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PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY ACTIVITY OF *EUPHORBIA HIRTA*: IMPLICATIONS FOR PHYSIOLOGICAL RECOVERY

Innocent YAKA [1,*], Dieu Donné Wilfrid Kpèdétin AGBODJOGBE [1] and Nicaise Tohossi KINNOUDO [2]

[1] Laboratory of Motor Skills, Performance, and Sports Health (LaMoP-2S), National Institute of Youth, Physical Education and Sports (INJEPS), University of Abomey-Calavi (UAC), Porto-Novo, Benin.
[2] Laboratory of the Para-University Institute for Research and Development in Health and Industry (APIPHARMA).
* Corresponding Author

ORCID Details
Innocent YAKA: https://orcid.ord/0009-0004-4177-9257
Dieu Donné Wilfrid Kpèdétin AGBODJOGBE: https://orcid.org/0009-0002-7962-9196
Nicaise Tohossi KINNOUDO: https://orcid.org/ 0009-0008-0767-2260

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ABSTRACT

Euphorbia hirta is a medicinal plant traditionally used to treat numerous conditions, particularly inflammation and pain. Its pharmacological properties justify scientifically evaluating its anti-inflammatory potential. This experimental study, conducted in Benin across the Atlantique and Zou departments, aimed to identify the primary phytochemical constituents and evaluate the anti-inflammatory activity of hydroethanolic extracts from *Euphorbia hirta* leaves. Anti-inflammatory activity was evaluated in vitro using the bovine serum albumin denaturation inhibition assay, with indomethacin serving as the reference anti-inflammatory drug. Absorbance values were measured via spectrophotometry to determine the inhibition percentage. Qualitative phytochemical screening was performed according to the methods of Raman and Harborne to identify the primary secondary metabolites present in the *Euphorbia hirta* extract. The hydroethanolic extracts of *Euphorbia hirta* demonstrated significant anti-inflammatory activity (analgesic, antipyretic, and antioxidant properties), reflecting a strong ability to inhibit protein denaturation. Phytochemical screening revealed the presence of alkaloids, tannins, anthocyanins, leucoanthocyanins, quinone derivatives, steroids, triterpenoids, free anthracene compounds, and reducing compounds, which may contribute to this biological activity. These findings confirm the potential of *Euphorbia hirta* as a natural source of bioactive compounds with anti-inflammatory activity. They support its potential application in developing natural post-exercise recovery strategies and encourage the utilization of local medicinal plants in health and sports medicine. However, in vivo studies and clinical trials remain necessary to validate these effects and elucidate the underlying mechanisms of action.

Keywords: *Euphorbia hirta*, Anti-Inflammatory Activity, Hydroethanolic Extract, Phytochemical Screening, Physical Recovery.

1 INTRODUCTION

Recovery is a cornerstone of physical conditioning and athletic performance. It allows the body to progressively restore its capacity following the demands of physical exertion and adapt to repeated training loads. Incomplete recovery can lead to accumulated fatigue, impaired performance, and an elevated risk of injury [1,2]. Intense physical effort triggers elevated oxidative stress and a transient inflammatory response, contributing to muscle damage that can delay key recovery processes when severe or prolonged [3].

Mitigating these effects requires effective post-exercise recovery strategies, such as active recovery, stretching, massage, hydrotherapy, and cold or hot water immersion [4,5]. However, access to these specialized modalities in Benin is often limited by financial constraints. Within the Beninese context, this reality justifies exploring accessible, cost-effective alternative methods tailored to local practice conditions, specifically those rooted in affordable traditional pharmacopeia. Medicinal plants represent a rich source of bioactive molecules. An ethnobotanical survey conducted across multiple markets in Benin involving 144 herbalists documented 358 plant species used in traditional medicine, underscoring the vital role of plant biodiversity in local therapeutic practices [6].

Furthermore, medicinal plants play a prominent role in traditional remedies for various ailments, particularly inflammatory conditions. Among them, *Euphorbia hirta* (family *Euphorbiaceae*) is widely utilized for its therapeutic properties and accessibility [7]. A comprehensive review of the genus *Euphorbia* highlights broad ethnomedicinal uses and diverse biological activities, including anti-inflammatory and antioxidant actions [8]. Phytochemical investigations have identified several classes of secondary metabolites in *E. hirta*, such as flavonoids, alkaloids, tannins, and triterpenoids [9].

The primary relevance of this plant to physiological recovery hinges on its biological properties, which may directly address the physiological strains of strenuous exercise. Experimental studies report notable antioxidant and anti-inflammatory activities in *E. hirta* extracts [10]. Additionally, research on ethanolic leaf extracts has demonstrated modulation of the inflammatory response [11]. These findings provide a sound biological framework for the hypothesis that specific constituents of *E. hirta* may actively participate in anti-inflammatory pathways.

Nevertheless, it is important to distinguish measured anti-inflammatory activity from proven clinical efficacy in athletic recovery. To date, available literature supports the pharmacological interest of the plant, but does not conclusively establish that *E. hirta* directly enhances recovery in athletes. This experimental study aims to bridge that specific knowledge gap.

This study aimed to identify the key phytochemical constituents and evaluate the anti-inflammatory activity of hydroethanolic leaf extracts of *Euphorbia hirta*.

2 MATERIALS AND METHODS

2.1 Study Setting and Design

This experimental study was conducted at the Laboratory of Motor Skills, Performance, and Sports Health (LaMoP-2S) within National Youth, Physical Education and Sports Institute (INJEPS) at the University of Abomey-Calavi (Benin), in collaboration with the laboratory of the Para-University Institute for Research and Development in Health and Industry (APIPHARMA). Field collection occurred in Benin across the Atlantique and Zou departments. *Euphorbia hirta* was selected based on its traditional applications and the documented pharmacological potential in the literature [6,12].

2.2 Plant Material

Fresh *E. hirta* leaves were harvested, thoroughly washed with water, drained, and shade-dried inside the laboratory at room temperature (25 ± 2°C) for two to three weeks. The dried plant material was ground using an electric mill. The resulting fine powder was utilized for hydroethanolic extraction and phytochemical screening. The leaves and the powder are shown in Figure 1.

Figure 1: *E. hirta* leaves in their natural habitat and powder (Field photograph)

2.3 Preparation of the Hydroethanolic Extract

Extraction was performed using the pulverized plant material and a hydroethanolic solvent mixture. Briefly, 50 g of plant powder was macerated in 500 mL of a distilled water/ethanol mixture under continuous agitation for 72 hours. The mixture was filtered to yield a crude liquid extract, which was subsequently dried in an oven at 50°C for 48 hours [13,14]. The final dried extract yielded a mass of 7.53 g, corresponding to an extraction yield of 15.06%.

2.4 Animal Models

Wistar rats (Figure 2) were sourced from the APIPHARMA animal facility and acclimatized for two weeks prior to testing. The animals were maintained on a standard commercial pellet diet (formulated for growing and lactating rabbits) providing a metabolizable energy content of ≥ 2,347 kcal/kg.

Figure 2: Wistar rats

2.5 Phytochemical Analysis

The harvested leaf powder underwent qualitative phytochemical screening following the tube reaction methods outlined by Houghton & Raman [13], which are widely accepted in the literature [14,16]. These methods rely on characteristic color reactions and precipitation tests for qualitative identification of chemical families. Specific assays were executed on the extracts to confirm the presence or absence of major secondary metabolite classes based on visual indicators (color shifts and precipitate formation).

2.6 Evaluation of Anti-Inflammatory Activity

2.6.1 Anti-Edema Activity

Fifteen rats were assigned to three groups as detailed in Table 1.

Paw edema was induced by injecting 0.1 mL of formalin into the subplantar aponeurosis of the right hind paw of each rat. Paw volume measurements were recorded at 0, 30, 60, 120, 180, 240, 300, 360 minutes, and 24 hours post-induction, following the protocol adapted from Gbenou et al. [17].

Table 1: Distribution of rats for anti-edematous activity

Group	Treatment	Dose
5 Negative control rats	Normal saline	1 mL/100g
5 Positive control rats	Indomethacin	100 mg/kg
5 Treated rats	Hydroethanolic extract	500 mg/kg

Thirty minutes after formalin injection, the treated group received an oral dose of 500 mg/kg of *E. hirta* hydroethanolic extract. Indomethacin served as the reference drug (100 mg/kg), while the control group received normal saline (2 mL/kg).

Paw volume was determined using a modified Bhatt plethysmometer setup based on fluid displacement [18]. Water levels were readjusted to the baseline mark on the primary syringe using an auxiliary syringe, and displaced water volumes were read directly from the syringe scale.

2.6.2 Analgesic Activity

Fifteen rats were allocated into three groups as shown in Table 2.

Table 2: Distribution of rats for analgesic activity

Group	Treatment	Dose
5 Negative control rats	Normal saline	2 mL/kg
5 Positive control rats	Aspegic	50 mg/kg
5 Treated rats	Hydroethanolic extract	500 mg/kg

Analgesic activity was assessed using the tail-flick test [19], which evaluates spinal nociceptive reflexes by applying a thermal stimulus to the rat's tail. Oral treatments were administered to each group: Group 1 received *E. hirta* hydroethanolic extract (500 mg/kg), Group 2 (reference group) received lysine acetylsalicylate (Aspegic, 50 mg/kg), and Group 3 (control group) received normal saline (2 mL/kg). One hour post-administration, each rat's tail was immersed in a water bath maintained at 50°C. The latency to tail withdrawal was measured and recorded as the reaction time [19].

2.7 Antipyretic Activity

Fifteen rats were grouped as outlined in Table 3.

Table 3: Distribution of rats for antipyretic activity

Group	Treatment	Dose
5 Negative control rats	Normal saline	2 mL/kg
5 Positive control rats	Aspegic	50 mg/kg
5 Treated rats	Hydroethanolic extract	500 mg/kg

Hyperthermia was induced via intraperitoneal (IP) injection of a 20% aqueous brewer's yeast (BY) suspension at a dose of 2 mL/kg. Baseline rectal temperatures were recorded 24 hours post-yeast injection. The control group received 2 mL/kg of distilled water, the reference group received Aspegic (50 mg/kg in 2 mL/kg distilled water), and the test group received 500 mg/kg of *E. hirta* hydroethanolic extract. Rectal temperatures were monitored at 0, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 24 h post-treatment administration [17,20].

2.8 Statistical Analysis

Statistical computations were performed using R software (version 4.4.0, R Foundation for Statistical Computing, Vienna, Austria) and Microsoft Excel. Quantitative values are reported as mean ± standard deviation (SD). Inter-group

comparisons were evaluated using analysis of variance (ANOVA). For time-course parameters measured repeatedly on the same animals, a two-way repeated-measures ANOVA was applied to determine main treatment effects, time points, and their interactions. Significant ANOVA results were subjected to Tukey's post-hoc test to identify specific pair-wise differences. The significance threshold was set at $p < 0.05$.

3 RESULTS

3.1 Phytochemical Screening

Table 4 presents the qualitative phytochemical screening results of the *Euphorbia hirta* extract.

Phytochemical evaluation of the *E. hirta* hydroethanolic extract confirmed a diverse array of secondary metabolites, including alkaloids, tannins (total, gallic, and catechic), anthocyanins, leucoanthocyanins, quinone derivatives, steroids, triterpenoids, free anthracene compounds, and reducing compounds.

Table 4: Phytochemical screening results of *Euphorbia hirta* extract

Chemical Groups	Result
Alkaloids	Present (+)
Total tannins	Present (+)
Gallic tannins	Present (+)
Catechic tannins	Present (+)
Flavonoids	Absent (−)
Anthocyanins	Present (+)
Leucoanthocyanins	Present (+)
Mucilages	Absent (−)
Coumarins	Absent (−)
Saponins	Absent (−)
Cyanogenic derivatives	Absent (−)
Quinone derivatives	Present (+)
Steroids	Present (+)
Triterpenoids	Present (+)
Free anthracene compounds	Present (+)
Reducing compounds	Present (+)
C-glycosides	Absent (−)
O-glycosides	Absent (−)
Cardiotonic glycosides	Absent (−)

3.2 Anti-Inflammatory Activity of Euphorbia hirta

3.2.1 Effects of Treatment on Paw Edema Progression

3.2.1.1 Percentage Increase in Paw Edema

The progression of edema volume in rats treated with the reference drug (indomethacin), the hydroethanolic (HE) extract of *E. hirta*, and the control group demonstrated progressive inhibition over 24 hours (Figure 3).

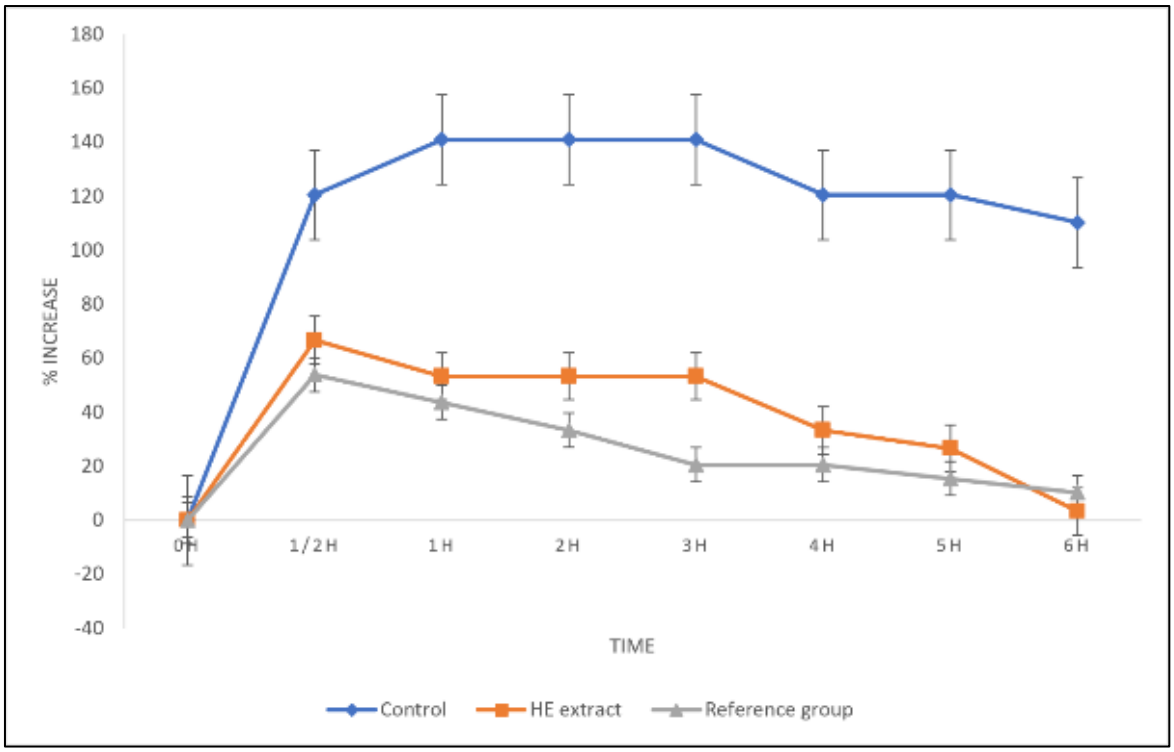

Figure 3: Percentage increase in edema in rats post-treatment

3.2.1.2 Percentage Inhibition of Edema

The percentage inhibition of paw edema achieved by the *E. hirta* HE extract versus the reference compound reached high levels over the 24-hour observation period (Figure 4).

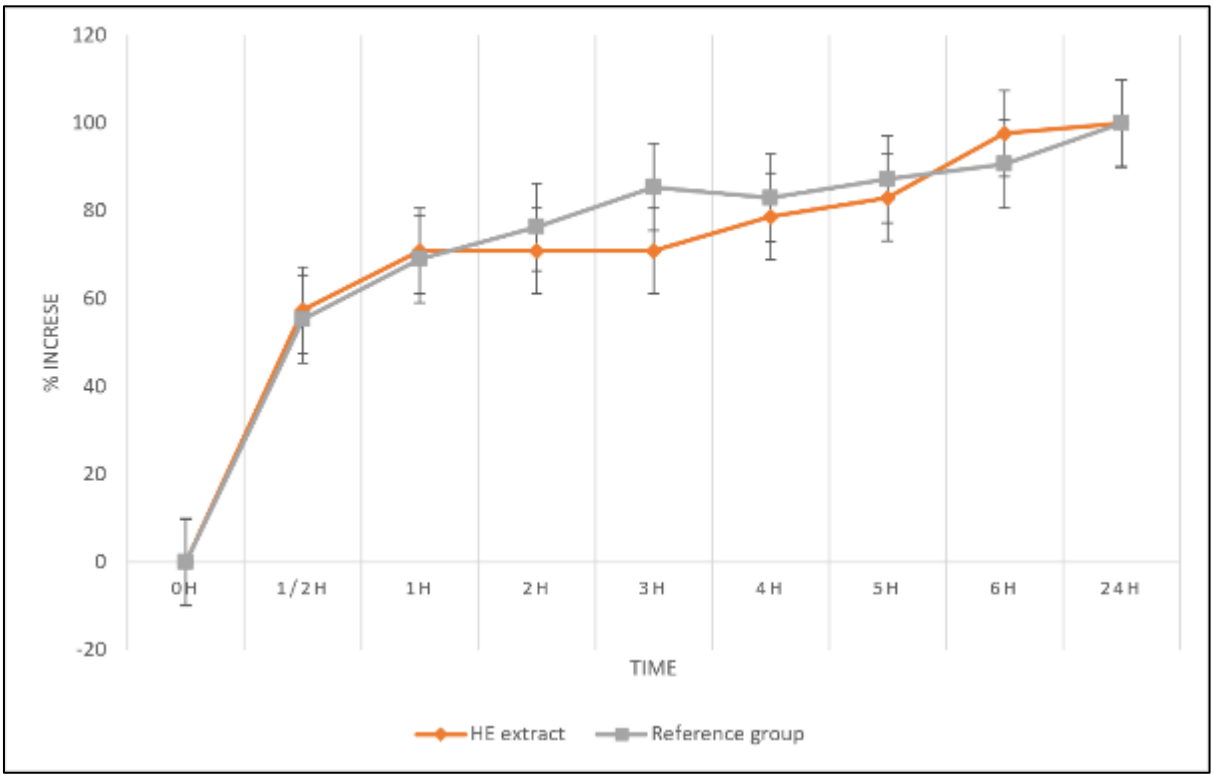

Figure 4: Percentage of edema inhibition in treated rats

3.2.2 Analgesic Activity

Thermal latency and reaction times of rats treated with *E. hirta* HE extract showed significant antinociceptive effects compared to negative control and reference groups (Figure 5).

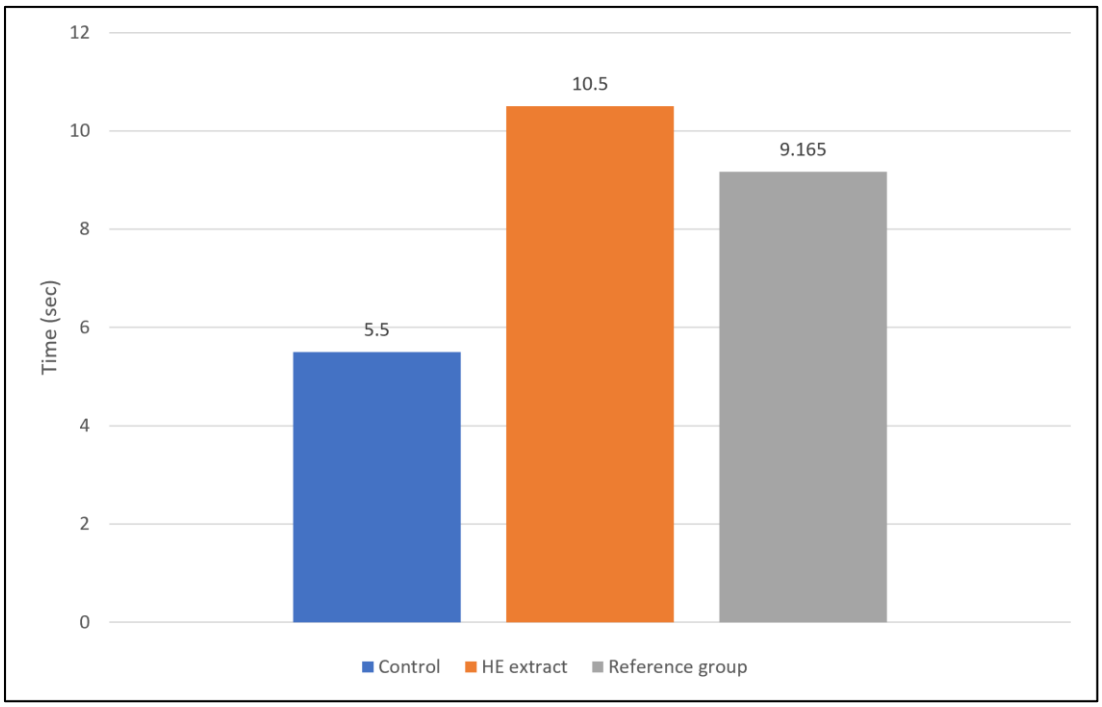

Figure 5: Analgesic reaction time

3.2.3 Antipyretic Activity

Core body temperature changes following brewer's yeast induction exhibited marked temperature reductions in the *E. hirta* HE extract group matching the standard antipyretic drug (Figure 6).

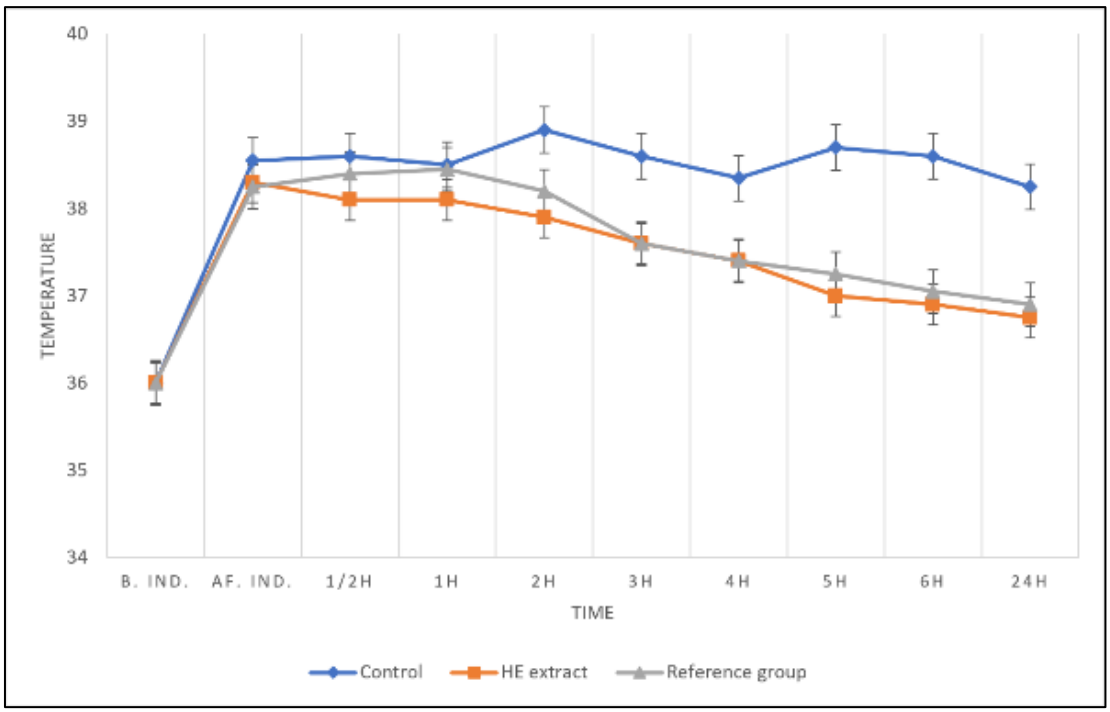

Figure 6: Temperature evolution in rats subjected to antipyretic activity testing

4 DISCUSSION

4.1 Phytochemical Screening

Phytochemical screening of the hydroethanolic extract of *Euphorbia hirta* identified several secondary metabolite classes, notably alkaloids, tannins (total, gallic, and catechic), anthocyanins, leucoanthocyanins, quinone derivatives, steroids, triterpenoids, free anthracene compounds, and reducing agents. Conversely, flavonoids, mucilages, coumarins, saponins, cyanogenic derivatives, C-glycosides, O-glycosides, and cardiotonic glycosides were not detected (Table 4).

The presence of tannins and alkaloids aligns with previous reports validating their occurrence in *E. hirta* [21]. These compounds, combined with established antioxidant, anti-inflammatory, antimicrobial, and analgesic properties, likely account for a major portion of the plant's biological activity [22]. Steroids and triterpenoids, common across the *Euphorbiaceae* family, further reinforce the plant's anti-inflammatory, immunomodulatory, and radical-scavenging profile [23]. Furthermore, quinone derivatives, free anthracene constituents, and reducing compounds enhance the overall antioxidant capacity of the extract [24,25].

The absence of certain expected secondary metabolites, particularly flavonoids, may be attributed to variations in harvest timing, geographic origin, exclusive use of leaf tissues, extraction parameters, or the specific solvent ratios applied [26]. Overall, these findings corroborate existing literature by demonstrating a rich composition of potential bioactive molecules, offering a promising pharmacological foundation. Subsequent analytical studies will be crucial to isolate, quantify, and verify the specific chemical constituents primarily driving these biological effects [21,26].

4.2 Anti-Edema Activity of E. hirta Extract

Formalin administration (0.1 mL) induced progressive paw edema in control animals, characteristic of an acute inflammatory response driven by increased vascular permeability and vasodilation [27]. This experimental model is highly relevant for screening novel anti-inflammatory interventions [25]. In contrast, rats treated with the hydroethanolic (HE) extract of *Euphorbia hirta* displayed a marked reduction in paw volume starting within the first hour, reaching nearly 70% inhibition [28]. This rapid onset is likely mediated by bioactive constituents such as tannins, alkaloids, anthocyanins, triterpenoids, and steroids that exhibit potent anti-inflammatory and antioxidant activities. *Euphorbia hirta* extracts are known to downregulate key pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, and nitric oxide [28].

By the third hour post-treatment, the HE extract maintained approximately 70% edema inhibition compared to 85% observed in the indomethacin reference group, confirming a robust and sustained response [29,30]. From the fourth hour onward, paw volume suppression in treated groups differed significantly from controls ($p < 0.05$). Complete (100%) edema resolution was observed in treated rats by hour 6 and persisted through 24 hours. In agreement with earlier studies, these dual anti-inflammatory and antioxidant actions may help mitigate oxidative stress, structural muscle disruption, and localized pain resulting from strenuous physical exertion [31]. Consequently, *E. hirta* hydroethanolic extract offers potential value as a complementary agent in post-exercise physiological recovery protocols, particularly when paired with modalities like massage or warm-water immersion [31].

4.3 Analgesic Activity of *E. hirta* Extract

Our results demonstrate significant analgesic properties for the hydroethanolic extract of *E. hirta*. Treated rats exhibited a thermal latency period of 10.50 ± 1.50 s on the hot plate, significantly exceeding the values observed in both the negative control group (5.50 ± 0.50 s) and the positive reference group (9.17 ± 0.84 s), indicating a clear reduction in pain sensitivity. This antinociceptive effect is likely linked to secondary metabolites with documented anti-inflammatory and radical-scavenging capacities [32]. Extracts of *E. hirta* may attenuate the generation of pro-inflammatory pain mediators such as NO, TNF-α, IL-1β, and IL-6 [33]. These combined properties suggest that *E. hirta* could serve as a natural aid to alleviate exercise-induced soreness and post-workout muscle discomfort in athletic populations [30].

4.4 Antipyretic Activity of *Euphorbia hirta* Extract

The hydroethanolic extract of *Euphorbia hirta* exhibited pronounced antipyretic activity. Following brewer's yeast-induced pyrexia, core rectal temperatures in control rats remained elevated at around 38.6 ± 0.3°C. In extract-treated animals, rectal temperatures steadily dropped from 38.20°C to 36.75°C over 24 hours, matching the temperature reduction produced by the standard reference drug (36.9°C). This hypothermic effect indicates modulation of systemic febrile signaling pathways.

The antipyretic response is likely driven by the identified anti-inflammatory phytoconstituents [30], which inhibit pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and nitric oxide production—key drivers of prostaglandin E2 (PGE2)

synthesis and hypothalamic thermoregulatory shifts [30]. These findings align with broad evidence that polyphenol-rich plant extracts exert integrated anti-inflammatory, antioxidant, and antipyretic actions [22,30]. Collectively, these biological effects point to the potential utility of *Euphorbia hirta* in facilitating physiological recovery and minimizing post-exercise systemic inflammation.

5 CONCLUSION

This study demonstrates that hydroethanolic extracts of *Euphorbia hirta* possess significant anti-inflammatory activity in vitro, as confirmed by the bovine serum albumin denaturation inhibition assay. These findings align with established pharmacological literature documenting the plant's anti-inflammatory potential [8,10]. Phytochemical profiling revealed a rich assortment of secondary metabolites, including alkaloids, tannins, anthocyanins, leucoanthocyanins, quinone derivatives, steroids, triterpenoids, free anthracene compounds, and reducing agents, that likely underpin the observed biological actions.

In sports medicine, these preliminary data support further exploration of *Euphorbia hirta* as a plant-derived therapeutic candidate for post-exercise recovery strategies. However, definitive claims regarding its direct capacity to enhance recovery in competitive athletes remain premature. Rigorous in vivo studies and controlled clinical trials are required to establish optimal dosing, safety parameters, bioavailability, and precise mechanisms of action.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

Statement of ethical approval

This study obtained ethical approval from both LaMoP-2S and APIPHARMA committees.

Author Contributions

- Innocent YAKA: Conceptualization, Methodology, Investigation, Writing original draft, Formal analysis
- Dieu Donné Wilfrid Kpèdétin AGBODJOGBE: Conceptualization, Supervision, Writing, review & editing, Validation
- Nicaise Tohossi KINNOUDO: Investigation, Data curation, Formal analysis, Visualization

Data availability statement

All the data used for this study are available, on demand to the corresponding author.

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