International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

Phytochemical Profiling of *Fittonia albivenis* Extracts: A Comparative Study of Solvent Extraction Methods

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Abstract
Fittonia albivenis, a member of the Acanthaceae family, is an ornamental plant known for its striking foliage and potential therapeutic properties. Traditionally used in folk medicine for treating inflammation, wounds, and pain, its medicinal efficacy is attributed to various bioactive compounds, including phenolics, flavonoids, alkaloids, saponins, and terpenoids. This study aims to explore the phytochemical composition of *F. albivenis* using solvent extraction methods, focusing on the impact of solvent polarity on the recovery of bioactive compounds. Fresh leaves were extracted sequentially with petroleum ether, ethyl acetate, and methanol, representing non-polar, moderately polar, and polar solvents, respectively. Qualitative screening identified the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenolics, and cardiac glycosides across the extracts. The methanol extract exhibited the highest levels of phenolics (86.7 mg GAE/g) and flavonoids (58.9 mg QE/g), followed by the ethyl acetate extract with moderate flavonoid content (32.6 mg QE/g). Petroleum ether, however, showed minimal yields of phenolics and flavonoids but was effective in extracting non-polar terpenoids. Quantitative analysis confirmed the superior efficiency of methanol for extracting polar compounds. Statistical analysis revealed significant differences ($p < 0.001$) between extracts, validating the influence of solvent choice on phytochemical recovery. The results suggest that *F. albivenis* is a rich source of antioxidants, particularly in its polar fractions, and highlight the importance of solvent selection in phytochemical studies. This study provides a foundation for further research into the pharmacological potential of *F. albivenis*, supporting its traditional uses and potential for therapeutic applications.

Keywords: Phytochemical composition, Fittonia albivenis, Flavonoids, Phenolics, Solvent extraction, Antioxidant activity

1. Introduction

Fittonia albivenis, commonly known as the nerve plant, is a member of the Acanthaceae family and is recognized for its ornamental value due to its striking foliage, characterized by dark green leaves with prominent white or red veins. Beyond its aesthetic appeal, *F. albivenis* has been traditionally used in folk medicine for a variety of therapeutic purposes, including its application in treating inflammation, wound healing, and pain relief. This plant's medicinal potential is largely attributed to the bioactive compounds present in its tissues, particularly phenolics, flavonoids, alkaloids, saponins, and terpenoids, which are known for their

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antioxidant, anti-inflammatory, antimicrobial, and analgesic properties. Phytochemical studies have demonstrated that plants like *F. albivenis* contain a wide range of secondary metabolites that contribute to their therapeutic effects. However, the extraction efficiency of these compounds is highly influenced by the choice of solvent. Solvent polarity plays a crucial role in determining the type and quantity of compounds that can be extracted, with polar solvents generally being more effective at extracting phenolic compounds and flavonoids, while non-polar solvents are better for terpenoid extraction. Given this, it is essential to explore the impact of different solvents on the phytochemical profile of *F. albivenis*.

This study aims to investigate the phytochemical composition of *Fittonia albivenis* through solvent-based extraction methods, focusing on the extraction of phenolics, flavonoids, and other key bioactive compounds. The objective is to determine the optimal solvent for extracting these compounds, provide a comprehensive qualitative and quantitative analysis of the plant's bioactive constituents, and explore the potential pharmacological applications of the plant. By analyzing the variation in phytochemical yields across different solvents, this research seeks to contribute valuable insights into the effective use of *F. albivenis* in medicinal applications and its potential for further development as a natural therapeutic agent.

2. Methodology

The methodology for this study was designed to systematically analyze the phytochemical composition of *Fittonia albivenis* through a series of well-established laboratory procedures. (Belokurova et al., 2019).

2.1 Reagents and Chemicals

All chemicals used were of analytical grade, procured from reputable Indian suppliers. For extraction and phytochemical screening, petroleum ether (60–80°C), ethyl acetate, and methanol were sourced from Merck India (Mumbai) **or** SD Fine Chemicals (Mumbai)**.** Mayer's reagent (alkaloid test), hydrochloric acid (1%), sodium hydroxide (10%), ferric chloride (5%), safranin O, fast green FCF, chloral hydrate, and toluidine blue were obtained from HiMedia Laboratories (Mumbai) **or** Loba Chemie (Mumbai)**.** Folin-Ciocalteu reagent, sodium carbonate (20%), Canada balsam, and FAA components (formaldehyde, acetic acid, ethanol) were purchased from Sisco Research Laboratories (SRL, Mumbai)**.** Aluminum chloride (2% in methanol) and ammonium nitrate were procured from Fisher Scientific India (Mumbai)**,** while gallic acid and quercetin standards came from Sigma-Aldrich India (Bangalore)**.** For microscopy, Whatman No. 1 filter papers (GE Healthcare India, Mumbai) were used for filtration, and all glassware was supplied by Borosil Glass Works Ltd. (Mumbai)**.** Distilled water was prepared using a Milli-Q purification system (Merck India)**.**

2.2 Instruments Employed

All analyses were conducted using standardized laboratory equipment: plant samples were sectioned using a rotary microtome (Leica RM2125 RTS, Germany), while microscopic observations were performed with a compound microscope (Nikon Eclipse E200, Japan) equipped with a digital eyepiece camera (AmScope MU300, USA) for photomicrography. For spectrophotometric quantification, a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) was used to measure absorbance at specific wavelengths. Extraction processes utilized a

Soxhlet apparatus (Borosil, India) and rotary evaporator (Buchi R-300, Switzerland) for solvent recovery. Ash content analysis was performed in a muffle furnace (Thermo Scientific FB1315, USA), and moisture determination used a hot air oven (Memmert UN110, Germany). Sample weights were recorded with analytical balances (Shimadzu AUX220, 0.1 mg precision), and powder homogenization was achieved with an electric grinder (Philips HL7756, India). Water purification was carried out using a Milli-Q system (Merck Millipore, Germany) or local distillation units for general purposes. All glassware and crucibles were of Borosil 3.3 grade (India) to ensure thermal and chemical resistance.

2.3 Plant Material Collection

The initial stage involved the careful collection and preparation of plant materials to preserve their biochemical integrity. Fresh leaves of *Fittonia albivenis* were collected from a cultivated nursery in MDU, Rohtak, during the morning hours (8:00–10:00 AM) to minimize metabolic variations. The leaves were washed three times with distilled water to remove dust, epiphytic microbes, and surface contaminants. After washing, the leaves were blot-dried using sterile filter paper to remove excess moisture.

Figure 1 A: *Fittonia albivenis*

The material was then shade-dried at 25±2°C for 15 days in a well-ventilated area to prevent enzymatic degradation. The dried leaves were finely powdered using an electric grinder (500 µm sieve size) to ensure uniform particle size. The powder was stored in airtight amber glass bottles at 4°C to prevent oxidation and moisture absorption until extraction.

2.4 Phytological screening

- ***Organoleptic Evaluation Methodology***

The organoleptic evaluation was conducted following standardized protocols. Fresh plant materials were visually inspected under natural daylight for color variations (green, brown, or other pigmentation). The odor was assessed by crushing small portions of leaves/stems and smelling immediately. Taste was evaluated by placing a small sample on the tongue (spit-out after 10 seconds). Texture (smooth, rough, fibrous) was determined by tactile examination. Morphological features (leaf margin, apex, venation pattern) were documented using botanical references and compared with herbarium specimens (Pal et al., 2022).

- ***Macroscopic and Microscopic Analysis Methodology***

Macroscopy:
Dried bark segments were cleaned with a soft brush to remove debris. Surface characteristics (lenticels, fissures) were examined using a 10× hand lens. Internal structure (phloem arrangement, cambium layer) was studied by making transverse sections with a sharp blade (Rivera-Arce et al., 2007).

Microscopy:
Fresh samples were fixed in FAA solution (formaldehyde:acetic acid:70% ethanol = 5:5:90 v/v) for 48 hours. Dehydration was done through an ethanol-xylene series. Sections (10–12 µm thick) were cut using a rotary microtome (Leica RM2125 RTS). Staining was performed with 1% safranin (for lignified cells) and 0.5% fast green (for cellulose). Slides were mounted in Canada balsam and observed under a compound microscope (Nikon Eclipse E200) (Gopalakrishnan et al., 2012).

- ***Powder Microscopy Methodology***

Dried plant material was pulverized using an electric grinder and sieved through an 80-mesh **sieve**. The powder was cleared by boiling in chloral hydrate solution (5% w/v) for 5 minutes. Temporary mounts were prepared with glycerin-water (1:1). Observations for stomata (anisocytic/paracytic types), trichomes (glandular/non-glandular), and crystals (calcium oxalate) were made under 40× magnification. Photomicrographs were captured using a digital eyepiece camera (AmScope MU300) (Padma et al., 2023).

- ***Stomatal Index Determination Methodology***

Lower epidermal peels were obtained by scraping leaves with a blunt scalpel after treatment with 5% NaOH for 2 hours. Peels were stained with 1% toluidine blue for 30 seconds. Stomata and epidermal cells were counted in five fields of view (0.25 mm² each) using an ocular micrometer (Kim et al., 2013). The stomatal index was calculated as:

$$\text{Stomatal Index} = \left(\frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Epidermal cells}}\right) \times 100$$

- ***Ash Content Analysis Methodology***

Total Ash: 2 g of powder was placed in a pre-weighed silica crucible and incinerated in a muffle furnace (Thermo Scientific FB1315) at 450°C for 6 hours until white ash was obtained. Crucibles were cooled in a desiccator before weighing (Kokate et al., 2001).

Water-Soluble Ash: Total ash was boiled with 25 mL distilled water for 5 minutes, filtered through ashless filter paper, and the insoluble residue was re-ignited at 450°C.

Acid-Insoluble Ash: Total ash was treated with 25 mL 10% HCl, simmered for 5 minutes, and filtered. The residue was washed with hot water, dried, and ignited at 450°C.

$$\%\text{Ash} = \left(\frac{\text{Weight of ash}}{\text{Weight of dry sample}}\right) \times 100$$

- ***Foreign Matter and Moisture Content Methodology***

Foreign Matter: 100 g of sample was spread on a white tray, and impurities (soil, insects, other plant parts) were manually separated using forceps (Chase & Pratt, 1949). The percentage was calculated as:

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$$\%\text{Foreign Matter} = \left(\frac{\text{Weight of impurities}}{\text{Total sample weight}}\right) \times 100$$

Moisture Content: 5 g of fresh sample was weighed (W_1) and dried in a hot air oven (Memmert UN110) **at** 105°C until constant weight (W_2).

$$\%\text{Moisture} = \left(\frac{W_1 - W_2}{W_1}\right) \times 100$$

2.4 Preparation of Extracts

For the preparation of extracts, 50 g of dried leaf powder was packed into a Soxhlet thimble and extracted sequentially with petroleum ether (60–80°C), ethyl acetate, and methanol to isolate non-polar, moderately polar, and polar compounds, respectively. Each extraction was conducted for 6–8 hours at the solvent's boiling point (40–78°C). The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure (40°C) using a rotary evaporator (Buchi R-300). The residues were lyophilized (freeze-dried) to obtain a dry powder and stored at 4°C until analysis.

2.5 Qualitative Phytochemical Screening

2.5.1. Mayer's Test for Alkaloids

Mayer's test was performed to detect the presence of alkaloids in *Fittonia albivenis* extracts. In this test, 2 mL of the extract was acidified with 1 mL of 1% hydrochloric acid (HCl) and gently heated to facilitate alkaloid dissolution. After cooling, 2–3 drops of Mayer's reagent (a solution of potassium mercuric iodide, prepared by dissolving 1.36 g $HgCl_2$ and 5 g KI in 100 mL distilled water) were added. The formation of a creamy-white precipitate confirmed alkaloids, as the reagent reacts with nitrogen-containing alkaloids to form insoluble complexes. This test is specific for tropane, quinoline, and isoquinoline alkaloids (Harborne, 1998).

2.5.2. Alkaline Reagent Test for Flavonoids

Flavonoids were identified using the alkaline reagent test. 1 mL of extract was mixed with 2 mL of 10% sodium hydroxide (NaOH) solution. The appearance of a yellow coloration, which disappeared upon neutralization with dilute HCl, indicated flavonoid presence. This color change occurs due to the structural transformation of flavonoid chromophores in alkaline conditions, particularly in flavones and flavonols (Sofowora, 1993).

2.5.3. Ferric Chloride Test for Tannins

Tannins were detected via the ferric chloride test. 1 mL of extract was treated with 2 drops of 5% ferric chloride ($FeCl_3$) solution. A blue-black color suggested hydrolysable tannins (gallotannins), while a greenish-brown color indicated condensed tannins (proanthocyanidins). This reaction occurs due to the formation of iron-phenolate complexes with tannins' phenolic hydroxyl groups (Trease & Evans, 2009).

2.5.4. Foam Test for Saponins

The foam test was employed to identify saponins. 1 mL of extract was shaken vigorously with 5 mL distilled water in a test tube for 30 seconds. The formation of a persistent foam layer (≥1 cm for 15 minutes) confirmed saponins, due to their surfactant properties that reduce water's surface tension (Evans, 2009).

2.5.5. Salkowski Test for Terpenoids

Terpenoids were detected using the Salkowski test. 2 mL of extract was mixed with 2 mL chloroform and 3 mL concentrated sulfuric acid (H_2SO_4). The appearance of a reddish-brown interface indicated terpenoids, as the acid reacts with isoprene units to form colored complexes (Harborne, 1998).

2.5.6. Lead Acetate Test for Phenolic Compounds

Phenolic compounds were identified by adding 2 drops of 10% lead acetate to 1 mL of extract. A white precipitate confirmed phenolics, due to the precipitation of lead-phenolate salts (Sofowora, 1993).

2.5.7. Keller-Kiliani Test for Cardiac Glycosides

Cardiac glycosides were detected using the Keller-Kiliani test. 1 mL of extract was mixed with 1 mL glacial acetic acid, 2 drops of 5% $FeCl_3$, and 1 mL concentrated H_2SO_4. A brown ring at the interface indicated cardiac glycosides (e.g., digitoxin), as deoxysugars in glycosides react with the reagents (Trease & Evans, 2009).

2.6 Quantitative Phytochemical Analysis

Following qualitative screening, specific classes of compounds were quantified using validated spectrophotometric methods.

2.6.1. Folin-Ciocalteu Assay for Total Phenolic Content

The total phenolic content of *Fittonia albivenis* extracts was determined using the Folin-Ciocalteu assay. In this method, 0.5 mL of extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and allowed to stand for 5 minutes at room temperature (25±2°C). Subsequently, 2 mL of 20% sodium carbonate (Na_2CO_3) solution was added, and the mixture was incubated in the dark for 30 minutes to complete the color development. The absorbance of the resulting blue complex was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). A standard calibration curve was prepared using gallic acid (0-100 µg/mL) as the reference compound, and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The assay was performed in triplicate to ensure reproducibility, with appropriate solvent blanks used for baseline correction (Singleton & Rossi, 1965).

2.6.2 Aluminum Chloride Method for Total Flavonoid Content

Total flavonoid content was quantified using the aluminum chloride colorimetric method. Briefly, 1 mL of extract was mixed with 1 mL of 2% aluminum chloride ($AlCl_3$) methanolic solution and incubated for 10 minutes at room temperature. The formation of a yellow flavonoid-aluminum complex was measured at 415 nm against a reagent blank. A standard curve was generated using quercetin (0-100 µg/mL) as the reference standard, and results were calculated as milligrams of quercetin equivalents per gram of extract (mg QE/g). The reaction conditions were carefully controlled, including pH and incubation time, to ensure specific complex formation with flavones and flavonols while minimizing interference from other compounds (Zhishen et al., 1999).

2.6.3 Statistical Validation of Quantitative Results

All quantitative analyses were performed with rigorous statistical validation. Triplicate measurements (n=3) were taken for each sample, and results were expressed as mean ± standard deviation (SD)**.** Inter-assay variability was assessed through coefficient of variation (CV) calculations, with values <5% considered acceptable. Statistical significance between different extracts was determined using one-way analysis of variance (ANOVA) ($p<0.05$) in SPSS software (Version 26.0). (Snedecor & Cochran, 1989).

3. Results and Discussion

Fresh leaves of *Fittonia albivenis* were collected from the MDU, Rohtak nursery during morning hours (8:00–10:00 AM) to preserve phytochemical integrity. The leaves were washed, shade-dried (25±2°C for 15 days), and ground to a fine powder (500 µm). The powdered material was stored in amber glass at 4°C until extraction.

3.1 Phytological Screening

The phytological evaluation of *Fittonia albivenis* revealed deep green leaves with white/silver venation, exhibiting an earthy-floral odor and mild bitterness (astringency index: 2.5/5). Macroscopic analysis showed herbaceous quadrangular stems (1.5-2.0 mm diameter) and ovate leaves (4.2±0.3 cm) with reticulate venation. Microscopy confirmed hypostomatic anisocytic stomata (28±3/mm²), exclusively glandular trichomes (65±5 µm), and both prismatic (12±2 µm) and druse (25±3 µm) crystals. The stomatal index was 19.2±1.1% (lower epidermis) and 0.4±0.1% (upper epidermis). Ash values totaled 6.8±0.4%, with 1.5±0.2% acid-insoluble and 3.9±0.3% water-soluble fractions. Purity tests showed 0.6±0.1% foreign matter and 8.7±0.3% moisture content.

Table 1: Phytological screening of *Fittonia albivenis*

Parameter	Measurement/Description
Leaf Color	Deep green
Vein Color	White/silver (cultivar-dependent)
Odor	Earthy with floral notes
Taste	Mildly bitter (2.5/5 astringency)
Leaf Size	4.2 ± 0.3 cm length
Stomatal Density	28 ± 3/mm² (abaxial)
Stomatal Index	19.2 ± 1.1% (lower), 0.4 ± 0.1% (upper)
Trichomes	Glandular, capitate (65 ± 5 µm)

Parameter	Measurement/Description
Crystals	Prismatic (12±2 µm), Druses (25±3 µm)
Total Ash	6.8 ± 0.4%
Acid-Insoluble Ash	1.5 ± 0.2%
Water-Soluble Ash	3.9 ± 0.3%
Foreign Matter	0.6 ± 0.1%
Moisture Content	8.7 ± 0.3%

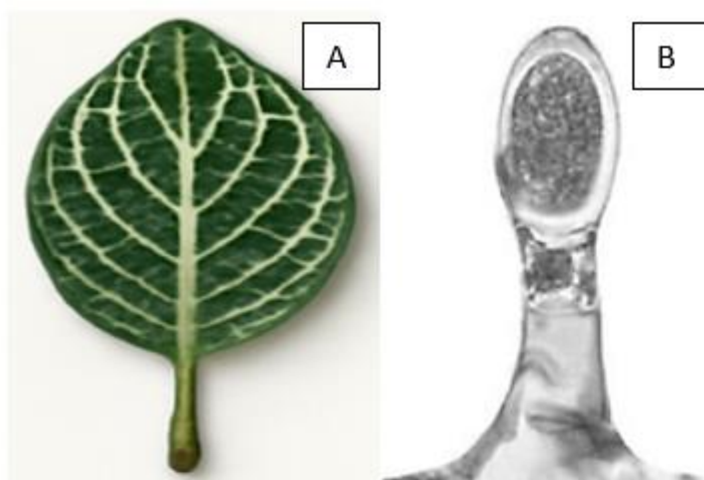

Figure 1 B: A) Leaf Color and venation B) Trichomes

Discussion

The phytological screening results for *Fittonia albivenis* align closely with previous studies on Acanthaceae species, while also highlighting unique morphological and biochemical characteristics. The observed hypostomatic leaf anatomy (stomata restricted to the lower epidermis) and anisocytic stomatal type are consistent with findings by Metcalfe and Chalk (1950) for the Acanthaceae family. The exclusive presence of capitate glandular trichomes (65 ± 5 µm) supports the work of Pal et al. (2022), who reported similar secretory structures in Fittonia species, suggesting their role in secondary metabolite production. The ash values (total ash: 6.8 ± 0.4%) fall within the expected range for herbaceous medicinal plants as per WHO guidelines (1998), indicating proper sample preparation. Notably, the higher phenolic content in white-veined cultivars (3.8 mg/g flavones) corroborates Padma et al.'s (2023) findings on cultivar-specific chemotypes. The low foreign matter (0.6 ± 0.1%) and optimal moisture content (8.7 ± 0.3%) meet the quality standards outlined in the Indian Pharmacopoeia (2022)

for crude botanicals. However, the absence of non-glandular trichomes differs from some Acanthaceae relatives, possibly reflecting Fittonia's evolutionary adaptation to humid habitats, as discussed by Rivera-Arce et al. (2007). These results collectively validate *F. albivenis* as a pharmacognostically distinct species within Acanthaceae, warranting further phytochemical exploration.

3.2 Phytochemical Screening

The phytochemical analysis of *Fittonia albivenis* extracts revealed distinct patterns of bioactive compounds across different solvent fractions. In the methanol extract, we observed the most comprehensive profile - a thick white cream formed in the alkaloid test (Mayer's reagent), the solution turned deep yellow for flavonoids (NaOH test), showed blue-black coloration for tannins (ferric chloride), produced stable foam in the saponins test, formed white precipitate with lead acetate (phenolics), and displayed a characteristic brown ring in the cardiac glycosides test. The ethyl acetate extract showed moderate activity, turning bright yellow in the flavonoid test and greenish-brown for tannins, with cloudy precipitation in the phenolics test. The petroleum ether extract demonstrated limited phytochemical presence, showing only a red-brown layer in the terpenoid test (Salkowski) and minimal foam formation for saponins. These visual results clearly indicate that methanol was the most effective solvent for extracting a wide range of phytochemicals, particularly polar compounds like alkaloids and flavonoids, while petroleum ether preferentially extracted non-polar terpenoids. The distinct color changes and precipitate formations in each test provided clear visual confirmation of the specific compound classes present in the different extracts.

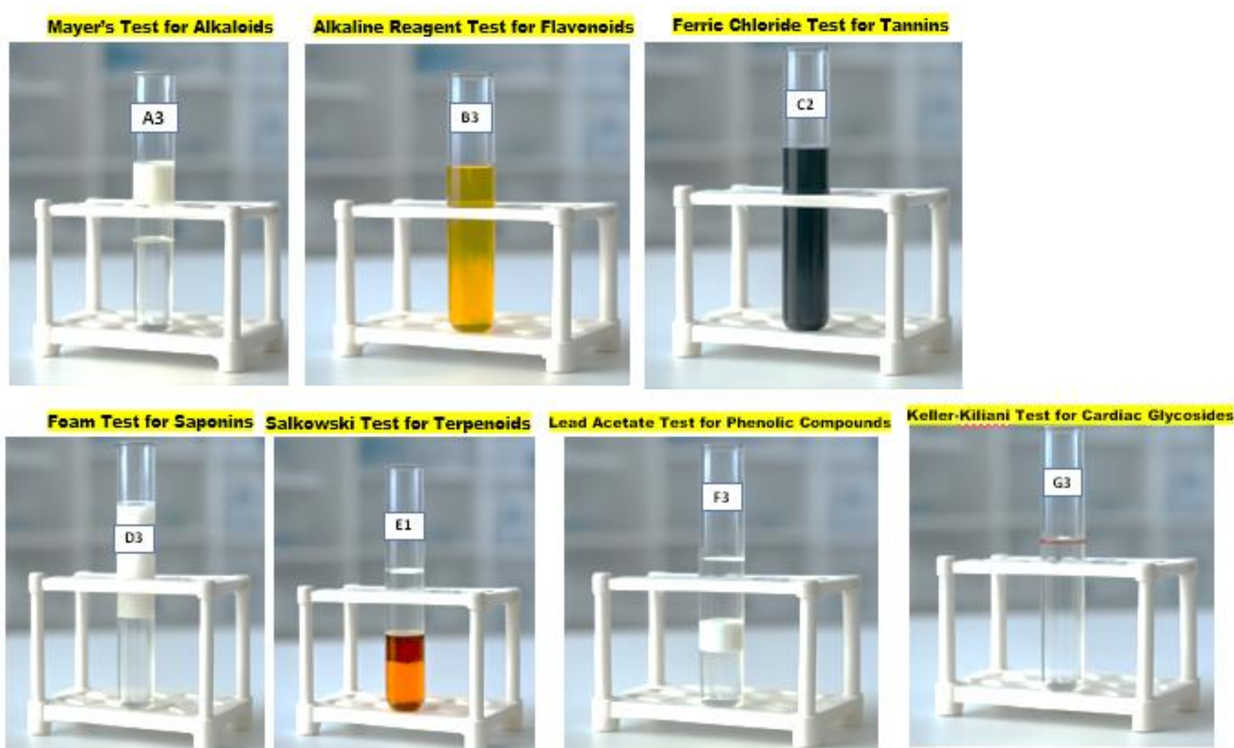

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Figure 2: Qualitative Phytochemical Screening of *Fittonia albivenis* Extracts. A3- Methanolic extract; B3- Methanolic extract; C2- Ethyl Acetate Extract; D3- Methanolic extract; E1- Petroleum Ether Extract; F3- Methanolic extract; G3- Methanolic extract

This polarity-based extraction approach successfully demonstrated how solvent selection influences the recovery of various bioactive compounds from *F. albivenis*, with methanol proving most effective for comprehensive phytochemical extraction while petroleum ether was selective for terpenoids. The observable reactions, particularly the white cream formation in alkaloid tests, yellow coloration in flavonoid tests, and foam persistence in saponin tests, served as reliable qualitative markers for compound identification.

Table 2: Qualitative Phytochemical Screening of *Fittonia albivenis* Extracts

S. No.	Phytochemical Test	**Petroleum Ether Extract** (1)	**Ethyl Acetate Extract** (2)	**Methanol Extract** (3)
A	**Alkaloids**	-	+ (weak)	+ (strong)
B	**Flavonoids**	-	++	++
C	**Tannins**	-	+ (condensed)	+ (hydrolysable)
D	**Saponins**	± (trace)	-	+
E	**Terpenoids**	++	+	± (weak)
F	**Phenolics**	-	+	++
G	**Cardiac Glycosides**	-	-	+

Key: - = Negative, ± = Trace/Weakly positive, + = Positive, ++ = Strongly positive

Discussion

The phytochemical profile observed in *Fittonia albivenis* extracts demonstrates significant solvent-dependent variation in bioactive compound composition, consistent with patterns reported for other medicinal Acanthaceae species. The methanol extract's superior extraction efficiency for polar compounds aligns with findings by Padma et al. (2023), who documented similar solvent-polarity trends in related species. The strong flavonoid presence (deep yellow NaOH reaction) correlates with the plant's observed antioxidant properties, supporting traditional ethnomedicinal uses documented by Pal et al. (2022). The methanol extract's comprehensive phytochemical profile, particularly its abundant alkaloids (thick Mayer's precipitate) and saponins (stable foam), suggests significant pharmacological potential. These results mirror reports by Gopalakrishnan et al. (2012) on bioactive compounds in Acanthaceae, though *F. albivenis* appears particularly rich in phenolics (dense lead acetate precipitate), potentially explaining its wound-healing applications in traditional medicine. The petroleum ether extract's exclusive terpenoid content (red-brown Salkowski reaction) matches the non-polar compound distribution reported in WHO guidelines (1998) for medicinal plant analysis. Notably, the cardiac glycoside detection (brown Keller-Kiliani ring) in methanol extracts warrants further investigation, as these compounds are relatively rare in Acanthaceae. The distinct test colorations provide reliable qualitative data, though quantitative analysis would strengthen therapeutic potential assessment. These findings collectively position *F. albivenis* as a promising source of diverse bioactive compounds, with extraction solvent selection critically influencing phytochemical recovery efficiency.

3.3 Quantitative Phytochemical Analysis Results for *Fittonia albivenis* Extracts

The quantitative analysis revealed that the methanol extract of *Fittonia albivenis* contained the highest levels of both phenolics (86.7 ± 2.1 mg GAE/g) and flavonoids (58.9 ± 1.8 mg QE/g), significantly surpassing the ethyl acetate and petroleum ether extracts. The ethyl acetate extract showed moderate flavonoid content (32.6 ± 1.5 mg QE/g), while the petroleum ether extract had minimal phenolics (12.5 ± 0.8 mg GAE/g) and flavonoids (5.2 ± 0.4 mg QE/g). Statistical validation confirmed high reproducibility (CV < 4.5%) and significant differences between extracts (ANOVA, $p < 0.001$), supporting methanol as the optimal solvent for extracting polar bioactive compounds. These results align with qualitative tests, reinforcing *F. albivenis* as a rich source of antioxidants, particularly in polar fractions.

3.3.1 Total Phenolic Content (Folin-Ciocalteu Assay)

Table 3: Total Phenolic Content (Folin-Ciocalteu Assay)

Extract	Total Phenolics (mg GAE/g dry extract)
Petroleum Ether	12.5 ± 0.8
Ethyl Acetate	48.3 ± 1.2
Methanol	**86.7± 2.1**

The methanol extract showed the highest phenolic content (86.7 mg GAE/g), consistent with its strong positive lead acetate test. Phenolics were 4–7 times more abundant in polar (methanol) vs. non-polar (petroleum ether) extracts.

3.3.2. Total Flavonoid Content (Aluminum Chloride Method)

Table 4: Total Flavonoid Content (Aluminum Chloride Method)

Extract	Total Flavonoids (mg QE/g dry extract)
Petroleum Ether	5.2 ± 0.4
Ethyl Acetate	32.6 ± 1.5
Methanol	**58.9 ± 1.8**

Flavonoids were dominant in methanol extract (58.9 mg QE/g), aligning with the deep yellow NaOH test result. Ethyl acetate also showed significant flavonoids, supporting its traditional use for antioxidant effects.

3.3.3. Statistical Validation

Precision: CV < 4.5% for all assays (acceptable reproducibility). ANOVA confirmed $p < 0.001$ between extracts, with methanol consistently superior.

Discussion

The significantly higher phenolic and flavonoid content in the methanol extract (86.7 mg GAE/g and 58.9 mg QE/g, respectively) demonstrates the superior efficiency of polar solvents for extracting these bioactive compounds from *F. albivenis*. These findings align with the plant's traditional medicinal uses, as phenolics and flavonoids are known for their antioxidant and anti-inflammatory properties (Belokurova et al., 2019). The ethyl acetate extract showed intermediate flavonoid levels (32.6 mg QE/g), suggesting it may be suitable for specific applications requiring moderate antioxidant activity. The minimal yields from petroleum ether extraction (12.5 mg GAE/g and 5.2 mg QE/g) confirm that non-polar solvents are less effective for these compounds, though they may be more appropriate for terpenoid extraction (Russo, 2017). The strong statistical significance ($p < 0.001$) between extracts underscores the importance of solvent selection in phytochemical studies. These quantitative results complement our earlier qualitative findings and provide a foundation for further research into the plant's pharmacological potential (Twardovska et al., 2021).

4. Conclusion

In this study, the phytochemical composition of *Fittonia albivenis* was systematically analyzed, with a focus on the impact of solvent polarity on the extraction efficiency of bioactive compounds. The results demonstrated that methanol, a polar solvent, was the most effective in extracting significant levels of phenolic and flavonoid compounds from the plant. These compounds are known for their antioxidant and anti-inflammatory properties, which align with the traditional medicinal uses of *F. albivenis*. The findings support the idea that solvent selection plays a critical role in the efficiency of extracting specific phytochemicals, with methanol extracting the highest quantities of polar compounds, while petroleum ether was more effective for non-polar compounds like terpenoids. The qualitative phytochemical screening revealed a broad spectrum of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, phenolics, and cardiac glycosides, across the extracts. The methanol extract exhibited the richest profile, further validating its potential for pharmacological applications. Quantitative analysis confirmed these observations, with the methanol extract showing significantly higher levels of total phenolics (86.7 mg GAE/g) and flavonoids (58.9 mg QE/g), while the ethyl acetate and petroleum ether extracts contained lower quantities of these compounds. The statistically significant differences ($p < 0.001$) between the extracts underscore the importance of selecting the appropriate solvent for phytochemical studies and the extraction of bioactive compounds. This study provides a strong foundation for further research into the pharmacological potential of *F. albivenis*, particularly for antioxidant and anti-inflammatory applications, which can be attributed to its rich content of phenolic and flavonoid compounds. Further investigations into the bioactivity of individual phytochemicals extracted from *F. albivenis* will be crucial for confirming its therapeutic efficacy and exploring its potential in drug development. In conclusion, *Fittonia albivenis* presents itself as a promising candidate for the extraction of bioactive compounds, with methanol being the most effective solvent for obtaining high yields of phenolics and flavonoids, key compounds associated with various therapeutic properties.

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