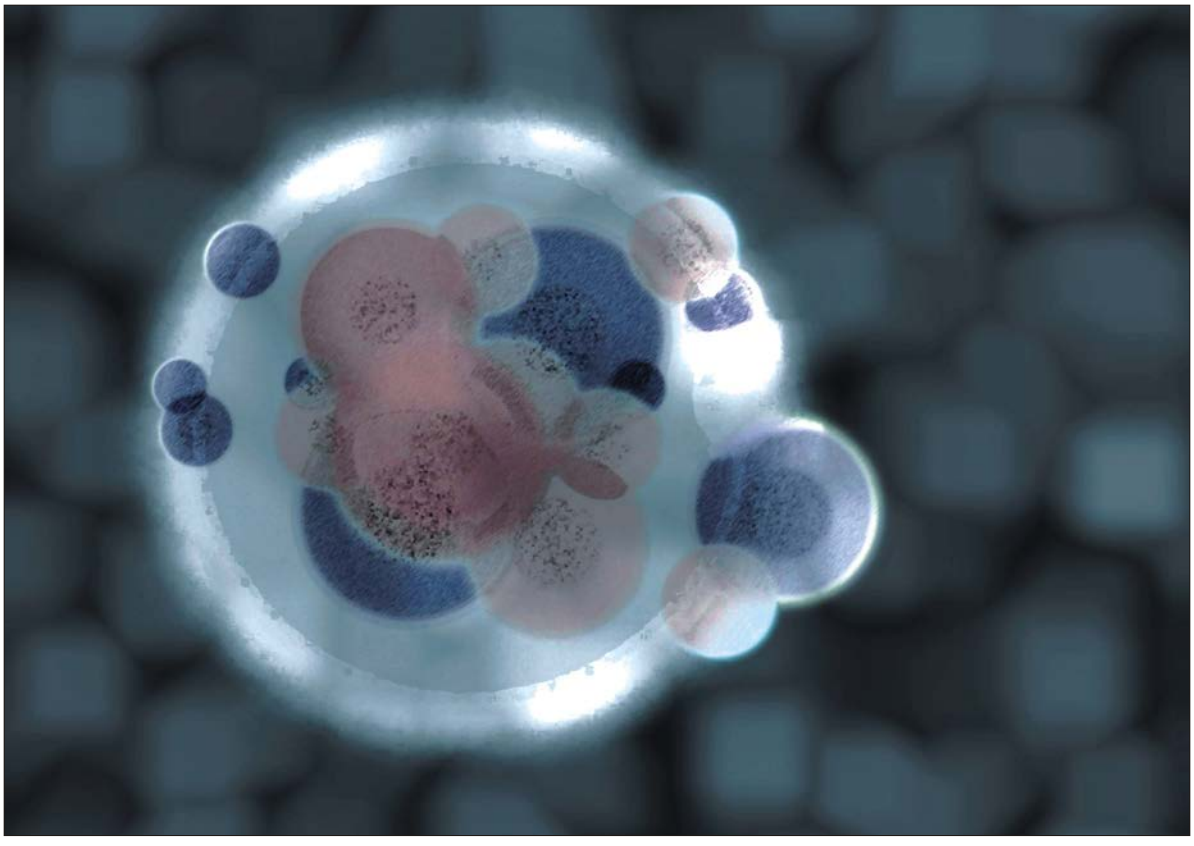

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Chemical Composition and Hypotensive Effects of Essential Oil of *Monodora myristica* Gaertn.

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Abstract: The composition of the essential oil from the fruit seeds of *Monodora myristica* Gaertn. (Annonaceae) was studied by capillary gas chromatography. The analysis using a combination of retention indices and combined Gas Chromatography/Mass Spectrometry (GC/MS) led to the identification of 30 components. The oil contains mainly monoterpenoids (93.2%) out of which 77.4% are monoterpene hydrocarbons and some sesquiterpenoids (5.8%) The major constituents were α-phellandrene (34.4%) and p-cymene (22.2%). The essential oil effects on the cardiovascular system were studied by recording the amplitude and frequency of the frog isolated heart contractions and by recording arterial blood pressure variations of the guinea pig. At the dose of 40, 80 and 120 μL kg^{-1} the essential oil induced a hypotensive effect on the blood pressure and at the dose of 0.01-0.05%. It reduced significantly the cardiac contractions of the isolated heart; however at 0.06% it totally stopped the cardiac contractions. Both these effects conducted in the present study could contribute to the anti hypertensive activity of the *Monodora myristica* essential oil.

Key words: Chemical composition, hypotensive, blood pressure, cardiac contractions

INTRODUCTION

Monodora myristica Gaertn. (Annonaceae) is a perennial tree growing in the tropical rainforest from Liberia to Angola. It is a wild plant among the most used as food and drug. In developing countries several plants give edible products: Fruits, seeds, leaves, flowers, nuts, oils, mushrooms and honey, which take a large place in the local diet and could strongly overcome or ameliorate prevailing food and health problems (Betti and Nzooh, 1998; Okwu, 2001; Tatsadjieu *et al*., 2003; Oboh, 2004; Okpeton *et al*., 2004; Tchiegang *et al*., 2005). The distinction between food and drug is not always clear. So, the seeds of *Monodora myristica*, in this case, possess these two properties and have carried us to pursue its study.

Data on *Monodora myristica* traditional uses are collected by personal contact with local traditional healers. In Central African Republic the seeds of fruit are used as condiment and drug in the treatment of headache and hypertension.

Earlier studies on *Monodora myristica* have reported the chemical composition and the evaluation of antimicrobial activities of essential oils collected in other countries (Cimanga *et al*., 2002; Tatsadjieu *et al*., 2003; Oussou *et al*., 2004; Ngueфack *et al*., 2004; Odoh, 2004; Agnaniet *et al*., 2004).

The present study investigate to the seeds essential oil composition of Central African species with the aim of evaluating the antihypertensive activity.

MATERIALS AND METHODS

Plant: The fruit seeds of *Monodora myristica* were collected in September 2006 from the forest of Lobaye near Boukoko (130 km south of Bangui, Central African Republic), voucher specimens were kept in the herbarium of the faculty of Sciences, University of Bangui.

Essential oil extraction: Five hundred gram of the air-dried seeds were communited and hydrodistillated for 4 h using a Clevenger-type apparatus. The essential oil was dried after decantation over anhydrous sodium sulfate.

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Analyses: GC analyses were performed on two fused silica capillary columns (25 m×0.25 mm), coated with OV-101 or Carbowax 20 M, the oven temperature was programmed from 50-200°C at 5°C min^{-1}; helium was used as a carrier gas at a flow rate of 0.8 mL^{-1} min^{-1}.

GC/MS analyses were carried out on a Hewlett Packard capillary GC-quadrupole MS system (model 5970) fitted with a (25 m×0.23 mm) fused silica column coated with DB-1 and using the same GC parameters. The volatile components were identified by comparison of their retention indices and their experimental mass spectra with those of reference compounds, further confirmation was done by referring to retention indices data generated from a series of alkanes: C_9-C_{30} (Adams *et al*., 2001; Jennings *et al*., 1980).

Animals: Quantitative assessment of antihypertensive activity was conducted in October, 2006.

Preparation of the isolated heart: Brain and spinal of the frog were destroyed. The back of the animal was turned and pinned on cork plank. With the scissors, the frog heart was laid bare by cutting away skin, muscular tissue and pericardium. The heart was intubated through arterial trunk and carefully isolated.

Perfusion and recording of the amplitude and the frequency of the isolated heart contractions: The classical experimental model of the heart isolated from frog was used (Lompo *et al*., 1991).

Statistical analysis: All data are presented as means±SEM. comparisons between data were performed by Student's t-test. Statistical significance was set at $p<0.05$.

Essential oil effects on heart contractions: Heart is initially perfused during approximately 5 min with the normal Ringer solution, then with the increasing concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) of essential oil of *Monodora myristica* on the one hand, on the other hand with a solution of vérapamil at the concentrations of 0.5×10^{-3}, 1.5×10^{-3}, 2×10^{-3} and 2.5×10^{-3} mg mL^{-1} during 2 min. Effects on amplitude of the contractions and on the heart rate are recorded.

Essential oil effects on arterial blood pressure: Guinea pig weighing 300-350 g (male or female). Animals were treated under urethane 2% (1 g kg^{-1}). The back of the animal was turned and neck region was dissected. Jugular vein was dissected and intubated with catheter supplied by syringe filled of heparinized Mac Ewen solution. Then carotid was dissected and intubated with catheter supplied with the blood pressure transducer itself connected to Gould recording system type 8000 S. After 1 h for stability, different doses of essential oil were administrated: 40, 80 and 120 µL kg^{-1}. Arterial blood pressure variations were recorded.

RESULTS

The essential oil was obtained in yield of 1.2%. The compounds identified in the essential oil are shown in Table 1.

Guinea pig blood pressure was reduced by essential oil at 40, 80 and 120 µL kg^{-1}. The blood pressure decreased in dose dependent manner. The effects were entirely or partially reversible according to the concentration used (Fig. 1).

The perfusion of the heart isolated from clamping plate with the essential oil of *Monodora myristica* to 0.04% causes a reduction in the amplitude (−56.31±2.45 %, $p<0.001$) and heart rate (−17.28±2.15%, $p<0.05$) (Fig. 2a-c). Washing with of Ringer standardizes the contractions. The vérapamil with the concentration of 1.5×10^{-3} mg mL^{-1} causes a reduction in the amplitude (−45.48±2.42%, $p<0.001$) and especially of the frequency of the contractions (−57.12±1.27%, $p<0.001$) (Fig. 4a-c). The concentration of 2.5×10^{-3} mg mL^{-1} causes the irreversible stop of the heart approximately 1 min after the perfusion (Fig. 4d-f).

Table 1: Volatiles compounds of essential oil of *Monodora myristica*

Compounds	IR	Percentage
α-thujène	926	1.8
α-pinène	934	6.3
Sabinène	970	0.1
β-pinène	974	0.3
β-myrcène	985	4.9
α-phellandrène	998	34.4
p-cymène	1015	22.2
Limonène	1023	4.0
β-phellandrène	1024	0.5
(E)-β-ocimène	1043	0.1
Linalool	1089	3.1
Cis-p-menth-2-enol	1113	0.4
Trans-p-menth-2-en-ol	1133	0.3
p-cymen-8-ol	1173	0.2
α-terpinéol	1182	1.0
Cis-pinocarvéol	1186	6.3
Pipéritol	1191	0.2
Unknow	1195	0.4
Thymoquinone	1221	0.2
Carvacrol	1289	3.6
Cis-pinocarvyl acétate	1302	0.1
a-santalène	1411	0.1
β-caryophyllène	1413	0.8
germacrène-D	1480	1.4
g-cadinène	1509	0.1
δ-cadinène	1518	0.3
germacrène D-4-ol	1570	1.1
caryophyllène oxyde	1576	0.3
epi-a-cadinol	1634	0.9
T-muurolol	1635	0.3
a-cadinol	1648	0.4
Total	-	99.0

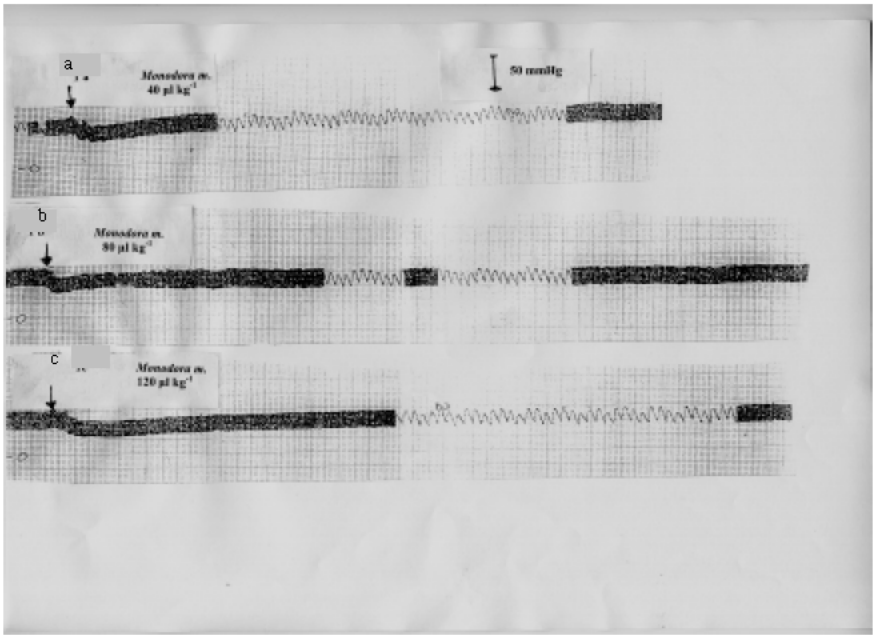

Fig. 1: Hypotensive effects of essential oil on arterial blood pressure; (a) Effects of *M. myristica* (40 μL kg^{-1}), (b) Effects of *M. myristica*(80 μL kg^{-1}) and (c) Effects of *M. myristica* (120 μL kg^{-1})

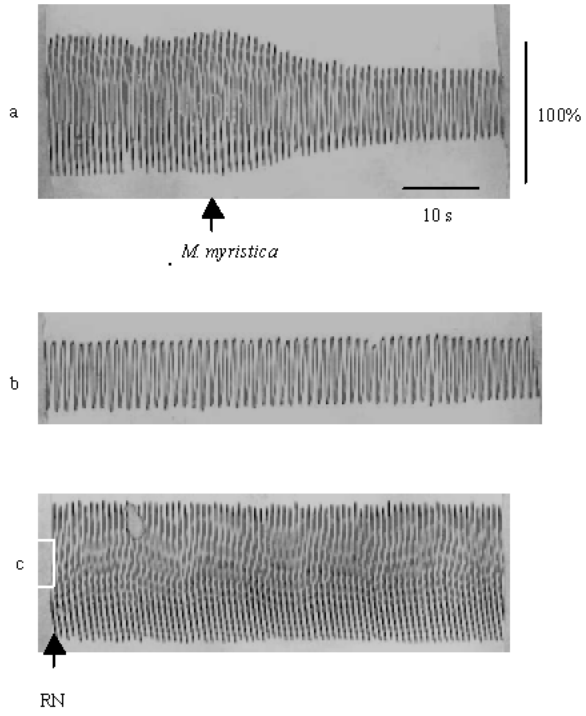

Fig. 2a-c: Effects of *M. myristica* (0.04 %) on the amplitude and the frequency contractions heart isolated from toad; (a) Pilot recording follow-up effects *M. myristica* (0.04 %) (b) continuation effects of *Mr. myristica* (0.04%) and (c) Return to normal ringer (RN)

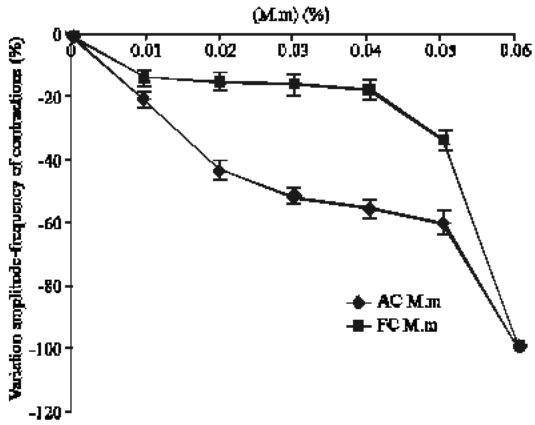

Fig. 3: Variation of the amplitude (AC) and frequency (FC) of contractions of the heart isolated from toad according to the concentration of essential oil of *Monodora myristica* (M. m)

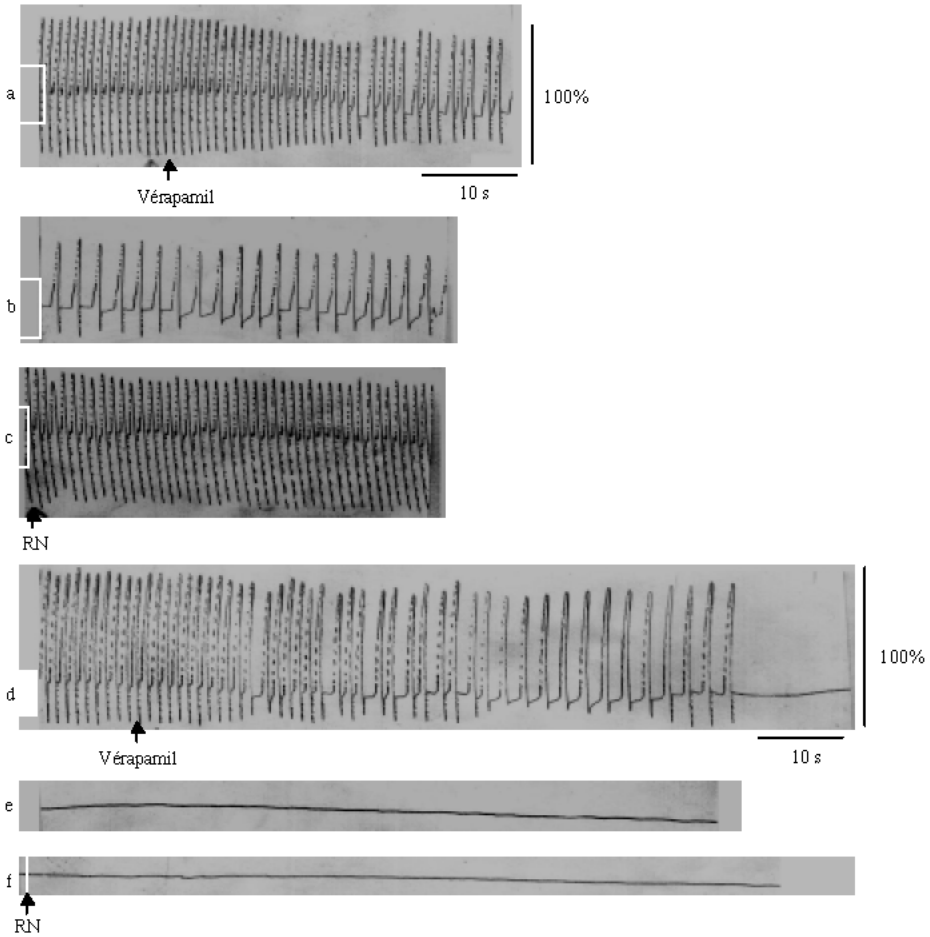

Fig. 4a-f: Effects of Vérapamil (1.5×10^{-3} and 2.5×10^{-3} mg mL^{-1}) on the contractions heart isolated from toad (a) Pilot recording follow-up effects of Vérapamil (1.5×10^{-3} mg mL^{-1}), (b) Continuation effects of Vérapamil (1.5×10^{-3} mg mL^{-1}), (c) Return to normal Ringer (RN) (d) Pilot recording follow-up effects of Vérapamil (2.5×10^{-3} mg mL^{-1}), (e) Continuation effects of Vérapamil (2.5×10^{-3} mg mL^{-1}) and (f) Return to normal Ringer (RN)

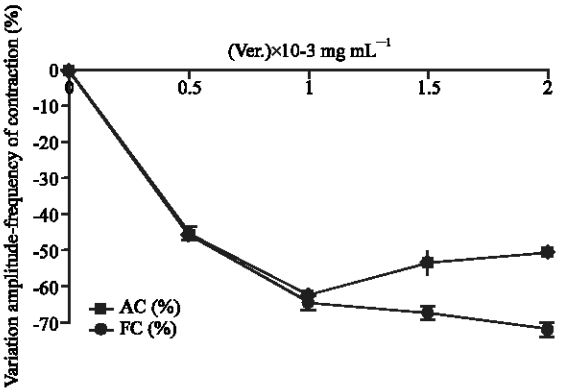

Fig. 5: Variation of amplitude (AC) and frequency (FC) contractions heart isolated of toad according to the concentration of vérapamil

The curves concentration-answers of the essential oil of *Monodora myristica* and the vérapamil are presented, respectively on Fig. 3 and 5. These curves show that the essential oil of *Monodora myristica* causes a reduction more marked in the amplitude than of the frequency of the contractions of the heart isolated from clamping plate, the vérapamil decreasing much more the frequency.

DISCUSSION

A total of 30 compounds were identified (99%). The essential oil contains exclusively terpenoid compounds with monoterpenoids (93.2%) being predominant. The monoterpene hydrocarbons and oxygenated monoterpenoids accounted for 77.4 and 15.8% of the oil, respectively. The amount of sesquiterpenoids (5.8%) was, however, significantly lower than in the Gabon essential oil (Agnaniet *et al*., 2004). Among the hydrocarbons α-phellandrene (34.4%) and p-cymene (22.2%) were found as major constituents of our essential oil and the species studied can be an interesting source of these compounds. It has reported that phellandrene along with pinene have been found as major components of the essential oil from Cameroon (Chalchat *et al*., 1997). Furthermore, phellandrene has not been detected as prevalent component in the essential oil from Democratic Republic of Congo (Cimanga *et al*., 2002). These results show that the chemical composition of centrafrican essential oil is different of those from other countries of the same region (Central Africa).

The *Monodora myristica* essential oil causes a normal arterial pressure decrease of the guinea-pig; it thus causes a hypotensor effect proportions depending.

At low doses, the frequency variations were less pronounced than the amplitude variations.

However, at 0.06% concentration, the frequency and the amplitude were reduced to 100% in 30 sec after treatment (heart activity was entirely abolished). Essential oil of *M. myristica* induced a significant diminution of the amplitude and the frequency on frog isolated heart contractions. It thus induces inotropic and chronotropic negatives effects which could contribute to the reduction in the cardiac flow, which reduction could partly explain the hypotensor effect of the essential oil *M. myristica* observed in the guinea-pig. The pressure difference between two points being directly proportional to the flow rate and the resistance (friction than impedes flow). These effects were similar to those induce by vérapamil, an anti calcique drug and could explain reduction cardiac rate flow. *M. myristica* essential oil reducing of the heart rate flow could partially explain its effects on arterial blood pressure and could contribute its use against hypertension.

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