



(RESEARCH ARTICLE)



## Evaluation of the anti-inflammatory activity of a gel based on *Azelia africana* (fabaceae) leaves

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### Abstract

*Azelia africana* is a plant used by traditional healers in the treatment of several inflammatory pathologies, in particular edema, rheumatism, lumbago. The effectiveness of its barks in treating inflammation is well established. Ethnobotanical data led researchers to formulate a stable topical hydrogel based on bark extract, the tolerance and effectiveness of which in animals have been found to be satisfactory. As excessive use of the bark may impair the survival of the plant, further leaf studies have been performed and the anti-inflammatory effect has been confirmed in rats. The objective of the present study was to evaluate the anti-inflammatory efficacy of a topical gel based on the hydro-ethanolic extract of these leaves in patients with inflammatory symptoms, compared to a diclofenac gel.

Using hydroxyethyl cellulose and glycerol a stable gel was produced and used for the evaluation of the anti-inflammatory activity of the leaves of the plant. The dry extract obtained from the leaves was rich in flavonoids (57.26 mg quercetin equivalent per gram), phenols, tannins, alkaloids, anthraquinones and steroids. We carried out a randomized pilot study on 52 patients who came for consultation at the « Cliniques Universitaires des Montagnes » and at the Bangangté District Hospital for inflammatory symptoms between June 1 and July 17, 2020; pregnant women were excluded from the study. Topical administration on 15 inflammatory pathologies revealed that the *Azelia africana* leaf extract gel exhibits a better onset of action ( $4.29 \pm 1.89$  min) than the diclofenac gel ( $16.96 \pm 4, 73$  min). The decrease in pain visual analogue scale (VAS): values for *Azelia africana* gel was greater than for diclofenac gel, with  $P < 0.05$  on day 2 and  $P < 0.001$  from day 3 to day 7. The difference in First Hour Sedimentation Rate was significant between the two gels ( $P < 0.05$ ); but this difference was no longer statistically significant after the second hour ( $P > 0.05$ ). In addition, *Azelia africana* leaf extract gel reduced swelling ( $P = 0.001$ ) and stiffness ( $P < 0.001$ ) better than diclofenac gel.

These results clarify the effectiveness of *Azelia africana* leaf extract gel in the management of inflammatory pathologies and / or symptoms.

**Keywords:** *Azelia africana*; Leaf extract; Topical hydrogel; Anti-inflammatory

### 1. Introduction

In Central Africa, and particularly in Cameroon, most of the population does not have access to modern health care [1]. The constant increase in the prices of pharmaceutical specialties and health services, the inaccessibility of generic drugs

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despite their supposedly competitive cost, constitute a major problem for the financially deprived populations. This justifies the growing interest in traditional herbal medicines [2]. In recent decades, inflammatory diseases have remained a major public health problem. This is the case with rheumatological conditions, affecting around 100 million people worldwide and accounting for 20% of medical consultations [3]. Treatment of inflammation is often based on the administration of non-steroidal anti-inflammatory drugs and corticosteroids. All NSAIDs, regardless of their route of administration, carry the risk of gastric toxicity. In most cases, herbal medicine can be helpful in reducing the initial doses of synthetic anti-inflammatory drugs [4]. *Afzelia africana* is a plant of the Fabaceae family used in traditional medicine in the treatment of several pathologies such as rheumatism and low back pain. The work carried out by Foutse in 2017 showed an anti-inflammatory activity in vitro of the aqueous extract of the bark superior to that of the tincture of *Arnica montana* [5]. In 2018, Kengne developed a topical anti-inflammatory hydrogel from the aqueous extract of the bark, which has been shown to be tolerant and effective in animals [6]. For the sake of the sustainability of the plant species, Tebue investigated the leaves of the plant and demonstrated their anti-inflammatory activity in 2019 [7]. In the present study, we aimed to evaluate the effectiveness of a topical gel based on the hydro-ethanolic extract of *Afzelia africana* leaves in the management of inflammation, in comparison to a gel of diclofenac.

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## 2. Equipment

The equipment used included, among others, for the preparation of the gel: *Afzelia africana* leaves, a rotavapor, hydroxyethyl cellulose, culture media solvents; and for activity assessment, survey sheets, goniometer, tape measure, visual analogue scale (VAS).

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## 3. Methods

Preparation and quality control of the gel.

The preparation and quality control of the gel were carried out according to a protocol inspired by that of Tebue [7]. This is how the leaves were washed, dried, pulverized and subjected to extraction with 70% ethanol. The extract obtained was concentrated in a Rotavapor and then dried in an oven at 50 ° C.

A screening was carried out according to the conventional protocol to highlight the main phytochemical groups present in the extract [8].

Used as a gelling agent, Hydroxyethylcellulose (Natrosol 250 \*) made it possible to formulate a stable gel.

Evaluation of the anti-inflammatory activity of the gel.

Ethical considerations: This study required obtaining ethical clearance N ° 2020/043 from the Institutional Ethics Committee of « Université des Montagnes », research authorizations to “Cliniques Universitaires des Montagnes” and the District Hospital of Bangangte as well as the free and informed consent of patients or parents or guardians in the case of minors.

Study population: it consisted of children over 5 years old and adults who came for consultation for inflammatory pathologies or symptoms of the musculoskeletal system, trunk and striated muscle. This was an open-label, randomized, bi-centric comparative study conducted from June 1 to July 17, 2020. For an attendance rate of 195 patients per month in a rheumatology and physiotherapy department, an NSAID prescription rate of 88 % [9], a margin of error of 5% and a test power of 80%, the minimum recommended sample size is 52 patients.

Administration of the product: The patients were divided into 2 groups: group A receiving *Afzelia africana* gel and group B receiving diclofenac gel. The choice of the gel to be administered to each patient was made by drawing lots. The local application was done 3 times a day for 07 consecutive days; every morning before applying the product, the pain was assessed on the Visual Analogue Scale (VAS): this is a 10 cm graduated ruler; on the left 0 is no pain and on the right end 10 is the worst pain imaginable. The Sedimentation Rate (SR) was determined before and at the end of treatment. The swelling was assessed using a tape measure before treatment and at the end of treatment; stiffness was measured using a goniometer. Observations were noted at the end of treatment according to the following criteria: aggravated state if there is an increase in at least one of the signs of inflammation with or without an increase in SR; steady state if there is no noticeable change; improved condition if there is a decrease in inflammatory signs with or without a decrease in SR; and cure: absence of inflammatory signs with or without return of SR to normal. In order to assess the tolerance of the product, clinical symptoms of intolerance were sought and data concerning all possible adverse events were recorded.

Statistical analysis: The progress of treatment in each group was analyzed using SPSS Excel and EPI-info software. The means of all scores recorded for all subjects in the groups and independent tests allowed for intergroup comparisons. The level of  $P < 0.05$  was used to determine significance.

## 4. Results

### 4.1. The extract

The dry extract powder was obtained with a yield of 11.43%; it was a dark brown, crystalline powder with a residual moisture of 4.32%.

Phytochemical screening showed a high content of flavonoids ( $57.26 \pm 0.12$  mg quercetin equivalents per gram), a good content of phenols, alkaloids, tannins, anthraquinones and steroids.

Microbiological testing of the extract did not reveal the presence of mesophilic bacteria, molds or yeasts.

### 4.2. The gel

After several tests, the gel retained had the formula in Table I

**Table 1** Percent formula of the gel

Excipients	Content	Role Rôle
Extract of <i>Afzelia africana</i> leaves	5%	Active principle
Natrosol 250 HHX	3%	Gelling agent
Glycerol	30%	Co solvent/Humectant
Benzyl alcohol	2%	Preservative
Distilled water	60g%	Solvent

The final product packaged in 30g tubes is shown in Figure 1



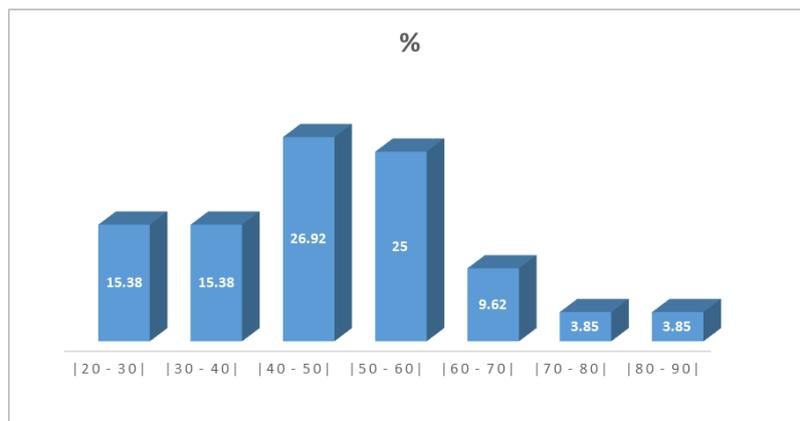
**Figure 1** Packaging of the gel based on the leaves of *Afzelia africana*

The gel stability test did not show any significant change in color (brown), odor (characteristic), homogeneity, pH (5.58) and flavonoid content (2.91 mg equivalent quercetin per gram) after 2 months when stored at  $25 \pm 3$  °C at a relative humidity of  $60\% \pm 5\%$ . After 2 months of storage there were no microorganisms in the gel: absence of yeasts and fungi, absence of mesophilic aerobic germs, absence of *Staphylococcus aureus* and absence of *Pseudomonas aeruginosa*.

### 4.3. Activity evaluation

Sociodemographic characteristics of the study population.

Age: Figure 2 shows the different age groups recorded

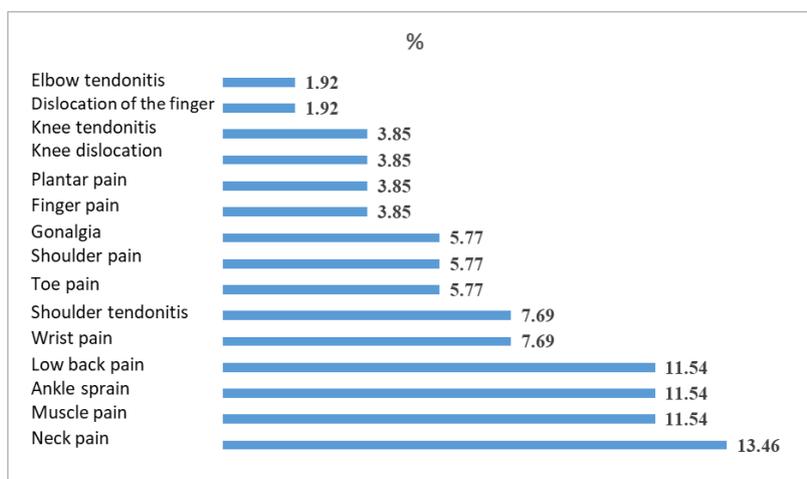


**Figure 2** Percentages of the different age groups included in the study

The patients were divided into 7 age groups. The most represented was that between 40 and 50 years (26.92%). The average age was  $47 \pm 14.9$  years with a minimum of 21 years and a maximum of 81 years.

Gender: The female sex was the most represented with a percentage of 61.54% against 38.46% for the male sex.

Reasons for consultation: Figure 3 shows the different recorded reasons for consultation



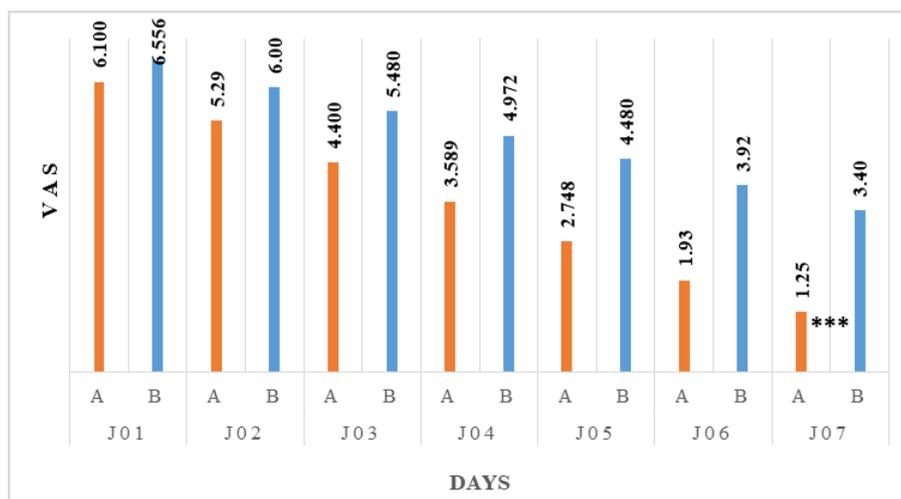
**Figure 3** Reasons for consultation recorded

The most common reasons for consultation were neck pain (13.46%), muscle pain, ankle sprains and back pain (11.54%).

Assessment of the gel. The gel based on *Afzelia africana* extract had a greater spreading capacity than the diclofenac gel. For both gels no side effects were observed after 7 days of treatment. *Afzelia africana* gel exhibited a better onset ( $4.29 \pm 1.89$  min) than diclofenac gel ( $16.96 \pm 4.73$  min).

Treatment monitoring. Evolution of pain. Figure 4 shows the evolution of pain VAS over seven days of treatment:

The feeling of pain at the end of treatment (Day7) is lower in patients using Afzelia gel than in those using diclofenac gel. This difference was found to be significant from Day2 to Day7 (with  $P < 0.05$  at Day2 and  $P < 0.001$  from Day3 to Day7). In fact, the pain VAS went on average from  $6.1 \pm 0.82$ cm to  $1.25 \pm 1.11$ cm for the Afzelia gel and from  $6.6 \pm 0.92$ cm to  $3.40 \pm 1.41$ cm for the diclofenac gel.



**Figure 4** Evolution of pain (VAS) in the two groups over 7 days of treatment  
A. africana gel; B: diclofenac gel; \* P <0.05; \*\* P = 0.001; \*\*\*p<0.0001

Variation of the sedimentation rate (SR) between Day0 and Day7. After the first hour, the Afzelia gel significantly reduced SR better (P < 0.05). But in the second hour, the difference in SR between the 2 gels was no longer significant (P > 0.05). Variation in swelling between Day 0 and Day 7. Swelling was present in only 32.69% of patients. Compared to diclofenac gel, Afzelia gel acted better on swelling and significantly

(p = 001) as shown in Table 2.

**Table 2** Comparative effects of the two gels on swelling

	Afzelia gel = 10	Diclofenac gel N = 7	P
Swelling	1.30 ± 0.36	0.69 ± 0.22	0,001

Evolution of the stiffness between Day 0 and Day 7. Many patients (86.54%) presented with loss of function in the affected limb or joint. The Afzelia gel significantly reduced (P < 0.001) stiffness with 22.48 ±, 96% against 11.95 ± 4.35% for the diclofenac gel (Table 3).

**Table 3** Comparative effects of the two gels on stiffness

	Afzelia Gel N = 23	Diclofenac Gel N=22	P
Stiffness (%)	22.48 ± 8.96	11.95 ± 4.35	< 0.001

Situation after treatment. Table 4 shows that after 7 days of treatment, Afzelia gel showed more recovery (48.15%) than diclofenac gel (24%).

**Table 4** Status of patients after treatment

End state	Afzelia africana Gel	diclofenac Gel
Improvement	14 (51.85%)	19 (76%)
Healing	13 (48.15%)	6 (24%)
Total	27 (100%)	25 (100%)

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## 5. Discussion

The sample size was 52 patients recruited from “Cliniques Universitaires des Montagnes” and Bangangté District Hospital over the short period from June 1 to July 17, 2020 (= 6 weeks). We were unable to have more patients due to the Covid-19 pandemic which caused the closure of the physiotherapy department. The sample size used in previous work varies from study to study: Hugh recruited 36 patients for 2 months [10]; Castanedo-Cázares recruited 74 patients for 4 days [11].

Hugh only used the VAS as a tool to assess pain and stiffness. Castanedo-Cázares and Bourreau [12] also used VAS as the only tool to assess the effectiveness of treatment. In the present study, pain, stiffness, swelling and sedimentation rate were assessed, using more tools: VAS, goniometer and tape measure.

The female sex was the most represented (61.54%); Dado Fall obtained a percentage of 52% for the male sex, but with a statistically insignificant difference compared to the female sex [13].

The most represented age group was between 40 and 50 years (26.92%) with an average of 47 years, which differs from the results of Bourreau who worked in 2004 on a population of mainly between 50 and 85 years [12], and Hugh with a population between 60 and 64 years in 2003 [10].

The *Afzelia africana* gel showed a greater decrease in the mean VAS values than the diclofenac gel. This difference was found to be significant from Day 2 to Day 7 (with  $P < 0.05$  at Day 2 and  $P < 0.001$  from Day 3 to Day 7). In fact, the pain VAS decreased by 56% on average for the diclofenac gel after the 7th day of treatment. These results are similar to those found by Moghadam in 2003 in a study comparing the effectiveness of diclofenac gel to the of capsanthin found in red pepper [13].

*Afzelia africana* gel showed a better mean time to action ( $4.29 \pm 1.89$  minutes) than diclofenac gel ( $16.96 \pm 4.73$  minutes). The value obtained for the diclofenac gel is high compared to that found by Sengupta when comparing its efficacy with that of nimesulide gel and piroxicam gel, on healthy volunteers [14].

They noticed that an average of 15 minutes elapsed before the patient could feel a reduction in pain.

*Afzelia africana* gel significantly decreased SR better than diclofenac gel for the 1st hour. After the 2nd hour the difference in activity for SR was no longer significant ( $P > 0.05$ ). The flavonoids, sterols and tannins present in the extract may be responsible for the anti-inflammatory and analgesic activity of the gel. Indeed, the work of Williams on the evaluation of the biological activity of the flavonoids of *Tanacetum parthenium* showed that the flavonoids act on inflammation by inhibiting cyclooxygenase and lipooxygenase [15] while hesperidin, luteolin, and quercetin are shown to be potent anti-inflammatories and myricetin, quercetin, naringenin, and catechin are effective antioxidants [16]. Quercetin has the ability to inhibit nitric oxide synthetase, lipooxygenase, phospholipase A2 and C as well as cyclooxygenase-2, which are pro-inflammatory enzymes [17]. Tannins are known to be potent inhibitors of cyclooxygenase-1 with antiphlogistic activity [18, 19]. The anti-inflammatory effect of *Afzelia africana* could therefore be explained by the synergistic and / or potentiating action of these secondary metabolites.

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## 6. Conclusion

The objective of the present study was to evaluate the clinical efficacy of a gel based on the hydro-ethanolic extract of the leaves of *Afzelia africana* (Fabaceae). We obtained a gel effective on acute inflammation and chronic inflammation with clinical efficacy at least comparable to that of diclofenac. This activity is probably due to the synergistic and / or potentiating action of the tannins, flavonoids and sterols present in the extract of *Afzelia africana*.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to announce.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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