Concentration and bioactivity of condensed tannins and total phenolics of *Lespedeza* species from a germplasm collection

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Keywords: Bioactivity; Condensed tannins; Phenolics, *Lespedeza*

Abstract. Interest in 'AU-Grazer' sericea lespedeza (*Lespedeza cuneata*) as an anti-parasitic bioactive forage has been growing in the United States, but the concentration and bioactivity of tannins and other phenolics from additional accessions of *L. cuneata*, as well as other *Lespedeza* species, have not been evaluated. A study was completed to determine the concentration of extractable condensed tannins (ECT), total CT (TCT), total phenolics (TP), and protein-precipitable phenolics (PPP) of 32 accessions of *L. cuneata* and 16 additional *Lespedeza* species from a germplasm collection. The plants were established in small pots in a greenhouse and then transplanted into small field plots at the Fort Valley State University Agricultural Research Station in Georgia. Once established in the field, samples from each plot were collected, freeze-dried, and ground for analysis. The ECT and TCT for *L. cuneata* accessions averaged 6.6 ± 1.4% and 9.1 ± 1.8%, respectively, while TP were 114.8 ± 33.2 mg/g plant material and PPP averaged 81.5 ± 25.3 mg binding CT/g plant material. For other *Lespedeza* species, ECT ranged from 3.7 ± 1.3 (L. striate) to 8.8 ± 1.3 (*L. frutescens*) and TCT from 6.0 ± 0.5 (*L. japonica*) to 10.8 ± 1.3 (*L. frutescens*). Total phenolics ranged from 45.3 ± 24.0 (*L. striate*) to 185.8 ± 43.9 (*L. virgata*), and PPP from 22.1 ± 71 (*L. tomentosa*) to 89.0 ± 23.6 (*L. virginica*). All *Lespedeza* species evaluated in this study had relatively high levels of CT, with several species as high or higher in TP and PPP (bioactivity) than *L. cuneata*, suggesting potential increased use of these plants as nutraceutical forages for animals.

Introduction

Parasitic infection with gastrointestinal nematodes (GIN), particularly *Haemonchus contortus*, is one of the leading economic constraints in small ruminant production systems worldwide. Usefulness of commercially available anthelmintics is rapidly decreasing due to a growing prevalence of resistant GIN strains (Kaplan and Vidyashankar 2004), and scientists and farmers have been searching for alternative, non-synthetic parasite management strategies.

Sericea lespedeza (*Lespedeza cuneata*), a warm-season perennial legume rich in condensed tannins (CT), has excellent anti-parasitic bioactivity against GIN and coccidia (*Eimeria* spp.) in sheep and goats (Terrill et al. 2012). There is an extensive *Lespedeza* germplasm collection at the USDA Plant Genetic Resources Conservation Unit (PGRCU) in Griffin, GA, but these plant resources have never been evaluated as to the concentration and bioactivity level of their CT. The objective of this work was to determine the concentration and bioactivity of CT and other phenolics of *Lespedeza* species and *L. cuneata* accessions from a germplasm collection.

Methods

Lespedeza seeds were obtained from a germplasm collection at the USDA Plant Genetic Resources Conservation Unit (PGRCU) in Griffin, GA. Twenty *Lespedeza* species were planted in 3 replicates of small plots at Fort Valley State University in Fort Valley, GA on June 22, 2016 after two-leaf germination was achieved in the greenhouse. After an establishment year, forage was harvested in July 2017, freeze-dried, ground, and analyzed for CT concentration and bioactivity level. Analyses for extractable and bound CT were completed using the procedure of Terrill et al. (1992) using CT purified from 'AU-Grazer' *L. cuneata* as the standard. Protein-precipitable phenolics (PPP) and total phenolics (TP) were analyzed as described by Courson (2019).

Results and Discussion

The *Lespedeza* species and accessions sampled from small field plots are shown in Table 1. The vast majority of the *Lespedeza* germplasm collection consisted of accessions of *L. cuneata* originating from Japan, United States, South Korea, and China. For anti-parasitic bioactivity, the most thoroughly researched *Lespedeza* species and cultivar is sericea lespedeza (*L. cuneata*), originally from Asia, and 'AU-Grazer', a cultivar developed from a breeding program at Auburn University in Alabama.

Table 1. *Lespedeza* species, number of accessions, and origin for plants started in a greenhouse and transplanted to field plots (n=3 plots/accession) in June of 2016 at Fort Valley State University.

Lespedeza Species	Number of accessions for each species	Origin
L. cuneata	39	Japan (21), United States (5), South Korea (4), China (2)
L. bicolor	2	China, U.S.
L. capitata	2	U.S.
L. cyrtobotrya	2	South Korea
L. daurica	2	China
L. japonica	1	South Korea
L. juncea	2	China, South Korea
L. pilosa	1	Japan
L. thunbergii	2	U.S., China
L. tomentosa	2	South Korea
L. virgata	2	Japan, South Korea
L. divaricata	2	South Korea
L. intermixta	1	South Korea
L. serala	1	U.S.
L. frutescens	1	U.S.
L. virginica	1	U.S.
L. striate	1	Asia

There was a species effect ($P < 0.001$) on extractable (ECT), protein-bound (PBCT), and total condensed tannins (TCT), with highest TCT concentrations (10-11%) in *L. virginica*, *L. capitata*, *and L. frutescens*, all of which are native to North America (Fig. 1). Lowest total CT concentrations (4-6%) were in *L. stiate* and *L. japonica*, while the *L. cuneata* accessions averaged 9% TCT. Highest concentrations of ECT (8-9%) were in *L. virginica*, *L. frutescens*, and *L. divaricata* (Fig. 1). There was also a species effect ($P < 0.001$) on total phenolics (TP) and protein precipitable phenolics (PPP), with highest TP values for *L. virgata*, *L. cyrtobotrya*, and *L. virginica*, while PPP concentrations were highest for *L. virginica*, *L. frutescens*, *and L. pilosa* (Fig. 2).

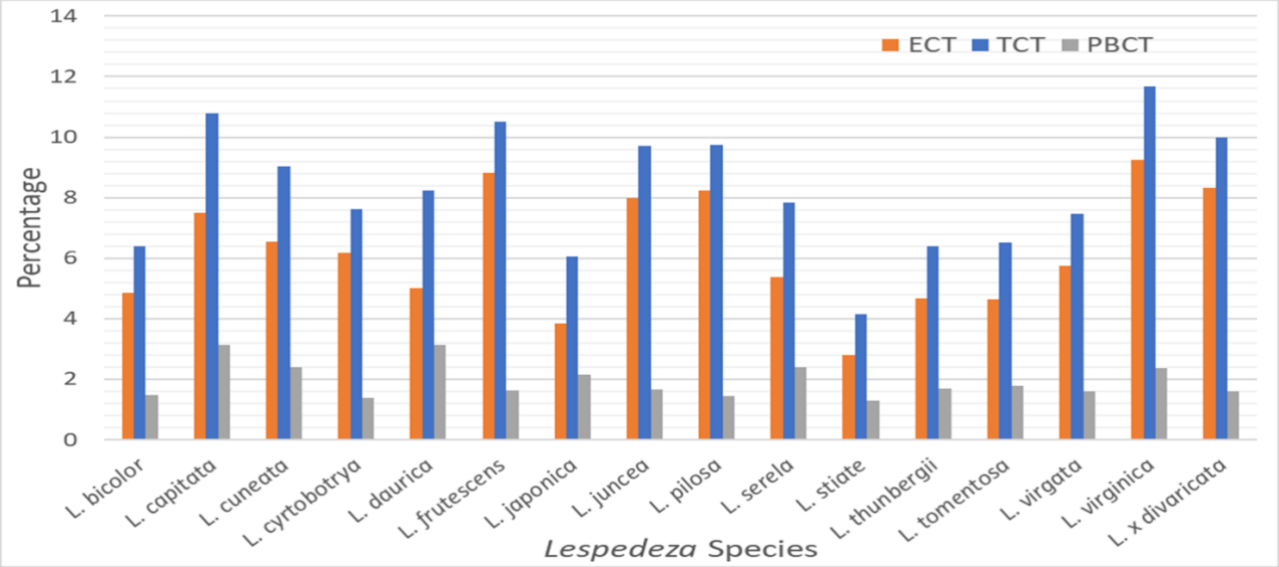

Figure 1. Total condensed tannins (TCT), extractable CT (ECT), and protein-bound CT (PBCT) in *Lespedeza* species from a germplasm collection.

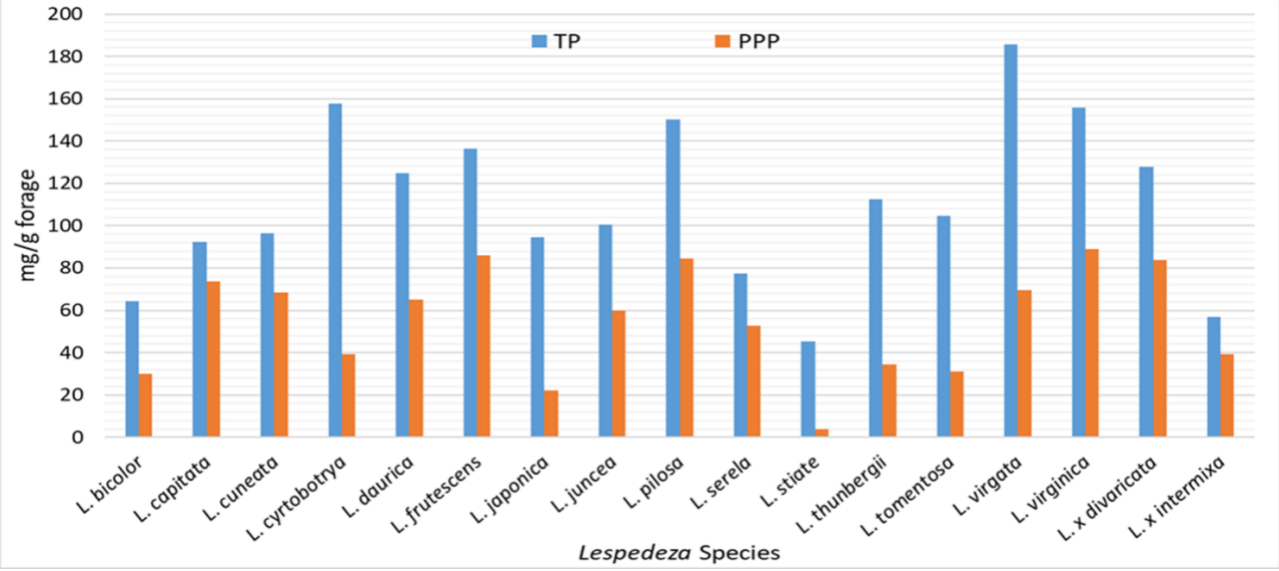

Figure 2. Total phenolics (TP) and protein-precipitable phenolics (PPP) in Lespedeza species from a germplasm collection.

The anti-parasitic bioactivity of *Lespedeza cuneata* in the diet of goats and sheep has been well-documented (Terrill et al., 2012). This plant and other non-native *Lespedeza* species have also been used extensively in herbal medicine in China (Ji et al. 2003) and Korea (Lee et al. 2011). High concentrations of tannins (ECT, TCT) and PPP (bioactivity) in *Lespedeza* species native to North America suggest their possible increased use in native species grazing systems for livestock in areas where they are well-adapted (Clewell 1966; Fant et al. 2010).

Conclusions and/or Implications

Most of the *Lespedeza* species evaluated in this study had high CT concentrations, with several species as high or higher in TP and PPP (bioactivity) than *L. cuneata*, suggesting increased use of these plants as nutraceutical forages for animals in areas where they are adapted.

Acknowledgements

The authors thank G. Dykes and V. Owen for their assistance with planting and harvesting field small plots.

References

Clewell, A.F. 1966. Native North American species of *Lespedeza* (Leguminosae). *Rhodora 68(775)*: 359-405.

Courson, E.M 2019. Nutritional Value and Bioactivity of Select Desmodium Species. Fort Valley State University Master's Thesis.

Fant, J.B., Banai, A., Havens, K. and Vitt, P. 2010. Hybridization between the threatened plant, *Lespedeza leptostachya* Englem. and its co-occurring congener *Lespedeza capitata* Michx.: morphological and molecular evidence. *Conservation Genetics 11(6):* 2195-2205.

Ji, M., Hongzhu, D., Lan, H., and Jianying, L. 2003. Resources of medicinal plants *Lespedeza* in Gansu Province. *Chinese Wild Plant Resources 22(6)*: 22-24.

Kaplan, R.M., and Vidyashankar, A.N. 2012. An inconvenient truth: global worming and anthelmintic resistance. *Veterinary Parasitology 186(1-2)*: 70-78.

Lee, K.I., Yang, S.A., and Kim, S.M. 2011. Antioxidative and nitric oxide production inhibitory activities of *Lespedeza bicolor* stem extracts depending on solvents. *Korean Journal of Medicinal Crop Science 19(5)*: 368-372.

Terrill, T.H., Rowan, A.M., Douglas, G.B., and Barry, T.N. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal. Sci. Food Agric. 58(3)*: 321-329.

Terrill, T.H., Miller, J.E., Burke, J.M., Mosjidis, J.A., and Kaplan, R.M. 2012. Experiences with integrated concepts for the control of *Haemonchus contortus* in sheep and goats in the United States. *Veterinary Parasitology 186(1-2)*: 28-37.