

**Isolation, Chemical Characterization, Evaluation of Analgesic and Anti-inflammatory Activities of Triterpenoids From the Tubers of *Raphionacme vignei* E. A. Bruce (Apocynaceae).**

**Running Title:** Triterpenoids Central Acting Analgesia

**Abstract:**

*Raphionacme vignei* E. A. Bruce (Apocynaceae) is a plant of the traditional African pharmacopoeia, whose parts are used in the treatment of various pathologies. Water-soaked *R. vignei* tubers are edible. The objective of this study was to isolate triterpenoids from the acetonic extract of *R. vignei* tubers, evaluate the analgesic and anti-inflammatory activities of each molecule. The isolated compounds, characterized by NMR and mass spectrometry, is composed of six triterpenoids: beta-amyrin dodecanoate **1**(DDQ1), lupeol dodecanoate **2**(DDQ2), beta-amyrin acetate **3**(DDQ3), lupeol acetate **4**(DDQ4), luepol **5**(DDQ5) and  $\beta$ -sitosterol **6**(DDQ6). These molecules (DDQ2, DDQ3, DDQ4, DDQ5, DDQ6) are anti-inflammatory in carrageenan induced rat paw edema, with better anti-inflammatory power for DDQ2 and DDQ4, which would be related to the presence of acetate function and cycle E. DDQ2 and DDQ4 are also analgesic in acetic acid induced contortions and the removal test of rat tail on the heating plate. The analgesic action of DDQ2 and DDQ4, superior to that salicylic acetyl acid, identical to that morphine, suggests a central action of these two molecules. The potent analgesic effect of DDQ2 and DDQ4, could be attributed to the presence of cyclopentane and isoprene substitution in position 19 of the lupane family. DDQ2 and DDQ4 represent a potential for the synthesis of structural analogues with analgesic and/or anti-inflammatory properties

**Key Words:** *Raphionacme vignei*, Tubers, Triterpenoids, Inflammation, Pain

## 1. INTRODUCTION

The inflammatory process is a defensive response of the body to various stimuli that may be of physical, chemical or biological origin. It can therefore result from the penetration of pathogenous agents into the body (bacteria, viruses, parasites). The mechanism of the anti-inflammatory reaction involves mediators besides exert deleterious reactions on the tissues [1-3]. Therapeutically, inflammation involves the use of non-steroidal anti-inflammatory drugs (NSAIDs), among which such as acetylsalicylic acid (ASA), and anti-steroid glucocorticoids. Inflammatory drugs limited by the many side effects they cause, such as ulcers gastroduodenal, hydrosodium retention, obesity, osteoporosis [4-7]. Pain is a common condition in many pathologies, when it increases intensively, affecting the patient's quality of life [8,9]. Commonly used analgesics belong to either the NSAID class, interacting with nociceptive transmission through a peripheral mechanism, or to opioids commonly referred to as morphine, which are central-acting analgesics [10-12]. These analgesics induce also adverse effects (gastrointestinal disorders, renal toxicity, sedation, respiratory depression), limiting their effectiveness and long-term use in therapy [13,14]. *Raphionacme vignei* E. A. Bruce (Apocynaceae) is a widespread plant in West Tropical Africa (Benin, Burkina, Cameroon, Ghana, Guinea, Ivory Coast, Mali). It is an herbaceous plant that grows at altitudes ranging from 0 to 1500 m, in an habitat of sandy to rocky outcrops or savannah. *R. vignei* tubers are tasted raw by local populations in African regions [15]. Previous work has highlighted the interest of

triterpenoid molecules in preventing the inflammatory process and regulating pain through a central and/or peripheral mechanism [16-18]. The purpose of that study was to isolate triterpenoids from *R. vignei* tubers and also evaluate their analgesic and anti-inflammatory activities.

## **2. MATERIALS AND METHODS**

### **2.1 Plant material:**

The tubers of *R. vignei* were harvested in July 2017 in the village of Maleme Niani, in Tambacounda region (Senegal). The plant has been authenticated in the Botanical Laboratory of the Faculty of Science and Technology (FST) of Assane Seck University of Ziguinchor (ASUZ). A reference specimen was deposited at the herbarium of the botanical Laboratory, under number 2017/021.

### **2.2 Animal Material:**

The animal material used consisted of Wistar-strain rats with an average weight of 150-250 g. The peripheral analgesic activity study was conducted in albino mice with an average weight of 40 g. The rats were bred from the pet farm in Department of Pharmacology of the Faculty of Medicine, Pharmacy and Odontology (FMPO) of Cheikh Anta Diop University (CADU) of Dakar. The mice came from the Pasteur Institut of Dakar.

### **2.3 Experimental procedures:**

#### **2.3.1 Extraction and fractionation:**

Samples of *R. vignei* tubers has been dried at room temperature (30 °C) away from sunlight for one month. The dried drug was sprayed with a crusher (type Bradender OHG Duisburg). The fine obtained powder (150 g), was used as a raw material for extraction. A successive exhaustion of the powder was carried out with solvents of increasing polarities (acetone,

ethanol and water). In fact, 150 g of powder were introduced into 1 L flask containing 500 ml of solvent. The mixture has been left in maceration for 24 hours. This procedure was performed for each solvent, after filtration and drying of the marc. The obtained filtrates gave respectively, after evaporation, an acetonetic, ethanolic, and aqueous residue (**Table 1**).

### **2.3.2 Phytochemical analysis:**

A phytochemical screening to identify secondary metabolites in the obtained extracts was carried out. This phytochemical and qualitative analysis of tubers extracts was performed as follows: sterols and terpenoids (Lieberman reagent), alkaloids (Bouchardat / Valser-Mayer / Dragendorff reagents), flavonoids (HCl concentrate + magnesium shavings), tannins (Stiasny reagent, FeCl<sub>3</sub> test), saponins (foam test), free or combined quinonic substances (Borntraegen reagent).

### **2.3.3 Purification of acetonetic extract:**

A 15 g mass of the dry residue of the acetonetic fraction was purified using a REVELERIS «Chromatography Flash System » (Reveleris Silica 80 g column (normal phase, flow rate: 60 ml/min, equilibration : 6.3 mn, retention time: 62.5 mn), slope detection: medium 'ELSD threshold: 100 mV, UV threshold: 0.2 AU (UV1 wavelength: 254 nm, UV2 wavelength: 254 nm, UV2 wavelength 215 nm), collection mode: collect peaks (volume per tube: 25 ml, uncorrected 25 ml, injection type: dry); ELSD motor: isopropanol (solvent A: Ether Petroleum (EP), solvent B: Dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>)). The purification was monitored by thin-layer chromatography (pre-induced kieselgel plates 60 F254 250 pm, Merck) with petroleum ether/ dichloromethane eluant. After plates elution, the molecules were detected by spraying with Dragendorff or 50% H<sub>2</sub>SO<sub>4</sub> reagent followed by heating. The isolated

molecules were characterized by analysis of UV (UV / VIS Philips PU 8720), IR (Nicolet Avatar 320 FT-IR), RMN ( $^1\text{H}$  and  $^{13}\text{C}$ , Bruker Avance DRX-600) and mass (Micromass ESI-Q-TOF spectra).

### **2.3.4 Pharmacological Testing:**

#### **2.3.4.1 Carrageenan-induced rat paw edema:**

Anti-inflammatory activity was evaluated *in vivo*, using 1% carrageenan-induced rat paw edema method [19]. The rats were released in batches of 5. They were then fasted 12 hours before the experiment.

Control group: Physiological water (10 ml/kg, *per os*)

Reference Group (Acetylsalicylic acid, 1 mg/kg, *per os* and 10 mg/kg, *per os*)

DDQ2 (1 mg/kg, 3 mg/kg and 10 mg/kg, *per os*)

DDQ3 (1 mg/kg, 3 mg/kg and 10 mg/kg, *per os*)

DDQ4 (1 mg/kg, 3 mg/kg and 10 mg/kg, *per os*)

DDQ5 (1 mg/kg, 3 mg/kg, *per os*)

DDQ6 (1 mg/kg, 3 mg/kg, *per os*)

For each rat, the thickness of the left posterior rat paw was measured using a digital slide stand, before the administration of the different treatments by gavage. A 100 µl injection of 1 % carrageenan into the foot pad of rat paw was carried out 1 h after gavage of the tested product. The obtained thicknesses has been measured every hour during 5 hours with a sliding foot. The significance of edema was assessed by determining the mean percent increase (% INC) in rat paw thickness according to the formula:  $\% \text{ INC} = (\text{PTh} - \text{PT0} / \text{PT0}) \times 100$

PTh = paw thickness at time T in hours.

PT0 = initial thickness.

#### **2.3.4.2 Acetic acid pain test:**

Peripheral analgesic activity was evaluated *in vivo* in mice using 3 % acetic acid pain model [20]. The mice were divided into batches of 5. They were then fasted 12 hours before the experiment. The different solutions were administered (n=5).

Control Group (Physiological Water, 10 ml/kg, *per os*)

Reference Group (Acetylsalicylic Acid, 1mg/kg, 3 mg/kg and 10 mg/kg, *per os*)

DDQ2 (1mg/kg and 3mg/kg, *per os*)

DDQ3 (1 mg/kg and 3 mg/kg, *per os*)

DDQ4 (1 mg/kg, 3 mg/kg and 10 mg/kg, *per os*)

Intraperitoneal (ip) injection of 3 % acetic acid solution at a dose of 10 ml/kg was carried out one hour after gavage of the tested product. Pain sensitivity was assessed by the number of contortions counted for 30 min after the first reaction.

#### **2.3.4.3 Hot plate test:**

Central analgesic activity was evaluated in the rat, using the tail thermal stimulation test described previously [21,22]. The rats were divided into groups of 5 and fasted 12 hours before the experiment. The treatments were administered intraperitoneally (ip):

- Lot 1: Physiological control (1 ml/kg, *ip*);
- Lot 2 : Morphine (1 mg/kg, *ip*);
- Lot 3: ASA (100 mg/kg, *ip*);
- Lots 4 and 5: DDQ2 (1 mg/kg, *ip*) and DDQ4 (1 mg/kg, *ip*)

Sensitivity to pain was assessed by measuring the time the rat's tail was removed. Measurements were made at T0, T30 min and T60 min.

#### **2.4 Statistical analyses:**

The results were expressed as mean  $\pm$  standar error of mean (sem). The Student test was used to highlight the existence of a significant difference with a p significance threshold of 0.05. n=5 is the number of experiments in each group.

### **3. RESULTS**

#### **3.1 Extraction yields:**



Table 1 gives the yields of the different fractions obtained. Each yield is calculated as the ratio of the mass of the extract to that of the dried starting powder after each extraction. This table shows that *R. vignei* tubers are very rich in polar compounds.

### 3.2 Phytochemical screening:

Phytochemically, sterols and polyterpenes are detected in all extracts. Alkaloids are detected in ethanolic and aqueous extracts whereas saponins, polyphenols and flavonoids are detected in the aqueous extract. However, any of the extracts do not contain tannins and anthraquinones.

### 3.3 Purification of acetonetic extract:

A "Flash chromatography system" in the normal phase of the acetonetic extracts of the tubers of *R. vignei* has led to the isolation and elucidation of six compounds. A 1.5 g mass of the dry residue of the acetonetic extract was purified using a REVELERIS "Chromatography Flash System" type device with a solvent gradient system: 100% EP (10 mn), 90-10% EP/CH<sub>2</sub>Cl<sub>2</sub> (10 mn), 70-30% EP/CH<sub>2</sub>Cl<sub>2</sub> (10 mn), 10-90% EP/CH<sub>2</sub>Cl<sub>2</sub> (10 mn), 20-80% EP/CH<sub>2</sub>Cl<sub>2</sub> (10 min), 100% CH<sub>2</sub>Cl<sub>2</sub> (5 min) and 100% MeOH (5 min) wash. The six (6) pure products were collected using an automatic collector. Compounds 1 (47.4 mg), 2 (45.7 mg), 3 (96 mg), 4 (34.6 mg), 5 (23.2 mg), and 6 (6.8 mg) were isolated. Known compounds 1-6 were readily identified by their spectral data and by comparison with the corresponding compounds reported in the literature [2-6]. These molecules correspond to: beta amyirin dodecanoate 1(DDQ1), lupol dodecanoate 2(DDQ2), beta amyirin acetate 3(DDQ3), lupol acetate 4(DDQ4), lupol 5 (DDQ5) and -sitosterol 6(DDQ6) (**fig. 2**).

### 3.4 Pharmacological Test Results:

### 3.4.1 Administration of physiological water (10 ml/kg, *per os*) and acetylsalicylic acid (ASA) (10 mg/kg, *per os*):

Administration of 100  $\mu$ l of 1 % carrageenan in the plantar pad of the rat's left leg induces inflammatory oedema which results in an increase in the thickness of the leg. The percentage of variations in the thickness of the leg is 34.39  $\pm$  8.81; 67.77  $\pm$  6.79 and 92.72  $\pm$  6.05 (n= 5), respectively at times T1h, T3h and T5h after administration of carrageenan. Administration of 10 mg/kg *per os* of ASA significantly prevents the development of carrageenan-induced inflammatory edema. The variation of the inflammatory edema of the leg is 21.79  $\pm$  2.27; 33.77  $\pm$  7.08; 30.96  $\pm$  7.25, respectively at T1h, T3h and T5h. This variation is significantly different from that observed in the control group ( $p < 0.05$ , n=5) (**fig. 3 A**).

### 3.4.2 Anti-inflammatory activity of triterpenoids from *R. vignei* tubers

At a dose of 1 mg/kg *per os*, DDQ2 significantly prevents inflammatory carrageenin edema. The variation in leg thickness is 30.62 $\pm$ 3.80 %; 33.68 $\pm$ 3.38 % and 45.02  $\pm$  4.87 %, respectively, at T1h, T3h and T5h. Between 1 and 3 mg/kg, the prevention of inflammatory edema is dose dependent. However, at the 10 mg/kg dose, there is a trend towards a decrease in anti-inflammatory activity. At this dose, the variation in leg thickness is 46.29  $\pm$  8.21 % (**fig. 3 A**).

DDQ3 is dose-dependent for inflammatory carrageenan edema between 1 and 3 mg/kg, *per os*. After 5 hours, edema changes were 55.87  $\pm$  6.62 % and 35.73  $\pm$  3.59 %, respectively, compared to 92.72  $\pm$  6.62% in the control group ( $p < 0.05$ , n=5). However, the prevention of inflammatory edema is less important at 10 mg/kg *per os*. At this dose, the variation of inflammatory edema is 50.47  $\pm$  4.74 % ( $p < 0.05$ , n=5) (**fig. 3 B**).

DDQ4 induces a dose-dependent prevention of inflammatory edema. Indeed, at doses of 1, 3 and 10 mg/kg *per bone*, the variations of inflammatory edema are respectively  $70.74 \pm 9.14$  %,  $39.91 \pm 3.15$  % and  $35.30 \pm 4.71$  % vs  $92.72 \pm 6.05$  in the control group ( $p < 0.005$ ,  $n = 5$ ) (**fig. 3 C**).

The anti-inflammatory activity of DDQ5 *per os* is inferior to previously observed with DDQ2, DDQ3 and DDQ4. At 1 and 3 mg/kg, the variations of inflammatory edema are respectively  $52.35 \pm 6.22$  % and  $42.18 \pm 3.41$  %. They are, however, significantly different from those of the control group ( $p < 0.05$ ,  $n = 5$ ) (**fig. 3 D**).

Similar results to DDQ5 were observed with DDQ6. The changes in inflammatory edema at 1 and 3 mg/kg were  $43.22 \pm 5.73$  % and  $41.96 \pm 4.64$  % vs  $92.72 \pm 6.62$  % in the control group, respectively ( $p < 0.05$ ,  $n = 5$ ). (**fig. 3 E**).

#### **3.4.3 Induction of pain by acetic acid after administration of physiological water:**

Intraperitoneal injection (ip) of a 3 % acetic acid solution, after gavage of mice with physiological water (10 ml/kg, *per os*), induces pain that results in contortions. The number of contortions is  $72.60 \pm 6.64$  (**fig. 4**).

#### **3.4.4 Prevention of pain after administration of acetylsalicylic acid (ASA):**

ASA (100 mg/kg, *per os*) prevents acetic acid-induced contorsions. The number of contortions is significantly different from the control ( $26.80 \pm 4.66$  versus  $72.60 \pm 6.64$ ) ( $P < 0.001$ ,  $n = 5$ ) (**fig. 4**).

### 3.4.5 Peripheral analgesic activity of triterpenoids of tubers of *R. vignei*:

The 3 % ip injection of acetic acid after DDQ2 treatment (1 and 3 mg/kg, *per os*) is associated with pain prevention. Indeed, at the dose of 1 and 3 mg/kg, the number of contortions was respectively  $30 \pm 5.43$  and  $40.25 \pm 3.75$ , compared to  $72.60 \pm 6.64$  in the control group ( $P < 0.001$ ,  $n=4$ ). Prevention of contortions after treatment with DDQ3 (1 and 3 mg/kg, *per os*) is less important than that observed with DDQ2 under the same conditions. However, the prevention of contortions remains significant in relation to the control group. Indeed, at the dose of 1 and 3 mg/kg per bone, the number of contortions is  $48.75 \pm 5.32$  and  $48.50 \pm 2.50$  vs  $72.60 \pm 6.64$  respectively ( $p < 0.05$ ,  $n=5$ ). DDQ4 prevents dose-dependent contortions induced in mice by acetic acid. In fact, at the dose of 300  $\mu\text{g/kg}$  and 1 mg/kg per bone, the number of contortions is respectively  $53.75 \pm 7.26$  and  $28.25 \pm 5.48$  vs  $72.60 \pm 6.64$  in the control group ( $p < 0.05$ ,  $n=5$ ) (**fig. 4**).

### 3.4.6 Central analgesic effect of DDQ2 and DDQ4:

On the model of the heating plate, the prior administration of DDQ2 (1 mg/kg, ip) or DDQ4 (1 mg/kg, ip), is associated with an increase in the withdrawal time of the tail of the animal on the heat beam. After 60 minutes after administration, the tail-retract latency is  $8.4 \pm 0.40$  s and  $7.04 \pm 0.48$  s vs  $4.8 \pm 0.48$  s respectively in the control group ( $p < 0.05$ ,  $n=5$ ) (**fig. 5 A-B**). Identical results were obtained with morphine administered under the same conditions. The latency observed after morphine administration was significantly different from that of the control group ( $7.85 \pm 0.74$  s vs  $4.8 \pm 0.48$  s) ( $p < 0.05$ ,  $n=5$ ). However, the pretreatment of rats with ASA (100 mg/kg, ip), a peripheral-acting analgesic, does not combine with a significant increase in tail withdrawal time ( $4.97 \pm 0.61$  s vs  $4.8 \pm 0.48$  s) (ns,  $n=5$ ) (**fig. 5 A-B**).

#### 4. DISCUSSION

Previous work had demonstrated the value of phytochemicals in the regulation of pain and the suppression of the inflammatory process [23-25]. Several triterpenoid compounds have an experimental interest in the prevention of pain by a central or peripheral mechanism. Some of these molecules also prevent inflammatory edema by a mechanism involving the prevention of prostaglandin production, the inhibition of COX2 expression, or interfering with the synthesis of inflammatory cytokines (IL-1, TNF $\alpha$ , IL-6) [16,18,26]. The objective of that study was to highlight the analgesic and anti-inflammatory activity of triterpenoids isolated from the tubers of *R. vignei*. The anti-inflammatory action of triterpenoids was evaluated on the model of inflammatory carrageenan edema. The analgesic action of these same molecules was tested on the acetic acid pain model and the rat tail removal test on the hot plate. Structurally, triterpenoids of *R. vignei* are characterized by the arrangement of ABC basic cycles. All these triterpenoids induce significant anti-inflammatory activity. This observation suggests the importance of the ABC cycle arrangement in initiating the anti-inflammatory response. The DDQ2 and DDQ4 triterpenoids prevent the different phases of the inflammatory process. They have in common the presence of an acetate function in position 3. In addition, DDQ2 and DDQ4 have a better anti-inflammatory activity among the tested triterpenoids. The anti-inflammatory power of DDQ2 and DDQ4, which is superior to other triterpenoids, may be related to the presence of acetate function and cycle E. However, the presence of a free alcohol function in position 3 may be correlated with an inferior anti-inflammatory activity, as is the case for DDQ5 and DDQ6. Carrageenan inflammatory edema involves

over-expression of COX2, resulting in increased production of pro-inflammatory prostaglandins. There is also a production of cytokines that amplifies the inflammatory response. Triterpenoids of *Ganoderma lucidum* induce a powerful anti-inflammatory activity. At the molecular level, triterpenoids of this plant involve the inhibition of NF- $\kappa$ B activity, the suppression of COX2 expression and inflammatory cytokines (IL-1, TNF $\alpha$ , IL-6) [17, 18]. The anti-inflammatory activity of DDQ2 and DDQ4 is superior to that of acetylsalicylic acid and close to that of glucocorticoids. If the hypothesis of an anti-inflammatory mechanism of DDQ2 and DDQ4, identical to that of glucocorticoids, were to be verified, their actions could involve mechanisms such as prevention of the production of inflammatory cytokines, inhibition of phospholipase A2 (PLA2), suppression of COX2 activity or expression. 25-methoxy hispidol A, an isolated triterpenoid of *Poncirus trifoliata*, identical to that of dexamethasone, implicates the inhibition of NF- $\kappa$ B activation and the production of inflammatory cytokines [16]. These results seem to support the hypothesis of an anti-inflammatory action of *R. vignei* triterpenoids, particularly DDQ4, which is likely to interfere with the production of inflammatory mediators such as IL-1, IL-6 and TNF $\alpha$ . Triterpenoids of *R. vignei* with anti-inflammatory action were evaluated on the model of acetic acid pain in mice. In fact, the ip injection of acetic acid promotes the production of prostaglandins which are mixed mediators of pain and inflammation, thus justifying the peripheral analgesic action of non-steroidal anti-inflammatory drugs such as acetylsalicylic acid (ASA) (Reference). In this study, DDQ2, DDQ3, and DDQ4 induce significant analgesic action on the test of acetic acid contortions. However, better analgesic activity was observed for DDQ2 and DDQ4. The analgesic action of these two molecules was much higher than that of AAS. The potent analgesic effect of

DDQ2 and DDQ4 on the contortion test suggested the probable existence of a possible central-origin mechanism of action for these molecules. The analgesic action of DDQ2 and DDQ4, greater than that of the other triterpenoids of *R. vignei*, is believed to be related to the presence of cyclopentane and isoprenic substitution at position 19 of the lupane family. Previous works on *Mitragyna speciosa* had demonstrated a central anti-inflammatory and analgesic action of mitragynine, a plant alkaloid [21]. DDQ2 and DDQ4 induce a powerful analgesic action on the model of the heating plate, comparable to that of morphine administered under the same conditions. The central analgesic action involves a variety of mechanisms that may involve the opioid, vanilloid or glutamatergic systems. Mitragynine induces its analgesic action by the interplay of opioid receptors such as morphine. Its action is sensitive to naloxone, a direct antagonist of morphine receptors [21]. Conversely, the centrally induced analgesic action of an ether fraction of oil in the leaves of *Melastoma malabathricum*, containing triterpenoids, is insensitive to naloxone, suggesting the probable involvement of the vanilloid or glutamatergic system [16, 27]. Studies of antagonism involving opioid, vanilloid, and glutamatergic systems would elucidate the central mechanism of analgesic action of triterpenoids of *R. vignei* tubers.

## 5. CONCLUSION

The triterpenoids of *R. vignei* are both analgesic and anti-inflammatory. DDQ2 and DDQ4 have better analgesic activity in acetic acid contortions and thermal pain models. The peripheral analgesic action of DDQ2 and DDQ4 would involve a prevention of inflammatory prostaglandins production. The powerful centrally analgesic effect of these molecules, would involve the opioid, glutamatergic or vanilloidergic systems.

## ETHICAL APPROVAL

The experimental protocols were conducted in accordance with the guidelines on the care and use of laboratory animals (Senegal National Ethical Committee for Health Research). All animals had received human care and its use was approved (02/18/2019) by the Research Ethical Committee of Cheikh Anta DIOP University of Dakar (approval n° 0373/2019/CER/UCAD).

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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**Table 1: Extraction yields**

<b>Fractions</b>	<b>Weight (g)</b>	<b>Yield (%)</b>
Acetone	6,32	4,21
Ethanol	0,365	0,24
H <sub>2</sub> O	11,98	8,09

UNDER PEER REVIEW



Method Name:  
Run Name: DDQ-hexane  
Run Date: 2017-10-23 14:38

Column: Reveleris® Silica 80g  
Flow Rate: 60 mL/min  
Equilibration: 6.3 min  
Run Length: 62.5 min  
Air Purge Time: 1 min

Slope Detection: Low  
ELSD Threshold: 20 mV  
UV Threshold: 0.05 AU  
UV1 Wavelength: 254 nm  
UV2 Wavelength: 215 nm

Collection Mode: Collect All  
Per-Vial Volume: 25 mL  
Non-Peaks: 25 mL  
Injection Type: Dry

ELSD Carrier: Iso-propanol  
Solvent A: Petroleum Ether  
Solvent B: Methylene chloride  
Solvent C: Methanol  
Solvent D: <No solvent chosen>

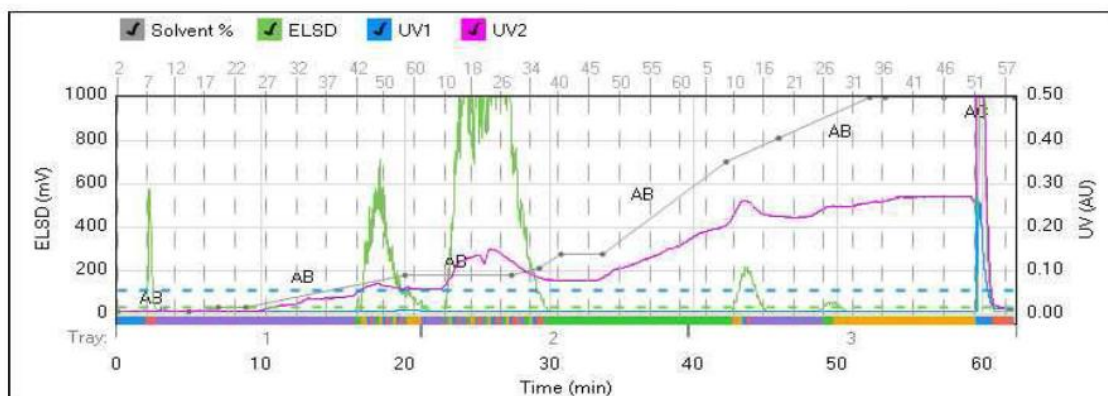
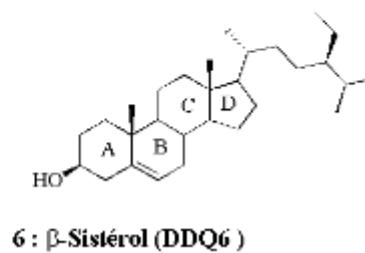
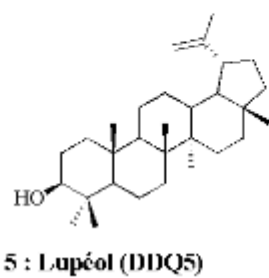
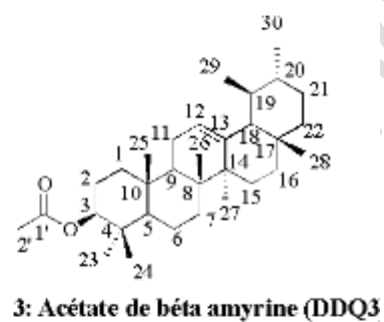
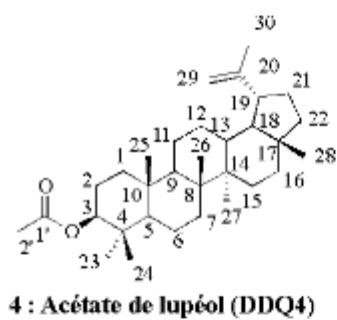
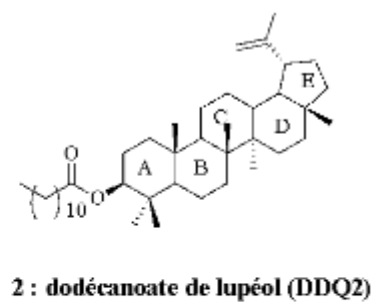
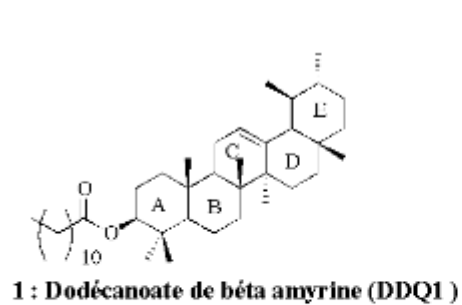
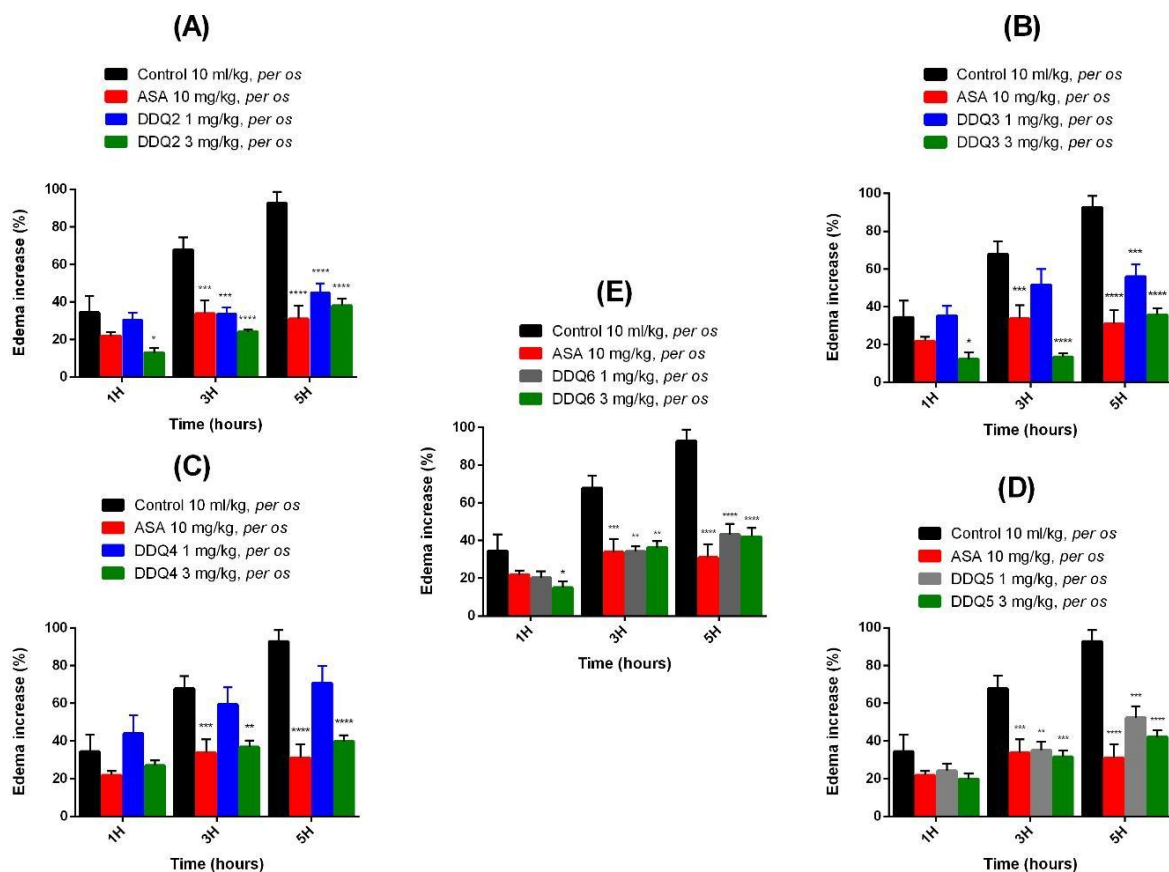


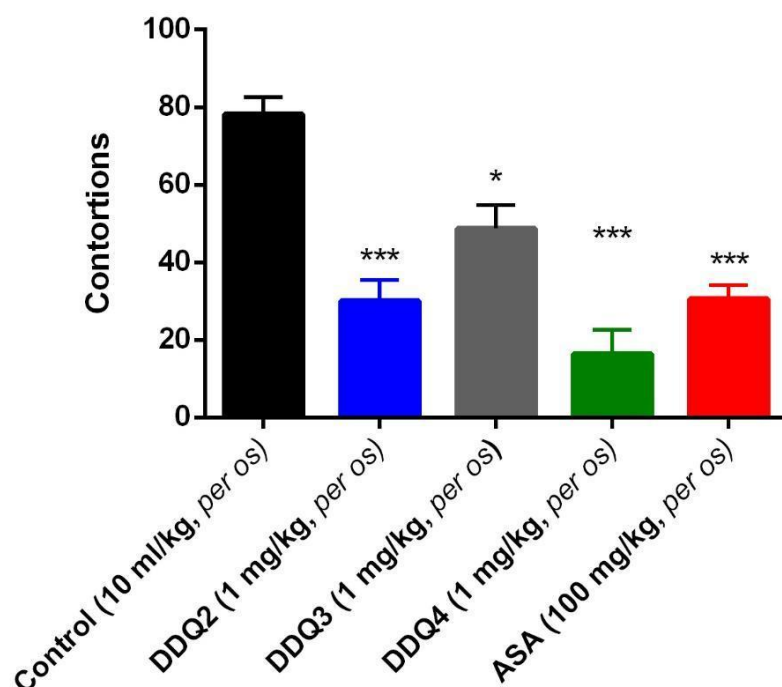
Figure 1: Flash *REVELERIS* purification chromatogram



**Figure 2: Molecular structures of triterpenoids isolated from *R. vinei* tubers**

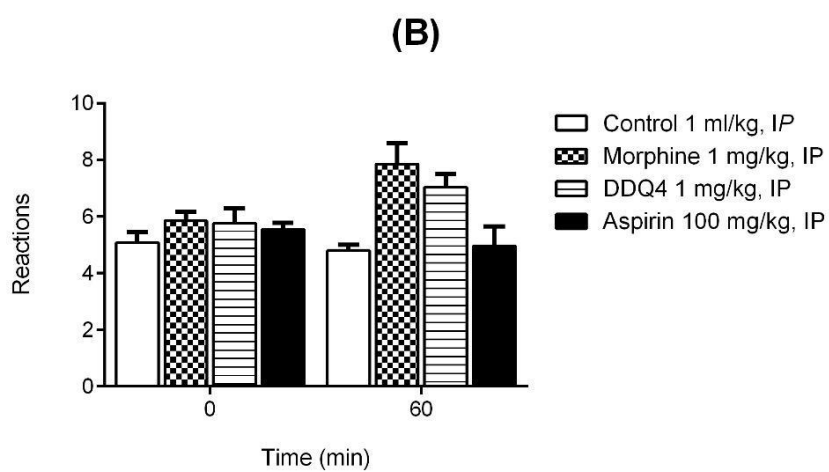
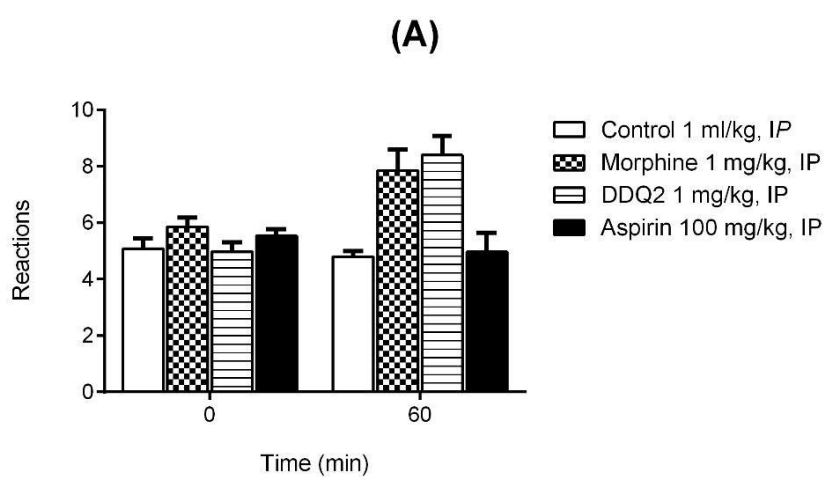


**Figure 3: Anti-inflammatory activity of triterpenoids from *R. vignei* tubers in carrageenan induced rat paw edema**



**Figure 4: Peripheral analgesic activity of DDQ2, DDQ3 and DDQ4 in acetic acid induced contortions**





**Figure 5: Central analgesic activity of DDQ2 and DDQ4 in hot plate tail withdrawal test**

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