WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 11, 135-146. Research Article ISSN 2277– 7105

ANTI-INFLAMMATORY ACTIVITY OF THE COMBINATION OF AQUEOUS EXTRACTS OF THE BARK OF *MUSANGA CECROPIOIDES* (CECROPIACEAE) AND FRUITS OF *PICRALIMA NITIDA* (APOCYNACEAE)

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Article Received on
07 July 2021,
Revised on 27 July 2021,
Accepted on 18 August 2021
DOI: 10.20959/wjpps202111-21424

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ABSTRACT

Musanga cecropioides (Cecropiaceae) and *Picralima nitida* (Apocynaceae) are nutraceutics plants widely used in tropical pharmacopoeia. The objective of the present work was to evaluate the anti-inflammatory activity of *Musanga cecropioides* barks and *Picralima nitida* fruits in combination. The combination of aqueous extracts of *Musanga cecropioides* and *Picralima nitida* was carried out according to the proportions 50/50, 70/30 and 30/70. The evaluation of the anti-inflammatory activity was demonstrated firstly by an *in-vitro* test according to the denaturation of hen egg albumin at concentrations of 50, 100, 200, 400 and 800 µg/ml, and secondly by an *in-vivo* test according to the carrageenan-induced swelling of the paw on an animal model, at concentrations of 50, 100, 200 and 400 mg/kg. The results of the phytochemical screening show that the extracts obtained contain the following families of compounds: alkaloids, sterols, phenols, flavonoids, anthraquinons, saponins and tannins. The combination of the aqueous extracts in 70/30 proportion significantly inhibits the denaturation of albumin (75.07%) compared to each extract. The similar effect was observed on oedema with a maximum at the third hour (71.27%). The combination of *Musanga cecropioides* bark extracts and *Picralima nitida* mature fruits optimizes the anti-inflammatory activity.

KEYWORDS: *Musanga cecropioides*, *Picralima nitida*, combination, protein denaturation, oedema, anti-inflammatory activity

1- INTRODUCTION

Inflammation is a transient biological response of the body to exogenous (trauma) or endogenous stimuli.[1] It is usually a beneficial process, with the aim of eliminating the pathogen and repairing tissue damage. Sometimes inflammation can be harmful due to the aggressiveness of the pathogen, its persistence, its location, abnormal regulation of the inflammatory process or quantitative and qualitative abnormalities of the cells involved in inflammation.[2] Current treatment involves steroidal (ISA) or non-steroidal (NSAID) anti-inflammatory drugs. These drugs can effectively reverse the inflammatory syndrome, but are also associated with sometimes very serious side effects.[3] These include gastric damage, esophagitis and drug-induced hepatitis.[4]

This controversy around synthetic anti-inflammatory drugs is growing, to the point where the use of non-toxic plants is becoming a relevant alternative. Some plants, due to their biological activity, can ensure the management of the inflammatory syndrome, with little or no harmful effect.[5] In this regard, plant extracts are used for the reversal of the inflammatory syndrome. In recent years, approaches to combine plants in order to optimize anti-inflammatory activity are gaining ground.[6,7] In tropical ecosystems, two nutraceutics plants seem to be representative in the pharmacopoeia, namely *Musanga cecropioides* and *Picralima nitida*.

Musanga cecropioides (Cecropiaceae) is a medium-sized tree reaching thirty metres in height, dioecious, deciduous. In pharmacopoeia, the leaves are used in the treatment of hypertension and asthma in West Africa and as an anti-diabetic in Central Africa.[8,9] Some therapeutic virtues attributed to *Musanga cecropioides* are supported by ethno-pharmacological research. For example, Ayinde et al[10] demonstrated the hypotensive effect of bark extracts. Toxicological studies have shown that aqueous extracts of bark are relatively harmless by the oral route.[11] Regarding the anti-inflammatory effect, Abimbola et al[12] showed that *Musanga cecropioides* leaf extract has the properties to reverse the inflammatory syndrome.

Picralima nitida (Apocynaceae) is a 35 m tall tree, containing white latex in all its hairless parts. The decoction of the fruits is a medicinal product used to treat cough or typhoid fever and malaria. Fakeye et al[13] showed that the bark of the roots and fruits of *Picralima nitida*

contain akuammigin, akuammicin, deacetylpicralin and the leaves akammin, picraphylin and melinonin. Other work has shown that *Picralima nitida* seed extracts have anti-inflammatory activity.[14] Recent work has shown that the combination of aqueous extracts of *Musanga cecropioides* barks and *Picralima nitida* fruits is devoid of acute and subchronic oral toxicity.[15]

However, few studies have investigated the anti-inflammatory activity of *Musanga cecropioides* barks and *Picralima nitida* fruits, let alone the combination of these two plants. The present work aims to evaluate the *in-vitro* and *in-vivo* anti-inflammatory activity of the combination of aqueous extracts of *Musanga cecropioides* trunk bark and *Picralima nitida* mature fruits.

2- MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant material

The fruits of *Picralima nitida* were collected (November 2019) in the village of Dogmoa, Nkongsamba Sub division, Moungo Division, Littoral Region of Cameroon, and subsequently identified at the National Herbarium of Cameroon by comparison with specimens N°41604/HNC. The *Musanga cecropioides* barks were collected in a peri-road forest in the village of Missolè in the Sub division of Dibamba, Sanaga Maritime Division, littoral region in November 2019, then identified at the National Herbarium of Cameroon by comparison with specimens N°289/HNC.

2.1.2. Animal material

The study was conducted on albino rats of the Wistar strain (*Rattus norvegicus*). The animals, weighing between 140 and 175 g, were supplied by the animal house of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. They were marked and caged, then acclimatized to the experimental conditions (room temperature, natural light cycle, sufficient ventilation) for one week before the start of the experiment. The animals were fed daily on a complete diet (55% corn meal, 13% soybean meal, 10% wheat meal, 12% fish meal, 5% bone meal, 5% vitamin and mineral complex) and free access to drinking water.

2.2 Methods

2.2.1. Preparation of extracts of *Musanga cecropioides* and *Picralima nitida*

Fresh peels of *Musanga cecropioides* and fresh mature fruits of *Picralima nitida* were cut and dried in the shade for 15 days. The separately prepared extracts were pulverized to small particles. The powder of each plant (1000 g) was macerated in distilled water (10 L) for 48 hours. The resulting mixture was filtered under vacuum on Buchner n°2 paper. The two filtrates obtained were evaporated at 50°C in an oven and the dry extracts were obtained. The combination was carried out in the following proportions: 50/50, 70/30, 30/70. The extraction yield by weight was determined according to the formula: R (%)= ($M1/M0$) × 100, and gave 8 and 6.85% for *Musanga cecropioides* and *Picralima nitida* respectively.

2.2.2. Phytochemical screening

Phytochemical screening was carried out using the usual methods for revealing families of secondary metabolites in aqueous extracts of *Musanga cecropioides* and *Pricralima nitida*.

2.2.3. Activity of aqueous extracts of *Musanga cecropiodes* and *Pricralima nitida*

2.2.3.1. Assessment of anti-inflammatory activity *in-vitro*

The evaluation of the *in-vitro* anti-inflammatory activity was conducted by protein denaturation using hen egg albumin.[16] The reaction mixture consisted of 0.2 mL of hen egg albumin, 2.8 mL of phosphate buffer (pH 6.4), and 2 mL of the aqueous extract of varying concentrations at different proportions, 50/50, 70 0/30, 30/70 (*Musanga cecropioides* and *Picralima nitida*). The final volume of the reaction mixture was 5 mL, giving final concentrations of 50, 100, 200, 400 and 800 µL. A similar volume of distilled water was used as control. Diclofenac sodium at final concentrations similar to those of the extracts was used as a reference drug. The different reaction mixtures were incubated at 37 ± 2°C in the water bath for 15 min and heated at 70 ± 5°C for 5 min. Afterwards, the mixtures were cooled, the absorbance was read at 660 nm.

The inhibition percentage of protein denaturation was calculated using the following formula:

$$IP = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

IP: Inhibition percentage

Abs control = absorbance control

Abs sample = absorbance sample

2.2.3.2. Evaluation of the anti-inflammatory activity *in -vivo*

The anti-inflammatory effect of the combination was assessed in rats by measuring the change in oedema induced by injection of carrageenan.[17] The control batches received distilled water (5 mL/kg body weight) and the reference substance, diclofenac sodium (50 mg/kg body weight), respectively, orally. The test batches were orally administered the combination of the aqueous extract of *Musanga cecropioides* bark and the mature fruit of *Picralima nitida* at doses of 50, 100, 200 and 400 mg/kg body weight respectively. One hour later, inflammation was induced in each rat in the plantar pad of the right hind paw by injection of 50 µL of a 1% carrageenan solution diluted in 0.9% NaCl. The diameters of the treated paw of each rat were measured 1 h before and then 1 h, 2 h and 3 h after carrageenan injection using a calliper[18]. Measurements of the change in diameter of the treated paw of each rat were made. The anti-inflammatory activity of the combination of the aqueous extracts and the reference substance was evaluated according to the following formula:

$$Inhibition\ (\%) = \frac{((Dn - Do)c - (Dn - Do)t)}{(Dn - Do)c} x\ 100$$

Dn: volume of the treated leg at time (t);

Do: volume of the paw before carrageenan injection;

(Dn - Do) c: mean difference in paw diameter variation of control rats;

(Dn - Do)t: mean difference in paw diameter variation of treated batches[19].

2.2.4. Statistical analysis

Data were entered into Excel (Microsoft Office 2013, USA) and analysed with Graph Pad Prism version 8.01. Quantitative data were presented as mean ± standard deviation. One- and two-factor ordered analysis of variance (ANOVA) was used to compare group means. Tukey and Bonferroni post hoc tests were used for multiple comparisons. The significance level was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Photochemical screening of extracts of *Musanga cecropiodes* and *Pricralima nitida*

The results of the phytochemical screening of the aqueous extracts of *Musanga cecropiodes* and *Picralima nitida* are presented in table 1. Anthraquinones, coumarins and anthocyanins were not detected in the aqueous extract of *Musanga cecropioides*. However, flavonoids were abundant. This extract also contained phenols, tannins and saponins. In the aqueous extract of

Picralima nitida, coumarins and anthocyanins were not detected, whereas alkaloids and saponins were present, as well as phenols, sterols, tannins, flavonoids and anthraquinones.

The identification of the families of compounds from the combination of the aqueous extracts of the 2 plants revealed the presence of alkaloids, sterols, phenol, flavonoids, saponins, coumarins and tannin. These results are in agreement with those of Adeneye et al[8] and Erharuyi et al[20], who worked on *Musanga cecropioides* and *Picralima nitida* respectively, with the exception of the presence of anthraquinones. In studying the influence of secondary metabolites present in plants on inflammation, Duwiejua et al[14], showed that alkaloids would be responsible for a decrease in the inflammatory syndrome. Ghedira[21] demonstrated that flavonoids and phenolic acid can exert several activities such as anti-inflammatory and analgesic activity.

Table 1: Phytochemical screening of aqueous extracts of *Musanga cecropiodes* and *Pricralima nitida.*

Chemical constituants	*Musanga cecropiodes*	*Pricralima nitida*
Alkaloids	+	+
Sterols	+	+
Phenols	+	+
Flavonoids	+	+
Anthraquinones	-	+
Coumarins	-	-
Saponins	+	+
Anthocyanins	-	-
Tannins	+	+

Sign (-): absent; Sign (+): present

3.2. Anti-inflammatory activity of the combination of extracts of *Musanga cecropiodes* and *Pricralima nitida*

3.2.1. *In-vitro* anti-inflammatory effect

The *in-vitro* anti-inflammatory effect is presented in figure 1. The combination of aqueous extracts of *Musanga cecropioides*, *Picralima nitida* (50/50, 70/30, 30/70) showed a dose-dependent inhibition ($p < 0.01$). From this result, it can be seen that the aqueous extract of the combination (70/30) of *Musanga cecropioides* barks and Picralima nitida mature fruits resulted in a higher percentage of inhibition compared to the other extracts at the concentration of 400 µg/mL of egg albumin. In addition, there was significant anti-inflammatory activity of the different combinations with optimum percentage inhibition (75.07%) observed for the 70/30 combination. The percentage of inhibition of denaturation

by the combination of aqueous extracts at 70/30 proportions is higher compared to the other extracts at a concentration of 800 µg/mL and reaches the maximum with a percentage of 75.07%. From the results, it appears that at this same concentration the aqueous extract of the combination has a lower percentage of inhibition than the reference molecule (diclofenac) which reaches a peak of inhibition at 78.54%. This anti-denaturation effect would be due to the interaction of the molecules present in the natural extracts with certain amino acids constituting the proteins, in the case of albumin (the model studied) these interactions would take place at two specific sites: tyrosine and threonine of the aliphatic chain.[19]

Protein denaturation is a process in which proteins lose their tertiary and secondary structure through the application of external stress such as heat or by certain compounds such as strong acids or bases and organic solvents.[22] The mechanism leading to this denaturation probably involves the breaking of electrostatic, hydrogen, hydrophobic or disulphide bonds.[23]

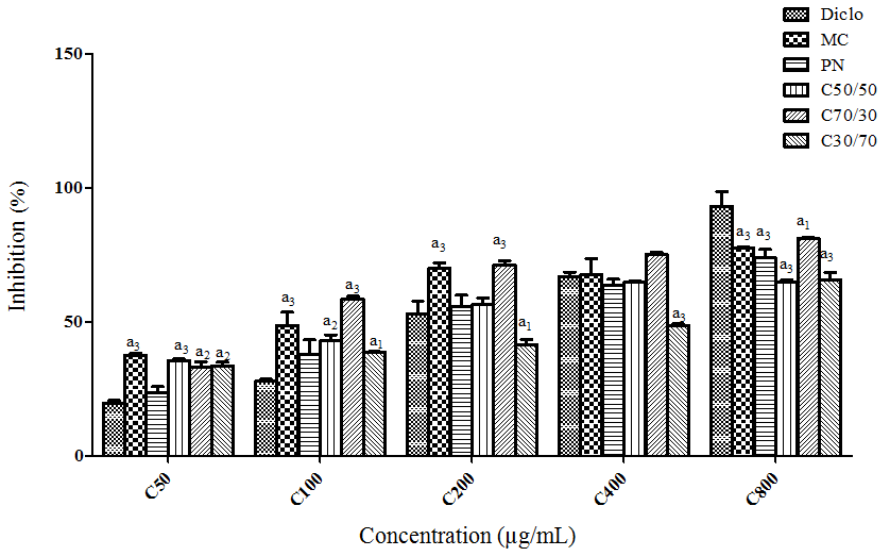

Figure 1: In vitro anti-inflammatory activity of the combination of *Musanga cecropiodes* and *Pricralima nitida* water extracts.

[a](p <0.05; p <0.01; p <0.001) significant difference from Control. Diclo (Diclofenac), MC (Musanga cecropioides aqueous extract), PN (Picrlima nitida aqueous extract), C (combination of aqueous extracts in various proportions)

3.2.2. *In vivo* anti-inflammatory effect in an animal model

Table 2 shows the percentage change in paw diameter (ΔD). From this table it can be seen that the combination of aqueous extract of *Musanga cecropioides* barks and mature *Picralima nitida* fruits resulted in a dose-dependent ($p < 0.01$) decrease in carrageenan-induced oedema

in rats. This result is comparable to that of diclofenac sodium used as a reference drug. From the first hour the combination of aqueous extracts at doses of 50, 100, 200 and 400 mg/kg showed 43.39, 40.67, 41.24 and 44.80 % inhibition respectively, similar to that of diclofenac sodium (44.04 %). The highest inhibition was obtained with a dose of 400 mg/kg (71.27%) the combination of aqueous extracts at the third hour after induction of oedema. This inhibition of oedema was statistically comparable to that obtained with the standard (diclofenac sodium). Diclofenac (50 mg/kg) used as a reference molecule also significantly inhibited oedema of the rat paw during the first two phases of carrageenan-induced acute inflammation. The maximum inhibition percentage was 77.77% in the second phase. The anti-inflammatory activity of the combination of aqueous extracts (70/30) was evaluated in the carrageenan-induced acute inflammation model.

Carrageenan is widely used as a phlogogenic substance to induce inflammation, and is used to screen compounds with anti-inflammatory activity.[24] Its local injection into the arch of the rat's foot provokes an inflammatory reaction, which manifests itself as a noticeable oedema after 30 min.[25] Carrageenan-induced oedema develops in a triphasic manner: the first phase lasts about one hour after induction and is due to the release of serotonin and histamine.[26] The second phase extends from the second hour to the third hour and is mediated by the kinin system.[27] The final phase begins three hours after injection and is attributed to prostaglandins, a product of cyclooxygenase.[28]

Pre-treatment of rats with the aqueous extract of the combination inhibited carrageenan-induced paw oedema during the first two phases at the different doses. In the first phase, the maximum percentage inhibition was 44.80% (1 hour) at 400 mg/kg body weight. This result corroborates that of Nguemfo et al[29] who noted an inhibition of carrageenan-induced rat paw oedema in the first phase by the methylene chloride fraction of *Allanblackia monticola*. The combination of the aqueous extracts also resulted in a significant inhibition of the second phase of the acute inflammation induced by carrageenan. This effect is believed to be due to the interference of the combination extract on the kinin system.

The steroids present in this extract would act by inhibiting kallikrein, thus preventing the conversion of hepatic kininogen to bradykinin and kallidin which are mediators of this phase.[30] This result is similar to that reported by Dimo et al[31], who concluded that the steroids present in the aqueous extract of *Kalanchoe crenata*, would be responsible for the anti-inflammatory effect in the second phase of the oedema induced by carrageenan.

Table 2: Effect of the combination of aqueous extracts of *Musanga cecropioides* trunk bark and *Picralima nitida* fruit on carrageenan-induced paw oedema in rats.

Batch	Dose (mg/kg)	Oedema (ΔD en mm)		
		1 h	2 h	3 h
Distilled water	0	0.41±0.04	0.38±0.03	0.35±0.03
Diclofenac	50	0.23±0.02[a] (44.04)	0.17±0.01[a] (55.18)	0.08±0.01[a] (77.77)
CAEMCPN	50	0.23±0.04[a] (43.39)	0.19±0.03[a] (50.81)	0.14±0.03[a] (59.06)
CAEMCPN	100	0.24±0.01[a] (40.67)	0.19±0.02[a] (49.92)	0.12 ± 0.03[a] (64.97)
CAEMCPN	200	0.22±0.02[a] (41.24)	0.19±0,01[a] (50.32)	0.12 ± 0.01[a] (66.49)
CAEMCPN	400	0.22±0.01[a] (44.80)	0.16±0.01[a] (56.58)	0.1±0.01[a] (71.27)

Values are mean oedema (ΔD in mm) ± standard deviation, n=5.

[a]p <0.01 significant difference from Control. Values in brackets represent percentages of inhibition. CAEMCPN (combination of the aqueous extracts of Musanga cecropioides and Picralima nitida)

CONCLUSION

The aqueous extracts of the bark of the trunk of *Musanga cecropioides* (Cecropiaceae) and of the mature fruits of *Picralima nitida* (Apocynaceae) contain pharmacological substances, potentially anti-inflammatory, including alkaloids, flavonoids, phenols, sterols, saponins and tannins. The combination of the aqueous extracts of *Musanga cecropioides* and *Picralima nitida* optimize the inhibition of protein denaturation, with an optimum of 70/30. This optimal combination reverses carrageenan-induced oedema similar to diclofenac in rats. These results show the interest of these plants in the treatment of inflammatory diseases and support their therapeutic uses in traditional medicine.

Funding: This study was not funded by any research funding agency or public or private sector funder.

ACKNOWLEDGEMENTS

The authors would like to thank Professor Dongmo of the Faculty of Science, University of Douala for his technical and logistical assistance during the anti-inflammatory tests.

Ethical considerations

Conflict of interest: The authors declare that they have no conflict of interest

Ethical approval: This study did not involve human participants. It was approved by the ethical committee of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. The work was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) requirements on animal experimentation.

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