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Phytochemical Screening, Anti-inflammatory and Analgesic Activities of Root Barks from *Acacia macrostachya* Reichenb. Ex DC. (Fabaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript. Author ACC managed the work, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author WLMEBK contributed to perform the inflammatory tests. Author TKT contributed to manage the interpretation of the data. Author CA contributed to perform the statistical analysis and analgesic testing. Author GLB contributed to perform the analgesic tests and read the manuscript. Author MTN contributed to realize the anti-inflammatory and analgesic tests. Author NO contributed to supervise the work. Author MK contributed to analyze the results, read and approved the manuscript. Author RWS designed the idea, supervised the work, analyzed the results, read and approved the manuscript.

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ABSTRACT

Aims: *Acacia macrostachya* Reichenb. ex DC. (Fabaceae) is used in traditional medicine for the treatment of many pathologies including diarrhea, malaria with convulsion and fevers, snake bites, vomiting, nausea, dysenteric syndrome and choleriformis, inflammatory diseases and old wounds.

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The purpose of this study was to carry out the phytochemical screening, to assess the general acute toxicity, the anti-inflammatory and analgesic activities of the dichloromethane extract of *A. macrostachya* root barks in order to develop new lower-toxic anti-inflammatory drugs.
Place and Duration of Study: The work was carried out in the Department of Traditional Pharmacopoeia and Pharmacy (MEPHATRA / PH) of the Institute for Research in Health Sciences (IRSS) and LABIOCA from Université Joseph KI-ZERBO in Ouagadougou between March and June 2020.
Methodology: Phytochemical screening was carried out through thin layer chromatography with specific reagents. Acute toxicity assay was carried out according to the "dose adjustment" method from the OECD guideline 423 2001. Analgesic effect was evaluated on the number of abdominal contortions induced by the intraperitoneal injection of acetic acid while the anti-inflammatory activity using the Carrageenan anti-edematous test was determined according to Winter.
Results: Phytochemical profile demonstrated the presence of alkaloids, coumarins, polyphenols, tannins, flavonoids, saponosides, triterpenes and sterols. At the dose of 200 mg/kg (bw), the extract inhibited acetic acid-induced pain by 67.08% and carragenaan-induced edema by 74.03%. *A. macrostachya* dichloromethane extract is slightly toxic with LD_{50} higher than 5000 mg/kg (bw).
Conclusion: The results of this study demonstrated the interest of *A. macrostachya* in the treatment of inflammatory pathologies and constitute a scientific basis of its traditional uses.

Keywords: Acacia macrostachya; phytochemical screening; acute toxicity; anti-inflammatory; analgesic.

1. INTRODUCTION

Inflammation is a defense mechanism response to the immune system on the body's part tissues, due to physical injury caused by chemical agents or pathogenic germs. Inflammation is generally a beneficial immune process, since it eliminates a pathogen but it causes pain, edema or fever which are unpleasant for the body. Pain remains one of the main reasons for seeking medical attention worldwide [1,2].

Inflammatory processes are involved in many degenerative diseases such as rheumatoid arthritis, gout, rheumatic polymyalgia, heart disease, asthma and cancer [3,4]. Nonsteroidal anti-inflammatory drugs are prescribed worldwide for the treatment of inflammations, but their use may be associated with a high frequency of intestinal side effects and erosions of the mucous membranes which can develop into ulcers leading to complications such as perforation, hemorrhage and increasing blood pressure [5].

In order to develop new less toxic anti-inflammatory drugs, researcher efforts have been increasingly focused on natural products especially medicinal plants. *Acacia macrostachya* Reichenb. ex DC. is a medicinal plant of the Fabaceae family used in traditional medicine for the treatment of many pathologies including diarrhea, malaria with convulsion and fevers, snake bites, vomiting, nausea, dysenteric syndrome and choleriformis, inflammatory diseases, old wounds, stomach cramps, uterine fibroid, intestinal parasites, dysmenorrhea, constipation and painful spasms [6].The previous studies demonstrated a powerful antioxidant activity of *Acacia macrostachya* with no toxicity against human DNA [7]. According to the success of the traditional uses of this plant for the treatment of inflammatory diseases, therefore it has been assumed that a high probability for this plant to possess antispasmodic, analgesic and anti-inflammatory properties. The leaves are consumed in large quantities to prevent the dissemination of venom in the blood, root barks are used as an aphrodisiac, and to treat blenorrhagia, and diarrhea accompanied by vomiting [6]. According to our bibliographic research, there is no scientific information on the analgesic and anti-inflammatory activities of the root barks of *A. macrostachya*.

The purpose of this study was to carry out phytochemical screening, assess the acute toxicity potential, and anti-inflammatory and analgesic properties of the dichloromethane root bark extract of *Acacia macrostachya*.

2. MATERIALS AND METHODS

2.1 Chemicals

Materials used for this study include: Methylene chloride, dimethyl sulfoxide, carrageenan, acetic acid, acetylsalicylic acid and paracetamol from sigma.

2.2 Plant Material

The plant material used in this study consists of the root barks of *A. macrostachya* (Fabaceae) harvested in February 2018 at Laongo, 25 miles from Ouagadougou Capital of Burkina Faso with the GPS coordinates 12°31'50", 52"N; 01°17'2,7"W and identified by Prof AmadéOuedraogo from the Laboratory of Ecology (University Joseph Ki Zerbo of Ouagadougou). A voucher specimen was deposited in the herbarium of University Joseph Ki-Zerbo (identification number 17252). The root barks were dried up at room temperature for two weeks under ventilation and then transformed into fine powder using an electric grinder. This powder (50 g) was macerated in dichloromethane (150 ml) for 24 hours under continuous agitation. After filtration, dichloromethane was evaporated using a rotary evaporator (BüchiRotavapor R-200) and lyophilized to obtain dried extract for the phytochemical and biological analyses.

2.3 Animals

Male and female NMRI mice weighing between 18 and 32 grams were used in this study. Animals are of the same breeds/strains and were supplied by the pet facility from the Department of Medicine, Traditional Pharmacopoeia and Pharmacy (MEPHATRA/PH) of the Institute for Health Science Research of Ouagadougou (Burkina Faso), and as well from the International Center for Research and Development on Livestock in the Subhumide area (CIRDES) by Bobo Dioulasso. All animals are tabulated in MEPHATRA/PH pet store at 25°c with 75% humidity and a 12/14 hour photoperiod for two weeks before starting the experiments. The *in vivo* testing was carried out in accordance with current laboratory animal care.

2.4 Phytochemical Screening

Phytochemical screening was carried out on thin layer chromatographic plates (60 f250, 20 x 20 glass support, fluka –silica gel) according to the methods described in the scientific literature [8,9]. This method uses the thin layer chromatography (TLC) to screen the principal chemical groups such as alkaloids, steroids, terpenes, flavonoids, tannins etc.

Several specific reagents were used to reveal these groups of compounds:

- The sulfuric vanillin reagent for terpenes and sterols;
- Neu's reagent for flavonoids;
- 5% methanolic $FeCl_3$ reagent for tannins and phenolic compounds
- The sulfuric anisaldehyde reagent for saponosides
- The 5% methanolic KOH reagent for coumarins
- Dragendor'ff reagent for alkaloids

2.5 Acute General Toxicity

The acute general toxicity test was carried out according to the "dose adjustment" method of the organization for cooperation and economic development (OCDE) guideline 423 2001 [10]. Six NMRI mice received orally 2,000 mg/kg body weight of *A. macrostachya* dichloromethane extract. After administration of these extracts, the animals were observed for two hours and afterwards, they were fed. They were then observed for 24, 48 and 72 hours, a week and two weeks, during this period the signs of toxicity including changes in morphology, mobility, tremors, weight, breathing, sensitivity to noise after metal shock, appearance of stool, mobility and death were noted [11].

2.6 Anti-inflammatory Activity: Carrageenan Anti-Edematous Test

The injection of carrageenan under the plantar fascia of the hind paw of the mouse causes an inflammatory reaction which can be reduced by any substance with anti-inflammatory power [12]. The mice were fasted for 17 hours before the test. A known volume, 0.05 ml of carrageenan (1% suspended in 0.9% NaCl) was injected under the plantar aponemosis of the hind paw, thus causing the appearance of edema in the metatarsal region. Seven lots of six mice were formed and tests were repeated 3 times. These lots were treated with the plant extract at 25, 50, 100, 200 and 400 mg/kg (per os) or the reference substance (acetylsalicylic acid) at 200 mg/kg, one hour before the injection of the carrageenan. The volume of the leg of each experimental animal was measured before, and 1, 3 and 5 hours after carrageenan injection. The volume variation of thetreated leg made it possible to evaluate the anti-inflammatory effect of the plant extract. The average volume of the edema of the treated paw was calculated from 3 deviation measurements not exceeding 4%. The anti-inflammatory activity was evaluated as a

percentage of the edema reduction in treated mice compared to the control group according to the following formula:

% inhibition = (A –B) / A x 100

A, represents the average difference of the increase volume in the paw of the mice in control group and B represents the average difference of the increase volume in the paw of the mice in treated groups.

2.7 Analgesic Activity: Acetic Acid Test

The intraperitoneal administration of acetic acid in mice causes abdominal contortions. The number of contortions observed after administration of a pharmacological substance made it possible to assess its analgesic peripheral effect. The analgesic effect was evaluated on the number of abdominal contortions induced by the intraperitoneal injection of acetic acid (0.6%) according to the method described by Sawadogo *et al*. [13]. The mice were fasted 17 hours before the experiment. Seven lots of six mice were formed and the tests were repeated 3 times. The control group received distilled water and the treated lots received the plant extract at 25, 50, 100, 200, and 400 mg/kg and the reference substance (paracetamol at 200 mg/kg) per os. One hour after the administration of the extracts, the animals received acetic acid intraperitoneally at 10 ml/kg. Five minutes after the acetic acid injection, the number of contortions was counted in each animal for 15 minutes. The analgesic effect was evaluated according to the following formula:

% inhibition = (1 - Wt / Wb) x 100

Wb represents the average of the number of contortions per mice in the control group and Wt is the average of the number of contortions per mice in the treated groups.

2.8 Statistical Analysis

Statistical analysis was performed using Graph Prism 5 software and One Way ANOVA “analysis of variance” followed by Dunnett’s tests were used as statistical processing. The differences are considered significant for p value less than 0.05. Values are expressed as mean ± SD, p <0.05 is considered significant compared to the control. (**) = $p < 0.01$, (***) = $p < 0.001$, and (****) = $p < 0.0001$ vs control.

3. RESULTS

3.1 Phytochemical Screening

The result of phytochemical screening by TLC highlighted the presence of alkaloids, coumarins, flavonoids, polyphenols, tannins, saponosides, triterpenes and sterols in *A. macrostachya* extract.

3.2 Acute General Toxicity

The results of the acute oral toxicity study in mice demonstrated no clinical signs of toxicity and death after the administration of 2000 mg/kg body weight (bw) of extract. All animals survived after 14 days of observation. According to the Global Classification and Harmonization System (GHS), the LD_{50} of the dichloromethane extract of *A. macrostachya* is higher than 5000 mg/kg.

3.3 Anti-inflammatory Effect

The result of anti-inflammatory effect of the dichloromethane extract of *A. macrostachya* at doses of 25, 50, 100, 200 and 400 mg/kg b.w. demonstrated significant decrease of the edema volume of treated mice after drug administration from one hour to five hours. The effective dose 50 (ED_{50}) value of the anti-inflammatory effect of *A. macrostachya* is about 52.89 mg/kg body weight. Our results show that the reference substance (acetylsalicylic acid) exhibited higher anti-inflammatory effect compared to the plant extract at 200 mg/kg, (Table 1).

3.4 Analgesic Effect

The analgesic effect induced by different doses of *A. macrostachya* dichloromethane extract compared to the paracetamol used as reference drug is reported in Table 2.

The analgesic effect of *A. macrostachya* dichloromethane extract reduced significantly the number of contortions in a dose-dependent manner. Thus the analgesic effect of the extract is more effective at 200 mg / kg and 400 mg / kg with respective inhibition percentages of 67.08 and 70.19%. The ED_{50} value of the analgesic effect of *A. macrostachya* is about 37.90 mg/kg body weight. The paracetamol at 200 mg / kg has an inhibition percentage of 73.76%. The results show that at the same dose of 200 mg / kg of body weight there is no significant difference between the activity of *A. macrostachya* and the reference substance (paracetamol).

Table 1. Anti-inflammatory effect of the dichloromethane extract of *A. macrostachya*

Sample	Dose (mg/Kg)	Volume of edema (mL)			Inhibition (%)		
		1 h	3 h	5 h	1 h	3 h	5 h
Control	0	0.28±0.06	0.43±0.07	0.46±0.12	0	0	0
A. macrostachya	25	0.26±0.01	0.38±0.01	0.31±0.02	7.58±0.01	10.57±0.01	32.97±0.02*
	50	0.23±0.01	0.35±0.02	0.26±0.02	15.97±0.01	17.75±0.02	43.12±0.02**
	100	0.22±0.03	0.31±0.03	0.15±0.03	21.76±0.03	26.63±0.03	67.39±0.03***
	200	0.17±0.04	0.25±0.05	0.12±0.02	39.32±0.04	41.12±0.05	74.03±0.02***
	400	0.16±0.01	0.22±0.02	0.09±0.01	41.12±0.01	49.35±0.01	79.59±0.01***
ASA	200	0.11±0.01	0.14±0.01	0.06±0.02	62.08±0.01	67.36±0.01	87.68±0.02***

Values are expressed as mean ± SD, p <0.05 is considered significant compared to the control. (**) = p < 0.01, (***) = p < 0.001, and (****) = p < 0.0001 vs control

Table 2. Analgesic effect of the root barks of *A. macrostachya*

Sample	Dose (mg/Kg)	Number of contortions	Percentage of inhibition (%)
Control	0	80.5 ± 3.32	0
A. macrostachya	25	52 ± 0.82	33.02 ±0.02 **
	50	34.75 ± 2.75	56.83 ± 0.02**
	100	31 ± 3.00	61.49 ± 0.04***
	200	26.5 ± 1.50	67.08 ± 0.02***
	400	24 ± 2.50	71.66 ± 0.04***
Paracetamol	200	23.25 ± 3.86	73.76 ± 0.01***

Values are expressed as mean ± SD, p <0.05 is considered significant compared to the control. (**) = p < 0.01, (***) = p < 0.001, and (****) = p < 0.0001 vs control

4. DISCUSSION

The results of this study show that the root barks of *A. macrostachya* have pharmacological properties such as anti-inflammatory and analgesic. Phytochemical screening revealed the presence of triterpenes and sterols, saponosides, tannins, alkaloids, coumarins, flavonoids and polyphenols in this plant. Our results corroborate with those of Tondé et al. [14] who proved that *A. macrostachya* is very rich in saponins, flavonoids, tannins, and alkaloids. Sawadogo et al., [7] also documented the presence of saponins, tannins, triterpenoids/steroids, antraquinons, flavonoids and alkaloids in *A. macrostachya*dichlomethane extract. These compounds are well known to possess anti-inflammatory, analgesic and antipyretic effects due to their inhibitory effect on the enzymes involved in the production of chemical mediators of inflammation and their antioxidant property [15]. The richness of *A. macrostachya* dichloromethane extract in phenolic compounds contribute to justify its use in traditional medicine for the treatment of pathology developing fever, pain and inflammation.

The dichloromethane root bark extract of *A. macrostachya* is considered to be practically non-toxic according to the Global Classification and Harmonization System (GHS). The LD_{50} of this extract would be higher than 5000 mg/kg. This result gives safe guarantee regarding the oral use of the extract.

A.macrostachya dichloromethane extract demonstrated significant inhibitory effect on the inflammatory edema induced by carrageenan in dose-dependent manner. This anti-inflammatory effect is lower in the initial phase (1 h) of edema and exhibited higher effect in the late phase (5 h). Carrageenan injection induces the release of several chemical mediators which are responsible of the inflammatory process. This inflammatory response is biphasic: the initial phase (0-1 hour) is characterize by the release of histamine and serotonin, the second phase (1-5 hours) by the bradykinin prostaglandins biosynthesis [16]. Moreover, the carrageenan-induced edema is sensitive to cyclooxygenase and lipoxygenase inhibitors [17]. Therefore, our results suggest that the anti-inflammatory effect of *A. macrostachya* root barks could be due to an antagonistic action on the biosynthesis of inflammation mediators including histamine, bradykinin, serotonin and prostaglandin. Indeed, the best edema inhibition effect of the extract was observed at the fifth hour.

Phytochemical screening revealed the presence of tannins, saponosides, flavonoids and triterpenes / steroids in *A. macrostachya* extract. According to Wang et al., [18], these components are responsible for the anti-inflammatory properties of plant extracts.

A. macrostachya dichloromethane extract exhibited significant analgesic effect in the acetic acid induced contortions in mice. Acetic acid injection caused tissue damages leading to the release chemical mediators including histamine, substance P, bradykinin, serotonin, and prostaglandins, as a result of cyclo-oxygenase-2 (COX-2) stimulation [19]. These chemical mediators are responsible of the abdominal contortions in mice. The acetic acid test is generally used to study the non-morphine analgesic effect of many compounds [17]. At different doses, the plant extract reduced significantly the number of abdominal contortions. The analgesic effect of the extract could be attributed to the presence of tannins, saponosides, flavonoids and triterpenes / steroids highlighted during chemical screening. Indeed, Zeashan et al., [20] reported that tannins, saponosides, flavonoids and triterpenes / steroids have anti-inflammatory and analgesic effects. Our further studies will focus on the inhibitory effects of *A. macrostachya* extracts against the enzymes and mediators implicated in the inflammation process.

5. CONCLUSION

This study made it possible to highlight the acute general toxicity, anti-inflammatory and analgesic properties of *A. macrostachya* root barks, plant used in traditional medicine for the treatment of inflammatory diseases. The results of this study constitute scientific basis for the traditional uses of this plant and prerequisite for further research and development of anti-inflammatory drugs. The low toxicity of this plant, especially by the oral route, provides a safe guarantee for its traditional and modern uses.

ETHICAL APPROVAL

The *in vivo* testing was carried out in accordance with current laboratory animal care and ethics guidelines for experimental pain research on conscious animals [21].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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