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RESEARCH ARTICLE

Phytochemical profiling and evaluation of antioxidant and anti-inflammatory activities of *Ipomoea alba* L.

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Abstract

Plant-based medicine has been one of the oldest therapeutic practices in India and continues to offer valuable treatments for various ailments. *Ipomoea alba*, commonly known as morning glory, belongs to the family Convolvulaceae and is native to the tropical and subtropical regions of North and South America. It is renowned for its large, fragrant, nocturnal blooms, this plant holds significant potential in traditional medicine, particularly for managing gastrointestinal disorders, inflammation and skin conditions. The nutrient content of *Ipomoea alba* leaves and seeds has demonstrated promising health benefits. This study investigated the phytochemical profile of *Ipomoea alba* leaves using three solvents: water, methanol and chloroform. Phytochemical analysis confirmed the presence of carbohydrates, proteins, alkaloids, flavonoids, saponins and tannins. HPLC analysis identified the presence of phenols in the aqueous extract, albeit in small quantities. Among the three extracts, the methanolic extract exhibited the highest antioxidant activity, as determined by DPPH, ABTS and FRAP assays. Anti-inflammatory activity, assessed using a proteinase inhibitory assay, demonstrated that the methanolic extract showed the greatest inhibition at lower concentrations compared to the aqueous and chloroform extracts. The results suggest that the antioxidant and anti-inflammatory properties of *Ipomoea alba* may hold potential applications in cancer prevention and treatment. Future studies will aim to evaluate its cytotoxic effects, thereby exploring its potential role in cancer therapy.

Keywords: anti-inflammatory activity; antioxidants; HPLC analysis; *Ipomoea alba*; phytochemicals

Introduction

Diet plays a vital role in disease prevention, as specific nutrients and bioactive compounds present in the food we consume are critical for maintaining overall health and resilience. Plant-based foods, in particular, are rich source of secondary metabolites-such as polyphenols, flavonoids, alkaloids and terpenoids-which provide a wide range of health-promoting effects. These naturally occurring compounds act as powerful antioxidants, neutralizing harmful free radicals in the body. Additionally, they exhibit anti-inflammatory and antimicrobial properties, contributing to the reduction of inflammation and the prevention of infections. Regular consumption of these bioactive compounds over time strengthens the immune system and offers protection against chronic diseases, including insomnia, cardiovascular conditions, respiratory issues and cancer (1).

The genus *Ipomea*, a member of the Convolvulaceae family, comprises approximately 650 species that flourish in warm and humid environments. One prominent species, *Ipomea alba*, is distinguished by its white, nocturnal blooms-its species name "alba" translating to "white" (Fig. 1). While some *Ipomea* species are considered weeds, others, such as sweet potatoes, have significant economic value (2).The *Ipomea* genus, including *Ipomea alba*, is known for its diverse biological activities. Key bioactive compounds identified in *Ipomea* species include lipids, phenolic substances, alkaloids, and glycolipids, which are the most common biologically active constituents derived from their plant extracts (3).

Ipomea alba is nutrient-rich and widely consumed as both a raw and cooked vegetable in various regions worldwide. Traditionally, it has been employed as a remedy for conditions such as snake bites, constipation and boils, particularly in regions like Southeast Asia and South America, where its medicinal properties have been extensively documented. For example, its efficacy in treating snake bites has been reported in rural India (4) and its use as a laxative is well recognized in folk medicine across various cultures (5). Additionally, *Ipomea alba* is valued for its antioxidant properties and its role in managing excess body weight. Despite its primary use as an ornamental plant, *Ipomea alba* is consumed for its bioactive compounds, such as ipalbine and ipalbidine.

Materials and Methods

Plant material

Plants were collected from the Mysuru district, Karnataka, India, in March 2023. Fresh leaves were thoroughly washed with tap water, followed by rinsing with distilled water. After washing, the leaves were shade-dried for 15 days at room

Fig. 1. *Ipomea alba.*

temperature. Once dried, leaves were ground into a fine powder using a pebble and mortar and subsequently stored in an airtight container.

Forty grams of the dried, powdered leaves of *Ipomoea alba* were subjected to successive extraction with distilled water, methanol and chloroform (200 mL each) for eight hours using a Soxhlet apparatus. The resulting solutions were filtered through Whatman No. 1 filter paper and evaporated to dryness in a hot air oven maintained at 40°C. This temperature was carefully selected to prevent the degradation of thermo labile compounds that might occur at higher temperatures. The dried extracts were then utilized for subsequent analysis (6).

Qualitative screening of phytochemicals

Standard phytochemical screening methods were employed to assess the plant extracts. The presence of alkaloids was determined using Wagners' reagent, while carbohydrates were assessed through Molischs' and Benedicts' tests. Flavonoids were identified using the Alkaline Reagent test and Lead Acetate tests. The presence of phenols was confirmed using Ferric Chloride and Liebermanns' tests, whereas proteins were detected through the Ninhydrin method. Saponins, steroids, tannins and quinones were screened using the foam, Liebermann-Burchard test, Braymer's test and HCl test, respectively (7, 8).

HPLC analysis of phenols

The analysis was carried out using a C18 column (4.6 mm × 250 mm) in isocratic mode. The mobile phase consisted of acetonitrile and water in a 7:3 ratio, with a flow rate of 1 mL/min. Standard gallic acid (0.4 mg/mL) and sample solutions (10 mg/mL) were prepared by dissolving them in the mobile phase. A volume of 20 µL of each solution was injected into the system and the elution was monitored at 254 nm (9, 10).

The phenolic content in the sample was calculated using the following formula:

$$\text{Phenolic content} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{standard amount}}{\text{dilution of standard}} \times \frac{\text{dilution of sample}}{\text{sample amount}} \times \text{Mean weight} \quad \text{(Eq. 1)}$$

Quantitative estimation of phytochemicals

Estimation of total proteins

The protein concentration in plant samples was estimated using Bovine serum albumin (BSA) as the standard reagent for constructing a standard curve. Initially, 0.5 mL of reagent 1 (prepared by mixing 50 mL of 2 % sodium carbonate in 0.1 N sodium hydroxide and 1 mL of 0.5 % copper sulfate in 1 % potassium sodium tartrate) was added to the sample extracts. The samples were left undisturbed for 10 min. Subsequently, 0.5 mL of freshly prepared Folin-Ciocalteu reagent was added and the mixture was incubated in the dark for 30 min. After incubation, the absorbance was measured at 660 nm. The protein content was expressed as mg BSA per g of fresh weight (11).

Estimation of total alkaloids

The total alkaloid content was determined using a caffeine standard assay. Accurately measured aliquots (ranging from 0.08 to 0.4 mL) of the caffeine standard solution were transferred into individual separatory funnels. To each funnel, 5 mL of pH 4.7 phosphate buffer and 5 mL of Bromocresol Green (BCG) solution were added, followed by shaking the mixture thoroughly with 1, 2, 3 and 4 mL of chloroform, respectively. The chloroform layers were collected into 10 mL volumetric flasks and diluted with chloroform to adjust the final volume (12).

Estimation of total flavonoids

The total flavonoid content was determined using the aluminium chloride colorimetric method. Various volumes of quercetin standard solutions (4-20 mg/mL) were added to 10 mL volumetric flasks containing 4 mL of distilled water. To each flask, 0.3 mL of 5 % sodium nitrate ($NaNO_2$) solution was added, followed by 0.3 mL of 10 % aluminum chloride ($AlCl_3$) solution after 5 min of incubation. After an additional 6 min, 2 mL of 1 mol/L NaOH solution was added. The final volume was adjusted to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min and the absorbance was measured at 510 nm. The total flavonoid content was calculated using a calibration curve and expressed as mg of quercetin per g of dry weight.

Estimation of antioxidants

DPPH assay

The DPPH (1,1-diphenyl-2-picryl hydrazyl radical) assay measures the ability of antioxidants to neutralize free radicals. DPPH is a stable radical that appears violet in color, which changes to yellow upon reduction. In this assay, varying concentrations of the test samples (0.1-0.5 mg) were prepared in test tubes, with the total volume adjusted to 0.1 mL using methanol. Subsequently, 3 mL of DPPH solution was added to

each tube, followed by incubation in the dark for 15 min. After the incubation period, the absorbance was measured at 517 nm using a spectrophotometer, with methanol serving as the blank. The percentage inhibition was determined using the following formula:

Percentage inhibition = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100. (Eq. 2)

Here, the absorbance of the control represents the reading without the test sample, while the sample absorbance includes the test compound (13, 14)

FRAP assay

The Ferric Reducing Antioxidant Power (FRAP) assay was performed using different concentrations (100-500 µg) of methanol, aqueous and chloroform extracts The total volume in each test tube was adjusted to 0.1 mL with methanol. To each tube, 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide [$K_3Fe(CN)_6$] were added. The mixtures were thoroughly vortexed and incubated at 50°C for 20 min using a vortex shaker. After incubation, 2.5 mL of 10 % trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was collected and mixed with 2.5 mL of deionized water and 0.5 mL of 0.1 % ferric chloride solution. The absorbance of the resulting solution was recorded at 700 nm using a UV-spectrophotometer, with a blank serving as the reference (15, 16).

ABTS assay

The free radical scavenging ability of the plant extracts was assessed using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline -6-sulfonic acid)) assay. Different concentrations of the extracts (ranging from 0.01 to 0.05 mg) were prepared in test tubes and the total volume was adjusted to 0.1 mL with methanol. To each tube, 3 mL of ABTS solution (adjusted to an absorbance of 1) was added, followed by 30-min incubation in darkness (17). After incubation, the absorbance was measured at 734 nm using a spectrophotometer, with methanol serving as the blank. The percentage of inhibition was determined using the formula:

Percentage inhibition =

[(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100 (Eq. 3)

Estimation of anti- inflammatory activity

Proteinase inhibitory action

A reaction mixture (2 mL) was prepared, containing 0.06 mg of proteinase, 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 1 mL of the test sample at varying concentrations. The mixture was incubated at 37°C for 5 min. Similarly, different concentrations of standard Trypsin were added to the reaction mixture and incubated under identical conditions. Afterward, 1 mL of 0.8 % casein was added to each tube, followed by 20 min incubation at 37°C. To stop the reaction, 2 mL of 70 % Perchloric acid was added. The resulting cloudy suspension was centrifuged and the absorbance of the supernatant was measured at 210 nm.

Statistical analysis

The statistical analyses were conducted using GraphPad Prism software. Results were evaluated using One-way Analysis of Variance (ANOVA), with a significance level set at $p < 0.05$. Post-hoc analysis was performed using Fishers' test. The coefficient of determination (R^2) for the model was calculated to be 0.9733, indicating a high degree of correlation.

Results and Discussion

The phytochemical analysis of *Ipomea alba* leaves revealed the presence of alkaloids, flavonoids, proteins, saponins, carbohydrates and tannins. However, the phytoconstituents sterols and quinones were not detected in any of the plant extracts. Notably, all extracts tested positive exclusively for saponins. A summary of the phytochemical constituents identified in the plants extracts is presented in Table 1.

High performance liquid chromatography (HPLC)

The HPLC analysis confirmed the presence of gallic acid, a phenolic compound, in the aqueous extract, with a retention time of 2.337 min, consistent with the standard reference, as shown in Fig. 2. The detection of phenolic compounds such as gallic acid in the aqueous extract can be attributed to their polar nature. Phenolic compounds possess hydroxyl (-OH) groups attached to an aromatic ring, making them more soluble in polar solvents such as water. This increased solubility facilitates a more efficient extraction of phenols in the aqueous phase compared to less polar solvents like chloroform or methanol, which exhibit lower extraction efficiency for these compounds.

Quantitative estimation of phytochemicals

Total alkaloids

The total alkaloid content was determined using a caffeine standard assay, a spectrophotometric method that allows the estimation of all alkaloids present in plant extracts. As shown in table 2, the chloroform extract of leaves contained the highest alkaloid content (9.234 ± 0.685 mg/mL), followed by

Table 1. Qualitative analysis of water, methonol and chloroform extracts of *Ipomea alba*

Phytochemical Constituents	Tests	Water	Methanol	Chloroform
Alkaloids	Wagner's Test	-	-	+
Carbohydrates	Molish,s Test	-	-	-
	Benedict's Test	+	+	+
Flavonoids	Lead Acetate Test	-	+	+
Phenols	Libermann's Test	-	-	-
Proteins	Ninhydrin Test	+	-	-
Saponins	Foam Test	+	-	+
Sterols	Libermann-Buchard Test	-	+	-
Tannins	Braymer's Test	+	-	-
Quinones	Sulphuric Test	-	-	-

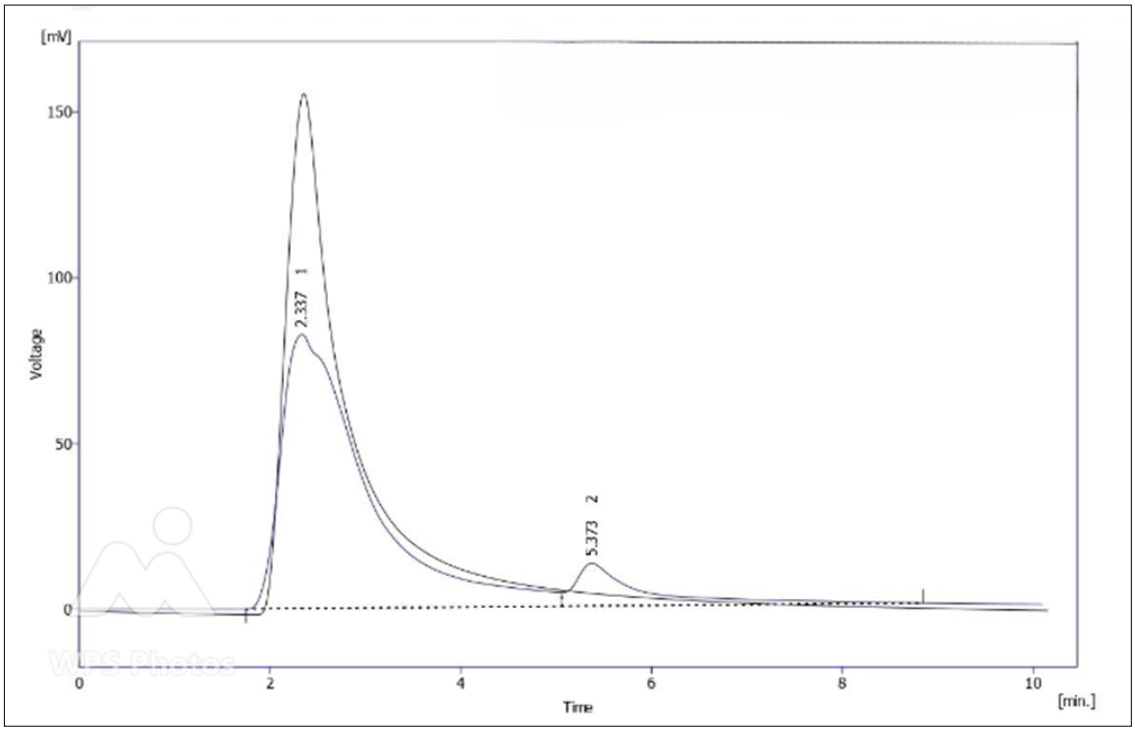

Fig. 2. HPLC analysis water overlay

the methanolic extract (0.245 ± 0.078 mg/mL) and the aqueous extract (0.142± 0.096 mg/mL). Water exhibited the lowest alkaloid content among the tested solvents.

Total flavonoids

Flavonoids represent a diverse group of polyphenolic compounds known for their potent radical-scavenging activity against oxidizing molecules, including singlet oxygen and free radicals implicated in various diseases (18). Consequently, the presence of flavonoids enhances the plant extracts' ability to neutralize free radicals (19).

The results in Table 2, the total flavonoid contents of the different *Ipomoea alba* leaf extracts was ranked as follows: the chloroform extract exhibited the highest flavonoid content (24.3 ± 4.80 mg/mL), followed by the aqueous extract (0.680 ± 0.13 mg/mL) and the methanolic extract (0.245 ± 0.078 mg/mL). The higher flavonoid content in the chloroform extract can be attributed to the solvents' moderate polarity, which enhances the extraction of semi-polar compounds like flavonoids. Conversely, more polar solvents such as water and methanol were less effective in extracting flavonoids, leading to lower yields in these extracts. The relative solubility of flavonoids in chloroform may therefore lead to a higher yield in this extract, while the aqueous and methanolic solvents may be less efficient in extracting flavonoids.

Total proteins

The total protein content in the fresh plant sample was estimated using the Lowry method. As shown in Table 2, the aqueous extract demonstrated the highest protein content (3.21 mg/mL), followed by the methanolic extract (0.357 mg/mL) and the chloroform extract (0.171 mg/mL). The higher protein yield in the aqueous extract is likely due to the water solubility of most proteins, facilitating more efficient extraction compared to organic solvents like methanol and chloroform.

Antioxidant assay

The antioxidant assay of *Ipomea alba* was estimated using FRAP, DPPH and ABTS assays for methanolic, aqueous and chloroform extracts (Table 3).

In the FRAP assay, the methanolic extract exhibited the highest antioxidant activity (0.564±0.043 nm), followed by the aqueous extract (0.552±0.028 nm) and the chloroform extract (0.427±0.015 nm) extract. However, the industrially available gallic acid standard demonstrated superior antioxidant activity (0.605±0.081nm) compared to the plant samples.

The DPPH assay showed that the standard gallic acid (35.84±13.57 %) had the highest antioxidant activity. Among the leaf extracts, the methanolic extract exhibited the highest activity (22.38±10.38%), followed by the chloroform extract (18.22±2.844 %) and the aqueous extract (15.32±3.95 %).

Table 2. Total Alkaloid, Flavonoid and Protein Content in Aqueous, Methanol and Chloroform extracts of *Ipomea alba*

Solvent	Water (mg/mL)	Methanol (mg/mL)	Chloroform (mg/mL)
Total alkaloids	0.142 ±0.096	0.245±0.078	9.234±0.685
Total Flavonids	0.680±0.134	0.484±0.094	24.3±4.80
Total Protiens	3.450±0.178	0.383±0.020	0.183±0.009

Table 3. Antioxidant activity of water, methanol and chloroform extracts of *Ipomea alba*

Solvent	Water (mg/mL)	Methanol (mg/mL)	Chloroform (mg/mL)
ABTS(% inhibition)	59.42±21.53	74.76±18.477	27.18±8.420
FRAP (Absorbance in nm)	0.552±0.028	0.564±0.043	0.427±0.015
DPPH(% inhibition)	15.32±3.95	22.38±10.38	18.22±2.844

Methanolic extract of leaves (22.38±10.38 %) showed higher values then followed by chloroform (18.22± 2.844 %) and aqueous (15.32±3.95 %) extract.

Similarly, in the ABTS assay, the methanolic extract showed the higher antioxidant activity (74.76 ± 18.47 %), followed by the aqueous extract (59.42 ± 21.53 %) and the chloroform extract (27.18 ± 8.42 %). Statistical analysis of different antioxidant assays revealed significant differences between the methanol, aqueous and chloroform extracts.

Anti- inflammatory activity

Proteinases play a crucial role in arthritic conditions, with neutrophils being a rich source of serine proteinases stored in lysosomal granules. Previous studies have established that leukocyte proteinases contribute significantly to tissue damage during inflammatory processes and proteinase inhibitors provide substantial protection in such cases (20).

In the current study, various plant extracts demonstrated notable anti-protease activity at different concentrations, as presented in Table 4. Comparative analysis revealed that the methanolic extract exhibited the highest inhibition at lower concentrations (20-60 µg/ml), followed by the aqueous extract. The chloroform extract consistently demonstrated the least inhibition (Fig. 3).

Conclusion

The study on *Ipomea alba* confirmed the presence of carbohydrates, proteins, alkaloids, flavonoids and saponins, with the methanolic extracts showing strong antioxidant activity. While this study highlights the significant antioxidant and anti-inflammatory properties of *Ipomea alba*, further investigating into its cytotoxicity is essential to gain a comprehensive understanding of *Ipomea albas*' full therapeutic potential.

Table 4. Results of the protein inhibitory assay evaluating the anti-inflammatory activity of water, methanol and chloroform extracts of Ipomea alba at varying concentrations (µg/mL)

Concentration of sample (µg/mL)	Percentage inhibition			
	Standard Trypsin	Methanol Extract	Aqueous Extract	Chloroform Extract
20	11.38	16.5	11.71	6.4
40	18.76	17	14.04	10.85
60	24.38	18	15.71	14.95
80	32.14	19	21.61	21
100	39.95	22.85	24.23	24.33

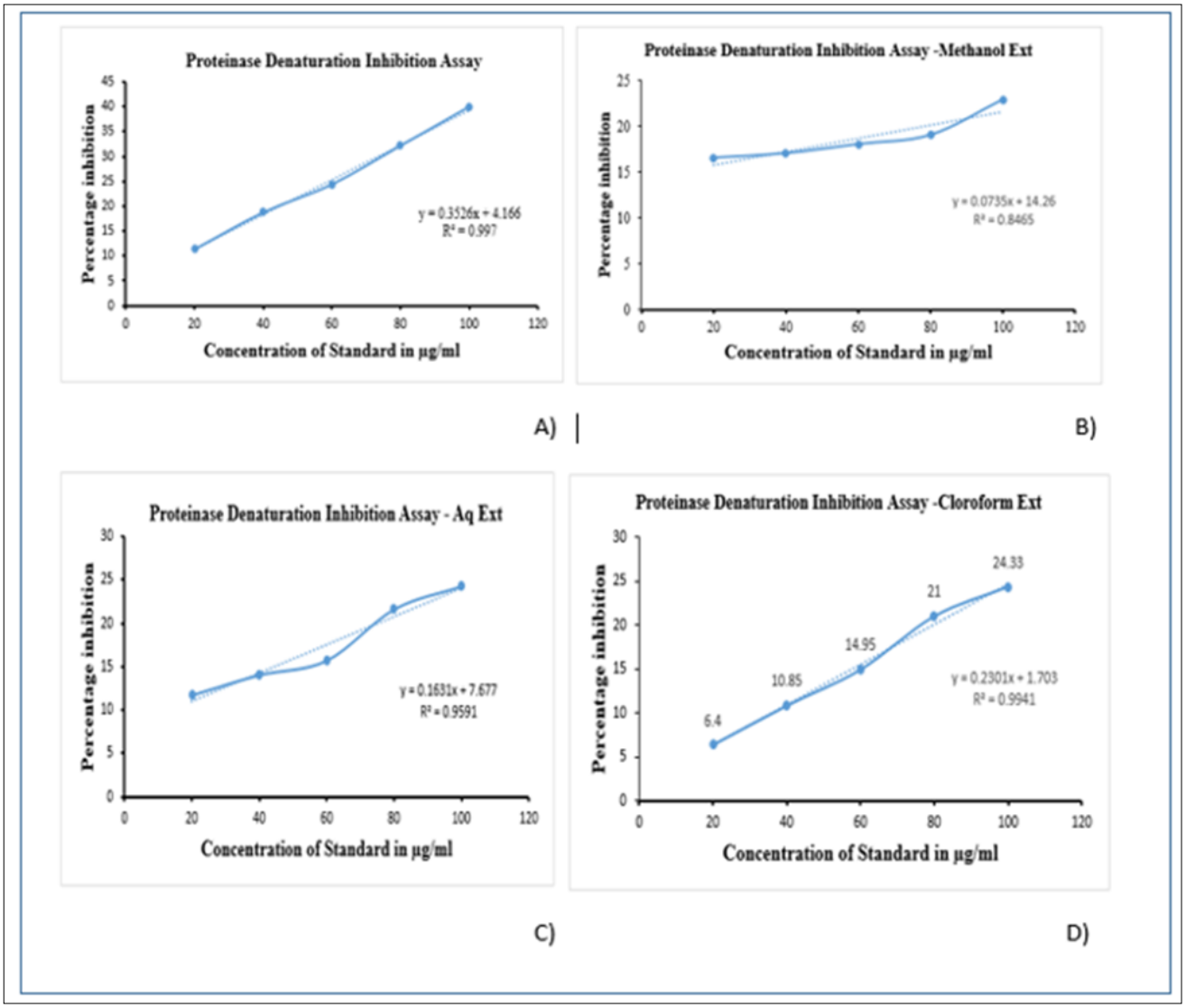

Fig.3. Protienase inhibitory assay of (A) standard, (B) aqueous extract, (C) methanol extract, (D) chloroform extract

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Authors' contributions

HKR carried out the phytochemical analysis, antioxidant and anti-inflammatory assays, performed statistical analysis and drafted the manuscript. JX conceived and participated in the design of the study. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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Generative AI tools were utilized to enhance language and readability in the preparation of this manuscript. All scientific content, including the research design, analysis and conclusions, are the sole work of the authors.

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