Phytochemical Profiling, Extraction Optimization and Antioxidant Efficacy of Achyranthes aspera.

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ABSTRACT

Background:*Achyranthes aspera* is a highly valued medicinal plant, yet its clinical use is often limited because its raw phytoconstituents have restricted topical efficacy. The optimization of its extraction methods and the targeted isolation of its bioactive secondary metabolites require systematic investigation to maximize therapeutic potential.

Methods: This study evaluated Continuous Shaking Extraction (CSE), Ultrasonic Extraction (USE), and Microwave-Assisted Extraction (MAE) using 95% methanol to optimize extract yield. Comprehensive qualitative and quantitative phytochemical profiling was conducted, assessing Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). Bioactive compounds were isolated using a stepwise silica gel column chromatography gradient and identified via Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR). Antioxidant potential was assessed using DPPH and ABTS radical scavenging assays.

Results: MAE emerged as the superior extraction technique, yielding 12.50% extract. The extract demonstrated a robust phytochemical profile, with a TPC of 0.575 mg GAE/100 mg and a TFC of 0.366 mg QE/100 mg. Column chromatography successfully isolated significant polyphenols, specifically Chlorogenic acid and a Quercetin derivative, validated by TLC and FTIR spectral analysis (exhibiting O–H stretching at 3462 cm^{-1} and C=C stretching at 1447 cm^{-1}). The methanolic extract exhibited potent radical scavenging activity, with an IC_{50} of 65.7 µg/mL in the DPPH assay.

Conclusion: *A. aspera* leaves possess a robust polyphenolic profile with highly potent antioxidant capabilities. Optimized MAE coupled with chromatographic isolation provides an efficient pathway for extracting therapeutic phytoconstituents.

Keywords: *Achyranthes aspera*, Microwave-Assisted Extraction, Column Chromatography, Chlorogenic Acid, Quercetin Derivative, Polyphenols, Antioxidant Activity.

1. INTRODUCTION

Plant biodiversity remains a significant basis of medicinal substances, providing therapeutic properties that stem from bioactive compounds produced during their secondary metabolism, such as flavonoids, alkaloids, tannins, and phenols [1]. Approximately 80% of the global populace continues to rely on herbal medications as their main system of behaviour due to their compatibility with human physiology and fewer side effects [2].These natural products are seen as rich sources of bioactive compounds and are considered one of the most impactful discoveries in modern medicine [3].The rising global prevalence of multidrug-resistant microbial strains has necessitated the exploration of natural, plant-derived alternatives. Because infection-induced oxidative stress is known to severely exacerbate chronic inflammatory pathways, finding therapeutic agents with dual properties is critical. Consequently, extensive research has recently focused on the phytochemical screening of

Indian medicinal plants to validate their synergistic antioxidant, antibacterial, antifungal and anti-inflammatory efficacies [4,5,6,7].

Achyranthes aspera, an herbaceous plant belonging to the Amaranthaceae family, has a widespread global distribution and is commonly found in regions including the tropical zones of Asia, Africa, Australia, and the Americas [8]. Renowned in traditional medicine, the plant exhibits significant pharmacological properties, including diuretic, purgative, and laxative effects, making it beneficial for various skin disorders, edema, and dropsy [9]. Previous phytochemical investigations indicate that *Achyranthes aspera* is known to contain triterpenoid saponins with oleanolic acid, ecdysterone, and long-chain alcohols [10].

Despite its recognized medicinal value, maximizing the recovery of its bioactive secondary metabolites requires the modernization of extraction protocols. Advanced techniques such as Microwave-Assisted Extraction (MAE) and Ultrasonic Extraction (USE) offer potential advantages over continuous shaking extraction methods [11]. Consequently, this study aims to systematically compare these extraction techniques, quantitatively profile the polyphenolic content, isolate specific bioactive phytoconstituents using gradient column chromatography,and evaluate the *in vitro* antioxidant potential of *Achyranthes aspera* leaf extracts.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

The leaves of *Achyranthes aspera* were collected, washed thoroughly with distilled water to remove superficial impurities, and air-dried in a shaded, well-ventilated area [12]. The dried leaves were pulverized into a fine powder. Physicochemical parameters, including loss on drying, total ash, acid-insoluble ash, water-soluble ash, and foaming index, were evaluated using standard pharmacopeial and World Health Organization methods [13].

2.2 Optimization of Extraction Methods

Three methodologies were employed using 95% methanol as the solvent:

- **Continuous Shaking Extraction (CSE):** Dried powder (5 g) in 100 mL methanol was subjected to continuous shaking at 110 ± 2 rpm for durations of 30, 180, and 360 minutes.
- **Ultrasonic Extraction (USE):** Utilizing acoustic cavitation, 1 g of powder in 20 mL methanol was sonicated at 60 kHz for 5, 15, and 30 minutes [14].
- **Microwave-Assisted Extraction (MAE):** 1 g of powder in 20 mL methanol was subjected to microwave treatment (180 W) for 1, 3, and 5 minutes [15].

2.3 Qualitative Phytochemical Screening

The optimized *Achyranthes aspera* leaf extract was subjected to preliminary qualitative phytochemical screening to identify the presence of major secondary metabolites. Standard chemical testing protocols were employed to detect the presence of alkaloids (using Dragendorff's and Mayer's reagents), flavonoids (via Aluminum chloride), phenolic compounds and tannins (using Ferric chloride), saponins (via the foam test), and terpenoids (utilizing the Sulfuric acid test).

2.4 Quantitative Phytochemical Assessment

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, measured at 760 nm and expressed as mg of Gallic Acid Equivalents (GAE) per 100 mg of extract [16]. The total flavonoid content (TFC) was quantified via a modified aluminum chloride colorimetric assay, measured at 510 nm and expressed as mg Quercetin Equivalents (QE) per 100 mg of extract [17].

2.5 Chromatographic Separation and Characterization

A 9.3 g methanolic extract of *A. aspera* was processed through a silica gel column (100–200 mesh) using a stepwise gradient starting with petroleum ether, followed by ethyl acetate, and finally methanol [18]. Thin Layer Chromatography (TLC) was performed on silica gel 60 F254 plates using a toluene: ethyl acetate: formic acid (6.7:0.75:0.15) mobile phase. To identify the characteristic functional groups, Fourier Transform Infrared (FTIR) spectroscopy was performed over the standard infrared region [19].

2.6 *In Vitro* Antioxidant Assays

Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays.

- **DPPH Assay:** The decrease in absorbance of the stable DPPH radical was measured at 517 nm [20].
- **ABTS Assay:** The ABTS radical cation was generated with potassium persulfate, and decolorization was measured at 734 nm [21]. Extract concentrations ranging from 10 to 500 µg/mL were utilized to calculate IC_{50} values.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Evaluation and Extraction Yield

The dried leaf powder exhibited a loss on drying of 9.5%, total ash of 8.4%, acid-insoluble ash of 3.5%, water-soluble ash of 1.8%, and a foaming index of 4 mL. Among the extraction techniques evaluated, Microwave-Assisted Extraction (MAE) demonstrated superior efficiency, yielding 12.50% of a dark greenish-brown semisolid extract. This significantly outperformed Ultrasonic Extraction (USE) at 10.45% and Continuous Shaking Extraction (CSE) at 9.33%.

Table 1: Physicochemical Evaluation of *A. aspera* Leaf Powder

Sr. No.	Parameter	Observed Value (% w/w)
1	Loss on Drying	9.50%
2	Total Ash	8.40%
3	Acid-Insoluble Ash	3.50%
4	Water-Soluble Ash	1.80%
5	Foaming Index	4 mL

Table 2: Comparison of Extraction Techniques and Yields for *A. aspera*

Sr. No.	Extraction Technique	Abbreviation	Method Type	Duration	Extraction Yield (%)
1	Continuous Shaking Extraction	CSE	Conventional	360 Minutes	9.33%
2	Ultrasonic Extraction	USE	Non-conventional	30 Minutes	10.45%
3	Microwave-Assisted Extraction	MAE	Non-conventional	5 Minutes	12.50%

3.2 Qualitative Phytochemical Assessment

Initial qualitative screening of the methanolic extract revealed the abundant presence of diverse bioactive secondary metabolites, which strongly indicates considerable

pharmacological potential. The standard chemical tests confirmed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids.

3.3 Phytochemical Profiling

Qualitative analyses validated the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids. The Gallic acid standard calibration curve demonstrated a strong linear connection ($R^2 = 0.988$), establishing the TPC of the extract at 0.575 mg GAE per 100 mg of extract. Concurrently, the Quercetin calibration curve ($R^2 = 0.983$) established a TFC of 0.366 mg QE per 100 mg of extract.

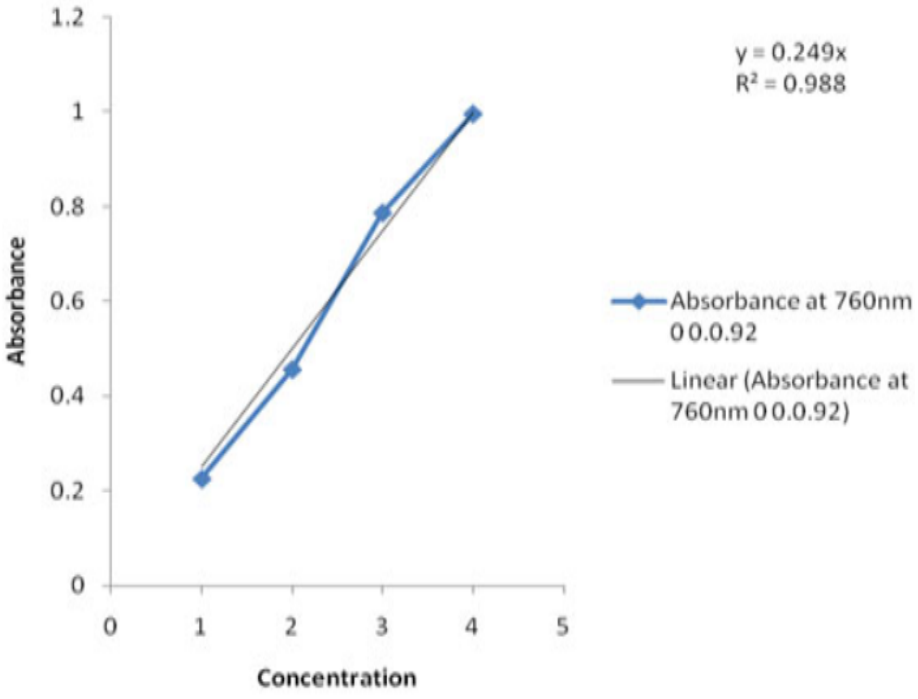

Figure 1: Standard Calibration Curves for Gallic Acid

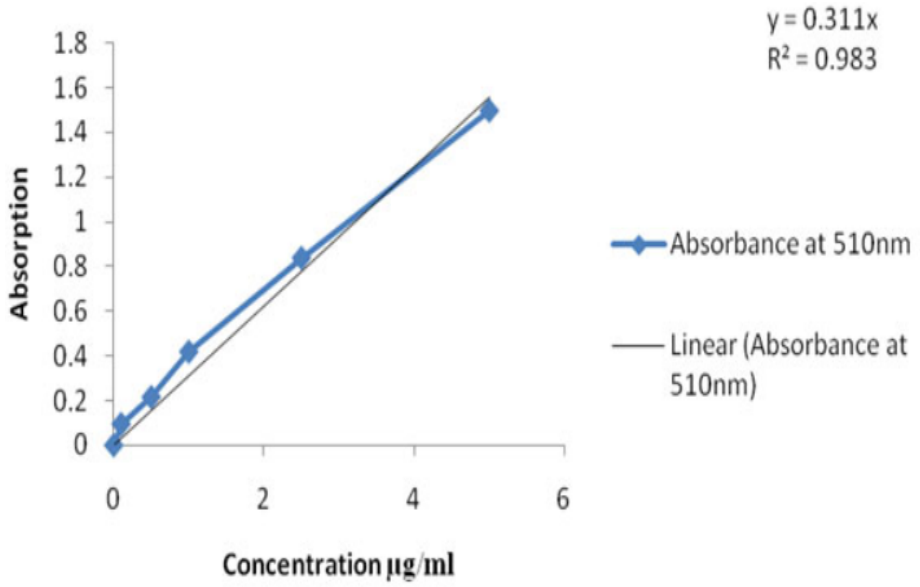

Figure 2: Standard Calibration Curves for Quercetin

3.4 Chromatographic Isolation of Bioactive Phytoconstituents

Column chromatography facilitated the successful separation of targeted bioactive compounds.

- **Fraction-1:** Eluted using an 80 Petroleum Ether: 20 Ethyl Acetate gradient, yielding a 450 mg residue. Subsequent chromatography and TLC analysis revealed a distinct pinkish-red spot with an Rf value of 0.45, identified as a Quercetin derivative (Flavonoid-III).
- **Fraction-2:** Eluted utilizing an 80 Ethyl Acetate: 20 Methanol ratio. TLC analysis produced a distinct spot with an Rf value of 0.36, positively identifying the isolate as Chlorogenic acid (Phenolic acid-VI).
- **Fraction-3:** Eluted utilizing a 70 Ethyl Acetate: 30 Methanol ratio. This highly polar fraction underwent subsequent TLC analysis and pooling, which led to the successful isolation and validation of several additional bioactive constituents, including Ferulic

Acid (Rf 0.74), Caffeic Acid (Rf 0.68), Oleanolic Acid (Rf 0.19), and Ursolic Acid (Rf 0.20).

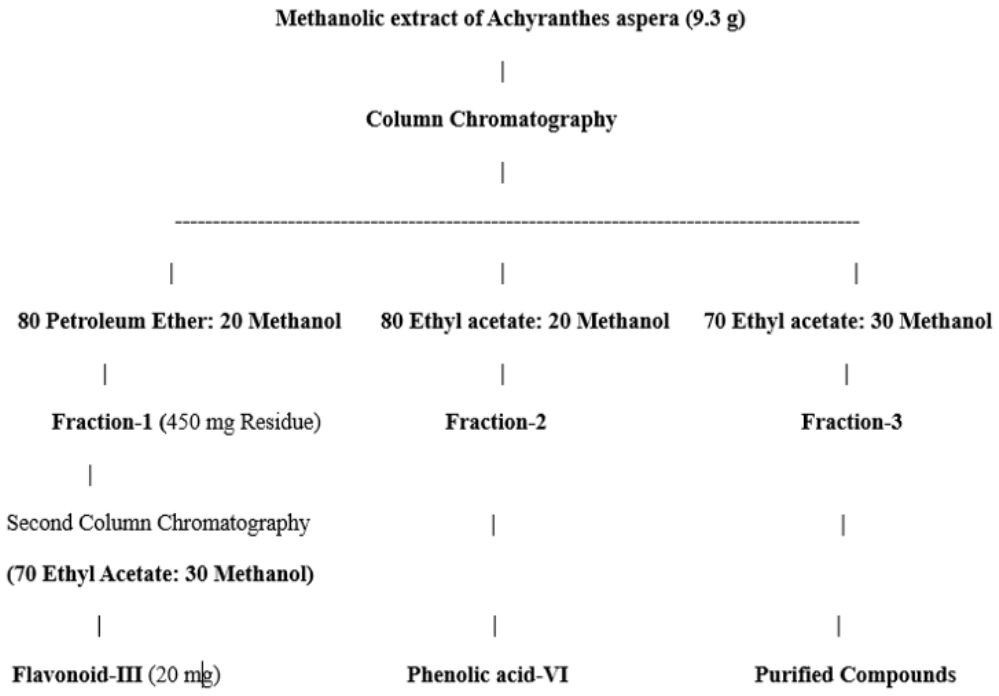

Figure 3: Flowchart of the Chromatographic Separation Process

Table 3: TLC Analysis and Identification of Isolated Fractions from *A. aspera* Extract

Spot No.	Isolated Fraction	Standard Reference	Standard Rf Value	Observed Rf Value	Identification
1	Fraction-1	Quercetin Derivative	0.45	0.45	Flavonoid-III
2	Fraction-2	Chlorogenic acid	0.36	0.36	Phenolic acid-VI
3	Fraction-3	Ferulic Acid	0.73	0.74	Phenolic acid
4	Fraction-3	Caffeic Acid	0.69	0.68	Phenolic acid

5	Fraction-3	Oleanolic Acid	0.19	0.19	Triterpenoid
6	Fraction-3	Ursolic Acid	0.2	0.2	Triterpenoid

3.5 FTIR Spectral Analysis

The FTIR spectral data strongly correlated with the quantitative and chromatographic findings. A prominent absorption band at 3462 cm^{-1} confirmed O–H stretching vibrations associated with phenolic compounds and alcohols. An aromatic C=C stretch was observed at 1447 cm^{-1}, while a strong C-O stretch at 1039 cm^{-1} indicated the presence of glycosidic linkages, demonstrating the presence of sugar moieties.

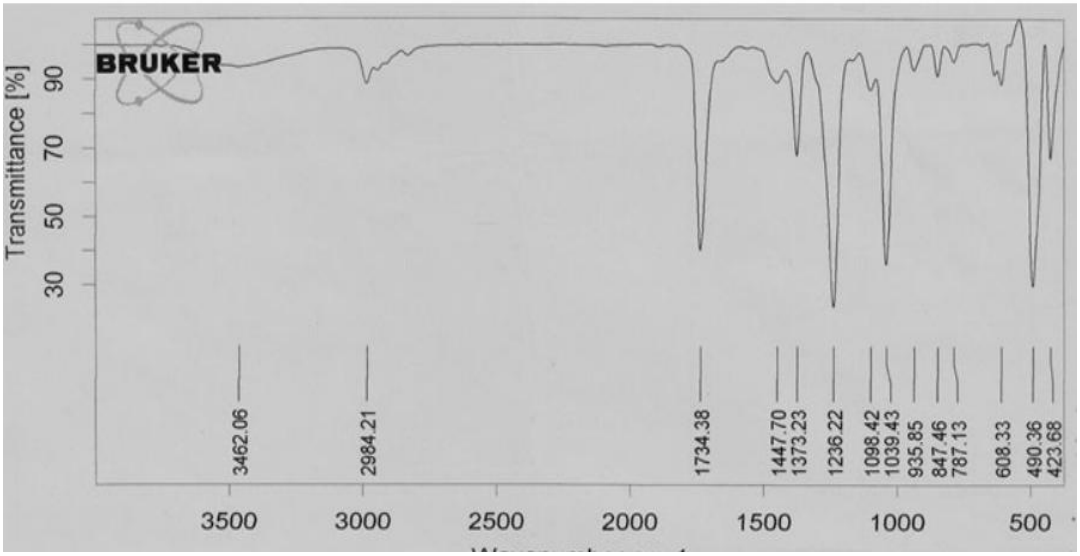

FIGURE 4: FTIR Spectrum of AAE

3.6 *In Vitro* Antioxidant Activity

The *A. aspera* extract exhibited highly potent, dose-dependent radical scavenging activity. In the DPPH assay, the extract achieved an IC_{50} of approximately 65.7 µg/mL. The extract demonstrated even higher scavenging efficiency in the ABTS assay, recording an IC_{50} of

approximately 47.7 µg/mL. This suggests a strong ability of the extract to neutralize a broader range of free radicals, directly attributable to the high concentrations of phenolic compounds and flavonoids.

Table 4: DPPHand ABTS Radical Scavenging Activity of AAE at Varying Concentrations

Concentration (µg/mL)	DPPH Radical Scavenging Activity (%)	ABTS Radical Scavenging Activity (%)
10	12%	18%
20	22%	30%
40	38%	45%
60	48%	58%
80	55%	65%
100	60%	70%
200	75%	80%
300	85%	90%
400	90%	95%
500	95%	98%

4. CONCLUSION

The methanolic extract of *Achyranthes aspera* possesses substantial therapeutic potential, driven by a rich profile of bioactive secondary metabolites. This study systematically confirms that Microwave-Assisted Extraction (MAE) is a superior methodology for maximizing phytochemical yield (12.50%). Through step-wise chromatographic separation and structural validation via FTIR, high-value polyphenols including Chlorogenic acid and a Quercetin derivative were successfully isolated. The remarkable radical scavenging activity

demonstrated in the *in vitro* assays establishes a strong scientific foundation for utilizing *A. aspera* phytoconstituents as highly effective natural antioxidant agents.

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