In Vitro Antioxidant and Anti-inflammatory Potential of the Optimized Combinations of Essential Oils from Three Cameroon Grew Ocimum L.

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Abstract: In the best of our knowledge, there is no report on the antioxidant and anti-inflammatory effect of the combination of essential oils from Ocimum genus. In our previous report, the combinations of Ocimum basilicum/Ocimum gratissimum, Ocimum basilicum/Ocimum urticaefolium and Ocimum gratissimum/Ocimum urticaefolium were optimized for their antifungal effect. The present report presents the spectral profile and antioxidant and anti-inflammatory effects of those optimized combinations. The optimized combinations were prepared following our previously described approach and analyzed by Fourier Transformed infrared spectroscopy for fingerprinting of essential oils involved in the combination. The radical scavenging activity was evaluated on diphenyl-picryl-hydrazyle (DPPH) and ferric reducing antioxidant power (FRAP). The antioxidant activity was determined using the protective model of Linseed oil accelerated oxidation. The anti-inflammatory activity was assayed using cyclooxygenase (COX) 1 and 2. The results indicated that the fingerprints of the combination are different from those in the essential oil analyzed alone. This denotes the physical expression of different hydrogen bonds in the mixture. Overall, the optimized combinations exerted a maximum reduction (OD = 4) effect on the ferric ion. As well, they scavenged the DPPH efficiently (90% inhibition). These combinations also successfully inhibited the auto-oxidation of flaxseed oil by reduction of cis double bonds, aldehyde and hydroxyl. The optimized combination of Ocimum basilicum and Ocimum gratissimum showed a lower inhibitory effect in comparison to the control consisting of enzyme without inhibitor. Of note, Ocimum gratissimum alone exerted an inhibitory effect on both isoform of cyclooxygenase with IC50 = 0.23 µL/mL and 0.27 µL/mL for cyclooxygenase 1 and 2 respectively. Also, the essential oil of Ocimum basilicum exhibited a better effect on cyclooxygenase 2 (IC50 = 0.22 µL/mL) but showed less action on cyclooxygenase 1 (IC50 = 0.55 µL/mL). The essential oil of Ocimum urticaefolium presented the best effect on cyclooxygenase 1 (IC50 = 0.06 µL/mL) but appeared to be nonactive on cyclooxygenase 2. Globally, the optimized combinations inhibited efficiently all the isoforms of cyclooxygenase with percentage inhibition of ≥67% for cyclooxygenase 2 and ≥98% for cyclooxygenase 1.

Key words: Optimized combination, essential oil, Ocimum, antiradical, antioxidant, anti-inflammatory.
1. Introduction

Living cells in an organism are involved in oxidative reactions for many purposes including cell communication, metabolism, renewal or death and defense mechanisms [1]. The oxidative reactions include all the reactions in which a compound from any cell compartment loses an electron or proton. This leads to conformation changes of compounds around the electronic deficient atom. The outcomes of these changes can be useful for cell normal live or can lead to dysfunctions. This phenomenon is at the basis of almost all human diseases including communicable infectious diseases and non-communicable diseases such as Alzheimer, Parkinson, diabetes, autoimmune diseases and inflammatory diseases [2].

During microbial infections, the resident granulocytes and neutrophil present at the infection site will increase their transcription of the proinflammatory cytokines including interleukins (1β, 6, 10, 12, 18) tumour necrosis factor alpha, leukotriene (through lipoxygenase pathway), prostaglandin (through cyclooxygenase pathway) amongst other. In addition to these peptides, the degranulation of neutrophils releases highly toxic chemicals that create a deleterious environment both for the host cells than for the infectious agent. These toxic compounds are mainly reactive oxygen species (H2O2, HO, NO, O…) and reactive nitrogen species (NO, NOO, NOOO) and are produced by hyper-consumption of glucose and Adenosine Triphosphate (ATP) leading to a so-called oxidative burst. These free radicals will liquify all surrounding cells, leading to the cardinal sign of the inflammation (redness, heat, pain, and edema). During non-infective disease as Alzheimer, diabetes, arthritis, the reaction is initiated by an unusual chemical resulting from the pathology high concentration of glucuronic acid in diabetes; immune system dysfunction, uric acid, Cohn’s disease for Arthritis [3-5].

The management of the inflammatory disease is done by two classes of anti-inflammatory drugs name steroidal and nonsteroidal anti-inflammatory drugs. The steroidal anti-inflammatory drugs, also called corticosteroids, are a group of drugs that exert their therapeutic effect by binding the corticoids receptors. This biding leads to the inhibition and inactivation of the proinflammatory peptide synthesis thereby inhibits the production of chemoattractant, the vasodilatation, and recruitment of immune cells. For this reason, some strong corticoids are used as an immunosuppressant. The non-specificity of their target and the presence of these targets in almost all cells and organs lead to a myriad of side effect. The side effect of corticosteroids includes skin atrophy and wound abnormal healing on bones, the long-term use of corticoids leads to the osteoporosis. On skeletal muscle, corticosteroids lead to the myopathy. In the central nervous system, this class of anti-inflammatory can lead to psychiatric disorder. In this sense, it has been reported that mood swings, euphoria, depression, and suicide attempts may occur. On the endocrine system, the uses of corticosteroids on diabetes can lead to the aggravation of diabetes. On the cardiovascular system, dyslipidaemia and hypertension are described in a patient with long-term uses of corticosteroids. On intestinal tract, corticosteroids have described leading to peptic ulcer, upper gastrointestinal bleeding, pancreatic and oral candidiasis [6, 7].

The second class of anti-inflammatory drugs is called nonsteroidal anti-inflammatory drugs (NSAID). This class of anti-inflammatory is designed to inhibit the effect of cyclooxygenases thereby arresting the production of prostaglandins, one group of the soluble inflammatory mediator. These groups of anti-inflammatories also suffer from a certain number of side effects including gastrointestinal ulceration and bleeding, hepatic dysfunction and failure in some case, skin hypersensitization and renal dysfunction. The common side effect of all the classes of anti-inflammatory drugs is the induction of
immuno-suppression thereby exposing the organism to various infectious agents in the regard of these sides’ effects, there is a need of new drugs with original mechanism of actions targeting more than one inflammatory mediator. As strong anti-inflammatory agent can also have an immune-suppression effect, the antimicrobial effect of the new drug is an important parameter to take in consideration [8, 9].

Essential oils are a complex mixture of volatile compounds belonging mainly to the Terpenoids (mono and sesquiterpenes) extracted by distillations methods or expression of pericarps in citrus. There is a myriad of papers reporting their antioxidants [10-12], anti-microbial [11, 13-16], anti-inflammation [17-19], anti-cancer [20-22].

Drugs combinations have been used as an alternative to resistant viral infections and the strategy was extended to all the infectious diseases. Nowadays, this strategy is increasingly used in the management of non-infectious diseases such as neurological disorder, diabetes and inflammation [23-25]. The interest of the combination resides on the fact that, combining two drugs that have synergistic effect is useful in many ways including reducing the toxicity, breaking down resistance by multipletarget in one go, enlarging the spectrum of the combination [26]. Three methods are mainly used namely isobole, checkerboard and experimental design [26-28]. The two first lack statistical application to validate the approaches even if, to strengthen the outcome, some authors had successfully combined the two (checkerboard + isobole) [28]. The principal limit of these two methods is the lack of optimal concentration that can guide the physician in drug prescription. Research by our group has recently used with success of all these methods to find the optimal combinations of essential from four Cameroon grew Ocimum [16]. Individually, these essential oils had shown a protective effect on flaxseed oil [16]. The present report describes the anti-radical, antioxidant and anti-inflammatory activities of these combinations.

2. Materials and Methods

2.1 Essential Oils Extraction and Combination Preparation

The leaves of Ocimum basilicum L. 428782HNC, Ocimum gratissimum L. 5817/SRF/Cam and Ocimum urticeafolium L. 49085 HNC were collected and extracted as previously reported [11, 16, 29] using a Clevenger-type apparatus. The optimized combinations were prepared as previously described [26].

2.2 Flaxseed Oil

Flaxseed oil was purchased in Bacau and the content was the same as previously reported [29].

2.3 Reagents

All reagents were purchased from Sigma-Aldrich, including anhydrous sodium thiosulfate ≥ 98.0%, potassium iodide 99%, starch from potatoes, Chloroform GC grade 99-99.4%, acetic acid 99.8-100% and Butylated Hydroxyl-Toluene 99.0%, DPPH 99%, Inhibitor Screening Assay Kit from Cayman Chemical Co. (Cat. No. 760111).

2.4 Infrared Analysis of the Combinations

Prior to the spectrum acquisition, the combination was prepared regarding the proportion of each essential oil in the combination. The following formula was used to prepare 100 µL each combination.

\[ \text{VEO}_x = \frac{\text{EO}_x}{(\text{EO}_x + \text{EO}_y)} \times 100 \]

\[ \text{VEO}_x = \text{volume of the essential required for 100 µL of the combination;} \]

\[ \text{[EO}_x] = \text{the concentration of the essential oil x in the optimized combination of essential oil x and y;} \]

\[ \text{[EO}_y] = \text{the concentration of the essential oil in the optimized combination of essential oil x and y.} \]

The spectrum was acquired as previously described [29]. Briefly, 10 µL of the prepared combinations placed on the attenuated total reflectance (ATR) sampling device. The apparatus was Tensor 27 Fourier transformed infrared spectrophotometer, placed in the
environment where the temperature was set at 25 °C and relative humidity was set at 30%. The spectra were acquired from 4,000 to 550 cm\(^{-1}\) with a resolution of 4 cm and 260 scans with the velocity of 10 KHz with interferogram size 14,220 points. The acquired spectrum was digitalized with OPUS software and Origin 5.1 software was used for spectral manipulation.

2.5 Chemometric Analysis

The chemometric analysis of the spectrum proceeded as previously reported [29]. Briefly, all spectra were processed using the Savitzky-Golay algorithm and all the spectra were divided into three regions. The first region from 550 cm\(^{-1}\) to 1,600 cm\(^{-1}\), corresponding to the fingerprint region, was submitted to the second derivative.

The interaction between the compounds from the essential oils in combination was analyzed by comparing the spectrum obtained with the spectrum predicted arithmetically from the spectrum of each essential oil alone.

2.6 Antioxidant Effect

2.6.1 Anti-free Radical Effect on DPPH Radical

The scavenging effect of the optimized combination was determined using the protocol previously described [11] with slight modifications. Briefly, 200 µL ethanolic solution of the optimized combination was added to 800 µL of ethanolic solution of DPPH (0.004%). After 30 min incubation in absolute darkness, the absorbance was read at 517 nm. Each experiment was performed in triplicate and the percentage of inhibition was calculated using the following formula.

\[
\%SC = \frac{Abs_{ref} - Abs_{comb}}{Abs_{ref}} \times 100
\]

\%SC = scavenging percentage of the testing on DPPH;

\(Abs_{ref}\) = absorbance of the negative control;

\(Abs_{comb}\) = absorbance of the combination.

2.6.2 Protective Effect on Flaxseed Oil

The test was performed as previously described [29] slight modifications. Briefly, 15 mL of the flaxseed oil was prepared with optimized combination and incubated in the oven at 60 °C with light and under agitation for 15 days. An aliquot was collected on days 0, 2, 3, 6, 9, 12 and 15 for analysis.

(1) Infrared Follow-Up of the Flaxseed Stability

Structural changes of the mixture aliquots were assessed as previously reported. Briefly, 10 µL of the aliquots was placed on the FTIR ATR device and the spectrum was acquired from 400 to 550 cm\(^{-1}\). The chemometric analysis consists of the assignment of the region as follows: cis-HC = CH-(769 to 646 cm\(^{-1}\)), trans-HC = CH-(1,066 to 914 cm\(^{-1}\)), C = O in aldehyde (1,739 to 1,724 cm\(^{-1}\)), unconjugated-HC = CH-(3,030 to 2,995 cm\(^{-1}\)) and -OO-H (3,660-3,066 cm\(^{-1}\)). All these regions were submitted to Gaussian decomposition. The following formula was used to quantify the depth of the observed changes.

\[
\%I = \frac{(A0-Ai)/A0}{(A0-Ai)} \times 100
\]

where \%I is the percentage inhibition of oxidation, \(A0\) is the area of the negative control at day 0, \(Ai\) is the absorbance of the samples at day \(i\).

(2) Chemical Detection of Peroxide Value

The peroxide value was measured at the end of the incubation days using the previous protocol [12]. Briefly, 300 µL of the samples were mixed with a solution of acetic acid/chloroform/tween 80 (2.75/1.75/0.5). Then 50 µL of saturated potassium iodide solution, 5 mL of distilled water and 50 µL of starch (10 g/L) were sequentially added and the mixture was titrated with sodium thiosulfate (0.02 M in distilled water). The following formula was used to quantify the peroxide value:

\[
PV (mM) = V \times C \times 50
\]

with \(V\) = consumption of 0.02 M sodium thiosulfate solution, and \(C\) = molar concentration of sodium thiosulfate solution.

2.7 Anti-cyclooxygenase 1 and 2 Activities

The COX-1 and COX-2 inhibitory effects were assessed using a colourimetric COX (ovine) Inhibitor Screening Assay Kit from Cayman Chemical Co. (Cat. No. 760111) as described previously [28]. The tests
were performed according to the Cayman Chemical Co. method. The effect of the essential oil was measured by quantification of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm followed by incubation of either ovine COX-1 or COX-2 with arachidonic acid. The enzymes were pre-incubated for 5 min at 25 °C with essential oils at different concentrations and then arachidonic acid was added (final concentration 1.1 mM) and TMPD followed by incubation for 5 min at 25 °C. The COX-inhibiting effect was calculated using the following equation:

\[
\%I_{COX} = \frac{([\text{prost}]_{\text{neg}} - [\text{prost}]_{\text{con}}) - ([\text{prost}]_{\text{HeI}} - [\text{prost}]_{\text{con}})}{([\text{prost}]_{\text{neg}} - [\text{prost}]_{\text{con}})} \times 100
\]

[prost]_{neg} was the concentration of prostaglandin in the negative control (without essential oils); [prost]_{con} was the concentration of prostaglandin in the blank (without TMPD); [prost]_{HeI} was the concentration in the well containing essential oils at concentration i.

2.8 Statistical Analyses

The data were statistically analysed using the Kruskal-Wallis test for classification of the spectrum and ANOVA for data comparison [29].

3. Results and Discussion

3.1 Results

Figs. 1-3 are the spectral signals of the optimized combination of the essential oils. From these figures, it was observed that the obtained signal differed from the predicted model. In fact, in addition to some shift in the signals, the intensity of the signal is more important in the optimized combination than in predicted spectrum (Figs. 1a, 2a and 3a). The

![Fig. 1 Spectral behaviour of the optimized combination of essential oils from Ocimum basilicum and Ocimum gratissimum: (a) second derivative of predicted (blue) over obtained (red) signal at fingerprint zone in combination of essential oils from leaves of Ocimum basilicum and Ocimum gratissimum; (b) second derivative of the difference between the predicted and the obtained signal at the fingerprint zone in the same combination.](image)
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**Fig. 2** Spectral behaviour of the optimized combination of essential oils from *Ocimum basilicum* and *Ocimum urticaefolium*: (a) second derivative of predicted (blue) over obtained (red) signal at fingerprint zone in combination of essential oils from leaves of *Ocimum basilicum* and *Ocimum urticaefolium*; (b) second derivative of the difference between the predicted and the obtained signal at the fingerprint zone in the same combination.

**Fig. 3** Spectral behaviour of the optimized combination of essential oils from *Ocimum gratissimum* and *Ocimum urticaefolium*: (a) second derivative of predicted (red) over obtained (blue) signal at fingerprint zone in combination of essential oils from leaves of *Ocimum basilicum* and *Ocimum urticaefolium*; (b) second derivative of the difference between the predicted and the obtained signal at the fingerprint zone in the same combination.
spectrum obtained by subtracting the predicted model to the observed model and subsequent peaks refinement by spectrum derivation presented in Figs. 1b, 2b and 3b revealed the difference in the intensity of vibration, appearance and disappearance of the signal in comparison to the predicted model.

3.1.1 Antiradical Effect of the Essential Oil

All essential oils involved in this study have a ferric reducing effect (Fig. 4) and were more efficient than both ascorbic acid and gallic acid at up to 4 µL/mL, all the essential oils reduced the ferric ion more efficiently than both ascorbic acid and gallic acid. Essential oils of Ocimum basilicum exhibit better activity than the other and that of Ocimum gratissimum and Ocimum urticaefolium had the same level of activity.

The optimized combination highlights a strong reducing effect (Fig. 5).

All the essential oils involved in this study have a DPPH scavenging capacity (Fig. 6). The essential oils of Ocimum gratissimum (IC50 = 0.64 µL/mL) have the same activity as Vitamin C (IC50 = 0.65 µL/mL). This essential oil had the lowest activity in comparison to that from Ocimum basilicum (IC50 = 0.018 µL/mL) and Ocimum urticaefolium (IC50 = 0.03 µL/mL).

All the combination showed an inhibition percentage of 90.69 for Ocimum basilicum and Ocimum gratissimum, 91.44 for Ocimum gratissimum and Ocimum urticaefolium and 92.05 for Ocimum basilicum and Ocimum urticaefolium (Fig. 10).

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**Fig. 4** Ferric reduction antioxidant power of the essential oils, ascorbic acid and gallic acid.

**Fig. 5** Ferric reduction antioxidant power of the optimized essential oil combinations.
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Fig. 6 DPPH scavenging potential of essential oils, Ascorbic acid and tannic acid.

\[ \text{OBOG} = \text{optimized combination of } Ocimum basilicum \text{ and } Ocimum gratissimum; \text{ OBOU} = \text{optimized combination of } Ocimum basilicum \text{ and } Ocimum urticaefolium; \text{ OGOU} = \text{optimized combination of } Ocimum gratissimum \text{ and } Ocimum urticaefolium. \]

3.1.2 Antioxidant Effect of Essentials Oil on Linseed Oil

In comparison to the control (untreated) (Fig. 7), the most important reduction of the Cis double bonds was observed with the optimized combination of \( O. \) basilicum/\( O. \) urticaefolium. The optimized combination of \( O. \) urticaefolium/\( O. \) gratissimum and that of \( O. \) basilicum/\( O. \) urticaefolium had prevented at the same level the disappearance of the non-conjugated double bonds. The appearance of Trans double bound was prevented by the optimized combinations of \( O. \) urticaefolium/\( O. \) gratissimum and that of \( O. \) basilicum/\( O. \) urticaefolium. The appearance of aldehyde was prevented by the combination of \( O. \) urticaefolium/\( O. \) gratissimum and that of \( O. \) basilicum/\( O. \) urticaefolium. Regarding the appearance of a hydroxyl group, the optimized combination of \( O. \) basilicum/\( O. \) urticaefolium had significantly reduced their quantity.

3.1.3 The Effect of Essential Oils on Cyclo-Oxygenase

From Fig. 8, the essential oil of \( O. \) basilicum exerts a strong effect on cyclooxygenase 2 (IC\textsubscript{50} = 0.55 ± 0.041 µL/mL) in comparison to cyclooxygenase 1
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COX1 = cyclooxygenase 1; COX2 = cyclooxygenase 2; OB = *Ocimum basilicum*; OG = *Ocimum gratissimum*; OU = *Ocimum urticaefolium*.

**Fig. 8** Inhibitor percentage 50 of *Ocimum basilicum*, *Ocimum gratissimum* and *Ocimum urticaefolium* on cyclooxygenase 1 and cyclooxygenase 2.

COX1 = cyclooxygenase 1; COX2 = cyclooxygenase 2; OBOG = optimized combination of *Ocimum basilicum*/*Ocimum gratissimum*; OBOU = optimized combination of *Ocimum basilicum*/*Ocimum urticaefolium*; OGOU = optimized combination of *Ocimum gratissimum*/*Ocimum urticaefolium*.

**Fig. 9** Inhibition percentage of optimized combinations of essential oil on cyclooxygenase 1 and cyclooxygenase 2.

**Fig. 10** DPPH scavenging potential of the optimized essential oil combinations.

IC\(_{50}\) = 0.2275 ± 0.0045 µL/mL. The essential oil of *O. gratissimum* had also highlighted an effect on both isoforms of cyclo-oxygenase. The essential oil of *O. urticaefolium* has a strong effect on cyclooxygenase 1 (IC\(_{50}\) = 0.061 ± 0.041 µL/mL) and no effect on cyclooxygenase 2. From the essential oil used in this study, the latest one is the most selective toward cyclooxygenase 1 followed by that of *Ocimum basilicum*.

From Fig. 9, it was observed that all the optimized combination has an anti-cyclo-oxygenase effect on both isoform 1 and isoform 2. The effect of these combinations was less efficient on cyclooxygenase 2 with the percentage of inhibition of 67.5% for the combination of *Ocimum gratissimum/Ocimum urticaefolium*.

### 3.2 Discussion

When two chemicals are mixed together, they can interact with each other or stay on their own. Since in almost all reports on the essential oil’s activity, the pharmacological effect is attributed to the main compounds, we logically assumed that, they were no interaction between compounds in our optimized combination and that the observed effects were just the sum of the single effect of each compound. Therefore, an additive model was considered to predict the behavior of the signal in infrared spectroscopy.

The spectrum of the optimized combination of *O. basilicum/O. gratissimum*, *O. basilicum/O. urticaefolium*, and *O. gratissimum/O. urticaefolium* is different from
the predicted spectrum in Figs. 1-3 respectively. To the best of our knowledge, there are no reports on the analysis of the optimized combination of the essential oils in general and neither on the Ocimum essential oils. Based on the analysis of the interaction between αs-casein-bound and chitosan [30], Protein-Phenolic interaction in food [31], that goes in the same line with Stuart description of FTIR-ATR mixture [32, 33], the shift observed in this study was logically attributed to the interaction between the compounds of essential oils.

These essential oils alone and the optimized combination were analyzed for their antioxidant effect in DPPH, FRAP and linseed oil protection. The global observation of the results highlights that the optimized combination exerts a better effect compared to essential oils alone (Figs. 4-7, 10, 11).

Regarding the ferric reduction antioxidant power, all essential oils highlight the reduction of ferric ion. The essential oil of O. basilicum has a better effect in comparison to the report by Politeo et al. in 2007 [34]. In fact, these authors, working with linalool/estragole chemotype had obtained very low response 1 g/L. At that concentration the essential oil used in the present study, linalool/eugenol chemotypes [16, 29], had a better effect than gallic acid and vitamin C. The essential oil of O. gratissimum has the second most important Ferric Reduction Antioxidant power in comparison to O. urticaefolium. In the best of our reading, there is no report on the Ferric Reducing Antioxidant Power of the essential oil of O. gratissimum. Regarding Ocimum urticaefolium, this is the first report on their anti-free radical effect as well.

The optimized combinations of these essential oils presented in Fig. 5 highlighted a strong ferric reducing antioxidant power. We did not find in the literature the Ferric Reducing Antioxidant Power of the combination of Ocimum essential oils.

These essential oils were also tested for their effect on the DPPH radical. The essential oils of O. urticaefolium and that of O. basilicum had exhibited the best activity (Fig. 6). For O. basilicum, the results obtained here showed the same result as that of Pripdeevech et al. [34] with linalool/eugenol/eucalyptol and methyl chavicol. This is the first report of O. urticaefolium DPPH scavenging activity. This essential oil is eugenol/β-bisabolene/elimicine chemotype [35]. These compounds have a good scavenging effect on DPPH [35]. For Ocimum gratissimum, the results obtained here are in the same line with that previously reported by our team [11]. Putting all these reports together highlights the fact that, the presence of chemical cannot alone justify clearly the global effect of essential oil. The antioxidant effect on flaxseed oil obtained here is the same as was previously reported by Hzounda et al. [29].

We did not find the report on the anti-cyclooxygenase effect of the essential oils from O. basilicum, O. gratissimum and O. urticaefolium. However, the result presented here goes in the same line than that was previously published. In fact, in the review by Silveira et al. [36], some phenylpropanoids (class of terpenoids that include eugenol and elimicine) had exerted an inhibitory effect on both isoforms of cyclooxygenase. In another way, these results highlight the selectivity of the essential oil of Ocimum

![Fig. 11 Peroxide value of the linseed oil with and without antioxidant submitted in accelerated auto-oxidation.](image-url)
*urticaefolium* over the cyclooxygenase 1.

We did not find the report of the effect of the combination on the essential oil. The results of the optimized combination highlight at least the synergistic effect on the cyclooxygenase isoforms. This finding is similar to the preliminary results [29].

### 4. Conclusions

The results from this report present good antioxidant and anti-cyclooxygenase effect of the optimized combinations of *O. basilicum/O. gratissimum*, *O. basilicum/O. urticaefolium*, and *O. gratissimum/O. urticaefolium*. This suggests the potential uses of these optimized combinations as antioxidant and anti-inflammatory agents.

### Author Contribution

HFJB designed and wrote the manuscript, NJP, ELG and NCC designed the anti-radical part and proofread the manuscript, JDPM, BE, FBF and LMI designed and proofread the manuscript.

### Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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