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Antimicrobial and antioxidant activities of isolated compounds from the root bark of *Croton pseudopulchellus* (Euphorbiaceae)

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Abstract

Background: Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms pose an enormous threat to public health. Hence, search for new antimicrobial is very important to overcome microbial resistance and emerging pathogenic bacteria and fungi. In this study, we designed to describe the isolation, structure elucidation, antimicrobial and antioxidant activities of components from the root bark of *Croton pseudopulchellus*.

Methods: Chromatography techniques were used for isolation and purification of compounds. Their structures were determined by means of spectroscopic and spectrometric data, as well as by comparison with literature data. The broth and agar dilution methods were used for antibacterial and antifungal assays. Antioxidant properties were evaluated using ferric reducing antioxidant power (FRAP) and diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays.

Results: Phytochemical investigation of the root bark extract of *Croton pseudopulchellus* led to the isolation of five secondary metabolites namely, 18-methoxycarbonyl-18-methoxycarbonyl-15,16-epoxy-*ent*-cleroda-3,13(16),14-triene-,20,19-olide (megalocarpoidolide B) (**1**), 7,8-dehydrocrotocorylifuran (**2**), vitexin (**3**), lupeol (**4**) and acetyl aleuritolic acid (**5**). Vitexin (**3**) showed promising antimicrobial activities with minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) values ranged between 16 and 32 µg/mL and interesting antioxidant properties very close to those of vitamin C and butyl hydroxyl toluene (BHT) used as reference antioxidant drugs. Compounds **1**, **2**, **3** and **4** were isolated from this plant for the first time.

Conclusion: These findings indicate that *C. pseudopulchellus* contains vitexin which has interesting antimicrobial and antioxidant properties, therefore confirming some of its uses in traditional medicine.

Keywords: *Croton pseudopulchellus*; Euphorbiaceae; vitexin; antibacterial; antifungal; antioxidant.

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Background

Microbial resistance to antibiotics is increasingly becoming a concern to public health. Drug discovery must be an ongoing process if chemotherapic agents that are effective against rapidly growing drug-resistant bacteria and fungi are to be obtained. The WHO has acknowledged the need to identify new antibiotics and/or new approaches to overcome the growing problems associated with such infectious agents. In recent years, pharmaceutical companies have focused on developing drugs from natural sources. The plants are alternative sources of the most effective and cheapest drugs [1]. The local use of natural plants as primary remedies due to their pharmacological properties is quite common, particularly in Africa [2].

The genus *Croton* belongs to the family Euphorbiaceae, and is a diverse and complex group of plants ranging from herbs and shrubs to trees. *Croton pseudopulchellus* Pax is a shrub usually growing from 2 - 4 metres tall, occasionally to 6 metres [3]. In East Africa, the leaves are applied directly to treat chest infections, or taken internally as infusions or decoctions to treat syphilitic ulcers, gonorrhoea, colds, and anthrax. A root-decoction is used in the treatment of asthma [4,5]. Previous phytochemical studies on this plant revealed the presence of terpenoids, but mostly diterpenoids [5,6]. *Ent*-kaur-16-en-19-oic acid, isolated as the major component from the stem bark of *C. pseudopulchellus*, showed a moderate *in vitro* antiplasmodial activity against the chloroquine sensitive strain of *P. falciparum* with an IC_{50} value of 31.77 mg/ml [5]. The acetone and ethanol leaf extracts of *C. pseudopulchellus* were highly cytotoxic to the non-cancerous cells with LC_{50} varying between 7.86 and 48.19 µg/mL. Regarding the anti-inflammatory activity, the acetone leaf extract of *C. pseudopulchellus* displayed NO inhibitory potency with an IC_{50} of 34.64 µg/mL, while the ethanol leaf extract of the same plant was active against 15-lipoxygenase with an IC_{50} of 0.57 µg/mL. The acetone and ethanol extracts of *C. pseudopulchellus* showed DPPH and ABTS radical scavenging activity [7]. The minimal inhibitory concentration of the acetone extract from *C. pseudopulchellus* was 0.1 mg/ml against a drug-sensitive strain of *Mycobacterium tuberculosis* H37Rv, by the radiometric method [8].

In the search for bioactive principles from Cameroonian medicinal plant, the root bark of *C. pseudopulchellus* was investigated. We report herein the isolation, structural elucidation, antimicrobial and antioxidant activities of five isolated secondary metabolites.

Methods

General experimental procedure

The ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AMX-500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) using $CDCl_3$ as solvent. Column chromatography was run with Merck silica gel 60 (0.063–0.200 mm) and TLC was carried out on silica gel GF254 pre-coated plates with detection accomplished by visualizing with a UV lamp at 254 and 365 nm, followed by spraying with 50% H_2SO_4 and then heating at 100ºC.

Plant material

The root bark of *C. pseudopulchellus* was harvested at Mougoudi, a village around Maroua city, in May 2017. The plant species was identified by Mr. Tapsou, a botanist at IRAD (*Institut de Recherche Agricole et de Développement*) of Maroua, Cameroon. The identification was confirmed at the Herbarium of Wildlife School, Garoua, Cameroon, by comparison with a specimen whose voucher number was HEFG No 1289.

Extraction and purification

The air-dried root bark of *C. pseudopulchellus* (409 g) was powdered and extracted thrice with MeOH at room temperature for 48, 96 and 144 h. The filtrate was evaporated to give 82.8 g of methanol extract. The obtained extract was partitioned with hexane to afford 16.2 g of hexane extract, and 60.8 g of MeOH residue extract. 20 g of MeOH residue extract was subjected to silica gel column chromatography eluted with CH_2Cl_2/MeOH gradient [2.5% (frs 1-14), 5% (frs 15-27), 7.5% (frs 28-47), 30% (frs 48-56), 50% (frs 57-65) to give 65 fractions of 300 mL each. These fractions were combined into five main fractions [A (500 mg) (frs 1-10), B (300.2 mg) (frs 11-30), C (875.6 mg) (frs 31-48), D (1.252 mg) (frs 49-55) and E (1.832 mg)(frs 56-65)] based on their TLC profile. Silica gel column chromatography of fraction B eluted with hexane-CH_2Cl_2 gradient yielded compounds **1** (10 mg) and **4** (15 mg). Purification of fraction C on silica gel eluted with hexane-EtOAc gradient afforded compounds **2** (17 mg) and **5** (8 mg). Compound **3** (9 mg) was obtained from fraction D following the same process as fraction C.

Antimicrobial evaluation

Tested microorganisms

The antimicrobial activity was performed against four bacterial and three fungal species. The selected microorganisms were the Gram-positive (*Staphylococcus aureus* ATCC25923) and Gram-negative (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* S2(1), *Shigella flexneri* SDINT) bacteria and yeast strains of *Candida albicans* ATCC10231, *C. tropicalis* PK233 and *Cryptococcus neoformans* H99. These microorganisms were taken from our laboratory collection. The fungal and bacterial strains were grown at 37 °C and maintained on Sabouraud Dextrose Agar (SDA, Conda, Madrid, Spain) and nutrient agar (NA, Conda) slants, respectively.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC)

The antibacterial and antifungal activities were evaluated by determining the MICs and MMCs as previously described [9]. MICs of isolated compounds were determined by broth micro dilution method. Each test sample was dissolved in dimethylsulfoxide (DMSO) to give a stock solution. This was serially diluted two-fold in Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for yeasts to obtain a concentration range of 512 to 0.50 µg/mL. Then, 100 µL of each sample concentration was added to respective wells (96-well micro plate) containing 90 µL of SDB/ MHB and 10 µL of inoculum to give final concentration ranges of 256 to 0.25 µg/mL. The final concentrations of microbial suspensions were $2.5x10^5$ cells/mL for yeasts and 10^6 CFU/mL for bacteria. Nystatin (Sigma-Aldrich, Steinheim, Germany) and ciprofloxacin (Sigma-Aldrich, Steinheim, Germany) were used as positive controls for yeasts and bacteria respectively. Microorganisms left untreated + 1% (v/v) DMSO + SDB / MHB were used as negative control. MICs were assessed visually and were taken as the lowest sample concentration at which there was

no growth or virtually no growth. The lowest concentration that yielded no growth after the sub-culturing was considered as the MMCs [9]. All the tests were performed in triplicate and repeated three times with similar results.

Antioxidant assay

Ferric reducing antioxidant power (FRAP) assay

The FRAP was determined by the Fe^{3+} - Fe^{2+} transformation in the presence of the compound **3** as previously described [10]. The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Butylated hydroxytoluene (BHT) was used as a positive control. All the tests were performed in triplicate and repeated three times with similar results.

Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The free radical scavenging activity of the compound **3** was evaluated according to described methods [11]. The EC_{50} (µg/mL), which is the amount of sample necessary to inhibit by 50% the absorbance of free radical DPPH was calculated [9]. Vitamin C was used as a positive control. All the analyses were carried out in triplicate and repeated three times with similar results.

Statistical analysis

Data were analyzed by one-way analysis of variance followed by Waller-Duncan Post Hoc test. The experimental results were expressed as the mean ± Standard Deviation (SD). Differences between groups were considered significant when $p < 0.05$. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 12.0) software

Results

Chemical analysis

The phytochemical analysis of the MeOH extract from the air-dried root bark of *C. pseudopulchellus* led to the isolation of five known compounds, including two clerodane diterpenoids (**1-2**), two pentacyclic triterpenoids (**4-5**) and one flavonoid (**3**) (Figure 1).

Compound **1** was obtained as a white amorphous powder. ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 173.1 (C_{20}), 167.1 (C_{18}), 142.9 (C_{15}), 139.4 (C_3), 138.5 (C_{15}), 136.1 (C_4), 124.2 (C_{13}), 110.8 (C_{14}), 75.5 (C_{19}), 51.7 ($C_{18\text{-}OCH3}$), 48.7 (C_9), 43.4 (C_{10}), 37.0 (C_8), 36.2 (C_5), 35.3 (C_6), 29.5 (C_7), 29.1 (C_{11}), 26.2 (C_2), 19.3 (C_1), 17.2 (C_{12}), 16.3 (C_{17}). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.39 (1H, t, *J* = 1.8 Hz, H_{15}), 7.28 (1H, s, H_{16}), 6.86 (1H, dd, *J* = 2.4, 5.5Hz, H_3), 6.32 (1H, brs, H_{14}), 4.85 (1H, d, *J* = 12.2 Hz, $H_{19\alpha}$), 4.41 (1H, dd, *J* = 12.2, 2.4 Hz, $H_{19\beta}$), 3.73 (3H, s, $H_{19\text{-}OCH3}$), 0.98 (3H, d, *J* = 6.7 Hz, H_{17}). Compound **1** was identified to 18-methoxycarbonyl-15,16-epoxy-ent-cleroda-3,13(16),14-triene-,20,19-olide (megalocarpoidolide B) previously isolated from the root of *Croton megalocarpoides* [12].

Compound **2** was obtained as a white amorphous powder. ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 175.9 (C_{20}), 171.9 (C_{19}), 166.4 (C_{18}), 144.2 (C_{15}), 140.2 (C_3), 139.4 (C_{16}), 134.9 (C_4), 130.4 (C_8), 127.1 (C_7), 125.6 (C_{13}), 108.0 (C_{14}), 72.0 (C_{12}), 52.8 (C_9), 52.2 ($C_{19\text{-}OCH3}$), 51.6 ($C_{18\text{-}OCH3}$), 50.3 (C_{10}), 45.6 (C_5), 42.2 (C_{11}), 33.2 (C_6), 26.4 (C_2), 19.6 (C_{17}), 19.2 (C_1). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.47 (1H, brs, H_{16}), 7.46 (1H, t, *J* = 1.8 Hz, H_{15}), 7.00 (1H, dd, *J* = 4.9, 1.8 Hz, H_3), 6.42 (1H, brs, H_{14}), 5.85 (1H, brd, *J* = 6.7 Hz, H_7), 5.52 (1H, t, *J* = 8.2 Hz, H_{12}), 3.72 (6H, s, $H_{18/19\text{-}OCH3}$), 3.25 (1H, dd, *J* = 16.8. 6.7 Hz, $H_{6\alpha}$), 1.68 (3H, brs, H_{17}). Compound **2** was identified as 7,8-dehydrocrotocorylifuran previously isolated from the root of *Croton megalocarpoides* [12].

Compound **3** was isolated as a yellow powder. ^{13}C-NMR (Pyridine d_6, 125 MHz) δ 182.7 (C_4), 164.6 (C_7), 164.3 (C_2), 162.4 (C_5), 162.0 ($C_{4'}$), 157.1 (C_9), 129.4 ($C_{2'/6'}$), 122.3 ($C_{1'}$), 116.6 ($C_{3'/5'}$), 105.9 (C_8), 105.1 (C_{10}), 103.0 (C_3), 98.9 (C_6), 83.3 ($C_{5''}$), 80.6 ($C_{3''}$), 75.3 ($C_{1''}$), 72.7 ($C_{2''}$), 72.0 ($C_{4''}$), 62.7 ($C_{6''}$). ^{1}H-NMR (Pyridine d_6, 500 MHz) δ 13.9 (1H, s,C_{5OH}), 8.30 (2H, d, *J* = 7.9 Hz, $H_{2'/6'}$), 7.24 (2H, d, *J* = 7.9 Hz, $H_{3'/5'}$), 6.77 (1H, s, H_6), 6.72 (1H, s, H_3), 5.96 (1H, d, *J* = 9.8 Hz, $H_{1''}$), 5.05 (1H, t, *J* = 8.9 Hz, $H_{2''}$), 4.70 (1H, t, *J* = 9.2 Hz, $H_{4''}$), 4.64 (1H, d, *J* = 11.3 Hz, $H_{6''\alpha}$), 4.55 (1H, dd, *J* = 11.3, 4.6 Hz, $H_{6''\beta}$), 4.48 (1H, t, *J* = 8.9 Hz, $H_{3''}$), 4.22 (1H, brs, $H_{5''}$). Compound **3** was identified as vitexin previously isolated from *Vitex lucens* [13].

Compound **4** was isolated as white needles. ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 150.9 (C_{20}), 109.3 (C_{29}), 79.0 (C_3), 55.3 (C_5), 50.4 (C_9), 48.3 (C_{18}), 48.0 (C_{19}), 43.0 (C_{17}), 42.8 (C_{14}), 40.8 (C_8),40.0 (C_{22}), 38.8 (C_4), 38.7 (C_1), 38.0 (C_{13}), 37.2 (C_{10}), 35.9 (C_{16}), 34.3 (C_7), 29.8 (C_{21}), 28.0 (C_{23}), 27.5 (C_{15}), 27.4 (C_2), 25.2 (C_{12}), 20.9 (C_{11}), 19.3 (C_{30}), 18.3 (C_6), 18.0 (C_{28}), 16.3 (C_{25}), 15.9 (C_{26}), 15.3 (C_{24}), 14.6 (C_{27}). Compound **4** was identified as lupeol [14].

Compound **5** was isolated as a white amorphous powder. ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 182.9 (C_{28}), 171.0 ($C_{3\text{-}OOC\text{-}}$), 160.5 (C_{14}), 116.7 (C_{15}), 80.9 (C_3), 55.9 (C_5), 51.3(C_{17}), 49.1 (C_9), 41.5 (C_{18}), 40.8 (C_7), 39.0 (C_8), 37.9 (C_{10}), 37.7 (C_4), 37.4 (C_1), 37.3 (C_{13}), 35.3 (C_{19}), 33.7 (C_{21}), 33.3 (C_{12}), 31.9 (C_{16}), 31.4 (C_{29}), 30.7 (C_{22}), 29.3 (C_{20}), 28.7 (C_{30}), 27.9 (C_{23}), 26.1 (C_{26}), 23.5 (C_2), 22.4 (C_{27}), 21.3 ($C_{3\text{-}OOC\text{-}CH3}$), 18.7 ($C_6$), 17.3 ($C_{11}$), 16.6 ($C_{24}$), 15.6 ($C_{25}$). Compound **5** was identified as acetyl aleuritolic acid [14].

Compound **5** is already described in *C. pseudopulchellus* [5], but compounds **1**, **2**, **3** and **4** are described here in this plant for the first time.

Antimicrobial activity

The isolated compounds from root bark of *C. pseudopulchellus* presented antimicrobial activity to at least three of the tested microorganisms (Table 1). The results revealed variability in the minimum inhibitory concentrations (MIC) and minimum microbicidal concentration (MMC) of each compound for given pathogenic microorganisms. Among the isolated compounds, compound **3** (MIC = 16 – 32 µg/mL) presented the lowest MIC values (i.e. the highest activities) whereas the other compounds (MIC = 64 – > 256 µg/mL) displayed the largest MIC values (i. e. the lowest activities). Interestingly, compound **3** was able to inhibit the growth of all the tested bacterial and yeast strains. By contrast, compounds **1** and **2** did not showed any antifungal activity against all the tested yeast strains. Compounds **4** and **5** showed selective antimicrobial activities against bacterial and yeast strains. Their inhibitory effects were noted on 42.85% (3/7) and 51.14% (4/7) of the tested microorganisms.

Antioxidant activity

Compound **3** was evaluated for its antioxidant activity using DPPH and FRAP assays (Figures 2 - 3). Compounds **1** - **2** and **4** - **5** were not tested in both DPPH and FRAP assays. The DPPH• radical scavenging activity and ferric reducing antioxidant power were observed with compound **3**. The lowest IC_{50} value reflects the highest DPPH radical scavenging activity. According to the results obtained, compound **3** displayed less DPPH• radical scavenging activity when compared to vitamin C used as reference antioxidant drug (Figure 2). Compound **3** displayed concentration dependent reducing power (Figure 3). The low concentrations of vitexin (≤ 4 µg/mL) were not significantly different than those of BHT whereas at high concentrations (> 4 µg/mL), the antioxidant power of vitexin was lesser than that of BHT.

Discussion

The results obtained from the present study revealed variability in the minimum inhibitory concentrations (MIC) of each compound for given pathogenic microorganisms. Indeed, low MIC is an indication of high efficacy of the compound while high MIC may indicate low efficacy or possible development of resistance by the microorganisms to the antimicrobial [15]. Among the tested compounds, compound **3** displayed the largest activity. This finding supports the possibility that the antimicrobial vitexin might be the response molecule for the ability to inhibit sensitive microorganisms of root bark of *C. pseudopulchellus*. The results obtained from the present study provide evidence that root bark of *C. pseudopulchellus* possesses antimicrobial agents against pathogenic yeasts and bacteria, suggesting that it may be used to cure diseases caused by the susceptible microorganisms.

The Gram-positive bacterium, *S. aureus* was found to be more sensitive compared to Gram-negative bacteria, *E. coli* and *S. flexneri*. Also, the findings of the present study showed that the antimicrobial activities varied with the bacterial and fungal strains. These variations may be due to genetic differences between the microorganisms. With regard to the MIC and MMC values, a lower MMC/MIC (≤ 4) value signifies that a minimum amount of pure compounds is used to kill the microbial species, whereas, a higher value signifies the use of comparatively more amount of compound for the control of any microorganism [11].

Antimicrobial cut-off points of pure compounds have been defined as follows: highly active: MIC below 1 µg/mL (or 2.5 µM), significantly active: 1 ≤ MIC ≤ 10 µg/ mL (or 2.5 ≤ MIC < 25 µM), moderately active: 10 < MIC ≤ 100 µg/mL (or 25 < MIC ≤ 250 µM), weakly active: 100 < MIC ≤ 1000 µg/mL (or 250 < MIC ≤ 2500 µM and not active: MIC > 1000 µg/mL (or > 2500 µM) [16]. Based on this, compound **3** can be considered moderately active against all the tested microorganisms whereas the other compounds were in general, weakly active. The strains of *E. coli* S2 (1) and *S. flexneri* [17 - 19] included in the present study were MDR clinical isolates and these were resistant to commonly used drugs such as tetracycline, streptomycin, ampicillin, nalidixic acid, *co*-trimoxazole and furazolidone. However, compounds **3** displayed moderated antibacterial activities against these bacterial strains, suggesting that its administration may represent an alternative treatment against multidrug resistant *E. coli* and *S. flexneri*. The mechanism of the active compound **3** (flavonoid glycoside) may be due to the disruption of microbial membranes and its ability to complex bacterial cell walls, extracellular and soluble proteins [20].

The results of antimicrobial activities corrobated those of Lall and Meyer [8] who demonstrated that the minimal inhibitory concentration of the acetone extract from *C. pseudopulchellus* was 0.1 mg/ml against a drug-sensitive strain of *Mycobacterium tuberculosis* H37Rv, by the radiometric method. Also, the susceptibilities of bacterial and fungal species to 3-acetyl aleuritolic acid, lupeol and vitexin have been documented in the literature [21-25]. However, the antimicrobial activities of megalocarpoidolide B and 7,8-dehydrocrotocorylifuran are reported here for the first time.

The findings of the present study also showed that compound **3** displayed antioxidant activity using DPPH and FRAP assays. These experimental findings imply that vitexin can protect the organism against free radical oxidative damages. Our results are consistent with those previously reported [7, 25,27].

Phenolic compounds such as vitexin are known to be potential antioxidant due to their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals [28,29].

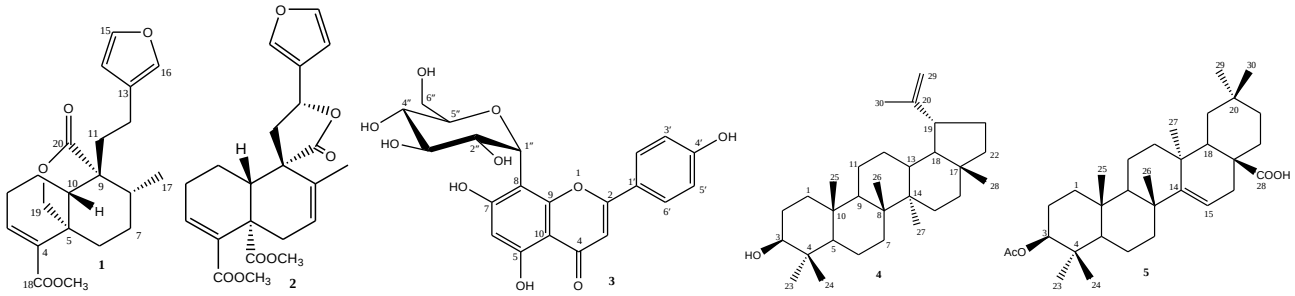

Figure 1. **Structures of isolated compounds.**

1: 18-methoxycarbonyl-15,16-epoxy-*ent*-cleroda-3,13(16),14-triene-,20,19-olide (megalocarpoidolide B); **2**: 7,8-dehydrocrotocorylifuran; 3: vitexin; **4**: lupeol; **5**: acetyl aleuritolic acid.

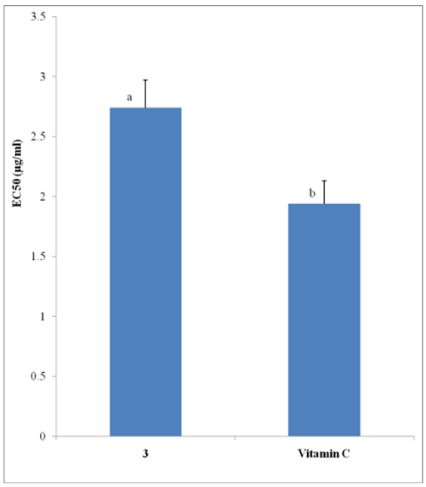

Figure 2. Equivalent concentrations of compound 3 scavenging 50% of DPPH radical (EC 50).
Results represent the mean ± standard deviation of the triplicate EC_{50} of each sample. Letters a - b indicate significant differences between samples according to one-way ANOVA and Waller Duncan test; $p < 0.05$. Compounds **1-2**, and **4-5** were not tested.

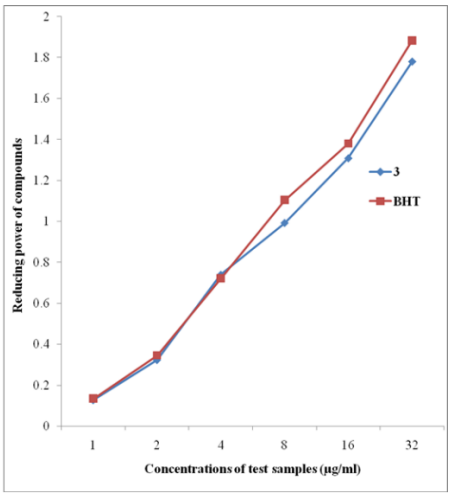

Figure 3. Reducing power activity of compound 3 and butylated hydroxytoluene (BHT).
Results represent the mean ± standard deviation of the triplicate reducing power at each concentration. Compounds **1-2** and **4-5** were not tested.

Table 1. Antimicrobial activity (in µg/ml) of isolated compounds from root bark of *C. pseudopulchellus* against bacterial and yeast strains

Crude extracts/ Isolates	Inhibition parameters	*S. aureus* ATCC25923	*P. aeruginosa* ATCC27853	*E. coli S2(1)*	*Shigella flexneri* SDINT	*C. albicans* ATCC10231	*C. tropicalis* PK233	*C. neoformans* H99
1	MIC	128	128	256	256	>256	>256	>256
	MMC	>256	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/	/
2	MIC	64	64	128	64	>256	>256	>256
	MMC	>256	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/	/
3	MIC	16	16	32	16	32	16	16
	MMC	16	16	32	32	32	32	16
	MMC/MIC	1	1	1	2	1	2	1
4	MIC	256	256	>256	>256	>256	>256	256
	MMC	>256	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/	/
5	MIC	128	128	>256	>256	>256	256	64
	MMC	>256	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/	/
Reference drugs*	MIC	1	4	16	32	2	0.5	1
	MMC	1	4	16	32	2	1	1
	MMC/MIC	1	1	1	1	1	2	1

*: Ciprofloxacin for bacteria; nystatin for fungi; /: not determined.; MIC: Minimum inhibitory concentration; MMC: Minimum Microbicidal Concentration

Conclusion

The phytochemical investigation of the root bark extract of *C. pseudopulchellus* led to the isolation of five secondary metabolites namely 18-methoxycarbonyl-15,16-epoxy-*ent*-cleroda-3,13(16),14-triene-,20,19-olide (megalocarpoidolide B) (**1**),7,8-dehydrocrotocorylifuran (**2**), vitexin (**3**), lupeol (**4**) and acetyl aleuritolic acid (**5**). The isolated compounds presented different degrees of antimicrobial activities. Vitexin showed the highest antioxidant, antibacterial and antifungal activities. These findings indicate that *C. pseudopulchellus* contains antimicrobial and antioxidant compounds, therefore confirming its uses in traditional medicine against infectious diseases.

Additional file

Supplementary file.doc: NMR spectra of the isolated compounds, available at: www.investchempharma.com-imcp39-supplementary-file.docx.

Abbreviations

^{13}C-NMR: Carbon Thirteen Nuclear Magnetic Resonance; ^{1}H-NMR: Proton Nuclear Magnetic Resonance; 2D NMR: Two-dimension Nuclear Magnetic Resonance; ATCC: American Type Culture Collection; BHT: Butylated hydroxytoluene; CC: Column Chromatography; DPPH: diphenyl-1-picrylhydrazyl; DMSO: Dimethylsulfoxide; EtOAc: Ethyl acetate; FRAP : Ferric reducing antioxidant power; MDR: Multi-Drug-Resistant; MeOH: Methanol; MHA: Mueller Hinton agar; MHB: Mueller Hinton broth; MIC: Minimum inhibitory concentration; MMC: Minimum Microbicidal Concentration; NA: Nutrient agar; IRAD: *Institut de Recherche Agricole et de Développement*; SDB: Sabouraud Dextrose Broth; TLC: Thin Layer Chromatography; $CDCl_3$: Deuterated chloroform; UV: Ultra-violet.

Authors' Contribution

TNSJ, TJD and LH designed the project. TNSJ, LH and MLA carried out the isolation and structural elucidation under the supervision of SN. TJD, ICK and SEE did the biological experiments. TNSJ, LH and TJD wrote and edited the manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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References

1. Ekom ES and Tamokou JD. 2018. Methanol leaves extract of *Psidium guajava* Linn. exhibited antibacterial and wound healing activities. *Int. J. Curr. Microb. Appl. Sci.* 7(7): 4008-4023
2. Tamokou JD, Ekom Endeguele S, Njouendou AJ, Fosso Mboutchom JA and Kengne IC. 2017. Antibacterial activities of methanol extracts of *Carica papaya, Ocimum gratissimum and Solanum torvum* under normal and osmotic stress conditions. *J. Compl. Altern Med Res.* 4(2): 1-11.
3. African Plant Database (version 3.4.0). Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute, Pretoria. Http://www.ville-ge.ch/musinfo/bd/cjb/africa/http://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php. Accessed May 07, 2019
4. Burkill HM. 1994. The Useful Plants of West Tropical Africa, vol. 2. Families E-I, Royal Botanic Gardens, Kew, p. 49.
5. Langat MK, Crouch NR, Pohjala L, Tammela P, Smith PJ, Mulholland DA. 2012. *Ent*-kauren-19-oic acid derivatives from the stem bark of *Croton pseudopulchellus* Pax. *Phytochemistry Lett.* 5:414–418.
6. Langat MK, Crouch NR, Nuzillard J-M, Mulholland DA. 2018. Pseudopulchellol: A unique sesquiterpene-monoterpene derived C-25 terpenoid from the leaves of *Croton pseudopulchellus* Pax (Euphorbiaceae). *Phytochemistry Lett*. 23:38-40.
7. Njoya ME, Eloff NJ, McGaw JL. 2018. *Croton gratissimus* leaf extracts inhibit cancer cell growth by inducing caspase 3/7 activation with additional anti-inflammatory and antioxidant activities. *BMC Complent Altern Med*. 18: 305.
8. Lall N, Meyer JJ. 1999. *In Vitro* Inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. *J Ethnopharmacol.* 66(3):347-354.
9. Tamokou J-D, Tala FM, Wabo KH, Kuiate JR, Tane P. 2009. Antimicrobial activities of methanol extract and compounds from stem bark of *Vismia rubescens*. *J. Ethnopharmacol.* 124:571–575.
10. Padmaja M, Sravanthi M, Hemalatha KPJ. 2011. Evaluation of antioxidant activity of two Indian medicinal plants. *Journal* of Phytology, 3(3): 86-91.
11. Djouossi MG, Tamokou JD, Ngnokam D, Kuiate JR, Tapondjou AL, Harakat D, Voutquenne-Nazabadioko L. 2015. Antimicrobial and ltern antioxidant flavonoids from the leaves of *Oncoba spinosa* Forssk. (Salicaceae*). BMC Complement. Altern. Med.* 15:134.
12. Ndunda B, Langat MK, Mulholland DA, Eastman H, Jacob MR, Khan SI, Walker LA, Muhammad I, Kerubo LO, Midiwo JO. 2016. New *ent*-Clerodane and Abietane Diterpenoids from the roots of Kenyan *Croton megalocarpoides* Friis &M.G. Gilbert. *Planta Med.* 82(11-12):1079-1086.
13. Briggs L and Cambi RC. 1958. The extractives of *Vitex Lucens*-I. *Tetrahedron* 3:269-273.
14. Shashi B, Mahato and Asish P. Kundu. 1994. ^{13}C NMR spectra of pentacyclic triterpenoids- a compilation and some salient features. *Phytochemtry* 37(6):1517-1575.
15. Shanmugam K, Holmquist L, Steele M, Stuchbury G, Berbaum K, Schulz O, Benavente Garc´ıa O, Castillo J, Burnell J, Garcia Rivas V, Dobson G and M¨unch G. 2008. Plant-derived polyphenols attenuate lipopolysaccharide-induced nitric oxide and tumour necrosis factor production in murine microglia intoxification in rats. *J. Ethnopharmacol.* 66: 355-361.
16. Tamokou JD, Mbaveng TA, Kuete V. 2017. Antimicrobial activities of African medicinal spices and vegetables. In: *Medicinal Spices and Vegetables from Africa: Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases*. Edited by Kuete V, 1st Ed., Academic Press, Cambridge, Elsevier, chapter 8, Pp 207-237.
17. Acharyya S, Sarkar P, Saha DR, Patra A, Ramamurthy T, Bag PK. 2015. Intracellular and membrane damaging activities of methyl gallate isolated from *Terminalia chebula* against multi-drug resistant *Shigella species. J Med Microbiol* 64(8): 901-909.
18. Jouda JB, Tamokou JD, Djama Mbazoa C, Douala-Meli C, Sarkar P, Bag PK, Wandji J. 2016. Antibacterial and cytotoxic cytochalasins from the endophytic fungus *Phomopsis* sp. harbored in *Garcinia kola* (Heckel) nut. *BMC Complement Altern Med* 16:462.
19. Tamokou JD, Fosso MJA, Njouendou AJ, Ekom SE, Kengne IC. 2017. Antibacterial activity of three Cameroonian medicinal plants traditionally used for the treatment of urinary tract infections and their synergistic effects with amoxicillin and serum. *J. Adv. Med. Pharm. Sci.* 15(2):1-14.
20. Cowan MM. 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 12:564–82.
21. Basile A, Giordano S, López-Sáez JA, Cobianchi RC. 1999. Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry* 52(8):1479-1482.
22. Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JJ, Lall N. 2008. Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana*. *J. Ethnopharmacol*. 116(1):194-197.
23. Tamokou JD, Kuiate JR, Tene M, Nwemeguela KTJ, Tane P. 2011. The antimicrobial activities of extract and compounds isolated from *Brillantaisia lamium*. *Iran. J. Med. Sci*. 36(1):24-31.
24. Tamokou JD, Mpetga Simo DJ, Lunga PK, Tene M, Tane P, Kuiate JR. 2012. Antioxidant and Antimicrobial Activities of Ethyl Acetate Extract, Fractions and Compounds from the stem Bark of *Albizia adianthifolia* (Mimosoideae). *BMC Complement. Altern. Med.* 12:99.
25. Adamczak A, Ozarowski M, Karpi´nski TM. 2020. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *J. Clin. Med*. 9:109.
26. An F, Yang G, Tian J, Wang S. 2012. Antioxidant effects of the orientin and vitexin in *Trollius chinensis* Bunge in D-galactose-aged mice. *Neural Regen. Res*. 7(33):2565-2575.
27. Khole S, Panat NA, Suryawanshi P, Chatterjee S, Devasagaya TPA, Ghaskadbi S. 2016.
Comprehensive assessment of antioxidant activities of apigenin isomers: vitexin and isovitexin. *Free Rad. Antiox.* 6(2):155-166.
28. Hall CA, Cuppett SL. 1997. Structure activities of natural antioxidants. In: Hudson BJL, editor. antioxidant methodology in vitro concepts. London: Elsevier Applied Science; p. 1-18.
29. Pietta P, Sionetti P, Mauri P. 1998. Antioxidant activity of selected medicinal plants. *J Agric Food Chem*. 46:4487–4490.