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Chemical composition, in vitro antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon

Florentine Marie-Chantal Ndoye Foe^{1*}, Tatiana Flore Kemegni Tchintang^{1,2,4}, Ascencion Maximilienne Nyegue^{2,3}, Jean-Pierre Abdou^{4,5}, Abel Joel Gbaweng Yaya^{4,5}, Alembert Tiabou Tchinda⁴, Jean-Louis Oyono Essame⁴ and François-Xavier Etoa²

Abstract

Background: In the Cameroonian traditional medicine, plants of the Capparidaceae, Euphorbiaceae and Liliaceae families are used to treat several metabolic diseases. These plants are rich in various compounds belonging to the glucosinolates and thiosulfates family. Till date, very little studies have been done aiming at assessing the antioxidant and inflammatory properties of the essential oils (EOs) of these plants. Essential oils are volatile extracts produced by secondary metabolism. They are usually constituted of terpenes and may also contain specific non terpenic components such as glucosinolates and thiosulfates for the species that are being considered in the present study. This study highlights and compares the chemical composition, antioxidant and anti-inflammatory properties of the essential oils of the stem barks of *Drypetes gossweileri* (Euphorbiaceae), roots of *Pentadiplandra brazzeana* (Capparidaceae), red bulbs of *Allium cepa* and *Allium sativum* (Liliaceae) collected in Cameroon (Central Africa).

Methods: The essential oils were extracted by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS). In vitro antioxidant activities were determined using the radical scavenging assay, total phenolic content, ferric reducing antioxidant power (FRAP) assay and determination of antioxidant activity index (AAI) according to the method described by Scherer and Godoy. The anti-inflammatory activities were evaluated using albumin denaturation method. Differences ($p < 0.05$) between the experimental and the control groups were evaluated using one way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons.

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* Correspondence: chantfoe@yahoo.com

¹Department of Biochemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon

Full list of author information is available at the end of the article



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Results: The main components of *Allium sativum* essential oil were diallyl trisulfide (41.62 %), diallyl disulfide (19.74 %), allyl methyl trisulfide (12.95 %), diallyl sulfide (7.1 %) and diallyl tetrasulfide (4.22 %). Those of *Allium cepa* essential oil were diallyl trisulfide (22.17 %), dipropyl trisulfide (11.11 %), 2-methyl-3,4-dithiaheptane (9.88 %), methyl propyl trisulfide (8.14 %), dipropyl tetrasulfide (8.07 %) and 2-propenyl propyl disulfide (5.15 %). *Drypetes gossweileri* and *Pentadiplandra brazzeana* essential oils presented similar chemical compositions as compared with benzylisothiocyanate content (63.19 and 97.63 % respectively), but differed in benzylcyanide content (35.72 and 0.86 % respectively). The essential oils were rich in phenolic compounds in the following order *Allium sativum* < *Allium cepa* < *D. gossweileri* < *P. brazzeana*. The essential oils exhibited high antioxidant and DPPH radical scavenging effect but low ferric reducing power activity. Moreover, the four essential oils showed anti-inflammatory activities (by heat denaturation of Bovine Serum Albumin). The anti-inflammatory activities of *P. brazzeana* and *A. cepa* essential oils were comparable but higher than those of *D. gossweileri* and sodium diclofenac used as a reference non-steroidal anti-inflammatory drug.

Conclusion: The essential oils of the plants were rich in organosulfur compounds. These compounds were probably responsible for their appreciable antioxidant and anti-inflammatory activities. Due to their antioxidant and anti-inflammatory properties, the essential oils of some of these plants might be used as natural additives in the pharmaceutical, cosmetic and agro-industries.

Keywords: Essential oils, Chemical composition, Sulphur-containing plants, In vitro antioxidant, Anti-inflammatory activity

Background

Allium species, especially *Allium* vegetables are characterized by their high content in thiosulfinates (TNs). The TNs or alkane (ene) thial-S-oxide are formed by the action of the enzyme alliinase (E.C.4.4.1.4) from their respective S-alk (en) yl cysteine sulfoxides. However, depending on the *Allium* species and the environmental conditions, the TNs formed are degraded to various polysulfides including diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta-, and hexasulfides, vinyl-dithiins, (*E*)- and (*Z*)-ajoene [1]. The organosulfur compounds in *Allium* are water-soluble (S-allylcysteine and S-allylmercaptocysteine) and lipid-soluble compounds (alliin, diallyl sulfide, triallyl sulfide, diallyl disulfide, diallyl polysulfides) [2, 3]. TNs exhibit different degrees of antimicrobial activity and are found to be effective antioxidants in terms of scavenging [1].

In damaged plant cells, notably those of Capparidaceae and Euphorbiaceae families, glucosinolates (GLs) are transformed by the endogenous myrosinase (EC 3.2.1.147) to produce a number of compounds depending on the precursor glucosinolate and the environmental conditions [4]. GLs form a variety of hydrolysis products including isothiocyanates, oxazolidine-2-thiones, nitriles, epithionitriles, and thiocyanates [5]. Certain GLs (sulforaphane and other isothiocyanates) are well known for their diverse biological activities ranging from bactericidal, nematocidal, fungicidal, insecticidal, antioxidant, antimutagenic, antiproliferative and allelopathic properties [6, 7].

Oxidation is a chemical reaction involving the transfer of an electron from electron rich to electron deficient

entity. The electron deficient molecule is termed an oxidizing agent [8]. An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Substances which protect biomolecules from free radical-mediated damage both in vitro and in vivo fall under this category. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated during irradiation by UV light, X and γ rays, products of metal-catalyzed reactions, which are present as pollutants in the atmosphere, produced by neutrophils and macrophages during inflammation process, by-products of mitochondria-catalyzed electron transport reactions and other mechanisms [9]. ROS at higher concentration (termed oxidative stress) are important mediators of cell structure damages including lipids, membranes, proteins and nucleic acids [10]. The harmful effects of ROS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes [11]. Thus, ROS are important mediators that provoke or sustain inflammatory process and consequently, their neutralization by antioxidants and free radical scavengers can attenuate inflammation [12, 13]. Both inflammation and free radical damage are inter-related aspects that influence each other.

Inflammation is a protective and defense mechanism of the body to a physical, chemical or biological aggression. It is important when it does not last for long. Its main purpose is to destroy the injurious agent and/or minimize its harmful effect by limiting its propagation [14]. Inflammatory response occurs in two distinct phases: an acute and a chronic. The acute phase is characterized by local vasodilatation, increased capillary

permeability and release of inflammatory mediators like histamine, serotonin and prostaglandins. The Chronic phase is characterized by infiltration of leukocytes and phagocytic cells [15]. This results in tissue degeneration and fibrosis. In the market, most of the available conventional drugs to relieve oxidative stress and to treat inflammatory-related diseases are either steroidal or non-steroidal anti-inflammatory chemicals. Though these drugs are potent enough, long-term administration is required for treatment of chronic diseases. Furthermore, these drugs might lead to severe side effects such as gastric intolerance, bone marrow depression, water and salt retention due to prolonged use [16]. It is in this light that natural remedies with very little side effects, proven efficacy and safety are sought for as substitutes for chemical therapies. Therefore, sulfur-containing plants selected for this study were chosen by virtue of their use in traditional medicine in West Cameroon in the treatment of several inflammatory skin disorders and inflammatory-related diseases.

Allium sativum L. and *Allium cepa* L. (Liliaceae) commonly known as garlic and onion respectively are bulbous herbs used as food item, spice and medicine in different parts of the world. *Allium sativum* can also be used in homemade cosmetics. It has been employed for its diverse biological activities including anti-carcinogenic, antiatherosclerotic, antithrombotic, antimicrobial, anti-inflammatory and antioxidant effects [17–21]. The major beneficial effects of *Alliums* are ascribed to the high content in organosulfur compounds produced when *Allium sativum* or *Allium cepa* tissue is damaged and the odorless precursors are converted by the alliinase enzyme and lachrymatory-factor synthase [22, 23]. The most important sulfur-containing compound in *Allium cepa* bulbs is the amino acid cysteine and its derivatives. *A. cepa* also contains high levels of flavonoids mainly quercetin as well as other phenolic compounds [24]. In contrast to *Allium cepa*, *Allium sativum* mainly contains allicin derivatives such as ajoene, vinylthiins, and alkenyl-sulphides such as diallyl disulfide and diallyl trisulfide depending on the type of solvent used for the extraction [22, 25].

Drypetes gossweileri is a dioic plant of the Euphorbiaceae family with an average size of about 30 to 40 m. The color of the stem bark turns from grey-green to yellowish-green and it gives a strong characteristic odor when cut [26]. It has been used in traditional medicine for treating fever, malaria, intestinal worms in Central Africa [27, 28]. Phytochemical studies conducted by Nguana et al. [29] revealed the presence of metabolites such as alkaloids, phenols, flavonoids, saponines, anthraquinones, tannins, anthocyanins, sterols, lipids and essential oils. Phytochemical analysis of the extracts from previous studies indicated the presence of steroids, triterpenoids, alkaloids, saponins with antimicrobial

properties [30]. Many research works describe the antibacterial [31, 32], antifungal [29, 33], antioxidant and antiradical [32, 34] effects of *D. gossweileri*. It is also used in pest management [35].

Pentadiplandra brazzeana Baill (Capparidaceae) is a climber found in Western and Central Africa [36], the berries of which are eaten and used as a sweetener of beverages [37]. Root, seed, and leaf extracts of *P. brazzeana* are known to contain benzyl-, 3-methoxybenzyl-, 4-methoxybenzyl-, 3,4-dimethoxybenzyl-, and indole-type glucosinolates [38]; and the essential oil obtained from its roots is mainly constituted of benzylisothiocyanate and benzylcyanide [32, 39–41]. Its root essential oil (EO) has been proven to possess free-radical scavenging [32, 40, 42], antibacterial [32, 42], antifungal [41, 42] and anti-inflammatory activities [42]. In previous studies, the aqueous extract of *P. brazzeana* root was shown to possess androgenic activity [43]. Moreover, the crude extract of *P. brazzeana* root as well as the compounds isolated from it (thiourea and urea) exhibited a moderately strong antiplasmodial activity against two *Plasmodium falciparum* strains [44].

The antioxidant and anti-inflammatory activities of some *Allium* species grown in other countries have been analyzed, but no information is available on the in vitro antioxidant activities of red bulb *A. cepa* and red bulb *A. sativum* species growing in Cameroon. Antioxidant activities have already been studied using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging method and the β -carotene bleaching test [40, 42], but a different method is used here to confirm it. In addition, anti-inflammatory activity has not yet been reported for *D. gossweileri* stem barks. Moreover, considering that antioxidants and free radical scavengers can also exert an anti-inflammatory effect [13], the essential oil of *D. gossweileri* stem barks were also assessed for these activities in comparison with that obtained from *A. cepa* and *A. sativum*, two plants well-known for their antioxidant and anti-inflammatory activities. Therefore, the purpose of this study was to determine the chemical composition, antioxidant and anti-inflammatory potential of the aforementioned sulfur-containing plants in order to confirm their efficacy in the treatment of inflammatory disorders as claimed in Cameroonian ethnomedicine.

Methods

Chemical reagents and solvents

Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and aluminum chloride were purchased from Sigma-Aldrich (Germany). Ascorbic acid and bovine serum albumin were purchased from Sigma-Aldrich

(China). All other reagents and solvents were of analytical grade.

Plant materials and extraction procedure

A. sativum red bulbs, *A. cepa* red bulbs, *D. gossweileri* stem barks and *P. brazzeana* roots were purchased in Yaounde (Mokolo and Mfoundi markets) in August 2013. *A. sativum* red bulbs and *A. cepa* red bulbs were harvested at Garoua (North Region of Cameroon). *D. gossweileri* stem barks and *P. brazzeana* roots were harvested at Hawae and Ngomedzap (Center Region of Cameroon) respectively by the vendors. Identification was done at the National Herbarium of Cameroon by comparison with voucher specimens 25742/SRF/Cam, 44810/HNC, 25749/SRF/Cam and 42918/SRF/Cam for *A. cepa*, *A. sativum*, *D. gossweileri* and *P. brazzeana* respectively.

EOs were extracted by hydrodistillation using a Clevenger-type apparatus for 5 h, dried over anhydrous sodium sulfate and then stored at 4 °C until bioassay. The extraction yields were calculated as the ratio of the mass of EO to the mass of the starting plant material and expressed as a percentage (w/w).

Chemical analysis of the essential oils

The EOs were analyzed by gas chromatography and gas chromatography coupled to mass spectrometry as described by Agnani et al. [45].

Gas chromatography

GC analysis was performed on a Varian gas chromatograph, model CP-3380, with flame ionization detector containing two silica capillary columns: HP5 J&W Agilent (5 %-Phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 μm film) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m × 0.25 mm i.d. × 0.25 μm film); N₂ was the carrier gas at 0.8 mL/min; injection type 0.1 μL of pure sample, split ratio 1:100; injector temperature 220 °C, detector temperature 250 °C; temperature program 50–200 °C at 5 °C/min, then kept at 200 °C for 10 min. The linear retention indices of the components were determined relative to the retention times of a series of n-alkanes. The entire set up was coordinated by COMPASS software system that ensured its functioning and follow-up of the chromatographic analysis.

Gas chromatography-mass spectrometry

GC-MS analyses were performed using a Hewlett-Packard GC 5890 series II equipped with a HP5 (5 %-Phenylmethylpolysiloxane) fused silica column (30 m × 0.25 mm; film thickness 0.25 μm) and a DB-Wax fused silica column (30 m × 0.25 mm; film thickness 0.25 μm) interfaced with a quadrupole detector (Model 5972);

temperature program (50–200 °C at 5 °C/min); injector temperature, 220 °C; MS transfer line temperature, 180 °C; carrier gas, helium at a flow rate of 0.6 mL/min; injection type, split, 1:10 (1 μL 10:100 CH₂Cl₂ solution); ionization voltage, 70 eV; electron multiplier 1460 eV; scan range, 35–300 amu; scan rate, 2.96 scan/s.

Qualitative analysis

The identification of the constituents was based on comparison of their relative retention times with either those of authentic samples or with published data in the literature [46] and matching their mass spectra with those obtained from authentic samples and/or the NBS75K and Wiley 7th NIST 98 EPA/NIH libraries spectra and literature data [46].

Quantitative analysis

The percentage composition of the essential oils was computed by the normalization method from the GC-FID peak areas, assuming an identical mass response factor for all compounds.

Determination of radical scavenging and antioxidant activity

In order to determine the radical scavenging and antioxidant activities of the EOs, the following assays were applied: total phenolic content determination, FRAP assay and DPPH assay.

Determination of total phenolic content (TPC)

The phenolic content in EOs was determined according to the method described by Singleton et al. [47] with slightly modifications. In effect, 10 μg/mL of EOs were used in the analysis. The reaction mixtures were prepared by mixing 60 μL of EO, 2 mL of 10 % Folin-Ciocalteu dissolved in water. For the blank, 60 μL of methanol, 2 mL of 10 % Folin-Ciocalteu reagent were mixed in water. After 30 min of incubation at room temperature, the absorbance was measured at 750 nm using a V-1100 spectrophotometer. Ascorbic acid was used as standard. Experiments were carried out in triplicate. The total phenolic content was expressed in μg ascorbic acid equivalent per mg of EO (μg AAE/mg) using the following equation based on the calibration curve:

$$\begin{aligned} \text{Absorbance} &= 0.02 \times \text{ascorbic acid } (\mu\text{g}) \\ &+ 0.04; R^2 \\ &= 0.99 \end{aligned}$$

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the EOs was determined in accordance with the method reported by Zhao et al. [48]

with slight modifications. 1 mL of each EO was mixed with 2.5 mL of 0.2 M phosphate buffer (pH = 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1 %). The mixture was incubated at 50 °C for 20 min. 2.5 mL of 10 % trichloroacetic acid was added and the tubes were centrifuged for 10 min at 3000 rpm. The supernatant (1.25 mL) was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1 % ferric chloride solution. The absorbance was measured at 700 nm in a V-1100 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. Experiments were carried out in triplicate. The results were expressed in μg ascorbic acid equivalent per mg of EO (μg AAE/mg) using the equation below based on the calibration curve:

$$\begin{aligned} \text{Absorbance} &= 0.29x \text{ ascorbic acid } (\mu\text{g}) + 0.77; R^2 \\ &= 0.98 \end{aligned}$$

DPPH Radical scavenging assay (RSA)

The free radical scavenging activity of the EOs was evaluated as described by Sanchez et al. [49] with slight modifications, based on the ability of test compounds to neutralize DPPH radical. The conversion of the stable violet DPPH radical into a yellow reduced form (DPPH/ H^+) is observed simultaneously. The test samples were prepared in methanol and 100 μL of each sample (1.25 to 10 $\mu\text{g}/\text{mL}$ for essential oils) was added to 1900 μL of freshly prepared 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (50 mg/L) in pure methanol. Ascorbic acid was used as a positive control and 2 mL of 50 mg/L DPPH/methanol solution was used as negative control. The content of each preparation was mixed and incubated at room temperature in a dark cupboard for 10 min. The absorbance was measured at 517 nm in a V-1100 spectrophotometer. All tests were carried out in triplicate. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical scavenging, using the equation here below:

$$\begin{aligned} \% \text{ RSA} &= \left[\frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}} \right] \\ &\times 100. \end{aligned}$$

Where A_{blank} is the absorbance of the control (containing all reagents except the test sample) and A_{sample} is the absorbance of tested EO solution.

The SC_{50} values (concentration of sample required to scavenge 50 % of free radicals) were calculated from the regression equations derived by the least-square method and prepared from the different concentrations of both ethanol extracts and essential oils. The higher the SC_{50} the lower the antioxidant activity of the assayed sample. Ascorbic acid was used as reference.

The antioxidant activity was then calculated and expressed as the antioxidant activity index (AAI): $AAI =$

final concentration of DPPH in the control sample ($\mu\text{g}\cdot\text{mL}^{-1}/SC_{50}$ ($\mu\text{g}\cdot\text{mL}^{-1}$). Scherer and Godoy's criteria were considered [50] depending on whether the EOs showed weak antioxidant activity ($AAI < 0.5$), moderate antioxidant activity (AAI , between 0.5 and 1.0), strong antioxidant activity (AAI , between 1.0 and 2.0) and very strong antioxidant activity when $AAI > 2.0$.

Determination of the anti-inflammatory activity: inhibition of albumin denaturation

Anti-denaturation assay was conducted as described by Biswakanth et al. [51] with slight modifications. The reaction mixture consisted of EO samples at different concentrations and 1 % bovine serum albumin (BSA) fraction prepared in saline phosphate buffer (pH = 7.4). The pH of the reaction mixture was adjusted to 6.8 using small amounts of glacial acetic acid. The test tubes were incubated at 72 °C for 5 min and then cooled for 10 min. The absorbance of these solutions was determined using a spectrophotometer (Mapada V-1100) at a wavelength of 660 nm. The experiment was performed in triplicate. The percentage inhibition of precipitation (denaturation of the protein) was determined on a percentage basis relative to the control using the formula:

$$\begin{aligned} \text{Percentage of inhibition denaturation} \\ &= 100 - \left[\left(\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right) \times 100 \right] \end{aligned}$$

Statistical analysis

All the grouped data were statistically evaluated with GraphPad InStat software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. P values less than 0.05 were considered to be statistically significant. All the results were expressed as mean \pm s.d. The regression equations and correlation coefficients were obtained by the least-square method.

Results and discussion

Extraction yields of essential oils

The extraction yields of the EOs are shown in Table 1. The yield ranged from 0.007 % for *A. cepa* to 0.2 % for *A. sativum* bulbs. The extraction yield of red bulb *A. cepa* was similar to that previously found by Mnayer et al. [52] (0.006 to 0.013 %) while the yield of *A. sativum* bulbs was similar to that obtained by the same author but, higher (0.09 %) than that observed in a previous study by Khadri et al. [53]. However, the yield obtained in this study represents only half of the yield obtained by Lawrence and Lawrence (0.4 %) [54].

As regards *D. gossweileri*, its stem bark gave a yield of 0.04 %. This yield was different from those obtained by

Table 1 Relative percentages of constituents and extraction yields of EOs from *A. sativum*, *A. cepa* bulbs, *D. gossweileri* stem barks and *P. brazzeana* roots

Components	RI	Relative percentage (%)			
		<i>A. cepa</i>	<i>A. sativum</i>	<i>D. gossweileri</i>	<i>P. brazzeana</i>
2,4-dimethylthiophene	884	-	0.63	0.03	-
Diallyl sulfide	888	1.38	7.10	0.11	-
2,5-dimethyl thiophene	908	0.82	-	-	-
Allyl methyl disulfide	918	-	-	0.01	-
Methyl propyl disulfide	922	-	1.19	-	-
N,N'-Dimethyl thiourea	951	-	-	0.01	-
Benzaldehyde	962	-	-	0.28	-
dimethyl trisulfide	964	-	0.58	-	-
2-Phenyl furan	978	-	0.48	-	-
Octyl aldehyde	990	2.26	-	-	-
phenylacetaldehyde	1026	2.69	-	0.06	-
Limonene	1029	-	0.62	-	-
2-propenyl propyl disulfide	1032	5.15	0.20	0.02	-
2,5-dimethyl-1,3,4-thiadiazole	1037	-	0.74	-	-
Terpinolene	1059	-	-	0.02	-
Phenylmethanol	1081	-	-	0.01	-
Diallyl disulfide	1088	-	19.74	-	-
Trans-propenyl propyl disulfide	1091	2.86	-	-	-
Linalool	1101	-	0.88	-	-
Dipropyl disulfide	1109	2.71	0.62	-	-
1-propenyl propyl disulfide	1114	3.77	-	-	-
3,5-dimethyl-1,2,4-trithiolane	1118	1.81	-	-	-
Campholenol	1124	-	-	0.03	0.31
Allyl methyl trisulfide	1149	-	12.95	-	-
Methyl propyl trisulfide	1153	8.14	-	-	-
Benzylcyanide	1157	2.52	-	35.72	0.86
3,4-Dihydro-3-vinyl-1,2-dithiin	1165	-	1.37	-	-
Dimethyl tetrasulfide	1224	1.32	1.59	0.02	-
2,5-Dimethylthiazole	1265	4.62	-	-	-
Diallyl trisulfide	1319	22.17	41.62	-	-
3-Methoxyoctane	1327	-	0.62	-	-
Dipropyl trisulfide	1334	11.11	-	-	-
1-propenyl propyl sulfide	1345	1.26	-	-	-
Allyl propyl sulfide	1377	-	1.30	-	-
Di-1-propenyl sulfide	1382	1.71	2.08	-	-
Benzylisothiocyanate	1393	-	-	63,19	97.63
p-methoxybenzylcyanide	1395	-	0.85	-	1,2
B-caryophyllene	1398	-	0.23	-	-
2-methyl-3-isothiazolone	1435	2.23	-	-	-
Germacrene D	1484	-	-	0.02	-
γ-cadinene	1506	-	0.39	-	-
2-methyl-3,4-dithiaheptane	1527	9.88	-	-	-

Table 1 Relative percentages of constituents and extraction yields of EOs from *A. sativum*, *A. cepa* bulbs, *D. gossweileri* stem barks and *P. brazzeana* roots (Continued)

B-sesquiphellandrene	1550	-	-	0.05	-
Diallyl tetrasulfide	1558	-	4.22	-	-
p-methoxybenzylisothiocyanate	1575	3.55	-	-	-
Dipropyl tetrasulfide	1649	8.07	-	-	-
Benzyl sulfide	1856	-	-	0.06	-
Methyl linolenate	2013	-	-	0.06	-
Total identified (%)		98.77	100	99.70	100
EOs extraction yields (%)		0.007	0.2	0.04	0.02

RI linear retention indices on a HP5 column; – not found; EO essential oils

Eyele et al. (0.2 %) [55]; Agnani et al. (0.19 %) [56]; Ngono (0.007 and 0.29 %) and [32] Mbouma (0.023 to 0.088 %) [33].

The roots of *P. brazzeana* gave a yield of 0.02 % which was similar to those found in previous studies by Koudou et al. [39], Ndoye [40], Ngono [32] and Tchingang et al. [41]. However, this yield is lower than those found by Koudou et al. [39] and Nyegue et al. [42], respectively 0.35, 0.2 and 0.13 %.

Chemical composition

The results of the chemical analysis are presented in Table 1.

GC-MS analysis of *Allium sativum* EO revealed 22 components representing 100 % of the total EO. The major components were diallyl trisulfide (41.62 %), diallyl disulfide (19.74 %), allyl methyl trisulfide (12.95 %), diallyl sulfide (7.1 %) and diallyl tetrasulfide (4.22 %). This chemical profile was closed to that obtained by Mnayer et al. [52], but with slight difference in that diallyl disulfide (37.90 %) was predominant compared to diallyl trisulfide (28.06 %), while the allyl methyl trisulfide (7.26 %) represented almost half of its content in the previous study [52]. Furthermore, studies conducted by Khadri et al. [53] showed that allyl methyl trisulfide (34.61 %) and diallyl disulfide (31.65 %) were rather the major constituents of the EO of *Allium sativum*. However, it is worth noting that, in the two previous studies [52, 53], allyl methyl disulfide was present in the chemical composition of the *Allium sativum* essential oil, contrarily to this study where it is practically absent. Nonetheless, different studies on the chemical composition of *A. sativum* essential oil showed that diallyl disulfide and diallyl trisulfide are the two major compounds of the EO of *A. sativum* [52, 57, 58].

Twenty one constituents representing 98.77 % of the total EO were identified in the *Allium cepa* EO. The main components were diallyl trisulfide (22.17 %), dipropyl trisulfide (11.11 %), 2-methyl-3, 4-dithiaheptane

(9.88 %), methyl propyl trisulfide (8.14 %), dipropyl tetrasulfide (8.07 %) and 2-propenyl propyl disulfide (5.15 %). The chemical composition of *A. cepa* used in this study differed greatly from previous reports in which dipropyl disulfide was reported to be the major compound present [52, 57].

Analysis of the chemical compositions of the EO of *D. gossweileri* stem barks revealed 17 compounds representing 99.70 % of the total EO. Benzylisothiocyanate was the main component, representing 63.19 % followed by benzylcyanide (35.72 %). These results are in agreement with those of Eyele et al. [55], Ngono [32] and Mbouma [33] who documented that benzylisothiocyanate (56.5 to 95.6 %) and benzylcyanide (4.3 to 32.3 %) were also the major compounds of *D. gossweileri* EO. However, Agnani et al., [56], reported that benzylcyanide (73.7 %) was the main compound in *D. gossweileri* stem bark EO from Gabon, instead of benzylisothiocyanate (5.2 %).

As for the EO of *P. brazzeana* roots, four components were identified, representing 100 % of the total oil. The main compound found was benzylisothiocyanate (97.63 %). Benzylisothiocyanate has been reported by Ndoye [40], Nyegue et al. [41], Ngono [32] and Tchingang et al. [41] to be the major compound present in *P. brazzeana* EO.

It was observed that the chemical composition of the aforementioned essential oils differs greatly among genus, and also among the same species. This is possibly due to analytical techniques, chemotypes, and differences in cultivars of the plants, culture climate and other growth conditions which might affect the chemical composition [38, 59].

Determination of radical scavenging and antioxidant activity

Determination of total phenolic content (TPC)

The Total Phenolic Contents (TPC) of *Allium sativum*, *Allium cepa*, *D. gossweileiri* and *P. brazzeana* essential oils (EOs) are presented in Table 2.

Table 2 Total phenolic, and ferric reducing power contents of essential oils of *A. cepa*, *A. sativum*, *D. gossweileiri*, *P. brazzeana*

	TPC (µg AAE/mg) Essential oil	FRAP (µg AAE/mg) Essential oil
<i>A. cepa</i>	429.33 ± 1.31 ^b	2.75 ± 0.02 ^b
<i>A. sativum</i>	463.38 ± 1.24 ^a	5.33 ± 0.01 ^a
<i>D. gossweileiri</i>	365.38 ± 0.66 ^c	0.76 ± 0.03 ^c
<i>P. brazzeana</i>	342.20 ± 0.99 ^d	0.08 ± 0.03 ^d

Values followed by the different superscript letter (a, b, c or d) within the same column are significantly different ($p < 0.05$) according to Tukey's HSD test. AAE ascorbic acid equivalent

From our literature review, there is no scientific investigation on the determination of the TPC of the EOs of *D. gossweileiri* stem barks and *P. brazzeana* roots. The results indicate that the TPC of the EOs ranged from 342.20 ± 0.99 to 429.33 ± 1.31 µg AAE/mg.

The highest level of phenolics were found in both *A. sativum* and *A. cepa* EOs (463.38 ± 1.24 and 429.33 ± 1.31 µg AAE/mg), while the lowest contents were recorded in the EOs of *D. gossweileiri* stem barks and *P. brazzeana* roots (365.38 ± 0.66 µg AAE/mg and 342.20 ± 0.99 µg AAE/mg). These results do not match with those of Abdel-Salam et al. [60] who showed that TPC of red *A. sativum* are lower than those of red *A. cepa*.

Generally, EOs possess significant secondary metabolites from plants, particularly the active lipophilic compounds. This might be due to the fact that phenolic compounds are often extracted in higher amounts by using polar solvents such as water [61]. Phenolic antioxidants are products of secondary metabolism in plants and their antioxidant activity is mainly due to their redox properties and chemical structure, which might play an important role in chelating transition metals and scavenging free radicals [62]. Consequently, the antioxidant activities of plant extracts are often explained by their total phenolic and flavonoid contents. Also, the sterical structures of antioxidants or free radicals are known to play a more important role in their abilities to scavenge different types of free radicals [63].

Ferric reducing antioxidant power (FRAP)

The ferric reducing power in the EOs of *A. cepa*, *A. sativum*, *D. gossweileiri*, *P. brazzeana* is presented in Table 2. Form our literature review; there is no scientific report on the determination of the ferric reducing power of *D. gossweileiri* and *P. brazzeana* EOs.

Their ferric reducing capacity ranged from 0.08 ± 0.03 to 2.75 ± 0.02 µg AAE/mg. The highest reducing power was found in *A. sativum* EO (5.33 ± 0.01 µg AAE/mg), followed by the *A. cepa* EO (2.75 ± 0.02 µg AAE/mg), *D. gossweileiri* EO (0.76 ± 0.03 µg

AAE/mg) and finally, EO (0.08 ± 0.03 µg AAE/mg) of *P. brazzeana*. These results are comparable to that of Benkeblia et al. [64] who found that *A. cepa* and *A. sativum* showed the highest reducing capacity with 107 and 196 %, respectively. It is worth mentioning that the ferric reducing antioxidant power of *D. gossweileiri* EO was 10 folds greater than that of *P. brazzeana* EO. This might be ascribed to the presence of more phenolic compounds [65] in *D. gossweileiri* than in *P. brazzeana*. The ability to reduce the ferricyanide complex of Fe³⁺ to the ferrous (Fe²⁺) form might be attributed to the hydrogen donating ability of phenolic compounds [66], which is indicative of the presence of a reducing agent [67]. In addition, the number and position of the hydroxyl groups in phenolic compounds also govern their antioxidant activity [68]. However, Amagase et al. [69] argued that this antioxidant activity could be attributed to the organosulfur compounds.

DPPH Radical scavenging assay (RSA)

Tables 3 and 4 show the radical scavenging activity (RSA) of the EOs of *A. cepa*, *A. sativum*, *D. gossweileiri* and *P. brazzeana*.

The stable free radical DPPH was used to test the ability of the EOs and ascorbic acid to donate hydrogen ion. The scavenging effects of the EOs on DPPH radical increased as the EO concentration increased. All the EOs were able to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow diphenylpicrylhydrazine with varying degrees of scavenging capacities. Great bleaching action (from purple to yellow) reflected a higher antioxidant activity and thus a lower SC₅₀. The SC₅₀ values increased in the following order: *P. brazzeana* < *A. sativum* < *D. gossweileiri* < *A. cepa* < ascorbic acid. These results indicate that EOs exhibited a significant DPPH radical scavenging activity about 10 folds more active than ascorbic acid. Thus, the EOs were found to be better antioxidants. These results are in agreement with the findings of Agnani et al. [56],

Table 3 DPPH radical scavenging activity and AAI of *A. cepa*, *A. sativum*, *D. gossweileiri*, *P. brazzeana* essential oils and ascorbic acid

	Regression curve's equations	Coefficient of determination : R ²	SC ₅₀ (µg/ml)	AAI
<i>A. cepa</i>	y = 192.3 × + 11.96	0.927	0.20	12.626
<i>A. sativum</i>	y = 191.6 × + 12.73	0.919	0.19	12.886
<i>D. gossweileiri</i>	y = 194.0 × + 11.10	0.931	0.20	12.821
<i>P. brazzeana</i>	y = 190.2 × + 14.15	0.904	0.19	13.298
Ascorbic acid	y = 19.12 × + 12.19	0.934	1.98	1.262

Linear regression analysis was used to calculate SC₅₀ value
SC₅₀ Scavenging concentration (µg/mL) at 50 %, AAI antioxidant activity index

Table 4 Relationship between TPC and FRAP, TPC and RSA

	Relationship between TPC and FRAP, FRAP = f (TPC)		Relationship between TPC and RSA, SC ₅₀ = f (1/TPC)	
	Regression curve Equation	Coefficient of determination : R ²	Regression curve equation	Coefficient of determination : R ²
Essential oil	y = 0.016x - 0.434	0.22	y = 0.782 x + 0.189	0.21

Ngono [32] and Mnayer et al. [52], but in disaccordance with those found by Ndoye [40], Nyegue et al. [42], Lee et al. [24] and Abdel-Salam et al. [60]. Mnayer et al. [52] showed that *Allium sativum* EO was a more effective DPPH radical scavenger (SC₅₀ = 7.67 mg/mL) than *Allium cepa* EO (SC₅₀ = 20.19 mg/mL). On the contrary, Lee et al. [24] and Abdel-Salam et al. [60] reported that *Allium cepa* EO exhibited higher DPPH radical scavenging activity than *A. sativum* EO. Moreover, Negue et al. [42] found that *P. brazzeana* EO exhibited a weak DPPH radical scavenging activity (RSA = 14 % for 1 g/L, SC₅₀ = 1.5 mg/mL and SC₅₀ = 3.23 mg/mL) in comparison with the results of the present study. However, Ngono [32] stated that although the RSA of both the EO of *P. brazzeana* roots and *D. gossweileri* stem barks were weak, the RSA of *P. brazzeana* EO was higher than that of *D. gossweileri* EO. The major components of the EO (benzylisothiocyanate and benzylcyanide) of these two plants could not be responsible for the antiradical activity, but it might be due to the synergetic action between different components or the contribution of some minor components [41]. It has been shown that benzylisothiocyanate and benzylcyanide tested individually were found to be less active than the whole EO.

Using Scherer and Godoy's criteria [49], EO showed very strong antioxidant activity (AAI > 2), while ascorbic acid showed a strong antioxidant activity index (1 < AAI < 2).

It is interesting to note that there were weak linear correlations between the total phenolic concentration and the FRAP values or DPPH radical scavenging percentage, with coefficients, R² = 0.22 and 0.21 respectively (Table 4). These results show that most antioxidant compounds might be organosulfur compounds. Indeed, the antioxidant activity of *Allium* species is attributed partly to its sulfur compounds, which represent the main constituents of these EO [57, 70, 71]. Amagase et al. [69] reported that diallyl polysulphides contributed to the antioxidant properties of the EOs. In addition, the antioxidant activity is also related to other minor bioactive compounds: dietary fibers, microelements (especially selenium) and polyphenols [72] which potentialize antioxidant activity. The different antioxidant activities between these EO might be due to the variability of the chemical composition and sulfides concentration. It might also be attributed to the presence and synergetic action of the different trace compounds.

Determination of the anti-inflammatory activity

Anti-denaturation of bovine serum albumin method was used to evaluate the anti inflammatory property of the EOs of *A. cepa*, *A. sativum*, *D. gossweileiri* and *P. brazzeana*. The results are summarized in Table 5. All the EOs protected the BSA against heat induced denaturation. The percentage of BSA protection against

Table 5 Effect of essential oil on BSA denaturation inhibitory activity, percentage inhibition compared to sodium diclofenac

	<i>A. cepa</i>	<i>A. sativum</i>	<i>D. gossweileri</i>	<i>P. brazzeana</i>	Diclofenac sodium
Concentration (µg/ml)	Inhibition percentage (%)				
3.125	7.33 ± 1.15 ^b	4.00 ± 0.00 ^a	4.00 ± 0.00 ^a	7.56 ± 2.00 ^b	7.33 ± 0.00 ^b
6.25	9.33 ± 1.15 ^a	6.00 ± 2.00 ^a	8.67 ± 2.31 ^a	16.477 ± 1.15 ^b	9.33 ± 1.15 ^a
12.5	14.00 ± 2.00 ^a	14.00 ± 2.00 ^a	13.33 ± 3.05 ^a	26.94 ± 2.00 ^b	14.00 ± 0.00 ^a
25	33.33 ± 1.15 ^c	22.66 ± 2.30 ^b	19.33 ± 1.15 ^a	38.95 ± 1.15 ^d	18.00 ± 0.00 ^b
50	54.67 ± 3.05 ^c	38.00 ± 2.00 ^b	28.00 ± 2.00 ^a	52.52 ± 1.15 ^c	24.00 ± 0.00 ^a
75	82 ± 1.04 ^e	57 ± 1.08 ^c	42 ± 0.11 ^b	72.87 ± 0.98 ^d	36 ± 2.00 ^a
100	-	76 ± 0.91 ^c	56 ± 0.76 ^b	-	48 ± 1.00 ^a
125	-	95 ± 1.02 ^c	70 ± 2.14 ^b	-	60 ± 1.73 ^a
Regression curve equations	y = 1.070x + 2.579 R ² = 0.991	y = 0.753x - 3.637 R ² = 0.976	y = 0.525x + 3.642 R ² = 0.991	y = 0.907x + 8.587 R ² = 0.973	y = 0.430x + 5.090 R ² = 0.982
IC ₅₀ value (µg/mL)	44.31	61.577	88.30	45.66	104.44

Linear regression analysis was used to calculate IC₅₀ value

Values followed by the same superscript letter (a, b, c, d or e) within the same line are not significantly different (p > 0.05) according to Tukey's HSD test

heat increased with increasing concentration. The present findings show a concentration dependent inhibition of protein (albumin) denaturation by EOs from 3.125 to 125 µg/mL. Sodium diclofenac at the same concentration range was used as the reference drug and it also exhibited a concentration dependent inhibition of protein denaturation. Indeed, the effect of sodium diclofenac against heat denaturation of BSA was found to be about 2 folds lower than that of *A. cepa* and *P. brazzeana* EO. *P. brazzeana* EO was found to be 2 folds more active than *D. gossweileri* EO. The anti-inflammatory activity of *P. brazzeana* EO was to a lesser extent comparable to that of *A. cepa*. These observations were confirmed by comparing their IC₅₀ values.

To the best of our knowledge, no study on the in vitro anti-inflammatory properties of the EOs of *D. gossweileri* has been published. However, [42] reported that the anti-inflammatory activity of the EO of *P. brazzeana* root is about 100 folds less active (35 ± 5 µg/mL) than that of nordihydroguaiaretic acid (0.23 ± 0.02 µg/mL) against the soybean 5-lipoxygenase enzyme. The IC₅₀ of *P. brazzeana* EO found in this study is slightly higher (45.66 µg/mL) than that reported by Nyegue et al. [42] using a different method. This means that the EO sample used by Nyegue et al. [42] was slightly more active than the present EO sample. This could be explained by the fact that the EO sample used by the aforementioned author contained more compounds (11) than the EO sample used in this investigation, which contained only 4 components (Table 1). Concerning the antiradical, anti-inflammatory activities of the EO of *P. brazzeana* and *D. gossweileri*, these activities might be due to the synergetic action of several components or the contribution of trace components. Nyegue et al. [42] reported that the two main components (benzylisothiocyanate and benzylcyanide) tested individually were found to be less active than the whole essential oil.

Conclusion

In order to valorize some organosulfur plants from Cameroon as dietary, cosmetic and pharmaceutical additives, the chemical composition, in vitro antioxidant and anti-inflammatory activities of the essential oils of *A. sativum*, *A. cepa*, *D. gossweileri* and *P. brazzeana* were investigated in this study. This paper reveals that the essential oils of *D. gossweileri* stem barks and *P. brazzeana* roots as well as those of the red bulbs of *A. sativum* and *A. cepa* are all effective antioxidant and anti-inflammatory compounds. The findings of this study support the popular use of these plants against inflammatory skin disorders and inflammatory-related diseases in West-Cameroon, though additional studies are necessary to test other

enzymatic systems that trigger the development of the inflammatory process and also address their mechanism of action.

Availability of data and materials

The datasets supporting the conclusions of this article are presented in this main paper. Plant materials used in this study have been identified at the Cameroon National Herbarium where voucher specimens are deposited.

Consent for publication

Not applicable in this section.

Ethic approval and consent to participate

Not applicable in this section.

Abbreviations

%: percentage; *A. cepa*: *Allium cepa*; *A. sativum*: *Allium sativum*; AAE: ascorbic acid equivalent; AAI: antioxidant activity index; BSA: bovine serum albumin; *D. gossweileri*: *Drypetes gossweileri*; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; EO: essential oils; eV: electron volt; FRAP: ferric reducing antioxidant power; GC: gas chromatography; GC-FID: gas chromatography-flame ionization detector; GC-MS: gas chromatography-mass spectrometry; GLs: glucosinolates; LRI: linear retention indices found on a hp5 column; mg: milligramme; mL: milliliter; *P. brazzeana*: *Pentadiplandra brazzeana*; pH: potential of hydrogen; R²: correlation coefficient; RSA: radical scavenging activity; SC₅₀: scavenging concentration at 50 %; TNs: thiosulfates; TPC: total phenolic content; w/w: weight: weight; µg: microgramme; µL: microliter.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NFFMC, TKTF, and EFX designed the study and were involved in the drafting and correction of the manuscript. NFFMC also coordinated the study. TKTF carried out the plant selection and collection, extraction of essential oils, in vitro antioxidant and anti-inflammatory assays, analysis and interpretation of data. NM performed GC, GC/MS analyses of the essential oils and participated in the correction of the manuscript. AJP and YGAJ carried out radical scavenging assays. TTA also participated in correcting the manuscript. All the authors read the manuscript, critically revised it for important intellectual content and approved the final version of the manuscript.

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Author details

¹Department of Biochemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon. ²Department of Microbiology, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon. ³Institute of Biomolecules Max Mousseron, "Equipe Glyco et novovecteurs pour le ciblage thérapeutique", Faculty of Pharmacy, University of Montpellier II, Montpellier, France. ⁴Institute of Medical Research and Medicinal Plants Studies, P.O. Box 8013, Yaounde, Cameroon. ⁵Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon.

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