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Pharmacognostic, Phytochemical Investigations, and *In Vitro* Anti-arthritic Activity of *Adiantum venustum* D. Don

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Abstract

The present investigation deals with pharmacognostical, physicochemical, phytochemical analysis, and *in vitro* anti-arthritic activity of ethanolic extracts of *Adiantum venustum* D. Don. The macroscopic and microscopic characters, physical constant values, extractive values, ash values, color analysis, and fluorescence analysis were performed. The stems were 13–14 cm in size while leaves were 3–5 mm. Stems were straight with nodes while leaves were triangular and fan shaped. Transverse section of aerial parts of *A. venustum* showed epidermis consists of closely packed cells with single layer. No intercellular space was found. Endodermis is indistinct. On microscopic examination, the powder showed wavy epidermis, parenchyma cells, Phloem, fiber, trichome, brownish matter, and crystals. Physical constants performed were loss on drying, ash content, acid insoluble ash, and water soluble ash. Extractive values in chloroform and alcohol were determined. Fluorescence studies of the powder were carried in UV, UVB, and day light with various solvents. Phytochemical screening of successive extracts showed positive reactions for steroids, flavonoids, carbohydrates, phenols, and tannins. *In vitro* anti-arthritic activity was performed by the inhibition of protein denaturation method. The ethanolic extracts of aerial parts exhibited remarkable anti-arthritic action. The protein denaturation was also found to be maximum at 75.293 ± 0.735 and 79.956 ± 0.9 % at a dose of 500 μg/mL by protein denaturation egg albumin and bovine serum albumin method, respectively.

Keywords: *Adiantum venustum* D. Don, Anti arthritic activity, Ethanolic extract, *In vitro*, Pharmacognstical evaluation, Physiochemical screening

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Introduction

Rheumatoid arthritis is a chronic inflammatory autoimmune joint disease. This disease can cause damage of cartilage and bone as well as disability.[1] Pain, swelling, morning stiffness, warmth, redness, and limits in the functions of the joints are the common symptoms of RA.[2,3] Treatment of disease involves measuring of disease activity with composite indices and applies a treatment-to-target strategy. Conventional, biological, and new non-biological disease-modifying antirheumatic drugs are commonly used in the treatment of RA.[4-6] The allopathic system of medicine for rheumatoid arthritis uses two conventional lines of the treatment. Current therapies for the treatment of RA generally focused on anti-inflammatory action. Although NSAIDs, DMARDs, and corticosteroids are highly efficient drugs in the treatment of rheumatoid arthritis, they have mild to serious side effects.[7-9] The major adverse drug reactions (ADRs) related with NSAIDs are gastrointestinal (ulceration or bleeding) and cardiovascular (myocardial infraction) effects. Gastrointestinal (GI) toxicity and upper GI adverse events such as perforation, ulceration, and bleeding are occurred in about 20% of patients taking long-term NSAIDs. Therefore, use of a safe medicine in the treatment of RA is still matter of concern and search of safe drugs for the treatment of rheumatoid arthritis for chronic use is still going on.[10-12]

The medicinal plants are commonly used in traditional medicine. In the past few decades, very intense pharmacological studies have been investigated for medicinal plants.[13-16] The main aim of the present investigation was to study the *in vitro* anti-arthritic in ethanolic extracts of *Adiantum venustum* D. Don.

Materials and Methods

Plant Material

The plant parts (stems and leaves) of *A. venustum* D. Don. were collected from the local market, Bhopal. The plant material was authenticated by the Department of Botany, Government Dr. Shyama Prasad Mukherjee College, Bhopal. A voucher specimen was deposited in the herbarium of the Department. The plant materials (leaves and stem) were air dried at room temperature under shade and then powdered to a fine grade using a laboratory scale mill and kept in air tight plastic bag until use.

Pharmacognostic Evaluation

Macroscopic evaluation

Ariel plant parts of *A. venustum* were subjected to color, odor, taste, determination of shape, size, surface characteristics, and appearance.

Microscopic evaluation

For microscopical examinations, aerial parts of *A. venustum* was soaked overnight in water, cut, cleared with chloral hydrate solution and water, and stained with a drop of hydrochloric

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acid and phloroglucinol solution and mounted in glycerin and observed under microscope. Photomicrographic images were taken using camera[17] (Nicon Coolpix L 24).

Powder Microscopy

Dried aerial parts of *A. venustum* were powdered and bleached for 30 min. Slides were prepared and examined under microscope fitted with a camera[18] (Nicon Coolpix L 24).

Color Reactions

Small quantity of powder of crude drug was treated with different chemical reagents and change in color was observed.[19]

Fluorescence Nature of Powder

Powdered crude drug was passed through sieve no. 120 and was treated with different chemical reagents and observed under day light, UV, UVB, and day light.[20]

Physicochemical Evaluation

Physicochemical values such as the foreign organic matter, percentage of total ash value, acid insoluble, water soluble and sulfated ash value, moisture content, foreign organic matter, foaming index, swelling index, and extractive values were performed according to the WHO guidelines on quality control methods for medicinal plant materials.[21-23]

Preparation of Extract

All the powdered plant materials (leaf and stem) were subjected to continuous Soxhlet with petroleum ether, benzene, chloroform, ethanol (90%), and aqueous after concentration and drying of extract in vaccum desicators.

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of all the extracts for the detection of various active ingredients was carried out using standard conventional procedures.[24]

Evaluation of Anti-arthritic Effect of *A. venustum* on Inhibition of Protein Denaturation Using Egg Albumin

The reaction mixture (5 mL) included egg albumin (0.2 mL), phosphate buffered saline, 2.8 mL (pH = 6.4), and 2 ml of *A. venustum* ethanolic extract and diclofenac sodium at various concentrations (100, 200, 300, 400, and 500 µg/mL), respectively. Equal volume of double-distilled water served as control. The mixtures were incubated at 37 ± 2°C in a biochemical oxygen demand incubator for 15 min and then heated at 70°C for 5 min. Their absorbance was measured at 660 nm.[25,26] The percentage inhibition of protein denaturation was appraised using under mentioned formula:

Percentage inhibition = [Abs control–Abs test sample ÷ Abs Test Control] × 100

Abs = Absorbance

Evaluation of Anti-arthritic Effect of *A. venustum* and *Oxalis corniculata* on Inhibition of Protein Denaturation Using Bovine Serum Albumin

Protein denaturation assay was done according to the method described by Gambhire *et al.* with some modifications as described in Gunathilake *et al.* The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH = 6.4), and 0.02 mL of *A. venustum* ethanolic extract and diclofenac sodium at various concentrations (100, 200, 300, 400, and 500 µg/ml), respectively, and the mixture was mixed and was incubated in a water bath (37°C) for 15 min, and then the reaction mixture was heated at 70°C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer. Phosphate buffer solution was used as the control.[27,28] The percentage inhibition of protein denaturation was calculated using the following formula:

% inhibition of denaturation = 100 × (1 − A2/A1)

Where A1 = Absorption of the control sample and A2 = Absorption of the test sample.

Results and Discussion

Pharmacognostic Evaluation

Macroscopic evaluation

Morphology of the aerial parts of *A. venustum* was done. Dried stems were blackish in color while leaves were green in color. The stems were 13–14 cm in size while leaves were 3–5 mm. Stems were straight with nodes while leaves were triangular and fan shaped. Leaves were petiolate and not lobed, upper margin was rounded and dentate, non-reticulate venetion. It occurs as ferns. It has aromatic odor with no taste.

Microscopic evaluation

Transverse section of aerial parts of *A. venustum* showed epidermis consists of closely packed cells with single layer. No intercellular space was found. Endodermis is indistinct. The xylem consists of small vessels and parenchyma. The pith in the center is large and made up of thin walled parenchymatous cells. The stele was observed in the center. Thick cuticle was present along with ground tissues which were circular in shape with no intracellular space [Figure 1].

Powder Microscopy

The powder was dark brown in color with aromatic odor and with no taste. On microscopic examination, the powder showed wavy epidermis, parenchyma cells, phloem, fiber, trichome, brownish matter, and crystals [Figure 2].

Color Reactions

The behavior of *A. venustum* powder on treatment with different chemical reagents showed dark brown when powder was as such, black with Conc. H_2SO_4, light brown with 1N HNO_3, yellowish-green with picric acid (saturated), dark brown with acetic acid, brown with 5% iodine, green with 5% $FeCl_3$, dark green with 10% NaOH and drop of $CuSO_4$ solution, brown with 40% NaOH and

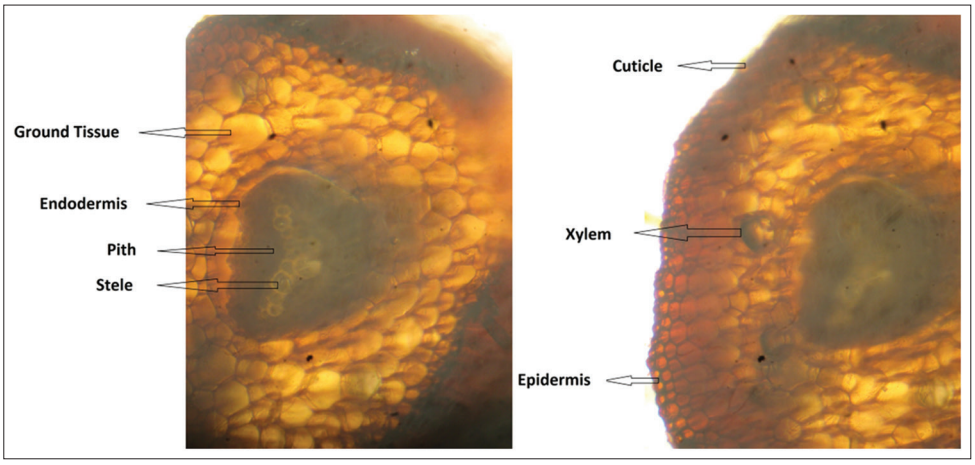


Figure 1: T.S. of aerial part (stem) of *Adiantum venustum*

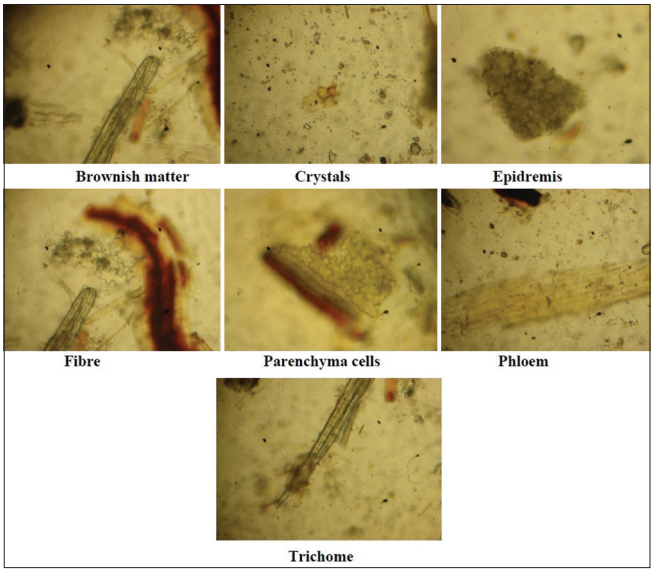


Figure 2: Powder microscopy of arial part (Stem) of *Adiantum venustum*

10% lead acetate, black with acetic acid and Conc. H_2SO_4, blackish-brown with Conc. HNO_3 and excess of ammonia, and blackish-brown with Acetic Acid and traces of $FeCl_3$ and transferred to Conc. H_2SO_4 [Table 1].

Fluorescence Nature

The fluorescence characteristics of powder after the treatment with different reagents emitted various color radiations under ultraviolet light [Table 2].

Physicochemical Evaluation

The results obtained for various physicochemical parameters of *A. venustum* are presented in Table 3. From the table, it can be seen that the loss on drying was found to be 9.13% ± 0.532, total ash value obtained was 16.15% ± 0.312, acid insoluble ash value obtained was 5.0% ± 0.707, water soluble ash value obtained was 4.81% ± 0.838, sulfated ash value obtained was 10.83% ± 0.849, alcohol soluble extractive value obtained was 1.44%± 0.111, water soluble extractive value obtained was 1.22% ± 0.003, foreign organic matter obtained was very low, swelling index gave no significant result, and foaming index was <100.

Table 1: Behavior of powder with different chemical reagents

Treatment	*Color of stems and leaves of Adiantum venustum*
Powder as such	Dark brown
Powder+Conc. H_2SO_4	Black
Powder+1N HNO_3	Light brown
Powder+Picric acid (saturated)	Yellowish-green
Powder+Acetic acid	Dark brown
Powder+5% Iodine	Brown
Powder+5% $FeCl_3$	Green
Powder 10% NaOH+drop of $CuSO_4$ solution	Dark green
Powder 40% NaOH+10% lead acetate	Brown
Powder+Acetic acid+Conc. H_2SO_4	Black
Powder+Conc. HNO_3+excess of ammonia	Blackish-brown
Powder+Acetic acid+traces of FeCl3 and transferred to Conc. H_2SO_4	Blackish-brown

Table 2: Fluorescence nature of powder of stems and leaves of *Adiantum venustum* under ultraviolet (UV) and visible radiations

Treatment	*UV*	*UV B*	*Day light*
Powder as such	Brownish-green	Colorless	Dull green
Powder+1N NaOH in methanol	Brownish-black	Colorless	Dark brown
Powder+1N NaOH in water	Dark brown	Colorless	Dark brown
Powder+50% HCl	Light brown	Colorless	Brown
Powder+50% HNO3	Dark brown	Colorless	Brown
Powder+50% H_2SO_4	Dark brown	Colorless	Brown
Powder+1 N NaOH in methanol+Nitrocellulose in amyl acetate	Blackish-brown	Colorless	Dark brown
Powder+1 N NaOH in water+Nitrocellulose in amyl acetate	Chocolate brown	Colorless	Dark brown
Powder+1 N HCl+Nitrocellulose in amyl acetate	Dark brown	Colorless	Dark brown

Preliminary Phytoprofile of Aerial Parts (Stems and Leaves) of *A. venustum*

A. venustum plant powder was subjected to successive solvent extraction. The different extracts obtained with their % yield, color, and consistency are recorded in Table 4.

Preliminary Phytochemical Screening of *A. venustum*

The extracts obtained from successive solvent extraction process were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents such as alkaloids, carbohydrates, proteins, amino acids, flavonoids, steroids, glycosides, saponins, and phenolics [Table 5].

Table 3: Physicochemical parameters of powder of aerial parts (stems and leaves) of *Adiantum venustum*

Parameters	*Value obtained on dry weight basis (%W/W)**
LOD	9.13%±0.532
Total ash value	16.15%±0.312
Acid insoluble ash value	5.0%±0.707
Water soluble ash value	4.81%±0.838
Sulfated ash value	10.83%±0.849
Extractive value	
Alcohol soluble extractive value	1.44%± 0.111
Water soluble extractive value	1.22% ± 0.003
Foreign organic matter	0.03 g
Swelling index	No significant result
Foaming index	<100

*Average of three determination±SD. N=3

In Vitro Anti-arthritic Activity by Inhibition of Protein Denaturation Method

The effects of ethanolic extract of plant parts (leaf and stem) of *A. venustum* on inhibition of protein denaturation are shown in Table 6 and Graph 1. Extracts of plant samples at different concentrations (dose levels) provided significant protection against denaturation of proteins. The maximum percentage inhibition was found 75.293 ± 0.735 at 500 µg/mL. It possesses significant activity comparable with that of the standard diclofenac sodium. The most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic, and disulfide bonding.

Similarly, the inhibitory effects on protein denaturation are shown in Table 7 and Graph 2. The present findings exhibited a concentration dependent impediment of protein denaturation by *A. venustum* as well as diclofenac sodium throughout the concentration range (100–500 µg/mL). Crude extract demonstrated 79.956 ± 0.9% inhibition of protein denaturation at 500 µg/mL, which was near to diclofenac, that is, 89.233 ± 0.780 at 500 µg/mL. From the results of the present study, it can be stated that ethanolic extracts of all the plant parts of *A. venustum* are capable of controlling the production of

Table 4: Extracts obtained with their % yield, color, and consistency of *Adiantum venustum*

Extracts	*Petroleum ether*	*Benzene*	*Chloroform*	*Ethanol*	*Aqueous*
Physical appearance	Greenish-brown sticky	Brown sticky	Brown sticky	Brown sticky gum	Brown syrup
% yield	3.20	2.18	3.24	6.12	11.08

Table 5: Preliminary phytochemical screening of *Adiantum venustum*

Active constituents test	*Petroleum ether extract*	*Benzene extract*	*Chloroform extract*	*Ethanol extract*	*Aqueous extract*
Alkaloids					
Dragondorff's test	+	+	+	+	+
Wagner's test	+	+	+	+	+
Hager's test	+	+	+	+	+
Carbohydrates					
Molisch's test	+	+	+	+	+
Barfoed's test	+	+	+	+	+
Proteins					
Biuret test	-	-	-	-	-
Amino acids					
Ninhydrin test	-	-	-	-	-
Flavanoids					
Shinoda test	+	+	+	+	+
Alkaline reagent test	+	+	+	+	+
Steroids and Trit erpenoids					
Salkowski test	+	+	+	+	+
Glycosides					
Borntrager's test	+	+	+	-	+
Legal's test	+	+	+	+	+
Baljet's test	+	+	+	+	+
Saponin glycosides					
Froth formation test	+	+	+	+	+
Phenolic compounds (Tannins)					
Ferric chloride test	+	+	+	+	+

Table 6: Percentage inhibition of protein denaturation using egg albumin by standard drug (diclofenac sodium) and plant extract

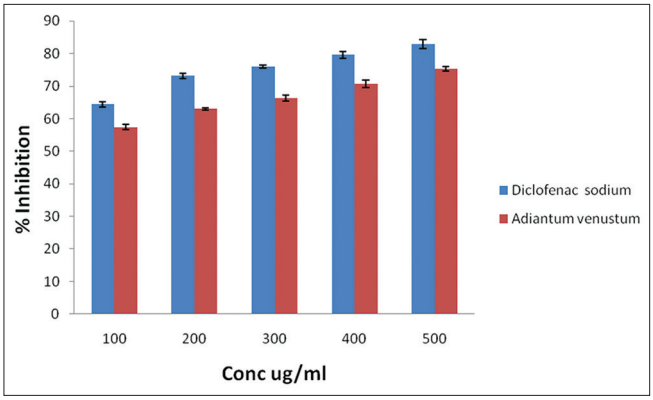
Conc. (µg/ml)	*% inhibition of protein denaturation*	
	*Diclofenac sodium**	*Adiantum venustum**
100	64.4±0.817	57.4±0.863
200	73.146±0.803	62.96±0.401
300	75.996±0.431	66.273±0.846
400	79.603±1.074	70.613±1.185
500	82.86±1.325	75.293±0.735

*Average of three determination±SD. N=3

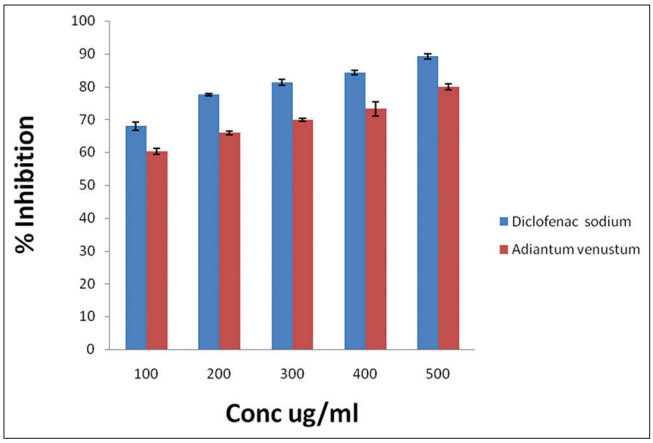
Table 7: Percentage inhibition of protein denaturation using BSA by standard drug (diclofenac sodium) and various plant extract

Conc. (µg/ml)	*% inhibition of protein denaturation*	
	*Diclofenac sodium**	*Adiantum venustum**
100	67.943±1.233	60.25±0.861
200	77.566±0.377	65.87±0.547
300	81.263±0.882	69.926±0.458
400	84.296±0.753	73.253±2.16
500	89.233±0.780	79.956±0.9

*Average of three determination±SD. N=3. BSA: Bovine serum albumin



Graph 1: Percentage inhibition of protein denaturation using egg albumin by standard drug (diclofenac sodium) and plant extract *Adiantum venustum*



Graph 2: Percentage inhibition of protein denaturation using bovine serum albumin by standard drug (diclofenac sodium) and plant extract *Adiantum venustum*

auto antigen and inhibits denaturation of protein in rheumatic disease.

Conclusion

It has been found that stems were blackish in color while leaves were green in color in the morphology. No intercellular space was found in microscopic evaluation. The stele was observed in the center. Fluorescence characteristics of powder emitted various color radiations under ultraviolet light. Various physicochemical evaluation such as total ash, water soluble ash, and sulfated ash were determined. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the changes in the anti-inflammatory activity, the ability of extracts on protein denaturation was studied. The ethanolic extract *A. venustum* showed maximum anti-inflammatory and anti-arthritic activities at 500 µg/mL, which was parallel to diclofenac at 500 µg/mL. The plant contains many secondary metabolites, for example, flavonoids, sitosteroids, alkaloids, tri-terpenoids, and phenolics. Hence, proper isolation of the active principles might help in the findings of new lead compounds in the fields of anti-arthritic and anti-inflammatory drug research. This established a significant scope to develop a broad spectrum use of *A. venustum* in herbal medicine and as a base for the development of novel potent drugs against inflammations and arthritis.

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