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Qualitative, Quantitative Phytochemical analysis and In-vitro Anti-inflammatory activity evaluation of Hydroalchoholic Extract of *Muehlenbeckia Platyclada* Leaves

Sarika Nigam[1], Yash Srivastav[2], Vishin Ashish Patil[3], Anil Kumar[4], Imran A Sheikh[5], Tapas Kumar Panigrahi[6] , Suhas Narayan Sakarkar[7] , Jyoti Joshi[8], Sujani Kamble[*9]

[1]Assistant Professor, Department of Pharmacy, ABESIT Group of Institutions, Ghaziabad, Uttar Pradesh, India

[2]Assistant Professor, Department of Pharmacy, Azad Institute of Pharmacy and Research, Lucknow, Uttar Pradesh, India

[3]Assistant Professor, Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India

[4]Head & Assistant Professor, Department of Chemistry (PG), Sahibganj College Sahibganj, Jharkhand, India

[5]Associate Professor, Anjuman I Islam's Kalsekar Technical Campus, School of Pharmacy, New Panvel Navi Mumbai , Maharashtra, India

[6]Associate Professor, Department of Pharmaceutical Chemistry, Royal College of Pharmacy, Raipur, Chhattisgarh, India

[7]Professor, Department of Pharmaceutics, Maharashtra Institute of Pharmacy Bramhapuri, Dist Chandrapur, Maharashtra, India

[8]Assistant Professor, Department of Pharmacy, Guru Ram Das Institute of Management and technology, Dehradun, Uttarakhand, India

[*9]Assistant Professor, Department of Pharmacognosy, Government College of Pharmacy, Bengaluru, Karnataka, India

*Corresponding Author: Sujani Kamble, Assistant Professor, Department of Pharmacognosy, Government College of Pharmacy, Bengaluru, Karnataka, India

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ABSTRACT

The dynamics of the consumer market evolved with globalization. But among its long-term impacts are changes in diet and lifestyle. Study in the chemical and pharmaceutical fields has led to the development and discovery of medicines that have saved millions of lives. However, extended use of these drugs has raised questions about their safety and potential toxicological consequences. To validate their traditional usage, researchers examined the herbs that were previously used in Chinese and Ayurvedic medicine. As a result, communities' reliance on complementary and alternative medicines has started to resurrect during the previous several decades. The purpose of this study is to investigate *Muehlenbeckia Platyclada*'s possible anti-inflammatory properties. Following the collection of the plant material, it underwent extraction, phytochemical screening, and further quantitative testing. The

hydroalcoholic extract of *Muehlenbeckia Platyclada* yields a percentage yield of 12.23%, according to the results. It is abundant in alkaloids, flavonoids, carbohydrates, saponins, diterpenes, proteins, and amino acids, according to the phytochemical screening. The total amount of flavonoids detected was 0.714 mg/100 mg, while the amount of alkaloids was assessed to be 0.519 mg/100 mg. The anti-inflammatory capability of the extract was determined to be 379.12µg/ml, whereas the IC 50 value of standard acetylsalicylic acid was 261.86µg/ml. According to the results, *Muehlenbeckia Platyclada* has strong anti-inflammatory properties.
KEY-WORDS: *Muehlenbeckia Platyclada,* Inflammation, Acetyl Salicylic Acid, Bradykinin, Pathogen

INTRODUCTION

The term "inflammatory" has Latin roots, "Inflammare," which means "burn." Numerous chemical changes might occur in the afflicted area following any kind of physical damage. It was once thought that inflammation was a unique disease caused by anomalies in body fluids. Most people agree that inflammation is a typical response to a disease or other disruption. The traditional signs of inflammation are heat, redness, swelling, pain, and loss of function. Many processes that may be categorized into three groups—the acute transitory phase, the delayed subacute phase, and the chronic proliferating phase—are often responsible for causing inflammation. Local edema develops in the early stages due to inflammatory exudates brought on by increased vascular permeability. The second phase is characterized by leukocyte and phagocyte migration through blood to vascular tissues, whereas the third phase is characterized by tissue fibrosis and disintegration. Endogenous mediators, including prostaglandins, histamine, serotonin, and bradykinin, are released in response to inflammation.[1]

Inflammation is not a straightforward process. Diagnosing a disease or injury is the initial stage in the inflammatory cascade. One popular method for doing this is to identify pathogen-associated molecular patterns (PAMPs), which are primarily focused on broad themes of pathogen-expressed molecules required for pathogen survival. Alarmins, also known as damage-associated molecular patterns (DAMPs), are organic molecules that the innate immune system detects as signs of injury or necrosis. One advantage of identifying these signals is a reduction in unintentional targeting of host cells and organs. Unlike adaptive immunity, the innate immune system is unable to distinguish between various pathogen strains and determine their level of toxicity [2].

Non-steroidal anti-inflammatory medicines (NSAIDs) are the medications most frequently administered globally to treat inflammation-related acute and chronic pain. The decrease of COX activity in the manufacture of prostaglandins and thromboxanes is a feature shared by the actions of the NSAID medication family.NSAIDs work primarily by inhibiting central and peripheral COX, which stops arachidonic acid from being converted into prostaglandin E2, thromboxanes, and prostacyclins. The actions of the COX-1 and COX-2 enzymes differ significantly from those of NSAIDs. COX-1 is a protein found in many cells, including those in the fetus and amniotic fluid. It is engaged in defense and control in addition to other physiological functions. Conversely, proinflammatory cytokines and inflammation activate COX-2. Although the drugs were helpful in the beginning, with the development of selective COX-2 inhibitors, there have been documented serious adverse effects on the kidneys, heart, and gastrointestinal tract [3,4].

The use of herbs has been utilized for medicinal purposes since ancient times. Their plethora of medicinal properties, which may contribute to the averting of ailments, have made them highly valued throughout. With good reason, China and India are known as the "Botanical Garden of the World" since they are the world's leading producers of medicinal plants. India holds a unique position in the world since it is the cradle of several well recognized traditional medicinal systems, such as homeopathy, yoga, naturopathy, Siddha, and Unani. [5]

Humans are aware of the importance of herbal medicine in the drug development process since it has been used for certain ailments from the beginning of human history. [6] Conventional wisdom derived from herbal medicine has consistently encouraged researchers to look for novel pharmaceuticals to enhance healthy living for both humans and animals. [7] Numerous phytochemicals, such as flavonoids, tannins, alkaloids, glycosides, saponins, and polyphenolics, are found in medicinal plants. The World Health Organization (WHO) states that herbal medicine would be a good place to get a range of medications for treating and preventing illnesses in people. The majority of people in wealthy nations utilize conventional medicine. Nonetheless, further research is needed to fully comprehend the effectiveness, safety, and characteristics of these therapeutic plants. [8]

The most recent research focused on the use of herbal remedies as an anti-inflammatory drug in particular. [9] *Muehlenbeckia Platyclada* , sometimes known as ribbon-bush, is a member of the same family. A medicinal plant family called Polygonaceae is used to treat fever, detoxification, fracture injuries, and dangerous snake bites. [10] When triggered by formyl-L-methionyl-L-leucyl-L-phenylalanine, the methanolic extract of this plant's leaves was discovered to have an impact on the formation of human neutrophil superoxide anion and neutrophil elastase delivery. In addition, the plant has long been utilized as an anthelmintic, antiulcerogenic, hypotensive, antihemorrhagic, sedative, diuretic, anti-inflammatory, antirheumatic, and abortive drug. [11]However, nothing is known regarding the pharmacological properties of the plant's root material. The current study concentrated on the phytochemical screening and in vitro anti-inflammatory efficacy of several solvent extracts of *Muehlenbeckia Platyclada* leaves due to its ethanobotanical significance.

MATERIALS AND METHODS

Gathering of Botanical Specimens

The leaves of *Muehlenbeckia Platyclada* were collected from a nearby region in Ghaziabad, Uttar Pradesh, India, taking into account the plant's geographical availability. Following a thorough cleaning with tab water, the leaves were then allowed to dry at room temperature. The materials were crushed and put through a 20-mesh filter once they had dried. The powdered drugs were kept out of direct sunlight and kept in sealed containers until needed.

Extraction by soxhlation process

The appropriate volume of air-dried powdered plant material was added to the Soxhlet apparatus, starting with petroleum ether and working up to hydroalcohol (ethanol: water; 75:25) for the powdered leaves of *Muehlenbeckia Platyclada*. Every time, the powdered material was removed and replaced with the next dissolvent compound once it had been air dried below 100oC. At 100oC, the extracted solvent was allowed to evaporate in the water bath. After the evaporation, the collected components were stored in a refrigerator for further analysis.

Qualitative phytochemicals analysis of extract

Table 1: Phytochemicals test[12]

Phytochemical	Test	Procedure
Alkaloids	Dragendroff's Test	The filtrates were subjected to a solution of potassium bismuth iodide, known as Dragendorf's reagent. The presence of alkaloids is shown by the formation of red precipitate.
Glycosides	Legal's Test	Sodium nitropruside was used to treat the extract with pyridine and sodium hydroxide. The presence of cardiac glycosides is indicated by the formation of a pink to blood red color.
Flavonoids	Alkaline Reagent Test	A few drops of sodium hydroxide solution were added to the extract. Flavonoids are indicated by the formation of a bright yellow color that becomes colorless when diluted acid is added.
Saponins	Froth Test	After diluting the extract with 20ml of distilled water, it was agitated for 15 minutes in a graduated cylinder. The presence of saponins is indicated by the formation of a 1 cm layer of foam.
Tannins	Gelatin Test	A 1% sodium chloride-containing gelatin solution was added to the extract. The presence of tannins is shown by the formation of white precipitate.
Phenols	Ferric Chloride Test	Three to four drops of ferric chloride solution were added to the extract. Phenols are present when blue black color begins to form.
Proteins and Amino acids	Xanthoproteic Test	A little amount of concentrated nitric acid was added to the extract. The development of a yellow hue signifies the existence of proteins.
Carbohydrates	Molisch's Test	In a test tube, filters were treated with two drops of an alcoholic α-naphthol solution. The presence of carbohydrates is shown by the formation of the violet ring at the junction.

Quantitative phytochemicals analysis of extract

Estimation of total alkaloids content

One milligram of the plant extract was dissolved in one milliliter of 2 N HCl, then filtered. After this solution was moved to a separating funnel, five milliliters each of phosphate buffer and bromocresol green solution were added. The mixture was collected in a 10-ml volumetric flask and diluted to the volume with chloroform after being vigorously agitated with 1, 2, 3, and 4 ml of the chloroform. In the same way as previously mentioned, a series of reference standard solutions containing 40, 60, 80, 100, and 120 µg/ml of atropine were created. Using a UV/Visible spectrophotometer, the absorbance of the test and standard solutions was measured at 470 nm in relation to the reagent blank. The alkaloid concentration was measured in milligrams of AE per 100 milligrams of extract.

Estimation of total flavonoids content

Using the aluminum chloride technique, the total flavonoid content was ascertained. 10 ml of methanol were used to dissolve 10 mg of quercetin, and different aliquots containing 5–25 µg/ml were made. After dissolving 10 mg of dried extract in 10 ml of methanol, the mixture was filtered. One milliliter (1 mg/ml) of this extract was used to estimate the flavonoids. After adding 1 ml of 2% AlCl3 solution to 3 ml of extract or each standard, the mixture was let to stand at room temperature for 15 minutes. The absorbance was then measured at 420 nm.[13]

Evaluation of *in vitro* anti-inflammatory activity

The *Muehlenbeckia Platyclada* extract's anti-inflammatory properties were assessed using the protein denaturation technique. The typical medication was acetylsalicylic acid, a non-steroidal anti-inflammatory medicine. The reaction mixture was combined with 0.2 mL of fresh hen's egg albumin and incubated at (37±1)°C for 15 minutes. It contained 2 mL of various concentrations of *Muehlenbeckia Platyclada* extract (100-500µg/mL) or standard Acetylsalicylic acid (100-500 µg mL-1). The reaction mixture was allowed to sit at 70°C in a water bath for ten minutes in order to cause denaturation. After cooling, double distilled water was used as a blank to measure the absorbance at 660 nm. [14]

RESULTS AND DISCUSSION

Muehlenbeckia Platyclada's hydroalcoholic extract yields a percentage of 12.23%. It is abundant in alkaloids, flavonoids, carbohydrates, saponins, diterpenes, proteins, and amino acids, according to the phytochemical screening. The calculated total alkaloid content was 0.519 mg/100 mg, but the observed flavonoid content was 0.714 mg/100 mg. The anti-inflammatory potential of the extract was determined to be 379.12µg/ml, whereas the IC 50 value of standard acetylsalicylic acid was 261.86µg/ml. Finally, we discovered that the hydroalcoholic extracts of Muehlenbeckia platyclade exhibited potent anti-inflammatory properties. Using herbal remedies that include anti-inflammatory ingredients may be a good option in light of the increasing need for substitutes to manage the many types of inflammatory processes.

Table 2:Percentage yield of hydroalcoholic extract of *Muehlenbeckia Platyclada*

S. No.	Name of Plant	Plant Part	Percentage Yield
1.	*Muehlenbeckia Platyclada*	Leaves	12.23%

Table 3: Result of Qualitative phytochemicals analysis of hydroalcoholic extract of *Muehlenbeckia Platyclada*

Phytochemical	Test	Result
Alkaloids	Dragendroff's Test	+ ve
Glycosides	Legal's Test	- ve
Flavonoids	Alkaline Reagent Test	+ ve
Saponins	Froth Test	+ ve
Tannins	Gelatin Test	- ve
Phenols	Ferric Chloride Test	- ve
Proteins and Amino acids	Xanthoproteic Test	+ ve
Carbohydrates	Molisch's Test	+ ve

Table 4: Result of total alkaloid content of hydroalcoholic extract of*Muehlenbeckia Platyclada*

S. No.	Extract	(Total alkaloid content mg/100mg)
1.	Hydroalcoholic	0.519

Table 5: Resultof total flavonoids content of hydroalcoholic extract of*Muehlenbeckia Platyclada*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.714

Table 6:% Inhibition of Acetylsalicylic acidandhydroalcoholicextractof *Muehlenbeckia Platyclada*

Concentration (µg/ml)	% Inhibition	
	Acetylsalicylic acid	Hydroalcoholic extract
100	18.52	15.34
200	41.32	26.64
300	62.06	44.87
400	77.12	53.38
500	90.32	65.32
IC_{50}	**261. 86**	**379.12**

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