

**ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES:
CHEMICAL CONSTITUENTS OF ESSENTIAL OILS OF *OCIMUM GRATISSIMUM*,
EUCALYPTUS CITRIODORA AND *CYMBOPOGON GIGANTEUS* INHIBITED
LIPOXYGENASE L-1 AND CYCLOOXYGENASE OF PGHS**

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ABSTRACT. The following studies report the inhibitory effect produced by chemical constituents of essential oils of three plants used in traditional medicine as anti-inflammatory and analgesic drugs, *in vitro*, on soybean lipoxygenase L-1 and cyclooxygenase function of prostaglandin H synthase (PGHS), the two enzymes involved in the production of mediators of inflammation. The essential oils were extracted from plants of three families: *O. gratissimum* (Labiatae), *C. giganteus* (Poaceae), and *E. citriodora* (Myrtaceae). Their chemical composition was established by GC/MS analyses. Among the three essential oils, one showed evident inhibition of L-1 with IC₅₀ value of 72 µg/mL for *Eucalyptus citriodora*. Only one essential oil that of *O. gratissimum* inhibited the two enzymes, cyclooxygenase function of PGHS and lipoxygenase L-1, with an IC₅₀ values, respectively, of 125 µg/mL and 144 µg/mL, whereas that of *C. giganteus* and *E. citriodora*, two of them had no effect on cyclooxygenase.

KEY WORDS: Essential oils, Soybean lipoxygenase (L-1), Cyclooxygenase function of prostaglandine H synthase-1, PGHS, *O. gratissimum* (Labiatae), *C. giganteus* (Poaceae), *E. citriodora* (Myrtaceae), *Enantia chlorantha*, Inhibition

INTRODUCTION

Plants are widely used in Cote d'Ivoire traditional medicine, especially among people who have little or no access to medical assistance. Some plants like *O. gratissimum*, *C. giganteus* and *E. citriodora* are respectively used for the treatment of boils, stomach-ache and tooth-ache. Accordingly, several studies aiming at studying the anti-inflammatory activity of aqueous or organic extracts of these medicinal plants have been described either *in vitro* on human monocyte or *in vivo* on rats or mice [1-4].

No study on the anti-inflammatory activity of essential oil of *O. gratissimum* has been described until now. Only anti-bacterial and anti-microbial activities are reported [5, 6]. However, a methanol extract and an aqueous suspension of *O. sanctum*, a plant which belongs to Labiatae as *O. gratissimum*, showed anti-inflammatory activity in rats [7, 8]. Singh *et al.* showed that the fixed oil of the same plant possessed significant anti-inflammatory activity against carrageenan which induced paw oedema in rats. In this case, pathways which blocked both lipoxygenase L-1 and cyclooxygenase were proposed [8].

Studies on *E. citriodora* plants are numerous. An ethanolic extract of *E. citriodora* has anti-inflammatory effect in mice [9]. More recently, a study of Juergens *et al.* showed that 1,8-cineol extracted from *E. citriodora*, presented anti-inflammatory activity and caused significant inhibition of the production of leukotriene B4 and thromboxane B2 [10].

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Very few investigations on anti-inflammatory activity of *C. giganteus* have been published. Kimbi *et al.* showed that a mixture of boiled water extracts of *C. giganteus* and *E. chlorantha* had strong effect against chloroquine resistant plasmodium [11].

The proteins implicated in production of mediators of inflammation, proposed in literature, were prostaglandin H synthase (PGHS) and lipoxygenase-5LO. Their natural substrate is arachidonic acid [12-14].

Indeed, it is well established that PGHS catalysed biotransformation of arachidonic acid into prostaglandins, thromboxanes and prostacyclins which are mediators of inflammation, hormonal modulation and platelet aggregation [15-20]. Lipoxygenase 5LO catalysed biotransformation of arachidonic acid into fatty acid hydroperoxides, lipoxines, and leukotrienes, compounds implicated in allergy and suffering. Lipoxygenase L-1 was a model of lipoxygenase 5LO and its natural substrate was linoleic acid [21-24].

In summary, several tests of aqueous extracts of leaves of the three plants, *in vitro*, on human monocytes and *in vivo* on rats and mice, have been described. But so far, there is no report on activity for their essential oils *in vitro* on enzymes.

The present study was initiated in order to investigate the effects *in vitro*, of chemical constituents of essential oil of *O. gratissimum*, *C. giganteus* and *E. citriodora* on both enzymes (cyclooxygenase function of prostaglandin H synthase and lipoxygenase L-1), involved in the inflammation [25] and to make up the mechanism of the reactions.

EXPERIMENTAL

Chemicals

Arachidonic acid, linoleic acid and hemin chloride were from Sigma. Phenol, ethanol and dimethylsulfoxide (DMSO) were from Prolabo.

Plants, essential oils and chromatographic analyses

Fresh leaves of *O. gratissimum*, *E. citriodora* and *C. giganteus* were picked up from fields of south of Cote d'Ivoire. The plants were identified by a resident botanist. The leaves were dried and subjected to distillation in a Clevenger apparatus.

GC analyses were performed on a DELSI 121 gas chromatograph (FID) with a fused silica WCOT capillary column (25 m x 0.3 mm) CPWAX 52 CB stationary phase, the temperature was programmed from 50 °C to 210 °C during 5 min. Initial hold and then to 210 °C at 2 °C/min, using N₂ as carrier gas. GC/MS analyses were performed on HEWLETT-PACKARD Model 5970-300, with a fused silica WCOT capillary column (50 m x 0.3 mm) CPWAX 51, temperature programmed from 50 °C to 230 °C at 3 °C/min, using (He) as carrier gas. Compounds were identified by their retention indices and by comparison on their mass spectra either with known compounds or from published spectra [26].

Biological materials

Prostaglandin H synthase (PGHS) was purified from sheep seminal vesicles microsomes by an already described procedure [27] and suspended in 0.1 M Tris-HCl buffer, pH 8.1, containing 30% glycerol and stored at -80 °C.

Lipoxygenase (L-1) was purified from soybean seeds by an adaptation of a previous described procedure [28] and stored at -20 °C, as a lyophilized powder.

The activity of PGHS (at 37 °C) or lipoxygenase L-1 (at 30 °C), in μmol of O_2 consumed per min and per mg of protein, was calculated by assuming that, 0.26 μmol of O_2 was dissolved per mL of buffer [27, 28].

Assay of enzyme activity

Assay of the cyclooxygenase activity of PGHS

Activity of the cyclooxygenase function of prostaglandin H synthase (PGHS) was measured at 37 °C, by monitoring oxygen consumption in the 1.3 mL incubation cell of a Gilson 516 H oxygraph equipped with a Clark electrode.

20 μL of a 0.03 μM solution of PGHS in 0.1 M Tris-HCl buffer (pH 8.1) were introduced into the cell, 3.25 μL of a 1 mM solution of hemin in 0.1 M NaOH (final concentration 2.7 μM) and 1.3 μL of a 1 M solution of phenol in EtOH (final concentration 1 mM) were added and the volume was completed to 1.3 mL with 0.1M Tris-HCl buffer (pH 8.1). The reaction was started by addition of 5.2 μL of 0.1 M arachidonic acid in EtOH (final concentration 400 μM).

Essential oil tests

For the reactions in the presence of essential oils, increasing amounts of 10, 20, 30, 40, and 50 μL of a 5 mg/mL solution of essential oil in EtOH were added before arachidonic acid.

Assay of the lipoxygenase-L-1 activity

Activity of L-1 was measured at 30 °C by monitoring oxygen consumption in the 1.3 mL incubation cell of a Gilson 516 H oxygraph equipped with a Clark electrode.

Essential oil tests

L-1 treated with linoleic acid. 10 μL of a 3.8 μM solution of soybean lipoxygenase-1 (final concentration 29 nM) were introduced into the cell, and the volume was completed to 1.3 mL with 50 mM Tris-HCl buffer (pH 9). The reaction was started by addition of 50 μL of 10 mM solution of linoleic acid (final concentration 0.38 mM). Reaction was started by addition of increasing amounts of 10, 20, 30, 40, and 50 μL of a 5 mg/mL solution of essential oil in EtOH.

L-1 not treated with linoleic acid. 10 μL of a 3.8 μM solution of L-1, were introduced into the cell and the volume completed to 1.3 mL with 50 mM Tris-HCl buffer (pH 9), then 20 μL of a solution 5 mg/mL (final concentration 77 mg/mL) of essential oil were added. To increasing times (10, 20, 30, and 60 min), the reaction was started by addition of 50 μL of 10 mM solution of linoleic acid.

RESULTS

There is no O_2 consumption by the essential oil alone in the buffer, nor by the essential oil in the presence of enzyme (L-1 or cyclooxygenase of PGHS), nor by the essential oil in the presence of the substrate (linoleic or archidonic acid). The essential oil yields of *O. gratissimum*, *C. giganteus* and *E. citriodora* [25] on distillation were respectively 0.8%, 1.2% [25] and 1.5%. The GC and GC/MS analysis showed predominant compounds whose names and percentage are given (Table 1).

Table 1. Chemical composition of essential oils of *E. citriodora*, *O. gratissimum*, and *C. giganteus*.

Constituents (%)	<i>E. citriodora</i> [25]	<i>O. gratissimum</i>	<i>C. giganteus</i>
α -Pinene	1.1	0.8	-
β -Pinene	0.8	-	-
α -Thujene	-	1.3	-
Myrcene	0.1	1	-
Limonene	0.8	0.3	14.18
1,8-Cineole	1.6	-	-
4-Terpineol	-	2.2	-
χ -Terpinene	0.6	2.3	-
α -Copaene	-	0.6	-
<i>p</i> -Cimene	0.5	-	0.06
Terpinolene	0.4	-	-
Citronellal	68.9	-	-
Linalool	4	-	-
Isopulegol	9	-	-
Caryophyllène oxyde	-	1.6	-
β -Caryophylene	0.7	-	-
Thymol	-	70.8	-
Citronellyl acetate	0.7	-	-
Citronellol	5.3	-	-
Globulol	0.11	-	-
<i>p</i> -Mentha-1,2,3-triene	-	-	0.21
cis-Limonene oxide	-	-	0.18
trans-Limonene oxide	-	-	0.44
trans-<i>p</i>-Mentha-2,8-dien-1-ol	-	-	26.18
cis-<i>p</i>-Mentha-2,8-dien-2-ol	-	-	11.93
Myrtenol	-	-	1.66
Verbenone	-	-	0.31
Cavacrol	-	1.1	-
Carvone	-	-	2.74
trans-Sabinene hydrate	-	0.3	-
cis-Sabinene hydrate	-	0.2	-
cis-Isopiperitenol	-	-	3.24
trans-isopiperitenol	-	-	4.15
Perillaldehyde	-	-	0.45
cis-<i>p</i>-Mentha-1(7),8-dien-2-ol	-	-	11.27
cis-Carvecol	-	-	2.13
trans-Carveol	-	-	0.5
trans- <i>p</i> -Mentha-1(7),8-dien-2-ol	-	-	13.3
Perillic alcool	-	-	0.01
Phenyl ethyle hexanoate	-	-	0.17

Test on lipoxygenase L-1

Increasing amounts of the essential oils of *O. gratissimum*, *E. citriodora* and *C. giganteus* from 40 μ g/mL to 192 μ g/mL, caused the inhibition of the activity of L-1 (Figure 1 and 2).

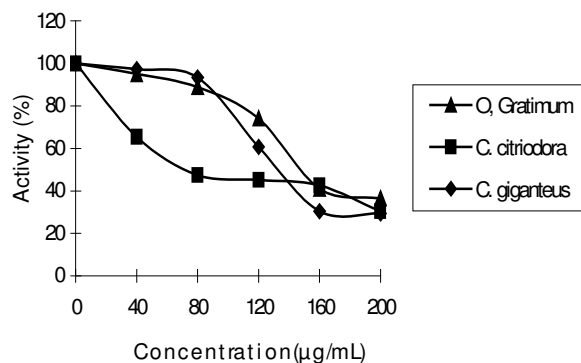


Figure 1. Inhibition of lipoxigenase L-1 by essential oils without treatment with linoleic acid.

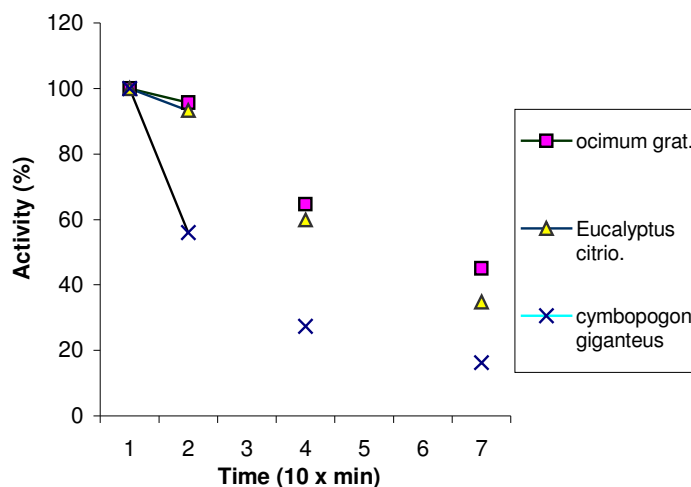


Figure 2. Inhibition of lipoxigenase L-1 by essential oils with treatment with linoleic acid.

The IC₅₀ values obtained, ranged from 72 µg/mL to 144 µg/mL (Table 2). The essential oils of *O. gratissimum* and *C. giganteus* were the weakest inhibitors with IC₅₀ values of 144 µg/mL and 130 µg/mL, respectively. The best inhibitors of L-1 were the essential oils of *E. citriodora* for which IC₅₀ value of 72 µg/mL was found.

Table 2. Inhibition of lipoxigenase L-1 and cyclooxygenase by essential oil of *O. gratissimum*, *E. citriodora* and *C. giganteus* without treatment with natural substrate.

Enzymes	<i>E. citriodora</i>	<i>C. giganteus</i>	<i>O. gratissimum</i>
Lipoxigenase L-1 IC ₅₀ value	72 (µg/mL)	130 (µg/mL)	144 (µg/mL)
Cyclooxygenase IC ₅₀ value	No reaction	No reaction	125 (µg/mL)

The influence of treatment of L-1 with linoleic acid before tests was examined. The times after which 50% of the initial activity of lipoxigenase was inhibited ($t_{1/2}$), was observed in each case. The best inhibition appeared at $t_{1/2}$ value of 15 min with (77 $\mu\text{g/mL}$) at 30 °C for the essential oil of *C. giganteus* (Figure 2).

Test on cyclooxygenase

The results obtained on inhibition of cyclooxygenase function of PGHS were different from those for lipoxigenase. Two of the essential oils from *E. citriodora* and *C. giganteus* had no effect at any concentration on the cyclooxygenase function of PGHS (Figure 3).

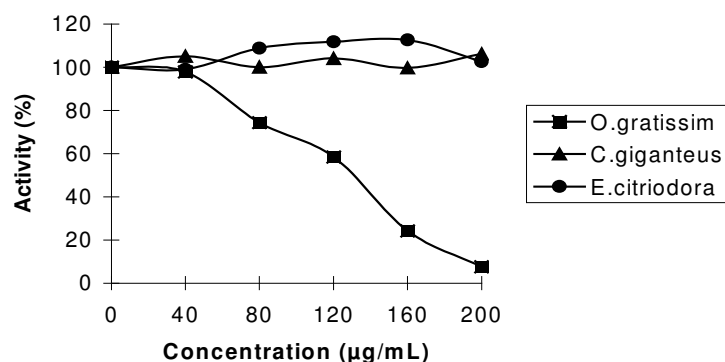


Figure 3. Inhibition of cyclooxygenase without treatment with arachidonic acid by chemical compounds of essential oils of *O. gratissimum*, *E. citriodora* and *C. giganteus*.

However, the essential oil of *O. gratissimum* was the only inhibitor of the enzyme with IC_{50} of 125 $\mu\text{g/mL}$ which was comparable to the value of IC_{50} found for the inhibition of L-1 without treatment with linoleic acid (Table 2). Chemical constituents of essential oil of *O. gratissimum* inhibited both enzymes.

DISCUSSION

Chemical constituents of essential oil of the three plants showed structures of terpenes, sesquiterpenes and oxygen compounds which had interaction *in vitro* with lipoxigenase L-1 and cyclooxygenase function of PGHS.

Lipoxigenase L-1 was inhibited by chemical constituents of all the essential oil tested. These results constituted the first report on tests of chemical constituents of essential oil of aromatic plants on lipoxigenase L-1 in the literature. The more efficient inhibitor of L-1 were chemical compounds of essential oil of *E. citriodora* with IC_{50} value of 72 $\mu\text{g/mL}$. This result is in agreement with the traditional medical used and with a previous one which showed inhibition of leukotriene LTB₄, product obtained in lipoxigenase-5 LO activity [10]. Treatment of Lipoxigenase by natural substrate before added essential oil, showed inhibition of the enzyme by chemical compound of essential oil of *C. giganteus* for $t_{1/2}$ value of 15 min. These results showed that essential oil of *C. giganteus* constituted irreversible inactivator after 15 minutes. This result is in agreement with a used of extract of *C. giganteus* to stop tooth-ache.

Cyclooxygenase function of PGHS was inhibited by only chemical constituents of essential oil of *O. gratissimum* with IC₅₀ value of 125 µg/mL. These results are in agreement with the used traditional medicine of extract of this plant to stop oedema [8] and boils. Chemical constituents of essential oil of *O. gratissimum* inhibited both enzymes with comparables values of IC₅₀. This aromatical plant can be used as anti-inflammatory and anti-ache drug in human medicine.

All these results showed *in vitro* interaction between chemical constituents of essential of aromatic medicinal plants and lipooxygenase L-1 and cyclooxygenase function of PGHS, implicated in inflammatory process. In order to clear up the molecular mechanism of anti-inflammatory activity of *O. gratissimum*, *E. citriodora* and *C. giganteus*, model reactions should be performed with compound isolated from essential oil of the plants. Also, it will thus be interesting as well to know whether the same essential oil of *O. gratissimum* is implicated in the inhibition of the activity of both enzymes.

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