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EVALUATION OF THE ANALGESIC ACTIVITY OF THE AQUEOUS EXTRACT OF THE TRUNK BARKS OF *DETARIUM MICROCARPUM* GUILL. AND PERR IN *MUS MUSCULUS*

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ABSTRACT

This study aims to scientifically prove the traditional use of *Detarium microcarpum* to relieve pain. Phytochemical screening revealed in the aqueous extract of Detarium microcarpum trunk barks, there are alkaloids, polyphenols, tannins, flavonoids, leucoanthocyanins, saponosides, polyterpenes and sterols. However, the study indicated an absence of quinones. The determination of trace elements revealed contents of zinc (7.94 ± 0.11 µg/g), potassium (15723.97 ± 0.68 µg/g), copper (38.42 ± 0.44 µg/g), magnesium (1001.14 ± 0.23 µg/g) and iron (49.84 ± 0.70 µg/g). The study of the acute oral toxicity of the aqueous extract of the trunk bark of *Detarium microcarpum* in *Mus musculus* revealed that the extract was not toxic up to a dose of 2000 mg/kg bw. The percentages of inhibition of the aqueous extract of the trunk bark of *Detarium microcarpum* at doses of 100 and 200 mg/kg bw were respectively 59.09 and 68.18% while the percentage of inhibition of EFFERALGAN 1000 mg at the dose of 150 mg/kg bw was 75.77 %. The analgesic effect of EFFERALGAN 1000 mg at a dose of 150 mg/kg bw was the same ($p > 0.05$) as that of the aqueous extract of the trunk bark of Detarium microcarpum at a dose of 200 mg/kg bw. However, the analgesic effect of EFFERALGAN 1000 mg at a dose of 150 mg/kg bw was significantly greater ($p < 0.05$) than that of the aqueous extract of the trunk bark of *Detarium microcarpum* at a dose of 100 mg/kg bw. kg pc.

KEYWORDS: *Detarium microcarpum*, Analgesic, phytochemical screening, trace elements.

INTRODUCTION

The use of plants for food, construction and medicine is an ancient practice. More than 80 % of the African population uses traditional medicine for treatment.[1] *Detarium microcarpum* Guill. and Perr is a plant commonly found in sub-Saharan Africa and belongs to the Caesalpiniaceae family. According to the bulletin of the French Society of Ethnobotany (SFE), a decoction of the bark of *Detarium microcarpum* is commonly taken to relieve pain such as headaches, sore throats, back pain and painful periods.[12] The objective of this study is to scientifically prove the traditional use of *Detarium microcarpum* bark in the treatment of pain. The specific objectives are

- Search for secondary metabolites and trace elements in the aqueous extract of *Detarium microcarpum* trunk bark,
- Study the acute toxicity of the aqueous extract of the trunk bark of *Detarium microcarpum*,
- Evaluate the analgesic effect of the aqueous extract of the trunk bark of *Detarium microcarpum*.

MATERIAL AND METHODS

Plant material

The plant material is composed of trunk bark of *Detarium microcarpum*.

Animal material

Mice (*Mus musculus*) aged 16 weeks and weighing between 21.1 and 30.3 grams constituted the animal material.

Preparation of the aqueous extract of the trunk bark of *Detarium microcarpum*

The trunk bark of *Detarium microcarpum* collected in Korhogo – Ivory Coast was dried away from light for 11 weeks. They were made into powder using an electric grinder. Then, one hundred (100) grams of *Detarium microcarpum* trunk bark powder were added to a saucepan containing one liter of boiling distilled water and this mixture was brought to a boil for 15 to 20 minutes. After cooling, a first filtration was carried out on a sieve and the filtrate obtained was filtered twice on hydrophilic cotton. In addition, the filtrate from the last

two filtrations was placed in a crystallizer and brought to an oven at 40 °C for complete drying. Finally, the dry mass at the bottom of the crystallizer was scraped and made into a fine powder and the latter constituted the aqueous extract of the trunk bark of *Detarium microcarpum*.[2]

Phytochemical screening

The phytochemical screening consisted of searching for secondary metabolites in the aqueous extract of the trunk bark of *Detarium microcarpum*, likely to possess biological activities.[10]

Test for alkaloids

Alkaloids were detected by Dragendorff and Bouchardat reagents. 6 mL of each plant extract of the two extracts were evaporated on a sand bath. The residue of each extract is taken up in 6 mL of alcohol (60°) and the alcoholic solution thus obtained was distributed in two tubes test. In the first tube were added 2 drops Dragendorff reagents (aqueous solution of iodo-bismuth potassium). The appearance of a precipitate or an orange color indicates the presence of alkaloids. In the second tube, are added 2 drops of Bouchardat reagent (aqueous solution of iodine-iodide). The appearance of a reddishbrown color indicates their presence.

Test for phenols

2 mL of each extract was added a drop of alcohol solution of ferric chloride (2%). The appearance of a blackish-blue or darker or lighter green color indicated the presence of phenolic compounds.

Test for flavonoids

2 mL of each plant extract are evaporated in a capsule, without carbonizing the residue. After cooling, the residue is taken up 5 mL of hydrochloric alcohol (obtained by mixing 10 mL of 96° ethanol,10 mL of distilled water and 10 mL of concentrated hydrochloric acid) diluted 2 times in a test tube. It is added 2 to 3 magnesium shavings (exotherm). This gives a pinkorange or purple. The addition of 3 drops isoamyl alcohol intensifies a pink-orange or purple, indicating the presence of flavonoids. The control is performed with the alcoholic solution of quercetin.

Test for tannins

Detection of catechin tannins 5 mL of each extract are evaporated. The dry residue was added 15 mL of reagent Stiasny (10 mL of 40% formalin added 5 mL of hydrochloric acid (HCl) concentrate). The mixture was kept in a water bath at 80°C for 30 minutes. It is cooled under running water. The observation of large flake precipitate characterizes catechin tannins. Test for gallic tannins The solution containing the flakes is filtered and the filtrate collected is then saturated with sodium acetate. To the mixture, 3 drops of ferric chloride 2%. The appearance of an intense black-blue color indicates the presence of gallic tannins.

Test for sterols and polyterpenes

They were detected by the reaction of Liebermann. 5 mL of each of the two extracts were evaporated on a sand bath. The residue was dissolved in 1 mL of hot acetic anhydride; we added 0.5 mL of concentrated sulphuric acid. The appearance at the interphase of a purple ring, turning blue to green indicated a positive reaction.

Test for leucoanthocyanins

2 mL of each extract were evaporated. After cooling, the residue was added 5 mL of hydrochloric acid and 1 mL of isoamyl alcohol. The solution was heated for 15 minutes in a water bath at 80°C for 30 minutes. The appearance of a cherry-red or purple characterizes the presence of leucoanthocyanins.

Test for quinones

Identification of quinones was used Borntraeger reagent (ammonia diluted 2 times) that allows the detection quinone substances. Evaporated to dryness in a capsule 2 mL of each plant extract. The residue was mixed in 5 mL of hydrochloric acid (HCl) diluted 1/5. The solution is in a boiling water bath for half an hour in a test tube. Then cooled in a cold water stream and the hydrolyzate is extracted with 20 mL of chloroform in a test tube. The chloroform phase was collected in a test tube and mixed with 1/2 mL of dilute ammonia 2 times. The appearance of a color ranging from red to purple indicates the presence of quinones.

Test for saponins

10 mL of each plant extract were put into a test tube of 160 mm height and 16 mm in diameter. This was stirred vigorously test tube for 10 seconds. The foam height is measured after 10 minutes resting. A height of more than 1 cm of foam, indicates the presence of saponins. The saponins may also be demonstrated by the persistence of the foam.

Dosage of trace elements

The determination of the mineral content of the aqueous extract of the trunk bark of *Detarium microcarpum* was carried out using the calcination mineralization method.[11]

Sample preparation

The aqueous extract of the trunk bark of *Detarium microcarpum* was dried for 24 hours in a Memmert-Germany oven at 60°C. Then, It was preserved in glass vials to be calcined later.

Mineralization by calcination

A quantity (0.4 g) of aqueous extract of the trunk bark of *Detarium microcarpum* was weighed into a porcelain crucible of 30 mL capacity and was placed for 5 hours in a muffle furnace (Naberthem-Germany) set at 550°C. After cooling, 2 mL of 0.5 N chloridryc acid were added to the ash obtained then brought to complete evaporation on a sand bath. The final residue recovered is filtered into a 100 mL volumetric flask and distilled water was

added to reach the gauge mark. Five (5) mL of the filtrate were taken for the determination of minerals by the atomic absorption spectrophotometer (AAS 20 type VARIAN, Australia). The operation was duplicated in order to have an average of the content. The wavelengths at which potassium, iron, zinc, magnesium, and copper were read were respectively 766.5 nm; 248.3 nm; 258 nm; 285.2 nm and 324.7 nm. The results of the optical densities of each mineral made it possible to determine the quantities of minerals (ppm) contained in the aqueous extract of the trunk bark of Detarium microcarpum. The mineral contents (T) were determined as follows :

$$T = \frac{(C_{ess} - C_{bl})\, V}{P_{ess}}$$

T : content in µg/g
Cess : sample concentration (mg/mL)
V: recovery volume of the test (mL)
Cbl : blank concentration in mg/mL
Pess : test portion (g or Kg).

Study of the acute toxicity of the aqueous extract of trunk bark of *Detarium microcarpum* in *Mus musculus*

The study of the acute toxicity of the aqueous extract of the trunk bark of *Detarium microcarpum* was carried out by the oral route.[13] The study was carried out with six female mice (*Mus musculus*) aged 20 weeks and weighing between 21 and 24.1 g. The trial design used in the study was that using an initial dose of 300 mg/kg body weight (bw). The aqueous extract of *Detarium microcapum* trunk barks was tested in a sequential process in which three female mice were used at each stage. The absence or manifestation of mortality linked to the aqueous extract of the trunk bark of *Detarium microcapum* at a dose of 300 mg/kg bw determined the next step, that is to say : administration of the dose immediately higher (2000 mg/kg bw) in three additional female mice and continuous observation for 14 days was made to note clinical signs and possible deaths. The dose administered obeyed the rule of 1 mL/100 g bw.

Evaluation of the analgesic activity of the aqueous extract of the trunk bark of *Detarium microcarpum*

An analgesic substance reduces the number of contortions. In this study, twenty-four (24) male and female mice with an average weight of 30.28 ± 0.8 grams, aged 22 weeks were divided into 4 batches each containing 6 mice. Each batch of mice was treated as follows

- Batch 1: 1 mL of distilled water (untreated batch)
- Batch 2: 100 mg/kg bw of the aqueous extract of the trunk bark of *Detarium microcarpum*
- Batch 3: 200 mg/kg bw of the aqueous extract of the trunk bark of *Detarium microcarpum*
- Batch 4: 150 mg/kg bw of EFFERALGAN 1000 mg.

One hour after these treatments, the induction of abdominal contortions was done by injecting 1% acetic acid intraperitoneally.[3] Each mouse in each batch received 1% acetic acid at a rate of 1 mL/100 g bw and was then placed alone in a transparent cage and the number of contortions was counted over a period of 30 minutes. The contortions per batch were summed and an average calculated.

The analgesic effect of the aqueous extract of the trunk bark of *Detarium microcarpum* was determined according to the percentage (%) of inhibition calculated as follows

$$\% \text{ of inhibition} = \frac{\text{Number of contorsions of the untreated batch} - \text{Number of contorsions of the test batch}}{\text{Number of contorsions of the untreated batch}} \times 100$$

Statistical analysis

The results were expressed as means followed by the standard deviation (SD) of the mean (Mean ± SD). The graphical representation of the data was carried out using the Graph Pad Prism 8.0.1 software. Statistical analysis of the results was carried out using analysis of variance (ONE WAY ANOVA). Differences between means were determined using Dunnett's multiple comparison test. The significance threshold is set at $p < 0.05$ for the expression of the results.

RESULTS

Phytochemical screening

The phytochemical screening revealed in the aqueous extract of the trunk bark of *Detarium microcarpum*, the presence of alkaloids, polyphenols, tannins, flavonoids, leucoanthocyanins, saponosides and polyterpenes and sterols (Table I). However, the study indicated an absence of quinones.

Table I: Phytochemical screening of the aqueous extract of *Detarium microcarpum*.

Phytochemical compounds	Trunk bark of *Detarium microcarpum* Aqueous extract of the trunk bark of *D. microcarpum*
Alkaloids Dragendorff	+
Alkaloids Bouchardat	+
Polyphenols	+
Catechin tannins	+
Gallic tannins	+

Flavonoids	+
Leucoanthocyanins	+
Sterols and polyterpenes	+
Quinones	-
Saponins	+

(+): Presence (-): Absence.

Dosage of trace elements

The determination of trace elements in the aqueous extract of the trunk bark of Detarium microcarpum revealed contents of zinc (7.94 ± 0.11 µg/g), potassium (15723.97 ± 0.68 µg/g), copper (38.42 ± 0.44 µg/g), magnesium (1001.14 ± 0.23 µg/g) and iron (49.84 ± 0.70 µg/g) (Table II).

Table II: Trace element content of the aqueous extract of the trunk bark of *Detarium microcarpum*.

Trace elements	Contents (µg/g of dry extract) Aqueous extract of *Detarium microcarpum*
Zinc	7,94 ± 0,11
Potassium	15723,97 ± 0,68
Copper	38,42 ± 0,44
Magnesium	1001,14 ± 0,23
Iron	49,84 ± 0,70

Toxicity

The study of the acute oral toxicity of the aqueous extract of the trunk bark of *Detarium microcarpum* in *Mus musculus* revealed that it was not toxic because at the dose of 2000 mg/kg bw, no mortality or any no significant change in behavior was reported.

Analgesic activity of the aqueous extract of the trunk bark of *Detarium microcarpum*

Following the injection of 1% acetic acid into mice from different batches, the number of contortions in untreated rats (batch 1) was 22.00 ± 1.00 contortions compared to 9.00 ± 0.58; 7.00 ± 0.58 and 5.33 ± 0.33 contortions respectively for batch 2 (batch treated with the aqueous extract 100 mg/kg bw); batch 3 (batch treated with the aqueous extract 200 mg/kg bw) and batch 4 (batch treated with EFFERALGAN 1000 mg to 150 mg/kg bw). The treatments carried out in rats from batches 2 ; 3 and 4 very significantly reduced ($p < 0.0001$) contortions compared to the contortions of rats in the untreated group (batch 1).

The percentages of inhibition of the aqueous extract of the trunk bark of *Detarium microcarpum* at doses of 100 and 200 mg/kg bw were respectively 59.09 and 68.18 % while the percentage of inhibition of EFFERALGAN 1000 mg at the dose of 150 mg/kg bw was 75.77%.

The analgesic effect of EFFERALGAN 1000 mg at a dose of 150 mg/kg bw was the same ($p > 0.05$) as that of the aqueous extract of the trunk bark of *Detarium microcarpum* at a dose of 200 mg/kg bw . However, the analgesic effect of EFFERALGAN 1000 mg at a dose of 150 mg/kg bw was significantly greater ($p < 0.05$) than that of the aqueous extract of the trunk bark of *Detarium microcarpum* at a dose of 100 mg/kg bw. kg pc. Figure 1 shows the analgesic activity of the aqueous extract of the trunk bark of *Detarium microcarpum* and EFFERALGAN on contortions induced by 1% acetic acid. Figure 1 shows the number of contortions recorded depending on the treatments.

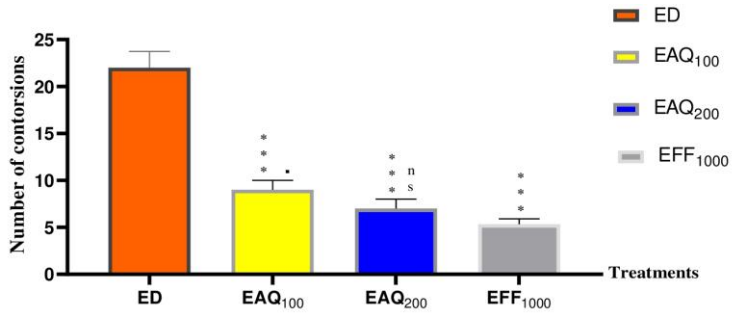

Figure1: Number of contortions recorded according to treatments

****p < 0.0001 :** very highly significant difference compared to the untreated batch (lot1)

***p < 0.05 :** significant difference compared to EFFERALGAN 1000 mg (lot 4)

ns : non-significant difference compared to EFFERALGAN 1000 mg (lot 4)

ED : batch of rats treated with distilled water

EADQ100 : batch of rats treated with the aqueous extract of the trunk bark of Detarium microcarpum 100 mg/kg bw

EAQ200 : batch of rats treated with the aqueous extract of the trunk bark of Detarium microcarpum 200 mg/kg bw

EFF1000 : batch of rats treated with EFFERALGAN 1000 mg at a dose of 150 mg/kg

DISCUSSION

Phytochemical screening revealed in the aqueous extract of the trunk bark of *Detarium microcarpum*, the presence of alkaloids, polyphenols, tannins, flavonoids, leucoanthocyanins, saponosides, polyterpenes and sterols. Except for tannins, the secondary metabolites found in the aqueous extract of the trunk bark of *Detarium microcarpum* are the same as those found in the aqueous extract of the stem bark of *Xylopia villosa*.[4]

These secondary metabolites participate in the life of the plant with the environment and they have varied roles. They can serve as defense for plants or, on the contrary, attract certain species with a beneficial role such as pollinators.[5]

The determination of trace elements in the aqueous extract of the trunk bark of *Detarium microcarpum* revealed the presence of zinc, potassium, copper, magnesium and iron. These trace elements are the same as those found in the aqueous extract of the stem barks of *Xylopia villosa*.[4]

The absence of mortality and abnormal behavior in mice after gavage of the aqueous extract of the trunk bark of *Detarium microcarpum* at a dose of 2000 mg/kg bw demonstrates that the lethal dose 50 of said extract is beyond 2000 mg /kg pc.[13]

Contortion is a violent and abnormal twisting of muscles and limbs. It is also a violent and disorderly movement. Administration of the aqueous extract of *Detarium microcarpum* trunk bark reduced writhing. The inhibitory effect exerted by the aqueous extract of the trunk bark of *Detarium microcarpum* on the contortions induced by 1% acetic acid is similar to that of *Bauhinia purpurea* Lam.[6] Indeed, *Bauhinia purpurea* is used in India, Malaysia and Pakistan to treat jaundice, cough, diabetes, ulcers, infectious diseases, convulsions and pain.[6] The reduction of contortions by the aqueous extract of the trunk bark of *Detarium microcarpum* on the contortions induced by 1% acetic acid is also similar to that of the aqueous extract of the stem bark of Pterocarpus erinaceus Poir.[7] The analgesic activity of *Detarium microcarpum* is also similar to species of the genus Desmodium, which are used in traditional Chinese and Indian medicines to treat dysentery, malaria, cough, fever, rheumatism and pain.[8] This analgesic effect of the aqueous extract of the trunk bark of *Detarium microcarpum* would also be linked to the magnesium present in the aqueous extract of *Detarium microcarpum* because magnesium would have an impact on the number of migraines and the intensity of the pain by reducing the frequency and duration.[9]

CONCLUSION

Detarium microcarpum is not toxic in *Mus musculus* up to a dose of 2000 mg/kg body weight. The presence, on the one hand, of secondary metabolites (alkaloids, polyphenols, tannins, flavonoids, leucoanthocyanins, saponosides and polyterpenes and sterols) and on the other hand, of trace elements (zinc, potassium, copper, iron and magnesium) in the aqueous extract of the trunk bark of *Detarium microcarpum* are the basis of the analgesic effect of said extract.

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