



FORMULATION AND EVALUATION OF A POLYHERBAL ANTIOXIDANT SYRUP

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ABSTRACT

Oxidative stress plays a significant role in the progression of various chronic diseases, necessitating the development of safe and effective natural antioxidant formulations. The present study focuses on the formulation and evaluation of a polyherbal antioxidant syrup using aqueous extracts of *Emblica officinalis*, *Zingiber officinale*, and *Ocimum sanctum*, with natural honey as a stabilizing and sweetening agent. The formulation was designed as a simple and cost-effective approach suitable for preparation using basic laboratory facilities. The prepared syrup was evaluated for organoleptic characteristics, physicochemical parameters, microbial quality, and short-term stability. The formulation exhibited a pH of 5.86 ± 0.03 , viscosity of 145.2 ± 2.5 cP, density of 1.12 ± 0.01 g/mL, and total soluble solids of 68°Brix, indicating appropriate stability and consistency for

oral use. The antioxidant assessment demonstrated 64.28% free radical inhibition along with appreciable reducing capacity, suggesting effective electron-donating potential. Spectral analysis revealed an absorption peak around 285 nm, indicating the presence of phenolic compounds contributing to antioxidant activity. Microbial studies confirmed the absence of pathogenic organisms, and stability evaluation over 30 days showed no significant changes in physicochemical properties. This study highlights the development of a stable and effective polyherbal syrup using readily available plant materials, offering a practical alternative to synthetic antioxidants. The formulation demonstrates potential as a nutraceutical preparation for managing oxidative stress and warrants further detailed investigation.

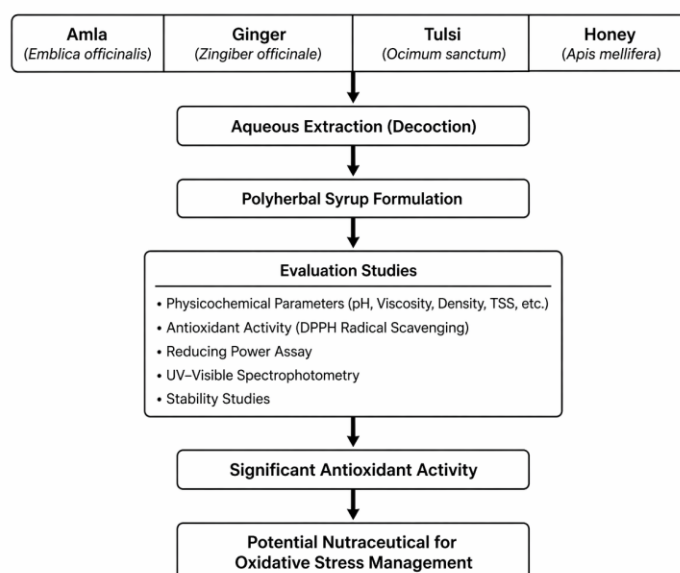


Fig. No. 1: Graphical abstract illustrating the formulation, evaluation, and antioxidant potential of the polyherbal antioxidant syrup.

KEYWORDS: Polyherbal syrup, Antioxidant activity, DPPH assay, *Emblica officinalis*, Stability studies Nutraceutical formulation.

1. INTRODUCTION

Oxidative stress is a well-recognized biochemical phenomenon arising from an imbalance between the generation of reactive oxygen species (ROS) and the body's intrinsic antioxidant defense mechanisms. Although ROS play a vital role in normal physiological processes such as cell signaling and immune response, their excessive accumulation can lead to oxidative damage of lipids, proteins, and nucleic acids. This damage has been strongly associated with the development and progression of several chronic disorders, including cardiovascular diseases, diabetes mellitus, cancer, neurodegenerative conditions, and inflammatory disorders.^[1-2] As a result, the role of antioxidants in mitigating oxidative stress has gained significant attention in both pharmaceutical and nutraceutical research.

In recent years, there has been a growing shift toward the use of natural antioxidants derived from plant sources due to concerns regarding the safety and long-term effects of synthetic antioxidants.^[3] Herbal materials are rich in bioactive compounds such as polyphenols, flavonoids, tannins, and vitamins, which exhibit strong free radical scavenging activity. In addition to their antioxidant potential, these phytoconstituents often provide complementary pharmacological benefits, including anti-inflammatory, antimicrobial, and

immunomodulatory effects. This multifaceted activity makes herbal formulations particularly attractive for preventive and therapeutic applications.

Polyherbal formulations, which combine multiple medicinal plants, are a cornerstone of traditional systems of medicine such as Ayurveda.^[4] The rationale behind such combinations lies in the concept of synergism, where the therapeutic effect of the combined ingredients is greater than that of individual components. By integrating different herbs with distinct yet complementary mechanisms of action, polyherbal formulations can provide enhanced efficacy, improved safety, and broader therapeutic coverage.^[5-8] Despite their widespread traditional use, there remains a need for systematic scientific evaluation of such formulations to validate their efficacy and ensure quality and consistency.

Among the various antioxidant-rich medicinal plants, *Emblica officinalis* (Amla), *Zingiber officinale* (Ginger), and *Ocimum sanctum* (Tulsi) have been extensively studied for their potent bioactivity. Amla is a rich source of vitamin C and hydrolyzable tannins, which contribute to its strong antioxidant and cytoprotective properties.^[9-10] Ginger contains bioactive compounds such as gingerols and shogaols, known for their antioxidant and anti-inflammatory activities. Tulsi, often referred to as the “queen of herbs,” is abundant in phenolic compounds like eugenol and rosmarinic acid, which play a crucial role in neutralizing free radicals and enhancing endogenous antioxidant defenses. Honey, a natural sweetening agent, further contributes to antioxidant activity due to the presence of flavonoids, phenolic acids, and enzymatic components, while also acting as a natural preservative.

The selection of an appropriate dosage form is equally important in ensuring patient compliance and stability of the formulation. Syrups offer several advantages, including ease of administration, improved palatability, and suitability for pediatric and geriatric populations. Moreover, the presence of sugar and honey not only enhances taste but also contributes to the preservation and stability of the formulation by reducing water activity. These attributes make syrup an ideal dosage form for delivering herbal antioxidants in a convenient and acceptable manner.

Although several herbal antioxidant products are available in the market, many lack adequate scientific validation, particularly in terms of formulation optimization, physicochemical characterization, and stability assessment. Furthermore, there is a growing need for simple, cost-effective formulations that can be developed and evaluated using basic laboratory

facilities. Addressing this gap, the present study focuses on the formulation and evaluation of a polyherbal antioxidant syrup using aqueous extracts of Amla, Ginger, and Tulsi, incorporated into a honey-based syrup system.

The objectives of this study include the preparation of a stable and palatable polyherbal syrup, evaluation of its physicochemical properties, assessment of its *in vitro* antioxidant activity using standard assays, and determination of its microbial safety and stability over time. By integrating traditional knowledge with modern analytical approaches, this work aims to provide a scientifically validated herbal formulation with potential nutraceutical applications in the management of oxidative stress.

2. MATERIALS AND METHODS

2.1 materials

Fresh fruits of *Embllica officinalis* (Amla), rhizomes of *Zingiber officinale* (Ginger), and leaves of *Ocimum sanctum* (Tulsi) were procured from a local herbal market and authenticated based on standard morphological characteristics. Natural honey (*Apis mellifera*) was obtained from a certified supplier and used as a sweetening and stabilizing agent.

All chemicals and reagents used in the study were of analytical grade. These included 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, potassium ferricyanide, trichloroacetic acid, ferric chloride, and phosphate buffer, which were procured from standard chemical suppliers. Distilled water was used throughout the study.

2.2 preparation of herbal extracts

Aqueous extracts of the selected herbs were prepared using the decoction method. The plant materials were washed thoroughly with distilled water to remove impurities and air-dried under shade. Each herb was then coarsely powdered using a mechanical grinder.

For extraction, accurately weighed quantities of plant materials were boiled with distilled water in separate containers. Amla (50 g) was boiled with 250 mL of water for 30 minutes, Ginger (30 g) with 200 mL for 20–25 minutes, and Tulsi (20 g) with 200 mL for 20 minutes. The decoctions were allowed to cool to room temperature and filtered through muslin cloth followed by Whatman No. 1 filter paper.

The filtrates were concentrated to reduced volumes under controlled heating and stored in amber-colored bottles at 4°C until further use.

2.3 Formulation of Polyherbal Syrup

The polyherbal syrup was prepared by incorporating the individual herbal extracts into a sugar-based syrup. A simple syrup base was prepared by dissolving 65 g of sucrose in 50 mL of distilled water with the addition of 0.2 g citric acid, followed by gentle heating until a clear solution was obtained.

The previously prepared extracts of Amla (10 mL), Ginger (10 mL), and Tulsi (5 mL) were mixed thoroughly. The sugar syrup was then added gradually with continuous stirring to ensure uniform mixing. Honey (20 mL) was incorporated as a natural sweetener and preservative.

The final volume was adjusted to 100 mL using distilled water. The prepared syrup was filtered, transferred into clean amber-colored bottles, and stored at room temperature for further evaluation.

2.4 Evaluation of Polyherbal Syrup

2.4.1 Organoleptic Evaluation

The prepared syrup was evaluated for colour, odour, taste, appearance, and consistency by visual inspection and sensory assessment.

2.4.2 Physicochemical Parameters

The pH of the formulation was measured using a calibrated digital pH meter. Viscosity was determined using a Brookfield viscometer at 25°C. Density was measured using a pycnometer, and Total soluble solids of the formulation were determined using a digital refractometer at 25°C. A few drops of the syrup were placed on the prism surface, and the °Brix value was recorded. All measurements were performed in triplicate ($n = 3$), and results were expressed as mean $\pm$ standard deviation.

2.4.3 Antioxidant Activity

(a) DPPH Radical Scavenging Assay

The antioxidant activity of the formulation was evaluated using the DPPH radical scavenging method. A 0.1 mM DPPH solution was prepared in methanol. An aliquot of the sample was

mixed with DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a UV–visible spectrophotometer.

The percentage inhibition of DPPH radicals was calculated using the standard formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

(b) Reducing Power Assay

The reducing power of the formulation was determined by measuring its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The reaction mixture containing sample, phosphate buffer, and potassium ferricyanide was incubated at 50°C , followed by the addition of trichloroacetic acid and centrifugation. The supernatant was mixed with ferric chloride, and absorbance was measured at 700 nm. Higher absorbance indicates stronger reducing power.

2.4.4 UV–Visible Spectral Analysis

The formulation was diluted appropriately and scanned in the wavelength range of 200–400 nm using a UV–visible spectrophotometer to identify the presence of phenolic compounds.

2.4.5 Microbial Analysis

Microbial quality was assessed using total plate count and fungal count methods. The presence of pathogenic organisms such as *Escherichia coli* and *Salmonella* spp. was evaluated using standard microbiological techniques.

2.5 Stability Studies

The stability of the formulated syrup was evaluated over a period of 30 days at room temperature. Samples were analyzed at predetermined intervals (0, 7, 15, and 30 days) for changes in pH, colour, odour, clarity, and precipitation.

3. RESULTS AND DISCUSSION

3.1 Organoleptic Characteristics

The formulated polyherbal syrup was evaluated for its sensory attributes, including colour, odour, taste, and overall appearance. The syrup exhibited a clear, homogeneous appearance with a brownish-yellow colour, which is characteristic of herbal formulations containing Amla and honey. The odour was pleasant with a mild herbal aroma, while the taste was sweet with a slight pungency attributed to Ginger.

These organoleptic properties indicate good acceptability of the formulation, which is particularly important for oral liquid dosage forms intended for routine consumption. The absence of turbidity or particulate matter further confirms the uniformity and quality of the prepared syrup. These characteristics confirm good patient acceptability.

Table No. 1: Organoleptic Properties of Polyherbal Syrup.

Parameter	Observation
Colour	Brownish – Yellow
Odour	Pleasant herbal
Taste	Sweet with mild pungency
Appearance	Clear and homogeneous
Consistency	Smooth

3.2 Physicochemical Properties

The physicochemical parameters of the formulation were found to be within acceptable limits. The pH of the syrup was recorded as 5.86 ± 0.03 , indicating a slightly acidic nature, which is favourable for both stability and microbial control. The viscosity (145.2 ± 2.5 cP) and density (1.12 ± 0.01 g/mL) values suggest appropriate flow properties and consistency, ensuring ease of administration.

The total soluble solids were found to be 68°Brix, which falls within the standard range for syrup formulations. This high sugar concentration not only enhances palatability but also contributes to preservation by reducing water activity.

These findings are consistent with previously reported herbal syrup formulations, where similar physicochemical characteristics were associated with improved stability and patient compliance.

The formulation showed stable physicochemical parameters suitable for oral administration and the slightly acidic pH supports stability and microbial control.

Table No. 2: Physicochemical Parameters.

Parameter	Value (Mean $\pm$ SD)
pH	5.86 ± 0.03
Viscosity (cP)	145.2 ± 2.5
Density (g/mL)	1.12 ± 0.01
Total Soluble Solids	68°Brix

3.3 Antioxidant Activity

3.3.1 DPPH Radical Scavenging Assay

The antioxidant activity of the polyherbal syrup was assessed using the DPPH radical scavenging assay, and the results are presented in Figure 2. The formulation exhibited 64.28% inhibition, indicating a strong free radical scavenging potential, although slightly lower than the standard ascorbic acid (83.02%).

The observed antioxidant activity can be attributed to the presence of phenolic compounds, flavonoids, and vitamin C derived from Amla, Ginger, Tulsi, and honey. These phytoconstituents act as hydrogen or electron donors, thereby neutralizing free radicals and preventing oxidative damage.

The results are in agreement with earlier studies reporting significant DPPH scavenging activity in polyherbal formulations, supporting the concept of synergistic interaction among multiple plant constituents. The formulation shows strong antioxidant activity due to polyphenols.

Table No. 3: DPPH Activity.

Sample	Absorbance	% Inhibition
Control	0.742	–
Ascorbic Acid	0.126	83.02%
Herbal Syrup	0.265	64.28%

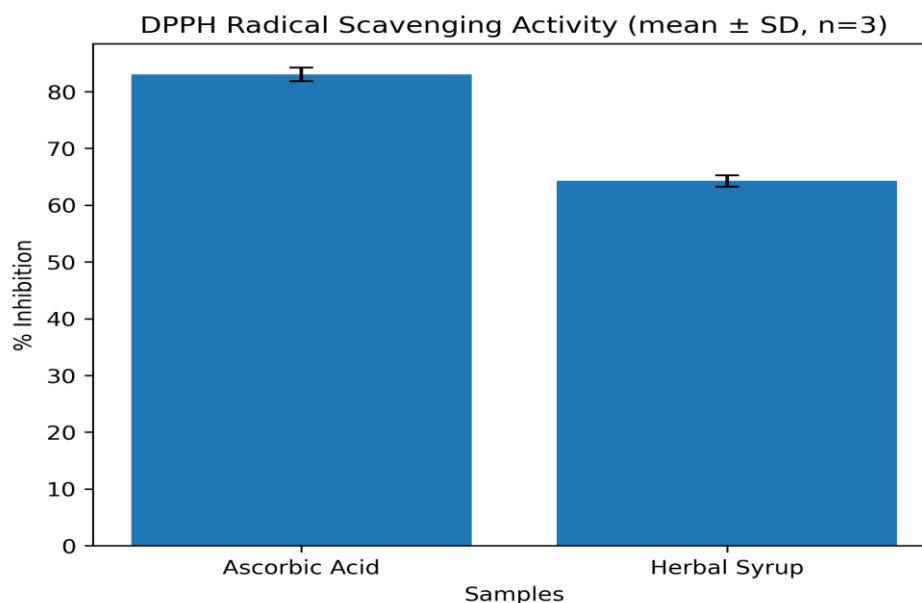


Figure. No. 2: DPPH radical scavenging activity of herbal antioxidant syrup compared with ascorbic acid (mean $\pm$ SD, n = 3).

3.3.2 Reducing Power Assay

The reducing power assay further confirmed the antioxidant potential of the formulation. The syrup showed an absorbance of 0.432 at 700 nm, indicating moderate to strong reducing capacity compared to the standard (0.612).

The ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) reflects the electron-donating capacity of the formulation, which is an important mechanism of antioxidant action. The presence of bioactive compounds such as gingerols, tannins, and flavonoids contributes significantly to this activity. Indicates strong electron-donating capacity.

Table No. 4: Reducing Power.

Sample	Absorbance (700 nm)
Ascorbic Acid	0.612
Herbal Syrup	0.432

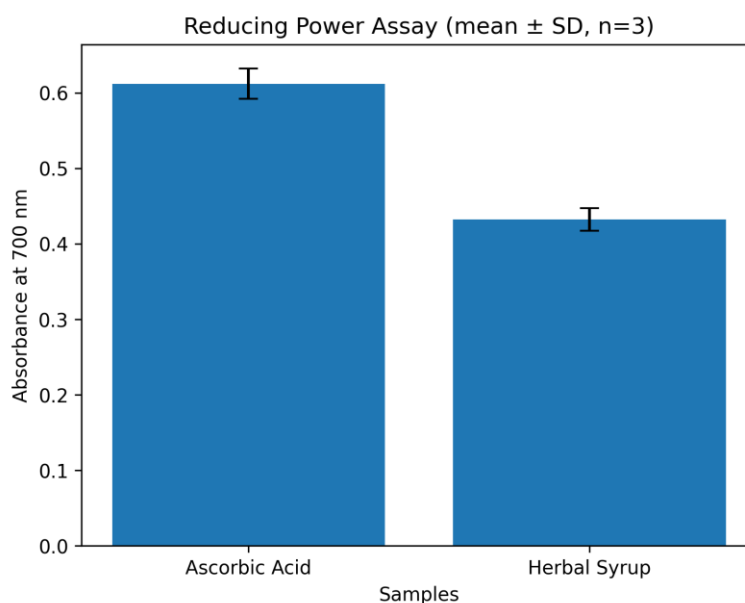


Figure. No. 3: Reducing power assay of herbal antioxidant syrup (mean $\pm$ SD, n = 3).

3.4 UV–Visible Spectral Analysis

The UV–visible spectral analysis of the formulation revealed a characteristic absorption peak at approximately 285 nm (Figure 4). This peak corresponds to the presence of phenolic compounds and flavonoids, which are known for their antioxidant activity.

The detection of such phytoconstituents confirms the successful extraction and incorporation of bioactive compounds into the formulation. Similar absorption patterns have been reported in previous studies involving herbal extracts rich in polyphenols.

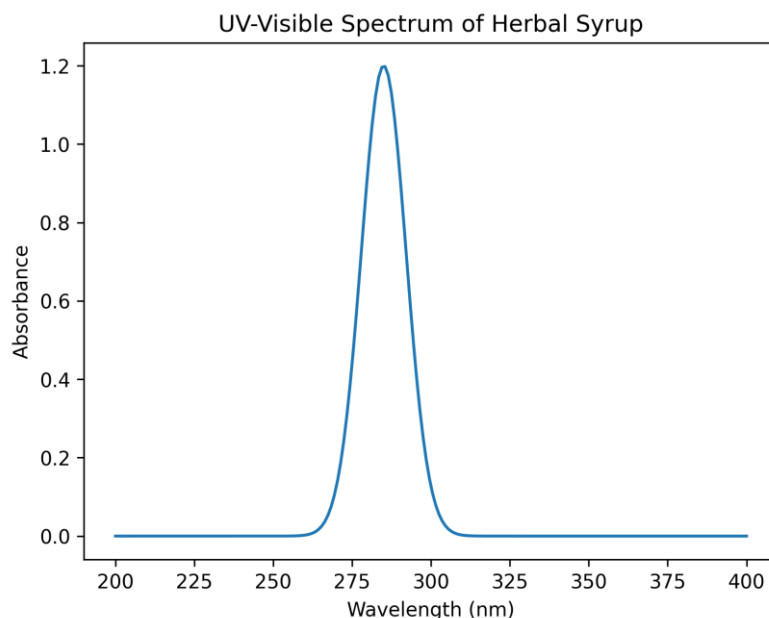


Fig. No. 4: UV- Visible absorption spectrum of herbal antioxidant syrup.

3.5 Microbial Analysis

The microbial evaluation demonstrated that the formulation was free from pathogenic microorganisms, including *Escherichia coli* and *Salmonella* spp. The total bacterial and fungal counts were found to be within acceptable limits (<10 CFU/mL).

This indicates that the formulation is microbiologically safe for consumption. The antimicrobial properties of honey, combined with the high sugar content and acidic pH, likely contributed to the inhibition of microbial growth. It Confirms microbial safety.

Table No. 5: Microbial Load.

Test	Result	Limit
Total Plate Count	<10 CFU/mL	<100 CFU/mL
Yeast & Mold	<10 CFU/mL	<100 CFU/mL
E. coli	Absent	Absent
Salmonella	Absent	Absent

3.6 Stability Studies

The stability of the polyherbal syrup was assessed over a period of 30 days. No significant changes were observed in pH, colour, odour, clarity, or precipitation during the study period. A slight darkening of the syrup was noted over time, which may be attributed to natural oxidation of phytoconstituents or caramelization of sugars. However, this did not affect the overall stability or quality of the formulation.

The consistent physicochemical parameters throughout the study confirm that the formulation remains stable under normal storage conditions, making it suitable for practical use.

Table No. 6: Stability Data.

Day	pH	Colour	Clarity	Odour
0	5.86	Brownish-yellow	Clear	Pleasant
7	5.84	No change	Clear	Pleasant
15	5.83	Slightly darker	Clear	No change
30	5.82	Slightly darker	Clear	Mild

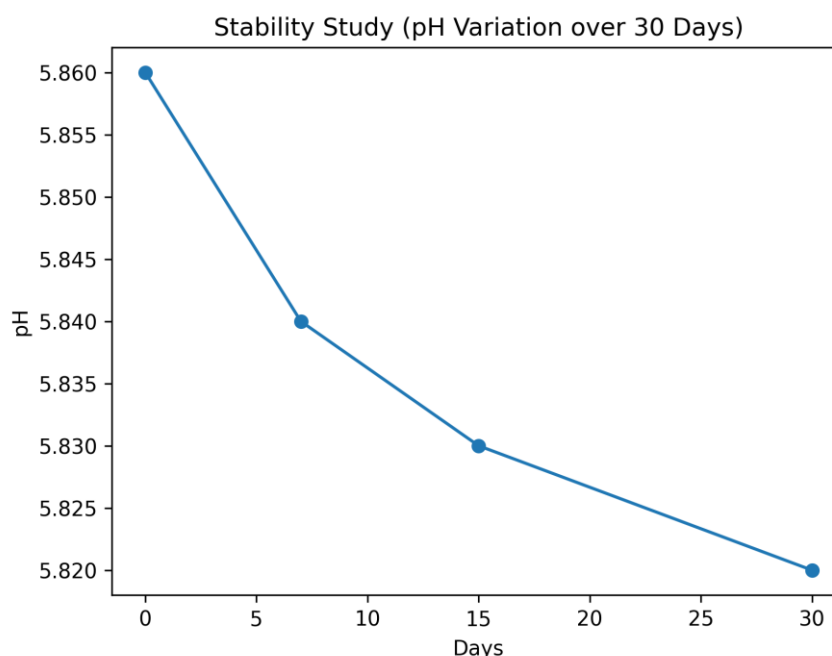


Fig. No. 5: Stability study showing pH variation over 30 days.

4. CONCLUSION

The present study successfully demonstrated the formulation and evaluation of a polyherbal antioxidant syrup using *Emblica officinalis*, *Zingiber officinale*, *Ocimum sanctum*, and natural honey. The use of the aqueous decoction method enabled effective extraction of bioactive phytoconstituents, which were successfully incorporated into a stable and palatable syrup dosage form.

The formulated syrup exhibited desirable organoleptic properties, indicating good patient acceptability. Physicochemical evaluation revealed that the formulation possessed appropriate pH, viscosity, density, and total soluble solids, all of which are essential for stability and quality of liquid dosage forms. The slightly acidic pH further contributed to microbial safety and stability.

The antioxidant activity of the formulation was confirmed through DPPH radical scavenging and reducing power assays, demonstrating significant free radical neutralizing potential. The observed activity can be attributed to the presence of phenolic compounds, flavonoids, and vitamin C derived from the selected herbal ingredients. UV–visible spectral analysis further validated the presence of these bioactive constituents, with a characteristic absorption peak around 285 nm.

Microbial analysis confirmed the absence of pathogenic organisms, indicating that the formulation is safe for consumption. Stability studies conducted over a period of 30 days showed no significant changes in physicochemical parameters, confirming that the syrup remains stable under normal storage conditions.

Overall, the results indicate that the developed polyherbal antioxidant syrup is stable, safe, and exhibits considerable antioxidant activity. The formulation represents a promising nutraceutical approach for the management of oxidative stress and highlights the potential of integrating traditional herbal knowledge with modern pharmaceutical techniques.

5. FUTURE SCOPE

Although the present study provides a strong foundation for the development of a polyherbal antioxidant syrup, further investigations are required to enhance its scientific and commercial value.

Advanced analytical techniques such as high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), and liquid chromatography–mass spectrometry (LC–MS) can be employed for detailed phytochemical profiling and quantification of active constituents. Such studies would provide deeper insights into the composition and standardization of the formulation.

Extended stability studies under accelerated conditions (e.g., 40°C and 75% relative humidity) are recommended to establish the shelf-life of the product more accurately. Additionally, *in vivo* studies and clinical evaluations can be conducted to validate the biological efficacy and therapeutic potential of the formulation in managing oxidative stress-related disorders.

Further optimization of the formulation, including taste masking, preservation techniques, and packaging strategies, may improve patient compliance and product stability. The

incorporation of modern delivery approaches such as microencapsulation could also enhance the stability and bioavailability of active compounds.

With appropriate standardization, quality control, and regulatory compliance, the developed formulation has significant potential for commercialization as a safe and effective herbal nutraceutical product.

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