Chapter

New Potential and Characterization of *Andrographis echioides* L. Ness Stem Extract as an Anti-Arthritic Agent

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Abstract

This chapter explored the phytochemical composition and anti-arthritic potential of *Andrographis echioides* stem extract. Qualitative and quantitative analyses revealed a diverse array of phytochemicals, with saponins emerging as the most abundant compound. The extract exhibited concentration-dependent anti-arthritic activity through two primary mechanisms: protein denaturation inhibition and red blood cell membrane stabilization. The extract's effectiveness was comparable to diclofenac sodium, a widely used non-steroidal anti-inflammatory drug. These findings suggest that the phytoconstituents present in the extract significantly contribute to its anti-arthritic properties. The study's results indicate that *Andrographis echioides* stem extract may hold promise as a natural alternative for arthritis treatment.

Keywords: anti-arthritic, phytochemical, secondary metabolites, protein denaturation, cell membrane

1. Introduction

Plants have been used in synthetic drug development and non-pharmacological applications due to their medicinal components. Traditional medicine has used herbal remedies to combat fungal and insect threats since prehistoric times [1]. Despite the widespread use of medicinal plants, their value remains unrecognized in many countries. Phytochemicals, found in plants, play a crucial role in human health by reducing platelet aggregation, activating detoxifying enzymes, exerting antioxidant effects, stimulating the immune system, and demonstrating antibacterial properties [2]. These compounds, including alkaloids, carotenoids, saponins, tannins, phenols, steroids, and flavonoids, have disease prevention capabilities, including antiparasitic, anticancer, antidiabetic, antiaging, antidepressant, and antimicrobial effects [3]. They also contribute to wound healing, accumulate bioactive substances in fruits and vegetables, and enhance stress tolerance [4]. Flavonoids are predominant in plants, with many fruits and vegetables containing anticarcinogenic varieties [5].

Anthocyanins, carotenoids, and lycopene are natural antioxidants found in various fruits and vegetables.

The therapeutic use of natural products, especially those derived from plants, has seen a resurgence in popularity in recent years. These products, depending on their specific attributes, can be categorized as pharmaceuticals, cosmetics, medical devices, nutritional supplements, or traditional foods [6]. Over the past two decades, a significant number of newly approved chemical entities have been small-molecule natural products (SMNPs) or SMNP-based compounds [7]. As a result, governments, international organizations, and businesses are actively exploring medicinal herbs and natural products as a valuable source for new drug compounds. This renewed interest in plant derivatives is partly due to the increased adverse effects associated with conventional medicines. However, plant-based products face several challenges, such as an unpleasant taste and strong odor, which can negatively impact patient compliance. Additionally, their limited solubility, slow dissolution rate, and instability at high pH levels restrict their therapeutic application, often requiring large dosages to achieve the desired effect [8]. Moreover, rapid oxidation and degradation during storage can reduce the concentration of active ingredients in plant derivatives, thereby diminishing their nutritional value.

Rheumatoid arthritis is an inflammation of the joints, primarily caused by a weak autoimmune system and swelling. Symptoms include weight loss, low fever, poor appetite, feeling unwell, and irritation around joints. The disease is not congenital and can be caused by environmental influences or infection [9]. Autoimmune diseases, such as lumps, atherosclerosis, and cancer, can be treated with antioxidants and a healthy diet. Citrus fruits, which contain essential nutrients like carotenoids, selenium, folic acid, potassium, and vitamin C, can also help protect against human malignancies [10]. Arthritis is a chronic condition that causes irregular function, inflammation, and pain. There are 100 types of arthritis, including rheumatoid arthritis, osteoarthritis, and infectious arthritis. Rheumatoid arthritis results from the immune system attacking joints, while osteoarthritis occurs when cartilage becomes tired or less movable [11]. Infectious arthritis is caused by bacteria and viruses, and antibiotics are used to cure it. Synovitis, a type of arthritis, is directly linked to rheumatoid arthritis. Treatment depends on the type and suitability of the individual, with the initial step being a general examination by a doctor. Herbal and nutritional supplements have become important in therapeutic practice and research for rheumatology and orthopedics [12].

Andrographis echioides is an herbaceous plant found in dry areas of southern Asian countries, with a flowering season from March to June and October to December. The plant has elongated leaves with glandular hairs, a slightly quadrangular stem, and a raceme-type inflorescence [13]. The flower's calyx is lanceolate with glandular hairs, and the capsules are ovoid, sparsely hairy, pointed above, and narrowed below. The plant has an average number of capsules per plant and four seeds per capsule. The plant's anatomy includes a well-defined epidermis with epidermal hairs, a well-defined hypodermis, and a chlorenchymatous cortex. The root is composed of a single layer of barrel-shaped epidermal cells, lacking stomata and cuticle, and a compactly arranged parenchymatous cortex [14]. *Andrographis echioides* has been found to have various pharmacological activities. It has been found to have diuretic activity, antimicrobial activity, anthelmintic activity, hepatoprotective and antioxidant effects, anti-inflammatory, analgesic, and antipyretic activity, and anti-ulcer activity. The plant's ethanol extract has shown potential anti-ulcer activity in all tested models [15]. Additionally, the plant's leaves have been subjected to Soxhlet extraction using

ethanol as a solvent, showing potential for controlling A. aegypti larvae. These findings suggest that *Andrographis echioides* may be a valuable tool for treating various diseases and infections. Further research is needed to isolate the active principles responsible for these promising properties.

2. Materials and methods

2.1 Plant collection and extraction

This investigation focused on the stem of *Andrographis echioides*, with specimens obtained from Trichy. The collected stems underwent cleaning and shade-drying processes. Subsequently, the dried stems were ground into a powder using a mechanical device and sieved through a 20-mesh screen. A hot percolation technique was employed to extract the ethanolic component from 500 grams of the powdered material. The mixture was maintained at ambient temperature for 24 hours with minimal agitation. Following filtration, the extract was subjected to further analysis at 35°C. Phytochemical screening of the *Andrographis echioides* stem was performed using anhydrous ethanol, chloroform, and aqueous solutions [16].

2.2 Preliminary analysis

Qualitative screening of 18 metabolites was performed using designated solvents to identify the presence of chemical constituents in the root extracts. Quantitative analyses were conducted to evaluate the quantity and concentration of phytonutrients in the extract. These quantitative screenings employed specific methodologies for each bioactive compound, encompassing flavonoids, tannins23, saponins, alkaloids, and phenols [17, 18].

2.3 Preparation of ethanolic extraction

Ethanol extracts were obtained by subjecting 500 g of dried, powdered plant material to continuous extraction with 1 L of ethanol in a Soxhlet apparatus for 10 hours. The resulting extracts underwent filtration using Whatman No. 42 filter paper (125 mm). Subsequently, the filtrate was concentrated and desiccated utilizing a rotary evaporator under reduced pressure conditions. The final dried samples, weighing 50 g, were transferred to labeled sterile containers and stored at a temperature of −20°C [19].

2.4 Protein denaturation model

The control solution was prepared using 1 ml of egg albumin, 2.8 ml of phosphate buffer, and 1000 μg/ml of distilled water and subsequently labeled. *Andrographis echioides* extracts were prepared at various concentrations (100, 200, 300, 400, and 500 μg/ml) and combined with corresponding solvent concentrations (900, 800, 700, 600, and 500 μg/ml), then labeled appropriately. The test solution consisted of 1 ml egg albumin, 2.8 ml phosphate buffer, and ethanol extract at concentrations of 100, 200, 300, 400, and 500 μg/ml.

A single drop of concentrated HCl was introduced to the test solution, which was then incubated at 37°C for 5 minutes. Following incubation, the solution was cooled.

Absorbance measurements for all solutions were obtained using a UV-visible spectrophotometer at 660 nm.

The extent of protein denaturation inhibition was quantified using the following equation:

$$\text{Percentage inhibition} = \left[VC - Vt / Vc\right] \text{x}\, 100$$

In this formula, Vt denotes the absorbance of the test sample, while Vc represents the absorbance of the control sample [20, 21].

2.5 Anti-arthritic analysis

The Human Red Blood Cells (HRBC) technique was employed to assess in vitro anti-arthritic activity. Blood specimens obtained from healthy volunteers were combined with an equivalent volume of sterilized Alsever's solution. All experimental samples underwent incubation at 37°C for a duration of 30 minutes, followed by centrifugation at 3000 rpm [22, 23].

$$\text{Percentage protection} = 100 - \left(OD\,\text{sample} / OD\,\text{control}\right) \times 100$$

3. Results

3.1 Phytochemical analysis

The examination delineates the active biomolecules identified within the stem extract. In light of this finding, the stem of *A. echioides* has been deemed a focal point for the biosynthesis of bioactive compounds. The identified compounds include terpenoids, flavonoids, steroids, alkaloids, saponins, tannins, cardiac glucosides, phlorotannins, proteins, coumarin, emodin, anthraquinone, anthocyanin, carbohydrates, leucoanthocyanin, phenols, alkaloids, and xanthoproteins. Among these constituents, with the exception of leucoanthocyanins and phlobatannins, all others exhibited the anticipated results (**Figure 1**, **Table 1**). The quantitative assessment of flavonoids, tannins, saponins, phenols, alkaloids, and terpenoids indicates potential outcomes. Notably, with the exception of tannins, the remaining phytonutrients, particularly saponins, displayed significant results (**Figure 2**, **Table 2**).

3.2 Protein denaturation model

The results show that *Andrographis echioides* stem extract inhibits protein denaturation in a dose-dependent manner, similar to diclofenac sodium. At 100 µg/mL, the extract (0.37%) is slightly less effective than diclofenac (0.27%), indicating notable anti-inflammatory potential (**Figures 3** and **4**, **Table 3**). The findings suggest that *Andrographis echioides* may serve as a natural anti-arthritic agent, supporting its traditional medicine use.

3.3 Anti-arthritic activity

The HRBC membrane stabilization assay demonstrates a dose-dependent anti-inflammatory effect of *Andrographis echioides*, comparable to diclofenac sodium.



Figure 1.
A, B, and C. Qualitative analysis of ethanolic extract of Andrographis echioides *stem.*

S.No	Phytochemical constituents	*Andrographis echioides*
1	Terpenoids	+++
2	Flavanoids	+++
3	Saponin	+++
4	Tannin	+++
5	Alkaloids	+++
6	Steroids	+++
7	Glycosides	+++
8	Phlobotannins	—
9	Protein	+++
10	Coumarin	+++
11	Emodin	++

S.No	Phytochemical constituents	*Andrographis echioides*
12	Anthroquinone	+++
13	Anthocyanin	+++
14	Carbohydrate	++
15	Leucoanthocyanin	—
16	Cardiac glycosides	+++
17	Xanthoproteins	+++
18	Phenol	++

(+ = slightly present, ++ = moderately present, +++ = strongly present, − = absence)

Table 1.
Qualitative analysis of the ethanolic extract of Andrographis echioides *stem.*

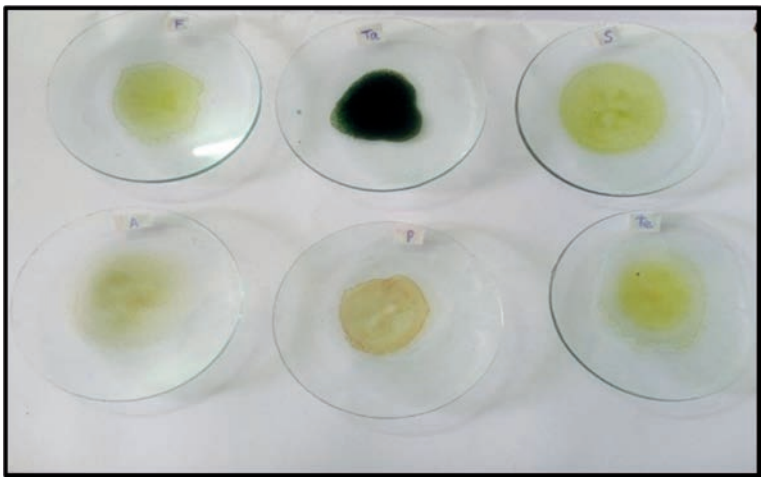


Figure 2.
Quantitative analysis of the ethanolic extract of Andrographis echioides *stem.*

S.No	Phytochemical constituents	Empty Value (mg/g)	Yield value (mg/g)	Final Value (mg/g)
1.	Phenol	18.830	18.846	0.016
2.	Flavonoid	20.656	20.669	0.013
3.	Tannin	20.286	20.326	0.04
4.	Saponin	21.046	21.088	0.42
5.	Alkaloids	19.325	19.595	0.27
6.	Terpenoids	18.771	18.785	0.014

Table 2.
Quantitative analysis of ethanolic extract of Andrographis echioides.

As the concentration increases, the membrane stabilization percentage improves, indicating its ability to prevent hemolysis and protect cell membranes from damage. At 100 µg/mL, the extract shows 0.22% stabilization, while diclofenac sodium exhibits 0.50%, with an overall 81.3% stabilization effect (**Figures** 5 and **6**, **Table 4**).

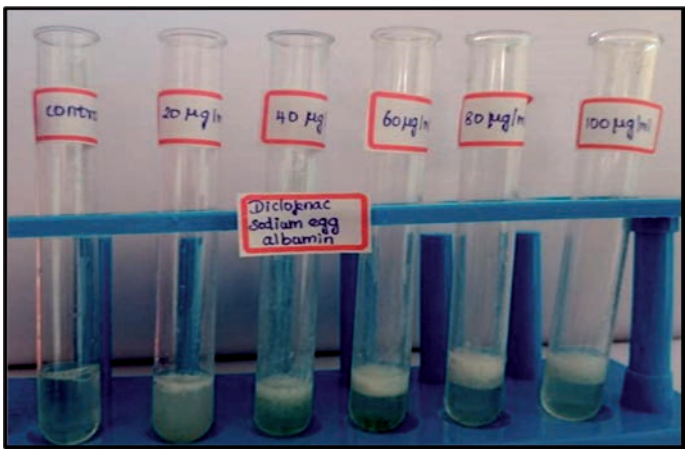


Figure 3.
Protein denaturation assay.

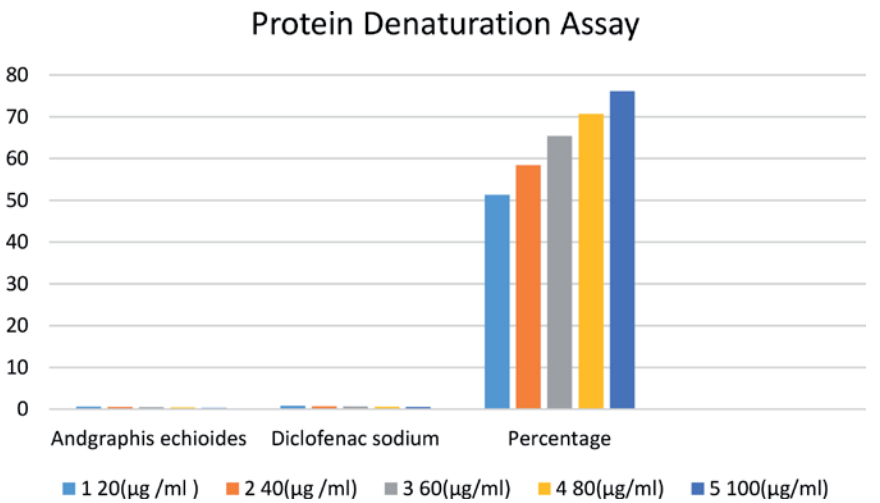


Figure 4.
Graph representation of protein denaturation assay.

S.No	Concentration	Protein denaturation (%)	
		Andrographis echioides	**Diclofenac sodium**
1	20 (µg/ml)	0.68	0.55
2	40 (µg/ml)	0.54	0.47
3	60(µg/ml)	0.50	0.39
4	80 (µg /ml)	0.43	0.33
5	100 (µg /ml)	0.37	0.27

Table 3.
Protein denaturation assay of Andrographis echioides *stem.*

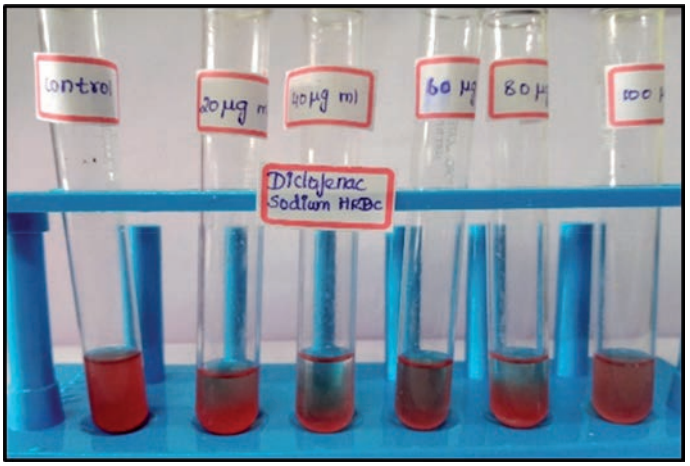


Figure 5.
HRBC membrane stabilization assay.

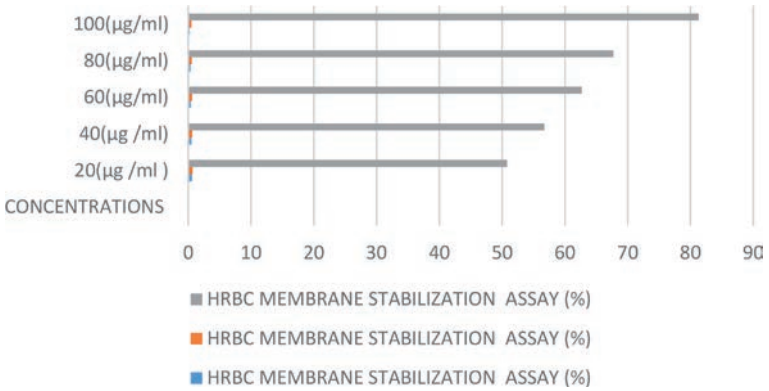


Figure 6.
Graph representation of HRBC membrane stabilization assay.

SI.No	Concentrations	HRBC Membrane Stabilization Assay (%)		
		Andgraphis echioides	**Diclofenac sodium**	**Percentage %**
1	20(µg /ml)	0.58	0.72	50.8%
2	40(µg /ml)	0.51	0.65	56.7%
3	60(µg/ml)	0.44	0.62	62.7%
4	80(µg/ml)	0.38	0.55	67.7%
5	100(µg/ml)	0.22	0.50	81.3%

Table 4.
In vitro anti-arthritic activity of the stem of Andrographis echioides *using HRBC.*

Although slightly less effective than diclofenac, *Andrographis echioides* still exhibits significant anti-arthritic potential, supporting its traditional use in inflammation management and suggesting its role as a natural alternative for arthritis treatment.

4. Discussion

Research conducted by Gurupriya and Cathrine in 2022 revealed that the stem of *Andrographis echoides* contains a variety of biologically active compounds, including steroids, terpenoids, saponins, tannins, alkaloids, and flavonoids. These compounds exhibit efficacy against microorganisms, liver toxins, inflammation, tumors, and free radicals [24]. An investigation focused on the phytochemical composition of Ocimum species ethanolic extract. Their analysis identified the presence of phenols, glycosides, flavonoids, terpenoids, and saponins in the extract [25]. Likely, further studies by Pavithra Kumari corroborated the presence of various phytochemicals in Ocimum species, specifically phenols, flavonoids, saponins, tannins, and alkaloids. Notably, this species exhibited a particularly high concentration of saponins compared to other phytochemicals [26].

A study reported on the quantitative analysis of moisture content, including volatile components, in the tested drug. Their findings emphasized the importance of the final drying process and the rate of moisture removal from secondary metabolites in determining the drug's therapeutic efficacy. Additionally, they highlighted the significance of saponin compounds for isolating diosgenin and their potential in anti-arthritic applications [27].

Barleria lupulina has demonstrated anti-inflammatory activity through the inhibition of protein denaturation. An ethanolic leaf extract of the plant was tested, and the results showed inhibition percentages of 53.78%, 55.25%, and 60.6% at different concentrations in the protein denaturation test. Diclofenac sodium, used as a standard, showed inhibition percentages of 63.3%, 75%, and 79%. The study finally suggested that *B. lupulina* has potential as a remedy for oxidative stress [28].

Another study evaluated the anti-inflammatory activity of ethanol extract and fractions of B. sapida stem bark using in vitro methods, specifically albumin denaturation and membrane stabilization assays. The results showed that the extract and fractions exhibited anti-inflammatory activity by inhibiting heat-induced albumin denaturation and stabilizing red blood cell membranes at concentrations of 200–1000 μg/mL and 50–250 μg/mL, respectively. The ethyl acetate fraction, which had the highest phenolic and flavonoid content, was a key contributor to this activity [29].

The study investigates the anti-inflammatory and anti-arthritic activities of *Martynia annua* ethanolic fruit extract. The extract was extracted using ethanol and phytochemically investigated. The results showed antioxidant activity of 49.1 and anti-hyaluronidase activity of 84.60, indicating its potential for anti-arthritic and anti-inflammatory properties. The extract's potential is significant for treating inflammation and rheumatoid arthritis [30]. Another study assessed the anti-arthritic and anti-inflammatory activities of the methanolic extract of *Peganum harmala* leaves in vitro and in vivo. The extract showed significant antioxidant, anti-inflammatory, and anti-arthritic activities primarily due to alkaloids, flavonoids, and phenols. It also attenuated polyarthritis, weight loss, anemia, and paw edema in polyarthritic rats. The results suggest that *P. harmala* extract has potential therapeutic benefits [31].

5. Summary and conclusion

This investigation demonstrates that the phytochemical screening of the ethanolic extract of *A. echioides* revealed an abundant presence of terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, proteins, coumarins, anthraquinones, anthocyanins, cardial glycosides, and xanthoproteins. Phenols, carbohydrates, and emodine were detected in moderate quantities, while phlobatannins and leucoanthocyanins were not observed. Quantitative assessment of the ethanolic extract indicated high saponin content (0.042 mg/g), low tannin levels (0.04 mg/g), and intermediate concentrations of alkaloids (0.27 mg/g), terpenoids (0.014 mg/g), and flavonoids (0.13 mg/g). The ethanolic extract of *A. echioides* demonstrated inhibition of protein (egg albumin) denaturation at concentrations ranging from 20 to 100 μg/ml. Diclofenac sodium, utilized as a reference drug, stabilized the HRBC membrane across the same concentration range. Similarly, the ethanolic stem extract of *A. echioides* exhibited HRBC membrane stabilization at equivalent doses. Both protein denaturation and HRBC membrane assays revealed maximal efficacy for stem extracts and diclofenac sodium at 100 μg/ml. These findings suggest that the phytoconstituents present in the ethanolic stem extract of *A. echioides* contribute to its anti-arthritic activity.

Acknowledgements

I extend my heartfelt thanks to the faculty and staff members of the Department of Botany for providing the necessary facilities and assistance. Finally, I acknowledge all the researchers whose work has contributed to the foundation of this study.

Conflict of interest

The authors declare that they have no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

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