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Cobra-repellent activity of *Eryngium foetidum* L. (Andu) and *Aquilaria agallocha* (Agil)

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Abstract

Foliage of Andu, *Eryngium foetidum* L., is traditionally used in Sri Lanka to repel cobras, *Naja naja naja* L. Application of aqueous suspension of crushed Andu leaves, spreading dried leaves and growing Andu plant in agricultural lands and residential areas are the traditional practices used to repel cobras. Burning of Agil wood is also used traditionally in Sri Lanka to repel cobras. These traditional practices indicate the presence of compounds in Andu and Agil that may have a repellent activity against cobra. Therefore, identification of volatile repellents present in Andu and Agil plants might possibly lead to a semiochemical based management program to control cobra population. Active compounds present in Andu and Agil were collected by steam distillation. Repellent activity of extracts obtained from steam distillation was assayed against cobra using a two-choice laboratory bioassay. A dilution series of the extracts (20, 10, 1, 0.1 and 0.01 mg/ 25 μL pentane) was prepared using pentane. For each bioassay, two baits (one day old chicks) were treated, one with 25 μL of the test extract and the other with 25 μL of pentane. It was visually observed that whether the cobra uses bait stations as prey within 15 minute time period. Each treatment was replicated five times. Bioassay showed that steam-distilled extracts from both plants associate with very strong repellent activity on cobra. Dose-response bioassay showed that the maximal activity associates with 0.1 mg equivalent of steam distillate of Andu and 1 mg equivalent of steam distillate of Agil. Thin layer chromatographic and gas-liquid chromatographic analysis of steam distillates showed that the extracts contain more than one compound. Therefore, the repellent activity of Andu and Agil may be associated with one or more compounds present in steam distillate.

Introduction

Cobra, *Naja naja naja* L. (Elapidae), is one of the deadly poisonous snakes in Sri Lanka (Wall, 1993). Venom of cobra mainly acts as a neurotoxin (Deraniyagala, 1955). Cobra can be found almost anywhere. Cobras live in holes, clumps of grass and dens of small mammals such as porcupine, hare, hedgehog, rats and mice. As cobra feeds on rodents and insects, they can be found quite often in agricultural areas especially in paddy fields.

Cobra also attacks poultry farms and feeds on eggs and chicks. Therefore, a high cobra population is a danger and a nuisance to mankind and animals. However, cobra plays an important role in the environment as well as in agriculture. By feeding on insects and rodents, cobras help in biological controlling of insects and rodent population and thus keep the ecosystem in balance. They are rampantly hunted for their skin. Their skin has a great commercial value and is used in the manufacture of shoes, purses, belts and other decorative pieces. Moreover, cobra venom is a potential source for medicines such as anti-venom (*http://www.cobras.org*). Hence, controlling and conservation of cobra population is very important. In search for new control tactics, it is thus appropriate to investigate traditional methodologies and to determine scientific basis of their action.

Since ancient times, control of cobra population was done by mechanical killing. This severely affects the environmental integrity. Foliage of Andu, *Eryngium foetidum* L. (Apiaceae), is traditionally used in Sri Lanka to repel cobras (B. Weerapperuma, Embilipitiya, Sri Lanka, pers. com.). Farmers spread a suspension of crushed Andu leaves in paddy fields, especially during the harvesting season to keep cobras away. Burning of Agil, *Aquilaria agallocha* wood and using their aromatic fumes have also been used to repel cobras (H.M. Gunapala, Maspotha, Kurunegala, Sri Lanka, pers. com.) as in Chilaw and Anuradhapura districts, farmers add Agil wood to their fires set at night in their cultivation lands to keep animals away. On this basis, we hypothesized that volatile repellents are present in Andu and Agil plants and their identification might possibly lead to a semiochemical based management program to control cobra population.

Materials and Methods

A. Source of active material

Andu plants were collected from Imbulpe, Balangoda. Arial parts of matured plants were taken. Immediately after collection, plants were stored in sealed polythene bags and kept in a freezer at -10 °C to minimize the loss of volatiles and to prevent microbial growth.

Mature Agil wood was collected from Sithulpawwa area. The wood was dried and stored in polythene bags. Prior to use the wood was cut into small pieces, dried and ground into a powder.

B. Source of test animals

Test animals were newly captured cobra, *Naja naja naja* L., of mix sex and age. They were collected from different places of Sri Lanka and kept in captivity at the National Zoological Gardens, Dehiwala.

C. Extraction of active material

Steam distillation was the method of choice used to extract active material from both plants. Since crushed Andu leaves are used traditionally to repel cobras, it is reasonable to suspect that the active materials are volatile and hence steam distillation is the most appropriate method. For steam distillation, fresh Andu plants were cut into small pieces and exposed to live steam in a steam distillation apparatus. For Agil, powdered wood was exposed to live steam in the same manner. Since burning of Agil wood is the traditional practice to repel cobras, collecting the fumes by burning the wood would have been a better method than steam distillation. However, because of the difficulties associated with collecting fumes, steam distillation was carried out as the next best method. The distillate from steam distillation was collected and extracted into diethyl ether. Ether extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. Both extracts were stored in a refrigerator at -4 °C until used.

D. Bioassay of extracts

A bioassay was developed to test the behavioral activity of the two extracts against cobra. The bioassay method consisted of visually observing the behavioral responses of cobra to a bait station treated with extracts and comparing it to the responses towards a solvent-treated control station. Baits were one-day old chicks. Cages (8x5x5 ft) covered with a wire mesh were used as bioassay chambers. There was a window (1x1 ft) for each cage through which test animals and bait stations can be introduced. Prior to bioassay, a randomly selected (mix age and sex) cobra was kept in a cage and starved for seven days. A dilution series of the extracts was prepared using pentane. For each bioassay, two bait stations were treated, one with 25 µL of the test extract and the other with 25 µL of pentane. Two bait stations were placed in two opposite corners of the cage (~6 ft apart). It was visually observed that whether the cobra uses bait stations as a prey within 15 minute time period. Each treatment was replicated five times. Dose-response bioassay was performed for the two extracts to determine the minimum quantity required to elicit repellency for cobras and to indicate the potency of the extracts.

Significant differences of the proportion of cobra responses between the solvent control and extracts from steam distillation each were tested separately using Cochran's Q test (Zar, 1984).

E. Analysis of extracts

Extracts obtained from steam distillation were analyzed using thin layer chromatography (TLC) and gas-liquid chromatography (GLC).

Results and Discussion

Maximal activity in 15-minute bioassay was obtained with 0.1 mg equivalent of steam distillate of Andu and 1 mg equivalent of steam distillate of Agil (Table 1). Results showed that the proportion of baits treated separately with Andu and Agil extracts were less significantly consumed by cobras than those of their respective blank solvent controls ($p<0.05$, after Cochran's Q tests). These preliminary bioassay results clearly indicate that there is a strong repellent activity of Andu and Agil extracts against cobra. GLC and TLC analysis of steam distilled volatile extracts showed the presence of more than one compound.

Table 1:Dose-response relationships for cobra in laboratory bioassay to Andu and Agil plant extracts

Extract obtain from Andu					Extract obtain from Agil				
Dose mg/25 µL pentane	No. of baits Tested		No. of baits consumed		Dose mg/ 25 µL pentane	No. of baits Tested		No. of baits consumed	
	C	S	C	S		C	S	C	S
20	5	5	0	0	20	5	5	1	0
10	5	5	2	0	10	5	5	2	0
1	5	5	2	0	1	5	5	5	1
0.1	5	5	5	0	0.1	5	5	5	3
0.01	5	5	5	5	0.01	5	5	5	5

C- control S- baits treated with extracts

Conclusion

Appearance of more than one compound on TLC and GLC analysis indicates that the repellent activity can be associated with one or more compounds in the steam distilled volatile extract. Further analysis is needed to identify the active compounds.

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References

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