The *Acacia* bark phytotoxic potential: a non-synthetic bio-herbicide

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Abstract

A natural technique to control weed emergence and decrease the use of synthetic herbicides could be the application of phytochemical substances with phytotoxic effects from *Acacia* bark. In Mediterranean area, *A. melanoxylon* and *A. dealbata* bark are an abundant waste material from non-native tree species control actions, requiring suitable disposal. The presence of phytotoxic substances in young harvested biomass is greater than in mature plant tissues, thus for organic substrates formulation aging treatment is only effective (phytotoxicity reduction after 8 weeks) for *A. melanoxylon* mature bark, which potentiate young bark for different valorization. This study explores *A. melanoxylon* and *A. dealbata* bark extracts from young debarked trees, regarding their bio-herbicide effect on germination and development of cress seeds. Extraction conditions were performed under fixed time (t: 45min) and a maximum temperature of 120°C, using the following solvents: ethanol, water and an equal volume of ethanol:water. The extract yield was greater in water extracts (40 g dry extract L^{-1}), followed by 50% ethanol:water (36 g dry extract L^{-1}) and ethanol (28 g dry extract L^{-1}). In water extract essay, the extract yield remained constant even after bark percentage gradual reduction over the extraction ratio conditions (liquid:bark ratio from 1:5, 1:10 and 1:15), suggesting a possible final liquor saturation. However, all bark extracts inhibited the tested seed growth (root index <8%) compared to deionized water control (root index = 100%). The bio-herbicide activity of water soluble *Acacia* bark phytochemical substances increases its phytotoxic effect as extract concentration increase.

Keywords: bark extracts, *A. melanoxylon*, *A. dealbata*, phytochemicals concentration, seed inhibition

INTRODUCTION

Weed infestation is considered a limiting factor for horticultural food production systems which decrease crop yield and therefore fruit yield. To feed the increasing global population and meeting the sustainable development goals, the use of synthetic herbicide for weed control must be reduced, while the search for alternative herbicide is growing worldwide (Mohammadi, 2013).

A natural and environmentally friendly method to control weed growth is the use of biochemicals produced from specific species which have a phytotoxic effect on target weeds, so called allelopathic phenomena (Jelassi et al., 2016). Most allelochemicals are secondary metabolites inherent to native species and activated for plant self-defense, which are commonly presented in external plant tissues (Feng et al., 2013). Tree barks contain great amount of those phytochemicals, thus making them potential alternative weed control options (Ogawa and Yazaki, 2018).

In Southern Mediterranean countries, some ecosystems are currently under Australian *Acacia* invasion risk (Vicente et al., 2013). *A. melanoxylon* and *A. dealbata* bark are an abundant waste material from non-native tree species control actions (e.g. ring-barking control method), requiring suitable disposal (Carneiro et al., 2013). A possible valorization of

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this biomass has been analyzed, and previous research (Chemetova et al., 2019; Souza-Alonso et al., 2018) revealed *Acacia* bark suitability as natural weed control. The allelopathic effect was attributed to the presence of naturally produced compounds such, phenolics, flavonoids and tannins (Chemetova et al., 2019; Ogawa and Yazaki, 2018).

The natural allelochemicals are mostly water-soluble, although different chemical composition resulted when extraction is fractioned among solvents of increasing polarity (Feng et al., 2013). A common biological essay for plant allelochemicals phytotoxicity activity evaluation are the germination tests using model-sensitive species (e.g. *Lepidum sativum* and/or *Lactuca sativa*) (Lorenzo et al., 2016). The application of *Acacia* bark extracts is a promising approach due to its simplicity, easy extraction method and ready availability of raw-material, however the minimum concentration of the extract that has phytotoxicity activity must be determine (Mohammadi, 2013).

The objective of this research was to evaluate the potential of *A. melanoxylon* and *A. dealbata* bark extracts from juvenile debarked trees, regarding their bio-herbicide effect on germination and growth of cress seeds.

MATERIALS AND METHODS

A. dealbata and *A. melanoxylon* bark collection was conducted during the winter season 2017-2018. Both barks were sampled from juvenile tree stands (up to 6 years old). *A. melanoxylon* bark was collected from Tapada da Ajuda, the Instituto Superior de Agronomia (ISA) Campus (38°42'27.5"N, 9°10'56.3"W) (Lisbon, Portugal), while *A. dealbata* was collected from Mata Nacional do Bussaco (40°22'36.7"N 8°22'05.4"W) (Luso, Portugal).

The bark samples were collected separately after on-site ring-barking method application, air-dried and ground in a knife mill (Fritsch pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany) with an output sieve of 10×10 mm^2, followed by 2×2 mm^2. The 0.4-0.25 mm fraction was used for extractive content chemical analysis, while the coarser bark from output sieve of 10×10 mm^2 was used for testing bio-herbicide effect.

The dry mass content was assessed by oven-drying samples at 105°C for 24 h, and the ash content was determined by combustion of the oven-dried sample at 550°C for 5 h in a muffle furnace, according to TAPPI Standard Methods (T211 om-02). Samples (around 3.5 g dry mass) were full extracted using a solvent sequence with crescent polarity (dichloromethane, ethanol, and water) in a Soxhlet apparatus (T204 cm-07) for total extractives determination. The extraction analysis was done in duplicate and results were reported as percentage of the dry mass material.

For bio-herbicide effect evaluation, the extraction was performed using a stainless-steel reactor (max ca. 4 L) with fluid recirculation. The conditions were set according to previous pre-tests (data not shown) under fixed time 45 min and a maximum temperature of 120°C using ethanol, water and an equal volume of ethanol water as solvents. For water extraction, different liquid-to-solid ratio (R) were tested: R1:5, R1:10 and R1:15. From the obtained liquors, solvents were left to evaporate, and distilled water was added as dissolution agent.

Phytotoxicity of extracts was evaluated using *Lepidium sativum* as model plant. According to European Standards (CEN, 2011), a total of 10 seeds were place on top of Petri dish filled with perlite and a filter paper impregnated with 50 mL of the water dissolved extracts (0.25, 0.5 and 1.5 g per Petri dish), the incubation was performed at room temperature (25°C) in the dark, for 3 days, and water as a control. The allelopathic effect was evaluated based on the average germination rate (GR), and root length (RL) to calculate root index (RI).

RESULTS AND DISCUSSION

Both *Acacia* barks presented equal ash content, around 3% dry basis, however extractives content was greater in *A. dealbata* compared to *A. melanoxylon* bark, 36 and 29% dry basis, respectively. According to Taflick et al. (2017), exhausted *Acacia* bark showed 26% of extractives even after the industrial hot water-based process for tannin extraction.

Figure 1 describes the extractives distribution, within the total extracted content (% total extractives), by solvent. In both *Acacia* species, ethanol extracts showed higher yields, three times greater than water extracts, and dichloromethane extracts registered the lowest yields, meaning that the most of *Acacia* extractives were composed by hydrophilic compounds, while lipophilic compounds were residual.

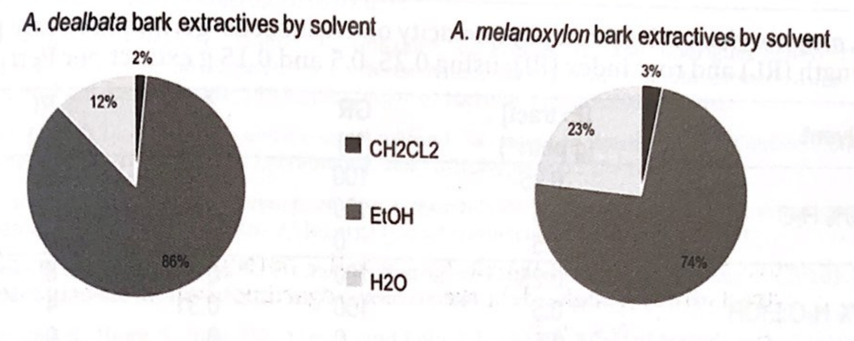

Figure 1. Soxhlet extraction: *A. dealbata* (left) and *A. melanoxylon* (right) bark extractives content (% total extractives) by solvent: dichloromethane (CH_2Cl_2), ethanol (EtOH), and water (H_2O).

The stainless-steel reactor extraction data focuses on *A. melanoxylon* bark as a representative species for further determination of the minimum concentration of *Acacia* extract that has bio-herbicide activity. Table 1 presents total water (100% H_2O) extract (g L^{-1}) variation with decreasing bark material in the reactor, and different solvents for the higher liquid-to-solid ratio R 1:5. The extract saturation may occur among the water extraction even after bark percentage successive reduction over the extraction ratio conditions successive reduction, from R1:5 to R1:15, the final extract difference was less than 12%, respectively, using same reaction condition. *Acacia* is widely known as tannin rich species (Souza-Alonso et al., 2018), and water is more suitable solvent for extracting tannin from the bark than ethanol (Taflick et al., 2017).

Table 1. Stainless-steel reactor extraction: *A. melanoxylon* bark total extract (g L^{-1}) using liquid-to-solid ratio (R1:5, R1:10 and R1:15); and water (100% H_2O), water:ethanol (50% EtOH:H_2O), and ethanol (100% EtOH) solvents.

Solvent	Liquid:solid ratio		
	R1:5 400 g:2000 mL	R1:10 200 g:2000 mL	R1:15 133 g:2000 mL
	Bark extracts (g L^{-1})		
100% H_2O	40.2	37.0	35.5
50% EtOH:H_2O	35.7	-	-
100% EtOH	27.5	-	-

Between solvents at R1:5, water presented the highest yield (40 g L^{-1}), while ethanol reduces the total final extract, reaching minimum of 28 g L^{-1} in 100% EtOH. Figure 1 reports ethanol as a solvent with greater extractive content, however, this finding may only be attributed to sequence of soxhlet extraction; while, when bark is extracted only one time (Table 1), there is evidence that water can extract most of semi-polar and polar substances. The influence of particle size may be also considered between extraction methods. Literature findings (Sowndhararajan et al., 2015) highlighted that polar solvents are responsible for the higher yields.

Phytotoxicity of extracts from R1:5 ratio extraction is presented in Table 2. All extracts presented an allelopathic effect on tested seeds (RI≤12%). Up to 0.5 g extract Petri^{-1} all seeds germinated (GR=100%), however the bio-herbicide activity of soluble substances rises its phytotoxic effect as extract concentration increases. The solvents 50% H_2O:EtOH and 100%

H_2O had a quicker bio-herbicidal effect among the concentration increment, reaching cress seed inhibition (GR and RI = 0%) at extract concentration 1.5 g Petri^{-1}, while ethanol allows cress seed germination (GR=86%) and showed the highest cress growth.

Table 2. *A. melanoxylon* bark extract phytotoxicity on cress seeds: germination rate (GR), root length (RL) and root index (RI); using 0.25, 0.5 and 0.15 g extract per Petri dish.

Solvent	[Extract] (g petri^{-1})	GR (%)	RL (cm)	RI (%)
100% H_2O	0.25	100	0.59	8
	0.5	100	0.23	3
	1.5	0	0	0
50% H_2O:EtOH	0.25	100	0.64	8
	0.5	100	0.31	4
	1.5	0	0	0
100% EtOH	0.25	100	0.88	12
	0.5	100	0.83	11
	1.5	86	0.45	6
Control H_2O	-	100	7.55	100

Acacia bark water soluble extract had greater yield (Table 1) and combining its inhibitory effect at minimum extract concentration of 30 g L^{-1}, could be a promising bio-herbicide which, simultaneously, promotes sustainable management of juvenile *Acacia* tree stands, and may alleviate the costs of invasive plant control.

CONCLUSIONS

A. melanoxylon and *A. dealbata* bark can be extracted using polar solvents. Water can extract most of semi-polar and polar substances. For the studied conditions, the extract saturation may occur among the water extraction even after bark ratio successive reduction. All bark extracts were phytotoxic for tested cress seeds. The bio-herbicide activity of water-soluble substances rises its phytotoxic effect as extract concentration increase, and 30 g L^{-1} extract may establish the minimum concentration of *Acacia* extract required for full cress seeds germination and growth inhibition. The tested innovative bio-herbicide approach contributes for synthetic herbicide replacement using natural allelochemical, which also addresses agroecological value of sustainable control and management of non-native plants.

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