

Antifungal Activity of Some Extractives and Constituents of *Aloe vera*

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ABSTRACT

Dried latex (*Aloe* drug) and extractives of *Aloe vera* obtained by hexane, ethyl acetate and methanol were tested with the aim of assessing their activity against some phytopathogenic fungi and understanding the chemical nature of the active principles present in them. The activity of the extractives against *Colletotrichum gloeosporioides*, *Colletotrichum capsici* and *Fusarium solani* was assessed by poisoned food technique and their activity against *Cladosporium cucumerinum* was assessed by thin layer chromatographic bioautography. Polar extractives obtained by methanol and ethyl acetate showed higher activity than non-polar extractive obtained by hexane. The extractives showed higher activity against *Colletotrichum* species than *F. solani*. Two constituents, namely aloin and aloe-emodin were identified as active principles by their activity against *Colletotrichum gloeosporioides* and *Cladosporium cucumerinum*.

Key words: Aloin, aloe-emodin, antifungal activity, aloe drug, *Colletotrichum gloeosporioides*

INTRODUCTION

Plants belonging to the genus *Aloe* have been known for their medicinal value (Ali *et al.*, 1999). Juice of *Aloe vera* is useful in treating wounds from thermal burns and radiation injury. This material is also used in the treatment of dry and moist epidermis, prophylactic action, prevention of kraurosis, dermatitis, eczema, psoriasis, neurodermatitis, herpes, subcutaneous infections (Hegggers *et al.*, 1993; Capasso *et al.*, 1998). The application of fresh Aloe pith relieves pain, burning and itching and has antiseptic action. *Aloe vera* gel is used in the treatment of seborrhea, acne vulgaris and alopecia (Behl *et al.*, 1993). *Aloe vera* is externally used for cicatrisation and internally as laxative. Hydro alcoholic extract is also part of some make-up products with cicatrisation effect. Several anthraquinones have been isolated from *A. vera* of which the most important are aloin and aloe-emodin (Shelton, 1991). The plant is reported to contain mono and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals (Newall *et al.*, 1996).

There are several reports about the antifungal activity of crude extractives of *Aloe vera* (Nebedum *et al.*, 2009; Rosca-Casian *et al.*, 2007; Subramanian *et al.*, 2006; Shamim *et al.*, 2004; Ali *et al.*, 1999; Saks and Barkai-Golan, 1995), but there is very little information about the chemical nature of the active principles, which contribute towards antifungal activity of the plant. The aim of the present study was to have a comparative investigation of the antifungal activity of the extractives obtained by solvents of varying polarity and to understand the chemical nature of

the active principles. Thus the activity of different extractives of *Aloe vera* was evaluated against *Colletotrichum gloeosporioides*, *Colletotrichum capsici* and *Fusarium solani* by poisoned food technique (Nene and Thapliyal, 2002; Nidiry and Babu, 2005). The activity of the extractives against *Cladosporium cucumerinum* was evaluated by Thin Layer Chromatographic (TLC) bioautography (Zhao *et al.*, 1998). The activities of two compounds, namely aloin and aloe-emodin present in *Aloe vera* were evaluated against *C. gloeosporioides* and *C. cucumerinum*.

MATERIALS AND METHODS

Plant material: *Aloe vera* L. Burm.f. (Liliaceae) leaves were harvested from the experimental plot of Indian Institute of Horticultural Research, Hesaraghatta, Bangalore, in April 2009, dried at 60°C and were powdered. The powdered plant material was Soxhlet extracted first with hexane, then with ethyl acetate and finally with methanol. The respective extractives were obtained by completely distilling out the solvents on a water bath. *Aloe* drug was obtained by drying the latex at 25°C.

Tested material: Hexane extractive, ethyl acetate extractive, methanol extractive of the dried leaves, *Aloe* drug (dried latex), Aloin (Barbaloin; 10-Glucopyranosyl-1,8-dihydroxy-3-[hydroxy-methyl]-9[10H]-anthracenone) from Sigma [Approx. 20%(HPLC)] and Aloe-emodin (1,8-dihydroxy-3-[hydroxy-methyl]anthracenone) from Sigma [Minimum. 95%(HPLC)].

Used organisms: *Cladosporium cucumerinum* IMI 249540 obtained from International Mycological Institute, UK, *Colletotrichum gloeosporioides* ITCC 4573 obtained from the Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi, India, *Colletotrichum capsici* and *Fusarium solani* isolated in Division of Plant Pathology, IIHR and maintained on a potato-dextrose-agar (PDA) medium.

Studied activity: Antifungal activity of Hexane, ethyl acetate, methanol extractives, aloe drug and aloin were studied by observing the mycelial growth inhibition of *Colletotrichum* species and *Fusarium solani* by poisoned food technique, surfactant Tween-80 being added at a level of 0.3% to the media in both the control and the treated samples. Phenol was used as a standard. In the case of aloe-emodin, the experiment was conducted by dissolving the required amount of the compound in 0.25 mL of acetone and incorporating to 30 mL of the medium, the same amount of acetone being added to the control also. The percent mycelial growth inhibition was calculated by the formula:

$$\frac{(C-T)}{C} \times 100$$

where, C is the mycelial diameter of the control and T is the mycelial diameter of the treated samples (Nene and Thapliyal, 2002; Nidiry and Babu, 2005). Antifungal activity against *C. cucumerinum* was determined by TLC bioautography. In this case, the extractives, aloin and aloe-emodin were spotted on a TLC plate, eluted with ethyl acetate, sprayed with the inoculums of *C. cucumerinum* and observations were taken after an incubation period of 4 days.

RESULTS AND DISCUSSION

The results provided in Table 1 show that the methanol extractive of *A. vera* exhibits highest antifungal activity against the mycelial growth of *Colletotrichum* species at both the concentrations. Non-polar extractives showed moderate activity. Aloe drug also showed antifungal activity against *Colletotrichum* species at both the concentrations. At low concentration (0.2%) of extractives, *Fusarium solani* did not show any inhibition. But at higher concentration (0.5%) it showed inhibition. Polar extractives (ethyl acetate and methanol) showed higher activity against

Table 1: Antifungal activity of *Aloe vera* extractives and constituents against the mycelial growth of *Colletotrichum* species and *Fusarium solani*

Test organisms	Extractives/constituents	Concentration (%)	Mycelial growth inhibition (%)
<i>*Colletotrichum gloeosporioides</i>	Hexane	0.200	9.9±0.2
		0.500	11.3±0.0
	Ethyl acetate	0.200	12.5±0.0
		0.500	16.4±0.1
	Methanol	0.200	17.6±0.1
		0.500	22.0±0.0
	Aloe drug	0.200	14.0±0.0
		0.500	21.6±0.0
	Aloin	0.050	40.2±0.1
		0.100	47.8±0.1
		0.200	53.1±0.2
	Aloe-emodin*	0.025	NI
		0.050	13.3±0.1
	Phenol (standard)	0.025	55.6±0.2
		0.050	78.3±0.3
<i>§Colletotrichum capsici</i>	Hexane	0.200	5.9±0.1
		0.500	7.1±0.1
	Ethyl acetate	0.200	8.7±0.0
		0.500	14.8±0.0
	Methanol	0.200	10.6±0.1
		0.500	18.5±0.1
	Aloe drug	0.200	7.8±0.1
		0.500	13.1±0.1
	Phenol (standard)	0.025	22.6±0.1
		0.050	51.1±0.1
<i>*Fusarium solani</i>	Hexane	0.200	NI
		0.500	2.8±0.1
	Ethyl acetate	0.200	NI
		0.500	7.9±0.1
	Methanol	0.200	NI
		0.500	9.8±0.0
	Aloe drug	0.200	NI
		0.500	5.2±0.0
	Phenol (standard)	0.025	21.3±0.1
		0.050	60.9±0.1

Concentrations of the extractives expressed as a percentage of the compounds in PDA (w/v). *Higher concentrations of aloe-emodin were not tried because of its poor solubility in acetone and water. *Observations were taken after an incubation of 5 days at 27±2°C. §Observations were taken after an incubation of 8 days at 27±2°C. The average of two replications is shown. NI- No inhibition

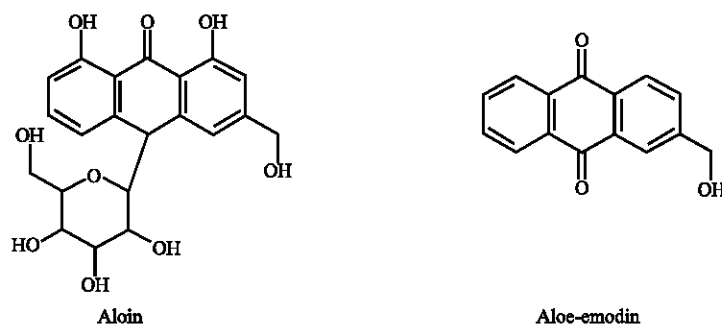


Fig. 1: Chemical structure of aloin and aloe-emodin

the mycelial growth of *F. solani* compared to non-polar extractive (hexane). Two compounds present in the extractives namely aloin and aloe-emodin (Fig. 1) showed moderate activity against *C. gloeosporioides*.

Activity of the extractives and the compounds against *C. cucumerinum* was determined qualitatively. Aloe-emodin, which had an R_f value of about 0.8, showed a very conspicuous inhibition spot. The inhibition spots corresponding to aloe-emodin were present in all extractives and the most conspicuous inhibition spot was observed in the case of ethyl acetate extractive.

The antifungal activity exhibited by the extractives of *Aloe vera* in the present study is consistent with the reports by earlier workers. It may be noted that Rosca-Casian *et al.* (2007) showed that hydroalcoholic plant extracts obtained from *A. vera* fresh leaves exhibited antifungal activity against the mycelial growth of *Botrytis gladiolorum*, *Fusarium oxysporum* f.sp. *gladioli*, *Heterosporium pruneti* and *Penicillium gladioli*. Saks and Barkai-Golan (1995) reported antifungal activity of the *Aloe vera* gel against *Penicillium digitatum*, *Alternaria alternata*, *Botrytis cinerea* and *Penicillium expansum*. Further, Subramanian *et al.* (2006), Nebedum *et al.* (2009) and Rodriguez *et al.* (2005) also reported antifungal activity of *Aloe vera* extractives against different phytopathogenic fungi. However, earlier investigators did not report any comparison of the efficacies of extractives obtained by different solvents. In the present study, we have reported the efficacies of different extractives obtained by different solvents indicating the chemical nature of the extractives and the active principles.

Present results show that polar extractives exhibit higher antifungal activity than non-polar extractives. The activity of the extractives is higher against *Colletotrichum* species than *Fusarium solani*. The antifungal activity of aloin and aloe-emodin against *C. gloeosporioides* and *C. cucumerinum* shows that they are two active principles, which contribute towards the antifungal property of the crude extractives. Both aloin and aloe-emodin are anthraquinone derivatives and antifungal activity of several anthraquinone derivatives in other plants has been reported (Agarwal *et al.*, 2000; Singh *et al.*, 2006). Further detailed studies may be required to understand the chemical nature of other active principles present in the plant.

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