Physicochemical Characterization and Bioactive Profiling of Cissus quadrangularis Salt (Pirandai uppu), a Traditional Siddha Formulation

G. Shiyamala[1], S. Selvakumar[2*] M. Maghimaa[3], Prakash Balu[4]

[1] Assistant Professor, Department of Microbiology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Tiruchengode, Namakkal-637205

[2] Assistant Professor, Department of Microbiology, Kavery Arts and Science College, Mecheri, Salem.

[3] Professor, Department of Pathology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennnai 602105. Tamil Nadu, India

[4] Professor, Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies, Chennai - 600117, Tamilnadu, India

***Corresponding Author**

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ABSTRACT

Cissus quadrangularis salt (Pirandai uppu) is a traditional Siddha formulation widely used for gastrointestinal, inflammatory, and bone-related disorders, yet scientific validation remains limited. This study aimed to standardize and evaluate the formulation through physicochemical characterization, phytochemical profiling, chromatographic analysis, and assessment of antibacterial and antioxidant activities. The salt was prepared using traditional incineration and aqueous extraction methods. Physicochemical parameters such as pH, solubility, and water-soluble ash content were determined, while qualitative phytochemical screening and thin-layer chromatography (TLC) were employed for bioactive profiling. Antibacterial activity was assessed against clinically relevant intestinal pathogens using the agar well diffusion method, and antioxidant potential was evaluated using the DPPH free radical scavenging assay. The formulation exhibited a strongly alkaline pH (10.3) and high water-soluble ash content (92%), indicating enhanced mineral bioavailability. Phytochemical analysis confirmed the presence of flavonoids, tannins, alkaloids, saponins, and cardiac glycosides. The salt demonstrated significant antibacterial activity against multidrug-resistant Shigella dysenteriae and Proteus spp. and showed strong antioxidant activity (90.45% DPPH inhibition; IC_{50} = 23.28 µg/mL). These findings scientifically support the traditional use of Pirandai uppu and provide baseline data for quality control and further investigations.

Keywords: Cissus quadrangularis, Pirandai uppu, Siddha medicine, physicochemical analysis.

INTRODUCTION

Traditional medical systems such as Siddha medicine have long utilized mineral- and plant-based formulations for the management of various ailments, emphasizing holistic healing and natural therapeutics [1]. Cissus quadrangularis, commonly known as Pirandai, is a well-documented medicinal plant extensively used in Siddha and Ayurvedic practices for the treatment of bone fractures, digestive disorders, and inflammatory conditions [2]. Several studies have reported its role in enhancing fracture healing and reducing inflammation, highlighting its pharmacological importance [3,4].

One of the traditional Siddha preparations of this plant is Pirandai uppu (Cissus quadrangularis salt), which is prepared through incineration followed by aqueous extraction, resulting in a mineral-rich formulation believed

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to possess therapeutic properties [5,6]. Such calcined formulations are commonly used in Siddha medicine due to their enhanced stability, bioavailability, and prolonged shelf life [7,8].

Despite its widespread traditional use, scientific validation of Pirandai uppu remains limited, particularly with respect to its physicochemical characteristics and phytochemical composition. Standardization and characterization of traditional formulations are essential to ensure quality, safety, and reproducibility, as emphasized by the World Health Organization[13]. Therefore, the present study aims to scientifically validate the traditional Siddha formulation Cissus quadrangularis salt (Pirandai uppu) through systematic physicochemical characterization, qualitative phytochemical screening, and thin-layer chromatographic (TLC) profiling. This investigation seeks to establish standardization parameters and provide experimental evidence supporting its traditional medicinal use.[8–13]

Materials and Methods

Collection and Preparation of Cissus quadrangularis

Fresh stems of Cissus quadrangularis L. were collected from the local region and authenticated by a qualified taxonomist. The plant material was thoroughly washed with tap water, followed by distilled water to remove adhering impurities. The stems were shade-dried at room temperature for 7–10 days and subsequently used for the preparation of ash and salt following traditional Siddha methodology.

Preparation of Cissus quadrangularis Ash and Salt (Pirandai Uppu)

The dried stems were incinerated in a clean earthen vessel under controlled conditions until complete combustion was achieved, yielding a fine greyish-white ash. The ash was cooled and sieved to remove coarse particles. For salt preparation, the ash was mixed with distilled water, stirred thoroughly, and filtered using Whatman No.1 filter paper. The filtrate was evaporated to dryness over a low flame to obtain pure white crystalline salt (Pirandai uppu). The prepared ash and salt were stored in airtight containers for further analysis (Figure 1).

Physicochemical Analysis

Physicochemical parameters such as color, taste, solubility in water, and pH were evaluated using standard pharmacopoeial procedures.

pH determination: A 1% aqueous solution of ash and salt was prepared, and pH was measured using a calibrated digital pH meter.

Water-soluble ash content: The percentage of water-soluble ash was determined by dissolving the ash in distilled water, filtering, drying the residue, and calculating the soluble fraction gravimetrically (Table 2, Figure 3).

Phytochemical Screening

Qualitative phytochemical analysis of ash and salt was carried out using standard chemical tests to detect the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and cardiac glycosides. The intensity of reactions was recorded as strongly present (++), present (+), or absent (−) based on color change or precipitate formation (Table 2, Figure 2).

Thin Layer Chromatography (TLC) Analysis

TLC profiling of Cissus quadrangularis salt was performed on silica gel 60 F_{254} plates to assess chemical complexity and formulation consistency. The salt extract was spotted on the plates and developed using an appropriate solvent system. Developed plates were visualised under UV light and iodine vapour to detect separated components, and distinct bands were recorded (Figure 5).

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Bacterial Isolates and Culture Conditions

Clinically relevant intestinal bacterial isolates, including Escherichia coli, Enterococcus faecalis, Klebsiella spp., Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus, Proteus spp., Pseudomonas spp., and Vibrio cholerae, were obtained from a recognised microbiology laboratory. Cultures were maintained on nutrient agar slants and sub-cultured before experimentation.

Gram Staining and Biofilm Formation Assay

Gram staining was performed using standard procedures to determine the Gram reaction of bacterial isolates. Biofilm formation was assessed using the tube method, where bacterial cultures were grown in nutrient broth, stained with crystal violet, and visually examined for biofilm adherence along tube walls. Results were recorded as positive or negative (Table 1).

Antibiotic Susceptibility Testing

Antibiotic susceptibility of the isolates was evaluated using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Antibiotic discs of kanamycin (KAN), streptomycin (STR), tetracycline (TET), and ampicillin (AMP) were placed on inoculated plates and incubated at 37 °C for 24 h. Resistance or sensitivity was determined by measuring the zone of inhibition as per CLSI guidelines (Table 1).

Antibacterial Activity of Cissus quadrangularis Salt

The antibacterial activity of Cissus quadrangularis salt was evaluated using the agar well diffusion method. Different concentrations of the salt (up to 1500 mg/mL) were prepared in sterile distilled water. Wells were punched into Mueller–Hinton agar plates seeded with test organisms, and samples were added accordingly. Plates were incubated at 37 °C for 24 h, and zones of inhibition were measured in millimeters (Table 3).

Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant potential of Cissus quadrangularis salt was assessed using the DPPH free radical scavenging assay. Various concentrations of the salt extract were mixed with DPPH solution and incubated in the dark. Absorbance was measured spectrophotometrically at 517 nm. Percentage inhibition was calculated, and IC_{50} values were determined from the dose–response curve (Figure 4, Table 3).

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean values. Graphical representation and IC_{50} calculations were carried out using standard analytical methods.

RESULTS AND DISCUSSION

Antibiotic Resistance and Virulence Characteristics of Intestinal Isolates

The intestinal bacterial isolates investigated in this study exhibited diverse Gram reactions, biofilm-forming capacities, and antibiotic resistance profiles (Table 1). A predominance of Gram-negative organisms was observed, consistent with the epidemiology of enteric infections. Most isolates demonstrated positive biofilm formation, a critical virulence factor associated with chronic infection, enhanced persistence, and reduced antibiotic susceptibility. Biofilm-associated resistance mechanisms are well documented and contribute significantly to treatment failure in gastrointestinal infections [12].

Notably, Shigella dysenteriae and Klebsiella spp. exhibited multidrug resistance to kanamycin, streptomycin, and tetracycline. This resistance pattern reflects the global rise in antimicrobial resistance among enteric pathogens and underscores the urgent need for alternative or adjunct therapeutic agents [13]. In contrast, Staphylococcus aureus remained sensitive to all tested antibiotics, consistent with previous regional surveillance studies reporting variable resistance patterns among Gram-positive isolates [14]. These findings provide a

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relevant clinical context for evaluating the antibacterial potential of traditional formulations such as Cissus quadrangularis salt.

Physicochemical Characterization of Cissus quadrangularis Ash and Salt

Physicochemical evaluation revealed clear differences between Cissus quadrangularis ash and its derived salt (Pirandai uppu) (Table 2). The salt exhibited a higher alkaline pH (10.3) compared to ash (9.1), indicating successful chemical transformation during traditional incineration and aqueous extraction processes. Alkalinity in Siddha salt formulations is often associated with improved mineral solubility and bioavailability, particularly of calcium-based constituents, which are essential for bone health and metabolic functions [15].

The significantly higher water-soluble ash content of the salt (92%) compared to ash (80%) suggests efficient conversion of insoluble inorganic components into bioavailable mineral forms. Similar observations have been reported in mineral-rich Siddha formulations, where increased water-soluble fractions correlate with enhanced therapeutic efficacy. The pure white crystalline appearance and improved solubility further indicate formulation purity and suitability for oral administration. These physicochemical parameters provide essential baseline data for quality control and standardization, in line with WHO recommendations for traditional medicines [16].

Qualitative Phytochemical Profile and Its Biological Relevance

Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides, with stronger expression in the salt compared to ash (Table 2, Figure 2). The enrichment of phytoconstituents in the salt may be attributed to the extraction and concentration processes involved in its preparation. Similar enhancement of bioactive compounds following traditional processing has been reported in earlier pharmacognostic studies [17].

Flavonoids and tannins are widely recognized for their antioxidant and antimicrobial properties, functioning through free-radical scavenging, metal chelation, and disruption of microbial membranes. Recent studies emphasize their role in mitigating oxidative stress-induced tissue damage and inflammatory responses [18,19]. Alkaloids and saponins further contribute to antimicrobial efficacy by interfering with cellular integrity and enzyme systems of pathogenic bacteria. The presence of these phytochemicals provides a mechanistic basis for the observed antibacterial and antioxidant activities of Pirandai uppu.

Antibacterial Activity of Cissus quadrangularis Salt

The antibacterial activity of Cissus quadrangularis salt demonstrated a clear concentration-dependent response, with maximum inhibition observed at 1500 mg/mL (Table 3). Shigella dysenteriae and Proteus spp. showed the highest susceptibility, exhibiting inhibition zones of 18.8 mm and 18.5 mm, respectively. These findings are particularly significant given the multidrug-resistant nature of these pathogens, as identified earlier in this study.

Comparable antimicrobial efficacy of mineral-rich herbal formulations against enteric pathogens has been reported in recent literature, supporting the role of combined phytochemical and inorganic constituents in broad-spectrum antibacterial activity [20]. The alkaline nature of the salt may further enhance antibacterial action by disrupting microbial cell wall integrity and metabolic processes. Collectively, these results suggest that Pirandai uppu could serve as a complementary therapeutic agent in managing resistant gastrointestinal infections.

Antioxidant Potential and Free Radical Scavenging Activity

The antioxidant activity assessed using the DPPH radical scavenging assay revealed strong dose-dependent activity, with a maximum inhibition of 90.45% and a low IC_{50} value of 23.28 µg/mL (Table 3, Figure 4). This level of activity is comparable to or exceeds that reported for several medicinal plant extracts rich in phenolics and flavonoids [21,22].

The potent antioxidant activity observed can be attributed to the high concentration of electron-donating phytochemicals, particularly flavonoids and tannins, which neutralize free radicals and reduce oxidative stress.

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Oxidative stress is a key contributor to inflammatory disorders and delayed tissue repair, and its mitigation supports the traditional therapeutic claims of Cissus quadrangularis formulations [23].

TLC Profiling and Formulation Standardization

TLC analysis revealed distinct and well-resolved bands with characteristic Rf values (Figure 5), confirming the chemical complexity and consistency of Cissus quadrangularis salt. TLC fingerprinting is widely recognized as a simple yet powerful tool for authentication and quality assessment of herbal and herbo-mineral formulations [23]. The observed chromatographic pattern corroborates the phytochemical screening results and can serve as a reference profile for batch-to-batch consistency and detection of adulteration. Recent studies highlight the importance of TLC and HPTLC profiling in establishing standardization protocols for traditional medicines, especially where complex mixtures are involved [24].

Scientific Validation of Traditional Use and Future Perspectives

The combined physicochemical stability, enriched phytochemical profile, significant antibacterial activity against resistant pathogens, and strong antioxidant potential provide compelling scientific support for the traditional use of Pirandai uppu. These findings align with earlier pharmacological and clinical reports on Cissus quadrangularis, which document its efficacy in fracture healing, inflammation control, and metabolic regulation [25].

However, the absence of quantitative mineral profiling and in vivo safety evaluation represents a limitation of the present study. Future investigations should focus on elemental analysis (e.g., calcium, phosphorus, magnesium) using advanced techniques such as ICP-OES, along with toxicity and bioavailability studies, to further strengthen translational relevance and regulatory acceptance [26].

Table 1. Identification and Antibiotic Resistance Profile of Intestinal Pathogens

Bacterial isolate	**Gram reaction**	**Biofilm formation**	**Antibiotic resistance pattern**
Escherichia coli	Gram-negative	Positive	KAN, STR
Enterococcus faecalis	Gram-positive	Positive	AMP
Klebsiella spp.	Gram-negative	Positive	KAN, TET
Salmonella typhi	Gram-negative	Positive	STR
Shigella dysenteriae	Gram-negative	Positive	KAN, STR, TET
Staphylococcus aureus	Gram-positive	Negative	Sensitive to all
Proteus spp.	Gram-negative	Negative	STR, TET
Pseudomonas spp.	Gram-negative	Negative	KAN, STR
Vibrio cholerae	Gram-negative	Negative	AMP

Abbreviations: KAN – Kanamycin; STR – Streptomycin; TET – Tetracycline; AMP – Ampicillin

Table 2. Physico-Chemical and Phytochemical Characteristics of Cissus quadrangularis Ash and Salt

Parameter	**Cissus ash**	**Cissus salt**
pH	9.1	10.3

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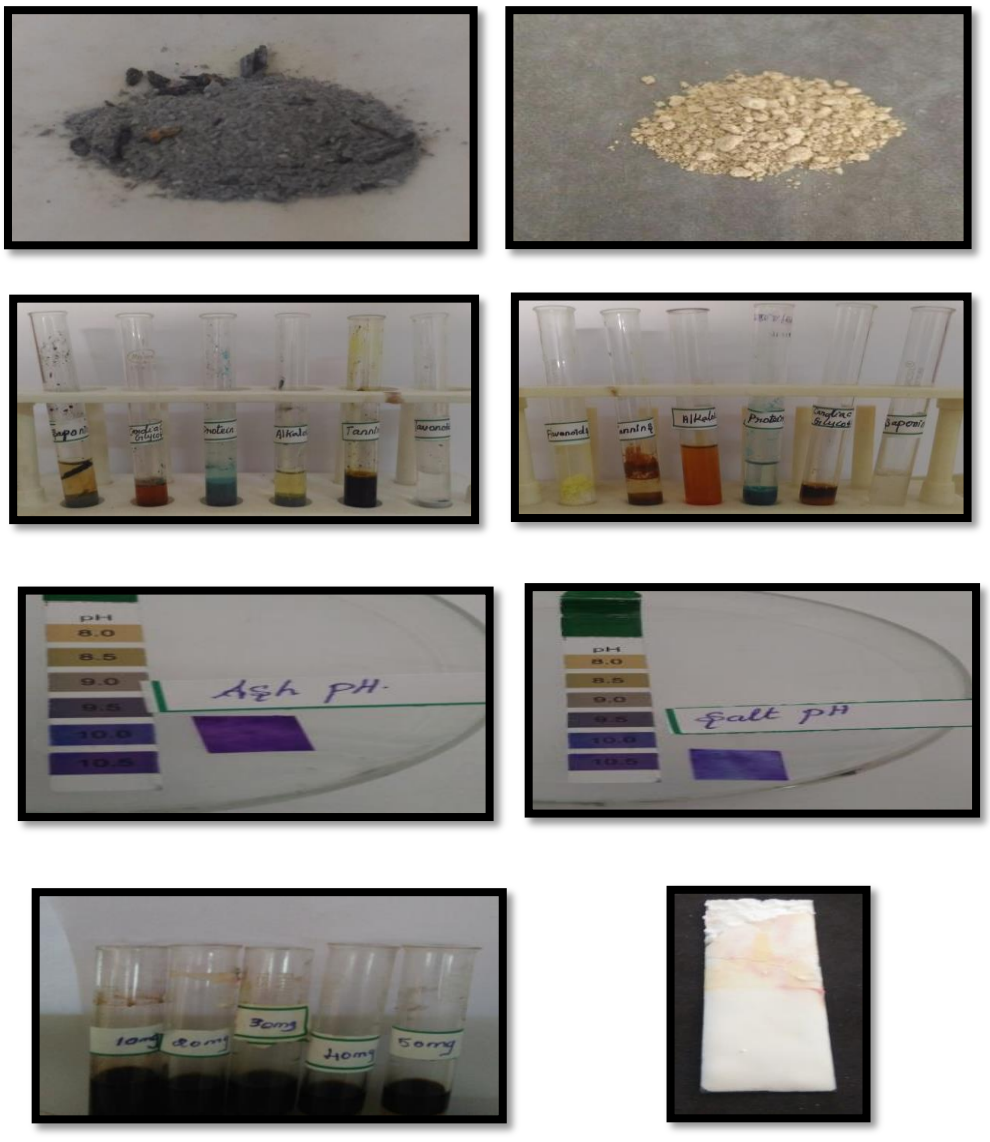
Solubility in water	Insoluble	Soluble
Color	Greyish white	Pure white
Taste	Sour	Bitter
Water-soluble ash (%)	80	92
Alkaloids	+	+
Flavonoids	+	++
Tannins	+	++
Saponins	−	+
Cardiac glycosides	−	+

Symbols: (++) strongly present; (+) present; (−) absent

Figure 1: Cissus Quadrangularis Ash and Salt Preparation

Cissus ash Cissus salt



ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Figure 2: Phytochemical Analysis of Cissus Ash and Salt

Cissus ash Cissus salt

Figure 3: Detection of Parameters

P^H in Cissus ash P^H in Cissus salt

Figure 4: Antioxidant Assay Figure 5: TLC

Table 3. Antibacterial and Antioxidant Activity of Cissus quadrangularis Salt

Test organism/assay	**Maximum zone of inhibition (mm)**	**Concentration**
Shigella dysenteriae	18.8	1500 mg/mL
Proteus spp.	18.5	1500 mg/mL
Klebsiella spp.	17.0	1500 mg/mL
Escherichia coli	15.2	1500 mg/mL
Staphylococcus aureus	14.6	1500 mg/mL
DPPH scavenging activity	**90.45%**	**50 µg/mL**
IC_{50} value	**23.28 µg/mL**	—

CONCLUSION

The present study provides scientific validation of the traditional Siddha formulation Cissus quadrangularis salt (Pirandai uppu) through comprehensive physicochemical characterization and bioactive profiling. The formulation exhibited strong alkalinity and high water-soluble ash content, indicating enhanced mineral availability and formulation stability. Qualitative phytochemical analysis confirmed the presence of therapeutically relevant constituents, including flavonoids, tannins, alkaloids, saponins, and cardiac glycosides, which are known to contribute to antioxidant and antimicrobial activities. TLC profiling established a reproducible chemical fingerprint, supporting formulation consistency and quality control. Biological evaluation demonstrated significant antibacterial activity against clinically important intestinal pathogens, including multidrug-resistant strains, along with potent free-radical scavenging activity. These findings collectively substantiate the traditional therapeutic claims associated with Pirandai uppu and highlight its potential as a natural antimicrobial and antioxidant agent. However, further studies involving quantitative mineral analysis, detailed safety and toxicity assessment, and mechanistic investigations are necessary to strengthen its translational and clinical relevance. Overall, this study establishes essential baseline data for standardization and supports the integration of traditional Siddha formulations into evidence-based medicinal research.

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