

STANDARDIZATION AND QUALITY ASSESSMENT OF DIGESTOSAP SYRUP  
THROUGH PHYTOCHEMICAL PROFILING AND LC-MS ANALYSISSreedevi A. P.<sup>1\*</sup>, Reeshma C. R.<sup>2</sup>, Sandhya V. R.<sup>3</sup>, Maneesha K. S.<sup>4</sup>, Anu Joy<sup>5</sup>, Sibi Narayanan<sup>6</sup> and  
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## ABSTRACT

**Introduction:** Digestosap, a polyherbal Ayurvedic syrup developed by Sitaram Ayurveda Pvt Ltd., is formulated to stimulate appetite and enhance digestive health. Given the increasing demand for safe and effective herbal remedies, rigorous quality assessment is essential to validate its therapeutic claims. This study employs a multifaceted analytical approach to evaluate Digestosap's quality, safety, and efficacy. **Materials & Methods:** Digestosap was subjected to organoleptic evaluation for sensory attributes, physicochemical analysis for parameters like pH, specific gravity, total sugar, and phytochemical screening to identify bioactive compounds. Thin Layer Chromatography (TLC) was used to confirm key constituents, while microbial limit testing assessed safety. LC-MS analysis characterized the phytochemical profile responsible for its digestive benefits. **Results & Discussion:** Phytochemical analysis revealed flavonoids, phenols, glycosides, and alkaloids, supporting digestive and carminative effects. TLC verified constituents from ingredients like Dhanyakam and Pippali. Microbial testing showed no harmful pathogens and low counts (<10 cfu/ml). LC-MS identified compounds such as cinnamic acid, guaiacol, and stigmasterol, linked to gut health, anti-inflammatory, and metabolic benefits. **Conclusion:** Digestosap syrup demonstrates high quality, safety, and therapeutic potential as a digestive aid. Its diverse phytochemical profile, validated through LC-MS, supports its efficacy in enhancing appetite and digestion. This study bridges traditional Ayurvedic knowledge with modern analytical science, establishing Digestosap as a promising herbal formulation for clinical use in digestive health management.

**KEYWORDS:** Digestosap Syrup, Phytochemical Analysis, LC-MS, Appetite Stimulation, Digestive Health, Ayurvedic Medicine, Thin Layer Chromatography.

## INTRODUCTION

Appetite plays a crucial role in maintaining adequate nutritional intake and supporting overall health.<sup>[1]</sup> However, poor appetite, clinically referred to as anorexia or appetite loss, is a common symptom seen in various pathological conditions such as gastrointestinal disorders, chronic infections, metabolic syndromes, and even psychological conditions like stress and depression. Left unaddressed, diminished appetite can lead to malnutrition, weight loss, micronutrient deficiencies, and a weakened immune system, thereby impairing the body's ability to heal and function optimally.<sup>[2]</sup>

In the search for safe and effective remedies, herbal formulations have garnered considerable attention due to their traditional use, bioactive constituents, and relatively favourable safety profiles.<sup>[3]</sup> Among these, herbal digestive syrups are commonly used in ethnomedicine and complementary systems of healthcare for their ability to stimulate digestion and enhance appetite. Digestosap is one such polyherbal formulation developed by Sitaram Ayurveda Pvt Ltd., composed of multiple medicinal plants reputed in Ayurvedic and traditional medicine systems for their carminative, digestive, and appetite-stimulating properties. To ensure the quality, safety, and efficacy of such a formulation,

comprehensive evaluation through physicochemical and phytochemical analyses is essential.<sup>[4]</sup>

Physicochemical studies provide fundamental data of the formulation, including pH, Specific gravity, Total sugar, refractive index and moisture % which directly affect patient acceptability, dosage precision, and shelf-life. Furthermore, phytochemical screening of Digestosap aids in the preliminary identification of classes of bioactive compounds such as alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, and glycosides. These compounds are known to contribute significantly to the therapeutic activities of herbal preparations, including appetite stimulation and gastrointestinal support. Moreover, microbial limit testing is conducted to ensure the microbiological quality of Digestosap, confirming its safety by quantifying permissible levels of microbial contaminants, in accordance with pharmacopeial standards.<sup>[5]</sup>

To deepen the understanding of its bioactive profile, Liquid Chromatography–Mass Spectrometry (LC-MS) is employed as an advanced analytical tool. LC-MS enables the detection, separation, and precise identification of a wide range of phytochemicals present in complex herbal matrices with high sensitivity and specificity. It offers a powerful approach to characterize secondary metabolites and marker compounds that could be responsible for the observed pharmacological effects of Digestosap. The

LC-MS analysis not only aids in the standardization of the formulation but also assists in establishing a chemical fingerprint, ensuring batch-to-batch consistency and quality control.

This study, therefore, aims to investigate the physicochemical parameters, conduct preliminary phytochemical screening, carry out microbial limit testing and perform a detailed LC-MS analysis of Digestosap to evaluate its overall quality profile and support its claimed therapeutic efficacy. Through this integrative approach, we seek to bridge traditional knowledge with modern analytical science, facilitating the validation and potential clinical application of Digestosap in digestive and appetite-related disorders.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Raw materials

The raw materials for Digestosap were procured from the local market in Thrissur. These materials are identified and authenticated at the Pharmacognosy Division of Sitaram Ayurveda Pvt Ltd.

### 2.2 Preparation of Digestosap

Digestosap syrup is formulated using a blend of powerful herbal ingredients, as detailed in Table 1. The ingredients, their botanical names, and the parts used are outlined in the table, the formulation is processed following traditional Ayurvedic methods.

**Table 1: Composition of Digestosap.**

Sl. No	Ingredients	Botanical name	Parts used
1.	Nirgundi	<i>Vitex negundo</i>	Leaves
2.	Hareethaki	<i>Terminalia chebula</i>	Fruit rind
3.	Sunti	<i>Zingiber officinale</i>	Rhizome
4.	Pippali	<i>Piper longum</i>	Fruit
5.	Maricham	<i>Piper nigrum</i>	Fruit
6.	Dhanyakam	<i>Coriandrum sativum</i>	Fruit
7.	Hingu	<i>Ferula asa-foetida</i>	Exudate
8.	Ajamodam	<i>Tachyspermum ammi</i>	Fruit
9.	Chincha	<i>Tamarindus indicus</i>	Fruit pulp
10.	Sita khanda	<i>Saccharum officinarum</i>	As such
11.	Saindhavam	Rock salt	As such
12.	Jeerakam	<i>Cuminum cyminum</i>	Fruit
13.	Maricham	<i>Piper nigrum</i>	Fruit
14.	Jathipathri	<i>Myristica fragrans</i>	Aril
15.	Misreya	<i>Foeniculum vulgare</i>	Fruit
16.	Pudhina	<i>Mentha arvensis</i>	Distillate

### 2.3 Preparation process

The preparation of the herbal syrup follows a series of carefully controlled steps aimed at preserving and enhancing the medicinal properties of the raw ingredients. Initially, decoction is prepared using the herbs for the syrup, which is subsequently filtered. Tamarind is processed and mixed with the decoction. The mixture is concentrated, by adding the syrup base. Pudina distillate is incorporated to enhance the therapeutic effects of the syrup. Finally, the syrup is

subjected to quality testing to ensure consistency, potency, and the preservation of its medicinal properties.

### 2.4 Organoleptic Analysis

The organoleptic properties like colour, odour, taste, and consistency, of Digestosap are analysed.<sup>[6]</sup>

### 2.5 Physicochemical Analysis

Digestosap was quantified for refractive index, moisture percentage, pH, TSS, specific gravity and total sugar, as

per the standards of the Ayurvedic Pharmacopoeia of India.<sup>[7]</sup>

## 2.6 Preliminary Phytochemical Analysis

Phytochemical analysis of Digestosap was performed to identify the presence of various components, such as sugars, reducing sugars, ketoses, amino acids, proteins, starch, quinones, glycosides, flavonoids, phenols, saponins, alkaloids, tannins, and coumarins.<sup>[8]</sup>

## 2.7 Thin Layer Chromatography (TLC)

Thin Layer Chromatographic (TLC) fingerprint analysis was conducted to separate distinct bands. A 10 ml sample was refluxed with 20 ml of methanol for 30 minutes, followed by filtration and complete evaporation using a water bath. The resulting residue was reconstituted in 1 ml of methanol. TLC was performed using silica gel G as the adsorbent, which was prepared as a slurry and uniformly coated onto clean glass plates (10 × 5 cm) using a commercial spreader.<sup>[9]</sup> The methanolic extract was applied to the coated plate with a fine capillary tube, approximately 2 cm from the lower edge, and allowed to air dry. The TLC plate was then placed at a 45° angle in a development chamber, ensuring that the spotted area remained above the level of the mobile phase. The chromatogram was developed 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.

## 2.8 Microbial Limit Test

The microbial limit test was conducted as per the standard procedure outlined in the Ayurvedic Pharmacopoeia of India. Samples were collected aseptically. Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) were prepared by dissolving the respective culture media in double-distilled water, adjusting the pH using 1N NaOH and 1N HCl, followed by heating, autoclaving, and pouring into sterile Petri dishes. The media were allowed to solidify under aseptic conditions. For microbial analysis, 1 ml of each drug sample was serially diluted with 9 ml of sterile diluent. From the appropriate dilutions, 0.1 ml was plated using the spread plate technique. NA plates were incubated at 37°C for 24–48 hours, while SDA plates were incubated at room temperature for 3–5 days. Isolated microbial colonies were examined microscopically and biochemically. The observed colony counts were compared against the microbial limit standards specified in the Ayurvedic Pharmacopoeia of India.<sup>[7]</sup>

## 2.9. LC-MS

For the LCMS-QTOF analysis of Digestosap (Sample Name: VLC03), chromatographic separation was performed using a C18 column (2.1 × 50 mm, 1.8 µm) maintained at 37 °C with a flow rate of 0.3 mL/min. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), applied in a gradient elution mode over a total runtime of 15 minutes. The

mass spectrometric detection was carried out using an ESI-QTOF system operating in both positive and negative ionization modes, with the following settings: gas temperature at 325 °C, gas flow rate of 5 L/min, nebulizer pressure at 30 psi, capillary voltage at 3500 V, and fragmentor voltage at 175 V. For sample preparation, 1.0 mL of Digestosap was accurately measured and diluted to 25 mL with methanol:water (1:1, v/v), followed by further dilution to achieve the desired concentration. The solution was filtered through a 0.22 µm syringe filter prior to injection into the LC-MS system.<sup>[10]</sup>

## 3. RESULTS

### 3.1 Organoleptic Analysis

Organoleptic parameters such as colour, odour, taste & consistency of Digestosap are illustrated in Table no 2.

**Table-2: organoleptic analysis of Digestosap.**

Sl.No	Parameters	Result
1	Colour	Dark Brown
2	Odour	Characteristic
3	Taste	Sweet
4	consistency	Viscous Liquid

### 3.2 Physicochemical Analysis of Digestosap

Table 3 displays the average values of the physicochemical parameters, derived from three consecutive trials. The parameters analyzed include Refractive Index (RI), pH, Total Soluble Solids (TSS), specific gravity, total sugar, & moisture content, all assessed to determine the Digestosap's stability and suitability for consumption.

**Table no 3: Physicochemical analysis of Digestosap.**

Sl.No	Parameters	Result
1	pH	3.94
2	Total soluble solids(TSS)	64
3	Specific Gravity	1.319
4	Total sugar	58.67%
5	Moisture	26.47 %
6	Refractive Index	1.457

### 3.3 Phytochemical Analysis

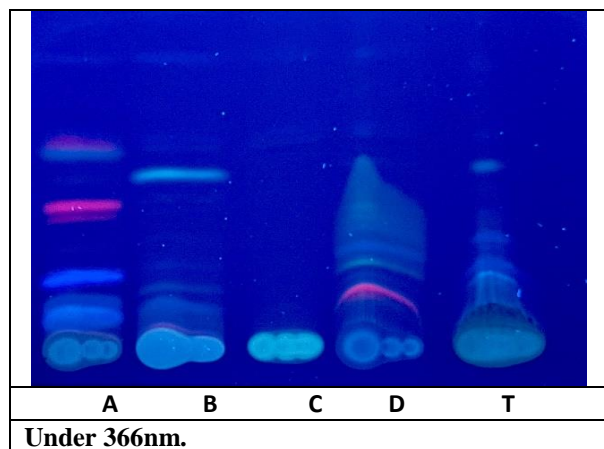
The presence of various phytochemicals present or absent in the aqueous and methanolic extract of Digestosap are shown in the table 4. These phytochemicals include carbohydrates, sugars, proteins, glycosides, steroids, flavonoids, phenols, and other compounds.

**Table no. 4: The phytochemical analysis of Digestosap.**

Sl No:	Organic Phytochemical constituents	Name of the test conducted	Digestosap	
			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	K I 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	H <sub>2</sub> SO <sub>4</sub> test	+	+

**3.4 Thin Layer Chromatography (TLC)**

Thin Layer Chromatography (TLC) was performed to identify and verify the active constituents in Digestosap syrup. The results confirmed the presence of key phytochemicals from its ingredients, including Ajamodam, Thippali, Chinch, & Dhanyakam.

**Figure 1-** A: Ajamodam; B: Dhanyakam; C: Chinch; D: Thippali; T: Digestosap.**Table No. 5: shows the R<sub>f</sub> value of Digestosap and the raw materials that aid in digestion.**

No	Samples	R <sub>f</sub> Values
1	A	0.23,0.34,0.56
2	B	0.15,0.44,0.49
3	C	0.48
4	D	0.22,0.31,0.44
5	T	0.17,0.28,0.45,0.49

**3.5 Microbial Limit Test**

The microbial analysis of Digestosap is performed to ensure the product's safety by identifying harmful microorganisms like *E. coli*, *Staphylococcus sp.*, and *Salmonella sp.* The findings of this analysis are shown in Table no 6.

**Table No 6: The Microbial Limit Test of Digestosap.**

Sl No	Test	Results
1	<i>E.coli</i>	Absent
2	<i>Staphylococcus sp.</i>	Absent
3	<i>Pseudomonas sp.</i>	Absent
4	<i>Salmonella sp.</i>	Absent
5	Total bacterial count cfu/g or cfu/ml (100000)	<10
6	Total yeast and mold cfu/g or cfu/ml(1000)	<10

**3.6 LC-MS**

The LC-MS analysis provides insight into the phytochemical profile of the Digestosap and support its claimed digestive properties by highlighting the presence

of compounds known for their Carminative effects. The chromatogram of Digestosap with multiple peaks were shown in fig 2 & corresponding compounds were given in table no 7.

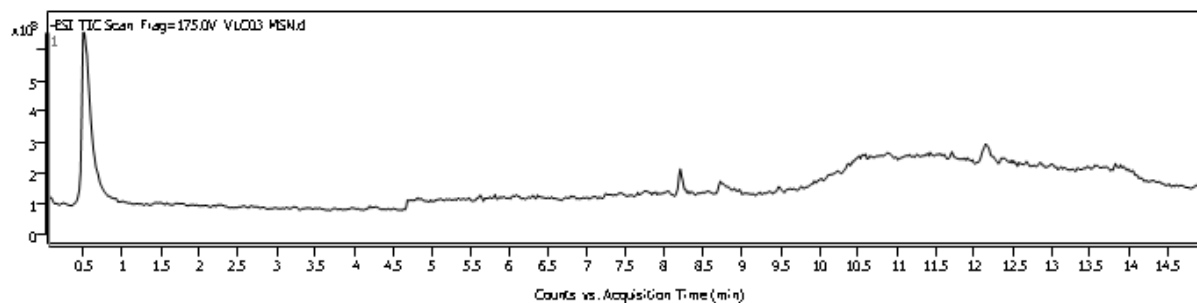


Figure 2.1-Negative mode.

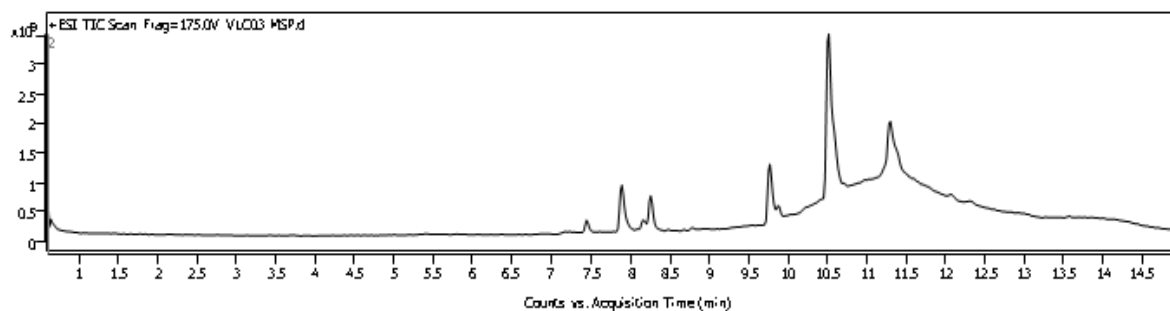


Figure 2.2-positive mode.

Figure 2: LC-MS chromatogram of Digestosap.

Table No 7: The LC-MS analysis of Digestosap.

Sl no	Constituent	Category	Chemical formula	Retention time(min)	Action
1	Cinnamic acid	Phenolic acid	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	8.607	Supports gut microbiota; improve digestive health <sup>[11]</sup>
2	Guaiacol	Phenolic compound	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>	0.981	Gastroprotective, anti-reflux agent <sup>[12]</sup>
3	2-Heptanone / 2-Nonanone	Volatile ketones	C <sub>7</sub> H <sub>14</sub> O	9.903	Antimicrobial <sup>[13]</sup>
4	Stigmasterol	Phytosterol	C <sub>29</sub> H <sub>48</sub> O	10.069	lipid metabolism regulation <sup>[14]</sup>
5	Genistein	Isoflavone	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	10.617	Anti-inflammatory; improves colonic function <sup>[15]</sup>
6	4-Hydroxycinnamic acid (p-Coumaric acid)	Phenolic acid	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	9.288	Digestive enzyme enhancer; immunomodulatory <sup>[16]</sup>
7	Phytol	Diterpenoid alcohol	C <sub>20</sub> H <sub>40</sub> O	11.182	Metabolic regulator (PPAR modulation); Supports digestion <sup>[17]</sup>
8	Vitexin & Cosmosiin	Phenolic acid	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	10.583	Antioxidant; protects against oxidative stress-related digestive issues <sup>[18]</sup>
9	Taxifolin	Phenolic compound	C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>	7.359	Antioxidant; gastroprotective <sup>[19]</sup>
10	Linoleic acid	Volatile ketones	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	11.197	Prebiotic effect; gut microbiota fermentation to beneficial CLA <sup>[20]</sup>
11	2-tert-Butyl-4-methoxyphenol	Aromatic ketone	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	13.490	Anti-inflammatory <sup>[21]</sup>
12	5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	Phytosterol	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	11.729	Anti-inflammatory; intestinal mucosa protection <sup>[22]</sup>

#### 4. DISCUSSION

A comprehensive analysis of Digestosap Syrup, a proprietary Ayurvedic formulation developed by Sitaram Ayurveda, provides valuable insights into its organoleptic, physicochemical, phytochemical, microbial, and LC-MS profiles. The findings contribute significantly to understand the formulation's quality, stability, and therapeutic potential. Furthermore, the study presents valid evidence for supporting the efficacy

and safety of Digestosap in improving appetite and metabolism, thus highlighting its therapeutic relevance.

The organoleptic analysis of Digestosap shows it has a dark brown colour, characteristic odour, sweet taste, and liquid consistency. The sweet taste improves palatability and help stimulate salivation and digestive secretions. Its liquid form allows for easy administration and quick

absorption. Overall, the sensory attributes of Digestosap are consistent with its intended use as a digestive aid.

The physicochemical analysis of Digestosap syrup highlights its suitability as a digestive formulation. Its acidic pH of 3.94 supports digestion and microbial stability, while TSS 64 and total sugar content 58.67% enhance palatability and preservation. The specific gravity of 1.319 ensures consistency and accurate dosing, and the low moisture content 26.47% minimizes microbial risk, contributing to shelf life. A refractive index of 1.457 confirms the syrup's concentration and purity. Overall, the formulation is stable, palatable, and effectively designed to support digestive health.

Phytochemical screening of Digestosap syrup confirmed the presence of various bioactive compounds linked to digestive health. Both aqueous and methanolic extracts tested positive for carbohydrates, sugars, reducing sugars, ketoses, flavonoids, phenols, glycosides, steroids, and quinones. These constituents are known to aid digestion and enhance metabolism. Flavonoids, phenols, and glycosides in particular provide antioxidant and anti-inflammatory benefits that may soothe the digestive tract. They are also associated with carminative effects, helping to relieve bloating by relaxing intestinal muscles and improving peristaltic movement.<sup>[22]</sup> Saponins and alkaloids, present only in the aqueous extract, help to relieve bloating-related abdominal discomfort by stimulating gut secretions.<sup>[23,24]</sup> Steroids found in both extracts contribute to reducing inflammation, thus indirectly supporting smoother digestion. The combined presence of these compounds indicates, the formulation is potential as an effective carminative and digestive tonic.

The TLC fingerprint of Digestosap revealed  $R_f$  values 0.17, 0.28, 0.45, 0.49, indicating the presence of phytochemicals derived from its herbal ingredients. The  $R_f$  values of 0.49 and 0.45 correlate with Dhanyakam and Thippali, respectively, suggesting the presence of appetite-stimulating compounds like flavonoids and piperine. The unique 0.28 spot represent synergistic compounds formed during formulation, while the 0.17 spot likely originates from minor constituents. Chinch, which shows an  $R_f$  of 0.48, is closely aligned with  $R_f$  0.49 of Digestosap, suggesting that its active constituents are well integrated into the formulation. Overall, Digestosap contains key phytochemicals that promote salivary and gastric secretions, supporting its role in enhancing appetite and digestion.

The microbial analysis of Digestosap syrup confirmed the absence of harmful pathogens including *E. coli*, *Staphylococcus sp.*, *Pseudomonas sp.*, and *Salmonella sp.*, indicating high safety standards. The total bacterial count was <10 cfu/ml, significantly below the permissible limit of 100,000 cfu/ml, while the total yeast and mold count was also <10 cfu/ml, well under the 1,000 cfu/ml threshold. These results demonstrate

effective microbial control and hygienic manufacturing practices. The low microbial load ensures product stability, extended shelf life, and safe consumption. Overall, the microbial quality of Digestosap supports its reliability as a safe and hygienic digestive formulation.

The LC-MS results of the digestive syrup formulation disclosed a carefully selected combination of bioactive compounds, each contributing specific benefits to digestive health. Cinnamic acid, identified with a retention time of 8.607 minutes, supports gut microbiota and improves digestive health through its antioxidant properties. Guaiacol (0.981 minutes), with its gastroprotective effects, acts as an anti-reflux agent, contributing to the overall reduction of digestive discomfort. The volatile ketones 2-Heptanone and 2-Nonanone (9.903 minutes) display antimicrobial properties, ensuring the balance of beneficial gut microbes. Stigmasterol (10.069 minutes), a phytosterol, regulates lipid metabolism, which is essential for optimal fat digestion. Genistein (10.617 minutes) is noted for its anti-inflammatory action, improving colonic function and reducing inflammation in the digestive tract. 4-Hydroxycinnamic acid (p-Coumaric acid) (9.288 minutes) enhances digestive enzyme activity, optimizing the breakdown of nutrients and providing immunomodulatory benefits. Phytol (11.182 minutes) acts as a metabolic regulator, modulating lipid and carbohydrate metabolism, which supports digestion. Vitexin and Cosmosiin (10.583 minutes) are antioxidants that protect against oxidative stress, improving digestive health and preventing cellular damage. Taxifolin (7.359 minutes) shows gastroprotective and antioxidant properties, reducing oxidative damage and supporting the integrity of the gastric mucosa. Linoleic acid (11.197 minutes) exerts a prebiotic effect, enhancing gut microbiota fermentation to produce beneficial compounds like conjugated linoleic acid (CLA). 2-tert-Butyl-4-methoxyphenol (13.490 minutes) provides anti-inflammatory effects, further aiding in the reduction of gastrointestinal irritation. Lastly, 5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (11.729 minutes) helps protect the intestinal mucosa and offers anti-inflammatory effects. These LC-MS results confirm the presence of compounds with a broad spectrum of digestive benefits, from antimicrobial to anti-inflammatory and metabolic regulation, ensuring a well-rounded approach to digestive health.

The analysis of Digestosap syrup demonstrates its significant potential as a digestive aid, containing a blend of bioactive compounds that promote digestive health, further reinforcing its effectiveness in enhancing digestive function and overall gut wellness.

## 5. CONCLUSION

The comprehensive analysis of Digestosap syrup underscores its potential as an effective and safe polyherbal formulation for enhancing digestive function and appetite. The organoleptic and physicochemical

properties confirm its palatability, stability, and suitability for consumption. Phytochemical screening and TLC analysis verified the presence of bioactive compounds, including flavonoids, phenols, and glycosides, which support its carminative and digestive properties. The absence of harmful pathogens and low microbial counts ensure its safety and shelf-life stability. LC-MS analysis further elucidated a diverse phytochemical profile, with compounds like cinnamic acid, genistein, and phytol contributing to gut health, anti-inflammatory effects, and metabolic regulation. By integrating traditional Ayurvedic principles with rigorous scientific validation, this study establishes Digestosap as a reliable digestive tonic, paving the way for its potential clinical application in managing appetite-related and digestive disorders.

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