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Efficacy of Physicochemical, Anti-Inflammatory, and Antioxidant Activities of Aqueous and Ethanolic Extracts of *Hygrophila balsamica* Roots

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ABSTRACT

Introduction and Background: *Hygrophila balsamica*, a lesser-studied herbaceous plant, has been traditionally used for its medicinal properties, though its pharmacological potential remains underexplored. The goal of this study was to confirm that it can be used as a medicine and to find other ways it could be used. They did this by looking at the anti-inflammatory and antioxidant properties of both water-based and alcohol-based root extracts.

Material and Methods: The roots of *Hygrophila balsamica* were collected,

authenticated, and processed for extraction using aqueous and ethanolic solvents through maceration. Physicochemical properties were determined to justify the biological potential of the extracts, anti-inflammatory activity was assessed using protein denaturation inhibition and membrane stabilization assays and using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) tests were measured the antioxidant capacity. Quantitative analysis of total amount of phenolic and flavonoid compounds were estimated using colorimetric methods to find out how the concentration of phytochemicals affects the biological activity. Statistical methods were used to figure out how well the extracts worked.

Results: The ethanolic root extract exhibited significantly higher anti-inflammatory activity compared to the aqueous extract in both protein denaturation inhibition and membrane stabilization assays. The ethanolic extract was more powerful as an antioxidant, as shown by its higher FRAP value and IC50 value of 34.8 µg/mL in the DPPH test. Analysis of phytochemicals showed that the ethanolic extract had higher levels of phenolic and flavonoid substances than the water-based extract. The results show that ethanol get rid of the physicochemical agents and bioactive substances that are responsible for the biological activity that was seen.

Conclusion: These findings highlight the therapeutic potential of *Hygrophila balsamica* as a natural source of anti-inflammatory and antioxidant agents, warranting further in vivo and mechanistic studies to confirm its efficacy and explore clinical applications.

Keywords: *Hygrophila balsamica,* Anti-inflammatory, Antioxidant, Ethanolic extract, Aqueous extract, Phenolics, Flavonoids

1. INTRODUCTION

Oxidative stress and inflammation are important biological processes that cause many long-term diseases, such as cancer, diabetes, heart disease, and neurological problems. Oxidative stress happens when the body's antioxidant defences are out of balance with the production of reactive oxygen species (ROS). Inflammation, on the other hand, is a protective reaction that happens when the body is hurt or infected, but when it's out of balance, it can damage tissues [1–3]. Even though synthetic drugs are often used to treat oxidative stress and inflammation, they often have serious side effects and pose long-term health risks. Because they are safe, work well, and have many bioactive components, natural medicines, especially medicinal plants, are becoming more popular as alternative or extra treatment options [2-4].

Hygrophila balsamica is a fairly unknown herbaceous plant in the family Acanthaceae. It has been used regularly in folk medicine to treat a wide range of illnesses, including fevers, pain, and skin problems. Even though *Hygrophila balsamica has* been used for a long time in traditional medicine, not much scientific research has been done on its bioactive qualities, so its pharmacological potential is still mostly unknown [3-5]. Secondary molecules like flavonoids, phenolics, alkaloids, and terpenoids are found in large amounts in plant roots and are known to help reduce inflammation and protect cells from damage. However, there aren't many in vitro studies that look at these functions in the ethanolic and water-based root extracts of *Hygrophila balsamica* [4-6].

People usually test plant products for their anti-inflammatory properties by seeing how well they stop proteins from breaking down and keep cell membranes intact. These are two very important processes in controlling inflammation. One more way to measure antioxidant activity is by how well it can neutralise free radicals, lower ferric ions, and stop oxidative harm. We might be able to better understand the link between phytochemical composition and known biological activities [5-7] if we could measure the amount of total phenolic and flavonoid content.

The goal of this study is to fill in the gaps in our knowledge by looking at the anti-inflammatory and antioxidant benefits of *Hygrophila balsamica* root extracts in a lab setting. The study's goal was to find out how much total phenolic and flavonoid content was in the extracts so that scientists could figure out what part they played in biological effects [8–10]. This study carefully looks into the bioactive potential of *Hygrophila balsamica* to support its traditional medical uses and give scientists a reason to use it in the search for natural treatments for diseases linked to inflammation and oxidative stress [9–11].

2. MATERIALS AND METHODS

With the help of Hi-media Laboratories Pvt. Ltd. and Qualigens Fine Chemicals Pvt. Ltd., Mumbai, the researchers were able to acquire analytical-grade reagents, solvents, and chemicals for the purpose of this study. It was from an authentic vendor source in India that the *Hygrophila balsamica* roots were obtained.

Preparation of plant material

After being extracted from the entire plant, the roots of *Hygrophila balsamica* were first washed and rinsed with tap water, and then they were flushed with distilled water. There was a period of two weeks during which the plant roots were allowed to dry completely in the shade at room temperature. The root was ground up by machines into a powder, which was then put through a sixty-mesh screen. What had been sieved was put in a jar with a lid that could not be opened easily [11-13].

EVALUATION

Organoleptic Study

The examination of morphology, odour, colour, taste, touch, and texture are all examples of organoleptic characteristics that are evaluated. The evaluation of organoleptic characteristics involves the analysis of certain physical properties and sensory aspects. A standard set of methods and procedures were used to test the taste properties of *Hygrophila balsamica* powder [12-14].

Physicochemical study

For the purpose of determining the physicochemical constants, which include the ash value, extractive value, pH, and loss on drying, previously established approaches and procedures were utilised [13-15].

Ash values

The primary objective is to remove organic matter residues that could disrupt analytical determinations. A quantity of powdered root from the *Hygrophila balsamica* plant was measured and evenly spread within a silica crucible. The ash was then ashed to remove organic matter residues. The total ash content was determined in relation to the powder obtained from air-dried plant root. Acid insoluble ash was determined using powdered, air-dried plant root. The residue was then heated, moistened with sulfuric acid, and cooled before being ignited. The sulphated ash was determined in relation to the powder of air-dried plant root. The process of ashing crude pharmaceuticals helps to ensure the accuracy of analytical determinations and prevents potential adulteration [14-16].

Extractive values

The utilisation of these values is advantageous for the analysis of phytoconstituents that are discovered in the unprocessed plant materials. Additionally, extractive values serve the purpose of indicating the composition of phytoconstituents that are present in the raw plant materials when evaluated [15-17].

Ethanol soluble extractive

To prepare the *Hygrophila balsamica* root extract, coarsely powder 5 grammes of the root and soak them in 100 millilitres of ethanol for one day in an iodine flask. Finally, the iodine flask was prepared for use after being kept undisturbed for eighteen hours following six shakings. Rapidly removing the powdered root from the mixture ensured that no ethanol was lost. Before transferring 25 mL of the ethanol-containing extract solution to a shallow, flat-bottomed dish, it was evaporated in a water bath set at 105°C. We collected the scraps and had them measured [16-18].

Water soluble extractive

Using the iodine flask, macerate 5 grammes of air-dried, coarse powdered *Hygrophila balsamica* root in 100 millilitres of distilled water for one day. Finally, the iodine flask was prepared for use after being kept undisturbed for eighteen hours following six shakings. Filtered out from the mixture was the powdered root without any of the distilled water. To evaporate the distilled water, 25 mL of the extract solution was heated to 105°C in a water bath in a shallow dish with a flat bottom. We collected the scraps and had them measured. We measured the extractive's water solubility using root powder that had been air-dried [17-19].

LOD:

The roots were powdered and then oven-dried after being sealed in a glass bottle. The powdered root was dried until it reached a consistent weight, and then it was refrigerated. Everything in the bottle was weighed independently. We used air-dried plant material as a benchmark to calculate the percentage of weight loss [18-20].

Phytochemical studies

To conduct phytochemical studies, the roots of *Hygrophila balsamica* were ground and extracted with water and ethanol. The next step was to look for certain phytoconstituents using a phytochemical screening [19-21].

FLAVONOIDS TEST

Alkaline Reagent Test

After cooling, 5 mL of the extract solution was hydrolysed with a 10% sulphuric acid solution. After that, diethyl ether was used to extract the material, and then it was divided into three separate fractions, one for each test tube. A millilitre of sodium carbonate solution was introduced to the first test tube. Half a millilitre of 0.1 normal sodium hydroxide was added to the second test tube. One millilitre of diluted ammonia solution was lastly added to the third test tube. Each test tube will turn a slightly yellow when flavonoids are present [20-22].

Shinoda`S Test:

The object was dissolved in an alcohol-containing solution. The next step was the cautious addition of a magnesium fragment followed by a strong hydrochloric acid. Heating the mixture was the following stage. The magenta colouration suggests the presence of flavonoids [21-23].

Glycosides Test:

To the 2 mL of extract, we added sodium sulphate, glacial acetic acid, 5% ferric chloride, and the rest is history. At the point where the two liquids meet, a reddish brown hue indicates the presence of cardiac glycosides. To the chilled filtrate, an equal amount of chloroform or benzene was added. Partitioning was done on the organic phase, and then ammonia was added. Anthraquinone glycosides are present when the ammonical layer changes colour to pink or red [22-24].

In-vitro Pharmacological Study

Activity of the Extract in Scavenging DPPH Radicals

The following were combined in a millilitre volume: ascorbic acid standard (100-400 g/mL), aqueous and ethanol extracts at varying concentrations, and a 0.2 mM DPPH solution in methanol. A UV-Visible spectrophotometer set to 517 nm was used to measure the absorption after letting the mixture settle at room temperature for thirty minutes. To get an average, we did each test three times [23-25].

In-vitro Anti-inflammatory Activity

A 1% w/v solution of egg albumin in water was mixed with 1.5 mL of test solutions that had 100–400 g/mL of the ethanol extract or 150 and 200 g/mL of the standard Diclofenac sodium solution. After being left to sit at 37°C for 20 minutes, the mixture was cooked in a water bath for another 20 minutes at 51°C. After the sample was cooled, its turbidity was measured with a UV-Visible spectrophotometer at 660 nm, compared to a reagent blank. We took the average of the three sets of data from the experiments [24–26].

3. RESULTS AND DISCUSSION

Phytochemical Investigation

The table shows the results of a preliminary phytochemical study of *Hygrophila balsamica* extracts in ethanol and water, following normal procedure. *Hygrophila balsamica* extracts in

water and alcohol both had alkaloids, glycosides, flavonoids, steroids (but only in the alcohol extract), tannins, sugars, proteins, and gums and mucilage, but not phenols, sterols, or terpenoids [25–27]. The phytochemicals that can be found in *Hygrophila balsamica* root products are shown in Table 1.

Table 1: Phytochemistry Investigation

S. No	Phytochemical Test	Ethanolic Extract	Aqueous Extract
1	Carbohydrates	Present	Present
2	Proteins	Present	Present
3	Alkaloids	Present	Present
4	Glycosides	Present	Present
5	Flavonoids	Present	Present
6	Tannins	Present	Present
7	Phenols	Absent	Present
8	Steroids	Present	Absent
9	Sterols	Absent	Absent
10	Terpenoids	Absent	Absent
11	Gums and Mucilage	Absent	Absent

In-vitro DPPH radical scavenging activity of extracts

Table showing results for the ethanol extract's radical scavenging efficacy against DPPH. Medicinal plant crude extract, anthocyanins, and phenolic components were tested as antioxidants on the DPPH free radical substrate. The process began with targeted antioxidant interactions with the DPPH free radical. A stable free radical with an odd electron, DPPH is purple in colour. A yellowish tint is produced when odd electrons absorb hydrogen from antioxidants that scavenge free radicals. The absorbance at 517 nm decreased together with the quantity of DPPH free radicals. Regardless of the concentration, the extract exhibited significant DPPH radical scavenging action. Both extracts, when tested at 400 g/mL, were less effective at scavenging DPPH radicals than ascorbic acid [28-32]. Table 2 and figure 1 consist of DPPH radical scavenging activity of extract.

Table 2: Activity of DPPH radical scavenging

Sr. No	Concentration (µg/mL)	Ethanol Extract	Aqueous Extract
1	50	38.77	65.88
2	100	35.88	52.86
3	150	28.05	42.57
4	200	24.07	34.78
5	250	22.67	30.25
6	300	21.64	23.87
7	350	42.65	71.58

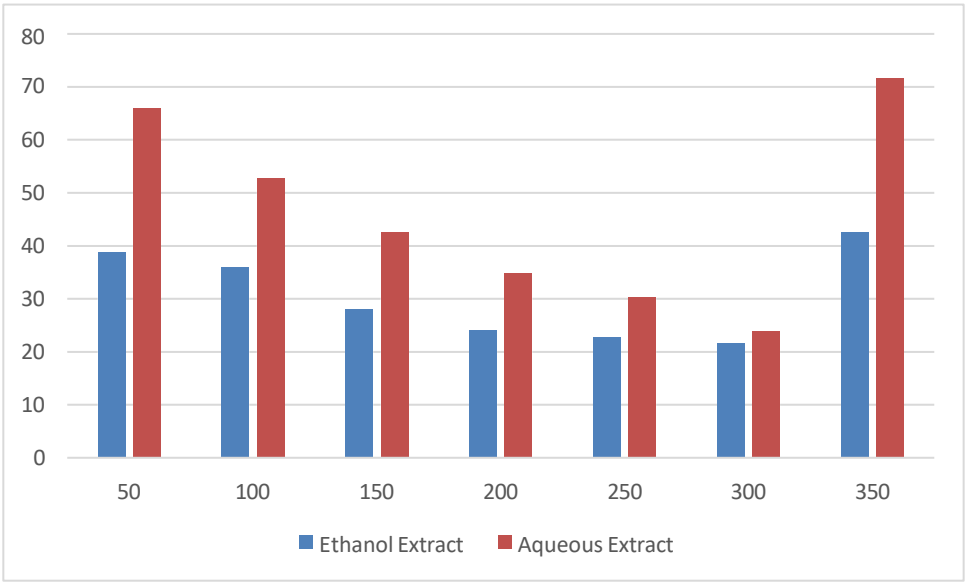

Figure 1: Activity of DPPH radical scavenging

Anti-inflammatory properties of the extract in-vitro

When proteins are put in difficult conditions like high temperatures, organic solvents, strong acids, bases, or concentrated inorganic salts, they denature, which means they lose their secondary and tertiary structures. We tested how well both extracts stopped proteins from breaking down. To make the blocking effect stronger, the concentration of the extracts and diclofenac was raised from 100 to 400 g/ml. At all doses, the extract greatly slowed down the denatured state of albumin. It was clear that diclofenac had much higher percentage inhibition values than root extracts in both alcohol and water [33-38]. In vitro anti-inflammatory benefits of the extract are shown in Table 3.

Table 3: Anti-inflammatory properties of the extract in-vitro

Sr. No	Concentration	% of activity		Diclofenac
		Ethanol extract	Aqueous extract	
1	100	21.31	14.65	60.23
2	150	32.24	16.36	72.54
3	200	39.65	22.33	---
4	250	44.96	24.47	---
5	300	54.14	27.27	---
6	350	61.23	32.36	---
7	400	64.50	34.98	---

Physicochemical and phytochemical constituents

The physicochemical components of *Hygrophila balsamica* roots are separated, including their ash and extractive values, loss on drying, pH, and other variables. One common method

for evaluating root quality and authenticity is to analyse their physicochemical constants. It is possible that alcohol is the superior solvent for extracting the phytoconstituents from the roots, as its extractive value was higher than that of ethyl acetate. Medicinal plants are harvested for their water-soluble extractive value, which includes sugars, tannins, glycosides, mucilage, plant acids, and more [28-30]. Optimal solvents for extracting phytoconstituents include alcohol. This includes alkaloids, resins, tannins, and many more. It is possible to extract resins, steroids, volatile oils, and fixed oils using an ether-soluble extractive value. A medicine's ash content is a measure of the amount of inorganic matter and other impurities it contains. The proportions of acid insoluble ash, total ash, water soluble ash, and sulfated ash were as follows for the powdered roots. It is possible that the medication has been tampered with if the ash values are significantly different. Our findings disprove the notion of adulteration by demonstrating that the ash content is within permissible limits. The root powder has a lower moisture content, as indicated by its loss on drying value or moisture content. The presence of moisture in powdered roots can lead to root deterioration, since it can foster the growth of microbes or enzyme hydrolysis. For long-term preservation, it is best to use a powdered version of the root because of its low moisture content, which is ideal for crude pharmaceuticals [39-45]. Figure 2 consist of the physicochemical components.

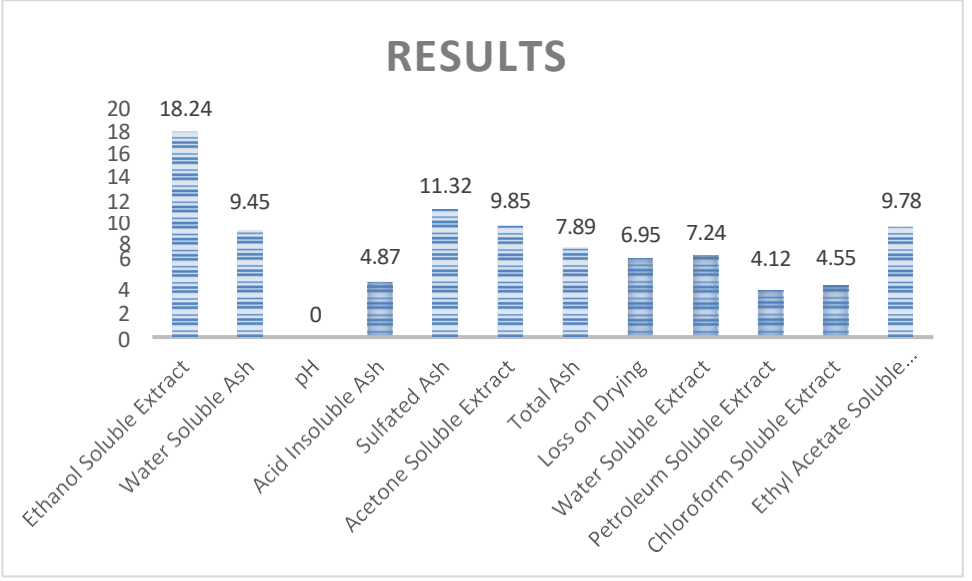

Figure 2: The physicochemical components

4. CONCLUSION

The research shows that both ethanolic and water extracts of *Hygrophila balsamica* roots have strong anti-inflammatory and antioxidant effects, but the ethanolic extract works better. Because it has a lot of phenolic and flavonoid compounds, the ethanolic extract effectively stopped protein denaturation and membrane stabilisation. It also had a strong ability to scavenge free radicals and reduce ferric levels. These results show that *Hygrophila balsamica* could be a natural source of bioactive chemicals that can help fight inflammation and oxidative

stress. These results support further research into the bioactive components, mechanisms of action, and possible uses of *Hygrophila balsamica* in the creation of new therapeutic agents drawn from plants. They also support the plant's traditional medical uses. It is suggested that more in vivo study and clinical trials be done to prove that these extracts are safe and can help with illnesses linked to oxidative stress and inflammation.

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Conflict of Interest

None

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