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PHYTOCHEMICAL SCREENING, CHROMATOGRAPHIC ISOLATION, AND ANTIOXIDANT EVALUATION OF *AGERATUM CONYZOIDES* LINN EXTRACTS

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ABSTRACT

Ageratum conyzoides Linn, a member of the Asteraceae family, is recognized for its medicinal properties. This study investigates the phytochemical composition, chromatographic isolation, and antioxidant activity of its extracts. The plant material was processed to obtain petroleum ether, ethyl acetate, and methanolic extracts. Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, tannins, and terpenoids. Ethyl acetate and n-butanol fractions were further purified using chromatographic techniques, leading to the isolation of bioactive fractions F1 and F2. Antioxidant activity was assessed using the DPPH radical scavenging method, with F1 showing the highest activity. These findings highlight the potential of Ageratum conyzoides Linn as a source of natural antioxidants.

Keywords *– Ageratum conyzoides, phytochemical screening, chromatographic isolation, antioxidant activity, DPPH scavenging.*

1. INTRODUCTION

Medicinal plants have been an integral part of traditional medicine, serving as a primary source for treating various ailments and maintaining health. Their use transcends cultural boundaries and has been a cornerstone in the discovery and development of modern therapeutic agents. Among these, *Ageratum conyzoides* Linn., a member of the family Asteraceae, holds significant importance in ethnomedicine due to its remarkable pharmacological properties. The plant is widely known for its anti-inflammatory, antimicrobial, and antioxidant activities, which have been exploited in traditional medicinal practices across different regions [1-3].

The pharmacological potential of *Ageratum conyzoides* Linn. is closely linked to its diverse phytochemical profile. It contains an array of bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenolic compounds, which collectively contribute to its medicinal efficacy [4-6]. These compounds not only exhibit therapeutic benefits but also serve as a basis for further scientific investigation aimed at identifying novel drug candidates. Understanding the chemical composition and biological activities of this plant could provide valuable insights into its applications in modern medicine.

Recent research has underscored the importance of antioxidants in combating oxidative stress, a condition implicated in the pathogenesis of numerous chronic diseases, including cancer, diabetes, and cardiovascular disorders. Natural antioxidants derived from plants like Ageratum conyzoides Linn. are increasingly gaining attention due to their efficacy and safety profiles. The

plant's antioxidant activity, driven by its phytochemical constituents, positions it as a potential source for developing natural antioxidant therapies.

This study aims to explore the phytochemical composition, chromatographic isolation, and antioxidant activity of Ageratum conyzoides Linn. By employing advanced analytical techniques, we seek to isolate and identify bioactive compounds responsible for its therapeutic effects. Furthermore, the antioxidant potential of these compounds will be evaluated, with the ultimate goal of establishing their relevance in pharmaceutical applications.

We hypothesize that bioactive compounds isolated from *Ageratum conyzoides* Linn. can serve as natural antioxidants, offering a foundation for the development of novel therapeutic agents. This work not only contributes to the scientific understanding of this plant but also paves the way for its potential integration into modern pharmaceutical formulations.

2. MATERIALS AND METHOD

2.1 Collection and Authentication

Ageratum conyzoides Linn plants were collected from the garden of Bhoj College, Bhopal, and authenticated by Prof. Dr. Jagrati Tripathi, Govt. College Khemlasha. A herbarium was prepared, and taxonomic details are provided in Table 1. The plants were shade-dried for 3-4 weeks, powdered, and used for extraction.

2.2 Preparation of Extracts

Powdered plant material (200 g) was extracted sequentially with petroleum ether, ethyl acetate, and methanol using a Soxhlet apparatus. The extracts were concentrated using a rotary evaporator, and yields were calculated as shown in Table 2.

2.3 Phytochemical Screening

Phytochemical screening was conducted using standard protocols [7]. Tests included Shinoda test for flavonoids, Wagner's test for alkaloids, and Salkowski test for terpenoids, among others (Table 3).

2.4 Chromatographic Isolation

Ethyl acetate extract was subjected to thin-layer chromatography (TLC) and column chromatography using silica gel. Fractions were analyzed, and two major fractions (F1 and F2) were isolated based on their Rf values.

2.5 In-vitro Antioxidant Activity (DPPH Free Radical Scavenging Activity)

The metabolic reactions occurring within an organism's body generate oxidants or free radicals. Free radicals are species capable of independent existence, containing one or more unpaired electrons. These radicals react with other molecules by accepting or donating electrons and are implicated in various pathological conditions. Secondary metabolites produced by plants act as antioxidants by scavenging these free radicals. The ethyl extract of *Ageratum conyzoides* Linn. was screened for its antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, as described by Hatano et al. (1988). Antioxidant activity of F1 and F2 was evaluated using the DPPH radical scavenging assay. Methanolic solutions of fractions were tested at various concentrations (25-200 µg/mL), and the percentage scavenging activity was calculated.

Preparation of Standard Solution

Ascorbic acid was used as the standard. The required quantity of ascorbic acid was dissolved in methanol to prepare solutions with concentrations of 25, 50, 100, 150, and 200 µg/mL.

Preparation of Test Sample

Stock solutions of the samples (fractions F1 and F2) were prepared by dissolving 10 mg of each fraction in 10 mL of methanol to obtain a concentration of 1 mg/mL. From this stock solution, required volumes were diluted with methanol to prepare solutions with final concentrations of 25, 50, 100, 150, and 200 µg/mL.

Preparation of Control (DPPH Solution)

A control solution was prepared by adding 0.5 mL of 1 mM DPPH to 2.5 mL of methanol. The absorbance of the control solution was measured immediately at 517 nm.

Protocol for Estimation of DPPH Scavenging Activity

a) Add 50 µL of DPPH solution to each test tube containing the prepared test samples.

b) Incubate the reaction mixture for 30 minutes at room temperature.

c) Measure the absorbance of the mixture at 517 nm using a UV-Visible spectrophotometer (Systronics), with methanol as the blank.

The percentage reduction in absorbance and IC50 values were calculated using the formula:

% Antioxidant Activity = (Control Absorbance −Sample Absorbance) / Control Absorbance × 100

All experiments were performed in triplicate to ensure accuracy and reproducibility.

3. RESULTS AND DISCUSSION

Phytochemical analysis confirmed the presence of flavonoids, alkaloids, saponins, tannins, and terpenoids in various extracts. The ethyl acetate extract exhibited the most diverse phytochemical profile.

Table-1: Yield of Extracts

Solvent	Weight of Powdered Material (g)	Volume of Solvent (mL)	Crude Extract Weight (g)	Yield (%)
Petroleum Ether	200	500	1.62	0.81
Ethyl Acetate	200	500	2.31	1.15
Methanol	200	500	1.82	0.91

Table-2: Preliminary Phytochemical analysis

S.No.	Tests	Ethyl Acetate	Petroleum Ether	Methanol
1	Test for Carbohydrates			
	Fehling's Test	_	+	+
2	Test for Alkaloid			
	Wagner's Test	_	_	+
3	Test for Flavonoids			
	Shinoda Test	+	_	+
	Alkaline Reagent Test	_	+	_
4	Test for Terpenoids			
	Salkowski Test	+	_	_
5	Test for Saponins			
	Foam Test	+	_	_
6	Test for Proteins	_	+	+

The IC50 values obtained for DPPH inhibition of two column fractions were 105.443µg/ml, 394.564 µg/ml and 251.57 µg/ml for first fraction (F1), second fraction (F2) and ascorbic acid respectively. The results revealed that fractions F1 are having higher percentage inhibition of DPPH absorbance and lower IC50 values of 105.443µg/ml when compared to IC50 value of ascorbic acid(251.57µg/ml).

The results underscore the rich phytochemical diversity of Ageratum conyzoides Linn, particularly in the ethyl acetate extract. Bioactive fractions F1 and F2 demonstrated significant antioxidant activity, with F1 outperforming F2 at all tested concentrations. This aligns with previous studies highlighting the antioxidant potential of flavonoids and phenolic compounds [8-10].

The chromatographic isolation techniques employed facilitated the identification of bioactive compounds. Fraction F1, exhibiting higher antioxidant activity, warrants further structural elucidation and pharmacological evaluation.

4. CONCLUSION

Ageratum conyzoides Linn., a medicinal plant belonging to the family Asteraceae, is a significant source of natural antioxidants. The present study highlights the phytochemical richness of its ethyl acetate extract and isolated fractions, which demonstrate noteworthy antioxidant activity. These findings corroborate the traditional medicinal uses of the plant, supporting its role in combating oxidative stress and associated pathological conditions.

The bioactivity observed is primarily attributed to the plant's diverse secondary metabolites, including flavonoids, alkaloids, terpenoids, and phenolic compounds, which possess free radical scavenging properties. The DPPH assay employed in this study serves as a reliable method for evaluating antioxidant potential, underscoring the ethyl acetate fraction's efficacy.

To further explore the pharmaceutical applications of *Ageratum conyzoides*, future studies should focus on the structural elucidation of its bioactive compounds using advanced spectroscopic techniques. Additionally, detailed investigations into the mechanisms of action of these compounds, including their interaction with cellular pathways, will provide a deeper understanding of their therapeutic potential. This work establishes a foundation for the development of plant-based antioxidants, which could serve as safer and more sustainable alternatives to synthetic compounds in medicine and food industries.

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