Evaluation of Anti-Inflammatory and Antioxidant Activities of Ethanolic and Palm Wine Extract of the Stems of *Phragmanthera capitata* (Sprengel) S. Balle (Loranthaceae) Collected on *Psidium guajava* L. in Cameroon

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Abstract: The objective of this study was to evaluate the anti-inflammatory and antioxidant activities of ethanolic and palm wine extract of *Phragmanthera capitata* stems widely used by traditional healers. Acute toxicity according to the OCDE Guideline 423 showed that the LD50 of ethanolic and palm wine extract was greater than 5000 mg/Kg body weight. Anti-inflammatory capacity was evaluated by hind paw oedema model using carrageenan-induced inflammation in rat. It has showed that palm wine and ethanolic extracts possessed a dose-dependent anti-inflammatory activity (at the first hour) with 21.47% and 41.24%, respectively, as a percentage inhibition for the 200 and 300 mg/Kg body weight of palm wine extract and 16.95% and 28.21% for the ethanolic extract of same dose as compared with 25.42% for aspirin. The phenol dosage by the Folin-Ciocalteu method showed that ethanolic extract (14420 mg EAA/g dry extract) had a high content relative to the palm wine extract (2570 mg EAA/g dry extract ). The antioxidant capacity was evaluated by the diphenyl-picryl test (DPPH), where the ethanolic extract showed strong antioxidant activity with a EC50 of 0.0085 mg/mL, when that of the wine extract was 0.049 mg/mL, after the ascorbic acid (standard) with 0.033 mg/mL. Ethanolic and palm wine extract of *Phragmanthera capitata* stems are non-toxic and have inflammatory and antioxidant activity that could justify its traditional use.

Keywords: *Phragmanthera capitata*, ethanol, palm wine, anti-inflammatory, antioxidant.

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process [1]. Sometimes inflammation can be harmful because of the aggressiveness of the pathogen, its persistence, the site of inflammation, abnormal regulation of the inflammatory process, quantitative or qualitative abnormality of the cells involved in the inflammatory process [2]. Inflammation is besides an important source of oxygenated radicals produced directly by activated phagocytic cells and even in normal conditions; mammals aerobic metabolism generates substances called reactive species of oxygen (ERO), which are involved in small quantities in physiological processes [3]. The excess of free radicals is the cause of numerous problems as asthma, cancers, cardiovascular diseases, hepatic disorders, degenerative diseases and other inflammatory processes by [4]. The decrease or the elimination of free radicals is made by the antioxidant system of the body. The use of chemical anti-inflammatory or antioxidant substances of synthesis is always accompanied with side effects, while the use of phytochemical compounds turns out useful and without side effects [2].

Medicinal plants are important for the pharmacological research and the elaboration of medicine, not only when the constituents of plants are directly used as therapeutic agents, but also as raw materials for the synthesis of medicine or as models for pharmacological active compounds [5]. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic...
drugs obtained from natural [1]. In developing countries, the wide-spread use of the Traditional Medicine is attributable not only to the fact that it is a cultural heritage, but also to its accessibility [6].

Loranthaceae family is widely used by traditional healers in Cameroon. Interest of this work has been given to a plant of the Loranthaceae family, Phragmanthera capitata. It was chosen on the basis of ethnobotanical surveys, which were used in traditional medicine for the treatment of arthritis, cancer, urinary infections, gynecological problems cardiovascular and prostate cancer [7, 8].

Interest of this work has been given to a plant of the Loranthaceae family, Phragmanthera capitata. It was chosen on the basis of ethnobotanical surveys, where it is used in traditional medicine for the treatment of arthritis, cancer, gynecological problems and cardiovascular diseases. With a view to contributing to improve the primary health care of the populations, the evaluation of the anti-inflammatory and antioxidant activities of ethanolic and palm wine extract of the stems Phragmanthera capitata (Sprengel) S. Balle (Loranthaceae) collected in Cameroon was undertaken. The aim of this study was to evaluate the anti-inflammatory and antioxidant activities of Phragmanthera capitata stems. The specific objectives were (1) to realize a phytochemical screening, (2) to evaluate acute toxicity of ethanolic and palm wine extract of Phragmanthera capitata stems, (3) to determine anti-inflammatory activity in vivo of these extracts on rats; (4) to estimate quantitatively the antioxidant activity in vitro of the same extracts.

MATERIALS AND METHODS

Materials

Plant material was Phragmanthera capitata stems collected in the morning just before the blooming on a guava (Psidium guajava), in the orchard of the chiefdom of Ndogbong, 3rd District of Douala, Department of Wouri, Coastal Region.

Females albino rats (Rattus norvegicus) aged about 4–12 weeks were used for acute toxicity and adult about 4-5 months were used for anti-inflammatory activity. They were housed in standard cages under standard environmental conditions of room temperature with 12 hours dark light cycle and provided with standard food for rodents and water ad libitum. These rats females were acclimatized at least 5 days before the beginning of the experiment to the Animal’s Laboratory of the Department of Pharmaceutical Sciences, Faculty of Medicine and the Pharmaceutical Sciences, University of Douala (FMPS). Rats lived in cages papered with shavings.

The laboratory equipment was constituted by apparatus (rotary evaporator IKA HB 10 BASIC, electronic precision balance METTLER PM 480, stews

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After administration rats were observed individually during 14 days with a particular attention during the first 30 min, at 4, 12 and at 48 hours. The observations concerned the modification of the skin, hairs, eyes, shivers, convulsions, salivation, diarrhea, lethargy, sleep and coma. Rats were weighed every 2 days, at the regular hours

The DL50 (lethal dose 50%), single dose required to kill half the members of a tested population, when it is orally administered is expressed by unit of weight of female rat (mg/Kg) [13].

**Anti-inflammatory activity**

Anti-inflammatory capacity was evaluated by hind paw oedema model using carrageenan-induced inflammation in rat as describe by Dieng et al., [14]. Injections of carrageenan into a plantar surface of the hind paw of the rat result an oedema of the metatarsal region. The intensity of this oedema, which reached its maximum of development in 3 hours, was estimated by the increase of the volume of the paw (with regard to the initial volume). The preventive oral administration of an extract having an anti-inflammatory activity reduces significantly the development of the oedema

The experiment took place as follows: rats females were deprived of food during 12 hours before the experiment; the diameter (Do) of the right hind paw of every rat, before treatment was measured with a caliper, one hour before the test. Extracts at 200 and 300 mg/Kg or aspirin (30 mg/Kg) or distilled water were administered by oral route by means of a stomach tube to 6 different groups of 3 rats; one hour after gavage, 0.1 ml of suspension of carrageenan 1% was injected unto a plantar surface of every rat; the diameter of the oedema was measured every hour, till the fifth hour. The percentage of inhibition of the oedema (PI) was calculated with the following formula:

\[
PI = \left( \frac{Dt - Do}{Dt - Do} \right) \text{control group} - \left( \frac{Dt - Do}{Dt - Do} \right) \text{treated group} \times 100
\]

Where,  
\[Dt = \text{diameter of the hind right paw in time } t;\]
\[Do = \text{diameter of the hind right paw in time } 0.\]

**Antioxidant activity**

Spectrophotometry is a quantitative analytical method which consisted in measuring the absorbance or the optical density of a chemical substance given in solution. More this substance is concentrated it absorbs the light within the limits of proportionalties expressed by the law of Beer-Lambert. The optical density of the solutions was determined by a spectrophotometer before hand calibrated on the wave length of absorption of the chemical species to be studied. When a light of intensity I0 got through a solution, a part of this one was absorbed by the substances in solution. The intensity I of transmitted light was thus lower than Io. The absorbance of the solution was defined as follows:

\[
A = \log \left( \frac{I_0}{I} \right), \text{ with the transmittance } T \text{ equal to } A = - \log T
\]

Absorbance is a positive value, without unit. It was higher, when the transmitted intensity was low [15].

**Total phenolic determination**

Total phenolic content was determined using the Folin-Ciocalteu reagent as describe by Merouane et al., [16].

**Antioxidant activity**

**DPPH radical scavenging assay**

Antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity describe by Ladoh et al., [3]. The percentage of inhibition (I) was calculated by the following:

\[
I \% = \left( \frac{Ac - As}{Ac} \right) \times 100
\]

Where,  
\[Ac = \text{absorbance of control} \]
\[As = \text{absorbance of the substance}\]

A curve of the concentrations of the extract according to the percentage of inhibition was drawn to obtain the index IC50. It was defined as the concentration of the substance (mg/mL) required to decrease the initial concentration of the DPPH of 50%. The ascorbic acid dissolved in the methanol was used as control.

**Statistical analysis**

The data registered in Excel (Service 2007 Microsoft, the USA) and analyzed with the software Statview version 5.0 (SAS Institute., Inc., USA). The quantitative data were presented in the form of average ± standard deviation (DS) in graphs and tables. The test t of Student on not matted series and the orderly analysis of the variance in a factor were used to compare the averages respectively between two and more than two groups. The test Post Hoc of Newman-Keuls was used to make the multiple comparisons even p <0.05.
The determination of the inhibition concentration (IC_{50}) was made by the software Excel 2010 by drawing the curve of the percentage of the inhibition according to the logarithm of the concentrations.

RESULTS AND DISCUSSION

Results

Phytochemical screening

Some compounds have being found with colored reactions (Table-1).

Oral acute toxicity

No sign such as convulsions, salivation, coma, vomiting, and shivers were observed at 2000 and 5000 mg/Kg body weight of ethanolic and palm wine extract. Saddles, eyes, pilosity and food feeding were normal. No death was observed. No significantly difference (p-value > 0.05) was found between the control group and tests groups at 2000 and at 5000 mg/Kg, whatever the day of the experiment.

Anti-inflammatory activity

Volume of oedema and percentage of inhibition were constituted in the table below (Table-2).

Table-1: Phytochemical screening of Phragmanthera capitata stems extract

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Hex E</th>
<th>E Ac</th>
<th>Eth E</th>
<th>Palm E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>reducing compound</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Hex E: Hexanic extract; E E: Ethyl acetate extract; Eth E: Ethanolic extract; Palm E: Palm wine extract

Table-2: Effect of different substances on oedema after carrageenan injection on right hind paw

<table>
<thead>
<tr>
<th>Substances</th>
<th>30 min</th>
<th>1 H</th>
<th>2 H</th>
<th>3 H</th>
<th>4 H</th>
<th>5 H</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA</td>
<td>1.87 ± 0.37а</td>
<td>2.20 ± 0.37***а</td>
<td>2.13 ± 0.18а</td>
<td>1.62 ± 0.20***а</td>
<td>2.20 ± 0.28***а</td>
<td>2.03 ± 0.28***а</td>
</tr>
<tr>
<td>Eth E 200</td>
<td>1.52 ± 0.49а</td>
<td>2.45 ± 0.43****а</td>
<td>3.72 ± 0.24а</td>
<td>2.85 ± 0.18а</td>
<td>2.57 ± 0.18а</td>
<td>2.18 ± 0.15а</td>
</tr>
<tr>
<td>Eth E 300</td>
<td>2.50 ± 0.28а</td>
<td>2.10 ± 0.40****а</td>
<td>1.95 ± 0.38а</td>
<td>2.02 ± 0.35а</td>
<td>1.48 ± 0.19а</td>
<td>1.10 ± 0.40а</td>
</tr>
<tr>
<td>Palm E 200</td>
<td>2.57 ± 0.40а</td>
<td>2.32 ± 0.36****а</td>
<td>2.62 ± 0.17а</td>
<td>2.27 ± 0.34а</td>
<td>2.25 ± 0.10а</td>
<td>2.13 ± 0.31а</td>
</tr>
<tr>
<td>Palm E 300</td>
<td>1.52 ± 0.25а</td>
<td>1.73 ± 0.18****а</td>
<td>1.68 ± 0.49а</td>
<td>1.35 ± 0.59а</td>
<td>1.43 ± 0.66а</td>
<td>1.32 ± 0.39а</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.93 ± 0.32а</td>
<td>2.95 ± 0.23а</td>
<td>2.97 ± 0.17а</td>
<td>3.05 ± 0.24а</td>
<td>2.77 ± 0.24а</td>
<td>2.25 ± 0.26а</td>
</tr>
</tbody>
</table>

Values are mean ± S E M. * p < 0.05, *** very significantly different p < 0.0001 significantly different from AAS or different substances Newman-Keuls test, p < 0.05; (-) no inhibition. Percentages of inhibition are in bracket. ASA: Aspirine; ETH E: Ethanolic extract; Palm E: Palm wine extract

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The volume of the oedema (inflammation) which was induced by the carrageenan increased with time till the third hour. There was a significant difference between all groups at the first hour (p˂0.05). The treatment of rats with stems extract of *Phragmanthera capitata* significantly (p˂ 0.0001) decreased inflammation compared with control group. The sizes of the oedema were 2.45 ± 0.43, 2.32 ± 0.36, 2.10 ± 0.40, 1.73 ± 0.18 and 2.20 ± 0.37 mm that respectively corresponded to the percentages of inhibition 16.95 %, 21.47%, 28.81 %, 41.24% and 25.42 % for Eth E 200, Palm E 200, Eth E 300, palm E 300 and ASA 30 mg/Kg. The two doses ethanolic extract had an inhibitive effect less important than palm wine extract and at 200 mg/Kg, this inhibitive effect was less than aspirin effect but higher than control group.

**Total Phenolic Determination**

The calibration curve of the ascorbic acid allowed determining the phenols content of ethanolic and palm wine extract (Figure-1).

![Calibration curve of ascorbic acid](image1)

**Fig-1: Calibration curve of ascorbic acid**

![Total phenols content of *Phragmanthera capitata* stems extracts](image2)

**Fig-2: Total phenols content of *Phragmanthera capitata* stems extracts**

Total phenols content of ethanolic and palm wine extract of *Phragmanthera capitata* stems determined by linear regression was respectively 14420 and 2570 mg EAA/g of dry extract.

**Antioxidant activity**

**DPPH radical scavenging assay**

Antioxidant activity of *Phragmanthera capitata* stems was determined by DPPH (1,1, diphenyl-2-picrylhydrazyl) free radical and the IC$_{50}$ was
determined with the curves $y = 4953.7x + 7.563$, $R^2 = 0.9774$ for ethanolic extract, $y = 1565.3x - 2.4273$, $R^2 = 0.9924$ for ascorbic acid and $y=1030.7x-0.7273$, $R^2 = 0.9957$ for palm wine extract (Figure-3).

Fig-4: Antioxidant activity of Phragmanthera capitata extract

Ethanoic extract had an antioxidant activity higher than palm wine extract and ascorbic acid (reference).

**DISCUSSION**

**Phytochemical screening**

The phytochemical characterization of Phragmanthera capitata stems extracts showed the presence of alkaloids, phenolic compounds, reduced compounds, tannins and saponosides. These results are similar to those found by Oluwole et al., [17] in Nigeria when they studied phytochemical and antimicrobial screening of leaves of Globimetula oreophila (Oliv.) Van Tiegh and Phragmanthera capitata (Spreng) S. Balle. Takem et al., [18] found other metabolites as terpene and glycosides. These slight variations in the phytochemical composition can be justified by the harvest hosts plants different, the used organ, the phenological stage and the abiotic conditions (season, climate and temperature). The presence of bioactive substances could justify the use of this plant in traditional medicine for the treatment of diseases as arthritis, diabetes, arterial high blood pressure, cardiac problems.

**Oral acute toxicity**

Traditional medicine for ethanolic and palm wine extract, no sign of toxicity has been observed both on zootechnic plan (hydric food, taken weight, taken evolution) as on the physiological plan (no salivation, neither of diarrhea, nor coma, nor convulsion). Oecd guidelines do not allow calculating exactly the dl50. However the death of a proportion of animals leads to the end of the study. The dl50 of the ethanolic and palm wine extract can be considered as being superior to 5000 mg/kg because no death was observed at the higher dose. According to lines 423 of the oecd, for a dl50 superior or equal to 2000 mg/kg, the headlines substance can be considered as being not toxic. The ethanolic and palm wine extract of phragmanthera capitata stems are not toxic. These results corroborate with thoses, who obtained a dl50 superior to 3000 mg/kg with mice during the evaluation of the anti-diarrhetic activity of the aqueous extract of the same loranthaceae [18].

**Anti-inflammatory activity**

In experimental conditions, the volume of the oedema induce by the carrageenan was maximal at the third hour [19]. The carrageenan caused a local inflammation when it was injected in paw plantar surface because of a tissular lesion. Injection of the carrageenan leads to the liberation of several chemical mediators who are responsible of the inflammatory process. This biphasic inflammatory answer of which the initial phase took place (approximately one hour), was due to the liberation of histamine, serotonin, and bradykinine. The second phase was due to the liberation of prostaglandins and lysosomials enzymes (2-4 h) [1]. These mediators increased the permeability of the capillaries region pulling formation of an exudates which was the cause of the localized oedema and compressed nerves and gave pain [20]. The ethanolic and palm wine extract had a significant anti-inflammatory activity at first hour with the percentages 16.95 % and 28.21 % for the respective doses 200 and 300 mg /kg body weight of ethanolic extract and 21.47% et 41.24% for palm wine extract at the same doses. This anti-inflammatory activity was maintained during the experiment with both extract. This would be justified by the inhibition of the synthesis of pro-inflammatory substances in the first phase of the inflammation. Palm wine extract had an important inhibition than ethanolic extract. This could be due to the difference of solubility of actives substances in the two solvents. Extract activity was dose dependent and
ethanolic extract at 200 mg/kg had an inhibitive effect less than aspirin. The extracts of phragmanthera capitata acted at first hour; this could justify the use of this plant on in the inflammatory diseases. The reduction of the oedema was maintained during the experiment in both extracts. The anti-inflammatory activity of this extract would be explained by the presence in phragmanthera capitata stems, of bioactive substances as polyphenolic compounds as tannins and simple phenols revealed by the phytochemical screening, which have anti-inflammatory activity. Tannins have antibacterial, antiviral, antioxidant properties and anti-inflammatory property (drug like arkogellules). Their anti-inflammatory action could be due to an effect on the leukocyte migration and to an antiphlogisthic action [12]. There is a very large variety of phenols, to simple compounds as the salicylic acid, molecule that gave aspirin by synthesis at complex substances as the polyphenolic compounds in which glucosides are bond. Phenols are anti-inflammatory and antiseptic. Phenolic acids, as rosmarinic acid, are strong antioxidant and anti-inflammatory and can have antiviral properties [21].

Aspirin (reference) reduced the inflammation by inhibition of cyclo-oxygenase enzyme responsible of the production of prostaglandins and thromboxane [22]. Aspirin gradually reduced oedema with a maximum at the third hour, acts on all the phases of inflammation until the elimination of the substance.

Total phenolic determination
Total phenols content of phragmanthera capitata determined by the method of folin-ciocalteu was 14420 mg eaa/g of dry ethanolic extract and 2570 mg eaa/g of dry palm wine extract. The difference between these results shows the effect of the solvent on the extraction of bio active substances. These results are different to those obtained by ladoh et al., [3] (445.2 mg eaa/g). An explication can be given by no identical experimental conditions (methanol as solvent, citrus sinensis as host plant and the period of collection of the plant).

Antioxidant activity
Dpph radical scavenging assay
Phragmanthera capitata extracts and ascorbic acid (reference) possessed an anti-radical activity. Ethanolic extract of stem showed an anti-radical activity (ec50 = 0.0085 mg/ml) higher than palm wine (ec50 = 0.049.21 mg/ml) extract and ascorbic acid (0.033mg/ml).

Antiradical activity of ascorbic acid was important than that palm wine extract. These results were different to those obtained by ladoh et al., [3] and can be explain by no identical experimental conditions (methanol as solvent, citrus sinensis as host plant, and the collect period of the plant). Antioxidant activity of this extract would be due to its wealth in bioactive substances revealed by the phytochemical screening. Ethanolic extract contented more phenol than palm wine extract and his capacity of trapping dpph free radical was more important. There was a correlation between phenolic content compounds and anti-radical activities [22].

CONCLUSION
The present study allowed highlighting the harmlessness of the ethanolic and palm wine extract of phragmanthera capitata stems. The anti-inflammatory test at 200 and at 300 mg/kg of body weight showed a dose dependent anti-inflammatory activity of both extract. Ethanolic and palm wine extract possessed a total phenols which allowed them to trap free radicals. The presence of these activities could justify the use of this plant in traditional medicine for the treatment of inflammatory diseases and the diseases related to oxidative stress.

ACKNOWLEGEMENTS
We thank dr etame loe gisèle (email: giseloetame@yahoo.fr; p.o. Box 15515 douala cameroon; tel: 237 699 833 818), general manager of industrial laboratory, genemark for their grant support.

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