Seasonal Variation and Analgesic Properties of Different Parts from *Curcuma zedoaria* Roscoe (Zingiberaceae) Grown in Brazil

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This work describes the seasonal variation of curcumenol (**1**) and dihydrocurdione (**2**), two active terpenoids from different parts (roots, mother rhizome and rugous rhizome) of *Curcuma zedoaria* grown in Brazil. The analysis was carried out by high resolution gas chromatography, using external standards for determination. The results showed that both terpenoids are present in all the parts studied. However, *C. zedoaria* exhibited about three times more terpenoids in the mother rhizome in autumn than in other parts and seasons studied. The antinociceptive activity of the dichloromethane extracts from different parts and collected in different seasons was studied using the acetic acid-induced abdominal constriction model in mice. The extracts obtained from mother rhizome collected in autumn and winter at doses of 10 mg/kg body weight, i.p., caused considerable antinociceptive activity inhibiting 91.1 and 93.4% of the abdominal constrictions, respectively, whereas compounds **1** and **2** caused inhibitions of 64.0 and 46.0%, respectively. These results confirm that both compounds contribute to explain the antinociceptive effect of the plant but suggest that other compounds are also acting as analgesics.

Key words: Curcuma zedoaria, Curcumenol, Dihydrocurdione

Introduction

Curcuma zedoaria R. Br. (Zingiberaceae), popularly known as "*zedoaria*" or "gajitsu", is frequently employed in some countries, including Brazil, to treat different diseases, such as cervical cancer, hepatitis, inflammations and dolorous processes (Lee and Lin, 1988; Sakai *et al.*, 1989; Rana and Avadhoot, 1992; Teske and Trentini, 1995; Syu *et al.*, 1998; Lai *et al.*, 2004). It is well documented that its main active principles are terpenoids, especially sesquiterpenoids, which also are produced by cultured cells (Hikino *et al.*, 1968; Shiobara *et al.*, 1985; Syu *et al.*, 1998; Sasaki *et al.*, 2003; Nishiyama *et al.*, 2005).

However these studies were focused on *C. zedoaria* produced in Asiatic countries little is known concerning the plant grown in Brazil. A previous study showed that this plant was effective as supplement to control dental plaque and gingivitis in humans (Sandrini *et al.*, 1997). We have recently verified that curcumenol (**1**), a sesquiterpene isolated from rhizomes of this plant, exhibits significant and potent antinociceptive action when evaluated in some pharmacological models of pain in mice (Navarro *et al.*, 2002).

In this present study we have determined by high resolution gas chromatography (HRGC) the quantity of curcumenol and dihydrocurdione present in different plant parts collected in different seasons as well as evaluated the analgesic profile of dichloromethane extracts and isolated compounds from *C. zedoaria* cultivated in the South of Brazil.

Material and Methods

Plant material

C. zedoaria was collected in Ilhota, Santa Catarina, Brazil in March, July, September and Decem-

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ber 2001, and authenticated by Prof. Renê Ferreira (Curso de Farmácia, UNIVALI). A voucher specimen was deposited at Barbosa Rodrigues Herbarium (Itajaí, SC) under number V. C. Filho 023.

Preparation of the samples

The extracts were prepared according to the procedures described previously by Vilegas and Lanças (1994) with minor modifications. Different parts (roots, mother rhizome and rugous rhizome) of this plant (100 g each) were powdered and macerated with 200 mL of dichloromethane for 7 d at room temperature. After evaporation of solvent under reduced pressure, all extracts were weighed after drying in a vacuum recipient with P_2O_5. Aliquots (10 mg) of each extract were dissolved in dichloromethane (0.3 mL) and filtered (0.45 μm HVLP membrane) prior to analysis. All the solvents were of analytical grade.

High resolution gas chromatography analysis

HRGC separation was carried out using a Shimadzu model CG-14B instrument equipped with a FID, denoted DB1 column (25 m, 0.25 mm i.d., 0.25 μm film thickness). Temperatures of the injection port and of the detector were 290 °C and 300 °C, respectively. The carrier gas was hydrogen at a pressure of 50 kPa and the oven temperature was held at 100 °C for 2 min, increased to 290 °C at 10 °C/min, and then held at 290 °C for 20 min. Aliquots of 1 μL were injected using the split mode (split ratio 1:30). Peaks were identified by comparison of their retention time with those of standards and their areas were integrated using a Software integrator acquired from Micro-Química. The calibration curve was constructed using the conditions described above, with standard samples of curcumenol (**1**) and dihydrocurdione (**2**) within the concentration range 0.03–0.93 mg/mL and 0.02–0.75 mg/mL, respectively (Fig. 2).

Pharmacological analysis

Swiss mice of both sexes (25–35 g) were housed temperature conditions in automatically controlled [(23 ± 2) °C and 12 h light-dark cycles)]. The animals were given access to water and Nuvital chow *ad libitum* unless otherwise indicated. The animals remained in the appropriate laboratory of UNIVALI until some hours before the experiments.

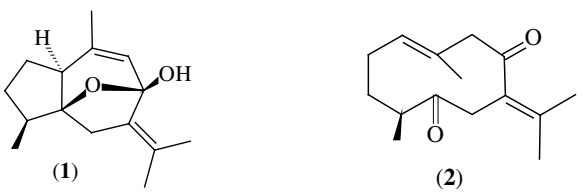
The abdominal constrictions induced by intraperitoneal injection of acetic acid (0.6%) were carried out according to the procedures previously described (Collier *et al.*, 1968; Souza *et al.*, 1998), with minor modifications. Animals were pretreated with extracts or compounds (10 mg/kg) intraperitoneally 30 min before the acetic acid injection. Control animals received a similar volume of 0.9% NaCl (10 mL/kg, i.p.). After the challenge, each mouse was placed in a separate glass funnel and the number of abdominal contractions of the abdominal muscles together with stretching was cumulatively counted over a period of 20 min. Antinociceptive or analgesic activity was expressed as the reduction of the number of abdominal contractions between control animals and mice pretreated with extracts or compounds.

Statistical analysis

The results are presented as mean ± s. e. m. and statistical significance between groups was analyzed by means of the t test or analysis of variance followed by Dunnett's multiple comparison tests, when appropriate. p values less than 0.05 were considered significant accompanied by their respective 95% confidence limits.

Results and Discussion

All the experiments were performed with dichloromethane extracts, directly obtained from the different parts of the plant, since showed to be suitable for HRGC analysis and due previous studies suggesting that non-polar compounds are those responsible for the antinociceptive properties of this plant (Navarro *et al.*, 2002). A comparative TLC of this extract using several eluent systems with specific reagents demonstrated a very similar chromatographic profile and high quantities of steroids and terpenoids. However, when analyzed by HRGC, the chromatographic profile indicated a great difference regarding concentra-



(**1**) (**2**)

Fig. 1. Structure of curcumenol (**1**) and dihydrocurdione (**2**).

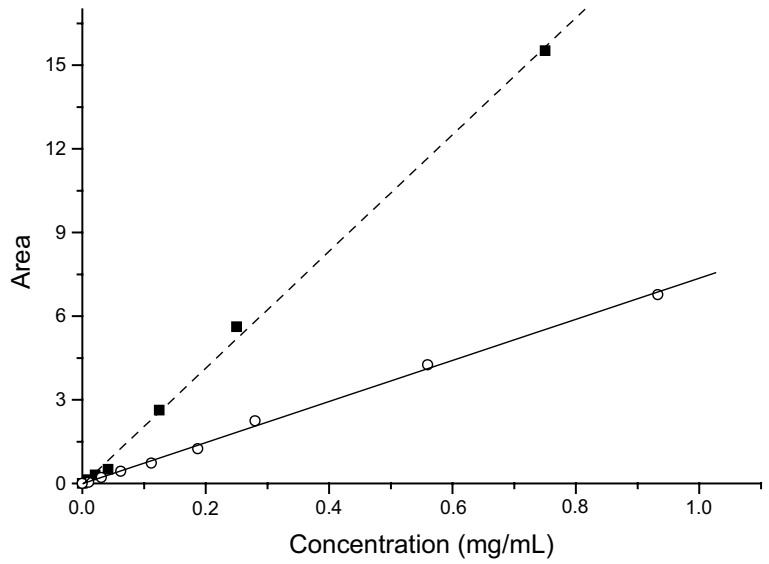


Fig. 2. Calibration curve (HRGC-FID) constructed using standard samples of curcumenol (○) within the concentration range 0.03–0.93 mg/mL and dihydrocurdione (■) within the concentration range 0.02–0.75 mg/mL (area in arbitrary units).

tions between the different parts, mainly in those collected in the autumn (results not shown). Considering that the sesquiterpenes curcumenol (**1**) and dihydrocurdione (**2**) (Fig. 1) seem to be the main components responsible for the analgesic action of this plant (Navarro *et al.*, 2002), we quantified them by HRGC. The quantitative analysis of compounds **1** and **2** was performed using external calibration over a range of 0.03–0.93 mg/mL and 0.02–0.75 mg/mL, respectively (Fig. 2). The yield of **1** and **2** was determined as a function of 100 g of dried plants. The results indicated that the production of **1** and **2** is about three times greater in mother rhizome in the autumn than in other plant parts and in other seasons (Tables I and II). Other terpenoids or steroids were detected by chromatographic methods, but studies are currently in progress for quantification and pharmacological evaluation.

In an attempt to relate the analgesic activity with the amount of compounds present in the different plant parts and seasons, we have initially prepared three extracts obtained by maceration with dichloromethane at room temperature. As can be observed in Fig. 3A, the mother rhizome, collected in autumn and winter, caused significant inhibition in the number of constrictions induced by acetic acid 0.6%, i.p., with inhibition of 91.1% and 93.4%, respectively. When collected in spring and summer, these parts caused inhibitions of 11.1% and 44.4%, respectively. Comparing with some drugs used clinically such as aspirin (35%)

Part	Autumn	Winter	Spring	Summer
Roots	15.70 ± 0.14	8.90 ± 0.15	8.70 ± 0.12	1.5 ± 0.10
Mother rhizome	33.10 ± 0.12	9.10 ± 0.08	5.90 ± 0.05	6.0 ± 0.01
Rugous rhizome	10.40 ± 0.03	3.10 ± 0.03	2.00 ± 0.04	2.9 ± 0.03

Table I. Contents of curcumenol (**1**) in different parts and seasons of *C. zedoaria* (mg/100 g dried plant).

Part	Autumn	Winter	Spring	Summer
Roots	8.50 ± 0.27	3.10 ± 0.15	4.40 ± 0.06	1.60 ± 0.10
Mother rhizome	25.00 ± 0.25	9.40 ± 0.17	6.10 ± 0.15	7.40 ± 0.14
Rugous rhizome	6.70 ± 0.03	2.90 ± 0.08	1.50 ± 0.06	1.50 ± 0.03

Table II. Contents of dihydrocurdione (**2**) in different parts and seasons of *C. zedoaria* (mg/100 g dried plant).

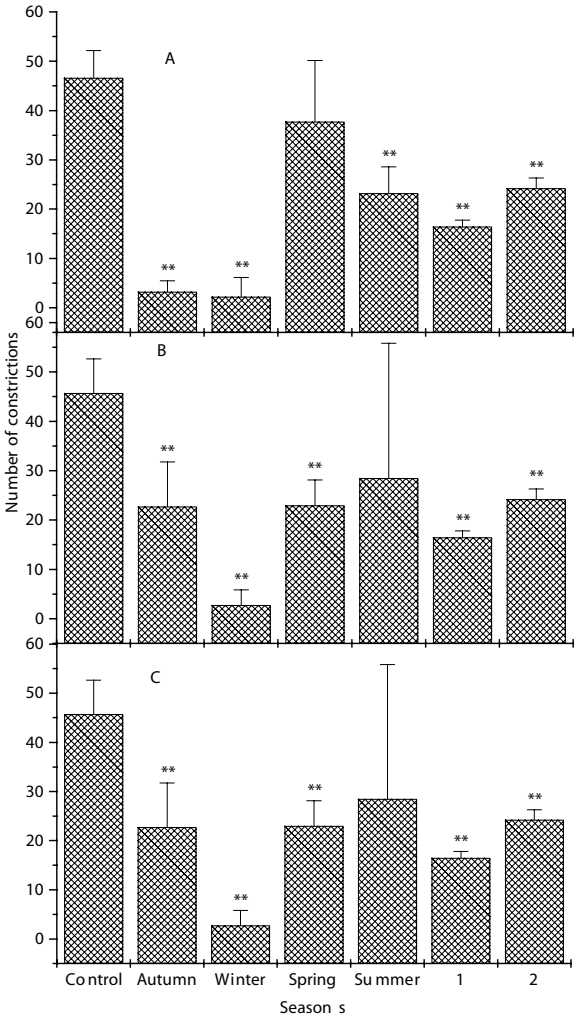


Fig. 3. Effect of dichloromethane extracts obtained from different parts and seasons of *C. zedoaria* against acetic acid-induced abdominal constrictions in mice, in comparison with curcumenol (**1**) and dihydrocurdione (**2**) at 10 mg/kg, i.p.: (A) mother rhizome; (B) rugous rhizome; (C) roots. Each column represents the mean ± s. e. m. of six experimental values.
** $p < 0.05$.

and paracetamol (38%), we observed that this part, when collected in autumn and winter, was about three-fold more potent that reference drugs. Fig. 3B shows the analgesic action of rugous rhizomes. As can be observed, this part exhibited a similar pharmacological profile to that verified for the mother rhizome, with inhibitions for autumn and winter of 80.8 and 92.1%, respectively. On the other hand, a variation is observed when compared with the plant part collected in winter, showing a significant analgesic effect, with inhibition of 89.3%, thus revealing more effectiveness than reference drugs. The results suggest that in this season, this part of the plant produces other substances with analgesic potential. Fig. 3C shows the results obtained for roots. As can be observed, they showed a profile similar to that of the previous parts studied. However, the parts collected in winter and spring showed a better pharmacological profile, with inhibitions of 89.3% and 46.5%, respectively. Although compounds **1** and **2** contribute to explain the activity of the plant with inhibition of 64.0 and 46.0% at 10 mg/kg, and considering, that the quantified compounds appeared in greater amounts in autumn, other constituents present mainly in winter and spring should be responsible for the effects observed. Although the writhing test is a non-specific model, it is widely used for analgesic screening because it involves local peritoneal receptors, which are generally associated with prostanoids as the increase of levels of PGE2 and PGF2a in peritoneal fluids, as well as products of the lipoxigenase (Vongtau *et al.*, 2004).

In summary, the results suggest that the mother rhizome collected in autumn could be used in phytotheraphy preparations that include *C. zedoaria.*

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