

Selective herbicidal activity of an indole alkaloid isolated from *Rhazya stricta* Decne. growing in Saudi Arabia

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Summary The herbicidal activity of the alkaloidal constituents of *Rhazya stricta* Decne. growing in Saudi Arabia was studied on wild radish, Italian rye grass, alfalfa and wheat. The biological activity was determined after a series of bioassay-guided fractionations of a crude alkaloidal sample taken from the plant. The active compound was isolated in a pure form using medium pressure liquid chromatography (MPLC) and high performance liquid chromatography (HPLC). It was subjected to compound identification using mass spectroscopy. Fragment ions similar to those published on 1,2 dehydroaspido-spermidine were identified. The results show that this compound has the ability to inhibit the growth of wild radish, a common weed, without affecting the early seedling growth of major crops such as wheat and alfalfa.

Keywords *Rhazya stricta*, 1,2 dehydroaspido-spermidine, indole alkaloids, herbicidal activity, wild radish, alfalfa, wheat.

INTRODUCTION

Rhazya stricta Decne. is one of the dominant species in the rangeland of Saudi Arabia. Through allelopathic activity, it has been shown to inhibit seedling growth (Allred 1968, Assaeed 1996 and Assaeed and Al-Doss 1996). This phenomenon has encouraged the search for and the development of natural products that have pest-controlling activities (Cardelling 1988, Narwal 1996, Chou and Chou 1999). Several studies found that alkaloidal extracts from plants have herbicidal activity (Wink 1983, Petroski *et al.* 1990, Wink and Latz-Bruning 1995, Elakovich and Yang 1996).

R. stricta contains many alkaloidal compounds (Chatterjee *et al.* 1974, Hassan *et al.* 1977, Atta-ur-Rahman *et al.* 1989 and 1991) that are able to affect the growth of pests such as *Culex pipiens* (El-Hag *et al.* 1996), nematodes e.g. *Meloidogyne javanica* (Al-Rajhi *et al.* 1997) and weeds (Al-Mutlaq 2001, Al-Mutlaq *et al.* 2002). In previous studies, Al-Mutlaq (2001) and Al-Mutlaq *et al.* (2002) reported that *Rhazya stricta* leaf leachates and total alkaloidal extracts affected seedling development of alfalfa (*Medicago sativa*), wild radish (*Raphanus sativus*) and Italian ryegrass (*Lolium multiflorum*) but not wheat (*Triticum aestivum*).

The aim of this work was to isolate and identify the compound (s) responsible for herbicidal effects on weeds from the total alkaloidal extract of *Rhazya stricta*.

MATERIALS AND METHODS

Experiments were conducted in the Pesticide Laboratory, College of Food and Agricultural Science, King Saud University. Seeds of Italian ryegrass, alfalfa, wild radish and wheat were used in this study.

Extraction and purification Twenty five kg of fresh leaf material were air-dried, powdered, and macerated twice in 90% aqueous ethanol. After filtration, the ethanolic extract was evaporated under reduced pressure and the residue dissolved in 300 mL of a solution of 10% aqueous ethanol. This solution was extracted three times with 100 mL petroleum ether (boiling range of 60–80°C). The remaining aqueous layer was treated with 10% acetic acid until a pH of 3 was achieved and partitioned with methylene chloride. The aqueous layer was rendered alkaline with 25% ammonium hydroxide until a pH of 11 was achieved after which the precipitated crude alkaloids were filtered, dissolved in ethanol, and evaporated. The alkaline filtrate was further extracted with methylene chloride. The organic layer was added to the precipitate. A bioassay of this extract showed significant inhibition in the growth of seedlings of wild radish, a moderate inhibition of alfalfa and Italian rye grass seedlings and slight inhibition of wheat seedlings.

Thin layer chromatography of the total alkaloidal extract on silica gel (20 × 20 cm), developed with *n*-hexane:methylene chloride:methanol (in proportions of 65:25:10), and visualised with Dragendorff reagent, revealed the presence of numerous alkaloidal spots. The extract was then subjected to vacuum liquid chromatography (VLC) on silica gel (for TLC) and eluted with 10% methylene chloride in hexane, followed by 100% methylene chloride, and finally with 5% methanol in methylene chloride. A bioassay of these three fractions showed that the inhibition of radish seedling growth was the greatest in the fraction eluted with 5% methanol in methylene chloride.

Further fractionation of the previous fraction by medium pressure liquid chromatography (MPLC) on Si gel and elution with a gradient of methanol in methylene chloride from 1% to 10%, gave three groups of fractions (A, B, and C). A bioassay of the three fractions showed that fraction A exhibited the greatest inhibition of radish seedling growth.

Finally, fraction A was subjected to a Waters high performance liquid chromatograph (HPLC) on a Novapak C-18 column (15 cm × 4.5 mm), eluted with methanol:water (in the ratio of 6:4) and detection was carried out using ultra violet light at 254 nm, which resulted in the isolation of five substances. A bioassay carried out on these substances indicated that substance one was the most active and selective in the inhibition of the growth of wild radish seedlings without affecting the growth of seedlings of the other plants.

Substance one (1) was then injected into an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector in the EI mode at 70 eV and gave one peak with ion fragments at m/z 280 (M^+), 251, 210, 194, 149, 125, 97, 83, and 56.

Bioassay test Seedling growth was tested by adding 5 mL of each tested sample (total alkaloids, fractionated alkaloids, and compound one) to a 9 cm diameter Petri dish containing a single filter paper of Whatman No. 1. Five uniformly sized seeds of each plant were distributed on the filter paper and the dish was covered with its lid and was kept at $25 \pm 1^\circ\text{C}$ for seven days. Samples were prepared by dissolving the alkaloidal fractions in the least amount of a mixture of 1% Tween 80 in ethanol, then completing the volume of 5 mL with distilled water, so that the final tested concentration was 150 ppm. In addition, control samples were similarly made by adding 0.5 mL of 1% Tween 80 in ethanol solution to 4.5 mL distilled water. Another control sample in water only was carried out. The experiment included three replicates of each sample. The root and shoot lengths were measured for the best four grown seedlings of each treatment. Probit analysis of the results was carried out according to Finney (1971). Regression analysis was used to fit shoot and root response to alkaloidal concentration using SAS programming (SAS 1988).

Table 1. Effect of the isolated compounds (1–5) on the lengths of the roots and shoots of wheat, alfalfa, Italian rye grass and wild radish seedlings after seven days of treatment.

Treatments	Root length in cm			
	Wheat	Alfalfa	Wild radish	Italian rye grass
Control	9.8 a*	4.4 a	7.7 a	7.8 ab
Blank	11.1 a	4.7 a	7.2 a	9.0 a
1**	7.2 b	4.7 a	2.3 c	7.3 b
2	6.9 b	2.7 a	4.9 b	5.7 c
3	3.9 c	2.5 b	0.4 d	2.0 d
4	10.5 a	4.0 a	1.5 cd	8.3 ab
5	8.8 a	4.2 a	1.3 cd	8.5 ab
LSD (0.05)	2.4	0.8	1.2	1.5
Treatments	Shoot height in cm			
	Wheat	Alfalfa	Wild radish	Italian rye grass
Control	8.4 a	2.7 ab	3.1 a	6.3 ab
Blank	7.7 abc	2.8 ab	2.7 a	6.6 a
1	7.1 bcd	2.4 b	1.3 b	6.0 abc
2	6.8 cd	2.6 b	2.8 a	5.7 abc
3	6.2 d	1.7 c	0.4 c	3.4 d
4	7.9 ab	2.8 ab	1.3 b	5.1 c
5	7.7 abc	3.1 a	1.0 bc	5.5 bc
LSD (0.05)	1.0	0.4	0.6	1.1

* Denote that numbers with the same letter in a column are not significantly different at $P < 0.05$.

** Concentrations of the extracted compounds = 150 ppm.

RESULTS

The active compound (1) was obtained in a partially pure form after a series of bioassay-guided fractionations of the crude alkaloidal fraction of *R. stricta*. VLC of the total alkaloids showed that the fraction eluted with 5% methanol in methylene chloride was the most active on wild radish seedlings. This fraction revealed several alkaloidal spots on TLC and was further fractionated using MPLC resulting in an active fraction that was eluted with 1% methanol in methylene chloride. HPLC of this fraction gave five pure compounds of which compound one was the most active and selective on wild radish seedlings in comparison with the growth of seedlings of wheat, alfalfa, and Italian rye grass. Table 1 shows that 150 ppm of compound one reduced the length of wild radish shoots and roots to 1.0 and 1.3 cm respectively in comparison with the control (3.1 and 7.7 cm respectively). The effect of this compound on the other seedlings was not significant in comparison with the control.

The mass spectrum of compound one showed the molecular ion at m/z 280, and fragment ions at 251, 210, 194, 149, 125, 97, 83, and 56.

DISCUSSION

Rhazya stricta is a native plant in sub-tropical areas especially in the Indian sub-continent. It is famous for the presence of indole alkaloids which have been shown to possess certain biological activities such as anticancer activity (Mukhobpadhyay *et al.* 1981 and 1983). Studies on the plant growing in Saudi Arabia showed that a total alkaloids fraction was selectively effective against the growth of alfalfa, and Italian ryegrass and not on wheat seeds (Al-Mutlaq *et al.* 2002). This study was therefore conducted to isolate and identify the compound(s) responsible for this herbicidal activity.

The molecular ion peak (M^+) of the mass spectrum of the isolated compound one corresponds with the molecular formula $C_{19}H_{24}N_2$ and fragment ions at 251, 210, 194, 149, 125, 97, 83, 56 which were similar to those published on 1,2 dehydroaspido-permidine (Figure 1, previously isolated from *R. stricta*, growing in India (Atta-ur-Rahman and Zaman 1986). Therefore, this compound one will be one of the compounds responsible for the herbicidal activity of *R. stricta*.

Table 1 shows the effect of the isolated compounds (1–5) on the seedling growth of each of wheat, alfalfa, Italian rye grass and wild radish. The results show that although all of the isolated compounds inhibited the growth of all types of seedlings, only compound one was very selective on wild radish with no effect on wheat, alfalfa and Italian ryegrass. Our previous studies (Al-Mutlaq 2001, Al-Mutlaq *et al.* 2002) have

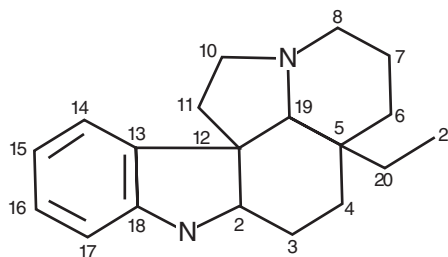


Figure 1. Structure of 1,2 dehydroaspido-permidine.

indicated that the plant extract and its total alkaloids were effective against the growth of wild radish, Italian ryegrass and alfalfa seeds, and were not effective on wheat. This study determined that one compound was controlling wild radish seedling growth. Other indole alkaloids than compound one appear to be responsible for the inhibition of Italian ryegrass and alfalfa seeds as indicated by Al-Mutlaq (2001) and Al-Mutlaq *et al.* (2002).

The selective herbicidal activity of 1,2 dehydroaspido-permidine on wild radish without affecting the growth of wheat, Italian rye grass and alfalfa is the first record for this compound. Therefore, this compound may be used as a promising selective herbicide for dicotyledonous plants in wheat and alfalfa crops.

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