Development of a Novel Polyherbal Formulation for Augmenting Milk Production in Healthy Dairy Cows

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ABSTRACT

Aim: The study was designed to develop and standardize a novel polyherbal formulation (PHF) for augmenting milk production in healthy dairy cattle.

Materials and methods: Five raw plant drugs, viz., tubers of Asparagus racemosus Willd. (Shatavari), whole plant of Eclipta alba (L.) Hassk. (Bhringraj), seeds of Trigonella foenum-graecum L. (Mathika), fruits of Foeniculum vulgare Mill. (Mishrey), and Anethum sowa Roxb. ex Flem. (Shatapusha) were used to prepare hydroalcoholic extracts using the Soxhlet method. Three in-house batches of PHF were prepared and standardized as per Ayurvedic Pharmacopoeia of India (API) methods. Pharmacognostic authentication and chemical identification were done by macroscopic and microscopic studies, phytochemical screening, physicochemical analysis, and high performance thin layer chromatography (HPTLC) fingerprinting. The safety studies of galactagogue preparation were performed through heavy metals, microbial contamination, aflatoxins, and pesticide residue analysis.

Results: Organoleptic studies revealed that all the batches appeared as semisolid in nature, blackish-brown in color, with a pleasant odor and slight bitter taste. Phytochemical screening confirmed the presence of similar secondary metabolites in the different batches of both raw drugs and PHF. Physicochemical analysis and HPTLC fingerprints at different illuminations showed that all three batches were uniformly composed and complied the pharmacopeial limits. Results of safety parameters advocated that all the three batches were safe and complied as per the WHO and API guidelines.

Conclusion: The present work first claims the standardization of this unique, cost-effective, nonhormonal, Ayurvedic galactagogue in-house preparation, i.e., PHF for augmenting milk yield in dairy herd. It proves that all the three batches have similar characteristics and uniformly composed. It serves as a reference for identification and distinguishing the galactagogue herbs.

Keywords: Dairy, Galactagogue, High performance thin layer chromatography, Polyherbal formulation, Standardization.

Journal of Drug Research in Ayurvedic Sciences (2019): 10.5005/jdras-10059-0064

INTRODUCTION

India is one of the world’s largest milk-consuming countries with inflated milk demands. Many adverse factors, viz., unidentified disease conditions, lack of proper feed management, unhealthy nutrition, and poor medication, have badly impacted the milk yield of lactating dairy cattle and now, it has become a challenging issue in front of Indian dairy industry. Moreover, applications of harmful chemotherapeutic agents and synthetic hormones like recombinant bovine somatotropin and oxytocin for the purpose to increase the milk production and getting more profits are reported to cause several serious health issues like hyperthyroidism, cystic ovary, and reproductive failures in dairy animals.¹⁻² On the other hand, the uses of nonhormonal polyherbal galactagogue do not cause such deleterious effects on cows, goat, and buffaloes.³⁻⁷ An effective feed management along with herbal agents potentiated milk production in the dairy herd as per the veterinary data. Marketed phytophagelactagogues are cost-effective than the synthetic hormonal galactagogues. Polyherbal formulations (PHFs) are the compositions of several varieties of herbs in different dosage forms; they are purely herbal, nonhormonal, safe, and environment friendly, without showing any side effects and upsetting total health of cattle. Ancient literature such as Charaka and Susruta Sanhita described some indigenous medicinal species like Asparagus racemosus Willd., etc., for inducing milk. Ayurvedic galactagogues were studied to be safe, capable to induce therapeutic actions in domestic animals at fixed doses,⁸ and used as nutritive food supplements in dairy herds.⁹⁻¹¹ They assist in the maintenance and augmentation of dairy cows’ milk production. Many traditional culture and folk claimed that they stimulate milk yield not only in dairy cattle but also in humans.¹² They stimulate, maintain, and enhance the milk yield through physiological actions.¹³ Supplementation of herbal galactagogue showed a significant hike, i.e., 14.24% in total milk yield in Surti buffaloes without affecting milk composition.¹⁴ Payapro, a herbal galactagogue, increased cow’s milk yield by 31.10%. Similarly, dairy cows fede with Lectovet, a PHF, not only increased the milk output but also enhanced the percentage of milk fat.¹⁵ The comparative study was designed to develop and standardize a novel polyherbal formulation (PHF) for augmenting milk production in healthy dairy cattle.
evaluation of feeding impact of two commercialized PHFs, i.e., Ruchamax and Payapro, in lactating cows revealed that the average milk yield was highest, i.e., 11.8 L/day, in Ruchamax-supplemented cows while moderate volume of milk, i.e., 9.3 L/day, was observed in Payapro-supplemented cows as compared to 7.1 L/day in control animals.35 Some of the Indian and exotic herbal species have been used as galactagogues in the domain of veterinary medicine such as Trigonella foenum-graecum L. (Fenugreek), Foeniculum vulgare Mill. (Fennel), Rubus idaeus L. (Raspberry leaf/Red Raspberry), Medicago sativa L. (Alfalfa), Withania somnifera (L.) Dunal (Mother’s milk tea, Ashwagandha), Asparagus racemosus Willd. (Shatavari), Galega officinalis L. (Goat’s rue), and Pimpinella anisum L. (Anise),3,16 which were employed either as a single drug itself or ingredients of some commercialized Ayurvedic preparations.17–19 Many more plant species like Leptadenia reticulata (Retz.) Wight and Arn., Nigella sativa L., including Asparagus racemosus Willd. were identified for their galactagogic properties.20 Moreover, some Nigerian herbs, viz., Vitex doniana Sweet, Kigelia Africana (Lam.) Benth., Allophylus afric anus P. Beauv., Alternanthera sessilis (L.) R. Br. ex DC (sissile joyweed), Secamone afzelii (Roem. and Schult.) K. Schum., Calotropis procera (Aiton) Dryand., Adansonia digitata L., Lecaniodiscus cupanioides Planch. ex Benth., Launaea taxacifolia (Willd.) Anim ex C. Jeffrey, etc., were reported to increase milk yield effectively.21 Globally, Trigonella foenum-graecum L. is a potent galactagogue that is commonly recommended for lactating mothers to increase the milk secretion.22,23 Chloroform extracts of Trigonella foenum-graecum L. seeds produced a mastogenic effect that stimulated the growth of mammary glands and induced estrogenic actions.21 In vitro assays of fenugreek seeds suggested some estrogen-like compounds embedded in this plant, i.e., phytoestrogen that stimulated pS2 (estrogen-induced protein) expression; likewise, another phytoestrogen, i.e., diosgenin was responsible to increase the milk flow without any toxic effects.11 Its seeds also influenced the maintenance of lactation in ruminants, i.e., buffaloes, goat, etc.24 Trigonella foenum-graecum L. supplemented to goat’s diet increased the milk production, i.e., 13% hike through growth hormone (PRL) stimulation.25 Some E2-like molecules, such as anethole and estragole, were secondly identified for galactagogic properties because of their structural resemblance to dopamine and inhibiting the antiserotory action of dopamine on prolactin resulted high milk secretion likewise; another constituent of Foeniculum vulgare Mill. named anol has been reported to increase the growth of mammary glands in immature female rabbits.26 Asparagus racemosus Willd., an Indian milk-enhancer herb for mammals, had been described in several classical texts such as Charaka and Susruta Samhita. Alcoholic extracts of this plant induced estrogenic effects in mammary glands of rats with increased milk yield26 and also played as an active constituent of some important galactagogue formulation like Ricalex. Some clinical trials have established a fact that Asparagus racemosus Willd. increased the PRL level28 and produced a lactogenic effect when supplemented in rat’s food.29 The galactagogue effect of roots of this herb was observed to be significant in human buffaloes.30 The phytochemical investigation of Asparagus racemosus Willd. reported some bioactive compounds, i.e., Shatavarins I–IV, a steroidal saponins31 that are responsible for producing estrogenic activity.28 A hypothetical opinion established the fact that Asparagus racemosus Willd. causes enlargement of the mammary gland through corticoids or PRL actions.27 Some Ayurvedic formulations were reported to enhance the milk production in lactating cattle as well as in humans. They significantly worked and were found safe for human breastfeeding medicines and veterinary dairy pharmaceuticals.32–36 The efficacy of PHF was preclinically evaluated in rats and further applied to stimulate the milk yield in lactating dairy animals. In this direction, plant drugs were utilized without adopting an appropriate uniform chemical standardization.37 However, for ensuring the safety, efficacy, quality, and authenticity of herbal and veterinary pharmaceutical, the use of standardized materials and preparations is essential as per the WHO guidelines.38,39 Now it has become acceptance criteria for phytogalactagogue so as to compensate the surging milk demand globally at a validated scientific platform. Previous studies reported that various regulatory bodies worked in this way to ensure that Ayurvedic drugs are prepared strictly in accordance with prescribed pharmacopeial standards so that consumers may get safe, pure, potent, and effective herbal medicines. Standardization of such polyherbal medicines provided a set of standards as the qualitative and quantitative values that guide to assurance the quality, efficacy, safety, and reproducibility. It plays a fundamental role in guaranteeing the quality and stability of herbal formulations and works as a regulatory tool in the quality control of herbs.40,41 Some investigations also revealed that due to the lack of standard data for the identification and authentication, quality control measures for PHF could not be established. Standardization of Ayurvedic milk inducing herbs determines their mechanisms of action and establishment of therapeutic dosage. It has vast importance in terms of their acceptability and quality enhancement for supporting the various interdisciplinary investigations. Commercially quality compliance through pharmacopeial standardization of such formulations has wide importance to accelerate market, gaining profits, and maintaining the goodwill of herbs internationally. Not only this, standardization of PHF scientifically supports various traditional claims through clinical studies on different animal models.39,42,43 In view of such objective, the present work was designed to formulate and standardized a novel, nonhormonal, and potent polyherbal galactagogue as PHF having milk-yielding properties consisting hydroalcoholic extract of five herbs, i.e., tubers of Asparagus racemosus Willd. (Shatavari), whole plant of Eclipta alba (L.) Hassk. (Bhringraj), seeds of Trigonella foenum-graecum L. (Methika), fruits of Foeniculum vulgare Mill. (Misreyaa), and Anethum sowa Roxb. ex Fleming (Shaptapushpa) for augmenting milk production without the side effects unlike synthetic hormonal galactagogue in healthy dairy cows, which may help in making up the high milk demand in India. So far, no one has established this unique combination of herbal gougue with standardization procedures. The study was designed including four criteria, namely, identification of the plant material by pharmacognostical characters, phytochemical screening of secondary metabolites, physicochemical analysis, and chromatographic profiling.

**Materials and Methods**

**Collection of Plant Materials**

Plant materials, i.e., tubers of Asparagus racemosus Willd. (Shatavari), whole plant of Eclipta alba (L.) Hassk. (Bhringraj), seeds of Trigonella foenum-graecum L. (Methika), fruits of Foeniculum vulgare Mill. (Misreyaa), and Anethum sowa Roxb. ex Fleming (Shaptapushpa) fruits were collected from local markets and their natural habitats, which were free from pollution. The raw drugs were authenticated by the research officer (pharmacognosy), RARIOED, Lucknow, U.P. (previous name NVARI, under CCRAS, New Delhi, India).
Solvents and Reagents
All solvents, reagents, and chemicals utilized for experiment were of analytical grade. Double distilled water was utilized throughout the analysis. For high performance thin layer chromatography (HPTLC) analysis, aluminum TLC plate, E-Merck, thickness of 0.2 mm, precoated Silica Gel 60 with fluorescent indicator F254 used as stationary phase.

Instrumentation
The HPTLC analysis was performed using CAMAG HPTLC assembly (Muttenez, Switzerland) attached with a semiautomatic sample applicator (spray-on technique) Linomat IV, Hamilton (Reno, Nevada, USA) Syringe (100 μL), twin trough development chamber, lighting system CAMAG TLC visualizer Reprostar 3 integrated into win CATS software of version 1.4.2. The Olympus CH20iBIMF microscope (trinocular with camera attachment) was used in pharmacognosy of the plant material. Photomicrographs in both the cases were taken using SONY digital camera, model no. DSC-350.

Identification and Authentication of Plant Materials
The collected plant materials were identified and authenticated taxonomically by detailed pharmacognostical studies including correct taxonomic identification and their parts’ macroscopic and microscopic characterizations.

Preparation of Hydroalcoholic Extract (50:50)
The raw plant materials were cleaned, dried, and then prepared as coarse powder. The 50% ethanol was added to the raw materials in a ratio 8:1, mixed thoroughly, and the mixture was macerated for 4 hours. Individual hydroalcoholic (ethanol:water, 50:50) extracts were prepared at 35–40°C and were evaporated in petri plates and rotary evaporator on water bath maintained at 60°C. The extract was scrapped and packed accordingly. The percentage yields of the extracts according to the weight were calculated.

Preparation of PHF
Hydroalcoholic extracts of each plant extract was mixed in the equal proportion to prepare the three batches of finished PHF.

Standardization of Crude Plant Ingredients and PHF
The well-authenticated plant materials were chemically standardized through phytochemical and physicochemical standard (API) evaluation methods. The organoleptic characters of sample extracts of individual drugs and the formulation were tested on the basis of their physical properties. The sensory perceptions were determined by the physical examination of color, odor, taste, and clarity of raw drugs as well as the PHF. Preliminary phytochemical screening was carried out using standard confirmatory tests to detect the presence of various categories of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, reducing sugars, and phenols. The physicochemical analysis of sample extracts was performed to determine chemical parameters like pH value, total ash, acid-insoluble ash, loss on drying, water-soluble extractive, alcohol-soluble extractive, and TLC profiles.

Optimization of Mobile Phase
The mobile phase were optimized with distinct combinations of solvent systems in contrasting ratios under standard chromatographic conditions with aim to get best separation and resolution of different samples tracks.

HPTLC Fingerprinting
The sample solution of PHF each 10 μL was applied in the form of bands indicated as tracks 1, 2, and 3 in Figure 1 on an E Merck aluminum plate that was precoated with silica gel 60 F254 of 0.2 mm thickness through a HPTLC Linomat IV semiautomatic applicator. The plate was developed in an optimized mobile phase, i.e., ethyl acetate:isopropyl alcohol:water, 65:25:10 (v/v/v), in a CAMAG twin trough glass chamber and dried in the oven for 5 minutes. The plate was visualized under UV 254 nm, 366 nm, and images were taken through the software-supported system. Further, the plate was post-derivatized in ASR through the dipping method, dried, and photodocumented.

Results
The collected plant materials for batches 1, 2, and 3 were identified and authenticated taxonomically through the standard pharmacognostical tools; this included correct taxonomic identification of plants species as well as macroscopic and microscopic characterization of official parts.

Macroscopy
Five raw drugs were subjected to macroscopical examination for the identification of shape, size, color, texture, and organoleptic characters as per the pharmacopeial standard method. Tubers of Asparagus racemosus Willd. are borne in a compact bunch, fleshy, and spindle-shaped. They are silvery white or light ash-colored externally and white internally, more or less smooth when fresh, longitudinal and wrinkles when dry. They have no well-marked odor but sweet with bitter in taste (Fig. 2A). The whole plant of Eclipta alba (L.) Hassk. is herbaceous annual, 30–50 cm high, erect, and branched. It has well-developed roots associated with a number of secondary branches arising from the main root. The stem is herbaceous, branched, and cylindrical. Leaves of the plant are 2.2–8.5 cm long and 1.2–2.3 cm wide (Fig. 2B). Seeds of Trigonella foenum-graecum L. are oblong, 0.2–0.5 cm long, and 0.15–0.35 cm broad. Seeds become mucilaginous when soaked in water, odor is pleasant, and have bitter taste (Fig. 2C). Seeds of Foeniculum vulgare Mill. are about 6 mm long, green, and beaked (Fig. 2D). Fruits of Anethum Sowa Roxb. ex Fleming are dark brown, often stalk attached, broadly oval, and compressed dorsally. Mericarps are 4 mm long, 2–3 mm broad and 1 mm thick, glabrous, traversed from the base to apex, and odor is faintly aromatic and warm with slightly sharp taste (Fig. 2E).

Microscopy
The microscopic analysis of crude drugs of PHF included the examination of size, shape, and relative position of different cells and tissues as well as the chemical nature of the cell walls and also incorporated the form and nature of the cell contents. Tuber of Asparagus racemosus Willd. has three anatomical zones: periderm, cortex/stele, and pith. The cells are thin walled, submersed, are radially oblique and oblong (Fig. 3A). The mature root of Eclipta alba (L.) Hassk. shows poorly developed cork, consisting of three to five rows of thin-walled, tangentially elongated cells, while the secondary cortex consists of outer one or two rows of tangentially elongated or rounded cells with air cavities, inner secondary cortex of tangentially elongated to irregular shaped, parenchymatous cells with conspicuous air cavities (Fig. 3B),
The transverse section of the leaf through midrib shows both upper and lower single-layered epidermis, externally covered with cuticle, and a few epidermal cells elongated outward to form uniseriate hairs; the epidermis is followed by the cortex (Fig. 3C). The mature stem has single-layered epidermis, externally covered with cuticle, few epidermal cells elongate to form characteristic nonglandular trichomes, the cork where formed, poorly developed consisting of rectangular cells, secondary cortex composed of large, rounded, or irregular-shaped parenchymatous cells (Fig. 3D). The seed of *Trigonella foenum-graecum* L. showed a layer of thick-walled, columnar palisade, covered externally with thick cuticle; cells are flat at base, mostly pointed but a few flattened at apex, supported internally by a tangentially wide bearer cells having radial rib-like thickenings; followed by four to five layers of tangentially elongated, thin-walled, parenchymatous cells (Fig. 3E). Transverse sections of fruits of *Foeniculum vulgare* Mill. show pericarp with outer epidermis of quadrangular to polygonal cells with smooth cuticle and a few stomata (Fig. 3F). The pericarp of *Anethum sowa* Roxb. ex Fleming fruits shows epidermis of polygonal tabular cells having thick outer wall and striated cuticle; mesocarp, parenchymatous, some cells lignified and show reticulate thickening; the endocarp consists of tabular cells sometimes with
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sinuous anticlinal walls (Fig. 3G). Botanical description and macro and microscopical characters of individual raw drugs of batches 2 and 3 were found similar to batch 1.

Percentage Yield of Extracts from Crude Drug
The percentage yield from the extract of seeds of *Trigonella foenum-graecum* L. for batches 1, 2, and 3 was obtained to be 12%, 16.8% and, 12.8%, respectively. The percentage yields from the fruit extract of *Foeniculum vulgare* Mill. of batches 1, 2, and 3 were calculated to be 15.2%, 18%, and 16.4%, respectively, while from the tuber extract of *Asparagus racemosus* Willd. percentage yields were 36%, 40%, and 44%, for batches 1, 2, and 3 respectively. The percentage yields of *Eclipta alba* (L.) Hassk. whole plant extract for batches 1, 2, and 3 were obtained to be 20%, 26%, 20.8%, respectively. From the fruit extracts of *Anethum sowa* Roxb. ex Flem for batches 1, 2, and 3, the percentage yields were found to be 18%, 18%, 17.2%, respectively.

Organoleptic Studies
Determination of organoleptic characters for PHF’s batches 1, 2, and 3 indicated that they appeared as semisolid in nature, blackish-brown in color, and having a pleasant odor with slight bitter taste.

Chemical Standardization of PHF
The physicochemical study of all the three batches of PHF as presented in Table 1 indicating that total ash and acid-insoluble ash were calculated in the range of 7.28–7.35% (w/w) and 0.28–0.36% (w/w), respectively. pH (10% aqueous solution) value was observed in range of 5.48–5.76. Presence of almost similar secondary metabolites found in all the batches of PHF indicated that three batches were observed in the close proximity.

Analysis of batches 1, 2, and 3 for the safety parameters like heavy metals (Pb, Cd, As, Hg), microbial contamination, pesticide residue analysis, and aflatoxins as given in Table 2 indicates that all the batches of formulation are safe and complied as per the WHO and API guidelines. Phytochemical screening confirmed the presence of various categories of phytoconstituents, viz., tannins, steroid, flavonoids, alkaloids, coumarins, quinone, saponins, carbohydrates, phenolics, furanoids, amino acids in batches 1, 2, and 3 of PHF as shown in Table 3 through standard phytochemical tests.

The HPTLC profile of the three batches of this formulation (Fig. 3) indicates seven prominent bands at RF 0.21 (black), 0.29 (black), 0.40 (black), 0.50 (black), 0.59 (black), 0.73 (black), and 0.86 (black) under UV 254 nm; nine bands at RF 0.21 (florescent blue), 0.30 (blue), 0.49 (blue), 0.55 (dark purple), 0.62 (blue), 0.66 (florescent blue), 0.78 (blue), 0.84 (blue), and 0.89 (florescent blue) under UV 366 nm; and seven bands at RF 0.10 (green), 0.19 (green), 0.30 (green), 0.49 (blue), 0.62 (pink), 0.71 (green), and 0.80 (dark purple) under white light after derivatization with the anisaldehyde-sulphuric acid reagent (ASR). These revealed that all the three batches are qualitative similar in respect of phytoconstituents.

Discussion
Plant-based galactogouges emerge as substituent of synthetic hormonal milk enhancer to compensate surging milk demands at a global scale. Phytogalactagogues are in high demand because they do not produce any side effects, enhance milk secretion efficiently, and work as high nutritive supplements with immense therapeutic potentials. Hence, standardization of PHF is a vital step that helps to establish uniformity, international acceptability, effective sale, and...
### Table 1: Physicochemical and safety parameters of PHF’s ingredients of batches 1, 2, and 3

| Parameters                              | Batches          | E. alba | F. vulgare | A. racemosus | A. sowa | T. foenum |
|-----------------------------------------|------------------|---------|------------|--------------|---------|-----------|
| Ash (%)                                 |                  | 21.61   | 15.30      | 21.03        | 8.25    | 9.32      | 9.03      | 10.84     | 3.54      | 3.09      | 3.99      |
| Acid-insoluble ash (%)                  |                  | 7.45    | 4.88       | 0.13         | 2.09    | 0.42      | 0.45      | 0.48      | 0.57      | 1.18      | 0.99      | 0.14      | 0.50      | 0.49      |
| LOD (%)                                 |                  | 14.42   | 7.83       | 6.70         | 8.44    | 7.93      | 8.00      | 13.54     | 8.27      | 8.22      | 8.92      | 4.02      | 10.55     | 9.41      | 6.82      | 9.56      |
| Water-soluble extractive values (%)     |                  | 13.65   | 15.38      | 15.33        | 30.92   | 15.22     | 13.66     | 70.08     | 78.8      | 83.25     | 15.49     | 15.78     | 15.76     | 22.91     | 27.1      | 24.2      |
| Alcohol-soluble extractive value (%)    |                  | 2.94    | 5.48       | 5.34         | 13.79   | 4.40      | 5.06      | 4.15      | 10.1      | 11.36     | 5.36      | 4.44      | 4.71      | 11.99     | 7.29      | 7.00      |
| pH (10% aq. solution)                   |                  | 6.93    | 6.77       | 6.73         | 5.60    | 5.61      | 5.34      | 5.74      | 5.43      | 5.67      | 5.70      | 5.86      | 5.83      | 4.94      | 4.8       | 5.0       |
| Microbiological analysis                |                  |         |            |              |         |           |           |           |           |           |           |           |           |           |           |           |
| Total viable aerobic count (cfu/g)      |                  | BDL     | BDL        | BDL          | 2.350   | 2.350     | 2.350     | 48.500    | 48.500    | 48.500    | 250       | 250       | 250       | 4.950     | 4.950     | 4.950     |
| Total Enterobacteriaceae (g)            |                  | Ab.     | Ab.        | Ab.          | Ab.     | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       |
| Total fungal count (cfu/g)              |                  | 3800    | <10        | <10          | <10     | <10       | <10       | <10       | <10       | <10       | <10       | <10       | <10       | <10       | <10       |
| Test for specific pathogen (/g)         |                  |         |            |              |         |           |           |           |           |           |           |           |           |           |           |
| E. coli, Salmonella spp.,               |                  | Ab.     | Ab          | Ab           | Ab.     | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       | Ab.       |
| Staphylococcus aureus,                  |                  |         |            |              |         |           |           |           |           |           |           |           |           |           |           |
| Pseudomonas aeruginosa                  |                  |         |            |              |         |           |           |           |           |           |           |           |           |           |           |
| Aflatoxins (by ELISA), ppb              |                  |         |            |              |         |           |           |           |           |           |           |           |           |           |           |
| B1                                      |                  | 2.60    | 2.60       | 2.60         | ND      | ND        | ND        | ND        | ND        | 2.99      | 2.99      | 2.99      | 2.19      | 2.19      | 2.19      |
| B2                                      |                  | 2.59    | 2.59       | 2.59         | ND      | ND        | ND        | ND        | ND        | 2.99      | 2.99      | 2.99      | 2.19      | 2.19      | 2.19      |
| G1                                      |                  | 1.77    | 1.77       | 1.77         | ND      | ND        | ND        | ND        | ND        | 1.99      | 1.99      | 1.99      | 1.46      | 1.46      | 1.46      |
| G2                                      |                  | 1.73    | 1.73       | 1.73         | ND      | ND        | ND        | ND        | ND        | 1.99      | 1.99      | 1.99      | 1.46      | 1.46      | 1.46      |
| Pesticide residue                       |                  | ND      | ND         | ND           | ND      | ND        | ND        | ND        | ND        | ND        | ND        | ND        | ND        | ND        | ND        | ND        |
The various traditional claims and previous phytogalactagogues research are utilized in this work for the development of novel PHFs in the veterinary pharmaceuticals as well as dairy farming with fixed aim to induce milk secretion in dairy cattle and other mammals of our interest. This study proposed its novelty in terms of pharmacognostical and chemical examination of three batches of five raw drugs, their hydroalcoholic extracts, along with PHFs for their standardization for the purpose of augmenting the milk production in healthy dairy cows. The study first applied standard botanical and pharmacognostical tools for the purpose to identify and authenticate plant materials. Assessment of sensory, macroscopic, as well as microscopic attributes is the initial examination part to authenticate the identity and purity of plant drugs before further analysis is undertaken as per the WHO recommendations. Powder microscopy through histological characterization of five raw drugs of PHF is mandatory for the correct and clear identification of powdered drug samples because it serves as diagnostic parameters. It is useful for the observations of the internal structure, constitution, and inclusions of plant cells.

### Table 2: Physicochemical and safety parameters of PHF of batches 1, 2, and 3

| Parameters               | PHF batch 1 | PHF batch 2 | PHF batch 3 |
|--------------------------|-------------|-------------|-------------|
| Ash (%)                  | 7.28        | 7.53        | 7.35        |
| Acid-insoluble ash (%)   | 0.28        | 0.26        | 0.36        |
| pH (10%aq. solution)     | 5.48        | 5.55        | 5.76        |
| Heavy/toxic metals       |             |             |             |
| Lead (ppm)               | 3.12        | 3.59        | 3.32        |
| Arsenic (ppm)            | 1.01        | 1.00        | 1.01        |
| Mercury (ppm)            | 0.25        | 0.24        | 0.26        |
| Cadmium (ppm)            | 0.03        | 0.03        | 0.04        |
| Microbiological analysis |             |             |             |
| Total viable aerobic count (cfu/g) | 375 | 560 | 330 |
| Total Enterobacteriaceae (g)       | Absent      | Absent      | Absent      |
| Total fungal count (cfu/g)         | Less than 10 | Less than 10 | Less than 10 |
| Test for specific pathogen (/g) E. coli, Salmonella spp., Staphylococcus aureus, Pseudomonas aeruginosa | Absent | Absent | Absent |
| Afatoxins (by ELISA) B1, B2, G1, G2 | ND | ND | ND |
| Pesticide residue            | ND | ND | ND |

upgrade the quality level of Ayurvedic galactagogues. Majority of commercialized herbal galactagogues have not been thoroughly standardized as per pharmacopoeial standards and evaluated scientifically. So, standardization of polyherbal galactagogue preparations along with their raw ingredients is quite essential in order to maintain the quality, efficacy, and optimizing the high milk productivity in dairy cattle for better milk profits. Under this subject, detection of microbial contamination, foreign matters adulteration in plant materials, and toxicity of herbal extracts were reported to be important segments of chemical standardization as far as quality, efficacy, and safety of herbal galactagogues are concerned. The present work scientifically favors various indigenous knowledge in the form botanicals in applications as Ayurvedic galactagogues. The various traditional claims and previous phytochemoalactagogues research are utilized in this work for the development of novel PHFs in the veterinary pharmaceuticals as well as dairy farming with fixed aim to induce milk secretion in dairy cattle and other mammals of our interest. This study proposed its novelty in terms of pharmacognostical and chemical examination of three batches of five raw drugs, their hydroalcoholic extracts, along with PHFs for their standardization for the purpose of augmenting the milk production in healthy dairy cows. The study first applied standard botanical and pharmacognostical tools for the purpose to identify and authenticate plant materials. Assessment of sensory, macroscopic, as well as microscopic attributes is the initial examination part to authenticate the identity and purity of plant drugs before further analysis is undertaken as per the WHO recommendations. Powder microscopy through histological characterization of five raw drugs of PHF is mandatory for the correct and clear identification of powdered drug samples because it serves as diagnostic parameters. It is useful for the observations of the internal structure, constitution, and inclusions of plant cells.
It is essential for the detection of contaminants of formulation and useful for finding the authenticity and quality of materials used in PHF. The results of botanical standardization showed that all three batches of the individual plant ingredients have shown similarity for different botanical characteristics. Macro-morphological and microscopic profiles were established for rapid identification and the quality control parameters of the different parts of raw drug ingredients of PHF. The tested organoleptic characters of crude drugs may be set as characteristics for the initial identifications. Raw ingredients, their mixture, and their hydroalcoholic extracts (50:50) along with PHF were qualitatively as well as quantitatively evaluated through physicochemical parameters. Chemical standardization was carried out to authenticate the three batches of in-house preparation. Phytochemistry plays a prime role in authentication, identification, and quality evaluation of raw drugs along with PHF. Phytoestrogens, steroidal saponins, and glycosidized molecules have reported to produce estrogenic and milk-stimulating actions. Therefore, phytochemical screening is foremost to confirm the presence of such categories of phytocompounds and may become one of the basis for screening botanicals selection for galactagogue properties. Due to this reason, phytochemical screening were carried out to detect the presence of different secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, etc. In this subject, some of the bioactive markers reported to have high milk-yielding properties were isolated and identified, namely, Diosgeni, Shatavari (I–IV), Anethole and Estragole in Trigonella foenum-graecum L., Asparagus racemosus Willd., Trigonella foenum-graecum Mill., and Pimpinella anisum L., respectively, as botanical galactagogues. Such investigations contributed to explore the use of the botanicals as herbal galactagogues as well as the applications in the development of Ayurvedic milk-yielding formulations for veterinary, dairy, and human consumptions. Phytochemical studies in Anethum sowa Roxb. ex Fleming detected tannin, flavonoids, coumarins, phenol, carbohydrates, and saponin in all the three batches. While steroids, acid, quinine, amino acid, protein, and furanoids were absent in batches 1, 2, and 3. Eclipta alba (L.) Hassk., known for its curative properties, is used as analgesic, antibacterial, antihelmentic, antihyperglycemic, antioxidant, and immunomodulator agent and also considered a good rejuvenator. Most of the phytoconstituents like tannins, flavonoids, coumarins, saponin, and phenol were confirmed in all the three batches of E. alba (L.). Steroids, alkaloid, quinines, carbohydrates, protein, amino acids, and furanoids were not observed in all the batches of Eclipta alba (L.) Hassk. In this sequence, another chief constituent of PHF, i.e., Asparagus racemosus Willd. that is known for the galactagogue properties beside it, has been recognized for the treatment of impotency and as a rejuvenative tonic for females. Moreover, it showed antibacterial, antioxidant, and anxiolytic properties. It was also studied for increasing milk secretion and yield. For this Asparagus racemosus Willd. was tested phytochemically, which revealed presence of tannin, steroids, flavonoids (by LET), coumarins, quinone, saponin, carbohydrate, phenol (by LET), amino acid, proteins, and furanoids in the tubers of Asparagus racemosus Willd. Quinine, amino acid, and protein were found in low concentrations in the tuber of Asparagus racemosus Willd. The important phytochemicals, namely, flavonoids and alkaloids that chiefly induce milk secretion, were not found in its three batches of PHF. Similarly, in Foeniculum vulgare Mill., all the phytochemicals as shown in Table 3 were present. Likewise, Trigonella foenum-graecum L. was tested positively for tannin, flavonoids, alkaloid, coumarins, saponin, carbohydrate phenol, amino acid, protein, and furanoids in all the three batches.

The quantitative tests method, i.e., physicochemical analysis, is an essential part of standardization to set the pharmacopoeial standards of PHF. Almost similar results of all the three batches for these parameters indicate that all the three batches of PHF are found in close proximity. Result of safety parameter of PHF showed that all the batches are safe as per the WHO and API guidelines. Moreover, the outcomes of aflatoxins, microbial load, and pesticidal residue analysis help to draw an inference that PHF with its ingredients are free from chemicals, toxins, and safe for consumption. Results of phytochemical screening of PHF with its ingredients shown in Table 1 revealed the presence of various secondary metabolites that are responsible for galactagogue function, medicinal and nutritive properties as literature per the past studies. It also showed that tannins, coumarins, saponins, carbohydrates, phenolics, and furanoids were present in higher concentration. The study confirmed the presence of analogous phytoconstituents in its five raw ingredients, which proved the presence of same raw ingredients in PHF 1, 2, and 3.

The generated data in the form of physicochemical results revealed that the raw ingredients complied with limits of the Ayurvedic Pharmacopoeia of India (API) and up to the mark as far as quality is concern. The chromatographic analysis was conducted in the form of the HTPLC fingerprinting profile under optimized chromatographic conditions for the identification and authentication of in-house phytoagalactagogue preparation. This study established reliable HPTLC fingerprint profile at different illuminations with prominent bands at specific Rf as presented in Figure 1, which represents the active constituents of five different herbs in the form of distinct bands that control milk-inducing function in lactating cattle. Fingerprint patterns at different illuminations with prominent bands and characteristics Rf may set as the quality standard of developed PHF, HPTLC profiles revealed a uniform chemical pattern in its three batches. The HPTLC fingerprints are suitable for rapid and simple authentication and comparison of three batches of PHF, which indicated that all the three batches are qualitatively similar in respect of phytoconstituents. The uniformity in composition of batches 1, 2, and 3 was determined through identification of similar band patterns and almost similar Rf values. Whereas the results of safety parameters indicate that all the three batches of PHF are safe as per the WHO and API guidelines. Results of the organoleptic, physicochemical, phytochemical, and HPTLC evaluations showed that all the three batches of this formulation are equivalent in respect of composition and quality. This work may be utilized as a base study for further preclinical and clinical trials in different animal models specifically dairy cattle. The outcomes in the form of scientific data could help to validate the traditional belief that some Ayurvedic herbs are potent to improve milk production in lactating cattle after the preclinical studies. The application of present work may be fruitful for healthy practices in veterinary and livestock, specifically to fulfill the massive dairy production demands.

**Conclusion**

The present study developed and standardized new PHF, a galactagogue in different batches to establish the quality medicine for the purpose of augmenting milk production in healthy dairy cows. This Ayurvedic veterinary medicine may not only be...
helpful to compensate the massive demands of milk in India but also have the therapeutic potentials to keep the cattle healthy and productive. The study could be utilized as a base model for further evaluating safety, toxicity, and clinical trials on dairy animals. Data may be useful for the standardization and routine quality control of different pharmaceuticals batches and other commercial veterinary as well as human galactagogues. This Ayurvedic formulation may work as an alternative for hormone replacement therapy (HRT) or exogenous estrogens; hence, it is safer and effective in the dairy economics and livestock management.

Acknowledgments

The authors are grateful to the Director General, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India, New Delhi, for providing financial support and necessary facilities to carry out the study under the IMR project, “Development of a polyherbal formulation for augmenting milk production in healthy dairy cows.” The authors are also thankful to the in-charges of peripheral bodies of CCRAS, i.e., Regional Ayurvedic Research Institute for Eye Diseases, Lucknow, U.P., and Regional Ayurvedic Research Institute of Drug Development, Gwalior, M.P., for their constant supports for conducting the project works.

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हिंदी सारांश

स्वस्थ दुःधार गायों में दुःध उत्पादन वृद्धि के लिए एक नए पॉलीहर्बल योग (पीएचएफ) का विकास

लक्ष्य: स्वस्थ दुःधार पशुओं में दुःध उत्पादन के लिए एक नए पॉलीहर्बल योग (पीएचएफ) का विकास और मानकीकरण हेतु इस अध्ययन की रूपरेखा तैयार की गई।

सामग्री और विधियाँ: पौंच प्राकृतिक पदार्थ औषधियाँ यथा एसपेरेगस रेसमोसस विल्ड के कंद (शतावरी), एक्सिग्ना अल्बा (एल.) हर्द (शुरुगार) का पूरा पीथा, ट्राज्नोल्या फेस्टन-योकेम एन (सेथिका) के बीज, कीलकुलस वल्लरी मिल (सिन्धू), और एलेंधुम तोया रोस्कब. एक्स फ्लोरिंग (शतपुष्पा) का प्रयोग सक्सेसलैट विधि का उपयोग कर हाइड्रोल्योहलिक सशर्त तैयार करने में किया गया। पीएचएफ के तीन आंतरिक बैंक तैयार किए गए और आयुर्विदिक फार्माकोपिया ऑफ इंडिया (एपीआई) विधियों के अनुसार मानकीकरण किया गया। फार्माकोग्नोस्टिक एवं केमिकल प्रमाणीकरण मैक्रोस्कोपिक और माइक्रोस्कोपिक अध्ययनों, फाइटोकेमिकल स्क्रीनिंग, फिजिकोकेमिकल एनालिसिस और हाई परफोर्मेंस थिन येसर क्रॉमेटोग्राफी (एपीटीएलसी) मिगराप्टिंग दुर्घासा किया गया। गैलेक्टागॉन तैयार करने के उपरांत अध्ययन विधि, हेवी एंट्रि, माइक्रोवियल संदर्भ, एफ्लाउटरिक्स और कीटनाशक अंशांश विश्लेषणों के माध्यम से किया गया।

परिणाम: ओर्ग्नोलैटिक अध्ययनों में पाया गया कि सभी बैंक हल्के कड़े स्वाद और सुखद गंध के साथ काले भूरे रंग में अर्थस स्नातित हुए। फाइटोकेमिकल स्क्रीनिंग ने प्राकृतिक औषधियों और पीएचएफ दोनों के विभिन्न बैंकों में समान संकेंद्री रोटेबॉरोट्सकटेस की उपस्थिति की पुष्टि की। विभिन्न स्पष्टकरणों में फिजियोकेमिकल विश्लेषण और एपीटीएलसी मिगराप्टिंग थेशियों को समान रूप से निर्मित कर अनुभव लिया। सुरक्षा मानकों के परिणामों की प्रमाणीकरण की गई कि सभी तीन बैंक सुरक्षित थे और डबलयूएसी और एपीआई रिटाइर्स के अनुसार अनुभव लिया गया।

निष्कर्ष: वर्तमान कार्य के आधार पर औषधि, लागत-प्रभाव, नाना हार्मॉनिक, आयुर्विदिक गैलेक्टागॉन आंतरिक तैयारी के मानकीकरण अर्थस दृष्टि पर समृद्ध में दुःध उत्पादन वृद्धि के लिए पीएचएफ का दावा करता है। यह साबित करता है कि सभी तीन बैंकों में समान विशेषताएं हैं और समान रूप से निर्मित हैं। यह गैलेक्टागॉन औषधियों की पहचान करने और उन्हें अलग करने के लिए एक संदर्भ के रूप में कार्य करता है।

मुख्य शब्द: दुःधार, गैलेक्टागॉन, हाई परफोर्मेंस थिन लेयर क्रॉमेटोग्राफी, पॉलीहर्बल योग, मानकीकरण।