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Pharmaceutical Standardization and Phytochemical Profiling of Bilvamuladi Elixir

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Abstract

Bilvamuladi Elixir is a classical Ayurvedic polyherbal formulation indicated for *Udarashoola* (abdominal colic) in children. This study aimed to standardize and evaluate the formulation using pharmaceutical procedures and modern analytical tools. Raw drugs—*Aegle marmelos*, *Ricinus communis*, *Plumbago zeylanica*, *Zingiber officinale*, *Ferula foetida*, and Saindhava Lavana—were authenticated and processed as per classical guidelines. The elixir was prepared through hydro-distillation and homogenization to obtain a stable pediatric dosage form. HPTLC fingerprinting showed multiple peaks representing phenolics, flavonoids, coumarins, and terpenoids linked to carminative and digestive actions. GC–MS analysis revealed (E)-cinnamaldehyde as a major constituent, along with eucalyptol and α-terpineol, supporting antimicrobial, anti-inflammatory, and antioxidant effects. FTIR confirmed functional groups of polyphenols and tannins. These findings correlate with Ayurvedic claims and support the formulation's role in relieving pediatric abdominal colic while establishing a scientific basis for its quality and efficacy.

Key words : Bilvamuladi Elixir; Abdominal colic; HPTLC standardization.

There has been a remarkable growth in the acceptance and utilization of Ayurveda medicine over the past few decades. Owing to its natural origin, holistic approach, and relatively fewer adverse effects, Ayurveda has gained popularity not only in developing nations but also across developed countries. From a global perspective, there is a growing shift toward traditional and herbal formulations, as the limitations, long-term adverse effects, and safety concerns of modern pharmacotherapy are becoming increasingly evident[25].

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Ayurvedic formulations are generally composed of multiple herbals or Herbo-mineral ingredients. Each constituent possesses diverse phytochemical components, and their pharmacological actions vary depending on the synergistic interaction of these components[16]. Hence, it becomes the prime responsibility of regulatory authorities to ensure that Ayurvedic medicines available to health seekers meet the prescribed standards of quality, safety, and efficacy. For this purpose, standardization techniques for both raw materials and finished formulations have been recommended. Among these, High-Performance Thin-Layer Chromatography (HPTLC) is widely employed to establish the identity, purity, and quality of herbal drugs[19].

Abdominal colic pain is one of the most common gastrointestinal complaints encountered in pediatric practice. It significantly affects feeding, sleep patterns, and overall growth and development of children, thereby imposing emotional and psychological stress on caregivers. Functional gastrointestinal disorders, improper digestion, gas accumulation, and intestinal spasms are commonly implicated in pediatric abdominal colic. Conventional management often provides symptomatic relief but may be associated with recurrence or undesirable side effects, necessitating safer and more holistic therapeutic options[20].

According to Indian classical Ayurvedic literature, Bilvamuladi Elixir is a well-established polyherbal formulation indicated for the management of Udarashoola (abdominal colic pain), particularly in children. The formulation contains Bilva (*Aegle marmelos*), Eranda (Ricinus communis), Chitraka (*Plumbago zeylanica*), Shunthi (*Zingiber officinale*), Hingu (*Ferula asafoetida*), and Saindhava Lavana (rock salt). These ingredients collectively possess Deepana, Pachana, Vatanulomana, Shoolahara, and Agnivardhaka properties. By correcting Agnimandya and pacifying Vata-dominant pathology, Bilvamuladi Elixir helps relieve intestinal spasm, improves digestion, facilitates the expulsion of flatus, and effectively alleviates abdominal colic pain in children[15,20].

Aims and objectives :

To evaluate the therapeutic potential and phytochemical evaluation profile of Bilvamuladi elixir with using high performance thin layer chromatography (HPTLC) in the management of abdominal pain.

Pharmaceutical Study: Pharmaceutical study started from collection of genuine raw materials, followed by its pre- processing and finally conversion to the product-Bilvamuladi elixir. The ingredients of Bilvamuladi elixir are Bilva, Erand, Chitrak, Shunti, Hingu, Saindhav All the ingredients were taken in equal proportion. The reference of this yoga is found in yogratnakar samhita shuladhikar adhaya, the decoction of roots of Bilva Erand Chitrak Shunti added with hingu and saindhav.

Collection of Raw Drugs :

The raw drugs were collected from a GMP certified pharmacy (figure 1). The roots of Bilva Erand and Chitraka in dried form, niryas of hingu and saindhav (rock salt) are collected. Authentication of drug done in Dravya Guna department of Parul institute of ayurved and research.

Table-1. Ingredient of Bilvamuladi elixir

Sr No.	Plants	Latin name	Family	Drug Form	Quantity
1.	*Bilva*	Aegle marmelos (L.)	Rutaceae	Root powder	1:1:1 as per requirements
2.	*Erand*	*Ricinus communis* L.	Euphorbiaceae	Root powder	
3.	*Chitrak*	*Plumbago zeylanica* L.	Plumbaginaceae	Root powder	
4.	*Visvab-hesaja*	Zingiber officinale Roscoe	Zingiberaceae	swaras	500 ml
5.	*Hingu*	*Ferula asa-foetida* L.	Apiaceae	niryas	$\frac{1}{18}$ parts of distillate
6.	*Saind-hava*	Rock Salt	-	powder	$\frac{1}{12}$ parts of distillate

Drug pre-processing :

All the ingredients were clean properly and dried in sunlight. Bilva, Erand, Chitraka, and Shunthi were made into coarse powder. Hingu and Saindhav were powdered separately into a fine powder.

Preparation of Bilvamuladi elixir :

According to the method described in rasa Tantrasara va siddha prayoga sangrah, one part of the drug was soaked in four parts of water and kept overnight in round bottom flask. The next day, the mixture was distilled using a hydro-distillation apparatus to obtain the distillate. Saindhav powder and hingu powder were added to this distillate and mixed well using a homogenizer. after that 5 ethanol was added, followed by the gradual addition of flavouring agent and corn sweetener. finally, the whole mixture was homogenized properly to get a uniform preparation.

HPTLC Interpretation of Bilvamuladi Elixir:

The HPTLC fingerprint analysis for methanol extract of Bilvamuladi Elixir on was carried out at concentrations 4.0 µL, 6.0 µL, 8.0 µL, 10.0 µL and 12.0 µL under Visualizations: 254 nm (UV-absorbing compounds) and 366 nm (fluorescent compounds). The test solution the sample was prepared by weighing 5 g of sample in a beaker and to it 100 mL of Methanol was added. The solution was sonicated for 15 minutes. It was then cooled and filtered with a simple filter paper. The Test solution thus obtained was used for HPTLC fingerprinting. The Chromatography was performed on 10 × 10 cm thin layer chromatography (TLC) plates coated with 0.2 mm layers of silica gel 60 F254 (Merck). The samples were applied to the plate as 6 mm wide bands by means of a Linomat 5 sample applicator (CAMAG, Switzerland). The plate was developed to a distance of 8.0 cm with Toluene:Ethyl acetate: Formic acid (5:4:1 v/v/v) as mobile phase in a CAMAG twin-trough chamber saturated with mobile phase vapor. The plate was then dried at room temperature for five minutes was scanned at 254 nm by the use of a CAMAG TLC scanner 3 using winCATS 4 software (CAMAG, Switzerland).

Table-2. Rf values obtained for Methanol extract of Bilvamuladi Elixir Under 254 nm visualisation

Spot	6.0 µL		8.0 µL		10.0 µL		12.0 µL	
No.	Rf value	Area%	Rf Value	Area%	Rf value	Area%	Rf value	Area%
1	0.175	8.26	0.250	5.54	0.247	4.96	0.251	5.66
2	0.257	7.08	0.540	11.44	0.544	11.86	0.546	12.28
3	0.525	11.95	0.790	83.02	0.769	83.18	0.821	82.07
4	0.774	72.71						

Table-3. Rf values obtained for Methanol extract of Bilvamuladi Elixir Under 366 nm visualisation

Spot	4.0 µL		6.0 µL		8.0 µL		10.0 µL		12.0 µL	
No.	Rf value	Area%	Rf value	Area%	Rf value	Area%	Rf Value	Area%	Rf value	Area%
1	0.035	100	0.032	100	0.031	100	0.031	66.98	0.029	100
2							0.192	33.02		

Ingredients: *Aegle marmelos, Ricinus communis, Plumbago zeylanica, Zingiber officinales, Ferula assa foetida,* Saindhava Lavana.

Interpretation and Discussion :
Under 254 nm

Table-4. Phytochemical Interpretation of HPTLC Fingerprint of Bilvamuladi Elixir under 254 nm (UV Detection)

Rf Range	Probable Phytochemical Class	Likely Plant Source(s)	Probable Pharmacological Role	Area % Relevance
0.17–0.25	Coumarins[22,24], alkaloids[4,7], simple phenolics[13,17]	*Aegle marmelos*, *Plumbago zeylanica*	Digestive stimulant, carminative, antimicrobial, anti-inflammatory	~5–8% : minor but active support
0.25–0.55	Resin acids[22,24], terpenoids[11,12,21]	*Ricinus communis*, *Ferula foetida asa*	Purgative, carminative, anti-spasmodic	~7–12% : moderate contributor
0.52–0.55	Piperidine derivatives[22,24], chromones[14,21]	*Ferula asa foetida*	Carminative, anti-flatulent, expectorant	~11–12% : consistent moderate peak
0.76–0.82	Major flavonoids[22,24], tannins[4,7,13,17], resin esters[14,21]	*Aegle marmelos*, *Zingiber officinale*, *Plumbago zeylanica*	Dominant anti-inflammatory, digestive stimulant & carminative activity	~72–83% : major band

Under 366 nm

Table-5. Phytochemical Interpretation of HPTLC Fingerprint of Bilvamuladi Elixir under 366 nm (Fluorescent Detection)

Rf Range	Probable Phytochemical Class	Likely Plant Source(s)	Probable Pharmacological Role	Area % Relevance
0.02–0.04	Fluorescent phenolics[22,24,4,13], lignans[17]	*Aegle marmelos*, *Ricinus communis*	Antioxidant, digestive stimulant	100% at most loads : dominant fluorescent band
0.18–0.20	Alkaloids[22,24] volatile terpenoids [1,10,14,21]	*Zingiber officinale*, *Ferula asa foetida*	Stimulant, anti-flatulent, anti-inflammatory	~33% (only at 10 µL) : secondary peak

GCMS Analysis :

How the analysis is done: In the present GC–MS analysis, only peaks with significant relative abundance (area % ≥ 3%) and high NIST library match confidence scores (>90%) were considered for phytochemical identification and pharmacological interpretation. Peaks with extremely low area % were excluded because they typically represent background noise, column bleed, solvent residues, or trace volatiles that do not contribute meaningfully to the chemical profile or therapeutic activity of the formulation. Compounds with poor spectral match scores, unidentified mass fragments, synthetic industrial contaminants, or structures not botanically plausible for the listed ingredients were also excluded to maintain analytical validity. This selective approach aligns with established GC–MS interpretation standards and ensures that only biologically relevant and phytochemically credible constituents are considered in correlating the chromatographic profile with the pharmacological potential of Bilvamuladi Elixir.

Table-6. GC–MS Identified Phytoconstituents of Bilvamuladi Elixir with Retention Time, Molecular Characteristics, and Pharmacological Activities

RT (min)	Area %	Chemical Compound	CAS No.	MW	Formula	Pub Chem ID	SMILES	Pharma-cological Activity
11.422	73.77	Cinnamald-ehyde (E-)	14371-10-9	132	C_9H_8O	637511	O=CH/C=C/c1ccccc1[1]	Antimicrobial, anti-inflam-matory, antioxidant
4.759	5.88	Eucalyptol	470-	154.	$C_{10}H_{18}O$	2758	CC1CCC2	Bronchodilator,

		(1,8-cineole)	82-6	25			(C(C1)O2)[2]	mucolytic, anti-inflammatory
8.890	4.81	1,6-Heptadien-4-ol, 4-propyl-	52939-61-4	154	$C_{10}H_{18}O$	142990	CC(C)=CC-C1CC(C)-(C)O1[3]	Unidentified phytochemical — potential terpene derivative
9.390	4.51	α-Terpineol	98-55-5	154	$C_{10}H_{18}O$	17100	C=CC(C=C)CO[4]	Antioxidant, antimicrobial, anti-inflammatory
5.320	3.86	Methyl 2,2,3-trimethylcyclo-pentyl ketone	17983-22-1	154	$C_{10}H_{18}O$	6438191	O=C(C1CCC-(C)(C)C1)[5]	Antimicrobial

Table-7. Correlation of GC–MS Identified Compounds with Probable Herbal Sources in Bilvamuladi Elixir

Compound	Mapped Herbal Source	Reason for Mapping
Cinnamaldehyde (E-)	*Zingiber officinale* & *Ferula asa foetida*	Ginger and hing both contain aromatic aldehydes; cinnamaldehyde-like compounds are reported in pungent rhizomes
Eucalyptol (1,8-cineole)	*Aegle marmelos* & *Zingiber officinale* volatile oil	Both contain cineole as part of monoterpene oil fraction
α-Terpineol	*Aegle marmelos*, *Ferula asa foetida*	Present in citrus-like and resinous oleo-gum exudates
Heptadienol derivative	*Plumbago zeylanica*	Known for long chain alcohols & terpenoid derivatives
Trimethylcyclo-pentyl ketone derivative	*Ferula asa foetida*	Hing contains resinous ketones and volatile terpenoids

The GC–MS chromatogram of Bilvamuladi Elixir demonstrates a phytochemical profile dominated by (E)-cinnamaldehyde (73.77%), a key aromatic aldehyde known for its potent antimicrobial, anti-inflammatory, and antioxidant effects, contributing to digestive stimulant and gut-protective actions relevant to the formulation[2,18]. Minor but pharmacologically relevant constituents include eucalyptol (5.88%), which supports bronchodilation, mucolysis, and anti-inflammatory effects[8,9] and α-terpineol (4.51%), a monoterpene alcohol exhibiting antimicrobial and anti-inflammatory properties[3]. Methyl cyclopentyl ketone and heptadienol derivatives, although present in smaller concentrations, add to the formulation's antimicrobial and aromatic tonicity.

FTIR Analysis :

Table-8. FTIR Spectral Interpretation of Bilvamuladi Elixir Showing Functional Groups and Phytochemical Correlation

Wavenumber (cm^{-1})	Probable Functional Group	Type of Vibration	Interpretation Relevant to Bilvamuladi Elixir
3341 cm^{-1}	O–H stretch (alcohols, phenols) / N–H stretch[6,23]	Broad stretching	Indicates presence of polyphenols & flavonoids from *Aegle marmelos* and *Zingiber officinale*, and possible phenolic acids from *Plumbago zeylanica*, supporting antioxidant and digestive stimulant actions
1635 cm^{-1}	C=O stretching (conjugated carbonyl) and/or C=C aromatic[1,6,23]	Stretching of carbonyl & aromatic ring	Confirms presence of tannins, coumarins and flavonoids typical of *Aegle marmelos* and essential oil phytoconstituents from *Ferula foetida* and *Zingiber officinale* correlating with anti-inflammatory, carminative, and gut-protective actions

The FTIR spectrum of Bilvamuladi Elixir shows a broad intense band at approximately 3341 cm^{-1}, which corresponds to O–H stretching of phenolic compounds and alcohol groups, indicating the presence of polyphenols, flavonoids and coumarins derived primarily from *Aegle marmelos* and *Zingiber officinale* [1,6,23]. The strong absorption band near 1635 cm^{-1} corresponds to C=O and aromatic C=C stretching, which is characteristic of tannins, conjugated carbonyls and essential oil constituents, thus supporting the presence of anti-inflammatory, carminative and antioxidant phytochemicals such as coumarins, phenolic acids and terpenoids from *Ferula foetida*, *Plumbago zeylanica* and *Ricinus communis*[1, 6,23].

The present study provides a comprehensive pharmaceutical and phytochemical evaluation of Bilvamuladi Elixir, a classical Ayurvedic polyherbal formulation traditionally indicated for *Udarashoola* (abdominal colic), with special relevance to pediatric gastrointestinal disorders. By integrating classical Ayurvedic concepts with modern analytical techniques such as HPTLC, GC–MS, and FTIR, the study establishes a scientific basis for the formulation's therapeutic potential and standardization.

From a pharmaceutical perspective, the preparation of Bilvamuladi Elixir was carried out using authenticated raw materials and classical references, with suitable modifications for a pediatric-friendly liquid dosage form. Hydro-distillation enabled efficient extraction of volatile and semi-volatile constituents from the major herbal components, while the post-distillation addition of Hingu and Saindhava preserved their pharmacological efficacy. This method ensures uniformity,

Figure 1. Raw Drugs used for preparation

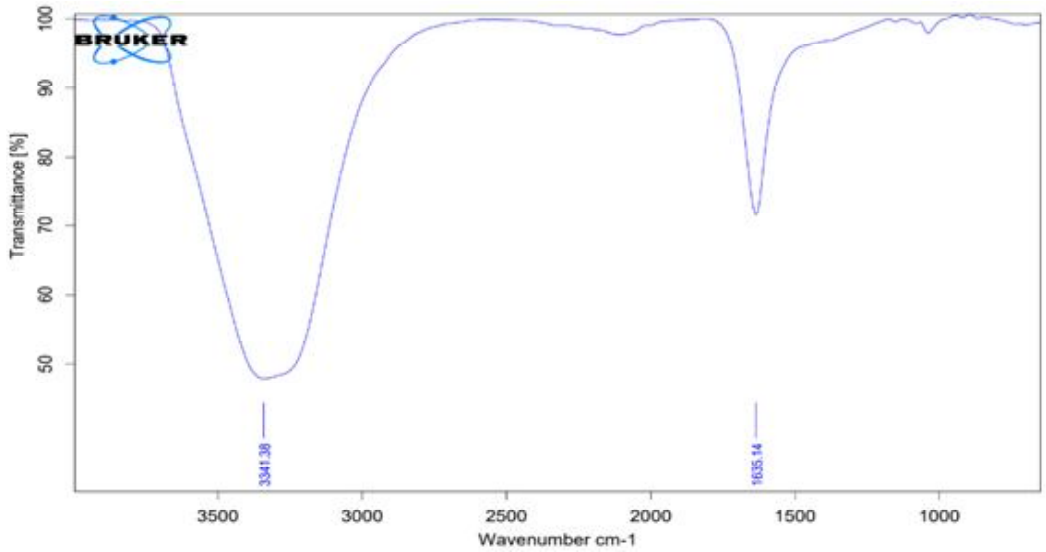

Figure 2. FTR analysis

stability, and palatability, which are essential for pediatric administration.

HPTLC fingerprinting of the methanolic extract revealed a consistent and reproducible chromatographic profile under both 254 nm and 366 nm visualizations. Under 254 nm, multiple well-resolved bands were observed across a wide Rf range, indicating the presence of diverse phytochemical classes such as phenolics, flavonoids, coumarins, terpenoids, and resinous compounds. The dominance of high-Rf bands

with large area percentages suggests a higher concentration of major bioactive constituents. These compounds are known to possess digestive stimulant, carminative, anti-inflammatory, and antispasmodic activities, which directly correlate with the formulation's classical indications in abdominal colic.

Under 366 nm visualization, intense fluorescent bands were observed, particularly at lower Rf values, indicating the presence of fluorescent phenolics and lignan-like compounds. These phytochemicals are associated with antioxidant and mucosal protective effects, suggesting an additional role of Bilvamuladi Elixir in reducing oxidative stress and supporting gastrointestinal mucosal integrity. The appearance of secondary fluorescent bands at higher sample concentrations further supports the presence of volatile terpenoids and alkaloidal fractions from ingredients such as Shunthi and Hingu, reinforcing their role in relieving flatulence and intestinal spasms.

GC–MS analysis provided detailed insight into the volatile chemical composition of the formulation. The chromatogram was dominated by (E)-cinnamaldehyde, which constituted the major proportion of the total peak area. This compound is well documented for its antimicrobial, anti-inflammatory, antioxidant, and digestive stimulant properties, supporting the formulation's *Deepana* and *Pachana* actions. Other identified compounds, including eucalyptol and α-terpineol, contribute bronchodilatory, carminative, and anti-inflammatory effects, which are beneficial in functional gastrointestinal disorders. Minor constituents, although present in lower concentrations, add to the overall synergistic pharmacological profile of the formulation.

FTIR analysis further confirmed the presence of key functional groups such as hydroxyl and carbonyl groups, indicating polyphenols, flavonoids, tannins, and conjugated aromatic compounds. These functional groups are consistent with the phytochemicals identified through HPTLC and GC–MS and support the formulation's antioxidant, anti-inflammatory, and gut-protective properties.

In conclusion, the analytical findings strongly support the classical Ayurvedic claims of Bilvamuladi Elixir in the management of pediatric abdominal colic. The formulation exhibits a well-defined, reproducible phytochemical profile with biologically relevant constituents that rationally explain its therapeutic actions. The study highlights the importance of integrating traditional knowledge with modern analytical techniques to ensure the quality, safety, and efficacy of Ayurvedic pediatric formulations.

References :

1. Ali BH, *et al*. (2008). Ginger and GI pharmacology. *Food Chem Toxicol. 46*(11): 409–420.
2. Anderson RA, CL Broadhurst, (2003). Polansky MM. Cinnamon improves glucose and lipid profiles. *Diabetes Care. 26*(12): 3215–3218.
3. Azhar I, *et al*. (2009). Antimicrobial and antioxidant activity of α-terpineol. *Phytother Res. 23*(11): 1521–1524.
4. Balasankara D, *et al*. (2018). Aegle marmelos phytochemistry and pharmacology. *Indian J Tradit Knowl. 17*(4): 652–660.
5. Benninga MA, *et al*. (2016). Childhood functional gastrointestinal disorders.

Gastroenterology. 150: 1443–1455.
6. Coates J. (2000). Interpretation of infrared spectra. Wiley.
7. Gupta RK, *et al*. (2010). Plumbagin digestive and anti-inflammatory activity. *Fitoterapia. 81:* 51–56.
8. Jayaprakasha GK, and LJ. Rao (2011). Chemistry and biological activities of cinnamaldehyde. *J Agric Food Chem. 59:* 4490–4501.
9. Juergens UR, *et al*. (2003). Antiinflammatory effects of eucalyptol. *Respir Med. 97:* 250–256.
10. Kamboj VP. (2000). Pharmacology of Aegle marmelos. *Fitoterapia. 71:* 403–412.
11. Kapoor LD. (2000). Handbook of Ayurvedic Medicinal Plants. CRC Press.
12. Khedkar R, *et al*. (2013) Ricinus communis pharmacology. *Pharmacogn Rev. 7:* 42–50.
13. Maity P, *et al*. (2009). Biological activities of Aegle marmelos. *J Ethnopharmacol*.
14. Mahendra P, and S. Bisht (2012). Ferula foetida pharmacology review. *J Ayurveda Integr Med. 3:* 125–129.
15. Murthy KRS. Sarangadhara Samhita. Chaukhambha; Varanasi.
16. Patwardhan B, and RA. Mashelkar (2009). Traditional medicine-inspired drug discovery. *Drug Discov Today. 14:* 804–811.
17. Pullaiah T, E. Chennaiah (2012). Phytochemical studies of Plumbago. Regency.
18. Ranasinghe P, *et al*. (2013). Cinnamomum zeylanicum pharmacology. *Evid Based CAM*.
19. Reich E, (2007). Schibli A. HPTLC for medicinal plants. Thieme.
20. Sharma P. Chakradatta. Chaukhambha; Varanasi.
21. Singh S, *et al*. (2015). Zingiber officinale pharmacology. *Int J Med Res*.
22. Stahl E. (1969). Thin Layer Chromatography. Springer.
23. Stuart B. (2004). Infrared Spectroscopy. Wiley.
24. Wagner H, and S. Bladt (1996). Plant Drug Analysis. Springer.
25. World Health Organization. WHO Traditional Medicine Strategy 2014–2023. Geneva (2013).