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EVALUATION OF IN VITRO, ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY ON THE AQUEOUS AND ETHANOLIC EXTRACT OF LEAVES OF HYGROPHILA BALSAMICA

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Abstract

Introduction and Background: The abundance of thousands of species of medicinal plants

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throughout a wide range of bioclimatic zones has earned India the title of "Emporium of medicinal plants." The current work seeks to characterize and investigate the phytochemical and pharmacological properties of Hygrophila balsamica leaf extracts both in aqueous and ethanolic mediums.

Material and Methods: The reagents, solvents, and chemicals employed in this study were of analytical grade and were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai and Qualigens Fine Chemicals Pvt. Ltd, Mumbai. The foliage of Hygrophila balsamica was procured from agricultural terrain in Karnataka, in the month of January. Department of Zoology, conducted the authentication process on the gathered leaves.

Results: The investigation recognizes the presence of alkaloids, glycosides, flavonoids, steroids, tannin, carbohydrate, and protein in the aqueous and ethanolic extracts, while specifically omitting gums, mucilage, phenols, sterols, and terpenoids. The findings from the extracts demonstrated notable antioxidant and anti-inflammatory properties, hence indicating promising prospects for medicinal applications.

Conclusion: The primary objective of this study is to conduct a phytochemical analysis and assessment of the leaves of Hygrophila balsamica in order to ascertain the validity of their traditional medicinal properties. The results of this study are expected to generate additional research in the fields of phytochemistry and therapeutic application.

Keywords: *Hygrophila balsamica,* phytochemical, physical chemical analysis, anti-inflammatory, and antioxidant properties

INTRODUCTION

India is referred to as the "Emporium of Medicinal Plants" due to the presence of thousands of medicinal plants in various bioclimatic regions. Around 80% of people worldwide, according to the World Health Organization, get their medical care from herbal sources. Only 17% of the approximately 2,50,000 species of higher plants in the world have been studied for their potential as medicines [1, 2].

Plants are the source of close to one-fourth of pharmaceutical medications. As an illustration, study on the often used local plant Glycyrrhiza glabra led to the discovery of carbenoxolone, the first medication helpful in treating gastrointestinal ulcers. Gefarnate was discovered as a result of research on cabbage. Foxglove (Digitalis purpurea), a traditional herbal remedy from Europe, was successful, as British physician William Withering discovered in the 18th century. Cardiac glycosides make the heart contract more forcefully and give it more time to rest in between beats [3, 4]. From foxglove leaves, more than 30 cardiac glycosides, including digitoxin and digoxin, have been discovered in the 20th century. Reserpine, an alkaloid still used today to treat high blood pressure, was first isolated from Rauwolfia root in 1949 by German chemists. A Chinese chemist discovered artemisinin, a sesquiterpene lactone from the wormwood plant, in 1972. Artemisinin is the main biologically active ingredient in treating malaria. Alkaloids from Catharanthus roseus (Madagascar periwinkle), which are employed in chemotherapy for children leukemia and the

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treatment of Hodgkin's disease, were found thanks to research on traditional plants conducted in the United States. The taxol chemical with anticancer properties was identified in the bark of Taxus brevifolia [5-7].

Flavoring medications are becoming the therapy of choice in Asian nations, especially for the agricultural population. The safety, effectiveness, and side effects of therapeutic plants vary. Ancient writings also describe the benefits of herbal remedies for age-related illnesses like dementia, osteoporosis, osteoarthritis, diabetes, immunological and liver disorders, for which there is no modern medicine or only palliative care. They are thought to be more compatible with the human body since they contain chemical components that are essential to the physiological processes of living things [8-10].

Analgesics, antibiotics, and non-steroidal anti-inflammatory medicines are some of the therapy options that are available for wound management. But the bulk of these treatments result in a variety of undesirable side effects. Numerous investigations into the potential of herbal medicines for the treatment of wounds have been conducted in recent years. In comparison to synthetic medications now on the market for the treatment of wounds, these natural therapies have demonstrated their efficacy. Pharmacological studies have revealed that a variety of natural herbs have strong wound-healing properties [11-13].

The primary objective of the present study is to provide a comprehensive analysis of the aqueous and ethanolic extract derived from the leaves of Hygrophila balsamica, with a particular focus on elucidating their phytochemical composition and evaluating their potential pharmacological activities. The objective of the present study is to examine the phytochemical composition, in-vitro pharmacological properties, and characterization of aqueous and ethanolic extracts derived from the leaves of Hygrophila balsamica.

MATERIALS AND METHODS

The reagents, solvents, and chemicals employed in this study were of analytical grade and were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai and Qualigens Fine Chemicals Pvt. Ltd, Mumbai. The foliage of Hygrophila balsamica was procured from agricultural terrain in Karnataka, in the month of January. Department of Zoology, conducted the authentication process on the gathered leaves.

Preparation of plant material

Hygrophila balsamica leaves that had been taken from the entire plant were first cleaned and rinsed with tap water, then with distilled water. For two weeks, the plant leaves were thoroughly dried in the shade at room temperature. The leaves were mechanically ground into a powder and sieved through a 60 mesh screen. The sieved material was kept in a container that was tightly sealed [14].

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Organoleptic Study

The evaluation of organoleptic characteristics pertains to the examination of physical attributes and sensory qualities, including but not limited to morphology, odor, color, taste, touch, and texture. The assessment of the sensory attributes of Hygrophila balsamica powder was conducted using accepted methodologies and protocols [15].

Physicochemical study

The physicochemical constants, namely ash value, extractive value, pH, and loss on drying, were assessed using known methodologies and procedures.

Ash Value

The determination of ash values is a valuable method for assessing the quality and purity of crude pharmaceuticals. It also provides crucial insights into potential adulteration, especially in relation to powdered plant sources. The primary objective of ashing crude pharmaceuticals is to completely remove any organic matter residues that could potentially disrupt the accuracy of analytical determinations. Upon combustion, the raw medicinal substances often yield an ash that predominantly consists of calcium, sodium, magnesium, and potassium silicates, as well as phosphates and carbonates [16, 17].

Ash value (Total)

A quantity of approximately 4 grams of powdered leaves from the Hygrophila balsamica plant was precisely measured and evenly spread within a silica crucible. The plant powder leaves included in the silica crucible were subjected to ignition at temperatures ranging from 500 to 600°C until all the powder leaves in the crucible turned entirely white, demonstrating the absence of carbon. The crucible was allowed to cool in ambient air and afterwards weighed. The total ash content was determined in relation to the powder obtained from air-dried plant leaves.

Acid-insoluble ash

A total of 25 mL of diluted hydrochloric acid was used to wash the ash from the crucible into a 100 mL beaker. Put the mixture in a beaker, cover it with a watch glass, and bring it to a boil over a hot plate. After 5 minutes, using ash-less filter paper, we filtered out the acid insoluble ash and washed the paper twice with hot distilled water to remove any lingering residue. The ashes were poured into the crucible, dried on a hot plate, and then burned until their weight remained constant. The crucible's residue was cooled in the desiccators and promptly weighed. We used the powdered air-dried plant leaves to determine the acid insoluble ash.

Water-soluble ash

The entire crucible's worth of ash was rinsed in a 100-mL beaker with 25 mL of distilled water. The mixture boiled for 5 minutes on a hot skillet after being sealed in a beaker with a watch glass. Ashless filter paper was used to filter the acid insoluble ash, and then it was cleaned twice with

hot distilled water, all within a 5-minute period. The leftovers were weighed after being dried on a hot plate in a crucible. After letting the crucible residue cool in desiccators, it was weighed. Acid insoluble ash was determined using powdered, air-dried plant leaves.

Sulphated Ash

Approximately 2 grams of plant leaf powder in coarse form were carefully measured and placed into a silica crucible. The silica crucible was then gently heated, ensuring that the plant material was totally incinerated. The residue was subjected to cooling, followed by moistening with 1 mL of sulfuric acid (H2SO4). The residue was heated until no white vapors were observed. The residue was subjected to a temperature of 800°C, resulting in the complete combustion of all-black particles. The crucible was cooled, followed by the addition of a small quantity of H2SO4, which was subsequently ignited. The residue in the crucible was allowed to cool in the desiccators and promptly weighed. The sulphated ash was determined in relation to the powder of air-dried plant leaves.

Extractive values

These values are beneficial for the analysis of phytoconstituents found in the unrefined plant materials. Furthermore, extractive values serve to indicate the composition of phytoconstituents present in the raw plant materials [18, 19].

Petroleum ether soluble extractive

Put 5 grams of the air-dried, coarse powder of Hygrophila balsamica leaves into the iodine flask, and soak them in 100 milliliters of petroleum ether for a day. After being left undisturbed for eighteen hours after being shook for six, the iodine flask was finally ready to be used. Without losing any of the petroleum ether, the powdered leaves were removed from the mixture right away. Using a flat-bottomed shallow dish, 25 mL of the filtrate extract solution was transferred, and the petroleum ether was evaporated in a water bath heated to 105°C. We gathered the leftovers and had them weighed. The extractive's solubility in petroleum ether was determined using air-dried leaf powder as a reference.

Chloroform soluble extractive

For one day, place 5 grams of the air-dried, coarse powder of Hygrophila balsamica leaves in 100 milliliters of chloroform in an iodine flask. After being left undisturbed for eighteen hours after being shook for six, the iodine flask was finally ready to be used. The mixture was filtered instantly to remove the leaf powder without wasting any of the chloroform. Using a water bath heated to 105 degrees Celsius, the chloroform in 25 milliliters of filtrate extract solution was evaporated. We gathered the leftovers and had them weighed. Extractive solubility in chloroform was determined using air-dried leaf powder as a standard.

Acetone soluble extractive

Leaves from 5 grams of air-dried Hygrophila balsamica were ground into a coarse powder and macerated in 100 milliliters of acetone in an iodine flask for 24 hours. After being left undisturbed for eighteen hours after being shook for six, the iodine flask was finally ready to be used. The powdered leaves were removed from the mixture and filtered instantly to prevent methanol loss. The acetone in the extract solution was evaporated in a water bath at 105 °C, and then 25 mL of the solution was transferred to a shallow dish with a flat bottom. We gathered the leftovers and had them weighed. Extractive solubility in acetone was determined using air-dried leaf powder as a standard.

Ethyl acetate soluble extractive

For one day, in the iodine flask, macerate 5 grams of the coarse powder of air-dried Hygrophila balsamica leaves in 100 milliliters of ethyl acetate. After being left undisturbed for eighteen hours after being shook for six, the iodine flask was finally ready to be used. The powdered leaves were removed from the mixture and filtered instantly to prevent methanol loss. Using a flat-bottomed shallow dish, we transferred 25 mL of the filtrate's extract solution and evaporated the ethyl acetate from the solution in a water bath heated to 105 °C. We gathered the leftovers and had them weighed. Extractive solubility in ethyl acetate was determined using air-dried leaf powder as a standard.

Ethanol soluble extractive

Put 5 grams of the air-dried, coarse powder of Hygrophila balsamica leaves into the iodine flask, and soak them in 100 milliliters of ethanol for a day. After being left undisturbed for eighteen hours after being shook for six, the iodine flask was finally ready to be used. Without losing any ethanol, the powdered leaves were removed from the mixture right away. The ethanol in the extract solution was evaporated in a water bath at 105°C, and then 25 mL of the solution was transferred to a shallow dish with a flat bottom. We gathered the leftovers and had them weighed. Extractive solubility in ethanol was determined using air-dried leaf powder as a standard.

Water soluble extractive

For one day, in the iodine flask, macerate 5 grams of the air-dried, coarse powder of Hygrophila balsamica leaves in 100 milliliters of distilled water. After being left undisturbed for eighteen hours after being shook for six, the iodine flask was finally ready to be used. Without losing any of the distilled water, the powdered leaves from the combination were filtered away. In a shallow dish with a flat bottom, 25 mL of the extract solution was heated to 105°C in a water bath to evaporate the distilled water. We gathered the leftovers and had them weighed. Using air-dried leaf powder, we were able to determine the extractive's water solubility.

LOD

The leaves were ground into a powder and then placed in a glass bottle with a stopper before being

dried in an oven. The powdered leaves were dried until their weight was uniform and then chilled. The bottle and its contents were weighed separately. The technique was continued until there was no more than a 0.5 mg change in weight. Percentage of weight loss was determined using air-dried plant material as a standard [20, 21].

Phytochemical studies

The leaves of Hygrophila balsamica were utilized for phytochemical investigations, whereby they were pulverized and subjected to extraction using water and ethanol. Subsequently, a phytochemical screening was conducted to identify several phytoconstituents.

FLAVONOIDS TEST
Alkaline Reagent Test

A volume of 5 mL of the extract solution underwent hydrolysis using a 10% sulfuric acid solution, followed by cooling. Subsequently, the substance was extracted using diethyl ether and subsequently partitioned into three distinct fractions, each allocated to an individual test tube. In the first test tube, 1 milliliter of diluted sodium carbonate was added. In the second test tube, 1 milliliter of 0.1 normal (N) sodium hydroxide was added. Lastly, in the third test tube, 1 milliliter of diluted ammonia solution was added. The presence of flavonoids is indicated by the development of a yellow tint in each test tube [22, 23].

Shinoda`S Test

The specimen was dissolved in an alcohol-based solution, then accompanied by the introduction of a magnesium fragment and strong hydrochloric acid in a gradual and controlled way. The mixture was then subjected to heat. The observation of the magenta coloration serves as an indication of the existence of flavonoids.

Test for gums and mucilage

The extract underwent treatment with 25 mL of 100% alcohol, followed by filtration of the resulting solution. The swelling properties of the filtrate were assessed.

Test for glycosides
Cardiac glycoside
To a 2 mL aliquot of extract, glacial acetic acid, one drop of 5% ferric chloride, and concentrated sulphuric acid were introduced. The manifestation of a reddish brown hue at the interface between the two liquid phases signifies the existence of cardiac glycosides [24, 25].

Anthraquinone glycosides

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An equal volume of benzene or chloroform was added to the cold filtrate. The organic phase was partitioned and subsequently treated with ammonia. The observation of a pink or red color change in the ammonical layer shows the existence of anthraquinone glycosides.

Thin Layer Chromatographic Profile (TLC)

The chromatographic approach developed by Tswett is widely regarded as the most valuable technology for isolating and separating elements of plants. All solids that are finely split possess the ability to adsorb other substances or have the potential to be adsorbed themselves. The phenomenon of selective adsorption constitutes the fundamental principle underlying chromatography.

.Selection of Mobile Phase

The selection of the solvent mixture for development was based on the specific phytoconstituents found in the extract. The solvents were examined in terms of their rising polarity. Multiple mobile phases were tested in order to achieve the optimal separation of the maximum number of phytoconstituents. After conducting numerous trials, the most effective solvent system was chosen.

Development of Chromatogram

The solution obtained from the extract was transferred into a capillary tube and subsequently applied as a spot on thin-layer chromatography (TLC) plates, positioned 2cm above the lowermost point of the plates. The initial points were allocated with equal sizes to the greatest extent feasible. The plates were subsequently introduced into a chromatographic tank utilizing a mobile phase. The plates were permitted to undergo development along three-fourths of their length before being extracted. The position of the solvent front was promptly identified and the plates were afterwards left to dry. Subsequently, the plates were subjected to examination under ultraviolet (UV) light or treated with various spraying reagents. The spots were detected and the corresponding Rf values were recorded [26, 27].

In-vitro pharmacological investigations on antioxidant

DPPH radical scavenging activity of extract activity

One milliliter of a 0.2 mM DPPH solution in methanol was mixed to one milliliter of various concentrations of aqueous and ethanol extract and standard, ascorbic acid (100-400 g/mL). After letting the mixture sit undisturbed for 30 minutes at room temperature, the absorbance was measured using a UV-Visible spectrophotometer set to 517 nm. Each test was repeated three times before an average was calculated [27, 28].

Extracts' in vitro anti-inflammatory action inhibits albumin denaturation

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Egg albumin aqueous solution (1% w/v) was combined with 1.5 mL of test solutions containing 100–400 g/mL of the ethanol extract or standard Diclofenac sodium (150 and 200 g/mL) solution, incubated at 37°C for 20 minutes, and then heated in a water bath at 51°C for 20 minutes. The turbidity was checked in a UV-Visible spectrophotometer after cooling by comparing the sample to a reagent blank at 660nm. The experiments were repeated three times, and the average was recorded [28, 29].

RESULTS AND DISCUSSION

Phytochemical Investigation

The findings of a preliminary phytochemical investigation of Hygrophila balsamica's aqueous and ethanol extract were recorded in Table using conventional protocol. Alkaloids, glycosides, flavonoids, steroids(only in ethanolic extract), tannin, carbohydrate, and protein were found in both the aqueous and ethanolic extracts of Hygrophila balsamica, while gums and mucilage, phenols, sterols, and terpenoids were not. Identification of Phytoconstituents in Hygrophila balsamica Leaf Extracts Prepared with Water and Ethanol

Table 1: Preliminary study on photochemistry

S. No	Tests	Presence or Absence in ethanolic extract	Presence or Absence in aqueous extract
1	Alkaloid	Present	Present
2	Gums and mucilage	Absent	Absent
3	Glycoside	Present	Present
4	Flavonoid	Present	Present
5	Phenol	Absent	Present
6	Steroid	Present	Absent
7	Sterols	Absent	Absent
8	Tannin	Present	Present
9	Terpenoid	Absent	Absent
10	Carbohydrate	Present	Present
11	Protein	Present	Present

TLC analysis

Table and Figure displayed the results of a TLC analysis performed on an ethanol extract of Hygrophila balsamica leaf material. TLC was used for qualitative chromatographic analysis of the ethanol extract to separate and identify the individual or combined phytoconstituents present in the extract. Three spots (Rf values of 0.2, 0.4, and 0.7) were observed in the ethanol extract, indicating the presence of these three types of phytoconstituents in the ethanolic extract of Hygrophila balsamica leaves. There are two distinct areas in the aqueous extract of leaves, indicating the

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presence of one main Phytoconstituent.

Table 2: Rf values of Hygrophila balsamica's ethanol extract

Solvents system	Distance traveled by solute	Distance traveled by solvent	Detecting agent	Rf value
Ethyl acetate: methanol: (5:5)	1.8	2.8	Iodine chamber	0.8
	2.1			0.7
	2.2			0.8

Table 3: Rf values of Hygrophila balsamica's aqueous extract

Solvents system	Distance traveled bysolute	Distance traveled bysolvent	Detectingagent	Rf value
Ethyl acetate: methanol:	2.4	2.8	Iodine chamber	0.68

Calculation of percentage inhibition

The following formula was used to determine the percentage of inhibition of various concentrations of aqueous and ethanol extracts of Hygrophila balsamica leaves compared to the standard drug for in-vitro methods including the assay of DPPH radical scavenging activity and the inhibition of albumin denaturation.

% inhibition = (A0 – A1 / A0) × 100

Where A0 - is the absorbance of control and

A1 - is the absorbance of test or standard

DPPH radical scavenging activity of aqueous and ethanol extracts in vitro

Tableau and figure detailing the ethanol extract's DPPH radical scavenging activity assay. Antioxidants such anthocyanins, phenolic compounds, and crude extract of medicinal plants were tested on the DPPH free radical as a substrate. Specific antioxidant interactions with the free radical DPPH formed the basis for the reaction. The purple-colored DPPH is a stable free radical that has an odd electron. The DPPH free radical's extra electron causes a sharp peak in absorption at 517 nm. When odd electrons take up hydrogen from free-radical-scavenging antioxidants, the result is a yellowish hue. Since the concentration of DPPH free radicals was decreased, so did the absorbance at 517nm.Concentrations between 100 and 400 g/mL showed a linear rise in DPPH radical scavenging activity. Significant DPPH radical scavenging activity was observed for the extract across all concentrations. At a concentration of 400 g/mL, both extracts had a lower proportion of DPPH radical scavenging action compared to ascorbic acid.

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Table 4: Hygrophila balsamica leaf aqueous and ethanol extract DPPH radical scavenging activity

Sr. No	Concentration (µg/mL)	% of activity (±SEM)		Ascorbic acid
		Ethanolextract	Aqueous extract	
1	50	23.87	21.64	28.33
2	100	30.25	22.67	37.88
3	150	34.78	24.07	42.32
4	200	42.57	28.05	53.87
5	250	52.86	35.88	65478
6	300	65.88	38.77	71.72
7	350	71.58	42.65	75.47

Anti-inflammatory action of ethanol extract inhibits albumin denaturation in vitro.

Denaturation occurs when a protein is subjected to extreme conditions, such as high temperatures, organic solvents, strong acids, bases, or concentrated inorganic salts, and the protein loses its secondary and tertiary structure. Both extracts were tested for their capacity to prevent protein denaturation. Increasing the concentration of the extracts and diclofenc from 100 to 400 g/ml boosted the inhibitory action. Significant suppression of albumin denaturation was seen at all doses of the extract. Diclofenac revealed much higher % inhibition values than aqueous and ethanolic leaf extracts [30].

Table 5: Hygrophila balsamica leaf aqueous and ethanol extract inhibits albumin denaturation by %.

Sr. No	Concentration	% of activity		
		Ethanol extract	Aqueous extract	Diclofenac
1	50	20.22	13.74	58.11
2	100	31.33	15.78	71.14
3	150	38.17	21.11	---
4	200	43.78	23.22	---
5	250	53.98	26.88	---
6	300	60.78	31.47	---
7	350	63.49	33.87	---

Physicochemical constituents

The leaves of *Hygrophila balsamica* are broken down into its component physicochemical, such as their ash and extractive values, loss on drying, and pH. Analysis of physicochemical constants is often used to determine the authenticity and quality of the leaves. Alcohol had a higher extractive value than ethyl acetate, suggesting that it may be the better solvent for releasing the leaves' phytoconstituents. Sugars, tannins, glycosides, mucilage, plant acids, etc., that dissolve in water are extracted from medicinal plants using their water-soluble extractive value. Many phytoconstituents, including alkaloids, resins, tannins, etc., can be extracted with alcohol as the

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optimum solvent. Fixed oils, volatile oils, steroids, and resins can all be extracted with the help of an ether-soluble extractive value. The ash content indicates the percentage of inorganic material and other contaminants in the medication. For powdered leaves, the percentages of acid insoluble ash, total ash, water soluble ash, and sulfated ash were as follows. If the ash values are off in any way, the medicine may have been tampered with. Our results show that the ash content is within acceptable ranges, ruling out the possibility of adulteration. Less moisture is present in the leaves powder, as measured by the moisture content or loss on drying value. Plant leaves can deteriorate due to the presence of moisture in the powdered leaves, which can promote the growth of microorganisms or enzyme hydrolysis. Low moisture content is really suited for high stability of crude pharmaceuticals, which is why a powdered form of the leaves is preferable for long-term preservation [31].

Table 6: The physicochemical components

Sr. No	Parameters	Results
1	Total ash	8.57
2	Water soluble ash	10.68
3	Acid insoluble ash	5.24
4	Sulfated ash	12.11
6	Petroleum soluble extract	4.56
7	Chloroform soluble extract	4.24
8	Acetone soluble extract	10.14
9	Ethyl acetate soluble extract	10.00
9	Ethanol soluble extract	20.32
10	Water soluble extract	8.35
11	Loss on drying	7.14
12	pH	8.55 & 4.32

CONCLUSIONS

The primary objective of this study is to conduct a phytochemical analysis and assessment of the leaves of Hygrophila balsamica in order to ascertain the validity of their traditional medicinal properties. The investigation reveals the presence of alkaloids, glycosides, flavonoids, steroids, tannin, carbohydrate, and protein in the aqueous and ethanolic extracts, whereas the absence of gums, mucilage, phenols, sterols, and terpenoids is observed. The analyzed samples exhibited notable antioxidant and anti-inflammatory properties, indicating promising prospects for medicinal applications. The results of this study are expected to inspire other investigations in the fields of phytochemistry and therapeutic application.

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None

Conflict of Interest

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None

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