

Phytochemical Profile, Adaptogenic and Neuroprotective Properties of *Annona senegalensis* Pers. (Annonaceae): A Medicinal Plant from Bankui Region of Burkina Faso

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This study aimed to evaluate the qualitative phytochemical profile of the hydroethanolic extract of *Annona senegalensis* leaves and to validate its biological properties. High-performance thin-layer chromatography (HPTLC) analysis, quantification of phytochemical compounds, followed by *in vivo* evaluation of antioxidant, analgesic and neuroprotective activities were performed using standardized methods. HPTLC analysis revealed a rich profile of phenolic and terpenic compounds, with rutin and quercitrin identified as prominent constituents and further confirmed by spectrophotometric quantification. The confirmed phytochemical profile is closely correlated with *in vitro* antioxidant properties (IC₅₀ of 27.81 ± 2.35 µg/mL) and significant inhibition of lipid peroxidation (70.84 ± 2.06%). The extract was well tolerated and showed anxiolytic and analgesic effects without producing sedation. The phenolic and terpenic constituents of the extract may contribute to its biological effects and to the ergogenic responses and metabolic adaptations observed under physical stress. These findings provide scientific support for the traditional use of *Annona senegalensis* Pers. in African medicine.

Keywords: *Annona senegalensis* Pers., HPTLC, Adaptogen, Neuroprotection.

INTRODUCTION

Natural plant-based substances have attracted increasing attention as potential alternatives to synthetic agents used to combat fatigue and maintain physical and mental performance. Their growing interest in pharmacological research is linked to the need for safer approaches to managing fatigue and stress, particularly in view of the adverse effects associated with some synthetic stimulants and psychotropic agents [1,2]. Fatigue, whether physical or mental, remains a major public health concern, affecting productivity, well-being and quality of life among working populations [3,4]. Plant-derived compounds that can enhance the body's ability to cope with physical and psychological stress are commonly investigated as

adaptogenic or ergogenic agents. Their potential to improve stress tolerance, sustain physical performance and support recovery has made them an important area of research in modern pharmacology [5-7].

In sub-Saharan Africa, traditional medicine offers a vast reservoir of largely untapped plant-based therapeutic resources. Among these heritage species, *Annona senegalensis* Pers. (Annonaceae) holds a prominent place [8,9]. This medicinal plant is well documented in African ethnobotanical literature for its broad therapeutic applications. Traditional uses extend from the treatment of gastrointestinal disorders and metabolic conditions to the management of asthenia, chronic fatigue and febrile pain [8,10]. These traditional uses point to the potential analgesic and tonic properties of the species, with a

possible role in supporting the body's homeostatic response to stress [11]. In the Bankui region, the plant is traditionally used to improve physical capacity and athletic performance [10]. However, the transition from traditional empirical use to rigorous scientific validation raises several methodological challenges. Indeed, very few experimental or clinical studies using standardized physical exertion models exist to validate its effectiveness in improving motor capacity or post-exercise recovery. This study aims to provide an integrated evaluation of the antifatigue, metabolic, anxiolytic and analgesic effects of *Annona senegalensis* leaf extract (ASE), with the goal of addressing the existing knowledge gap and providing scientific evidence for its traditional use as a natural ergogenic agent and potential metabolic modulator.

EXPERIMENTAL

Preparation of plant extract: The leaves of *Annona senegalensis* Pers. were collected in the Bankui region during the rainy season (July 2024). The botanical identity of the species has been formally verified in the national herbarium of Burkina Faso at the National Center for Scientific and Technological Research and a reference specimen has been deposited under specimen no. 8767. The plant material was cleaned with distilled water and dried in the dark and dry at a controlled room temperature for two weeks. The dried leaves were then ground using a mechanical grinder to obtain a fine, homogeneous powder. Leaf powder (25 g) was subjected to cold maceration in 250 mL of an ethanol/water mixture (80:20, v/v) with continuous mechanical stirring for 24 h. The macerate was filtered through Whatman No. 1 filter paper. The filtrates were concentrated in an oven at a maximum temperature of 40 °C. The crude extract of *Annona senegalensis* obtained (ASE) was stored at 4 °C.

Standardized colorimetric and precipitation methods: The detection of major families of secondary metabolites was performed on the ethanolic extract using standardized colorimetric and precipitation methods [12]. Alkaloids were detected by Dragendorff's reagent, phenolic compounds and tannins by FeCl₃ reagent, flavonoids by the cyanidin reaction (Shinoda test), saponins by measuring the foam index, terpenoids by the Libermann-Burchard test and sugars by Fehling's reagent [13].

Analysis of phenolics and terpene by HPTLC: The chromatographic profiles were performed by following the methods as described earlier [14,15]. HPTLC analysis of terpene compounds was performed on 60F₂₅₄ silica gel plates (200 × 100 mm, Merck) on a glass support (Merck, Germany). For each sample, 5.0 µL of extract was applied as 8.0 mm bands using a TLC Sampler 4 autosampler (ATS 4, CAMAG, Muttenz, Switzerland). Development was carried out in an ADC 2 automated chamber (CAMAG) over a migration distance of 70 mm, after chamber saturation for 20 min and plate activation for 10 min (MgCl₂ at 33% relative humidity). Two distinct mobile phase systems were used: (i) for polyphenols: Tetrahydrofuran/toluene/formic acid/water (7:3:1:1 v/v/v/v) and (ii) for terpenes: ethyl acetate/ethanol/formic acid/water (8:1:1:1 v/v/v/v).

After 5 min of automatic drying, the specific detection of the compounds was performed automatically using a

CAMAG Derivatizer sprayer (2 mL): **Polyphenols/flavonoids:** Successive spraying of Neu's reagent (green nozzle, level 3) with heating at 100 °C for 3 min, followed by spraying of PEG 400 reagent. **Terpenes:** Spraying of vallilin reagent (0.5 mL anisaldehyde, 85 mL methanol, 10 mL glacial acetic acid, 5 mL conc. sulphuric acid) followed by heating between 100 and 110 °C. Acquisition of the chromatographic profiles was centralized on a TLC Visualizer 2 system controlled by vision CATS software (version 3.2), under white light, UV 254 nm and UV 366 nm.

Determination of phytochemicals: The quantification of *Annona senegalensis* leaf extract (ASE) secondary metabolites were carried out by spectrophotometry according to standardized protocols: total polyphenols were determined by the Folin-Ciocalteu method at 760 nm and expressed in milligrams of gallic acid equivalent per 100 g (mg GAE/100 g) [16,17], while total alkaloids were evaluated at 470 nm after complexation with green bromocresol (BCG) in buffered medium (pH 4.7) and chloroform extraction, with contents expressed in milligrams of atropine equivalent (mg EATr/) [4,12,13,18]. Vitamin C was quantified at 530 nm by the KMnO₄ decolorization method and expressed in milligrams of ascorbic acid equivalent (mgEA), while terpenoids, total tannins and sugars were quantified by respective spectrophotometric methods based on the vanillin-perchloric acid reaction (548 nm), polyvinylpyrrolidone (PVP) precipitation coupled to the Folin-Ciocalteu reagent and the universal phenol-sulfuric acid method [18-20].

in vitro Antioxidant activities: The evaluation of antioxidant potential was carried out by complementary spectrophotometric methods. Radical scavenging activity was determined by the DPPH test at 517 nm after 30 min of incubation in the dark, the results being expressed as 50% inhibitory concentration (IC₅₀) [21], while reducing power was measured via the FRAP test at 700 nm by evaluating the ability to reduce the ferric complex [22]. In parallel, the membrane-protective effect of the extract was quantified on liver homogenate by the inhibition of lipoperoxidation (LPO) was measured at 532 nm by the assay of thiobarbituric acid reactive substances (TBARS) [13,23].

Experimental animals and ethics: Non-inbred Swiss mice, weighing between 20 and 30 g, were obtained from Laboratory of Animal Physiology of Université Joseph KI-ZERBO. The animals were housed in standardized polypropylene cages under a controlled 12 h light/dark cycle, at a constant ambient temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%. They had *ad libitum* access to standard rodent pelleted food and distilled water throughout the experiment. All animal handling and experimentation procedures were approved by the Animal Ethics Committee of Joseph KI-ZERBO University (approval code CE-UJKZ/2025-16).

Assessment of acute oral toxicity: The acute toxicity test was performed according to OECD guideline no. 423 [24]. Twelve nulliparous female mice, 9 weeks old, with a mean body weight of 27 ± 2.44 g, were used for the acute toxicity study. The animals were randomly divided into two groups of six mice each. Water was withheld for 4 h before treatment. Group 1 served as the control and received distilled water, whereas Group 2 received a single oral dose of ASE at

2000 mg/kg body weight. The animals were observed continuously for 1 h following treatment and were then provided with food and water. Further observations were performed at 24, 48 and 72 h and once daily for 14 days. Mortality and changes in mobility were recorded throughout the observation period. Body weight was measured on days 1, 3, 7 and 14. On day 14, all animals were euthanized and the liver, kidneys, heart, spleen and lungs were collected and weighed to determine their absolute organ weights.

Analgesic activity: This test was performed according to the method described previously [22,25]. The peripheral analgesic potential of the extract was evaluated using a chemotherapy induced abdominal contortion model in mice. Nociception was induced by intraperitoneal injection of a 0.6% (v/v) acetic acid solution at a dose of 10 mL/kg body weight. A total of 24 male NMRI mice were randomly assigned to four experimental groups (n = 6 per group): (i) a negative control group receiving distilled water orally; (ii) a positive control group receiving diclofenac (10 mg/kg body weight, orally) as the reference non-steroidal anti-inflammatory drug and (iii–iv) two test groups receiving ASE at doses of 200 and 400 mg/kg body weight, respectively, by oral administration. One hour after treatment with ASE, diclofenac or distilled water, each mouse received an intraperitoneal injection of 0.6% acetic acid. Five minutes after the injection, the number of abdominal contortions, defined as contractions of the abdominal muscles accompanied by extension of the hind limbs, was counted individually for each mouse over a 20 min observation period. The antinociceptive effect was expressed as the percentage inhibition of pain-related responses, calculated by comparing the number of abdominal contortions in each treated group with that of the negative control group. The analgesic effect was evaluated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Nte} - \text{Nt}}{\text{Nte}} \times 100$$

where Nte: number of contortions in the negative control group, Nt: number of contortions in the test group or the positive control group.

Elevated plus-maze test: The elevated plus-maze test was performed according described methods [26,27]. Anxiety was assessed using a device consisting of two open arms (30 × 5 cm) and two arms enclosed by 15 cm high walls, intersecting at the center and raised 50 cm above the ground. Thirty minutes after oral administration of the respective treatments viz. distilled water, diazepam (2 mg/kg body weight), or ASE at doses of 200 and 400 mg/kg body weight-behavioural testing was conducted in a noise-attenuated room under dim lighting. A total of 20 male NMRI mice were randomly assigned to four experimental groups (n = 5): (i) a negative control group receiving the vehicle (distilled water, orally); (ii) a positive control group treated with a reference (diazepam, 2 mg/kg body weight, ip); (iii–iv) two test groups receiving the ASE at doses of 200 and 400 mg/kg (orally).

Each mouse was individually positioned on the central platform, facing an open arm. During a standardized 5 min session, the number of entries and the total time spent with

arms open (indices of anxiolysis), the total number of entries (an indicator of spontaneous locomotor activity) and the frequency of head dipping (a marker of curiosity and exploration) were automatically recorded. The maze was cleaned with 70% v/v ethanol after each session to remove olfactory cues. An arm entry was defined as a mouse having entered one arm of the maze with all four limbs. Behaviours were defined as protected if they occur in the closed arms or center and unprotected when they were exhibited in the open arm region of the maze [28].

Neuromuscular test grip test: The grip strength test was performed according to the protocol described above [29,30], using a low-force measurement system. Each mouse was placed on a metal grid (2 mm in diameter, 7.5 cm long) connected to a force sensor and then gradually pulled backward by its tail until it released its grip. This assessment was repeated five times per animal and the maximum tension of each trial was recorded using a digital dynamometer. This test was carried out after one week of gavage feeding (oral route) for the following experimental groups (n = 5 for each group): (i) Group 1: Distilled water (negative control); (ii) Group 2: Vitamin C (positive control); (iii) Group 3: ASE (200 mg/kg body weight); and (iv) Group 4: ASE extract (400 mg/kg body weight).

Forced swim test: The forced swim test was performed according to the reported protocols [31,32]. Twelve weeks old mice, with a mean weight of 29.30 ± 3.31 g, were used. The swim was performed in plastic containers (50 cm long, 35 cm high and 25 cm wide) filled with water to a depth of 30 cm, at a temperature of 28 ± 1 °C. The mice (n = 12) were randomly divided into two equal groups (n = 6): a negative control group (distilled water) and an experimental group receiving an extract of *A. senegalensis* (200 mg/kg, orally) for 10 consecutive days. After these 10 days of supplementation, a metallic foil representing 9% of body weight was attached to the mice's tails for a swim-to-exhaustion test. Mice were considered exhausted if they did not surface to breathe after 7 sec. The time it took them to swim to exhaustion was used as a performance indicator.

Post-exercise serum biochemical analyses: Immediately after the forced swim test, blood samples were collected from the mice. Glucose concentrations and catalytic activities of serum creatine kinase total and lactate dehydrogenase (LDH) were determined by standardized enzymatic and kinetic methods on a Cobas c analyzer (model 311/501/502, Roche/Hitachi) using dedicated commercial reagent kits from Roche Diagnostics GmbH (Germany).

Statistical data processing: Results are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using appropriate parametric methods. An independent-samples Student's t-test was used for comparisons between two groups, while one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was applied for comparisons of multiple groups with the negative control. Statistical calculations were performed using dedicated statistical software. A *p*-value < 0.05 was considered statistically significant. Exact *p*-values are provided in the relevant figure and table legends to ensure transparent reporting of the results.

RESULTS AND DISCUSSION

Phytochemical profile: The qualitative phytochemical screening of ASE revealed a diverse range of secondary metabolites (Table-1). Tannins, flavonoids, saponins, terpenoids, cardiac glycosides, alkaloids and reducing sugars were detected in the extract. The TLC chromatogram (Fig. 1) showed several well-resolved bands with different migration characteristics, reflecting the presence of compounds with a broad range of polarities. The terpenoid and steroid profiles (Fig. 1.1 and 1.2) showed a visible spot with intense cyan fluorescence at 366 nm after vanillin-sulphuric acid and anisaldehyde-sulphuric acid derivatization, with a R_f value of ≈ 0.40 . This band may represent a phytosterol or pentacyclic triterpene, but confirmation requires authentic standards or spectroscopic analysis.

TABLE-1
PHYTOCHEMICAL SCREENING OF THE HYDRO-ETHANOLIC LEAVES EXTRACT OF *Annona senegalensis*

Phytochemical constituents	Test/reagents used	ASE
Tannins	Ferric chloride test (FeCl_3)	Presence
Flavonoids	Alkaline reagent/Shinoda test	Presence
Saponins	Frothing test	Presence
Terpenoids	Liebermann-Burchard test	Presence
Cardiac glycosides	Keller-Kiliani test	Presence
Alkaloids	Dragendorff's reagent	Presence
Reducing sugars	Fehling's test	Presence

Distinct fluorescence-quenching bands were observed at R_f values of approximately 0.28 and 0.56. These bands are consistent with the presence of phenolic or flavonoid constituents. The band at $R_f \approx 0.28$ showed intense blue-white fluorescence and may correspond to a flavonol glycoside such

as rutin, whereas the band at $R_f \approx 0.56$ was tentatively assigned to quercitrin. Such assignments should be regarded as tentative unless supported by reference standards or complementary spectroscopic data [10].

Previous phytochemical investigations have reported variation in the abundance of quinones and alkaloids in *A. senegalensis* leaves [33]. Ziyaev et al. [34], through detailed phytochemical isolation, identified several alkaloids belonging to the aporphinoid and benzyltetrahydroisoquinoline groups. Reported compounds include the noraporphins anonaine, normuciferin and asimilobine; the aporphine and oxo-aporphine alkaloids roemerin, nuciferin, lysicamine, liriodenine, *N*-oxyroemerin and benzyltetrahydroisoquinoline alkaloid coclaurine. These constituents provide a plausible phytochemical basis for some of the biological activities previously associated with the species [34,35]. Regional studies have reported considerable variation in the phytochemical composition of *A. senegalensis* leaves. Samples from Togo contained sterols, triterpenes, carotenoids, flavonoids such as rutin and isoquercitrin, anthocyanins, saponins and tannins, whereas epicatechin and related compounds were more prominent in samples from Burkina Faso [36,37]. Such differences may reflect geographical, environmental or extraction related factors.

Quantitative analysis (Table-2) showed that ASE contained appreciable amounts of total polyphenols (98.39 ± 1.49 mg GAE/100 g extract) and total flavonoids (4.32 ± 0.40 mg QE/100 mg extract). The extract also contained terpenoid compounds (30.75 ± 1.15 g β -sitosterol equivalents/100 g), total sugars (93.76 ± 16.86 mg sucrose equivalents/100 mg) and vitamin C (17.33 ± 0.13 mg EAA/100 mg). ASE exhibited antioxidant activity in the DPPH assay, with an IC_{50} of 27.81 ± 2.35 $\mu\text{g/mL}$, together with an iron-reducing antioxidant capacity of 610.06 ± 0.00 $\mu\text{mol EAA/g}$. The extract

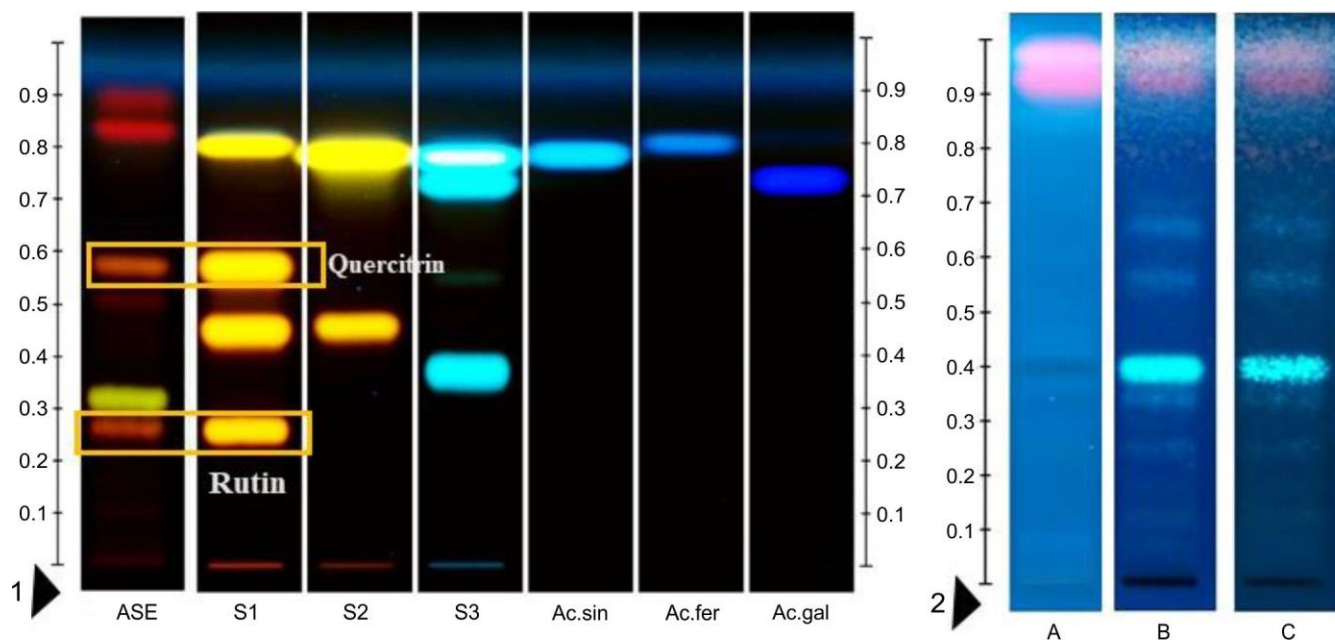


Fig. 1. HPTLC profiles of polyphenols, terpenes and steroids in the hydroethanolic leaf extract of *A. senegalensis* (ASE). (1) Polyphenol profile visualized with NEU reagent, showing rutin and quercitrin alongside reference standards; (2) Terpene and steroid profiles visualized without reagent (A), with anisaldehyde (B) and vanillin-sulphuric acid (C)

TABLE-2
RESULTS OF QUANTITATIVE EVALUATION OF *Annona senegalensis* HYDROETHANOLIC

Parameter type	Metabolic parameters	Value (Mean $\pm$ SD)
Phytochemicals	Alkaloids (μ g Atropine equivalent/100 g of powder)	366.70 $\pm$ 33.55
	Polyphenols (mg EAG/100 mg of extracts)	98.39 $\pm$ 1.49
	Total flavonoids (mg EQ/100 mg of extract)	4.32 $\pm$ 0.40
	Terpenoids (g equivalent β -sitosterol/100 g of extract)	30.75 $\pm$ 1.15
	Vitamin C (mgEAA/100 mg of extract)	17.33 $\pm$ 0.13
	Total sugar (mg Sucrose equivalent/100 mg of dry extract)	93.76 $\pm$ 16.86
Antioxidant capacities	LPO (% inhibition)	70.84% $\pm$ 2.06%
	FRAP (μ mol EAA/g)	610.06 $\pm$ 0.00
	DPPH (μ g/mL)	27.81 $\pm$ 2.35

EAG: Gallic acid equivalent; EQ: Quercetin equivalent; EAA: Ascorbic acid equivalent; LPO: Lipid peroxidation; CAT: Catalase; FRAP: Ferric reducing antioxidant power; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; (IC₅₀): Half-maximal inhibitory concentration.

showed 70.84 $\pm$ 2.06% inhibition of lipid peroxidation, with a corresponding reduction in oxidative membrane damage.

The antioxidant findings are consistent with previous work by Diallo *et al.* [13], who reported a high DPPH radical scavenging activity for *A. senegalensis* leaves, with 95.06 $\pm$ 0.10% inhibition. The coexistence of phenolic, flavonoid, terpenoid and alkaloid constituents provides a plausible phytochemical basis for the antioxidant activity observed in ASE.

Acute toxicity and physiological tolerance: As shown in Fig. 2, oral administration of ASE leaves did not trigger any sign of systemic toxicity or behavioural anomalies over the 14-day monitoring timeline. Throughout the experiment, both cohorts displayed a steady and healthy weight progression Student's t-test demonstrated that these minor differences were statistically non-significant ($p > 0.05$) at every single temporal checkpoint (D1, D3, D7 and D14). Administration of an oral limit dose of 2,000 mg/kg did not induce any mortality, with the LD₅₀ being greater than 2,000 mg/kg. In accordance with OECD Guideline 423, this extract is classified as non-toxic or low-risk product [38]. The changes in hepatic, renal, cardiac, pulmonary and splenic function remained non-significant ($p > 0.05$). This high physiological

tolerance confirms that the bioactive constituents of the extract do not disrupt basal nutrient absorption or the homeostatic mechanisms of organic metabolism.

Previous experimental toxicological studies [39-41] conducted on different organs of *A. senegalensis* generally conclude that the plant has an excellent safety profile in cases of acute exposure, although caution is advised during sub-acute or prolonged administration at high doses. It was showed that a single oral limit dose of up to 5,000 mg/kg body weight in mice resulted in no mortality, behavioural abnormalities, or histopathological changes in liver tissue, placing the median lethal dose (LD₅₀) well above this threshold [42]. Extracts of *A. senegalensis* leaves protect vital organs against xenobiotic-induced cellular damage by promoting hepatocyte regeneration and reducing transaminases in response to aflatoxin [43] and by normalizing the lipid profile in liver cancer by reducing total cholesterol [39]. It also appears to be nephro-protective by reversing gentamicin-induced renal toxicity [44]. However, some authors report that oral administration of an aqueous leaf extract (100-300 mg/kg body weight) for 21 days to male Wistar rats induced a significant reduction in the testis weight/body weight ratio and total testicular protein

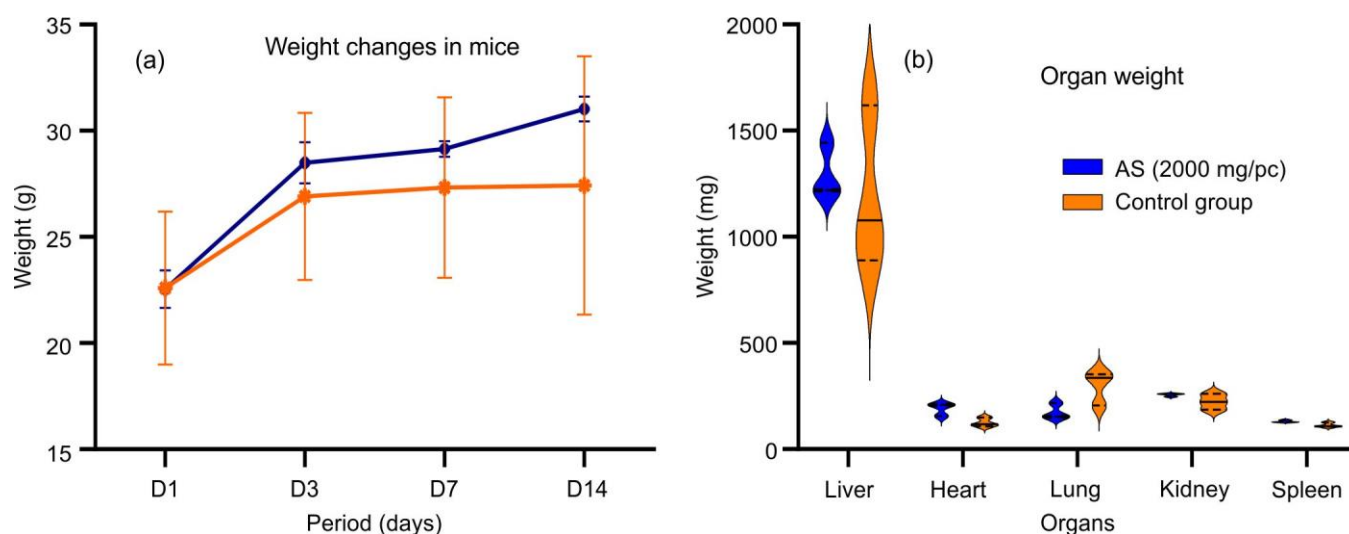


Fig. 2. Effect of *Annona senegalensis* hydroethanolic leaf extract on body weight during the 14-day observation period. Left: body weight (g) recorded on days 1, 3, 7 and 14. Right: absolute weights (mg) of vital organs (liver, heart, lung, kidney and spleen) at study completion. Data are mean $\pm$ SD ($n = 3$ /group). Statistical comparison between treated and control groups was performed using Student's t-test (ns, $p > 0.05$)

content [45]. Furthermore, the root bark of *A. senegalensis* exhibits more pronounced acute toxicity than other parts of the plant. Other researchers [40,46] have indicated that prolonged administration of stem bark or root extracts causes severe liver toxicity, even at moderate doses, leading to the metabolic alterations, elevated liver enzymes and liver tissue necrosis.

Peripheral analgesia and preservation of motor performance: Intraperitoneal injection of acetic acid in the control group induced a standardized pain syndrome, characterized by a high mean of 45.00 ± 2.55 abdominal contortions (Table-3). This experimental model validates its sensitivity based on the results of the reference group. Administration of diclofenac (a non-steroidal anti-inflammatory drug) significantly reduced ($p < 0.05$) the number of contortions to 27.80 ± 2.95 , which represent an inhibition rate of 38.2%. At a dose of 400 mg/kg, ASE caused a drastic and highly peripheral decrease in the pain score. A maximum inhibition of 43.1% was observed. At this concentration, the extract showed higher analgesic activity than diclofenac (43.1% vs. 38.2%). Similar activity has been reported for the aqueous extract, with 63.36% inhibition following oral pretreatment at 200 mg/kg [38]. Bakrou *et al.* [47] reported an 87.37% protective effect, when compared with 45.07% for aspirin. The antinociceptive activity may be associated with phytoconstituents such as flavonoids, tannins and terpenoids [33]. Such activity may be relevant to pain associated with muscle contusions and exercise induced microtrauma, although its role in improving athletic performance requires further investigation [10].

TABLE-3
ANALGESIC EFFECT OF ASE AND DICLOFENAC

Experimental Group	Number of writhings (mean $\pm$ SD)	Inhibition (%)
Distilled water	45.00 ± 2.55	0.0%
Diclofenac	27.80 ± 2.95	38.2%***
ASE (200 mg/kg)	39.40 ± 5.03	12.4% ^{ns}
ASE (400 mg/kg)	25.60 ± 2.07	43.1%***

Data are reported as mean $\pm$ standard deviation. Statistical significance compared to the negative control group (distilled Water) was analyzed using one-way ANOVA followed by a post-hoc test (ns: non-significant, $p > 0.05$; *** $p < 0.001$). Abbreviations: AS: *Annona senegalensis* leaf extract

Preservation of motor performance and functional safety: A major pharmacological advantage of ASE may lie in the absence of deleterious side effects on the musculo-skeletal system. Two behavioural tests confirm this biological safety *in vivo*. The grip test (Fig. 3) showed preserved

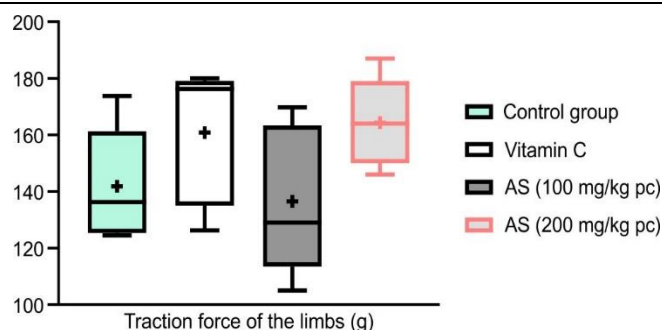


Fig. 3. Limb grip strength following ASE administration ($n = 5/\text{group}$). One-way ANOVA showed no significant difference among groups ($F = 1.94$, $p = 0.164$)

neuromuscular strength at 200 mg/kg, with a score of 164.50 ± 15.31 g and no significant difference from the control group ($p > 0.05$). The elevated cross-maze test yielded a comparable number of entries, which support the absence of detectable impairment in motor coordination or exploratory activity. The analgesic effect was therefore not accompanied by apparent muscle relaxation or neurobehavioral impairment under the tested conditions. These findings support the traditional use of the plant for managing bruises and related pain, although its effects on muscle recovery and physical performance require further investigation.

Anxiolytic activity and adaptive resistance to muscle fatigue: Table-4 presents the elevated plus maze (EPM) results, an established *in vivo* model for assessing anxiety-related and sedative-like behavioural responses in rodents. ASE increased the time spent in the open arms at 400 mg/kg (147.20 ± 102.68 s) compared with the control (53.00 ± 4.00 s) and diazepam (123.40 ± 44.97 s). The extract-treated animals further showed increased head-dipping behaviour, with 17.00 ± 6.36 entries compared with 4.25 ± 0.96 in the control group. These findings are consistent with previous reports [42,44] attributing the behavioural effects of the plant to its flavonoid content, while saponins have been associated with nootropic effects [48].

The total number of EPM entries remained comparable between the extract and control groups (9.40 vs. 8.60) and shows no clear evidence of generalized locomotor suppression under the tested conditions. The traction test similarly showed preserved forelimb grip strength, with a value of 164.50 ± 15.31 g at 200 mg/kg. The behavioural profile observed in the present study is therefore compatible with an anxiolytic like effect without marked motor impairment or sedation at the tested doses. Previous studies [36] reported reduced locomotor activity, prolonged pentobarbital-induced sleep and

TABLE-4
ANXIOLYTIC PROPERTIES

	Open arm entries (n)	Time in open arms (s)	Total entries (n)	Head-dippings (n)
Control	3.80 ± 3.56	53.00 ± 24.00	8.60 ± 5.59	4.25 ± 0.96
ASE (200 mg/kg)	$3.00 \pm 2.92^{\text{ns}}$	$116.20 \pm 91.48^{\text{ns}}$	$9.00 \pm 5.15^{\text{ns}}$	$11.00 \pm 10.22^{\text{ns}}$
ASE (400 mg/kg)	$4.60 \pm 2.07^{\text{ns}}$	$147.20 \pm 102.68^*$	$9.40 \pm 2.70^{\text{ns}}$	$17.00 \pm 6.36^{**}$
Diazepam (1 mg/kg)	$13.20 \pm 6.61^{**}$	$123.40 \pm 44.97^*$	$28.80 \pm 9.12^{**}$	$23.40 \pm 14.02^{**}$

Data are presented as mean $\pm$ standard deviation (SD) for $n = 5$ mice per group. Statistical significance compared to the negative control group (distilled Water) was analyzed using one-way ANOVA followed by a post-hoc test (ns: non-significant, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$). Abbreviations: AS: *Annona senegalensis* leaf extract; ED: distilled Water (Vehicle); s: seconds; n: number.

attenuated apomorphine-induced stereotypy, with responses varying by dose and experimental conditions.

The observed activity may be related to the combined effects of alkaloids and polar flavonoid glycosides [49,50], with rutin and quercitrin detected by HPTLC. Flavonoids have been proposed to interact with the γ -aminobutyric acid-A (GABA_A) receptor system [51,52], but such receptor-level effects cannot be established from the present data. Since rutin and quercitrin were detected rather than individually isolated and pharmacologically tested, their involvement remains a literature-based hypothesis.

Active adaptive strategy and cellular protection stress mechanisms: Fig. 4 presents the behavioural and plasma biochemical responses of mice treated with *A. senegalensis* leaf extract (200 mg/kg) immediately after the forced swimming test. The treated group showed a shorter active swimming time than the control group (74.90 ± 14.87 vs. 123.20 ± 70.81 s). Plasma glucose remained comparable between groups (199.62 ± 29.38 vs. 213.20 ± 44.79 mg/dL), while CK2 levels were similar (2497.80 vs. 2509.80 U/L) and LDH was lower in the treated group (1260.34 ± 372.80 vs. 1406.16 ± 165.16 U/L). None of these differences reached statistical significance ($p > 0.05$). The stable CK2 and LDH values provide no evidence of marked acute muscle injury under the tested conditions.

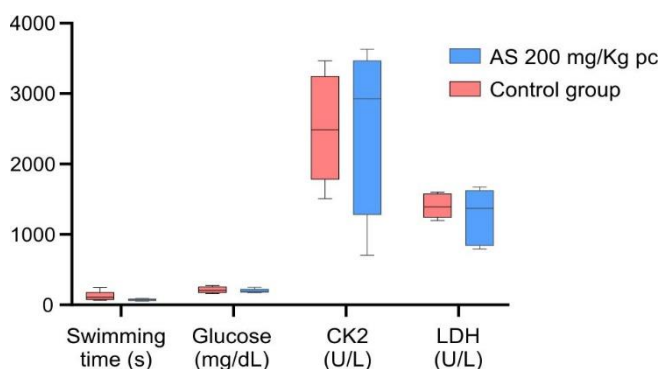


Fig. 4. Effects of ASE on FST parameters and muscle-stress biomarkers. No significant differences were observed between groups ($p > 0.05$; ns = not significant)

The forced swimming test is widely used to assess fatigue related behaviour and responses to acute stress [30,53]. The present biochemical profile may reflect preserved metabolic and muscular homeostasis following exercise. The antioxidant properties of the extract, particularly its flavonoid and tannin content, may contribute to this response, although the present study does not establish a direct mechanistic relationship [54]. As a whole, the EPM, grip-strength, analgesic and forced-swimming findings suggest that the extract produces distinct behavioural and physiological responses under different experimental conditions. Its anxiolytic-like and antinociceptive effects, together with the absence of significant changes in selected post-exercise biochemical markers, provide preliminary support for its traditional use in stress, pain and fatigue-related conditions. The antioxidant findings from DPPH, FRAP and lipid-peroxidation assays provide preliminary evidence of antioxidant activity. Since endogenous markers such as SOD, CAT and MDA were not measured, the underlying cellular mechanism remains unresolved.

Conclusion

The present study provides preliminary evidence of the pharmacological potential of *Annona senegalensis* Pers. leaf extract (ASE). Phytochemical screening revealed a diverse profile of secondary metabolites, with measurable polyphenols, flavonoids, terpenoids, sugars and vitamin C. ASE exhibited appreciable antioxidant activity in DPPH, FRAP and lipid-peroxidation assays. Oral administration at 2000 mg/kg produced no mortality or significant alterations in the evaluated physiological and biochemical parameters during the 14-day observation period, which support its tolerance under acute exposure conditions. ASE produced peripheral antinociceptive activity, with 43.1% inhibition of acetic acid-induced writhing at 400 mg/kg. The extract did not significantly impair grip strength or general exploratory behaviour at the tested doses. Increased open-arm exploration in the elevated plus maze was compatible with an anxiolytic-like behavioural effect, although the underlying mechanism remains to be established. Following forced swimming, glucose, CK2 and LDH levels remained statistically comparable with the control group, however, no evidence of marked acute metabolic or muscular injury under the experimental conditions is provided. The findings support the traditional relevance of *A. senegalensis* leaves in pain, stress and oxidative processes, justifying further pharmacological investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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