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Original Research Article

OPTIMIZATION OF THE ANTI-INFLAMMATORY ACTIVITY OF CURCUMIN IN COMBINATION WITH EXTRACTS OF *PIPER CAPENSE* AND SOME AFRAMOMUM SPECIES

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ABSTRACT

Apart from its broad pharmacological potential, curcumin has low bioavailability, which affects its efficient activity in the body. To improve its bioavailability or optimize its pharmacological properties, its combination with extracts of certain plants is considered to be one of the avenues to be explored. The present study aimed to boost the anti-inflammatory potential of curcumin by combining it with extracts of *Piper capens* and *Aframomum* species namely *Aframomum melegueta*, *Aframomum angustifolium* and *Aframomum alboviolaceum*). To achieve this, an anti-inflammatory screening was carried out using the rat paw oedema inhibition test. This screening involved aqueous and hydro-ethanolic plant extracts alone and then in combination with natural curcumin. The phytochemical screening was performed following standard protocols. The findings of the anti-inflammatory screening showed that the aqueous extracts of *A. alboviolaceum* and the hydro-ethanolic extracts of *A. melegueta* seeds had interesting anti-inflammatory activity (p-value = 0.0000 and p-value = i0.0085 respectively). As for the formulated recipes, the findings revealed that recipes based on curcumin with the aqueous extracts of *P. capens*, or with the hydro-ethanolic extracts of *A. angustifolium* leaves and *A. melegueta* seeds had an optimized anti-inflammatory activity compared with curcumin. Phytochemical analyses revealed the presence of flavonoids, anthocyanins, tannins, quinones, terpenoids, alkaloids, phenolic acids and coumarins. The hydro-ethanolic extracts of *A. melegueta* seeds and *A. angustifolium* leaves had high levels of total polyphenols. In conclusion, the extracts of *P. capense*, *A. angustifolium* and *A. melegueta*, can be used as adjuvants to boost the anti-inflammatory potential of curcumin.

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INTRODUCTION

Curcumin, considered to be the main active ingredient in *Curcuma longa*, has a broad pharmacological potential (antioxidant, anti-inflammatory, anticancer, antimicrobial, antidiabetic, etc.) although its bioavailability is low [1-6]. The metabolic and physiological pathways, mechanisms of action and mediators involved, as well as the regulations, are just as multiple and complex, giving curcumin its high potential for action. It can act on several targets simultaneously [2, 5-7]. Regarding curcumin's anti-inflammatory activity, the literature reports that this potential is linked through the downregulation of various cytokines, such as TNF-α, IL-1, IL-6, IL-8, IL-12 and monocyte chemoattractant protein (MCP)-1 (also known as CCL2) [8-9]. In addition, curcumin has been shown to exert anti-inflammatory effects by suppressing the activation of nuclear factor Nk (NF-kB) [2, 10].

Several sources reported that curcumin has a low bioavailability i.e. serum concentrations of unchanged curcumin after administration were very low, even at high doses [11-13]. The low plasma or tissue levels of curcumin after administration are thought to be related to its poor absorption, rapid metabolism, chemical instability and rapid systemic elimination [2, 12, 14]. This low bioavailability does not allow considerable activity. Digestive, blood and cellular enzymes are the major "obstacles" to overcome to observe efficient physiological activity of curcumin [9, 15-17]. According to Agrawal et al. [11], the co-administration of curcumin with piperine, an alkaloid extracted from certain species of plants in the piperaceae family (*Piper nigrum*, *Piper longum*, etc.), can improve the serum concentration of curcumin. In fact, piperine is likely to inhibit the liver and intestinal enzymes involved in the metabolization of curcumin in the body [18-21]. Furthermore, the literature reports that co-administration of curcumin with certain essential oils could also improve the bioavailability of curcumin by increasing its blood concentration and also prolong its retention [11, 17, 22-24].

Piper capense belongs to the Piper genus and is rich in alkaloids, including piperine [25-27]. This plant is a potential candidate for improving the bioavailability of curcumin and optimizing the pharmacological activities of this active ingredient in turmeric [28-29]. In the Democratic Republic of the Congo (DRC), *Aframomum* species selected in the current study are used as pepper substitutes in food. According to previous studies, *Aframomum* species are rich in essential oils like diterpenoids and sesquiterpenoids as well in alkaloids and flavonoids [28-31]. Given their chemical composition, they may optimize the pharmacological properties of curcumin. Moreover, *Aframomum* extracts have been reported to possess the anti-inflammatory potential [30, 32-33]. They may therefore optimize the anti-inflammatory activity of curcumin through synergistic activity.

This study was carried out with a view to potentiating the anti-inflammatory activity of curcumin in combination with the extracts of other aromatic plants consumed locally in DRC.

MATERIALS AND METHODS

Plant Material

P. capense and *A. melegueta* seeds were bought at the Somba Zikida market in Kinshasa city from Kongo Central province. Those of *A. albolaceum* were bought at the Kianza market in the commune of Ngaba from the Plateau de Bateke in Kinshasa. In addition, the leaves of *A. angustifolium* were collected in the province of Nord Ubangi. All these samples were collected in DRC.

After collection, the species used in the current study were identified by a specialist botanist at the Hebarium of the Institut National d'Etudes et de Recherches Agronomiques (INERA, Faculty of Sciences and Technologies), University of Kinshasa. The identification of *P. capense*, *A. melegueta*, *A. alboviolaceum*, and *A. angustifolium*, was carried out by referring to the herbariums of the botanical garden of Meise (consulted online) and then those preserved at the herbarium of INERA, Faculty of Sciences and Technologies, University of Kinshasa of which collection numbers are: Malaisse F. 11388; Pauwels 4401, Robyns 4246, Robyns 4284 respectively. These plants were dried in the room temperature for two weeks and then were grounded to obtain the powder.

Extraction of Plants Material

Qualitative Phytochemical Screening

This test is used to identify the presence of large groups of secondary metabolites using specific reagents. The identification is performed by observing changes in coloration or the formation of precipitates resulting from the reaction between the chemical groups sought and the specific reagents used [34-36].

Phytochemical screening by Thin Layer Chromatography (TLC)

Phytochemical screening by Thin Layer Chromatography (TLC) was carried out according to the standard protocol described in the literature [36].

Preparation of the Samples

The anti-inflammatory activity was performed using the extracts of *P. capense, A. melegueta, A.* alboviolaceum and *A. angustifolium* which was evaluated individually and the recipes formulated using extracts of these plants and pure curcumin. One gram of pulverized extract from each sample was put in 10 mL of methanol for 24 hours at laboratory temperature and then the macerates were filtered using Whatman paper N = 1. These filtrates were used to test for flavonoids, phenolic acids, iridoids, anthocyanins and anthraquinones (10 µL deposit). Then, another one gram of pulverized extracts was macerated in 10 mL of dichloromethane. The mixture was placed in an ultrasonic bath for 45 minutes, and the macerates were filtered using Whatmann paper n°1. The filtrates were used to test for terpenoids and coumarins (10 µL deposit). At last, one gram of pulverized extracts was macerated in 1 mL of 10% ammonia

and 5 mL of ethyl acetate respectively. The resulting reaction mixtures were incubated under thermal agitation for 30 minutes. The filtrates (10 µL) were used to test for alkaloids.

Determination of Polyphenol Compounds

Fifty mg of the powder of each sample was weighed and placed in a flask to which 50 mL of distilled water or 80% ethanol was added. The flasks containing the mixtures were placed in the ultrasound bath for 45 minutes. The macerates obtained were then filtered using Whatmann number 1. The filtrates were used to determine the content of polyphenolic compounds. The determination of total polyphenol content, flavonoid content and tannin content was carried out as described previously [36-38].

Determination of Total Polyphenol Content

The Folin-Ciocalteu method was used to determine the total polyphenol content as previously described [39]. A reaction mixture consisting of: 0.2 mL of the extract to be analyzed, 2 mL of distilled water and 0.2 mL of Folin reagent was prepared. The mixture was incubated for 3 minutes and 0.4 mL sodium carbonate (20%) was added. The resulting solution was shaken well and then left to stand for an hour at laboratory temperature and protected from light. The same operation was carried out for the blank, except that instead of the extract 0.2 mL of 80% ethanol was added. After incubation, the reading of the absorbances was performed using a UV-visible spectrometer (Jenway 7615) at 725 nm. The experiment was performed in triplicate.

Furthermore, a range of gallic acid was prepared as controls (0.5 mg/mL to 0.03125 mg/mL). The content of polyphenolic compounds was expressed in mg gallic acid equivalent per gram of dry matter (mg/GAE/g) using the following calibration line equation (Equation 1):

$Y = 7.2852x + 0.0581$……Equation 1

$R^2 = 0.99$

where Y is the absorbance of the extract and
X is the gallic acid equivalent (mg/GAE/g).

Determination of Flavonoid Content

Flavonoids were determined using the method described by Mbadiko et al.. [39]. Flavonoids form a yellow complex with aluminium trichloride, absorbing at 415 nm. Therefore, a reaction mixture comprising 1 mL of the extract and 1 mL of 2% $AlCl_3$ was prepared.

The resulting solution was shaken well and then incubated at laboratory temperature in the dark for one hour. The same procedure was performed for the control, except that 1 mL of 80% ethanol or distilled water was added instead of the extract. After incubation, the absorbances were read using a spectrophotometer at 415 nm.

A range of standard quercetin (0.5 mg/mL to 0.03125 mg/mL) was used to calculate the flavonoid concentration in the extracts. The experiment was performed in triplicate. The results were expressed as mg quercetin equivalent per gram of dry matter (mg EQ/g DM) using the following equation for the calibration line (Equation 2):

$Y = 22.382x - 0.15$……Equation 2

y = absorbance of the extract,
x = quercetin equivalent (mg/g).
$R^2 = 0.9826$

Determination of Condensed Tannin Content

The vanillin method was used to determine the condensed tannin content as described in the literature. Condensed tannins are polymers of flavan-3-ols, also known as catechins, and flavan-3,4-diols known as leuco-anthocyanidins, or a mixture of the two. The reaction between vanillin and free flavan-3-ols and the terminal units of proanthocyanidins forms a red complex of which the intensity is proportional to the levels of flavanols present in the medium. This complex shows an absorption maximum at 500 nm [36, 40].

A reaction mixture consisting of 0.5 mL of aqueous or 80% hydro-ethanolic extracts, 1.5 mL of vanillin and 1.5 mL of chloridric acid was prepared. The resulting reaction solutions were then shaken well and incubated for 30 minutes at laboratory temperature and protected from light. The absorbances were read using a spectrophotometer (Jenway 7615) at 500 nm.

A standard range of concentration with catechin (0.01 to 0.1 mg/mL) was prepared in order to calculate the concentration of tannins for different extracts. The experiment was performed in triplicate. The equation for the following calibration line was used to determine the tannin content, expressed as mg catechin equivalent per g of corresponding dry matter (mg/CE/g DM) (Equation 3):

$y = 34.358x - 0.0132$……Equation 3
$R^2 = 0.9994$, where x is absorbance and y is catechin equivalent (mg/g).

Anti-inflammatory Activity

Animal Material

In this study, 69 adult Wistar rats 3-month aged and weighing between 100 and 250 grams each, were grouped into homogeneous batches of 3 rats/cage. The temperature was controlled and the light/dark cycle was 12 hours. Water was available at all times, as well as conventional rat food. Prior to doing the trials, the rats were given seven days to become used to the lab environment. Every experiment was conducted from 8:00 to 15:00 in a quiet room. Each series of trials had a different group of three rats.

Preparation of Extracts

The anti-inflammatory activity was performed using the extracts of *P. capense*, *A. melegueta*, *A. alboviolaceum* and *A. angustifolium* was evaluated individually and the recipes formulated using extracts of these plants and pure curcumin.

Exactly 10 g of each sample was macerated in 100 mL of distilled water or 80% ethanol for 24 hours. The macerates

obtained were filtered using Whatmann paper n°1 and the filtrates were transferred to petri dishes and placed in an oven at 40°C to evaporate the solvents and obtain the dry extracts. These were used to prepare the analytical solutions for each plant at a concentration of 100 mg/mL. As for the recipes based on the above-mentioned aromatic plant extracts and pure curcumin, 66.66 mg of pure curcumin and 33.34 mg of total dry extract of the aromatic plants (2:1) were weighed. Later, one mL of ethanol 80% was added to obtain an analysis recipe with a concentration of 100 mg/mL. The pure (natural) curcumin was purchased from Tokyo Chemical Industry (TCI CO 43 A, Lot W4NRA-KV).

Procedure for the Anti-inflammatory Test

The rat paw oedema inhibition test, generally used to assess the anti-inflammatory potential of the extracts, was used in the present study [41-43].

The rats were kept for 16 hours in an incubator with exclusive access to water. The rats were then weighed at the time of experimentation and the volumes of their hind legs (V0) were determined at time T0. Then 30 minutes later, 0.04 mL of 1% formalin was injected subcutaneously into the aponeurosis of the left hind paw of each rat under study.

and then administered orally 1 mL of the extract studied or pure curcumin (prepared with 80% ethanol) or recipes based on curcumin and the plant extracts.

The rats were divided into 21 groups, with 3 rats per group:

- 20 groups received orally 1 mL of the extracts or recipes formulated; with a dose of 100 mg/Kg,
- 2 groups received 1 mL of the standard molecules: curcumin (100 mg/Kg) and aspirin (20 mg/mL),
- One batch (control) received 1 mL of distilled water.

Following administration of the extracts or recipes, the development of oedema in the left hind paw, compared with the right hind paw, was monitored over a 6-hour experimental period with 1-h intervals. A caliper was used to determine the volume of paw oedema during the experiment.

The percentage of oedema inhibition was calculated using the formula below (Equation 4):

$$\% = \frac{P_0 - P_t}{P_0} x\ 100 \text{.....Equation 4}$$

The results obtained enabled us to assess the effect of our extracts on the development of oedema in the mouse.

The percentage of oedema inhibition was calculated according to the following formula (Equation 5):

$$P_t = \frac{V_t - V_0}{V_0} x\ 100 \text{.....Equation 5}$$

Where: P_0: average % increase in the control leg

P_t: average % increase in the treated leg

V_0: initial volume of the mouse leg

V_t: volume after injection of 1% formalin and treatment

Ethical Consideration

This study was approved by the Ethics Committee of the Life Sciences Department (Faculty of Science and Technology, University of Kinshasa, Kinshasa, DRC) under the number 007/CDB/GMK/SCI/UNIKIN.

Statistical Analysis

GraphPad Prism 6.0 and Statistix 8.0 software were used for all statistical analyses. For data presented as mean ± standard deviation, the ANOVA one-way test was used to compare sample means, followed by the LSD multiple comparison test. The significance level was at α = 0.05 and the interval of confidence was 95%.

RESULTS

Phytochemical Screening

Qualitative Phytochemical Screening

The results of the phytochemical screening in solution are presented briefly in Table 1. It shows that all the extracts analyzed contain total polyphenols, leuco-anthocyanins, bound quinones, alkaloids and catechic tannins. Flavones were only found in *P. capense* seed extracts, while flavonones were found in extracts of *A. melegueta*, *A. alboviolaceum* and *A. angustifolium*. Anthocyanins were found in extracts of *A. alboviolaceum*, *A. angustifolium* and *P. capens*. Gallic tannins were found in extracts of *A. melegueta*. Saponins were found in extracts of *A. angustifolium*. Steroids and free quinones were found in *P. capens* seed extracts, while terpenoids were found in *A. angustifolium* extracts.

Phytochemical TLC Screening

The figures 1-7 show the chromatoplates after phytochemical analysis of extracts of *P. capense* (PC), *A. melegueta* (AF), *A. alboviolaceum* (AA) and *A. angustifolium* (Ag) by TLC.

Polyphenolic Compound Content

The results of the determination of polyphenolic compounds in the aqueous and hydro-ethanolic extracts of the plants studied are given in Table 2.

Table 2 shows that the hydro-ethanolic extracts of *A. melegueta* contain a high level of total polyphenols (839.59 ± 0.26 mg/EAG/g), followed by those of the leaves of *A. angustifolium* (651.90 ± 0.53 mg/EAG/g), the aqueous extracts of *A. melegueta* (250.23), the hydro-ethanolic extracts of *P. capense* (186.85 mg/EAG/g). While the hydro-ethanolic extracts of *A. alboviolaceum* (94.990 mg/EAG/g), followed by aqueous extracts of *P. capense* (62.500 mg/EAG/g); followed by aqueous extracts of A. *angistifolium* (61.150 mg/EAG/g) and aqueous extracts of *A. alboviolaceum* (38,780 mg/EAG/g).

Significant levels of flavonoids (p-value = 0.0000) were found in the hydro-ethanolic extracts of *A. alboviolaceum* (59.18±0.91 mg/QE/g), followed by extracts of *A. angustifolium* leaves (51.85±0.31 mg/QE/g), followed by extracts of *P. capense* seeds (41.46±4.2 mg/QE/g), followed by aqueous extracts of *P. capense* seeds (29.60±2.5 mg/QE/g), followed

by aqueous extracts of *A. angustifolium* leaves (23.67±1.90 mg/QE/g), followed by hydroethanol extracts of *A. melegueta* seeds (22.30±0.25 mg/QE/g), followed by aqueous extracts of *A. alboviolaceum* (15.28±0.11 mg/QE/g) and aqueous extracts of *A. melegueta* (14.98±0.49 mg/QE/g).

As for tannins, high levels were found in ethanolic extracts of *P. capense* seeds (49.00±0.09 mg/CE/g), followed by aqueous extracts of *A. melegueta* seeds (41.80±0.27 mg/CE/g), followed by aqueous extracts of *A. angustifolium* leaves (30.87±0.09 mg/CE/g), followed by ethanolic extracts of *A. melegueta* seeds (28.36±0.20 mg/CE/g) and *A. angustifolium* leaves (27.47±0.65 mg/CE/g) followed by aqueous extracts of A*. alboviolaceum* (14.86±0.20 mg/CE/g), followed by aqueous extracts of *P. capense* (10.75±1.57 mg/QE/g) and ethanolic extracts of *A. alboviolaceum* seeds (10.53±0.10 mg/QE/g).

Anti-inflammatory Activity using the Rat Paw Oedema Inhibition Test

The results of the anti-inflammatory activity of aqueous extracts of different species and recipes are presented in Table 3. Table 3 shows that for the extracts analyzed individually, aqueous extracts of *A. alboviolaceum* seeds showed interesting activity after 60 minutes of the experiment (% inhibition: 84.75±4.57) compared with aqueous extracts of *A. angustifolium* leaves (% inhibition: 67.14±2.91), *A. melegueta* seeds (% inhibition: 56.67±2.57) and *P. capense* seeds (% inhibition: 51.33±12.39), curcumin (% inhibition: 80.19±2.28) or aspirin used as reference compounds (% inhibition: 76.25±0.21). Comparing the activity of the extracts analyzed separately with that of the recipes based on curcumin and the plant extracts, Table 3 shows that the recipe based on curcumin and the aqueous extract of *P. capens* seeds showed high activity (% inhibition: 91.29±4.6), followed by the aqueous extracts of *A. alboviolaceum* seeds (% inhibition: 84.75±4.57) and the recipe based on curcumin and aqueous extracts of *A. alboviolaceum* seeds (% inhibition: 82.50±2.64). The multiple comparison test revealed that the inhibition percentages of the last two extracts were close to that of curcumin (% inhibition: 80.19±2.28). Moreover, the aqueous extracts of *P. capense* analyzed separately had very low anti-inflammatory activity; although the recipe based on curcumin and aqueous extracts of *P. capense* showed significant activity exceeding all the other extracts.

Table 4 shows the results of the anti-inflammatory activity of hydro-ethanol extracts or recipes based on curcumin and hydro-ethanolic extracts of our plants. It shows that recipes based on curcumin and hydro-ethanolic extracts of *A. angustifolium* leaves or *A. melegueta* seeds have an interesting anti-inflammatory activity (% inhibition: 87.00±0.15 and 82.89±4.63 respectively). Their action lasts for 6 hours. Hydro-ethanolic extracts of *A. melegueta* and *A. angustifolium* appear to enhance the anti-inflammatory activity of curcumin. Although the multiple comparison test revealed that there was no significant difference in the anti-inflammatory activity of the extracts over 5 hours of experiments.

Furthermore, by comparing the average inhibition percentages of the hydro-ethanolic extracts of the plants analyzed individually, Table 4 reveals that after one hour of experimentation, the extracts of *A. melegueta* seeds show an interesting activity (% inhibition: 80.63±10.46), compared to those of *A. alboviolaceum* seeds (% inhibition: 77.18±5.46), *P. capense* seeds (% inhibition: 76.88±4.54) and *A. angustifolium* leaves (% inhibition: 73.63±6.41). In addition, unlike the activity of the extracts of *Aframomum*, which increased over time, that of *P. capense* was not interesting between 2 and 6 hours of the experiment. However, the multiple comparison test showed that there was no significant difference compared to the anti-inflammatory activity of the extracts of the plants analyzed individually after 5 hours of experimentation.

Table 1: Phytochemical composition of different extracts used

Extracts	**Phytocompounds**		***Aframomum***			***Piper capense* (Seeds)**
			Melegueta (seeds)	*alboviolaceum* (seeds)	*angustifolium* (Leaves)	
Aqueous	Total polyphenols		+	+	+	+
	Flavonoids	Flavones	-	-	-	+
		Flavonones	+	+	+	-
		Flavonols	-	-	-	-
	Anthocyanins		-	+	+	+
	Leucoanthocyanins		+	+	+	+
	Bound quinones		+	+	+	+
	Alkaloids		+	+	+	+
	Tannins	Catechic	+	+	+	+
		Gallic	+	-	-	-
	Saponins		-	+	+	-
Hydro-ethanol	Steroids		+	-	-	+
	triterpenoids		-	-	+	-
	Free quinones		+	-	-	+

Legend: '**+**' : present; '**-**' : absent

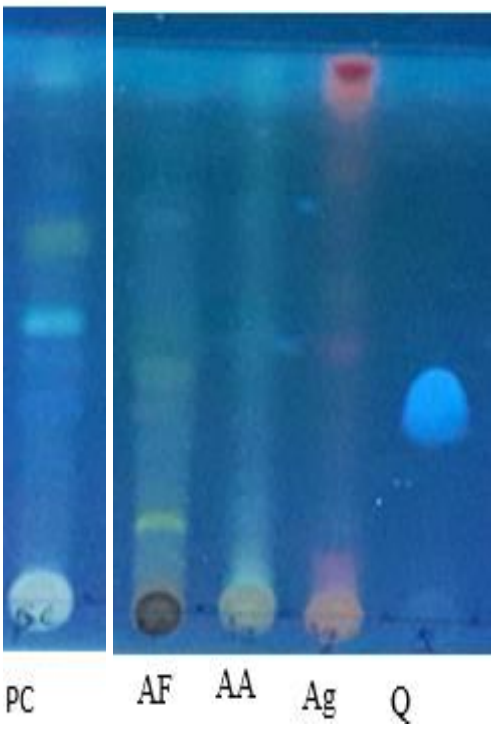

Figure 1.1: Alkaloid chromatoplates
Legend: AF: *A. melegueta*; AA: *A. alboviolaceum*; AAg: *A. angustifolium*; PC: *Piper capense*; Q; Quinine

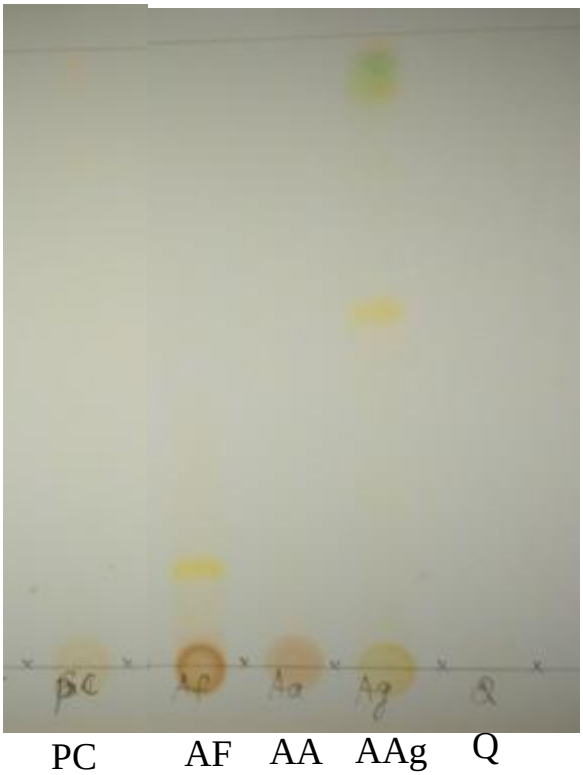

Figure 1.2: Chromatoplates of alkaloids observed under visible light

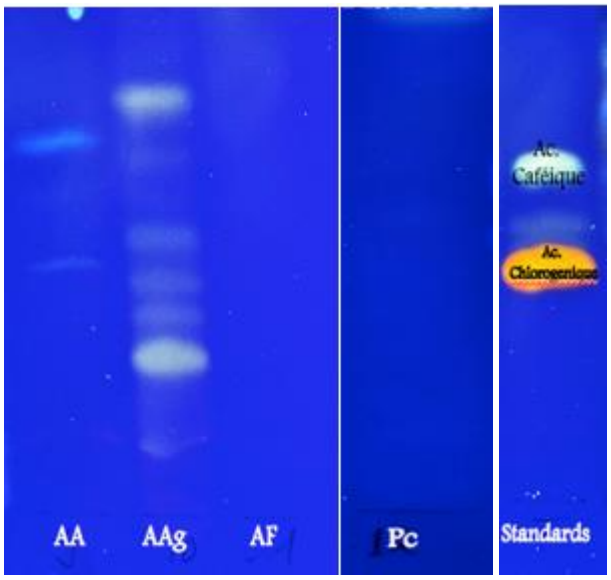

Figure 2.1: Chromatoplates of flavonoids and phenolic acids developed after analysis in mobile phase 1

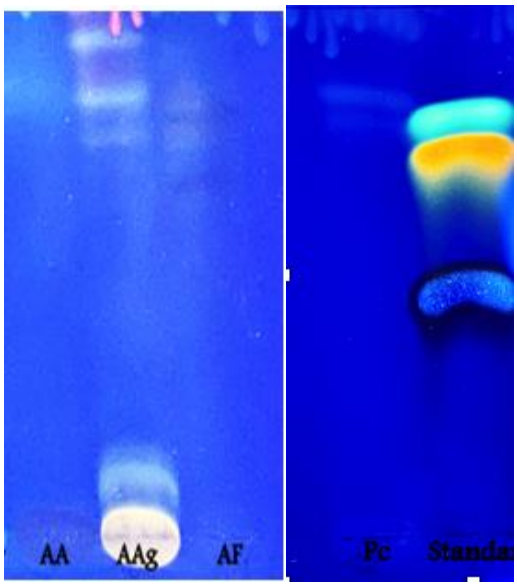

Figure 2.2: Chromatoplates of flavonoids and phenolic acids developed after analysis in mobile phase 2

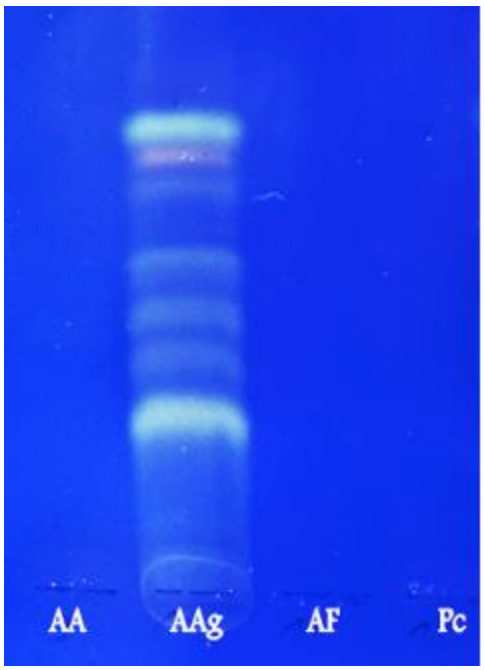

Figure 2.3: Chromatoplates of flavonoids and phenolic acids developed after analysis in mobile phase 3

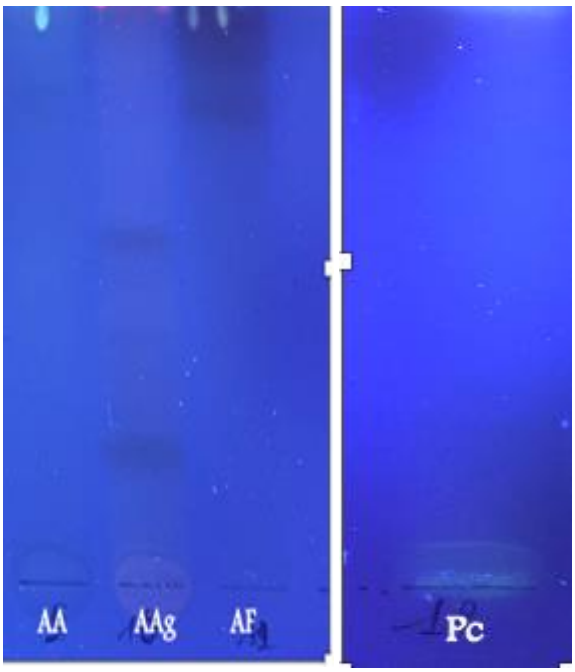

Figure 3: Chromatoplates of anthraquinones

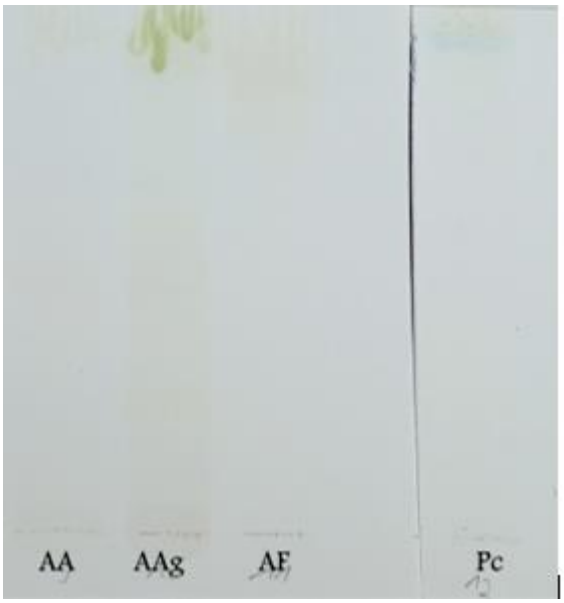

Figure 4: Iridoid chromatoplate

Figure 5: Anthocyanin chromatoplate

Terpenoid

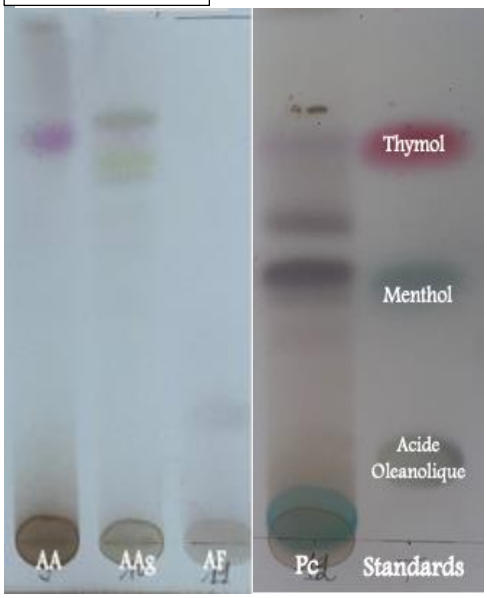

Figure 6: Terpenoid chromatoplate

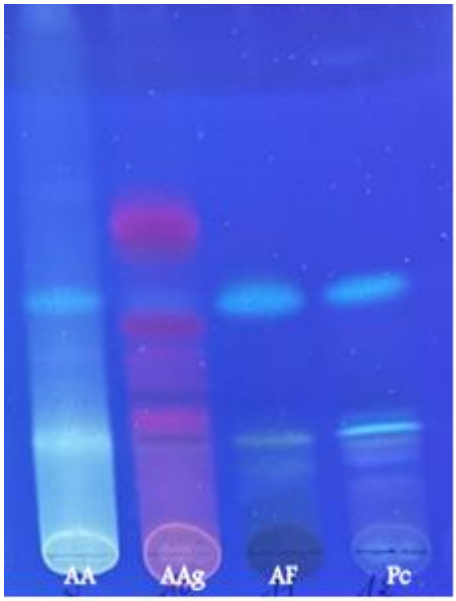

Figure 7: Coumarins chromatoplate

Table 2: Determination of polyphenolic content in extracts of *P. capense* and some species of *Aframomum* genus

Polyphenolic compounds	***P. capens***		***A. melegueta***		***A. alboviolaceum***		***A. angustifolium***	
	Aqueous extract	**Hydro-ethanolic extracts**	**Aqueous extract**	**Hydro-ethanolic extracts**	**Aqueous extract**	**Hydro-ethanolic extracts**	**Aqueous extract**	**Hydro-ethanolic extracts**
Total polyphenols **(mg/EAG/g)**	62.50 ± 0.29^{f}	186.85 ±0.18^{d}	250.26± 0.13^{c}	839.59 ± 0.26^{a}	38.78 ± 2.29^{g}	95.25 ± 0.26^{e}	61.30±0.80^{f}	651.90 ±0.53^{b}
Flavonoids **(mg/QE/g)**	29.60±2.8^{d}	41.46±4.2^{c}	14.98±0.49^{f}	22.30±0.25^{e}	15.28±0.11^{f}	59.18±0.91^{a}	23.67±1.90^{e}	51.85±0.31^{b}
Tannins **(mg/CE/g)**	10.75±1.57^{f}	49.00±0.09^{a}	41.80±0.27^{b}	28.36±0.20^{d}	14.86±0.20^{e}	10.53±0.10^{f}	30.87±0.09^{c}	27.47±0.65^{d}

Legend: GAE: Gallic acid equivalent; QE: Quercetin equivalent; CE: Catechin equivalent. The increasing order of the degree of concentration of polyphenolic compounds is as follows: a>b>c>d>e>f. The same letters on the values show that the difference is not significant. For total polyphenols: comparison of total polyphenol content in aqueous and hydroethanolic extracts: P-value: 0.0000; F: 306244,12; critical comparison value: 2,6962; For flavonoids: comparison of total flavonoids content in aqueous and hydroethanolic extracts: P-value: 0.0000; F: 221,58; critical comparison value: 5,5116; For tannins: comparison of total tannins content in aqueous and hydroethanolic extracts: P-value: 0.0000; F: 1567,64; critical comparison value: 1.7525.

Table 3: The anti-inflammatory activity of aqueous extract of different species and recipes based on curcumin and their combination in a 2:1 ratio

Extracts or recipes	Dose (mg/Kg)	% inhibition (n = 3)					
		1 h	2 h	3 h	4 h	5 h	6 h
A. melegueta (Seeds/ Aqueous extracts)	100	56.67±2.57^{d,e}	67.57±2.02^{d,e}	71.61±2.57^{d}	79.91±0.82^{c,d}	86.92±0.32^{c,d}	96.72±1.66^{a}
A. angustifolium (Leaves/ Aqueous extracts)	100	67.14±2.91^{c,d}	69.63±2.60^{d}	70.82±2.38^{d}	72.79±3.52^{d}	74.49±3.08^{d}	77.74±2.57^{c,d}
A. Alboviolaceum (Seeds/ Aqueous extracts)	100	84.75±4.57^{ab}	87.98±4,38^{a,b}	89.96±4.04^{a,b}	90.81±4.68^{a,b}	93.90±3.45^{a,b}	97.15±2.95^{a}
P. capense (Seeds/ Aqueous extracts)	100	51.33±12.39^{e}	61.09±3.53^{e}	69.70±4.19^{d}	72.62±5.55^{d}	76.32±5.31^{d}	81.94±10.05^{c,d}
Curcumin-*A. meleguea* (Seeds/Aqueous extracts)	100	75.99±1.18^{b,c}	80.94±1.29^{b,c}	84.27±1.28^{b,c}	87.43±1.23^{a,b,c}	89.32±1.23^{a,b,c}	92.62±1.80^{a,b,c}
Curcumin-*A. angustifolium* (Leaves/Aqueous extract)	100	76.52±1.86^{b,c}	79.81±3.20^{c}	82.54±1.74^{b,c}	84.92±5.22^{b,c}	86.24±2.76^{b,c}	89.42±1.34^{a,b,c}
Curcumin-*A. alboviolaceum* (Seeds/Aqueous extracts)	100	82.50±2.64^{a,b}	84.68±1.80^{b,c}	85.98±2.45^{b,c}	87.02±1.76^{b,c}	88.60±3.50^{b,c}	89.62±3.20^{a,b,c}
Curcumin - *P. capense* (Seeds/Aqueous extracts)	100	91.29±4.6^{a}	93.44±3.47^{a}	94.79±2.61^{a}	95.87±1.92^{a}	96.96±1.24^{a}	97.66±0.82^{a}
Curcumin	100	80.19±2.28^{a,b,c}	82.84±1.79^{b,c}	84.79±2.60^{b,c}	87.34±2.36^{a,b,c}	90.82±2.06^{a,b,c}	92.96±2.16^{a,b}
Aspirin	20	76.25±0.21^{b,c}	78.00±0.87^{c}	80.31±0.79^{c}	82.75±0.64^{b,c}	83.66±0.76^{b,c}	85.38±0.61^{b,c,d}
p-value		0.0000	0.1703	0.0000	0.0000		0.0000
F		20.51	39.80	30.06	18.19		9.70
LSD		13.794	7.844	7.703	6.692		10.819

Legend: The letters on the inhibition percentage average indicate the levels of inhibition of inflammation by the extracts. These levels follow the following ascending order: a>ab>abc>bc>c>cd>d>de>e>f>g. Averages with the same letters indicate a similar level of activity (p-value = 0.0000).

Table 4: The anti-inflammatory activity of the hydro-ethanolic extracts and recipes based on curcumin and hydro-ethanolic extracts combined in a 2:1 ratio.

Sample or recipes	Dose (mg/Kg)	% inhibition (n=3)					
		1 h	2 h	3 h	4 h	5 h	6 h
A. Melegueta (Seeds/ hydro-ethanolic extracts)	100	80.63±10.46^{a}	84.32±9.58^{a}	86.70±8.59^{a}	91.18±7.21^{a}	93.23±6.20^{a}	93.92±5.90^{ab}
A. angustifolium (Leaves/ hydro-ethanolic extracts)	100	73.63±6.41^{a}	80.71±5.43^{a}	83.24±4.82^{a}	87.17±3.65^{a}	89.77±2.67^{a}	90.88±2.39^{ab}
A. alboviolaceum (Seeds/ hydro-ethanolic extracts)	100	77.18±5.72^{a}	80.47±5.68^{a}	84.00±5.61^{a}	87.82±5.17^{a}	89.69±4.98^{a}	90.84±5.10^{ab}
P. capense (Seeds/ hydro-ethanolic extracts)	100	76.88±4.54^{a}	79.95±3.17^{a}	81.54±3.54^{a}	82.99±3.38^{a}	83.75±3.53^{a}	84.10±3.11^{b}
Curcumin-*A. melegueta* (Seeds/ hydro-ethanolic extracts)	100	82.89±4.63^{a}	87.02±3.71^{a}	88.11±5.19^{a}	89.90±4.65^{a}	91.42±4.74^{a}	94.82±4.70^{a}
Curcurmin-*A. angustifolium* (Lesaves/ hydro-ethanolic extracts)	100	87.00±0.15^{a}	89.60±0.79^{a}	89.99±0.53^{a}	91.84±0.98^{a}	93.38±1.29^{a}	95.20±0.85^{a}
Curcumin-A. alboviolaceum (Seeds/ hydro-ethanolic extracts)	100	81.42±3.72^{a}	83.81±2.7^{a}	87.55±4.85^{a}	89.77±5.31^{a}	91.52±4.63^{a}	94.64±3.96^{a}
Curcumin-*P. capense* (Seeds/ hydro-ethanolic extracts)	100	80.16±0.66^{a}	83.49±0.6^{a}	84.81±0.48^{a}	87.12±1.58^{a}	88.63±1.77^{a}	91.62±2.59^{ab}
Curcumin	100	80.19±2.28^{a}	82.84±1.7^{a}	84.79±2.60^{a}	87.34±2.36^{a}	90.82±2.06^{a}	92.96±2.16^{ab}
Aspirin	20	76.25±0.21^{a}	78.00±0.87^{a}	80.31±0.79^{a}	82.75±0.64^{a}	83.66±0.76^{a}	85.38±0.61^{b}
p-value		0.1355	0.1225	0.2649	0.1409	0.035	0.0085
F		1.78	1.84	3.37	1.76	2.60	3.57
LSD		14.303	12.82	12.90	11.66	10.675	10.28

Legend: The letters on inhibition percentage average indicate the levels of inhibition of inflammation by the extracts. These levels follow the following ascending order: a>ab>b. Averages with the same letters indicate a similar level of activity (p-value = 0.0000).

DISCUSSION

Phytochemical Screening

Qualitative phytochemical screening revealed that these different plants did not have the same secondary metabolite composition. This test showed that leuco-anthocyanins, bound quinones, alkaloids and catechic tannins were present in most of the plants studied.

While TLC results revealed the presence of flavonoids in the extracts of *A. angustifolium* and A. *melegueta*, as well as phenolic acids in the extracts of all the plants. The analysis of various chromatoplates developed showed that these plants contained terpenoids, coumarins and alkaloids. Some studies reported that plants containing polyphenols, sterols, alkaloids, saponins, coumarins, and terpenes have anti-inflammatory properties, and most of these metabolites act by blocking the cyclooxygenase (Cox) and lipoxygenase pathways [44-47].

The analysis of polyphenolic compounds revealed that the hydro-ethanolic extracts of *A. melegueta* seeds had a high content of total polyphenols compared to other plant extracts. Flavonoids were also present in large quantities in the hydro-ethanolic extracts of *A. alboviolaceum* seeds compared with other extracts analyzed. High levels of tannins were reported in the hydro-ethanolic extracts of P. capense.

Polyphenolic compounds have anti-inflammatory potential by inhibiting Cox: Polyphenolic compounds have anti-inflammatory potential by inhibiting Cox activity [45-48]. Yet, active plant substances with anti-inflammatory properties act at several levels of the inflammatory reaction by inhibiting: the arachidonic acid metabolism, signal transduction mechanisms involved in inflammatory cell activation, synthesis of pro-inflammatory cytokines, expression of adhesion molecules, activation of nuclear factor kappa-B and production of reactive oxygen species [48-53]. This is the case with *C. longa*, which contains curcumin, a polyphenol that inhibits the production of prostaglandin E2 and the expression of Cox 2 [1,5, 7]. However, it has no effect on the expression of Cox 1 but it inhibits IL-6 and IL-8 gene expression and reduces NO production and inducible NO synthase (NOS) enzyme expression in a dose-dependent manner as well the nuclear factor kappa-B [51-54].

Anti-inflammatory Activity using the Rat Paw Oedema Inhibition Test

The aqueous extracts of *A. alboviolaceum* revealed an interesting anti-inflammatory activity compared with other extracts analyzed separately. The activity of these extracts was spread over six hours of the experiment. As for the recipes prepared with curcumin and total aqueous extracts of different plants, the one based on curcumin and total aqueous extracts of *P. capense* showed interesting activity. This shows that aqueous extracts of *P. capense* can optimize the anti-inflammatory action of curcumin. This interesting activity is thought to be associated with the presence of alkaloids like piperine, in *P. capense* extracts which, according to the literature, are likely to improve the absorption and bioavailability of curcumin, by inhibiting the action of intestinal and hepatic enzymes involved in the rapid metabolization of curcumin [2, 11, 19, 54-56].

In view of the results reported in Table 3, it is evident that the aqueous extract of *A. alboviolaceum* would also be a good candidate in improving the bioavailability and anti-inflammatory activity of curcumin.

Regarding the recipes prepared with curcumin and hydro-ethanolic extracts of the plants studied, those based on curcumin and total hydro-ethanolic extracts of *A. angustifolium* or *A. melegueta* leaves had an interesting activity compared with curcumin analyzed separately. The optimization of the action of curcumin associated with the hydro-ethanolic extracts of these plants would be linked either to the synergy of action between curcumin and the phytocompounds of these plants, or to the susceptibility of these phytocompounds to improve the absorption and bioavailability of curcumin. There is no information in the literature about the optimization of curcumin action by combining it with the total extracts of the plants analyzed in this study.

CONCLUSION

Based on these findings, the present work was carried out to demonstrate that aqueous and hydro-ethanolic extracts of *P. capense* and some *Aframomum* species have anti-inflammatory activity and they can, in combination with curcumin, boost its anti-inflammatory potential. The results of the anti-inflammatory screening showed that the aqueous extracts of *A. alboviolceum* and the hydro-ethanolic extracts of *A. melegueta* analyzed individually showed interesting activity compared to other extracts. This activity is thought to be associated with the presence of polyphenolic compounds, terpenoids and alkaloids, which were identified by phytochemical screening. About the recipes analyzed, the aqueous extracts of *P. capense* and curcumin or hydro-ethanolic extracts of *A. angustifolium* leaves or *A. melegueta* seeds and curcumin showed interesting anti-inflammatory activity. Based on our results, it is evident that extracts of *P. capense*, *A. angustifolium* and A. *melegueta* are likely to optimize the anti-inflammatory potential of curcumin. These extracts are thought to contain phytocompounds which, in synergy with curcumin, can boost its anti-inflammatory potential, or which can be used as adjuvants to improve the absorption and bioavailability of curcumin.

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AUTHORS' CONTRIBUTION

CMM: Study conception, Investigation, Data curation & analysis, Original Draft preparation, JPKN: Data curation & analysis, Interpretation of data, Revision of the original draft,

GNB: Preparation of samples, Investigation, Data curation, Original draft preparation, JPMN: Preparation of samples, Analysis and Interpretation of data, GKM: Investigation, Data Curation, JBI: Investigation, Analysis and Interpretation of data; MCAY: Data analysis, Supervision, NKN: Data analysis, Revision of draft, Supervision; PKM: Data curation & analysis, Revision of the original draft; TFM: Study conception, Supervision; PTM: Study conception, Supervision. All authors read and approved the final version of this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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