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### Study of the Analgesic and Toxic Effects in Vivo of a Selective Ethyl Acetate Extract of the Leaves of *Gossypium Barbadense* L. (Malvaceae) and Structural Characterization of an Isolated Phytoconstituent

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#### ABSTRACT

*Gossypium barbadense* L. is a medicinal Malvaceae used as an indispensable intractant in the endogenous medication of skin super infection caused by shingles in Côte d'Ivoire. The objective of this study was to study the analgesic and toxic effects in vivo of the extract with ethyl acetate GB2.3, obtained from the hydroalcoholic crude extract of the leaves of *Gossypium barbadense* L., and to characterize a phytoconstituent of the extract. The results obtained showed that the extract showed a significant analgesic effect by inhibition at 73.66% of the pain induced by acetic acid in mice and that it had insignificant toxicity at the dose of 100 mg/kg body weight. A phytoconstituent was isolated with a yield of 15.21% by HPLC, whose molecular structure was determined by spectrometry (MS, IR, NMR <sup>1</sup>H, <sup>13</sup>C).

**Keywords:** *Gossypium barbadense*, analgesic effect, toxicity, structure, spectrometry

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## INTRODUCTION

Zoster-induced superinfection, like all skin conditions, is a major public health problem in tropical countries<sup>1-2</sup>. The pathogenic species in question are of the genus *Staphylococcus*, namely *Staphylococcus aureus* and *Streptococcus pyogenes*<sup>3-5</sup>. In Sub-Saharan Africa in general, and in Côte d'Ivoire in particular, we are witnessing the uncontrolled all-out proliferation of bacteria due to antibiotic resistance<sup>6</sup>, which is one of the forms of drug resistance in full progression, which crystallizes medical research. It is a global scourge which constitutes a real worrying public health problem. This is why, in order to find new molecules of antibacterial plant origin, a bio-guided phytochemical investigation was carried out on *Gossypium barbadense* L. (Malvaceae), a plant of the Ivorian flora used in the treatment of secondary infection caused by shingles. Previous studies by Koffi *et al.*<sup>7</sup> have shown that the ethyl acetate extract from the crude hydroethanolic extract of the leaves of *G. barbadense* has good antibacterial and antioxidant potential. In this investigation, therefore, the aim was to assess the analgesic activity and toxicity of the ethyl acetate extract and also to isolate and characterize the phytoconstituents present.

## MATERIALS AND METHOD

### Plant material

The analyte is a selective ethyl acetate extract coded GB2.3, obtained from the crude hydro ethanolic extract of the leaves of *G. barbadense*.<sup>7</sup>

### Animals

*Mus musculus* mice, male and female, of homogeneous parental strains *Swiss*, 8 weeks old, weighing between 18 and 32 g, coming from the pet store of the UFR Biosciences of the Félix Houphouët-Boigny University (Abidjan / Côte d'Ivoire), were used for the experiment. The animals were treated in accordance with the requirements of bioethics.<sup>8</sup>

### Analgesic test

Before the experimentation, 4 lots of 5 mice were fasted for 16 h after being acclimated.<sup>9</sup>

- The first batch (negative control) received 0.1 mL of distilled water intraperitoneally (ip).
- The second and third batches received intraperitoneally ip 10 mg/mL of GB2.3, ie 100 mg/kg of body weight.
- The fourth batch (positive control) received intraperitoneally (ip) 10 mg/mL of Paracetamol (N- (4-hydroxyphenyl)-acetamide), ie 100 mg/kg of body weight. The animals received after 30 min by ip injection 0.1 ml acetic acid (CH<sub>3</sub>CO<sub>2</sub>H, 1%).<sup>10</sup>

The percentage of cramp inhibition was calculated according to the formula<sup>9</sup> :

$$\% \text{ of inhibition} = \frac{W_{c1a} - E_{ppa}}{W_{c1a}} \times 100$$

**W<sub>c1a</sub>** : Average number of contortions of mice in the white control lot.

**E<sub>ppa</sub>** : Average number of contortions of mice treated with plant extracts and Paracetamol.

### Toxicity test

The *in vivo* toxicity test was performed out in accordance with code 423<sup>11</sup>. Before the experimentation, 2 lots of 3 acclimatized mice were fasted for 16 h. The GB2.3 extract (1 mL) at the doses of 300 mg / kg and 2000 mg / kg of body weight was administered by gavage to the mice of the different batches, and the mortality was observed over 21 days. The operation is repeated three times.

### Isolation by HPLC

A mass (112.87 mg) of GB2.3 was dissolved in 20 mL of distilled water. The solution obtained is filtered through a 0.45 µm diameter membrane (MILLEX GV®), then introduced into the column of the chromatograph for the separation of phytoconstituents. The elution conditions by gradient are shown in Table 1.

**Table 1: Elution conditions**

T(min)	0.1% TFA in H <sub>2</sub> O	0.1% TFA in MeCN
0.3	95	5
50	0	100
52	0	100
53	95	5

TFA : trifluoroacetic acid ; MeCN : acetonitrile

The analysis of the fractions obtained was carried out by CCM.

### Structural analysis

Fourier transform and NMR IR spectrometries (<sup>1</sup>H, <sup>13</sup>C, COZY, DEPT, HSQC and HBMC) were performed respectively on Thermo Electron-Nicolet (Nexus 870) and Bruker Avance III (600 MHz) spectrometers. DMSO-d<sub>6</sub> was the diluting solvent.

## RESULTS AND DISCUSSION

### Analgesic activity

Peritoneal injection of acetic acid (1%) caused abdominal contractions or stretching of the hind legs in mice. The effects of the GB2.3 extract tested were highlighted with regard to the number of abdominal contractions or stretching of the hind legs. From this point of view, the GB2.3 extract revealed a manifest analgesic effect. Otherwise, compared to Paracetamol (pain inhibited 100%), the ability of the GB2.3 extract to inhibit pain is established. In fact, 73.66% of the pain induced by

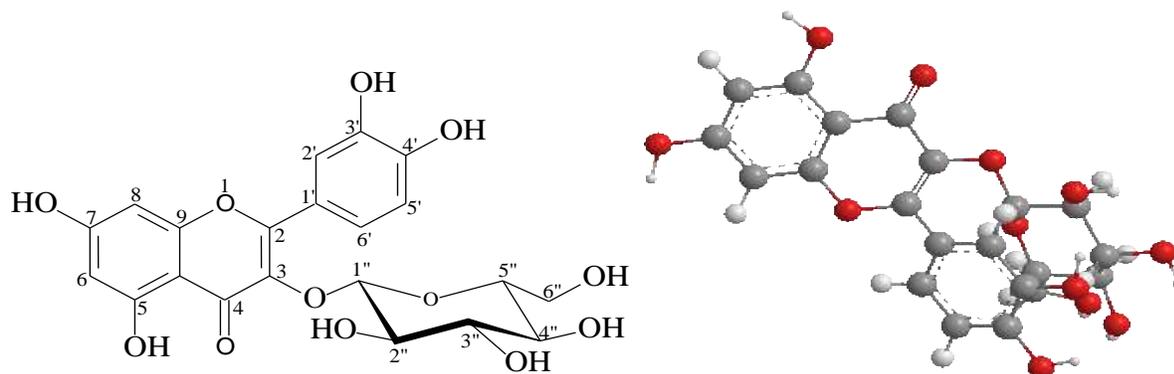
acetic acid in mice is inhibited by GB2.3. Kouakou-Siransy *et al.*<sup>12</sup> have shown that the methanol extract of the leaves of *Gossypium hirsutum sp* has a significant analgesic effect at a dose of 37.5 mg/kg of body weight. Acetic acid involves the peripheral mechanisms of pain. It induces the release of many chemical mediators involved in pain such as histamine, prostaglandins (PGE<sub>2</sub> and PGE<sub>α</sub>), serotonin, bradykinin<sup>13</sup>. These mediators have been demonstrated in high proportions in the peritoneal exudates of rodents after injection of acetic acid<sup>14</sup>. Paracetamol acts on the mechanisms pain by intervening in the biosynthesis of prostaglandins<sup>15</sup>. Thus, the fraction tested having an analgesic effect at a dose of 10 mg / kg of body weight, could have an inhibiting effect on the release of the mediators involved in peripheral pain. Koffi *et al.*<sup>7</sup> reported that the GB2.3 ethyl acetate extract is rich in flavonoids and has a significant anti-free radical property against the stable radical DPPH. The presence of flavonoids in GB2.3 seems to be at the origin of the analgesic effect of the leaves of *G. Barbadense* observed. Indeed, some studies showed the analgesic effect of flavonoids and other secondary metabolites identified in extracts of plant matrices<sup>16-18</sup>.

### **Toxicity**

After administration of the doses of the GB2.3 extract to animals, some clinical signs have been observed, in particular the acceleration of the heart rate, breathing difficulties, slight twisting of the hind legs, immobility, agitation, convulsions, the reduction of activities and the dormancy on the belly with the hind legs apart. At the 2000 mg / kg of body weight dose, only one of the three mice used per batch did not survive. What represents a mortality rate of 33.33% per batch. The surviving animals returned to normal appearance the following days. What could be justified by the capacity of mice to metabolize the phytoconstituents of plant extracts<sup>19</sup>. The various mortalities made it possible to classify *G. Barbadense* in categories 5. Thus, the median lethal dose (LD<sub>50</sub>) of *G. barbadense* is between 2000 and 2500 mg / kg of body weight. According to the toxicity scale drawn from the work of Hodge & Sterner<sup>20</sup>, we can conclude that GB2.3 has a low toxicity.

### **Molecular structure of the phytocompound isolated from the GB2.3 extract**

The HPLC made it possible to isolate with 15.21% yield, the phytocompound (orange-yellow powder), tested positive for the Neu reagent by TLC, indicating its belonging to the family of flavonoids. The results of the spectral analyzes (**Table 2**), compared with the spectral data drawn from the literature<sup>21-22</sup>, suggests a molecular structure similar to that of Quercetin 3-O-β-D-glucopyranoside



Its mass spectrum in negative mode shows the peak of the pseudo molecular ion ( $m/z= 464.3 [M-H]^{-}$ ) corresponding to the crude formula  $C_{21}H_{20}O_{12}$  with a number of unsaturations equal to 12. Its IR spectrum shows the presence of the  $\nu_{O-H}$  valence adsorption bands at  $3400-3450\text{ cm}^{-1}$  (narrow and strong alcoholic and / or phenolic free OH);  $\nu_{C-H}$  (aliphatic) at  $1362\text{ cm}^{-1}$ ;  $\nu_{C-H}$  (aromatic) at  $2900-2950$ ;  $\nu_{C=C}$  (aromatic) at  $1500-1605\text{ cm}^{-1}$ ;  $\nu_{C=O}$  at  $1013-1060\text{ cm}^{-1}$ ;  $\nu_{C-O-C}$  at  $1200\text{ cm}^{-1}$ ;  $\nu_{C-C=O}$  ( $\alpha, \beta$ -unsaturated conjugate) at  $1605\text{ cm}^{-1}$ ;  $\nu_{C=O}$  at  $1656\text{ cm}^{-1}$ . The  $^1H$  and  $^{13}C$  NMR spectra respectively indicate the presence of 20 protons and 21 carbons (Table 2), corresponding to the crude formula.

**Table 2:**  $^1H$ ,  $^{13}C$ , HSQC and HMBC NMR spectral data of the isolated phytochemical

Position	RMN $^1H$ $\delta$ (ppm)	RMN $^{13}C$ $\delta$ (ppm)	DEPT 135	HSQC	HMBC (H $\rightarrow$ C)
1	-				
2	-	156.81	C		
3	-	133.85	C-O-		H-1''
4	-	177.94	C=O		
10	-	104.48	C		H-6 ; H-8
5	-	161.74	C		H-6 ; HO-C-5
6	6.21(d ; $J=2.0Hz$ )	99.12	CH	H-6 C-6	
7	-	164.58	C		H-6 ;H-8 ; HO-C-7
8	6.42(d ; $J=2.0Hz$ )	93.97	CH	H-8 C-8	
9	-	156.67	C		H-8
1'	-	121.67	C		H-1' ; H-5'
2'	(7.62 – 7.57) (m)	116.71	CH	H-2' C-2'	HO-C-3'
3'	-	145.29	C		H-2' ; H-5' ; HO-C-4'
4'	-	148.94	C		H-2' ; H-5' ; HO-C-4'
5'	(6.90 – 6.81) (m)	115.69	CH	H-5' C-5'	HO-C-4'
6'	(7.62 – 7.57) (m)	122.07	CH	H-6' C-6'	H-2'
1''	5.47 (d ; $J=7.4Hz$ )	101.41	CH	H-1'' C-1''	
2''	(3.40 – 3.21)	74.59	CH	H-2'' C-2''	
3''	(3.40 – 3.21)	78.03	CH	H-3'' C-3''	
4''	(3.40 – 3.21)	70.45	CH	H-4'' C-4''	
5''	(3.40 – 3.21)	77.02	CH	H-5'' C-5''	

6''	(3,40 – 3.21)	61.49	CH <sub>2</sub>	2H-6'' C-6''
5-OH	12.64	-		
7-OH	10.83	-		
4'-OH	9.68	-		
3'-OH	9.15	-		

The DEPT spectra made it possible to count 11 protonated carbons (CH, CH<sub>2</sub>) including 1 C<sub>secondary</sub> and 10 C<sub>tertiaries</sub>. DEPT spectra 45, 90 and 135, allow to glimpse 10 C<sub>quaternaries</sub> of which C = 0 at 177.94 ppm; C-O at 61.49 ppm as well as 5 C<sub>tertiaries</sub> of the pyranic nucleus between 78.03 and 70.45 ppm. The <sup>1</sup>H NMR spectrum shows 2 doublets at 6.21 and 6.42 ppm corresponding to 2 aromatic H (H-6 and H-8) of ring A of a flavonoid substituted at C-5 and C-7, coupling with a coupling constant  $J = 2Hz^{23-25}$ ; multiplets at 7.62 - 7.57 ppm) and 6.90 - 6.81 ppm correspond respectively to the aromatic protons H-2', H-6' and H-5' of the B nucleus of a flavonoid<sup>23-24</sup>. The signals appearing at 12.64; 10.83; 9.68 and 9.15 ppm are those of the H groups of OH groups respectively in C-5, C-7, C-4' and C-3' in nuclei A and B. The existence of several signals between 3.21 and 3.59 ppm attests to the presence of the pyran nucleus of a glucose<sup>26</sup>. The proton H-1'' resonating at 5.47 ppm (d,  $J = 7.4 Hz$ ) is that of the anomeric carbon of β-D-glucopyranose. The isolated phytochemical Quercetin 3-O-β-glucopyranoside is said to have antiviral activity against Arbovirus MAYV<sup>27</sup>. Arboviruses are also vectors of pathologies known as arboviruses, which are zoonoses and anthrozooses.

## CONCLUSION

The results obtained in this work have shown, on the one hand, that the ethyl acetate GB2.3 extract of *Gossypium barbadense* L. exhibits an analgesic effect, and on the other hand that it has negligible toxicity. These significant results are rational justifications attesting to the use of *G. barbadense* in endogenous traditional therapy in Côte d'Ivoire in the treatment of skin infection induced by shingles. Spectral analyzes made it possible to characterize the isolated compound as Quercetin 3-O-β-glucopyranoside, a phytoconstituent of the GB2.3 extract.

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