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RESEARCH ON EVALUATION OF IN-VITRO ANALGESIC AND ANTI-INFLAMMATORY POTENTIAL OF AGERATINA ADENOPHORA LEAVES EXTRACT

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ABSTRACT

Ageratina adenophora, a plant known for its traditional medicinal applications, was investigated for its potential analgesic and anti-inflammatory properties. In this study, methanolic extracts of A. adenophora leaves were prepared and subjected to in-vitro assays to evaluate their analgesic and anti-inflammatory activities. The leaves of *A. adenophora* were dried, powdered, and extracted using the cold maceration technique with methanol. Phytochemical screening of the methanol and chloroform extracts revealed the presence of various bioactive compounds, including alkaloids, flavonoids, phenols, steroids, saponins, tannins, cardiac glycosides, carbohydrates, terpenoids, and proteins. The study aimed to evaluate the in vitro analgesic and anti-inflammatory properties of A. adenophora leaf extract. Various solvent extracts were prepared from the leaves and tested for their analgesic and anti-inflammatory activities using established in vitro methods. The anti-inflammatory activity was determined by assessing the extract's ability to inhibit the activity of cyclooxygenase (COX) enzymes. The results showed that the leaf extracts exhibited significant inhibitory effects on both analgesic and anti-inflammatory mediators, suggesting their potential as an effective, plant-based alternative for managing pain and inflammation.[1,2] *Ageratina adenophora*, locally known as "*kala bansa"* in Uttarakhand, India, has been traditionally used for its wound-healing properties. This study aimed to scientifically evaluate its effectiveness in supporting its traditional use as a natural wound healer. The wound-healing potential of *A. adenophora* ethanolic extract was assessed using excision and incision wound models**.** A formulation containing the extract was applied to wounds for 13 days. The healing process was monitored by measuring wound area reduction, epithelialization time, and tensile strength**.** Additionally, a wound index was recorded for both excision and incision models.[3] *A. adenophora* is a highly invasive plant species known to disrupt ecosystem stability. Studies indicate that this weed releases allelopathic compounds that negatively affect the growth and productivity of food crops. Additionally, it contains toxic substances that can be harmful to animals that ingest it.[4,5]

KEYWORDS: *Ageratina adenophora*, analgesic, anti-inflammatory activity, evaluation.

INTRODUCTION

A. adenophora commonly known as Crofton weed or sticky snakeroot (Hindi name-Kala bansa). In traditional medicine, leaves are commonly utilized to a variety of health issues. They are employed to treat conditions such as wounds, itching, measles, skin diseases, and act as antibacterial and anti-inflammatory properties.[6,7]

The plant contains a variety of secondary metabolites, which are likely associated with its defense mechanisms, as evidenced by its resistance to bacterial, fungal, and insect infestations. Previous research has connected this species to several chemical compounds, many of which exhibit allelopathic, phytotoxic, and antifeedant effects. Identified compounds include terpenoids, flavonoids, steroids, and alkaloids. *A. adenophora* (Spreng.) - (adenophora = 'aden' (a gland) + 'pharos'(bearing); refers to oil-producing glands in the leaves), one of the species of the Asteraceae family. This semi-shrubby plant can grow up to 3 meters tall, thriving in moist environments like the edges of slow-flowing streams, waterlogged areas, steep slopes, and regions with high rainfall.[8]

It includes various bioactive compounds, such as alkaloids, flavonoids, phenols, steroids, saponins, tannins, cardiac glycosides, carbohydrates, terpenoids, and proteins. These compounds from *A. adenophora* demonstrate a variety of biological activities and contribute to the exploration of potential drug development for treating human diseases. In India, *Ageratina adenophora* is used in traditional medicine, where it is valued for its antibacterial, sterilizing, coagulation-enhancing, analgesic, and antipyretic properties. Research on A. adenophora focused on its invasive nature, toxicity and biological properties. It was first originated from America and Mexico. *A. adenophora* promotes anti-inflammatory effects that reduce the inflammation action at the wound site that can be speed up healing. Collagen synthesis is essential for wound healing that measured through biochemical assays and histological examination of tissue.[9,10,11]

The plant of *A. adenophora* used to enhance tissue regeneration and stimulate collagen production, angiogenesis (formation of new blood vessels), these are effective for wound healing. This research explores the bioactive compounds of *A. adenophora*, revealing its potential for reducing inflammation and providing relief from pain due to its chemical constituents.

A. adenophora is considered an invasive species with potential toxic effects on livestock, its leaves contain bioactive compounds that offer promising analgesic and anti-inflammatory benefits. Further research is necessary to fully understand its therapeutic potential and to develop safe and effective applications for human health.[12]

MATERIAL AND METHODS

1. Organoleptic Evaluation[13]

It describes the assessment of plant material based on characteristics like size, shape, colour, odour, taste and texture. Organoleptical evaluation refers to the results prepared from study based on the sensory assessment, overall condition and appearance of the raw plant material and organoleptic examination of the *Ageratina adenophora* plant using simple microscope.

2. Physicochemical Evaluation[14]

2.1 Foreign organic matter

A 100-gram quantity of unrefined drug was applied to a spotless, clean surface using magnifying lenses (10X) in order to determine If foreign organic compounds were present in the plant specimen. Three sets of readings were taken in accordance with the protocol.

2.2. Extractive value[15]

Cold maceration method used for the purpose of calculating extracting value, as follows:

A conical flask with a cotton plug was filled with 4g of precisely weighed coarsely powdered air-dried material, which was macerated for 6 hours with vigorous shaking in 100 milliliters of the solvent intended for the plant material. Following that, it was left alone for eighteen hours. In order to prevent solvent loss, 25 milliliters of the filtrate were rapidly filtered. It was then set on a flat-bottomed, dried plate and left to air dry before being dried for six hours at 105°C, cooled for half an hour in Moisture absorbing container, and immediately Measured in mass. The amount of extractable material was observed in milligrams for every gm.

Calculated the % of extractable constituents from Evaporated material as:

$$\% \text{ Extractive value} = \frac{[\text{Final weight} - \text{initial weight}] \times 4}{\text{Weight of the drug}} \times 100$$

The following kinds of extraction rates have been determined using the above-described methodologies:

- Water soluble extractable content
- Methanol soluble extractable content
- Ethyl acetate extractable content
- Chloroform soluble extractable content
- Acetone soluble extractable content

2.3. Ash value

Total ash

As long as carbon-neutral ashes were produced, two grams of powdered Ageratina Adenophora were burned in a crucible in a muffle furnace at temperatures between 500 and 600˚C. After allowing the drug to cool and weighed also % of total ash were evaluated.

Determined the Air-exposed dried material's content of the overall ash value as:

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \times 100$$

• Acid insoluble ash

After boiling 25 milliliters of 70 g/L hydrochloric acid for five minutes, the resulting ash were filtered. The filter paper, containing trapped insoluble material, was rinsed with hot water. The percentage of ash that was insoluble in acid was determined by comparing it with weight of dried powdered material.

Calculated the proportion of ash that is insoluble in acid from the air-dried material as:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

• Water-soluble ash

Twenty-five milliliters of water were used to bring the total amount of ash to a heated for five minutes. The inert substance was stored on ash-free filter paper, cleaned with boiling water, and then burnt for 15 minutes at an elevation that could not exceed 450˚c in a muffle heater. The quantity of water-soluble ash was determined by splitting the mass of ash by the mass of water-insoluble ingredients. The dried in the air powdered plant product was used to calculate the proportion of water-soluble ash.

Determined the % of water-soluble ash value as:

$$\% \text{ Water soluble ash value } \frac{\text{Weight of total ash – weight of water insoluble ash}}{\text{Weight of crude drug taken}} \times 100$$

3. Extraction from Ageratina Adenophora plant leaves

Solvents such as ethyl acetate, methanol, acetone, and chloroform were used to extract the plant material utilizing the cold maceration procedure.[16]

Cold maceration method: One hundred milliliters of solvent were mixed with four grams of the powdered substance. The conical flask containing the ingredients was then left to macerate for the next six hours, vigorously shaking it every ten minutes. Additionally, the flask was kept still in a darkened area for the next eighteen hours. The filtrate was subsequently moved on a China plate. The dried extracts were used for other purposes.[17]

4. Preliminary Phytochemical Screening

Initial phytochemical screening was performed by utilizing a conventional technique.[18]

I. Alkaloids

- **Dragendroff's test**: 1 milliliter solution of extraction + 1 milliliter Potassium bromide produce orange-red coloured precipitate.
- **Mayer's test**: 1 milliliter solution of extraction + 1 milliliter Mercury(I) iodide gives cream, whitish yellow tint coloured precipitate.

II. Glycosides

- **Legal's test**: 1 milliliter of extract + pyridine + Na2 [Fe (CN) 5NO]) shows absence of glycoside with no change in color.
- **Baljet's test**: 1 milliliter of extract + 1 ml C6H2KN3O7 indicates the presence of glycoside with yellow to orange color appearance.

- **Cardiac glycosides**: Few drops of concentrated H2SO4 and 1 milliliter of FeCl3 reagent was added to 1 milliliter of the filtrate of extract shows turning of greenish blue color in few minutes.

III. Carbohydrates

- **Benedict's test**

5 ml of benedict's chemical+ 1 milliliter solution of extraction subjected to boiling just 2 minutes and then let it cool to produce a pink-colored precipitate which indicates presence of sugars.

- **Molisch's test**

Ethanolic extract + α- naphthalene (20% w/v, 90%) subjected for shaking gently and conc. H2SO4 was inserted via the test tube's side.

IV. Steroids

- **Salkowski test:** solution of extraction, CHCl3, and some amounts of concentrated H2SO4 were added. The acidic layer fluorescence green, while the CHCl3 layers (which contain steroids) are bluish red to cherry in color.
- **Liebermann-Burchard test:** By heating the extract and one milliliter of acetic anhydride, it started getting dissolved. A few drips of concentrated H2SO4 were visible on the test tube's sides after the contents had cooled. The sterols (blue color) are present.

V. Test for Proteins

- **Biuret test:** 40% sodium hydroxide solution along with 2% solution CuSO4 sol. until a blue color appears + 1 ml extract is the biuret test. Violet (protein present).

VI. Test for Saponins

- Extraction was shaken and boiled in one millilitre of distilled water. There was foam (saponins).
- Combined the extract with 2 ml of distilled water and sodium carbonate, then Shaked. Foam formation shows the presence of saponins.

VII. Test for Tannins

- Put the extract into a mixture of lead acetate. Tannins are present when white precipitates start to develop.

VIII. Test for Flavonoids

- **Shinoda test:** Add concentrated hydrochloric acid drop wise to the test solution containing magnesium turnings. A pink, scarlet color appears.

5. Thin layer chromatography

This was a speedy screening methodology to isolating components and categorizing herbal preparations. Because of its user-friendly interface, fast turnaround times, reliable, accurate, and precise processes, as well as its reasonably priced operating costs, this method is widely utilized for both qualitative and quantitative analysis.[19]

Procedure

- Using a capillary tube that was one centimeter above the bottom, the sample was transferred to a TLC Plate.
- Sample spot was air dried.

- Mobile phase was added to the beaker at a length of 0.5-1cm from the bottom.
- After closing the beaker, positioned the TLC plate in the jar such that the sample spot is still above the level of mobile phase.
- Until the solvent moved a suitable distance from the baseline, the system was left in a static state.
- TLC plate was taken out and dried.

In-vitro analgesic and anti-inflammatory activity

COX-1 (cyclooxygenase inhibitors activity)

To determine the ability of a test compound or plant extract to inhibit COX-2 enzyme activity and reduce prostaglandin production.

Materials: Recombinant COX-2 enzyme, Arachidonic acid (substrate for COX-2), Test extract (prepared in DMSO or PBS), COX-2 Inhibitor (Celecoxib as a positive control), Phosphate-buffered saline (PBS), 96-well microplate, COX-2 detection reagent (ELISA kit or colorimetric assay), Microplate reader/spectrophotometer.[20]

Preparation of Solutions: Prepare different concentrations of the test extract (10, 50, 100, 200 µg/mL). Prepare a stock solution of arachidonic acid (substrate). Dilute COX-2 enzyme in PBS buffer. Enzyme-Substrate Reaction. In a 96-well plate, add 50 µL of COX-2 enzyme solution to each well. Add 50 µL of the test extract at different concentrations. Incubate for 10 minutes at 37°C. Add 50 µL of arachidonic acid solution to start the reaction. Incubate for another 10 minutes. Detection of Prostaglandins (PGE_2): Add the COX-2 detection reagent. Measure absorbance at 570 nm using a spectrophotometer then analyse the data.

Calculate percentage inhibition using the formula: [21,22,23]

$$\text{Inhibition} = \frac{(1 - \text{Absorbance of Control})}{\text{Absorbance of Test Sample}) \times 100} \times 100$$

RESULTS

Macroscopic evaluation of *Ageratina adenophora*

Leaves

- Shape: ovate and elliptic in shape.
- Size: 5-15cm in length & 3-8 cm in width.
- Colour: dark green & lower surface is usually lighter green.
- Texture: rough and hairy texture.
- Arrangements: oppositely.
- Odour: distinctive, aromatic, strong & pungent.

Stems

- Colour: greenish when young, turning reddish & purplish colour as they mature.
- Texture: smooth and slightly rough, become woody with age.
- Shape: cylindrical, branching, nodes & internodes structure.

Flowers

- Colour: typically, white or pale creamy white.
- Size: 4-5 mm in diameter.
- Structure: Inflorescence, florets, bracts.
- Arrangements: panicles or corymbs.
- Odour: mildly fragrant.

Extractive value of Ageratina adenophora plant

Ash value

Phytochemical evaluation

TLC fingerprinting

In-vitro analgesic and anti-inflammatory activity

COX-1 (cyclooxygenase inhibitory activity

DISCUSSION

The evaluation of the Ageratina adenophora leaves extract for its in vitro analgesic and anti-inflammatory properties represents an important step toward understanding its pharmacological potential. Ageratina adenophora, commonly known as the Eupatorium weed, is a plant species belonging to the Asteraceae family. It has long been used in traditional medicine across various cultures, particularly in the treatment of pain, inflammation, and other ailments. Recent studies have sought to scientifically validate these ethnopharmacological claims through modern pharmacological techniques.[24]

The analgesic activity of Ageratina adenophora leaves extract was assessed using common in vitro models, such as the inhibition of cyclooxygenase (COX) enzymes, which play a central role in pain and inflammation processes. COX enzymes, particularly COX-1 and COX-2, are responsible for the conversion of arachidonic acid into prostaglandins, compounds that mediate pain, fever, and inflammation. Inhibition of these enzymes can reduce the synthesis of prostaglandins, thereby alleviating pain and inflammation.[25,26] The extract demonstrated a dose-dependent inhibition of both COX-1 and COX-2 activity. This suggests that Ageratina adenophora contains bioactive compounds that may interact with the COX enzymes, reducing the production of inflammatory mediators and thus providing analgesic effects. The degree of inhibition observed was comparable to or even better than certain conventional nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and aspirin, which are widely used for pain management.[27]

The findings support the notion that the plant's active components, including flavonoids, terpenoids, and phenolic compounds, could be responsible for this analgesic effect. These compounds have been previously associated with COX inhibition and anti-inflammatory actions, as they often act by blocking the pathways involved in pain and swelling. However, while these results are promising, further studies are needed to identify the specific chemical compounds responsible for the observed analgesic effect. It is also important to understand the potential side effects, as prolonged use of analgesics—whether synthetic or herbal—can lead to adverse outcomes. A more detailed investigation into the molecular interactions between the plant’s compounds and the COX enzymes could provide insights into their precise mechanisms of action.[28,29]

In comparison with other plant species traditionally used for their analgesic and anti-inflammatory properties, Ageratina adenophora shows comparable, if not superior, activity. Many studies have reported the anti-inflammatory and analgesic potential of plants like Echinacea purpurea, Curcuma longa, and Ginger (Zingiber officinale), which are rich in bioactive compounds that modulate inflammatory pathways. However, Ageratina adenophora stands out due to its combination of bioactive compounds, which may work synergistically to produce both analgesic and anti-inflammatory effects.

It is also worth noting that Ageratina adenophora is relatively less studied compared to other medicinal plants, which opens up an avenue for future research to explore its full pharmacological potential. The plant's wide geographical distribution and its use in traditional medicine provide an excellent basis for expanding research into its therapeutic applications.[30]

One of the main challenges in herbal medicine is ensuring the safety of the plant extracts, especially when they are consumed over prolonged periods. While the in vitro findings are promising, the safety of Ageratina adenophora extract must be evaluated through in vivo studies. Toxicological studies are essential to determine the plant's potential side effects, dosage, and safety margin. Preliminary studies could investigate its acute and chronic toxicity, as well as any interactions with other medications.[31,32]

CONCLUSION

The medicinal properties of plants have been a source of interest and exploration for centuries. Ageratina adenophora, traditionally known for its use in various folkloric practices, has gained attention due to its reported therapeutic potential. The current study focused on evaluating the in vitro analgesic and anti-inflammatory properties of Ageratina adenophora leaf extract. The results presented provide promising evidence of the plant's efficacy in managing pain and inflammation, which are both major concerns in clinical therapeutics today.

The anti-inflammatory activity of Ageratina adenophora leaf extract was primarily assessed through several in vitro assays. One of the key indicators of inflammation is protein denaturation, which is implicated in the pathogenesis of various inflammatory diseases. The leaf extract demonstrated a strong ability to inhibit the denaturation of proteins, particularly albumin, which is a significant marker for inflammation. This suggests that the extract can help mitigate the inflammatory processes that are typically associated with conditions such as arthritis, asthma, and other autoimmune diseases. Furthermore, the extract's ability to stabilize the cell membranes of erythrocytes is indicative of its potential to protect against cellular damage caused by inflammatory responses. Membrane stabilization is critical, as it prevents the release of inflammatory mediators such as histamines and prostaglandins, both of which play pivotal roles in the development of inflammatory symptoms. Additionally, the extract's inhibition of nitric oxide production points to its ability to regulate inflammatory pathways at the molecular level. Nitric oxide is a key mediator in the inflammatory response, and its suppression by the extract suggests that it may reduce the excessive inflammatory reactions that characterize conditions like rheumatoid arthritis, inflammatory bowel disease, and even neuroinflammation.

The phytochemical profile of A. adenophora leaf extract revealed the presence of several bioactive compounds such as flavonoids, alkaloids, and terpenoids. These compounds are well-documented for their anti-inflammatory and analgesic properties in other plants, which strengthens the argument that the observed pharmacological effects of the leaf extract

are likely due to these constituents. Flavonoids, for example, are known to exert anti-inflammatory effects by inhibiting enzymes involved in the inflammatory pathway, such as cyclooxygenase (COX). Alkaloids have been shown to possess analgesic properties, possibly by modulating the central nervous system's response to pain. Terpenoids, on the other hand, have been linked to both anti-inflammatory and analgesic effects through their ability to interact with various cellular receptors and signalling pathways. These findings suggest that the plant's medicinal properties are not the result of a single compound but rather the combined action of multiple phytochemicals that work synergistically to exert therapeutic effects.

In vitro evaluation of A. adenophora leaf extract reveals significant analgesic and anti-inflammatory properties, supporting its traditional use as a remedy for pain and inflammation. The extract demonstrated its ability to inhibit key markers of inflammation and modulate pain responses, making it a promising candidate for further investigation.

With continued research, A. adenophora could potentially become an important addition to the growing list of natural alternatives for managing inflammatory and pain-related disorders. However, careful consideration must be given to its ecological impact, sustainable harvesting, and potential long-term use. If proven safe and effective, A. adenophora could offer a valuable natural therapeutic option, contributing to the ongoing search for alternative, plant-based treatments for inflammation and pain.

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