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Development of HPLC based method to study the bioactive component of *Andrographolide paniculata* in serum of Nicobari Fowl

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ABSTRACT

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The bioactive component of andrographolide in *Andrographis paniculata* (Kalmegh) was quantified using HPLC. Aqueous extract prepared from 30 g & 50 g of *Andrographis paniculata* was used as water supplement in Nicobari fowls. The serum samples collected in 3 and 24 hours after supplementation were processed to comprise of Serum: phosphate buffer (pH 5.8): Methanol at the ratio of 1 ml: 0.5 ml: 1 ml and injected into HPLC. The concentration of andrographolide in serum from 30 g and 50 g group at 3 hours interval were 67.056 ppm and 151.31 from 50 g, respectively; while nil concentration at 24 hrs after feeding. The HPLC based method developed to quantify the andrographolide in serum is simple, sensitive and might be base reference for further studies on ethno veterinary medicine

Key words: *Andrographis paniculata,* andrographolide in serum, herbal feed additive, HPLC method, Nicobari fowl, quantification

Herbal enrichment of eggs is given much more concentration to produce organic eggs by incorporation of bioactive principles of herbs depending upon the herb fed to the hen. Further, herbal feed additives as an alternative to antibiotic growth promoters in poultry have been in use for decades in view of antibiotic residue free production of poultry produce. Those herbs exert immunomodulatory action; which confer birds with greater general immunity from various diseases (Dhenge *et al*., 2009). There is evidence suggesting that herbs, spices, and various plant extracts have digestion stimulating and antimicrobial properties (Sharifi *et al*., 2013). *Andrographis paniculata* (Kalmegh) is one of herbal plants having antimicrobial and growth promoting activity and one of the most widely used plants in ayurvedic formulations (Hooker, 1885; Chopra *et al.,* 1992). *Andrographis paniculata* Nees (Family Acanthaceae) is abundantly available medicinal plants. Kalmegh possesses growth promoting, immuno-stimulatory, antiviral, antioxidant and hepato-protective effect in poultry (Mathivanan *et al.,* 2006; Mathivanan and Kalairasi, 2007; Sapcota *et al.,* 2005). But there is only limited evidence about the level of bioactive principle in serum to exert optimal effects in poultry and the method of its quantification in serum. Quantification of bioactive components present in herbs and in serum as well is very much prerequisite in feeding of medicinal herbs to determine the efficacy of the concern herb. Main Bioactive compounds are *Andrographolide* and *Diterpenoid lactone*. Andrographolide is used as standard to analyze Kalmegh.

Various authors (Maiti *et al.,* 1959; Gaind *et al.,* 1963; Sharma *et al.,* 1992; Srivastava *et al.,* 2004; Du *et al*., 2003; Chen *et al.,* 2007) reported gravimetric, colorimetric, spectrophotometric and high performance liquid chromatographic methods for estimation of andrographolide in *Andrographis paniculata*. However, no biological study is reported to quantify the andropholide in serum on feeding of *Andropholide paniculata* to poultry. Hence, the aim of this study was to develop the method to quantify andrographolide using reverse phase high performance liquid chromatography (HPLC) in the serum of Nicobari fowl fed with Kalmegh (*Andrographis paniculata* Nees).

Standard andrographolide and methanol (HPLC grade) used as solvent for preparation of standard and samples were procured from Sigma. Stock solution (1 mg/ml) and working standard solution (100 mg/ml) of andrographolide in methanol was prepared. The Kalmegh leaves were dried under sun shade and powdered. The powdered dried plant of *Andrographis paniculata* (0.5 g) was weighed and diluted with 7 ml of methanol. The extract was sonicated in ultrsonic homogenizer for 10 min. This extract was filtered with Whatman's no.1 filter paper. The volume was made upto 10 ml with methanol. The extract was again filtered using 0.2 µm syringe filter. The alcoholic extract was used for High Performance Liquid Chromatography analysis of Andrographolide. A quantity of 30 and 50 g of Kalmegh powder were separately soaked overnight in a beaker of 200 ml water. Next day morning soaked powder was filtered and the aqueous extract was used for supplementation in the water of experimental birds.

A total of 270 laying Nicobari fowls belonging to same batch were selected at 35 weeks of age. Birds were managed under deep litter system and 16 hours light with 3 lux of intensity per sqft. All birds were fed *ad libitum* feed as per Bureau of Indian Standards (BIS, 2007)

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Table 1: The concentration (ppm) and peak areas of Andrographolide present in Kalmegh powder and its efficacy to enrich the serum with Andrographolide in Nicobari fowl

Samples	Retention Time (RT)	Peak area	Conc. (ppm)
Kalmegh powder	4.381	17355436	339±3.81
Serum collected 3 hrs interval feeding of kalmegh extract prepared from 30 gm of kalmegh	4.371	7983943	67.056±15.03
Serum collected 3 hrs interval feeding of kalmegh extract prepared from 50 gm of kalmegh	4.397	3790720	151.31±16.73
Control group		Not detectable amount	
Serum collected 24 hrs interval feeding of kalmegh extract		Not detectable amount	

Standard peak area-2890309 (1 µg/ml); *-Average of three determinations

recommendation. Birds were assigned to 1 of 3 dietary treatments (3 replicates of 30 birds in each replicate).T3: Control without supplementation; T2: Birds were given water supplemented with Kalmegh extract (10 ml/ bird/ day) prepared out of 30 gm. T3: Birds were given water supplemented with Kalmegh extract (10 ml/bird/day) prepared out of 50 gm.

The blood samples were collected at three and 24 hours after feeding of Kalmegh extracts and control groups. The serum samples were processed for High Performance Liquid Chromatography (HPLC) to quantify andrographolide. Serum of about 1 ml was mixed with 0.5 ml of phosphate buffer (pH 5.8) and 1 ml methanol was added. The tubes were vortexed at 8000 g for 5 min. The supernatant was separated and kept for injection into HPLC.

Chromatographic separation was performed on a Shimadzu liquid chromatographic system (LC-2010A, HT) that was equipped with multi-wavelength UV array detector, a column oven (35°C), and a degasser, an auto-injector and low pressure gradient quaternary pumps. A reverse phase C-18 column (250 x 4 mm, 5 µl (waters phenomenex) was used for the separation. Class VP series version 6.01 (Shimadzu) data station was applied for the data collecting and processing. Mobile phase of a mixture of methanol and water (65: 35) was delivered at a flow rate of 1 ml min-1 with detection at 223 nm. The column temperature was maintained at 25°C. 20 µl of standard preparation was injected using a rheodyne syringe and the chromatogram was recorded. The process was repeated for serum sample. Peak corresponding to the retention time of phyto-constituent was identified and evaluation was viapeak areas with linear regression.

The extracted and quantified amount of andrographolide in Kalmegh powder was 339 ppm (Table 1 & Fig. 1).

Wide range of andrographolide concentration in Kalemgh has been reported by various authors viz., 47 ppm (Meenu *et al.,* 2012), 237 ppm (Monika, 2010) and 500 ppm (Mamatha, 2011). The andrographolide content recorded in the present study is within the range reported by different authors. This wide variation in the amount depends on species, method and time of collection,

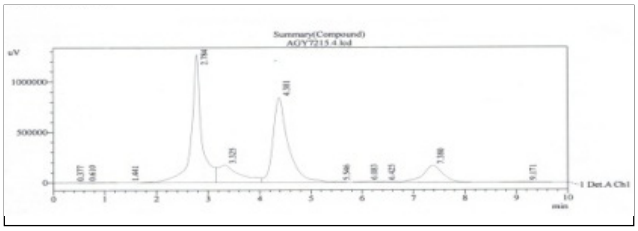

Fig. 1: HPLC chromatogram of *Andrographolide paniculata* sample

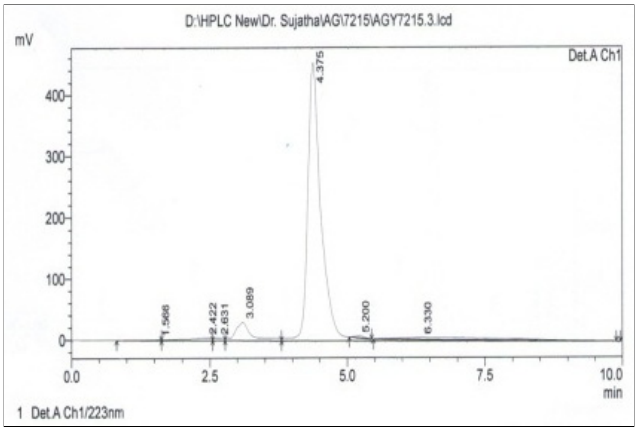

Fig. 2: HPLC Chromatogram

growing region and season (Meenu *et al*., 2012).

The concentration of andrographolide in serum (Table 1 & Fig. 2) from 30 g and 50 g group, collected at 3 after supplementation were 67.056 ppm and 151.31 ppm respectively. There was nil concentration of andrographolide in the control serum and sample collected at 24 hrs after feeding supplementation. The yield was confirmed by repeating the quantification for ten days.

The sample mixture comprising of serum: Phosphate buffer: Methanol at the ratio of 1.0:0.5:1.0 ml in the present study is agreed by Kumaran *et al*. (2003) with little change at ratio of 0.5:0.5:2. The quantity of serum needed for extraction of phytocontitutent was double and methanol was just half in the present study as compared to the Kumaran *et al.* (2003). The method used by them was for the recovery of standard andrographolide in rabbit serum. That might be reason for the change of ratio in the serum sample mixture. The

maximum recovery and the precision of the assay values indicated that the developed HPLC method for quantification of serum andrographolide was very sensitive and reliable. This method will be very useful for further clinical trials with phyto-constituents in veterinary field.

The developed HPLC method might be utilized for the quantitative determination of andrographolide in serum samples of poultry fed with *Andrographis paniculata* herb. The method developed is simple, sensitive and statistically validated. The HPLC based method developed in this study for the quantification of andrographolide in serum can be the base reference for estimation of any bioactive components of medicinal plants in serum in further studies on ethno veterinary medicine. The HPLC based method developed to quantify the andrographolide in serum is simple, sensitive and might be base reference for further studies on ethno veterinary medicine.

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