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PHYTOCHEMICAL SCREENING, AND QUALITY STANDARDIZATION OF A POLYHERBAL UNANI FORMULATION FOR GASTROINTESTINAL HEALTH

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ABSTRACT

Background: Polyherbal formulations are an important component of Unani medicine and may provide complementary effects through the combined phytochemical properties of multiple medicinal plants. However, systematic phytochemical profiling and quality standardization are essential to ensure consistency, safety, and reproducibility. **Objective:** The present study aimed to evaluate the preliminary phytochemical profile, Quality Standardization, and stability of a polyherbal Unani formulation developed for gastrointestinal health. **Methods:** The formulation contained *Aegle marmelos*, *Syzygium cumini*, *Phyllanthus emblica*, *Terminalia bellirica*, *Rosa damascena*, *Trachyspermum ammi*, *Piper longum*, *Piper nigrum*, *Plantago ovata* husk, and *Cassia angustifolia*. Methanolic, aqueous, and chloroform extracts were subjected to preliminary phytochemical screening. The finished formulation was evaluated for organoleptic characteristics, moisture content, ash values, extractive values, pH, swelling index, bulk density, microbial contamination, pesticide residues, and preliminary stability. **Results:** Phytochemical screening revealed

alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, and steroids in different extracts. The formulation showed a brown, homogeneous appearance with characteristic aromatic odour and astringent/spicy taste. Moisture content was 6.23%**,** total ash 8.62%**,** acid-insoluble ash 1.27%**,** alcohol-soluble extractive 18.72%**,** and water-soluble extractive 24.36%**.** The pH was 5.86**,** swelling index 8.40 mL/g**,** and bulk density 0.80 g/mL**.** Microbial contamination and pesticide residues complied with the applicable specifications. Preliminary six-month stability assessment demonstrated retention of acceptable organoleptic, physicochemical, and microbiological characteristics. **Conclusion:** The formulation demonstrated a diverse phytochemical profile and satisfactory preliminary quality characteristics. Further HPTLC/HPLC fingerprinting, quantitative marker analysis, extended stability studies, and pharmacological and clinical investigations are warranted to establish comprehensive standardization, safety, and therapeutic efficacy.

KEYWORDS: Polyherbal formulation; Unani medicine; phytochemical screening; quality standardization; and gastrointestinal health.

INTRODUCTION

Polyherbal formulations constitute an important component of traditional systems of medicine, including Unani medicine, where combinations of medicinal plants are frequently used to address complex gastrointestinal disorders. Gastrointestinal health depends on coordinated regulation of intestinal motility, secretion, mucosal integrity, microbial balance, and bowel consistency (Bai et al., 2025). Consequently, traditional formulations intended for gastrointestinal health may contain several botanicals with complementary pharmacological properties, including carminative, astringent, antimicrobial, antidiarrhoeal, laxative, anti-inflammatory, and mucilage-forming activities (Rana & Smriti, 2026).

The polyherbal Unani formulation investigated in the present study contains *Aegle marmelos*, *Syzygium cumini*, *Phyllanthus emblica*, *Terminalia bellirica*, *Rosa damascena*, *Trachyspermum ammi*, *Piper longum*, *Piper nigrum*, *Plantago ovata* husk (Ispaghula), *Cassia angustifolia* and other ingredients. The combination represents several phytochemical classes, particularly tannins, phenolics, flavonoids, alkaloids, terpenoids, volatile oils, anthraquinone glycosides, and polysaccharides (Karole et al., 2019).

Aegle marmelos (bael) has traditionally been associated with gastrointestinal applications, and published evidence describes antidiarrhoeal, antimicrobial, anti-inflammatory, and ulcer-

healing activities. Its reported phytochemistry includes coumarins, alkaloids, flavonoids, tannins, and other secondary metabolites (Monika et al., 2023). *Syzygium cumini* contains phenolics and tannins and has been investigated for gastroprotective, antidiarrhoeal, antioxidant, and antimicrobial properties (Ayyanar & Subash-Babu, 2012).

Phyllanthus emblica is rich in polyphenols, tannins, phenolic acids, flavonoids, and other bioactive compounds and has demonstrated antimicrobial potential against several pathogenic bacteria (Prananda et al., 2023). Similarly, species of *Terminalia*, including *T. bellirica*, contain hydrolysable tannins, phenolics, flavonoids, and terpenoids and have demonstrated antimicrobial and gastrointestinal-related biological activities. Recent experimental work specifically reported antibacterial activity of *T. bellirica* fruit extracts against gastrointestinal pathogens (Tiwana et al., 2024).

The aromatic ingredients may provide additional gastrointestinal and antimicrobial properties. *Trachyspermum ammi* contains volatile oil rich in thymol and other monoterpenes and is traditionally used as a carminative and antispasmodic agent. Its antimicrobial activity has also been documented (Bairwa et al., 2012). *Piper nigrum* and *Piper longum* contain characteristic piperamides and volatile constituents, while *Piper* species have demonstrated antimicrobial activity in experimental studies (Salehi et al., 2019).

The formulation also contains Ispaghula husk, derived from *Plantago ovata*. Psyllium husk contains a high proportion of soluble fibre and forms a viscous gel in the presence of water, supporting regulation of bowel movement and stool consistency. Evidence supports its use in both constipation and diarrhea-related bowel disturbances (Przybyszewska et al., 2024). *Cassia angustifolia* (senna) contains anthraquinone glycosides, particularly sennosides A and B, and is primarily recognized for its laxative action in constipation (Thaker et al., 2023). The hepatoprotective (liver-protecting) action of *Rosa damascena* (Damask rose) is primarily attributed to its rich content of bioactive phytochemicals, notably flavonoids (quercetin and kaempferol) (Alam et al., 2008).

Although the individual medicinal plants have been extensively investigated, scientific characterization of the finished polyherbal formulation is essential because the biological activity and quality of a multi-ingredient product cannot be inferred solely from the properties of its individual ingredients. WHO quality-control guidance emphasizes identity, purity, physicochemical evaluation, chromatographic characterization, and appropriate testing

of herbal materials and finished products (Dubey & Dixit, 2023).

Therefore, the present study was designed to evaluate the preliminary phytochemical profile, and Quality standardization characteristics of the selected polyherbal Unani formulation intended for gastrointestinal health. The study provides preliminary evidence for its phytochemical composition, microbial growth-inhibitory potential, and quality consistency.

MATERIALS AND METHODS

Study material and formulation

A representative batch of the polyherbal Unani formulation was obtained from a reliable source and examined as the finished product. The formulation contained *Aegle marmelos*, *Syzygium cumini*, *Phyllanthus emblica*, *Terminalia bellirica*, *Rosa damascena*, *Trachyspermum ammi*, *Piper longum*, *Piper nigrum*, *Plantago ovata* husk, *Cassia angustifolia*, and other ingredients according to the manufacturer's formulation specification.

The identity, batch number, manufacturing date, expiry date, and storage conditions were recorded. Whenever possible, authenticated raw materials and voucher specimens should be maintained for individual botanical ingredients.

Preparation of extract

The formulation was powdered uniformly and passed through a suitable sieve. Approximately 100 g of the powdered formulation was extracted with 600 mL of methanol, aqueous, and Chloroform by maceration for 72 h at room temperature with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at a temperature not exceeding approximately 40–45°C. The concentrated extract was stored in an airtight container at 4°C until analysis (Bhogale & Prabh, 2025).

Preliminary phytochemical screening

Preliminary phytochemical analysis was performed using standard methods described by Harborne (1998), Evans (2009), and Kokate et al. (2018). The extract was screened for alkaloids (Mayer’s, Wagner’s, Dragendorff’s, and Hager’s tests), flavonoids (Shinoda test), tannins and phenolic compounds (ferric chloride test), saponins (froth test), cardiac glycosides (Keller-Killiani test), terpenoids (Salkowski test), steroids (Liebermann-Burchard test), carbohydrates (Molisch’s test), and proteins (Biuret test). The appearance of characteristic colour development, precipitate formation, or persistent frothing was recorded

as a positive reaction. Phytochemical screening was considered qualitative and was not interpreted as quantitative determination of individual compounds.

Quality Standardization Parameters of the Polyherbal Unani Formulation

The finished formulation was evaluated for selected quality parameters following established WHO approaches for medicinal plant materials and herbal preparations (WHO, 2011) and established protocols (Haruna et al., 2023; AOAC, 2006; De & Sharma, 2023; Khandelwal, 2008; Kokate et al., 2018; and Ratajczak et al., 2015). The following parameters were determined: Organoleptic characteristics: colour, odour, taste, and appearance, Moisture content/loss on drying, Total ash, Acid-insoluble ash, Water-soluble ash, Alcohol-soluble extractive value, Water-soluble extractive value, Microbial load, pH of an aqueous dispersion, Swelling index, particularly relevant because the formulation contains Ispaghula husk.

Stability Study of the Polyherbal Unani Formulation

The stability of the polyherbal Unani formulation was evaluated under controlled storage conditions to assess changes in its physical, physicochemical, and microbiological quality characteristics during storage. The stability protocol was designed with reference to the principles described in the WHO guidelines for quality control of herbal materials and stability evaluation of pharmaceutical products (World Health Organization [WHO], 2011, 2024).

The formulation was packed in a well-closed, moisture-protective container and stored at room temperature (25 ± 2°C/60 ± 5% RH)**.** Samples were evaluated initially (0 month), 1 month, 3 months, and 6 months for appearance, odour, moisture content, total ash, pH, water-soluble extractive, swelling index, bulk density, and microbial quality. The formulation was considered stable when no significant change from the initial values occurred and all parameters remained within the predetermined specifications.

Fig. Polyherbal Unani Formulation.

RESULTS

Table 1: Preliminary phytochemical screening.

The formulation extract showed positive reactions for several major phytochemical groups.

Phytochemical Test		Methanol	Aqueous	Chloroform
Alkaloids	Mayer's test	+	+	-
	Wagner's test	+	+	-
	Dragendorff's test	+	+	-
	Hager's test	+	+	-
Flavonoids	Shinoda test	+	+	-
	Alkaline reagent test	+	+	-
	Ferric chloride test	+	+	-
	Lead acetate test	+	+	-
Tannins	Ferric chloride test	+	+	+
	Gelatin test	+	+	+
	Lead acetate test	+	+	+
	Potassium dichromate test	+	+	+
Saponins	Froth test	+	+	-
	Foam test	+	+	-
Glycosides	Legal's test	+	+	-
	Bornträger's test	+	+	-
				-
Phenolic compounds	Ferric chloride test	+	+	-
	Folin–Ciocalteu test	+	+	-
	Ellagic acid test	+	+	-
Terpenoids	Salkowski test	+	+	+
	Liebermann–Burchard test	+	+	+
	Copper acetate test	+	+	+
	Vanillin–sulfuric acid test	+	+	+
Steroids	Liebermann–Burchard test	+		-
	Salkowski test	+		-
Carbohydrates	Molisch's test	-	-	-
	Benedict's test	+	+	-
	Fehling test	+	+	-
Proteins	Biuret test	-	-	-
	Ninhydrin test	-	-	-
	Xanthoproteic test	-	-	-

Key: + = weakly positive; ++ = moderately positive; +++ = strongly positive; − = not detected.

The strong reactions for phenolics and tannins may be attributable in part to tannin-rich ingredients such as *A. marmelos*, *S. cumini*, *P. emblica*, and *T. bellirica*. Published investigations support the occurrence of tannins, phenolics and flavonoids in these botanicals.

Table 2: Quality Standardization Parameters of the Polyherbal Unani Formulation.

Sl No	Test parameter	Recommended specification	Results
1	Appearance	Brown, homogeneous powder; free from visible foreign matter	Complies
2	Odour	Characteristic odour of the formulation	Complies
3	Taste	Characteristic astringent/spicy taste	Complies
4	Moisture content / Loss on drying	Should comply with the applicable monograph; <10% w/w may be adopted as a product-specific specification when justified	6.23%
5	Foreign matter	Should comply with the applicable monograph; <1.0% may be adopted as a product-specific specification when justified	Complies
6	Total ash	Should comply with the applicable monograph**;** <15% w/w may be used as a product-specific specification when justified	8.62%
7	Acid-insoluble ash	Should comply with the applicable pharmacopoeial/individual monograph specification; a product-specific limit should be established based on validated batch data.	Complies
8	Alcohol-soluble extractive	Should comply with the established product specification	18.72%
9	Water-soluble extractive	Should comply with the established product specification; ≥20% may be adopted where scientifically justified	24.36%
10	pH	pH of the aqueous dispersion should comply with the established product specification	5.86
11	Swelling index	Should comply with the established specification for the formulation containing Ispaghula husk	8.40 mL/g
12	Bulk density	Should comply with the validated finished-product specification**;** ≥0.6 g/mL may be adopted as an in-house specification where justified	0.8gm/mL
13	Microbial contamination	Total aerobic microbial count, total yeast and mould count, and specified microorganisms should comply with the applicable pharmacopoeial requirements	Complies
14	Pesticide residues	Should comply with applicable pharmacopoeial/regulatory limits	Complies
15	Storage condition	Store in a well-closed container under conditions demonstrated to maintain product quality and stability	Complies

Table 3: Stability profile of the polyherbal Unani formulation during storage.

Parameter	Specification	0 Month	1 Month	3 Months	6 Months	Stability status
Appearance	Brown, homogeneous powder	Complies	Complies	Complies	Complies	Stable
Odour	Characteristic aromatic	Complies	Complies	Complies	Complies	Stable
Moisture content (% w/w)	NMT 10.0%	6.23	6.31	6.47	6.58	Complies
Total ash (% w/w)	NMT 15.0%	8.62	8.65	8.71	8.78	Complies
Acid-insoluble ash (% w/w)	NMT 2.0%	1.27	1.29	1.31	1.34	Complies
Alcohol-soluble extractive (%)	Product-specific specification	18.72	18.61	18.48	18.35	Complies
Water-soluble extractive (%)	NLT 20.0%	24.36	24.21	24.03	23.82	Complies
pH	Product-specific specification	5.86	5.84	5.81	5.79	Complies
Swelling index (mL/g)	Product-specific specification	8.40	8.34	8.26	8.19	Complies
Bulk density (g/mL)	NLT 0.60 g/mL	0.80	0.80	0.79	0.78	Complies
Total aerobic microbial count	Applicable pharmacopoeial limit	Complies	Complies	Complies	Complies	Stable
Total yeast and mould count	Applicable pharmacopoeial limit	Complies	Complies	Complies	Complies	Stable
Specified microorganisms	Must comply with applicable limit	Complies	Complies	Complies	Complies	Stable

DISCUSSION

The present study provides preliminary phytochemical, physicochemical, microbiological, and stability data for a polyherbal Unani formulation developed for gastrointestinal health. The formulation contains *Aegle marmelos*, *Syzygium cumini*, *Phyllanthus emblica*, *Terminalia bellirica*, *Rosa damascena*, *Trachyspermum ammi*, *Piper longum*, *Piper nigrum*, *Plantago ovata* husk, and *Cassia angustifolia*. The presence of multiple medicinal plants provides a diverse phytochemical matrix, supporting the polyherbal concept in which different botanicals may contribute complementary properties (Karole et al., 2019).

Preliminary phytochemical screening demonstrated alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, and steroids in different extracts. The variation among methanolic, aqueous, and chloroform extracts indicates solvent-dependent extraction of phytoconstituents. The detection of phenolics, flavonoids, and tannins is particularly relevant because *A. marmelos*, *P. emblica*, and *T. bellirica* are recognized sources of polyphenolic constituents. *A. marmelos* has been extensively associated with diverse

phytochemicals and traditional gastrointestinal applications (Monika et al., 2023), while *P. emblica* contains polyphenols, tannins, phenolic acids, and flavonoids (Prananda et al., 2023). However, qualitative tests cannot establish the concentration or identity of individual compounds and should therefore be considered preliminary.

Terpenoids were detected in all three extracts, which may partly reflect contributions from aromatic ingredients such as *T. ammi* and *Piper* species. Previous studies have documented the phytochemical diversity of *T. ammi* and *Piper* species (Bairwa et al., 2012; Salehi et al., 2019). Nevertheless, chromatographic analysis is necessary to confirm specific marker compounds.

The physicochemical results demonstrated satisfactory quality. The formulation showed a brown, homogeneous powder with characteristic aromatic odour and astringent/spicy taste. Moisture content was 6.23%**,** total ash 8.62%**,** acid-insoluble ash 1.27%**,** alcohol-soluble extractive 18.72%**,** and water-soluble extractive 24.36%**.** These parameters provide useful information regarding purity, inorganic residue, and soluble constituents and are consistent with recognized quality-control approaches for herbal materials (World Health Organization [WHO], 2011).

The aqueous dispersion showed a pH of 5.86**,** indicating mildly acidic characteristics. The swelling index of 8.40 mL/g is particularly relevant because the formulation contains *Plantago ovata* husk, a mucilage-rich material with substantial water-absorbing capacity. The bulk density of 0.80 g/mL further indicates satisfactory physical characteristics for handling and packaging of the powdered formulation.

Microbial contamination and pesticide-residue testing complied with the applicable specifications, supporting satisfactory preliminary microbiological quality. This is important because herbal materials may be exposed to microbial contamination during cultivation, processing, and storage. Ratajczak et al. (2015) emphasized the importance of microbiological quality control in non-sterile pharmaceutical products.

The preliminary six-month stability assessment showed that the formulation maintained acceptable organoleptic, physicochemical, and microbiological characteristics under the tested storage conditions. No substantial deterioration was observed, although longer-term and accelerated stability studies are necessary to establish definitive shelf life. For

comprehensive standardization, future studies should incorporate HPTLC/HPLC fingerprinting and quantitative marker analysis**,** including suitable markers such as piperine, thymol, and sennosides, where scientifically justified (WHO, 2011).

Overall, the findings indicate satisfactory preliminary quality and phytochemical characteristics of the polyherbal Unani formulation. However, the present study does not establish direct gastrointestinal therapeutic efficacy. Further quantitative phytochemical, toxicological, pharmacological, and clinical investigations are required to confirm its safety, consistency, mechanism of action, and therapeutic effectiveness.

CONCLUSION

The present study demonstrated that the investigated polyherbal Unani formulation possesses a diverse phytochemical profile characterized by alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, and steroids in different solvent extracts. The formulation also demonstrated satisfactory physicochemical characteristics, including 6.23% moisture content, 8.62% total ash, 18.72% alcohol-soluble extractive, 24.36% water-soluble extractive, pH 5.86, 8.40 mL/g swelling index, and 0.80 g/mL bulk density. The formulation complied with the established quality specifications for the evaluated parameters, including microbial contamination and pesticide residues. These findings indicate satisfactory preliminary quality and batch characteristics of the tested polyherbal preparation.

However, qualitative phytochemical screening alone is insufficient to establish chemical standardization or therapeutic efficacy. Future investigations should incorporate HPTLC/HPLC fingerprinting, validated quantitative marker-compound assays, microbial counts with numerical CFU values, aflatoxin and heavy-metal analysis, pesticide-residue quantification, and appropriate pharmacological and clinical investigations**.** Such additional investigations would provide a stronger scientific basis for establishing reproducible quality, safety, and therapeutic relevance of the formulation.

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