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FORMULATION AND EVALUATION OF A POLYHERBAL IMMUNE BOOSTER SYRUP

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ABSTRACT

The increasing demand for natural immune-supportive products has promoted the development of herbal formulations with antioxidant, antimicrobial, and immunomodulatory potential. The present study aimed to formulate and evaluate a polyherbal immune booster syrup containing betel leaf (*Piper betle*), carrot (*Daucuscarota*), amla (*Emblica officinalis*), and tulsi (*Ocimum sanctum*) extracts. The syrup was prepared using betel leaf extract (5 mL), carrot juice (10 mL), amla juice (5 mL), tulsi extract (5 mL), honey/sorbitol (3 mL), sodium benzoate (0.1 g), citric acid (0.05 g), and purified water q.s. to 30 mL. The formulation was evaluated for organoleptic, physicochemical, phytochemical, and microbial parameters.

The prepared syrup showed dark brown color, characteristic herbal odor, sweet taste, thick smooth viscous texture, and uniform liquid appearance without precipitation. The pH was found to be 5.5, indicating suitability for oral use and formulation stability. Viscosity was satisfactory, and no significant changes were observed during stability testing. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds, suggesting antioxidant and immune-enhancing potential.

Microbial analysis showed satisfactory safety with a total viable count of **1.8×10^2 CFU/mL** and total yeast and mold count of **<10 CFU/mL**, both within acceptable limits. Pathogenic microorganisms such as *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were absent.the formulated polyherbal syrup demonstrated acceptable quality, stability, microbial safety, and valuable phytoconstituents. It may serve as a promising natural supplement for enhancing immunity and promoting overall health. Further pharmacological and clinical studies are recommended.

INTRODUCTION

The immune system plays a crucial role in protecting the human body against infectious agents, environmental stressors, and various pathological conditions. A well-functioning immune system is essential for maintaining health and preventing disease [1]. In recent years, increasing awareness regarding preventive healthcare, recurrent infections, lifestyle-related disorders, and post-viral complications has significantly increased the demand for natural products that support immune function [2]. Herbal formulations have gained considerable attention due to their long history of traditional use, therapeutic benefits, safety profile, and lower incidence of adverse effects compared with many synthetic preparations [3].

Polyherbal formulations are based on the concept of combining multiple medicinal plants to produce synergistic therapeutic effects [4]. Such combinations may provide broader pharmacological activity through the presence of diverse phytoconstituents including flavonoids, alkaloids, tannins, phenolic compounds, vitamins, and essential oils [5]. These bioactive molecules are known to exhibit antioxidant, anti-inflammatory, antimicrobial, adaptogenic, and immunomodulatory properties, which collectively contribute to strengthening host defence mechanisms [6].

Betel leaf (***Piper betle*** Linn.) is an important medicinal plant widely used in traditional systems of medicine. It contains chavicol, eugenol, hydroxychavicol, and other phenolic compounds known for antimicrobial, antioxidant, and anti-inflammatory activities [7]. Carrot (***Daucuscarota*** Linn.) is a rich source of β-carotene, vitamins, and minerals that contribute to immune enhancement and protection against oxidative damage [8]. Amla (***Emblica officinalis*** Gaertn.) is recognized as one of the richest natural sources of vitamin C and contains tannins and polyphenols with potent antioxidant and rejuvenating effects [9]. Tulsi (***Ocimum sanctum*** Linn.), often referred to as holy basil, possesses adaptogenic, antimicrobial, anti-inflammatory, and immunomodulatory properties, making it highly valuable in immune-supportive preparations [10].

The oral syrup dosage form is widely preferred due to its ease of administration, pleasant taste, accurate dosing, and suitability for pediatric and geriatric populations [11]. Syrups containing herbal extracts offer an effective method for delivering active phytoconstituents in a stable and patient-friendly manner. Addition of natural sweeteners such as honey can further improve palatability while providing supplementary antimicrobial and soothing effects [12].

Considering the medicinal value of these herbs, the present study was undertaken to formulate and evaluate a polyherbal immune booster syrup containing betel leaf extract, carrot juice, amla juice, and tulsi extract [13]. The formulation was designed to provide a synergistic blend of antioxidant and immunomodulatory constituents. The prepared syrup was subjected to organoleptic evaluation, physicochemical characterization, phytochemical screening, and microbial assessment to determine its quality, safety, and suitability as a natural immune-supportive product [14].

MATERIALS AND METHODS

Herbal Ingredients

Fresh betel leaves (*Piper betle*), tulsi leaves (*Ocimum sanctum*), amla fruits (*Emblica officinalis*), fresh carrots (*Daucuscarota*), lemon, and honey were procured from the local market. Sodium benzoate and citric acid were used as preservative and pH-adjusting agent, respectively. Purified water was used as the vehicle for formulation.

Formulation of Polyherbal Immuno Booster Syrup

The herbal immuno booster syrup was prepared in a 30 mL batch size using the ingredients listed below [15].

Table 1. Formula Composition for 30 mL Batch

Ingredient	Quantity	Activity
Betel leaf extract	5 mL	Antioxidant, anticancer
Carrot juice	10 mL	Rich in β-carotene, immunity enhancer
Amla juice	5 mL	Vitamin C source, antioxidant
Tulsi extract	5 mL	Adaptogen, immune modulator
Honey or Sorbitol	3 mL	Sweetener, preservative
Sodium benzoate	0.1 g	Preservative
Citric acid	0.05 g	pH adjuster
Purified water	q.s. to 30 mL	Vehicle

Method of Preparation

Betel leaves, tulsi leaves, and amla fruits were thoroughly washed and processed separately to obtain aqueous or hydroalcoholic extracts, while fresh carrot juice was prepared by crushing cleaned carrots and filtering to remove fibrous matter. Measured quantities of betel leaf extract, tulsi extract, amla juice, and carrot juice were mixed in a clean beaker. Honey or sorbitol was then added gradually with continuous stirring until a uniform mixture was formed. Sodium benzoate was incorporated as a preservative, and citric acid was added slowly to adjust the pH to 4.5–5.5. Purified water was added to make the final volume up to 30 mL, and the syrup was filtered through muslin cloth or Whatman filter paper to obtain a clear preparation. Finally, the syrup was filled into clean amber-colored glass bottles and properly labeled with product details.

Evaluation of Herbal Syrup

Organoleptic Evaluation [16]

The prepared syrup was visually examined for sensory characteristics including:

- **Color:** Uniform and appealing appearance
- **Odor:** Characteristic herbal aroma
- **Taste:** Palatable and acceptable
- **Appearance:** Clear or slightly opaque, free from suspended particles

Physicochemical Evaluation

The formulation was subjected to physicochemical testing to ensure quality and stability.

- **pH:** Determined using a calibrated digital pH meter
- **Viscosity:** Measured using Brookfield viscometer
- **Specific Gravity:** Determined using pycnometer
- **Total Solids:** Estimated by evaporation method

Preliminary Phytochemical Screening

The syrup was screened for the presence of major phytoconstituents using standard qualitative tests.

- **Alkaloids:** Mayer's or Dragendorff's test
- **Flavonoids:**Shinoda test
- **Tannins:** Ferric chloride test
- **Saponins:** Foam test
- **Phenols:**Folin–Ciocalteu reagent test

Microbial Load Testing

Microbial quality of the formulation was assessed to ensure product safety and shelf life.

- **Total Viable Count:** Determined by plate count method
- **Pathogen Detection:** Tested for absence of ***Escherichia coli***, ***Salmonella*** spp., and ***Staphylococcus aureus*** using selective media [17].

RESULTS AND DISCUSSION

The formulated herbal syrup containing betel leaf, carrot juice, tulsi, amla, lemon, and honey was successfully prepared and evaluated for its

organoleptic, physicochemical, phytochemical, and microbial characteristics. The formulation showed acceptable quality parameters with desirable appearance, consistency, and stability.

Table 2. Organoleptic and Physicochemical Evaluation of Herbal Syrup

Test	Observation
Color	Dark Brown
Odor	Betel leaf fragrance, herbal aroma
Texture	Thick, smooth, viscous
Taste	Sweet
Appearance	Liquid
pH	5.5
Viscosity	Satisfactory
Stability	Stable

The syrup exhibited a dark brown color, which may be due to the presence of natural pigments and polyphenolic compounds from herbal ingredients. It possessed a characteristic betel leaf fragrance with a pleasant herbal aroma, indicating retention of volatile constituents. The texture was thick, smooth, and viscous, which is desirable for syrup formulations as it improves palatability and ease of swallowing. The sweet taste was mainly due to honey, which also contributes soothing and preservative properties. The liquid appearance was uniform without any precipitation or phase separation, indicating proper mixing of ingredients.

The pH of the syrup was found to be 5.5, which lies within the acceptable acidic range for oral herbal preparations. This pH helps maintain product stability and prevents microbial growth. The viscosity was satisfactory, ensuring better mouthfeel and consistency. Stability studies showed no significant changes in physical characteristics, confirming good formulation stability.

Table 3. Preliminary Phytochemical Screening of Herbal Syrup

Test	Observation
Test for Alkaloids	Present
Test for Flavonoids	Present
Test for Tannins	Present
Test for Saponins	Present
Test for Phenols	Present

Preliminary phytochemical screening confirmed the presence of important secondary metabolites in the herbal syrup. Mayer's test indicated the presence of alkaloids, which are known for therapeutic and antimicrobial properties. The Shinoda test produced a brown color confirming flavonoids, which possess antioxidant and anti-inflammatory activities. Ferric chloride test showed a dark blue-black color indicating tannins that provide astringent and antimicrobial effects. Foam test confirmed the presence of saponins, while the green color in the Folin–Ciocalteu test indicated phenolic compounds with strong antioxidant potential.

Table 4. Microbial Evaluation of Herbal Syrup

Test	Observation
Total Viable Count	1.8×10^2 CFU/mL
Total Yeast and Mold Count	< 10 CFU/mL
Pathogen Detection (***E. coli***, ***Salmonella***, ***Staphylococcus aureus***, ***Pseudomonas aeruginosa***)	Absent

Microbial evaluation by total viable count using the plate count method showed a count of **1.8×10^2 CFU/mL**, which is within acceptable limits for herbal oral liquid preparations. The total yeast and mold count was found to be **less than 10 CFU/mL**, indicating minimal fungal contamination. Pathogen detection tests confirmed the absence of harmful microorganisms such as ***E. coli***, ***Salmonella***, ***Staphylococcus aureus***, and ***Pseudomonas aeruginosa***, demonstrating the microbiological safety of the formulation. The formulated herbal syrup exhibited desirable organoleptic

properties, acceptable physicochemical parameters, presence of beneficial phytoconstituents, and satisfactory microbial quality. These results support its potential as a natural health-promoting syrup with antioxidant, antimicrobial, and immunity-enhancing benefits.

CONCLUSION

The present study successfully formulated and evaluated a polyherbal immune booster syrup containing betel leaf, carrot, amla, and tulsi extracts. The developed syrup exhibited desirable organoleptic properties, acceptable physicochemical characteristics, and good stability. Preliminary phytochemical screening confirmed the presence of beneficial bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which are known for their antioxidant, antimicrobial, and immunomodulatory activities. Microbial evaluation demonstrated that the formulation was safe for consumption, with microbial counts within permissible limits and absence of pathogenic organisms. The results indicate that the formulated syrup has strong potential as a natural immune-supportive health supplement. Further in vivo studies and clinical investigations are recommended to establish its therapeutic efficacy and long-term safety.

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