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Research Article

STANDARDIZATION AND SAFETY EVALUATION OF KOFSAP SUGAR-FREE SYRUP: INTEGRATING AYURVEDIC PRINCIPLES WITH MODERN ANALYTICAL TECHNIQUES

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ABSTRACT

Ayurveda, a holistic medicinal system, treats cough as an imbalance of *Kapha* and *Vata doshas*, employing therapies to soothe respiratory passages, reduce inflammation, and promote mucus expulsion. Kofsap Sugar-Free Syrup, developed by Sitaram Ayurveda, is a polyherbal formulation designed for effective cough relief without added sugars, catering to diabetic and health-conscious individuals. **Materials and Methods**: The study assessed Kofsap SF syrup quality, safety, and efficacy through organoleptic, physicochemical, phytochemical, microbial, and Gas Chromatography-Mass Spectrometry (GC-MS) analyses, adhering to Ayurvedic Pharmacopoeia of India standards. **Results**: Kofsap SF syrup exhibited acceptable organoleptic traits, including a dark brown colour, characteristic herbal odour, and slightly sweet-astringent taste. Physicochemical analysis confirmed stability with a pH of 5.94, refractive index of 1.339, and low moisture content (5.35%). Phytochemical screening identified carbohydrates, proteins, glycosides, steroids, flavonoids, phenols, saponins, and alkaloids. GC-MS analysis revealed key bioactive compounds, including cyclohexanol (36.665%) and thymol (25.460%), with antitussive, anti-inflammatory, and antimicrobial properties. Microbial tests confirmed safety, with no pathogens detected and a total bacterial count below 10 cfu/mL. **Conclusion**: Kofsap SF syrup demonstrates high quality, safety, and therapeutic potential, validated by comprehensive standardization. Its stability, rich phytochemical profile, and absence of contaminants position it as an effective Ayurvedic remedy for cough relief, aligning traditional wisdom with modern healthcare demands.

INTRODUCTION

Ayurveda, the ancient system of medicine originating from India, emphasizes a holistic approach to health, focusing on the balance of bodily energies *(Doshas)* and the individual's constitution *(Prakriti)* to maintain wellness and prevent disease. This traditional practice integrates physical, mental, and spiritual aspects of health, offering personalized therapeutic interventions. Disruptions in this balance manifest as ailments, with cough being a common respiratory condition attributed to an imbalance of *Kapha* and *Vata doshas*.

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Excess *Kapha* results in mucus accumulation and congestion, while aggravated *Vata* contributes to airway irritation, dryness, and inflammation. [1] Ayurvedic interventions for cough focus on pacifying these *Doshas*, promoting expectoration, and soothing respiratory tissues through a combination of herbal formulations, such as decoctions and syrups, and supportive therapies like steam inhalation and gargling.[2]

Cough is a prevalent respiratory symptom globally, significantly impacting the quality of life and healthcare systems. A systematic review and meta-analysis reported a global prevalence of chronic cough at approximately 9.6%, with higher rates observed in Europe and America compared to Asia and Africa. Factors contributing to this variation include environmental exposures, smoking, occupational

hazards, and comorbid conditions such as obesity and rhinitis. [3]

In contrast, modern medicine categorizes cough as either productive or non-productive and employs pharmacological agents such as expectorants, antitussives, or antihistamines for management. While effective, these treatments often carry side effects, including drowsiness, gastrointestinal disturbances, or, in some cases, dependency, prompting a global shift toward safer, natural alternative. This growing preference for herbal remedies has revitalized interest in Ayurveda, which offers time-tested solutions with minimal adverse effects.

Kofsap Sugar-Free Syrup, a proprietary Ayurvedic formulation developed by Sitaram Ayurveda, embodies this synergy of tradition and modernity. Crafted with a blend of potent herbs known for their expectorant, anti-inflammatory, and bronchodilator properties, Kofsap is designed to alleviate cough, reduce airway inflammation, and support overall respiratory health. Its sugar-free composition caters specifically to diabetic patients and health-conscious individuals, ensuring accessibility to a broader demographic without compromising efficacy.

Herbal formulations play a pivotal role in the Ayurvedic management of *Kasa*. However, the efficacy and safety of these formulations depend on consistent quality and standardization. Standardization refers to the process of establishing consistent parameters for the quality, purity, and potency of herbal products, ensuring batch-to-batch uniformity and therapeutic efficacy. This involves comprehensive evaluation of raw materials, including authentication of plant species, assessment of active constituents, and adherence to good manufacturing practices.

The importance of standardization is underscored by the challenges associated with herbal medicines, such as variability in plant sources, differences in harvesting and processing methods, and potential contamination or adulteration. These factors can significantly affect the safety and therapeutic outcomes of herbal products. Therefore, implementing rigorous standardization protocols is essential to ensure the reliability and acceptance of herbal formulations in both traditional and modern healthcare systems. [4,5]

As more people turn to natural and safer alternatives for managing cough, the need to standardize Ayurvedic formulations has become increasingly important to ensure they meet today's expectations for quality and safety. Kofsap SF syrup is a thoughtfully developed herbal remedy that brings together the time-tested principles of Ayurveda with modern formulation techniques. Designed to be both pleasant-tasting and easily absorbed, it offers a convenient option for those seeking effective relief without added sugar. This study takes a closer look at Kofsap, using a range of analytical methods to assess its consistency, safety, and therapeutic potential.

The investigation encompasses organoleptic assessment, physicochemical analysis, and phytochemical profiling. Additionally, microbial analysis ensures the formulation's safety by confirming the absence of harmful pathogens, while Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses provide detailed insights into the syrup's chemical composition and volatile bioactive compounds. Through these rigorous standardization protocols, this study aims to establish Kofsap SF syrup as a reliable, high-quality Ayurvedic remedy for cough management, bridging the gap between traditional knowledge and modern healthcare needs.

MATERIALS AND METHODS

Collection of Raw Materials

Raw materials for Kofsap SF syrup were sourced from certified suppliers in Thrissur and authenticated at the Pharmacognosy Division of Sitaram Ayurveda Pvt. Ltd.

Preparation of Kofsap Sugar-Free Syrup

Kofsap Syrup was formulated using a blend of Ayurvedic herbs processed per traditional methods. The composition, including botanical names, and parts used, is detailed in Table 1.

Table 1: Composition of Kofsap Sugar-Free Syrup

S.No.	Ingredients	Botanical Name	Parts Used
1	*Prsniparni*	*Desmodium gangeticum*	Root
2	*Salaparni*	*Pseudarthria viscida*	Root
3	*Badra*	*Aerva lanata*	Root
4	*Bruhati*	*Solanum anguivi*	Root
5	*Gokshura*	*Tribulus terrestris*	Root
6	*Gambari*	*Gmelina arborea*	Root

7	*Bilva*	*Aegle marmelos*	Root
8	*Patala*	*Stereospermum colais*	Root
9	*Syonaka*	*Oroxylum indicum*	Root
10	*Agnimantha*	*Premna corymbosa*	Root
11	*Nagara*	*Zingiber officinale*	Rhizome
12	*Maricha*	*Piper nigrum*	Seed
13	*Pippali*	*Piper longum*	Fruit
14	*Vasa*	*Justicia beddommi*	Root
15	*Kantakari*	*Solanum surratense*	Root
16	*Samanga*	*Mimosa pudica*	Whole plant
17	*Tulsi*	*Ocimum sanctum*	Leaves
18	*Karpooram*	*Cinnamomum camphora*	As such
19	*Pudiha*	*Mentha arvensis*	As such
20	*Ajamoda*	*Trachyspermum ammi*	As such
21	*Sucralose*		As such

Preparation Process

Kofsap Syrup was prepared using traditional Ayurvedic decoction methods. Herbal roots, rhizomes, seeds, fruits, leaves, and whole plants were added in water to extract bioactive compounds. Menthol, thymol, and camphor were incorporated as per formulation requirements. The decoction was filtered, concentrated, and formulated into a sugar-free syrup base, ensuring stability and palatability. The final syrup was bottled under controlled conditions, with pre- and post-bottling analyses to verify quality and efficacy.

Organoleptic Analysis

Organoleptic properties colour, odour, taste, and consistency of Kofsap-SF Syrup were evaluated as per Ayurvedic Pharmacopoeia of India (API) guidelines. [6]

Physicochemical Analysis

Physicochemical parameters pH, total soluble solids, specific gravity, total sugar, moisture %, refractive index were tested per API standards to assess stability and quality. [7]

Preliminary Phytochemical Analysis

Phytochemical screening of aqueous and methanolic extracts of Kofsap SF Syrup assessed the presence of carbohydrates, proteins, glycosides, steroids, flavonoids, phenols, saponins, alkaloids, and other constituents using standard tests. [8]

Thin Layer Chromatography (TLC)

Thin Layer Chromatographic (TLC) finger printing was performed to separate and identify distinct bands. A 10 ml sample was refluxed with 20 ml of methanol for 30 minutes, then filtered. The filtrate was evaporated to dryness using a water bath, and the resulting residue was reconstituted in 1 ml of methanol. TLC was carried out using silica gel G as the stationary phase, prepared as a slurry and uniformly coated onto clean glass plates (10 × 5 cm) with a commercial spreader. The methanolic extract was applied to the coated plate using a fine capillary tube, approximately 2 cm from the lower edge, and allowed to air dry.

The plate was then placed in a developing chamber at a 45° angle, ensuring the sample spot remained above the level of the mobile phase. Chromatographic development was carried out using a solvent system of toluene and ethyl acetate in a 9:1 ratio. After development, the plate was examined under UV light at 366 nm to visualize the separated bands.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed to identify bioactive compounds. The sample was extracted with hexane, filtered through a 0.2µm Nylon syringe filter, and analysed using a 7890A GC with a 5975C detector and DB-5MS column (30m × 0.250mm, 0.25µm film). Injection volume was 2µL (splitless), with helium as the carrier gas (1mL/min). The oven temperature ramped from 100°C (1 min hold) to 200°C at 10°C/min, then to 300°C at 20°C/min (10 min hold). Electron Impact ionization (70 eV) was used, with the injector at 300°C. Spectra were compared with the NIST-08 database. [9]

Microbial Limit Test

Microbial analysis followed API protocols, testing for pathogens (E. coli, Salmonella, Staphylococcus, Pseudomonas) and total bacterial and yeast counts using spread plate techniques on nutrient agar and Sabouraud Dextrose Agar (SDA). [7]

RESULTS AND DISCUSSION

Organoleptic Properties

Kofsap SF syrup exhibited a dark brown colour, characteristic herbal odour, slightly sweet-astringent taste, and liquid consistency which is tabulated in the Table number 2. The dark brown colour reflects the rich herbal composition, enhancing visual appeal and indicating authenticity. The characteristic odour, derived from herbs like *Ocimum sanctum* and *Zingiber officinale*, aligns with consumer preference for natural fragrances, avoiding synthetic additives. The slightly sweet-astringent taste, despite the sugar-free formulation, ensures palatability promoting compliance. The liquid consistency facilitates easy administration, aligning with Ayurvedic principles of patient-friendly delivery.

Table 2: Organoleptic Analysis of Kofsap Sugar-Free Syrup

S.No.	Parameters	Result
1	Colour	Dark brown
2	Odour	Characteristic
3	Taste	Slightly sweet and astringent
4	Consistency	Liquid

Physicochemical Properties

Physicochemical analysis mentioned in table no 3 is confirmed Kofsap SF syrup stability and quality. The pH of 5.94 indicates a slightly acidic nature, suitable for oral consumption and minimizing irritation to the throat, a critical factor for cough remedies. The absence of total sugar validates its sugar-free status, making it ideal for diabetic patients. The specific gravity 1.019 and refractive index 1.339 suggest appropriate formulation consistency, reflecting the presence of dissolved herbal extracts. Low moisture content 5.35% minimizes microbial growth, ensuring shelf-life stability. In the table no 3 total soluble solids of 5 indicate a balanced concentration of active ingredients, optimizing therapeutic efficacy without excessive viscosity.

Table 3: Physicochemical Analysis of Kofsap Sugar-Free Syrup

S.No.	Parameters	Result
1	pH	5.94
2	Total Soluble Solids (TSS)	5
3	Specific Gravity	1.019
4	Total Sugar	Not Detected
5	Moisture %	5.35
6	Refractive Index	1.339

Phytochemical Profile

Phytochemical screening, as presented in Table 4, has revealed a diverse array of bioactive compounds. Carbohydrates and proteins, detected in both aqueous and methanolic extracts *via* Molisch's and Biuret tests, suggest metabolic and structural support, aiding tissue repair in inflamed respiratory passages. The methanolic extract contained glycosides (Keller-Killiani test), steroids (Salkowski test), and flavonoids (alkaline reagent test), indicating anti-inflammatory, bronchodilator, and antioxidant properties, essential for alleviating cough and airway irritation.[10] Phenols, present in both extracts, provide antimicrobial and antioxidant effects, potentially reducing microbial-induced cough.[11] Saponins (aqueous extract, foam test) and alkaloids (aqueous extract, Wagner's reagent) support expectoration and respiratory health by loosening mucus and soothing airways. [12]

Table 4: Phytochemical Analysis of Kofsap Sugar-Free Syrup

S.No.	Constituent	Test	Aqueous Extract	Methanolic Extract
1	Carbohydrate	Molisch's Test	+	+
2	Sugar	Benedict's Test	-	-

3	Reducing Sugar	Fehling's Test	-	-
4	Ketose	Seliwanoff's Test	-	-
5	Protein	Biuret Test	+	+
6	Starch	KI Test	+	+
7	Glycoside	Keller-Killiani Test	-	+
8	Steroid	Salkowski Test	-	+
9	Terpenoid	Salkowski Test	-	-
10	Flavonoid	Alkaline Reagent	-	+
11	Phenol	Phenol Reagent Test	+	+
12	Saponin	Foam Test	+	-
13	Alkaloid	Wagner's Reagent	+	-
14	Tannin	Ferric Chloride Test	-	-
15	Coumarin	NaOH Test	-	-
16	Amino Acids	Ninhydrin Test	-	-
17	Quinone	H2SO4 Test	-	-

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was employed to profile the phytochemical components of Kofsap-SF, a polyherbal cough syrup, in comparison with individual herbal constituents commonly associated with its formulation. TLC analysis of Kofsap-SF mentioned in the table no 5 which revealed three R_f values 0.14, 0.19, and 0.32 indicating the presence of multiple phytochemicals. The Rf 0.32 band was common to several key herbal ingredients such as *Vasa*, *Tulsi*, and *Samanga*, suggesting the presence of shared bioactive compounds potentially responsible for the formulation's antitussive effect. In contrast, the bands at 0.14 and 0.19 were unique to Kofsap-SF, indicating formulation- synergistic components. Overall, the TLC fingerprinting supports the polyherbal nature of Kofsap-SF and provides preliminary confirmation of the inclusion of key antitussive herbs such as *Vasa*, *Tulsi*, and *Samanga*.

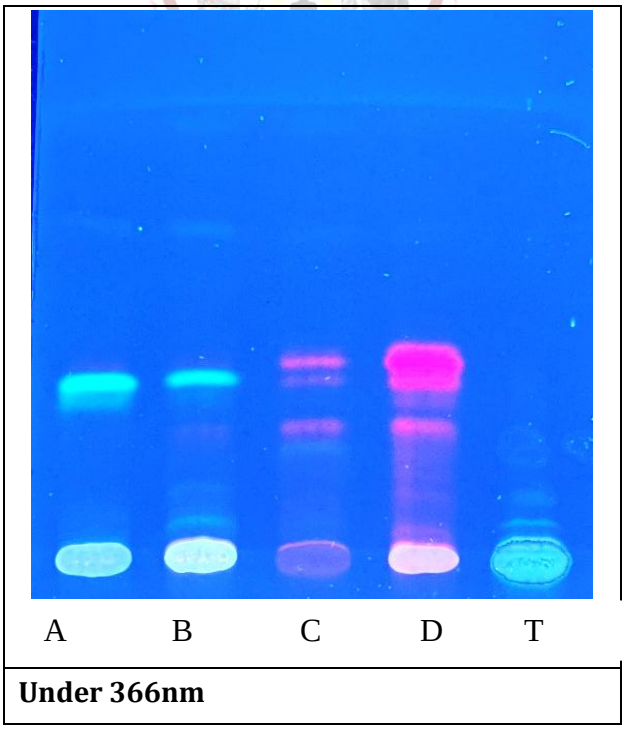


Figure 1- A: *Vasa*, B: *Kandakari*, C: *Samanga*, D: *Tulsi*, T: Kofsap SF syrup

Table 5: Rf value of Kofsap- SF and the raw materials that act as antitussive

No	Samples	Rf Values
1	A	0.29, 0.32
2	B	0.34
3	C	0.17, 0.23, 0.32, 0.35
4	D	0.32, 0.34
5	T	0.14, 0.19, 0.32

Gas Chromatography-Mass Spectrometry (GC-MS) Profile

Table no 6 shows the pharmacological action of the 9 mostly present active constituents in the formulation. The GC–MS analysis of the ethanolic extract reveals the presence of multiple bioactive compounds with well-documented pharmacological properties. Cyclohexanol, 1-methyl-4-(1-methylethyl), identified as the major constituent with the highest peak area (36.665%), is known for its antitussive and anti-inflammatory effects, indicating its potential role in respiratory and inflammatory conditions. [13,14] Thymol, comprising 25.460% of the extract, is a well-established compound with multifaceted actions including antibacterial, antifungal, antioxidant, anti-inflammatory, and antitussive activities, making it a key contributor to the extract's therapeutic profile.[15] Other aliphatic hydrocarbons such as dodecane, tetradecane, and hexadecane derivatives exhibit antimicrobial and antioxidant activities, suggesting supportive roles in managing microbial infections and oxidative stress. The presence of compounds like eicosane and octacosane adds value due to their anti-inflammatory, analgesic, and antitumor properties. Additionally, heptacosane's reported P-glycoprotein inhibition suggests a possible role in overcoming drug resistance. These findings collectively support the extract's potential as a source of pharmacologically active compounds with broad-spectrum therapeutic relevance. The GC-MS chromatogram of Kofsap-SF syrup is illustrated in figure no 2.

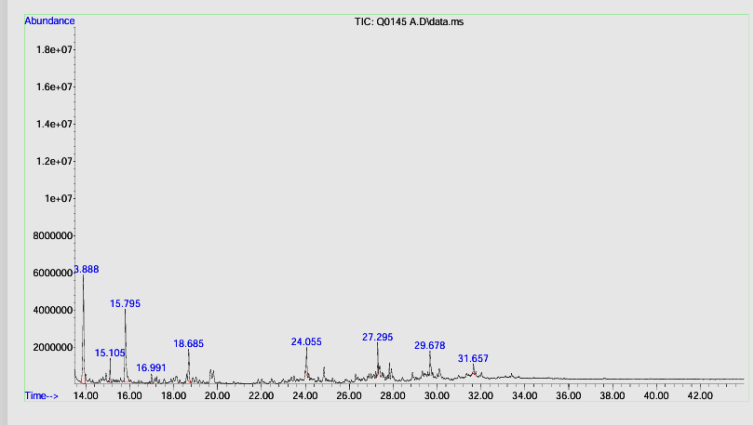


Figure 2- GC-MS chromatogram of Kofsap-SF syrup

Table 6: GC-MS Analysis of Kofsap Sugar-Free Syrup

No	Constituent	Chemical formula	Area	Action
1.	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{20}O$	36.665	Potential antitussive,[13] anti-inflammatory [14]
2.	Thymol	$C_{10}H_{14}O$	25.460	Antitussive, anti-inflammatory, antibacterial, antifungal, antioxidant [15]
3.	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	4.553	Antioxidant, antimicrobial [16]
4.	Tetradecane	$C_{14}H_{30}$	2.477	Broad spectrum antibacterial and antifungal [17]
5.	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	9.205	Antimicrobial [18]
6.	Hexadecane, 2,6,11,15-tetramethyl-	$C_{20}H_{42}$	6.336	Anti-inflammatory, anti-bacterial [19]
7.	Eicosane, 2-methyl-	$C_{21}H_{44}$	5.843	Antifungal, anti-inflammatory, and

				analgesic agent [20]
8.	Heptacosane	$C_{27}H_{56}$	7.023	P-gp Inhibition [21]
9.	Octacosane	$C_{28}H_{58}$	2.438	Antibacterial and antitumor effects, [22] as well as anti-inflammatory and analgesic properties.[20]

Microbial Safety

Microbial analysis of Kofsap SF mentioned in the table no 6 confirmed its safety, with no detection of pathogens (E. coli, Staphylococcus sp., Pseudomonas sp., Salmonella sp.). The total bacterial count was below 10cfu/mL, and no yeast or mold was detected, reflecting stringent manufacturing standards. These results ensure no risk of contamination-related adverse effects, critical for a respiratory remedy administered orally.

Table 7: Microbial Analysis of Kofsap SF Syrup

S.No.	Test	Results
1	E. coli	Absent
2	*Staphylococcus sp.*	Absent
3	*Pseudomonas sp.*	Absent
4	*Salmonella sp.*	Absent
5	Total Bacterial Count (cfu/mL)	<10
6	Total Yeast and Mold (cfu/mL)	Nil

CONCLUSION

The comprehensive standardization of Kofsap Sugar-Free Syrup, a polyherbal Ayurvedic formulation, reaffirms its potential as a safe, effective, and palatable remedy for cough. The formulation brings together traditional Ayurvedic wisdom and modern scientific validation, ensuring both therapeutic consistency and consumer trust. Organoleptic and physicochemical analyses confirmed its stability, sugar-free nature, and suitability for a wide demographic, including diabetic and health-conscious individuals. The presence of a rich phytochemical profile- featuring glycosides, flavonoids, phenols, saponins, and alkaloids- supports its multi-pronged action in reducing inflammation, easing expectoration, and soothing irritated respiratory tissues. TLC profiling and GC-MS analysis further validated the inclusion of bioactive compounds with established antitussive, anti-inflammatory, and antimicrobial activities, particularly highlighting constituents like thymol and cyclohexanol derivatives. Importantly, microbial safety was ensured through rigorous testing. Together, these findings substantiate the formulation's quality, safety, and therapeutic potential. Kofsap SF syrupthus represents a well-standardized, evidence-based Ayurvedic alternative for cough management, aligning with modern demands for efficacy, safety, and accessibility in herbal medicine.

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