RESEARCH PAPER

Phytochemical Profiling and Comparative Quantitative Analysis of Feronia limonia (Linn.)

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ABSTRACT

Phytochemical study of plant products has gained popularity in both plant biochemistry and organic chemistry in recent years. One of the current issues in phytochemistry is carrying out all of the above operations with little amounts of material. Feronia limonia Linn. contains a number of secondary metabolites with medicinal potential. This study examines the key phytochemicals found in the leaves of the medicinally important plant Feronia limonia on a qualitative and quantitative level. The goal of this study is to evaluate phytochemicals by analysing diverse extracts quantitatively and qualitatively. Quantification and phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and glycosides. So it can be concluded that phytocomponents of Feronia limonia serve as potential plant source in the field of pharmaceutical as well as LED developments.

Keywords: Feronia limonia, phytochemical analysis, flavonoids, phenols.
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Introduction

Nature has long served as a vital source of therapeutic agents for human health and well-being. Since ancient times, plants have been utilized in the treatment of various ailments, forming the foundation of traditional medicine systems across cultures. Over the years, numerous medicinal plants have been scientifically investigated for their pharmacological properties, leading to the discovery of novel drugs and the development of new therapeutic agents.[1] In addition to their medicinal value, these plants have also contributed to the advancement of food additives, agrochemicals, and industrial compounds.

Phytochemicals are bioactive compounds naturally occurring in plants play a central role in this context. These substances function as part of the plant's defence mechanism against diseases and environmental stressors and have shown promising potential in disease prevention and treatment in humans. [2]. Primary and secondary components are the two types of phytochemicals that act in plant metabolism. Only a small percentage of the world's 2,50,000 to 5,00,000 plant species have been studied for phytochemicals. [3]

In recent years, phytochemical research on plant products has grown in prominence in both plant biochemistry and organic chemistry. It examines the chemical structure, distribution, and biological function of plant components, as well as the variety of organic compounds accumulated by plants. As a result, developments in phytochemistry are closely tied to the effective use of existing techniques and the constant development of new approaches to unsolved problems as they occur. Completing all of the above duties with such a small amount of material is one of the difficulties in phytochemistry. To solve a biological problem, such as plant growth regulation, plant-animal interactions biochemistry, or the genesis of ancient plants, researchers must first find a spectrum of complex chemical compounds that may only be available in microgram quantities.[4]

As a result, comprehensive plant species screening with the goal of discovering new bioactive compounds can aid in the treatment of a variety of fungal and bacterial illnesses that affect economically important crops and animals, including humans. Plants are beneficial because

they contain chemical components that have physiological effects on the human body. Alkaloids, tannins, flavonoids, and phenolic compounds are the most important bioactive components in plants. Because of their low mammalian toxicity, target selectivity, and biodegradability, these insecticides outperform synthetic pesticides. [5]

Ethnobotanical and Historical Significance of *Feronia limonia* L.

Feronia limonia L., commonly known as wood apple, belongs to the family Rutaceae. In Sanskrit, the plant is referred to as *Kapittha*, a name cited in numerous ancient texts. The *Puranas* describe the tree metaphorically as resembling a cosmic egg, symbolizing the origin of creation. Historical records, including those by the 7th century Buddhist scholar Xuanzang (circa 602–644 A.D.), reference wood apple as a notable indigenous fruit of India.[6]

The species holds deep religious significance, with its leaves traditionally offered to Lord Shiva during worship. Classical Ayurvedic texts by Charaka and Sushruta also document the therapeutic uses of *F. limonia* for a range of ailments including urinary tract infections, toxicosis, diarrhoea, ringworm, and chronic skin disorders.

F. limonia is a slow-growing, deciduous tree that can attain a height of up to 20 meters. It bears round to ovate fruits with a hard, woody pericarp, typically measuring 5-7.6 cm in diameter.[7] Two main varieties are recognized one producing larger, sweeter fruits, and the other yielding smaller, more acidic ones. The name "wood apple" is derived from the fruit's woody shell and sticky, aromatic pulp.

The leaves are embedded with oil glands and are traditionally used in Ayurvedic formulations for their hepatoprotective properties. According to the Encyclopaedia of Medicinal Plants, the pulp of the fruit, when mixed with honey, cardamom, and cumin, is used as a remedy for liver cirrhosis in malnourished children, as well as for treating piles and diarrhoea.[8]

Phytochemical analyses have revealed that *F. limonia* contains a wide array of biologically active secondary metabolites, including coumarins, alkaloids, volatile oils, sterols, tannins, and flavonoids, which contribute to its growing scientific relevance. Beyond its medicinal applications, the tree also provides durable wood used in construction and other utilitarian purposes.

Figure No.1: *Feronia limonia* L. Plant

Regional Names of *Feronia limonia* L.

Feronia limonia L., commonly known as wood apple in English, is recognized by various vernacular names across different regions and languages of the Indian subcontinent:
Sanskrit: Kapittham
Marathi: Kavatha
Hindi: Kowit, Kathbel
Punjabi: Bilin, Kainth
Tamil: Vilamaram, Vilangai
Telugu: Velaga
Bengali: Kathbel, Kavat
Assamese: Beal
Gujarati: Kavit, Kotha, Kondu
Kannada: Belada, Bekalu, Bilvara, Aminamara, Beladahannu
Malayalam: Vila
These diverse regional names reflect the plant's widespread traditional use and cultural significance across India and neighbouring countries.

Geographical Distribution and Cultivation of *Feronia limonia* L.

Feronia limonia L. is a tropical angiosperm species native to India, Bangladesh, parts of Sri Lanka, Pakistan, and select regions of Southeast Asia. As a fruit-bearing plant adapted to tropical climates, it thrives in dry, arid conditions with moderate monsoonal rainfall. Beyond its native range, *F. limonia* is also cultivated in several Southeast Asian countries, including Thailand, Malaysia, and Cambodia. In India, the species is widely grown across various states, including Maharashtra, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Karnataka, Kerala, and in certain areas of the Western Himalayas. Among these, Maharashtra stands out as the leading producer of *Feronia limonia* in the country.

Morphological Characteristics of *Feronia limonia* L.

Feronia limonia L., commonly known as wood apple, is a deciduous tree exhibiting a short, cylindrical stem with a height ranging from 75 to 95 cm and a girth of approximately 60 to 120 cm. The branches are armed with thorns, contributing to its distinctive morphology. The leaves are
pinnately compound, measuring 7–10 cm in length, and comprise small, ovate to oblong leaflets. These leaflets possess marginal toothed oil glands, which are a notable feature of the species. The inflorescence is a loose, lateral panicle bearing polygamous flowers, which include both male and bisexual forms. The fruit is globose to ovate, characterized by a hard, woody pericarp that encases the pulp and seeds. Seeds are white in color and embedded within the aromatic, edible pulp.

Traditional Uses of *Feronia limonia* L.

In the Ayurvedic system of medicine, the fruit of *Feronia limonia* is valued for its high vitamin C content and is traditionally employed as an anti-scorbutic agent. It is also used as a tonic for the liver, heart, and lungs, as well as a carminative. Additionally, it is administered in the treatment of hiccups and for soothing sore throats. The fruit pulp has been traditionally utilized in the management of sterility, and in the treatment of breast and uterine cancers. When applied as a poultice, the pulp serves as an antidote for venomous bites.

Ripe fruits are commonly used in the treatment of renal disorders, whereas unripe fruits possess anti-diarrhoeal properties. The leaves of the plant are employed in traditional medicine for the treatment of flatulence and haemorrhoids, and are known for their astringent, anti-diabetic, anti-emetic, and expectorant properties. [19,23,22, 33,29]
Materials and Methods[10,11,12]

Plant Material Collections and Drying

Fresh leaves of the plant were discovered and gathered in the Ahmednagar District of Maharashtra, India. Dried in the shade at ordinary room temperature. Store crude medicine powder in sealed bottles away from light and humidity until utilized for extract extraction.

Authentication

The plant specimen was authenticated by Dr. D.L.Shirodkar Botanist, Botanical Survey of India, Pune. The plant sample voucher specimen received for future reference (Voucher Specimen Number PBPLA-1)
Crude Extract preparation:
In the Soxhlet system, 100 grammes of dry powdered leaf material were subjected to 12 hours of successive organic solvent extraction. Graded polarity solvents such as hexane, chloroform, ethyl acetate, ethanol, and distilled water were utilised in the extraction operation. The solvent is kept at a boiling temperature throughout the extraction process, and all laboratory criteria are met. The pure dry extract was labelled and stored in the refrigerator until further testing. (Harborne et al., 1998, Gokhale et al., 2020 & Trease et al., 2002).

Phyto-chemical Screening:

The physical properties of concentrated dried extracts of *Feronia limonia* leaves, as well as their percent yield, were investigated. It was also used to do preliminary qualitative screening of

phytochemicals using well-established methods. (Mukherjee et al., 2002, Harborne et al., 1998 & Khandelwal et al., 2005).

Thin Layer Chromatography

To identify pure phytocomponents present in extracts need to isolate them by chromatographic techniques. Once their phytochemical nature is identified it will be easy to characterize them for better pharmacological action. The chromatographic analysis was performed by steps well defined procedures.[18,20,28]

Quantification of phyto-chemicals in crude extract of *Feronia limonia* .

Quantitative phytochemical analysis: The phytochemicals which are extracted may show response in qualitative analysis. The phytochemicals present in the all extracts was determined and quantified by standard procedures as follows.

Determination of total phenolic compounds: 100 milligrams of the sample extract was properly weighed and diluted in 100 mL of distilled water. 1 ml of this solution was transferred to a test tube, followed by the addition of 0.5 ml 2N Folin-Ciocalteu reagent and 1.5 ml 20 percent Na_2CO_3 solution, and lastly the volume was filled up to 8 ml with distilled water, vigorous shaking, and let to stand for 2 hours. At 765 nm, the absorbance was measured. Using a standard calibration curve derived from varying diluted quantities of gallic acid, these data were utilised to determine the total phenolic content. Under the same circumstances, the absorption of a standard gallic acid solution (0.5 mg/ml) in methanol was determined. All of the tests were performed in duplicate.[11,39]

Determination of total flavonoids: The approach relies on the production of a flavonoids-aluminium combination with a maximum absorptivity of 415nm. A mixture of 100 l of sample extracts in methanol (10 mg/ml) and 100 l of 20 percent aluminium trichloride in methanol was used. A drop of acetic acid was added, followed by a 5ml methanol dilution. The absorbance was measured at 415 nm after 40 minutes. 100 mL of sample extracts and a drop of acetic acid were used to make blank samples, which were then diluted to 5 mL with methanol. Under the same circumstances, the absorption of a standard quercetin solution (0.5 mg/ml) in methanol was determined. All of the tests were performed in duplicate.[11]

Determination of total alkaloids:

The 1gm test extract was macerated with 20 ml of ethanol and 20% H_2SO_4 (1:1 v/v). The filtrate (1 ml) was added to 5 ml of 60% H_2SO_4. After 5 min, 5 ml of 0.5% formaldehyde in 60% H_2SO_4 was mixed with the above mixture and allowed to stand for 3 hr. The absorbance was read at 565 nm.[11,39]

Determination of total tannins: 0.25 ml Folin Phenol reagent, 0.5 ml 35 percent sodium carbonate solution, 3.75 ml distilled water, 0.5 ml test extract, 3.75 ml distilled water, 0.25 ml Folin Phenol reagent, 0.25 ml Folin Phenol reagent, 0.25 ml Folin Phenol reagent, 0.25 ml Folin Phenol rea at 725nm, the absorbance of the aforementioned combination was measured. Standard solutions were tannic acid dilutions (0 to 0.5 mg/ml). Tannic acid content is measured in milligrams per millilitre of extract.[4,15]

Determination of total glycosides:

The extract (1 gram) was macerated in 50 ml distilled water before being filtered. 4 ml alkaline pirate solution was added to the filtrate (1 ml). After boiling for 5 minutes, the mixture was allowed to cool. At 490 nm, the absorbance was measured.[10,32]

Test for Terpenoids:

The 1gm test extract was macerated in 50 ml ethanol before being filtered. 2.5 ml filtrate, 2.5 ml aqueous phosphomolybdic acid solution (5%), and 2.5 ml concentrated H_2SO_4 Allow 30 minutes for the mixture to settle before adding 12 mL of ethanol. At 700 nm, the absorbance was measured.[1,17]

Test for Steroids:

The 1gm test extract was macerated in ethanol for 20 minutes before being filtered. 2 ml chromagen solution was added to the filtrate (2 ml), and the solution was allowed to stand for 30 minutes. At 50 nm, the absorbance was measured. The difference in colour intensity or absorbance between the test and blank samples is proportional to the concentration of the particular phytocomponents found in the test extract. All of the above quantitative data is presented in milligrams per gm of dried sample. [38, 7, 37,27,40].

Results and Discussion

Phyto-chemical screening test:

In this work, the qualitative analyses of *Feronia limonia* leaves crude extracts were examined, and the findings were presented. Table 1 shows the physical properties and % yield of all extracts. The data shows that the percentage yield of chloroform, ethyl acetate, and ethanol extracts were higher.

Table No. 1: Characteristics of Plant extracts

Feronia limonia Leaves Extract particulars	Percent Yield (%W/W)	Characteristics	
		Colour	Consistency
Hexane extract (FLH)	06.21%	Dark Green	Solid
Chloroform extract (FLC)	06.70%	Brown	Solid
Ethyl acetate extract (FLEA)	11.01%	Dark brown	Semisolid
		Dark yellowish	

Appearance/

The extract variety yielded positive results for a variety of phytochemicals reported in table 2. In *Feronia limonia* leaves, polarity gradient solvent selection resulted in greater separation of distinct complex metabolites.

Table No. 2: Qualitative analysis of *Feronia limonia Linn.* Leaves extract

Sr. No.	**Tests**	**FLH**	**FLC**	**FLEA**	**FLET**	**FLA**
1	Tests for Acidic compounds	-	-	+	+	-
2	Test for carbohydrate					
	Molish's test	-	-	-	+	+
	Fehling test	-	+	+	+	+
	Benedicts test	-	-	+	+	-
	Barfoed test	-	+	-	-	-
	Selivanoffs test	-	-	+	-	-
	Osazone formation test	-	-	-	-	-
3	Test for Proteins					
	Biuret Test	-	-	-	+	+
	Millons Test	-	-	-	-	-
4	Test for amino acids					
	Ninhydrine test	-	+	-	+	+
5	Test for Steroids					
	Salkowski test	+	+	-	-	-

	Libermann test	-	-	-	+	-
	Libermann-Burchard reaction	-	-	-	-	-
	Test for Glycosides					
6	Anthraquinone glycoside test	-	+	+	+	-
	Cardiac glycoside test	-	+	-	-	-
	Cynogentic glycosides test	-	-	-	+	-
7	Test for Terpenoids:	-	+	-	+	-
8	Test for Saponin					
	Foam test	-	+	-	-	+
	Test for Alkaloids					
	Dragondorff's test	-	-	-	+	-
9	Mayer's test	-	-	-	-	-
	Hager's test	-	-	+	+	-
	Wagner's test	-	-	-	+	-
	Test for Tannins and Phenolic compounds					
10	5% $FeCl_3$ test	-	+	-	+	-
	Lead acetate solution	-	+	+	+	-
	Test for Flavonoids					
11	Shinoda test	-	+	+	+	-
	Sulphuric acid test	-	-	+	+	-

Abbreviation: FLH- *Feronia limonia* leaves Hexane extract; FLC- *Feronia limonia* leaves Chloroform extract; FLEA- *Feronia limonia* leaves Ethyl Acetate extract; FLET- *Feronia limonia* leaves Ethanol Extract, FLA- *Feronia limonia* leaves Aqueous Extract., (-):Absent, (+): Presence

As per above observation all solvents showed their efficiency to separate variety of phytocomponents based on affinity. As it observed phytochemical complexity need to isolate and quantify them for better characterization.

In *Feronia limonia* Linn. leaves Hexane extracts showed presence of steroids, Chloroform extracts showed saponins, steroids, carbohydrates, glycosides, saponins, Ethyl acetate extract showed acidic compounds, glycosides, alkaloids, Phenolic compounds as well as flavonoids., Ethanol extract showed glycosides, alkaloids, Phenolic compounds as well as flavonoids, Aqueous extracts showed proteins and saponins positive test.

Thin Layer Chromatography

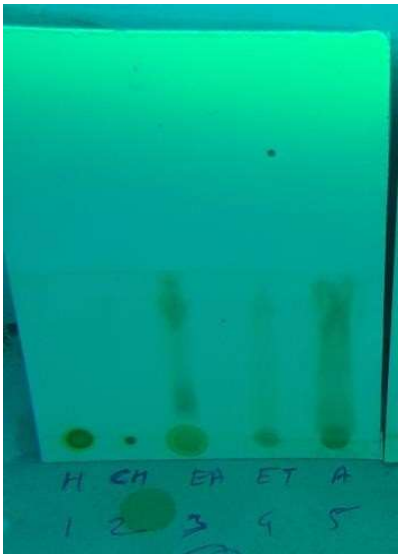


Figure No.2: TLC for alkaloids under Long UV light *Feronia limonia,*

Table No. 3: Thin Layer Chromatography of *Feronia limonia* leaves extracts: Result

Sr. No.	Chemical constituent	Mobile Phase	Visualization Spraying reagent	Color of spot	Rf- value	Std. Rf value
1.	Alkaloids	Toluene : Ethyl acetate: Formic acid (50:40:10)	10% H_2SO_4 in ethanol	Violet - blue	Chloroform:0.43 Ethanol : 0.89 Ethyl acetate : 0.85	0.82
2.	Glycoside	Ethyl acetate : Methanol : Water (100 : 16.5 : 13.5)	Under UV - 365	Violet - blue	Ethanol : 0.70 Ethyl acetate: 0.62	0.65
3.	Flavonoid	Toluene : Ethyl acetate : Glacial acetic acid : Water (100:11:11:26)	Anisaldehyde – Sulfuric acid.	Yellowish green	Hexane: 024 Ethanol : 0.82 Chloroform : 0.89	0.80
4.	Tannin	Ethyl acetate: Formic acid : Acetic acid : Water (100:11:11:26)	5 % $FeCl_3$ in 0.1N HCl	Black	Ethanol : 0.60	--
5.	Steroids	Ethyl acetate :	Vanillin –	Pink	Ethanol : 0.74	0.7

		Methanol : Acetic acid (70 : 20 : 10)	Sulfuric acid.		Chloroform : 0.45 Ethyl acetate : 0.83	0
6.	Saponin	Ethyl acetate: Formic acid : Acetic acid : Water (100:11:11:26)	Anisaldehyde – Sulfuric acid.	Green	Aqueous: 0.65	-

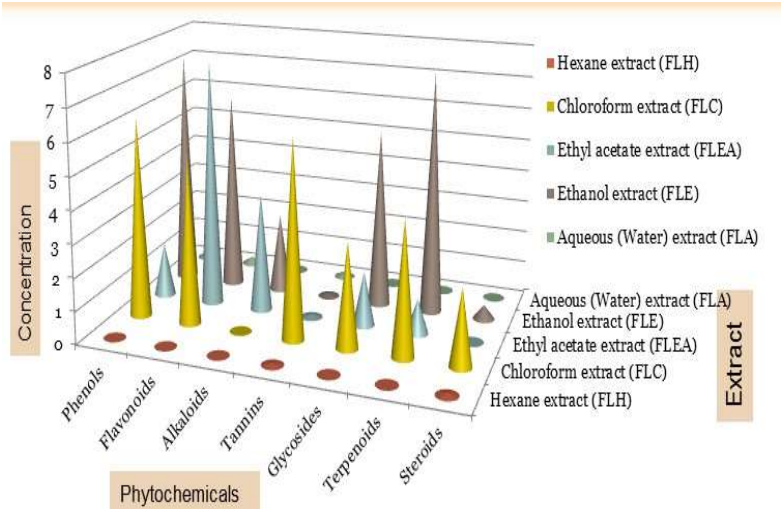
Table No. 03: Thin Layer Chromatography observation and result

Quantification of phyto-chemicals in crude extract of *Feronia limonia*

In quantitative examination of all assays, extracts of *Feronia limonia* leaves indicated good findings for seven phytochemicals, including phenol, flavonoids, alkaloids, tannin, glycosides, terpenoids, and steroids. The findings were depicted in Table 4 and Graph 1. Although alkaloids, phenolic chemicals, flavonoids, and glycosides are important secondary metabolites that contribute to the plant's therapeutic properties, they are also important primary metabolites. Additional analytical methods were used to determine the phytochemical contents of the extract.

Table No.4: Quantitative Analysis of *Feronia limonia* Linn. Leaves extracts

Extracts/ Test	Phytochemical Mean ± STD						
	Phenols	Flavonoids	Alkaloids	Tannins	Glycosides	Terpenoids	Steroids
Feronia limonia Linn. Leaves extracts							
Hexane extract (FLH)	-	-	-	-	-	-	-
Chloroform extract (FLC)	6.20 ± 0.062	5.21 ± 0.030	-	6.11 ± 0.121	3.23 ± 0.012	4.11 ± 0.030	2.34 ± 0.012
Ethyl acetate extract (FLEA)	1.18 ± 0.013	7.18 ± 0.043	3.68 ± 0.015	0.08 ± 0.003	1.68 ± 0.023	1.08 ± 0.013	-
Ethanol extract (FLET)	7.31 ± 0.052	6.15 ± 0.078	2.45 ± 0.165	0.10 ± 0.004	5.51 ± 0.012	7.44 ± 0.055	0.44 ± 0.051
Aqueous (Water) extract (FLA)	0.16 ± 0.020	0.22 ± 0.043	-	0.16 ± 0.003	--		



Graph No. 1: Quantitative phytochemical analysis of *Feronia limonia* Linn. Leaves

The highest concentration of phenols (7.31 mg /g), flavonoids (6.15 mg /g), glycosides (5.51 mg /g) and terpenoids (7.44 mg /g) observed in ethanol extract. The highest concentration of flavonoids (7.18 mg /g), alkaloids (3.68 mg /g) in ethyl acetate extract. The steroids (2.34 mg /g) observed in chloroform extract.

Conclusion

The present study demonstrated that Feronia limonia leaves contain a diverse array of phytochemicals across successive solvent extracts. Qualitative screening confirmed the presence of primary metabolites such as carbohydrates, proteins, and lipids, alongside key secondary metabolites including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and glycosides. The richness of these phytoconstituents suggests that the leaves actively participate in biosynthetic pathways, leading to the formation of complex bioactive derivatives.

Quantitative analysis revealed that the ethyl acetate and ethanol extracts contained the highest concentrations of bioactive compounds, highlighting the influence of solvent polarity on extraction efficiency. Among the metabolites, alkaloids are particularly noteworthy due to their metabolic significance and protective roles in biological systems. Flavonoids and phenolic compounds demonstrated notable antioxidant, anti-inflammatory, and anticarcinogenic potential, while tannins exhibited antifungal and antibacterial activity. The combined presence of these compounds supports the therapeutic relevance of F. limonia and underscores the advantages of plant-derived metabolites over synthetic agents in terms of efficacy and reduced side effects.

Overall, the findings indicate that the bioactive components of Feronia limonia, particularly those in ethanol and ethyl acetate extracts, may play significant roles in physiological and pharmacological activities. These extracts hold promise as potential sources of novel phytopharmaceuticals, and future studies focusing on bioactivity-guided fractionation, structural characterization, and in vivo validation could further establish their therapeutic and nutraceutical applications.

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