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Turmeric (*Curcuma longa* L.) volatile oil inhibits key enzymes linked to type 2 diabetes

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Abstract
Anti-diabetic capacity of *Curcuma longa* volatile oil in terms of its ability to inhibit glucosidase activities was evaluated. Turmeric volatile oils inhibited glucosidase enzymes more effectively than the reference standard drug acarbose. Drying of rhizomes was found to enhance α-glucosidase ($IC_{50} = 1.32–0.38$ μg/ml) and α-amylase ($IC_{50} = 64.7–34.3$ μg/ml) inhibitory capacities of volatile oils. *A*r-Turmerone, the major volatile component in the rhizome also showed potent α-glucosidase ($IC_{50} = 0.28$ μg) and α-amylase ($IC_{50} = 24.5$ μg) inhibition.

Keywords: Curcuma longa, *volatile oil, α-amylase, α-glucosidase, ar-turmerone*

Introduction

Glucosidase inhibitors have been used to control postprandial hyperglycaemia (PPH) in type 2 diabetes patients (Gin and Rigalleau 2000). Acarbose and miglitol, the conventionally used glucosidase inhibitors for the management of PPH in diabetic patients, are known to be associated with several side effects (Fujisawa et al. 2005); therefore, search for glucosidase inhibitors from natural sources with lesser side effects attains more interest in scientific community. The role of plants as source of glucosidase inhibitors has recently been reviewed by Kumar et al. (2010). Turmeric (*Curcuma longa* L.) rhizome is recommended for the treatment of diabetes and related disorders in Indian traditional medicine 'Ayurveda' (Chattopadhyay et al. 2004). Anti-diabetic potential of *C. longa* rhizome in terms of its ability to inhibit glucosidase activity has been demonstrated by many studies (Kumar et al. 2005; Srinivasan 2005; Du et al. 2006). In this study, α-amylase and α-glucosidase inhibitory potentials of *C. longa* volatile oil were evaluated for the first time. As the dried turmeric rhizomes are preferentially stored, transported and used by consumers, the effects of drying of rhizome on anti-diabetic effects of its essential oil were also studied.

Materials and methods

Chemicals, reagents and plant material

α-Amylase (EC 232-588-1), α-glucosidase (EC 232-604-7) and other chemicals for the study were purchased from Sigma Aldrich (St Louis, MO, USA). Authenticated samples of fresh mature rhizomes of *C. longa* var. *Alleppey* were obtained from a field in Nedumangad, Thiruvananthapuram (India), in March 2009 and representative samples were deposited in the herbarium of the institute (Voucher No. 07/SIP/2009). The samples were kept at 4°C till analysed.

Extraction of volatile oil and ar-turmerone

Fresh rhizomes (2 kg) were washed and divided into two halves and one portion was dried in an air oven (50°C, 36 h). Volatile oils were extracted from both fresh and dry rhizome using Clevenger apparatus.

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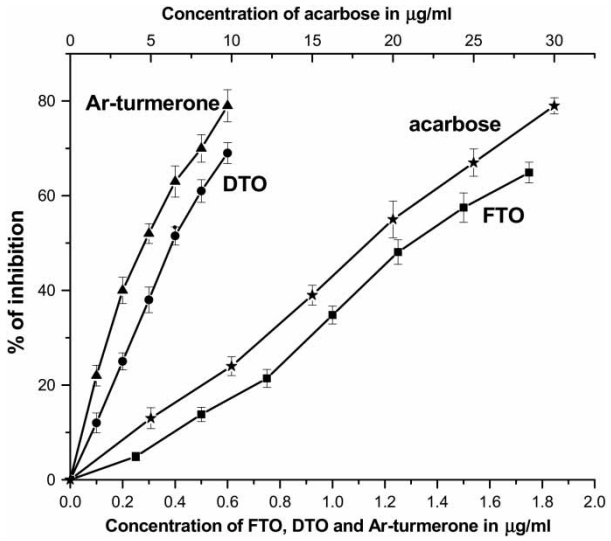

Figure 1. α-Glucosidase inhibition capacity of *C. longa* volatile oils obtained from fresh (FTO) and dried (DTO) rhizomes, *ar*-turmerone and acarbose (mean ± SD, $n = 5$).

Volatile oils obtained from fresh and dry rhizomes were respectively termed as FTO and DTO. Composition of volatile oils was analysed using GC-MS (Sacchetti et al. 2005). Five hundred milligrams of volatile oil in 25 ml hexane was submitted to flash column chromatography [BUCHI C-615, 2.3 × 40 cm silica (60–120 mesh) column, mobile phase: gradient of 0–60% of ethyl acetate in hexane (10 ml/min)]. Fraction 3 (20% ethyl acetate in hexane) yielded a single compound, which was identified as ar-turmerone (92% purity) by spectral and chromatographic analysis (Lee et al. 2003).

α-Amylase inhibition assay

Extract/acarbose (10 µl, 10–100 µg) in dimethyl sulphoxide and 20 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase (0.5 mg/ml) were incubated at 25°C for 10 min and 25 µl of 1% starch in 0.02 M sodium phosphate buffer was added. After incubation at 25°C for 10 min, 50 µl of 3,5-dinitrosalicylic acid was added to the wells and incubated in boiling water for 5 min. The reaction mixtures were cooled to room temperature, diluted with 500 µl water and absorbance at 540 nm was measured using a multimode plate reader (Synergy 4, Biotek, Winoosky, Vermond, USA) (Apostolidis et al. 2007).

α-Glucosidase inhibition assay

α-Glucosidase (20 µl, 1 U/ml) was premixed with 25–500 µg of extract/acarbose and made up to 500 µl with 50 mM phosphate buffer (pH 6.8). Then it was incubated for 5 min at 37°C and 1 mM pNPG (200 µl) in 50 mM of phosphate buffer (pH 6.8) was added to initiate the reaction and the mixture was further incubated for 20 min at 37°C. The reaction was terminated by the addition of 500 µl of 1 M sodium carbonate and made up to 1.5 ml with water. The absorbance of the mixtures at 405 nm was measured using a multimode plate reader (Synergy 4, Biotek, Winoosky, Vermond, USA) (Apostolidis et al. 2007).

Statistical analysis. Results were presented as mean ± SD of five analyses. Significant differences between means were determined by ANOVA followed by Turkey's pair-wise comparison test at a level of $p < 0.05$ using MS Office Excel 2003 with inerSTAT (Mexico City, Mexico) as an add-in.

Results and discussion

Anti-diabetic efficacy

Volatile oils obtained from both fresh (FTO) and dried (DTO) rhizomes exhibited potent glucosidase inhibitory efficacies in dose-dependent manner. DTO ($IC_{50} = 0.38 \pm 0.09$ µg/ml) showed a 3.5 times more α-glucosidase inhibitory capacity than that of FTO ($IC_{50} = 1.32 \pm 0.08$ µg/ml; Figure 1). α-Glucosidase inhibitory potential of both FTO and DTO was significantly higher ($p < 0.05$) than that of reference glucosidase inhibitor acarbose ($IC_{50} = 18.12 \pm 1.23$ µg). FTO, DTO and acarbose showed IC_{50} values of 64.7 ± 5.9, 34.3 ± 6.2 and 296.3 ± 12.7 µg/ml, respectively, for α-amylase inhibition (Figure 2). This study showed that volatile oil from turmeric rhizome was a potent glucosidase inhibitor and drying of rhizome resulted in significant ($p < 0.05$) enhancement in its glucosidase inhibitory potential. Composition analysis indi-

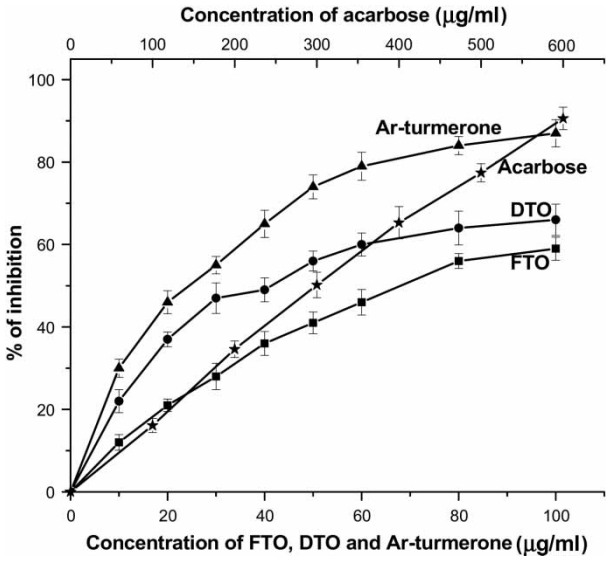

Figure 2. α-Amylase inhibition capacity of *C. longa* volatile oils obtained from fresh (FTO) and dried (DTO) rhizomes ar-turmerone and acarbose (mean ± SD, $n = 5$).

cated a significant increase ($p < 0.05$) in proportion of the major volatile component *ar*-turmerone (45.0–58.0%) in volatile oils on drying the rhizomes (Supplementary Table I; online version only) The high α-glucosidase ($IC_{50} = 0.28 \pm 0.05$ μg/ml) and α-amylase ($IC_{50} = 24.5 \pm 4.8$ μg/ml) inhibitory potentials shown by *ar*-turmerone indicated it as a major glucosidase inhibiting potential in the volatile oils. Some of the other constituents in the volatile fraction might also contribute to the inhibitory potential. Reports on the glucosidase inhibition potential of volatile oils of *Eucalyptus camaldulensis* (Basak and Candan 2010) and *Myrtus communis* (Sepici et al. 2004) are available. Anti-diabetic potential of turmeric fractions other than volatile oils are also available (Kumar et al. 2005; Srinivasan 2005; Du et al. 2006). The high α-amylase and α-glucosidase inhibitory potentials of turmeric volatile oils were demonstrated in this study. Detailed studies to find out the glucosidase inhibition capacities of individual turmeric volatile components and to explore the effect of storage and processing conditions on the anti-diabetic potential of turmeric rhizome volatile fraction are also warranted by this study.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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