritis. In certain rheumatic diseases, the destruction of the joint is due to the production of auto- antigens, that may be produced due to the denaturation of proteins. RA is characterized by chronic inflammation in joints with concomitant destruction of cartilage and bone. Several anti- arthritic drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. In the present study the ethanolic and aqueous extracts of *E.aureum* (Linden & Andre) G.S Bunting inhibited heat induced protein denaturation and may be one of the reasons of possessing anti- arthritic activity.

4. CONCLUSION

At the end of the study, the conclusion that deduced is that *E.aureum* is a plant containing several groups of chemical compounds with anti-inflammatory and anti-arthritic potential. The preliminary phytochemical screening of the plant extract indicated the presence of alkaloids, flavonoids, glycosides, tannins, terpenoids, steroids, phytosterols, phenolic compounds etc., which may be responsible for the anti-arthritic activity by reducing inflammation. *In-vitro* inhibition of protein denaturation induced by heat was the method employed to establish the anti-arthritic potential of the plant, as the denaturation of protein due to the production of auto-antigens is one of the causes of RA. The aqueous extract showed more activity compared to the ethanolic extract. The *in-vitro* studies have shown that *E.aureum* exhibits significant anti- arthritic activity. Anti-arthritic activity is may be due to the presence of flavonoids, alkaloids and phenolic compounds. Since, the free radical scavenging and cytokine inhibition of these compounds are already proven, that may be the mechanism by which inflammation is reduced in case of arthritis.

From the study we conclude further isolation of flavonoids, alkaloids, Tannins and Polyphenolic compounds can be attempted. This may be a stepping stone towards new drug discovery, Characterization and Formulation of different ayurvedic and allopathic medicines.

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