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ISSN: 2836-2322

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Pharmacy and Drug Development

Research Article

Quantitative Screening of Bioactive Fractions in Three Tropical Medicinal Plants (*Ageratum conyzoides, Acmella oleracea, and Aegle marmelos*) from Gujarat, India

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Received Date: 18 May 2026; **Accepted Date:** 09 June 2026; **Published Date:** 15 June 2026

Abstract

This study evaluated and compared the quantitative phytochemical composition of Ageratum conyzoides, Acmella oleracea, and Aegle marmelos leaf powders to determine their potential as potent bioactive resources for pharmacology and animal nutrition. Testing was conducted in strict triplicate (n=3) at the Sumitra Research Institute in Gujarat, India. Standard spectrophotometric and gravimetric assays were used to quantify six primary secondary metabolite fractions: phenols, flavonoids, tannins, saponins, alkaloids, and steroids. The results revealed highly significant variations ($p<0.001$) in the quantitative distribution of all secondary metabolites across the three plant species. Ageratum conyzoides leaf powder contained the highest concentrations of all measured fractions, yielding phenols (985.30 mg/g), flavonoids (655.10 mg/g), tannins (216.30 mg/g), saponins (111.80 mg/g), alkaloids (95.60 mg/g), and steroids (108.50 mg/g). This was followed systematically by Acmella oleracea leaf powder, which exhibited intermediate values with phenols (611.50 mg/g), flavonoids (409.20 mg/g), tannins (107.50 mg/g), saponins (87.66 mg/g), alkaloids (70.59 mg/g), and steroids (58.38 mg/g). Aegle marmelos leaf powder consistently recorded the lowest baseline concentrations, with values of 475.20 mg/g phenols, 275.10 mg/g flavonoids, 80.56 mg/g tannins, 30.80 mg/g saponins, 43.72 mg/g alkaloids, and 29.07 mg/g steroids. These findings demonstrate that Ageratum conyzoides serves as an exceptional source of natural polyphenolic antioxidants and defensive compounds. Meanwhile, Acmella oleracea provides a balanced alkaloid-rich intermediate profile, and Aegle marmelos offers a mild, non-astringent chemical matrix with low anti-nutritional factors. Ultimately, this comparative screening provides a validated empirical roadmap for selecting and dosing these botanical powders as safe, sustainable phytogenic alternatives to synthetic chemical modifiers..

Key words: Phytochemical quantification, Polyphenols, Secondary metabolites, Asteraceae, Phytobiotics.

Introduction

The explore-and-exploit paradigm of modern ethnopharmacology and animal nutrition is increasingly focused on mapping the chemical architecture of tropical flora [1, 2]. Over the past few decades, the indiscriminate reliance on synthetic drugs, chemical preservatives, and subtherapeutic antibiotic growth promoters in agricultural systems has triggered global challenges [3]. These include the rapid proliferation of antimicrobial-resistant pathogens, tissue residue accumulation, and severe environmental imbalances [4]. In response, research has shifted toward identifying safe, biodegradable, and biologically active alternatives derived from the secondary metabolic pools of plants [5, 6]. Tropical ecosystems, such as those found across the Indian subcontinent, host a vast array of medicinal plants that synthesize complex chem-

ical defense networks [7]. These networks offer exceptional antioxidant, anti-inflammatory, and antimicrobial properties that can be leveraged for both human health and livestock production [8].

Despite the immense therapeutic potential of tropical medicinal plants, their practical application in clinical pharmacology and livestock feed formulation remains severely limited by a lack of quantitative standardization [7]. Historically, much of the existing literature on ethnobotanical flora has relied on qualitative, presence-or-absence screening methods. This lack of precision makes it difficult to predict how these plants will perform in practical settings [9]. Without exact quantification of primary bioactive fractions such as phenols, flavonoids, alkaloids, and saponins formulating reliable doses is practically impossible. Under-dosing fails to trigger any beneficial physiological or therapeutic response, while over-dosing risks introducing anti-nutritional factors (such as bitter tannins or cell-permeabilizing saponins) that can depress appetite, reduce nutrient availability, or cause systemic toxicity [10, 11]. Furthermore, secondary metabolite profiles fluctuate drastically based on geographic location, soil composition, and climate. This variability makes it essential to establish rigorous baseline profiles for specific regional ecosystems, such as the semi-arid and coastal zones of Gujarat, India [11].

Ageratum conyzoides (Billygoat-weed), a resilient member of the Asteraceae family, is widely recognized for its high adaptability and its ability to synthesize potent volatile oils and polyphenolic compounds. These components provide robust tissue-protective and radical-scavenging capabilities [12]. Acmella oleracea (Toothache plant), another prominent Asteraceae species, is valued for its unique concentrations of lipophilic alkamides, particularly spilanthol, which exerts strong local anesthetic, sialagogue, and insecticidal effects [13]. In contrast, Aegle marmelos (Bael), belonging to the Rutaceae family, possesses a completely different biochemical blueprint. It is rich in specialized coumarins, alkaloids like aegeline, and essential oils that support metabolic regulation and gastrointestinal homeostasis [14].

To address these challenges, this study evaluates three distinct plant species known for their diverse taxonomic heritages and functional properties: Ageratum conyzoides, Acmella oleracea, and Aegle marmelos. Screening these three distinct medicinal plants under uniform processing and environmental conditions allows researchers to map their varying capacities to produce vital secondary metabolites. Quantifying the precise concentrations of phenols, flavonoids, tannins, saponins, alkaloids, and steroids provides a clear chemical roadmap for researchers and professionals [14]. This data makes it possible to predict the exact antioxidant potency, antimicrobial strength, and palatability index of each leaf powder. Ultimately, this comparative analysis bridges the gap between traditional ethnobotanical knowledge and modern, evidence-based applications. It gives pharmacologists, animal nutritionists, and feed manufacturers a validated framework to select and utilize regional plant resources effectively, maximizing their functional value while ensuring total safety for human and animal systems.

This study evaluates three distinct plant species known for their diverse taxonomic heritages and functional properties: Ageratum conyzoides, Acmella oleracea, and Aegle marmelos.

Materials and Methods

Experimental Location and Environmental Conditions

The entire experimental protocol—including processing, quantitative phytochemical profiling, and analytical testing was conducted at the Sumitra Research Institute in Gujarat, India (coordinates approx. 22.3094 ◦ N, 72.1362 ◦ E). The plant samples were processed and analyzed during the dry post-monsoon phase, which typical ambient laboratory conditions mirror. The regional weather profile during this analytical timeframe was characterized by a mean ambient temperature of 28.5◦C ± 2.3 ◦C, an average relative humidity of 45 % ± 5 %

Collection, Authentication, and Processing of Plants

Fresh, healthy, and disease-free leaves of three distinct plant species were collected simultaneously to minimize seasonal variations in secondary metabolite accumulation: Ageratum conyzoides (L.) (Family: Asteraceae), Acmella oleracea (L.) (Family: Asteraceae) and Aegle marmelos (L.) (Family: Rutaceae). The botanical identities of all three species were authenticated by an expert taxonomist at the Herbarium of the Sumitra Research Institute, and voucher specimens were deposited in the institute's repository for future reference. The collected leaves were thoroughly washed under running tap water to eliminate dust and soil debris, followed by a final rinsing with distilled water. The washed leaves were spread uniformly in a clean, well-ventilated room and allowed to air-dry at room temperature (25 ◦C± 2 ◦C) for 14 days to preserve heat-labile bioactive compounds. The completely dried leaves were pulverized into a fine, homogeneous powder using a mechanized laboratory blender. The resulting leaf powders were sieved through a 40-mesh standard screen, transferred to airtight, light-resistant amber glass containers, and stored in a desiccator at room temperature until needed for analysis.

Quantifying Phytochemicals

Total Phenolic Content

Total phenolic content was determined colorimetrically using the Folin-Ciocalteu reagent method as modified for crude plant material. A known mass of leaf powder was extracted with 80 % aqueous methanol. An aliquot of the extract (0.5 mL) was mixed with 2.5 mL of 10 % Folin-Ciocalteu reagent, followed by the addition of 2.0 mL of 7.5 % (w/v) sodium carbonate (Na2CO3) solution. The reaction mixtures were incu-

bated in the dark at room temperature for 30 minutes. The absorbance was measured at λ=765 nm using a UV-Visible Spectrophotometer.

Total Flavonoid Content

The aluminum chloride (AlCl3) colorimetric assay was employed to quantify total flavonoids. The leaf powder extract was mixed with 0.1 mL of 10 % AlCl3, 0.1 mL of 1 M potassium acetate (CH3 COOH), and 2.8 mL of distilled water. After a 40-minute incubation period at ambient temperature, the absorbance of the reaction mixture was measured at an optical density of 415 nm.

Determination of Tannins

Tannin content was evaluated via the vanillin-HCl method. The leaf matrix was extracted using 4% HCl in methanol. To 1.0 mL of the filtered extract, 5.0 mL of a freshly prepared vanillin-HCl reagent (1% vanillin and 8% HCl mixed in a 1:1 ratio) was added. The mixture was allowed to stand for 20 minutes in a water bath set to 30 ∘C. Absorbance was read at an optic density of 500 nm.

Determination of Total Saponins

Saponin content was quantified using the classic spectrophotometric vanillin-sulfuric acid method. The defatted sample was extracted with aqueous vanillin reagent in the presence of concentrated sulfuric acid (H2SO4). The mixture was heated in a water bath at 60 ∘C for 10 minutes, then cooled rapidly in an ice bath. The absorbance was monitored at optical density (λ) of 544 nm against a blank.

Determination of Total Alkaloids

Alkaloid quantification was performed using the gravimetric method described by Harborne [15]. Five grams of the leaf powder was weighed into a beaker, and 200 mL of 10% acetic acid in ethanol was added. The beaker was covered and allowed to stand for 4 hours. The mixture was filtered, and the filtrate was concentrated on a water bath to one-quarter of its original volume. Concentrated ammonium hydroxide (NH4 OH) was added dropwise to precipitate the alkaloids completely. The precipitate was collected on a pre-weighed filter paper, washed with 1% ammonium hydroxide solution, dried in an oven at 60 ∘C to a constant mass, and weighed. The alkaloid percentage was converted to mg/g dry weight.

Determination of Total Steroids

Total steroid concentrations were measured using a modified colorimetric method. The sample was extracted with chloroform. An aliquot of the extract was treated with Liebermann-Burchard reagent (a freshly prepared mixture of acetic anhydride and concentrated sulfuric acid). The mixture was incubated at room temperature for 15 minutes until a green color developed, and the absorbance was measured spectrophotometrically at λ=640 nm. A standard curve generated from cholesterol was used to determine the total steroid content.

Statistical Analysis

All experimental data derived from the triplicate runs (n=3) were subjected to a one-way Analysis of Variance (ANOVA) using SPSS Software (Version 26.0). Means were separated using Tukey's Honestly Significant Difference (HSD) post-hoc test. Differences among means were considered statistically significant at $P<0.05$.

Results and Discussion

The quantitative distribution of secondary metabolites across the three leaf powders reveals highly significant variations ($P<0.05$). Ageratum conyzoides uniformly displays the highest concentration across all six measured phytochemical fractions, followed systematically by Acmella oleracea, while Aegle marmelos contains the lowest levels. This comparative study highlights profound variations in the quantitative secondary metabolic profiles of Ageratum conyzoides, Acmella oleracea, and Aegle marmelos leaf powders. These distinct profiles reveals their varying potential for use in pharmacology, phytogenic animal feed formulation, and as natural alternatives to synthetic antibiotics. Phenols and flavonoids represent the primary antioxidant framework in botanical systems. Their mechanism of action relies heavily on the radical-scavenging capabilities of their phenolic hydroxyl groups [18, 19, 20]. In this study, Ageratum conyzoides demonstrated an exceptionally high phenolic content (985.3 mg/g) and flavonoid profile (655.1 mg/g). This dense concentration aligns with previous literature characterizing A. conyzoides as a hyper-accumulator of polyphenolic compounds, notably precocenes and polymethoxyflavones [21]. This high concentration gives the plant strong free-radical scavenging properties and allows it to protect cellular membranes from oxidative damage [22, 23]. By comparison, Acmella oleracea contained intermediate concentrations (phenols: 611.5 mg/g; flavonoids: 409.2 mg/g). These values support its traditional use as an anti-inflammatory agent, since polyphenols regularly downregulate pro-inflammatory cascades such as the NF-κB and COX-2 pathways [24]. While Aegle marmelos exhibited the lowest baseline levels in this dataset (phenols: 475.2 mg/g; flavonoids: 275.1 mg/g), these values are still biologically significant. They match established baselines for the Rutaceae family and contribute to its validated antidiabetic and tissue-protective properties [25]. Tannins and saponins serve vital roles as chemical defense compounds in plants, protecting them against fungal pathogens and herbivores. When used in animal nutrition, they modulate gastrointestinal environments [26].

Ageratum conyzoides contained high levels of tannins (216.3 mg/g) and saponins (111.8 mg/g). High-tannin leaf powders can exert anthelmintic effects by binding to surface proteins of nematodes, though excessively high levels can some-

times reduce protein digestibility in monogastric animals due to protein-precipitation properties [27]. The saponin fraction (111.8 mg/g in A. conyzoides vs. 87.66 mg/g in A. oleracea) suggests these extracts possess strong surfactant properties [28]. These properties can permeabilize cell membranes, making them effective against protozoa and bacteria [25].

Aegle marmelos leaf powder showed low concentrations of both groups (tannins: 80.56 mg/g; saponins: 30.80 mg/g), suggesting it could serve as a mild, non-astringent phytogenic additive with minimal risk of reducing feed palatability or nutrient intake [29]. Alkaloids and steroids are highly potent secondary metabolites that exert direct physiological effects on animal and human systems. The alkaloid content was highest in Ageratum conyzoides (95.60 mg/g), followed by Acmella oleracea (70.59 mg/g), and lowest in Aegle marmelos (43.72 mg/g). In Acmella oleracea, this alkaloid profile is historically dominated by bioactive alkamides, primarily spilanthol. Spilanthol is responsible for the plant's signature local anesthetic, tingling, and sialagogue (saliva-stimulating) effects [37]. The alkaloid pool in Aegle marmelos typically includes compounds like aegeline, which contributes to metabolic homeostasis and lipid regulation [30]. Steroids followed an identical distribution trend: 108.5 mg/g (A. conyzoides) > 58.38 mg/g (A. oleracea) > 29.07 mg/g (A. marmelos). Plant sterols, such as β-sitosterol and stigmasterol, stabilize lipid bilayers and possess anti-inflammatory and cholesterol-lowering properties [31]. The superior steroid and alkaloid concentration in A. conyzoides highlights its potential as a potent physiological modifier, though its application requires careful dosing to avoid pyrrolizidine alkaloid toxicity [28. 31]. Conversely, A. oleracea and A. marmelos present safer, more balanced metabolic profiles for continuous dietary or therapeutic inclusion.

Conclusion

In conclusion, this quantitative comparative analysis highlights significant differences in the secondary metabolic architecture of Ageratum conyzoides, Acmella oleracea, and Aegle marmelos leaf powders. The outcome confirms that Ageratum conyzoides is a superior bio-resource for all six evaluated phytochemical classes. It contains exceptional concentrations of phenols (985.3 mg/g) and flavonoids (655.1 mg/g), which provide a robust natural antioxidant and free-radical scavenging framework. Acmella oleracea serves as an effective intermediate option, striking a balance between cellular protection and functional bio-motility. Its profile is supported by high levels of alkaloids (70.59 mg/g), which align with its documented anesthetic and anti-inflammatory properties. While Aegle marmelos displayed the lowest baseline values in this study, it maintains a clean, non-astringent chemical matrix with low tannin and saponin concentrations. This balance minimizes the risk of introducing bitter anti-nutritional factors into target treatments. Ultimately, these findings provide animal scientists, pharmacologists, and biochemists with a clear, empirical roadmap to select and dose these botanical leaf powders effectively. This information helps maximize their therapeutic value as natural health modulators, safe metabolic modifiers, and sustainable alternatives to synthetic chemical additives.

Phytochemical Fraction (mg/g)	Ageratum conyzoides	Acmella oleracea	Aegle marmelos	SEM	P-value
Phenols	985.30^{a}	611.50^{b}	475.20^{c}	14.25	<0.001
Flavonoids	655.10^{a}	409.20^{b}	275.10^{c}	10.80	<0.001
Tannins	216.30^{a}	107.50^{b}	80.56^{c}	4.92	<0.001
Saponins	111.80^{a}	87.66^{b}	30.80^{c}	2.04	<0.001
Alkaloids	95.60^{a}	70.59^{b}	43.72^{c}	1.88	<0.001
Steroids	108.50^{a}	58.38^{b}	29.07^{c}	2.11	<0.001

Means within the same row carrying different superscript letters (a, b, c) differ significantly (P<0.05) based on Tukey's HSD test.
Table 1: Quantitative Phytochemical Composition of Selected Leaf Powders

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Citation: Alagbe, John Olujimi, Anorue Daniel N. Quantitative Screening of Bioactive Fractions in Three Tropical Medicinal Plants (*Ageratum conyzoides, Acmella oleracea, and Aegle marmelos*) from Gujarat, India. *J. Pharm. Drug. Dev.* Vol. 5 Iss. 1. (2026) DOI: 10.58489/2836-2322/047