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Research Article

Phytochemical Analysis and *In Vitro* Assesment of the Anti-Inflammatory and Antioxidant Activities of *Adiantum Capillus Veneris*

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A B S T R A C T

Introduction: This study examines the anti-inflammatory and antioxidant activities of different extracts of Adiantum capillus-veneris. We conducted an in vitro screening of the anti-inflammatory and antioxidant potentials of hexane, ethyl acetate, butanol and aqueous extracts.

Methods: The extracts were evaluated for their anti-inflammatory properties including membrane stabilization assay, inhibition of albumin protein denaturation and proteinase inhibitory assay in vitro models. Three different tests were done to determine the antioxidant potential: DPPH free radical scavenging, hydrogen peroxide scavenging, and hydroxyl radical scavenging. Also, phytochemical screening was performed for identification of bioactive compounds.

Results: The butanol extract displayed the most marked therapeutic activity, as evidenced by significant inhibition of haemolysis (89.19%) and protein denaturation inhibition (88.49%) at 500 µg/mL. In the proteinase inhibitory assay also, the butanol extract (80.75%) was more effective than other extracts ($p < 0.01$). Among all tested extracts, the butanol extract had the most potent scavenging activity on free radicals, showing 84.07% DPPH radical inhibition and 81.13% hydroxyl radical scavenging activity at 300 µg/mL. With the ethyl acetate extract showing less activity. The extract did much less well than ascorbic acid in hydrogen peroxide scavenging results. Alkaloids, flavonoids, sterols and phenols were identified by phytochemical screening.

Conclusion: It implies that the butanol extract of Adiantum capillus-veneris had potent antioxidant and anti-inflammatory activities. The extract could be a possible candidate for a therapeutic agent for many anti-inflammatory activities.

Keywords: *Adiantum Capillus-Veneris*, Anti-Inflammatory, Antioxidant, Butanol Extract, Membrane Stabilization, Protein Denaturation, Proteinase Inhibition, Free Radical Scavenging

Introduction

WHO has acknowledged the significant role of traditional medicine in delivering essential healthcare.[1] For almost a millennium, various plant species have been explored as potential sources for creating medicinal compounds. Even now, a significant portion of the drugs used today are derived from plants. The ancient practices of the Chinese traditional medicine and the Indian Ayurvedic system were documented over a span of approximately one thousand years, dating back to the first millennium BC[2]. Medicinal herbs have been utilised since ancient times to alleviate symptoms and manage illness.[33] Currently, researchers are increasingly exploring the pharmacological impacts and possible mechanisms of various medicinal plants through both *in vitro* and *in vivo* studies.

Adiantum capillus-veneris, a member of the Pteridaceae family, is frequently known as a Maidenhair Fern, as well as Geuwtheer and Dumtuli in Kashmiri vernacular. The plant thrives in regions ranging from warm climates to tropical areas, characterised by abundant humidity levels. It thrives in moist, shady environments such as forests, near waterfalls, and along streams or rivers. The plant is found extensively across different areas including southern Europe, the Atlantic coast up to Ireland, southern alpine valleys, central to southern America, Australia and Iran. In India, it is found in various parts, including Kashmir.

In traditional tales, it's been observed that consuming a brew made from *Adiantum capillus-veneris* fronds can be helpful in purging the respiratory tract and easing breathing difficulties, asthma, nasal congestion, and thoracic discomfort within the respiratory realm.[44] Doctors in ancient times also employed eye drops to treat the condition referred to as fistula lacrymalis.[55] Oral powders derived from *Adiantum capillus-veneris* were extensively employed for addressing gastrointestinal concerns such as jaundice, diarrhoea, and abdominal spasms.[66] The *Adiantum capillus-veneris* was identified as a substance that helps to prevent headaches. It was additionally intended to be efficient for breaking down kidney stones and serving as a diuretic substance when taken orally. The Maidenhair fern was also introduced as a powerful anti-inflammatory agent. As a result, it was applied to fistulas as an ointment.[77]

The Maidenhair fern has been demonstrated to possess anti-diabetic,[88] neuropharmacological,[99] cholesterol-lowering,[1010] anti-obesity,[1111] anti-bacterial,[1212] and anti-fungal activities. Haider et al. indicated the anti-inflammatory capabilities of *Adiantum capillus-veneris*.[1313] According to reports, more than 124 substances have been extracted from the Adiantum genus since the 1960s, encompassing terpenes, flavonoids, phenylpropanoids, steroids, alicyclic acids, lipids, and lengthy chain substances.

Inflammation serves as the body's protective response prompted by physical traumas, bacterial invasions, scalds, and other detrimental factors that may jeopardise the host's health.[1414] It can be categorised as either acute or chronic inflammation. Acute inflammation usually develops quickly after injury, often within two hours, while chronic inflammation continues as a sustained response to a prolonged medical issue.[15],[1615,16] It's been claimed that long-lasting inflammation is the primary reason for worldwide mortality.[1717] In medical terms, inflammation is defined as an abnormal event characterised by discomfort, enlargement, heat, redness, and compromised tissue operation.[1818] This process involves changes in blood flow, increased permeability of blood vessel walls, and tissue harm caused by the activation and migration of white blood cells, as well as the generation of reactive oxygen species and nearby inflammatory agents like prostaglandins, leukotrienes, and platelet-activating factors spurred by enzymes such as phospholipase A2, cyclooxygenases, and lipoxygenases.[19,20] [19,20]

Despite their efficacy in treating chronic inflammatory ailments, conventional steroidal and non-steroidal anti-inflammatory medications have failed to effectively address persistent inflammatory conditions such as rheumatoid arthritis. These traditional anti-inflammatory medications have also been linked to adverse effects.[21],[22] [21,22] So, the goal of this research was to analyse the phytochemicals in ACE (*Adiantum capillus-veneris extracts*) and to assess the inflammation and antioxidant activity by utilising in vitro models

Study Design

This study follows a preclinical in vitro experimental design, specifically an analytical comparative study, where different solvent extracts of *Adiantum capillus-veneris* were evaluated for their anti-inflammatory and antioxidant properties using in vitro assays

Study Duration

The study was conducted over a period of six months from 5th October 2021 to 5 thApril 2022

Ethical and Institutional Permissions

Since this study involves in vitro assays and does not include animal or human subjects, ethical clearance from an Institutional Animal Ethics Committee (IAEC) or Institutional Review Board (IRB) was not be required

However, permission for plant usage have been obtained from relevant authorities by the supervisor of Department of taxonomy, University of Kashmir, under voucher specimen number 3751-(KASH) archived in the university of Kashmir Herbarium

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Methodology

The complete *Adiantum capillus-veneris* specimen was obtained from the Gulmarg region of Baramulla district, located in Jammu and Kashmir, India. Akhtar H Malik, the supervisor at the Taxonomy Department of the University of Kashmir, authenticated it, utilising voucher specimen number 3751-(KASH) archived in the University of Kashmir Herbarium.

Preparation of Extracts

After gathering the *Adiantum capillus-veneris* plant, it underwent a drying procedure in a sheltered setting for a span of numerous days. The dried plant matter was initially pulverised into a rough powder and then underwent consecutive extraction steps employing hexane, ethyl acetate, butanol, and water, utilising the cold maceration method.

Qualitative Phytochemical Screening

The extraction technique yielded extracts, namely hexane, ethyl acetate, butanol, and aqueous. These extracts were then submitted to a preliminary phytochemical screening to identify different phytoconstituents.

Phytochemical Analysis of *Adiantum capillus-veneris* to Identify and Quantify Its Bioactive Compounds

Extract Preparation

Adiantum capillus-veneris whole plant was collected and then dried for a few days in the shade. Following a rough processing step, the plant's dried material was extracted utilising the cold maceration technique with ethyl acetate, butanol, hexane, and water in sequential order. A few grams of dried powdered samples were subjected to the tests of tannins, reducing sugars, phenolic compounds, glycosides, flavonoids, carbohydrates, alkaloids, and amino acids.

Qualitative Phytochemical Screening

The aqueous, butanol, ethyl acetate, and hexane extracts of *Adiantum capillus-veneris*, acquired through the extraction process, were subjected to initial phytochemical analysis to detect various plant compounds.

Test for Alkaloids

To find out whether there were any alkaloids in the filtrates, several tests were carried out. In Dragendroff's test, the filtrates were treated with Dragendroff's reagent, which caused a red precipitate to develop that acted as an alkaloid indicator.[23] [23] In Hager's test, alkaloids were detected when a small amount of filtrate was mixed with a few drops of Hager's reagent. This resulted in the production of a creamy white precipitate. Wagner's test included adding Wagner's reagent to a portion of the filtered extract; the presence of alkaloids was indicated by the formation of a reddish-brown precipitate along the tube's walls.[24] [24] In the end, Mayer's test required mixing the extract with Mayer's reagent, which produced a cream-coloured precipitate that acted as a sign of alkaloids.[2525] These unique assays, which produced various precipitate forms, were essential markers for identifying alkaloids in the samples.

Test for Steroids and Terpenoids

Salkowski test

To perform the Salkowski test, shake the crude extract with two millilitres of chloroform and then pour concentrated H_2SO_4 down the test tube's side. When a reddish-brown hue shows up at the interface, terpenoids are present.

Liebermann Burchard Test

In a test tube, small amounts of acetic anhydride were introduced following the combination of the extract and chloroform. A water bath was used to heat the combination, then ice water was used to chill it. The exterior of the experimental tube was treated with H_2SO_4. The formation of brown rings at the two layers and the ensuing greening of the top layer indicate the presence of steroids. However, the development of a deep red colour indicates the existence of triterpenoids.

Test for Tannins

Ferric Chloride Test

The sample underwent treatment with 1 mL of a solution containing 5% $FeCl_3$. Detection of tannins was signalled by the precipitate transitioning to a hue of either blue-black or greenish-black.

Test for Saponins

Foam Test

After incorporating 0.5 mL of distilled water into the test mixture, vigorous shaking ensued. The formation of enduring foam provided evidence for the presence of saponins.

Test for Glycosides

Anthraquinone Glycoside (Borntrager's Test)

1 mL of 5% sulfuric acid was introduced into the mixture. The mixture was warmed in a bain-marie and subsequently filtered. The filtrate was then agitated with an equivalent amount of chloroform and allowed to settle for a duration of 5 minutes. Next, the bottom layer of chloroform was vigorously mixed with an identical amount of diluted ammonia. The ammoniacal layer may become pink to crimson in colour, indicating the existence of anthraquinone glycosides.

Cardiac Glycosides (Keller Kiliani Test)

Distilled water was used to stir the extract. In this experiment, glacial acetic acid was mixed with a small

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quantity of ferric chloride, and then H_2SO_4 was cautiously introduced to the test tube. A brown circle close to the boundary indicates the presence of cardiac glycosides, whereas a purple stripe might be observed beneath the brown circle.

Identification of Phenolic Substances

Iodine Test

To 1 mL of the extract, a small amount of iodine solution was introduced. If phenolic compounds are present, a fleeting crimson hue will emerge.

Ferric Chloride Test

A small quantity of 5% solution of ferric chloride was employed to get this. Phenolic compounds are denoted by a deep green and also bluish-black hue.

Identifying Proteins and Amino Acids

Ninhydrin Test

A small amount of ninhydrin solution (consisting of 10 mg of ninhydrin dissolved in 200 mL of acetone) was introduced to 2 mL of the filtrate; the violet solution signalled the existence of amino acids.

Xanthoproteic Test

Add 10% ammonium hydroxide solution to the plant extract. When there are proteins present, a solution becomes yellow.

Detection of Flavonoids

Shinoda Test

Three drops of HCl, 0.5 g of magnesium turnings, and 5 mL of 95% ethanol were added to the dry extract. Flavonoids are identified by the generation of a rosy hue.

Alkaline Reagent Test

Mix 1 mL of the extract with 2 mL of 2% sodium hydroxide solution and a small amount of diluted hydrochloric acid. The tint of the material seemed to be a lively yellow, yet it turned transparent when diluted acidic solution was introduced.

Carbohydrates

Molisch's Test

Combine 2.3 mL of extract with a small amount of Molisch's reagent, shake vigorously, and subsequently introduce concentrated sulfuric acid along the edges of the test tube. The formation of a purple circle at the juncture of two fluids indicates the existence of sugars.[26 26]

Fehling's Test

1 mL solution of Fehling (A and B) was combined and subjected to boiling for a duration of 1 minute. The solution to be tested was added to the experiment tube in equal parts, and it was then allowed to boil for five minutes. The emergence of a crimson hue verified the existence of sugars that undergo reduction.

Benedict's Test

2 mL of Benedict's solution was introduced to a small quantity of the sample solution within a test tube. The mixture underwent a boiling period lasting 2 minutes in a water bath, after which it was left to stand. The occurrence of red precipitate serves as an indication of the existence of reducing sugars.

In vitro Anti-Inflammatory Activities

Anti-Inflammatory Activity

Membrane Stabilisation Assay (Preparation of Erythrocyte Suspension)

With minor adjustments, the method described in Shinde et al. was used to produce a red blood cell suspension[27].[27] A robust individual volunteered their entire blood sample for examination. In the centrifugal compartments treated with heparin, the blood underwent centrifugation at 3000 revolutions per minute for a duration of five minutes. Following that, three rinses of the blood were conducted utilising an equivalent amount of standard saline solution containing 0.9% NaCl. Post-centrifugation, the blood volume was gauged and merged with an isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4) to create a 10% (v/v) mixture. The ingredients of the buffer solution were NaCl (9.0 g/L), NaH_2PO_4 (0.2 g/L), and Na_2HPO_4 (1.15 g/L)

Heat-Induced Haemolysis

The examination was carried out employing the methodology delineated by Okoli et al.,[28 28] with various modifications as specified[2929]. To summarise, a blend was concocted by combining 0.05 mL of 'blood cell suspension and 0.05 mL of AC extracts of the whole plant with 2.95 mL of phosphate buffer at a pH of 7.4'. The mixture underwent incubation in a vigorously stirred water bath for 20 minutes at 54 °C. After the incubation interval, the blend was centrifuged at a velocity of 2500 revolutions per minute for three minutes. Consequently, the optical density of the fluid above the sediment was ascertained at a wavelength of 540 nm utilising a UV/VIS spectrophotometer. The study encompassed a control incorporating a phosphate buffer solution. The degree of haemolysis was determined utilising the equations provided below:

$$\%\text{inhibition of hemolysis} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs Control})} \times 100$$

Impact on the Denaturation of Proteins

The protein denaturation analysis was performed in compliance with minor adjustments made in alignment

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with the recommendations of Banerjee et al et al.[3030] The composition of the resulting mixture (5 mL) was as follows: 0.022 mL of extract, 4.78 mL of pH 6.43 phosphate-buffered saline (PBS), & 0.2 mL of 1% bovine albumin. Following a thorough mixing process, the mixture was immersed for fifteen minutes in a water bath which was heated to 37 °C. Afterwards, the response underwent heating at 70 °C for 5 minutes. After cooling, the cloudiness was measured at a wavelength of 600 nm with a UV/VIS spectrometer. The standard was a phosphate buffer solution. The percent decrease in protein denaturation was calculated utilising the provided equation.

$$\% \text{ Inhibition of Denaturation} = \frac{(\text{Abs Control} - \text{Abs Sample})}{(\text{Abs Control})} \times 100$$

Proteinase Inhibitory Activity

The proteinase inhibitory activity of the AC (*Adiantum capillus-veneris extracts*) was evaluated using the adapted method outlined by Gunathilake et al., [29]based on the procedure modified byjuvekar et al[31].[31] Essentially, the reaction mixture consisted of 0.06 mg of trypsin, 1 mL of a 20 mM tris-HCl buffer (pH 7.4), and 1 mL of the experimental sample. The solution was subjected to incubation at a temperature of 37 °C for about five minutes. Following this, 1 mL of casein with a concentration of 0.8% (weight/volume) was introduced into the solution. After that, the mixture was incubated for a further twenty minutes. When the incubation period ended, the reaction was halted by adding 2 mL of 70% perchloric acid. The liquid's absorption over the sediment was measured at 210 nm after the mixture's centrifugation and compared to a buffer blank solvent. The phosphate buffer solution acted as the reference standard. The subsequent equation was employed to determine the extent of protein denaturation inhibition.

$$\% \text{ Inhibition of Proteinase} = \frac{(\text{Abs Control} - \text{Abs sample})}{(\text{Abs Control})} \times 100$$

In Vitro Antioxidant Potential of *Adiantum capillus-veneris* through Various Assays

DPPH Radical Scavenging Assay

The DPPH radical method is widely utilised to evaluate the antioxidant potential of natural substances by gauging their capability to counteract harmful radicals. This assessment hinges on measuring how well antioxidant compounds can deactivate the steady radical. In this study, the DPPH radical was employed to assess the in vitro ability of extracts to scavenge free radicals. The procedure was mainly adapted from the technique outlined by Hussen and Endalew et al [32]. [32] A blend comprising 1.0 mL of extracts with concentrations ranging from 50–300 µg/mL and 1.0 mL of 0.8 mmol/L DPPH solution was prepared. The solution was swiftly stirred and allowed to stand for 30 minutes. The absorbance was subsequently gauged at 517 nm wavelength, with a reagent blank serving as the comparison. Ascorbic acid was utilised as the standard.

$$\% \text{ Inhibition of Scavenging} = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \times 100$$

The above equation was used to calculate the inhibition % for scavenging a DPPH radical.

"In this context, Abs C denotes the absorbance of DPPH+ dissolved in methanol, while Abs S represents the absorbance of DPPH radical combined with the sample (such as extract or standard)."

H_2O_2 Scavenging Activity

The assessment of hydrogen peroxide removal was carried out following the procedure detailed by Ruch and associates.[3333] The essence of this approach lies in the reduction of H_2O_2 absorption as H_2O_2 undergoes oxidation. 3.4 mL of phosphate buffer were mixed with a 43 millimolar (mM) solution of hydrogen peroxide (H_2O_2) that was created in a 0.1 molar (M) phosphate buffer having a pH of 7.4. 1 mL of extract, each at different concentrations. This mixture was subsequently introduced into 0.6 mL of hydrogen peroxide solution with a concentration of 43 mM. At 230 nanometers, the absorbance of the resultant combination was measured. The solution was devoid of H_2O_2 and consisted only of a sodium phosphate buffer. The samples and criteria were employed to compute the ratio of H_2O_2 elimination using the subsequent formula.

$$\% \text{ Inhibition} = \left(1 - \frac{As}{Ac}\right) X\ 100$$

Here, A_C represents the absorbance of the control sample, while As denotes the absorbance when extracts are present.

Hydroxyl Radical Scavenging

The examination was conducted in accordance with the protocol outlined by Halliwell and colleagues.[34 34] This procedure relies on identifying substances reactive to thiobarbituric acid (primarily malondialdehyde), which produces a pink colour when it reacts with thiobarbituric acid, formed when deoxyribose breaks down upon encountering hydroxyl radicals. The hydroxyl radical is generated through the Fe^{3+}- ascorbate - H_2O_2 system, known as the Fenton reaction.

The reaction mixture comprises 10 mM ferric chloride, 25 mM deoxyribose, 100 mM ascorbic acid, 2.8 mM hydrogen peroxide (H_2O_2) in a buffer solution of 10 mM KH_2PO_4 (pH 7.4), and various concentrations of plant extracts ranging from 50 to 300 µg/mL. This mixture was then incubated at 37 °C for 1 hour. A mixture of 1% thiobarbituric acid and 3% trichloroacetic acid (1 mL each) was combined, and the resulting solution was heated to 100 °C for 20

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minutes. The intensity of colour change was measured using spectrophotometry at a wavelength of 532 nm. The results were quantified as the percentage of inhibition of deoxyribose oxidation, calculated using a specified formula.

$$\% \text{ inhibition} = \frac{(\text{Abs Control} - \text{Abs sample})}{(\text{Abs Control})} \times 100$$

Statistical Analysis

The results are displayed as ± the standard deviation. One way Anova-way ANOVA was utilised to compare the differences among experimental groups, followed by Dunnetts test tests (control versus test) using the GraphPad Prism 5 programme.

Results

Extraction of *Adiantum capillus-veneris* in Different Solvents

The table 1 details the extraction of *Adiantum capillus-veneris* using various solvents—aqueous, butanol, ethyl acetate, and hexane—expresseding.

Heat-Induced Haemolysis

Figure 1.The figure illustrates the percentage inhibtion of heat induced haemolysis of red blood cells at various concentrations of Adiantum capillus veneris ranging from 100-500µg/ml.

Hexane, ethyl acetate, butanol and aqueous extracts displayed the capacity to suppress haemolysis in a manner reliant on concentration. The reduction in hemolysis observed with the hexane extract ranged from 41.24% to 79.60% at concentrations of 100–500 µg/mL, while the inhibition percentage for the aqueous extract varied from 37.43% to 63.59%. The butanol extract demonstrated notably greater inhibition of hemolysis (89.19%) compared to the ethyl acetate extract (83.14%) at a concentration of 500 µg/mL, along with other extracts. The standard medication, diclofenac sodium, demonstrated the highest suppression of 96.71% when administered at a concentration of 500 µg/mLas depicted in table 3The sequence of the inhibition percentage from extracts: butanol extract > ethyl acetate extract > hexane extract > aqueous extract.

Table 1.Percentage yield of Different extracts

Aqueous Extract	Butanol Extract	Ethyl Acetate Extract	Hexane Extract
Amount of grinded AC taken = 375 g	Amount of grinded AC taken = 375 g	Amount of grinded AC taken = 375 g	Amount of grinded AC taken = 375 g
Weight of extract obtained = 31.4 g	Weight of extract obtained = 38.5 g	Weight of extract obtained = 28.7 g	Weight of extract obtained = 6.32 g
% yield = 8.3 g	% yield = 10.2 g	% yield = 7.6 g	% yield = 1.68 g

Table 2.Results of qualitative Phytochemical screening of whole plant of AC

Phytoconstituents	Hexane Extract	Ethyl Acetate Extract	Butanol Extract	Aqueous Extract
Alkaloids				
Dragendorff's test	-	-	+	-
Hagers test	-	+	+	+
Wagners test	-	-	+	-
Mayers test	-	+	+	-
Test for steroids & terpenoids				
Salkowski test	-	-	+	-
Liebermann-Burchard test	-	-	+	+
Test for tannins	-	-	-	-
Test for saponins	-	-	+	-
Test for glycosides				
Anthraquinone glycosides	-	-	+	-
Cardiac glycosides	-	-	+	+
Test for phenols				
Iodine test	-	-	+	-
Ferric chloride (1%) test	-	-	+	-

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Detection of proteins and amino acids				
Ninhydrin test	-	-	+	+
Xanthoproteic test	-	-	-	+
Detection of flavonoids				
Shinoda test	-	+	+	-
Alkaline reagent test	-	-	+	+
Detection for carbohydrates				
Molisch's test	-	-	-	-
Fehling's test	-	+	+	+
Benedicts test	-	-	+	+

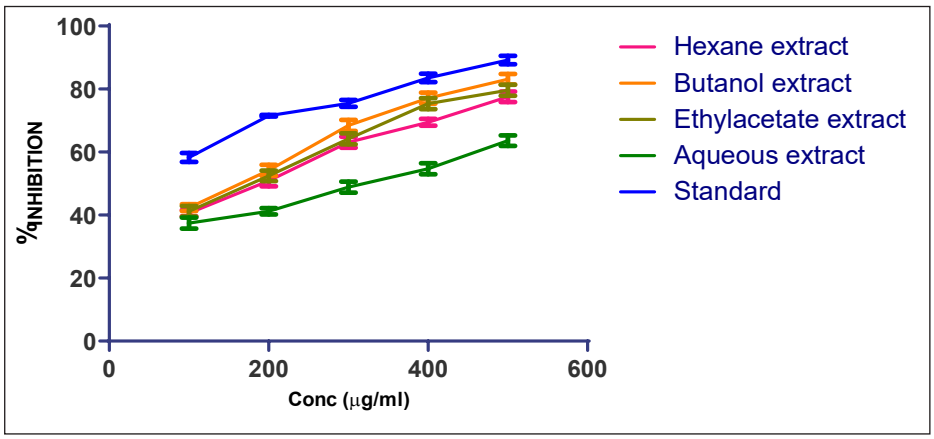

Figure 1.Heat Induced Haemolysis *Adiantum Capillus Veneris*

Table 3.Results of heat induced haemolysis activity of different extracts obtained from *Adiantum capillus veneris* and Diclofenac sodium as Standard

Treatment	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	IC_{50} µg/mL
Standard (diclofenac sodium)	46.06 ± 0.007***	69.66 ± 0.001***	77.34 ± 0.007***	83.70 ± 0.07***	91.94 ± 0.007***	75.61±0.19***
Hexane extract	38.48 ± 0.011***	49.32 ± 0.011***	61.24 ± 0.011***	70.09 ± 0.006***	76.24 ± 0.006***	204.81+0.97***
Ethyl acetate extract	39.05 ± 0.01***	49.86 ± 0.011***	61.85 ± 0.010***	73.59 ± 0.73***	76.73 ± 0.011***	196.57+ 0.81***
Butanol extract	42.005 ± 0.011***	52.57 ± 0.011***	66.39 ± 0.011***	75.70 ± 0.006***	80.75 ± 0.011***	165.20± 0.77***
Aqueous extract	36.82 ± 0.011***	43.18 ± 0.006***	48.74 ± 0.006***	53.49 ± 0.011***	62.27 ± 0.010***	317.44±0.82****

Note: Each value denotes the average + standard deviation from three separate experiments. *P<0.05;P<0.01;***P<0.001;nsP>0.05

Proteinase Inhibitory Activity

Figure 2. The Proteinase inhibitory activity of *Adiantum capillus veneris* extracts was measured . The values are the averages of three readings. The data are shown as means +_the standard deviations of three repeated tests.

The various extracts obtained from Adiantum capillus-veneris proficiently hindered protein denaturation in a manner contingent upon dosage. The figure demonstrates the inhibitory effect of different samples, ranging in concentrations from 100 to 500 µg/mL, on protein denaturation. Butanol extract demonstrated a notably superior proteinase inhibition rate of 80.75% in contrast to the other extracts as depicted in table 4

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Table 4.Anti-proteinase activity shown by various extarcts obtained from whole plant of *Adiantum capillus veneris*

Treatment	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	IC_{50} µg/mL
Standard (diclofenac sodium)	91.29 ± 0.006***	92.79 ± 0.011***	94.59 ± 0.011***	95.52 ± 0.006***	96.71 ± 0.0105***	11.59±0.62***
Hexane extract	41.24 ± 0.010***	52.42 ± 0.010***	64.24 ± 0.011***	75.41 ± 0.011***	79.60 ± 0.011***	173.21+0.7**
Ethyl acetate extract	42.36 ± 0.006***	54.18 ± 0.011***	68.43 ± 0.011***	77.09 ± 0.11***	83.14 ± 0.010***	154.59+1.3**
Butanol extract	58.28 ± 0.008***	71.50 ± 0.002***	75.41 ± 0.007***	83.51 ± 0.008***	89.19 ± 0.008***	45.88+0.63***
Aqueous extract	37.43 ± 0.011***	41.24 ± 0.006***	48.88 ± 0.0011***	54.74 ± 0.011***	63.59 ± 0.010***	312.61+1.30***

Note: Each value denotes the average + standard deviation from three separate experiments. *P<0.05;P<0.01;***P<0.001;nsP>0.05

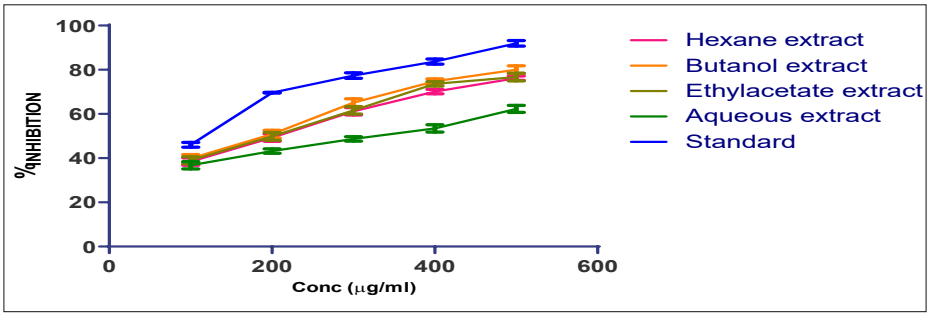

Figure 2.Proteinase Inhibitory *Adiantum Capillus Veneris*

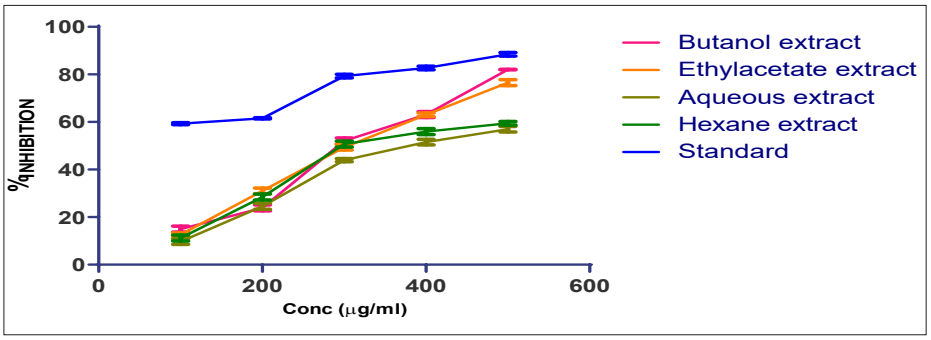

Figure 3.Albumin Denaturation *Adiantum Capillus Veneris*

Denaturation of Albumin Protein by Adiantum capillus-veneris

Figure 3. The figure illustarates the percentage inhibition of protein denaturation at various concentrations of Adiantum capillus veneris ranging from 100-500µg/ml

Each of the specimens efficiently halted protein denaturation in a manner proportional to the dosage. The diagram depicts the diverse inhibitory effects of different extracts at concentrations ranging from 100–500 µg/mL on protein denaturation. The extent of inhibition in protein denaturation by the hexane extract fluctuates between 11.198% and 59.46% within the concentration range of 100–500 µg/mL, whereas the aqueous extract exhibits inhibition levels ranging from 9.74% to 56.98%. The butanol extract demonstrated a notably greater level of inhibition at 88.49%, whereas the ethyl acetate displayed relatively lower inhibitory levels at 82.05%. as depicted in table 5 The sequence in which the *Adiantum capillus-veneris* extracts were restrained was as follows: butanol extract > ethyl acetate extract > hexane extract > aqueous extract.

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Table 5.Results of Albumin Denaturation Inhibition activity of different extracts obtained from whole plant of *Adiantum capillus veneris*

Treatment	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	IC_{50}µg/ml
Standard (diclofenac sodium)	81.55 ± 0.006***	85.42 ± 0.011***	89.31 ± 0.011***	92.19 ± 0.006**	95.04 ± 0.011***	3.4+0.7***
Hexane extract	11.198 ± 0.011*	28.48 ± 0.011*	50.68 ± 0.011*	55.99 ± 0.011*	59.46 ± 0.0066*	370±1***
Ethyl acetate extract	14.99 ± 0.011**	23.86 ± 0.011**	52.07 ± 0.011**	63.11 ± 0.011**	82.05 ± 0.001**	314.16±1.7***
Butanol extract	59.303 ± 0.003***	61.53 ± 0.002***	79.28 ± 0.007***	82.70 ± 0.0068***	88.49 ± 0.0066***	5.9±0.6ns
Aqueous extract	9.74 ± 0.011*	24.56 ± 0.011*	43.92 ± 0.006*	51.52 ± 0.0105*	56.98 ± 0.01*	402.6±1.5***

Note: Each value denotes the average + standard deviation from three separate experiments. *P<0.05;P<0.01;***P<0.001;nsP>0.05

Antioxidant Activity

Figure 4.DPPH radical scavenging activity of Hexane, Ethylacetate, Butanol and Aqueous extracts of *Adiantum capillus veneris.*

All the samples exhibited a scavenging of DPPH radicals that varied with concentration, as illustrated in the figure. The scavenging capability of the hexane and aqueous samples was 62.68% and 78.90% correspondingly at a concentration of 300 µg/mL. Meanwhile, at the identical concentration, the butanol sample displayed a scavenging rate of 84.07%, whereas the ethyl acetate sample demonstrated relatively lower scavenging activity at 81.6%.

Hydrogen Peroxide

Figure 5. Hydrogen peroxide scavenging activity of Hexane, Ethylacetate, Butanol and Aqueous extracts of Adiantum capillus veneris

The Hydrogen peroxide Scavenging capacity of the extracts , another significant indicator of antioxidant activity was also found to be appreciable for all the extracts. The hydrogen peroxide capability of various extracts obtained from whole plant of Adiantum capillus veneris and ascorbic caid standard is shown in table 7 and represented graphically in Fig 5.

Hydroxyl Radical

The figure 6 illustrates all the samples exhibited a scavenging of hydroxyl radicals dependent on their concentration. The hexane and aqueous samples displayed a scavenging effect of 56.81% and 59.46%, respectively at a dosage of 300 µg/mL against hydroxyl radicals. Conversely, the butanol sample exhibited a higher scavenging effect of 81.13% while the ethyl acetate sample showed 66.23% on hydroxyl radicals as shown in table 8 The sequence of scavenging effect on hydroxyl radicals is as follows: butanol extract > ethyl acetate extract > aqueous extract > hexane extract.

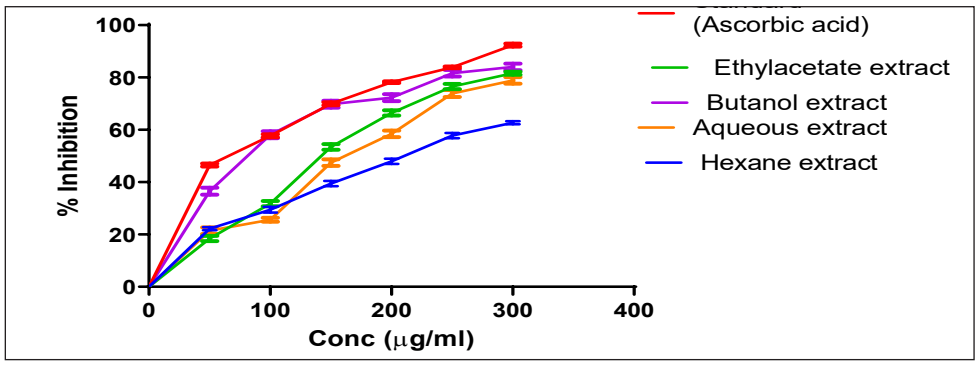

Figure 4.DPPH *Adiantum Capillus Veneris*

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Table 6.Results of DPPH radical scavenging activity shown by extracts of *Adiantum capillus veneris*

Treatment	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL	300 µg/mL
Standard (ascorbic acid)	46.51 ± 0.011***	57.74 ± 0.011***	69.96 ± 0.011***	78.17 ± 0.006***	83.86 ± 0.006***	92.36 ± 0.011***
Hexane extract	22.20 ± 0.006**	29.45 ± 0.011**	39.49 ± 0.010**	47.9 ± 0.011**	57.7 ± 0.010**	62.68 ± 0.006 **
Ethyl acetate extract	18.50 ± 0.18***	31.80 ± 0.010***	52.66 ± 0.006***	66.47 ± 0.010***	76.55 ± 0.011***	81.6 ± 0.006***
Butanol extract	36.5 ± 0.011***	58.16 ± 0.011***	69.80 ± 0.011***	72.27 ± 0.011***	81.60 ± 0.010***	84.07 ± 0.01***
Aqueous extract	21.45 ± 0.010***	25.64 ± 0.006***	47.46 ± 0.011***	58.45 ± 0.011***	73.91 ± 0.011***	78.90 ± 0.01***

Note: Each value denotes the average + standard deviation from three separate experiments. *P<0.05;P<0.01;***P<0.001;nsP>0.05

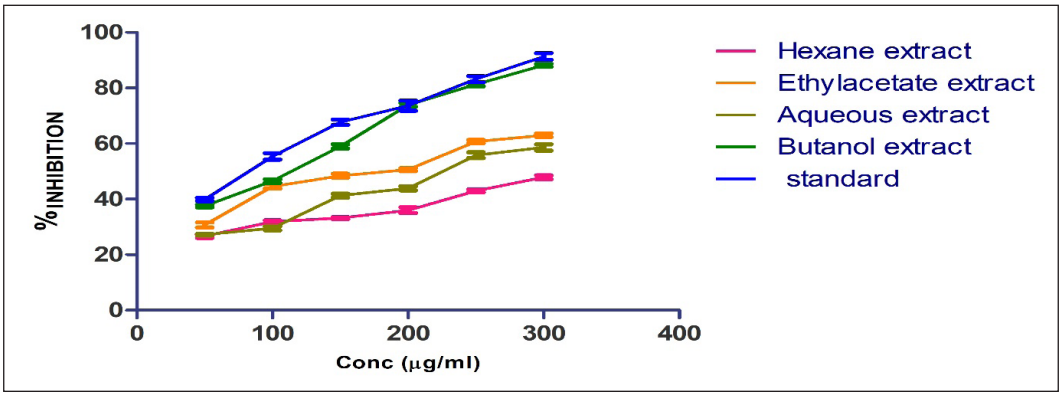

Figure 5.Hydrogen Peroxide *Adiantum Capillus Veneris*

Table 7.Results of hydrogen peroxide Scavenging capacity shown by various extracts of *Adiantum capillus veneris*

Treatment	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL	300 µg/mL
Standard (ascorbic acid)	39.87 ± 0.007***	55.36 ± 0.013***	67.68 ± 0.011***	73.61 ± 0.021***	83.17 ± 0.012***	91.30 ± 0.013***
Hexane extract	26.69 ± 0.011***	31.89 ± 0.085***	33.20 ± 0.006***	35.99 ± 0.018***	42.94 ± 0.008***	47.85 ± 0.012***
Ethyl acetate extract	30.71 ± 0.307***	44.57 ± 0.010***	48.41 ± 0.009***	50.61 ± 0.506***	60.70 ± 0.60***	62.99 ± 0.008***
Butanol extract	37.53 ± 0.0066***	46.51 ± 0.0086***	58.96 ± 0.011***	73.91 ± 0.009***	81.33 ± 0.09***	88.14 ± 0.0075***
Aqueous extract	27.17 ± 0.0035***	29.51 ± 0.0088***	41.26 ± 0.008***	43.88 ± 0.009***	55.82 ± 0.012***	58.59 ± 0.014***

Note: Each value denotes the average + standard deviation from three separate experiments. *P<0.05;P<0.01;***P<0.001;nsP>0.05

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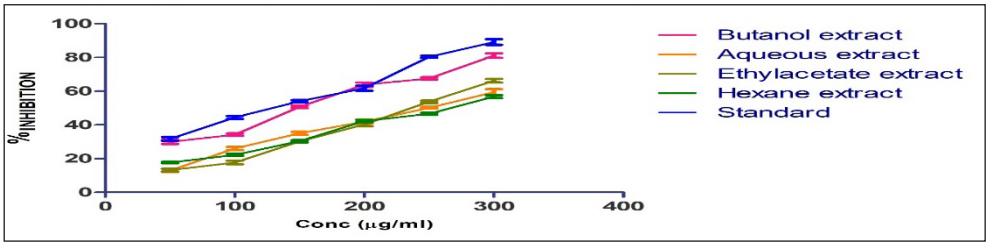

Figure 6.Hydroxyl radical savenging activity of Hexane, Ethylacetate, Butanol and Aqueous extracts of *Adiantum capillus veneris*

Table 8.Results of Hydroxyl radical scavenging activity shown by various extracts of *Adiantum capillus veneris*

Treatment	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL	300 µg/mL
Standard (ascorbic acid)	31.67 ± 0.021***	44.43 ± 0.016***	54.06 ± 0.013***	61.79 ± 0.029***	80.41 ± 0.012***	89.13 ± 0.03***
Hexane extract	17.70 ± 0.007*	22.24 ± 0.011*	30.34 ± 0.011*	42.33 ± 0.012*	46.66 ± 0.011*	56.81 ± 0.016*
Ethyl acetate extract	13.22 ± 0.013*	17.71 ± 0.017*	30.23 ± 0.011*	40.28 ± 0.017*	53.76 ± 0.011*	66.23 ± 0.016*
Butanol extract	29.95 ± 0.012***	34.23 ± 0.007***	50.67 ± 0.007***	63.85 ± 0.013***	67.56 ± 0.007***	81.13 ± 0.013***
Aqueous extract	13.00 ± 0.013**	26.01 ± 0.013**	35.05 ± 0.012**	41.96 ± 0.016**	50.16 ± 0.006**	59.46 ± 0.024**

Note: Each value denotes the average + standard deviation from three separate experiments. *P<0.05;P<0.01;***P<0.001;nsP>0.05

Discussion

Membrane Stabilisation Assay

The extracts from the AC plant showcased notable anti-inflammatory properties by stabilising the membrane of red blood cells, hindering the discharge of lytic enzymes, and other substances involved in inflammation. The AC plant butanol extract exhibited nearly identical efficacy when compared to diclofenac.

Inhibiting the breakdown of red blood cells could offer valuable insights into inflammation since the membrane of these cells resembles that of lysosomes.[35 35] Enhancing the integrity of these cell barriers might delay or obstruct the degradation and subsequent release of the cytoplasmic contents, thus lessening tissue damage and thereby lowering the inflammatory response.[3636] Substances that offer substantial defence for cell membranes against harmful agents are crucial for impeding the advancement of inflammation.

Protein Denaturation Assay

The literature extensively documents the alteration of protein structures, a consequence of inflammation seen in conditions like arthritis. Mizushima highlights that one of the main methods NSAIDs operate is by shielding against protein structural changes.[3737] This prevention of protein alteration might play a crucial role in NSAIDs' anti-inflammatory impact on treating rheumatism. As part of investigating the mechanism behind this anti-inflammatory effect, researchers examined how plant extracts could hinder protein structural changes. The highest level of inhibition, reaching 88.49%, was noticed in the butanol extract when it was at a concentration of 500 µg/mL.

Proteinase Inhibitory Activity

Proteases have been linked to inflammatory responses in arthritis. Neutrophils transport numerous serine proteases within their lysosomal granules.[38 38] Enzymes from white blood cells have a notable impact on the progression of tissue harm in inflammatory reactions.[39] stated that proteinase inhibitors offered a considerable degree of defence.[39] AC demonstrated notable anti-proteinase efficacy across various doses, as evidenced in the table. It displayed the highest suppression of 80.75% with the 500 µg/mL butanol extract. At a concentration of 500 µg/mL, Diclofenac sodium exhibited its highest level of inhibition, reaching a maximum of 91.94%.

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DPPH Assay

The decline in DPPH concentration signifies the antioxidative potential of plant extracts. The order in which extracts demonstrated the elimination of free radicals was as follows: butanol extract > ethyl acetate extract > aqueous extract > hexane extract. Particularly, the butanol extract displayed exceptional efficacy in scavenging DPPH, exhibiting an inhibition rate of 84.07%, surpassing that of other extracts. DPPH, a radical with a nitrogen centre, exhibits notable stability. The technique of scavenging DPPH radicals stands as the most utilised method to gauge the extent of free radical scavenging.[40] [40] The reduction in DPPH was noted by a change in colour from violet to yellow, indicating the ability of the extracts to donate a proton to the DPPH radical.[4141] The intensity of the colour was evaluated at 517 nm, with Ascorbic acid serving as the standard benchmark.

H_2O_2 Assay

The outcomes for H_2O_2 radical elimination were akin to those of DPPH radical removal. The AC samples demonstrated efficient scavenging of H_2O_2 radicals in a manner that depends on the dosage. The AC butanol extract displayed the most potent radical scavenging activity (88.14 ± 0.0075%), with the ethyl acetate extract ranking next (62.99 ± 0.008), followed by the aqueous extract (58.59 ± 0.014), and finally, the hexane extract (47.85 ± 0.012), which exhibited the lowest activity. The buildup of H_2O_2 in organisms initiates the creation of hydroxyl radicals, leading to significant harm to cell membranes. Compounds that impede these radicals have the potential to serve as therapeutic agents to alleviate the symptoms associated with oxidative stress. Ascorbic acid was employed as the reference medication to evaluate antioxidant effectiveness in the test for scavenging free radicals. Hydrogen peroxide usually demonstrates minimal reactivity, but occasionally triggers hydroxyl radicals within cells, thereby presenting a threat to cellular health. The removal of Hydrogen peroxide is highly crucial for maintaining the integrity of food systems[42],[43],[44].[44] Therefore, compounds that can eliminate the free radicals within the cell could function as antioxidants.

Hydroxyl Radical Assay

The different extracts of AC suppressed the hydroxyl radicals in a dosage-dependent fashion. Hydroxyl radicals exhibit high reactivity and have a brief life span.[45] [45] They possess the capacity to initiate detrimental effects on essential macromolecules like proteins and nucleic acids. In the Haber-Weiss/ Fenton process, hydroxyl radicals arise because of the interaction between hydrogen peroxide and iron ions.[46],[4746,47] The vigorous behaviour of hydroxyl radicals leads to widespread damage to both the cell and its components, ultimately deeply affecting the entire organism.[48] [48] Hence, it is crucial to eliminate hydroxyl radicals, which induce harmful impacts.

Conclusion

The findings of the current research suggest that the butanol extract demonstrates anti-inflammatory characteristics in comparison to the other extracts examined. These actions might result from the abundant presence of polyphenolic substances like alkaloids, flavonoids, phenols, and steroids. The extracts function as antioxidants, either inhibiting free radicals or acting as scavengers, potentially serving as primary antioxidant agents. They additionally hindered the alteration of albumin due to heat, inhibited proteinase function, and fortified the membrane of erythrocytes. The refinement of every bioactive substance is essential, and this purified variant can be utilised, potentially exhibiting enhanced efficacy. The research suggests that the component from the AC plant could serve as the primary compound for developing a powerful medication to alleviate inflammation.

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Authors' Contribution: ZS conducted the experiments, analyzed the data, drafted the manuscript and manuscript revision. SS and MAZ supervised the research, provided guidance, and reviewed the manuscript. TA contributed to data analysis, interpretation.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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