

Full Length Research Paper

## Effect of essential oil of *Chromoleana odorata* (Asteraceae) from Ivory Coast, on cyclooxygenase function of prostaglandin-H synthase activity

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Aqueous extracts of *Chromoleana odorata* are commonly used in traditional medicine as anti-inflammatory drug against pains or as cataplasm to stop hemorrhage in Ivory Coast. In this work, we want to test the volatiles as essential oil extracted from the fresh leaves of *C. odorata*, on the cyclooxygenase function of prostaglandin-H synthase (PGHS), a protein implicated in the inflammatory process. Our goal is to make up the *in vivo* therapeutic activity and understand the mechanism of its biologic activity. This essential oil is extracted from leaves of *C. odorata* by hydrodistillation to be submitted to *in vitro* test. The chemical constituents of the essential oil of *C. odorata* increase the activity of cyclooxygenase function of PGHS with an AC<sub>50</sub> value of 87.5 µg/mL. Studies are in progress to identify all the constituents implicated in the activity.

**Key words:** *Chromoleana odorata*, essential oil, cyclooxygenase, PGHS.

### INTRODUCTION

Extracts of *Chromoleana odorata* (L.) R. M. King and H. Rob. are used in folk medicine of Ivory Coast as cataplasms to stop hemorrhages or as anti-inflammatory drugs against pains (Bedi et al., 2001). The biological activity of extracts of *C. odorata* has been shown by several studies in the world. Indeed, the extracts of fresh leaves of *C. odorata* have been used in the treatment of malaria in Ghana and Benin (Ayensu et al., 1978). They have also been employed as cataplasms to stop accidental hemorrhages in Thailand (Tiratana and Suwaimuraks, 1991). Aqueous extracts of this plant presented an anti-microbial activity against gonococcus in Guatemala (Caceres et al., 1995).

The chemical composition of essential oils of *C. odorata* from Ivory Coast was studied (Bedi et al., 2001, 2004). The major components were found to be alpha-

pinene (21.15%), geigerene (11.68%) and pregeigerene (19.61%). The chemotype of the essential oil from Ivory Coast was comparable to that of Cameroon (Lamaty et al., 1992). Geigerene and pregeigerene appeared in the chemotype of littoral countries of Africa from the West to the Equatorial seaside (Ayensub et al., 1978; Bedi et al., 2004; Lamaty et al., 1992; Sohounhloue et al., 1996). Only the chemotype of the essential oil coming from Benin presented different major products: beta-caryophyllene (21%) and germacrene D (15.30%) (Sohounhloue et al., 1996). Such chemical products of aromatic plant were implicated *in vitro* biological reaction in the literature. Recently, the pair geigerene/pregeigerene (7.5%) presented in *C. odorata* from Nigeria was screened for antimicrobial activity and showed antibacterial activity against *Bacillus cereus* (MIC = 39 g/mL) and antifungal activity against *Aspergillus niger* (MIC = 78 g/mL) (Moses et al., 2010). The main compounds of the essential oil of the subterranean part of *Cacalia tangutica* (Maxim.) Hand. -Mazz were: a-

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zingiberene (13.49%), germacrene D (10.76%),  $\alpha$ -pinene (8.54%), caryophyllene(Z-) (6.36%), linalool (6.16%), b-myrcene (4.89%), b-ocimene(Z-) (4.40%) and ocimene(Z-) (3.58%). The major constituents which were also presented in the oil of *C. odorata*, presented a broad antimicrobial spectrum and had better antimicrobial activity against yeast and gram-positive bacteria. The minimum inhibitory concentration values were 0.16 - 5.00 g/L and minimum bactericidal concentration values were 0.16 - 5.00 g/L (Yang et al., 2008). The volatiles components of the leaves of *Stachys schtschegleevii* from Iran showing  $\alpha$ -pinene (36.4%) (major), germacrene-D (18.6%), limonene (8.2%) and piperitone (6.2%) as the main constituents, exhibited moderate activity against six Gram- positive and -negative bacteria (Sonboli et al., 2005). No study showing direct reaction between the chemical constituents of essential oil of *C. odorata* and the cyclooxygenase function of PGHS was not described yet.

But it has already been reported that the constituents of the essential oils of aromatic plants could react with enzymes to cause their inhibition. The reaction would take place by chemical reaction with the active site of the protein or by synergy reaction (Sirois et al., 1984) or by anti-oxidant effect (Dorman et al., 1995).

Studies based on the activity of the essential oils of *C. odorata* on a model of 15-lipoxygenase (L15), an enzyme involved in inflammatory processes, was investigated in the last years. The chemical components of the essential oil of this plant inhibited *in vitro*, Lipoxygenase L1 of seeds of soja, a model of the human Lipoxygenase (L15), with an IC<sub>50</sub> value of 60  $\mu$ g/mL (Bedi et al., 2004). No study of reaction of the essential oil of *C. odorata* with cyclooxygenase function of PGHS is established yet.

It is well established that the cyclooxygenase function of PGHS catalyses the biotransformation of arachidonic acid into prostaglandin G<sub>2</sub>. Prostaglandin G<sub>2</sub> is a precursor of two other mediators: prostacycline (vasodilatator or anti-aggregation mediator) and thromboxanes A<sub>2</sub> (vasoconstrictor or aggregation mediators). These two compounds are involved in platelet aggregation processes during haemostatic activity (Collen et al., 2004). It was established that aggregation effect activated the production of thromboxane A<sub>2</sub> (Collen et al., 2004; Kohayakawa et al., 1986). The present work aims at studying the effect of essential oil of *C. odorata* on the cyclooxygenase function of PGHS.

## EXPERIMENTALS

### Plant material

Fresh leaves of *C. odorata* were picked up in a forest area in the morning. Their botanic and family names were established by Professor Ake Assi, a resident botanist at the University of Cocody-Abidjan. After washing by distilled water, 500 g of leaves were further treated by hydrodistillation into a special apparatus.

### Distillation material

500 g of fresh leaves were introduced in a Clevenger apparatus. After boiling for 2 h, the distillate was collected. It was then dried over MgSO<sub>4</sub> and samples were prepared to be analyzed by spectroscopic methods or to be used in biochemical assays. The yields of hydrodistillation were estimated based on a mass of 500 g of fresh leaves.

### Biological material

Prostaglandin H synthase (PGHS) was purified from sheep seminal vesicles microcosms by methods consigned in literature (Mahy et al., 1991) and was then suspended in 0.1 M Tris-HCl buffer, pH = 8.1, containing 30% glycerol and stored at - 80°C. Arachidonic acid and hemin chloride, phenol and ethanol were commercial.

### Assay of the cyclooxygenase activity of PGHS

The cyclooxygenase activity was measured at 37°C, by monitoring oxygen consumption in the 1.3 mL incubation cell of a Gilson 516H oxygraph equipped with a Clark electrode. 20  $\mu$ L of a 0.03  $\mu$ M solution of PGHS in 0.1 M Tris-HCl buffer, pH = 8.1 were introduced into the cell, 3.25  $\mu$ L of a 1 mM solution of hemin in 0.1 M NaOH (final concentration 2.7  $\mu$ M) and 1.3  $\mu$ M 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 0.1 M Tris- HCl buffer, pH=8.1. The reaction was started by addition of 5.2  $\mu$ L of 0.1 M arachidonic acid in EtOH (final concentration 400  $\mu$ M) (Mahy et al., 1991).

The cyclooxygenase activity of PGHS (at 37°C, in  $\mu$ mol of oxygen consumed per min and per mg of protein), was calculated assuming that 0.26  $\mu$ mol of oxygen was dissolved per mL of buffer (Mahy et al., 1991).

### Reaction of cyclooxygenase of PGHS with the essential oil of *C. odorata*

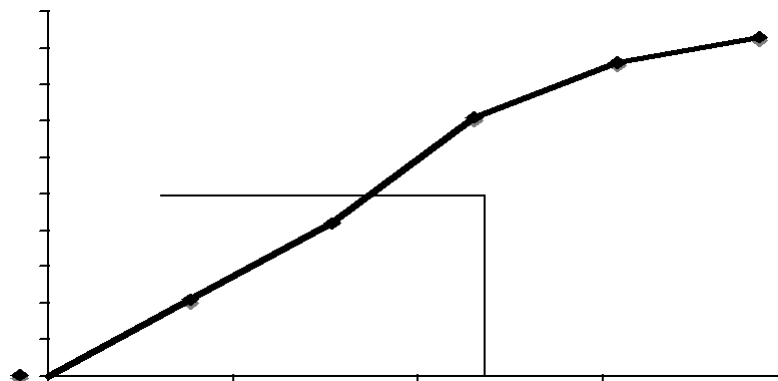
Increasing amounts of a 5 mg/mL solution of essential oil of *C. odorata* in EtOH (10, 20, 30, 40 and 50  $\mu$ L final concentration: 38.5, 77, 115.5, 154 and 192.5  $\mu$ g/mL), respectively, were added to the addition of arachidonic acid and the oxygen consumption was then monitored. The cyclooxygenase activity of the enzyme measured at 37°C was X  $\mu$ mol of oxygene consumed per min and per mg of protein.

## RESULTS

No oxygen consumption was observed either by the essential oil alone in the buffer, or by the essential oil in the presence of cyclooxygenase of PGHS, or by the essential oil in the presence of the arachidonic acid substrate. When 5.2  $\mu$ L of 0.1 M arachidonic acid in EtOH were added into the cell that contained both cyclooxygenase of PGHS and the essential oil, oxygen consumption was observed. The results obtained are reported in Figure 1.

At 0  $\mu$ g/mL of essential oil and 0  $\mu$ M of arachidonic acid, we have 0% activity of protein. At 0  $\mu$ g/mL of essential oil and 400  $\mu$ M of arachidonic acid, we have 100% activity of protein. When 400  $\mu$ M of arachidonic acid were added on the increasing concentration of essential

% activity



**Figure 1.** Effect of essential oil of *C. odorata* on the cyclooxygenase activity of PGHS.

oil, the activity of protein increases. For the concentrations between 154 and 192.5  $\mu\text{g}/\text{mL}$  of essential oil, the values of the activity of the protein begins to decrease and the protein is destroyed. At 50% of activity of enzyme, the concentration of essential oil, which constitutes the  $\text{AC}_{50}$  value, is 87.5  $\mu\text{g}/\text{mL}$ .

## DISCUSSION

The first study of reaction between the essential oil of *C. odorata* with the cyclooxygenase function of PGHS is presented here. At 0  $\mu\text{g}/\text{mL}$  of essential oil and 400  $\mu\text{M}$  of arachidonic acid, 100% activity of protein was obtained. This result indicates that the natural substrate of the protein is the arachidonic acid. When 400  $\mu\text{M}$  of arachidonic acid were added on each concentration of essential oil, the activity of protein increases. Such behavioral process of making up the activity of proteins has already been mentioned in the literature. The aggregation effect activated the production of thromboxane  $\text{A}_2$  a precursor of aggregation effect (Collen et al., 2004; Kohayakawa et al., 1986; Wei et al., 2007).

When the concentration of essential oil in the cell is increased, the activity of the protein is constant rather than decrease; probably the protein is destroyed because no reaction is observed when the essential oil added.

The graphic determination of the concentration of the essential oil needed for increasing the activity of the protein of 50% ( $\text{AC}_{50}$ ) is 87.5  $\mu\text{g}/\text{mL}$ . The value is higher than those obtained with plant containing the same terpenes as previously described (Moses et al., 2010; Yang et al., 2008). This result explains that an important concentration of essential oil from plant material is required for traditional treatment. It is likely that a high concentration of the chemical components of the

essential oil produces probably an activation of COX1, which leads to the *in vitro* production of Tromboxane  $\text{A}_2$ , involved in platelets aggregation (Collen et al., 2004).

Consider that, the activation observed could be interpreted by a possible production of tromboxane  $\text{A}_2$  or prostacyclines, *in vivo* (Wei et al., 2007) and these results should be in agreement with the observed biomedical behavior of the extract of *C. odorata* in stopping bleeding (Triratana et al., 1991).

The results give indication on the therapeutic action of the essential oil of *C. odorata* but it is difficult to understand all the aspect of this study now. Reactions between terpenes from essential oil of *C. odorata* and the enzyme could be a chemical reaction with the active site or a synergy reaction (Sirois et al., 1984) or an anti-oxidant effect (Dorman et al., 1995). The isolate of the components were not implicated in this activity (terpens, thromboxanes, prostacyclines...), so it will be intersecting, in the future, to make up HPLC analyses to identify the constituents concerned. Studies are required to confirm the *in vivo* production of the thromboxane  $\text{A}_2$  or prostacyclines. Physiological analyses based on anti thrombotique *in vitro* tests of essential oil on platelets are in progress to give some indications on the haemostatic or vasoconstrictor effect of leaves of *C. odorata*.

## Conclusion

The essential oil of *C. odorata*, a famous medicinal plant of Ivory Coast increase the activity of cyclooxygenase function of PGHS, a protein implicated in inflammation process. The concentration value of essential oil required to increase the activity of the cyclooxygenase function of PHGS is 87.5  $\mu\text{g}/\text{mL}$ . We must work to identify all the constituents implicated in this activity.

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