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EXPLORING THE ANTIOXIDANT, ANTI-INFLAMMATORY, AND PHYTOCHEMICAL PROPERTIES OF *MOLLUGO CERVIANA*.

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ABSTRACT

Plants are considered to be the most important resource as they are loaded with biologically active constituents. The present research work was carried out to investigate the phytochemical, antioxidant, and anti-inflammatory potential of the Plant *Mollugo cerviana*, a common weed in South India. Phytochemical screening of the Plant extract reveals the presence of bioactive constituents such as tannins, steroids, flavonoids, alkaloids, and phenolic compounds that has good pharmacological activity. The antioxidant activity was of the plant extract was evaluated using the DPPH assay, that demonstrates a significant ability to scavenge free radicals, indicating strong antioxidant potential. In addition, the anti-inflammatory effect was assessed using the albumin denaturation assay, where the plant extract effectively inhibited protein denaturation, which is considered to be a key mechanism in inflammation. These findings suggest that *Mollugo cerviana* has promising antioxidant and anti-inflammatory properties, supporting its use in treating oxidative stress and inflammation-related conditions. The results offer scientific validation for the traditional uses of *Mollugo cerviana* and highlight its potential in pharmaceutical and herbal medicine development.

INTRODUCTION

Mollugo cerviana, commonly known as carpetweed, was well known for its therapeutic uses and now it has attracted the scientific interest due to its rich array of bioactive compounds that offers potential therapeutic properties. This plant was traditionally used in folk medicine for treating Jaundice, inflammation, and many types of Infections. The plant also exhibits a rich biological profile loaded with the secondary metabolites. These metabolites have demonstrated significant biological

activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Alkaloids, one of the major groups of bioactive derivatives in *Mollugo cerviana*, are known for their wide range of pharmacological effects including antimicrobial and anticancer effects. Alkaloids from plants like *Berberis vulgaris* and *Camellia sinensis* have shown potential ability in treating infections and cancer (Zhao *et al.*,, 2020; Li *et al.*,, 2020). The high amount of Alkaloid content in plant extracts suggests that it could be a promising source for the development of alkaloid-based therapies, particularly in combating Cancer.

Flavonoids contribute to its antioxidant and anti-inflammatory properties. Flavonoids are known for their ability to scavenge free radicals and mitigate oxidative stress, which is linked to chronic diseases like cancer, cardiovascular disease, and neurodegenerative disorders (Kumar *et al.*,, 2021; Yadav *et al.*,, 2023). The presence of flavonoids in plants supports its potential in the prevention and management of oxidative stress-related conditions. Phenolic compounds also contribute significantly to the plant's antioxidant potential. These compounds neutralize free radicals, reducing oxidative damage associated with aging and chronic diseases (Jiang *et al.*,, 2022; Bhagat *et al.*,, 2021). Similarly, tannins are known for their antimicrobial and anti-inflammatory properties, traditionally used in treating infections and wounds (Khan *et al.*,, 2021; Kouadio *et al.*,, 2020). Steroids suggest the plant's potential as a natural alternative to synthetic steroids, offering anti-inflammatory and immunosuppressive effects, which are commonly used in conditions like rheumatoid arthritis and asthma (Ahmed *et al.*,, 2022; Rathore *et al.*,, 2021). The phytochemical properties of the plant are the key factors in determining the pharmacological and biological activity which are used in lead identification and drug designing. The present research work is designed to evaluate the phytochemical, antioxidant and anti-inflammatory activity of the plant *Mollugo Cerviana*

MATERIALS AND METHODS

1. Phytochemical Analysis

1.1. Quantification of Total Alkaloid Content

Dissolved 1 mg of *Mollugo cerviana* ethanolic extract in DMSO, added 1 ml of 2N HCl, filter, and transferred to a separating funnel. Added bromocresol green solution, phosphate buffer, and

Shaked with chloroform. Collected in a 10 ml flask, diluted with chloroform. Measured the absorbance at 470 nm using a UV/Visible spectrophotometer to calculate the alkaloid content as mg of standard reference (SR) plant extract.

1.2. Determination of Total Flavonoid Content

Mixed 1 ml of the sample with 1 ml of quercetin solution (50 mg/ml) in test tubes. Added 4 ml distilled water and 0.3 ml of 5% sodium nitrite solution. After 5 minutes, added 0.3 ml of 10% aluminium chloride, followed by 2 ml of 1M sodium hydroxide to the mixture. Diluted the mixture to 10 ml with distilled water and mixed to develop an orange-yellow colour. Measured the absorbance at 510 nm using a UV-visible spectrophotometer. quercetin is used as a standard to express flavonoids

1.3. Determination of Total Phenol Content

Mixed 1 ml of the sample with gallic acid standard (0.007 mg/ml to 1 mg/ml) in test tubes. Added 5 ml distilled water, 0.5 ml Folin-Ciocalteu reagent and the mixture was uniformly agitated. After 5 minutes, added 1.5 ml of 20% sodium carbonate, diluted to 10 ml, and incubated for 2 hours. Measured absorbance at 750 nm and expressed total phenols as mg GAE per 100 g dry mass.

1.4. Estimation of Total Tannin Content

Added 0.1 ml of sample extract to a 10 ml volumetric flask with 7.5 ml distilled water, 0.5 ml Folin-Ciocalteu reagent, and 1 ml of 35% sodium carbonate solution. Diluted to 10 ml, shaked well, and incubated for 30 minutes at room temperature. The absorbance was measured at 700 nm using a UV/Visible spectrophotometer

1.5. Quantification of Steroids

Added 1 mL of test sample to 2 mL of 4N sulfuric acid, 2 mL of 0.5% iron (III) chloride, and 0.5 mL of potassium hexacyanoferrate (III) solution. The solution was heated at 72°C in a water bath for 30 minutes, then diluted to 10 mL with distilled water. The absorbance was measured at 780 nm.

2. **ANTI-INFLAMMATORY ACTIVITY**

The method for evaluating protein denaturation inhibition, (slightly modified from Mizushima and Kobayashi and Sakat et al) involves adding 500 µL of 1% BSA solution to various concentrations (500, 250, 100, 50, and 10 µg/mL) of the test sample. This mixture is incubated at room temperature for 10 minutes and then heated at 51°C for 20 minutes. After cooling to room temperature, the

absorbance is measured at 660 nm. Acetyl salicylic acid is used as a positive control.

The percent inhibition is calculated using the formula:

$$\% \text{ Inhibition} = 100 - \left(\frac{A1 - A2}{A0}\right) \times 100$$

Where:

- A1 = Absorbance of the control
- A2 = Absorbance of the test sample
- A0 = Absorbance of the positive control

A dose-response curve is plotted to determine the IC50 value, the concentration required for 50% of maximum scavenging capacity. All experiments are conducted in triplicate and results averaged.

3. DPPH RADICAL SCAVENGING ACTIVITY

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed to evaluate the antioxidant potential of the plant extract.

Prepared a 0.1 mM DPPH solution in methanol. Added 100 µl of this solution to 300 µl of the sample at varying concentrations (500, 250, 100, 50, and 10 µg/ml) and the mixture was shaked vigorously and the absorbance was measured at 517 nm using a UV-VIS spectrophotometer.

The percentage of DPPH inhibition was calculated by

$$\%\text{Inhibition} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100$$

RESULTS

PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of *Mollugo cerviana* revealed the presence of significant bioactive compounds, including alkaloids, flavonoids, phenols, tannins, and steroids. These findings provided an insight into the potential therapeutic applications of the plant in future studies Quantitative estimation was carried out using appropriate standards and the results were tabulated.

Standard calibration curves were formulated for the quantitative estimation of various phytochemicals using the mean value of the active molecules with

appropriate standards. The total alkaloid content was determined using atropine as the standard, which showed a linear regression equation of y = 0.5506x + 0.1505 with a high correlation coefficient (R^2 = 0.9872), indicating a strong linear relationship. The total flavonoid content was estimated using quercetin as the standard, yielding a regression equation of y = 0.6428x + 0.3488 and an R^2 value of 0.8038, reflecting moderate linearity. Phenolic content estimation using gallic acid showed excellent linearity with a regression equation of y = 0.5145x + 0.0752 and R^2 = 0.9748. Tannin content was calculated using tannic acid as the standard, giving a regression equation of y = 0.528x + 0.6027 with a good correlation coefficient (R^2 = 0.8734). Additionally, steroid content estimation using cortisone acetate as the standard exhibited a strong linear relationship with the equation y = 0.5082x + 0.2231 and R^2 = 0.9752. These standard curves confirm the reliability and accuracy of the respective assays for the quantification of phytochemical constituents in the tested samples. The standard graphs were represented in **Fig 1**.

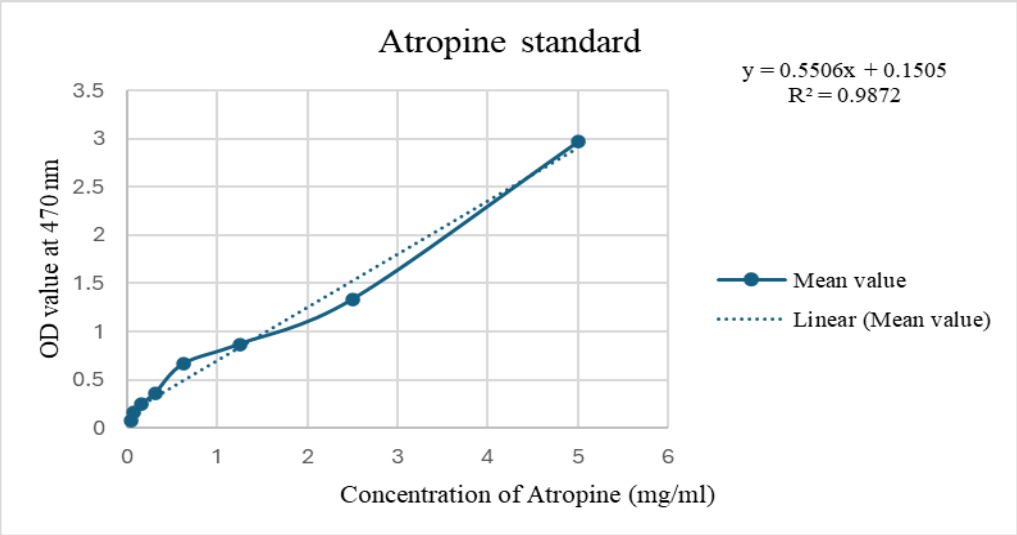

Fig 1A. Calibration Curve of Atropine used in Alkaloid Estimation

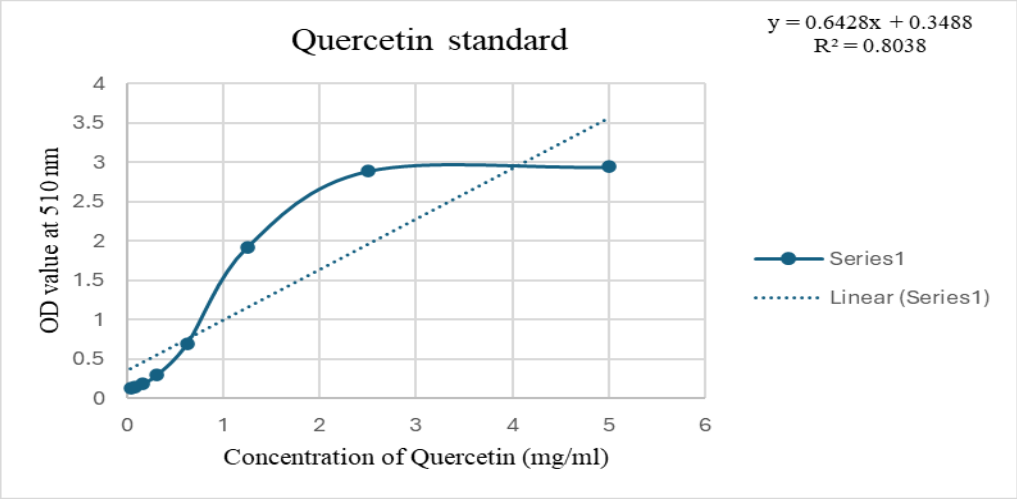

Fig 1B. Calibration Curve of Quercetin used in Flavonoid Estimation

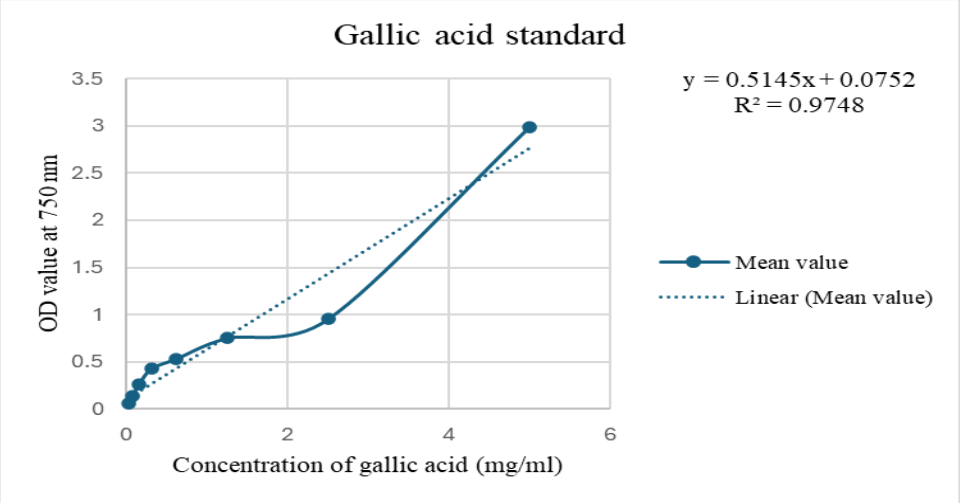

Fig 1C. Calibration Curve of Total Phenol used in Gallic Acid Estimation

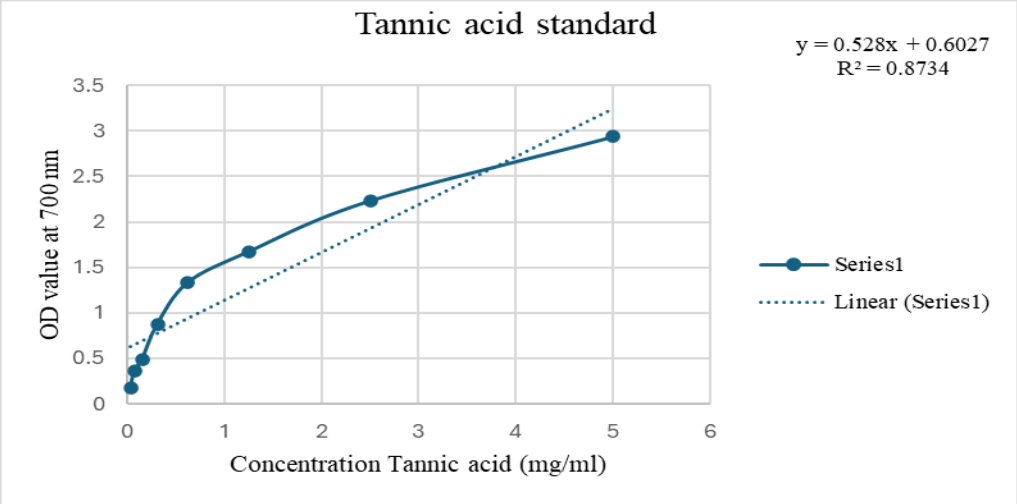

Fig 1 D: Calibration Curve of Tannic Acid used in Tannic Acid Acid Estimation

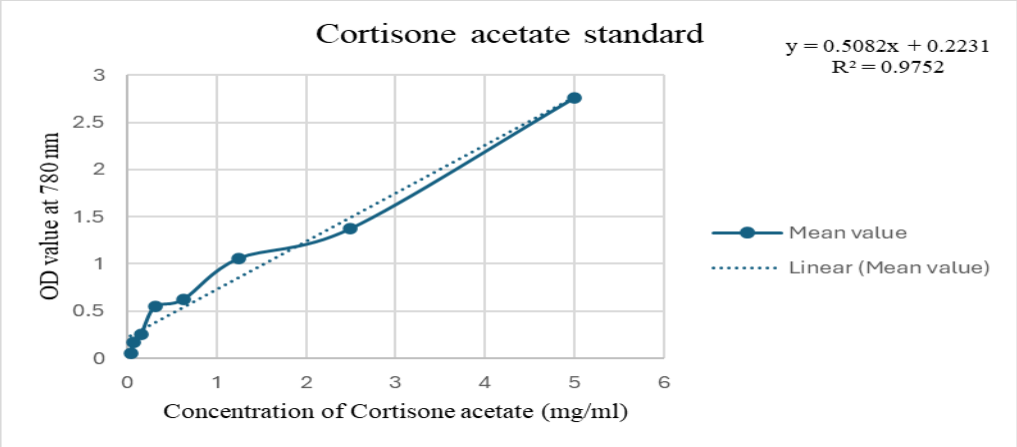

Fig 1 E: Calibration Curve of Cortisone used in Steroid Estimation

All the experiments were performed in triplicates, and the mean values of the total quantities of secondary metabolites were calculated and presented in Table 1. The values clearly indicate there is an enormous accumulation of secondary metabolites in the Plant extract.

Ethanolic Extract of *Mollugo Cerviana*	**Alkaloids**	**Flavonoids**	**Phenolic content**	**Tannins**	**Steroid Content**
	0.565	0.9311	2.3706	2.1804	4.6639

Table 1: Mean values representing the secondary metabolite production in the ethanolic plant extract of *Mollugo Cerviana*

ANTI-INFLAMMATORY ACTIVITY

The heat induced denaturation of albumin was measured at 660 nm and the results revealed that the plant extract has a high impact on denaturation process. At the highest tested concentration of 500 µg/ml, the absorbance dropped to 1.567, compared to the control's mean absorbance of 1.828. These results proves that the

plant extract had a noticeable inhibitory effect on protein denaturation.

The percentage inhibition of albumin denaturation indicates a dose-dependent response. The inhibition at lower concentrations progressively decreased, which is consistent with the expected behaviour of compounds in bioassays where higher concentrations are generally more effective. In comparison, the standard, acetyl salicylic acid, demonstrated a much stronger inhibitory effect, despite its moderate effect, the extract's ability to inhibit protein denaturation indicates its potential as an anti-inflammatory agent, though its potency is lower than that of the standard. (Fig 2)

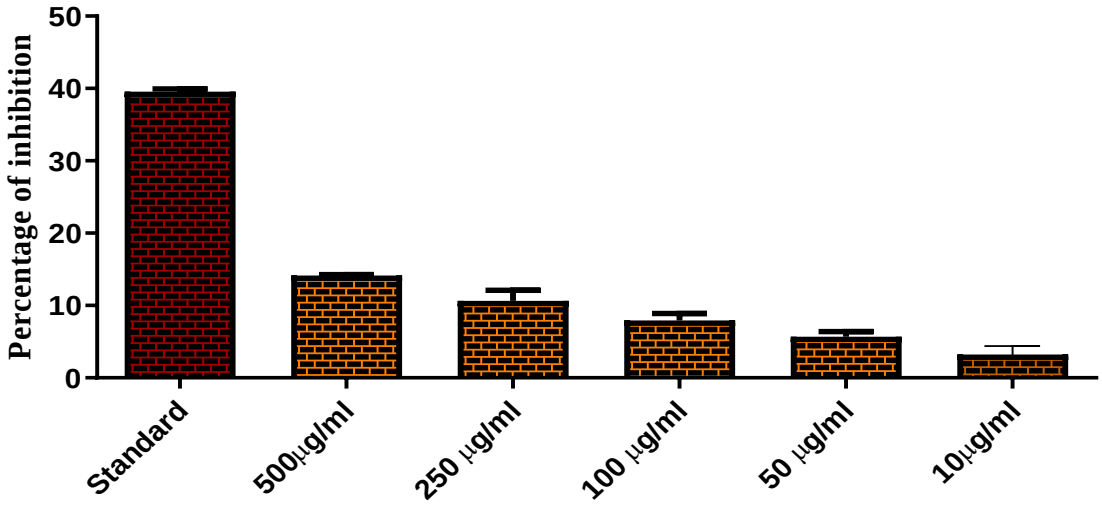

Fig 2: Percentage of Albumin Denaturation in Antioxidant Assay

The IC50 value of the Plant extract was found to be 121.7 µg/ml indicates that the plant extract requires a relatively high concentration to achieve significant inhibition

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

The antioxidant activity of the Plant extract was well documented by the antioxidant activity with Ascorbic acid as a Standard which has an inhibition percentage of around 72%, serving as a strong reference for comparison., The IC50 of the Plant extract was found to be 97.09 µg/ml, indicating its relatively

potent activity in scavenging free radicals. The percentage of Antioxidant activity is represented in **Fig 3.**

The 95% confidence interval for the IC50 ranged from 79.29 to 118.9 µg/ml, providing a statistical reliability to the result. The DPPH radical scavenging assay remains an essential tool in the analysis of antioxidant activities of natural and synthetic compounds. The ability to rapidly assess the antioxidant potential of new substances is invaluable in fields ranging from pharmacology to food sciences. The high degree of reproducibility, along with the straightforward analysis of results, ensures that the DPPH assay will continue to be a cornerstone of antioxidant research.

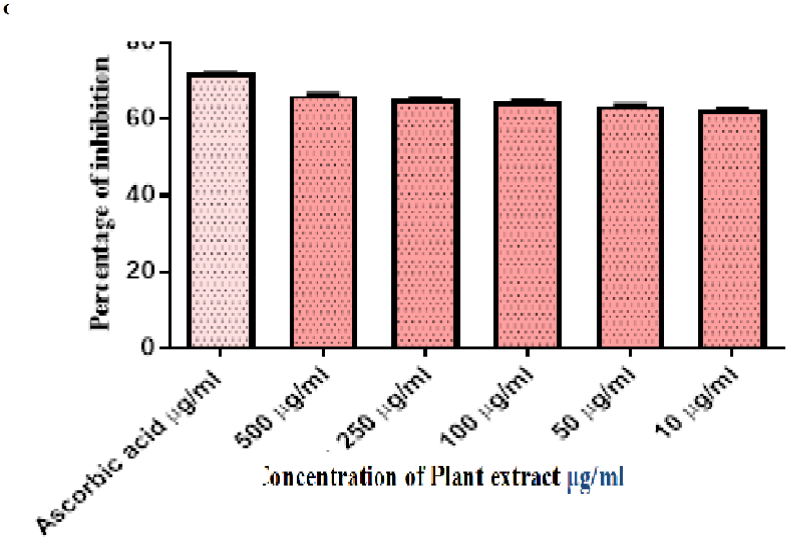

Fig 3: Percentage of inhibition in DPPH ASSAY

DISCUSSION

Mollugo cerviana is a medicinal plant that contains a variety of bioactive compounds, including alkaloids, flavonoids, phenols, tannins, and steroids, each contributing to its potential therapeutic properties. The alkaloid content (0.565 mg/g) suggests that the plant may have antimicrobial, anti-inflammatory, and anticancer effects, similar to other plants like *Berberis vulgaris* and *Camellia sinensis* (Zhao *et al.*,, 2020; Li *et al.*,, 2020). Alkaloids are well-known for their ability to target various diseases, including infections and cancers, and the presence of these compounds in *Mollugo cerviana* positions it as a potential source for the development of alkaloid-based drugs. The flavonoid

content (0.931 mg/ml) in *Mollugo cerviana* supports its potential as an antioxidant and anti-inflammatory agent, which are crucial in managing diseases related to oxidative stress, such as cancer, cardiovascular diseases, and neurodegenerative disorders (Kumar *et al.*,, 2021; Yadav *et al.*,, 2023). Flavonoids possess free radical scavenging properties and can mitigate oxidative damage, thus playing a protective role in preventing chronic diseases.

Additionally, the total phenolic content (2.3706 mg/g) of *Mollugo cerviana* underlines its significant antioxidant potential. Phenolic compounds, particularly gallic acid, are known for their ability to neutralize free radicals, reducing oxidative stress, which is a central mechanism in aging, cancer, and cardiovascular diseases (Jiang *et al.*,, 2022; Bhagat *et al.*,, 2021). This high phenolic content positions *Mollugo cerviana* as a strong candidate for further research aimed at developing natural antioxidants. Tannins (2.1805 mg/g) also contribute to the plant's therapeutic properties, offering both antimicrobial and anti-inflammatory effects. Traditionally, tannins have been used in the treatment of wounds, infections, and inflammation, supporting their relevance in modern herbal medicine (Khan *et al.*, 2021; Kouadio et al., 2020). Steroids (4.663 mg/g) in the plant are known to exhibit potent anti-inflammatory and immunosuppressive properties, suggesting that *Mollugo cerviana* could serve as an alternative or complementary treatment to synthetic steroids for conditions like rheumatoid arthritis, asthma, and autoimmune diseases (Ahmed *et al.*,, 2022; Rathore *et al.*,, 2021).

When compared to other medicinal plants, the bioactive compounds in *Mollugo cerviana* align with those found in well-known therapeutic plants such as *Berberis vulgaris* and *Azadirachta indica*, which are rich in flavonoids and phenols and have demonstrated antioxidant and anti-inflammatory properties (Díaz *et al.*,, 2020; Patil *et al.*,, 2021). This similarity further supports the plant's broad therapeutic potential, particularly for managing oxidative stress, inflammation, and microbial infections. The combination of alkaloids, flavonoids, phenols, tannins, and steroids creates a synergistic effect, enhancing the plant's ability to treat a variety of diseases.

The study also examined the anti-inflammatory potential of *Mollugo cerviana* by testing its ability to inhibit albumin denaturation, a process that contributes to inflammation. At a

concentration of 500 µg/ml, the extract demonstrated a 14.13% inhibition of albumin denaturation, which was lower than the standard acetyl salicylic acid (39.55%) but still indicative of the plant's potential anti-inflammatory activity (Mizushima & Kobayashi, 1968; Sakat *et al.*,, 1994). The goodness of fit for the dose-response curve on anti-inflammatory activity was assessed, with an R-squared value of 0.9311, indicating a strong correlation between the concentration of the extract and the inhibition of albumin denaturation. The plant extract demonstrated significant anti-inflammatory activity through the inhibition of albumin denaturation, with the highest inhibition observed at 500 µg/ml. While the extract's IC50 value was higher than that of the standard (acetyl salicylic acid), the results suggest that it possesses moderate anti-inflammatory properties. Further studies, including optimization of concentration and formulation, are needed to enhance its potency and explore its potential for clinical applications.

Furthermore, the antioxidant potential of *Mollugo cerviana* was evaluated using the DPPH radical scavenging assay, which showed significant activity with an IC50 value of 97.09 µg/ml. While less potent than ascorbic acid, this finding suggests that the plant extract possesses valuable antioxidant properties (Chanda & Dave, 2009; Karami *et al.*,, 2016). The steep dose-response curve (HillSlope: -1.573) and the 95% confidence interval for the IC50 value (79.29 to 118.9 µg/ml) confirm the reliability of the antioxidant effect (Petersen *et al.*,, 2012).

CONCLUSION

In conclusion, *Mollugo cerviana* exhibits considerable therapeutic potential due to its diverse bioactive compounds, including alkaloids, flavonoids, phenols, tannins, and steroids. These compounds contribute to its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, positioning the plant as a valuable resource for managing diseases related to oxidative stress, inflammation, and infections. The alkaloids and flavonoids in *Mollugo cerviana* suggest its potential for treating infections and chronic diseases like cancer and cardiovascular disorders. The plant's steroid content offers a natural alternative to synthetic steroids for inflammation and autoimmune conditions. Despite promising results, further research is required to isolate the specific compounds responsible for these effects and understand their mechanisms of action. In vivo studies are essential to assess the safety and clinical

efficacy of *Mollugo cerviana*. Overall, it holds promise for developing plant-based therapies to complement or replace synthetic treatments in various medical fields.

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