Evaluation of Anti-Inflammatory and Antioxidant Activities of Ethanolic Extract of 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 Phragmanthera capitata stems widely used by traditional healers. Acute toxicity according to the OECD Guideline 423 showed that the LD 50 of ethanolic extract was greater than 5000mg/kg body weight. Anti-inflammatory capacity was evaluated by hind paw oedema model using carrageenan-induced inflammation in rat. It has showed that ethanolic extract possess a dose-dependent anti-inflammatory activity at the first hour with 16.95 % and 28.21 %, respectively, as a percentage inhibition for the 200 and 300 mg / kg body weight extract. The phenol dosage by the Folin-Ciocalteu method showed that ethanolic extract had 14420 mg mg EAA/g of dry extract. The antioxidant capacity was evaluated by the diphenyl-picryl test (DPPH), and the EC 50 was 0.0085 mg / ml for ethanolic extract when EC 50 of the ascorbic acid (reference) was 0.033 mg/ml. Phragmanthera capitata stems ethanolic extract is non toxic and have inflammatory and antioxidant activity that could justify its traditional use.

Keywords: Phragmanthera capitata, ethanol, anti-inflammatory, antioxidant, Psidium guajava.

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, or quantitative or qualitative abnormality of the cells involved in the inflammatory process [2].

Medicinal plants are important for the pharmacological research and the elaboration of medicine, not only when the constituent 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 [5, 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 ethnomedical 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 ethnomedical surveys, were it is used in traditional medicine for the treatment of arthritis, cancer, gynecological problems and cardiovascular diseases. An explanation could be found by the phytochemical screening of the aqueous extract which showed the presence of bioactive substances coming from plants secondary metabolism as glycosides, alkaloids, tannins, saponines, phenols, anthraquinones and flavonoids [9, 10]. It has been demonstrated that aqueous extract of Phragmanthera capitata possesses anti-diarrhoeic, anti-secretory, gastroprotective and anti-ulcer properties, an antipyretic and analgesic potential, steroidalogenetic and spermatogenic activity, antibacterial and anti-lipogenic properties, one [9-13]. With a view to contributing to improve the primary health care of the populations, the evaluation of the anti-inflammatory and antioxidant activities of the stems of Phragmanthera capitata (sprengel) s balle (lorantaceae) 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 make a phytochemical screening, (2) to evaluate acute toxicity of ethanolic extract of Phragmanthera capitata stems; (3) to determine anti-inflammatory activity of this extract in vivo on rats; (4) to estimate quantitatively the antioxidant activity of the same extract in vitro.

MATERIALS AND METHODS

Material

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, at Douala 3rd district, department of Wouri, Littoral region. Plant identifications and authentications were carried out at the National Herbarium of Cameroon where a specimen number after deposit of the sample N/REF 075/IRAD/DG/CRRA-NE/SSRB/12/2016 was assigned.

Females albino rats (Rattus norvegicus) aged about 4–12 weeks were used for acute toxicity and adult about 04- 05 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 pet store of the Faculty of Medicine and the Pharmaceutical Sciences of the University of Douala. 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 MEMMERT, UV spectrophotometer UVILINE 910, water bath WATER HWS-24), of technical material (spatula, bucket in stainless steel 15 l, cotton wool, filter paper N°4, glass , pipette 1, 5 ml, sounds of oro-gastric force-feeding, food for rats, micropipettes 1000 µl, calipers, syringes 1, 2, 5, 10 ml, to becher 250 ml, sterile flasks 60 ml, not sterile flasks 60, 125 ml, cages (goals) for rats, feeding-bottles), reagents (Folin-Ciocalteu reagent , sodium carbonate, DPPH (1,1diphényl-2-picryl-hydrazyl), ascorbic acid, BHT: 3,5-ditertiobutyl-4-hydroxytoluee, sulphuric acid, acetylsalicyclic acid, ammoniac, iron chloride, acetic anhydride, hydrochloric acid, liqueur of Fehling A, liqueur of Fehling B, shavings of magnesium, potassium hydroxide, bismuth nitrate, acetic acid, potassium iodide) and of solvents. (Hexane, ethyl acetate, ethanol, physiological water, water for injectable preparation).

METHODOLOGY

This experimental study took place from November 16 th, 2016 to June 31st, 2017, in the Phytochemistry Laboratory, in the Pet store and in the Experimental Pharmacology Laboratory of the Faculty of Medicine and the Pharmaceutical Sciences of the University of Douala (FMPS).

Extraction

The stems of Phragmanthera capitata collected in the morning, were dried in the sunshade during two weeks then sprayed: 200 g of powder was macerated successively during 48 hours, in solvents of increasing polarity, then filtered and concentrated in the rotary evaporator; and 1 kg of powder was macerated in 5 l of ethanol 95 %, filtered and concentrated in the rotary evaporator.

Phytochemical screening

Phytochemical screening is a set of reactions based on the haste or the color, made in tube or on Passover of Chromatography on Thin Layer who allow to highlight families of compounds such as alkaloids, coumarines, flavonoids.
Tests for the presence of alkaloids, saponins, tannins, reducing compound, resins and phenols were performed on the crude extracts [14-17].

**Oral acute toxicity**

Acute toxicity was evaluated according to the OECD (Organisation for Economic co-operation and development) Guideline 423 limit test at 2000 mg/kg and 5000 mg/kg [18].

A single administration of ethanolic the extract of Phragmanthera capitata was made on rats females deprived of food during 12h but not water. These rats aged from 8 to 12 weeks were marked for an individual identification and distributed in group of three.

After administration rats were observed individually during 14 days with a particular attention during the first 30 min, at 4, 12 and at 48 h. 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 DL 50 (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) [18].

The experiment took place as follows: rats females were deprived of food during 12h 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 (200 and 300mg/kg) or aspirin (30mg/kg) or distilled water were administered by oral route by means of a stomach tube to 4 groups of 03 rats; one hour after gavage, 0.1 ml of suspension of carrageenan 1 % was injected into 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:

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

Dt: diameter of the hind right paw in time t; Do: diameter of the hind right paw in time 0.

**Antioxidant activity**

Spectrophotometry is a quantitative analytical method which consists 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 proportionalities expressed by the law of Beer-Lambert. The optical density of the solutions is determined by a spectrophotometer beforehand calibrated on the wavelength of absorption of the chemical species to be studied. When a light of intensity I0 gets through a solution, a part of this one is absorbed by the substances presents in solution. The intensity I of transmitted light is thus lower than I0. The absorbance of the solution is defined as follows:

\[
A = \log (\text{Io/I}), \text{ with the transmittance T equal to } A = - \log T
\]

Absorbance is a positive value, without unit. It is higher when the transmitted intensity is low [20].

**Total phenolic determination**

Total phenolic content was determined using the Folin-Ciocalteu reagent, with slight modifications [21].

**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 with slight modification [3]. The percentage of inhibition (I) was calculated by the following:

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

Ac = absorbance of control; As = absorbance of the substance
A curve of the concentrations of the extract according to the percentage of inhibition was drawn to obtain the index IC 50. It is 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 Stat view version 5.0 (SAS Institute, Inc., USA). The quantitative data were presented in the form of average ± standard deviation (DS) in graphs and table. The test t of Student on not mated 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): 

<table>
<thead>
<tr>
<th>compounds</th>
<th>Hex E</th>
<th>E E</th>
<th>Eth E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Phénols</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>reducing compound</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Hex E: hexanic extract; E E: ethyl acetate extract; Eth E: ethanolic extract

Oral acute toxicity

No sign such as convulsions, salivation, coma, vomiting, and shivers were observe at 2000 and 5000 mg / kg body weight. Saddles, eyes, pilosity and food feeding were normal. No death was observed. No statistically significant 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 are constituted in the table below (Table 2).

<table>
<thead>
<tr>
<th>Substances</th>
<th>30 mn</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 ± 0.37a</td>
<td>2.20 ± 0.37***</td>
<td>2.13 ± 0.18a</td>
<td>1.62 ± 0.20***</td>
<td>2.20 ± 0.28***</td>
<td>2.03 ± 0.28***</td>
</tr>
<tr>
<td>Eth E 200</td>
<td>1.52 ± 0.49a</td>
<td>2.45 ± 0.43***</td>
<td>3.72 ± 0.24a</td>
<td>2.85 ± 0.18a</td>
<td>2.57 ± 0.18a</td>
<td>2.18 ± 0.15a</td>
</tr>
<tr>
<td>Eth E 300</td>
<td>2.50 ± 0.28a</td>
<td>2.10 ± 0.40***</td>
<td>1.95 ± 0.38a</td>
<td>2.02 ± 0.35a</td>
<td>1.48 ± 0.19a</td>
<td>1.10 ± 0.40a</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.93 ± 0.32a</td>
<td>2.95 ± 0.23a</td>
<td>2.97 ± 0.17a</td>
<td>3.05 ± 0.24a</td>
<td>2.77 ± 0.24a</td>
<td>2.25 ± 0.26a</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
The volume of the oedema (inflammation) induces by the carrageenan increases with time till the third hour. There is a significant difference between all groups at the first hour ($p<0.05$). The treatment of rats with stems extract of Phragmanthera capitata significantly ($p< 0.0001$) decreases inflammation compared with control group. The sizes of the oedema are $2.45 \pm 0.43$; $2.10 \pm 0.40$; $2.20 \pm 0.37$ mm what corresponds respectively to the percentages of inhibition 16.95 %, 28.81 % and 25.42 % for Eth E 200; Eth E 300 and for ASA 30 mg / kg. The ethanolic extract at 300 mg / kg an inhibitive effect more important aspirin.

**Total Phenolic Determination**

The calibration curve of the ascorbic acid allowed to determine the phenols content of ethanolic extract (Figure-1).

![Fig-1: Calibration curve of ascorbic acid](image)

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

Total phenols content of ethanolic extract of *Phragmanthera capitata* stems determined by linear regression was 14420 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 curve (Figure-2).

![Fig-2: Antioxidant activity of ethanolic extract of Phragmanthera capitata stems](image)

**Fig-2: Antioxidant activity of ethanolic extract of Phragmanthera capitata stems**

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DISCUSSION

Phytochemical screening

The phytochemical characterization of Phragmanthera capitata stems showed the presence of alkaloids, phenolic compounds, reduced compound, tannins and saponosides. These results are similar to those found by Oluwole et al., in 2013 in Nigeria when they studied phytochemical and antimicrobial screening of leaves of Globiometula oreophila (Oliv) van Tiegh and Phragmanthera capitata (Spreng) S. Balle [10]. Takem et al., found other metabolites as terpenes and glycosides [9]. 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 as demonstrated by many people [10-12]. It could justify the use of this plant in traditional medicine for the treatment of diseases as arthritis, diabetes, arterial high blood pressure and cardiac problems.

Oral acute toxicity

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, no coma, no convulsion). OECD guidelines do not allow calculating exactly the DL 50. However the death of a proportion of animals leads to the end of the study. The DL 50 of the ethanolic extract can be considered as being superior to 5000 mg/kg because no death was observed at the higher dose. According OECD 423 guidelines, a DL50 superior or equal to 2000mg/kg, the headlines substance can be considered as being not toxic. The ethanolic extract of Phragmanthera capitata stems is not toxic [18]. These results corroborate the works of Takem and collaborators who in 2014 obtained a DL 50 superior to 3000mg/kg with mice during the evaluation of the anti-diarrheic activity of the aqueous extract of the same Loranthaceae [9].

Anti-inflammatory activity

In experimental conditions, the volume of the oedema induces by the carrageenan is maximal at the third hour [22]. The carrageenan causes a local inflammation when it is injected in plantar surface paw because of a tissular lesion. Injection of the carrageenan leads the liberation of several chemical mediators who are responsible of the inflammatory process. This biphasic inflammatory answer of which the initial phase, takes approximately one hour, is due to the liberation of histamine, serotonin, and bradykinine and the second phase is due to the liberation of prostaglandins and lysosomials enzymes (2-4 h) [1]. These mediators increase the permeability of the capillaries region pulling formation of an exudates which is the cause of the localized oedema which compresses nerves and gives pain [23]. The ethanolic extract has a significant anti-inflammatory activity at first hour with the percentages 16.95 % and 28.21 % for the respective doses 200 and 300mg / kg body weight. This anti-inflammatory activity is maintained during the experiment with the both doses of extract. This would be justified by the inhibition of the synthesis of pro-inflammatory substances in the first phase of the inflammation. Ethanolic extract at 300mg / kg has presented the biggest inhibition compared to the dose 200mg/kg. The results could be attributed to the presence of bioactive substances which would inhibit inflammation. The activity of the plant is dose dependent and it is the ethanolic extract in the dose 200 mg/kg which presents a lesser activity than that of the aspirin. The extracts of Phragmanthera capitata act at first hour, what justifies the use of plant on in the inflammatory diseases. The reduction of the oedema is maintained during the experiment in both doses. The
anti-inflammatory activity of this extract would be explain 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 antiphlogistic action [15]. There is a very large variety of phenols, to simple compounds as the salicylic acid, molecule gave aspirin by synthesis at complex substances as the phenolic 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 [24].

Aspirin (reference) reduced the inflammation by inhibition of cyclo-oxygenase enzyme responsible of the production of prostaglandins and thromboxane [25]. Aspirin gradually reduces oedema with a maximum at the third hour, acts on all the phases of inflammation until the substance is eliminated.

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. These results are different to those obtained by Ladoh et al., 2015 [3]. (445.2 mg EAA/g) an explication can be given by not identical experimental conditions methanol as solvent, citrus sinensis as host plant, and the collect period.

Antioxidant activity

DPPH radical scavenging assay

Phragmanthera capitata Ethanolic extracts and ascorbic acid (reference) possess an anti-radical activity. Ethanolic extract of stem showed an anti-radical activity (ec 50 = 0.0085 mg/ml) higher than ascorbic acid (0.033mg/ml). These results are different to those obtained by ladoh et al., [3], an explication can be given by not identical experimental conditions methanol as solvent, citrus sinensis as host plant, and the collect period. Antioxidant activity of this extract would be due to its wealth in bioactive substances revealed by the phytochemical screening. There is a correlation between phenolic content compounds and anti-radical activities [26].

CONCLUSION

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

REFERENCES


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