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ANTIOXIDANT AND ANALGESIC ACTIVITIES OF ETHYL ACETATE EXTRACT FROM STEM OF GNIDIA DAPHNIFOLIA L.F (THYMELACEAE)

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Abstract
Gnidia daphnifolia L.f is among the *Gnidia* species endemic to Madagascar. It is a medicinal plant traditionally used in a treatment of several diseases including diarrhea, dysentery, fatigue and yellow fever or hepatitis. The aim of this work is to carry out the antioxidant of different extract and analgesic activity of ethyl acetate extract of *Gnidia daphnifolia* L.f stems. Different extraction methods such as Soxhlet extraction, reflux extraction and cold maceration have been carried out to obtain extracts. Phytochemical screening of the hydroethanolic extract allowed the presence of polyphenols, triterpene and steroids. Hexane, ethyl acetate and methanolic extracts were showed an antioxidant property. Methanolic extract had a significant antioxidant activity between these three extracts. Analgesic properties of ethyl acetate extract were conducted on experimental animals. At 50 et 100 mg/kg body weight of ethyl acetate extract, the results are significant compared of negative control. This extract inhibited both the pain induced by the acetic acid injection and the immersion of the mice's tail in thermostatic water. The high-dose of this extract produced is toxic for the mice. So, these pharmacological properties could be due the presence of these families chemical compounds showed in phytochemical screening.

KEYWORDS: *Gnidia daphnifolia* L.f, analgesic, antioxidant, *Writhning*, *Tail-flick.*

1. INTRODUCTION

Free radicals have been implicated as a factor at the origin of several human pathologies such as cancer, cardiovascular and neurodegenerative diseases, inflammation, diabetes, osteoarthritis, atherosclerosis and especially aging.[1,2] In addition, pain is one of the frequent causes in the medical consultation. It is considered a sign of the disease. According to its duration, it is divided into two classes: acute pain and chronic pain.[3,4] Faced with this situation, the majority of the world's population, especially in developing countries, is treated with traditional herbal remedies. This holds that the plant kingdom becomes an important alternative way to explore in order to discover a variety of active principles.

Gnidia daphnifolia L.f is a medicinal plant, endemic to Madagascar, traditionally used for the treatment of diarrhea, dysentery and yellow fever.[5,6] It belongs to the Thymelaceae family and the *Gnidia* genus. *Gnidia daphnifolia* L.f is a tree about 2 m tall, with glabrescent branches, outer bark reddish brown.[7] It is characterized by its fibrous stems, with which the Antaimoro papers have been manufactured. It spreads in the Mananara basin, tributary of Mandrare and also in the western slopes of the mountains between Andohahela and Elakelaka (mountain of Apiky above Mahamavo), Ankarana, and Tsaratanana.[8]

The present study aims to evaluate the antioxidant activity using the biautography method and the analgesic properties of the hydroethanolic extract of the stems of *Gnidia daphnifolia* L.f by referring to the inhibition of pain induced by acetic acid and hot water temperature at 55-56°C.

2. MATERIALS AND METHODS

2.1. MATERIALS

Other technical materials, various materials were used for the realization of this work: plant and animal materials, some chemicals.

1.1.1. Plant materials

Gnidia daphnifolia L.f, identified by Jaqueline Razanatsoa, Botanist in Botanical and Zoological park of Tsimbazaza, Antananarivo, were collected in May 2018 at Ramena, Region of Diana (Madagascar). The stems of this plant were dried an oven with air until complete dehydration. Then, it was triturated until in order to obtain a finely granulated powder in which hydroethanolic extract was realized.

1.1.2. Animals

Male and female Swiss mice weighing 17-20 g and aged 3-4 weeks were used to evaluate analgesic activity of plant extract. The animals were kept in plastic cages at room temperature with free access to rations and water.

1.1.3. Drugs and chemicals products

The following chemical compounds were used: Paracetamol, acetylsalicylic acid and acetic acid.

2.2. METHODOLOGY

Several methods were applied such as preparation of hydroethanolic extract, phytochemical screening, and evaluation of the antioxidant and analgesic activities.

1.1.4. Preparation of hydroethanolic and ethyl acetate extract

The hydroethanolic extract was obtained from maceration in ethanol-water (70/30; v/v) of this powder (50 g) for 7 days. The mixes were stirred frequently. Afterward, the solution was filtered through cotton wool. Then, the solvent was evaporated and hydroethanolic extract was reached. For the ethyl acetate extract, the reflux extraction technique was applied.

1.1.5. Phytochemical screening

Phytochemical screening is a process that allows highlighting the presence of chemical families in an extract such polyphenols, alkaloids and terpenes. The methods described by FONG and *al.* were applied.[9]

The characterization of saponins is based on the appearance of foams after the agitation of the extracts. The search for polysaccharides was made by reaction with ethyl alcohol. Alkaloids were detected using general alkaloid characterization reagents. Three reagents were used, namely the reagents of Dragendorff, Wagner and Mayer. The flavonoids were characterized by the Wilstater and Bate-Smith tests, the tannins and polyphenols by the ferric chloride test. The Lieberman-Burchard test made it possible to characterize triterpenes and steroids.

1.1.6. Antioxidant activity

Various methods can be applied to assess the antioxidant activity of an extract, in this study, the Biautography method which is based on the DPPH (2.2-diphenyl-1-picrylhydrazyl) free radical scavenging test, was applied. The DDPH was dissolved with methanol (violet color). The extracts were deposited on the TLC plate and the latter was soaked in a Petri dish containing DPPH° solution. A change in color from purple to yellow indicates that the extract is active.[10]

1.1.7. Analgesic activity

Analgesic activity of plant extract was evaluated with periphery and central components by the *Writhning* and *Tail-flick* tests.

2.2.4.1. *Writhning* test

The peripheral analgesic activity of the M_2 extract was evaluated according to the number of stretching movements of the hind legs and torsions of the dorsal-abdominal musculature induced by the intraperitoneal injection of 3% acetic acid according to the method described by Kazunaga and *al*.[11] Collier and *al*.[12] Sawadogo and *al*.[13]

The mice were divided into five groups of three mice. Each batch consists of male and female mice. The mice of the negative control batch received distilled water orally, on the other hand the mice of the other batches received the ethyl acetate extract 50 mg/kg and 100 mg/kg of body weight (BW) and Paracetamol at 100 mg/kg BW (positive control), by oral administration.

Two hours after the administration of the extracts, the animals received intraperitoneally 3% acetic acid at a rate of 10 ml/kg BW.

Five minutes after the injection of acetic acid, the number of pain syndromes was recorded for 30 minutes.

The analgesic effect was evaluated according to the formula:

$$\%\text{pain inhibition} = 1 - \frac{Wt}{\text{Wb}}$$

Wt represents the mean number of contortions of the mice of the negative control batch and Wb is the mean of the number of contortions of the mice of the treated batch.

2.2.4.2. *Tail-flick* test

The method described by Amour and Smith[14] and Gray et al.[15] was applied to evaluate the central analgesic effect of the hydroethanolic extract.

The mice were divided into five groups consisting of three mice. A group of three mice receiving distilled water orally was examined as a negative control. The mice from the other batches received the extract at different doses of ethyl acetate extract (50 and 100 mg/kg of BW) and aspirin at a dose of 150 mg/kg BW.

The hot water bath at a temperature of 55°C to 56°C was used as an experimental device to produce pain. The mouse tail was put in hot water at its mid-length. The animals automatically removed their tails if they sensed the nociceptive disorder. The soaking time was timed.

For the determination of the nociceptive thresholds, three tests were carried out. The first is used for the habituation of the animals. The average calculated on the last two measurements consists in determining this threshold.

The percentage of pain inhibition was determined according to this formula

$$\%\text{pain inhibition} = \frac{M - T}{T}$$

M: reaction latency time of the processed batch (in seconds);
T: negative control batch reaction latency time (in seconds).

2.2.4.3. Statistical analysis

The results are expressed as the average ± SEM the data obtained was statistically analyzed using the Student's "T" test on the "Microsoft Excel 2016" software. The differences considered significant are at p less than 0.05 ($p < 0.05$) and p less than 0.01 ($p < 0.01$) when compared with the negative control.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical screening

Phytochemical studies of the hydroethanolic extract of the stems of *Gnidia daphifolia* L.f made it possible to detect the presence of polyphenols and terpenes. Alkaloids, saponins and polysaccharides are absent.

3.1. Antioxidant activity

For the different extracts, a qualitative study on CCM was carried out to determine their antioxidant activity. After the TLC plate was soaked in the methanolic solution of the radical DPPH, the results of this test was assigned in the Table 2.

***Table 1: Chemical composition of stem hydroethanolic extract of Gnidia daphnifolia* L.f.**

Chemical Families	Saponines	Polysaccharides	Alkaloids	Flavonoids	Tannins and Polyphenols	Quinone	Steroids and terpenoids
Hydroethanolic Extract	-	-	-	++	+++	++	+++
++ abundant, +++ highly abundant, - absence							

Table 2: Antioxidant activity of different extracts of Gnidia daphnifolia L.F stems.

Sample	Zone diameter (cm)	Results and comparison
Hexane extract M_1	0.4	Weakly active extract
AcOEt extract M_2	0.6	Moderately active extract
MeOH extract M_3	0.9	Highly active extract

The comparison of the antioxidant activity of different extracts shows that the methanolic extract has the largest trapping zone diameter; hence it exhibits high antioxidant activity compared to the other two extracts.

The antioxidant activity of these extracts increases with their polarity, the more polar the extract, the better it shows activity. This activity of the methanolic extract M_3 could be due to the presence of many antioxidant compounds such as phenolic compounds.

3.2. Analgesic activity

4.3.1. Writhning test

The inhibitor capacity of the Aspirin, and the ethyl acetate extract form *Gnidia daphnifolia* L .f stems on the painful syndrome inducing by the injection of the acetic acid are shown in Table 3.

For the mice in the negative control batch that received distilled water, the average number of abdominal cramps is 7 ± 2.8 during 30 min which corresponds to 0% of pain inhibition. In the presence of Aspirin (150 mg/kg of BW), no abdominal torsion was recorded, so the percentage of pain inhibition is 100%. The statistical analysis of the results between Aspirin and negative control show a significant effect ($n=3$, $p<0.05$), which it validates the methodology and the results obtained. After the injection of acetic acid on the mice treated with plant extract, the number of abdominal pain varies depending on the dose received.

For 50 mg/kg BW of ethyl acetate extract, 15±0.2 of pain syndrome was listed with 78.57% of pain inhibition and 54±0.4 for the dose 100 mg/kg BW. The difference is significant for the dose 50 mg/kg BW by comparing this results with negative control ($n=4$, $p<0.05$). But for the other dose, pain inhibition takes on negative value. This case could be related of the toxicity of the plant and this explains the appearance of opposite effect.

4.3.2. Tail-flick test

Immersion of the mice's tail in a thermostatic water bath produced a pain syndrome which the mice automatically pull its tail out of the water bath. The Table 4 shows the effect of Paracetamol and ethyl extract from *Gnidia daphnifolia* L.f stems on the latency time of mice's tail output in the water bath.

The average latency time is 4.36±0.10 seconds for the negative control batch mice that received distilled water, which corresponds to 40% of pain inhibition. In the presence of Paracetamol 100 mg/kg BW, this time

increases significantly (n=4, p<0.05). This significant difference indicates the validation of the procedures and the results obtained.

Concerning the mice treated with 50 mg/kg and 100 mg/kg BW of ethyl acetate extract from *Gnidia daphnifolia* L.f stem, the latency time are respectively 7.94±0.22 seconds, so the pain inhibition is 82.11% and 4.61±0.28 seconds which corresponds to 5.73% of pain inhibition.

According to the statistical analysis, the comparison of the results of the extract has a significant threshold compared to the negative control. But, the extract at 50 mg/kg BW is highly significant (n=3, p<0.01) than that 100 mg/kg BW.

4. CONCLUSION

The three extract of *Gnidia daphnifolia* L.f stems have antioxidant properties. This property is probably linked by the presence of phenolic compounds.

The significant results obtained during this study showed that *Gnidia daphnifolia* L.f stems have also analgesic properties (central and peripheral). Oral administration of high-dose ethyl acetate extract is very toxic to mice. So, this plant should be used with precautions.

In-depth chemical and pharmacological studies will be considered to isolate and identify precisely the compounds responsible of these activities and to understand their level of action as well as their mechanism of action.

Table 3: Results of Wirthning test.

Group	Number of abdominal cramps (average ± ESM)	Pain inhibition (%)
Distilled water Negative control	7.0 ± 2.8	0
Aspirin Positive control	0	100
Gnidia daphnifolia L.f 50 mg/kg BW	1.5 ± 0.2	78.57
100 mg/kg BW	54.0 ± 0.4	-617.4

Table 4: Results of Tail-flick test.

Group	Latency time (average ± ESM) in seconds	Pain inhibition (%)
Distilled water Negative control	4.36 ± 0.10	0
Paracetamol Positive control	6.29 ± 0.38	44.26
Gnidia daphnifolia L.f 50 mg/kg BW	7.94 ± 0.22	82.11
100 mg/kg BW	4.61 ± 0.28	5.73

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