ISSN 1983-3466

Structure-function activity of capsaicin analogues as antifungal and antioxidant compounds

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Key words: Capsaicin analogues; *Colletotrichum gloeosporioides; Penicillium expansum;* DPPH.

Introduction

The capsaicin [(E)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide] has several biological activities including antioxidant and antimicrobial [1,4]. Application of capsaicin (CAP) has been restricted due to its burning sensation or pungency. Thus, it has been synthetized nonpungent analogues of CAP [2]. The aim of the present work was extend the knowledge on CAP analogues [N-benzilbutanamide, CAP-1; N-(3-methoxybenzyl)butanamide, CAP-2; N-(4-hydroxy-3-methoxybenzyl)butanamide,CAP-3; N-(4-hydroxy-3-methoxybenzyl)hexanamide, CAP-4; N-(4-hydroxy-3-methoxybenzyl)tetradecanamide, CAP-5]. The results were expected to provide more information on the structure-activty relationships of capsaicin while providing a contribution to the molecular details involved in the antioxidant and antifungal activity of CAP analogues.

Materials and Methods

Capsaicinoids were acquired from SP-Pharma (58.61% capsaicin, 33.76% dihydrocapsaicin and 7.63% nordihydrocapsaicin). Capsaicin was purchased from Sigma-Aldrich. The capsaicin analogues were prepared by condensation of vanillylamine with acyl chlorides [2] and their chemical identities and purities confirmed by LC/ESI-MS.

DPPH assay. The free radical scavenging activity was measured by scavenging of the DPPH radical. DPPH results were defined as the concentrations sufficient to obtain 50% of the maximum effect (EC_{50}) [3].

Antifungal assay. Capsaicin analogues (6.25 - 800 µM) were evaluated by liquid growth inhibition assay. The MIC is the lowest concentration of tested substance preventing visible antifungal growth. *In vivo* antifungal assay was carried out fresh "Fuji" apples using method previously described [4].

Results and Discussion

As shown in Table 1, the CAP, CAP-SP-Pharma, CAP-4 were as potent as commercial antioxidant BHT ($p < 0.05$). However, the absence of phenolic moiety in the molecule of CAP-1 led to lost of antioxidant activity CAP-5 showed to be 1.5-fold less active than CAP, suggesting that hydrophilicity influence in antioxidant activity. However, hidrophilicity was not enough to explain the similar antifungal activity of capsain and its analogues against *C. gloeosporioides* (Table 1). The capsaicin analogue, CAP-3 and CAP-4 displayed better antifungal activity toward *P. expansum*. In fact, acyl chains shorter than 8 carbons improved antifungal activity, but showed a low efficacy in the reduction of rots produced by *P. expansum* in apple fruit.

Table 1 – Antioxidant and antifungal results of amides.

Amide	DPPH*	*C. gloeosporioides* CBMAI864	*P. expansum* CCT7549#
	EC_{50} µg/mL	MIC µM	
CAP	96.29±5.50[a]	800	800
CAP SP-Pharma	90.94±2.87[b]	400	400
CAP-1	ND	800	800
CAP-2	-	800	800
CAP-3	98.97±3.31[bc]	800	600
CAP-4	91.96±3.17[bc]	800	600
CAP-5	148.39±21.99[a]	800	800
BHT	85.78±5.48[c]	-	-

* ND not detected. Each value is the mean ± deviation, n = 9. Numbers with different letters are significantly different by Tukey test ($p < 0.05$)

Conclusion

The present work describes, for the first time, the antifungal effect exhibited by capsaicin analogue against *P. expansum* and *C. gloeosporioides*. In general, acyl (around six carbons) and phenolic group of capsaicin analogues are importante for the antioxidante and antifungal activity.

Acknowledgements

The authors are grateful to Cleber W. Liria for LC/ESI-MS analysis of analogues.

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