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Chemical composition, cytotoxicity and *in vitro* antitrypanosomal and antiplasmodial activity of the essential oils of four *Cymbopogon* species from Benin ☆

Salomé Kpoviessi [a,b,c], Joanne Bero [b], Pierre Agbani [d], Fernand Gbaguidi [a,c], Bénédicta Kpadonou-Kpoviessi [a], Brice Sinsin [d], Georges Accrombessi [a], Michel Frédérich [e], Mansourou Moudachirou [c], Joëlle Quetin-Leclercq [b,*]

[a] *Laboratory of Physic and Synthesis Organic Chemistry (LaCOPS), University of Abomey-Calavi (UAC), Faculty of Sciences and Technics (FAST), BP: 4521 Cotonou, Benin*
[b] *Pharmacognosy Research Group, Louvain Drug Research Institute, Université catholique de Louvain, B1 7203 Av. E. Mounier 72, B-1200 Bruxelles, Belgium*
[c] *Laboratory of Pharmacognosy and Essential oils (LAPHE), University of Abomey-Calavi (UAC), Faculty of health Sciences (FSS), Faculty of Sciences et Technics (FAST), 01 BP: 188 Cotonou, Benin*
[d] *Laboratory of Applied Ecology (LEA), University of Abomey-Calavi (UAC), Faculty of Agronomic Sciences (FSA), 03 BP: 1974 Cotonou, Benin*
[e] *Université de Liège, Drug Research Center, Laboratoire de Pharmacognosie, Av. de l'Hôpital 1, B36, B-4000 Liège, Belgium*

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ABSTRACT

Ethnopharmacological relevance: *Cymbopogon* species are largely used in folk medicine for the treatment of many diseases some of which related to parasitical diseases as fevers and headaches. As part of our research on antiparasitic essential oils from Beninese plants, we decided to evaluate the *in vitro* antiplasmodial and antitrypanosomal activities of essential oils of four *Cymbopogon* species used in traditional medicine as well as their cytotoxicity.

Materials and methods: The essential oils of four *Cymbopogon* species *Cymbopogon citratus* (I), *Cymbopogon giganteus* (II), *Cymbopogon nardus* (III) and *Cymbopogon schoenantus* (IV) from Benin obtained by hydrodistillation were analysed by GC/MS and GC/FID and were tested *in vitro* against *Trypanosoma brucei brucei* and *Plasmodium falciparum* respectively for antitrypanosomal and antiplasmodial activities. Cytotoxicity was evaluated *in vitro* against Chinese Hamster Ovary (CHO) cells and the human non cancer fibroblast cell line (WI38) through MTT assay to evaluate the selectivity.

Results: All tested oils showed a strong antitrypanosomal activity with a good selectivity. Sample II was the most active against *Trypanosoma brucei brucei* and could be considered as a good candidate. It was less active against *Plasmodium falciparum*. Samples II, III and IV had low or no cytotoxicity, but the essential oil of *Cymbopogon citratus* (I), was toxic against CHO cells and moderately toxic against WI38 cells and needs further toxicological studies. Sample I (29 compounds) was characterised by the presence as main constituents of geranial, neral, β-pinene and *cis*-geraniol; sample II (53 compounds) by *trans*-*p*-mentha-1(7),8-dien-2-ol, *trans*-carveol, *trans*-*p*-mentha-2,8-dienol, *cis*-*p*-mentha-2,8-dienol, *cis*-*p*-mentha-1(7),8-dien-2-ol, limonene, *cis*-carveol and *cis*-carvone; sample III (28 compounds) by β-citronellal, nerol, β-citronellol, elemol and limonene and sample IV (41 compounds) by piperitone, (+)-2-carene, limonene, elemol and β-eudesmol.

Conclusions: Our study shows that essential oils of *Cymbopogon* genus can be a good source of antitrypanosomal agents. This is the first report on the activity of these essential oils against *Trypanosoma brucei brucei*, *Plasmodium falciparum* and analysis of their cytotoxicity.

1. Introduction

Aromatic plants are used since ancient times for their medicinal properties (Bakkali et al., 2008). Essential oils may be used as alternatives or adjuvants to current antiparasitic therapies and the emergence of parasites resistant to current chemotherapies highlights the importance of plant essential oils as potential novel

☆ *Chemical compounds studied in this article.*

β-Myrcene (PubChem CID: 31253); Limonene (PubChem CID: 22311); β-Citronellal (PubChem CID: 7794); Geranial (PubChem CID: 638011); Neral (PubChem CID: 643779); β-Pinene (PubChem CID: 14896); Piperitone (PubChem CID: 6987); (+)-2-Carene (PubChem CID: 78249); cis-*p*-Mentha-1(7),8-dien-2-ol (PubChem CID: 6429040); Nerol (PubChem CID: 643820).

* Corresponding author. Tel.: +32 2 764 72 54; fax: +32 2 764 72 93.
E-mail address: joelle.leclercq@uclouvain.be (J. Quetin-Leclercq).

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antiparasitic agents (Anthony et al., 2005; Moon et al., 2006; Nibret and Wink, 2010; Cheikh-Ali et al., 2011; Monzote et al., 2011; Palmeira-de-Oliveira et al., 2012).

Cymbopogon species are commonly used in folk medicine for the treatment of many diseases. *Cymbopogon citratus* is used for the treatment of nervous and gastrointestinal disturbances, and as an antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (Santin et al., 2009). Decoctions of the leaves and flowers of *Cymbopogon giganteus* are used as an effective treatment against skin disorders, conjunctiva, headaches and hepatitis (Adjanohoun et al., 1979; Adjanohoun and Aké Assi, 1979, 1985). *Cymbopogon nardus* is used for cooking, perfumery, rheumatism and in the treatment of fevers, intestinal parasites and of digestive and menstrual problems (Konwar and Gohain, 1999; Abena et al., 2007). *Cymbopogon schoenathus* is used as an embrocation, a diuretic, an insecticide, an aphrodisiac, for fever, snake-bite and for the treatment of rheumatism. The smoke from the burning grass is said to dispel temporary maniacal symptoms (IUCN, 2005, Khadri et al., 2010). Essential oils of these species are known for antimicrobial, antifungal, antioxidant, analgesic, anti-nociceptive, neurobehavioral and insecticidal properties (Bassolé et al., 2011; Innsan et al., 2011, Khadri et al., 2010; Abena et al., 2007; Jirovetz et al. 2007; de Billerbeck et al., 2001; Viana et al., 2000; Onawunmia et al., 1984) and as repellent against mosquitos, the major vector of malaria (Nonviho et al., 2010; Samarasekera et al., 2006; Tyagi et al., 1998). Their direct activity against *Trypanosoma brucei* and *Plasmodium falciparum* was not very documented excepted for essential oil from *Cymbopogon nardus* of Malaysia whose *in vitro* antitrypanosomal activity was recently reported by Muhd Haffiz et al. (2013). Furthermore, they are used in traditional medicine for the treatment of symptoms given by malaria or sleeping sickness (as fevers, headaches,…). So it seemed interesting to study the antiplasmodial and antitrypanosomal activities of these essential oils and its components.

Trypanosoma brucei is the parasite responsible for human African trypanosomiasis or sleeping sickness, an illness affecting 300,000–500,000 people, while up to 60 million people in 36 countries are at risk of contracting the disease (World Health Organisation (WHO), 2002). This parasite is transmitted by the bite of infected tsetse flies of the genus Glossina.

Malaria is also a disease caused by a protozoan parasite of *Plasmodium* species and still remains a major public health problem in the world. Five hundred million people are exposed to this disease, with an annual death rate that the World Health Organisation (WHO/World Health Statistic, 2011) estimates to more than 800,000 people in 2009.

These two parasitic diseases are the cause of considerable mortality and morbidity throughout the world (WHO/World Health Statistic, 2011) and parasites develop resistance to most of the drugs used. Some of these drugs need a long course parenteral administration, show toxicity and a variable efficacy between strains or species. These reasons led to the search for new antitrypanosomal and antiplasmodial compounds and it is known that plants used in traditional medicine are a source of new leads with a new mechanism of action (Hoet et al., 2004, Bero et al., 2011).

The present study investigates the *in vitro* antitrypanosomal and antiplasmodial activity of essential oils from four plants of *Cymbopogon* genus used in traditional medicine in Benin. Oils from fresh leaves of each plant were prepared and analysed by GC/FID and GC/MS. They were evaluated for their antitrypanosomal and antiplasmodial activities and their selectivity was assessed by analysing their cytotoxicity against Chinese Hamster Ovary cells (CHO) and a human non cancer fibroblast cell line (WI38).

2. Materials and methods

2.1. Plant material

Fresh leaves of *Cymbopogon citratus* (DC) Stapf, *Cymbopogon giganteus* (Hochst.) Chiov., *Cymbopogon nardius* (L.) Rendle and *Cymbopogon schoenantus* (L.) Spreng. (Poaceae) were collected in March 2011, from the Botanical Garden of the Abomey-Calavi University. Voucher specimens (nos. AA6387, AA6388, AA6389 and 6390/HNB respectively) of these leaves were conserved at the University of Abomey-Calavi Herbarium.

2.2. Chemicals and drugs

DMEM and Ham's-F12 culture media were purchased from Life technologies corporation (Grand Island, NY 14072, USA); Dulbecco's Phosphate Buffered Saline (DPBS 1 ×) from Invitrogen (Grand Island, NY 14072, USA); tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) (MTT), (S)-(+)-camptothecin, suramine, chloroquine, artemisinin, dimethyl sulfoxide (DMSO), α-pinene, β-pinene, camphene, *p*-cymene, myrcene, α-terpinene, γ-terpinene,1,8-cineol, terpinolene, borneol, citronellyl acetate, terpine-4-ol, α-terpineol, geraniol, verbenone, carvacrol, thymol, bornyl acetate, α-copaene, β-caryophyllene, fenchone, thujone, *trans*-pinocarveol, *trans*-verbenol, lavandulol, myrtenal, *trans*carveol, carvone, aromadendrene, *allo*-aromadendrene, γ-gurjunene, *cis*-ocimene, camphor and *n*-alkanes "C_7–C_{28}" were obtained from Sigma-Aldrich (Steinhein, Germany), Acros Organics (New jersey, USA), and Fluka Chemie (Buchs, Switzerland); α-thujene, sabinene, γ-3-carene, limonene, linalool, α-humulene, *cis*-pinane, α-phellandrene, *p*-cymenene, myrtenyl acetate and valencene were purchased from extrasynthese (Genay, France). All compounds were of analytical standard grade. Ter-Butyl methyl ether (TBME) was an analytical grade solvent purchased from Fluka Chemie, and anhydrous Na_2SO_4 was of analytical reagent grade from UCB (Bruxelles, Belgium).

2.3. Isolation of essential oils

Five hundred grams (500 g) of fresh leaves were steam distillated for 3 h in a modified Clevenger-type apparatus (Bruneton, 1993). The extraction was carried out in triplicate. The oils were preserved in a sealed vial at 4 °C. The essential oil yields were calculated based on the fresh plant material.

2.4. Chemical analysis of essential oils

2.4.1. GC/FID and GC/MS analysis

The GC/FID analysis was carried out on a FOCUS GC (Thermo Finigan; Milan, Italy) using the following operating conditions: HP 5MS column (30 m × 0.25 mm, film thickness: 0.25 μm) (J&W Scientific Column of Agilent Technologies, No. US167072Ã, USA); injection mode: splitless; injection volume: 1 μL (TBME solution); split flow: 10 mL/min; splitless time: 0.80 min; injector temperature: 260 °C; oven temperature was programmed as following: 50–250 °C at 6 °C/min and held at 250 °C for 5 min; the carrier gas was helium with a constant flow of 1.2 mL/min; FID detector temperature was: 260 °C. The data were recorded and treated with the ChromCard software.

The GC/MS analysis was carried out using a TRACE GC 2000 series (Thermo-Quest, Rodano, Italy), equipped with an autosampler AS2000 Thermo-Quest. The GC system was interfaced to a Trace MS mass spectrometer (ThermoQuest) operating in the electronic impact mode at 70 eV. HP 5 MS column (30 m × 0.25 mm, film thickness: 0.25 μm) was used in the same operating conditions as above. The coupling temperature of the GC was