GC-MS Analysis and cardiovascular activity of the essential oil of *Ocotea duckei*

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RESUMO: “Análise CG-EM e atividade cardiovascular dos óleos essenciais de *Ocotea duckei*”. O óleo essencial obtido da destilação por vapor de água das folhas, caule, raiz e frutos de *Ocotea duckei* teve sua composição química analisada através de CG-EM. A atividade farmacológica desses óleos também foi avaliada, mostrando significantes efeitos cardiovasculares. Quarenta e nove substâncias foram identificadas, constituindo-se por uma mistura complexa de monoterpenos (45%) e sesquiterpenos (55%). Os frutos forneceram (1,9%) mais óleo essencial do que os caules (1,0%), raízes (0,8%) e folhas (0,7%). O principal componente encontrado nas folhas foi o trans-caryophylleno (60,54%), nas cascas do caule, β-eudesmol (27,51%) e nos frutos, dl-limoneno (30,12%). O componente predominante do óleo essencial das raízes foi elemol (24,31%). Em ratos normotensos, não anestesiados, o óleo essencial de diferentes partes de *Ocotea duckei* (folhas, frutos, caule e raiz) induziu significante (*p* < 0,05) hipotensão seguido de bradicardia.


ABSTRACT: The essential oils obtained by steam distillation from the roots, stems, leaves and fruits of *Ocotea duckei* had their composition analyzed by GC-MS. The pharmacological activity of these oils was also evaluated showing significant cardiovascular effects. Forty-nine substances were identified, consisting of a complex mixture of monoterpenes (45%) and sesquiterpenes (55%). The fruits yielded (1.9%) more essential oil than the stems (1.0%), roots (0.8%) and leaves (0.7%). The main component in the oil of the leaves was trans-caryophyllene (60.54%), in the stem bark β-eudesmol (27.51%) and in the fruits, dl-limonene (30.12%). The predominant essential oil component in the roots was elemol (24.31%). In non-anesthetized normotensive rats, the essential oils from different parts of *Ocotea duckei* (leaves, fruits, stem and roots) induced significant (*p* < 0.05) hypotension followed by bradycardia.

Keywords: *Ocotea duckei*, Lauraceae, essential oil, GC-MS analysis, cardiovascular activity.

INTRODUCTION

The family Lauraceae is constituted by *circa* 50 genera and approximately 500 species, most of them trees. Its main distribution centers are South America and Southeastern Asia and the plants of this family are known for the production of a wide diversity of secondary metabolites (Brunnmitt, 1992; Silva et al., 1989; Barbosa-Filho et al., 1989a,b; Barbosa-Filho et al., 1989b, 1988). *Ocotea duckei* is a member of the Lauraceae found in Northeastern Brazil, where it is popularly known as “louro de cheiro”. There is no reported use in folk medicine for this plant. A morphoanatomical study of the leaves of *Ocotea duckei* was carried out in order to contribute to separate it from the other species of the same genus (Coutinho et al., 2006a,b). Previous reports on *Ocotea duckei* described the isolation of alkaloids (Dias et al., 2003; Silva et al., 2002; Morais et al., 1998a) and lignoids (Morais et al., 1996; Morais et al., 1998b; Morais et al., 1999; Barbosa-Filho et al., 1999). Yangambin, the main lignoid isolated from this species has shown many pharmacological properties, such as: (a) a selective platelet activating factor (PAF) receptor antagonist, observed in several *in vitro* and *in vivo* experimental models (Castro-Faria-Neto et al., 1995a,b; Herbert et al., 1997), (b) an effective pharmacological
agent against cardiovascular collapse and mortality in endotoxin shock (Tibiriçã et al., 1996; Ribeiro et al., 1996; Araújo et al., 2001), (c) an anti-allergic effect (Serra et al., 1997), (d) increased sleeping time induced by pentobarbital and the blockage of convulsions induced by pentylenoptetrazole (Almeida et al., 1995; Pachê et al., 1993), (e) yangambin was not mutagenic when tested using strains of Salmonella typhimurium (Marques et al., 2003), (f) topical treatment of eggs and first instars with yangambin as well as feeding larvae with a yangambin-treated diet resulted in inhibition of postembryonic development, morphological alteration, and oviposition reduction (Cabral et al., 2007a,b), (g) yangambin presented antileishmanial activity against promastigotes forms of L. chagasi and L. amazonensis (Monte-Neto et al., 2007) and antimicrobial activity against Escherichia coli (Antunes et al., 2006).

This is the first report of the composition of the essential oils obtained from the fruits, leaves, stem and roots of Ocotea duckei, as well as the pharmacological activity on the cardiovascular system.

MATERIAL AND METHODS

Plant material

The plant was collected in March 2005, near the city of Santa Rita, State of Paraíba, Brazil. A voucher specimen (Agra 4309) is deposited in the Herbarium Prof. Lauro Pires Xavier (JPB) in the Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba.

Isolation of the essential oils

The fresh material (1000 g) of each part of the plant was extracted using a steam distillation process in a Clevenger apparatus for 3 h at a controlled temperature (40 °C) according to the literature (Matos et al., 1999). The yields in terms of percentage of the fresh weight of the different parts of the vegetal were determined.

Analysis of the essential oils

The essential oils were analyzed by GC-MS, using a Hewlett Packard HP 5890 coupled to a HP 5971A mass selective detector, using the following conditions: Column: SP 2100 dimethylpolysiloxane DB-5 fused silica capillary column 30 m x 0.25 mm (0.1 µm film thickness). The conditions of the experiment were: carrier gas: He, (1 mL/min.); programmed column temperature 35 - 180 °C at 4 °C/min. then 180 – 280 °C at 20 °C/min. Electron impact MS was used at 70 eV. 1 µL of each essential oil was analyzed. The concentration of their components was calculated using the individual peak areas for each substance. Each substance was identified by spectrometric analysis using the Wiley data base, retention times and Kovats indexes (Alencar et al., 1994). Visual analysis of the ther mass spectra and comparison with literature data were used for confirmation of the results.

Animals

The animals used for all experiments were male Wistar rats (250 – 300 g body weight) housed in conditions of controlled temperature (21 ± 1 °C) and exposed to a 12 hours light-dark cycle with free access to food (Purina-Brazil) and tap water.

Measurement of arterial blood pressure in non-anaesthetized rats

Rats were anaesthetized using sodium pentobarbital (60 mg.kg⁻¹, i.p.), and a polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery. Another catheter was inserted into the inferior vena cava via the left femoral vein for administration of drugs. Both catheters were filled with heparinized saline and led under the skin to exit between the scapulae. Two days later, experiments were performed on non-anaesthetized rats accustomed to their environment, and provided with food and water ad libitum. The arterial catheter was connected to a precalibrated pressure transducer (Statham P23 ID; Gould, Cleveland, OH, USA). The transducer signal was fed to an amplifier-recorder (Model TBM-4M, WPI, Sarasota, FL, USA) and to a personal computer (Pentium 166 MHz) equipped with an analog-to-digital converter board (CIO-DAS16/JR, Computer Boards, Inc., Mansfield, MA, USA). Using CVMS software (WPI, Sarasota, FL, USA), data were sampled every 500 Hz and stored on a CD-ROM. Beat-to-beat time series were generated and processed off-line in another Personal computer. For each cardiac cycle, the computer calculated mean arterial pressure (MAP), and pulse interval (referred to as heart rate). After haemodynamic parameters had stabilized different doses of the essential oil from different parts of Ocotea duckei (OEOD - 1, 5, 10 and 15 mg.kg⁻¹, i.v., randomly) were administered. Successive injections were separated by a time sufficient to allow full recovery of arterial pressure, usually 15 - 20 min.

Drugs

The drugs used were heparin sodium salt (Roche), sodium nitroprusside, sodium pentobarbital (Jansen). OEOD was dissolved in 0.1% cremophor plus saline for in vivo experiments.

Data analysis

Values are expressed as mean ± S.E.M. When
Among the 49 identified substances, 45% were monoterpenes and 55% were sesquiterpenes. The fruits of Ocotea duckei yielded the largest amount of essential oil (1.9% of the fresh weight), when compared with the other parts of the plant, stem bark (1.0%), roots (0.8%), leaves (0.7%). Significant quantitative differences were observed regarding the presence of trans-caryophyllene, which is present as the major component of the leaf oil (60.54% of the total oil) and very little in the roots (0.61% of the total oil). It is not found in the stem or fruits. d,l-limonene, was found in considerable concentration in the fruit oil (30.12%), being less present in the stem bark’s oil (6.65%), and not detected in roots or leaves. The main components in the fruit oil are β-pinene (12.25%) and α-pinene (9.89%). β-Eudesmol is the main component in the stem bark oil (27.51%). This same substance is also found in an important amount in the roots (13.44%). The root oil showed elemol (24.31%) and β-elemene (16.69%) as its main components. α-Pinene, was the only substance found in all parts of the plant: fruits (9.89%), stem bark (9.02%), roots (2.89%) and leaves (0.62%). Table 1, shows the composition of all the oils obtained from the plant parts, as well as molecular weights, retention times and Kovat’s index for each component.

In six non-anaesthetized rats, baseline values of mean arterial blood pressure and heart rate were 109 ± 2 mmHg and 360 ± 9 bpm, respectively. The essential oil from the leaves (1, 5, 10, and 15 mg.kg⁻¹ i.v., randomly, n = 6) induced significant (p < 0.05) hypotension (7 ± 1, 15 ± 2, 21 ± 2 and 37 ± 3 %, respectively), followed by intense bradycardia (3 ± 1, 9 ± 2, 18 ± 4 and 53 ± 4 %, respectively). OEOD from the fruits (1, 5, 10, and 15 mg.kg⁻¹ i.v., randomly, n = 6) also induced a marked hypotension (6 ± 1, 8 ± 3, 18 ± 3 and 26 ± 3 %, respectively), which was followed by bradycardia (3 ± 2, 3 ± 1, 12 ± 3 and 35 ± 2 %, respectively). OEOD from stem bark and OEOD from the roots (1, 5, 10, and 15 mg.kg⁻¹ i.v., randomly, n = 6), were both able to induce hypotension (8 ± 1, 25 ± 3, 38 ± 7, 27 ± 5 % and 4 ± 2, 20 ± 2, 33 ± 4, 25 ± 5 %, respectively), and bradycardia (5 ± 2, 22 ± 2, 53 ± 3, 49 ± 4 % and 3 ± 1, 30 ± 4, 57 ± 3 and 35 ± 2 %, respectively). Interestingly, for all of the essential oils tested, the hypotensive effect was more potent on diastolic arterial blood pressure compared with the effect induced on systolic pressure (data not shown).

It is well established that the parasympathetic nervous system plays a significant role in the control of cardiac activity and arterial blood pressure (Higgins et al., 1973). For all of the OEOD tested, hypotension was always followed by bradycardia, indicating a marked participation of cardiac parasympathetic pathways in these responses. It is well reported in the literature that trans-caryophyllene is a calcium channel blocker (Sensch et al., 1993). Thus, we hypothesized that hypotensive and bradycardiac effects might be due to the presence of trans-caryophyllene in the essential oils; however, this may not be the case, since in the essential oils from stem bark and from the fruits hypotension and bradycardia were comparable to that induced by leaves and roots, which do not contain this compound. Furthermore, hypotensive activity for α-terpineol was demonstrated in conscious rats (Saito et al., 1996). Nevertheless, this substance was not found in the essential oil from the leaves, which induced hypotension and bradycardia similar to those induced by the other OEOD. Finally, since α-pinene was found in all of the essential oils studied, it is a probable candidate for hypotension and bradycardia. As a matter of fact α-pinene was reported to induce spasmyolytic effect (El Tantawy et al., 1999). However, considering that OEOD from the leaves was able to induce the most important hypotensive and...
bradycardic effects, and since the lowest amount of the compound was found in the OEOD from the leaves, this might not be the only compound responsible for the cardiovascular effects observed. Whatever the underlying additional mechanisms, the results shown herein suggest that the hypotensive and bradycardiac action of OEOD from different parts of the plant might be due to the presence of one and/or to the association of different compounds present in the essential oil of Ocotea duckei. Furthermore, for all of the OEOD tested we suggest that the hypotensive response is probably due to a decrease in total peripheral resistances associated with a cardiac parasympathetic stimulation (direct and/or indirect), which might in turn reduce cardiac output and consequently decrease mean arterial pressure.

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**REFERENCES**


