

New Anti-inflammatory Balm Based on a POACEAE Weed: *Eleusine indica* (L) Gaertn

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Abstract— *Eleusine indica* (L) Gaertn, also known as “Indian Eleusine” or “Tsiavotraombilahy”, “Tsipipihina” (Malagasy name) is an annual herbaceous plant of the POACEAE family. Native to tropical and subtropical regions, it is often considered a weed, especially in agricultural areas and lawns areas. The plant has rapid proliferation and has the ability to compete with other crops. In the folk medicine, a decoction of the whole plant is used to treat sprains and strains. Previous studies have revealed its anti-inflammatory, antioxidant and cytotoxic properties. This study aims to promote the unsuspected virtue of *Eleusine indica* plant, often ignored, through the formulation of an anti-inflammatory balm to treat osteoarthritis, joint diseases and rheumatic pain. Currently, age-related degenerative diseases, in particular inflammatory syndromes, are frequently encountered in everyday practice. However, the cost of medicines in Madagascar is very high, hence the need to develop an effective and affordable remedy to treat patients. This study concerns the formulation and quality controls of a new anti-inflammatory balm according to the standards of the European Pharmacopoeia. The results obtained confirmed the possibility of exploiting the plant extract as an active ingredient of an anti-inflammatory balm which is affordability and easy for use. The product obtained is stable, non-toxic with satisfactory physicochemical and microbiological qualities. This absence of toxicity supports the safe use of the product. Preclinical tests will be necessary before its commercialization. These results suggest that formulated balm may represented an effective therapeutic approach with good safety against inflammatory pain. This research illustrates the possibilities of valorizing so-called wild plants with medicinal properties, both for the health coverage of the population and for its socio-economic benefits.

Keywords— *Eleusine indica*, wild grass, rheumatism, balm, anti-inflammatory, toxicity, formulation.

I- INTRODUCTION

Currently, age-related degenerative diseases, including inflammatory joint syndromes, constitute a health, social and economic problem. Worldwide, approximately 1.71 billion people suffer from osteoarticular conditions such as low back pain, osteoarthritis, trauma and rheumatoid arthritis (Cieza *et al.*, 2020). These conditions limit the movements of affected subjects in 80 % of cases and 25 % are unable to perform daily tasks (Dembelé, 2022). The treatment of these conditions is very expensive, which is why patients in developing countries like Madagascar (around 60 % of the population) resort to using herbal remedies for treatment (WHO, 2010; Rafidisaona, 2021). Also, current treatment of inflammation uses steroidal and non-steroidal anti-inflammatory drugs, which although effective, most often have side effects that may prevent their long-term use (Belkacemi, 2014).

Medicinal plants represent an inexhaustible source of natural bioactive compounds. According to the World Health Organisation (2002), more than 80 % of the world's population uses herbal medicine for treatment. Secondary metabolites of plants, particularly phenolic compounds, are the subject of numerous *in vivo* and *in vitro* studies. The aim of this study is the valorization of *Eleusine indica* (POACEAE) by developing an effective remedy within everyone's reach to relieve rheumatic and joint diseases.

In Africa, a large number of medicinal plants are used in the treatment of joint and inflammatory pain (Birnbbaum, 2021; Denou *et al.*, 2016). The species *Eleusine indica* (L) Gaertn (POACEAE) is a weed, known as “Goosefoot”, “Tsiavotraombilahy” or “Tsipipihina” (Malagasy name), native to tropical and subtropical regions (Kashyap *et al.*, 2023). This wild herb is commonly used in traditional medicine in many parts of the world for deworming, mitigate cough and lung infections, dysentery, heart attacks, and high blood pressure, spleen and liver problems, blood and kidney stones as well as dislocation of bones and lumbago (Sukor, *et al.*, 2022; Kashyap *et al.*, 2023).

In Malagasy Traditional medicine's, boiled plant decoctions are commonly used as for the treatment of rheumatic conditions, sprains and strains, and relieve pain caused by straining abdominal muscles (Quansah, 1988; Abdul *et al.*, 2015).

Previous scientific investigations on the plant have identified its main bioactive constituents such as vitamin D, alkaloids and hydrocyanic acids as well as its anti-inflammatory, antioxidant and cytotoxic properties (Hilu *et al.*, 1978; Iberahim *et al.*, 2015). Also, aqueous extract of *Eleusine indica* has been reported to possess a wide array of biological activities, which were antioxidant and free radical scavenging properties, anti-inflammatory, antimicrobial, antipyretic, antiviral and hepatoprotective (Iqbal, M., & Gnanaraj, C., 2012).

Thus, based on these previous results, the present work aims to enhance the unsuspected virtue of *Eleusine indica*, through the formulation of an anti-inflammatory balm that can contribute to the treatment of painful conditions, joint diseases and rheumatic pain. As a matter of fact, there is a progressive demand for topical drug route over the other routes due to various advantages of topical drug delivery systems such as reduced risk of systemic adverse reactions, avoidance of first pass metabolism and gastro-intestinal tract variability, direct administration to desired site of action, non-invasiveness and prevention of drug-drug interactions.

Their treatment regimen is straightforward and may be easily discontinued by just taking the application off the skin's surface (Mepheron & Cimino, 2013). Then, the objectives are to formulate an anti-inflammatory balm, and to determine the physicochemical quality control parameters as well as to verify the skin biotolerance of the final product.

II- MATERIALS AND METHODS

The plant material (*Eleusine indica* (L) Gaertn) consists of the whole plant (coded E2023), were collected in May 2023 from Antananarivo, Madagascar (Fig. 1). After harvesting, they were washed, then dried and ground into powder.



Fig. 1. *Eleusine indica* (L) Gaertn plant

A. Preparation of the plant extract

According to literature data on the use of *Eleusine indica*, an extraction by reflux heating at 100 °C with distilled water from the dried plant powder was carried out.

After cooling, the decoction was filtered and then evaporated to dryness under reduced pressure. The residue thus obtained is weighed and stored in hermetically sealed boxes. The extract thus obtained was used as an active ingredient for the formulation of the balm. The extraction yield was calculated and used in the determination of proportionality of the active ingredient in the developed product (Rakotoarisoa *et al.*, 2020).

B. Skin toxicity test

In vivo experiments were carried out on SWISS mice weighing 20 g to 30 g, supplied by the animal facility of the National Center for Pharmaceutical Research Applications (CNARP). They had free access to tap water and were fed a standard diet.

The tests were carried out according to the guideline of the Organization for Economic Co-operation and Development or OECD (OECD, 2001b; OECD, 2008b). The 3Rs (Replacement, Reduction, and Refinement) principles were used in the implementation of this procedure.

All animals were acclimatized in the cage with a temperature of ± 23°C for one week. The total study duration was 72 hours from the application day. It allows the effect of the product coming into contact with the skin to be assessed and categorized (Zuang *et al.*, 2013).

The procedure of Draize acute primary irritation test was carried out on male mice. Application site was evaluated for scoring the reaction namely erythema and oedema. (Bene *et al.*, 2017, Yapi *et al.*, 2019, Rakotoarisoa *et al.*, 2023).

C. Galenic formulation and quality controls

According to the formulation methods described in the European Pharmacopoeia and Good Manufacturing Practice, E2023 balm was prepared manually.

The formulation study requires several tests before finding the final formula that will compose the product. The preformulation test began with the preparation of the different raw materials and then the search for the appropriate base support before the incorporation of the active principle. Thus, the aqueous plant extract was ground until completely homogeneous with a pestle in a porcelain mortar with the necessary quantities of base. The balm thus prepared was packaged in PET boxes with sufficient quantities for 50 g.

Final formulation was evaluated for their physicochemical parameters such as homogeneity, pH, odor, color, stability, appearance, sterility, and phase separation for 60 days at room temperature. These quality controls were determined according to the methods recommended for external use products described in the Pharmacopoeia (Bene *et al.*, 2017; Rakotoarisoa *et al.*, 2024).

III- RESULTS

All experimental measurements were carried out in triplicates and the results were expressed as the mean ± standard deviation. Statistical significance of each test is estimated by Student's t test. At (p <0.05), the values were considered significantly different at 95 %.

A. Extraction result

A yield of 2.5 % of aqueous extract of *Eleusine indica* was obtained in the form of a brown powder with an unpleasant odor.

Based on the manufacturing process, anti-inflammatory balm with proportion of aqueous extract was made. (Fig. 2).

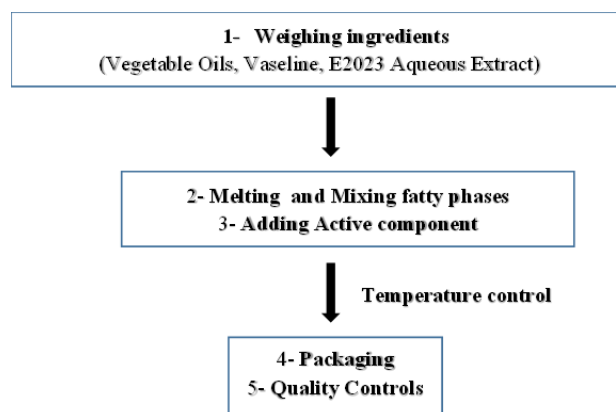


Fig. 2. Manufacturing process of balm (E2023).

B. Evaluation of the formulation developed

The investigations of the skin hypersensitivity test with the main characteristics and physicochemical parameters of E2023 balm formulated are given in Table 1.

During skin toxicity test, no dermal irritation reaction was observed on the back of each animal. The Primary Irritation Index (PII) was zero after 24 hours and 72 hours of application of the balm E2023 (Fig.3).

TABLE I. Physicochemical parameters of *Eleusine indica* balm (E2023).

Physicochemical and organoleptic parameters of balm E2023		Value of the Primary Irritability Index (PII) according to the Draize scale (24 h -72 h)
Color	Light brown	Erythema : 0
Odor	Good	
Consistency	Semi-solid	Oedema : 0
Homogeneity	Good	
pH	Compliant (6 to 7)	PII : 0
Phase separation	Nothing	Non-toxic for skin
Stability (after 60 days at 20° C)	No change	

SKIN REACTIONS in mice



Evaluation of the Primary Irritability Index

Fig. 3. Skin test reactions in mice with balm (E2023).

The results of the microbiological tests after incubation of each petri dish show the total absence of bacterial germs and yeasts for up to 72 hours (Fig. 4).



Fig. 4. Microbiological test of balm (E2023) in petri dish

The formulated herbal balm (E2023) had physical stability during the short-term investigation, since all the testing parameters were almost similar on manufacture date as well as after 60 days.

These results demonstrate the sterility and stability of the balm preparation (E2023) in compliance with the conditions of Good Manufacturing Practice (Fig. 5).



Fig. 5. Anti-inflammatory balm (E2023) with *Eleusine indica* extract.

IV- DISCUSSION

This study involved the formulation of an anti-inflammatory balm made from the aqueous extract of *Eleusine indica*. It consists of both the valorization of this wild herb and the demonstration of the possibility of its exploitation as a phytomedicine.

The main quality parameters obtained from the developed anti-inflammatory balm are in agreement with some data from the literature. Balm is a traditional or Ayurveda form of topical semi-solid, anhydrous preparation, commonly used for relieving body aches and pains. Balms are preferable for topical use as they are water impermeable, occlusive, moisture protecting, chemically, physically stable even without preservatives and longer acting with slow absorption (Ramesh *et al.*, 2010).

The results of microbiological controls on the finished product showed the possibility of using it for cutaneous use as well as good conservation. Despite the low yield of active ingredient obtained, the developed product can be exploited for commercial purposes because this plant is a wild herb that grows easily in fields and gardens. It is a rapidly growing weed, which can produce almost 50,000 seeds per plant (Kashyap *et al.*, 2023).

The safety test revealed no signs of skin hypersensitivity or side effects in albino mice upon contact with the product. Therefore, its use will not have any side effects for humans because this animal is phylogenetically closer to humans (Yapi *et al.*, 2019). The formulation investigations are very encouraging, but despite this, product efficacy tests will still be necessary to establish its spectrum of activity.

V- CONCLUSION

The formulation and quality controls of the new anti-inflammatory balm (E2023) were carried out in compliance with the standard and quality of products for external use. The new product thus developed can be categorized as an improved Traditional Remedy made from the POACEAE family (*Eleusine indica*).

Based on the results obtained and those of previous studies on the plant species, E2023 balm could be used in the treatment of painful and inflammatory joint and muscle conditions. However, *in vivo* efficacy tests will be required to confirm its mechanism of action. This new product could be used by the entire population and contribute to solving the problem of lack of access to medication and towards more holistic and environmentally friendly care.

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