Food and Chemical Toxicology 57 (2013) 57–68

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Cytotoxic mechanism of *Piper gaudichaudianum* Kunth essential oil and its major compound nerolidol

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ARTICLE INFO

Article history:
Received 6 December 2012
Accepted 7 March 2013
Available online 20 March 2013

Keywords:
P. gaudichaudianum essential oil
Nerolidol
Saccharomyces cerevisiae
DNA repair
ROS
Cytotoxicity

ABSTRACT

Piper gaudichaudianum Kunth is used in popular medicine as anti-inflamatory and against liver disorders. One of the most studied components of the plant is the essential oil for which chemical analysis revealed (*E*)-nerolidol as major compound. Recently, we have shown that *P. gaudichaudianum* essential oil possesses strong cytotoxic effects in mammalian V79 cells. The aim of this study was to analyze the cytotoxicity and mutagenicity of *P. gaudichaudianum* essential oil and nerolidol using *Saccharomyces cerevisiae* as model study. Treatment of the XV185-14c and N123 strains with essential oil and nerolidol led to cytotoxicity but did not induce mutagenicity. Our results revealed an important role of base excision repair (BER) as the *ntg1*, *ntg2*, *apn1* and *apn2* mutants showed pronounced sensitivity to essential oil and nerolidol. In the absence of superoxide dismutase (in *sod1*Δ mutant strain) sensitivity to the essential oil and nerolidol increased indicating that this oil and nerolidol are generating reactive oxygen species (ROS). The ROS production was confirmed by DCF-DA probing assay in Sod-deficient strains. From this, we conclude that the observed cytotoxicity to *P. gaudichaudianum* essential oil and nerolidol is mainly related to ROS and DNA single strand breaks generated by the presence of oxidative lesions.

1. Introduction

Piper gaudichaudianum Kunth is a plant that belongs to the Piperaceae family. This family is composed of 7 genera and approximately 1100 species. The genus *Piper* (Piperaceae) is well known for its aromatic herbs, which are largely distributed in tropical and subtropical regions of the world. Some Piper species are also employed in folk medicine as analgesics for pain management, toothaches and wound treatment (Guerrini et al., 2009). *P. gaudichaudianum* Kunth is widely distributed in the Brazilian Atlantic forest, from the Northeast to southern of Brazil, and reaching Argentina and Paraguay. This plant is popularly known by the names "Iaborandi", "Jaborandi", "Paripaioba" and "Pariparoba" (Di Stasi and Hiruma-Lima, 2002).

According to popular medicine, the leaves infusion and fresh leaves of *P. gaudichaudianum* are used to relieve toothache, while the fresh roots are used as anti-inflammatory and against liver disorders (Di Stasi and Hiruma-Lima, 2002). Other previously described biological activities using different plant material from this species include fungicidal and larvicidal effects (Lago et al., 2004; Morais et al., 2007) as well as anti-inflammatory and analgesic activities of leaf extracts (Di Stasi and Hiruma-Lima, 2002; Moreira et al., 2001). Furthermore, the essential oil of *P. gaudichaudianum* leaves has anti-inflammatory and larvicidal activity (Morais et al., 2007). We have recently demonstrated that this essential oil has strong cytotoxic, genotoxic and mutagenic effects in mammalian V79 cells, and these effects are likely related to its oxidative potential (Péres et al., 2009).

The previously published studies on *P. gaudichaudianum* have presented analyses of its leaves and its essential oil phytochemical

Abbreviations: BER, *Saccharomyces cerevisiae* strains defective in base excision repair; Can, canavanine; DCF, 2′,7′-dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; DMSO, dimethylsulfoxide; GC × GC/TOF-MS, Comprehensive two dimensional gas chromatography/time-of-flight mass spectrometer analyses; GX X GC, Comprehensive two-dimensional gas-chromatography; H_2O_2, hydrogen peroxide; His, histidine; Hom, homoserine; HR, *Saccharomyces cerevisiae* strains defective in homologous recombination; Lys, lysine; MM, minimal medium; NER, *Saccharomyces cerevisiae* strains defective in nucleotide excision repair; NHEJ, *Saccharomyces cerevisiae* strains defective in non-homologous end-joining; 4-NQO, 4 nitroquinoline-oxide; OH·, hydroxyl radical; PRR, *Saccharomyces cerevisiae* strains defective in post-replication repair; ROOH, hydroperoxides; ROS, reactive oxygen species; SC, synthetic complete medium; TLS, *Saccharomyces cerevisiae* strains defective in translesion synthesis; TIC, typical two-dimensional separation/total ion chromatogram; YPD, Complete liquid medium.

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0278-6915
http://dx.doi.org/10.1016/j.fct.2013.03.013

profile, reporting triterpenes, flavonoids and alkaloids as the major constituent compounds. Among the terpenes identified were (*E*)-nerolidol, d-limonene, linalool, β-caryophyllene, α-humulene and cymene (Andrade et al., 1998; Péres et al., 2009; Poser et al., 1994; Rorig and Poser, 1991).

(*E*)-nerolidol, one of the major sesquiterpenes present in the essential oil of *P. gaudichaudianum* that was identified previously by our research group (Péres et al., 2009), is used to enhance flavor and aroma, is approved by the U.S. Food and Drug Administration, and has been studied as a topical skin penetration enhancer for the transdermal delivery of therapeutic drugs (Lapczynski et al., 2008; Williams and Barry, 2004). Studies related to the biological activity of nerolidol showed an antifungal effect against *Microsporum gypseum* (Lee et al., 2007), activity against L3 larvae of *Anisakis* type I (Navarro-Moll et al., 2011), and antimalarial (Lopes et al., 1999), antileishmanial and antiulcer activities (Arruda et al., 2005; Klopell et al., 2007). In addition, (*E*)-nerolidol and α-humulene have been shown to be cytotoxic on renal cell adenocarcinoma ACHN, the hormone-dependent prostate carcinoma LNCaP, the amelanotic melanoma C32 and the MCF-7 breast cancer cell line (Legault et al., 2003; Loizzo et al., 2007a,b; Sylvestre et al., 2007).

In view of the absence of knowledge about the exact cytotoxic mechanisms of *P. gaudichaudianum* essential oil, the aim of the present study was to investigate the cytotoxic effects of the essential oil and compound nerolidol (a racemic mixture of *cis* and *trans* isomers) using *Saccharomyces cerevisiae* strains deficient in the major DNA repair proteins. Induced mutagenesis was also tested in the haploid XV185-14c and N123 *S. cerevisiae* strains. The oxidative potential of the essential oil and nerolidol was also estimated by cytotoxic assay with superoxide dismutase and catalase yeast deficient strains and 2′,7′-dichlorofluorescein (DCF) fluorescent assay, which detects intracellular ROS generation. In addition, we performed a more detailed chemical analysis of *P. gaudichaudianum* essential oil by comprehensive two dimensional gas chromatography/time-of-flight mass spectrometer analyses (GC × GC/TOF-MS).

2. Materials and methods

2.1. Chemicals

Amino acids (L-histidine, L-threonine, L-methionine, L-tryptophan, L-leucine, L-lysine, L-arginine), 4 nitroquinoline-oxide (4-NQO), hydrogen peroxide (H_2O_2), L-canavanine, nitrogen bases (adenine and uracil), 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and dimethylsulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Yeast extract, bacto-peptone, bacto-agar, yeast nitrogen base with no amino acids and glucose were acquired from Difco Laboratories (Detroit, MI, USA). Nerolidol (a racemic mixture of cis and trans isomers) used in the analytical and biological assays was also acquired from Sigma–Aldrich.

2.2. Sample

Leaves from *P. gaudichaudianum* were collected in Riozinho (Rio Grande do Sul province, Brazil) in February, 2010. The voucher specimen was identified and deposited at the Herbarium of the Department of Botany, Federal University of Rio Grande do Sul, Porto Alegre, Brazil (Voucher No.: ICN 128412). The leaves of *P. gaudichaudianum* were air-dried and the oil was obtained by hydrodistillation process for 4 h, using a Clevenger type apparatus, in accordance with the method recommended by British Pharmacopoeia (2011), producing 0.55% (w/v) of essential oil. The distilled oil was dried over anhydrous sodium sulfate and stored in closed dark vials at 4 °C until use. The oil was yellow and had a distinct sharp odor. The essential oil and the standard nerolidol were diluted (1:100 v/v) in hexane prior to GC × GC/TOF-MS.

2.3. Analysis by GC × GC/TOF-MS

The GC × GC/TOF-MS analyses were performed using an Agilent 6890GC system with an Pegasus III TOFMS analyzer (LECO Corporation, St. Joseph, MI, USA). The primary GC column was DB-5 (methyl silicon wit 5% of phenyl substituted groups)

Table 1
Saccharomyces cerevisiae strains used in this study. BER: base excision repair; NER: nucleotide excision repair; HR: homologous recombination; NHEJ: non-homologous end-joining; TLS: translesion synthesis.

Strain	Genotype	DNA repair pathway affected	Source
BY4741 (WT)	*MATα*; *his*3Δ1; leu2Δ0; *met*15Δ0; *ura*3Δ	–	Euroscarf[a]
apn1Δ	BY4741; with *apn1*::*kanMX4*	BER	Euroscarf
rad1Δ	BY4741; with *rad1*::*kanMX4*	NER	Euroscarf
rad10Δ	BY4741; with *rad10*::*kanMX4*	NER	Euroscarf
rad18Δ	BY4741; with *rad18*::*kanMX4*	PRR	Euroscarf
rad30Δ	BY4741; with *rad30*::*kanMX4*	TLS	Euroscarf
rad50Δ	BY4741; with *rad50*::*kanMX4*	NHEJ	Euroscarf
rad52Δ	BY4741; with *rad52*::*kanMX4*	HR	Euroscarf
rev1Δ	BY4741; with *rev1*::*kanMX4*	TLS	Euroscarf
rev3Δ	BY4741; with *rev3*::*kanMX4*	TLS	Euroscarf
ku70Δ	BY4741; with *ku70*::*kanMX4*	NHEJ	Euroscarf
SJR751 (WT)	*MATα*; *ade*2-101$_{oc}$; *his*3Δ200; *ura*3ΔNco; *lys*2Δ*Bgl*; *leu*2-R	–	RL Swanson[b]
ntg1Δ	SJ751; with *ntg1*::LEU2	BER	RL Swanson
ntg2Δ	SJ751; with *ntg2*::*his*G	BER	RL Swanson
ntg1Δ ntg2Δ	SJ751; with *ntg1*::LEU2 *ntg2*::*his*G	BER	RL Swanson
ntg1Δ ntg2Δ apn1Δ	SJ751; with *ntg1*::LEU2 *ntg2*::*his*G *apn1*::*HIS3*	BER	RL Swanson
ntg1Δ ntg2Δ apn1Δ rad1Δ	SJ751; with *ntg1*::LEU2 *ntg2*::*his*G *apn1*::HIS3 *rad1*::*his*G	BER/NER	RL Swanson
ntg1Δ ntg2Δ apn1Δ rev3Δ	SJ751; with *ntg1*::LEU2 *ntg2*::*his*G *apn1*::HIS3 *rev3*::*kanMX4*	BER/TLS	RL Swanson
ntg1Δ ntg2Δ apn1Δ rad52Δ	SJ751; with *ntg1*::LEU2 *ntg2*::*his*G *apn1*::HIS3 rad52::*URA3*	BER/HR	RL Swanson
EG103 (SOD-WT)	MATα; leu2-3 112his3-Δ1 trp1-289 ura3-52	–	E Gralla[c]
EG118 (*sod1Δ*)	like EG103, except *sod1*::URA3	–	E Gralla
EG110 (*sod2Δ*)	like EG103, except *sod2*::TRP1	–	E Gralla
EG133 (sod1Δ sod2Δ)	like EG103, except *sod1*::URA3 e *sod2*::TRP1	–	E Gralla
EG223 (*ctt1Δ*)	like EG103, except *ctt1*::TRP1	–	E Gralla
EG (sod1Δ ctt1Δ)	like EG103, except *sod1*::URA3 e *ctt1*::TRP1	–	E Gralla
XV185-14c	*MATα*; *ade*2-2; *arg*4-17; *his*1-7; *lys*1-1; *trp*5-48; *hom*3-10	–	RC Von Borstel[d]
N123	MATa; his1-7	–	JAP Henriques[e]

[a] Strains obtained from Euroscarf, Frankfurt, Germany.
[b] RL Swanson, Atlanta, Georgia, USA.
[c] E Gralla, Los Angeles, California, USA.
[d] RC Von Borstel, Edmonton, Alberta, Canada.
[e] JAP Henriques, Porto Alegre, Rio Grande do Sul, Brazil.