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Antimicrobial Activity of Phenylpropanoyle from the Rhizome of *Zingiber officinale* Roscoe (Zingiberaceae)

Talla Emmanuel[1*], Bealem Aristide[1], Tatsadjieu Ngoune Leopold[2], Ngassoum Martin Benoît[3], Wansi Jean Duplex[4], Yaya Gbaveng[1], Dabole Bernard[5], Meli Alain[7], Mbafor Tanyi Joseph[5] and Alain Roch[6]

[1]Department of Chemistry, Faculty of Science, University of Ngaoundere, PO Box 454, Ngaoundere, Cameroon.
[2]Department of Microbiology, Institute of Technology, PO Box 454, Ngaoundere, Cameroon.
[3]Department of Applied Chemistry, National School of Sciences Agribusiness, PO Box 454, Ngaoundere, Cameroon.
[4]Department of Chemistry, Faculty of Science, University of Douala, PO Box 24157, Douala, Cameroon.
[5]Department of Organic Chemistry, Faculty of Science, University of Yaoundé 1, PO Box 812, Yaoundé, Cameroon.
[6]Département of General Chemistry, Organic and Biomedical, Faculty of Science, University of Mons, B-7000 BP Mons, Belgium.
[7]Département of Chemistry, Ecole Normale Supérieure, University of Maroua, BP55, Maroua, Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Author TE performed the experiments, analyzed the data, managed the literature searches and wrote the first draft of the manuscript. Author BA performed the experiments, analyzed the data, and managed the literature. Author TNL performed the experiment of antimicrobial activities. Author NMB managed the analyzed of the study. Author WJD managed the study and corrected the manuscript. Author YB participated to the perform of the experiments. Author DB managed the analysis of the study. Authors MA, MTJ and AR managed the analyzed of the study.

Original Research Article

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ABSTRACT

Aim: This study was carried out to determine the phytochemical and the antimicrobial activities of *Zingiber officinale* (Zingiberaceae) Rhizome.

**Corresponding author: Email: tallae2000@yahoo.fr;*

Methodology: The air-dried and powdered rhizome (1.0 kg) of *Z. officinale* was extracted with ethyl acetate (1.5 L) at room temperature for 72 h. After evaporation under reduced pressure, 46.5 g of crude extract was obtained. The CH_2Cl_2 extract was purified by column chromatography over silica gel 60 (230-400 mesh) and preparative TLC using a gradient system of hexane, ethyl acetate and MeOH.
Agar diffusion test plates with *Escherichia coli* CIP 548, *Listeria monocytogenes* CIP 82110, *Enterococcus faecalis* CIP 76117, *Bacillus cereus* CIP 6624, *Staphylococcus aureus* CIP 7625, *Pseudomonas fluorescents* CIP 6913, *Bacillus substilis* ATCC 6636, *Candida lusitaniae* ATCC 200950, *Candida tropicalis* ATCC 750, *Cryptococcus neoformans* CIP 95026, *Aspergillus flavus*, and *Aspergillus parasiticus* as test strains were performed.
Results: A new nonyl-3-phenylpropanoate (1), together with known compounds, 8-hepthyl-12- propyldecanol (2) were isolated from the ethyl acetate extract of the rhizome of *Zingiber officinale.* The structures of the two compounds were determined by comprehensive analyses of their 1D and 2D NMR, mass spectral data, chemical reactions, and comparison with previously known analogue.
The two compounds isolated from ethyl acetate extract showed antibacterial and antifungal activity and the Minimal Inhibitory Concentration (MIC) against the test fungi varied from 3.34 mM to 5.08 mM for compound 1 and from 3.81 mM to 5.08 mM for compound 2.
Conclusion: One new compound, nonyl-3-phenylpropanoate, isolated from ethyl acetate extract of Zingiber officinale rhizome exhibited antibacterial and antifungal activities.

Keywords: Zingiber officinale; Zingiberaceae; Phenylpropanoyle; Antimicrobial activity.

ABBREVIATIONS

TLC: Thin Layer Chromatography; TOF-MS-ES ($[M+Na]^+$: Time Of Flight-Mass Spectrometry Electrospray; ^{1}H-NMR: Proton Nuclear Magnetic Resonance; HMBC: Heteronuclear Multiple Bond Correlation ; COSY: Correlation Spectroscopy; ^{13}C-NMR: Carbon 13 Nuclear Magnetic Resonance; EI-MS: Electron Impact –Mass Spectrometry; DEPT: Distorsionless Enhancement by Polarisation Transfer; HMQC: heteronuclear Multiple Quantum Coherence; TMS: Tetramethylsilane ; MeOH: Methanol;

1. INTRODUCTION

In continuation of our research for antimicrobial molecules from Cameroonian rainforest medicinal plants. rhizomes of *Zingiber officinale* (Zingiberaceae), was studied. Ginger known as rhizomes of *Zingiber officinale* (Zingiberaceae) is one of the spices valued for its aroma and pungency characteristic. It grows in the tropical region, especially in the southern and eastern part of Asia. That spice is commercialized in the dry form. In Cameroon, ginger is often consumed in the form of a drink commonly called “Njinjia”. Ginger is a stimulant, carminative and frequently used for dyspepsia, gastroparesis, constipation and colic [1]. Ginger oil has proved to prevent skin cancer in mice [2] some studies have demonstrated that gingerols can be used to fight against ovarian cancer [3,5]. [10]-gingerol and [12]-gingerol isolated from ginger rhizome have been reported to show antibacterial activity against periodontal bacteria [6]. Previous phytochemical investigations on the rhizome of *Zingiber officinale* yielded 6-gingerol, zingerone, shogaol, butyl hydroxyl toluene, butyl hydroxyl anisole [7,8]. In this report, we describe the isolation and structural elucidation of

one new phenylpropanoyle, together with the antimicrobial activities of isolated compounds and extract.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

Infrared spectra were recorded on a JASCO 302-A spectrophotometer. HR-TOF-MS-ESI was recorded on a Bruker FTICR 4.7 T mass spectrometer. EI-MS were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance. The ^{1}H- and ^{13}C-NMR spectrum were recorded at 500 MHz and 125 MHz respectively on Bruker DRX 500 NMR spectrometers. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear ^{1}H connectivities were determined by using the COSY experiment. One-bond ^{1}H-^{13}C connectivities were determined with HMQC gradient pulse factor selection. Two- and three-bond ^{1}H-^{13}C connectivities were determined by HMBC experiments. Chemical shifts are reported in δ (ppm) using TMS as internal standard and coupling constants (J) were measured in Hz. Column chromatography was carried out on silica gel (70-230 mesh, Merck). TLC was performed on Merck precoated silica gel 60 F_{254} aluminium foil, and spots were detected using ceric sulphate spray reagent. Phenolic compounds were detected using $FeCl_3$ reagent. The purity of the compounds was investigated by means of ^{1}H-NMR and TOF-MS-ESI. The degree of purity of all tested compounds was $>$ 95%, and of the positive control 99.9%. All other substances, if otherwise not specified, were purchased from Sigma-Aldrich (Germany). All reagents used were of analytical grade.

2.2 Plant Material

The rhizome of *Zingiber officinale* was collected at the Meiganga locality in the Adamawa region (Cameroon) in July 2009 and identified by Mr. Nana Victor a botanist of National Herbarium of Cameroon (HNC). A specimen was deposited at the Laboratory of chemistry of the natural substances under reference number N° 43125/HNC.

2.3 Extraction and Isolation

The air-dried and powdered rhizome (1.0 kg) of *Z. officinale* was extracted with ethyl acetate (1.5 L) at room temperature for 72 h. After evaporation under reduced pressure, 46.5 g of crude extract was obtained. The CH_2Cl_2 extract was purified by column chromatography over silica gel 60 (230-400 mesh) and preparative TLC using a gradient system of hexane, ethyl acetate and MeOH. 200 sub-fractions (ca. 250.0 mL each) were collected and pooled on the basis of TLC analysis leading to five main fractions (A–C).

Fraction A (10.2 g, combined from sub-fractions 1-50) was chromatographed over a silica gel 60C column with a hexane-ethyl acetate gradient. A total of 30 fractions of ca. 100.0 mL each were collected and combined on the basis of TLC. Fractions 5-10 were further chromatographed on silica gel 60H with a mixture of hexane-ethyl acetate (4:1) for elution to yield 8-hepthyl-12- propyl-decanol **(**41.0 mg). Fraction B (7.0 g, combined from sub-fractions 51-70) was chromatographed over a silica gel 60C column with a hexane-ethyl acetate gradient, a total of 20 fractions of ca. 100.0 mL each was collected and combined on the basis of TLC. Fractions 7-15 were further chromatographed over a silica gel 60H with a mixture of hexane-ethyl acetate (5:2) to yield nonyl-3-phenylpropanoate (17.0). Fraction C

(6.5 g, combined from sub-fractions 71-100) was chromatographed on a silica gel 60C column with a hexane-ethyl acetate gradient. 25 fractions of ca. 100.0 mL each were collected and combined on the basis of TLC.

2.3.1 Chemical derivatives

*Methanolysis of compound (**1**)*: Compound **1** (6.5 mg) was added to a mixture of HCl (3.5 mL, 1N) and dry MeOH (6.0 mg), and refluxed for 16 h with magnetic stirring. 10.0 mL H_2O was added and the refluxed mixture was extracted with *n*-hexane (3 x 10 mL). The methyl-3-phenylpropanoate (**1a**) (2.0 mg) was obtained after purification of the *n*-hexane extract over a silica gel column with *n*-hexane- as solvent. The MeOH/H_2O phase was evaporated under reduced pressure.

Nonyl-3-phenylpropanoate (**1**): Yellow green oil ($CHCl_3$); R_f 0.75, silica gel 60 F_{254}, Hexane/ethyl acetate (6/1); IR (KBr): υ_{max}, 1640, 1300, 1045 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; HR-TOF-MS-ES ($[M+Na]^+$ at *m*/*z* = 299.1987, calcd 299.2043).

Methyl-3-phenylpropanoate (1a): White oil ($CHCl_3$); R_f 0.70, silica gel 60 F_{254}, Hexane/ethyl acetate (6/1); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.40 (t, *J* = 7.0 Hz, $\underline{CH_2}$-CO), 2.83 (t, *J* = 7.0 Hz, $\underline{CH_2}$-CH2-CO), 3.55 (s, Me), 7.09 (brs, C_6H_5-); EI-MS (%): *m*/*z* 164 (10, $C_{10}H_{12}O_2$) $[M]^+$; 150 (81, $C_9H_{10}O_2$); 149 (49, $C_9H_9O_2$); 134 (78, $C_9H_{10}O$); 119 (31, C_9H_{11}); 105 (20, C_8H_9); 91 (56, C_7H_7); 78 (100, C_6H_6); 43 (25, C_3H_7).

2.4 Antimicrobial Assays

2.4.1 Microorganisms used

Escherichia coli CIP 548, *Listeria monocytogenes* CIP 82110, *Enterococcus faecalis* CIP 76117, *Bacillus cereus* CIP 6624, *Staphylococcus aureus* CIP 7625, *Pseudomonas fluorescents* CIP 6913, *Bacillus substilis* ATCC 6636, *Candida lusitaniae* ATCC 200950, *Candida tropicalis* ATCC 750, *Cryptococcus neoformans* ATCC 95026, *Aspergillus flavus*, and *Aspergillus parasiticus* were obtained from the Microbiological Laboratory of National Advanced School of Agro-industrial Sciences, University of Ngaoundere, Cameroon.

2.4.2 Standard antibacterial and antifungal used

Tetracycline disc (10µg) and griseofulvin purchased from ABTECH Biologicals LTD Liverpool were used respectively as standard antibacterial and antifungal. The stock solution of griseofulvin was prepared by dissolving the powder in DMSO, after which a volume containing 25 µg was loaded on sterile filter paper (Whatman n°1) during the diffusion technique on solid media.

2.4.3 Studied activity

Compounds 1 and 2 were tested for antimicrobial activity using the diffusion technique on solid media. Sterile, 6 mm diameter filter paper (Whatman n°1) were placed on plates of Mueller-Hinton agar (Difco) or Sabouraud Dextrose Agar (for fungi) which had been surface spread with 0.1 ml of logarithmic phase bacteria at a density adjusted to a 0.5 McFarland turbidity standard (10^8 colony forming units [CFU]/ml) or 0.1 ml of yeast (10^5 CFU /ml). *A. flavus* and *A. parasiticus* were subcultured in potato dextrose agar (Merck, Germany) to enhance sporulation. Seven day-old cultures were covered with 1ml distilled water and the

colonies were probed with the tip of a sterile Pasteur pipette to obtain a mixture of mycelium and conidia. The suspensions were transferred to sterile tubes, allowed to sediment for 30 minutes and adjusted to 10^5 cells/ml using haemocytometer. 0.1 ml of the suspension was spread on plates of Sabouraud Dextrose Agar and exposed to air dry. Then, 6 mm diameter sterile filter paper (Whatman n°1) was placed on plates.

Stocks solutions of tested compounds were prepared by dissolving each compound in the solvent CH_2Cl_2/MeOH (9:1) in order to obtain the concentration of 8.9 mM and 6.78 mM respectively for compound 1 and compound 2, after which, 15 µl of solution were loaded on the filter paper. In the control plates, 15 µl of solvent were loaded on the filter paper. The plates were then incubated for 24 h at 37°C for bacteria and 72 h at 25°C for fungi. The results were recorded by measuring the zones of growth inhibition surrounding the filter paper.

The macrobroth dilution method in broth media, Mueller-Hinton for bacteria and Sabouraud for yeast, was used to determine the Minimal Inhibitory Concentration (MIC). In these experiments, 0.4 ml of a suspension containing 1 x 10^6 CFU/ml was added to 3.6 ml of susceptibility test broth containing appropriate dilutions of the compound in glass test tubes (13 by 100 mm) fitted with loose plastic nonscrew caps. All tubes were incubated in air at 37°C for 24 h before being read. The MIC was considered the lowest concentration of the compound that prevented visible growth.

For the filamentous fungi (*A. flavus* and *A. parasiticus*), the MICs were performed by the agar disc diffusion Method as previously described by Tatsadjieu et al. [9]. Sabouraud agar medium with different concentrations of each compound (0.67 mM to 6.69 mM for compound 1 and 0.5 mM to 5.08 mM for compound 2) were prepared. About 20 ml of the medium were poured into glass Petri–dishes (9 cm x 1.5 cm). Each Petri–dish was inoculated at the centre with a mycelial disc (6 mm diameter) taken at the periphery of *A. flavus* or *A. parasiticus* colony grown on Sabouraud agar for 48 h. Control plates (without compound 1 or 2) were inoculated following the same procedure. Plates were incubated at 25°C and the colony diameter was recorded each day. Minimal Inhibitory Concentration was defined as the lowest concentration of compound in which no growth occurred.

3. RESULTS AND DISCUSSION

The ethyl acetate extract of rhizome of *Z. officinale* was separated by repeated column chromatography and preparative TLC to obtain one new and one known compound (Fig.1). The known compound was identified as 8-hepthyl**-**12-propyl-decanol (2). The structure was confirmed by spectra comparison with authentic and published values [10,11].

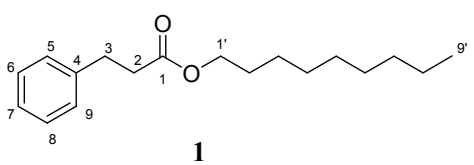
Compound 1 was obtained as yellow oil. The molecular composition was found to be $C_{18}H_{28}NaO_2$ by HR-TOF-MS-ES ($[M+Na]^+$ at m/z = 299.1987, calcd 299.2043). The presence of the carbonyl ester functions was revealed by strong absorptions at 1640 cm^{-1} in the IR spectrum. Its ^{1}H-NMR spectrum (Table 1) showed a singlet of five aromatic protons at δ = 7.08, two coupled triplets of methylene protons at δ = 2.85 and δ = 2.32 (t, J = 7.1 Hz, each 2H) for H-3 and H-2, respectively. The ^{13}C-NMR exhibited a carbonyl group of ester at δ = 171.2 and two methylenes at δ = 31.2 (CH_2-$\underline{CH_2}$-COO) and 33.8 ($\underline{CH_2}$-CH_2-COO). All these data suggested the presence of a 3-phenylpropanoyl moiety.

Furthermore, in the ^{1}H-NMR spectrum, a terminal methyl at δ = 0.96 (brs, 3H) and methylenes at δ= 4.12 (t, J = 7.4 Hz, -$\underline{CH_2}$-O-, 2H), 1.57 (m, -$\underline{CH_2}$-CH_2-O-, 2H), and 1.28

(brs, 12H) were also were observed. These data suggested the presence of a long chain linked to 3-phenylpropanoyle. Its presence was further confirmed by the ^{13}C-NMR spectrum (Table1), which showed characteristic signals at δ = 61.4 (C-1′), 29.4 ($(CH_2)_n$) and 14.1(CH_3).

To confirm the linkage between the long chain and the 3-phenylpropanoyl, the HMBC experiment was used. In the HMBC spectrum, correlation of H-3 (δ = 2.85) with C-1(δ = 171.2), C-2 (δ = 31.2) and C-5/C-9 (δ = 129.6); of H-1' (δ = 4.12) with C-1 (δ = 171.2), C-3' (δ = 29.4), suggested that the long chain is linked to 3-phenylpropanoyl across an ester function.

Methanolysis of 1 yielded the methyl-3-phenylpropanoate and nonanol identified by EI-MS. From the above spectroscopic studies, compound 1 was characterized as nonyl-3-phenylpropanoate.



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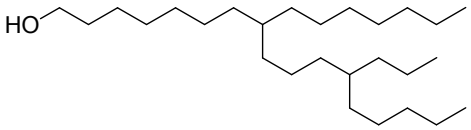

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Fig.1. Structures of compounds isolated from *Z. officinale*

The two compounds isolated from ethyl acetate extract were tested for their antimicrobial activity on Gram-positive and negative bacteria and fungi. The results of the diffusion test showed that compound 1 and compound 2 showed antibacterial and antifungal antimicrobial activities (Table 3).

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignments for (1) $CDCl_3$

Attribution	1	
	^{13}C	^{1}H
1	171.2	-
2	31.2	2.32 (*t*, *J* = 7.1)
3	33.8	2.85 (*t*, *J* = 7.1)
4	139.9	-
5	129.6	7.08 (*brs*)
6	128.7	7.08 (*brs*)
7	127.9	7.08 (*brs*)
8	128.7	7.08 (*brs*)
9	129.6	7.08 (*brs*)
1'	61.4	4.12 (*t, J =7.4*)
$-(CH_2)_7-$	29.4	1.28 (*brs*)
CH_3-	14.1	0.96 (*brs*)

Assignments were based on HMQC, HMBC and NOESY experiments

Table 2. Profile of ethyl acetate compounds isolated from the extract

Products	Mass (g)	Frontal ratio	Color	Aspect	Family of compounds	Yield (%)
1	0.4	0.3	Yelow	oil	Phenolic	1.48
2	0.1	0.4	Yellow- green	oil	Phénolic	0.37

Table 3. Diameter of inhibition (mm) of compounds isolated from ethyl acetate extracts

	Inhibition zone (mm)*					MIC (mM)	
Microorganisms	**Tetracycline**	**Griseofulvin**	**Compound 1**	**Compound 2**	**Control**	**Compound 1**	**Compound 2**
Bacteria							
E. coli CIP548	14.0±0.5	-	30.5±0.5	29.0±1.0	00.0±0.0	>6.69	>5.08
L. monocytogenes CIP 82110	18.0±0.0	-	49.0±1.0	41.5±0.5	00.0±0.0	>6.69	>5.08
P. fluorescens CIP 6913	18.0±0.0	-	33.5±0.5	24.0±1.0	00.0±0.0	>6.69	>5.08
S. aureus CIP 7625	22.0±0.5	-	14.5±0.5	5.5±0.5	00.0±0.0	>6.69	>5.08
B. cereus CIP 6624	14.0±0.0	-	20.3±1.2	23.0±0.8	00.0±0.0	>6.69	>5.08
Enterococcus faecalis CIP 76117	13.0±0.0	-	28.5±0.5	18.5±0.5	00.0±0.0	>6.69	>5.08
B. subitlis ATCC 6636	14.0±0.0	-	30.5±0.5	26.0±1.0	00.0±0.0	>6.69	>5.08
Fungi							
A. flavus	-	22.0±1.0	31.5±1.5	10.5±0.5	00.0±0.0	3.34	5.08
A. parasticus	-	18.0±0.0	15.5±0.5	5.5±0.5	00.0±0.0	5.01	3.81
C. tropicalis ATCC 750	-	20.0±0.5	29.0±1.0	19.5±0.5	00.0±0.0	5.01	5.08
C. lusitaniae ATCC 200950	-	15.0±0.0	34.0±1.0	19.5±0.5	00.0±0.0	5.01	5.08
C. neoformans CIP 95026	-	16.0±1.0	32.5±0.5	23.0±1.0	00.0±0.0	5.01	5.08

**Values are means of three replicates. Compound 1: methyl-3-phenylpropanoate Compound 2: 8-hepthyl-12-propyl-decanol*

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication N° 85-23, revised 1985) were followed, in full compliance with the European Union guidelines for experimentations on laboratory animals (Council Directive 86/609/EEC of 24/11/86). All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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