Trypanosoma brucei Inhibition by Essential Oils from Medicinal and Aromatic Plants Traditionally Used in Cameroon (Azadirachta indica, Aframomum melegueta, Aframomum daniellii, Clausena anisata, Dichrostachys ciriea and Echinops giganteus)

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Abstract: Essential oils are complex mixtures of volatile components produced by the plant secondary metabolism and consist mainly of monoterpenes and sesquiterpenes and, to a minor extent, of aromatic and aliphatic compounds. They are exploited in several fields such as perfumery, food, pharmaceutics, and cosmetics. Essential oils have long-standing uses in the treatment of infectious diseases and parasitosis in humans and animals. In this regard, their therapeutic potential against human African trypanosomiasis (HAT) has not been fully explored. In the present work, we have selected six medicinal and aromatic plants (Azadirachta indica, Aframomum melegueta, Aframomum daniellii, Clausena anisata, Dichrostachys ciriea, and Echinops giganteus) traditionally used in Cameroon to treat several disorders, including infections and parasitic diseases, and evaluated the activity of their essential oils against Trypanosoma brucei TC221. Their selectivity was also determined with Balb/3T3 (mouse embryonic fibroblast cell line) cells as a reference. The results showed that the essential oils from A. indica, A. daniellii, and E. giganteus were the most active ones, with half maximal inhibitory concentration (IC50) values of 15.21, 7.65, and 10.50 µg/mL, respectively. These essential oils were characterized by different chemical compounds such as sesquiterpene hydrocarbons, monoterpen hydrocarbons, and oxygenated sesquiterpenes. Some of their main components were assayed as well on T. brucei TC221, and their effects were linked to those of essential oils.
Keywords: essential oils; African trypanosomiases; Trypanosoma brucei; Cameroon; aromatic and medicinal plants

1. Introduction

For centuries, people living in Africa have been facing various infectious tropical illnesses, among which African trypanosomiases are some of the most frequent relevant parasitic diseases. African trypanosomiases, commonly called sleeping sickness in humans (HAT; Human African Trypanosomiasis) and Nagana in domestic livestock, affect a huge number of people living in poverty in 36 sub-Saharan countries and hence have a key socioeconomic impact [1–3]. After a century of outbreaks, due to political instability and a lack of funding, around 70 million people and 50 million cattle are still at risk of exposure in Africa [4].

Trypanosomiasis is transmitted by the bite of insects from the Glossina spp. (Glossinidae) and is fatal in humans, if untreated. While taking a blood meal, infected Glossina flies can spread extracellular protozoans from the species Trypanosoma brucei. There are three morphologically indistinguishable subspecies of T. brucei. The subspecies T. b. gambiense is responsible for a chronic form of the human disease, while T. b. rhodesiense causes an acute form, which more rapidly leads to death. Both subspecies are infective to humans, whereas T. b. brucei is only infective to animals. During the early stage of the disease or the hemolymphatic phase, the parasite is restricted to the blood and lymph, and, after months or years, it invades the central nervous system, resulting in various neurological symptoms, including sleeping disturbance [3].

As for other neglected tropical diseases, the chemotherapeutical arsenal against HAT is based on limited, expensive, and often toxic medicines that are administered parentally in a context of poverty and a lack of qualified personnel in healthcare centers. The few drugs that are available are pentamidine and suramin for the early stage of the disease and eflornithine (also in combination with nifurtimox) and melarsoprol, an organoarsenic compound, for the late stage, when the parasite infects the brain. Although melarsoprol can cause severe reactive encephalopathy, it remains a first line treatment for infections by T. b. gambiense in many rural areas because of the high cost of eflornithine [1]. Overall, the scenario described above highlights the critical nature of the current situation and the urgent need to explore new sources of potentially effective and safe compounds for therapy.

It has been estimated that a large part of the African population relies on herbal medicines as the first-line treatment for different ailments. In this regard, the African flora represents a valuable source of anti-infectious compounds to be exploited as drugs [5–7]. In the fight against Trypanosoma infections, new therapeutic options can be provided by plant extracts, essential oils, and plant-borne compounds [6,8–10]. Essential oils are volatile mixtures distilled from aromatic plants and composed of several dozens of components such as terpenoids, phenylpropanoids, and aliphatic compounds [11,12]. In recent years, an increasing interest on essential oils as alternative/integrative therapies in the treatment of HAT has been observed [13]. Essential oils and representative components from lemongrass (Cymbopogon citratus (DC.) Stapf), oregano (Origanum vulgare L.), thyme (Thymus vulgaris L.), clove (Syzygium aromaticum (L.) Merr. and L.M. Perry), basil (Ocimum basilicum L.), and yarrow (Achillea millefolium L.) exhibited efficacy both in in vitro and in vivo models of trypanosomal infections [14]. Essential oils are composed of a plethora of chemical compounds, which have various modes of action on microorganisms, and, additionally, essential oils do not induce any form of resistance [15].

In the present study, we selected a panel of Cameroonian medicinal and aromatic plants as potential sources of anti-trypanocidal compounds. We focused on Azadirachta indica A. Juss (Meliaceae), Aframomum melegueta K. Schum. (Zingiberaceae), Aframomum daniellii (Hook. F.) K. Schum. (Zingiberaceae), Clausena anisata (Willd.) Hook.f. ex Benth. (Rutaceae), Dichrostachys cinerea (L.) Wight and Arn. (Mimosaceae), and Echinops giganteus A. Rich. (Asteraceae).
A. indica, also known as ‘neem tree’, is considered by many people living in Africa as a miraculous plant for a wide range of uses in ethnopharmacology such as anthelmintic, antimalarial, anti-inflammatory purposes and for healing skin diseases. Most of these properties were then confirmed by scientific reports [16–18]. All parts of the plant can be used for medicinal purposes, including the seed oil extracted by mechanical pressure [19,20]. Notably, the ethanolic extract obtained from neem stem bark exhibited activity against T. b. brucei [21], whereas the leaf essential oil has been barely investigated to date.

A. melegueta, also known as ‘alligator pepper’ or ‘grain of paradise’, is a perennial plant native to western Africa, and its seeds are used as a spice in food due to their aromatic flavor and pungent taste or as ingredients of ethnomedical preparations for the treatment of snakebites, stomachaches, and diarrhea [22]. Antimicrobial, anti-inflammatory, anticancer, and antioxidant properties have been reported for alligator pepper [23]. Concerning seed volatile constituents, they showed repellent activity against adults of the maize weevil Sitophilus zeamais [24].

A. daniellii, also known as ‘African cardamom’, is an herbaceous plant traditionally used in Africa as a spice due to the pungent taste of its seeds, whereas, for medicinal purposes, the plant is employed as a laxative and for curing parasitic and other microbial infections [25,26]. The anti-inflammatory effect of its seed essential oil and the preservative properties in stored grains have also been reported [27,28].

C. anisata is an evergreen tropical tree up to 10 m tall with leaves containing secretory glands and emitting a strong smell [29]. In Africa, it is considered highly effective against insects and has also been used in the treatment of malaria [30].

D. cinerea is a tree growing in tropical areas in countries such as Cameroon, Kenya, South Africa, and Tanzania, where a decoction of its leaves and roots is used against venereal disease, eye inflammations, skin diseases, and snake bites. The root is used for chest complaints and the twigs for gonorrhoea and syphilis. The essential oil was toxic to mosquito vectors of bancroftian filariasis [26].

E. giganteus is a perennial herb widely used in African traditional medicine for the treatment of various ailments [31,32]. In previous studies, the root methanolic extract showed significant antibacterial [33], antifungal [34], and antioxidant effects [35]. The cytotoxicity of the crude methanol extract from the roots has also been demonstrated [36,37].

Overall, these Cameroonian plants are also traditionally used to control populations of arthropod pests [38–40]. In this research, we shed light on the growth inhibitory potential of the essential oils obtained from the leaves of A. indica, A. daniellii, and C. anisata; the seeds of A. melegueta and D. cinerea; and the roots of E. giganteus against T. brucei TC221. Selected pure constituents from the above mentioned essential oils were also evaluated.

2. Materials and Methods

2.1. Plant Material

Leaves of A. indica were collected during the dry season (January 2016) from a tree in the city of Guidiguis (north of Cameroon), about 70 km from Maroua. The leaves were air-dried in the shade for one week and kept in papers. Fruits (pods) of A. melegueta were collected in a forest near Foumbam (western Cameroon) in December 2015. Once harvested, the seeds were removed from their pods and dried at room temperature over a period of three weeks in the absence of sunlight. At the end of the drying process, the seeds were placed into paper bags before hydrodistillation. Leaves of C. anisata were collected in the village of Baffou, Menoua Division, Western Cameroon. Leaves of A. daniellii and fruits of D. cinerea and roots of E. giganteus were collected from Bamougoum and Bafoussam’s market (Cameroon, Western Region), respectively. The pericarp of D. cinerea was removed and the seeds used; the roots of E. giganteus were washed with water and sliced into small pieces. These plant parts were dried at room temperature for one week. The botanical identification of the five species was performed by a taxonomist at the Cameroon National Herbarium (Yaoundé, Cameroon), and the voucher specimens were archived with the following codes: 4447 SRFK (A. indica),
43117 HNC (A. melegueta), 43130 HNC (A. danielli), 44242/HNC (C. anisata), 42920 HNC (D. cinerea), and 23647 SRF (E. giganteus). The botanical names were also checked against The Plant List database (www.theplantlist.org).

2.2. Isolation of Essential Oil

The dry leaves of A. indica, A. daniellii, and C. anisata; the seeds of A. melegueta and D. cinerea; and the roots of E. giganteus were cut into small pieces and subjected to hydrodistillation using a Clevenger-type apparatus until no more oil was obtained. The essential oils obtained were dried using Na$_2$SO$_4$ and stored at $-20^\circ$C in vials sealed with teflon caps and protected from light before use. The oil yields were calculated on a dry weight basis (%, w/w).

2.3. Chemicals

For the identification of volatiles, the following analytical standards purchased from Sigma Aldrich (Milan, Italy) were used: $\alpha$-pinene, $\beta$-pinene, sabinene, 1,8-cineole, camphene, myrcene, $\alpha$-phellandrene, $\delta$-3-carene, $\pi$-cymene, limonene, $\gamma$-terpinene, terpinolene, linalool, trans-pinocarveol, terpinen-4-ol, $\alpha$-terpineol, myrtenal, citronellol, isobornyl acetate, (E)-caryophyllene, $\alpha$-humulene, (E)-$\beta$-ionone, and caryophyllene oxide. The reference drug suramin was purchased from Sigma Aldrich.

2.4. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Essential Oils

The chemical constituents of the Cameroonian essential oils were analyzed on an Agilent 6890 N gas chromatograph coupled to a 5973 N mass spectrometer (Santa Clara, CA, USA) and equipped with a HP-5 MS capillary column (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 $\mu$m film thickness; J & W Scientific, Folsom, CA, USA). For the separation of the volatile constituents, the following temperature program was used: 5 min at 60 $^\circ$C then 4 $^\circ$C/min up to 220 $^\circ$C, then 11 $^\circ$C/min up to 280 $^\circ$C held for 15 min. The injector and detector temperatures were: 280 $^\circ$C; carrier gas: Helium; flow rate: 1 mL/min; split ratio: 1:50; acquisition mass range: 29–400 m/z; and mode: electron-impact (EI, 70 eV). The essential oil was diluted 1:100 in n-hexane, and then 2 $\mu$L of the solution was injected into the GC-MS system. For the identification of essential oil components, co-injection with the authentic standards available in our laboratory (purchased from Sigma-Aldrich) was performed, together with a comparison of the retention indices and the mass spectra of those occurring in the ADAMS, NIST 08, and FFNSC2 libraries [41–43]. The percentage values of the volatile components were the means of three chromatographic analyses and were determined from the peak areas without the use of correction factors.

2.5. T. brucei and Mammalian Cell Culture and Growth Inhibition Assay

The cell culture conditions and the growth inhibition assay on T. brucei and Balb/3T3 cells were performed as described before [44]. T. brucei TC221 bloodstream forms and mouse embryonic fibroblast Balb/3T3 cells (ATCC no CCL-163) were cultivated in vented plastic flasks at 37 $^\circ$C with 5% CO$_2$. For T. brucei, the growth medium was HMI-9 [45] supplemented with 10% (v/v) fetal bovine serum (Gibco, Waltham, MA, USA), whereas the Balb/3T3 cells were grown in Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, glutamine (0.584 g/L), and 10 mL/L 100× penicillin-streptomycin (Gibco).

The essential oils or pure compounds identified from these oils were dissolved in dimethyl sulfoxide (DMSO) and serially diluted with growth medium in white 96-well microtiter plates. 20,000 bloodstream forms of T. brucei or Balb/3T3 cells were added to each well in a final volume of 200 $\mu$L. In the case of mammalian cells, we also tested 2000 cells/well with similar results. To avoid any damage to the cells, the concentration of DMSO in the solution was never higher than 1% (no cell growth inhibition was observed with this concentration of DMSO). Cell viability was verified by a drug-free control for each compound.
The plates were incubated for 48 h in the CO$_2$ incubator; then 20 µL of 0.5 mM resazurine (Sigma Aldrich) was added to each well, and the plates were incubated for an additional 24 h before the fluorescence was measured with an Infinite M200 microplate reader (Tecan group, Ltd., Männedorf, Switzerland) equipped with 540 and 590 nm excitation and emission filters. The half maximal inhibitory concentration (IC$_{50}$) values were calculated on a log inhibitor versus the response curves by non-linear regression using the GraphPad prism 5.04 software (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results and Discussion

3.1. Chemical Composition of Essential Oils from Cameroonian Plants

The chemical composition of the essential oils hydrodistilled from the six Cameroonian medicinal and aromatic plants is reported in Table 1. In the essential oil obtained from neem leaves, a total of thirteen components were identified, accounting for 98.3% of the total composition. The oil was almost entirely composed by sesquiterpene hydrocarbons (97.4%), with germacrene B (74.0%) and γ-elemene (18.3%) as the predominant components. The minor constituents were (E)-caryophyllene (2.4%) and β-elemene (0.9%). This work represents one of the few studies on the chemical composition of neem leaf essential oils. Neem trees have been largely investigated for the oil obtained by mechanical pressure or solvent extraction from their seeds. Earlier, El-Hawary et al. [46] studied the composition of Egyptian neem leaves, reporting β-elemene (33.39%), γ-elemene (9.89%), germacrene D (9.72%), caryophyllene (6.8%), and bicyclogermacrene (5.23%) as the major compounds, while Dastan et al. [47] found γ-elemene (20.8%), germacrene B (20.3%), trans-caryophyllene (13.5%), hexadecanal (12.8%), and methyl linolate (10.5%) as the major compounds in neem leaf oil from Iran.

A total of fifty-nine components were identified in the essential oil of alligator pepper, accounting for 99.4% of the total composition. The oil was dominated by oxygenated monoterpenes (83.3%), with 1,8-cineole (58.5%) and α-terpineol (19.4%) as the major compounds. Monoterape hydrocarbons gave a minor contribution (14.9%), with β-pinene (7.1%) and α-pinene (2.0%) as the most representative compounds. Interestingly, sesquiterpenoids, which are reported as volatile marker compounds of alligator pepper, were detected in only low levels here (0.6%). The chemical composition of the $A. melegueta$ seed essential oil showed a significant variability depending on the geographic origin and genetic characteristics of the samples. For example, samples from Nigeria exhibited humulene (26.23%), (E)-ocimene (23.22%), (E)-caryophyllene (19.17%), and (S)-2-heptyl acetate (16.22%) as the major volatile constituents [24]. On the other hand, the seeds from the Central African Republic contained high levels of β-pinene (>30%) and about 50% sesquiterpene hydrocarbons [48], and an oil sample from Cameroon was made up of β-caryophyllene (8.5%), α-humulene (31.3%), and their epoxides (17.9% and 27.7%, respectively) [49].

Fifty-seven compounds were identified in the essential oil from African cardamom leaves, accounting for 99.3% of the total composition. This oil was mainly made up of monoterape hydrocarbons (59.8% in leaves), accompanied by lower amounts of sesquiterape hydrocarbons (20.0%), oxygenated monoterpenes (11.0%), and oxygenated sesquiterpenes (8.4%). The major compounds were sabinene (43.9%) and (E)-caryophyllene (16.6%), whereas other components occurring at noteworthy levels were β-pinene (5.8%), terpinen-4-ol (3.7%), and α-pinene (2.4%). This study was the first report on the leaf essential oil from $A. daniellii$.

The essential oil extracted from the leaves of $C. anisata$ was characterized by high levels of phenylpropanoids (84.0%), which were mainly represented by (E)-anethole (64.6%), with minor contributions by (E)-methyl isoegenol (16.1%) and methyl chavicol (2.0%). Terpenoids constituted a minor part of this oil, being represented mostly by p-cymene (2.9%), γ-terpinene (2.4%), myrcene (2.0%), and germacrene D (2.2%).
Table 1. Chemical composition of the essential oils from *Azadirachta indica*, *Aframomum melegueta*, *Aframomum danielli*, *Clausena anisata*, *Dichrostachys cinerea*, and *Echinops giganteus*.

| No. | Component a | RI calc. b | RI lit. c | % d | **Azadirachta indica** | **Aframomum melegueta** | **Aframomum danielli** e | **Clausena anisata** | **Dichrostachys cinerea** e | **Echinops giganteus** e | ID f |
|-----|-------------|-------------|-----------|-----|-----------------------|-------------------------|-------------------------|----------------------|--------------------------|--------------------------|------|
| 1   | isopentyl acetate | 873         | 869       | tr e | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 2   | 2-methyl butyl acetate | 876         | 875       | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 3   | 2-heptanone | 891         | 892       | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 4   | 2-heptanol | 901         | 894       | 0.2  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 5   | α-thujene | 916         | 924       | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 6   | α-pinene | 921         | 932       | 2.0  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 7   | α-fenchene | 938         | 945       | 0.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 8   | camphene  | 939         | 946       | 0.3  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | RI,MS |
| 9   | α-pinene | 963         | 974       | 7.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 10  | α-pinene | 979         | 988       | 0.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 11  | α-pinene | 982         | 988       | 0.2  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 12  | α-pinene | 996         | 1004      | 0.3  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 13  | β-3-carene | 1003        | 1008      | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 14  | α-pinene | 1009        | 1014      | 0.3  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 15  | α-pinene | 1016        | 1020      | 1.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 16  | α-pinene | 1020        | 1024      | 1.5  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 17  | α-pinene | 1021        | 1026      | 38.5 | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 18  | α-pinene | 1031        | 1030      | 0.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 19  | α-pinene | 1037        | 1032      | 0.4  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 20  | α-pinene | 1041        | 1044      | 0.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 21  | α-pinene | 1050        | 1054      | 0.9  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 22  | α-pinene | 1057        | 1065      | 1.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 23  | α-pinene | 1071        | 1067      | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 24  | α-pinene | 1079        | 1086      | 0.8  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 25  | α-pinene | 1086        | 1089      | 0.2  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 26  | α-pinene | 1081        | 1083      | 5.1  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 27  | α-pinene | 1089        | 1098      | 0.9  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 28  | α-pinene | 1094        | 1094      | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 29  | α-pinene | 1096        | 1095      | 1.8  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 30  | α-pinene | 1105        | 1100      | tr   | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 31  | α-pinene | 1108        | 1114      | 0.3  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| 32  | α-pinene | 1113        | 1118      | 0.2  | 1.0                   | 1.0                     | 0.2                     | Tr                   | Tr                       | Tr                       | Std  |
| No. | Component          | RI calc. | RI lit. | Azadirachta indica | Aframomum melegueta | Aframomum daniellii | Clausena anisata | Dichrostachys cinerea | Echinops giganteus | ID     |
|-----|--------------------|----------|---------|-------------------|---------------------|---------------------|-------------------|---------------------|---------------------|--------|
| 34  | $\alpha$-campholenal | 1123     | 1122    | tr                | tr                  | tr                  | 0.1               | RL,MS               |                     | RI,MS  |
| 35  | trans-pinocarveol   | 1128     | 1135    | 0.2               | 0.3                 | 0.3                 | 0.1               | Std                 |                     |        |
| 36  | trans-$p$-menth-2-en-1-ol | 1131   | 1136    | 0.1               | tr                  | tr                  | 0.1               |        | Std                 |        |
| 37  | cis-\(\beta\)-terpineol | 1142   | 1140    | tr                | tr                  | tr                  | 0.1               | Std                 | 5.3                 | Std    |
| 38  | cis-verbenol        | 1142     | 1137    |                  | 0.2                 | 0.2                 | 0.1               | Std                 |                     | RI,MS  |
| 39  | trans-pinocamphone  | 1151     | 1158    | tr                | 0.4                 | 0.4                 | 0.1               | Std                 |                     | RI,MS  |
| 40  | pinocarvone         | 1152     | 1160    | tr                | 0.1                 | 0.1                 | 0.1               | Std                 | 0.1                 | Std    |
| 41  | borneol             | 1156     | 1165    | 0.2               | 0.7                 | 0.7                 | 0.1               | Std                 |                     | Std    |
| 42  | $p$-menth-1,5-dien-8-ol | 1158  | 1166    | 1.1               | rl                  | rl                  | 0.1               | Std                 |                     | Std    |
| 43  | cis-pinocamphone   | 1162     | 1172    | 1.4               | 3.7                 | tr                  | 7.5               | Std                 |                     | Std    |
| 44  | umbellulone         | 1166     | 1167    | 3.8               | 0.1                 | 0.1                 | 0.1               | Std                 |                     | Std    |
| 45  | terpen-4-ol         | 1167     | 1174    | 1.1               | 3.7                 | tr                  | 7.5               | Std                 |                     | Std    |
| 46  | cis-pinocarveol     | 1175     | 1182    | tr                | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 47  | cryptone            | 1183     | 1183    | tr                | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 48  | $p$-cymen-8-ol      | 1178     | 1179    | tr                | tr                  | tr                  | 0.3               | Std                 |                     | Std    |
| 49  | $\alpha$-terpineol  | 1181     | 1186    | 19.4              | 0.2                 | 0.2                 | 0.2               | Std                 |                     | Std    |
| 50  | myrtenal            | 1184     | 1195    | 0.2               | 0.1                 | 0.1                 | 0.1               | Std                 |                     | Std    |
| 51  | myrtenol            | 1186     | 1194    | 0.2               | 0.4                 | 0.4                 | 0.4               | Std                 |                     | Std    |
| 52  | cis-piperitol       | 1199     | 1195    | tr                | 0.1                 | 0.1                 | 0.1               | Std                 |                     | Std    |
| 53  | $\gamma$-terpineol  | 1195     | 1199    | tr                | 0.1                 | 0.1                 | 0.1               | Std                 |                     | Std    |
| 54  | methyl chavicol     | 1196     | 1195    | 2.0               | 2.5                 | 2.5                 | 2.5               | Std                 | 2.5                 | Std    |
| 55  | trans-piperitol     | 1205     | 1207    | tr                | tr                  | tr                  | 0.1               | Std                 |                     | Std    |
| 56  | trans-carveol       | 1217     | 1215    | 0.2               | 0.2                 | 0.2                 | 0.2               | Std                 |                     | Std    |
| 57  | cis-carveol         | 1228     | 1226    | tr                | 0.2                 | 0.2                 | 0.2               | Std                 |                     | Std    |
| 58  | thymol methyl ether | 1224     | 1232    | tr                | tr                  | tr                  | 0.1               | Std                 |                     | Std    |
| 59  | nerol               | 1229     | 1227    | 0.2               | 0.2                 | 0.2                 | 0.2               | Std                 |                     | Std    |
| 60  | citronellol         | 1231     | 1233    | 0.3               | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 61  | carvone             | 1240     | 1239    | tr                | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 62  | carvacrol methyl ether | 1237  | 1241    | tr                | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 63  | neral               | 1241     | 1235    | 0.2               | 0.2                 | 0.2                 | 0.2               | Std                 |                     | Std    |
| 64  | piperitone           | 1250     | 1249    | 0.3               | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 65  | (Z)-anethole        | 1250     | 1249    | tr                | 0.3                 | 0.3                 | 0.3               | Std                 |                     | Std    |
| 66  | $p$-anisaldehyde    | 1251     | 1247    | 0.7               | 0.7                 | 0.7                 | 0.7               | Std                 |                     | Std    |
| 67  | geraniol            | 1251     | 1249    | 18.2              | 18.2                | 18.2                | 18.2              | Std                 |                     | Std    |
| 68  | trans-ascaridol glycol | 1262  | 1266    | tr                | 18.2                | 18.2                | 18.2              | Std                 |                     | Std    |
| 69  | (E)-cinnamaldehyde  | 1267     | 1267    | tr                | 0.1                 | 0.1                 | 0.1               | Std                 |                     | Std    |
Table 1. Cont.

| No. | Component       | RI calc. | RI lit. | % | Azadirachta indica | Aframomum melegueta | Aframomum daniellii | Clausena anisata | Dichrostachys cinerea | Echinops giganteus | ID   |
|-----|-----------------|----------|---------|---|-------------------|---------------------|--------------------|-----------------|----------------------|------------------|------|
| 70  | phellandral     | 1269     | 1273    | 0.1 |                   |                      |                    |                 |                      |                  | RI,MS |
| 71  | isobornyl acetate | 1276     | 1283    |      |                   |                      |                    |                 |                      |                  | Std   |
| 72  | (E)-anethole    | 1287     | 1282    |      |                   |                      |                    |                 |                      |                  | Std   |
| 73  | thymol          | 1291     | 1289    | 0.1 |                   |                      | 64.6               |                 |                      |                  | Std   |
| 74  | trans-sabinyl acetate | 1291 | 1289 | tr |                      |                      |                    |                 |                      |                  | Std   |
| 75  | methyl myrtenate | 1292     | 1293    |      |                   |                      |                    |                 |                      |                  | Std   |
| 76  | carvacrol       | 1301     | 1298    | 0.5 |                   |                      |                   | 0.7             |                      |                  | Std   |
| 77  | cis-pinocarvyl acetate | 1303 | 1311 | 0.1 |                      |                      |                    |                 |                      |                  | Std   |
| 78  | myrtenyl acetate | 1316     | 1324    | 1.9 |                   |                      |                    |                 |                      |                  | Std   |
| 79  | silphiperfol-5-ene | 1318   | 1326    |      |                   |                      |                    | 2.1             |                      |                  | RI,MS |
| 80  | δ-elemene       | 1326     | 1335    | 0.1 |                   |                      | 0.1                |                 |                      |                  | RI,MS |
| 81  | presilphiperfol-7-ene | 1328 | 1334 | tr |                      |                      |                    |                 |                      |                  | RI,MS |
| 82  | silphinene      | 1333     | 1340    |      |                   |                      |                    |                 |                      |                  | RI,MS |
| 83  | 7-epi-silphiperfol-5-ene | 1336 | 1349 | tr |                      |                      |                    |                 |                      |                  | RI,MS |
| 84  | α-terpinyl acetate | 1341    | 1346    | tr  |                   |                      | 0.3                |                 |                      |                  | RI,MS |
| 85  | α-copaene       | 1362     | 1374    | 0.2 |                   |                      | 0.2                | tr              |                      |                  | Std   |
| 86  | β-bourbonene    | 1369     | 1387    | 0.3 |                   |                      | 3.0                |                 |                      |                  | RI,MS |
| 87  | modheph-2-ene   | 1362     | 1382    |      |                   |                      | 23.0               |                 |                      |                  | RI,MS |
| 88  | silphiperfol-6-ene | 1373   | 1377    |      |                   |                      |                    |                 |                      |                  | RI,MS |
| 89  | β-bourbonene    | 1377     | 1387    |      |                   |                      | 0.1                |                 |                      |                  | RI,MS |
| 90  | β-cubebeene     | 1377     | 1387    | tr  |                   |                      |                    |                 |                      |                  | RI,MS |
| 91  | α-isocomene     | 1379     | 1387    |      |                   |                      | 2.4                |                 |                      |                  | RI,MS |
| 92  | β-elemene       | 1380     | 1389    | 0.9 |                   |                      | 0.1                |                 |                      |                  | Std   |
| 93  | decanoic acid   | 1380     | 1386    |      |                   |                      |                    | 2.8             |                      |                  | RI,MS |
| 94  | anisyl methyl ketone | 1382   | 1380    |      |                   |                      | 0.1                |                 |                      |                  | RI,MS |
| 95  | iso-longifolene | 1383     | 1389    |      |                   |                      |                    |                 |                      |                  | RI,MS |
| 96  | β-isocomene     | 1400     | 1407    |      |                   |                      | 2.1                |                 |                      |                  | RI,MS |
| 97  | α-gurjunene     | 1400     | 1409    |      |                   |                      | 0.3                |                 |                      |                  | Std   |
| 98  | (E)-caryophyllene | 1402   | 1417    | 2.4 |                   |                      | 16.6               | 0.8             |                      |                  | Std   |
| 99  | methyl eugenol  | 1406     | 1403    |      |                   |                      | 0.3                |                 |                      |                  | Std   |
| 100 | α-trans-bergamotene | 1425   | 1432    |      |                   |                      | 0.1                |                 |                      |                  | RI,MS |
| 101 | isoamyl benzoate | 1433     | 1433    |      |                   |                      |                    |                 |                      |                  | RI,MS |
| 102 | γ-elemene       | 1427     | 1434    | 18.3|                   |                      | 0.1                |                 |                      |                  | RI,MS |
| 103 | α-humulene      | 1436     | 1452    | 0.4 |                   |                      | 1.5                | 0.8             |                      |                  | Std   |
| 104 | geranyl acetone | 1449     | 1453    |      |                   |                      |                    | 1.2             |                      |                  | RI,MS |
| 105 | (E)-β-farnesene | 1450     | 1454    |      |                   |                      |                    |                 |                      |                  | RI,MS |
| No. | Component [a] | RI calc. b | RI lit. c | Azadirachta indica | Aframomum melegueta | Aframomum daniellii e | Clausena anisata | Dichrostachys cinerea e | Echinops giganteus e | ID f |
|-----|---------------|------------|----------|-------------------|-------------------|-------------------|-----------------|-------------------|-------------------|------|
| 106 | germacrene D  | 1465       | 1484     | 0.5               | 0.3               | 2.2               | 0.3             | RI,MS             |                   |      |
| 107 | selina-4,11-diene | 1467     | 1474     |                   | 0.1               |                   | 0.1             | RI,MS             |                   |      |
| 108 | β-selinene    | 1469       | 1489     | tr                |                   |                   |                 |                   | RI,MS             |      |
| 109 | ar-curcumene  | 1472       | 1479     |                   | tr                |                   | 0.1             | RI,MS             |                   |      |
| 110 | bicyclogermacrene | 1480   | 1500     | 0.1               | 0.1               |                   |                 | RI,MS             |                   |      |
| 111 | benzaldehyde, 3,4-dimethoxy- | 1482 | 1489     | 0.2               |                   |                   |                 | RI,MS             |                   |      |
| 112 | (E)-β-ionone  | 1481       | 1487     | 0.5               |                   |                   |                 |                   | Std               |      |
| 113 | epi-cubebel   | 1489       | 1493     |                   | 0.1               |                   |                 | RI,MS             |                   |      |
| 114 | α-zingiberene | 1492       | 1493     |                   | 0.1               |                   |                 | RI,MS             |                   |      |
| 115 | (Z)-α-bisabolene | 1493   | 1506     | 0.1               |                   |                   |                 | RI,MS             |                   |      |
| 116 | silphiperfolan-6-α-ol | 1496     | 1507     | 0.1               |                   |                   |                 | 1.0               | RI,MS             |      |
| 117 | β-bisabolene  | 1498       | 1505     | 0.9               |                   |                   |                 |                   | RI,MS             |      |
| 118 | (E)-methyl isoeugenol | 1499 | 1491     | 16.1              |                   |                   |                 |                   | RI,MS             |      |
| 119 | cameroonan-7-α-ol | 1500     | 1510     | 7.1               |                   |                   |                 |                   | RI,MS             |      |
| 120 | β-bisabolene  | 1506       | 1505     | 0.3               |                   |                   |                 |                   | RI,MS             |      |
| 121 | 7-epi-α-selinene | 1507    | 1520     | tr                |                   |                   |                 |                   | RI,MS             |      |
| 122 | (E,E)-α-farnesene | 1508    | 1505     | 0.3               |                   |                   |                 |                   | Std               |      |
| 123 | trans-calamenene | 1508   | 1521     | tr                |                   |                   |                 |                   | RI,MS             |      |
| 124 | δ-cadinene    | 1510       | 1522     | 0.2               | tr                | 0.1              | 0.3             | RI,MS             |                   |      |
| 125 | silphiperfolan-7-β-ol | 1510 | 1519     | 2.5               |                   |                   |                 | RI,MS             |                   |      |
| 126 | β-sesquiphellandrene | 1520  | 1521     | tr                |                   |                   |                 |                   | RI,MS             |      |
| 127 | selina-3,7(11)-diene | 1531 | 1545     | 0.2               |                   |                   |                 | RI,MS             |                   |      |
| 128 | silphiperfolan-6-β-ol | 1535 | 1546     | 1.7               |                   |                   |                 |                   | RI,MS             |      |
| 129 | hedyccaryl    | 1536       | 1546     | 1.5               |                   |                   |                 |                   | RI,MS             |      |
| 130 | germacrene B  | 1546       | 1559     | 74.0              | 0.3               |                   |                 | RI,MS             |                   |      |
| 131 | elemicin      | 1556       | 1555     | 3.0               |                   |                   |                 | RI,MS             |                   |      |
| 132 | (E)-nerolidol | 1556       | 1561     | 0.7               |                   |                   |                 | Std               | RI,MS             |      |
| 133 | isoxoromadendrene epoxide | 1560 | 1572     | 1.8               |                   |                   |                 | RI,MS             |                   |      |
| 134 | prenopsan-8-ol | 1564   | 1575     | 3.2               |                   |                   |                 | RI,MS             |                   |      |
| 135 | caryophyllene oxide | 1564 | 1582     | 2.2               | 1.1               |                   |                 | Std               |                   |      |
| 136 | (3Z)-hexenyl benzoate | 1566 | 1565     | 0.3               |                   |                   |                 | RI,MS             |                   |      |
| 137 | spathulenol   | 1568       | 1576     | 0.1               |                   |                   |                 | MS               | RI,MS             |      |
| 138 | presilphiperfolan-8-ol | 1578 | 1585     | 22.7              |                   |                   |                 |                   | MS               |      |
| 139 | guaiol        | 1583       | 1600     | 0.5               |                   |                   |                 | RI,MS             |                   |      |
| 140 | humulene epoxide II | 1590 | 1608     | 0.1               | tr                |                   |                 | RI,MS             |                   |      |
| 141 | 10-epi-γ-eudesmol | 1600  | 1622     | tr                | 0.3               |                   |                 | RI,MS             |                   |      |
Table 1. Cont.

| No. | Component a | RI calc. b | RI lit. c | Azadirachta indica | Aframomum melegueta | Aframomum daniellii e | Clausena anisata e | Dichrostachys cinerea e | Echinops giganteus e | ID f |
|-----|-------------|------------|------------|-------------------|---------------------|---------------------|-------------------|-------------------|-------------------|------|
| 142 | eremoligenol | 1611       | 1629       | 0.4               | tr                  | 0.1                 | 0.4               | 1.0               |                   | RI, MS |
| 143 | γ-eudesmol   | 1615       | 1630       | 0.4               | tr                  | 0.4                 | 0.4               | 1.5               |                   | RI, MS |
| 144 | 1,10-di-epi-cubenol | 1619 | 1618 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 145 | β-eudesmol   | 1631       | 1649       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 146 | α -acorenol  | 1628       | 1632       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 147 | caryophylla-4(12),8(13)-dien-5-ol h | 1630 | 1639 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 148 | epi-α -muurolol | 1635 | 1640 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 150 | α -muurolol  | 1640       | 1644       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 151 | intermedeol  | 1639       | 1666       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 152 | α-cadinol    | 1647       | 1652       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 153 | ageratocromene | 1655 | 1658 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 154 | α-bisabolol  | 1673       | 1685       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 155 | 3-oxo-β-ionone | 1678 | 1685 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 156 | cyperotundone | 1688       | 1695       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 157 | (2E-6Z)-farnesol | 1709 | 1698 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 158 | curcuphenol  | 1716       | 1717       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 159 | (2E-6Z)-farnesal | 1718 | 1713 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 160 | (2E-6E)-farnesal | 1737 | 1740 | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | RI, MS |
| 161 | n-tricosane  | 2300       | 2300       | 0.2               | 1.5                 | 1.0                 |                   |                   |                   | Std |

Oil yield (%) | 0.01 | 0.3 | 0.2 | 2.0 | 0.4 | 1.8
Total identified (%) | 98.3 | 99.4 | 99.3 | 99.6 | 76.0 | 94.3
Grouped compounds (%)
Monoterpene hydrocarbons | 14.9 | 59.8 | 0.6 | 50.6
Oxycanated monoterpenes | 83.3 | 11.0 | 0.6 | 50.6
Sesquiterpene hydrocarbons | 97.4 | 0.2 | 20.0 | 5.2 | 54.7
Oxycanated sesquiterpenes | 97.4 | 0.2 | 20.0 | 5.2 | 54.7
Phenylpropanoids | 84.0 | 0.2 | 12.7 |
Others | 0.9 | 0.6 | 0.2 | 12.7 |

a Components are reported in order of their elution from an HP-5MS capillary column; b Retention index (RI) experimentally determined using a mixture of C₈-C₃₀ of n-alkanes; c Retention index taken from ADAMS [41] and/or NIST 08 [42] for an apolar capillary column; d Relative percentage values are means of three determinations with a Relative Standard Deviation (RSD%) below 15% for the most abundant components; e Analytical data are taken from Pavela et al. [26]; f Identification methods: standard (std), based on the comparison of RT (retention time), RI, and MS (mass spectrometry) with authentic compounds; RI, based on correspondence of calculated RI with those reported in ADAMS and NIST 08; MS, based on comparison with the WILEY, ADAMS, FFNSC2, and NIST 08 MS databases; g traces, % <0.1; h Correct isomer not identified. ID: Identity.
In the essential oil of *D. cinerea* seeds, a total of forty-nine volatile components were identified, accounting for 76.0% of the oil’s composition. Oxygenated monoterpenes were the most abundant constituents (50.6%), with geraniol (18.2%), terpinen-4-ol (7.5%), linalool (4.0%), and umbellulone (3.8%) as the most representative compounds. Oxygenated sesquiterpenes gave a lower contribution (12.1%), with none of the identified components exceeding 1.8%. Among other components occurring in the oil, it is worth noting the presence of ligustrazin (5.1%), elemicin (3.0%), and decanoic acid (2.8%). To our knowledge, no previous research has reported the chemical composition of *D. cinerea* seed essential oil.

Thirty-five volatile compounds, all belonging to the sesquiterpene class (94.3%), were identified in the root essential oil from *E. giganteus* (54.7% sesquiterpene hydrocarbons and 39.6% oxygenated sesquiterpenes). The major compounds were tricyclic sesquiterpenoids, which are characterized by multiple rearrangements of the caryophyllene cation [50,51], namely, silphiperfol-6-ene (23.0%), presilphiperfolan-8-ol (22.7%), presilphiperfol-7-ene (7.8%), and cameroonan-7-α-l (7.8%). Another noteworthy constituent occurring in the oil was (E)-caryophyllene (6.3%). The cameroonan-7-α-l, is responsible for the patchouli-like smell of the root oil [52]. The reported composition was quite consistent with that previously reported by Menut et al. [49] for root samples collected in Cameroon.

### 3.2. Inhibition of *Trypanosoma brucei* Proliferation

Essential oils are complex mixtures of volatile compounds with multitarget actions, the antitrypanosomal effects of which are largely unknown and barely explored. On this basis, we decided to test the in vitro inhibitory effects of a pool of essential oils taken from medicinal and aromatic plants growing in Cameroon. Some of them are known for their traditional uses in the treatment of infectious diseases and malaria [53,54] and, in the case of the Neem tree, also against *T. b. brucei* [21].

Based on the chemical analysis performed, they exhibited different chemical profiles characterized by diverse functionalized groups such as monoterpenic hydrocarbons (African cardamom), oxygenated monoterpenes (*A. melegueta* and *D. cinerea*), sesquiterpene hydrocarbons (*A. indica*), sesquiterpene hydrocarbons and oxygenated sesquiterpenes (*E. giganteus*), and phenylpropanoids (*C. anisata*). In this context, the main aim of our work was to identify the chemical scaffolds of possible natural lead compounds against trypanosomiasis.

Testing the essential oils obtained from Cameroonian plants, we obtained various degrees of inhibition on *T. brucei* proliferation, varying from not active (*A. melegueta*, *C. anisata*, and *D. cinerea*), to moderately active (*A. danielli*, *E. giganteus* and *A. indica*). Notably, the IC_{50} values on *T. brucei* were 7.65, 10.50, and 15.21 µg/mL for the essential oils from *A. danielli*, *E. giganteus*, and *A. indica*, respectively (Table 2). Furthermore, the most active oils were also evaluated for the growth inhibitory effects on Balb/3T3 cells as a reference. No effect on mammalian cells was observed with concentrations as high as 100 µg/mL, showing a noteworthy selectivity against *T. brucei* in comparison to mammalian cells, with selectivity indexes above 6.57 in all cases.

The inhibitory effects on *T. brucei* exhibited by the three essential oils highlights three classes of active compounds, i.e., monoterpenic hydrocarbons (for *A. danielli*) and sesquiterpene hydrocarbons (for *A. indica* and *E. giganteus*) (Figure 1). *E. giganteus* also contains high amounts of oxygenated compounds.

The toxicity of monoterpenic hydrocarbons against *T. brucei* can be attributed to the high hydrophobicity of this class of compounds, which are able to easily cross the cell membrane, causing the destabilization of phospholipid bilayers and the alteration of their permeability, leading to cell damage and death [44,55]. Among these compounds, the most abundant was sabinene (43.9%) (Figure 1) which showed an IC_{50} value against *T. brucei* of 5.96 µg/mL, which is close to that of the African cardamom essential oil (7.65 µg/mL) (Table 2). Another component of this oil with detectable antitrypanosomal activity was β-pinene, showing an IC_{50} value of 11.4 µg/mL. The antitrypanosomal activity of sabinene was already reported in a previous study, although its mechanism of action on the protozoal cell has not been elucidated [56].
Table 2. Inhibitory effects of essential oils from Cameroonian plants against Trypanosoma brucei brucei TC221 and Balb/3T3 cells.

| Treatment                     | IC₅₀ (µg/mL) | Selectivity Index (SI) |
|-------------------------------|-------------|------------------------|
|                               | T. b. brucei (TC221) | Balb/3T3 |
| Essential oils                |             |                        |
| Aframomum danielli           | 7.65 ± 1.1  | >100                   | >13.1   |
| Dichrostachys cinerea        | >100        | -                      | -       |
| Echinops giganteus           | 10.50 ± 1.7 | >100                   | >9.52   |
| Azadirachta indica           | 15.21 ± 0.97| >100                   | >6.57   |
| Aframomum melegueta          | >100        | -                      | -       |
| Clausena antisata            | >100        | -                      | -       |
| Pure compounds               | µg/mL (µM)  | µg/mL (µM)             |         |
| Sabinené                     | 5.96 ± 1.3 (43.8) | >100       | >16.7   |
| β-Pinene                     | 11.4 ± 2.6 (83.7) | >100       | >8.77   |
| 1,8-Cineole                  | >100        | -                      | -       |
| Terpinen-4-ol                | >100        | -                      | -       |
| (E)-Caryophyllene            | 8.25 ± 1.3 (40.4) | >100       | >12.1   |
| Reference drug               | µg/mL (µM)  | µg/mL (µM)             |         |
| Suramin                      | 0.0286 ± 0.0008 (0.0220) | -       | -       |

IC₅₀: half maximal inhibitory concentration.

E. giganteus as well as A. danielli essential oils contained the bicyclic sesquiterpene (E)-caryophyllene (Figure 1), which exhibited good inhibitory properties on T. brucei (IC₅₀ value of 8.25 µg/mL). However, the content of this component is only 6.3% in E. giganteus and can therefore not explain the good activity of this essential oil. It is rather tricyclic sesquiterpenes that are the major constituent. This is the first report documenting the antitrypanosomal activity of the tricyclic sesquiterpenes-containing E. giganteus essential oil. The latter was a rich source of compounds such as silphiperfol-6-ene, presilphiperfolan-8-ol, presilphiperfol-7-ene and cameroonan-7-α-l with an unusual skeleton (Figure 1) [26]. To date, these compounds have not been biologically investigated. Previously, the tricyclic sesquiterpene ledol was suggested to be responsible for the trypanocidal properties of Hagenia abyssinica (Bruce ex Steud.) J.F.Gmel. essential oil [13].

Among the other pure compounds, 1,8-cineole and terpinen-4-ol were inactive against T. brucei (IC₅₀ > 100 µg/mL) (Table 2), and this also explained the lack of activity of the A. melegueta and D. cinerea essential oils, which are dominated by these compounds.

Finally, we demonstrated for the first time that the leaf essential oil from the neem tree, which showed an IC₅₀ value of 15.21 µg/mL, can be a source of sesquiterpenes such as germacrene B and γ-elemene. Since this oil is completely dominated by sesquiterpenes (97.4%), it can be assumed that they are responsible for its antitrypanosomal activity. Further studies are needed to elucidate their mode of action and the possibility of them acting as lead compounds for the discovery of antitrypanosomal drugs.

![Chemical structures of the main essential oil constituents in (a) Aframomum danielli, (b) Echinops giganteus, and (c) Azadirachta indica.](image-url)
4. Conclusions

In conclusion, our biological investigation into the essential oils distilled from medicinal and aromatic plants growing in Cameroon identified some terpenoids as possible lead compounds of natural antitrypanosomal drugs. Further research is encouraged to disclose their mechanisms of action and in vivo efficacy.

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