ORIGINALE ARTICLE

Phytochemical Analysis, Anti-fatigue and Antioxidant Activities of *Nuxia capitata* Baker (Stilbaceae)

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ABSTRACT

Background: *Nuxia capitata* Baker (Stilbaceae) is traditionally used as an anti-fatigue remedy, but its efficacy is not well-documented. **Objectives** : This study aimed to validate the traditional use of this plant. **Methods** : Hexane (7.42%), ethyl acetate (2.01%), and methanol extracts (5.12%) were obtained by maceration. Antioxidant activity was evaluated using biotographie with DPPH. The forced swimming endurance test and modified string test were performed to assess anti-fatigue activities. Compounds from the active extract were isolated by liquid chromatography. **Results**: All extracts demonstrated potent antioxidant activity. Treatment with hexane or ethyl acetate extracts improved performance in the modified string test, with the ethyl acetate extract at 100 mg/kg showing the highest effect. Swimming times were significantly longer in all treated mice except those treated with hexane extract or high doses of extracts (200 mg/kg). Fractionation of the ethyl acetate extract led to the isolation of six known compounds, including three triterpenoids (ursolic acid, oleanolic acid, and taraxasterol acid) and three flavones (cirsimaritin, negletein, and quercetin). **Conclusion** : This study significantly enhances the understanding of the chemistry and pharmacology of *N. capitata*, suggesting that the plant is a promising treatment for combating fatigue and oxidation-related diseases.
Keywords : *Nuxia capitata*, phytochemical, Antioxidant, anti-fatigue.

INTRODUCTION

Fatigue is a complex physiological phenomenon characterized by a state of weariness and decreased capacity for work, often indicating an imminent or existing threat to overall health. The etiology of fatigue is multifaceted, involving various biochemical and physiological processes. Among the mechanisms underlying physical fatigue, two predominant factors have been extensively studied: energy depletion and free radical accumulation [1-3].

Energy depletion primarily involves the exhaustion of readily available energy sources, such as glucose and glycogen, leading to a decrease in ATP production. This metabolic stress triggers a cascade of events that ultimately result in reduced muscle function and overall physical performance. Concurrently, intense physical exertion leads to an increased production of reactive oxygen species (ROS), which can overwhelm the body's natural antioxidant defenses, resulting in oxidative stress. This oxidative damage can further exacerbate fatigue by impairing cellular functions and accelerating muscle damage.

In the quest for natural remedies to combat fatigue, traditional medicinal plants have garnered significant attention from researchers worldwide. One such plant is "*Nuxia capitata*" Baker, a member of the Stilbaceae family, which is endemic to Madagascar. Locally known as "Valanirana" or "Karambitona," this species has been traditionally employed to address various common ailments, including muscle fatigue [4]. Despite its longstanding use in folk medicine, scientific investigations into the pharmacological properties and phytochemical composition of "*N. capitata*" remain scarce, with only a handful of studies reported in the literature [4-6].

The limited scientific exploration of "*N. capitata*" presents a significant gap in our understanding of its potential therapeutic applications. To the best of our knowledge, no comprehensive pharmacological or chemical studies have been conducted

on this species to date. This lack of scientific evidence underscores the need for rigorous investigation to validate its traditional uses and explore its potential as a source of bioactive compounds.

In light of this research gap, our study was designed with the primary objective of providing scientific justification for the traditional use of "*N. capitata*" in alleviating fatigue. We aimed to accomplish this through a systematic approach encompassing several key aspects:

1. Evaluation of the antioxidant properties of various extracts derived from "*N. capitata*", given the crucial role of oxidative stress in fatigue development.
2. Assessment of the anti-fatigue potential of these extracts using established in vivo models.
3. Phytochemical investigation of the most promising extract, with the goal of isolating and characterizing the primary metabolites responsible for the observed biological activities.

By elucidating the chemical constituents and biological activities of "*N. capitata*", this research seeks to bridge the gap between traditional knowledge and scientific evidence. Furthermore, it aims to contribute to the broader field of natural product research, potentially uncovering novel compounds with therapeutic potential in the management of fatigue and related disorders.

2. MATERIALS AND METHODS

2.1 Plant Material: The leaves of *N. capitata* were collected in Fonenana at Imeritsiantosika municipality of Analamanga region, Madagascar on October 2020. Plant sample was identified by the botanist at the Botanical and Zoological Park of Tsimbazaza (PBZT). The leaves of the plant were dried, powdered and stored in an airtight container for future use.

2.2 Animals and management: Adult female SWISS mice weighing between 24 – 33 g were used. They were kept in standard polypropylene cages with 12 h light-dark cycle and fed standard diet (LFL 14/20) ad libitum with free access to water. Then adaptive trainings were made for 5 minutes once per week in an experimental animal room. Eighteen hours (18 h) prior to the final experiment, mice were fasted. All the experimental protocols had been approved by the Ethics Committee for Animal Experimentation of the Faculty of Sciences, University of Antananarivo - Madagascar (No. 02/2022).

2.3 General experimental procedures: All organic solvents were distilled before use. Extracts were fractioned by liquid chromatography over silica gel (Merck MN silica gel 60 M: 0.04–0.063 nm), eluted under atmospheric pressure. The NMR data including ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC were recorded in deuterated solvents (CD_3OD, $CDCl_3$, Pyridine) on Bruker 600 NMR spectrometer (proton at 600.19 MHz and carbon ^{13}C at 125.78 MHz). All chemical shifts (δ) are quoted in parts per million (ppm) using tetramethylsilane (TMS) as internal standard. Structures of isolated compounds were confirmed by comparison of their spectroscopic data with literature values. For mass spectrometry, compounds were dissolved in MeOH, CH_3CN or water and directly introduced through an integrated syringe pump into the ESI source. Stepwise control of the compounds has been achieved using electrospray ionisation mass spectrometry (ESI-MS) using a ZQ 4000 quadrupole mass spectrometer (Waters-Micromass, Manchester, UK), provided with a pneumatically assisted electrospray (Z-spray) ion source. Capillary voltage is around 3.5kV, and the cone voltage varies from 20 to 150 V depending on analyzed compounds. Thin layer chromatography (TLC) was performed on aluminium silica gel plates (Macherey-Nagel, SIL G/UV254, 0.20 mm). Spots were visualized on TLC either by UV lamp (254 and 366 nm) or by heating after spraying with 20% H_2SO_4 (v/v) solution.

2.4 Phytochemical screening

Hydroalcoholic extract of the dried and powdered leaves were prepared, and phytochemical screening were made as described previously [7]. Thus, specific reagents were used and the presence of alcaloids, flavonoids, leucoanthocyanins, tannins, polyphenols, coumarins, steroids, triterpenoids, saponins and polysaccharids were evaluated qualitatively.

2.5 Extraction, fractionation and isolation

The dried and powdered leaves (500 g) were macerated in a series of solvents according to a rule of increasing polarity: hexane (1.5 L for 4 days), ethyl acetate (1.5 L for 4 days) and methanol (1.5 L for 4 days). The obtained solutions were filtered and concentrated under reduced pressure, to get three extracts (*N. capitata* extracts): Hexane extract (37.1 g, 7.42%), ethyl acetate extract (10.05 g, 2.01%), and methanol extract (25.6 g, 5.12%). The ethyl acetate extract (3 g) was separated by chromatography on a silica column (60 g silica gel 60, 80 x 2 cm) using the eluent cyclohexane in gradient with ethyl acetate to give 500 fractions of 10 ml. Purification by crystallization of fractions having the same TLC appearance was carried out. Five products: **1** (18,2 mg, white powder), **2** (20,5 mg, white powder), **3** (6,9 mg, yellow powder), **4** (11

mg, yellow powder) and **5** (13 mg, yellow powder), respectively from fractions 215-225 eluted with cyclohexane/ AcOEt 50/50, 226-238 eluted with cyclohexane/ AcOEt 40/60, 245-248 eluted with cyclohexane/AcOEt 10/90, 347 eluted with AcOEt and 350 eluted with AcOEt were obtained. Their structures were determined by concerted analysis of its ^{1}H, ^{13}C, DEPT, HSQC, HMBC NMR spectra, mass spectra and by comparison with data from the literature.

2.4 Evaluation of antioxidant activity

Antioxidant activity was evaluated by TLC biautography and revelation of the plates with 2,2-diphenyl-1-picryl hydrazyl (DPPH) as previously described [8]. Briefly, DPPH solution in methanol at 2 mg/ml and TLC plates loaded with each tested product were prepared. Spots were visualized on TLC using the DPPH solution. The presence of antioxidant compounds was visualized as yellow bands against a purple background.

2.4 Evaluation of anti-fatigue activities in mice

Modified String test as described previously was used [3]. Metal bar (2 mm diameter × 30 cm long) were fixed by a metallic support column at each end. The bars are 35 cm above the floor. Hind legs of all tested mice were tied and all drugs were solubilized in DMSO 5%. DMSO or drugs were administered orally (10 ml/kg). Mice were randomly divided into the following 11 groups (3mice/group): Group 1: control-mice treated with DMSO 5%; Group 2: reference-mice treated with 5 mg/kg of caffeine; Group 3–11: mice treated with 50, 100 or 200 mg/kg of *N. capitata* extracts. Hind legs were tied and one hour after the gavage treatment, mice were suspended on the horizontal bar. The endurance times were recorded and mice were regarded as being exhausted when they fall off.

Forced Swimming Endurance Test was also conducted as described previously with minor modification [3,9-11]. The swimming exercise was carried out in an acrylic plastic pool (27 cm3), filled with 13 cm depth of water at 25°C. Mice were randomly divided into 11 groups (3 mice/group) and DMSO or the corresponding agents were administered orally (10 ml/kg) to mice. One hour (1 h) after the gavage treatment, the tests were made and the endurance times were noted. Records were made from the beginning of the test until exhaustion, determined by loss of coordinated movements and failure to return to the surface within 8 s. All the results are expressed as the mean ± standard deviation of the Mean (SD). Data were analyzed using Student's t-test. The difference is significant if $p<0.05$.

3. RESULTS

3.1 Preliminary phytochemical screening

N. capitata Baker leaves contained various constituents such as flavones, anthocyanins, coumarins, triterpenoids, insatured sterols, saponins and other phenolic compounds.

3.2 Antioxidant activity

All the tested products including hexane, ethyl acetate and methanol extracts of *N. capitata* show a yellow coloration when sprayed with DPPH (Figure 1). It indicates that these products had an antioxidant property.

Figure 1: Antioxidant activity of hexane (hexane), ethyl acetate (AcOEt) and methanol extracts (MeOH) of *N. capitata* leaves evaluated by TLC biautography and revelation of the plates with 2,2-diphenyl-1-picryl hydrazyl (DPPH) solution in methanol at 2 mg/ml.

3.3 Anti-fatigue activity of *N. capitata* extracts

The performances of mice treated with 5% of DMSO, caffeine at 5 mg/kg and *N. capitata* extracts at 50, 100 or 200 mg/kg during the modified string test are presented in Figure 2. The mice treated with DMSO (control) stayed suspended on the

horizontal bar during 0.49 ± 0.2 minutes. In contrast, mice treated with 100 mg/kg of hexane or ethyl acetate extracts remained suspended on the horizontal bar during 2.79 ± 0.3 and 3.68 ± 0.4 seconds, respectively. Difference observed are statistically significant when compared to control ($p < 0.05$, n=3). Similarly, the mice treated with 5 mg/kg of caffeine, a known anti-fatigue product, showed significant differences compared with the control ($p < 0.05$, n=3).

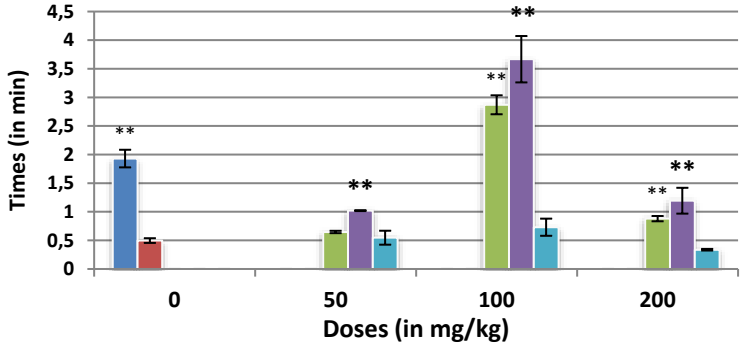

Figure 2: Performances of mice treated with 5% of DMSO ( ), caffeine at 5 mg/kg ( ), hexane ( ), ethyl acetate ( ) and methanol extracts ( ) during the modified string test. Values are expressed as the means ± SEM. **p<0.05, compared with the control group (DMSO 5%).

The performances of mice during the forced swimming endurance test were presented in Figure 3. As shown, only the methanol extract was active at the maximum tested doses (200 mg/kg). In addition, swimming time of all treatment and reference groups were longer than that of the control group (DMSO 5%) (n=3, p < 0.05), except for the mice treated with hexane extract or 200 mg/kg of ethyl acetate extract.

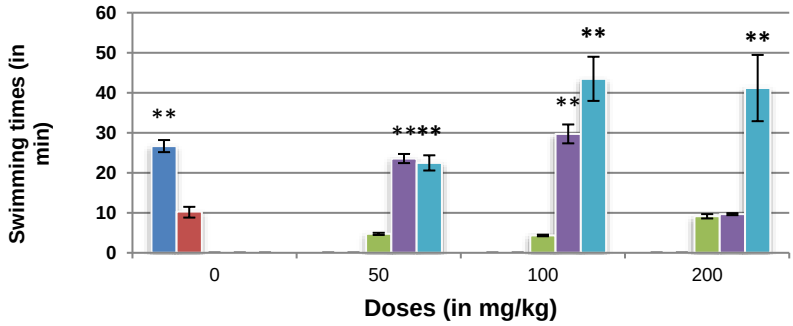

Figure 3: Effects of 5% of DMSO ( ), caffeine at 5 mg/kg ( ), hexane ( ), ethyl acetate ( ) and methanol extracts ( ) of *Nuxia capitata* Baker on exhaustive swimming times. Values are expressed as the means ± SEM. **p<0.05, compared with the control group (DMSO 5%).

3.4 Spectral data and identification of isolated compounds

Compound **1** yielded mixtures of triterpenes which exhibited a single spot when analysed by TLC in several solvents. The ^{13}C-NMR spectra of compound **1** showed sixty-seven signals indicating a triterpene mixture. In particular was observed six signals for olefinic carbons, being one secondary (δ 105.7), two tertiary (δ 123.4, δ 126.2) and three quaternary carbons (δ 145.2, δ 139.7, δ 154.2). Comparison with literature data suggested the presence of triterpenes type urs-12-ene (δ 126.2, δ 139.7), urs-20(30)-ene (δ 105.7, δ 154.2), olean-12-ene (δ 123.4, δ 145.2). Also was observed an intense signal at δ 78.8, typical of an oxymethine, and three signals for three carbonyl acid (δ 180.8, 180.6, 180.0) at the position 28. There were no others signals for oxygenated carbons. Thus the components of **1** were identified as a mixture of ursolic acid, oleanolic acid and taraxasterol acid [12,13]. The presence of triterpens was corroborated by ^{1}H NMR that showed signals for oxymethine (δ 3.47) and olefinic protons of an c acid (δ 5.53), oleanolic acid (δ 5.52) and taraxasterol acid (δ 4.79 and 4.81). Compound **2** is identified as an ursolic acid [13]. Compounds **3**, **4** and **5** are flavones identified respectively to cirsimaritin [12], negletein [14], and quercetin [15,16]. Their chemical structures are shown in figure 4.

Ursolic acid: δ (ppm) RMN ^{1}H (600.19 MHz, Pyridine): 5.53 (1H, br, H-12), 3.47 (1H, dd, H-3), 2.61 (1H, s, H-18), 1.24(3H, s, H-23), 1.23 (3H, s, H-27), 1.05 (3H, s, H-25), 1.03 (3H, s, H-26), 1.00 (3H, d, H-29), 0.96 (3H, d, H-30), 0.90 (3H, s, H-24). δ (ppm) ^{13}C NMR (Pyridine, 125.78 MHz): 180.8 (C-28), 139.7 (C-13), 126.2 (C-12), 78.5 (C-3), 56.4 (C-5), 54.2 (C-

18), 48.7 (C-9), 43.2 (C-17), 42.9 (C-14), 40.5 (C-19), 40.2 (C-20), 39.8 (C-8), 39.7 (C-1), 38.0 (C-4), 37.8 (C-22), 34.2 (C-10), 33.5 (C-15), 31.7 (C-7), 29.1 (C-21), 28.7 (C-23), 27.8 (C- 2), 25.2 (C-16), 24.3 (C-27), 21.6 (C-11), 19,4 (C-30), 17.8 (C-6), 17.7 (C-29), 16.4 (C-26), 16.2 (C-24), 15.7 (C-25).

Oleanolic acid: δ (ppm) RMN ^{1}H (600.19 MHz, Pyridine): 5.52 (1H, br, H-13), 3.47 (1H, dd, H-3), 1.07 (3H, s, H-27), 0.98 (3H, s,H-23), 0.97 (3H, s, H-30), 0.96 (3H, br. s,H-25), 0.95 (3H, s, H-29), 0.91(3H, s, H-24, H-26). δ (ppm) RMN ^{13}C (Pyridine, 125.78 MHz): 180.7 (C-28), 145.2 (C-13), 123.4 (C-12), 78.5 (C-3), 56.0 (C-5), 46.5 (C-19, C-17), 42.5 (C-18), 41.6 (C-14), 38.8 (C-4) , 37.7 (C-1) , 37.1 (C-9) , 33.5 (C-29), 33.3 (C-21) , 32.9 (C-22), 33.8(C-7), 29.1 (C-23), 28.6 (C-2), 28.4 (C-15) , 26.2 (C-27), 24.1 (C-11), 24.0 (C-30), 23.8 (C-16), 17.7 (C-26), 16.7 (C-24),15.8 (C-25).
Taraxasterol acid: δ(ppm) RMN ^{1}H (600.19 MHz, Pyridine): 4.84 (1H, s, H-30β), 4.79 (1H, s, H-30α), 3.23 (1H, dd, H-3), 1.04(3H,s,H-29, H-26), 1.00 (3H,s,H-23), 0.97 (3H,s,H-27), 0.91 (3H,s,H-25, H-24). δ (ppm) ^{13}C NMR (Pyridine, 125.78 MHz): 180.0 (C-28), 154.2 (C-20), 105.7 (C-30), 78.5 (C-3), 55.9 (C-5), 48.5 (C-9), 48.2 (C-18), 47.4 (C-17), 42.6 (C-14), 42.5 (C-13), 40.9 (C-8), 39.6 (C-4), 39.3 (C-1, C-22), 39.2 (C-16, C-19), 37.9 (C-10), 29.1 (C-23), 28.4 (C-2), 26.2 (C-29), 25.2 (C-15), 24.1 (C-12), 23.9 (C-21), 23.8 (C- 11), 19.1 (C-6), 16.6 (C-26), 16.7 (C-25),15.9 (C-24), 15.7 (C-27).

Cirsimaritin: δ(ppm) RMN ^{1}H (600.19 MHz, CD_3OD, $CDCl_3$): 7.53 (d, H-2′), 6.71 (d, H-3′), 6.71 (d, H-5′), 7.53 (d, H-6′), 6.39 (d, H-8), 6.36 (s, H-3), 3.74 (7-OCH3), 3.66 (7-OCH3). δ(ppm) RMN ^{13}C (125.78 MHz, CD3OD, CDCl3): 164.7 (C-2), 103.0 (C-3), 182.9 (C-4), 105.8 (C-4a), 152.7 (C-5), 132.1 (C-6), 158.6 (C-7), 90.7 (C-8), 153.6 (C-8a), 121.6 (C-1′), 127.9 (C-2′), 115.9 (C-3′), 160.5 (C-4′), 115.9 (C-5′), 127.9 (C-6′), 60.9 (6-OCH3), 56.0 (7-OCH3).

Negletein: δ(ppm) RMN ^{1}H (600.19 MHz, CD_3OD, $CDCl_3$): 7.93 (d, H-2′), 7.54 (d, H-3′), 7.56 (d, H-4′), 7.54 (d, H-5′), 7.93 (d, H-6′), 6.72 (d, H-3), 6.65 (s, H-8), 4.05 (7-OCH3). δ(ppm) RMN $_{13}$C (125.78 MHz, $CDCl_3$): 164.0 (C-2), 105.6 (C-3), 182.5 (C-4), 105.9 (C-4a), 145.4 (C-5), 129.4 (C-6), 152.7 (C-7), 90.7 (C-8), 150.8 (C-8a), 131.4 (C-1′), 126.2 (C-2′), 128.9 (C-3′), 131.6 (C-4′), 128.9 (C-5′), 126.2 (C-6′), 50.4 (7-OCH3) [M+Na]+ m/z 286.2 (calculated for $C_{16}H_{12}O_5Na^+$ 307.2).

Quercetin: δ(ppm) RMN ^{1}H (600.19 MHz, CD_3OD): 7.77 (s, H-2′), 7.63 (d, H-6′), 6.87 (d, H-5′), 6.37 (d, H-8), 6.18 (d, H-6). δ(ppm) RMN ^{13}C (125.78 MHz, CD_3OD): 147.5 (C-2), 177.3 (C-4), 104.4 (C-4a), 157.7 (C-8a), 94.2 (C-8), 165.2 (C-7), 99.0 (C-6), 162.3 (C-5), 123.7 (C-1′), 116.4 (C-2′), 145.7 (C-3′), 148.4 (C-4′), 116.4 (C-5′), 121.3 (C-6′).

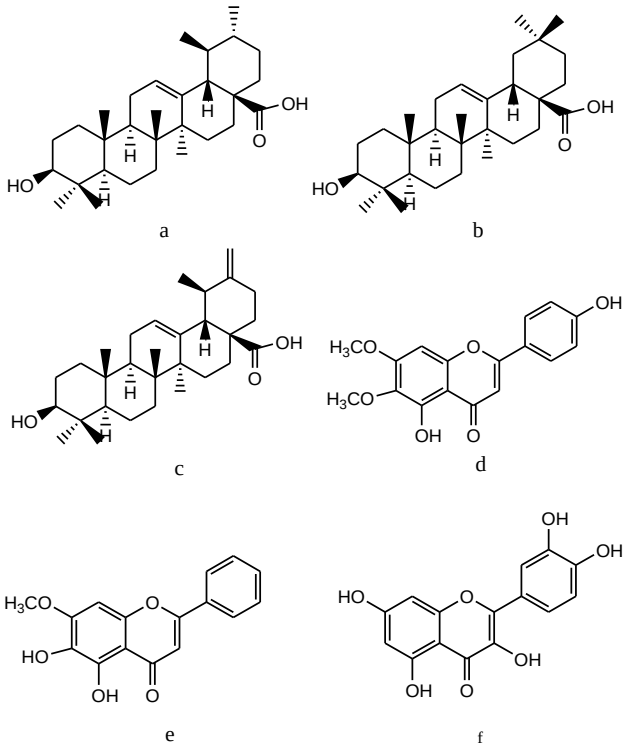

Figure 4: Structures of isolated compounds: Ursolic acid (a), oleanolic acid (b), taraxasterol acid (c), cirsimaritin (d), negletein (e) and quercetin (f)

4. DISCUSSION

In the present study, we conducted biological assays and phytochemical investigations to validate the traditional use of *N. capitata* leaves and to identify compounds from the most active extract. Hexane, ethyl acetate, and methanol extracts were prepared from the leaves by maceration to prevent the degradation of thermolabile components. The TLC DPPH method

of qualitative detection revealed that all these extracts possess antioxidant activity. Results from the forced swimming endurance test and the modified string test demonstrated that extracts from the leaves of *N. capitata* are capable of reducing fatigue during short-term and long-term physical exercises, respectively. In both models of exhaustion, all the extracts showed low effects at the maximum tested dose (200 mg/kg). Among these, the ethyl acetate extract was the most active during the modified string test, whereas the methanol extract had no significant effect on this model of exhaustion. Additionally, the endurance times of mice treated with the methanol or ethyl acetate extracts were significantly prolonged during the swimming test (long-term physical exercise), with the methanol extract exhibiting the highest effect. These results suggest that the anti-fatigue effect of extracts from *N. capitata* leaves depends on the doses and extraction methods used. Under the same experimental conditions, caffeine, a known anti-fatigue compound, showed a significant effect, thereby validating these results ($p < 0.05$, n=3). The effect of caffeine on endurance performance time is well-documented in the literature [17-19].

The determination of active components provides baseline information on the potential usefulness of medicinal plants [20]. Therefore, we also focused our work on the phytochemical screening and isolation of compounds from the ethyl acetate extract. Our results confirmed the presence of flavones, anthocyanins, coumarins, triterpenoids, unsaturated sterols, saponins, and other phenolic compounds, which were previously reported as major constituents of the *Nuxia* genus [5,6]. From the ethyl acetate extract, we isolated three flavones (cirsimaritin, negletein, and quercetin) and three triterpenoids (ursolic acid, oleanolic acid, and taraxasterol acid). Taraxasterol acid and the three flavones were obtained for the first time from *Nuxia*, whereas ursolic acid and oleanolic acid had been previously isolated from this genus [6]. Many of these identified compounds, including flavonoids, have been reported to possess antioxidant and/or anti-fatigue activities [1-3,22,23]. Given that water is commonly used to prepare the *N. capitata*-based traditional remedy for combating fatigue, it is likely that phenolic compounds, including flavonoids, are major constituents.

These findings corroborate the use of *N. capitata* in popular medicine and suggest that compounds from this plant act as radical scavengers, among other possible mechanisms, protecting cells from damage induced by radical accumulation during physical exercise. Radicals induce oxidative stress during physical exercise and play an important role in fatigue [1-3].

However, the study's limitations include a notably small sample size of only three mice per group, raising concerns about the statistical power and reproducibility of the results. To enhance the validity of future research, larger sample sizes are necessary.

5. CONCLUSION

Extracts from *Nuxia capitata* have been shown to enhance performance and endurance during physical exercise, with effects varying based on dose and extract polarity. The identification of specific chemical compounds from the most active extract provides insights into the plant's anti-fatigue mechanisms. These findings validate the traditional use of *N. capitata* as an anti-fatigue remedy. However, further research is necessary to confirm its clinical relevance, identify all active compounds, and fully elucidate the underlying mechanisms. Future studies should include larger sample sizes, more rigorous statistical analyses, and comprehensive clinical trials to ensure the efficacy and safety of *N. capitata* in medical applications.

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