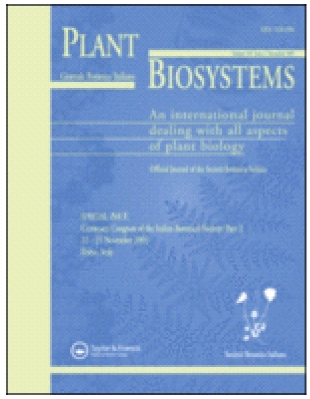

Leucaena leucocephala (Lam.) De Wit. as an alternative fodder resource in Mediterranean agroforestry systems

Journal:	*Plant Biosystems*
Manuscript ID	TPLB-2021-0262
Manuscript Type:	Rapid Report
Date Submitted by the Author:	29-Jul-2021

Complete List of Authors:	Badalamenti, Emilio; University of Palermo, Department of Agricultural, Food and Forest Sciences Maggio , Antonella; University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies Dardi, Angelo; University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies Palumbo Piccionello, Antonio ; University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies La Mantia, Tommaso; University of Palermo, Department of Agricultural, Food and Forest Sciences

Keywords:	animal nutrition, fodder legume, HPLC, toxicity, woody species

Leucaena leucocephala (Lam.) De Wit. as an alternative fodder resource in Mediterranean agroforestry systems

Emilio Badalamenti[1], Antonella Maggio[2*], Angelo Dardi[2], Antonio Palumbo Piccionello[2], Tommaso La Mantia[1]

[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy
[2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy
Corresponding author: antonella.maggio@unipa.it

Emilio Badalamenti – ORCID 0000-0002-8178-354X
Antonella Maggio – ORCID 0000-0002-0861-626X
Antonio Palumbo Piccionello – ORCID 0000-0003-4402-5798
Tommaso La Mantia – ORCID 0000-0002-7494-742X

Abstract

Leucaena leucocephala is worldwide used for wood production, reforestation and for feeding livestock. To assess the potential use of Leucaena for animal nutrition, we analysed the composition of methanolic extracts of leaf samples of two sicilian varieties, also determining the presence of mimosine, toxic for animals.

Key words: animal nutrition, fodder legume, HPLC, toxicity, woody species

Introduction

In the Mediterranean, the reduction in the coming decades of forage resources, both in quantity and quality, is expected (Ergon et al. 2018). Indeed, the combination of increased temperatures and reduced rainfall due to climate change could seriously lower soil water availability, with detrimental effects on carbon and nutrient balance in plants (Chang et al. 2017; Obermeier et al. 2018). One expected consequence is the seasonal shift towards winter by Mediterranean plants to reach more favourable ecological conditions (Rapacz et al. 2014), especially reducing fodder availability in summer. To overcome this shortage, different strategies could be employed, such as the use of different species or the identification of genotypes more resistant and adapted to the rapidly changing environmental conditions and increasing drought events (Dono et al. 2016; Dalzell 2019). For instance, Komainda et al. (2019) compared the productivity of different forage legumes to identify the most drought-tolerant species, while suggesting the replacement of the less drought-tolerant ones. *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) (hereinafter *Leucaena*), native to southern Mexico and Central America, is one of the most widely used trees in agroforestry systems, in fast-growing plantations, for restoration of degraded lands and for forage production throughout tropical and subtropical latitudes (Bageel et al. 2020). *Leucaena* may be cultivated under a wide range of rainfall conditions (750-1,800 mm of annual amount) and may withstand up to six months of drought periods (Binggeli et al. 1998). The high resistance to biotic damage and abiotic stresses makes *Leucaena* a promising species for Mediterranean areas. Moreover, due to fast-growing traits and high resistance to frequent coppicing, *Leucaena* is an ideal candidate for wood and biomass production (National Research Council 1980), capable of reaching very high yields in intensive cultivation systems, and promising results have been recently achieved also in Mediterranean conditions (Fernández et al. 2020). It is also appreciated by livestock for high palatability and high nutritive value of foliage (Garcia et al. 1996). However, the species holds two main shortcomings: a high invasive potential (Wolfe and Van Bloem 2012) and the presence of antinutritional factors in leaves. However, the invasiveness seems to be restricted to the shrub-like subspecies ‘common leucaena’ (*Leucaena leucocephala* (Lam.) de Wit subsp. *leucocephala*), while not affecting the tree-like subspecies ‘giant leucaena’ (*Leucaena leucocephala* subsp. *glabrata* (Rose) S. Zárate) (Bageel et al. 2020). Conversely, the major barrier for a wider use of the species is the high leaf content of mimosine, a non-protein amino acid whose degradation products (i.e. isomers of hydroxypyridone or DHP) are toxic to herbivores, mostly ruminants, and can cause alopecia, loss of appetite, salivation, reproduction issues and reduced productivity (Halliday et al. 2013). Up to now, *Leucaena* has been mostly used in tropical and sub-tropical systems, while being rarely tested under Mediterranean conditions. With this contribution, we preliminarily assessed the potentialities of

Leucaena for fodder production in Mediterranean agroforestry systems. Particularly, we quantified the mimosine content and we performed qualitative analyses (to assess the nutritive value of leaves) in two varieties occurring in Sicily (Hawaii and Perù), also testing leaf samples obtained from both varieties and subject to oven drying.

Materials and Methods

Plant material

Plant material was collected from mature *Leucaena* individuals belonging to cultivars Hawaii and Perù, as previously identified (Badalamenti et al. 2020). Fresh leaves were stored and air-dried before analyses. Moreover, we analysed leaf samples composed of both varieties and oven-dried for 48 h (hereinafter Mixed/Dry). Five mature individuals per cultivar were considered and leaves were collected from different branches in each selected plant. All the analyses were carried out at the STeBiCeF Department, University of Palermo.

Sample preparation

The plant matrices were firstly subject to methanol extraction to remove chlorophylls and other possible interfering substances and exploiting the poor solubility of mimosine in this organic solvent. The resulting plant matrices were then subject to water extraction to perform the quantitative assessment of mimosine content, as well as the qualitative characterization of the polar fraction.

Quantification of mimosine content

Mimosine content was quantified using first colorimetric method and UV-Vis spectroscopy (UV) and then High Performance Liquid Chromatography (HPLC) and MS-TOF.

For the first method, we considered as reference the calibration curve of the stock solution of L-mimosine (by diluting 5.12 mg of "L-Mimosine from Koa hoale seeds" (Sigma-Aldrich®) in 10 ml of H_2O), which was obtained by adding 1 ml 0.1 N HCl and 0.4 ml $FeCl_3$ (0.5%) to different concentrations of the stock solution (1, 5, 10, 25, 50 μg ml^{-1}). The quantification of mimosine content via UV analysis was performed in a Win Aspect spectrophotometer, analyzing the absorbance at λ = 535 nm (Ilham et al. 2015). Mimosine content is reported as g Kg^{-1} of leaf dry matter.

To obtain a more reliable estimate of leaf mimosine content, we also performed an HPLC-MS-TOF analysis. The calibration curve to mimosine has been performed with the standard solution of mimosine at 1, 5, 10, 20, 25, 50 μg ml^{-1}. The HPLC analysis was carried out in an Agilent

Technologies 1260 apparatus, using HPLC Column ZORBAX Extend C18. The resulting spectra were then analyzed by means of the specific data analysis and processing program Agilent MassHunter qualitative.

Qualitative analysis

The methanolic and acqueous extracts as previously described were also analyzed by HPLC/MS analyses (High Performance Liquid Chromatography coupled to Mass Spectrometry) in reverse phase, and conducted either in negative and positive ion mode, supported by Agilent MassHunter Qualitative Analysis software B.06.00. This analysis was performed to characterize the amino acidic, polyphenolic and glycidic components of *Leucaena* foliar tissue.

Statistical analysis

A one-way ANOVA was carried out to determine the effect of *Leucaena* variety (Hawaii, Perù and mixed/dry ST) on mimosine content, as well as the effect of different methods to quantify mimosine content (colorimetric and HPLC). Significance was determined at the 95% level of confidence. Before performing ANOVA, normality and homogeneity of variance of data were verified. Statistical analysis was performed using Systat Software, Inc. 2009 (version no. 13.00.05, San Jose, CA, USA).

Results and discussion

Mimosine content

A number of methods have been employed to reduce the adverse effects of mimosine on animal health such as the inoculation with *Synergistes jonesii*, a rumen bacterium capable of degrading toxic substances in not-harmful products (Klieve et al. 2002; Shelton et al. 2019), or physical treatments (ensiling, heating, ecc.) to reduce leaf mimosine content. Regardless of method, the lower the mimosine content in *Leucaena* leaves is the lower the potential toxic effects for animal health are. Hence, the search for low mimosine varieties is still ongoing and worthy to be pursued. The concentration of mimosine in plant tissues depends on plant traits (tissue age, plant organ, etc.), as well as on environmental conditions, such as light, soil pH and salts concentrations in soils (Vestena et al. 2001; Ghosh and Samiran 2007; Honda and Borthakur 2019). As mimosine content is higher in younger organs, shoots and individuals, as well as in more stressful conditions, it is suspected to play an evolutionary role, for instance as a defence against herbivores (Honda and Borthakur 2019). The toxic effect depends on the effective quantity of mimosine taken with the diet, which, in turn, depends on its concentration in plant tissues, which is affected by variety and plant portion. Xuan et al. (2006) reported that mimosine content was higher in young leaves and mature seeds than in xylem and mature leaves, where it ranged from 0.11% to 0.47%, respectively. In effect, the lower concentration of mimosine in mature plants is a common observation (Vestena et al. 2001; Xuan et al. 2006; Zayed et al. 2014; Honda and Borthakur 2019). In our experiment, we found quite low mimosine content in leaves. The percentage on a dry matter basis ranged from 1.1% to 1.5% and from 0.9% to 1.0% in the Hawaii and Perù varieties, with UV and HPLC analyses, respectively. However, with both methods, the mixed and dry treatment significantly reduced mimosine content (0.4-0.7 %; $p < 0.05$) (**Fig. 1**). Our results confirm the positive effect of heating on lowering the concentration of this toxic substance and reducing its toxic effects on livestock (Halliday et al. 2013; Honda and Borthakur 2019). We sampled dried leaves in mature individuals, conditions that further contributed to the low mimosine content found in our study. We also found a significantly lower mimosine content ($p < 0.05$) via HPLC analysis than via the traditional colorimetric method, further proving the higher selectivity of more advanced techniques. Regardless of the method, we found very low values compared with those more commonly found in literature, where mimosine content generally accounted for more than 2% of total dry matter, even reaching up to 8-12% (Soedarjo and Borthakur 1996; Vestena et al. 2001; Garcia et al. 2008; Barros-Rodríguez et al. 2014; Bageel et al. 2020). However, results in line with ours were found in four *Leucaena* varieties in Japan, where mature leaves had a mimosine content of about 0.5% (Xuan et al. 2006). The cultivar K8 showed a low mimosine content also in Brazil (Soltan et al. 2017), with percentages ranging from 0.2% to 1.5%.

Qualitative analysis

Overall, 67 different metabolites were detected in *Leucaena* leaf tissues via HPLC/MS analyses (**Fig. 2, Table S1**). Polyphenols are one of the more representative phytochemicals found in *Leucaena* leaves. Thirty-two phenolic compounds were detected (about 48% of total compounds), with flavones accounting for the largest part, and it is well kwown that they are particularly beneficial for ruminant health (Jiang et al. 2016). They are important plant secondary metabolites with anti-inflammatory and anti-oxidative properties and a primary protective function against photodamage and as a chemical defense against herbivores and pathogens (Olagaray and Bradford 2019; Acet 2020). In accordance to Xu et al. (2018), the most abundant polyphenols in *Leucaena* leaves were quercetin-3-O-α-rhamnopyranoside, quercetin, geraldone, apigenin, kaempferol, kaempferol-3-O-α-rhamnopyranoside and myristicin. In addition, we detected 10 amino acids, including three essential amino acids (leucine, phenylalanine and tryptophan) (Karau and Grayson 2014). The high protein (22-28% of dry matter) and β-carotene content, making *Leucaena* comparable to alfaalfa (*Medicago sativa* L.) (Garcia et al. 1996; González-García et al. 2009), is accompanied by a well balanced amino acidic composition, including essential amino acids (Ter Meulen et al. 1979), suggesting that *Leucaena* could be a good alternative forage both for ruminant and non ruminant livestock. *Leucaena leucocephala* holds many positive traits as a multipurpose tree in Mediterranean agroforestry systems, and it seems to be worthy of interest for feeding livestock in the inner and/or marginal Mediterranean regions, where inadequate nutritional supplies are one of the major issues for animal nutrition (Bianchetto et al. 2015; Papanastasis et al. 2008). Indeed, the low productivity of animals is not infrequently attributable to the low nitrogen and high fiber content of local plants and crop residues that form the most common food base on local tradional farms. Hence, the integration of the ordinary diet with woody forage resources has been viewed as a possible way to alleviate the nutritional deficiencies of basic diets (Papanastasis et al. 1997). In Mediterranean contexts, one relevant advantage of *Leucaena* is the persistence of foliage during summer, which is the most critical phase for Mediterrean plants and fodder resources. However, woody species may also cointain antinutrional factors (e.g., tannins or toxic compounds) in plant tissues, which have to be carefully assessed before deciding to introduce a new species in the diet. Based on this study, we deem that *Leucaena* could represent a promising fodder resource for feeding livestock in Mediterranean areas, possessing high nutritive and protein foliage (Honda et al. 2019), with a very low mimosine content, as well as being a low-demanding and easily cultivated species, with a good potential for wood and biomass production in agroforestry systems.

References

Acet T. 2020. Determining the phenolic components by using HPLC and biological activity of *Centaurea triumfetti*. Plant Biosyst. 155(9):1-12.

Badalamenti E, Pasta S, Sala G, Catania V, Quatrini P, La Mantia T.2020. The paradox of the alien plant *Leucaena leucocephala* subsp. *glabrata* (Rose) S. Zárate in Sicily: another threat for the native flora or a valuable resource? Int J Plant Biol. 11:8637.

Bageel A, Honda MDH, Carrillo JT, Borthakur D. 2020. Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*): a versatile tree-legume for sustainable agroforestry. Agrofor Syst. 94:251-268.

Barros-Rodríguez M, Sandoval-Castro CA, Solorio-Sánchez J, Sarmiento-Franco LA, Rojas-Herrera R, Klieve AV. 2014. *Leucaena leucocephala* in ruminant nutrition. Invited review. Trop Subtrop Agroecosyst. 17:173-183.

Bianchetto E, Buscemi I, Corona P, Giardina G, La Mantia T, Pasta S. 2015. Fitting the stocking rate with pastoral resources to manage and preserve Mediterranean forestlands: a case study. Sustainability 7:7232-7244.

Binggeli P, Hall JB, Healey JR (1998) An Overview of Invasive Woody Plants in the Tropics. School of Agricultural and Forest Science Publication Nº.13. University of Wales, Bangor.

Chang J, Ciais P, Viovy N, Soussana J-F, Klumpp K, Sultan B. 2017. Future productivity and phenology changes in European grasslands for different warming levels: Implications for grassland management and carbon balance. Carbon Bal & Manag. 12:1-21.

Dalzell SA. 2019. *Leucaena* cultivars - current releases and future opportunities. Trop Grasslands Forrajes Trop. 7:56-64.

Dono G, Cortignani R, Dell'Unto D, Deligios P, Doro L, Lacetera N, Mula L, Pasqui M, Quaresima S, Vitali A, Roggero PP. 2016. Winners and losers from climate change in agriculture: insights from a case study in the Mediterranean basin. Agric. Syst. 147:65-75.

Ergon A, Seddaiu G, Korhonen P, Virkajarvi P, Bellocchi G, Jorgensen M, Ostrem L, Reheul D, Volaire F. 2018. How can forage production in Nordic and Mediterranean Europe adapt to the challenges and opportunities arising from climate change? Eur J Agron. 92:97-106.

Fernández M, Alaejos J, Andivia E, Madejón P, Díaz MJ, Tapias R. 2020. Short rotation coppice of leguminous tree *Leucaena* spp. improves soil fertility while producing high biomass yields in Mediterranean environment. Ind Crop Prod. 157:112911.

García D, Wencomo H, Gonzáles M, Medina M, Cova L. 2008. Caracterización de diez cultivares forrajeros de *Leucaena leucocephala* basada en la composición química y la degradabilidad ruminal. Rev MVZ Córdoba. 13:1294-1303.

Garcia GW, Ferguson TU, Neckles FA, Archibald KAE.1996. The nutritive value and forage productivity of *Leucaena leucocephala*. Anim Feed Sci Technol. 60:29-41.

Ghosh MK, Samiran B. 2007. Mimosine toxicity − a problem of *Leucaena* feeding in ruminants. Asian J Anim Vet Adv. 2:63-73.

González-García E, O. Cáceres O, Archimède H, Santana H. 2009. Nutritive value of edible forage from two *Leucaena leucocephala* cultivars with different growth habit and morphology. Agroforest Syst. 77:131-141.

Halliday MJ, Padmanabha J, Mcsweeney CS, Kerven G, Shelton HM. 2013. *Leucaena* toxicity: a new perspective on the most widely used forage tree legume. Trop Grasslands Forrajes Trop. 1:1-11.

Honda MDH, Borthakur D. 2019. Mimosine content of *Leucaena leucocephala* under various environmental conditions. Trop Grasslands Forrajes Trop. 7:164-172.

Honda MDH, Ishihara KL, Pham DT, Borthakur D. 2019. Highly expressed genes in the foliage of giant leucaena (*Leucaena leucocephala* subsp. *glabrata*), a nutritious fodder legume in the tropics, Plant Biosyst. 154(2):1-10.

Ilham Z, Hamidon H, Rosji NA, Ramli N, Osman N. 2015. Extraction and quantification of toxic compound mimosine from *Leucaena leucocephala* leaves. International Symposium on Applied Chemistry (ISAC 2015). Procedia Chem. 16:164-170.

Jiang N, Doseff AI, Grotewold E. 2016. Flavones: From Biosynthesis to Health Benefits. Plants. 5(2):27.

Karau A, Grayson I. 2014. Amino Acids in Human and Animal Nutrition. In: Zorn H, Czermak P. (eds) Biotechnology of Food and Feed Additives. Advances in Biochemical Engineering/Biotechnology, vol 143. Springer, Berlin, Heidelberg. https://doi.org/10.1007/10_2014_269

Klieve AV, Ouwerkerk D, Turner A, Roberton R. 2002. The production and storage of a fermentor-grown bacterial culture containing *Synergistes jonesii*, for protecting cattle against mimosine and 3-hydroxy-4(1H)-pyridone toxicity from feeding on *Leucaena leucocephala*. Aust J Agr Res. 53:1-5.

Komainda M, Küchenmeister K, Küchenmeister F, Breitsameter L, Wrage-Mönnig N, Kayser M, Isselstein J. 2019. Forage legumes for future dry climates: Lower relative biomass losses of minor forage legumes compared to *Trifolium repens* under conditions of periodic drought stress. J Agron Crop Sci. 205(5):460-469.

National Research Council. 1980. Firewood crops: shrub and tree species for energy production. National Academy of Sciences: Washington DC.

Obermeier WA, Lehnert LW, Ivanov MA, Luterbacher J, Bendix J. 2018. Reduced summer aboveground productivity in temperate C_3 grasslands under future climate regimes. Earth's Future. 6:716-729.

Olagaray KE, Bradford BJ. 2019. Plant flavonoids to improve productivity of ruminants – A review. Anim Feed Sci Techn. 251:21-36.

Papanastasis VP, Yiakoulaki MD, Decandia M, Dini-Papanastasi O. 2008. Integrating woody species into livestock feeding in the Mediterranean areas of Europe. Anim Feed Sci Tech. 140(1-2):1-17.

Papanastasis, VP, Platis PD, Dini-Papanastasi O. 1997. Productivity of deciduous woody species and fodder species in relation to air temperature and precipitation in a Mediterranean environment. Agroforest. Syst. 37:187-198.

Rapacz M, Ergon Å, Höglind M, Jørgensen M, Jurczyk B, Østrem L, Rognli OA, Tronsmo AM. 2014. Overwintering of herbaceous plants in a changing climate: still more questions than answers. Plant Sci. 225:34-44.

Shelton HM, Kerven GL, Dalzell SA. 2019. An update on leucaena toxicity: Is inoculation with *Synergistes jonesii* necessary? Trop Grasslands Forrajes Trop. 7:146-153.

Soedarjo M, Borthakur D. 1996. Simple procedures to remove mimosine from young leaves, seeds and pods of *Leucaena leucocephala* used as food. Int J Food Sci Technol. 36:103-109.

Soltan YA, Morsy AS, Lucas RC, Abdalla AL. 2017. Potential of mimosine of *Leucaena leucocephala* for modulating ruminal nutrient degradability and methanogenesis. Anim Feed Sci Technol. 223:30-41.

Ter Meulen U, Struck S, Schulke E, El-Harith EA. 1979. A review on the nutritive value and toxic aspects of Leucaena leucocephala. Trop Anim Prod. 4:113-126.

Vestena S, Fett-Neto AG, Cortes Duarte R, Gui Ferreira A. 2001. Regulation of mimosine accumulation in *Leucaena leucocephala* seedlings. Plant Sci. 161:597-604.

Wolfe BT, Van Bloem SJ. 2012. Subtropical dry forest regeneration in grass-invaded areas of Puerto Rico: Understanding why *Leucaena leucocephala* dominates and native species fail. For Ecol Manage. 267:253-261.

Xu Y, Tao Z, Jin Y, Yuan Y, Dong TTX, Tsim KWK, Zhou Z. 2018. Flavonoids, a potential new insight of *Leucaena leucocephala* foliage in ruminant health. J Agric Food Chem. 66:7616-7626.

Xuan TD, Elzaawely AA, Deba F, Fukuta M, Tawata S. 2006. Mimosine in *Leucaena* as a potent bio-herbicide. Agron Sustain Dev. 26(2):89-97.

Zayed MZ, Zaki MA, Ahmad FB, Ho W-S, Pang S-L. 2014. Comparison of mimosine content and nutritive values of *Neolamarckia cadamba* and *Leucaena leucocephala* with *Medicago sativa* as forage quality index. Int J Scientific Technology Res. 3:146-150.

Figures

Figure 1

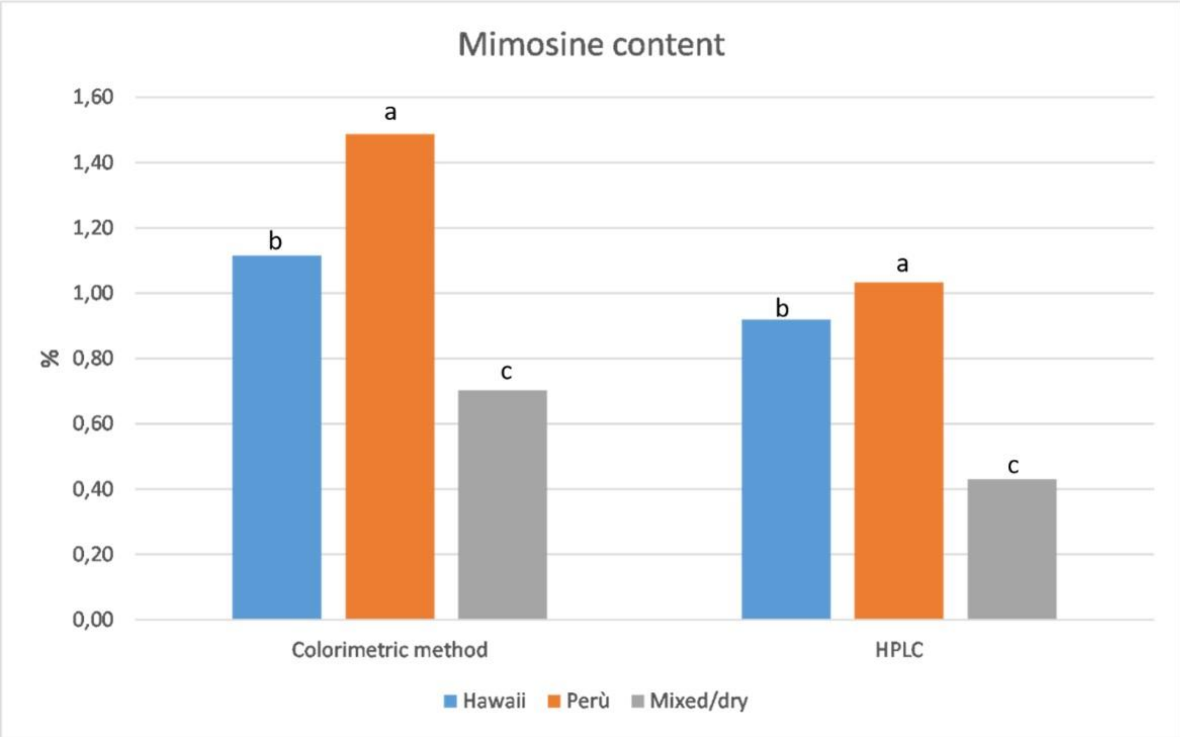

Figure 2

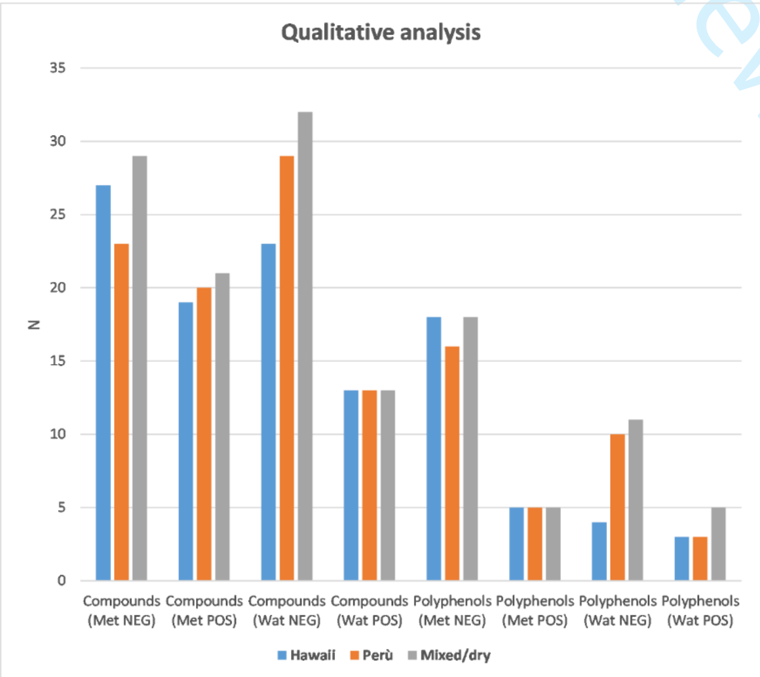

Figure captions

Figure 1 Mimosine content in *Leucaena* leaves sorted by variety and according to the quantification method (colorimetric or HPLC). Means with different letters are significantly different at $P < 0.05$, after Tukey's HSD range test.

Figure 2 Results of qualitative analysis in *Leucaena* leaves sorted by variety and according to the different extraction methods (Methane or Water). Met = Methanolic extract, Wat = Water extract, NEG = Negative, POS = Positive.

Figure 1 Mimosine content in *Leucaena* leaves sorted by variety and according to the quantification method (colorimetric or HPLC). Means with different letters are significantly different at $P < 0.05$, after Tukey's HSD range test

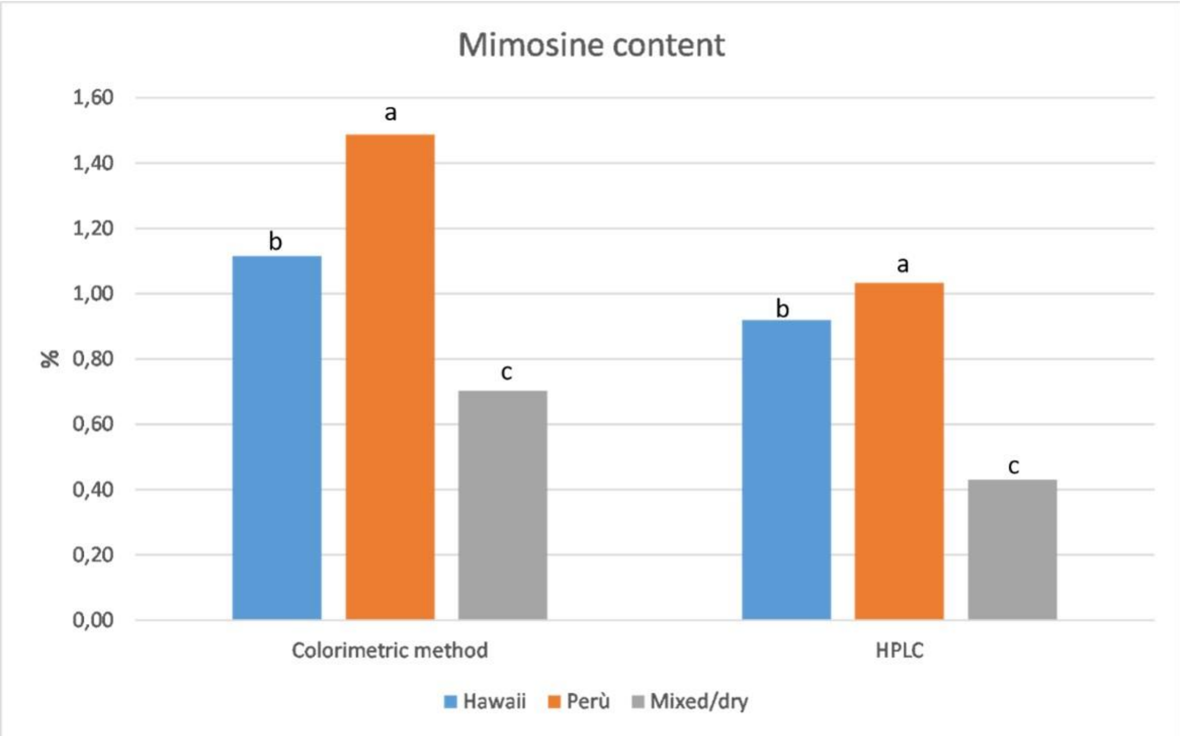

Figure 2 Results of qualitative analysis in *Leucaena* leaves sorted by variety and according to the different extraction methods (Methane or Water). Met = Methanolic extract, Wat = Water extract, NEG = Negative, POS = Positive

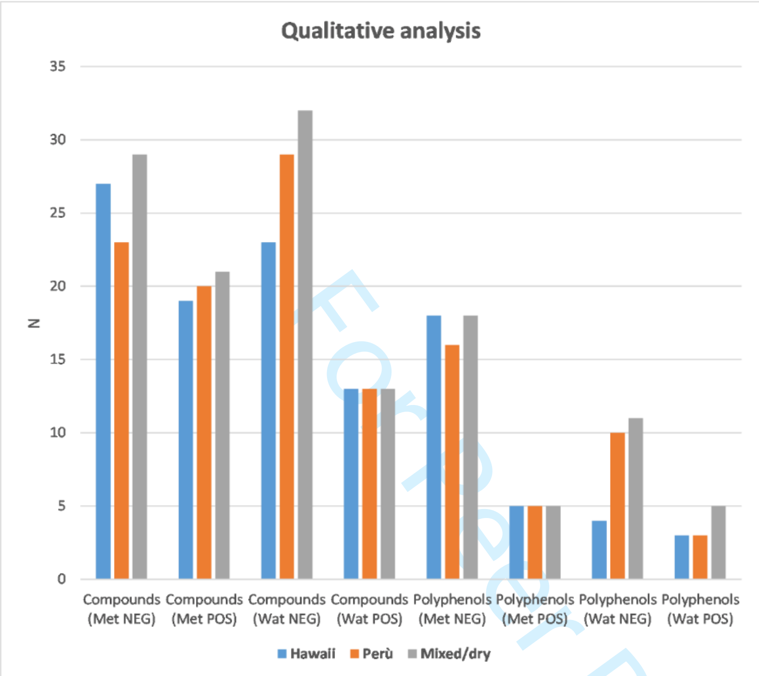

Table S1 Composition of water and methanolic extracts. H = Hawaii, Pe = Perù, MD = Mixed/Dry, P = Present; *non essential amino acids

Compound	Mass (M-H+) Positive mode	Mass Negative mode	Retention time (minutes)	H Water extract	Pe Water extract	MD Water extract	H Metanolic extract	Pe Methanolic extract	MD Methanolic extract
Carbohydrates									
Gluconic acid		195,0467	3,43	P	P	P			
Disaccharides [M+FA]		387,1075	3,46				P	P	P
Arabinose		149,0446	3,57				P	P	P
Aldaric acid ($C_6H_{10}O_8$)		209,0262	3,59	P	P	P			
Pentose ($C_5H_{10}O_5$)		149,0447	3,94	P	P	P			
Aldaric acid ($C_6H_{10}O_8$)		209,0262	4,17	P	P	P			
Aldopentose		149,0569	4,24	P	P	P			
Amino acids									
Asparagine*	133,0615	131,0462	3,12				P	P	P
Mimosine*	199,0726	197,0559	3,15	P	P	P	P	P	P
Glutamine*	147,0771		3,16				P	P	P
Allysine*	146,0822		3,25	P	P	P	P	P	P
Proline*	116,0711		3,35				P	P	P
Pipecolic acid*	130,0869		3,58	P	P	P	P	P	P
Phenylalanine	166,0871		4,28				P	P	P
Tetrahydropicolinate*	172,0602		4,86				P	P	P
Leucine	132,1023		5,11	P	P		P	P	P
Tryptophan	205,0985		15,61				P	P	P
Other									
Deamminated isomer of mimosine ($C_8H_9O_4$)	184,0619		4,23	P	P	P			
Rabelomycin		337,0741	4,94	P		P			
Fludarabine	286,0945		5,24	P	P	P			
Anthraquinone	404,1223		5,39	P	P	P			
Rabelomycin		337,07	5,40				P		P

Citroside A $C_{19}H_{30}O_8$ [M+Na]	409,1843	421,1613	22,96				P	P	P
Artomunoxantrione		443,11	27,70	P	P	P			
Silybin		481,1128	32,42				P		P
Alkaloids									
Choline	104,1071		2,83				P	P	P
Nipecotic acid	130,0868		5,40				P	P	P
Carboxylic acids									
Malic acid		133,012	4,36	P		P			
Citric acid		191,0192	4,44	P	P	P	P	P	P
α-Ketoglutaric acid		145,0136	5,83	P	P	P			
Hydroxy glutarate		147,0295	6,30		P	P			
Succinic acid		117,0188	6,38	P	P	P			
Maleic acid		115,0037	6,56	P		P			
Fumaric acid		115,0036	6,71	P	P	P			
Hydroxybutyric acid		103,0400	6,98			P			
Phenols									
Gallic acid		169,0133	8,78			P			
Quinic acid		191,0547	12,07	P	P	P			
Caffeoyl glucarate isomer		371,0577	16,74		P	P			
Gallic acid 3-O-(6-galloylglucoside)		483,0757	19,42				P	P	P
Caffeoyl glucuronide		355,0628	19,49		P	P			
Caffeoyl glucuronide isomer		355,0628	19,89		P	P			
Caffeoyl glucuronide isomer		355,0628	20,15		P	P			
Caffeoyl-hydroxycitric acid		369,0423	20,73	P	P	P			
Catechin		325,0478	20,80				P		P
Chlorogenic acid		353,085	20,93		P	P			
Caffeoyl glucuronide isomer		355,0628	21,86	P	P	P			
Caffeoyl-hydroxycitric acid		369,0423	23,67	P	P	P			
Epicatechin		289,0702	23,70				P		P

Epigallocatechin gallate	459,1054	457,0741	24,70						P
Mirecitine 3-rutinoside		625,1373	27,44				P	P	P
Rutin	611,1659	609,1395	28,97	P	P	P	P	P	P
Myristicin	465,1067	463,0752	29,41				P	P	P
Vicenin 2		593,1453	29,91				P	P	P
Apigenin	271,0644	269,0429	30,32	P	P	P			
Quercetin 3 arabinoside	435,0786	445,41	30,45				P	P	P
Quercetin 3 arabinoside	435,08	445.4000	30,61				P	P	
Quercitrin	449,1113	447,0909	30,66	P	P	P	P	P	P
Apigenin	271,0625	269,0434	31,75		P	P	P	P	P
Kaempferol 7-xyloside		417,0803	31,79				P	P	P
Quercitrin	191,02		31,92	P		P			
Kaempferol-3-O-α-rhamnopyranoside		431,0959	32,03				P	P	P
Geraldone	285,0793	283,0615	32,35				P	P	P
7,4'-Dihydroxyflavone		253,0495	34,09				P	P	P
Apigenin glucuronide methyl		465,1159	36,18				P	P	P
Cyanidin		285,0396	36,92				P	P	P
Quercetin-3-O-α-rhamnopyranoside		301,0337	37,32				P	P	P