GMR
The Original

# PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTIOXIDANT, IN VITRO ANTI-INFLAMMATORY AND TOXICITY POTENTIAL OF *ARQUITA ANCASHIANA* EXTRACT

**Arun A. Kondapure*[1], Dr. Kailasam Koumaravelou[2]**

[1.] Research Scholar, School of Pharmacy, PRIST Deemed to be University, Thanjavur, Tamil Nadu, India – 613403.
[2.] Director, PRIST School of Pharmacy, Department of Pharmacy, Thanjavur, Tamil Nadu, India-613403

**ABSTRACT**
Inflammation is a crucial biological response involved in various acute and chronic disorders. Medicinal plants are traditionally used for the management of inflammatory conditions due to the presence of bioactive phytoconstituents. The present study was designed to evaluate the phytochemical profile, antioxidant activity, in vitro anti-inflammatory activity, and toxicity profile of the plant extract of *Arquita ancashiana.* The plant material was extracted using soxhlet and maceration extraction methods and subjected to preliminary phytochemical screening. Antioxidant activity was evaluated by the DPPH radical scavenging assay, while anti-inflammatory activity was assessed using protein denaturation and proteinase inhibitory assays. Toxicity evaluation was performed according to standard OECD guideline 402. The extract showed the presence of important phytoconstituents such as alkaloids, flavonoids, phenols, tannins, and saponins. The extract exhibited concentration-dependent antioxidant and anti-inflammatory activity in the selected in vitro models. The toxicity study indicated that the extract was safe at the tested dose level. The findings suggest that the selected plant extract possesses promising antioxidant and anti-inflammatory potential, which may be attributed to its phytochemical constituents. Further in vivo and mechanistic studies are required to confirm its therapeutic potential.

**KEYWORDS:** *Arquita ancashiana,* phytochemical screening, antioxidant activity, anti-inflammatory activity, protein denaturation, proteinase inhibition, toxicity study.

## INTRODUCTION

Inflammation is an essential biological response to harmful stimuli, including pathogens, injuries and irritants, essential for immune defense and tissue repair[1]. However, prolonged or chronic inflammation is linked to several diseases, such as arthritis, cardiovascular disorders, diabetes and cancer. The rising incidence of inflammatory conditions is connected to lifestyle changes, pollutants and aging populations. While usual anti-inflammatory drugs like NSAIDs and corticosteroids are widely used, their long-term application often results in harmful effects such as gastrointestinal irritation, immunosuppression, and organ toxicity[2]. This has led to growing interest in plant-derived therapies as safer alternatives and fewer side effects.

Plants have been extensively explored for their anti-inflammatory potential due to presence of flavonoids, saponins, alkaloids, terpenoids, and polyphenols. Phytochemicals modulate key inflammatory pathways by inhibiting TNF-α, IL-1β, IL-6, and COX enzymes[3,4]. Several medicinal plants have demonstrated significant anti-inflammatory effects[5,6], reinforcing the therapeutic promise of botanical sources. Compounds obtained from plants also exhibit antioxidant properties, which contribute to their efficacy in controlling inflammation and related conditions, positioning them as attractive candidates for further research and pharmaceutical development[5].
*Arquita ancashiana*, a historically used medicinal plant shows excellent anti-inflammatory effects. Preliminary studies indicate that presence of flavonoids, alkaloids, saponins, and tannins, shows pharmacological activity[6].

## MATERIAL AND METHODS

### Materials:

The leaves of Arquita ancashiana were collected in March 2021 and authenticated by Dr. Vikas B. Awale, Head of the Department of Botany, Dr. Patangrao Kadam Mahavidyalaya, Sangli. The taxonomic identity of the plant was authenticated and a plant herbarium specimen was stored in the laboratory for future reference.

### Extraction procedure

Aqueous & ethanolic Extraction of leaves of Arquita ancashiana was carried out by Maceration and Soxhlet extraction method [7,8,9].

### Phytochemical Investigation of extracts:

Qualitative tests were conducted for all the extracts to identify the various phytoconstituents [10-14]. The various tests and reagents used are given below.

**Table1: Qualitative tests for identification of various phytoconstituents.**

| Sr. No. | Phytochemical | Test | Procedure | Positive Observation |
|---|---|---|---|---|
| 1 | Alkaloids | Hager's test | Filtrate + Hager's reagent | Creamy white precipitate |
| | | Wagner's test | Filtrate + Wagner's reagent | Brown/reddish precipitate |
| | | Dragendorff's | Filtrate + Dragendorff's reagent | Orange-red precipitate |
| | | Mayer's | Filtrate + Mayer's reagent | Creamy/White precipitate |
| 2 | Carbohydrates | Molisch's test | Filtrate + α-naphthol + conc. $H_2SO_4$ | Violet ring |
| | | Benedict's | Filtrate + Benedict's reagent and boil in a water bath. | Brick red precipitate |
| | | Barfoed's | Filtrate + Barfoed's reagent and heat for 1–2 minutes. | Red precipitate (indicates monosaccharides) |
| 3 | Flavonoids | Shinoda | Filtrate + Magnesium ribbon + Conc. $H_2SO_4$ | Pink, scarlet, or crimson red color |
| | | Lead acetate test | Filtrate + lead acetate | Yellow precipitate |
| 4 | Glycosides | Keller-Kiliani | Filtrate + glacial acetic acid + $FeCl_3$+ $H_2SO_4$ | Brown ring (turning blue/green) |
| | | Legal's | Filtrate + Sodium nitroprusside in pyridine and make alkaline. | Pink to blood red color |
| | | Baljet's | Filtrate + Sodium picrate solution. | Yellow to orange color |
| 5 | Saponins | Foam Test | Shake the extract vigorously with distilled water in a test tube. | Persistent "honeycomb" froth (10+ mins) |
| 6 | Steroids & Terpenes | Salkowski | Filtrate + Chloroform+ $H_2SO_4$ | Reddish-brown (chloroform) / Yellow fluorescence |
| | | Liebermann | Filtrate +acetic anhydride, + conc. $H_2SO_4$ | Blue-green color |
| 7 | Proteins | Ninhydrin | Filtrate + 0.2% Ninhydrin solution. | Violet or purple color |
| 8 | Phenols & Tannins | 5% $FeCl_3$ | Filtrate + Add a few drops of neutral Ferric chloride solution | Dark green or bluish-black color |

**In Vitro Antioxidant activity by DPPH method [15-16]**

The antioxidant activity of the test samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay in a 96-well microplate, with Ascorbic acid used as the reference standard. A freshly prepared DPPH solution (0.1 mM) was made in methanol and protected from light. Stock solutions of the test samples and standard were prepared and serially diluted to obtain concentrations ranging from 100 to 1000 µg/mL. In each well of the 96-well plate, 100 µL of DPPH solution was mixed with 100 µL of test sample or standard at different concentrations. For control, 100 µL of DPPH solution was mixed with 100 µL of methanol, while the blank contained methanol only. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to allow complete reaction. After incubation, the decrease in absorbance was measured at 517 nm using a microplate reader. All experiments were performed in triplicate (n = 3). The percentage of DPPH radical scavenging activity was calculated using the formula: (Absorbance of control − Absorbance of sample) / Absorbance of control × 100.

**In vitro anti-inflammatory activity by using following methods**

**Protein denaturation inhibition assay[17-19]**

The reaction mixture (5 mL) consisted of 0.5 mL of egg albumin, 2.5 mL of phosphate buffered saline (PBS, pH 6.4), and 2.0 mL of the test sample prepared at different concentrations ranging from 100 to 1000 µg/mL. A similar volume of double-distilled water served as the control. The reference standard used was Diclofenac sodium (1 mg/mL), which was treated in the same manner as the test samples. The reaction mixtures were incubated at 37°C ± 2°C for 15 minutes in an incubator, followed by heating at 70°C for 5 minutes in a water bath to induce protein denaturation. After cooling to room temperature, the absorbance of each sample was measured at 660 nm using the vehicle as a blank.

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100

**Proteinase inhibition assay [20-22]**

The proteinase inhibitory activity of the test samples was evaluated using a modified trypsin–BSA digestion method, with Diclofenac sodium used as the reference standard. Stock solutions of the standard and test samples were prepared and serially diluted with 20 mM Tris–HCl buffer (pH 7.4) to obtain concentrations ranging from 100 to 1000 µg/mL. The reaction mixture, with a total volume of 2 mL, consisted of 1 mL of Tris–HCl buffer and 0.06 mg of trypsin enzyme. To this, 1 mL of either the test sample or standard solution at the desired concentration was added, and the mixture was incubated at 37°C for 5 minutes. Following this, 1 mL of 4% (w/v) bovine serum albumin (BSA) was added as a substrate, and the reaction mixture was further incubated at 37°C for 20 minutes. The reaction was terminated by adding 2 mL of 70% perchloric acid (or alternatively 5% trichloroacetic acid), resulting in the formation of a precipitate. The mixture was then centrifuged at 3000 rpm for 10 minutes to obtain a clear supernatant. The absorbance of the supernatant was measured at 210 nm using a UV–Visible spectrophotometer, with buffer serving as the blank. A control was maintained containing all reagents except the test or standard, which was replaced with buffer. All experiments were performed in triplicate (n = 3), and the percentage inhibition of proteinase activity was calculated using the formula

(Abs control -Abs sample) /Abs control x 100

**Acute Dermal Toxicity Studies of extract as per OECD 402 Guidelines:**

The acute dermal toxicity study of ethanolic extract of *Arquita ancashiana* was carried out in accordance with standard guidelines (OECD 402).

**Experimental Animals**

Healthy adult Wistar rats of either sex (male and female), weighing 180–220 g, were used for the study. The animals were housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and 12 h light/dark cycle) with free access to standard pellet diet and water. The animals were acclimatized for one week prior to the experiment.

**Preparation of Animals**

Approximately 24 hours before the experiment, the dorsal fur of the animals was removed carefully using electric clippers without damaging the skin.

**Treatment Protocol**

The animals were divided into four groups (n = 3 per group). The extract was applied topically to the shaved dorsal area at a limit dose of **2000 mg/kg** body weight. The applied area was left uncovered, and care was taken to prevent ingestion of the formulation.

**Observation Parameters**

The animals were observed continuously for the first 24 hours after application and daily thereafter for a total period of 14 days. Observations included:

- Signs of dermal irritation (erythema and edema)
- Behavioural changes
- Signs of systemic toxicity
- Mortality

Body weight of the animals was recorded at regular intervals during the study period.

**Evaluation of Dermal Toxicity**

Dermal reactions, if any, were assessed visually and scored based on standard criteria. The absence or presence of erythema and edema was recorded.[23-26]

**Table 2: Grouping of Acute Dermal Toxicity Study.**

| Groups | Treatment | Dose (mg/kg) | No. of animals |
|---|---|---|---|
| G1 (Male) | Arquita ancashiana ethanolic extract | 2000 | 3 |
| G2 (Female) | Arquita ancashiana ethanolic extract | 2000 | 3 |

## RESULTS

**Phytochemical Investigation of extracts:**

Qualitative phytochemical analysis revealed that the ethanolic extract of *Arquita ancashiana* showed a stronger presence of major secondary metabolites. The ethanolic extract of *A. ancashiana* tested positive for alkaloids (Wagner's, Hager's, Dragendorff's and Mayer's), flavonoids, saponins, steroids/terpenes, proteins, phenols/tannins and carbohydrates, indicating a rich phytochemical profile. Aqueous extracts lacked alkaloids, flavonoids, glycosides steroids and Terpenes and proteins. These findings suggest that *Arquita ancashiana* contains a broader and more potent array of bioactive compounds, which may correlate with its higher biological efficacy observed in subsequent studies.

**Table 3: Preliminary phytochemical screening of the extracts**

| Sr, No. | Phytochemicals | Test | Arquita ancashiana (Aqueous) | Arquita ancashiana (Ethanolic) |
|---|---|---|---|---|
| 1 | Alkaloids | Hager's | - | + |
| | | Wagner's | - | + |
| | | Dragendorff's | - | + |

| | | | | |
|---|---|---|---|---|
| | | Mayer's | - | + |
| 2 | Carbohydrates | Molisch's | + | + |
| | | Benedict's | + | + |
| | | Barfoed's | + | + |
| 3 | Flavonoids | Shinoda | - | + |
| | | Lead acetate | - | + |
| 4 | Glycosides | Keller–Kiliani | - | + |
| | | Legal's | - | + |
| | | Baljet's | - | + |
| 5 | Saponins | Foam test | + | + |
| 6 | Steroids & Terpenes | Salkowski | - | + |
| | | Liebermann–Burchard | - | + |
| 7 | Proteins | Ninhydrin | - | + |
| 8 | Phenols & Tannins | 5% $FeCl_3$ | + | + |

Key: + = Present, – = Absent.

**Antioxidant Activity by DPPH Method:**

The antioxidant capacity of *Arquita ancashiana* was evaluated via the DPPH assay, with ascorbic acid serving as the reference standard. The standard demonstrated potent radical-scavenging activity with an $IC_{50}$ < 100 µg/mL, reaching a maximum inhibition of 86.67% at 1000 µg/mL, which effectively validated the experimental conditions. A concentration-dependent increase in percent inhibition was observed across all tested samples, indicating that the scavenging power rises with concentration from 100 to 1000 µg/mL.

Among the crude extracts, the ethanolic preparations exhibited moderate antioxidant potential with $IC_{50}$ values of 178.37 µg/mL. In contrast, the aqueous extracts displayed significantly weaker activity, characterised by high $IC_{50}$ values of 943.97 µg/mL for *A. ancashiana*. The disparity between the solvent types suggests that the primary antioxidant constituents (specific phenolic/flavonoid compounds) are more efficiently solubilised and recovered using ethanol.

**Table 4: DPPH antioxidant activity of the extract**

| Sr. No. | Sample | Conc (µg/mL) | Mean OD | % Inhibition | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 1 | Control | - | 0.988 ± 0.010 | - | - |
| 2 | Ascorbic Acid (Standard) | 100 | 0.252 ± 0.006 | 74.53 | **< 100** |
| | | 200 | 0.231 ± 0.003 | 76.59 | |
| | | 400 | 0.213 ± 0.003 | 78.44 | |
| | | 800 | 0.167 ± 0.006 | 83.10 | |
| | | 1000 | 0.132 ± 0.011 | 86.67 | |
| 3 | *A. ancashiana* (Ethanol) | 100 | 0.476 ± 0.003 | 51.86 | **178.37** |
| | | 200 | 0.528 ± 0.112 | 46.52 | |
| | | 400 | 0.452 ± 0.004 | 54.28 | |
| | | 800 | 0.341 ± 0.012 | 65.49 | |
| | | 1000 | 0.320 ± 0.002 | 67.65 | |
| 4 | *A. ancashiana* (Aqueous) | 100 | 0.784 ± 0.007 | 20.61 | **943.97** |
| | | 200 | 0.762 ± 0.003 | 22.91 | |
| | | 400 | 0.690 ± 0.008 | 30.20 | |
| | | 800 | 0.571 ± 0.015 | 42.17 | |
| | | 1000 | 0.453 ± 0.004 | 54.15 | |

Data represent mean ± SD, n = 3

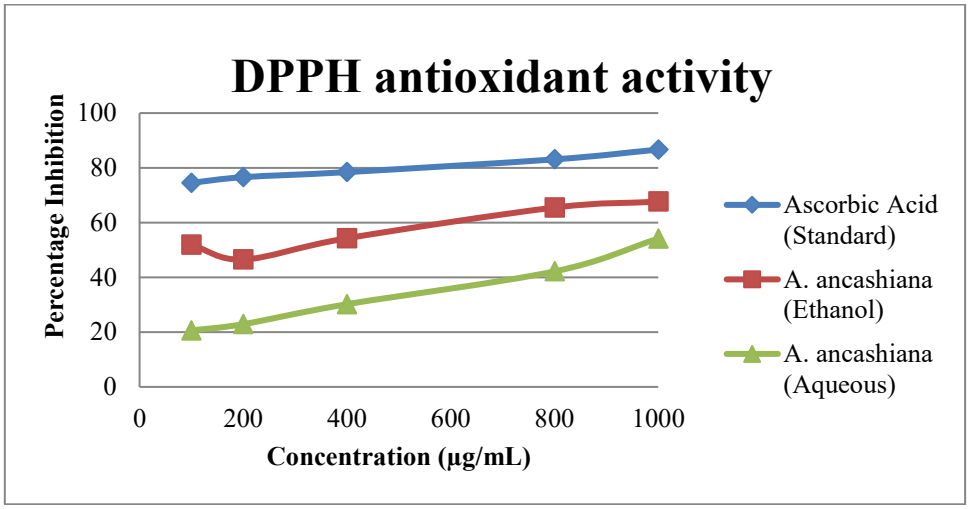


**Fig.no.1: %** Inhibition of DPPH at different Concentration of *Arquita ancashiana* (Aqueous Extract, Ethanolic Extract)

**In vitro Anti-Inflammatory Activity of Extract.**

**Protein Denaturation Inhibition Assay:**

The anti-inflammatory potential of the extracts was evaluated by assessing their ability to inhibit thermal denaturation of egg albumin. Diclofenac sodium was employed as the standard drug, which exhibited highly potent, concentration-dependent inhibition with an $IC_{50}$ of 105.12 µg/mL. Among the tested plant extracts, the ethanolic extracts showed superior protective effects ($IC_{50}$ of 188.96 µg/mL.) compared to the aqueous versions ($IC_{50}$ of 255.73 µg/mL). The aqueous extracts demonstrated lower activity confirming that the bioactive compounds responsible for stabilising protein structures are more efficiently extracted using ethanol.

**Table 5: Protein denaturation inhibition assay**

| Sr. No. | Sample | Conc (µg/mL) | Absorbance | % Inhibition | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 1 | Control | - | 0.850 ± 0.012 | - | - |
| 2 | Diclofenac sodium (Standard) | 100 | 0.456 ± 0.009 | 46.35 | **105.12** |
| | | 200 | 0.202 ± 0.008 | 76.24 | |
| | | 400 | 0.129 ± 0.007 | 84.82 | |
| | | 800 | 0.065 ± 0.008 | 92.35 | |
| | | 1000 | 0.051 ± 0.007 | 94.00 | |
| 3 | *A. ancashiana* (Ethanol) | 100 | 0.552 ± 0.010 | 35.06 | **188.96** |
| | | 200 | 0.381 ± 0.011 | 55.18 | |
| | | 400 | 0.297 ± 0.010 | 65.06 | |
| | | 800 | 0.228 ± 0.010 | 73.18 | |
| | | 1000 | 0.195 ± 0.009 | 77.06 | |
| 4 | *A. ancashiana* (Aqueous) | 100 | 0.585 ± 0.012 | 31.18 | **255.73** |
| | | 200 | 0.456 ± 0.011 | 46.35 | |
| | | 400 | 0.361 ± 0.011 | 57.53 | |
| | | 800 | 0.298 ± 0.011 | 64.94 | |
| | | 1000 | 0.263 ± 0.010 | 69.06 | |

Data represent mean ± SD, n = 3.

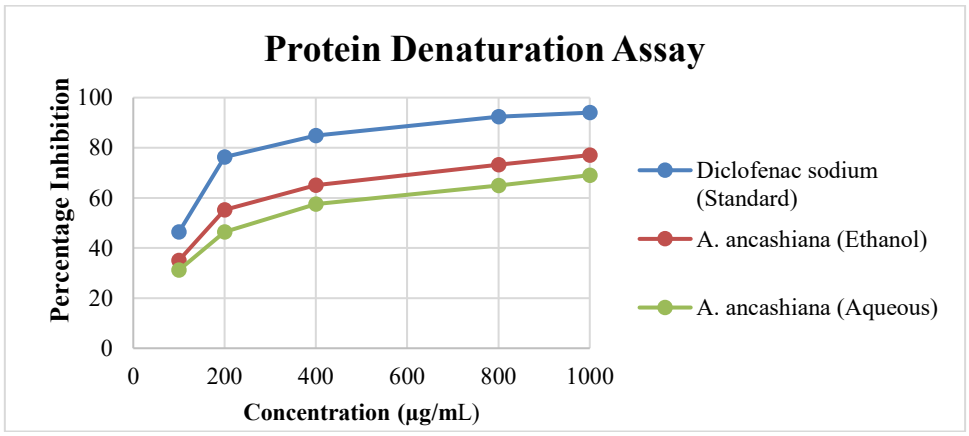


**Fig.no 2:** % Inhibition of Protein Denaturation Activity of Extracts of *Arquita ancashiana* (Aqueous Extract, Ethanolic Extract)

**Proteinase Inhibition Assay:**
Diclofenac sodium showed high potency with an $IC_{50}$ of 99.89 µg/mL and maximum inhibition of 76.57% at 1000 µg/mL. Among the plant extracts, *A. ancashiana* (Ethanol) showed the most significant activity ($IC_{50}$ 419.60 µg/mL), The aqueous extracts demonstrated considerably lower efficacy, with $IC_{50}$ values of 902.70 µg/mL for *A. ancashiana* indicating that the protease-inhibitory phytochemicals have higher solubility in organic solvents.

**Table 6: Proteinase inhibition assay**

| Sr. No. | Sample | Conc (µg/mL) | Absorbance | % Inhibition | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|
| 1 | Control | - | 0.905 ± 0.007 | - | - |
| 2 | Diclofenac sodium (Standard) | 100 | 0.451 ± 0.005 | 50.17 ± 0.52 | **99.89** |
| | | 200 | 0.378 ± 0.004 | 58.23 ± 0.48 | |
| | | 400 | 0.316 ± 0.004 | 65.08 ± 0.44 | |
| | | 800 | 0.253 ± 0.003 | 72.02 ± 0.41 | |
| | | 1000 | 0.212 ± 0.003 | 76.57 ± 0.39 | |
| 3 | *A. ancashiana* (Ethanol) | 100 | 0.595 ± 0.006 | 34.25 ± 0.58 | **419.60** |
| | | 200 | 0.523 ± 0.005 | 42.20 ± 0.55 | |
| | | 400 | 0.461 ± 0.005 | 49.00 ± 0.52 | |
| | | 800 | 0.409 ± 0.004 | 54.75 ± 0.50 | |
| | | 1000 | 0.382 ± 0.004 | 57.79 ± 0.47 | |
| 4 | *A. ancashiana* (Aqueous) | 100 | 0.631 ± 0.006 | 30.27 ± 0.58 | **902.70** |
| | | 200 | 0.569 ± 0.005 | 37.12 ± 0.55 | |
| | | 400 | 0.511 ± 0.005 | 43.54 ± 0.52 | |
| | | 800 | 0.464 ± 0.004 | 48.73 ± 0.50 | |
| | | 1000 | 0.439 ± 0.004 | 51.49 ± 0.47 | |

Data represent mean ± SD, n = 3.

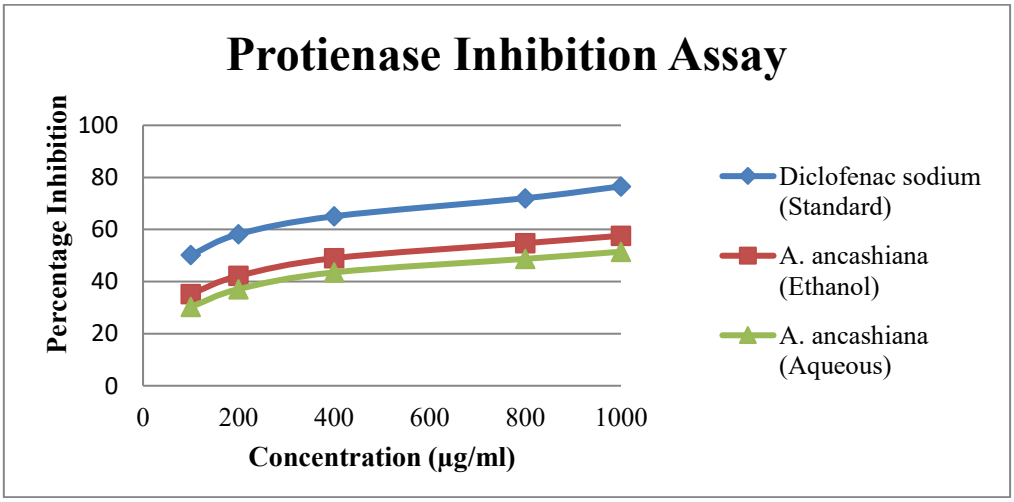


**Fig.no 3:** % Inhibition of Proteinase Inhibition Assay at different Concentration of Araquita *ancashiana* (Aqueous Extract, Ethanolic Extract)

**In Vivo Studies:**
**Acute Dermal Toxicity Study of the extracts**
The ethanolic extract of *Arquita ancashiana* (G1-male, G2-female) was applied topically to shaved dorsal skin of healthy adult Wistar rats (n = 3 per group; both sexes evaluated) at the OECD limit dose of 2000 mg /kg body weight as a single application. Throughout the 14 days observation period there was no mortality, no signs of dermal irritation (Draize erythema and oedema scores = 0), no abnormal clinical or behavioural finding, and a normal, sex-appropriate progressive gain in body weight and feed intake in all groups. Accordingly, the median lethal dermal dose ($LD_{50}$, dermal) is greater than 2000 mg/kg in both sexes, placing the extract in GHS Category 5 / Unclassified.
**Effect on body weight (Day 0, 7 and 14):**

A progressive and physiologically normal gain in body weight was observed in all groups. Male groups (G1) showed the typically higher absolute weights and larger 14 day weight gain (~22.7 g, ~10.8%) than the female groups (G2 ~16.7, ~8.8 %), consistent with the expected growth pattern of adult Wistar rats. No animal showed weight loss, stagnation or abnormal weight fluctuation.

**Table 7: Effect of extract on body weight (g) of Wistar rats over 14 days.**

| Group | Day 0 (g) | Day 7 (g) | Day 14 (g) | Total gain (g) | % gain (D0-D14) |
|---|---|---|---|---|---|
| G1 – *A. ancashiana* extracts 2000 mg/kg (Male) | 210.33 ± 7.51 | 220.67 ± 7.57 | 233.00 ± 8.19 | 22.67 ± 1.53 | 10.78 |
| G2 – *A. ancashiana* extracts 2000 mg/kg (Female) | 188.67 ± 6.43 | 196.00 ± 6.56 | 205.33 ± 7.51 | 16.67 ± 1.53 | 8.84 |

Values are mean ± SD; n = 3.

**Effect on feed intake:**

Feed intake remained within the normal physiological range and increased steadily over the 14 days period in parallel with body weight gain. There was no evidence of anorexia, hyperphagia or palatability related rejection of the formulations.

**Table 8: Effect of extract on feed intake (g/animal/day)**

| Group | Day 0 | Day 7 | Day 14 | Mean |
|---|---|---|---|---|
| G1 – *A. ancashiana* extracts (Male) | 18.00 ± 0.87 | 18.83 ± 0.76 | 19.50 ± 0.50 | 18.78 ± 0.71 |
| G2 – *A. ancashiana* extracts (Female) | 15.33 ± 0.76 | 16.00 ± 0.50 | 16.67 ± 0.58 | 16.00 ± 0.61 |

Values are mean ± SD; n = 3.

**General behavioural and clinical observations:**

Each animal was observed continuously for the first 4 h post-application, at 24 h, and thereafter at least once daily for 14 days following the OECD 402 (2017) functional observational battery. No abnormality of clinical concern was detected in any group; no animal died during the study.

**Table 9: Consolidated general behavioural and clinical observation chart.**

| Observation parameter | G1 *A. ancashiana* (Male) | G2 *A. ancashiana* (Female) |
|---|---|---|
| Skin and fur (colour, texture, alopecia) | Normal | Normal |
| Erythema at application site (Draize 0–4) | 0 | 0 |
| Oedema at application site (Draize 0–4) | 0 | 0 |
| Eyes (clarity, lacrimation, ptosis) | Normal | Normal |
| Mucous membranes | Normal pink | Normal pink |
| Salivation | Absent | Absent |
| Piloerection | Absent | Absent |
| Respiration | Normal | Normal |
| Circulation (cyanosis/pallor) | Normal | Normal |
| Tremors / convulsions | Absent | Absent |
| Gait and posture | Normal | Normal |
| Righting / corneal / pinnal reflexes | Present | Present |
| Grip strength / muscle tone | Normal | Normal |
| Somatomotor activity | Normal | Normal |
| Alertness / arousal | Alert | Alert |
| Aggression / irritability on handling | Absent | Absent |
| Stereotypy | Absent | Absent |
| Grooming behavior | Normal | Normal |
| Urination / defecation | Normal | Normal |
| Diarrhea | Absent | Absent |
| Food and water intake | Normal | Normal |
| **Mortality (Day 0–14)** | **0/3** | **0/3** |

Under the conditions of this study, the ethanolic extract of *Arquita ancashiana* applied topically at a single limit dose of 2000 mg/kg did not produce mortality, dermal irritation, behavioural deviation or any treatment related change in body weight or feed intake in either sex. The $LD_{50}$ (dermal) for extract is therefore > 2000 mg/kg in both sexes (GHS Category 5 / Unclassified), supporting a wide margin of cutaneous safety and further investigation in repeated dose efficacy studies.

## DISCUSSION

The present study was carried out to evaluate the phytochemical profile and pharmacological activities of the extract of *Arquita ancashiana*. The preliminary phytochemical investigation revealed the presence of secondary metabolites like flavonoids, saponins, steroids/terpenes, proteins, phenols/tannins and carbohydrates. These phytoconstituents are responsible for various pharmacological activities like antioxidant and anti-inflammatory activity.

The antioxidant activity by DPPH assay observed in the study may be attributed to the presence of phenolic and flavonoid compounds, which are able to donate hydrogen atoms or electrons and neutralize free radicals. The results of protein denaturation and proteinase inhibitory assays suggest that the extract shows anti-inflammatory activity. Inhibition of protein denaturation is an important indicator of anti-inflammatory potential of the extract. The observed activity may be due to the presence of bioactive constituents that stabilize proteins and inhibit inflammatory mediators. In acute dermal toxicity of the extract, the median lethal dermal dose ($LD_{50}$, dermal) is greater than 2000 mg/ kg in both sexes, placing the extract in GHS Category 5 / Unclassified.

All the observed findings suggest that the extract of *Arquita ancashiana* has significant therapeutic potential but need to be evaluated for long-term safety.

## CONCLUSION

The present study concluded that the extract of *Arquita ancashiana* possesses significant phytochemical and pharmacological potential. The presence of bioactive constituents such as flavonoids, saponins, steroids/terpenes, proteins, phenols/tannins and carbohydrates may be responsible for the observed antioxidant and anti-inflammatory activity.

Among the crude extracts, the ethanolic extract exhibited moderate antioxidant potential with $IC_{50}$ values of 178.37 µg/mL. In contrast, the aqueous extracts displayed significantly weaker activity, characterised by high $IC_{50}$ values of 943.97 µg/mL. In protein denaturation inhibition assay, among the tested plant extracts, the ethanolic extracts showed superior protective effects ($IC_{50}$ of 188.96 µg/mL.) compared to the aqueous versions ($IC_{50}$ of 255.73 µg/mL). While studying proteinase inhibition assay, *A. ancashiana* (Ethanol) extract showed the most significant activity ($IC_{50}$ 419.60 µg/mL). The aqueous extracts demonstrated considerably lower efficacy, with $IC_{50}$ values of 902.70 µg/mL. In acute dermal toxicity of the ethanolic extract, the median lethal dermal dose ($LD_{50}$, dermal) is greater than 2000 mg/ kg in both sexes, placing the extract in GHS Category 5 / Unclassified.

These results suggest that extracts of *Arquita ancashiana* could serve as an effective alternative to conventional anti-inflammatory treatment.

## REFERENCES:


1. El-Gabalawy H, Guenther LC, Bernstein CN. Epidemiology of immune-mediated inflammatory diseases: incidence, prevalence, natural history, and comorbidities. J Rheumatol. 2010;37(1):2-10. doi:10.3899/jrheum.091461.
2. Maddipati KR. Distinct etiology of chronic inflammation: implications on degenerative diseases and cancer therapy. Front Immunol. 2024; 15:1460302. doi:10.3389/fimmu.2024.1460302.
3. Azab A, Nassar A, Azab AN. Anti-inflammatory activity of natural products. Molecules. 2016;21(10):1321. doi:10.3390/molecules21101321.
4. Yuan G, Wahlqvist ML, He G, Yang M, Li D. Natural products and anti-inflammatory activity. Asia Pac J Clin Nutr. 2006;15(2):143-152.
5. Wahab SMA, Jantan I, Haque MA, Arshad L. Exploring the leaves of Annona muricata L. as a source of potential anti-inflammatory and anticancer agents. Front Pharmacol. 2018; 9:661. doi:10.3389/fphar.2018.00661.
6. Acero N, Muñoz-Mingarro D, Navarro I, León-González AJ, Martín-Cordero C. Phytochemical analysis and anti-inflammatory potential of *Acanthus mollis* L. rhizome hexane extract. Pharmaceuticals. 2023; 16(2):159. doi:10.3390/ph16020159.
7. Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020;12(1):1-10. doi:10.4103/jpbs.JPBS_175_19.
8. Handa SS, Khanuja SPS, Longo G, Rakesh DD. An overview of extraction techniques for medicinal and aromatic plants. Trieste: International Centre for Science and High Technology; 2008.
9. Azwanida NN. A review on the extraction methods use in medicinal plants, their advantages and disadvantages. J Mar Sci Res Dev. 2015;5(4):1-6.
10. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.
11. Khandelwal KR. Practical pharmacognosy: techniques and experiments. 19th ed. Pune: Nirali Prakashan; 2008.
12. Trease GE, Evans WC. Pharmacognosy. 16th ed. Edinburgh: Saunders Elsevier; 2009.
13. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 49th ed. Pune: Nirali Prakashan; 2014.
14. Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. Bristol: Wright Scientechnica; 1975.
15. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi:10.1016/S0023-6438(95)80008-5.
16. Bobo-García G, et al. Solvent selection for DPPH radical scavenging assay in a 96-well plate: a comprehensive study. J Food Compost Anal. 2015; 43:1-7. doi:10.1016/j.jfca.2015.04.011.

17. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol. 1968;20(3):169-173.
18. Chandra S, et al. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed. 2012;2(1): S178-S180. doi:10.1016/S2221-1691(12)60154-3.
19. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. In vitro anti-inflammatory properties of selected edible leaves. Biomedicines. 2018;6(4):107.
20. Oyedapo OO, Famurewa AJ. Antiprotease and membrane stabilizing activities of extracts of *Fagarazanthoxyloides*, *Olaxsubscorpioides* and *Tetrapleura tetraptera*. Int J Pharmacogn. 1995;33(1):65-69.
21. Sakat S, Tupe N, Ade A. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Bio Sci. 2010;1(2):1-14.
22. Leelaprakash G, Dass SM. In vitro anti-inflammatory activity of methanol extract of *Enicostemmaaxillare*. Int J Drug Dev Res. 2011;3(3):189-196.
23. Organisation for Economic Co-operation and Development. Test No. 402: acute dermal toxicity. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2017. doi:10.1787/9789264070585-en.
24. Ahmad J, et al. Development and characterization of a nanoemulgel for topical delivery: acute dermal toxicity and safety evaluation. J Drug Deliv Sci Technol. 2019; 53:101132.
25. Draize JH. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther. 1944; 82(3):377-390.
26. Soni RK, et al. Acute and sub-acute toxicity studies of *Paederia foetida* in rats. Int J Pharm Sci Res. 2013;4(4):1417.