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Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species

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ABSTRACT

This study was conducted to evaluate the phytochemical content and allelopathic potential of various organic extracts from seeds and pods of three Tunisian species of *Acacia* (*Acacia cyclops*, *Acacia mollissima* and *Acacia cyanophylla*) which was evaluated on lettuce germination and seedling growth. The total flavonoid contents in the organic extracts were closely ranged (3.1–24 mg RE/100 g DW), *n*-butanol extracts of Acacia pods were the richest ones in phenol and condensed tannin contents. For allelopathic activity, the EtOAc extract of seeds from *A. cyanophylla* inhibited the germination of the target seeds. The seedling growth was inhibited by all organic extracts with disproportion. The chromatographic separation methods led to isolation of a triterpenoid saponin named mollisside B from *A. cyclops* pods *n*-butanol extract. The allelopathic effect of this compound was evaluated on *Lactuca sativa* L. At a concentration of 200 μg/mL, the isolate has influenced on seedling growth with percentages of inhibition of 63.68% and 88.21% for shoot and root length, respectively.

1. Introduction

During the last two decades, the science of allelopathy has attracted a great number of scientists from the diverse fields world wide and is now viewed with multifaceted approach (Rice, 1984; Putnam and Tang, 1986; Inderjit Dakshini and Foy, 1999; Singh et al., 2001). Allelopathy intervenes in agriculture and the quality of food production for humans. It interferes on reducing environmental damage and health hazards from chemical inputs, minimizing the soil erosion and reducing reliance on synthetic herbicides (Min et al., 2003).

Many plants synthesize toxic substances for defense against other plants and microorganisms including viruses, bacteria and fungi. Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. It is a natural and environmentally friendly technique that might prove useful in controlling weeds, increasing crop yields, and decreasing the use of synthetic pesticides. These biochemicals are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms (El ayeb et al., 2013). Most of the allelochemicals that are classified as plant secondary metabolites are generally considered as not playing any role in primary metabolic processes for plant survival. Some allelochemicals are intermediates for lignifications and can also activate plant defense after exposure to pathogens (Omezzine et al., 2013).

Acacia is the second largest genus in the leguminosae family (Sulaiman et al., 2013), comprising more than 1350 species worldwide (Nasri et al., 2012), with members found in almost all habitats, approximately 800 are found in Australia, 130 in Africa and 20 in India. Although the genus *Acacia* is quite large and widespread in the warm subarid and arid parts of the world, relatively little is known about the chemistry of most species. *Acacia* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases. This plant contains variety of bioactive components such as phenolic acids, alkaloids, terpenes, tannins and flavonoids which are responsible for numerous biological and pharmacologi-

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cal properties like hypoglycemic, anti-inflammatory, anti-bacterial, anti-platelet aggregatory, anti-hypertensive, analgesic, anticancer, and anti-atherosclerotic (Sulaiman et al., 2013).

Members of the genus *Acacia* have been reported to contain saponins, in fact, many species have been examined for these substances. The presence of saponins has been reported from *Acacia pulchella*, *Albizia anthelmintica*, *Atethmia sinuata* (syn. *concinna*) and *Acacia delibrata*. A triterpenoid trisaccharide, acaciaside and a phytosterol, α-spinasterol, have been characterized from *Acacia auriculiformis*. Extraction of the leaves of *Acacia myrtifolia* yielded three triterpene glycosides: myrtifoliosides A, B and C. Recently several saponic triterpene glycosides from *Acacia victoriae* have been examined for their ability to decrease tumor cell proliferation and to induce apoptosis (Seigler, 2003).

These compounds have been associated with a variety of biological activities including allelopathy, poor digestibility in ruminant (Oleszek, 1996), deterrence to insect foraging (Tava and Odoardi, 1997) and beneficial antifungal properties (Nagata et al., 1985). Further, plant species containing saponins have long been used in folk medicine and have been shown to possess anti-inflammatory, hemolytic, cholesterol lowering and anticancer properties (Jurzysta and Nowacki, 1979; Malinow et al., 1982; Waller and Yamasaki, 1996).

Several studies also investigated the allelopathic potential of saponins. For example, Oleszek (1996) evaluated the allelopathic activity of saponins from *Medicago sativa* against wheat and effects on seedling growth were observed. Also, Waller and Yamasaki (1996) assayed soyasaponin I, isolated from mungbean (*Vigna radiata*), pointing out an autotoxic effect on shoot elongation and a stimulating effect on shoot and root growth of *Lactua sativa* L.

The present study was conducted to investigate whether the profiles of flavonoids, phenols, condensed tannins and saponins varied among various extracts obtained from pods and seeds of three Tunisian species of *Acacia*, *Acacia cyclops*, *Acacia mollissima* and *Acacia cyanophylla* and if there are correlations between them and allelopathic potentialities. The allelopathic activity of organic extracts was evaluated on lettuce (*L. sativa* L.).

The *n*-butanol extract of pods from *A. cyclops* rich in saponins led to the isolation of an allelochemical of which the potential allelopathic was also evaluated.

2. Material and methods

2.1. Plant material

Mature pods and seeds of three species of *Acacia* namely *A. cyclops*, *A. mollissima* and *A. cyanophylla* were collected in December 2010 from three different regions in Tunisia, Beja (a city of the north-west of Tunisia with a semi arid climate), Kelibia and Hammamet (coastal cities of the north-east of Tunisia with a Mediterranean climate). A voucher specimen of each species has been deposited at the Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Faculty of Science of Monastir, Tunisia (*A. cyclops* (A.cy-10), *A. mollissima* (A.m-10) and *A. cyanophylla* (A. cyn-10)).

Pods and seeds were separated then hand-picked to eliminate damaged ones and the selected ones were dried in shade for three days, carefully cleaned and grounded to powder.

Table 1
Yield, in percent of dry matter, of organic extracts of the different parts of *A. cyclops*, *A. mollissima* and *A. cyanophylla*.

Plant part	Solvent extract	Plant	% Yield
Seeds	EtOAc	*A. cyclops*	0.69
		A. mollissima	0.27
		A. cyanophylla	0.04
	BuOH	*A. cyclops*	2.51
		A. mollissima	0.71
		A. cyanophylla	0.28
Pods	EtOAc	*A. cyclops*	0.91
		A. mollissima	0.83
		A. cyanophylla	0.78
	BuOH	*A. cyclops*	7
		A. mollissima	5.2
		A. cyanophylla	4.3

2.2. Organic crude extract

2.2.1. Organic crude extract preparation

Pods and seeds of *A. cyclops*, *A. mollissima* and *A. cyanophylla* were dried, ground then extracted with acetone-water mixture (1:1) at 25 °C. The aqueous solutions thus obtained after evaporation solvent under vacuum, were successively extracted with ethyl acetate (EtOAc) and *n*-butanol (BuOH) to yield the corresponding extracts (Table 1).

2.3. Phytochemical analysis of organic extracts

2.3.1. Determination of total flavonoid contents (TFC)

Total flavonoid content in each organic extract was determined using a spectrophotometric method (Lamaison et al., 1991) with slight modifications, based on the formation of the complex flavonoid-aluminum. To 0.5 mL diluted organic extract (1 mg/mL in methanol), 0.5 mL of 2% aluminum chloride ($AlCl_3$) dissolved in methanol was added. The sample was incubated for 15 min at room temperature and the absorbance of the reaction mixture was mea-

Table 2
Total flavonoids (TFC), total phenolic (TPC), total condensed tannins (CTC) contents and saponins in different organic extracts of *A. cyclops*, *A. mollissima* and *A. cyanophylla* plant parts.

Plant part	Solvent extract	Plant	TFC (RE)*	TPC (GAE)*	CTC (CCE)*	Saponins**
Seeds	EtOAc	*A. cyclops*	7.25 ± 0.54^{a}	12.84 ± 0.96^{b}	12.55 ± 1.96^{a}	132.34^{c}
		A. mollissima	15.25 ± 0.56^{b}	11.78 ± 0.46^{b}	34.56 ± 1.52^{b}	77.45^{a}
		A. cyanophylla	3.60 ± 0.00^{a}	2.63 ± 0.08^{a}	8.38 ± 0.59^{a}	69.56^{a}
	BuOH	*A. cyclops*	4.20 ± 1.66^{a}	43.73 ± 0.54^{c}	89.36 ± 1.42^{c}	197.76^{d}
		A. mollissima	24.13 ± 0.98^{c}	43.89 ± 0.76^{c}	171.82 ± 2.0^{e}	82.89^{b}
		A. cyanophylla	3.10 ± 0.09^{a}	14.83 ± 0.95^{b}	78.40 ± 1.58^{c}	74.91^{a}
Pods	EtOAc	*A. cyclops*	15.55 ± 1.23^{b}	76.96 ± 0.78^{d}	121.94 ± 2.57^{d}	161.08^{c}
		A. mollissima	14.67 ± 0.81^{b}	62.37 ± 0.80^{d}	97.94 ± 2.35^{c}	91.82^{b}
		A. cyanophylla	14.99 ± 0.40^{b}	65.12 ± 2.51^{d}	125.84 ± 1.80^{d}	89.29^{b}
	BuOH	*A. cyclops*	20.83 ± 2.39^{c}	426.36 ± 2.99^{e}	2518.60 ± 1.98^{h}	285.65^{e}
		A. mollissima	15.05 ± 0.63^{b}	348.63 ± 3.90^{d}	1516.32 ± 2.94^{g}	101.03^{b}
		A. cyanophylla	12.58 ± 0.20^{b}	318.26 ± 1.38^{d}	1348.60 ± 2.16^{f}	93.50^{b}

RE rutin equivalents,GAE gallic acid equivalents, CCE cyanidin chloride equivalents.
Means in each column followed by different letters are significantlydifferent ($p < 0.05$).
** mg saponins/g organic extract.