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RESEARCH ARTICLE

Angiotensin-converting enzyme inhibitory activity of *Viscum triflorum* is host plant-dependent

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Abstract

Context: *Viscum triflorum* DC. (Viscaceae) is a hemiparasitic plant used in traditional medicine on Réunion Island as a remedy to treat hypertension.

Objective: The *in vitro* angiotensin-converting enzyme (ACE) inhibitory activity of extracts of *V. triflorum* and the corresponding host plant species were examined to evaluate the use as a remedy against hypertension, and to investigate whether the host plants have an influence on the activity.

Materials and methods: Aqueous, ethanol and acetone extracts of 24 leaf samples of *V. triflorum* and the corresponding host plants, representing 10 plant species, were prepared. The ACE inhibitory activities of the extracts were measured by HPLC using dansyltriglycine as substrate.

Results and conclusion: Water extracts of *Viscum* samples from only two of the 10 host plants, namely *Acacia heterophylla* Willd. (Fabaceae) and *Sophora denudata* Bory (Fabaceae), showed significant inhibitory activity, ≥50% inhibition in a concentration of 0.33 mg crude plant extract in 1 mL test solution. From the two mentioned host plant species activity was only detected in the water extract from one of the six samples of *A. heterophylla*. Three host species showed pronounced activity without any detection of activity in the samples of *V. triflorum*. The results support the traditional use provided that *V. triflorum* is collected from *A. heterophylla* or *S. denudata*.

Keywords: ACE, réunion, traditional medicine, *Acacia heterophylla*, *Sophora denudata*

Introduction

The genus *Viscum* L. comprises about 150 species, all evergreen hemiparasitic plants rooted in the tissue of the host plants (Heide-Jørgensen, 2008). The best known species is the European *Viscum album* L. (Viscaceae). This species is used for several medical purposes and the main areas of therapeutic applications are against hypertension, arteriosclerosis, cancer, and arthrosis (Wagner et al., 1986).

It has been previously demonstrated that *V. album* from different host plant species have different biological activities. *In vitro* studies of *V. album* have shown a host plant-dependent anti-inflammatory effect (Yesilada et al., 1998), antioxidant activity (Vicaş et al., 2008); and three preparations produced from *V. album* from different host trees exhibited different effects on human leukemia cells (Hülsen et al., 1986).

Several species of *Viscum* and other species belonging to the Viscaceae and Loranthaceae families are used for medical purposes such as a remedy to treat hypertension. *Viscum triflorum* DC. (Viscaceae) is used for several purposes in traditional medicine on Réunion Island and infusions are among other things used as an antihypertensive (Lavergne and Véra, 1989; Lavergne, 1990). In Argentine folk medicine, *Ligaria cuneifolia* (R. et P.) Tiegh. (Loranthaceae) is used as a substitute for the European mistletoe and infusions of *L. cuneifolia* leaf and stem are used to treat high blood pressure (Fernández et al., 1998). The Japanese mistletoe, *Taxillus yadoriki* Danser (Loranthaceae) has been prescribed

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as a hypotensive or antidiabetic folk medicine in Japan (Fukunaga et al., 1989).

The use of varieties of *V. album* as a hypotensive remedy has been supported in *in vivo* studies. Crude aqueous leaf extract of *V. album* produced significant decrease in blood pressure in renal artery-occluded hypertensive rats, salt-induced hypertensive rats, and normotensive rats (Ofem et al., 2007) and aqueous leaf extract of Korean mistletoe, *V. album* L. var. *coloratum* Ohwi, was effective in the treatment of spontaneously hypertensive rats (Kim, 2006).

In a previous study, we have shown that different samples of the hemiparasitic epiphytes *Cassytha filiformis* L. (Lauraceae) and *V. triflorum* showed different ability to inhibit the angiotensin-converting enzyme (ACE) that plays an important role in the regulation of blood pressure and diuresis (Adsersen and Adsersen, 1997). We had no opportunity to examine the corresponding host plants.

V. triflorum is an epiphytic branch parasite with *Acacia heterophylla* Willd. (Fabaceae) and *Dombeya* species (Sterculiaceae) as the most common host trees, it is indigenous to Réunion Island. The objective of the present study was to investigate the *in vitro* ACE inhibitory activity of samples of *V. triflorum* and their corresponding host plants to address the following questions:
Does the activity of *V. triflorum* depend on the host plant species on which it grows?
Does activity of the host imply activity in *V. triflorum*?
Is the traditional use of *V. triflorum* as a remedy against hypertension justified?

Materials and methods

Plant material

Leaf samples were collected at Maido, Forêt de Bebour and Plaine de Chicots, Réunion Island, in February 1997. Samples of *V. triflorum* were collected from 24 host plants representing 10 different plant species, all endemic to Réunion Island, and air-dried immediately after the collection. Voucher specimens of *V. triflorum* and the host plants are deposited at the Faculty of Pharmaceutical Sciences, University of Copenhagen. Identification was done by Thierry Pailler, Laboratoire de Biologie Végétale, l'Université de la Réunion. The material was collected according to the general collection authorization to this laboratory. The plant material was analyzed at the Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen in 1997.

Preparation of crude extracts

Of the dried powdered plant material, 1 g was extracted with 10 mL water, ethanol (96%), or acetone separately for half an hour in an ultrasonic bath. The extracts were filtered and concentrated *in vacuo* except for the water extracts, which were freeze-dried. Test solutions were made by dissolving 1 mg dry extract in 1 mL HEPES assay buffer, or buffer with 10% ethanol or acetone, corresponding to a final concentration of 0.33 mg crude plant extract in 1 mL test volume.

ACE inhibitory activity assay

In vitro ACE inhibitory activity was measured as described by Elbl and Wagner (1991) and later modified by Hansen et al. (1995). Dansyl L-glutamic acid, dansylglycine, and ACE from rabbit lung (EC 3.4.15.1; 3 U/mg protein) were obtained from Sigma. Dansyl triglycine was synthesized in our laboratory as described by Hansen et al. (1995). Captopril was used as a positive standard; the IC_{50} value was 12.0 ± 2.6 nM.

Instrument

HPLC determination of ACE inhibitory activity was performed using a Shimadzu LC 10 AS pump, a Shimadzu SIL 10 A injector, a Shimadzu SPD 10 A UV spectrophotometric detector, a Shimadzu SCL 10 A system controller, and a Shimadzu C-R 6A chromatopac integrator.

Results

Samples were considered active if ACE inhibition was 50% or more in one of the three extracts in a concentration of 0.33 mg crude extract in 1 mL test solution. Results are presented in Table 1.

Viscum samples from two of the 10 host plant species showed pronounced ACE inhibitory activity in the water extracts. All six samples of *V. triflorum* with *A. heterophylla* as host plant showed activity with inhibition in the range 64–87%. Only one of the corresponding host plants showed activity with 52% inhibition in the water extract. *V. triflorum* with *Sophora denudata* Bory (Fabaceae) as host was active in the water extract with 86% inhibition; there was no inhibition in the extracts from the host.

Pronounced ACE inhibitory activity was detected in the host species *Monimia rotundifolia* Thou. (Monimiaceae), *Dombeya ciliata* Cordem. (Sterculiaceae), and *Dombeya filcunea* Baill. (Sterculiaceae) without any detection of activity in the corresponding samples of *V. triflorum*. The two samples of *M. rotundifolia* showed activity in the water and ethanol extract and one of the samples was active in the acetone extract as well. Of the 11 *Dombeya* samples, representing four species, two species showed activity and only in the ethanol extract namely one of the three samples of *D. filcunea* and both samples of *D. ciliata*.

Discussion

Our bioassay analyses show that the ACE inhibitory activity of *V. triflorum* is host plant-dependent but apparently there is no connection between ACE inhibitory activity in the host and the corresponding sample of *V. triflorum*, only one of the six samples of *A. heterophylla* showed activity as well.

All samples of *V. trifolium* from *A. heterophylla* and the sample from *S. denudata* showed pronounced ACE

Table 1. Angiotensin-converting enzyme (ACE) inhibitory activity of studied plant species.

Plant species	Family	Voucher number[a]	Inhibition of ACE (%)[b]		
			Water	Acetone	Ethanol
Viscum triflorum DC.	Viscaceae	PEM001	73	3	18
Viscum triflorum DC.	Viscaceae	PEM003	77	6	2
Viscum triflorum DC.	Viscaceae	PEM015	64	2	4
Viscum triflorum DC.	Viscaceae	PEM017	69	3	5
Viscum triflorum DC.	Viscaceae	PEM021	75	12	4
Viscum triflorum DC.	Viscaceae	PEM023	87	34	19
Acacia heterophylla Willd.	Fabaceae	PEM002	52	24	20
Acacia heterophylla Willd.	Fabaceae	PEM004	18	17	7
Acacia heterophylla Willd.	Fabaceae	PEM016	16	15	10
Acacia heterophylla Willd.	Fabaceae	PEM018	17	9	9
Acacia heterophylla Willd.	Fabaceae	PEM022	15	13	8
Acacia heterophylla Willd.	Fabaceae	PEM024	18	14	9
Viscum triflorum DC.	Viscaceae	PEM025	89	6	13
Sophora denudata Bory	Fabaceae	PEM026	34	14	8
Viscum triflorum DC.	Viscaceae	PEM009	20	2	3
Viscum triflorum DC.	Viscaceae	PEM031	2	0	7
Viscum triflorum DC.	Viscaceae	PEM041	10	13	11
Dombeya reclinata Cordem.	Sterculiaceae	PEM010	13	16	36
Dombeya reclinata Cordem.	Sterculiaceae	PEM032	14	23	39
Dombeya reclinata Cordem.	Sterculiaceae	PEM042	15	11	25
Viscum triflorum DC.	Viscaceae	PEM011	16	2	8
Viscum triflorum DC.	Viscaceae	PEM033	15	10	10
Viscum triflorum DC.	Viscaceae	PEM039	21	1	10
Viscum triflorum DC.	Viscaceae	PEM045	10	15	13
Dombeya ficulnea Baill.	Sterculiaceae	PEM012	24	1	30
Dombeya ficulnea Baill.	Sterculiaceae	PEM034	26	17	51
Dombeya ficulnea Baill.	Sterculiaceae	PEM040	16	11	27
Dombeya ficulnea Baill.	Sterculiaceae	PEM046	23	17	37
Viscum triflorum DC.	Viscaceae	PEM007	20	22	1
Viscum triflorum DC.	Viscaceae	PEM029	11	3	6
Dombeya ciliata Cordem.	Sterculiaceae	PEM008	38	34	54
Dombeya ciliata Cordem.	Sterculiaceae	PEM030	34	25	52
Viscum triflorum DC.	Viscaceae	PEM005	9	30	2
Viscum triflorum DC.	Viscaceae	PEM027	12	0	10
Dombeya pilosa Cordem.	Sterculiacae	PEM006	31	35	32
Dombeya pilosa Cordem.	Sterculiacae	PEM028	32	23	33
Viscum triflorum DC.	Viscaceae	PEM043	21	12	15
Claoxylon glandulosum Baill.	Euphorbiaceae	PEM044	22	6	15
Viscum triflorum DC.	Viscaceae	PEM013	14	7	6
Viscum triflorum DC.	Viscaceae	PEM035	12	5	4
Monimia rotundifolia Thou.	Monimiaceae	PEM014	88	61	72
Monimia rotundifolia Thou.	Monimiaceae	PEM036	71	16	66
Viscum triflorum DC.	Viscaceae	PEM037	17	7	12
Viscum triflorum DC.	Viscaceae	PEM047	13	12	15
Melicope borbonica (Bory) T.G. Hartley	Rutaceae	PEM038	17	6	10
Melicope borbonica (Bory) T.G. Hartley	Rutaceae	PEM048	15	6	13
Viscum triflorum DC.	Viscaceae	PEM019	35	2	11
Melicope obtusifolia (DC.) T.G. Hartley	Rutaceae	PEM020	29	18	7

[a]Voucher numbers of samples of *V. triflorum* and corresponding host plants are consecutive numbers.
[b]Mean of two determinations.

inhibitory activity in the water extract. Both host plant species belongs to the plant family Fabaceae (subfamily Mimosoideae and Faboideae, respectively).

In *V. album*, several subspecies have been recognized. The nominal subspecies *V. album* ssp. *album* parasitizes angiosperm species, whereas *V. album* ssp. *abietis* Abrom. is found on *Abies* (fir) and *V. album* ssp. *austriacum* Vollm. is found on *Pinus* (pine). Apart from this, it has not been possible to demonstrate host specificity, but there is some evidence that *V. album* plants parasitizing different

host species can be separated by their DNA (Zuber and Widmer, 2000). This has not been studied in *V. triflorum*. If a similar pattern can be detected here the results above may be due to differentiation in the parasite, rather than a direct induction from the host plant.

The activity levels were only consistently high in *V. triflorum* from the Fabaceous trees *A. heterophylla* and *S. denudata*. The results justify the use of *V. triflorum* as an antihypertensive remedy provided that it has been collected from the two mentioned species.

Many *Viscum* spp. and other Loranthaceae are in focus as drugs or producers of chemicals of pharmacological interest. Our results show that it is important in such studies to take into account the identity of the host plants and the host–parasite relations.

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Declaration of interest

The authors report no declarations of interest.

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