Insecticidal activity of the essential oil and polar extracts from *Ocimum gratissimum* grown in Ivory Coast: efficacy on insect pests and vectors and impact on non-target species

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Abstract

Ocimum gratissimum L. is an aromatic herb cultivated in Western Africa for culinary and medical pest control purposes. The current research evaluated the insecticidal activity of white wild basil essential oil, ethanolic and water extracts against pests and insect vectors, i.e., the tobacco cutworm *Spodoptera littoralis*, the housefly *Musca domestica,* and the filariasis vector *Culex quinquefasciatus*. Furthermore, the toxicity of the essential oil and polar extracts against the non-target earthworm *Eisenia fetida* was assessed. The chemical profiles of the essential oil and polar extracts were obtained by GC-MS and HPLC-DAD analyses. Acute toxicity experiments were conducted on larvae of *C. quinquefasciatus* and *S. littoralis* and adults of *M. domestica* and *E. fetida*, to determine the LC_{50} and LC_{90} values of the oil and polar extracts. Chronic toxicity was evaluated on *S. littoralis* feeding on tomato discs treated with essential oil and polar extracts. The essential oil was dominated by thymol (50.0%) and *p*-cymene (16.8%), whereas ethanolic and aqueous extracts were characterized by carvacrol (13%) and thymol (11%), and shikimic acid (3%) and rosmarinic acid (2%), respectively. The essential oil was significantly more active on target insects than extracts, showing LC_{50}/LD_{50} of 39.6 µl.L^{-1} on *C. quinquefasciatus*, 72.2 µg.$adult^{-1}$ on *M. domestica* and 30.2 µg $larva^{-1}$ on *S. littoralis*. Furthermore, the essential oil and ethanolic extract at sublethal doses (10 and 70 µg cm^{-2}, respectively) affected the survival of *S. littoralis* larvae from the third day on. White wild basil oil $LD_{50,90}$ at day 5 were 2.8 and 12.3 µg cm^{-2}. Finally, the essential oil and polar extracts were not toxic to *E. fetida* over the positive control α-cypermethrin. Overall, our study showed that the essential oil of white wild basil is a potential candidate as a functional ingredient in insecticidal formulations to manage agricultural moth pests and insect vectors of public importance.

48 **Keywords**: essential oil; polar extracts; *Culex quinquefasciatus; Spodoptera littoralis*;
49 botanical insecticides; non-target organisms.
50

1. Introduction

Ocimum gratissimum L. (Lamiaceae), also known as white wild basil, clove basil, or African basil, is an aromatic herb of the genus *Ocimum* belonging to the Lamiaceae family. It is a pantropical species, native to southern Africa and Madagascar, and now widespread in all inter-tropical regions, because of its ecological adaptability (Lebrun and Stork, 1997). This species is also known under the vernacular names of 'awlomagnin' by the Akans people in Ivory Coast and 'baumier' in the Democratic Republic of Congo (Aké-Assi, 2011). *Ocimum gratissimum* is a bushy, branched shrub, with leaves 6- to 12-cm long, 3-cm wide, ovate, cuneate at the base, slightly pubescent under the veins. This plant is commonly used in the folk medicine of tropical countries as antinociceptive (Rabelo et al., 2003), antidiabetic (Abo et al., 2008), spasmolytic (Montalvo and Domínguez, 1997), expectorant (Akinmoladun et al., 2007), diuretic (Duarte et al., 2005), antibacterial (Nakamura et al., 1999), relaxant (Madeira et al., 2002), antidiarrheal (Offiah and Chikwendu, 1999) and anti-asthmatic (Costa et al., 2012) agent. Moreover, it is also used against snake bites (Owuor et al., 2005), to repel mosquitoes (Githinji and Kokwaro, 1994) and for insect control (Vieira and Simon, 2000). Mixed with *Capsicum annuum* L., *Otostegia integrifolia* Benth., *Prunus persica* (L.) Batsch and *Schinus molle* L., *O. gratissimum* has been used to treat malaria (Giday et al., 2007). Alone it is used against worms and to treat several disturbs related to other parasitic diseases (Kpoviessi et al., 2014).

Ocimum gratissimum is also cultivated for the exploitation of its essential oil (EO) which is obtained mainly from the leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been detected as the major components in the various EO chemotypes reported so far (Vieira et al., 2000). On the other hand, only a few studies have been

76 conducted on the composition of polar extracts where rosmarinic acid and flavonoids have
77 been evidenced as the main characteristic compounds (Grayer et al., 2000).
78 The *O*. *gratissimum* EO is active against several bacterial species such as
79 *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*,
80 whereas the leaf aqueous extract has been reported as effective against *P*. *aeruginosa*
81 (Talabi and Makanjuola, 2017). This EO is also active against fungi, including
82 *Trichophyton rubrum* (Castell.) Sabour., *T*. *mentagrophytes* (C.P. Robin) Sabour. (Silva et
83 al., 2005), *Cryptococcus neoformans* (San Felice) Vuill. (Lemos, et al., 2005), and
84 *Candida albicans* (C.P. Robin) Berkhout (Duarte et al., 2005).
85 It is worth noting that *O*. *gratissimum* EO is also accredited with insecticidal
86 effects. In Nigeria this plant, known under the name 'nchu anwu' (i.e. meaning "repellent
87 against mosquitoes"), is cultivated in house gardens to keep mosquitoes away (Oparaocha
88 et al., 2010). The EO showed larvicidal and repellent effects on mosquitoes such as *Aedes*
89 *aegypti* L. and *Aedes albopictus* (Skuse) (Cavalcanti et al., 2004; Oparaocha et al., 2010).
90 *Ocimum gratissimum* EO showed toxicity against third instar larvae of *Spodoptera*
91 *frugiperda* (J.E. Smith) and the tick *Rhipicephalus microplus* (Canestrini) (Lima et al.,
92 2018), and protected stored products from *Sitophilus zeamais* (Motschulsky) infestation
93 (Nguemtchouin et al., 2013).
94 The eco-friendly management of insect pests and vectors is crucial in current
95 Integrated Pest/Vector Management programs (Athanassiou et al., 2018; Pavela et al.,
96 2019a). In particular, the use of EOs and other plant-borne products has been recently
97 considered as extremely promising (Isman, 2015; Pavela and Benelli, 2016), both against
98 insect of economic importance, as well as against other arthropod pests (Stevenson et al.,
99 2017; Benelli and Pavela, 2018a,b). In this framework, given the traditional uses of *O*.
100 *gratissimum* in the preparation of insecticides, herein we investigated the acute and chronic

toxicity of its EO, ethanolic and aqueous extracts, against three target insects of high economic importance: the filariasis vector *Culex quinquefasciatus* Say, recently investigated also for its potential to vector Zika virus (Benelli and Romano, 2017; van den Hurk et al., 2017), the housefly *Musca domestica* L. and the Egyptian cotton worm *Spodoptera littoralis* (Boisduval).

2. Materials and Methods

2.1 Plant material

Leaves mixed with young branches and flowers were purchased in a local market of Abidjan, Ivory Coast, in August 2017. A voucher specimen was identified at the National Floristic Center of the University of Félix Houphouët-Boigny, Ivory Coast, and given the codex Adjanohoun and Aké-Assi n°225, Akoupé, Fofie no. 05. The plant name was checked against the Plant List database (http://www.theplantlist.org). Before distillation and solvent extractions, the plant material was cleaned and dried in the shade at room temperature for one week, then reduced into a powder using an electric mill.

2.2 Chemicals and reagents

The analytical standards of gallic acid, (+)-catechin hydrate, (-)-epicatechin, 3-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, shikimic acid, rutin, quercitrin, hyperoside, rosmarinic acid, carnosol, carnosic acid, cinnamic acid, naringin, eugenol, thymol and carvacrol were purchased from Sigma-Aldrich (Milan, Italy). The stock standard solutions were prepared by dissolving 10 mg of the analyte in 10 mL of methanol and stored in a glass-stoppered bottle at 4°C in the dark. Standard working solutions, at various concentrations, were daily prepared by appropriate dilution of aliquots

126 of the stock solutions in water. HPLC-grade ethanol and acetonitrile were purchased from
127 Sigma-Aldrich (Milan, Italy), while HPLC-grade formic acid 99-100% was bought from
128 J.T. Baker B.V. (Deventer, Holland). For sample preparation and chromatographic
129 analysis, deionized water ≥ 18 MΩ/cm resistivity purified with a Milli-Q system
130 (Millipore, Bedford, USA) was used. The analytical standards used for GC-MS peak
131 assignment were purchased from Sigma-Aldrich. All solvents and solutions were filtered
132 through a 0.45-µm PTFE filter from Supelco (Bellefonte, PA, USA) before use.
133
134 *2.3 Preparation of plant extracts*
135 *Ocimum gratissimum* powder (50 g) was extracted under magnetic stirrer with 500
136 mL of ethanol 96% for 3 h and with boiling water for 30 min. Afterwards, the extracts
137 were dried with a rotavapor, freeze-dried (yield 1.1 and 7.3%, w w^{-1} dry weight, for
138 ethanolic and water extract respectively) and stored at 4°C until use. For HPLC analysis,
139 the samples were prepared by re-dissolving 20 mg of the extract with 2 mL of methanol.
140 The sample solutions were filtered through a 0.45 µm pore size nylon membrane filter
141 (Phenex, Phenomenex, Torrance, CA, USA) before injection into HPLC-DAD. Each
142 sample was analysed in triplicate.
143
144 *2.4. Isolation of essential oil*
145 The powder (690 g) was inserted into a 10 L flask filled with 6 L of deionized
146 water and subjected to hydrodistillation for 3 h. At the end, the organic layer was separated
147 from the aqueous one and collected into amber vials sealed with PTFE-silicon septa.
148 Residual water drops were removed using anhydrous Na_2SO_4. The oil yield was estimated
149 (1.5%) on a dry weight basis (w w^{-1}).
150

151 *2.5 HPLC analysis of polar constituents*

152 HPLC-DAD studies were performed using a Hewlett-Packard HP-1090 Series II
153 (Palo Alto, CA, USA), equipped with a vacuum degasser, a binary pump, an autosampler
154 and a model 1046A HP photodiode array detector (DAD) following a previous developed
155 method with some modifications (Caprioli et al., 2016; Zorzetto et al., 2015). The
156 chromatographic separation was accomplished on a Synergi Polar-RP C18 (4.6 mm x 250
157 mm, 4 µm) analytical column from Phenomenex (Chesire, UK). The column was preceded
158 by a security cartridge. The mobile phase for HPLC-DAD (diode array detector) analyses
159 was a mixture of (A) water with 0.1% formic acid (v v^{-1}) and (B) acetonitrile with 0.1%
160 formic acid, flowing at 0.8 mL min^{-1} in gradient conditions: 0 min, 20% B; 0-15 min, 60%
161 B; 15-20 min, 60% B; 20-25 min, 20% B, 25-30 min, 20% B. The column temperature was
162 set at 30°C and the injection volume was 5 µL. UV spectra were recorded in a wavelength
163 range of 230-350 nm for the 18 compounds, where 230 nm was used for quantification of
164 shikimic acid, 256 nm for rutin, hyperoside and quercitrin, 272 nm for gallic acid, 280 nm
165 for (+)-catechin hydrate, (-)-epicatechin, carnosic acid, carnosol, cinnamic acid, naringin,
166 eugenol, thymol and carvacrol, and 325 nm for 3-O-caffeoylquinic acid, 5-O-
167 caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid and rosmarinic acid.
168
169 *2.6. GC-MS analysis*

170 The chemical composition of *O. gratissimum* EO was studied using an Agilent
171 6890N equipped with a 5973N single quadrupole mass spectrometer. Volatile components
172 were separated on an apolar column HP-5MS (J & W Scientific, Folsom, CA) equipped
173 with a 5% phenylmethylpolysiloxane coating, 30 m length, 0.25 mm internal diameter and
174 0.1 mm film thickness. The oven temperature was programmed at 60°C for 5 min, then
175 increased to 220°C at 4°C min^{-1}, and finally to 280°C at 11°C min^{-1} held for 15 min.

176 Carrier gas, flow rate, split ratio, temperatures of injector and detector, mass scan range,
177 and ionization voltage were the same of those reported in previous works (Benelli et al.,
178 2018a,b,c; Venditti et al., 2018; Kamte et al., 2018). The oil was injected after dilution
179 (1100) in *n*-hexane. Data were elaborated by the NIST Mass Spectral Search Program for
180 the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library v. 2.3 and the MSD
181 ChemStation software (Agilent, Version G1701DA D.01.00). Identification of EO
182 components was made by comparison with available analytical standards and by
183 combination of temperature-programmed retention index (RI) (Van den Dool and Kratz,
184 1963), and mass spectrum (MS) of peaks, with those stored in NIST 17 (2017), FFNSC2
185 (2012), ADAMS (2007) and home-made libraries. Relative percentages of EO constituents
186 were obtained by peak area normalization without using response factors.
187
188 *2.7. Insect rearing*
189 *Culex quinquefasciatus* larvae and *M. domestica* adults were reared with the
190 method of Pavela et al. (2018). *Spodoptera littoralis* early 3rd instar larvae were obtained as
191 described by Sut et al. (2017). All insect species were maintained at 25±1°C, 70±3% R.H.
192 and 16:8 h (L:D). These laboratory conditions were also used for the toxicity tests detailed
193 below.
194
195 *2.8. Insecticidal activity on Culex quinquefasciatus larvae*
196 Acute toxicity assays against mosquito larvae were done diluting the *O.*
197 *gratissimum* EO or extracts in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Czech
198 Republic) and then treating *C. quinquefasciatus* 3rd instar larvae as detailed by Benelli et
199 al. (2017a,b). Herein, the *O. gratissimum* EO or extracts were tested at the following
200 concentrations 5.0, 10.0, 20.0, 30.0, 40.0, 50.0; 60.0; 80.0 and 100.0 mg L^{-1}, to estimate the

lethal concentration (LC) values (4 groups each composed by 25 larvae per each tested concentration of each product). Negative control was distilled water plus the same amount of DMSO used to test *O*. *gratissimum* EO and extracts. α-cypermethrin (Vaztak®) was the positive control (concentrations: 0.001, 0.002, 0.003, 0.005, 0.007, 0.009, 0.015, 0.025 and 0.050 µg L^{-1}). Mortality was noted after 24 h (Benelli et al., 2018a).

2.9. Insecticidal activity on Musca domestica adults

Topical application assays were conducted testing *O*. *gratissimum* EO or extracts on *M*. *domestica* adult females (3–6 days old). According to Benelli et al. (2018b), 1 µL of acetone (Sigma-Aldrich, Germany) carrying *O*. *gratissimum* EO or extracts at doses of 50, 60, 70, 80, 90, 100, 110, 120, 150, 180 and 200 µg $adult^{-1}$ (4 groups, each composed by 80 houseflies, were tested for each dose), was applied through a microelectric applicator on the pronotum of CO_2-anesthetized fly adults. Acetone was the negative control. α-Cypermethrin (Vaztak®) at 0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8 and 1.0 µg $adult^{-1}$ was the positive control. After the treatment, *M*. *domestica* females were moved to a recovery box (10×10×12 cm) for 24 h, then mortality was recorded.

2.10. Insecticidal activity on Spodoptera littoralis larvae

The insecticidal activity of *O*. *gratissimum* EO or extracts on 3rd instar larvae of *S*. *littoralis* (weight 20-25 mg) was evaluated through topical application of the samples diluted in acetone (Sigma-Aldrich, Germany) using the method of Sut et al. (2017). Moth larvae were treated on the dorsum with 1 µL of acetone containing the *O*. *gratissimum* EO or extracts (doses of 10, 20, 30, 40, 50, 100, 150, 200, 250 and 300 µg $larva^{-1}$, 4 groups, each composed by 20 larvae, were tested per each concentration). Pure acetone was the negative control. α-Cypermethrin (Vaztak®) tested at 0.02, 0.03, 0.04, 0.05, 0.06, 0.07,

226 0.08, 0.09 and 0.1 µg larva^{-1} was the positive control. Post-treatment, *S. littoralis* larvae
227 were moved to a recovery box (10×10×7 cm, see Pavela et al., 2017a) for 24 h, then
228 mortality was checked.
229
230 *2.11. Chronic toxicity on Spodoptera littoralis larvae*
231 Chronic toxicity of the *O. gratissimum* EO and extracts was evaluated relying to the
232 method of Pavela et al. (2017b) with minor modifications. EO and extracts were dissolved
233 in methanol using a concentration series, and the solutions were uniformly applied (10 µl
234 cm^{-2}) using an electronic micropipette to leaf discs (2 cm^{-2}) prepared from tomato leaves.
235 In this way, we obtained food contaminated with EO or extracts in concentrations ranging
236 from 2.5 to 100 µg cm^{-2} (detailed in Table 4). After the evaporation of solvent, the
237 contaminated food was orally administered *ad libitum* to *S. littoralis* 2nd instar larvae
238 placed in Petri dishes (5 cm in diameter) having an agar bottom layer (3-4 mm thick) to
239 maintain a stable moisture level. Food was replaced daily. Mortality was assessed over 5
240 days. For compounds leading to >50% mortality, LD causing 50% and 90% mortality were
241 determined (Pavela et al., 2017b). For each tested concentration of the EO or extract, we
242 tested 4 groups, each composed by 15 larvae. Post-treatment, each group was moved into
243 plastic boxes (15x15x7 cm) in a growth chamber (25±1°C, 16:8 L:D) for 5 days, and larval
244 mortality was checked daily.
245
246 *2.12. Toxicity on Eisenia foetida*
247 Following OECD (1984) protocol, we tested the toxicity of *O. gratissimum*
248 extracts, EO, and α-cypermethrin on *E. fetida* adults. The latter was reared using artificial
249 soil as detailed by Pavela (2018). *Ocimum gratissimum* EO or extracts were added to the
250 soil at 200 mg kg^{-1}. α-Cypermethrin (Vaztak®) at 10 and 20 mg kg^{-1} of dry soil was the

positive control. Distilled water was the negative control. In the experiments, the selected EO, extracts, only water, or α-cypermethrin diluted in water were mixed into the soil and 10 earthworm adults were added; the experiment was performed in four replicates. The samples (650 g) were stored in glass pots (1 L) covered with gauze and kept in laboratory at 20±1°C, R.H. 80–85%, 16:8 (L:D) and 600 lux (Pavela, 2018). Mortality of *E. fetida* was determined after 5 and 10 days of exposure.

2.13. Statistical analysis

When mortality in the control ranged from 1 to 20%, we corrected experimental mortality with Abbott's formula (Abbott, 1925); if control mortality was >20%, experiments were repeated. $LD_{50(90)}$ or $LC_{50(90)}$ for the targeted organisms, with associated 95% CL and chi squares, were estimated using probit analysis (Finney, 1971). Moreover, in chronic toxicity assays where *S. littoralis* larvae ingested insecticide-treated tomato leaf discs and toxicity on *E. fetida*, data were transformed by arcsine√ then analysed using ANOVA followed by Tukey's HSD test ($P<0.05$).

3. Results and Discussion

3.1. Quantification of polar constituents in Ocimum gratissimum ethanolic and aqueous extracts

In the present work, a simultaneous analysis of eighteen compounds, namely shikimic acid, gallic acid, (+)-catechin hydrate, (-)-epicatechin, 3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, rutin, hyperoside, quercitrin, rosmarinic acid, carnosol, carnosic acid, eugenol, cinnamic acid, naringin, carvacrol and

thymol, in the ethanolic and aqueous extracts of *O*. *gratissimum* was achieved by HPLC-DAD. Quantitative data (mg kg^{-1}) of the analysed compounds are reported in Table 1. The two white wild basil extracts proved to be rich in the monitored polar constituents, displaying a total content of 262961.2 and 64635.1 mg kg^{-1}in the ethanolic and aqueous extract, respectively. The major constituents in the ethanolic extract were carvacrol (129862.2 mg kg^{-1}), thymol (105063.8 mg kg^{-1}) and rosmarinic acid (13390 mg kg^{-1}). On the other hand, the main constituents of the aqueous extract were rosmarinic acid (18732.9 mg kg^{-1}), shikimic acid (28731.2 mg kg^{-1}) and carvacrol (2544.2 mg kg^{-1}) (Fig. 1). Other secondary metabolites occurring at noteworthy levels in the ethanolic extract were shikimic acid (9056.1 mg kg^{-1}), eugenol (2586 mg kg^{-1}) and 3-caffeoylquinic acid (651.2 mg kg^{-1}); instead other abundant compounds in the aqueous extract were 3,5-di-O-caffeoylquinic acid (7097.6 mg kg^{-1}) and gallic acid (2873.1 mg kg^{-1}).

The level of carvacrol (129862.2 mg kg^{-1}) and thymol (105063.8 mg kg^{-1}) found in the ethanolic extract are higher if compared with those reported in other species of the Lamiaceae family, such as *Thymus lanceolatus* Desf., in which they were detected with concentration of 569.7 and 420.4 mg kg^{-1}, respectively (Caprioli et al., 2018). Also, the level of rosmarinic acid found in the aqueous extract (18732.9 mg kg^{-1}) was slightly higher with respect to those reported by Caprioli et al. (2018) in *T*. *lanceolatus* (15440.9 mg kg^{-1}) and much higher than those earlier reported in *Ocimum basilicum* L. (level ranging from 80 to 4790 mg kg^{-1}) by Kwee and Niemeyer (2011). On the other side, the amount of rosmarinic acid found in *O*. *gratissimum* is just two times lower than those detected in *Rosmarinus euriocalix* Jord. & Fourr. (Bendif et al., 2017) extracts (range 27811-43359 mg kg^{-1}) and comparable with those found by Del Baño et al. (2003) in *Rosmarinus officinalis* L.

300 The level of shikimic acid found in the aqueous extract (28731.2 mg kg^{-1}) was
301 higher if compared with those reported by Caprioli et al. (2018) in *T*. *lanceolatus* (1026.7
302 mg kg^{-1}), and by Bendif et al. (2017) in *R*. *euriocalyx* extracts (53-1853 mg kg^{-1}). Worthy
303 of mention is also chlorogenic acid, a hydroxycinnamic acid derivative widespread in
304 plants. Its levels were comparable with those reported in *T*. *lanceolatus* (1011.3 mg kg^{-1})
305 by Caprioli et al. (2018) and *R*. *eriocalyx* (range 62-10351 mg kg^{-1}) by Bendif et al. (2017).
306
307 *3.2. Ocimum gratissimum essential oil composition*
308 The hydrodistillation of the dry aerial parts of *O*. *gratissimum* gave 1.5% of a dark
309 yellow oil, whose composition is reported in Table 2. A total of 42 components were
310 identified in the oil by GC-MS accounting for 99.9% of the whole composition. The white
311 wild basil EO was dominated by the fraction of monoterpenes (87.4%), with oxygen-
312 containing compounds (55.0%) more abundant than pure hydrocarbons (32.4%). In this
313 fraction, the major compound was thymol (50.0%), followed by its biogenetic precursors
314 *p*-cymene (16.8%) and γ-terpinene (5.1%) (Fig. 2). Other noteworthy monoterpenes found
315 in the oil include α-thujene (2.7%) and myrcene (2.0%). Sesquiterpene hydrocarbons
316 represented a minor group (12.0%), with β-selinene (5.1%) and (*E*)-caryophyllene (3.1%)
317 as the most representative compounds.
318 Based on this profile, the EO from Ivorian *O*. *gratissimum* belonged to the thymol-
319 chemotype which has already been reported for other accessions from Brazil, Cameroon
320 and Republic of Guinea (Vieira et al., 2000; Lima et al., 2018; Nguemtchouin et al., 2013;
321 Kéita et al., 2000).
322
323 *3.3. Insecticidal activity and of toxicity on earthworms*

In acute toxicity assays, the white wild basil EO was significantly more active than plant extracts on target insects, showing LC_{50}/LD_{50} of 39.6 $\mu L\ L^{-1}$ on *C. quinquefasciatus*, 72.2 $\mu g\ adult^{-1}$ on *M. domestica* and 30.2 $\mu g\ larva^{-1}$ on *S. littoralis* (Table 3). However, the *O. gratissimum* EO provided a significantly lower efficacy compared with α-cypermethrin. Despite that, this EO can be considered as promising for further development of contact botanical insecticides since its LC_{90} is below 100 ppm (Pavela, 2015; Pavela et al., 2019a). Good efficacy of EOs in terms of acute toxicity is generally known and is justified by the mechanism of action of their individual major compounds. In our case, thymol and *p*-cymene may be the principal components exerting good insecticidal efficacy compared with other monoterpenes (Pavela and Benelli, 2016; Burt, 2004). Thymol is capable of altering the membrane permeability by interacting with the polar portion of lipid bilayer. This causes loss of membrane potential, with leakage of ions and enzymes from the cell (Sikkema et al., 1995). Other reports accredited thymol to impair enzymes involved in the synthesis of ATP (Di Pasqua et al., 2010). Thus, thymol can cross easily the insect cuticle, entering the insect body, where it may promote cell lysis (Bennis et al., 2004).

Thymol was already found to exhibit larvicidal activity, for instance against *Anopheles stephensi* Liston (LC_{50} = 48.9 ppm), *Ae. aegypti* (LC_{50} = 17.5 ppm), *Culex pipiens* L. (LC_{50} = 36 ppm) and *C. quinquefasciatus* (LC_{50} = 15.1 ppm) (Pandey et al. 2009; Tabanca et al. 2013; Traboulsi et al. 2002; Pavela et al., 2008). From a mechanistic point of view, thymol may interact with GABA-gated chloride channels and octopamine receptors giving neurotoxic effects (Priestly et al., 2003; Enan, 2005). Also, anti-AChE activity has been assigned to thymol for the inhibitory effects on larvae of *Anisakis simplex* (López et al., 2018). Thymol is endowed with powerful acaricidal activity on the ticks *R. microplus* and *Ixodes ricinus* (L.) (Lima et al., 2018; Tabari et al., 2017)

348 It is thus logical that the *O. gratissimum* extracts did not led to acute toxicity
349 against the tested insect pests and vectors, given that they contained predominantly
350 polyphenols which, instead of exerting neurotoxic effects on insects, cause various types of
351 food intake inhibition or growth of the larvae. This effect is manifested by chronic
352 mortality (Nasr et al., 2010; Pavela et al., 2017b, Gabaston et al., 2018) as confirmed also
353 in our tests on *S. littoralis* larvae. As found in our assays, both the EO and the extracts did
354 cause chronic mortality of *S. littoralis* larvae (Table 4). However, significant differences
355 were found, as follows from the estimated lethal doses. The *O. gratissimum* EO provided
356 the highest efficacy – causing larval mortality that increased as a function of time and
357 concentration. LD_{50} for the EO ranged between 5.4 and 2.8 $\mu g\ cm^{-2}$, for days 1 and 5 from
358 larval exposure, respectively (Table 4). The ethanolic extract exhibited LD_{50} ranging from
359 105.1 to 18.2 $\mu g\ cm^{-2}$ for days 1 and 5 from larval exposure, respectively. The mortality
360 rate caused by the aqueous extract was the lowest, and although it could be seen from the
361 second day, the LD_{50} (99.62 $\mu g\ cm^{-2}$) could be estimated only on day 5 from the beginning
362 of the experiment.
363 Besides target organisms, we also tested the effect of the *O. gratissimum* EO and
364 extracts on invertebrate species representatives of non-target organisms, namely *E. fetida*
365 earthworm adults. Earthworms are generally known to be able to consume a wide range of
366 contaminated organic materials, including industrial waste and sewage sludge (Lim et al.,
367 2016). However, they are very sensitive to soil contamination with insecticides (Wang et
368 al. 2012; Datta et al. 2016; Vasantha-Srinivasan et al. 2018). Generally, insecticides have a
369 negative effect on the survival of earthworms, especially in concentrations over 25 $mg\ kg^{-1}$
370 (Rodriguez-Campos et al. 2014; Datta et al. 2016). Notably, neither the EOs nor the polar
371 extracts were toxic to *E. fetida* in our tests (Table 5). On the contrary, the positive control
372 α-cypermethrin caused fatal earthworm mortality even in significantly lower doses, as

expected. The high tolerance of earthworms about soil contamination with EOs has also been confirmed by other studies (Vasantha-Srinivasan et al., 2016, 2018; Benelli, 2018; Pavela, 2018). Both the EO and extracts of *O*. *gratissimum* can be thus considered as friendly to the environment, although clearly, additional tests will have to be done on other non-target species, with special reference to non-target invertebrates. Moreover, regarding the effects of major compounds from *O*. *gratissimum* EO on non-target organisms, it should be noted that thymol can be considered as relatively safe having no impact on the physiology of mealworm beetles, honey bees, shellfish and mosquito fish *Gambusia affinis* Baird & Girard (Mattila et al., 2000; George et al., 2009; Lahlou, 2002; Tabari et al., 2017).

4. Conclusions and perspectives for future research

Despite the traditional use of white wild basil, *O*. *gratissimum*, in Western Africa both for culinary and medical purposes, little is still known about the toxicity of botanical preparations exploiting this plant species against three major insect species of high economic importance, such as the tobacco cutworm *S. littoralis*, the housefly *M. domestica,* and the filariasis and arbovirus vector *C. quinquefasciatus*. Results from the present study strongly support the traditional uses of white wild basil in Africa to control pests and vectors. Notably, the *O*. *gratissimum* EO may represent a potential candidate ingredient in insecticidal formulations, in order to combat agricultural pests and insect vectors of public importance, with limited toxicity on non-target invertebrates, such as earthworms. In this framework, further studies focusing on the nano- and microemulsion of these botanicals (Pavela et al., 2019b,c), followed by their bioactivity evaluation on

selected pests and non-target organisms – including biocontrol agents – in the field are ongoing.

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Conflict of Interest

The Authors declare no competing interests.

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684

Table

Table 1. Concentrations of eighteen selected compounds in the ethanolic and water extracts of *Ocimum gratissimum* flowering aerial parts.

No.	Compound	Ret. Time (min)	Concentration (mg kg^{-1} dw)[a] Ethanolic extract	Concentration (mg kg^{-1} dw)[a] Water extract
1	Shikimic acid	3.4	9056.1±980.7	28731.2±448.6
2	Gallic acid	4.9	n.d.[b]	2097.6±62.7
3	5-O-Caffeoylquinic acid	5.3	n.d.	n.d.
4	3-O-Caffeoylquinic acid	6.7	651.2±57.3	657.9±5.3
5	(+)-Catechin hydrate	7.5	253.8±43.1	1345.2±107.7
6	(−)-Epicatechin	7.9	254.1±42.7	1495.1±104
7	Rutin	8.2	582.7±101.3	453±23.7
8	Hyperoside	9.2	88.1±12.9	657.3±11.0
9	Naringin	9.9	121.4±22.1	139.6±6.1
10	Quercitrin	10.3	108±19.9	183.1±7.8
11	3,5-di-O-Caffeoylquinic acid	10.8	451.8±75.4	7097.6±60.5
12	Rosmarinic acid	11.8	13390±75.4	18732.9±581.2
13	Cinnamic acid	15.2	491.5±79.4	20.6±2.1
14	Eugenol	18.1	2586±419	230.9±6.2
15	Carvacrol	19.5	129862.2±15964.1	2544.2±93.9
16	Thymol	20.2	105063.8±15288.5	248.9±22.2
17	Carnosic acid	23.02	n.d.	n.d.
18	Carnosol	23.5	n.d.	n.d.

Total content	262961.2±34995.3	64635.1±1543

[a] Values are means of three replicates with relative standard deviations in the range 1.6-18.4 %. [b] n.d. means not detected

Table 2. Chemical composition of the essential oil from flowering aerial parts of *Ocimum gratissimum*.

No.	Compound	RI calc.[b]	RI lit.[c]	%[d]	ID[e]
1	α-thujene	920	924	2.7±0.5	A,B
2	α-pinene	926	932	0.9±0.2	A,B,C
3	camphene	939	946	0.1±0.0	A,B,C
4	sabinene	965	969	0.3±0.1	A,B,C
5	β-pinene	968	974	0.3±0.0	A,B,C
6	3-octanone	986	979	0.1±0.0	A,B
7	myrcene	988	988	2.0±0.4	A,B,C
8	3-octanol	999	988	Tr[f]	A,B
9	δ-3-carene	1007	1008	0.1±0.0	A,B,C
10	α-terpinene	1014	1014	1.5±0.3	A,B,C
11	*p*-cymene	1022	1020	16.8±2.9	A,B,C
12	limonene	1025	1024	0.8±0.2	A,B,C
13	(*Z*)-β-ocimene	1037	1032	0.1±0.0	A,B,C
14	γ-terpinene	1055	1054	5.1±0.9	A,B,C
15	*cis*-sabinene hydrate	1063	1065	0.4±0.1	A,B
16	*p*-cymenene	1086	1089	1.5±0.3	A,B
17	*trans*-sabinene hydrate	1095	1098	0.2±0.0	A,B
18	linalool	1100	1095	0.1±0.0	A,B,C
19	1,3,8-*p*-menthatriene	1108	1110	Tr	A,B
20	*trans*-thujone	1113	1112	0.1±0.0	A,B
21	*cis*-*p*-menth-2-en-1-ol	1119	1118	0.1±0.0	A,B
22	borneol	1160	1169	0.2±0.0	A,B,C
23	terpinen-4-ol	1166	1174	0.1±0.0	A,B,C
24	*p*-cymen-8-ol	1172	1179	1.9±0.4	A,B
25	*α*-terpineol	1187	1186	0.2±0.0	A,B,C
26	thymol methyl ether	1234	1232	0.5±0.1	A,B
27	thymol	1297	1289	50.0±4.8	A,B,C
28	carvacrol	1303	1298	1.1±0.2	A,B,C

29	α-cubebene	1344	1345	0.1±0.0	A,B
30	α-copaene	1368	1374	0.5±0.1	A,B
31	β-cubebene	1383	1387	Tr	A,B
32	β-elemene	1386	1389	0.2±0.1	A,B,C
33	(*E*)-caryophyllene	1409	1417	3.1±0.5	A,B,C
34	*trans*-α-bergamotene	1431	1432	0.2±0.0	A,B
35	α-humulene	1443	1452	0.5±0.1	A,B,C
36	γ-muurolene	1470	1478	Tr	A,B
37	germacrene D	1472	1484	0.3±0.1	A,B
38	β-selinene	1476	1489	5.1±0.8	A,B
39	α-selinene	1486	1498	1.6±0.4	A,B
40	α-bulnesene	1498	1509	0.1±0.0	A,B
41	δ-cadinene	1517	1522	0.3±0.1	A,B
42	caryophyllene oxide	1571	1582	0.4±0.1	A,B,C
	Total identified (%)			99.9±0.1	
	Grouped compounds (%)				
	Monoterpene hydrocarbons			32.4	
	Oxygenated monoterpenes			55.0	
	Sesquiterpene hydrocarbons			12.0	
	Oxygenated sesquiterpenes			0.4	
	Others			0.1	

[a] The order of components is according to the elution from a HP-5MS (30 m x 0.25 mm i.d. x 0.1 μm f.t.) capillary column. [b] Linear retention index calculated using the Van den Dool and Kratz (1963) formula. [c] Linear retention index value taken from Adams (2007). [d] Relative percentage values are mean of three determinations ± SD. [e] Identification method: A, comparision of the calculated RI with that of Adams (2007); B, MS matching with ADAMS, FFNSC2 and NIST 17 libraries; C, comparison with analytical standard (Sigma-Aldrich). [f] Tr, traces, % < 0.1.

Table 3. Acute toxicity of *Ocimum gratissimum* essential oil, ethanolic and aqueous extracts against selected insects of medical and agricultural importance, *Culex quinquefasciatus, Musca domestica* and *Spodoptera littoralis*.

Treatment	***C. quinquefasciatus* larvae**					***M. domestica* adults**					***S. littoralis* larvae**				
	LC_{50} (mg L^{-1})	**95% LCL-UCL**	**LC_{90} (mg L^{-1})**	**95% LCL-UCL**	**χ^2**	**LD_{50} (µg $adult^{-1}$)**	**95% LCL-UCL**	**LD_{90} (µg $adult^{-1}$)**	**95% LCL-UCL**	**χ^2**	**LD_{50} (µg $larva^{-1}$)**	**95% LCL-UCL**	**LD_{90} (µg $larva^{-1}$)**	**95% LCL-UCL**	**χ^2**
O. gratissimum essential oil	39.6	28.1-40.6	54.9	49.7-62.8	4.71 ns	72.2	56.5-78.9	120.9	118.6-136.8	0.23 ns	30.2	26.9-33.7	46.2	40.3-58.4	1.98 ns
O. gratissimum aqueous extract	Not effective: no mortality at 100 ppm					Not effective: no mortality at 300 µg $adult^{-1}$					Not effective: no mortality at 300 µg $adult^{-1}$				
O. gratissimum ethanolic extract	Not effective: no mortality at 100 ppm					Not effective: no mortality at 300 µg $adult^{-1}$					Not effective: no mortality at 300 µg $adult^{-1}$				
Positive control: α-cypermethrin	0.005	0.003-0.008	0.012	0.011-0.015	3.253 ns	0.18	0.15-0.19	0.75	0.62-0.83	4.231 ns	0.03	0.02-0.05	0.08	0.07-0.11	3.525 ns

$LC_{50(90)}$, lethal concentration killing 50% or 90% of the treated population;
$LD_{50(90)}$, lethal dose killing 50 or 90 of the treated population;
LCL = 95% lower confidence interval;
UCL = upper confidence interval;
ns = not significant (α=0.05).

Table 4. Chronic toxicity of *Ocimum gratissimum* essential oil, ethanolic and aqueous extracts against *Spodoptera littoralis* 3rd instar larvae in ingestion assays on botanical-treated tomato leaf disks over five days.

Treatment	Dose (µg cm^{-2})	*S. littoralis* larval mortality (%±SD)				
		Day 1	Day 2	Day 3	Day 4	Day 5
O. gratissimum essential oil	100	100.0±0.0i	100.0±0.0j	100.0±0.0j	100.0±0.0h	100.0±0.0h
	70	100.0±0.0i	100.0±0.0j	100.0±0.0j	100.0±0.0h	100.0±0.0h
	50	100.0±0.0i	100.0±0.0j	100.0±0.0j	100.0±0.0h	100.0±0.0h
	35	95.5±4.5h	100.0±0.0j	100.0±0.0j	100.0±0.0h	100.0±0.0h
	20	86.7±4.7g	86.7±4.7i	89.8±5.9i	92.3±4.7g	96.7±8.2fg
	15	76.7±4.7f	76.7±4.7g	86.7±8.2hi	93.3±5.9g	93.3±5.9g
	10	56.7±1.8e	66.5±8.2fg	76.7±6.8gh	81.8±7.8f	81.8±7.8f
	5	46.6±4.9e	46.7±2.5f	63.3±9.4g	70.1±5.2f	70.1±5.2e
	2.5	33.3±2.6d	33.3±2.6e	43.3±5.5f	46.7±2.5e	46.7±2.5d
Lethal dose (µg cm^{-2})	LD_{50} (CI_{95})	5.4 (4.2-7.8)	4.6 (3.9-5.8)	3.3 (2.6-4.1)	2.6 (2.2-3.3)	2.8 (2.1-3.5)
	LD_{90} (CI_{95})	25.1 (19.9-32.8)	18.3 (17.2-26.9)	16.7 (13.4-19.8)	15.6 (10.7-19.2)	12.3 (9.7-17.3)
	χ^2	2.245 ns	3.526 ns	5.095 ns	0.407 ns	3.111 ns
O. gratissimum ethanolic extract	100	46.5±3.9e	66.3±3.8fg	82.7±5.8h	92.8±4.7g	92.8±4.7g
	70	36.7±5.2d	50.3±4.7f	62.8±5.5g	73.3±5.7f	82.9±4.9f
	50	23.3±4.7c	33.3±9.4e	46.7±5.9f	53.3±5.8e	56.7±6.8d
	35	16.7±.2.2bc	26.7±8.2d	36.7±4.7e	46.7±9.2de	46.7±9.2d
	20	10.3±2.1b	16.5±3.1c	18.5±7.2c	18.5±7.2c	18.5±7.2c
	15	3.3±0.5a	10.3±4.7b	10.3±4.7b	10.3±4.7b	12.5±5.5bc
	10	0.0±0.0a	10.0±0.0b	10.0±0.0b	10.0±0.0b	10.0±0.0b
	5	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	2.5	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
Lethal dose (µg cm^{-2})	LD_{50} (CI_{95})	105.1 (89.4-153.3)	65.9 (57.9-77.4)	48.5 (42.7-53.8)	40.1 (36.8-43.3)	18.2 (15.6-22.9)

	LD_{90} (CI_{95})	445.4 (328.5-987.2)	262.8 (187.5-297.3)	132.6 (128.7-143.3)	104.2 (98.7-127.8)	84.5 (75.8-92.3)
	χ^2	0.191 ns	2.345 ns	1.894 ns	5.246 ns	3.513 ns
	100	3.3±0.5a	13.3±5.5bc	28.3±4.7d	35.8±7.6d	48.9±5.2d
	70	0.0±0.0a	0.0±0.0a	0.0±0.0a	12.3±5.3bc	28.7±6.5c
	50	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.0±0.0b
	35	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
O. gratissimum aqueous extract	20	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	15	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	10	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	5	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	2.5	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	LD_{50} (CI_{95})	N.D.	N.D.	N.D.	N.D.	99.6 (89.6-118.7)
Lethal dose (µg cm^{-2})	LD_{90} (CI_{95})	N.D.	N.D.	N.D.	N.D.	205.5 (158.4-238.1)
	χ^2	N.D.	N.D.	N.D.	N.D.	0.484 ns
Negative control	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
ANOVA *F, P*	-	561.15, <0.001	728.23, <0.001	691.11, <0.001	758.33, <0.001	561.15, <0.001

Within each column, different letters indicate significant differences (ANOVA, Tukey's HSD test, $P<0.05$; % = arcsine$\sqrt{}$ transformed data).
N.D. = not determined.
ns = not significant ($\alpha=0.05$).

Table 5. Toxicity of *Ocimum gratissimum* essential oil, ethanolic and aqueous extracts on the earthworm *Eisenia fetida* in botanical contaminated-artificial soil assays.

Treatment	*E. fetida* adult mortality (%)	
	5th day	10th day
O. gratissimum essential oil 200 mg kg^{-1}	0.0±0.0[a]	0.0±0.0[a]
O. gratissimum aqueous extract 200 mg kg^{-1}	0.0±0.0[a]	0.0±0.0[a]
O. gratissimum ethanolic extract 200 mg kg^{-1}	0.0±0.0[a]	0.0±0.0[a]
Negative control	0.0±0.0[a]	0.0±0.0[a]
Positive control: *a*-cypermethrin 20.0 mg kg^{-1}	95.5±5.5[c]	100.0±0.0[c]
Positive control: *a*-cypermethrin 10.0 mg kg^{-1}	77.3±5.2[b]	85.7±3.8[b]
ANOVA *F, P*	613.15, <0.0001	572.22, <0.0001

Different letters indicate significant differences (ANOVA, Tukey's HSD test, $P<0.05$; % = arcsine√ transformed data).

Figure

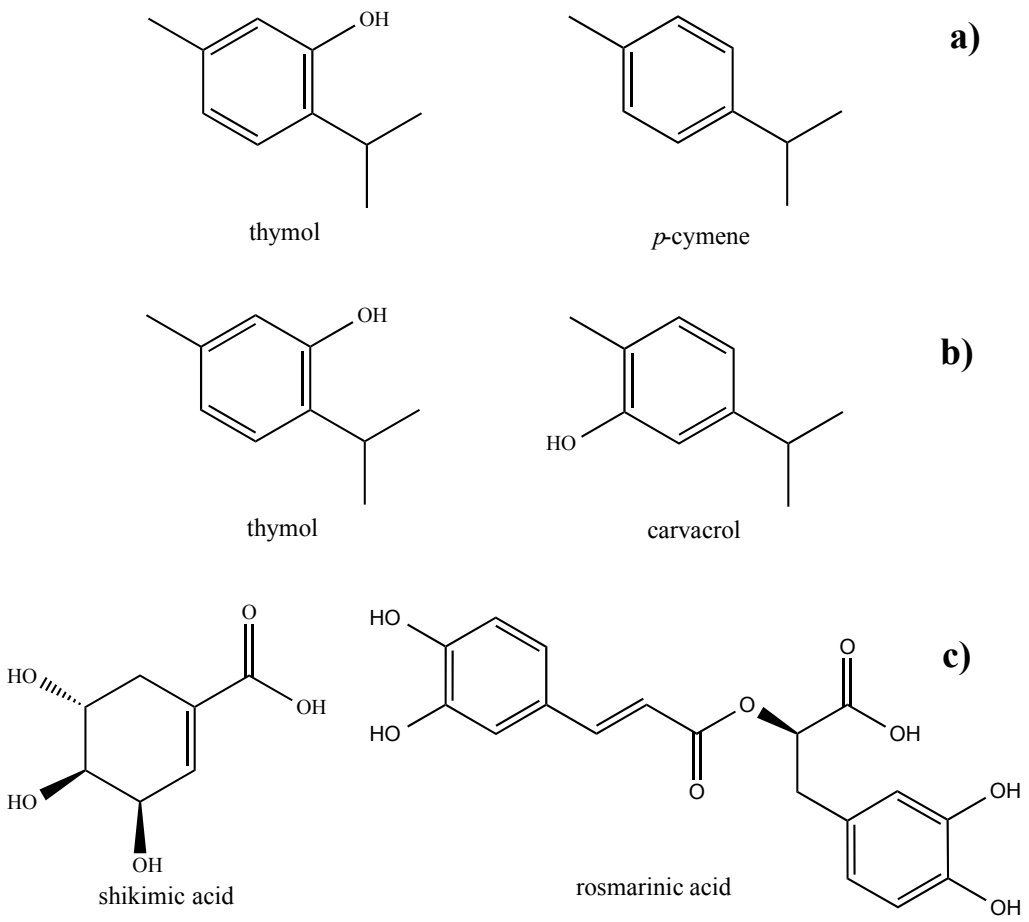

Fig. 1. Chemical structures of the major compounds in the essential oil **(a)**, ethanolic extract **(b)**, and aqueous extract **(c)** of white wild basil (*Ocimum gratissimum*) growing in Ivory Coast.

Figure

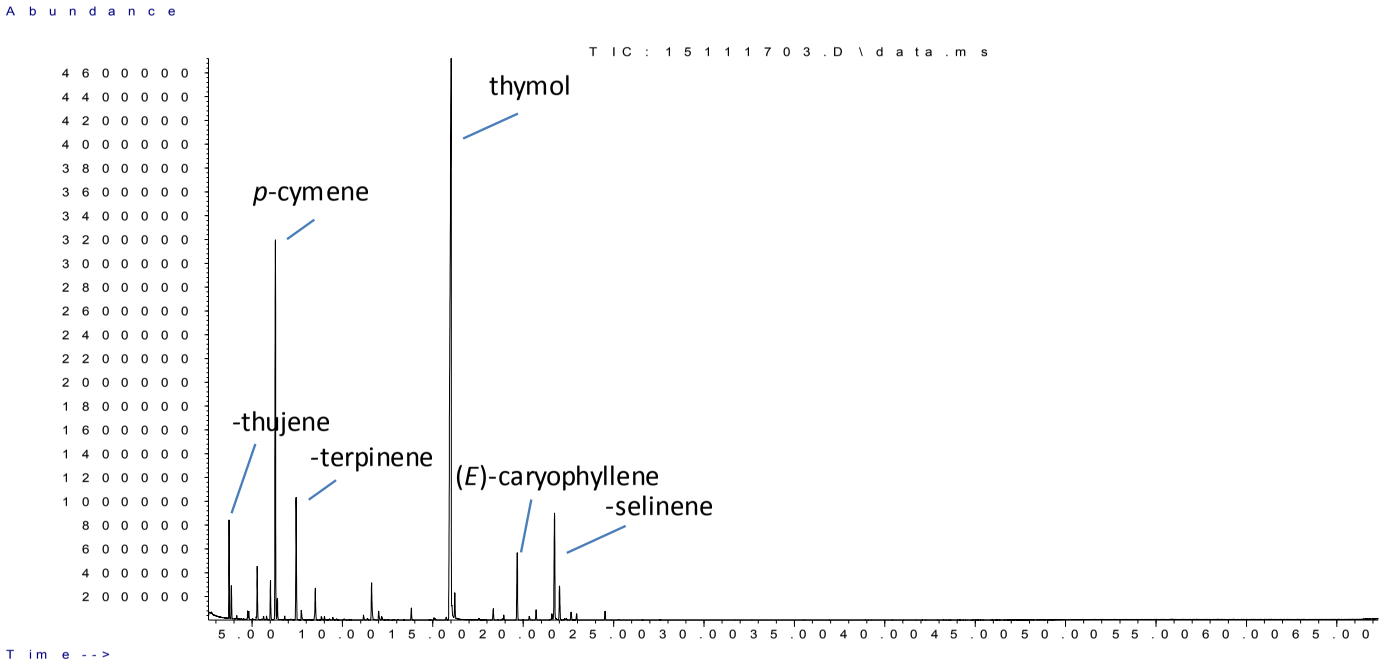

Fig. 2. GC-MS chromatogram of the essential oil from *Ocimum gratissimum* growing in Ivory Coast.