Research Article

Anti-inflammatory potential of *Secamone afzelii* extract: A study of Ivorian traditional plant on experimental rats

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Received: 4 September 2022 Revised: 14 October 2022 Accepted: 26 October 2022

Abstract

Objectives: *S. afzelii* aqueous leaves extract is used in traditional Ivorian medicine to fight against gastric disorders, colic, dysentery and kidney problems. This study aimed was to compare decoction and methanolic phytochemical composition of *S. afzelii* extracts from aerial parts and evaluate anti-inflammatory potential of this plant, in order to bring out solid scientific argument which can justify its use in many pathologies. **Methods**: Phytochemical screening of aqueous decoction and hydro methanolic extracts obtained from leaves of *S. afzelii* was evaluated with standard methods. Aqueous decoction was submitted first in rat's paw irritation test induced by formalin and secondary rat's paw oedema induced by carrageenan test was performed to appreciate anti-inflammatory activity. **Results**: Qualitative phytochemical screening showed presence of tannins, saponins, flavonoids, sterols, terpenoids and coumarins in both extracts. All doses evaluated in formalin test (25-50-100 and 200 b.wt.) have reduced significantly production of chemical inflammation mediators respectively up to 59.09%; 54.22%; 54.10% and 62.88% with an effect comparable to ketoprofen which was 55.56%. Concerning carrageenan-induced oedema test, strong limited oedema evolution was observed at 10 b.wt., with 60.28%, 66.92%, 61.30% and 45.29% respectively at 1st, 2nd, 3rd and 4th hours. However, these effects are not superior to ketoprofen, which decrease during 4 hours up 71.35, 73.69, 68.87 and 57.06%. **Conclusion**: Aqueous leaves and hydro methanolic extracts of *S. afzelii* revealed presence of flavonoids, saponins, sterols, terpenoids, tannins and coumarins. Aqueous leaves of *S. afzelii* possessed good anti-inflammatory potential.

Keywords: *Secamone afzelii,* phytochemical screening, inflammation, formalin, carrageenan

Introduction

Vegetables plant kingdom is an inexhaustible source of drugs used for primary health care, particularly in low-income countries due to the high cost of conventional drugs (Zabri et al., 2009). *S. afzelii* (Roem. & Schult.) K. Schum, a creeping wood plant climber of Asclepiadaceae family (Gill, 1992; Magid et al., 2016), is used in traditional Ivorian medicine to treat painful manifestations of gastric origin, colic, dysentery and kidney problems (Abere et al., 2012). Previous phytochemical studies carried out by our team and other researchers revealed that this plant was rich in secondary metabolites like flavonoids (Zabri, 2008; Magid, 2016), coumarins (Zabri et al., 2009) and tannins (Mensah, 2014).

In contrast, few studies have reported the power of *S. afzelii* against inflammatory manifestations, so that the objective of this study was to screen phytochemical constituents of decoction and hydro methanolic extracts and evaluate anti-inflammatory potential of this plant, in order to provide scientific evidence which would justify its use against several pathologies.

Material and methods

Plant material

Aerial parts of *S. afzelii* were collected in july 2022 on the town of Cocody-Abidjan (Ivory Coast). The plant has been identified at the Floristic National Center (CNF) of the

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DOI: https://doi.org/10.31024/ajpp.2022.8.5.3

University Félix Houphouët-Boigny Cocody (UFHB), Ivory Coast, where a voucher specimen under the number: UJCJ002631 has been deposited.

Experimental animals

Study was conducted out on male and female rats *(Rattus norvegicus)* whose weight varied between 100 g and 210 g. These animals were provided from laboratory of Pharmacology, Clinical Pharmacy and Therapeutics (UFR SPB of Felix Houphouët-Boigny University of Abidjan - Ivory Coast). All animals under experimentation were maintained in controlled environmental conditions of 24 ± 1°C with a 12 h cycle of light and dark. The animals were kept in spacious and hygienic cages and had free access to water and pellet food provided by Animal Feed Manufacturing Society (FACI®) of Ivory Coast.

Chemicals and solvents

- Methanol (Pharmivoire®, Ivory Coast)
- Distilled water (Pharmivoire®, Ivory Coast)
- NaCl 0.9% (Pharmivoire®, Ivory Coast)
- Carrageenan (Sigma, Germany)
- Formalin (Sharlau, Germany)
- Ketoprofen (Sigma-Aldrich, France)

Phytochemical screening

Two extracts were obtained from leaves of *S. afzelii*. The first is obtained from dried powdered leaves and the second from fresh leaves. The dried and powdered aerial part of *S. afzelii* (100 g) were macerated with aqueous-MeOH 80% during 24 h with 500 mL. Fresh aerial parts of *S. afzelii* were boiled (500 g in 1.5 L of water) during 1 hour to comply with traditional healers' procedures. Aqueous decoction and methanolic were evaporated under vacuum pressure to obtain 12 g and 2 g of dry extracts respectively. Qualitative phytochemical screening was done to evaluate major phytochemical constituents such as flavonoids, coumarins, saponins, alkaloids, sterols, terpenoids, and tannins using standard procedure of analysis as described in literature (Mamyrbekova-Bekro et al., 2008; Bekro et al., 2008). Detection of alkaloids was carried out using Dragendorff reagent. Catechic tannins characterization was realized using Stiasny reagent. Gallic tannins were detected by adding Stiasny reagent to sodium acetate and $FeCl_3$. Flavonoids were detected with cyanidin reaction. Coumarins were detected by adding KOH. Concerning detection of triterpenes and sterols, Liebermann-Büchard reagent was used. Saponins were characterized by measuring index of foam.

Preparation of Formaldehyde (2.5%v/v) solution

1ml of 37% formaldehyde solution was homogenized in 13.8 ml of physiological saline solution (0.9% NaCl).

Preparation of Carrageenan (1%w/v) solution

10 mg of pure carrageenan powder was homogenized in 10 ml of physiological saline solution (NaCl 0.9%).

Pharmacological tests

Formalin induced rat paw irritation test

The method performed was described by Dubuisson with some modifications (Tjolsen, 1992; Dubuisson, 1997). This test specific to confirm analgesic activity is based on determine licking time of rat's paw previously treated with substances evaluated after cause paw irritation by injection of formalin 2.5%. The licking time of the paw was counted for five minutes after the application of formalin, and then counted for fifteen minutes after ten minutes corresponding to the intermediate period of latency. A control and reference group were performed and receive respectively water and ketoprofen. The injection of a phlogogenic substance (formalin) under posterior plantar aponeurosis paw set off an inflammatory syndrome painful, manifests itself into two phases: (i) a neurogenic phase (phase 1) ranging from 0 to 5 minutes followed by (ii) a late or inflammatory phase (phase 2) ranging from 15 to 30 minutes after the application of the stimulus.

Preventive administration of substances with analgesic properties decrease paw licking time by inhibiting pain during phase 1, while those with anti-inflammatory properties stop extension of inflammatory phenomena during phase 2. On experimentation day, 36 rats were divided into six (6) homogeneous weight groups of six (6) rats per group. The solutions were administered to animals using an oral gavage at rate of 1ml/100g p.c., as follows:

- Group 1 (Control): rats treated with NaCl 0.9%

-Group 2 (Reference): rats treated with 10 b.wt. of ketoprofen;

- Group 3 (Test 1): rats treated with 25 b.wt. of extract;

- Group 4 (Test 2): rats treated with 50 b.wt. of extract;

- Group 5 (Test 3): rats treated with 100 b.wt. of extract;

- Group 6 (Test 4): rats treated with 200 b.wt. of extract;

In each group percentage of inflammatory syndrome was calculated according to the following formula:

$$\%\ \text{of inhibition} = \frac{(\text{Average licking time})_{Control} - (\text{Average licking time})_{test}}{(\text{Average licking time})_{Control}} \times 100$$

Carrageenan-induced oedema test

Method performed was described by Winter (Winter, 1962). This specific test to confirm the anti-inflammatory activity ~~is~~ based on measure oedema volume of rat's paw previously treated with substances evaluated after having

caused oedema with injection of carrageenan 1%. Oedema volume evolution was measured at 1h, 2h, 3h and 5h after injection of 1% carrageenan. Preventive administration of an anti-inflammatory substance reduces oedema volume of rat's paw. A control and reference group were performed and receive respectively water and ketoprofen. On experimentation day, 30 rats were divided into six (6) homogeneous weight groups of six (6) rats per group. The solutions were administered to animals using an oral gavage at rate of 1ml/100g p.c., as follows:

- Group 1 (Control): rats treated with NaCl 0.9%;
- Group 2 (Reference): rats treated with 10 b.wt. of ketoprofen;
- Group 3 (Test 1): rats treated with 5 b.wt. of extract;
- Group 4 (Test 2): rats treated with 10 b.wt. of extract;
- Group 5 (Test 3): rats treated with 20 b.wt. of extract;

In each group percentage of inflammatory syndrome was calculated according to the following formula:

$$\%\ \text{of inhibition} = \frac{\text{A(Ct} - \text{Co)}_{\ Control} - \text{A(Ct} - \text{Co)}_{test}}{\text{A(Ct} - \text{Co)}_{\ Control}} \times 100$$

A = Average of the values obtained

C_t = *Circumference of paw at a time t*

C_0 = *Initial circumference of the paw*

Ethical approval

Experimental procedures were carried under approval by the Ethics Committee for Animal Resources of the University (Ivory Coast). All test realize were in strict accordance with animal's care guidelines and declarations of European Union concerning handling of laboratory animals (Louhimies, 2002).

Statistical Method

Data were analysed using Graph Pad Prism® 8.0 software. Kruskal-wallis statistical test compared mean values ± SD (standard deviation) by analysis of variance (ANOVA) at risk α = 0.05. Codification for statistically significant difference was: * = $p \geq 0.01$; ** = 0.001; $p \leq 0.01$; *** = $p \leq 0.001$

Results

Phytochemical constituents

Phytochemical screening showed presence of tannins, saponins, flavonoids, sterols, terpenoids, and coumarins in aqueous decoction and methanolic extracts. However, no alkaloids were detected in both extracts (Table 1).

Table 1. Results of phytochemical screening of aqueous decoction and methanolic extracts of aerial part of *S. afzelii*

Class of compounds	Flavonoids	Coumarins	Saponins	Alkaloids	Tannins	Sterols and triterpenes
Aqueous extract	+	+	+	-	+	+
Methanol extract	+	+	+	-	+	+

(+) shows presence and **(-)** confirms absence of phytoconstituents

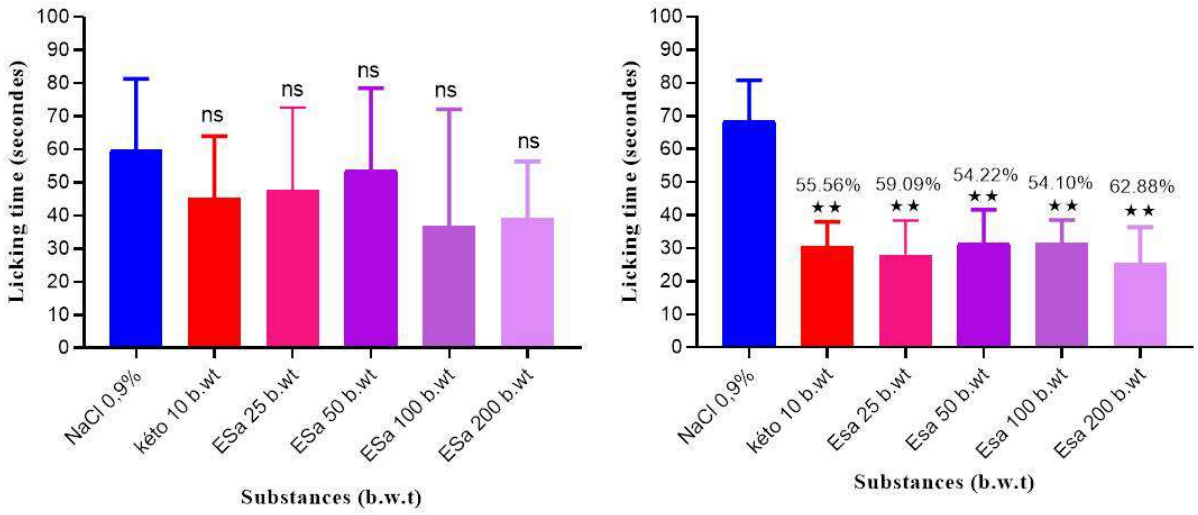

Figure 1. Effect of extracts after administration of formalin agent. Test de Kruskal-wallis, Values expressed on average ± SD (standard deviation); Neurogenic phase: No significant difference rat paw licking time compared to Control; Inflammatory phase: significant difference rat paw licking time compared to Control: Kéto 10 b.wt.**(p = 0,0003) ; ESa 25 b.wt.;**(p = 0,0013) ; ESa 50 b.wt.; **(p = 0,0016) ; ESa 100 .;**(p =0,0023) ; ESa 200 b.wt.; **(p =0,0026)

Secondary metabolites recognised were same in both extracts, so that our next investigation on animals' experimentation were performed using decoction because it is the most preparation used according to traditional healers'. Thus, aqueous extract was used to prepare stock solution and range concentration tested at 5, 10, 20, 25, 50, 100 and 200 b.wt.

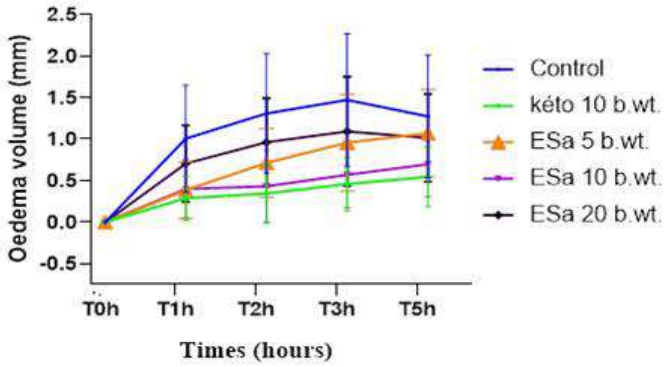

Figure 2: Effect of extracts after formalin injection

Effect of extract after formalin administration

On first time (T 5min) extract under study did not significantly inhibit rats' pain. In contrast, during late time (T 30min), all doses tested 25-50-100 and 200 b.wt., had significantly limited production of chemical inflammation mediators respectively up to 59.09%; 54.22%; 54.10% and 62.88% with an effect comparable to ketoprofen which was 55.56%.

Effect of extract after carrageenan administration

Extract dose at 5 b.wt., significantly inhibited oedema induced by carrageenan during 2 hours with an effect close to 61.56% and 45.52% respectively at 1st and 2nd hours. Concerning dose at 10 b.wt., oedema inhibition was lasted

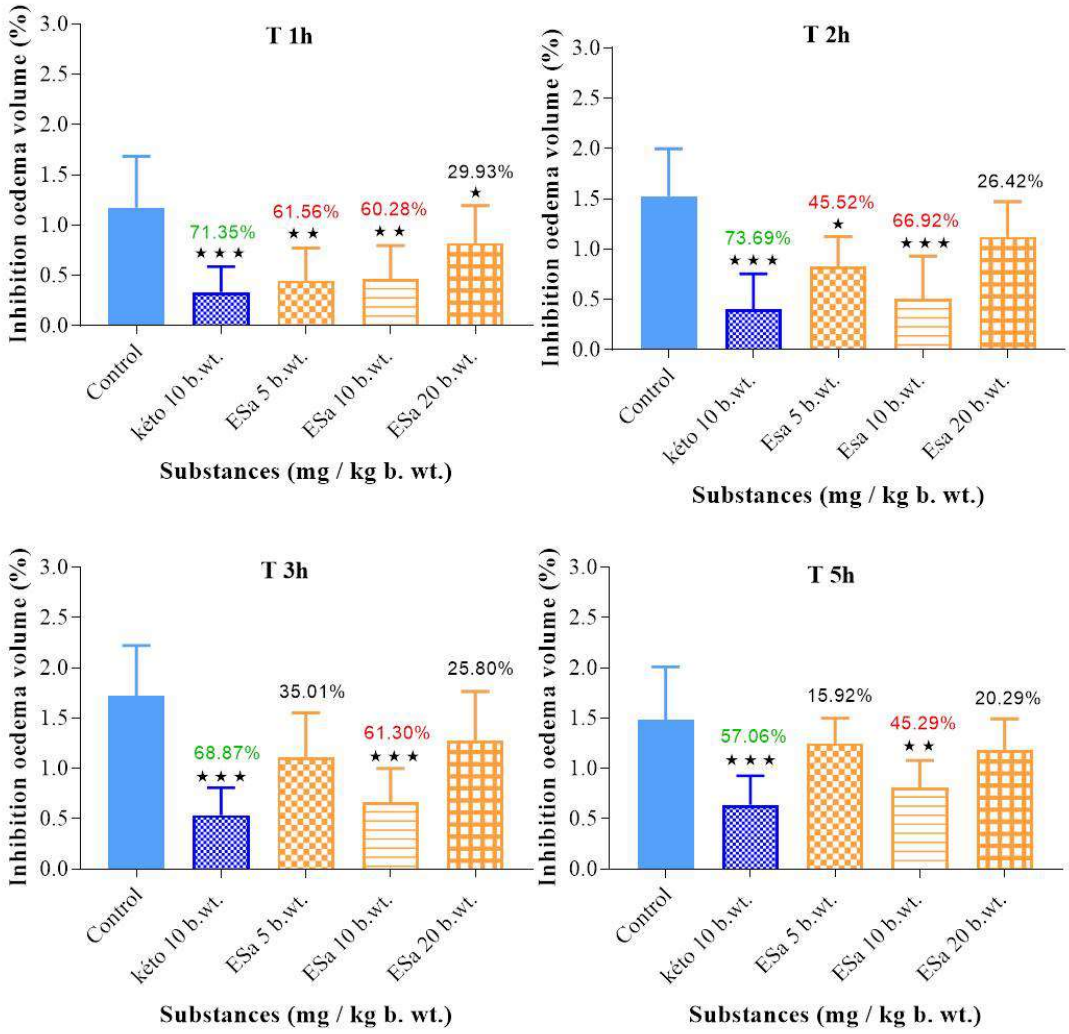

Figure 3: Evolution of inhibition paw volume oedema after of administration of extracts. Test de Kruskal-wallis, Values expressed on average ± SD (standard deviation). Values expressed on average ± SD (standard deviation); ***, **, *: Significant difference rat paw licking time compared to Control: T 1H: Kéto 10 b.w.t. ***(p = 0,0013); ESa 5 b.w.t **(p = 0,0080); ESa 10 b.w.t **(p = 0,0094); ESa 20 b.w.t (p =0,0194); T 2H: Kéto 10 b.w.t ***(p = 0,0003); ESa 5 b.w.t *(p = 0,0153); ESa 10 b.w.t **(p=0,0004); T 3H: Kéto 10 b.w.t ***(p = 0,0002); ESa 10 b.w.t *** (p = 0,0007); T 5H: Kéto 10 b.w.t ***(p = 0,0009); ESa 10 b.w.t ** (p = 0,0081.

for 4 hours, up to 60.28%, 66.92%, 61.30% and 45.29% respectively at 1st, 2nd, 3rd and 4th hours.

Discussion

Phytochemical analysis

Phytochemical analysis of samples was reported in Table 1. Results showed that flavonoids, sterols, terpenoids, tannins, coumarins and saponins were present in methanol and aqueous decoction extracts. These results are similar with to other researchers (Zabri et al., 2008; Zabri et al., 2009; Magid et al., 2016). Presence of these constituents may support reported medicinal use of this plant. These secondary metabolites have also been detected in *Secamone genus* (Sekandi et al., 2020), a plant of the same family of *S. afzelii*, which confirms its chemotaxonomics.

Polyphenolic compounds including flavonoids, tannins and coumarins receive increased attention because in recent years a significant number of studies have demonstrated their potential benefits for human health. Among polyphenols, most abundant flavonoids and coumarins are known for their antioxidant, anti-inflammatory and anti-microbial activity (Maleki et al., 2019; Bansal et al., 2013; Garcıa-Lafuente et al., 2009; Kim et al., 2004). Tannins are biomolecules known for their astringent properties which bind and precipitate proteins and various other organic compounds, including amino acids and alkaloids responsible of its multiple biological activities, such as anti-inflammatory (Wu et al., 2023; Park et al., 2013; Okuda et al., 2011).

Triterpenoids and sterols are a family of organic compounds found in plants and trees that exhibit interesting biological properties such as analgesic and anticonvulsant activities. Also, several studies have shown that these terpene compounds reduce inflammatory symptoms (Ge et al., 2022; Prakash et al., 2017; Kashyap et al., 2016).

Plants containing saponins are used to heal wounds and also possess anti-inflammatory activities (Costa et al., 2011; Zheng et al., 2022).

Anti-inflammatory effect

Formalin-induced rat paw irritation test is non-specific test widely used to screen analgesic, anti-inflammatory and relaxant substances (Koster, 1969). Injection of this phlogogenic substance into rat paw had directly stimulated free nerve endings (neurogenic phase: phase 1) with production of full chemical pain mediators such as substance P, histamine, serotonin and bradykinin (Parada, 2001), which secondarily accelerate prostaglandins, cytokines and molecules like IL-1β, IL-6, TNF-α (Minghetti, 2004; Bartels, 2010) production responsible of the inflammation (inflammatory phase: phase 2) (Hunskaar, 1987). Narcotic analgesics (Tjolsen, 1992) inhibit both phases (phase 1 and 2), while nonsteroidal anti-inflammatory drugs such as ketoprofen only inhibit late phase (phase 2).

Results obtained after realise this formalin test showed that extract evaluated did not significantly inhibit rats' pain (phase 1) allowing us to think that aqueous extract of *S. afzelii* had no analgesics properties. It was at second phase (phase 2) that all doses tested revealed significant anti-inflammatory effect by limited production of chemical inflammation mediators respectively involve in the persistence of pain (Figure 1). Hypothesis that doses under 25 b.wt. could still be effective, it was important to performing carrageenan test using lower range doses around 5, 10 and 20 b.wt., not only to find most effective dose but also to confirm extract anti-inflammatory potential because carrageenan test is a specific test to evaluate anti-inflammatory properties of substances. Carrageenan test confirms that dose of 5 b.wt., significantly inhibited oedema induced by carrageenan during 2 hours. Most remarkable effect was observed at 10 b.wt., which lasted for 4 hours like reference substance (kétoprofène 10 b.wt.) (Figure 2, 3). These results prove that it makes sense to support high probability that aqueous extract of *S. afzelii* possess anti-inflammatory properties and this potential could be attributed to polyphenolic (Mensah et al., 2014, Bansal et al., 2013; Zabri et al., 2009; Kim et al 2004) and triterpenoid compounds (Jeong et al., 2014; Prakash et al., 2017) which acts in synergy to developed biopharmacological effects responsible of its anti-inflammatory potential. Ours results are similar compared to those obtained by Mensah (Mensah, 2014). The charm of our investigations is that effect was revealed at low doses close to 10 b.wt., while those of Mensah were obtained at higher concentrations up to 300 b.wt. with 44.26% inhibition of oedema induce by carrageenan.

Conclusion

Aqueous leaves of *S. afzelii* revealed presence of flavonoids, saponins, sterols, terpenoids, tannins and coumarins and possessed good anti-inflammatory potential. Our study provided scientific evidence which support traditional use of *S. afzelii* aqueous extract in treatment of pathologies with low to moderate inflammatory components. However, biological other activities must be carried out for more effective and efficient use of this plant. Production of a finished product with acceptable microbiological quality followed by clinical study in humans will be the next steps to make, before allow in security *S. afzelii* aqueous extract as a potential phytomedicine candidate.

Conflicts of interest

The authors have declared that no competing interests exist.

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