International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

PHYTOCHEMICAL AND ANTI INFLAMMATORY SCREENING OF EXTRACT OF *RHYNCHOSIA BEDDOMEI*

Harish Kumar Dubey, Harsha Matoli, Dr. Kapil Purohit
Gurukul Institute of Pharmaceutical Science and Research, Gwalior (M.P.)

***Correspondence Info:**
Harish Kumar Dubey
Gurukul Institute of Pharmaceutical Science and Research, Gwalior (M.P.)
Email: dubey_harish@yahoo.co.in

***Article History:**

Received: 18/07/2023
Revised: 10/08/2023
Accepted: 26/08/2023

ABSTRACT

The present study aimed to evaluate the phytochemical composition and anti-inflammatory activity of the root extract of *Rhynchosia beddomei*. The roots of *R. beddomei* were subjected to extraction using a suitable solvent, and the resulting extract was assessed for its potential anti-inflammatory properties. Preliminary phytochemical screening was carried out to identify the presence of various secondary metabolites in the extract. The phytochemical analysis revealed the presence of a diverse range of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids. These bioactive compounds are known for their pharmacological significance and have been associated with various therapeutic properties. Furthermore, the anti-inflammatory activity of the root extract was evaluated using in vitro and/or in vivo experimental models. The extract exhibited significant anti-inflammatory effects, as evidenced by the suppression of inflammatory markers and mediators. This anti-inflammatory potential could be attributed to the presence of bioactive phytochemicals in the extract. In conclusion, the root extract of *R. beddomei* demonstrated promising anti-inflammatory activity, likely due to its rich phytochemical composition. These findings highlight the potential of *R. beddomei* as a source of natural anti-inflammatory agents. Further investigations are warranted to isolate and identify specific bioactive compounds responsible for the observed anti-inflammatory effects and to elucidate the underlying molecular mechanisms.

Keywords: *Rhynchosia beddomei*, anti-inflammatory activity, phytochemical screening, secondary metabolites, natural products.

INTRODUCTION

Inflammation is a ubiquitous type of defence that is widely described as a nonspecific reaction to tissue malfunction and is used by both the innate and adaptive immune systems to battle pathogenic intruders. The inability to resist self-harm is a distinguishing trait of inflammatory reactions in comparison to other aspects of antiparasite defences. Importantly, collateral damage from inflammation is not the same as immunopathology, which is a particular immune-mediated attack on target tissue that the immune system no longer recognises as self. Autoimmune pathology is caused by dysregulation of adaptive immune components such as antibodies and cell-mediated processes, and it is influenced by both hereditary and environmental factors. Although inflammation-induced collateral damage can certainly contribute to immunopathology (e.g., rheumatoid arthritis, multiple sclerosis, diabetes), the damage induced by inflammation represents a fundamental biological trade-off between

damage control and self-maintenance and is not activated by the presence of self-antigens (Medzhitov, 2008; Ahmed, 2011; Trowbridge *et al*., 1997).

Inflammation has a complex mechanism of action. The identification of infection or injury is the initial step in the inflammatory cascade. This is often accomplished through the identification of pathogen-associated molecular patterns (PAMPs), which are specifically focused towards generic motifs of pathogen-expressed molecules that are required for pathogen survival. Alarmins, also known as damage-associated molecular patterns (DAMPs), are endogenous molecules that are recognised by the innate immune system and signal injury or necrosis. The detection of these signals has the advantage of reducing unintentional targeting of host cells and tissues. Unlike adaptive immunity, the innate immune system is incapable of distinguishing between pathogen strains and determining if such strains are virulent (Netea *et al*., 2017; Cronstein, 1992).

NSAIDs are the most commonly used medications in the world, used to treat acute and chronic pain caused by an inflammatory process. NSAIDs are a class of medications whose actions are all connected to the suppression of COX action in the formation of prostaglandins and thromboxanes.NSAIDs work primarily by inhibiting COX, both central and peripheral, interfering with the conversion of arachidonic acid to prostaglandins E2, prostacyclins, and thromboxanes. COX-1 and COX-2 enzymes, which function in separate areas, are involved in the action of NSAIDs. COX-1 is found in most cells, including foetal and amniotic fluid, and is involved in physiological functions such as regulatory and protective effects. In contrast, inflammation and proinflammatory cytokines activate COX-2. Despite initial effectiveness, severe cardiovascular and renal consequences, as well as gastrointestinal problems in high doses, have been recorded quickly following the debut of selective COX-2 inhibitors (Vane and Botting, 1998; Moses and, 2002; Bertone Vonkeman *et al*., 2010)

Unlike current allopathic pharmaceuticals, which have singular active ingredients that target a single pathway, herbal remedies function through a symphonic approach. A plant includes a plethora of distinct chemicals that work together to target certain aspects of the intricate biological system. For millennia, medicinal plants have been a source of a wide range of biologically active substances, which have been widely used as raw material or as pure compounds to treat a variety of illness conditions. Because of the toxicity and side effects of allopathic treatments, the usage of herbal remedies is becoming more widespread. Medicinal plants are crucial in the production of powerful medicinal medicines. Over 1.5 million traditional medicine practitioners use medicinal plants in preventative, promotional, and curative applications13. India, having the world's largest repository of medicinal plants, may continue to play a significant role in the production of raw materials, either directly for crude medications or as bioactive chemicals in the manufacture of pharmaceuticals and cosmetics, among other things. (Arif *et al*., 2009; Dasilva1999; Tiwari, 2008).

Rhynchosia beddomei Baker (Fabaceae) is a viscous hairy under shrub found in South India's Deccan and Carnatic regions.

Based on folklore medications, the Adivasi tribes employed leaves to cure rheumatic aches, wound healing, boils, obesity, diabetes, and as an abortifacient. Flavonoids, particularly C-glycosylflavones, are abundant in Rhynchosia species (Rammohan *et al*., 2020). Recognizing this plant immense importance this study deals with anti - inflammatory activity on roots extract *of Rynchosia beddomei*.

Inflammation is a ubiquitous type of defence that is widely described as a nonspecific reaction to tissue malfunction and is used by both the innate and adaptive immune systems to battle pathogenic intruders. The inability to resist self-harm is a distinguishing trait of inflammatory reactions in comparison to other aspects of antiparasite defences. Importantly, collateral damage from inflammation is not the same as immunopathology, which is a particular immune-mediated attack on target tissue that the immune system no longer recognises as self. Autoimmune pathology is caused by dysregulation of adaptive immune components such as antibodies and cell-mediated processes, and it is influenced by both hereditary and environmental factors. Although inflammation-induced collateral damage can certainly contribute to immunopathology (e.g., rheumatoid arthritis, multiple sclerosis, diabetes), the damage induced by inflammation represents a fundamental biological trade-off between damage control and self-maintenance and is not activated by the presence of self-antigens (Medzhitov, 2008; Ahmed, 2011; Trowbridge *et al*., 1997).

Inflammation has a complex mechanism of action. The identification of infection or injury is the initial step in the inflammatory cascade. This is often accomplished through the identification of pathogen-associated molecular patterns (PAMPs), which are specifically focused towards generic motifs of pathogen-expressed molecules that are required for pathogen survival. Alarmins, also known as damage-associated molecular patterns (DAMPs), are endogenous molecules that are recognised by the innate immune system and signal injury or necrosis. The detection of these signals has the advantage of reducing unintentional targeting of host cells and tissues. Unlike adaptive immunity, the innate immune system is incapable of distinguishing between pathogen strains and determining if such strains are virulent (Netea *et al*., 2017; Cronstein, 1992).

NSAIDs are the most commonly used medications in the world, used to treat acute and chronic pain caused by an inflammatory process. NSAIDs are a class of medications whose actions are all connected to the suppression of COX action in the formation of prostaglandins and thromboxanes.NSAIDs work primarily by inhibiting COX, both central and peripheral, interfering with the conversion of arachidonic acid to prostaglandins E2, prostacyclins, and thromboxanes. COX-1 and COX-2 enzymes, which function in separate areas, are involved in the action of NSAIDs. COX-1 is found in most cells, including foetal and amniotic fluid, and is involved in physiological functions such as regulatory and protective effects. In contrast, inflammation and proinflammatory cytokines activate COX-2. Despite initial effectiveness, severe cardiovascular and renal consequences, as well as gastrointestinal problems in high doses, have been recorded quickly following

the debut of selective COX-2 inhibitors (Vane and Botting, 1998; Moses and, 2002; Bertone Vonkeman *et al*., 2010)

Unlike current allopathic pharmaceuticals, which have singular active ingredients that target a single pathway, herbal remedies function through a symphonic approach. A plant includes a plethora of distinct chemicals that work together to target certain aspects of the intricate biological system. For millennia, medicinal plants have been a source of a wide range of biologically active substances, which have been widely used as raw material or as pure compounds to treat a variety of illness conditions. Because of the toxicity and side effects of allopathic treatments, the usage of herbal remedies is becoming more widespread. Medicinal plants are crucial in the production of powerful medicinal medicines. Over 1.5 million traditional medicine practitioners use medicinal plants in preventative, promotional, and curative applications13. India, having the world's largest repository of medicinal plants, may continue to play a significant role in the production of raw materials, either directly for crude medications or as bioactive chemicals in the manufacture of pharmaceuticals and cosmetics, among other things. (Arif *et al*., 2009; Dasilva1999; Tiwari, 2008).

Rhynchosia beddomei Baker (Fabaceae) is a viscous hairy under shrub found in South India's Deccan and Carnatic regions. Based on folklore medications, the Adivasi tribes employed leaves to cure rheumatic aches, wound healing, boils, obesity, diabetes, and as an abortifacient. Flavonoids, particularly C-glycosylflavones, are abundant in Rhynchosia species (Rammohan *et al*., 2020).

Recognizing this plant immense importance this study deals with anti -inflammatory activity on roots extract *of Rynchosia beddomei*.

MATERIALS & METHODS

Collection of plant material

Roots of *Rhynchosia beddomei* were cleaned by tab water and a portion was dried at room temperature. The dried samples were ground and passed through a sieve (20 meshes). The powdered drugs were kept in sealed containers and protected from light until used. Another portion of sample was used for maceration (Singh, 2008).

Defatting & extraction of plant material

50.0 gram of powdered Roots of *Rhynchosia beddomei* were coarsely powdered and subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place. Defatted dried powdered of *Rhynchosia beddomei* has been extracted with hydroalcoholic (Ethanol: Water: 80:20v/v) as a solvent using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Phytochemical screening

The phytochemical analysis was performed as per the standard protocol.

Estimation of total phenolic content

The total phenolic content was estimated according to the Folin Ciocalteu method. The aliquots of the extract was taken in a test tube and made up to the volume of 10 ml with distilled water. The different concentration ranging from 5-25 micro gram per ml were also made. Then 1 ml of Folin-Ciocalteu reagent (1:1 with water) and 1ml of sodium carbonate solution were added to all standard & extact. After mixing, solution was

incubated at room temperature for 10 mins and the absorbance was recorded at 765 nm against the reagent blank. Using fallic acid as standard curve was prepared. Using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent equivalent in µg/mg of extract. Gallic acid is used as a standard compound and the total phenols were expressed as mg/100mg gallic acid equivalent using the standard curve equation: $y = 0.015x + 0.015$, $R^2 = 0.998$, Where y is absorbance at 765 nm and x is total phenolic content in the hydroalcoholic extract of *Rhynchosia beddomei*. The results were expressed as the number of equivalents of Gallic acid (mg/100mg of extract).

Total flavonoid content

Total flavonoid contents of all the extracts were determined and expressed as quercetin equivalent in µg/mg of extract. An aliquot (10ml) of extracts or standard solution of quercetin 5-25 µg was added with 1 ml of NaNO2, 1 ml of 2% $AlCl_3$. The mixture was incubated for 15 min at room temperature. The total volume was made up to 10ml by adding distilled water. The solution was mixed well and the absorbance was measured at 420 nm. Using the standard curve, the total flavonoid content was calculated.

Flavonoids content was calculated from the regression equation of the standard plot $y = 0.024x - 0.002$, $R^2 = 0.999$) and is expressed as quercetin equivalents (QE). Total flavonoids content was 0.612mg/100mg quercetin equivalent in hydroalcoholic extract of *Rhynchosia beddomei*. Flavonoids are the most common and widely distributed group of plant's phenolic compounds.

***In-vivo* anti-inflammatory activity**

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 ℃, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments.

Acute toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five rodents were administered 2000mg/kg of Hydroalcoholic extract of leaves of *Rhynchosia beddomei* orally and observed continuously for a period of 14 days, every hourly for 24 hours, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion etc [OECD guideline 2001]. The experimental dose of the extracts was selected as 100 and 200 mg/kg/p.o.

Experimental designs (Deng and Chow, 2010).

Group –1: Carrageenan control (0.1 ml of 1% w/v)

Group –2: Carrageenan (0.1 ml of 1% w/v) + Indomethacin

Group –3: Carrageenan control (0.1 ml of 1% w/v) + extract of *Rhynchosia beddomei* 100 mg/kg

Group –4: Carrageenan (0.1 ml of 1% w/v) + extract of *Rhynchosia beddomei* 200 mg/kg

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the

mean (SEM). Data were analyzed by one-way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The presence of alkaloids in the hydroalcoholic extract of *Rhynchosia beddomei* as indicated by the positive Hager's test suggests the potential for alkaloid-mediated biological activities. Alkaloids are known for their diverse pharmacological properties, including analgesic and anti-inflammatory effects. The positive Legal's test for glycosides indicates the presence of these compounds in the extract. Glycosides are often associated with cardioprotective and antioxidant activities. Further characterization of the specific types of glycosides present could provide insights into the potential health benefits of the extract.

The positive Fehling's test for carbohydrates suggests the presence of these energy-providing molecules in the extract. Carbohydrates are essential for cellular metabolism and may contribute to the overall nutritive value of the extract. The positive response to the lead acetate test confirms the presence of flavonoids in the hydroalcoholic extract. Flavonoids are well-known for their antioxidant and anti-inflammatory properties. Their presence could contribute to the observed anti-inflammatory activity of the extract. The absence of a positive response to the copper acetate test indicates the absence of diterpenes in the extract. Diterpenes are a class of compounds with various biological activities, including antimicrobial and anti-inflammatory effects. Their absence suggests that other phytoconstituents might be responsible for the observed activities. The positive Froth test result suggests the presence of saponins. Saponins have been linked to diverse biological effects, including anti-inflammatory, immune-modulating, and antimicrobial activities. The presence of saponins aligns with the potential anti-inflammatory activity of the extract.The positive Xanthoproteic test indicates the presence of proteins in the extract. Proteins play a crucial role in various physiological processes and could contribute to the overall nutritional value of the extract.

The positive result in the Ferric Chloride test suggests the presence of phenolic compounds. Phenols are well-known for their antioxidant properties and their potential to scavenge free radicals, which could contribute to the extract's overall biological activity.

The total phenolic content of the hydroalcoholic extract of *Rhynchosia beddomei* was found to be 0.412 mg/100 mg of dried extract. Phenolic compounds are well-known for their antioxidant properties, and their presence in plant extracts has been associated with various health benefits. The relatively low phenolic content observed in this extract suggests that while phenolics are present, they might not be the predominant group of compounds contributing to the antioxidant activity. However, even a modest phenolic content can contribute to the overall biological activity of the extract.

The total flavonoid content of the hydroalcoholic extract of *Rhynchosia beddomei* was determined to be 0.612 mg/100 mg of dried extract. Flavonoids are potent antioxidants that are often responsible for the free radical scavenging and anti-inflammatory effects of plant extracts. The presence of flavonoids in the extract, as indicated by the

measured content, supports the potential health-promoting properties attributed to *Rhynchosia beddomei*.

The presence of phenolic and flavonoid compounds aligns with the positive outcomes of the preliminary phytochemical screening, which indicated the presence of phenols and flavonoids. These compounds are likely contributing to the observed antioxidant and potentially anti-inflammatory properties of the extract.

The study demonstrates the potential anti-inflammatory activity of the *Rhynchosia beddomei* extract against carrageenan-induced paw edema in rats. The extract exhibited dose-dependent effects, with the highest dose (200 mg/kg) showing the most significant suppression of edema, especially at the later time points. These findings support the traditional use of *Rhynchosia beddomei* as an anti-inflammatory agent and warrant further investigation to elucidate the underlying mechanisms responsible for its observed effects.

Table 1: Preliminary phytochemical screening of *Rhynchosia beddomei*

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Hager's Test	Present
2	Glycosides	Legal's Test	Present
3	Carbohydrates	Fehling's Test	Present
4	Flavonoids	Lead acetate	Present
5	Diterpenes	Copper acetate Test	Absent
6	Saponins	Froth Test	Present
7	Proteins	Xanthoproteic Test	Present
8	Phenols	Ferric Chloride Test	Present

Table 2: Estimation of total phenolic and flavonoids content of *Rhynchosia beddomei*

S. No	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.412	0.612

Table 3: Effect of extract of *Rhynchosia beddomei* on paw edema induced by carrageenan in rats by different timelines

Groups	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr
Group-I	0.1 ml of 1% (w/v)	3.8±0.05	4.2±0.06	4.5±0.03	4.7 ±0.05	5.1 ±0.05
Group-II	10 mg/kg	1.2 ±0.05	1.0 ±0.05	0.8±0.07	0.6±0.04*	1.05 ±0.06**
Group-II	100 mg/kg	2.5 ±0.06	2.6 ±0.05	2.7±0.12	2.8 ±0.15	2.8 ±0.05
Group-III	200 mg/mg	1.9 ±0.12	1.8 ±0.05	1.6 ±0.1	1.4 ±0.25*	1.2 ±0.05*

CONCLUSION

In conclusion, this study aimed to investigate the extraction, phytochemical screening, and anti-inflammatory activity of the root extract of *Rhynchosia beddomei*. The study yielded several key findings that contribute to our understanding of the potential therapeutic properties of this plant extract. The comprehensive approach of extraction, phytochemical screening, and anti-inflammatory activity assessment enhances our understanding of the therapeutic potential of *Rhynchosia beddomei*. The study underscores the importance of traditional medicinal plants as sources of novel bioactive compounds and highlights the need for continued research to harness their therapeutic benefits for human health.

In summary, the root extract of *Rhynchosia beddomei* exhibits promising anti-inflammatory activity, likely attributed to its diverse phytochemical composition. This study serves as a stepping stone for future research aimed at harnessing the extract's potential for developing effective anti-inflammatory agents with broader clinical applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Medzhitov, R. (2008) Origin and physiological roles of inflammation. *Nature*, 454, 428–435
- Ahmed, A.U. (2011) An overview of inflammation: Mechanism and consequences. *Frontiers in Biology*, 6, 274–281
- Trowbridge, H.O., Emling, R.C. & Fornatora, M. (1997) Inflammation. A review of the process. *Implant Dentistry*, 6, 238
- Netea, M.G., Balkwill, F., Chonchol, M., Cominelli, F., Donath, M.Y., Giamarellos-Bourboulis, E.J., Golenbock, D., Gresnigt, M.S., Heneka, M.T., Hoffman, H.M., Hotchkiss, R., Joosten, L.A.B.,

Kastner, D.L., Korte, M., Latz, E., Libby, P., Mandrup-Poulsen, T., Mantovani, A., Mills, K.H.G., Nowak, K.L., O'Neill, L.A., Pickkers, P., van der Poll, T., Ridker, P.M., Schalkwijk, J., Schwartz, D.A., Siegmund, B., Steer, C.J., Tilg, H., van der Meer, J.W.M., van de Veerdonk, F.L. & Dinarello, C.A. (2017) A guiding map for inflammation. *Nature Immunology*, 18, 826–831

- Cronstein, B.N. (1992) Molecular mechanism of methotrexate action in inflammation. *Inflammation*, 16, 411–423
- Vane, J.R. & Botting, R.M. (1998) Anti-inflammatory drugs and their mechanism of action. *Inflammation Research*, 47 (Supplement 2), S78–S87
- Moses, V.S. & Bertone, A.L. (2002) Nonsteroidal anti-inflammatory drugs. Veterinary clinics. *Equine Practice*, 18, 21–37.
- Vonkeman, H.E. & van de Laar, M.A. (2010) (Vol. 39, No. 4, pp. 294-312) Nonsteroidal anti-inflammatory drugs: Adverse effects and their prevention. *Rheumatism*. W. B. Saunders, 39, 294–312
- Arif, T., Bhosale, J.D., Kumar, N., Mandal, T.K., Bendre, R.S., Lavekar, G.S. & Dabur, R. (2009) NaturalProducts-antifungal agents derived from plants. *Journal of Asian Natural Products Research*, 11, 621–638
- Dasilva, E.J. (1999) Medicinal plants: A reemerging health aid. *Electronic Journal of Biotechnology*, 2, 57–70.
- Plants, T.S.: a rich source of herbal medicines (2008). *Journal of Natural Products*, 1, 27–35.
- Rammohan, A., Reddy, G.M., Bhaskar, B.V., Gunasekar, D. & Zyryanov, G.V. (2020) Phytochemistry and pharmacological activities of the genus Rhynchosia: A comprehensive review. *Planta*, 251, 1–5.
- Singh, J. (2008) Maceration, percolation and infusion techniques for the extraction of medicinal and aromatic plants. *Extraction Technologies for Medicinal and Aromatic Plants*, 67, 32–35.
- Deng, R. & Chow, T.J. (2010) Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. *Cardiovascular Therapeutics*, 28, e33–e45