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Original article

Standardization of a galactogogue herbal mixture based on its total phenol and flavonol contents and antioxidant activity

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Abstract

Background and objectives: Dairy companies always try to increase the quantity and quality of milk production. According to the positive impact of different plants on milk production of cattle, this study was carried out to standardize a galactogogue herbal mixture based on its total phenol and flavonol contents and antioxidant activity. **Methods**: A mixture of *Galega officinalis* L. and *Nigella sativa* L. powders (4:1 w/w), which had shown milk stimulating activity on Holstein cows in a previous study, was extracted with hydro alcoholic solvent (80% aq. methanol) and its 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity and total phenol and total flavonol contents were measured according to standard methods. **Results**: The inhibition concentration 50% (IC_{50}) value of radical scavenging activity in DPPH test and the total phenol and total flavonol contents were found to be 154.29 µg/mL, 77.720±0.104 µg of gallic acid equivalent (GAE) and 0.231±0.018 µg of quercetin equivalent (QE) per mg of dry extract, respectively. **Conclusion:** This herbal extract can be standardized based on its antioxidant activity or total phenol and total flavonol contents and the standardized extract can be formulated as a veterinary galactogogue supplement.

Keywords*:* antioxidant activity, *Galega officinalis* L., *Nigella sativa* L., total flavonol, total phenol

Introduction

Qualitative and quantitative optimization of milk production is an important key point in dairy companies. Many factors like food quality and quantity, caging system, milking procedure, sanitary programs and diseases can affect milk productive performance of animals, especially milk yield, fat and protein percentage [1].

Many investigations have confirmed the positive impact of herbal additives on general health and productive performance of cattle over the past years and have led to an increasing interest in their utilization. Increasing food intake, improving digestibility, stimulating the immune system, securing nutrient requirements and increasing milk yield, are some of these beneficial effects [2-4].

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Asparagus racemosus Willd., *Carum carvi* L., *Trigonella foenum-graceum* L., *Mentha piperita* L., *Viola tricolor* L., *Matricaria chamomilla* L., *Urtica dioica* L., *Achillea millefolium* L. and *Thymus vulgaris* L. are some examples of medicinal plants with positive effects on cattle's health and productivity, usually in mixture formulations [5,6].

Galega officinalis L. (Fabaceae) is an important species of *Galega* genus. It is an herbaceous plant with odd-pinnate lanceolate leaves and bluish-white flowers, which is naturally distributed in Europe and Asia. It is commonly known as Goat's rue, which reflects traditional usage of its aerial parts and seeds as galactogogue in domestic animals. Moreover, the genus name, *Galega*, has been derived from "gala" (means milk) and "agein" (means production). *Nigella sativa* L. (Ranunculaceae) is an herbaceous plant with filamentous leaves and white flowers. It is naturally distributed in the Mediterranean region, Middle East and India. Its black prismatic seeds, which are commonly known as Black cumin or Black seed, have been traditionally used as a milk increasing agent [7].

There is no defined mechanism of action for the milk increasing activity of these two plants. There is a possibility that phenolic compounds like kaempferol and quercetin, phytosterols like diosgenin and phytochemicals with dopamine antagonistic activity like anethole, participate in this action. Moreover, natural antioxidants with free radical scavenging activity have shown different beneficial effects on productive performance of cattles [8-13].

Regarding the mentioned traditional and folklor antecedent and a previous veterinary study, which had been shown the milk stimulating activity of *G. officinalis* and *N. sativa* mixture in Holstein cows [14] along with the probable milk increasing role of phenolic phytochemicals with antioxidant activity, this study was aimed to standardize the hydro alcoholic extract of this herbal mixture, based on its antioxidant activity and total phenol and flavonol contents.

Experimental

Plant material

Dried aerial parts of *G. officinalis* and seeds of *N. sativa* were purchased from Zardband Co., Tehran, Iran and authenticated in Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The plant material was powdered and mixed according to the previously reported proportion for Holstein cows (4:1 w/w of *G. officinalis*: *N. sativa*).

Extraction

Mixture of dried and powdered aerial parts of *G. officinalis* and seeds of *N. sativa* (300 g), was percolated with 80% aq. methanol. The extract was collected every 48 hours for 3 times and was concentrated (EXT).

Antioxidant activity

1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of EXT was measured. The concentration of the extract which caused 50% inhibition of free radicals (IC_{50}) was calculated based on the plot of the inhibition percentage against sample concentrations [15-17].

Assay of total phenol and flavonol

Total phenol content of EXT was evaluated and expressed as gallic acid equivalent (µg GAE/mg EXT) using Folin-Ciocalteau method. Briefly, 750 µL of Folin-Ciocalteau reagent (diluted 10-fold with distilled water) was mixed with 200 µL of defined concentrations of the extract. After 5 min, 750 µL of sodium bicarbonate solution (60 g/L) was added to mixture. The mixture was stored at room temperature for 90 min. Subsequently, the absorbance was measured at 725 nm using a UV spectrophotometer. Finally, total phenol content was calculated using the calibration standard curve of gallic acid (25-150 mg/L in methanol). Total flavonol content was measured and expressed as quercetin equivalent (µg QE/mg EXT). All measurements were done in triplicate [16-19].

Statistical analysis

All values were expressed as mean ± SD. Data analysis was done using Excel software.

Results and Discussion

The extraction yield was 37.49% w/w. The plot of percentage inhibition of DPPH free radicals against sample concentration is shown in figure

1. The IC_{50} value of radical scavenging activity for EXT was 154.29 µg/mL based on this curve.
The calibration curve for gallic acid in total phenol assay is shown in figure 2 (y=0.0068x-0.0215, R^2=0.9912). According to the calibration curve, the mean value of total phenol content was measured in the hydro alcoholic extract as 77.720±0.104 µg GAE/mg EXT. Moreover, the total flavonol content was 0.231±0.018 µg QE/mg EXT based on methenamine method.
Galega officinalis aerial parts and *Nigella sativa* seeds have been traditionally used as galactagogue for centuries. Different pharmacological and clinical studies have separately evaluated and confirmed their positive effect on milk production in mammalians [8-10]. A recent study has shown that powdered mixture of *G. officinalis* aerial parts and *N. sativa* seeds (4:1 w/w, 200 g per cow per day) could positively affect the milk quantity and quality of 20 days and 150 days post-partum Holstein cows [14].
The results of the present study about total phenols, total flavonoids and the antioxidant effects of *G. officinalis-N. sativa* mixture demonstrated that the mixture is rich in total phenolics and flavonoids with antioxidant activity. The positive impact of these two plants on milk quantity and quality can be attributed to their active phytochemicals like phenols, flavonoids, saponins, terpenes and sterols. Phytochemical investigations on *G. officinalis* have shown the presence of phenolics like flavonol triglycosides, kaempferol, galein, medicagol and quercetin, triterpenes like galegadiol, sophoradiol and soyasapogenol, saponins and phytosterols like beta-sitosterol, stigmasterol and campesterol. The seeds of *N. sativa* are rich source of phenolics like vanillic acid, gallic acid, trans-cinnamic acid, apigenin, quercetin and catechin, triterpenes like beta-amyrin, isoflavonoids like diosgenin, saponins like melantin, and phytosterols like beta sitosterol, stigmasterol, gramisterol and campestrol. The role of flavonols like kaempferol and quercetin, phytosterols like diosgenin and phytochemicals with dopamine antagonistic activity like anethole, as active compounds in charge for galactogogue effect, is featured [20-22].

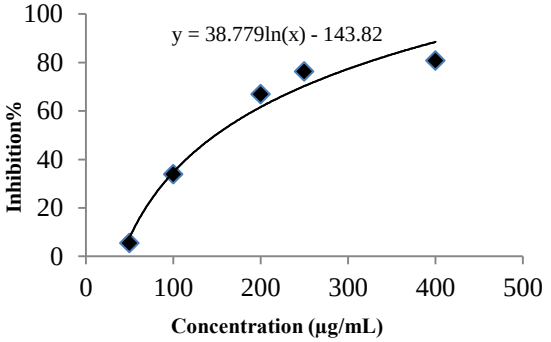

Figure 1. DPPH free radical inhibition percentage of hydro alcoholic extract of *G. officinalis* and *N. sativa* mixture

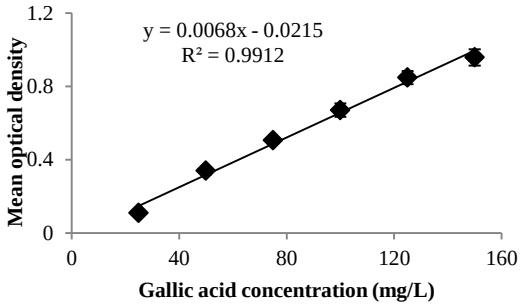

Figure 2. Calibration curve of gallic acid in total phenol assay

Destructive effects of oxidative stress on animal health were previously described. Reactive oxygen metabolites can damage cells and change their metabolic functions like steroid genesis. They also cause impairment in immune system and aggravate some diseases like mastitis in cattle. Using herbal antioxidants, is a suitable choice for strengthening the antioxidant defense system of animals and overcoming these problems [11,12]. Moreover, phenolics and flavonoids can reduce the incidence of extensive foam formation in cattle rumen, which causes pressure on lung, respiratory failure or death, increase milk quality and quantity and decrease the incidence of sub-clinical helminthic infections. These positive effects can help decrease the total bacterial count (TBC) and somatic cell count (SSC) in milk [13].

The above mentioned study about the positive effect of Goat's rue and black cumin mixture on milk quantity and quality of Holstein cows and the importance of phenolic compounds with antioxidant activity in milk increasing of these two plants, encouraged us to standardize the mixture based on total phenol and flavonol contents and antioxidant activity.

The antioxidant activity, total phenol and flavonoid contents of Goat's rue and Black cumin have been separately reported. Both plants have shown considerable antioxidant activity in previous investigations. Total phenolic content and IC_{50} value of radical scavenging for crude methanol extract of *N. sativa* seed, have been 27.8±0.11 µg GAE/mg EXT and 2.26 mg/mL in a previous study. In another study, the total phenol content of the aqueous extract of *G. officinalis* aerial parts, has been 24.455±1.3 µg QE/mg EXT [23-25]. The IC_{50} value of free radical scavenging activity for two standard antioxidant agents vitamin E and butylated hydroxyl anisole (BHA), have been 14.23 and 7.9 µg/mL in a previous study with the same method that we have used in the current investigation [26].

In conclusion, the extract of *Galega officinalis* and *Nigella sativa* mixture (4:1 w/w) can be standardized based on the antioxidant activity and phenol and flavonol contents and these extracts could be used as galactogogue supplements for cattle dietary ration.

Acknowledgments

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

DPPH: 1,1-diphenyl-2-picrylhydrazyl
GAE: gallic acid equivalent
QE: quercetin equivalent
EXT: extract
IC: inhibitory concentration
BHA: butylated hydroxyl anisole
TBC: total bacterial count
SCC: somatic cell count