

Original Research

# Analysis of the Improvement Effect of Nervonic Acid Extracted from *Xanthoceras sorbifolium* Bunge Oil on Antioxidant Response and Inflammatory Response in Parkinson's Disease

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## Abstract

**Objective:** An extract of *Xanthoceras sorbifolium* Bunge (XSB) oil called nervonic acid (NA) was studied for its potential to ameliorate oxidative stress and inflammation in people living with Parkinson's disease (PD). Recrystallization column chromatography was performed to isolate NA from the XSB oil. Twenty-five C57BL/6 mice (8–10 weeks old) were randomly assigned to one of five groups (control, model, low, medium, and high dosage). **Methodology:** Except for the control group, all of the experimental animals received an intraperitoneal injection of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The next phase was administering varied doses of NA produced from XSB oil to mice. Control, model, low-dose, medium-dose, and high-dose groups were created at random from SH-SY5Y and PC-12 cell cultures. Our study's control groups exhibited typical normative conduct. **Research:** Polymerase chain reaction (PCR) was used to examine oxidative stress (OS) and inflammatory factors (IFs) in cells. By the time recrystallization column chromatography had finished its analysis, the concentration of NA had increased by a factor of roughly 26. **Results:** The model and high-dose groups showed similar levels of apoptosis in behavior ( $p > 0.05$ ). All three NA treatment groups showed decreases in IFs and increases in superoxide dismutase (SOD) and GSH-Px mRNA ( $p < 0.05$ ). NA, an antioxidant and anti-inflammatory chemical, has shown promising results in PD animal and cell models. **Conclusions:** NA synthesized from XSB oil will soon be available for use in the treatment of Parkinson's disease. With the use of deep learning, patients will be able to arrest their health deterioration and enjoy an improved standard of living.

**Keywords:** Parkinson's disease; *Xanthoceras sorbifolium* Bunge; nervonic acid; inflammatory factors; oxidative stress

## 1. Introduction

Bradykinesia, rigidity, postural gait instability, and resting (or non-resting) tremor are some of the most notable clinical signs of Parkinson's disease [1]. Although difficulties with movement are the most noticeable sign of Parkinson's disease (PD), the sickness may also damage other sections of the brain, making it difficult for patients to pay attention, retain details, or even do the most basic of activities [2]. The sole choice for patients is long-term, ongoing medication to lower the severity of their symptoms since there is presently no therapy that can reverse the effects of PD in clinical settings [3]. Scientists need to develop a fresh means of diagnosing PD as soon as possible, and a therapy for the condition, so that people with PD may live with less anxiety [4].

Nervonic acid, often known as NA, is a substance implicated in the development and progression of several neurological disorders. Validation of NA's effectiveness in treating PD mice and protecting them from dyskinesia is encouraging for the future of NA as a therapy option for illnesses of the nervous system [5]. The most dependable sup-

ply of NA, shark brains, are now illegal to harvest, making it harder for people to synthesize their own NA. Therefore, it is quite concerning that the synthesis of NA for therapeutic purposes is on the rise [6].

The woody oil plant species *Xanthoceras sorbifolium* Bunge (XSB) is endemic to China but is only found in a small number of provinces and autonomous regions. Included in this group are provinces like Henan and Ningxia and Gansu and the Northeast and Liaoning and Inner Mongolia [7]. Using a cold pressing technique, XSB may provide edible oil rich in unsaturated fatty acids (FAs) (like oleic acid, linoleic acid, NA, etc.). The risk of developing cardiovascular disease, diabetes, and other health problems is reduced in those who regularly drink XSB oil, according to studies [8]. XSB may be refined into edible oil and has the potential to be utilized as a raw material in many different industries. It is a key ingredient in many modern products, including lubricants, paints, plasticizers, and even cosmetics [9]. In recent years, China has seen a consistent increase in the area planted with XSB and, therefore, in the yield per acre. If we can devise a method that is both trust-



worthy and efficient, we will not only be able to effectively solve the problem of a large demand and a small supply of NA that exists today, but we will also be able to give patients new hope and a guarantee for the future treatment of patients suffering from a wide range of diseases affecting the nervous system [10].

Therefore, the purpose of this study is to provide a benchmark for the future acquisition of NA and the treatment of PD by extracting NA from XSB oil and evaluating its effect on PD. To determine whether NA derived from XSB has a beneficial impact on PD, this study aims to confirm this. The current clinical limitations of PD therapy will be alleviated, treatment efficacy will increase, and patients' quality of life will improve.

## 2. Literature Review

He *et al.* [11], argue that "Oriental olive oil" is a particularly specific kind of oil that is pressed from the seeds of the oily woody shrub *amellia oleifera*. The chemical composition of olive oil from the Far East is not dissimilar to that of olive oil from Europe [12]. Camellia oil's long-term usage is associated with lower chances of hypertension, liver, heart, and brain illnesses. Camellia oil has shown promise as a possible immune system booster. It has been investigated as a possible therapeutic for skin issues and even as a counter to the effects of aging on the skin [13]. The production process not only makes it very resistant to heat, but also gives it powerful antioxidant properties. This suggests it has commercial potential as an oil in all-natural skin care products. Camellia shells are a rich source of the basic ingredients used to produce chemicals including furfural, xylitol, tannin, and charcoal, which remove fluoride from water [14].

Ikram *et al.* [15], described that a fatty acid called N-(24)-hydroxy-N-(9)-nervonic acid has been identified. Because both are monounsaturated fatty acids, there is a structural resemblance to lignoceric acid (24:0). Aryal *et al.* [16], presented that the chemical is also known as selacholeic acid and cis-15-tetracosenoic acid. These two designations refer to the same underlying idea. The Latin term *nervus*, from which we get the English word "nerve", really means "sinew" in Latin. Homem *et al.* [17], argue that it occurs spontaneously as a consequence of the elongation of oleic acid to 18:19. Erucic acid, a chemical, might be considered a direct precursor. Nervonic acid is abundant in the white matter of animal brains and in peripheral nervous tissue, as well as in the myelin sheath that protects nerve fibers. Jayaraj *et al.* [18], research that Cerebrosides are a kind of glycosphingolipid that are abundant in the nervous system. This includes the brain, spinal cord, and other neural tissues. They are thought to account for around 40% of the fatty acids found in sphingolipids [19].

According to Hayes D *et al.* [20], Walnuts (*Juglans regia*), a tree species prized for its nuts and its woody oil, play an important role in Chinese agriculture. When it comes to

both planted area and total yield, the country ranks first in the world. Due to the high oil content, walnuts are sometimes referred to as "oil depot on the tree". Walnut oil, because of its high concentration of bioactive components, is an excellent dietary supplement and health care product for many different reasons, including its capacity to enhance the appearance of the skin and the body's overall health and fitness [21]. In recent decades, the economic worth of walnuts has soared due in large part to the growing demand for new uses for walnut byproducts. The pomace, husk, and oil cakes from walnut trees may be processed into a variety of valuable components. The by-products of the walnut tree may be used to create activated carbon and color [22].

Chen *et al.* [8], considered that *paeonia suffruticosa*, popularly known as "Celestial Beauty, the king of flowers", is a species of flower that is native to the area of China between the Yangtze and Yellow Rivers and is quickly increasing in population there. Similar to flaxseed oil, it contains the linolenic acid that has been shown to have positive benefits on cardiovascular health, brain and nerve function, and even cancer and inflammation. Peony seeds provide abundant and high-quality oil when crushed. Ingredients in peony seed oil may have been used in medicine, cosmetics, and the food sector [23].

## 3. Materials and Methods

### 3.1 Study Area

This research was conducted between October 2021 and February 2022 at Beijing Yanqing Market Supervision Inspection and Testing Monitoring Center (Beijing).

### 3.2 Preparation of XSB Oil

XSB, ordered from Lingbao Jiulin Garden Engineering Co., Ltd. (Sanmenxia, Hebei, China), was squeezed by a hydraulic oil press with a pressing pressure of 10–55 MPa. With this method, the oil yield of XSB seeds can reach about 25%.

### 3.3 Preparation of Mixed FAs

Anhydrous sodium sulfate (Yuanze Biotechnology Co., Ltd., Shanghai) was added to XSB oil, which was placed for 24 hours and then filtered to obtain dry XSB oil. Then 40 g of XSB oil and 250 mL of sodium hydroxide-ethanol solution (72068, Merck, Darmstadt, Germany) were stirred continuously at 100 °C for 2 h. After cooling, petroleum ether (cas693-65-2, Yunbaihui Biotechnology Co., Ltd., Jinan, China) was added to extract unsaponifiable and the petroleum ether layer was discarded. Then sodium chloride solution (72068, Merck, Darmstadt, Germany) was utilized to adjust the pH value of the FA salt layer to 2–3, followed by standing, aqueous phase removal, washing with water till neutrality, and addition of anhydrous sodium sulfate (1614807, Merck, Darmstadt, Ger-

many) for drying. Finally, XSB oil mixed fatty acid was prepared by recovery of petroleum ether via rotary evaporation at 45 °C.

### 3.4 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Intraperitoneally

Using a synthetic analogue of meperidine may cause parkinsonism over time. Substantia nigra neurons were selectively harmed by the neurotoxin. Both the neurotoxicity caused by 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the transport of N-methyl-4-phenylpyridine was mediated by the mitochondria.

### 3.5 NA Extraction

20.0 g XSB FA solution was immersed in 85% ethanol according to the ratio of 1:9 g/mL and refrigerated at 5 °C for 2 h for 4 times of repeated crystallization to obtain recrystallized mixed FAs, which were then placed in the upper layer of silica gel chromatography column and eluted according to the ratio of petroleum ether to ethyl acetate of 9:1 to obtain fractions for vacuum decompression, and the final sample was NA.

### 3.6 Animal Data

Twenty-five 8–10 week old C57BL/6 mice (Kexing Zhongwei Biotechnology Co., Ltd., Beijing, China) were randomly split into 5 groups: The PD mouse model was developed by giving MPTP intraperitoneally once daily to all mice for 7 days (excluding the control group). Three groups of animals were administered either 20 mg/kg, 40 mg/kg, or 60 mg/kg of XSB oil-extracted NA (gavage once a day for 10 days), while two control groups received an equal amount of normal saline.

The collected mouse is then placed on a clean towel or other stable surface. The interscapular region is held still with the tentacles of the thumbs and fingers before needles are inserted. In this instance, three milliliters is just right. Subcutaneous (under the skin) injections are often administered in the genital area.

### 3.7 Mouse Behavior Examination

**Pole-climbing test:** The mouse was put on a rotarod, which was spun at a speed of 5 rpm/min–20 rpm/min for 180 seconds, and the time the mouse spent on the rotarod (latency) was recorded. The rotarod's diameter was 9 mm, and its length was 1 m. The wooden pole was fastened vertically in the cage. Every half an hour, we ran the experiment again to get an average. In an open-field test, one mouse was given five minutes to explore a box that measured 50 by 50 by 40 centimeters and was outfitted with a camera to capture its every move. The number of times the mouse groomed itself and the number of times it stood up (meaning its front two legs left the floor or climbed a wall) were both noted. After each experiment, 75% alcohol was used to disinfect the testing area.

### 3.8 Behavioral Euthanasia

When severe behavioral issues are the sole source of suffering, it may be compassionate to put an animal to sleep. This is what is meant by the term “behavioral euthanasia”. Animal health and annoying behaviors like leash pulling and guest jumping are generally disregarded.

### 3.9 TUNEL Staining

After the behavioral tests were finished, the mice's throats were slit while they were still under anesthetic. Each group's substantia nigra tissues were dehydrated with gradient ethanol, paraffin-embedded, and sectioned at 4 μm before undergoing standard dewaxing to water and Terminal Deoxynucleotidyl Transferase mediated dUTP Nick-End Labeling (TUNEL) staining (ab66110, Abcam, Cambridge, UK). Apoptotic neurons were represented by red fluorescence in the visual field.

### 3.10 Cell Data

The American Type Culture Collection (ATCC) provided both the SH-SY5Y and PC-12 cell lines, and they have completed short tandem repeat (STR) and mycoplasma testing, which confirmed that these cells are free of contamination. They were maintained in a 37 °C, 5% CO<sub>2</sub> incubator with a MEM/F12 culture mix containing 10% foetal bovine serum and 1% porcine serum. A different media would be used every three days.

### 3.11 Cell Grouping and Model Building

A control group, a model group, and groups given low, medium, and high doses were created from the collected cells. All groups (excluding the control group) used 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) to establish PD cell models. Both the control and experimental groups were first administered saline before the quantities of NA were varied between 5 and 20 mol/L.

The SH-SY5Y neuroblastoma cell line is often used as an in vitro model in studies of Parkinson's disease. A bone marrow biopsy from a 4-year-old with metastatic neuroblastoma was used to establish the first version of this cell line. It has undergone three further cycles of clonal selection since then. The SK-N-SH cell line was the seed from which these cells sprang.

### 3.12 Polymerase Chain Reaction (PCR) Detection

40 cycles of denaturation (94 degrees Celsius for 30 seconds), annealing (94 degrees Celsius for 5 seconds), and extension were performed on RNA that had been extracted using the Trizol method (60 degrees Celsius for 30 seconds). As a consequence of this procedure, cDNA-based constructs were created. Two-Cycle Time (2-CT) analysis primers (Table 1) were developed and synthesized by Thermo Fisher Scientific (Shanghai, China).

**Table 1. Primer sequences.**

| Genes                           | F (5'-3')              | R (5'-3')              |
|---------------------------------|------------------------|------------------------|
| <i>IL-6</i>                     | GATGTTGCTGCTTCACTTC    | CCTTGTTGGCTTATGTTCTG   |
| <i>IL-8</i>                     | GGGCTGCATCTAAAGTAAATGG | CAGAACACTGCTGTAGAAGGTA |
| <i>TNF-<math>\alpha</math></i>  | CTCTTCTCATTCCTGCTTG    | CTCCACTTGGTGGTTTGCT    |
| <i>SOD</i>                      | CACAACTGGTTCACCGCTTG   | GCCCAACCAGACAGAGAATGA  |
| <i>GSH-Px</i>                   | CGTGCAATCAGTTCGGACC    | CCAGGCATCTCCCTTCCATTC  |
| <i><math>\beta</math>-actin</i> | CTAAGGCCAACCGTGAAAAG   | ACCAGAGGCATACAGGGACA   |

IL-6, interleukin-6; IL-8, interleukin-8; TNF- $\alpha$ , tumor necrosis factor alpha; SOD, superoxide dismutase; GSH-Px, Glutathione peroxidase.

**Table 2. Fatty acid composition before and after recrystallization-column chromatography.**

| Before and after extraction | Types of fatty acids | Retention time (min) | Content (%) |
|-----------------------------|----------------------|----------------------|-------------|
| Before extraction           | Linoleic acid        | 17.534               | 45.14       |
|                             | Oleic acid           | 17.036               | 28.22       |
|                             | Erucic acid          | 22.841               | 8.36        |
|                             | Eicosaenoic acid     | 19.894               | 6.28        |
|                             | Palmitic acid        | 14.362               | 5.64        |
|                             | nervonic acid (NA)   | 26.354               | 2.58        |
|                             | Stearic acid         | 16.357               | 2.03        |
|                             | Tetracosanoic Acid   | 19.228               | 1.04        |
|                             | Others               | -                    | 0.89        |
| After extraction            | Linoleic acid        | 17.962               | 5.98        |
|                             | Oleic acid           | 17.061               | 4.15        |
|                             | Erucic acid          | 22.686               | 8.33        |
|                             | Eicosaenoic acid     | 19.542               | 2.42        |
|                             | Palmitic acid        | 14.811               | 7.54        |
|                             | NA                   | 26.238               | 66.97       |
|                             | Stearic acid         | 16.706               | 2.04        |
|                             | Tetracosanoic Acid   | 25.171               | 1.95        |
|                             | Others               | -                    | 0.62        |

### 3.13 Lactate Dehydrogenase (LDH) Toxicity Assay

Cells were washed three times, and the final concentration was adjusted to  $1 \times 10^7$ /mL with complete RPMI-1640. The Lactate Dehydrogenase (LDH) release rate was measured according to the recommendations of the LDH kit (ab102526, Abcam, Cambridge, UK).

### 3.14 Flow Cytometry

Cells were trypsin digested and resuspended in 100  $\mu$ m of binding buffer before being stained with 5  $\mu$ L of Annexin V-FLTC (ab108194, Abcam, Cambridge, UK) and 5  $\mu$ L of PI (ab14083, Cambridge, UK) for 15 minutes at RT in the dark. One way that fluorescence was measured was using a flow cytometer (CytoFLEX S, Beckman Coulter, Brea, CA, USA).

### 3.15 Western Blot

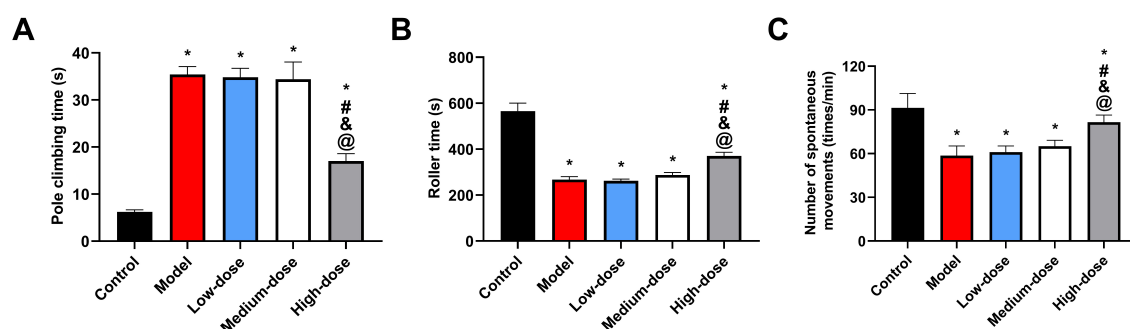
Total protein was extracted from lysed cells using Radio-immunoprecipitation assay (RIPA) (89901, Thermo Fisher, Shanghai, China). Following that, the proteins were transferred to a polyvinylidene difluoride (PVDF) mem-

brane using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12% gel). Nonfat dried milk (5%), used to seal the membrane, was mixed with water and let to sit for 2 hours before being poured into a container and chilled to 4 degrees Celsius. Abcam, an American business, was where we ultimately purchased the antibody (ab52939, ab278716, ab211061, ab32537, ab17942, ab8227, 1:1000, Abcam, Cambridge, UK). We utilized an enhanced chemiluminescence (ECL) luminescence reagent to process the film in the darkroom.

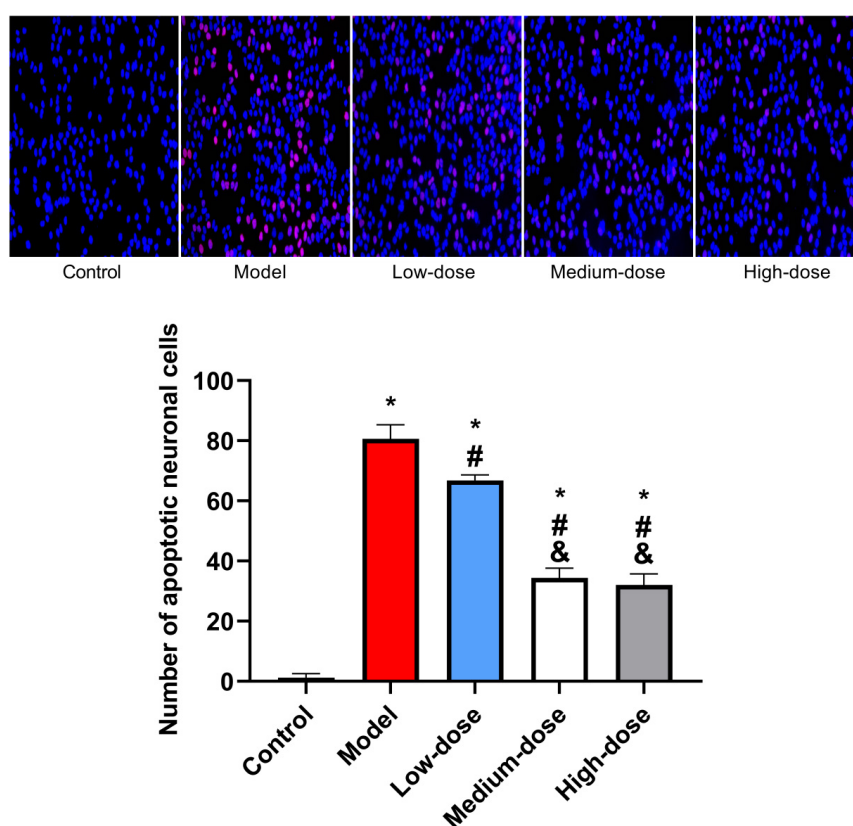
### 3.16 Statistics and Methods

Statistical analysis was performed in SPSS (v22.0, IBM Corp., Armonk, NY, USA), and the findings were shown as (mean standard deviation). We used a mixed-model analysis of variance (ANOVA) followed by a Bonferroni-Seltzer test to determine the statistical significance between the groups. If the *p*-value is less than 0.05, then there is a statistically significant difference between the groups.





**Fig. 1. Alterations in behavioral disorders in a mouse model.** (A) Comparison of pole climbing time. (B) Comparison of latency. (C) Comparison of the number of spontaneous movements. \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $^{\#}p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $^{\&}p < 0.05$ . @: Compared with the Medium-dose group, there is a difference  $^@p < 0.05$ .



**Fig. 2. Apoptosis of mouse neurons by TUNEL staining.** \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $^{\#}p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $^{\&}p < 0.05$ . TUNEL, Terminal Deoxynucleotidyl Transferase mediated dUTP Nick-End Labeling.

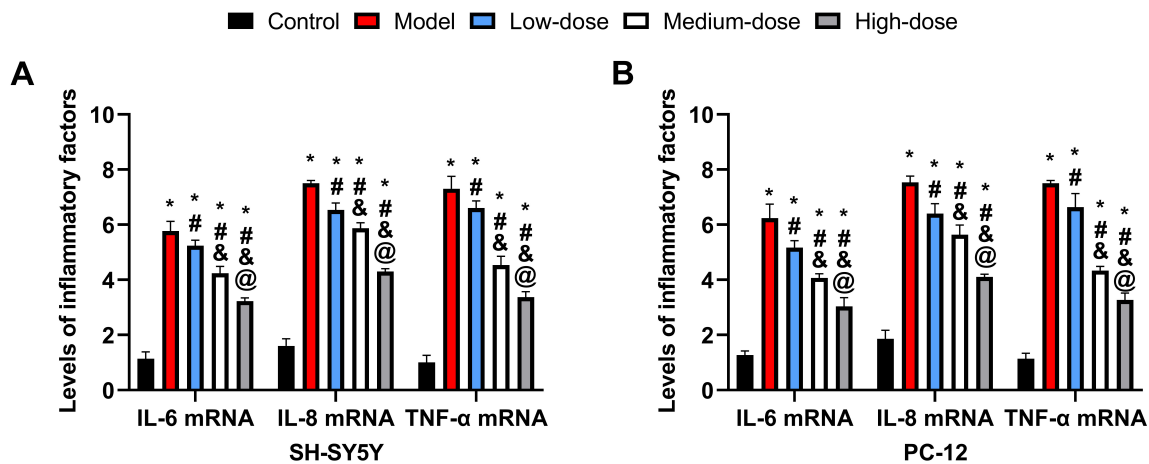
## 4. Results

### 4.1 Summary of Results

The high-dose group showed obviously improved behavior test results than the model group, with a lower neuronal apoptosis rate ( $p < 0.05$ ). Notable reductions in IFs in cells and increases in *SOD* and *GSH-Px* mRNA were observed in the three NA-treated groups ( $p < 0.05$ ).

### 4.2 NA Extraction Efficacy

Compared with the original Wenguan fruit oil, the NA content was greatly improved after low-temperature recrystallization, and the content of linoleic acid is reduced (Table 2).



**Fig. 3. Cellular inflammatory reaction.** (A) Comparison of inflammatory factor levels in SH-SY5Y. (B) Comparison of inflammatory factor levels in PC-12. \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $#p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $&p < 0.05$ . @: Compared with the Medium-dose group, there is a difference  $@p < 0.05$ .

#### 4.3 Impact of XSB Oil-Extracted NA on Mouse Behavior Disorders

Early on, we found that the latency times and durations of the rats' spontaneous movements were much shorter in the model group compared to the control group ( $p < 0.05$ ). This data confirmed the accuracy of the models. Results showed that XSB oil-extracted NA had a substantially improved impact on the behavior disorders, with the high-dose group's pole climbing time being considerably lower compared to the model group, and with latency and open-field autonomous movement durations being enhanced ( $p > 0.05$ , Fig. 1A–C).

#### 4.4 Influence of XSB Oil-Extracted NA on Apoptosis of Mouse Neurons

Neuronal apoptosis was observed to be significantly reduced in the low-, medium-, and high-dose groups relative to the control group. Yet, in contrast to the control group, the model group was shown to have statistically significant higher levels of neuronal apoptosis ( $p < 0.05$ ). Neuronal apoptosis occurred least often in the high-dose group ( $p < 0.05$ , Fig. 2). Therefore, a NA extract obtained from XSB oil may be able to protect neurons in PD rats.

#### 4.5 Influence of XSB Oil-Extracted NA on Cellular Inflammatory Reaction

The model group had substantially higher levels of interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α) mRNA than the control group ( $p < 0.05$ ), indicating that PD may expedite the escalation of the inflammatory response. NA generated from XSB oil showed a significant anti-inflammatory impact, as the mRNA levels of these genes reduced with increasing NA dose in the three groups of cells intervened by NA ( $p < 0.05$ , Fig. 3A,B).

#### 4.6 Impact of XSB Oil-Extracted NA on Cellular Antioxidant Capacity

SOD and GSH-Px mRNA levels were significantly reduced in the model group compared to the other groups ( $p < 0.05$ ), indicating that PD development may diminish cellular antioxidant capabilities. NA produced from XSB oil effectively protected against PD cells' antioxidant activity, as shown by increased SOD and GSH-Px mRNA levels ( $p < 0.05$ , Fig. 4A,B) in the low-, medium-, and high-dose groups compared to the control group.

#### 4.7 Impact of XSB Oil-Extracted NA on LDH Release Rate

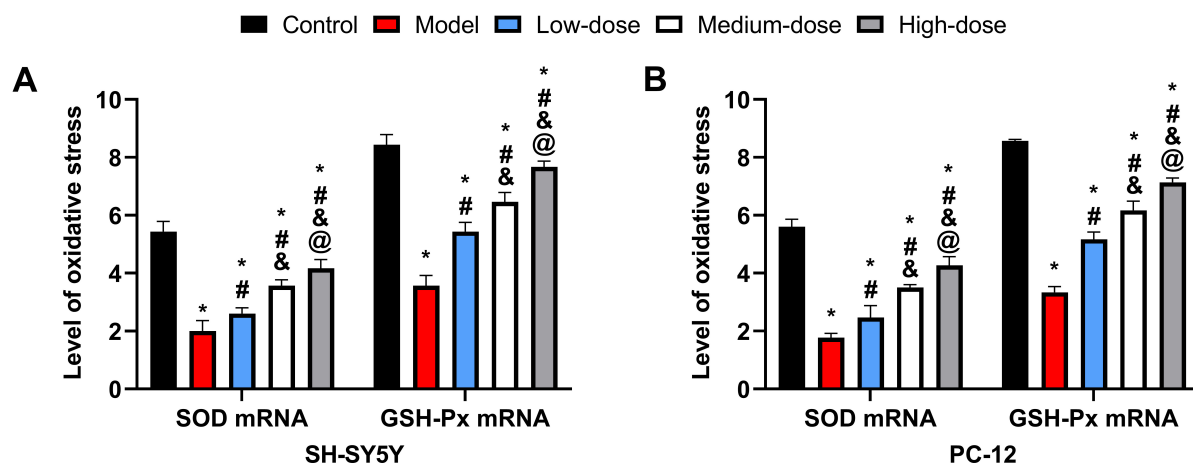
There was a statistically significant difference ( $p < 0.05$ ) in LDH release between the model and control groups, but there was no difference between the model and low dosage groups. As expected, the high-dose group had a much greater LDH release rate than the model group and the control group, but their rate was much lower than the medium-dose groups ( $p < 0.05$ , Fig. 5A,B). Extracts of NA from XSB oil have been demonstrated to efficiently block LDH release from PD cells.

#### 4.8 Influence of XSB Oil-Extracted NA on Apoptosis

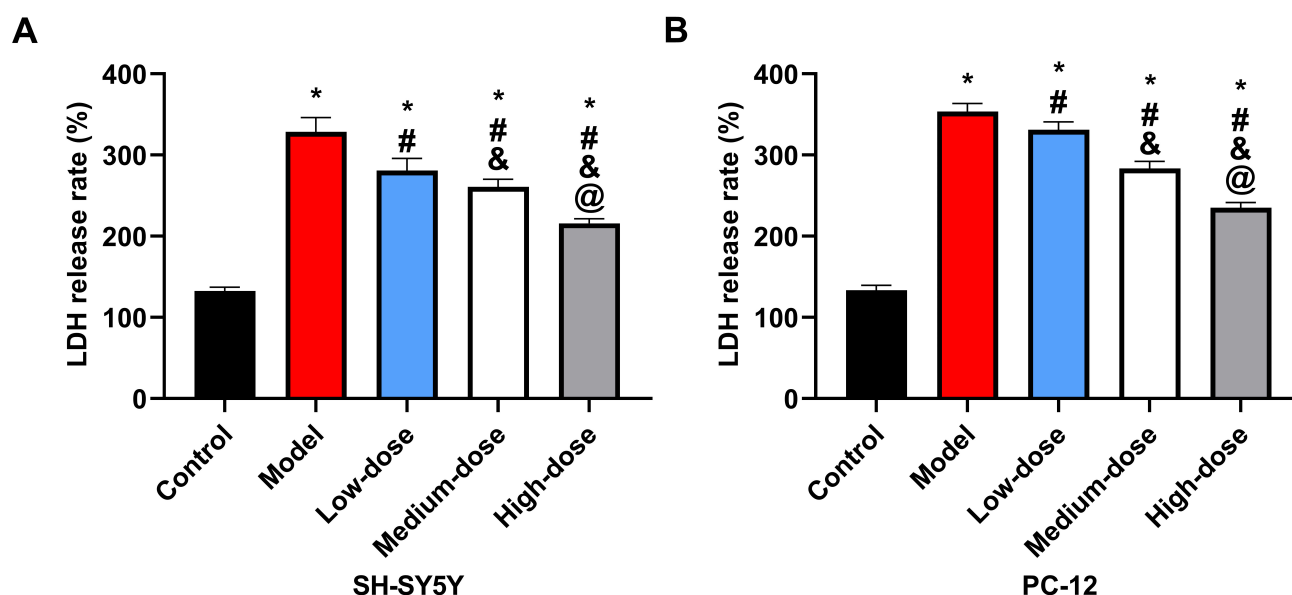
Among the six groups, the control group had the lowest apoptosis rate ( $p > 0.05$ ), whereas the model group had the highest. The comparison of the six groups led to this result. Apoptosis in three separate cell lines was decreased after treatment with NA extracted from XSB oil ( $p < 0.05$ , Fig. 6). Our data suggest that NA prepared from XSB oil may similarly be able to suppress apoptosis in PD cells.

#### 4.9 Influence of XSB Oil-Extracted NA on Ras/MEK/ERK Axis

Protein levels of Ras, MEK/p-MEK, and ERK/p-ERK were all found to be lower in the model group ( $p < 0.05$ ).



**Fig. 4. Cellular oxidative stress response.** (A) Comparison of oxidative stress levels in SH-SY5Y. (B) Comparison of oxidative stress levels in PC-12. \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $\#p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $\&p < 0.05$ . @: Compared with the Medium-dose group, there is a difference  $@p < 0.05$ .



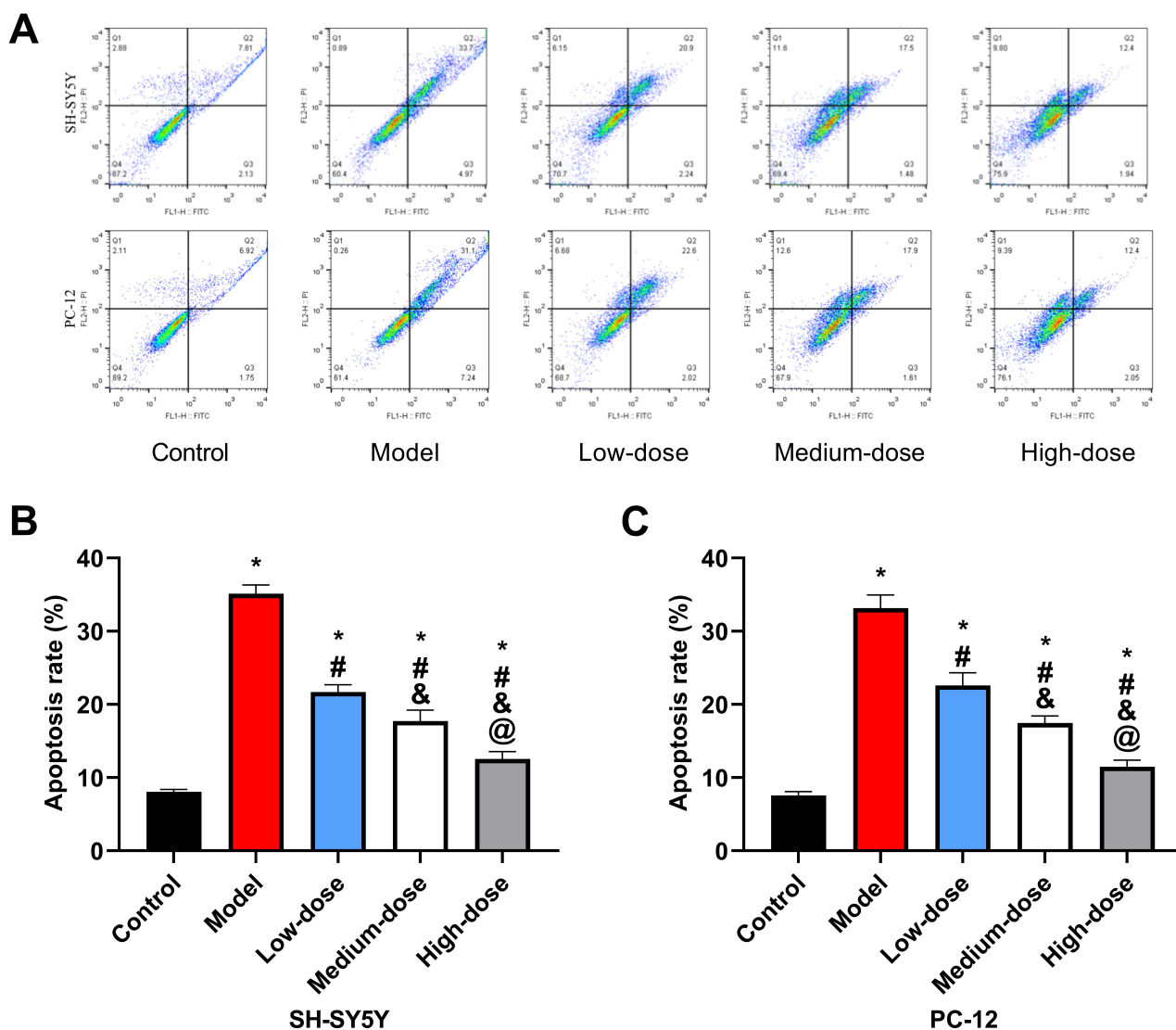
**Fig. 5. LDH release rate of cells.** (A) Comparison of LDH release rate in SH-SY5Y. (B) Comparison of LDH release rate in PC-12. \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $\#p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $\&p < 0.05$ . @: Compared with the Medium-dose group, there is a difference  $@p < 0.05$ . LDH, Lactate Dehydrogenase.

compared to the other 4 groups ( $p > 0.05$ ), as shown in Fig. 7A,B. In the 3 NA-treated groups of mice, these protein levels rose in a dose-dependent manner, with the highest levels seen in the high-dose group ( $p < 0.05$ ).

## 5. Discussion

Although NA is widely recognized as a breakthrough in the therapeutic treatment of neurological illnesses, its acquisition remains a pressing issue owing to its limited availability and high level of complexity. Low-temperature crystallization, the urea inclusion technique, metal salt pre-

cipitation, etc. are being employed for NA separation and extraction in clinical settings. However, there is still much debate regarding the best approach to extraction since there are no widely accepted standards. We investigated the NA separation and extraction strategy to better understand its clinical application implications and to give a more intuitive reference and guiding meaning for the clinic. We also constructed PD animal and cell models for validation to demonstrate the long-lasting impact of XSB oil-extracted NA in PD.



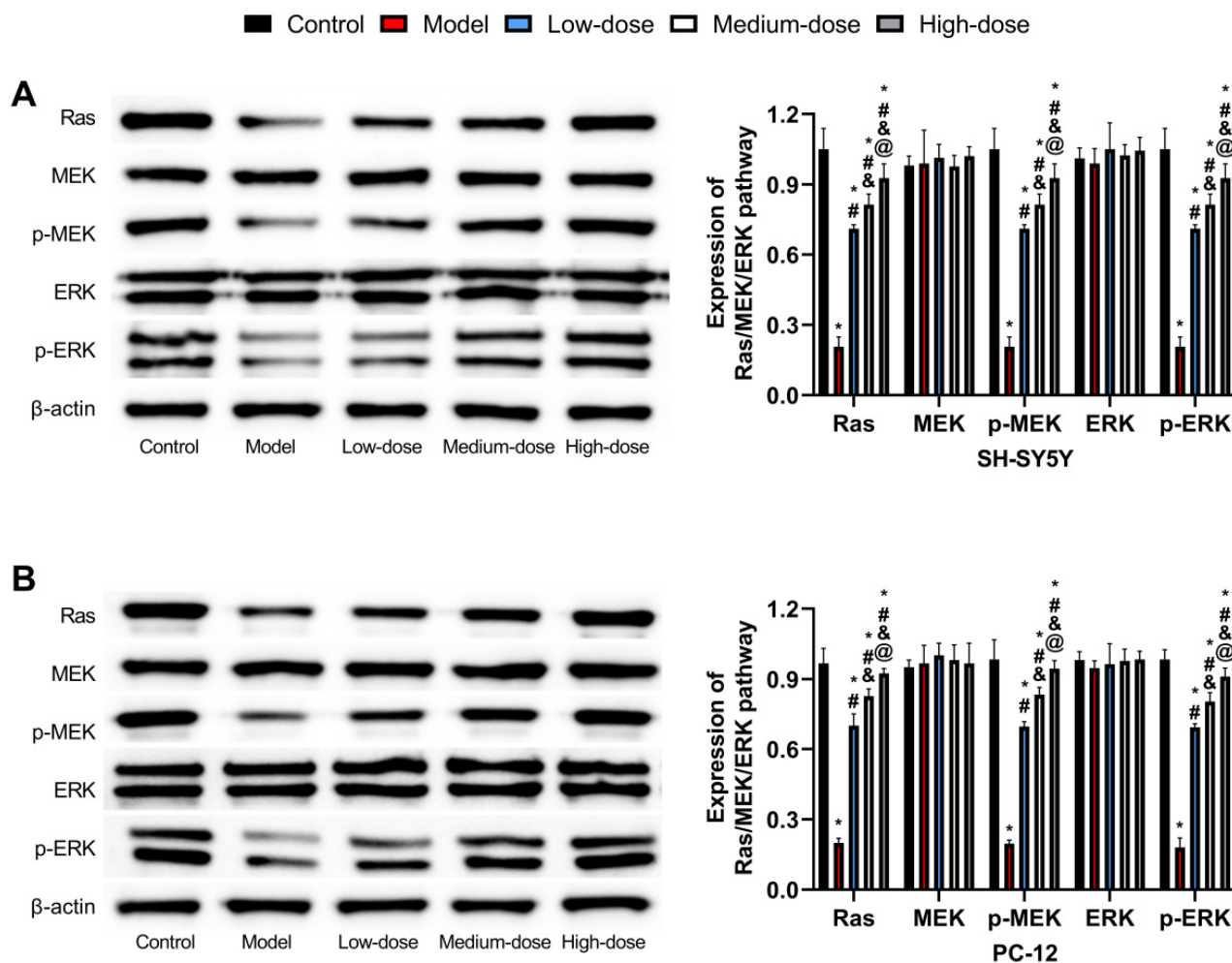
**Fig. 6. Apoptosis rate detected by flow cytometry.** (A) Results of flow cytometry. (B) Comparison of apoptosis rate in SH-SY5Y. (C) Comparison of apoptosis rate in PC-12. \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $\#p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $\&p < 0.05$ . @: Compared with the Medium-dose group, there is a difference  $@p < 0.05$ .

After optimizing the purification procedure in this experiment, the NA content reached 22.53% after selecting low temperature crystallization settings. The concentration may rise to around 30.81% after 4 rounds of recrystallization. Previous research showed that the NA percentage of XSB oil produced by straight pressing was only 2%–4%. The NA content was raised by a factor of 26 after being subjected to the recrystallization-column chromatography technique of processing. However, this approach has the limitation that it can only be utilized in the lab and cannot be industrialized.

The question of whether or not NA derived from XSB oil has the same stellar performance as regular NA remains unanswered. As a result, we conducted further tests on animals to investigate the effects of NA derived from XSB oil

on the abnormal behavior of PD mice. While the medium- and low-dose groups showed no statistically significant improvement in their mice's behavior, the high-dose group clearly exhibited reduced PD-related behavioral disorders, as evidenced by a shorter pole climbing time and statistically increased latency and spontaneous movement times. We hypothesized that since the XSB oil-extracted NA in this experiment was not completely pure, there were some variations with respect to the conventional NA. NA derived from XSB oil at the same dosage is impacted by other FAs compared with the conventional NA, which may explain why it has no discernible effect on behavioral impairments in PD mice. When the NA from XSB oil is administered in high enough doses, it plays a significant role in the therapy and repair of PD, which in turn helps to alleviate the





**Fig. 7. Western blot detection of *Ras/MEK/ERK* signaling pathway expression.** (A) Comparison of *Ras/MEK/ERK* signaling pathway expression in SH-SY5Y. (B) Comparison of *Ras/MEK/ERK* signaling pathway expression in PC-12. \*: Compared with the control group, there is a difference  $*p < 0.05$ . #: Compared with the model group, there is a difference  $\#p < 0.05$ . &: Compared with the Low-dose group, there is a difference  $\&p < 0.05$ . @: Compared with the Medium-dose group, there is a difference  $@p < 0.05$ .

behavioral abnormalities seen in mice. Previous research has shown that NA may prevent neuronal death in animals with PD [24]. Thus, we also analyzed the effect of NA on neuronal apoptosis in each strain of mice. NA derived from XSB oil also exhibited a protective effect on neuron cells, with certain therapeutic application potential, as shown by the data showing a significant improvement in apoptosis in the high-dose group of mouse neuron cells.

Our cell assays also showed that the MPP<sup>+</sup>-induced PD cell model exhibited pathological alterations typical of PD, including an increase in the rates at which LDH is released and apoptosis occurs. Consistent with previous studies, we found that NA produced from XSB oil greatly alleviated the pathological changes of PD when a specified dosage was obtained, with the highest-dose group showing the most considerable improvement. A breakthrough that might one day lead to a permanent clinical cure for Parkinson's disease. A insight into NA's function in PD may be gained by considering the data that it stimulates

the *Ras/MEK/ERK* axis. Moreover, the expression of proteins implicated in the *Ras/MEK/ERK* axis was analyzed to confirm that NA produced from XSB oil had an impact on PD model cells. Protein levels of Ras, mitogen-activated protein kinase, and extracellular signal-regulated kinase are all significantly decreased in a mouse model of Parkinson's disease. One hypothesized mechanism by which NA from XSB oil impacts PD cells is via reactivation of the *Ras/MEK/ERK* axis.

However, whether the same substantial benefit is shown in human trials remains unknown because of variations between animal models and humans and the limited clinical application research due to the difficulty of acquiring NA. More tests are required to determine how NA influences PD. Finally, different methods may also have positive NA extraction effectiveness, since there is no universal NA extraction guide at home and overseas. They want us to move rapidly on doing more studies on the therapeutic potential of NA so that we can make informed recommen-

dations. Lastly, the following were the experimental conditions that inhibited the detection of superoxide dismutase (SOD) and Glutathione peroxidase (GSH-Px): Extensive future study is needed to find markers like malondialdehyde (MDA) and reactive oxygen species (ROS) to completely understand the function that NA plays in the oxidative stress damage found in PD.

## 6. Conclusions

NA extracted from XSB oil can ameliorate the pathological changes of PD mice and cell models, reduce inflammatory reaction and improve antioxidant capacity, possibly, which is expected to become a new clinical treatment scheme for PD that can provide a more reliable guarantee for patients' health.

## Availability of Data and Materials

The datasets used during the current study are available from the corresponding author.

## Author Contributions

JZ designed the research study. DH performed the research. YC provided help and advice on data collection. DH analyzed the data and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Beijing Yanqing Center for Diseases Prevention and Control (approval number: 2021-YL021-02).

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## Conflict of Interest

The authors declare no conflict of interest.

## References

- [1] Weintraub D, Aarsland D, Chaudhuri KR, Dobkin RD, Leentjens AF, Rodriguez-Violante M, *et al.* The neuropsychiatry of Parkinson's disease: advances and challenges. *The Lancet. Neurology.* 2022; 21: 89–102.
- [2] Tolosa E, Garrido A, Scholz SW, Poewe W. Challenges in the diagnosis of Parkinson's disease. *The Lancet. Neurology.* 2021; 20: 385–397.
- [3] Vijiaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. *The Lancet. Neurology.* 2021; 20: 559–572.
- [4] De Miranda BR, Goldman SM, Miller GW, Greenamyre JT, Dorsey ER. Preventing Parkinson's Disease: An Environmental Agenda. *Journal of Parkinson's Disease.* 2022; 12: 45–68.
- [5] Liu F, Wu R, Ma X, Su E. The Advancements and Prospects of Nervonic Acid Production. *Journal of Agricultural and Food Chemistry.* 2022; 70: 12772–12783.
- [6] Fan Y, Meng HM, Hu GR, Li FL. Biosynthesis of nervonic acid and perspectives for its production by microalgae and other microorganisms. *Applied Microbiology and Biotechnology.* 2018; 102: 3027–3035.
- [7] Zang E, Qiu B, Chen N, Li C, Liu Q, Zhang M, *et al.* *Xanthoceras sorbifolium* Bunge: A Review on Botany, Phytochemistry, Pharmacology, and Applications. *Frontiers in Pharmacology.* 2021; 12: 708549.
- [8] Chen X, Lei Z, Cao J, Zhang W, Wu R, Cao F, *et al.* Traditional uses, phytochemistry, pharmacology and current uses of underutilized *Xanthoceras sorbifolium* bunge: A review. *Journal of Ethnopharmacology.* 2022; 283: 114747.
- [9] Zong JW, Zhang ZL, Huang PL, Chen NY, Xue KX, Tian ZY, *et al.* Growth, Physiological, and Photosynthetic Responses of *Xanthoceras sorbifolium* Bunge Seedlings Under Various Degrees of Salinity. *Frontiers in Plant Science.* 2021; 12: 730737.
- [10] Lu Y, Wang H, Liu Z, Zhang T, Li Z, Cao L, *et al.* A naturally-occurring phenomenon of flower color change during flower development in *Xanthoceras sorbifolium*. *Frontiers in Plant Science.* 2022; 13: 1072185.
- [11] He X, Li DZ, Tian B. Diversity in seed oil content and fatty acid composition in *Acer* species with potential as sources of nervonic acid. *Plant Diversity.* 2020; 43: 86–92.
- [12] Liu F, Wang P, Xiong X, Zeng X, Zhang X, Wu G. A Review of Nervonic Acid Production in Plants: Prospects for the Genetic Engineering of High Nervonic Acid Cultivars Plants. *Frontiers in Plant Science.* 2021; 12: 626625.
- [13] Widowati W, Ginting CN, Lister INE, Girsang E, Amalia A, Wibowo SHB, *et al.* Anti-aging Effects of Mangosteen Peel Extract and Its Phytochemical Compounds: Antioxidant Activity, Enzyme Inhibition and Molecular Docking Simulation. *Tropical Life Sciences Research.* 2020; 31: 127–144.
- [14] Javed M, Belwal T, Huang H, Xu Y, Ettoumi FE, Li L, *et al.* Generation and stabilization of CO<sub>2</sub> nanobubbles using surfactants for extraction of polyphenols from *Camellia oleifera* shells. *Journal of Food Science.* 2022; 87: 4027–4039.
- [15] Ikram M, Park TJ, Ali T, Kim MO. Antioxidant and Neuroprotective Effects of Caffeine against Alzheimer's and Parkinson's Disease: Insight into the Role of Nrf-2 and A2AR Signaling. *Antioxidants.* 2020; 9: 902.
- [16] Aryal S, Skinner T, Bridges B, Weber JT. The Pathology of Parkinson's Disease and Potential Benefit of Dietary Polyphenols. *Molecules.* 2020; 25: 4382.
- [17] Homem SG, Moreira EAM, da Silva AF, Barni GC, da Rosa JS, Oliveira DDL, *et al.* Relationship between oropharyngeal dysphagia, nutritional status, antioxidant vitamins and the inflammatory response in adults and elderly: A cross-sectional study. *Clinical Nutrition ESPEN.* 2020; 38: 211–217.
- [18] Jayaraj RL, Beiram R, Azimullah S, Meeran MFN, Ojha SK, Adem A, *et al.* *Lycopodium Attenuates* Loss of Dopaminergic Neurons by Suppressing Oxidative Stress and Neuroinflammation in a Rat Model of Parkinson's Disease. *Molecules.* 2019; 24: 2182.
- [19] Satoh T, Trudler D, Oh CK, Lipton SA. Potential Therapeutic Use of the Rosemary Diterpene Carnosic Acid for Alzheimer's Disease, Parkinson's Disease, and Long-COVID through NRF2 Activation to Counteract the NLRP3 Inflammasome. *Antioxidants.* 2022; 11: 124.

- [20] Hayes D, Angove MJ, Tucci J, Dennis C. Walnuts (*Juglans regia*) Chemical Composition and Research in Human Health. *Critical Reviews in Food Science and Nutrition*. 2016; 56: 1231–1241.
- [21] Poggetti L, Ferfua C, Chiabà C, Testolin R, Baldini M. Kernel oil content and oil composition in walnut (*Juglans regia* L.) accessions from north-eastern Italy. *Journal of the Science of Food and Agriculture*. 2018; 98: 955–962.
- [22] Ma Q, Wang Y, Zhu L, Bi C, Li S, Li S, *et al.* Characterization of the Complete Chloroplast Genome of *Acer truncatum* Bunge (Sapindales: Aceraceae): A New Woody Oil Tree Species Producing Nervonic Acid. *BioMed Research International*. 2019; 2019: 7417239.
- [23] Deng R, Gao J, Yi J, Liu P. Could peony seeds oil become a high-quality edible vegetable oil? The nutritional and phytochemistry profiles, extraction, health benefits, safety and value-added-products. *Food Research International*. 2022; 156: 111200.
- [24] Lewkowicz N, Piątek P, Namiecińska M, Domowicz M, Bonikowski R, Szemraj J, *et al.* Naturally Occurring Nervonic Acid Ester Improves Myelin Synthesis by Human Oligodendrocytes. *Cells*. 2019; 8: 786.