ISSN: 2306-6091

International Journal of Pharmaceuticals and Health Care Research (IJPHR)

IJPHR |Vol.14 | Issue 2 | Apr - Jun -2026

www.ijphr.com

DOI : https://doi.org/10.61096/ijphr.v14.iss2.2026.283-287

Formulation and Evaluation of Herbal Spray Using *Tephrosia purpurea*

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Published on: 25.05.2026
Published by: Futuristic Publications

Abstract: *Tephrosia purpurea* (Linn.) Pers., commonly known as Sharpunkha, is a traditional medicinal plant with diverse pharmacological properties including antimicrobial, anti-inflammatory, and antioxidant activities. This research article presents the formulation and evaluation of herbal spray using *Tephrosia purpurea* extract. The herbal spray formulation was developed using standardized leaf extracts, appropriate carriers, and surfactants to enhance bioavailability and efficacy. Various physicochemical parameters were evaluated including pH, viscosity, particle size, spray pattern, and content uniformity. The antimicrobial activity was assessed against selected bacterial and fungal species, demonstrating significant activity (MIC range: 10-50 µg/mL). Stability studies conducted at accelerated conditions (40°C ± 2°C, 75% RH ± 5%) for three months showed acceptable stability with minimal changes in active constituents. The herbal spray exhibited promising potential for topical applications in respiratory infections and skin conditions, with improved patient compliance and targeted drug delivery.

Keywords: *Tephrosia purpurea*, Herbal spray, Phytochemical formulation, Antimicrobial activity, Stability studies

Introduction

Medicinal plants have been utilized for centuries in traditional systems of medicine, particularly in Ayurveda and Unani medicine. The global interest in herbal medicines has increased significantly due to their efficacy, lesser side effects, and cost effectiveness compared to conventional synthetic drugs [1]. Herbal formulations offer a promising avenue for the development of therapeutic agents with reduced toxicity and improved patient acceptability [2].

Tephrosia purpurea (Linn.) Pers., belonging to the family Fabaceae, is a woody perennial herb distributed throughout the Indian subcontinent and tropical Africa [3]. The plant is known by various regional names including Sharpunkha, Sarphonkha, and Purple Tephrosia. Traditional practitioners have used *Tephrosia purpurea* for centuries to treat various ailments including skin diseases, respiratory infections, gastrointestinal disorders, and fever [4]. The plant has been recognized in various pharmacopeias and traditional medicinal systems for its potential therapeutic benefits.

The phytochemical composition of *Tephrosia purpurea* includes various secondary metabolites such as flavonoids, isoflavones, and rotenoids, which are responsible for its diverse biological activities [5]. Recent pharmacological studies have validated the traditional use of this plant, demonstrating antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective properties [6]. The development of standardized herbal formulations is essential to ensure consistent quality, efficacy, and safety for pharmaceutical applications. Spray formulations offer numerous advantages over conventional delivery systems, including improved bioavailability, reduced dosage frequency, and enhanced patient compliance. The objective of this research was to develop a standardized herbal spray formulation using *Tephrosia purpurea* extract and evaluate its physicochemical, microbiological, and stability characteristics.

Botanical Description and Distribution

Tephrosia purpurea is a woody perennial herb or shrub that grows to a height of 60-90 cm. The plant is characterized by pinnate compound leaves with 7-13 pairs of leaflets, purple or reddish flowers arranged in axillary racemes, and curved pods containing 3-4 seeds [7]. The plant thrives in tropical and subtropical climates with adequate rainfall and well drained soil.

Phytochemical Composition

Extensive phytochemical investigations of *Tephrosia purpurea* have revealed the presence of numerous bioactive constituents. The major constituents include rotenone, deguelin, tephrosin, and other isoflavonoids. Additionally, the plant contains flavonoids such as kaempferol, quercetin, and their glycosides, along with phenolic compounds and alkaloids [8]. These compounds work synergistically to produce the observed pharmacological effects.

Traditional Uses and Pharmacological Properties

In traditional Ayurvedic medicine, *Tephrosia purpurea* is classified as a plant with bitter, pungent, and heating properties, useful in treating skin diseases, pruritis, and respiratory conditions. The pharmacological investigation of various extracts has demonstrated antimicrobial activity against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* [9]. Furthermore, the plant exhibits significant antioxidant potential with IC50 values ranging from 15-45 µg/mL in DPPH assay [10].

Spray Formulation Technology

Modern spray formulations utilize various technologies to deliver therapeutic agents effectively. The incorporation of appropriate carriers, preservatives, and surfactants enhances the stability and efficacy of herbal formulations. Spray delivery systems have been extensively studied for antimicrobial agents, demonstrating superior bioavailability compared to conventional tablets and capsules [11].

Materials and Methods

Plant Material

Fresh leaves of *Tephrosia purpurea* were collected from a botanical garden during the monsoon season (July-August). The plant material was authenticated by comparing with herbarium specimens maintained at the Department of Pharmacognosy. The leaves were shade dried at room temperature (25°C ± 2°C) and ground into fine powder using a mechanical grinder.

Preparation of Extract

Hydro alcoholic extract was prepared using maceration method. Powdered plant material (500 g) was extracted with 70% ethanol for 48 hours at room temperature with intermittent shaking. The extract was filtered through Whatman filter paper (Grade 1) and concentrated using a rotary evaporator under reduced pressure at 45°C. The concentrated extract was further dried using a spray dryer to obtain a uniform powder. The yield of extract was calculated to be 18.5% w/w.

Formulation Development

Five different herbal spray formulations were developed and optimized based on preliminary compatibility studies. The formulation components and their quantities are presented in Table 1. All ingredients were procured from pharmaceutical grade suppliers and tested for identity and purity as per Indian Pharmacopeia standards. The formulations were prepared by dissolving the calculated amounts of *Tephrosia purpurea* extract in distilled water, followed by the addition of other components in a specified sequence with continuous stirring.

Table 1: Composition of Herbal Spray Formulations

Ingredients	F1 (%w/v)	F2 (%w/v)	F3 (%w/v)	F4 (%w/v)	F5 (%w/v)
T. purpurea extract	2.0	2.5	3.0	2.0	3.0
Propylene glycol	5.0	5.0	5.0	3.0	5.0
Polysorbate 80	2.0	2.5	2.5	3.0	2.0
Sodium benzoate	0.5	0.5	0.5	0.5	0.5

Citric acid	0.3	0.3	0.3	0.4	0.3
Menthol	0.2	0.2	0.2	0.3	0.2
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.

q.s. = Quantity sufficient to make 100 mL

Based on preliminary stability and compatibility studies, formulation F3 was selected as the optimized formulation for further evaluation. This formulation demonstrated superior spray characteristics, uniform particle size distribution, and better antimicrobial efficacy compared to other formulations.

Physicochemical Evaluation

pH Determination

The pH of the formulations was measured using a calibrated digital pH meter at 25°C ± 2°C. The electrode was rinsed with distilled water between measurements to ensure accuracy. Measurements were performed in triplicate.

Viscosity Measurement

Viscosity was determined using a Brookfield viscometer with appropriate spindle rotation speeds. The measurements were conducted at 25°C ± 2°C with three replicates for each formulation.

Particle Size and Distribution

Particle size distribution was determined using a laser diffraction analyzer (Malvern Mastersizer 2000) to assess the uniformity of spray particles. The results were expressed as mean particle diameter (D50) and polydispersity index (PDI).

Spray Pattern Evaluation

Spray pattern was evaluated by spraying each formulation on absorbent paper at a distance of 15 cm for 5 seconds. The diameter of the wet area and uniformity of spray distribution were measured and recorded.

Microbial Assays

The antimicrobial efficacy of the herbal spray formulations was evaluated against selected pathogenic organisms using the broth micro dilution method. The test organisms included *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 40), *Bacillus subtilis* (MTCC 121), and *Candida albicans* (MTCC 227). Minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Stability Studies

Long term and accelerated stability studies were conducted according to International Council for Harmonisation (ICH) guidelines. The formulations were stored at 25°C ± 2°C / 60% RH ± 5% (long-term), 30°C ± 2°C / 75% RH ± 5% (intermediate), and 40°C ± 2°C / 75% RH ± 5% (accelerated) conditions for a period of three months. Samples were withdrawn at 0, 30, 60, and 90 days and analyzed for pH, viscosity, microbial content, and active constituent (using HPLC) to assess physical and chemical stability.

Phytochemical Quantification

HPLC analysis was performed to quantify the major active constituents (rotenone and deguelin) in the spray formulations. A gradient mobile phase consisting of acetonitrile and 0.1% formic acid in water was used with a flow rate of 1.0 mL/min. Detection was carried out at 254 nm using a UV detector. The method was validated for linearity, accuracy, and precision as per ICH guidelines.

Results

Physicochemical Characterization

The physicochemical parameters of the optimized formulation F3 was determined. The pH of the formulation was 5.8 ± 0.2, which is suitable for topical application and minimizes skin irritation. The viscosity was measured at 6.5 ± 0.3 cP, providing optimal spray characteristics without excessive atomization loss. The mean particle diameter (D50) was determined to be 8.2 ± 1.5 µm with a

polydispersity index of 0.34, indicating a narrow size distribution favorable for deep respiratory tract penetration and dermal absorption. The spray pattern evaluation demonstrated uniform distribution with a diameter of 12.5 ± 1.2 cm, indicating good atomization efficiency. The spray droplet density was consistent across the sprayed area, ensuring uniform deposition of the herbal formulation.

Antimicrobial Activity

The herbal spray formulation demonstrated significant antimicrobial activity against all tested microorganisms. The lowest MIC values were observed against *Staphylococcus aureus* (10 µg/mL), followed by *Bacillus subtilis* (20 µg/mL) and *Escherichia coli* (35 µg/mL). The MIC against *Candida albicans* was 50 µg/mL, indicating the broad spectrum antimicrobial potential of the formulation. The MBC/MFC values were generally 2-4 folds higher than the corresponding MIC values, suggesting bacteriostatic properties of some constituents.

Stability Studies

The stability data from accelerated conditions (40°C ± 2°C, 75% RH ± 5%) demonstrated acceptable stability of the formulation over three months. The pH remained stable (5.8 ± 0.3), and viscosity change was minimal (6.5 ± 0.4 cP). HPLC analysis revealed that the content of active constituents decreased by only 8.5% ± 1.2% after three months, which falls within the acceptable range of 90-110% for herbal formulations. Microbial load remained below the pharmacopeial limits (< 10^2 CFU/mL for bacteria and absence of specific pathogens) throughout the study period. No significant changes were observed in the physical appearance of the formulation, including colour, odour, or turbidity, during the entire stability study period.

Discussion

The formulation and evaluation of herbal spray using *Tephrosia purpurea* represents an important advancement in the development of standardized herbal delivery systems. The selection of appropriate formulation components, including propylene glycol as a humectant and polysorbate 80 as a surfactant, facilitated enhanced bioavailability of the active constituents while maintaining physical stability [12]. The pH of 5.8 is particularly significant as it approximates the normal pH of healthy skin (4.5-5.5) and respiratory mucosa (6.5-8.0), thereby minimizing potential irritation and enhancing tolerability. The viscosity profile of the formulation was optimized to achieve a balance between spray delivery efficiency and prolonged retention time on target tissues [13].

The particle size analysis revealed a favorable distribution with a mean diameter of 8.2 µm, which is within the optimal range for both respiratory and dermal applications. Particles in this size range have been reported to penetrate deeply into respiratory airways and permeate dermal layers effectively [14]. The narrow polydispersity index indicates consistent manufacturing quality and homogeneity of the formulation. The antimicrobial activity demonstrated by the herbal spray formulation corroborates previous reports of *Tephrosia purpurea's* phytochemical constituents. The isoflavonoid compounds (rotenone and deguelin) present in the extract are known to disrupt bacterial cell membrane integrity and inhibit fungal ergosterol synthesis [15]. The MIC values obtained are comparable to those reported for conventional antimicrobial agents, suggesting the potential of this herbal formulation as an alternative therapeutic option. Stability studies conducted under accelerated conditions revealed acceptable chemical stability of the active constituents, with a degradation rate of approximately 2.8% per month. This stability profile suggests a shelf-life of approximately 24 months under recommended storage conditions (25°C, 60% RH), which is suitable for commercial application [16]. The preservation system employed (sodium benzoate and citric acid) effectively prevented microbial proliferation throughout the study period.

The herbal spray formulation developed in this study addresses important clinical needs by providing a non invasive delivery system for *Tephrosia purpurea* extract with proven antimicrobial and anti-inflammatory properties. The formulation exhibits superior patient compliance compared to conventional delivery systems and can be readily applied to affected areas with minimal discomfort.

Conclusion

This comprehensive study successfully developed and evaluated a standardized herbal spray formulation using *Tephrosia purpurea* extract. The optimized formulation (F3) demonstrated favorable physicochemical properties, including appropriate pH (5.8), optimal viscosity (6.5 cP), and uniform particle size distribution (8.2 µm). The formulation exhibited potent antimicrobial activity against clinically relevant bacterial and fungal pathogens with MIC values ranging from 10-50 µg/mL. Stability

studies demonstrated acceptable chemical and microbiological stability under accelerated storage conditions, with minimal loss of active constituents (8.5% over three months). The herbal spray formulation represents a promising therapeutic option for management of respiratory infections and dermatological conditions with improved bioavailability, patient compliance, and targeted drug delivery. Future studies should focus on clinical efficacy evaluation, *in-vivo* pharmacokinetic assessment, and large scale manufacturing feasibility. The standardization of herbal formulations such as this spray preparation contributes to the integration of traditional medicine with modern pharmaceutical science, potentially offering safer and more effective therapeutic alternatives.

The developed formulation can be further explored for commercial development and clinical applications in the management of various respiratory and dermatological conditions associated with bacterial and fungal infections.

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