Heliyon 10 (2024) e28783

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Exploring the antioxidant potential of *nekemias* species extracts on edible oils: *In vitro* assessment and lipid oxidation inhibition

Yuan Zeng , Zhengwen Yu *, Chunyan Jiang , Jiayu Liu , Huanchun Yang , Hongli Pan

School of Life Sciences, Guizhou Normal University, Guiyang, 550025, China

ARTICLE INFO

Keywords:
Nekemias plant species
Antioxidant properties
Lipid oxidation
Oleic acid system
Natural antioxidants

ABSTRACT

Synthetic antioxidants have long been used to protect edible oils from oxidation. However, concerns about their potential health risks and environmental impact have led to a growing interest in natural antioxidants. In this study, we explore the antioxidant properties of extracts from four *Nekemias* plant species: *Nekemias grossedentata* (AGR), *Nekemias megalophylla* (AME), *Nekemias chaffanjonii* (ACH), and *Nekemias* cantoniensis (ACA) by obtaining the values for different tests. We investigate their bioactive compound content and evaluate their antioxidant capabilities on six edible oils categorized into three lipid systems based on their fatty acid compositions: oleic acid, linoleic acid, and linolenic acid. Our findings demonstrate that AGR and AME extracts, rich in bioactive compounds, exhibit strong antioxidant activities *in vitro*, effectively inhibiting lipid oxidation, especially in oleic acid-rich oils like camellia oil. The antioxidant effects of these extracts are comparable to synthetic antioxidants such as TBHQ and superior to natural antioxidant Tea Polyphenols (TP). While the extracts also show antioxidant potential in linoleic and linolenic acid systems, the stability of their effects in these oils is lower than in oleic acid system. These results suggest that *Nekemias* species extracts have the potential to serve as natural additives for extending the shelf life of edible oils, contributing to the exploration of natural antioxidants.

1. Introduction

Edible oils are highly susceptible to oxidation, leading to rancidity and a decline in quality, which can have adverse effects on flavor, aroma, and nutritional value [1]. To combat oxidation and maintain the quality of edible oils, antioxidants are commonly added [2]. However, concerns about the safety of synthetic antioxidants such as BHA, BHT, PG, and TBHQ have prompted the search for natural alternatives [3–7]. Consequently, there is a growing interest in the development of natural antioxidants to replace synthetic ones in the food industry.

In recent years, research on plant-derived natural antioxidants has gained traction [8,9]. Several natural antioxidants, including Tea Polyphenols (TP), Vitamin E (VE), and Nordihydroguaiaretic Acid (NDGA), have emerged as potential alternatives to synthetic preservatives and antioxidants in various food products [10]. Extracts from plants rich in phenols and flavonoids, such as *Rosa canina*, green tea, and vine tea, have been shown to effectively replace synthetic additives in mayonnaise, biscuits, and edible oil lipid fractions [11–16]. As part of this growing trend, we explore the antioxidant properties of extracts from *Nekemias* species, a group of plants known for their rich chemical components and potential health benefits [17–19].

Nekemias species, including *Nekemias grossedentata*, *Nekemias megalophylla*, *Nekemias chaffanjonii,* and *Nekemias cantoniensis*, are

* Corresponding author.
E-mail address: yuzhengwen2001@126.com (Z. Yu).

https://doi.org/10.1016/j.heliyon.2024.e28783
Received 3 October 2023; Received in revised form 23 March 2024; Accepted 25 March 2024
Available online 27 March 2024

distributed widely in various regions in China [20]. These plants are rich in flavonoids, particularly dihydromyricetin, which is a potent natural antioxidant [21]. While *N. grossedentata*, commonly known as vine tea, is primarily found in regions south of the Yangtze River, *N. megalophylla*, also known as large-leaved mountain grape or mildewed tea, is mainly distributed in northeastern Yunnan, Guizhou, Sichuan, Hubei, Shanxi, and southern Gansu [22,23]. *N. chaffanjonii* is distributed in several provinces, including Anhui, Jiangxi, Hubei, Hunan, Guangxi, Sichuan, Guizhou, and Zhejiang [24], while *N. cantoniensis* is mainly found in Jiangxi, Hunan, Guangdong, Guangxi, and Hainan [25]. Despite their rich chemical composition and potential health benefits, the antioxidant activities of most *Nekemias* species have not been extensively investigated, making them an intriguing subject of study as natural antioxidants.

In this study, we comprehensively examine the bioactive components and antioxidant properties of extracts from four *Nekemias* plant species. We assess their ability to inhibit lipid oxidation in different edible oils, categorizing them into three systems based on their fatty acid compositions: oleic acid, linoleic acid, and linolenic acid. Our results demonstrate that these extracts, particularly those from *N. grossedentata* and *N. megalophylla*, exhibit significant antioxidant effects *in vitro* and effectively inhibit lipid oxidation, especially in oleic acid-rich oils. The antioxidant effects of these extracts are comparable to synthetic antioxidants like TBHQ and superior to natural antioxidant TP. While the extracts also show antioxidant potential in linoleic and linolenic acid systems, the stability of their effects in these oils is lower than in oleic acid systems.

This study highlights the promising potential of *Nekemias* species extracts as natural additives for extending the shelf life of edible oils. By replacing synthetic antioxidants with natural alternatives, we can mitigate potential health risks and contribute to the development of safer and more sustainable food products. Furthermore, this research adds to the growing body of knowledge on plant-derived natural antioxidants, paving the way for their broader application in the food and pharmaceutical industries.

2. Materials and methods

2.1. Preparation of edible oil

Six edible oils (without any chemical antioxidant) with varying proportions of oleic acid were used in this study: camellia oil (CLO), olive oil (OLO), sunflower oil (SFO), sesame oil (SMO), perilla seed oil (PSO), and linseed oil (LSO). These oils were provided by the 1509 Engineering Research Center for Development and Utilization of Substitute Tea Plants in Guizhou Universities, College of Life Science, Guizhou Normal University. The main fatty acid components of these oils were determined following a previous method [26]. Briefly, 0.1 g of each oil sample was accurately weighed and placed in a 100 mL garden bottom flask. 5 mL of extract was added for shaking and dissolving, followed by the addition of 5 mL of 0.5 mol/L KOH-methanol solution. The mixture was shaken for 1 min, and a small amount of anhydrous sodium sulfate was added. The flask was sealed and placed in an oven at 50 °C for approximately 1 h. The reactant was transferred to a separating funnel, and about 3 mL of water was added. The upper layer was separated, and the solvent was recovered under reduced pressure to obtain fatty acid methyl esters. Two hundred milliliters of ethyl acetate was added for dissolution, and 1 μL was taken for analysis using GC-MS (QP2010, Japan).

2.2. Preparation of nekemias plant extracts

The raw materials of *Nekemias* spieces were provided by 1509 Engineering Research Center for Development and Utilization of Substitute Tea Plants in Guizhou Universities, College of Life Science, Guizhou Normal University. The four samples, *N. grossedentata* (AGR), *N. megalophylla* (AME), *N. chaffanjonii* (ACH) and *N. cantoniensis* (ACA), were obtained by drying (101-2AB, Tianjin, China) and grinding (CXP-100, Shanghai, China) the raw materials. To extract the bioactive compounds from the plant materials, 10 g of dried powder from each plant was added to 500 mL of 70% ethanol and subjected to ultrasonic disruption (DK-1500D, Guangdong, China) for 30 min at room temperature. The resulting mixture was filtered through a Brinell funnel, and the filtration process was repeated three times. The filter liquor was collected and the ethanol was removed by evaporation in 79 °C using a rotary evaporator (EYE-LA N1001, Kunming, China). The resulting thickened liquid was mixed with 20 mL of acetone and evaporated again to obtain dry powder samples of the extracts.

2.3. Components analysis

According to recent reports, *Nekemias* plants are known to contain various bio-active compounds, including dihydromyricetin-based flavonoids, polyphenols, and polysaccharides [27,28]. To determine the components of the samples, 1 g of the dried powder from each sample was dissolved in 50 mL of 70% ethanol. The resulting solution was then subjected to specific chemical assays to determine total flavonoids, phenolics, four flavonoid active compounds.

2.3.1. Total flavonoids content

To determine the total flavonoids content (TFC) of the four *Nekemias* species extracts, a method with modifications was used [29]. A standard curve was prepared using rutin, and its concentration was measured by UV spectrophotometer (UV759CRT, shanghai, China) at 500 nm. In brief, 50 mg of rutin was dissolved in methyl alcohol to prepare a reference standard solution. Different volumes of the standard solution were taken and mixed with deionized water and 5% $NaNO_2$. The mixture was allowed to stand for 6 min, and then Al $(NO_3)_3$ was added. The final volume was adjusted to 25 mL using deionized water, and the absorbance was measured at 500 nm. A linear regression equation was obtained by the least square method. To measure the total flavonoids content of the sample, 3 mL of the

sample solution was added to a measuring flask, and deionized water was added to 6 mL. The chromogenic reaction was then carried out as described above. The results were compared with the rutin reference standard solution to calculate the total flavonoids content of the sample in milligrams rutin equivalents (RE)/g extract.

2.3.2. Total phenolics content

The determination of total phenolics content (TPC) was described in previous methods [30,31]. The procedure involved preparing a standard solution of gallic acid by dissolving 0.11 g of gallic acid in a 100 mL volumetric flask with deionized water. The standard solution was then diluted to create a series of gradients. To determine the phenolics content of the *Nekemias* extract, 1 mL of the extract was mixed with 5 mL of 10% folinphenol reagent solution in a test tube. The mixture was then allowed to react for 3–8 min, after which 4 mL of 7.5% sodium carbonate was added. The resulting mixture was diluted to a fixed volume with 70% ethanol before measuring the absorbance at 765 nm using a spectrophotometer. The concentration of phenolics in the extract was then determined by comparing the absorbance of the extract with the standard curve generated earlier. The total phenolics content of the extract was expressed as mg gallic acid equivalents (GAE) per g matter of *Nekemias* extracts.

2.3.3. Contents of four active compounds

The contents of four active compounds in the sample solution were analyzed by high performance liquid chromatography [32,33]. The chromatographic separation and identification of component were performed using an Agilent 1200 high performance liquid chromatography system, including G1311A pump, G1315 DAD detector, and Agilent chromatography workstation. All tests were carried out at room temperature. A 5 μL sample was injected, the mobile phase was constituted by 100% acetonitrile (solvent A), 0.1% (v/v) phosphoric acid (solvent B) and100% methanol (solvent C). The solutions were filtered through a 0.45 μm membrane filter before use. The flow rate was 1.0 mL/min, dihydromyricetin (DMY) was detected at 292 nm, myricetrin and rutin were detected at 254 nm, and myricetin was monitored at 375 nm. The quantification of each compound uses an external standard method. Calibration linear curve for each compound was constructed through the regression peak area (Y) and concentration (C). The results were expressed as a percentage content of each compound.

2.4. Antioxidation evaluation of nekemias species extracts in vitro

2.4.1. Ferric reducing antioxidant power (FRAP)

The ferric ion reducing antioxidant power (FRAP) was measured according to the method as described previously [34]. To do this, 0.2 mL of a diluted sample solution was mixed with 3 mL of FRAP solution and heated in a 37°Cwater bath for 10 min. After heating, the absorbance was measured at 593 nm. In this experiment, $FeSO_4$ solution was used as a control to measure the antioxidant activity of the sample. The antioxidant activity of the sample was expressed as mg $FeSO_4$ equivalents per gram of the sample.

2.4.2. Scavenging rate determination of DPPH & ABTS

The DPPH free radical-scavenging activity was performed according to a reported method [35]. 1 mL of the sample solution is diluted and mixed with 3 mL of DPPH radical solution. The mixture is then allowed to react for 30 min in the dark. The absorbance of the control (A_1) is measured at 517 nm, and the absorbance of the blank (A_0) is obtained using 1 mL of absolute ethanol instead of the sample solution. The absorbance of the sample (A_2) is also measured after mixing 1 mL of the sample solution with 3 mL of absolute ethanol. Using these values, the DPPH clearance is calculated using the formula:

$$\text{DPPH bleaching} = [A_0 - (A_1 - A_2)] \ / \ A_0 \times 100\ \% \tag{1}$$

The DPPH scavenging activity and IC_{50} value can be calculated using the regression equation derived from the DPPH clearance values. Each sample is analyzed in triplicate to ensure the accuracy of the results.

The scavenging activity on ABTS was decribed in the determination for TEAC assay [36]. In brief, the ABTS solution and $K_2S_2O_8$ solution are mixed together to prepare the working solution. The sample extracts are then added to this solution, and the reaction mixture is heated in a water bath at 37 °C for 10 min. The absorbance of the reaction mixture is measured at 734 nm immediately after heating. This value is denoted as A_i. To determine the background absorbance, two control samples are prepared. In the first control sample, the sample extract is replaced with phosphate buffer saline (PBS). This control sample measures the absorbance of ABTS cation solution without any sample extract. The absorbance of this sample is denoted as A_c. In the second control sample, the sample extract is added to PBS instead of the ABTS cation solution. This control sample measures the absorbance of the sample extract without the ABTS cation solution. The absorbance of this sample is denoted as A_j. The scavenging rate of the sample extract is calculated using the following formula:

$$\text{ABTS scavenging rate} = \left[1 - \left(A_i - A_j\right) \ / \ A_c\right] \times 100\% \tag{2}$$

The IC_{50} value, which is the concentration of the sample extract required to scavenge 50% of the ABTS radical cation, is also calculated using this method.

2.5. Thermal oxidation and POV assays of bulk oil

Oven-enhanced storage method was used to simulate the accelerated oxidation process of lipid in edible oil. First, 20 g of edible oil

was added to a triangular flask and 0.05% of the sample extracts from four *Nekemias* species were added to the oil and mixed thoroughly. Positive controls were used, including oil + TBHQ (0.05%) and oil + TP (0.05%), while six edible oils without antioxidants were used as blank controls. The supplier of antioxidants TBHQ and TP is Beijing Xinda Food Additives Co., Ltd. The oil samples were then placed in an oven at (60 ± 1)°C for enhanced storage. The samples were stirred once every 24 h, and the peroxide value (POV) was measured every three days. Each experiment and assay were carried out in triplicate.

Peroxide value (POV) is an important index and widely used to evaluate the degree of lipid oxidation [37,38]. It is worth mentioning that the modified titration method in this experiment is referred to previous report [39]. Briefly, 1 mL of oil was accurately measured and added in 250 mL iodine vial, 20 mL of glacial acetic acid and chloroform mixture with volume ratio of 3 to 2 were added to completely dissolve the oil, then 0.5 mL of saturated potassium iodide solution was added before shaking for 0.5 min and performing by dark treatment for 3 min. After removal, 50 mL of deionized water was added, 1% starch was used as an indicator, and 0.002 mol/L sodium thiosulfate standard solution was used for titration. At the same time, positive control and blank control were made. The results were expressed as the amount of peroxide oxygen per 1 kg of fat or oil:

$$\mathrm{POV}\ (\mathrm{mM}/\mathrm{kg\ oil}) = \mathrm{c} \times (\mathrm{V_{oil}} - \mathrm{V_{blank}}) \times 0.1269 \times 100 / \mathrm{m} \quad (3)$$

where c is the concentration of sodium thiosulfate standard solution, V_{oil} is volume of the sample, V_{blank} is the volume of the control, and m is the oil sample weight.

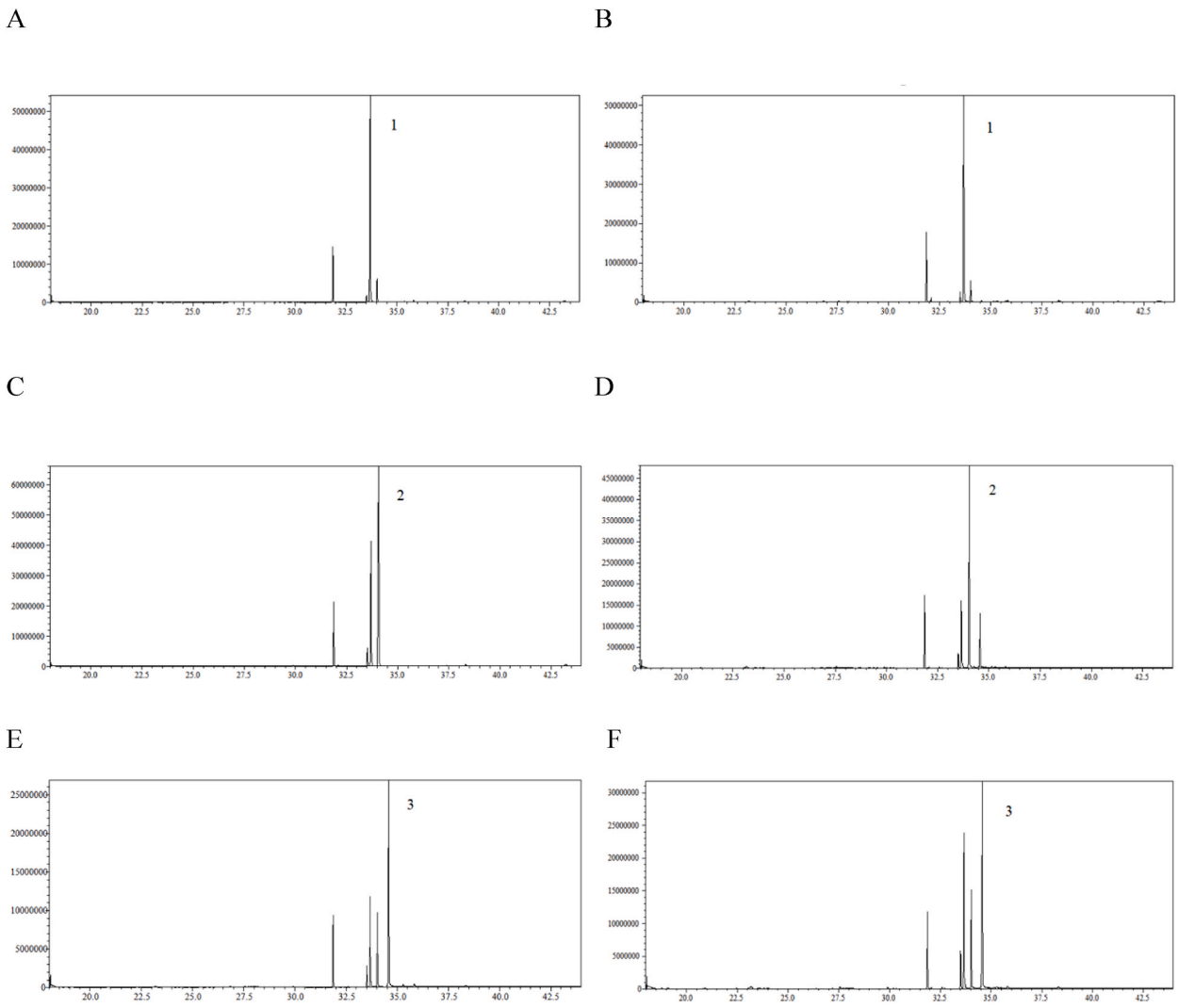

Fig. 1. Mass spectrum of compounds of camellia oil (A), olive oil (B), sunflower oil (C), sesame oil (D), perillaseed oil (E) and linseed oil (F). Note: The numbers 1 to 3 represent oleic acid, linoleic acid, and linolenic acid.

2.6. Reagents and statistical analysis

All reagents used in this study were analytical grade, among which HPLC grade acetonitrile and methanol were purchased from Merck, Germany, and other analytical reagents were purchased from Tianjin Kemeiou Chemical Reagent Co., Ltd. All data of sample extracts were presented as mean ± SD in triplicate. The results and multiple group comparison undertaken by one-way analysis of variance (ANOVA), post-hoc Duncan's test and Pearson correlation analysis ($P < 0.05$*, $P < 0.01$**) using IBM SPSS version 26.

3. Results

3.1. Contents analysis of edible oil's components

According to the determination of six edible oils in Fig. 1(A-F), CLO and OLO were rich in oleic acid, with the contents of 74.63% and 69.96%, SFO and SMO contained 56.01% and 52.84% linoleic acid respectively, PSO and LSO mainly contained linolenic acid, with the content of 47.43% and 39.58% severally (Fig. 1 and Table 1). Thus, these edible oils could also be divided into three oil systems, which was oleic acid, linoleic acid and linolenic acid (Table 1).

3.2. Major components analysis of sample extracts

In recent years, studies have demonstrated that the extracts, particularly from *N. grossedentata*, mainly contained phenolics, flavonoids, and other rich antioxidant active ingredients represented by dihydromyricetin [28]. In this study, the contents of total flavonoids, total phenolics and four flavonoid active compounds were determined in four extracts at the same concentration. The results in Fig. 2(a)-(c) and Table 2 indicated that the total flavonoids content in the extracts ranged from 109 to 675 mg RE/g extract, with the most flavonoids content 675 mg RE/g extract for AGR, followed by AME, ACA, and ACH with 418, 185, and 109 mg RE/g extract, respectively. On the other hand, the total phenolics content was in the order of AGR, AME, ACA and ACH with 421, 236, 71 and 49 mg RE/g extract. As for flavonoid components, the main compound was dihydromyricetin, followed by myricetrin, rutin and myricetin. In addition, the most contents are dihydromyricetin and myricetrin with 37% and 15% for AGR, which contained approximately the same amount to AME. Despite ACH contained 6% myricetin, which is the most.

3.3. Antioxidant activity in vitro

The antioxidant activities of the plant extracts *in vitro* are commonly measured by using DPPH radical scavenging method, ABTS and ferric reduction assays [40]. In this study, the antioxidant activities of the sample extracts were determined *in vitro* using FRAP, DPPH and TEAC assays. According to Fig. 3 (a) and Table 2, the results of the FRAP assay indicated that the reducing capability of AGR extract, which contained the most total flavonoids and phenolics, was stronger than TBHQ but inferior to TP at the same concentration. Furthermore, the reducing power of the other extracts decreased sequentially from AME and ACA to ACH, which was consistent with the contents of bioactive components in these samples.

The scavenging activity of the extracts in DPPH and TEAC tests were inferior to TP and TBHQ at the same concentration which were depicted in Fig. 3 (b) and (c). The results showed that the AGR extract had the highest DPPH and ABTS free radical clearance rate among the four samples, with rates exceeding 43%. And the antioxidant ability of AGR extraction was similar to AME but significantly better than ACA and ACH extracts, despite there was no significant difference between ACA and ACH extracts. The results were also confirmed by the IC_{50} values, which were also calculated to quantify the antioxidant activity further, with values ranging from 204 to 250 μg/mL and 176–300 μg/mL for DPPH and TEAC assays (Table 2).

In addition to test whether there is a correlation between these components and antioxidant activities of the four extracts, corresponding analysis were performed. As shown in Table 3, it was found that there were significant differences respectively between total flavnoids, total phenolics, myricetrin, and antioxidant activity, with correlation coefficients which exceeded 0.9. We also found that dihydromyricetin and rutin showed significant differences in clearing DPPH and ABTS, whose correlation coefficients were above 0.8.

Table 1
Components of six edible oils.

Components	Palmitic acid, methyl ester (%)	Stearic acid, methyl ester (%)	Oleic acid (%)	Linoleic acid, methyl ester (%)	Linolenic acid, methyl ester (%)
Rt	31.863	33.502	33.666	34.040	34.575
Camellia oil	16.27	2.16	**74.63**	6.94	–
Olive oil	20.60	3.28	**69.96**	6.16	–
Sunflower oil	13.69	3.94	26.36	**56.01**	–
Sesame oil	16.72	3.30	13.91	**52.84**	–
Perillaseed oil	13.89	4.23	17.81	16.64	**47.43**
Linseed oil	12.56	6.14	24.68	17.04	**39.58**

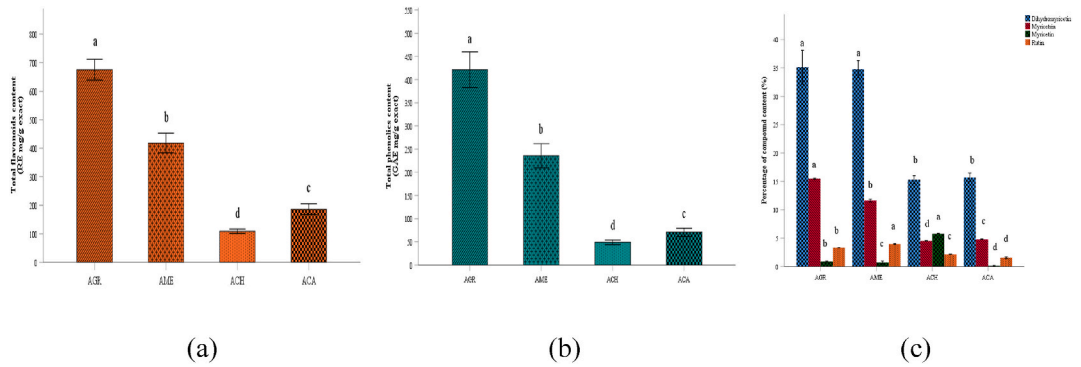

Fig. 2. The contents of total flavonoids (a), total phenolics (b) and four active flavonoid compounds (c) of four leaf samples from *Ampelopsis* species. Note: AGR (*N. grossedentata*), AME (*N. megalophylla*), ACH (*N. chaffanjonii*), ACA (*N. cantoniensis*). Different lowercase letters showed significant differences among the samples ($P < 0.05$).

Table 2
Antioxidant activities four exacts of *Nekemias* species using the DPPH assay, ABTS assay, and FRAP assay.

Samples	IC_{50}/DPPH (μg/mL)[a]	IC_{50}/ABTS (μg/mL)[b]	FRAP value (mmol $FeSO_4$/g exact)
TP	173 ± 0.47^{e}	174 ± 2.83^{d}	1.53 ± 0.02^{a}
TBHQ	137 ± 0.00^{f}	172 ± 3.68^{e}	0.33 ± 0.02^{d}
AGR	204 ± 0.94^{d}	176 ± 4.19^{d}	0.52 ± 0.01^{b}
AME	224 ± 0.47^{c}	196 ± 2.16^{c}	0.36 ± 0.02^{c}
ACH	250 ± 0.29^{a}	300 ± 3.40^{a}	0.24 ± 0.00^{f}
ACA	244 ± 0.26^{b}	266 ± 2.62^{b}	0.30 ± 0.01^{e}

Data are presented as the mean ± standard deviation ($n \geq 3$).
Note: TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and four extracts of AGR (*N. grossedentata*), AME (*N. megalophylla*), ACH (*N. chaffanjonii*) and ACA (*N. cantoniensis*). Data are presented as the mean ± standard deviation ($n \geq 3$). Different lowercase letters showed significant differences among the samples ($P < 0.05$).
[a] The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance at 517 nm by 50%.
[b] The antioxidant activity was evaluated as the concentration of the test sample needed to decrease the absorbance at 734 nm by 50%.

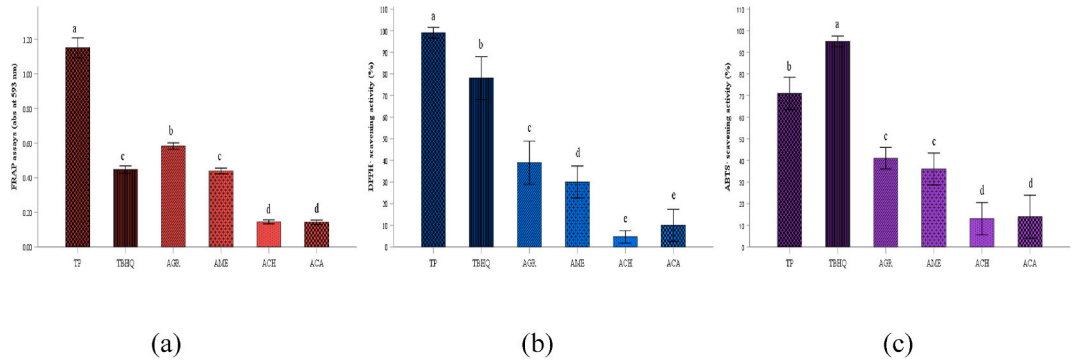

Fig. 3. The comparison of antioxidant abilities of four sample extracts from *Nekemias* species *in vitro* in terms of FRAP(a), DPPH(b), ABTS(c) based on TP and TBHQ under the same condition. Note: TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and four extracts of AGR (*N. grossedentata*), AME (*N. megalophylla*), ACH (*N. chaffanjonii*) and ACA (*N. cantoniensis*). Different lowercase letters showed significant differences among the samples ($P < 0.05$).

3.4. POV analysis in three oil systems

To compare the effectiveness of these antioxidants in inhibiting lipid oxidation in different types of oils, POV was used to assess the antioxidant properties of the sample extracts and the common antioxidants TP and TBHQ on six edible oils containing oleic acid, linoleic acid and linolenic acid in this study.

The effectiveness of antioxidants in inhibiting lipid oxidation across different types of oils was assessed using peroxide values (POV) in this study. As depicted in Fig. 4(a) and (b), oils rich in oleic acid, particularly camellia oil (CLO) containing 74.63% oleic acid, treated with antioxidants AGR and AME, exhibited less oxidation compared to those treated with ACH and ACA. For instance, at 18

Table 3
Bivariate correlation analysis between four main flavonoid compounds and three antioxidant capacities based on Pearson correlation coefficient test.

Compounds	FRAP	DPPH	ABTS
TFC	0.984**	0.980**	0.955**
TPC	0.980**	0.970**	0.951**
DMYC	0.720*	0.866**	0.928**
MRC	0.943**	0.990**	0.988**
MIC	−0.557	−0.587	−0.496
RC	0.588	0.818**	0.891**

Note: TFC, total flavonoids content; TPC, total phenolics content; DMYC, dihydromyricetin content; MRC, myricetrin content; MIC, myricetin compound; RC, rutin content; FRAP, ferric reducing antioxidant; DPPH·, DPPH radicals scavenging rate; ABTS·, ABTS radicals scavenging rate.

days, the POV of AGR and AME in CLO were both 0.28 g mM/kg oil, while in olive oil (OLO), they were 0.39 and 0.54 mM/kg oil, respectively, which was close to that of TBHQ but significantly lower than TP (Table 4 (a)). Notably, the oxidation rate of OLO was slower and more stable than that of CLO during the period from 15 to 27 days. Overall, in the oleic acid system, there was no significant difference between the antioxidant abilities of AGR and AME, which were comparable to TBHQ, followed by TP, ACA, and ACH.

Furthermore, in sunflower oil (SFO), all four extracts demonstrated comparable antioxidant effects, which were superior to TP but not as effective as TBHQ (Fig. 4(c)). Specifically, the POV was 1.07 g mM/kg oil for the blank control, 1.03 mM/kg oil for TP, mM/kg oil for TBHQ, 0.95 mM/kg oil for AGR, 0.82 g mM/kg oil for AME, 0.84 mM/kg oil for ACH, and 0.86 mM/kg oil for ACA at 18 d. The POV values reached a peak on day 15 and exhibited better antioxidant ability before 18 days compared to later periods, indicating a temporary boost in antioxidant activity. Conversely, in the linolenic acid system, the extracts in perilla seed oil (PSO) and linseed oil (LSO) showed less inhibition effects, as their POV with different treatments was close to TP before 18 days and even higher than the control after 18 days (Fig. 4(e) and (f)).

As for linolenic acid system, the extracts in PSO and LSO showed less inhibition effects, as their POV with different treatments was close to TP before 18 d, and even higher than the control after 18 d (Fig. 4 (f) and Table 4 (c)). Moreover, it can be observed that the antioxidant effects of the six treatments in linoleic acid and linolenic acid systems were not as stable as those in oleic acid system after the oven thermal oxidation treatment. The antioxidant effects in sesame oil (SMO) and perilla seed oil (PSO) were less stable compared to oleic and linoleic acid systems (Fig. 4(d) and (e)). The observed differences in the trends of POV values across various oil systems can be attributed to variations in the composition of fatty acids and other compounds present in each type of oil. For instance, both sesame oil (SMO) and PSO exhibited peaks in POV values during oven storage at 60 °C, suggesting a temporary increase in oxidation during this period. These fluctuations indicate a less predictable antioxidant behavior in oils rich in linolenic acid compared to oleic and linoleic acid systems.

Overall, the varying tendencies observed in Fig. 4 and Table 4 can be attributed to differences in the composition of fatty acids and other compounds present in each type of oil, as well as the effectiveness of the antioxidants used in inhibiting lipid oxidation under different conditions. Further research may be needed to fully understand the mechanisms underlying these observed trends and to optimize antioxidant strategies for different oil systems.

4. Discussion

Nekemias species exhibit varying antioxidant properties, influenced by factors such as species, growth environments, and growth stages. In this study, we analyze the antioxidant capacity of extracts from four *Nekemias* plants under identical growth conditions and stages. Additionally, we explore the potential bioavailability and biological activity of these compounds.

Prior research has highlighted the antioxidant potential of *Nekemias* species. Vine tea extract, rich in flavonoids and phenolics, has shown significant antioxidant activity *in vitro* [41–43]. Green leaves and tender tip leaves from *N. grossedentata* have demonstrated strong reducing and free radical scavenging abilities due to their flavonoid content, including dihydromyricetin and myricetrin [15,16]. In the study, we found that the *N. grossedentata* (AGR) extract exhibited robust antioxidant activity, similar to *N. megalophylla* (AME), surpassing *N. carnosifolia* (ACA) and *N. heterophylla* (ACH) extracts. These observations suggest a correlation between specific components, such as dihydromyricetin and myricetrin, and antioxidant activity.

Antioxidants in *Nekemias* extracts, such as polyphenols and flavonoids, function by donating hydrogen atoms or electrons to free radicals in the plant oils [44,45]. These antioxidants possess stable atomic structures with sites for electron or hydrogen donation. Free radicals, with their chemically unstable atomic structures due to unpaired electrons, initiate oxidative reactions in fatty acids [46]. Antioxidants react with free radicals, stabilizing them and halting further lipid peroxidation. This process interrupts the chain reaction of oxidative damage [47].

Oleic acid (monounsaturated fatty acid) is less prone to oxidation compared to polyunsaturated fatty acids due to the absence of multiple double bonds. Linoleic acid (polyunsaturated fatty acid with 2 Double Bonds) is more susceptible to oxidation due to its multiple double bonds, which create vulnerable sites for free radical attack. Linolenic acid (polyunsaturated fatty acid with 3 double bonds) is the most vulnerable to oxidation among the three fatty acids due to its higher number of double bonds. Antioxidants "quench" free radicals by stabilizing them through chemical reactions, preventing further oxidative damage to the vegetable oils containing oleic, linoleic, or linolenic acid [48].

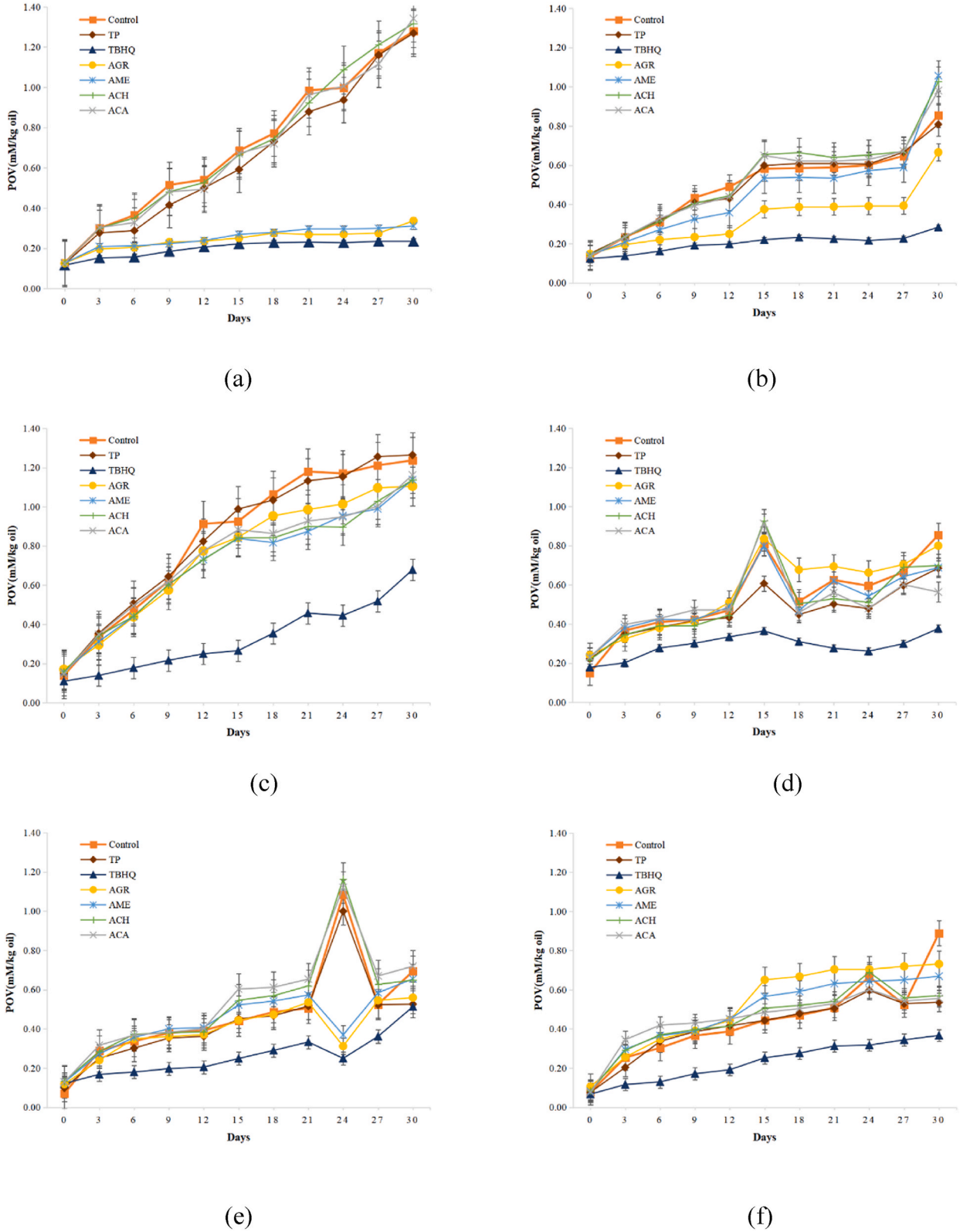

Fig. 4. Peroxide values in camellia oil (a), olive oil (b), sunflower oil (c), sesame oil (d), perilla seed oil (e) and linseed oil (f). Note: Control (blank control), TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and four extracts of AGR (*N. grossedentata*), AME (*N. megalophylla*), ACH (*N. chaffanjonii*) and ACA (*N. cantoniensis*).

Table 4
Determination of peroxide values (POV) in oleic acid system (a), Linoleic acid system (b) and Linolenic acid system (c) under 60 °C incubation for 30 day.

	Con.	TP	TBHQ	AGR	AME	ACH	ACA
POV (mM/kg oil)							
Camellia oil (Oleic acid)							
0d	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.00^{a}	0.13 ± 0.01^{a}	0.13 ± 0.00^{a}
3d	0.30 ± 0.02^{ab}	0.28 ± 0.01^{b}	0.15 ± 0.00^{d}	0.20 ± 0.01^{c}	0.21 ± 0.01^{c}	0.30 ± 0.02^{ab}	0.30 ± 0.01^{a}
6d	0.36 ± 0.01^{a}	0.29 ± 0.02^{c}	0.16 ± 0.00^{e}	0.20 ± 0.00^{d}	0.21 ± 0.00^{d}	0.35 ± 0.01^{a}	0.33 ± 0.01^{b}
9d	0.52 ± 0.03^{a}	0.42 ± 0.03^{b}	0.19 ± 0.02^{c}	0.23 ± 0.00^{c}	0.22 ± 0.01^{c}	0.48 ± 0.01^{a}	0.48 ± 0.03^{a}
12d	0.54 ± 0.03^{a}	0.50 ± 0.02^{a}	0.21 ± 0.03^{b}	0.24 ± 0.01^{b}	0.24 ± 0.02^{b}	0.53 ± 0.03^{a}	0.49 ± 0.08^{a}
15d	0.69 ± 0.06^{a}	0.59 ± 0.04^{b}	0.22 ± 0.01^{c}	0.25 ± 0.02^{c}	0.27 ± 0.01^{c}	0.66 ± 0.01^{a}	0.67 ± 0.01^{a}
18d	**0.77 ± 0.03^{a}**	**0.73 ± 0.02^{b}**	**0.23 ± 0.01^{d}**	**0.28 ± 0.01^{c}**	**0.28 ± 0.00^{c}**	**0.74 ± 0.02^{ab}**	**0.72 ± 0.02^{b}**
21d	0.99 ± 0.02^{a}	0.88 ± 0.04^{c}	0.23 ± 0.01^{e}	0.27 ± 0.01^{de}	0.30 ± 0.01^{d}	0.92 ± 0.00^{b}	0.96 ± 0.02^{ab}
24d	**1.00 ± 0.06^{b}**	**0.94 ± 0.06^{b}**	**0.23 ± 0.02^{c}**	**0.27 ± 0.01^{c}**	**0.30 ± 0.02^{c}**	**1.09 ± 0.06^{a}**	**1.01 ± 0.03^{ab}**
27d	1.17 ± 0.08^{a}	1.16 ± 0.05^{a}	0.24 ± 0.02^{b}	0.28 ± 0.01^{b}	0.30 ± 0.02^{b}	1.21 ± 0.13^{a}	1.12 ± 0.04^{a}
30d	1.28 ± 0.15^{a}	1.27 ± 0.07^{a}	0.23 ± 0.03^{b}	0.34 ± 0.06^{b}	0.31 ± 0.01^{b}	1.32 ± 0.18^{a}	1.34 ± 0.10^{a}
Olive oil (Oleic acid)							
0d	0.13 ± 0.01^{cd}	0.15 ± 0.00^{a}	0.12 ± 0.00^{d}	0.15 ± 0.01^{ab}	0.14 ± 0.00^{abc}	0.14 ± 0.00^{abc}	0.14 ± 0.01^{bc}
3d	0.23 ± 0.00^{a}	0.23 ± 0.00^{a}	0.14 ± 0.01^{c}	0.20 ± 0.01^{b}	0.21 ± 0.01^{b}	0.24 ± 0.01^{a}	0.23 ± 0.00^{a}
6d	0.31 ± 0.02^{a}	0.32 ± 0.04^{a}	0.16 ± 0.01^{c}	0.22 ± 0.01^{d}	0.27 ± 0.01^{b}	0.31 ± 0.01^{a}	0.33 ± 0.01^{a}
9d	0.43 ± 0.06^{a}	0.41 ± 0.01^{a}	0.19 ± 0.01^{c}	0.23 ± 0.00^{c}	0.33 ± 0.03^{b}	0.41 ± 0.03^{a}	0.39 ± 0.04^{a}
12d	0.49 ± 0.03^{a}	0.43 ± 0.01^{b}	0.20 ± 0.01^{e}	0.25 ± 0.01^{d}	0.36 ± 0.02^{c}	0.44 ± 0.03^{b}	0.44 ± 0.01^{b}
15d	0.58 ± 0.02^{b}	0.60 ± 0.01^{b}	0.22 ± 0.01^{e}	0.38 ± 0.03^{d}	0.53 ± 0.03^{c}	0.65 ± 0.03^{a}	0.65 ± 0.02^{a}
18d	**0.59 ± 0.01^{b}**	**0.61 ± 0.01^{b}**	**0.23 ± 0.02^{e}**	**0.39 ± 0.02^{d}**	**0.54 ± 0.03^{c}**	**0.66 ± 0.03^{a}**	**0.62 ± 0.04^{ab}**
21d	0.59 ± 0.02^{ab}	0.61 ± 0.02^{a}	0.22 ± 0.00^{d}	0.39 ± 0.02^{c}	0.53 ± 0.04^{b}	0.64 ± 0.02^{a}	0.62 ± 0.05^{a}
24d	**0.60 ± 0.01^{bc}**	**0.61 ± 0.02^{bc}**	**0.22 ± 0.02^{e}**	**0.39 ± 0.01^{d}**	**0.57 ± 0.02^{c}**	**0.65 ± 0.01^{a}**	**0.63 ± 0.03^{ab}**
27d	0.65 ± 0.01^{a}	0.67 ± 0.03^{a}	0.23 ± 0.02^{d}	0.39 ± 0.01^{c}	0.59 ± 0.00^{b}	0.67 ± 0.00^{a}	0.67 ± 0.01^{a}
30d	0.85 ± 0.08^{bc}	0.81 ± 0.02^{cd}	0.28 ± 0.02^{e}	0.67 ± 0.12^{d}	1.06 ± 0.09^{a}	1.03 ± 0.09^{a}	0.98 ± 0.05^{ab}
POV (mM/kg oil)							
Sunflower oil (Linoleic acid)							
0d	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.11 ± 0.01^{c}	0.17 ± 0.01^{c}	0.16 ± 0.02^{b}	0.16 ± 0.02^{a}	0.15 ± 0.01^{a}
3d	0.34 ± 0.01^{ab}	0.35 ± 0.02^{a}	0.14 ± 0.01^{d}	0.30 ± 0.01^{c}	0.31 ± 0.02^{bc}	0.34 ± 0.01^{a}	0.35 ± 0.01^{a}
6d	0.47 ± 0.02^{ab}	0.51 ± 0.02^{a}	0.18 ± 0.02^{c}	0.44 ± 0.04^{b}	0.44 ± 0.05^{b}	0.44 ± 0.01^{b}	0.50 ± 0.02^{ab}
9d	0.62 ± 0.02^{ab}	0.64 ± 0.01^{a}	0.22 ± 0.01^{c}	0.58 ± 0.02^{b}	0.60 ± 0.04^{ab}	0.60 ± 0.06^{ab}	0.62 ± 0.04a^{b}
12d	0.91 ± 0.04^{a}	0.82 ± 0.04^{ab}	0.25 ± 0.02^{c}	0.77 ± 0.10^{b}	0.73 ± 0.08^{b}	0.73 ± 0.05^{b}	0.77 ± 0.10^{b}
15d	0.92 ± 0.02^{a}	0.99 ± 0.07^{a}	0.27 ± 0.01^{b}	0.84 ± 0.15^{a}	0.84 ± 0.07^{a}	0.84 ± 0.08^{a}	0.88 ± 0.11^{a}
18d	**1.07 ± 0.14^{a}**	**1.03 ± 0.10^{ab}**	**0.35 ± 0.01^{c}**	**0.95 ± 0.01^{ab}**	**0.82 ± 0.14^{b}**	**0.84 ± 0.12^{ab}**	**0.86 ± 0.15^{ab}**
21d	1.18 ± 0.10^{a}	1.13 ± 0.14^{ab}	0.46 ± 0.04^{d}	0.99 ± 0.01^{bc}	0.87 ± 0.08^{c}	0.90 ± 0.04^{c}	0.93 ± 0.15^{c}
24d	**1.17 ± 0.13^{a}**	**1.15 ± 0.20^{ab}**	**0.45 ± 0.06^{c}**	**1.01 ± 0.13^{ab}**	**0.95 ± 0.11^{b}**	**0.90 ± 0.13^{b}**	**0.95 ± 0.12^{b}**
27d	1.21 ± 0.07^{ab}	1.26 ± 0.01^{a}	0.52 ± 0.04^{c}	1.10 ± 0.09^{ab}	0.99 ± 0.18^{b}	1.03 ± 0.16^{b}	1.00 ± 0.12^{b}
30d	1.24 ± 0.14^{a}	1.26 ± 0.07^{a}	0.68 ± 0.09^{b}	1.11 ± 0.04^{a}	1.14 ± 0.33^{a}	1.14 ± 0.03^{a}	1.16 ± 0.05^{a}
Sesame oil (Linoleic acid)							
0d	0.15 ± 0.01^{b}	0.22 ± 0.02^{a}	0.18 ± 0.00^{b}	0.24 ± 0.03^{a}	0.23 ± 0.00^{a}	0.22 ± 0.01^{a}	0.23 ± 0.01^{a}
3d	0.37 ± 0.01^{ab}	0.35 ± 0.01^{bc}	0.20 ± 0.05^{d}	0.32 ± 0.04^{c}	0.38 ± 0.01^{ab}	0.35 ± 0.00^{bc}	0.40 ± 0.01^{a}
6d	0.41 ± 0.02^{ab}	0.38 ± 0.01^{ab}	0.28 ± 0.03^{c}	0.38 ± 0.02^{c}	0.42 ± 0.01^{ab}	0.39 ± 0.04^{ab}	0.43 ± 0.04^{a}
9d	0.42 ± 0.03^{ab}	0.42 ± 0.04^{ab}	0.30 ± 0.00^{c}	0.41 ± 0.03^{b}	0.42 ± 0.03^{ab}	0.39 ± 0.04^{b}	0.47 ± 0.01^{a}
12d	0.47 ± 0.03^{a}	0.43 ± 0.06^{ab}	0.33 ± 0.03^{b}	0.51 ± 0.13^{a}	0.49 ± 0.03^{a}	0.45 ± 0.06^{ab}	0.47 ± 0.02^{a}
15d	0.81 ± 0.08^{a}	0.61 ± 0.20^{b}	0.36 ± 0.03^{c}	0.84 ± 0.04^{a}	0.80 ± 0.06^{ab}	0.93 ± 0.10^{a}	0.91 ± 0.06^{a}
18d	**0.52 ± 0.07^{b}**	**0.45 ± 0.05^{b}**	**0.31 ± 0.07^{c}**	**0.68 ± 0.10^{a}**	**0.47 ± 0.04^{b}**	**0.50 ± 0.07^{b}**	**0.46 ± 0.04^{b}**
21d	0.63 ± 0.01^{ab}	0.50 ± 0.05^{c}	0.28 ± 0.04^{d}	0.69 ± 0.08^{a}	0.62 ± 0.08^{ab}	0.53 ± 0.04^{b}	0.56 ± 0.03^{b}
24d	**0.60 ± 0.11^{ab}**	**0.48 ± 0.06^{b}**	**0.26 ± 0.05^{c}**	**0.66 ± 0.07^{a}**	**0.54 ± 0.10^{ab}**	**0.51 ± 0.09^{b}**	**0.48 ± 0.02^{b}**
27d	0.66 ± 0.09^{a}	0.60 ± 0.05^{a}	0.30 ± 0.03^{b}	0.70 ± 0.04^{a}	0.64 ± 0.08^{a}	0.69 ± 0.02^{a}	0.60 ± 0.09^{a}
30d	0.85 ± 0.15^{a}	0.69 ± 0.05^{bc}	0.38 ± 0.04^{d}	0.80 ± 0.04^{ab}	0.69 ± 0.09^{bc}	0.70 ± 0.00^{bc}	0.56 ± 0.04^{c}
POV (mM/kg oil)							
Perilla seed oil (Linolenic acid)							
0d	0.07 ± 0.00^{e}	0.10 ± 0.01^{d}	0.12 ± 0.00^{bc}	0.12 ± 0.00^{c}	0.13 ± 0.00^{ab}	0.13 ± 0.01^{a}	0.13 ± 0.00^{ab}
3d	0.29 ± 0.00^{b}	0.25 ± 0.01^{d}	0.17 ± 0.00^{e}	0.24 ± 0.00^{d}	0.27 ± 0.00^{c}	0.28 ± 0.01^{b}	0.32 ± 0.00^{a}
6d	0.34 ± 0.00^{d}	0.30 ± 0.01^{e}	0.18 ± 0.00^{f}	0.35 ± 0.01^{c}	0.36 ± 0.00^{c}	0.37 ± 0.01^{b}	0.37 ± 0.01^{b}
9d	0.38 ± 0.02^{c}	0.35 ± 0.00^{d}	0.20 ± 0.01^{e}	0.36 ± 0.00^{d}	0.40 ± 0.01^{b}	0.38 ± 0.00^{c}	0.38 ± 0.01^{c}
12d	0.39 ± 0.01^{bc}	0.36 ± 0.01^{d}	0.21 ± 0.00^{e}	0.37 ± 0.00^{d}	0.41 ± 0.01^{a}	0.38 ± 0.00^{c}	0.40 ± 0.01^{ab}
15d	0.44 ± 0.00^{d}	0.45 ± 0.00^{d}	0.25 ± 0.01^{e}	0.44 ± 0.00^{d}	0.52 ± 0.01^{c}	0.55 ± 0.01^{b}	0.60 ± 0.01^{a}
18d	**0.49 ± 0.01^{d}**	**0.47 ± 0.00^{e}**	**0.29 ± 0.00^{f}**	**0.47 ± 0.00^{e}**	**0.54 ± 0.00^{c}**	**0.57 ± 0.00^{b}**	**0.61 ± 0.01^{a}**
21d	0.51 ± 0.00^{e}	0.52 ± 0.01^{e}	0.33 ± 0.00^{f}	0.54 ± 0.00^{d}	0.57 ± 0.00^{c}	0.62 ± 0.00^{b}	0.65 ± 0.01^{a}
24d	**1.08 ± 0.05^{ab}**	**1.00 ± 0.07^{b}**	**0.25 ± 0.01^{d}**	**0.31 ± 0.01^{cd}**	**0.37 ± 0.02^{c}**	**1.16 ± 0.07^{a}**	**1.12 ± 0.11^{a}**
27d	0.52 ± 0.00^{e}	0.52 ± 0.00^{e}	0.36 ± 0.01^{f}	0.55 ± 0.01^{d}	0.58 ± 0.01^{c}	0.63 ± 0.01^{b}	0.67 ± 0.00^{a}
30d	0.69 ± 0.01^{a}	0.53 ± 0.00^{d}	0.51 ± 0.01^{d}	0.56 ± 0.01^{c}	0.65 ± 0.02^{b}	0.65 ± 0.03^{b}	0.72 ± 0.00^{a}
Linseed oil (Linolenic acid)							
0d	0.08 ± 0.00^{b}	0.08 ± 0.00^{b}	0.07 ± 0.00^{c}	0.11 ± 0.00^{a}	0.08 ± 0.00^{b}	0.08 ± 0.00^{b}	0.08 ± 0.01^{b}
3d	0.25 ± 0.00^{c}	0.20 ± 0.00^{d}	0.12 ± 0.01^{e}	0.26 ± 0.01^{c}	0.30 ± 0.00^{b}	0.29 ± 0.00^{b}	0.35 ± 0.00^{a}

(*continued on next page*)

Table 4 (*continued*)

	Con.	TP	TBHQ	AGR	AME	ACH	ACA
6d	0.30 ± 0.01^{e}	0.34 ± 0.02^{d}	0.13 ± 0.00^{f}	0.35 ± 0.00^{cd}	0.37 ± 0.00^{bc}	0.37 ± 0.01^{b}	0.42 ± 0.00^{a}
9d	0.37 ± 0.04^{b}	0.39 ± 0.04^{ab}	0.17 ± 0.01^{c}	0.39 ± 0.01^{ab}	0.39 ± 0.00^{ab}	0.40 ± 0.01^{ab}	0.43 ± 0.00^{a}
12d	0.39 ± 0.02^{c}	0.42 ± 0.01^{b}	0.19 ± 0.00^{d}	0.44 ± 0.00^{a}	0.45 ± 0.00^{a}	0.41 ± 0.01^{b}	0.45 ± 0.00^{a}
15d	0.44 ± 0.01^{e}	0.44 ± 0.00^{e}	0.25 ± 0.00^{f}	0.65 ± 0.00^{a}	0.57 ± 0.00^{b}	0.51 ± 0.00^{c}	0.48 ± 0.01^{d}
18d	$\mathbf{0.47 \pm 0.01^{d}}$	$\mathbf{0.48 \pm 0.01^{d}}$	$\mathbf{0.28 \pm 0.01^{e}}$	$\mathbf{0.67 \pm 0.00^{a}}$	$\mathbf{0.59 \pm 0.01^{b}}$	$\mathbf{0.52 \pm 0.01^{c}}$	$\mathbf{0.50 \pm 0.02^{c}}$
21d	0.51 ± 0.01^{d}	0.51 ± 0.01^{d}	0.31 ± 0.00^{e}	0.70 ± 0.01^{a}	0.63 ± 0.01^{b}	0.54 ± 0.01^{c}	0.53 ± 0.01^{c}
24d	$\mathbf{0.66 \pm 0.09^{a}}$	$\mathbf{0.60 \pm 0.05^{a}}$	$\mathbf{0.32 \pm 0.02^{b}}$	$\mathbf{0.70 \pm 0.04^{a}}$	$\mathbf{0.64 \pm 0.08^{a}}$	$\mathbf{0.69 \pm 0.02^{a}}$	$\mathbf{0.60 \pm 0.09^{a}}$
27d	0.52 ± 0.01^{e}	0.53 ± 0.00^{e}	0.34 ± 0.01^{f}	0.72 ± 0.01^{a}	0.65 ± 0.00^{b}	0.56 ± 0.00^{c}	0.54 ± 0.00^{d}
30d	0.89 ± 0.01^{a}	0.54 ± 0.01^{e}	0.37 ± 0.01^{f}	0.73 ± 0.01^{b}	0.67 ± 0.00^{c}	0.57 ± 0.00^{d}	0.56 ± 0.00^{d}

Note: Control (blank control), TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and four extracts of AGR (*N. grossedentata*), AME (*N. megalophylla*), ACH (*N. chaffanjonii*) and ACA (*N. cantoniensis*).
Different lowercase letters showed significant differences among the samples ($P < 0.05$).

We extended our investigation to assess the ability of *Nekemias* extracts to delay lipid oxidation in edible oils containing different fatty acids, including oleic acid, linoleic acid, and linolenic acid. The results revealed variations in antioxidant effects depending on the oil system and oleic acid content. Oleic acid systems benefited the most from AGR and AME extracts, with camellia oil exhibiting the highest efficacy. In linoleic acid systems, all four extracts showed similar antioxidant effects, while sesame oil exhibited initial effectiveness. However, perilla seed oil and linseed oil, rich in linolenic acid, displayed reduced antioxidant effects over time.

While our study highlights the antioxidant potential of *Nekemias* extracts, further research is needed to assess their applicability in clinical settings. *In vitro* experiments provide valuable insights, but *in vivo* studies and clinical trials are essential to understand their effects in real organisms. Acknowledging these limitations, *Nekemias* extracts show promise as natural antioxidants with diverse applications in health and food industries.

5. Conclusion

In conclusion, this research sheds light on the promising antioxidant potential of *Nekemias* species extracts, particularly *N. grossedentata* and *N. megalophylla*, as natural alternatives to synthetic antioxidants. These extracts exhibited robust antioxidant activity *in vitro*, linked to their high contents of total flavonoids, total phenolics, dihydromyricetin, and myricetrin. Importantly, these extracts demonstrated effective inhibition of lipid oxidation in various edible oils, with the most significant impact observed in oleic acid-rich oils, such as camellia oil. Their antioxidant effects in oleic acid systems were on par with the synthetic antioxidant TBHQ and even surpassed the natural antioxidant TP. These findings have significant implications for the food industry, as *Nekemias* species extracts could serve as valuable natural additives to prolong the shelf life of edible oils, mitigating the potential health risks associated with synthetic antioxidants. Moreover, this study contributes to the growing body of research on plant-derived natural antioxidants, offering potential solutions to the pressing need for safer and more sustainable alternatives to synthetic food additives. Future research should focus on exploring the application of *Nekemias* extracts in real-world food products and conducting *in vivo* studies to better understand their effects in living organisms. Additionally, investigations into the broader pharmacological and health-promoting properties of these extracts could unveil further opportunities for their utilization in both the food and pharmaceutical industries. Overall, this study represents a significant step toward harnessing the natural antioxidant potential of *Nekemias* species, contributing to the development of healthier and safer food products.

Funding

This research was funded by the National Natural Science Foundation of China [Grants No. 32060068 and No. U1812401] and Natural science research project of Guizhou Provincial Department of Education [Qianjiaoji [2022] 009].

CRediT authorship contribution statement

Yuan Zeng: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zhengwen Yu:** Supervision, Resources, Project administration, Methodology, Funding acquisition. **Chunyan Jiang:** Supervision, Methodology. **Jiayu Liu:** Methodology, Data curation. **Huanchun Yang:** Methodology, Data curation. **Hongli Pan:** Methodology, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zhengwen Yu reports financial support was provided by the National Natural Science Foundation of China [Grants No. 32060068 and No. U1812401] and Natural science research project of Guizhou Provincial Department of Education [Qianjiaoji [2022] 009].

References

[1] L. Fan, N.A.M. Eskin, The use of antioxidants in the preservation of edible oils, Handbook of Antioxidants for Food Preservation (2015) 373–388, https://doi.org/10.1016/b978-1-78242-089-7.00015-4.

[2] M. Safi, I.V. Shkhiyants, I.G. Fuks, et al., Effects of antioxidants on oxidation of various oils, Chem. Technol. Fuels Oils 16 (1980) 388–391, https://doi.org/10.1007/BF00727158.

[3] I. Marmol, C. Sanchez-de-Diego, N. Jimenez-Moreno, C. Ancin-Azpilicueta, M.J. Rodriguez-Yoldi, Therapeutic applications of rose hips from different rosa species, Int. J. Mol. Sci. 18 (2017) 1137 [Google Scholar] [CrossRef] [PubMed].

[4] D. Samec, M. Maretic, I. Lugaric, A. Mesic, B. Salopek-Sondi, B. Duralija, Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis, Food Chem. 194 (2016) 828–834 [Google Scholar] [CrossRef] [PubMed].

[5] R. Al Akeel, Y. Al-Sheikh, A. Mateen, R. Syed, K. Janardhan, V.C. Gupta, Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains, Saudi J. Biol. Sci. 21 (2014) 147–151 [Google Scholar] [CrossRef] [PubMed] [Green Version].

[6] R. Siwach, J. Tokas, R. Seth, Use of lycopene as a natural antioxidant in extending the shelf-life of anhydrous cow milk fat, Food Chem. 199 (2016) 541–546 [Google Scholar] [CrossRef] [PubMed].

[7] C.J. Hugo, A. Hugo, Current trends in natural preservatives for fresh sausage products, Trends Food Sci. Technol. 45 (2015) 12–23 [Google Scholar] [CrossRef].

[8] Y. Yang, X. Song, X. Sui, B. Qi, Z. Wang, Y. Li, L. Jiang, Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability, Ind. Crops Prod. 80 (2016) 141–147 [Google Scholar] [CrossRef].

[9] C.E. Ochoa-Velasco, J.A. Guerrero-Beltran, The effects of modified atmospheres on prickly pear (*Opuntia albicarpa*) stored at different temperatures, Postharvest Biol. Technol. 111 (2016) 314–321 [Google Scholar] [CrossRef].

[10] X. Xu, A. Liu, S. Hu, I. Ares, M.R. Martínez-Larrañaga, X. Wang, M. Martínez, A. Anadón, M.A. Martínez, Synthetic phenolic anti-oxidants: metabolism, hazards and mechanism of action, Food Chem. 353 (2021 Aug 15) 129488, https://doi.org/10.1016/j.foodchem.2021.129488. Epub 2021 Mar 5. PMID: 33714793.

[11] O.I.A. Soltan, H.S.S. Gazwi, A.E. Ragab, A.S.M. Aljohani, I.M. El-Ashmawy, G.E.-S. Batiha, A.A. Hafiz, S.M. Abdel-Hameed, Assessment of bioactive phytochemicals and utilization of *Rosa canina* fruit extract as a novel natural antioxidant for mayonnaise, Molecules 28 (2023) 3350, https://doi.org/10.3390/molecules28083350.

[12] S. Mildner-Szkudlarz, R. Zawirska-Wojtasiak, W. Obuchowski, M. Gośliński, Evaluation of antioxidant activity of green tea extract and its effect on the biscuit's lipid fraction oxidative stability, J. Food Sci. 74 (8) (2009 Oct) S362–S370, https://doi.org/10.1111/j.1750-3841.2009.01313.x. PMID: 19799681.

[13] Gorji S. Ghorbani, M. Calingacion, H.E. Smyth, M. Fitzgerald, Effect of natural antioxidants on lipid oxidation in mayonnaise compared with BHA, the industry standard, Metabolomics 15 (8) (2019 Jul 25) 106, https://doi.org/10.1007/s11306-019-1568-4. PMID: 31346792.

[14] X. Zhang, Y. Xu, H. Xue, G.C. Jiang, X.J. Liu, Antioxidant activity of vine tea (*Ampelopsis grossedentata*) extract on lipid and protein oxidation in cooked mixed pork patties during refrigerated storage, Food Sci. Nutr. 7 (5) (2019 Apr 2) 1735–1745, https://doi.org/10.1002/fsn3.1013. PMID: 31139386; PMCID: PMC6526652.

[15] C. Jia, J. Li, M. Zhang, W. Ma, S. Zhao, R. Liu, X. Li, Antioxidant properties of the extracts of vine tea (*Ampelopsis grossedentata*) with the different color characteristics and inhibition of rapeseed and sunflower oil oxidation, Lebensm. Wiss. Technol. (2020) 110292, https://doi.org/10.1016/j.lwt.2020.110292.

[16] C. Jia, M. Zhang, W. Ma, J. Li, S. Zhao, S. Xiong, X. Hu, X. Li, Evaluation of antioxidant properties of the different tissues of vine tea (*Ampelopsis grossedentata*) in stripped canola oil and sunflower oil, J. Food Sci. 85 (4) (2020 Apr) 1082–1089, https://doi.org/10.1111/1750-3841.15092. Epub 2020 Mar 9. PMID: 32147839.

[17] T. Zeng, Y. Song, S. Qi, et al., A comprehensive review of vine tea: origin, research on Materia Medica, phytochemistry and pharmacology[J], J. Ethnopharmacol. (2023) 116788.

[18] X. Kou, N. Chen, Pharmacological potential of *ampelopsin* in Rattan tea, Food Sci. Hum. Wellness 1 (1) (2012) 14–18, https://doi.org/10.1016/j.fshw.2012.08.001.

[19] Y. Zhou, F. Shu, X. Liang, H. Chang, L. Shi, X. Peng, J. Zhu, M. Mi, *Ampelopsin* induces cell growth inhibition and apoptosis in breast cancer cells through ROS generation and endoplasmic reticulum stress pathway, PLoS One 9 (2) (2014 Feb 13) e89021, https://doi.org/10.1371/journal.pone.0089021. PMID: 24551210; PMCID: PMC3923868.

[20] D.Y. Wang, Isolation and structural elucidation of grossedentatasin, Journal of Zhang Zhou Teachers College 16 (4) (1999) 212–215.

[21] Z.Y. Luo, X.F. Fu, M.C. Wu, Progress on the study of *Ampelopsis grossedentata*, Food Sci. (N. Y.) 26 (8) (2005) 513–517. Author 1, A.; Author 2, B. Book Title, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.

[22] X. Xie, J. Wang, H. Zhang, Characterization and antitumor activities of a water-soluble polysaccharide from *Ampelopsis megalophylla*, Carbohydr. Polym. 129 (2015 Sep 20) 55–61, https://doi.org/10.1016/j.carbpol.2015.04.037. Epub 2015 Apr 27. PMID: 26050888.

[23] X.F. Xie, J.W. Wang, H.P. Zhang, Q.X. Li, B.Y. Chen, Chemical composition, antimicrobial and antioxidant activities of essential oil from *Ampelopsis megalophylla*, Nat. Prod. Res. 28 (12) (2014) 853–860, https://doi.org/10.1080/14786419.2014.886208. Epub 2014 Feb 13. PMID: 24524337.

[24] W.W. Li, L.S. Ding, B.G. Li, New phenol clycoside form *Ampelopsis chaffanjonij*, Natural Product Research and De-velopment 10 (2) (1988) 19–20.

[25] N. Van Thu, T.D. Cuong, T.M. Hung, H. Van Luong, M.H. Woo, J.S. Choi, J.H. Lee, J.A. Kim, B.S. Min, Antiinflammatory compounds from *Ampelopsis cantoniensis*, Nat. Prod. Commun. 10 (3) (2015 Mar) 383–385. PMID: 25924510.

[26] X. Li, Y. Liang, H. Feng, et al., Analysis of Meitan Cuiya Tea Aroma Components by GC-MS[C]//2017 3rd International Forum on Energy, Environment Science and Materials (IFEESM 2017), Atlantis Press, 2018, pp. 559–563.

[27] L. Ye, H. Wang, S.E. Duncan, W.N. Eigel, S.F. O'Keefe, Antioxidant activities of Vine Tea (*Ampelopsis grossedentata*) extract and its major component dihydromyricetin in soybean oil and cooked ground beef, Food Chem. 172 (2015 Apr 1) 416–422, https://doi.org/10.1016/j.foodchem.2014.09.090. Epub 2014 Sep 28. PMID: 25442572.

[28] S.A. Baba, S.A. Malik, Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii Blume*, J. Taibah Univ. Sci. 9 (4) (2015) 449–454, https://doi.org/10.1016/j.jtusci.2014.11.001.

[29] P. Stratil, B. Klejdus, V. Kubáň, Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods, J. Agric. Food Chem. 54 (3) (2006) 607–616, https://doi.org/10.1021/jf052334j.

[30] K. Farhadi, F. Esmaeilzadeh, M. Hatami, M. Forough, R. Molaie, Determination of phenolic compounds content and antioxidant active in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran, Food Chem. 199 (2016) 847–855 [Google Scholar] [CrossRef] [PubMed].

[31] X.C. Wei, C. Zheng, Y.J. Xu, Study of new technology on extraction of dihydromyricetin from Ampelapsis, Food Sci. Technol. 39 (9) (2007) 103–107.

[32] Y.F. Li, S.H. Ouyang, Y.Q. Chang, T.M. Wang, W.X. Li, H.Y. Tian, H. Cao, H. Kurihara, R.R. He, A comparative analysis of chemical compositions in Camellia sinensis var. Pu'anensis Kurihara, a novel Chinese tea, by HPLC and UFLC-Q-TOF-MS/MS, Food Chem. 216 (2017) 282–288 [CrossRef].

[33] Y. Zhao, W. Lai, A. Xu, J. Jin, Y. Wang, P. Xu, Characterizing relationships among chemicals, sensory attributes and in vitro bioactivities of black tea made from an anthocyanins-enriched tea cultivar, LWT–Food Sci. Technol. 132 (2020), https://doi.org/10.1016/j.lwt.2020.109814.

[34] I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1) (1996) 70–76, https://doi.org/10.1006/abio.1996.0292.

[35] L. Liu, Y. Sun, T. Laura, X. Liang, H. Ye, X. Zeng, Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C, J. Tseng. Food Chem. 112 (1) (2009) 35–41, https://doi.org/10.1016/j.foodchem.2008.05.038.

[36] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (9–10) (1999 May) 1231–1237, https://doi.org/10.1016/s0891-5849(98)00315-3. PMID: 10381194.

[37] E. Ghohestani, J. Tashkhourian, B. Hemmateenejad, Colorimetric determination of peroxide value in vegetable oils using a paper based analytical device, Food Chem. 403 (2023 Mar 1) 134345, https://doi.org/10.1016/j.foodchem.2022.134345. Epub 2022 Sep 20. PMID: 36174343.

[38] M. Aleman, R. Bou, F. Guardiola, E. Durand, P. Villeneuve, C. Jacobsen, A.M. Sorensen, Antioxidative effect of lyophilized caffeic acid in fish oil enriched mayonnaise and milk, Food Chem. 167 (2015) 236–244 [Google Scholar] [CrossRef] [PubMed][Green Version].

[39] F. Eertmans, V. Bogaert, D. Volckaert, et al., Validation of a potentiometric peroxide value (POV) assay for analysis of mineral oil with low oxidative content[J], J. Chem. Pharmaceut. Res. 5 (2013) 395–402.
[40] R. Razavi, R.E. Kenari, Antioxidant evaluation of Fumaria parviflora L. extract loaded nanocapsules obtained by green extraction methods in oxidative stability of sunflower oil[J], J. Food Meas. Char. 15 (3) (2021) 2448–2457.
[41] Y. Zhang, W. Yang, H. Xiong, Basic constituent of *Ampelopsis grossedentata*, Natural Product Research and Devel-opment 13 (2001) 46–48.
[42] K. Xie, X. He, K. Chen, J. Chen, K. Sakao, D.X. Hou, Antioxidant properties of a traditional vine tea, Ampelopsis grossedentata, Antioxidants 8 (8) (2019 Aug 9) 295, https://doi.org/10.3390/antiox8080295. PMID: 31395833; PMCID: PMC6719964.
[43] R.C.V. Carneiro, L. Ye, N. Baek, et al., Vine tea (*Ampelopsis grossedentata*): a review of chemical composition, functional proper-ties, and potential food applications[J], J. Funct.Foods 76 (2021) 104317.
[44] M.S. Brewer, Natural antioxidants: sources, compounds, mechanisms of action, and potential applications[J], Compr. Rev. Food Sci. Food Saf. 10 (4) (2011) 221–247. Food lipids: chemistry, nutrition, and biotechnology[M]. CRC press, 2017.
[45] E. Choe, D.B. Min, Mechanisms and factors for edible oil oxidation[J], Compr. Rev. Food Sci. Food Saf. 5 (4) (2006) 169–186.
[46] E. Choe, D.B. Min, Mechanisms of antioxidants in the oxidation of foods[J], Compr. Rev. Food Sci. Food Saf. 8 (4) (2009) 345–358.
[47] T. Waraho, D.J. McClements, E.A. Decker, Mechanisms of lipid oxidation in food dispersions[J], Trends Food Sci. Technol. 22 (1) (2011) 3–13.
[48] M. Machado, L.M. Rodriguez-Alcala, A.M. Gomes, et al., Vegetable oils oxidation: mechanisms, consequences and protective strategies[J], Food Rev. Int. 39 (7) (2023) 4180–4197.