Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (Giles)

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**Abstract**

**Background:** Insecticide resistance in sub-Saharan Africa and especially in Benin is a major public health issue hindering the control of the malaria vectors. Each *Anopheles* species has developed a resistance to one or several classes of the insecticides currently in use in the field. Therefore, it is urgent to find alternative compounds to conquer the vector. In this study, the efficacies of essential oils of nine plant species, which are traditionally used to avoid mosquito bites in Benin, were investigated.

**Methods:** Essential oils of nine plant species were extracted by hydrodistillation, and their chemical compositions were identified by GC-MS. These oils were tested on susceptible “kisumu” and resistant “ladji-Cotonou” strains of *Anopheles gambiae*, following WHO test procedures for insecticide resistance monitoring in malaria vector mosquitoes.

**Results:** Different chemical compositions were obtained from the essential oils of the plant species. The major constituents identified were as follows: neral and geranial for *Cymbopogon citratus*, Z-carveol, E-p-mentha-1(7),8-dien-2-ol and E-p-mentha-2,8-dienol for *Cymbopogon giganteus*, piperitone for *Cymbopogon schoenanthus*, citronellal and citronellol for *Eucalyptus citriodora*, p-cymene, caryophyllene oxide and spathulenol for *Eucalyptus tereticornis*, 3-tetradecanone for *Cochlospermum tinctorium* and *Cochlospermum planchonii*, methyl salicylate for *Securidaca longepedunculata* and ascaridole for *Chenopodium ambrosioides*. The diagnostic dose was 0.77% for *C. citratus*, 2.80% for *E. tereticornis*, 3.37% for *E. citriodora*, 4.26% for *C. ambrosioides*, 5.48% for *C. schoenanthus* and 7.36% for *C. giganteus*. The highest diagnostic doses were obtained with *S. longepedunculata* (9.84%), *C. tinctorium* (11.56%) and *C. planchonii* (15.22%), compared to permethrin 0.75%. *A. gambiae* cotonou, which is resistant to pyrethroids, showed significant tolerance to essential oils from *C. tinctorium* and *S. longepedunculata* as expected but was highly susceptible to all the other essential oils at the diagnostic dose.

**Conclusions:** *C. citratus*, *E. tereticornis*, *E. citriodora*, *C. ambrosioides* and *C. schoenanthus* are potential promising plant sources for alternative compounds to pyrethroids, for the control of the *Anopheles* malaria vector in Benin. The efficacy of their essential oils is possibly based on their chemical compositions in which major and/or minor compounds have reported insecticidal activities on various pests and disease vectors such as *Anopheles*.

**Keywords:** Malaria, *A. gambiae*, Essential oils, Diagnostic dose, Knock-down times, Insecticide, Benin

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Background
Malaria is a life-threatening disease affecting 3.3 billion people worldwide with 80% of cases and 90% of deaths occurring in sub-Saharan Africa (SSA). Of those affected, children under the age of five and pregnant women are the most vulnerable [1]. The key factor for the reduction of human mortality is their protection against the malaria parasite vectors, the Anopheles mosquito, by the use of long-lasting insecticidal nets (LLIN) and application of indoor residual spraying (IRS) as recommended by the WHO [1]. Pyrethroids are the only insecticides recommended by WHO for the treatment of bednets [1] while in West Africa, Anopheles gambiae Giles is the major vector of malaria [2]. This mosquito species is now subjected to selection pressure, and has become resistant to dichlorodiphenyltrichloroethane (DDT) and pyrethroids because of the use of these insecticides both for agricultural and public health purposes [2,3]. During the last decade, the emergence of resistance to synthetic insecticides in Anopheles populations has been widely described in many African countries such as Benin [4-10], Ivory Coast [2,11], Niger [12], Burkina Faso [3,13,14], Mali [15], Nigeria [16], Kenya [17], Cameroun [18-20], Zanzibar [21], Uganda [22], Equatorial Guinea [23], and Ghana [24]. Because of the widespread occurrence of this resistance, there is a reduction of the efficacy of synthetic insecticides used in the field. Therefore, it is pertinent to explore the pesticidal activity of natural products [25] such as essential oils. Indeed the use of essential oils has been recognized as a potential alternative in the control of vectors of mosquito-borne diseases [26-30] such as A. gambiae. A lot of research on the pesticidal activities of essential oils has been conducted and has proven that essential oils could be considered as potential bioactive compounds against various pests and mosquitoes [28,31-34].

The objective of the current study was to investigate the chemical composition and the insecticidal activities of essential oils of nine aromatic plants traditionally used in Benin as a natural method of protection against A. gambiae, in order to confirm the traditional knowledge of Benin’s population and to find new valuable sources of active molecules against this malaria vector. Indeed these plant species have been reported to possess repellent and insecticidal activities against various mosquitoes, stored product beetles and other pests such as Anopheles species [30,35], Culex quinquefasciatus [36], Callosobruchus species [37,38], Sitophilus species [39,40] and Tribolium castaneum [41]. These plants include Chenopodium ambrosioides L. (Amaranthaceae), Securidaca longipedunculata Fresen. (Polygalaceae), Cochlospermum planchonii Hook. f. Ex Planch. (Bixaceae), Cochlospermum tinctorium A. Rich. (Bixaceae), Eucalyptus tereticornis Sm. (Myrtaceae), Cymbopogon citrrodora Hook. (Myrtaceae), Cymbopogon citratus (DC.) Stapf (Poaceae), Cymbopogon schoenanthus (L.) Spreng. (Poaceae) and Cymbopogon giganteus Chiov. (Poaceae).

For this purpose, the standard WHO susceptibility test has been used with various concentrations of essential oils under laboratory conditions [42].

Methods
Plant material
Nine plant species belonging to five botanical families were collected in several regions of Benin. Collected plant material was dried, free from light, at room temperature. All these plants have been the subject of classification and botanic description at the National Herbarium of the University of Abomey-Calavi, Benin [43]. Locations of collection, organs extracted and nature of soil are summarized in Table 1.

Essential oils
Essential oils from all plants were extracted by hydrodistillation using a Clevenger-type apparatus for two to four hours until essential oils were extracted completely from the organs. The extracted oils were dried over anhydrous magnesium sulfate and stored at 4°C, away from UV rays, before use. Each essential oil was diluted tenfold with diethyl ether. One milliliter of each diluted solution was charged into a sampler flask for GC-MS analysis.

Chemical analyses of essential oils by gas chromatography coupled with mass spectrometry
The GC-MS analysis of the essential oils was performed on an Agilent 6890 GC Plus automatic sampler system, coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium) and equipped with a capillary HP-5MS column fused with silica (length: 30 m; diameter: 0.25 mm, film thickness: 0.25 microns) in the split mode 1:100. The oven temperature was programmed at 60°C for 3 min and to 350°C at a rate of 5°C/min. The injector was kept at 250°C programmed with a rate of 10°C/s. Helium was used as carrier gas at a flow rate of 1 ml/min. All analyses were performed at constant flow. The mass detector conditions were: transfer line temperature 260°C; ionization mode electron impact: 70 eV. The identification of sample compounds was carried out in single runs. The Kovats retention indices were calculated for all volatile constituents using a series of n-alkanes C7-C17 [44]. Quantification of each compound was performed using percentage peak area calculations. The identification of oil volatile compounds was done by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference compounds [44-47]. The relative concentration of each
| Plants species       | Locations | Soils          | Organs       | Certification numbers |
|---------------------|-----------|----------------|--------------|-----------------------|
| Cymbopogon citratus | Cotonou   | Sandy          | Leaves       | AA6463/HNB            |
| Cymbopogon giganteus| Koudo     | Ferralitic     | Leafy stems  | AA6464/HNB            |
| Cymbopogon schoenanthus| Nalohou 2 | Gravely ferruginous | Leafy stems | AA6465/HNB            |
| Cochlospermum planchonii | Mount Kassa | Ferruginous | Roots       | AA6466/HNB            |
| Cochlospermum tinctorium | Mount Kassa | Ferruginous | Roots       | AA6467/HNB            |
| Eucalyptus citriodora | Abomey Calavi | Ferrallitic | Leaves      | AA6468/HNB            |
| Eucalyptus tereticornis | Abomey Calavi | Ferruginous | Leaves      | AA6469/HNB            |
| Chenopodium ambrosioides | Savalou   | Ferruginous | Leafy stems | AA6470/HNB            |
| Securidaca longepedunculata | Savalou | Ferruginous | Root bark    | AA6471/HNB            |
compound in the essential oil was quantified according to the peak area integrated by the analysis program (Chemstation data analysis).

Mosquito collection
Larvae of the resistant strain of A. gambiae were collected three times a week, from March 2012 to June 2012, from the natural breeding site in “Ladjî” in Cotonou. Ladji is a neighborhood of the outskirts of Cotonou, the capital of Benin. Larvae were brought and reared in the insectary of the Cotonou Research Center of Entomology (CREC). Larvae were fed with honey juice and the adults were placed in cages and fed in the same way. Emerging adult female mosquitoes of the resistant strain were used to carry out the susceptibility tests, whereas a susceptible strain of A. gambiae “Kisumu” originating from Kenya and maintained at the insectary, were used as a reference.

Bioassay on adult mosquitoes
Susceptibility tests were carried out using WHO insecticide susceptibility test-kits and standard procedures [42]. Four replicates of batches of 20-25 unfed females, aged 2-5 days old were exposed to filter papers (Whatman N°1) 12 cm × 15 cm, impregnated with 2 ml of various doses of the essential oil, diluted in acetone. The susceptible strain A. gambiae “Kisumu” was used as reference to determine the diagnostic doses. The filter papers of the control holding tubes were impregnated with acetone only. Tests were carried out at 25°C (± 2°C) and 70-80% relative humidity. Six doses were tested for each essential oil: 0.25% (w/v), 0.50% (w/v), 1% (w/v), 2% (w/v), 4% (w/v) and 8% (w/v). The number of mosquitoes knocked down was recorded every 5 min. Permethrin 0.75% was used as reference insecticide. Permethrin impregnated papers were obtained from the WHO reference center of “the Vector Control Research Unit of The University Sains Malaysia”. After the exposure time, mosquitoes were transferred to holding tubes and were fed with 10% honey juice for 24 hours. Subsequently the mortality was recorded. Data were plotted to determine the diagnostic doses to which the resistant strain from Ladjî was exposed.

Data analysis for bioassay
Times at which 50% or 95% of mosquitoes fell down on their back or on their side, i.e. knockdown time (KDT$_{50}$ and KDT$_{95}$) was calculated by means of the log time-probit using SPSS 17.0 software. The relation between the KDT, the mortality and the doses were assessed by probit regression. The diagnostic concentration which is the double of the minimum concentration at which 100% of the susceptible strain died [48] was also taken into account. All the results obtained for KDT (KDT$_{50}$ and KDT$_{95}$) and lethal doses (LC$_{50}$ and LC$_{99}$), were expressed with 95% confidence limits.

Results and discussion
Chemical composition of essential oils
Chemical compositions and essential oil yields, expressed as oil wt./wt. of dried organ extracted, showed a large variation (Tables 2, 3, 4, 5 and 6). These yields varied from 0.2% for Cochlospermum species to 4.6% for E. citriodora.

C. citriodora
The oil yield of C. citriodora was 1.7% (w/w). This value is higher than the one obtained with C. citriodora (1.3%) collected in northern Brazil [49]. Essential oil of C. citriodora was characterized by myrcene (12.4%), neral (33.1%) and geranial (44.3%). This result corroborates with previous results [49], which showed that the aerial part of C. citriodora contains myrcene (10.7%), neral (30.8%) and geranial (53.9%). Furthermore, myrcene, neral and geranial were also the main compounds identified in C. citriodora characterized in Burkina Faso, Brazil and Portugal [50-52].

C. giganteus
The oil yield of C. giganteus was 1.4% (w/w) and the main constituents of its essential oil were limonene (9.6%) and a set of monoterpenic alcohols: E-p-mentha-1(7),8-dien-2-ol (19.6%), E-p-mentha-1,2,8-dienol (19.3%), Z-p-mentha-2,8-dienol (10.2%), Z-p-mentha-1(7),8-dien-2-ol (2.1%), Z-carveol (17.0%) and E-carveol (6.0%) together with p-menth-6-en-2,3-diol (3.2%) and carvone (3.2%). This composition is similar to that of C. giganteus from Burkina Faso which was characterized by E-p-mentha-1(7),8-dien-2-ol, E-p-mentha-2,8-dienol, Z-p-mentha-2,8-dienol and Z-p-mentha-1(7),8-dien-2-ol [52] and from many West and Central African countries [28,53-55].

C. schoenanthus
The yield of C. schoenanthus essential oil was 2.6% (w/w). This result is similar to Ketoh et al. [56]. In the current work the major constituents were δ-2-carene (15.5%) and piperitone (58.9%); these results are consistent with the results obtained by Koba et al. and Ketoh et al. [38,56,57].

E. citriodora
Essential oil from E. citriodora was extracted with a yield of 4.6%. The main compounds detected by GC-MS were citronellal (52.8%), citronellol (20.0%), citronellyl acetate (9.0%) and neo-isopulegol (7.8%). Components like citronellal, citronellol and isopulegol were also detected in E. citriodora essential oil analyzed in Colombia [27,41].
Table 2 Chemical composition and oil yields of *Cymbopogon* species

| KI<sub>exp</sub><sup>a</sup> | KI<sub>lit</sub><sup>b</sup> | ID<sup>c</sup> | Compounds<sup>d</sup> | Cymbopogon citratus | Cymbopogon giganteus | Cymbopogon schoenanthus |
|-----------------------------|-----------------------------|-------------|-------------------------|-------------------|-------------------|-------------------------|
| 978                         | 974                         | MS, RI      | Sulcatone               | 0.5               |                   |                         |
| 982                         | 988                         | MS, RI      | Myrcene                 | 12.4              |                   |                         |
| 984                         | 988                         | MS, RI      | 2,3-Dehydro-1,8-cineole | 0.1               | 0.1               |                         |
| 995                         | 1001                        | MS, RI      | δ-2-Carene              | 0.1               | 15.5              |                         |
| 998                         | 1002                        | MS, RI      | α-Pheillandrene         | 0.2               |                   |                         |
| 1009                        | 1014                        | MS, RI      | α-Terpineene            | 0.2               |                   |                         |
| 1017                        | 1020                        | MS, RI      | p-Cymene                | 0.5               | 0.1               |                         |
| 1020                        | 1024                        | MS, RI      | Limonene                | 9.6               | 3.6               |                         |
| 1029                        | 1032                        | MS, RI      | Z-β-Ocimene             | 0.3               |                   |                         |
| 1039                        | 1044                        | MS, RI      | E-β-Ocimene             | 0.2               |                   |                         |
| 1081                        | 1083                        | MS, RI      | L-Fenchone              |                   | 0.2               |                         |
| 1082                        | 1082                        | MS, RI      | m-Cymenene              | 0.2               |                   |                         |
| 1085                        | 1090                        | MS, RI      | 6,7-Epoxymyrcene        | 0.2               |                   |                         |
| 1093                        | 1095                        | MS, RI      | Linalool                | 1.1               |                   |                         |
| 1114                        | 1118                        | MS, RI      | Z-p-menth-2-en-1-ol     |                   | 1.4               |                         |
| 1116                        | 1119                        | MS, RI      | **E-p-Mentha-2,8-dienol** | 19.3             |                   |                         |
| 1129                        | 1133                        | MS, RI      | Z-p-Mentha-2,8-dienol   | 10.2              |                   |                         |
| 1132                        | 1136                        | MS, RI      | **E-p-menth-2-en-1-ol** |                   | 0.7               |                         |
| 1138                        | 1144                        | MS, RI      | Neo-Isopulegol          | 0.1               |                   |                         |
| 1145                        | 1148                        | MS, RI      | Citronellal             | 0.1               | 0.7               |                         |
| 1148                        |                             | MS          | 4-Isopropenylcyclohexane|                   | 0.4               |                         |
| 1160                        | 1166                        | MS, RI      | p-menta-1,5-dien-8-ol   |                   | 0.1               |                         |
| 1168                        | 1173                        | MS, RI      | Rose furan epoxide      | 0.2               |                   |                         |
| 1180                        | 1179                        | MS, RI      | p-Cymen-8-ol            |                   | 0.1               |                         |
| 1183                        | 1186                        | MS, RI      | α-Terpineol             | 1.5               |                   |                         |
| 1183                        | 1187                        | MS, RI      | **E-p-Mentha-1(7),8-dien-2-ol** | 19.6             |                   |                         |
| 1188                        | 1195                        | MS, RI      | Z-Piperitol             |                   | 0.3               |                         |
| 1194                        |                             | MS          | *p-Menth-6-en-2,3-diol* |                   | 3.2               |                         |
| 1201                        | 1209                        | MS, RI      | E-Piperitol             |                   | 0.2               |                         |
| 1212                        | 1215                        | MS, RI      | E-Carveol               | 6.0               |                   |                         |
| 1216                        | 1224                        | MS, RI      | 2,3-Epoxyneral          | 0.1               |                   |                         |
| 1224                        | 1226                        | MS, RI      | **Z-Carveol**           |                   | 17.0              |                         |
| 1225                        | 1227                        | MS, RI      | Z-p-Mentha-1(7),8-dien-2-ol | 2.1             |                   |                         |
| 1237                        | 1235                        | MS, RI      | Neral                   |                   | 33.1              |                         |
| 1237                        | 1239                        | MS, RI      | Carvone                 |                   | 3.2               |                         |
| 1253                        | 1249                        | MS, RI      | **Piperitone**          |                   | 0.1               |                         |
| 1257                        | 1249                        | MS, RI      | Geraniol                | 1.0               |                   |                         |
| 1266                        | 1269                        | MS, RI      | Perilla aldehyde        | 0.8               |                   |                         |
| 1268                        | 1264                        | MS, RI      | **Geranial**            |                   | 44.3              |                         |
| 1286                        | 1293                        | MS, RI      | 2-Undecanone            | 0.1               |                   |                         |
| 1351                        | 1359                        | MS, RI      | Geranic acid            | 1.0               |                   |                         |
| 1376                        | 1379                        | MS, RI      | Geranyl acetate         | 0.8               |                   |                         |
E. tereticornis

E. tereticornis essential oil was extracted with a yield of 1.0%. This yield is lower than the one obtained from leaves of E. tereticornis (3.4%) isolated in Nigeria [58]. In our study, this oil was characterized by the presence of \(\beta\)-cymene (16.7%), cryptone (11.4%), spathulenol (13.5%), caryophyllene oxide (14.2%). Furthermore, compounds such as 4-terpineol (4.4%), phellandral (4.2%), cumin aldehyde (3.1%), \(\beta\)-phellandrene (2.9%), 1,8-cineole (2.2%) and humulene epoxide II (2.2%) were detected in significant amounts. \(\beta\)-Cymene, \(\beta\)-phellandrene, 1,8-cineole, 4-terpineol, cryptone and spathulenol were also identified in the oil of E. tereticornis analyzed in Benin, by Alitonou et al. [59] but in different amounts. Other differences were that it did not contain cumin aldehyde, humulene epoxide II and phellandral, whereas \(\alpha\)-phellandrene, bicyclogermacrene and \(\alpha\)-, \(\beta\)- or \(\gamma\)-isomers of eudesmol were detected. The presence of these main compounds was also noticed in the oil extracted in Argentina [60,61]. In contrast, the major constituents of the fresh leaf oil analyzed in India and Ethiopia were \(\alpha\)-pinene and 1,8-cineole [62,63], whereas the main compounds in the essential oil from Nigeria were \(\alpha\)- and \(\beta\)-pinene [58], the one from Cuba were 1,8-cineole and \(p\)-cymene [64], and the one from Algeria contained \(\alpha\)-pinene, 1,8-cineole, \(\beta\)-ocimene, alloanadendrene and 4-terpineol [65].

C. tinctorium

Essential oil yield of C. tinctorium was 0.2% (w/w) which is higher than the one obtained in Burkina Faso (0.10%) by Benoit-Vical et al. [66]. The essential oil extracted from C. tinctorium in the current study is dominated by 3-tetradecanone (48.3%), 3-hexadecanone (7.4%), 2-tridecanone (3.4%), cyclododecanone (7.8%), dodecyl acetate (2.0%), methyl tetradecanoate (2.3%) and 1-tetradecanol acetate (4.3%). This chemical composition is similar to the result obtained by Benoit-Vical et al. [66], in the tubercle essential oil which contained 3-tetradecanone (48.3%), 3-hexadecanone (7.4%), 2-tridecanone (3.4%), cyclododecanone (7.8%), dodecyl acetate (2.0%), methyl tetradecanoate (2.3%) and 1-tetradecanol acetate (4.3%).

C. planchonii

The essential oil of C. planchonii was extracted with a yield of 0.20% (w/w) lower than in the tubercle from Burkina Faso (0.30%) [66]. In the current study the composition of

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**Table 2 Chemical composition and oil yields of Cymbopogon species (Continued)***

| 1382 | 1389 | MS, RI | \(\beta\)-Elemene | 0.4 |
|---|---|---|---|---|
| 1408 | 1417 | MS, RI | \(E\)-Caryophyllene | 0.1 |
| 1421 | 1431 | MS, RI | \(\beta\)-Gurjunene | 0.1 |
| 1443 | 1452 | MS, RI | \(\alpha\)-Humulene | 0.1 |
| 1486 | 1495 | MS, RI | 2-Tridecanone | 0.1 |
| 1490 | 1500 | MS, RI | \(\alpha\)-Murolene | 0.1 |
| 1503 | 1513 | MS, RI | \(\gamma\)-Cadinene | 0.1 |
| 1513 | 1522 | MS, RI | \(\delta\)-Cadinene | 0.1 |
| 1540 | 1546 | MS, RI | Elemol | 5.3 |
| 1571 | 1582 | MS, RI | Caryophyllene oxide | 0.1 |
| 1595 | 1607 | MS, RI | 5-Epi-7-epi-\(\alpha\)-eudesmol | 0.3 |
| 1609 | 1622 | MS, RI | 10-Epi-\(\gamma\)-eudesmol | 0.2 |
| 1609 | 1615 | MS, RI | Selina-6-en-4-ol | 0.4 |
| 1636 | 1640 | MS, RI | Phenyl ethyl hexanoate | 0.1 |
| 1623 | 1630 | MS, RI | \(\gamma\)-Eudesmol | 1.1 |
| 1626 | 1629 | MS, RI | Eremoligenol | 1.9 |
| 1634 | 1640 | MS, RI | Hinesol | 0.7 |
| 1643 | 1649 | MS, RI | \(\beta\)-Eudesmol | 1.2 |
| 1646 | 1652 | MS, RI | \(\alpha\)-Eudesmol | 2.1 |
| Yields | 1.7 | 1.4 | 2.6 |
| Total identified | 95.2 | 93.4 | 98.4 |

*\(K_{\text{exp}}\) = retention indices are determined using n-alkanes (C\(_7\)-C\(_{17}\)).

\(K_{\text{lit}}\) = retention indices of reference compounds from literature.

ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2007); RI = comparison of calculated RI with those reported in the literature.

Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.
| KI_{exp} | KI_{lit} | ID | Compounds | E. citriodora | E. tereticornis |
|--------|--------|----|-----------|-------------|----------------|
| 918    | 926    | MS, RI | α-Thujene | 1.7          |                |
| 925    | 926    | MS, RI | α-Pinene | 0.1          | 0.7            |
| 964    | 969    | MS, RI | Sabinene | 0.5          |                |
| 968    | 974    | MS, RI | β-Pinene | 0.2          | 0.1            |
| 982    | 986    | MS, RI | Myrcene  | 0.1          | 0.3            |
| 998    | 1002   | MS, RI | α-Phellandrene | 0.5   |                |
| 1009   | 1014   | MS, RI | α-Terpineone | 0.1   | 0.5            |
| 1017   | 1020   | MS, RI | p-Cymene  | 0.1          | 16.7           |
| 1020   | 1024   | MS, RI | Limonene  | 0.2          |                |
| 1021   | 1025   | MS, RI | β-Phellandrene | 2.9   |                |
| 1023   | 1026   | MS, RI | 1,8-Cineole (Eucalyptol) | 1.5 | 2.2            |
| 1045   | 1044   | MS, RI | 2,6-dimethyl-5-Heptenal | 0.2 |                |
| 1050   | 1054   | MS, RI | γ-Terpineone | 0.1| 0.7            |
| 1080   | 1086   | MS, RI | Terpinolene | 0.1         |                |
| 1081   | 1089   | MS, RI | p-Cymene | 0.3          |                |
| 1093   | 1095   | MS, RI | Linalool  | 0.4          | 0.1            |
| 1104   | 1106   | MS, RI | Z-Rose oxide | 0.1 |                |
| 1109   | 1112   | MS, RI | E-Thujone | 0.2          |                |
| 1114   | 1118   | MS, RI | Z-p-Menth-2-en-1-ol | 0.5 |                |
| 1132   | 1136   | MS, RI | E-p-Menth-2-en-1-ol | 0.4 |                |
| 1139   | 1144   | MS, RI | Neo-Isopulegol | 7.8 |                |
| 1150   | 1148   | MS, RI | Citronellal | 52.8 | 0.1            |
| 1150   | 1154   | MS, RI | Sabina ketone | 0.1 |                |
| 1152   | 1155   | MS, RI | Iso-Isopulegol | 3.0 |                |
| 1166   | 1167   | MS, RI | Umbellulone | 0.1          |                |
| 1170   | 1174   | MS, RI | Terpinen-4-ol | 4.4 |                |
| 1181   | 1183   | MS, RI | Cryptone  | 11.4         |                |
| 1184   | 1186   | MS, RI | α-Terpineol | 0.3 | 0.5            |
| 1200   | 1192   | MS, RI | α-Phellandrene Epoxide | 0.2 |                |
| 1223   | 1224   | MS, RI | m-Cumenol | 0.5          |                |
| 1224   | 1223   | MS, RI | Citronellol | 20.0 |                |
| 1232   | 1238   | MS, RI | Cumin aldehyde | 3.1 |                |
| 1236   | 1239   | MS, RI | Carvone | 0.1          |                |
| 1239   | 1244   | MS, RI | Carvotanacetone | 0.1 |                |
| 1246   | 1249   | MS, RI | Piperitone | 0.2          |                |
| 1267   | 1273   | MS, RI | p-Menth-1-en-7-al | 4.2 |                |
| 1268   | 1274   | MS, RI | Neo-isopulegyl acetate | 0.3 |                |
| 1276   | 1283   | MS, RI | α-Terpinen-7-al | 0.3 |                |
| 1296   | 1298   | MS, RI | Carvacrol | 1.4          |                |
| 1301   | 1308   | MS, RI | p-Cymen-7-ol | 0.3 |                |
| 1326   | 1330   | MS, RI | 3-Oxo-p-menth-1-en-7-al | 0.2 |                |
| 1346   | 1350   | MS, RI | Citronellyl acetate | 9.0  |                |
| 1382   | 1389   | MS, RI | β-Elemene | 0.1          |                |
this essential oil was dominated by 3-tetradecanone (24.7%), ethyl tetradecanoate (11.4%) and isoamyl dodecanoate (14.1%), accompanied by 2-tridecanone (6.8%), dodecyl acetate (4.9%), 2-pentadecanone (5.7%), n-tetradecanol (3.0%), 3-hexadecanone (2.0%) and 4-octadecanone (2.2%). In the sample from Burkina Faso, 3-tetradecanone (69.8%) was the main compound, followed by tetradecyl acetate (14.4%), dodecyl acetate (4.7%), 3-hexadecanone (2.5%) and n-tetradecanol (1.0%) [66]. The sample characterized by Ouattara et al. [67] was similar to the previous one with major components involving 3-tetradecanone, 3-tetradecenone, dodecyl acetate, tetradecyl acetate, 2-tridecanone and β-elemene.

S. longepedunculata

The essential oil of S. longepedunculata was extracted with a yield of 0.7% (w/w). This yield is higher than the result obtained (0.30%-0.52%) by Alitonou et al. [68] in Benin and Adebayo et al. in Nigeria [69]. The S. longepedunculata essential oil was characterized by only one major constituent, namely methyl salicylate (99.4%). Indeed Nebie et al. [70] has shown that the essential oil of S. longepedunculata from Burkina Faso contains only one compound which is methyl salicylate. The same compound was also found in the methanol extract of S. longepedunculata from Ghana [71] and in essential oil extracted in Nigeria and Benin [68,69].

C. ambrosioides

C. ambrosioides essential oil was extracted with a yield of 1.3% (w/w). Its essential oil contained mainly ascaridole (41.9%). Some other components involving α-terpinene (16.5%), p-cymene (14.4%) and isoascaridole (7.5%) were identified as well. This chemical composition is similar to the one of a sample from China [39] but very different from a sample analyzed in India whose major components were m-cymene (43.9%) and myrtenol (13.3%) [72].

Adult bioassay on susceptible strains of Anopheles gambiae

The resistant status of mosquito samples was determined according to the WHO criteria summarized as follows [42]:

- 98-100% mortality indicates susceptibility of the mosquito strain to the tested essential oil
- Mortality less than 98% is suggestive of the existence of a resistance to the essential oil that needs to be confirmed by two additional tests
- Mortality less than 90% suggests resistance in the mosquito population

KDT50 and KDT95

KDT50 and KDT95 calculated with 95% confidence limits are summarized in Table 7. The lowest KDT50 and KDT95 values were obtained with C. citratus and are
Table 4 Chemical composition and oil yields of Cochlospermum species

| KI<sub>exp</sub><sup>a</sup> | KI<sub>lit</sub><sup>b</sup> | ID<sup>c</sup> | Compounds<sup>d</sup> | C. planchonii | C. tinctorium |
|-----------------|-----------------|---------|-----------------|--------------|--------------|
| 1025            | 1020            | MS, RI  | p-Cymene        | 0.3          |              |
| 1100            | 1099            | MS, RI  | Undecane        | 0.1          |              |
| 1154            | 1148            | MS, RI  | Citronellal     | 0.2          |              |
| 1183            | 1178            | MS, RI  | Naphthalene     | 0.1          |              |
| 1258            | 1249            | MS, RI  | Piperitone      | 0.1          |              |
| 1309            | 1305            | MS, RI  | Undecanal       | 0.2          |              |
| 1382            | 1389            | MS, RI  | β-Elemene       | 1.0          | 0.6          |
| 1388            | 1398            | MS, RI  | Cyperene        | 0.1          | 0.1          |
| 1400            | 1408            | MS, RI  | Dodecanal       | 0.1          | 0.1          |
| 1405            | 1411            | MS, RI  | Z-α-Bergamotene | 0.1          |              |
| 1408            | 1417            | MS, RI  | E-Caryophyllene | 0.1          |              |
| 1425            | 1432            | MS, RI  | E-α-Bergamotene | 0.6          |              |
| 1437            | 1445            | MS, RI  | Epi-β-Santalene | 0.1          |              |
| 1492            | 1469            | MS, RI  | 1-Dodecanol     | 0.5          |              |
| 1493            | 1506            | MS, RI  | Z-α-Bisabolene  | 0.3          |              |
| 1499            | 1505            | MS, RI  | β-Bisabolene    | 2.2          |              |
| 1500            | 1499            | MS, RI  | 2-Tridecanone   | 6.8          | 3.4          |
| 1505            | 1514            | MS, RI  | Z-γ-Bisabolene  | 0.1          |              |
| 1512            | 1509            | MS, RI  | Tridecanal      | 0.3          |              |
| 1513            | 1511            | MS, RI  | 6-Amorphene     | 0.1          |              |
| 1515            | 1524            | MS, RI  | Methyl dodecanoate | 0.5      |              |
| 1522            | 1529            | MS, RI  | E-γ-Bisabolene  | 0.3          |              |
| 1578            | 1576            | MS, RI  | Dodecanoic acid | 1.2          |              |
| 1576            | 1574            | MS, RI  | Cyclododecanone | 7.8          | 48.3         |
| 1585            | 1582            | MS, RI  | Caryophyllene oxide | 0.3      | 0.1          |
| 1598            |                 | MS      | 3-Tetradecanone | 24.7         | 48.3         |
| 1599            | 1607            | MS, RI  | Dodecyl acetate | 4.9          | 2.0          |
| 1602            | 1611            | MS, RI  | Tetradecanal    | 0.3          |              |
| 1663            | 1658            | MS, RI  | Neo-Intermedeol | 0.1          |              |
| 1680            | 1685            | MS, RI  | α-Bisabolol     | 0.1          |              |
| 1695            | 1671            | MS, RI  | n-Tetradecanol  | 3.0          |              |
| 1702            | 1697            | MS, RI  | 2-Pentadecanone | 5.7          | 0.7          |
| 1717            | 1722            | MS, RI  | Methyl tetradecanoate | 2.3      |              |
| 1775            | 1780            | MS, RI  | Tetradecanoic acid | 0.5        |              |
| 1786            |                 | MS      | 3-Hexadecanone  | 2.0          | 7.4          |
| 1795            | 1795            | MS, RI  | Ethyl tetradecanoate | 11.4     |              |
| 1798            |                 | MS      | 1-Tetradecanoyl acetate | 4.3      |              |
| 1818            | 1822            | MS, RI  | Hexadecanal     | 0.6          |              |
| 1846            | 1844            | MS, RI  | Isoamyl dodecanoate | 14.1      |              |
| 1874            |                 | MS      | Pentadecanol    | 2.0          |              |
respectively 2.1 min and 13.9 min at 0.5% and 1.2 min, 6.6 min at 1% whereas for permethrin (0.75%) these values were 11.3 min and 21.6 min. The highest KDT_{50} and KDT_{95} values were recorded with *C. planchonii* at 8% and were 11.3 min and 20.6 min, respectively.

**Mortality rates**
Mortality rates to different essential oils are shown in Table 7. At 0.25%, the mortality rate of *A. gambiae* “Kisumu” varies from 0.0% to 72.5%. However, the mortality rates increased with the dosage. At 0.50%, mortality has reached 100% for *C. citratus*, whereas it was at 5.6% for *S. longepedunculata*. At 1%, mortality was still 100% for *C. citratus* whereas it was 6.7% for *C. schoenanthus*. At 2%, the mortality rate was 29.6% for *S. longepedunculata* and 100% for *C. citratus, E. citriodora, E. tereticornis* and *C. ambrosioides*. At 4% and 8%, the mortality rates varied from 79.2% to 100% for *C. tinctorium* and *C. planchonii*. To summarize these results, the susceptibility tests on the sensitive strain *A. gambiae* “kisumu” have demonstrated its susceptibility status on essential oils tested. The most efficient essential oil was *C. citratus* at 0.50%, followed by *E. tereticornis* at 1%, *E. citriodora* and *C. ambrosioides* at 2%, *C. schoenanthus*, *C. giganteus*, *C. planchonii* and *S. longepedunculata* at 4%.

**Diagnostic concentrations**
The diagnostic concentration is defined as twice the lethal concentration (LC) for 99% mortality (LC_{99}) on sensitive strains [42]. Lethal concentration for 50% mortality (LC_{50}), lethal concentration for 99% mortality (LC_{99}) expressed with 95% confidence limits and diagnostic concentrations for all essential oils tested are summarized in Table 8.

The lowest diagnostic concentration of 0.77% for *C. citratus* was not significantly different from the diagnostic dose of permethrin (0.75%). Other interesting values were also obtained for *E. tereticornis* (2.80%), *E. citriodora* (3.37%), and *C. ambrosioides* (4.26%). These plant species were followed by *C. schoenanthus* and *C. giganteus* whose diagnostic concentrations were 5.48% and 7.36%, respectively. The highest diagnostic doses were obtained with *S. longepedunculata* (9.84%), *C. tinctorium* (11.56%) and *C. planchonii* (15.22%).

All diagnostic doses obtained above were tested on the resistant strain of *A. gambiae* and results obtained were

### Table 4 Chemical composition and oil yields of Cochlospermum species (Continued)

| 2003     | MS           | 4-Octadecanone | 2.2 |
|----------|--------------|----------------|-----|
| Yields   | 0.2          | 0.2            |     |
| Total identified | 81.9 | 82.5          |     |

a: ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2007); RI = comparison of calculated RI with those reported in the literature.
b: Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.

c: KI_{lit} = retention indices of reference compounds from literature.

### Table 5 Chemical composition and oil yield of Securidaca longepedunculata

| Kl_{exp} | Kl_{lit} | ID | Compounds | %  |
|----------|----------|----|-----------|----|
| 1194     | 1190     | MS, RI | Methyl salicylate | 99.4 |
| 1439     | MS       | Methyl 4-methoxysalicylate | 0.5 |
| Yield    | 0.7      | Total identified | 99.9 |

a: KI_{exp} = retention indices are determined using n-alkanes (C7-C17).
b: KI_{lit} = retention indices of reference compounds from literature.
c: ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2007); RI = comparison of calculated RI with those reported in the literature.

d: Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.

### Table 6 Chemical composition and oil yield of Chenopodium ambrosioides

| Kl_{exp} | Kl_{lit} | ID | Compounds | %  |
|----------|----------|----|-----------|----|
| 1012     | 1014     | MS, RI | α-Terpinene | 16.5 |
| 1018     | 1020     | MS, RI | β-Cymene | 14.4 |
| 1021     | 1024     | MS, RI | Limonene | 0.4 |
| 1050     | 1054     | MS, RI | γ-Terpinene | 0.3 |
| 1114     | 1119     | MS, RI | E-p-Menth-2,8-dien-1-ol | 0.2 |
| 1145     | 1148     | MS, RI | Citronellal | 0.1 |
| 1175     | 1178     | MS, RI | Naphthalene | 0.1 |
| 1235     | 1234     | MS, RI | Ascaridole | 41.9 |
| 1247     | 1252     | MS, RI | E-Piperitone epoxide | 1.1 |
| 1288     | 1289     | MS, RI | Thymol | 0.4 |
| 1299     | 1299     | MS, RI | Isoascaridole | 7.5 |
| 1347     | 1349     | MS, RI | Thymol acetate | 0.2 |
| 1477     | 1477     | MS, RI | E-β-Ionone | 0.1 |
| 1581     | MS       | 3-Tetradecanone | 0.4 |
| Yield    | 1.3      | Total identified | 83.6 |

a: KI_{exp} = retention indices are determined using n-alkanes (C7-C17).
b: KI_{lit} = retention indices of reference compounds from literature.
c: ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2007); RI = comparison of calculated RI with those reported in the literature.

d: Compounds are listed in order of their retention time; the names and the percentages of main compounds are indicated in bold.
| Essential oils and controls | 0.25% | 0.50% | 1% |
|-----------------------------|-------|-------|----|
|                             | KDT50 (min) | KDT95 (min) | Mortality (%) | Susceptibility | KDT50 (min) | KDT95 (min) | Mortality (%) | Susceptibility | KDT50 (min) | KDT95 (min) | Mortality (%) | Susceptibility |
| Cymbopogon citratus         | 62.0   | 119.8 | 55.6 | R  | 2.1 | 13.9 | 100 | S | 12 | 6.6 | 100 | S |
| Cymbopogon giganteus        | -      | -     | 10.7 | R  | 204.4 | 366.5 | 10.0 | R | 33.5 | 100.2 | 29.6 | R |
| Cymbopogon schoenanthus     | -      | -     | 0.0  | R  | 263.5 | 440.7 | 6.4  | R | 490 | 1178 | 6.7  | R |
| Eucalyptus citriodora       | -      | -     | 4.3  | R  | 125.2 | 207.3 | 10.4 | R | 32.2 | 61.7 | 75.5 | R |
| Eucalyptus tereticornis     | 124    | 498   | 72.5 | R  | 5.4  | 18.2 | 86.7 | R | 2.5 | 16.1 | 98  | S |
| Cochlospermum tinctorium    | 443    | 1156  | 23.3 | R  | 10.6 | 19.4 | 44.0 | R | 8.1 | 16.7 | 72.7 | R |
| Cochlospermum plachonii     | 345    | 741   | 13.0 | R  | 25.1 | 52.8 | 236  | R | 20.9 | 39.9 | 24.5 | R |
| Securidaca longepedunculata | -      | -     | 5.9  | R  | 314.1 | 539.5 | 5.6  | R | 762 | 913  | 86  | R |
| Chenopodium ambrosioides    | 736.3  | 12900 | 9.8  | R  | 1278 | 2013 | 188  | R | 1109 | 1789 | 41.9 | R |
| Permethrin 0.75%            | 11.3   | 216   | 100  | S  | 11.3 | 216 | 100  | S | 11.3 | 216 | 100  | S |
| Negative control            | 0      | 0     | 0    | -  | 0   | 0   | 0    | - | 0   | 0   | 0    | - |

| Essential oils and controls | 2% | 4% | 8% |
|-----------------------------|----|----|----|
|                             | KDT50 (min) | KDT95 (min) | Mortality (%) | Susceptibility | KDT50 (min) | KDT95 (min) | Mortality (%) | Susceptibility | KDT50 (min) | KDT95 (min) | Mortality (%) | Susceptibility |
| Cymbopogon citratus         | -  | 3.8 | 100  | S  | -  | 3.8 | 100  | S | -  | -  | -  | -  |
| Cymbopogon giganteus        | 62 | 9.5 | 62.7 | R  | 26 | 3.8 | 100  | S | -  | -  | -  | -  |
| Cymbopogon schoenanthus     | 69 | 145 | 83.0 | R  | 26 | 3.8 | 100  | S | -  | -  | -  | -  |
| Eucalyptus citriodora       | 45 | 9.0 | 100  | S  | 26 | 3.8 | 100  | S | -  | -  | -  | -  |
| Eucalyptus tereticornis     | -  | 3.8 | 100  | S  | -  | 3.3 | 100  | S | -  | -  | -  | -  |
| Cochlospermum tinctorium    | 69 | 123 | 78.1 | R  | 5.2 | 9.1 | 79.2 | R | -  | -  | -  | -  |
| Cochlospermum plachonii     | 163| 313 | 58.5 | R  | 12.1| 21.1| 98.3 | S | 11.3| 206| 100| S |
| Securidaca longepedunculata | 10.9| 81.0| 296  | R  | 26 | 3.8 | 98.2 | S | 26 | 3.8 | 100 | S |
Table 7 Knock down times (KDT), mortality and susceptibility of essential oils on sensitive *Anopheles gambiae* “Kisumu” (Continued)

| Essential Oil | KDT50 (s) | KDT95 (s) | Mortality (%) | Susceptibility | KDT50 (s) | KDT95 (s) | Mortality (%) | Susceptibility |
|---------------|-----------|-----------|---------------|----------------|-----------|-----------|---------------|----------------|
| Chenopodium ambrosioides | 12.8 | 25.2 | 100 | S | 2.6 | 3.8 | 100 | S |
| Permethrin 0.75% | 11.3 | 21.6 | 100 | S | 11.3 | 21.6 | 100 | S |
| Negative control | 0 | 0 | 0 | - | 0 | 0 | 0 | - |

\*The KDT (KDT50 and KDT95) values were expressed with 95\% confidence limits.
\[S = \text{Susceptible defined as 98\%-100\% of mortality; } RS = \text{Resistance suspected defined as } 90\%-97\% \text{ of mortality; } R = \text{Resistance defined as <90\% of mortality}.\]
Concerning the diagnostic doses tested, the lowest KDT 50 and KDT 95 were observed with *C. schoenanthus* (2.58 min, 3.84 min), followed by *S. longepedunculata*, *C. giganteus*, *E. tereticornis*, *C. ambrosioides* and *E. citriodora* for which the KDT 50 and KDT 95 were lower than 5 min and 6 min, respectively. A moderate knock down effect (KDT50 ≥ 12 min and KDT95 ≥ 20 min) was observed with *C. planchonii*, *C. tinctorium* and *C. citratus*.

The KDT 50 observed was 6 to 35 fold lower than for permethrin, the positive control, and the KDT 95 was 5 to 38 fold lower than permethrin. This observation demonstrates the promising insecticidal properties of these plants species on the *A. gambiae* resistant strain used.

Apart from the essential oil from *C. tinctorium* and *S. longepedunculata* for which resistance was suspected because the mortality was less than 97%, the resistant strain of *A. gambiae* was susceptible to all essential oils at diagnostic doses tested (Table 9). The resistance to permethrin in southern Benin has been demonstrated and was explained by the massive use of DDT in house spraying and agriculture, during the WHO malaria eradication program, which has permitted the apparition and the increase of *kdr* mutation in *A. gambiae* populations in Benin [7,10].

We have noticed that the knock-down times, the mortalities, and the resistance status of *A. gambiae* did not only depend on the values of doses used but mainly on the chemical composition of essential oils used.

Larvicidal activity of *C. citratus* essential oil on *Aedes aegypti* at lower concentrations (LC 50 = 0.28 μl/ml and LC 99 = 0.56 μl/ml) and its major component citral (neral and geranial) has been demonstrated by Freitas et al. [73]. *C. citratus* has also demonstrated a very good repellency against *A. aegypti* [30]. *C. citratus* has shown in the current study the best insecticidal activity against *A. gambiae*, but its KDT 50 and KDT 90 were higher than

### Table 8 LC50, LC99 and diagnostic concentration for all essential oils tested

| Essential oils         | LC50*  | LC99*  | Diagnostic concentration | Diagnostic concentration | Diagnostic concentration |
|------------------------|--------|--------|--------------------------|--------------------------|--------------------------|
|                        | %      | %      | mg/ml                    | mg/cm²                   |
| *Cymbopogon citratus*  | 0.237  | 0.386  | 0.77                     | 7.7                      | 0.085                    |
| *Cymbopogon giganteus* | 1.600  | 3.682  | 7.36                     | 73.6                     | 0.82                     |
| *Cymbopogon schoenanthus* | 1.570 | 2.739  | 5.48                     | 54.8                     | 0.60                     |
| *Eucalyptus citriodora* | 0.900  | 1.685  | 3.37                     | 33.7                     | 0.37                     |
| *Eucalyptus tereticornis* | 0.148 | 1.401  | 2.80                     | 28.0                     | 0.31                     |
| *Cochlospermum tinctorium* | 1.16  | 5.781  | 11.56                    | 115.6                    | 1.28                     |
| *Cochlospermum planchonii* | 2.314 | 7.608  | 15.22                    | 152.2                    | 1.69                     |
| *Securidaca longepedunculata* | 2.489 | 4.919  | 9.84                     | 98.4                     | 1.09                     |
| *Chenopodium ambrosioides* | 0.997  | 2.131  | 4.26                     | 42.6                     | 0.47                     |

LC50: lethal concentration for 50% mortality; LC99: lethal concentration for 99% mortality.

*The lethal doses (LC50 and LC99) values were expressed with 95% confidence limits.

### Table 9 KDT50, KDT95 and mortality of essential oils tested on the resistant strain of *Anopheles gambiae*

| Essential oils         | Diagnostic doses (% | KDT50*  | KDT95*  | Mortality (%) | Susceptibility b |
|------------------------|---------------------|---------|---------|---------------|-----------------|
|                        |                     | (min)   | (min)   |               |                 |
| *Cymbopogon citratus*  | 0.77                | 15.77   | 26.00   | 100           | S               |
| *Cymbopogon giganteus* | 7.36                | 2.92    | 5.53    | 100           | S               |
| *Cymbopogon schoenanthus* | 5.48   | 2.58    | 3.84    | 100           | S               |
| *Eucalyptus citriodora* | 3.37                | 4.02    | 5.93    | 100           | S               |
| *Eucalyptus tereticornis* | 2.80   | 3.56    | 5.27    | 100           | S               |
| *Cochlospermum tinctorium* | 11.56  | 12.01   | 20.67   | 90.4          | RS              |
| *Cochlospermum planchonii* | 15.22  | 11.35   | 30.72   | 100           | S               |
| *Securidaca longepedunculata* | 9.84  | 2.87    | 4.47    | 94.8          | RS              |
| *Chenopodium ambrosioides* | 4.26   | 3.96    | 5.84    | 98.0          | S               |
| *Permethrin 0.75%*     | 0.75                | 90.87   | 145.37  | 62.3          | R               |

KDT50: 50% knock down in mosquito’s population; KDT95: 95% knock down in mosquito’s population.

*The KDT (KDT50 and KDT95) values were expressed with 95% confidence limits.

*S = Susceptible defined as 98-100% of mortality; RS = Resistance suspected defined as 90-97% of mortality; R = Resistance defined as <90% of mortality.*
some of the other essential oils. The same conclusion has been found by Phasomkusolsil et al. [33] when the essential oil was tested on A. aegypti, C. quinquefasciatus and Anopheles dirus. The topical application of C. citratus showed high toxicity against Sitophilus oryzae [50]. C. citratus essential oil and citral have been shown to be potential anti-Leishmania agents [51]. Insecticidal and larvicidal activities of C. citratus have been attributed to citral that has demonstrated 100% mortality against A. aegypti, at 2.5 μl/ml with LC50 = 0.02 μl/ml and LC90 = 0.28 μl/ml respectively [73], and its repellent effect at 15% (v/v) is comparable to 5% C. citratus essential oil [74]. In conclusion, the presence of geranial and neral is potentially responsible for the insecticidal activity of the essential oil of C. citratus, as demonstrated in the current work.

C. giganteus essential oil, rich in limonene and (Z and E)-p-mentha-1(7),8-dien-2-ol such as the current sample from Benin, has proven to be toxic by fumigation to Callosobruchus species [28]. The insecticidal properties noticed in this study against A. gambiae might also be attributed to these main compounds.

Several studies on essential oils, rich in piperitone such as the essential oil from C. schoenanthus have demonstrated insecticidal activity against some pests. This is the case for Cymbopogon olivieri, which demonstrated good larvicidal activity against A. stephensi with LD50 = 321.9 mg/l [75]. Exposure of Callosobruchus maculatus to C. schoenanthus essential oil for 24 hours resulted in 90% of adult mortality at 6.7 μl/l [56]. Piperitone has been reported to be powerful against ants of Crematogaster spp [76]. Adults, newly laid eggs and neonate larvae of C. maculatus with an LC50 recorded at 1.6 ± 0.14 μl/l and all eggs were aborted at 6.7 μl/l with a total inhibition of the neonate larvae penetration in the seed [38]. Also in 2008, piperitone isolated from Artemisia judaica L., was studied against the third larvae of Spodoptera littoralis (Boisd) and has revealed a high insecticidal and antifeedant activity against this pathogen, with a LD50 = 0.68 μg/larvae [77]. Following this previous research we could attribute the insecticidal activity of C. schoenanthus to its main compound, i.e. piperitone.

The essential oil from E. citriodora, rich in citronellal, citronellol and isopulegol, has been revealed to be repellent against Tribolium castaneum at 0.084 ml/l and was more active than the commercial product IR3535 at 0.686 ml/l [41]. The insecticidal activity of E. citriodora has been demonstrated at 5 mg/ml against Lutzomyia longipalpis [27]. E. citriodora has also demonstrated larvicidal activity against C. quinquefasciatus [36] and acaricidal activity against larvae of Amblyomma cajennense and Anocentor nitens [78]. The presence of citronellal, citronellol and isopulegol could well explain the insecticidal activity of E. citriodora against A. gambiae.

The essential oil from E. tereticornis leaf extract has shown a larvicidal activity against A. stephensi at 160 ppm which has provoked 100% of oviposition deterrence [35]. The sensitivity of adults of A. aegypti has been shown, resulting from the presence of 1,8-cineole, α-pinene and p-cymene and is correlated to the amount of 1,8-cineole in the extract [26,60]. The insecticidal activity of the essential oil of E. tereticornis observed in the current work, might be explained by the presence of one of its major components (p-cymene) but also by a minor compound (1,8-cineole), which both have demonstrated insecticidal activity.

In essential oils from Cochlospermum species one minor compound (2-tridecanone) has been found to have insect repellent properties. Indeed, 2-tridecanone has demonstrated repellent activity against the granary weevil Sitophilus granarius and S. zeamais at 100 ppm and 500 ppm on wheat [79]. Its repellent activity was confirmed against ticks since 0.63 mg/cm2 was repellent to 87% of Amblyomma americanum after 12 hours and to 72% of Dermacentor variabilis after 15 hours [80]. The weak insecticidal activity of the essential oils of these two Cochlospermum species could be due to the low abundancy of 2-tridecanone.

Root powder, the methanol extract, and the main volatile component of S. longipedunculata (methyl salicylate) have proven to exhibit repellent and toxic effects against S. zeamais. In the same study, methyl salicylate has demonstrated a dose dependent fumigant effect with an LD100 of 60 μl in a 1-l container after 24 hours exposure on S. zeamais, Rhyzopertha dominica and Prostephanus truncatus and after 6 days exposure, 100% mortality could be recorded with 30 μl in a 1-l container [31].

The C. ambrosioides essential oil has demonstrated a larvicidal activity against A. arabiensis and A. aegypti after 24 hours exposure with LC50 and LC90 equal to 17.5 ppm and 33.2 ppm for A. arabiensis and 9.1 ppm and 14.3 ppm for A. aegypti under laboratory conditions [81]. Contact and fumigant toxicity of isolated compounds from this plant species have shown that ascarirole (LC50 = 0.84 mg/l) followed by isoascaridole (LC50 = 2.45 mg/l) were the most efficient insecticidal compounds by fumigation and contact with LC50 = 0.86 mg/l (ascaridole) and 2.16 mg/l (isoascaridole). The crude oil was less active with LC50 = 3.08 mg/l by fumigation and 2.12 mg/l by contact [39]. The insecticidal activity of C. ambrosioides, noticed in the study, might be explained by the presence of ascarirole and isoascaridole, which were among its major constituents.

**Conclusions**

The current study has dealt with the insecticidal properties of essential oils of nine plant species traditionally used in Benin for their repellency against A. gambiae bites. This
research has shown that the essential oils from all the plant species studied, have insecticidal properties against this vector of malaria. The most promising was C. citratus followed in order of effectiveness by E. tereticornis, E. citriodora, C. ambrosioides, C. schoenanthus, C. giganteus and C. planchonii. The chemical composition of each plant essential oil has been elucidated by GC-MS and correlated with the insecticidal properties of these plant species. To our knowledge, it was the first time that diagnostic doses of essential oils on A. gambiae were determined, using the WHO susceptibility test protocol. These doses were presented in %, mg/ml and mg/cm² to facilitate further research on these plant species. KDT₅₀ and KDT₉₅, LC₅₀ and LC₉₉ and results obtained have proven that all essential oils from these plant species are more effective against the resistant strain of A. gambiae than permethrin at the diagnostic doses tested. C. citratus, E. tereticornis, E. citriodora and C. ambrosioides as well as essential oil isolated components, such as citral, piperitone, 1,8-cineole, citronellal, 2-tridecanone, methyl salicylate, which possess demonstrated insecticidal properties, may be included in malaria vector control programs. These plants, occurring in the natural environment of local populations, could be obtained at lower cost and represent today a valuable source of bioactive compounds for the protection of the population against malaria.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AD8, FA, DCKS, SM, NDK and MCA have defined the study. HPY has conducted the identification and the harvesting of the plant species. AD8 and PMB have performed the experiments and interpretation of data. SM, NDK, DCKS, FA, PMB, MCA and AD8 drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

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