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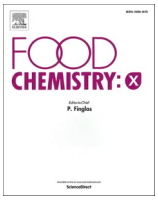

Comparative study on the antioxidant efficacy of *Adinandra nitida* extracts in inhibiting lipid oxidation in edible oils

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ABSTRACT

Exploring natural antioxidants is essential to delay lipid oxidation. This study investigated the inhibitory effect of *Adinandra nitida* (AN) extract in six edible oils, compared to TP and TBHQ. Methods included extract preparation, bioactive compounds analysis, in vitro antioxidant activities by FRAP, DPPH, and ABTS assays, fatty acid composition detection, and POV determination. The results showed that AN was rich in total flavonoids, total phenols and had better iron ion reduction ability than TBHQ. In oleic and linoleic acid-rich oils, AN significantly delayed early-stage lipid oxidation, outperforming TP and TBHQ. In linolenic acid-rich oils, AN maintained a stable effect. Molecular docking studies revealed strong binding interactions between main compounds and fatty acids, with Camelliaside A in (7.83) showing higher binding energy to linolenic acid than TBHQ (7.64), supporting the antioxidant effects. These findings suggest AN as a promising natural alternative to synthetic antioxidants, enhancing oil stability and shelf life.

1. Introduction

In the current landscape of increasing health consciousness, there is a growing demand for natural, safe, and effective antioxidants. These compounds play a crucial role in mitigating oxidative processes that lead to the deterioration of consumables, particularly edible oils. While synthetic antioxidants like BHA, TBHQ, and BHT have traditionally been used for this purpose, concerns about their long-term safety and potential health risks have prompted a shift towards natural alternatives (Shipp & Abdel-Aal, 2010). Among these, tea extracts have garnered significant attention due to their safety and exceptional antioxidant properties. Tea extracts, rich in polyphenolic compounds such as catechins and caffeine, exhibit strong free radical-scavenging abilities (Unachukwu et al., 2010; Zhang, Ho, et al., 2019; Zhao et al., 2020), which protect cells and tissues from oxidative damage—a key factor in the development of various chronic diseases (Senanayake, 2013). Moreover, tea has demonstrated efficacy in inhibiting lipid oxidation (Mildner-Szkudlarz et al., 2009; Zhang, Xu, et al., 2019), further enhancing its appeal as a natural antioxidant.

Adinandra nitida Merr. ex H. L. Li, commonly known as Shiya tea or Shibi tea, is a species from the Pentaphylacaceae family that typically grows on cliffs and is predominantly found in Southeast China, including Guangxi, Guangdong, Guizhou, Sichuan, and Yunnan Provinces (Chen et al., 2015). This evergreen shrub has been consumed as a traditional tea and herbal medicine in China for centuries (Chen, Ma, et al., 2017). It is recognized for its health benefits, primarily due to its high content of flavonoids and tea polyphenols, with minimal caffeine content, comprising over 20 % of these beneficial compounds (Chen et al., 2022; Liu et al., 2010). Recent studies have highlighted the multiple pharmacological properties of *Adinandra nitida*, including antioxidant, antibacterial, anti-inflammatory, antitoxic, antitumor, and analgesic effects (Chen, Ma, et al., 2017; Gao et al., 2010). Additionally, it has been shown to lower blood pressure and blood lipid levels, suggesting its potential in treating obesity and diabetes (Yuan et al., 2019; Zhao et al., 2021). This plant thrives in well-drained soils on cliffs and mountainous regions, making it a resilient species adapted to specific ecological niches (Liu et al., 2010). Its historical and medicinal significance, combined with its health benefits, underscore its importance as a natural therapeutic agent with promising applications in modern medicine.

Despite the well-documented benefits of tea extracts, their application in extending the shelf life of food, particularly edible oils, has been relatively underexplored. This study aims to address this gap by investigating the antioxidant properties of tea extracts from *Adinandra nitida*. The research will comprehensively analyze the bioactive compounds in

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these extracts, evaluate their in *vitro* antioxidant potential, and assess their ability to delay lipid oxidation in six distinct edible oils. The performance of these natural extracts will be compared to conventional antioxidants such as TBHQ and TP (tea polyphenols). To ensure a thorough analysis, the edible oils will be categorized into oleic acid, linoleic acid, and linolenic acid systems (Table of Contents).

In a world where health and sustainability are increasingly prioritized, this research seeks to redefine the role of tea extracts as natural antioxidants, contributing to the improvement of food preservation methods and nutritional quality. By harnessing the power of nature, this study aims to present a sustainable and healthier alternative to synthetic additives, paving the way for a tastier and healthier future.

2. Materials and methods

2.1. Chemicals and equipment

All reagents used in this study were sourced to meet analytical grade standards. Specifically, high-performance liquid chromatography (HPLC) grade acetonitrile and methanol were procured from Merck, Germany. Additional analytical reagents were obtained from Tianjin Kemeiou Chemical Reagent Co., Ltd. The antioxidants TBHQ and TP, crucial for our experiments, were supplied by Beijing Xinda Food Additives Co., Ltd.

2.2. Preparation of tea extracts

Tea material were sourced from the 1509 Engineering Research Center for Development and Utilization of Substitute Tea Plants at the College of Life Science, Guizhou Normal University. Fresh tea leaves were air-dried in a controlled environment to reduce moisture content. The leaves were then further dried in a drying oven (Model 101-2AB, Tianjin Taisite Instrument Co., Ltd., China) at 60 °C for 24 h to achieve complete dehydration.

After drying, the leaves were ground into a fine powder using a laboratory grinder (Model CXP-100, Shanghai Xingyue Instrument Co., Ltd., China) at 20,000 rpm for 5 min to achieve a particle size of approximately 100 μm. For extraction, 10 g of the tea powder was mixed with 500 mL of 70 % ethanol (*v*/v) in a 1 L Erlenmeyer flask. The mixture was sonicated using an ultrasonic cleaner (Model DK-1500D, Guangdong Dalishen Ultrasonic Technology Co., Ltd., China) at 40 kHz and 150 W for 30 min at 25 °C to enhance extraction efficiency.

The mixture was then filtered through a Büchner funnel using Whatman No. 1 filter paper. The filtration was repeated three times to ensure clarity. The filtrate was concentrated using a rotary evaporator (Model N-1001, Eyela, Tokyo Rikakikai Co., Ltd., Japan) at 40 °C under reduced pressure (0.09 MPa) to remove the majority of the ethanol. The concentrated extract was mixed with 20 mL of acetone to precipitate impurities, followed by another round of evaporation under the same conditions. The final product, a dry powder of concentrated tea extract, was stored at −20 °C in an airtight container until further analysis.

2.3. Determination of bioactive components

1 g of dry powder was mixed with 30 % (*v*/v) aqueous ethanol for reflux extraction. The ethanol concentration was adjusted in a 100 mL volumetric flask to the desired level. This solution was analyzed to quantify total flavonoids, total phenolics, and primary bioactive compounds.

2.3.1. Total flavonoids content

The total flavonoids content (TFC) was measured using a modified method (Cabrera et al., 2003; Farhadi et al., 2016). 3 mL of tea solution was diluted with deionized water to 6 mL in a 250 mL flask. After adding 1 mL of 5 % $NaNO_2$ solution, the mixture was shaken and left to stand for 6 min. Then, 10 mL of NaOH solution was added, and deionized water was used to reach the calibration mark. The solution was shaken again and allowed to sit for 15 min. The flavonoid content was measured at 500 nm using a UV spectrophotometer (UV759CRT, Shanghai, China). A rutin standard solution was used for the color reaction, and a linear regression equation was derived to calculate the total flavonoids as mg rutin equivalent (RE) per g of extract.

2.3.2. Total phenolics content

The total phenolics content (TPC) was determined using the Folin-Ciocalteu method (Abdel-Aal et al., 2022; 34. Jiang et al., 2013). 1 mL of tea extract was mixed with 5 mL of 10 % Folin-Ciocalteu reagent in a test tube and allowed to react for 3 to 8 min. Then, 4 mL of 7.5 g/100 mL Na_2CO_3 solution was added, and the mixture was left at room temperature for 60 min. The absorbance was measured at 765 nm using a spectrophotometer. A standard curve was prepared using gallic acid, and the phenolic content was expressed as mg gallic acid equivalents (GAE) per g of tea extract.

2.4. Antioxidant activities in vitro of tea extracts determination using FRAP, DPPH and ABTS assays

The Ferric Reducing Antioxidant Power (FRAP) assay was performed with minor modifications from the original method (Benzie & Strain, 1996). Briefly, 0.2 mL of the diluted tea extract was mixed with 3 mL of freshly prepared FRAP reagent. The mixture was incubated in a water bath at 37 °C for 10 min. After incubation, the absorbance was measured at 593 nm using a spectrophotometer. $FeSO_4$ solution was used as the standard, and the antioxidant capacity of the tea extracts was expressed as mg of $FeSO_4$ equivalents per g of the sample (mg $FeSO_4$/g).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity was evaluated following the protocol method (Liu et al., 2009). A 1 mL aliquot of the diluted tea extract was added to 3 mL of 0.1 mM DPPH radical solution. The reaction mixture was allowed to stand in the dark at room temperature for 30 min. The absorbance was recorded at 517 nm, with A_1 representing the absorbance of the DPPH solution with the sample, A_0 representing the absorbance of the DPPH solution with ethanol instead of the sample, and A_2 representing the absorbance of the sample solution with ethanol instead of DPPH. The DPPH radical scavenging activity was calculated using the formula:

$$\text{DPPH} \bullet \text{ scavenge rate} = [A_0 - (A_1 - A_2)]/A_0 \times 100\% \quad (1)$$

The DPPH scavenging activity and IC_{50} value were determined via a regression equation based on the DPPH clearance values. All samples underwent triplicate analysis for result accuracy.

The Trolox Equivalent Antioxidant Capacity (TEAC) assay, based on the scavenging of $ABTS^{\bullet+}$ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cations, was conducted as per the described method (Re et al., 1999). The ABTS radical cation was generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to react in the dark at room temperature for 12–16 h before use. The working solution was diluted with phosphate-buffered saline (PBS) to achieve an absorbance of 0.700 ± 0.020 at 734 nm. Tea extract samples were then mixed with this $ABTS^{\bullet+}$ working solution and incubated in a 37 °C water bath for 10 min. After incubation, the absorbance of the reaction mixture was measured at 734 nm (Ai). To account for background absorbance, two control samples were prepared: one with PBS replacing the tea extract (Ac) and another with the tea extract replacing the ABTS solution (Aj). The ABTS radical scavenging activity was calculated using the formula:

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

The IC_{50} value, indicating the concentration of tea extract required to scavenge 50 % of $ABTS^{\bullet+}$ radicals, was determined from the dose-response curve.

2.5. Preparation and determination of edible oils

In this study, six different types of edible oils were analyzed: camellia oil, olive oil, sunflower oil, sesame oil, perilla seed oil, and linseed oil. These oils were sourced from the 1509 Engineering Research Center for Development and Utilization of Substitute Tea Plants at Guizhou Universities, College of Life Science, Guizhou Normal University.

The analysis of the main components of these oils was conducted following a method previously established in the literatures (Li et al., 2018; Zeng et al., 2024). Initially, 0.1 g of each oil sample was accurately weighed and placed in a 100 mL round-bottom flask. To this, 5 mL of an extracting solution was added to dissolve the sample. This was followed by the addition of 5 mL of 0.5 mol/L KOH-methanol solution, and the mixture was shaken for 1 min. It was then incubated in an oven at 50 °C for about an hour. After incubation, the mixture was transferred to a separation funnel, to which approximately 3 mL of water was added. The upper layer was then separated, and the solvent was reclaimed under reduced pressure to obtain the fatty acid methyl ester.

For further analysis, 200 mL of ethyl acetate was used to dissolve the fatty acid methyl esters, and 1 μL of this solution was subjected to GC–MS analysis using a QP2010 instrument (Japan). The GC analysis utilized a ZB-1701 mid-polar column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:20. The injection port temperature was maintained at 240 °C, and the temperature program varied from 32 °C to 240 °C. MS analysis was performed in Electron Impact (EI) mode with an ionization voltage of 70 eV and an ion source temperature of 200 °C. A solvent delay time of 18 min was set, and the mass range from 33 to 450 u was scanned. Compound identification was carried out using standard spectral libraries, and quantification was achieved using the peak area normalization method. This approach ensured the accurate determination of the main components of each edible oil.

2.6. POV assays

The study aimed to assess accelerated lipid oxidation in edible oils using an oven-enhanced storage method combined with peroxide value (POV) assays (Ghohestani et al., 2022). Initially, 20 g of various edible oils were mixed with 0.05 % tea sample extracts in triangular flasks. For comparison, control groups were established with oils mixed with TBHQ (50 mg/100 mL) and TP (50 mg/100 mL), while another set with no antioxidants served as blank controls. These samples were then subjected to accelerated aging in an oven maintained at 60 ± 1 °C. To ensure homogeneity, the samples were stirred every 24 h, and their POV was measured at three-day intervals. The assays were conducted in triplicate to ensure reliability.

POV is a critical indicator of lipid oxidation and was measured using a modified titration method (Shantha & Decker, 1994; Zeng et al., 2024). Each assay involved taking 1 mL of oil, which was then dissolved in a 20 mL mixture of glacial acetic acid and chloroform (3:2 volume ratio). The solution was treated with 0.5 mL of saturated potassium iodide and shaken for 30 s, followed by a 3-min dark treatment to stabilize the reaction. Afterward, 50 mL of deionized water and 1 g/100 mL starch indicator were added. The titration was conducted using a 0.002 mol/L sodium thiosulfate solution until the endpoint was reached, indicated by a clear disappearance of the blue color.

The resultant POV values were calculated using the formula:

$$\mathrm{POV(mmol/kg\ oil)} = c \times (V_{oil} - V_{blank} 01269100/m \quad (3)$$

where c represents the concentration of the sodium thiosulfate solution, V_{oil} is the titrated volume of the oil sample, V_{blank} is the volume from the blank control, and m is the mass of the oil sample.

2.7. Calculation of binding energy of compound and oil at single-point energy level

The study into the interactions between key fatty acids and certain phenolic compounds commenced with the construction of molecular structures for oleic acid (cis-9-octadecenoic acid), linoleic acid (cis, cis-9,12-octadecadienoic acid), linolenic acid (cis, cis, cis-9,12,15-octadecatrienoic acid), and several bioactive compounds from tea (epigallocatechin gallate - EGCG, epicatechin gallate - ECG, epigallocatechin - EGC, epicatechin - EC) as well as the synthetic antioxidant TBHQ. These structures were crafted using Chem3D 14.0 and saved in SDF format to maintain their integrity.

Following structure creation, these molecules were processed using the Ligprep module within the Maestro 13.1 software suite. This step involved protonation state prediction and conformational optimization under the OLPS4 force field, ensuring that the molecules were in their most likely forms under physiological conditions.

To explore how these compounds interact with oleic acid, we utilized an Induced-Fit Docking approach. Each compound was docked with oleic acid to form initial small molecule-oil complexes, providing insights into potential binding modes and interactions.

For quantitative analysis of these interactions, quantum chemical calculations were performed using the Gaussian 16 software, a leading tool in computational chemistry. We utilized Density Functional Theory (DFT) at the B3LYP/3-21G level, a popular choice for its balance of computational efficiency and accuracy in modeling molecular interactions.Further, single-point energy calculations were conducted using the same computational approach. The binding energy (ΔG) for each complex was calculated using the formula:

$$\Delta G = E_{complex} - E_{compound1} - E_{compound2} - E_{compound3} \quad (4)$$

2.8. Reagent procurement and statistical analysis

For the statistical analysis of data from sample extracts, values were presented as means along with their standard deviations to assess variability. We employed a series of statistical tests to analyze the data rigorously: one-way ANOVA was used to detect differences among groups, post-hoc Duncan's test was applied for multiple comparisons, and Pearson correlation analysis was conducted to explore the relationships between variables. Significance was noted at levels $*p < 0.05$ and $**p < 0.01$. All statistical computations were carried out using IBM SPSS version 26, which helped ensure a comprehensive analysis while avoiding redundancy, thus maintaining the integrity and clarity of our findings.

3. Results

3.1. Major components analysis of sample extracts

In this study, the total flavonoid content (TFC) and total phenolic content (TPC) of *Adinandra nitida* (AN) tea extracts were determined, as shown in Fig. 1. The results indicated that the TFC and TPC of AN were 880 mg/g and 393 mg/g, respectively. The TFC was notably higher than the TPC, emphasizing the substantial differences in the composition of bioactive compounds in the samples. This disparity highlights the unique chemical characteristics of AN, particularly its richness in flavonoids such as Camelliaside A and B, which are recognized as key active compounds (Chen et al., 2022).

3.2. Antioxidant activity in vitro

The antioxidant activity of AN tea extracts was evaluated using a series of established assays, including FRAP, DPPH free radical-scavenging activity, and TEAC. These were compared with tea polyphenols (TP) and tert-butylhydroquinone (TBHQ), as shown in Fig. 2

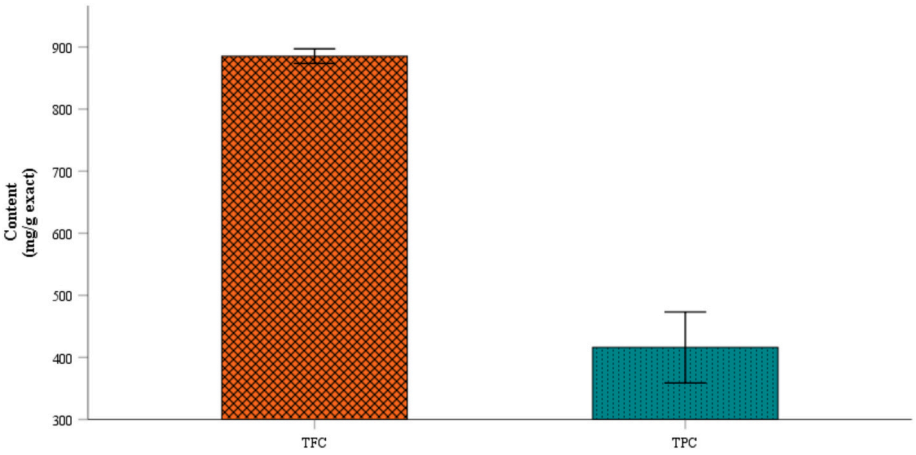


Fig. 1. The contents of total flavonoids and total phenolics of *Adinandra nitida*.

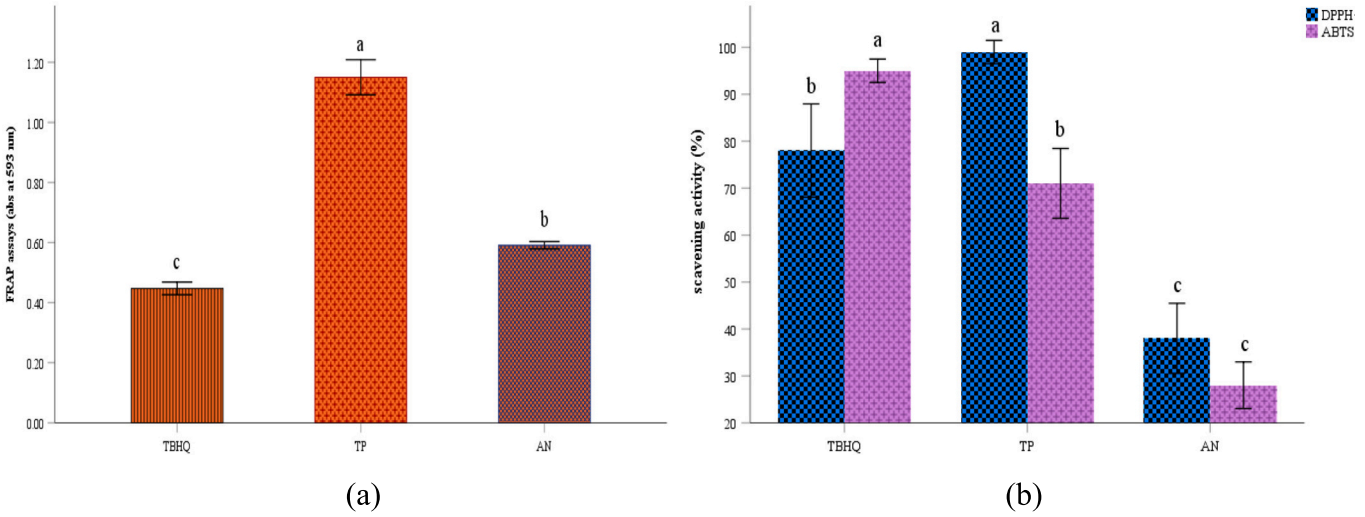


Fig. 2. The comparison of antioxidant abilities of tea sample extracts in vitro in terms of FRAP(a), DPPH and ABTS(b) based on TP and TBHQ under the same condition.
Note: TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and tea extract of AN (*Adinandra nitida*). Different lowercase letters showed significant differences among the samples. ($P < 0.05$).

and Table 1. In FRAP assays, the AN extract exhibited a value of 0.5861 mmol $FeSO_4$/g, which was lower than TP (1.1717 mmol $FeSO_4$/g) but higher than TBHQ (0.4429 mmol $FeSO_4$/g). However, the scavenging rates in both DPPH (38 %) and TEAC (28 %) assays were significantly lower than those of TP (99 %) and TBHQ (71 %), suggesting that while the AN extract showed superior iron ion reduction ability, its radical scavenging abilities were less effective than those of TP and TBHQ.

Table 1
Antioxidant activities of two sample 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)
TBHQ	107 ± 0.00	132 ± 3.68	0.33 ± 0.02
TP	73 ± 0.47	144 ± 2.83	1.53 ± 0.02
AN	123 ± 3.30	162 ± 1.70	0.52 ± 0.01

Data are presented as the mean ± standard deviation ($n \geq 3$).
Note: TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and tea extract of AN (*Adinandra nitida*). 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 %.

3.3. Main components of edible oil'

The fatty acid composition of six different edible oils was analyzed, categorizing them into three primary groups: oleic acid-rich oils (camellia oil and olive oil), linoleic acid-rich oils (sunflower oil and sesame oil), and linolenic acid-rich oils (perilla seed oil and linseed oil). The analysis revealed that camellia and olive oils had oleic acid contents of approximately 74.63 % and 69.96 %, respectively, while sunflower and sesame oils had linoleic acid contents of about 56.01 % and 52.84 %, respectively (Fig. 3). Perilla seed and linseed oils were characterized by linolenic acid contents of 47.43 % and 39.58 %, respectively, as detailed in Table 2. This categorization facilitated a clearer understanding of how different fatty acids interact with the antioxidant compounds in the oils.

3.4. POV analysis in three oil systems

The peroxide value (POV) analysis during an accelerated oxidation process at 60 °C over 30 days revealed significant differences in the

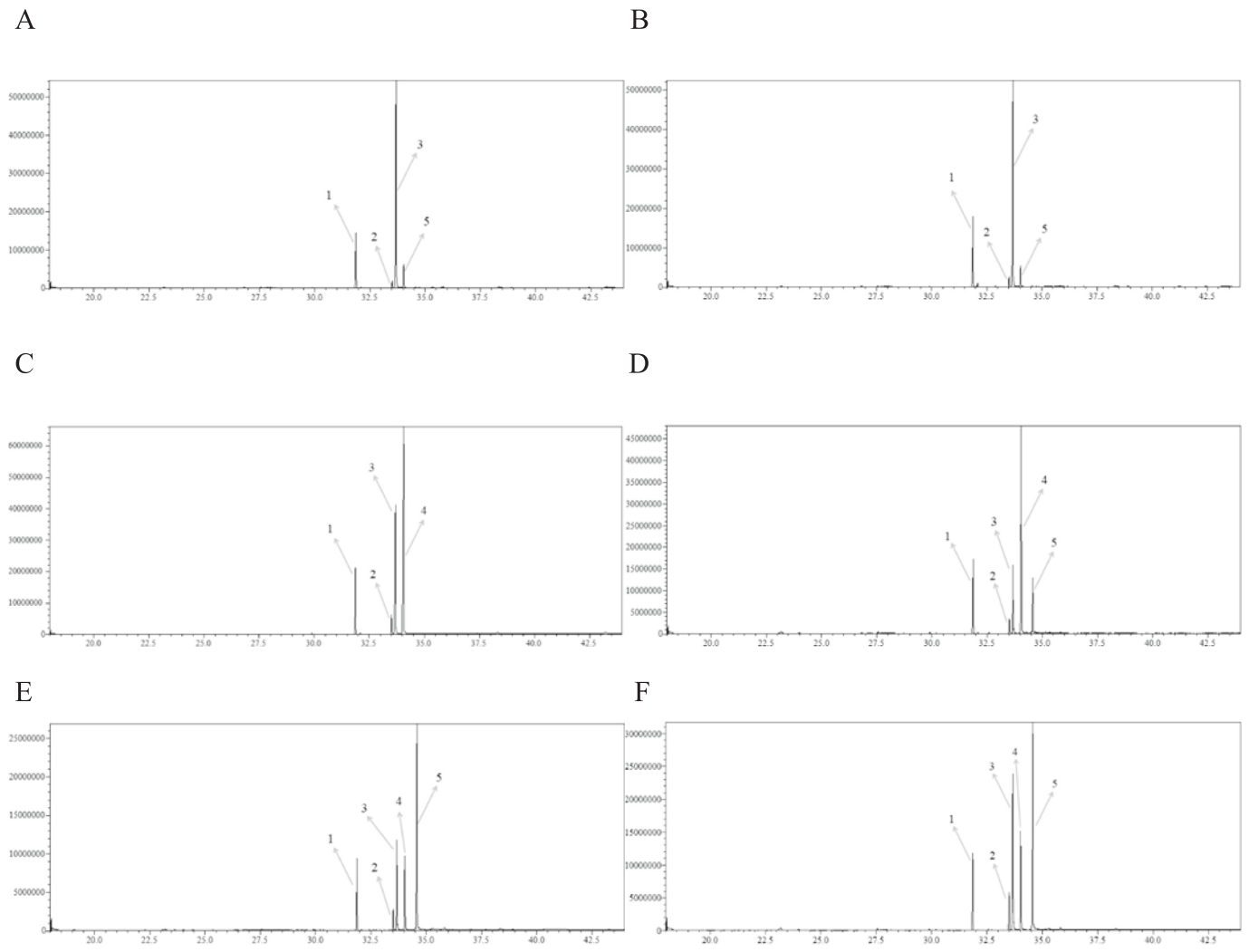


Fig. 3. GC Chromatograms of camellia oil (A), olive oil (B), sunflower oil (C), sesame oil (D), perillaseed oil (E) and linseed oil (F).
Note: The numbers 1 to 5 represent palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, and linolenic acid methyl ester.

Table 2
Composition of different fatty acids of six studied edible oils.

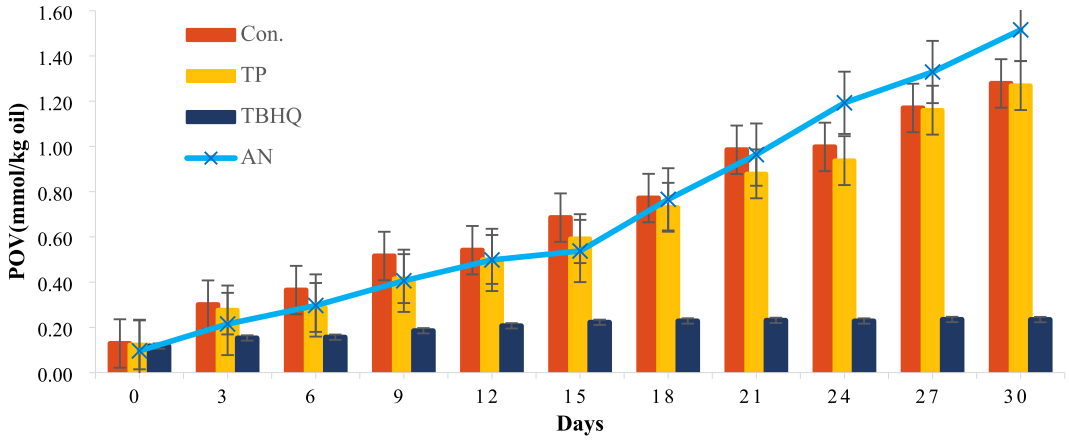
Components	Palmitic acid, methyl ester (%)	Stearic acid, methyl ester (%)	Oleic acid (%)	Linoleic acid, methyl ester (%)	Linolenic acid, methyl ester (%)
Rt	31.863	33.502	33.666	34.040	34.575
Camellia oil	16.27	2.16	**74.63**	6.94	–
Olive oil	20.60	3.28	**69.96**	6.16	–
Sunflower oil	13.69	3.94	26.36	**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**

antioxidant efficacy of AN extract compared to TP and TBHQ across different oil systems. In oleic acid-rich oils, such as camellia and olive oils, AN extract demonstrated superior antioxidant effects, particularly in the early stages of oxidation. By the 15th day, the POV for AN in camellia oil was 0.54 mmol/kg, lower than TP's 0.59 mmol/kg. Similar trends were observed in sunflower and sesame oils, where AN extract outperformed TP in delaying lipid oxidation.

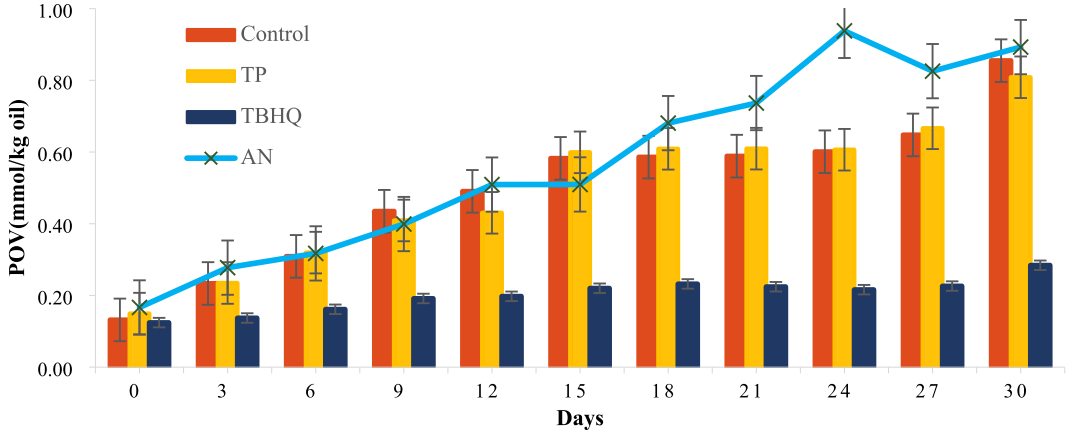
Fig. 4 and Table 3 illustrate the peroxide values observed during an oven experiment, which simulated oxidation at 60 °C for 30 days in three different oleic acid systems. The results highlighted notable changes in the antioxidant properties of AN extracts compared to TP and TBHQ. In oils with higher oleic acid content, such as camellia oil and olive oil, as well as oils with higher linoleic acid content, like sunflower oil and sesame oil, AN extract demonstrated superior antioxidant effects and a significant ability to delay the oxidative process compared to TP, particularly in the early stages of lipid oxidation.

By the 15th day, the peroxide values for AN in camellia oil, olive oil, sunflower oil, and sesame oil were 0.54, 1.00, 0.8, and 0.47 mmol/kg, respectively. These values were slightly lower than those for TP, which were 0.59, 0.60, 0.99, and 0.61 mmol/kg, respectively. Notably, in sesame oil at 15 days, the POV value for AN was the smallest, demonstrating similar stable antioxidant properties to TP. These results suggest that AN extract is a promising antioxidant in the pre-oxidative stage of edible oils with high oleic and linoleic acid content.

In oils rich in linolenic acid, such as linseed oil and Perilla seed oil, AN extract also showed effective antioxidant properties. For instance, the POV value for Perilla seed oil was 0.72 mmol/kg at 24 days and 0.47 mmol/kg at 21 days, which were lower than those for the TP group (1.00 and 0.51 mmol/kg, respectively). Although the POV values for Perilla seed oil (0.59–0.66 mmol/kg) after 24 days and flaxseed oil (0.64–0.71 mmol/kg) after 21 days were higher than those for TP, the values at other times were comparable to or even smaller than those for TP. This



(a)



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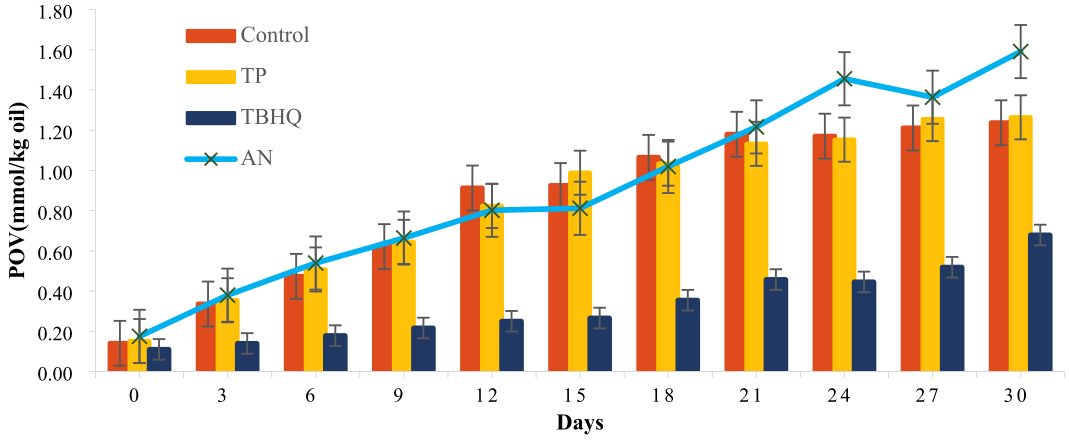
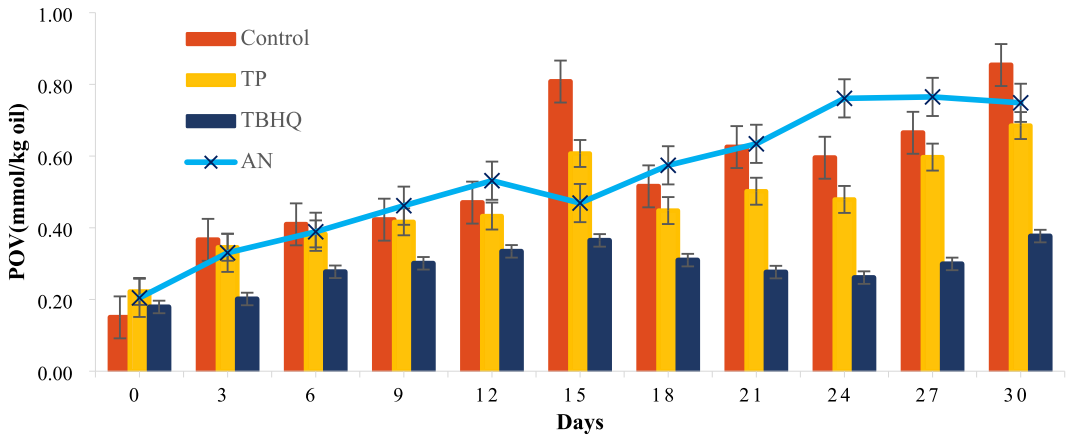


(c)

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 tea extract of AN (*Adinandra nitida*).



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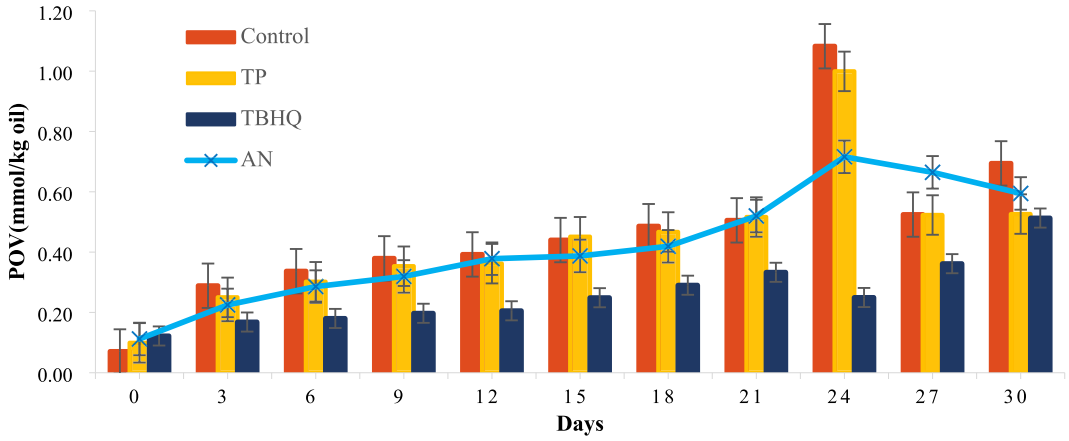

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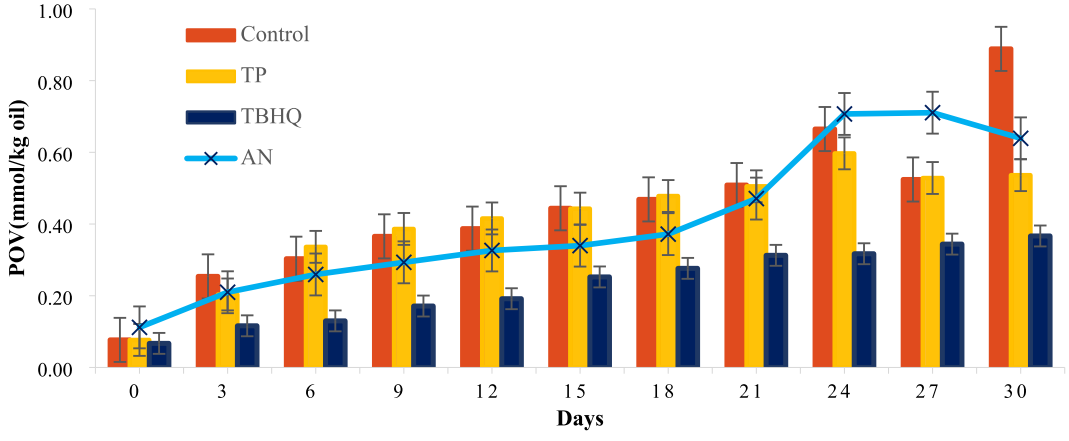

(f)

Fig. 4. (*continued*).

indicated that AN extract was effective in inhibiting lipid oxidation in cooking oils rich in linolenic acid.

Overall, these findings highlight the potential of AN extract as an effective antioxidant in various edible oils, particularly in the early stages of lipid oxidation in oleic and linoleic acid-rich edible oils and in the inhibition of linolenic acid-based oils. The AN extract's ability to maintain lower peroxide values compared to TP and TBHQ suggests its suitability for enhancing the stability and shelf-life of oils rich in oleic, linoleic, and linolenic acids.

Table 3
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	AN	TBHQ
POV (mmol/kg oil)				
Camellia oil (Oleic acid)				
0d	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.10 ± 0.01^{b}	0.12 ± 0.01^{a}
3d	0.30 ± 0.02^{a}	0.28 ± 0.01^{d}	0.22 ± 0.01^{c}	0.15 ± 0.00^{b}
6d	0.36 ± 0.01^{a}	0.29 ± 0.02^{c}	0.30 ± 0.00^{b}	0.16 ± 0.00^{b}
9d	0.52 ± 0.03^{a}	0.42 ± 0.03^{c}	0.41 ± 0.00^{b}	0.19 ± 0.02^{b}
12d	0.54 ± 0.03^{a}	0.50 ± 0.02^{b}	0.50 ± 0.01^{a}	0.21 ± 0.03^{a}
15d	**0.69 ± 0.06^{a}**	**0.59 ± 0.04^{c}**	**0.54 ± 0.01^{b}**	**0.22 ± 0.01^{b}**
18d	0.77 ± 0.03^{a}	0.73 ± 0.02^{b}	0.77 ± 0.01^{a}	0.23 ± 0.01^{a}
21d	0.99 ± 0.02^{a}	0.88 ± 0.04^{c}	0.96 ± 0.01^{a}	0.23 ± 0.01^{b}
24d	1.00 ± 0.06^{b}	0.94 ± 0.06^{c}	1.19 ± 0.01^{a}	0.23 ± 0.02^{b}
27d	1.17 ± 0.08^{b}	1.16 ± 0.05^{c}	1.33 ± 0.03^{a}	0.24 ± 0.02^{b}
30d	1.28 ± 0.15^{b}	1.27 ± 0.07^{c}	1.52 ± 0.01^{a}	0.23 ± 0.03^{b}
Olive oil (Oleic acid)				
0d	0.13 ± 0.01^{c}	0.15 ± 0.00^{c}	0.17 ± 0.01^{a}	0.12 ± 0.00^{b}
3d	0.23 ± 0.00^{b}	0.23 ± 0.00^{c}	0.28 ± 0.01^{a}	0.14 ± 0.01^{b}
6d	0.31 ± 0.02^{a}	0.32 ± 0.04^{b}	0.32 ± 0.00^{a}	0.16 ± 0.01^{a}
9d	0.43 ± 0.06^{a}	0.41 ± 0.01^{b}	0.40 ± 0.01^{a}	0.19 ± 0.01^{a}
12d	0.49 ± 0.03^{a}	0.43 ± 0.01^{c}	0.51 ± 0.01^{a}	0.20 ± 0.01^{b}
15d	**0.58 ± 0.02^{a}**	**0.60 ± 0.01^{c}**	**0.51 ± 0.00^{b}**	**0.22 ± 0.01^{a}**
18d	0.59 ± 0.01^{a}	0.61 ± 0.01^{b}	0.68 ± 0.10^{a}	0.23 ± 0.02^{a}
21d	0.59 ± 0.02^{b}	0.61 ± 0.02^{c}	0.74 ± 0.00^{a}	0.22 ± 0.00^{b}
24d	0.60 ± 0.01^{b}	0.61 ± 0.02^{c}	0.94 ± 0.03^{a}	0.22 ± 0.02^{b}
27d	0.65 ± 0.01^{b}	0.67 ± 0.03^{c}	0.83 ± 0.04^{a}	0.23 ± 0.02^{b}
30d	0.85 ± 0.08^{a}	0.81 ± 0.02^{b}	0.89 ± 0.02^{a}	0.28 ± 0.02^{a}

(a)

	Con.	TP	AN	TBHQ
POV (mmol/kg oil)				
Sunflower oil (Linoleic acid)				
0d	0.14 ± 0.01^{b}	0.15 ± 0.01^{c}	0.17 ± 0.00^{a}	0.11 ± 0.01^{b}
3d	0.34 ± 0.01^{b}	0.35 ± 0.02^{c}	0.38 ± 0.00^{a}	0.14 ± 0.01^{ab}
6d	0.47 ± 0.02^{b}	0.51 ± 0.02^{c}	0.54 ± 0.01^{a}	0.18 ± 0.02^{ab}
9d	0.62 ± 0.02^{b}	0.64 ± 0.01^{c}	0.66 ± 0.01^{a}	0.22 ± 0.01^{ab}
12d	0.91 ± 0.04^{a}	0.82 ± 0.04^{c}	0.80 ± 0.01^{b}	0.25 ± 0.02^{b}
15d	**0.92 ± 0.02^{a}**	**0.99 ± 0.07^{c}**	**0.81 ± 0.03^{b}**	**0.27 ± 0.01^{a}**
18d	1.07 ± 0.14^{a}	1.03 ± 0.10^{b}	1.02 ± 0.05^{a}	0.35 ± 0.01^{a}
21d	1.18 ± 0.10^{a}	1.13 ± 0.14^{b}	1.22 ± 0.04^{a}	0.46 ± 0.04^{a}
24d	1.17 ± 0.13^{b}	1.15 ± 0.20^{c}	1.46 ± 0.07^{a}	0.45 ± 0.06^{b}
27d	1.21 ± 0.07^{b}	1.26 ± 0.01^{c}	1.36 ± 0.03^{a}	0.52 ± 0.04^{b}
30d	1.24 ± 0.14^{b}	1.26 ± 0.07^{c}	1.59 ± 0.03^{a}	0.68 ± 0.09^{b}
Sesame oil (Linoleic acid)				
0d	0.15 ± 0.01^{a}	0.22 ± 0.02^{c}	0.20 ± 0.00^{b}	0.18 ± 0.00^{a}
3d	0.37 ± 0.01^{a}	0.35 ± 0.01^{c}	0.33 ± 0.01^{b}	0.20 ± 0.05^{ab}
6d	0.41 ± 0.02^{a}	0.38 ± 0.01^{b}	0.39 ± 0.02^{a}	0.28 ± 0.03^{a}
9d	0.42 ± 0.03^{a}	0.42 ± 0.04^{b}	0.46 ± 0.03^{a}	0.30 ± 0.00^{a}
12d	0.47 ± 0.03^{ab}	0.43 ± 0.06^{c}	0.53 ± 0.02^{a}	0.33 ± 0.03^{b}
15d	**0.81 ± 0.08^{a}**	**0.61 ± 0.20^{b}**	**0.47 ± 0.02^{b}**	**0.36 ± 0.03^{ab}**
18d	0.52 ± 0.07^{ab}	0.45 ± 0.05^{c}	0.57 ± 0.05^{a}	0.31 ± 0.07^{b}
21d	0.63 ± 0.01^{a}	0.50 ± 0.05^{c}	0.63 ± 0.05^{a}	0.28 ± 0.04^{b}
24d	0.60 ± 0.11^{b}	0.48 ± 0.06^{c}	0.76 ± 0.02^{a}	0.26 ± 0.05^{b}
27d	0.66 ± 0.09^{ab}	0.60 ± 0.05^{c}	0.77 ± 0.04^{a}	0.30 ± 0.03^{b}
30d	0.85 ± 0.15^{a}	0.69 ± 0.05^{c}	0.75 ± 0.03^{ab}	0.38 ± 0.04^{b}

(b)

	Con.	TP	AN	TBHQ
POV (mmol/kg oil)				
Perilla seed oil (Linolenic acid)				
0d	0.07 ± 0.00^{d}	0.10 ± 0.01^{a}	0.11 ± 0.00^{b}	0.12 ± 0.00^{c}
3d	0.29 ± 0.00^{a}	0.25 ± 0.01^{d}	0.22 ± 0.02^{c}	0.17 ± 0.00^{b}
6d	0.34 ± 0.00^{a}	0.30 ± 0.01^{d}	0.29 ± 0.00^{c}	0.18 ± 0.00^{b}
9d	0.38 ± 0.02^{a}	0.35 ± 0.00^{d}	0.32 ± 0.01^{c}	0.20 ± 0.01^{b}
12d	0.39 ± 0.01^{a}	0.36 ± 0.01^{d}	0.38 ± 0.01^{b}	0.21 ± 0.00^{c}
15d	0.44 ± 0.00^{a}	0.45 ± 0.00^{c}	0.39 ± 0.00^{b}	0.25 ± 0.01^{a}

(*continued on next page*)

Table 3 (*continued*)

	Con.	TP	AN	TBHQ
POV (mmol/kg oil)				
Perilla seed oil (Linolenic acid)				
18d	0.49 ± 0.01^{a}	0.47 ± 0.00^{c}	0.42 ± 0.03^{b}	0.29 ± 0.00^{a}
21d	0.51 ± 0.00^{a}	0.52 ± 0.01^{b}	0.52 ± 0.04^{a}	0.33 ± 0.00^{a}
24d	**1.08 ± 0.05^{a}**	**1.00 ± 0.07^{c}**	**0.72 ± 0.05^{b}**	**0.25 ± 0.01^{a}**
27d	0.52 ± 0.00^{b}	0.52 ± 0.00^{c}	0.66 ± 0.06^{a}	0.36 ± 0.01^{b}
30d	0.69 ± 0.01^{a}	0.53 ± 0.00^{c}	0.59 ± 0.03^{b}	0.51 ± 0.01^{c}
Linseed oil (Linolenic acid)				
0d	0.08 ± 0.00^{b}	0.08 ± 0.00^{c}	0.11 ± 0.01^{a}	0.07 ± 0.00^{b}
3d	0.25 ± 0.00^{a}	0.20 ± 0.00^{c}	0.21 ± 0.01^{b}	0.12 ± 0.01^{b}
6d	0.30 ± 0.01^{b}	0.34 ± 0.02^{d}	0.26 ± 0.01^{c}	0.13 ± 0.00^{a}
9d	0.37 ± 0.04^{a}	0.39 ± 0.04^{c}	0.29 ± 0.02^{b}	0.17 ± 0.01^{a}
12d	0.39 ± 0.02^{a}	0.42 ± 0.01^{c}	0.33 ± 0.02^{b}	0.19 ± 0.00^{a}
15d	0.44 ± 0.01^{a}	0.44 ± 0.00^{c}	0.34 ± 0.01^{b}	0.25 ± 0.00^{a}
18d	0.47 ± 0.01^{a}	0.48 ± 0.01^{c}	0.37 ± 0.02^{b}	0.28 ± 0.01^{a}
21d	**0.51 ± 0.01^{a}**	**0.51 ± 0.01^{b}**	**0.47 ± 0.04^{a}**	**0.31 ± 0.00^{a}**
24d	0.66 ± 0.09^{a}	0.60 ± 0.05^{b}	0.71 ± 0.07^{a}	0.32 ± 0.02^{a}
27d	0.52 ± 0.01^{b}	0.53 ± 0.00^{c}	0.71 ± 0.07^{a}	0.34 ± 0.01^{b}
30d	0.89 ± 0.01^{a}	0.54 ± 0.01^{d}	0.64 ± 0.04^{b}	0.37 ± 0.01^{c}
(c)				

Note: Control (blank control), TP (tea polyphenol, as control), TBHQ (tertiary butyl hydroquinone, as control), and tea extract of AN (*Adinandra nitida*). Different lowercase letters showed significant differences among the samples ($P < 0.05$).

3.5. Binding energy of compound-oil complexes at single-point energy level

Based on the existing results, this study also calculated the binding energies of small molecules such as Camelliasid A, Camelliasid B and TBHQ with three different fatty acids, and found that their binding modes and affinities were different.

The binding modes and energies of various small molecules with Camelliaside A, Camelliaside B, and tert-butylhydroquinone (TBHQ) were investigated (Fig. 5 (a), (b) and Table 4). Camelliaside A formed three hydrogen bonds with cis-9-octadecenoic acid (2.1, 2.4, and 2.6 Å) through hydroxyl/phenolic hydroxyl groups and the carboxyl group of the acid, along with hydrophobic interactions from the hydrocarbon side chain, resulting in a binding energy of −10.91 kcal/mol. In comparison, Camelliaside B formed two hydrogen bonds with cis-9-octadecenoic acid (2.1 and 2.3 Å) and also engaged in hydrophobic interactions, yielding a

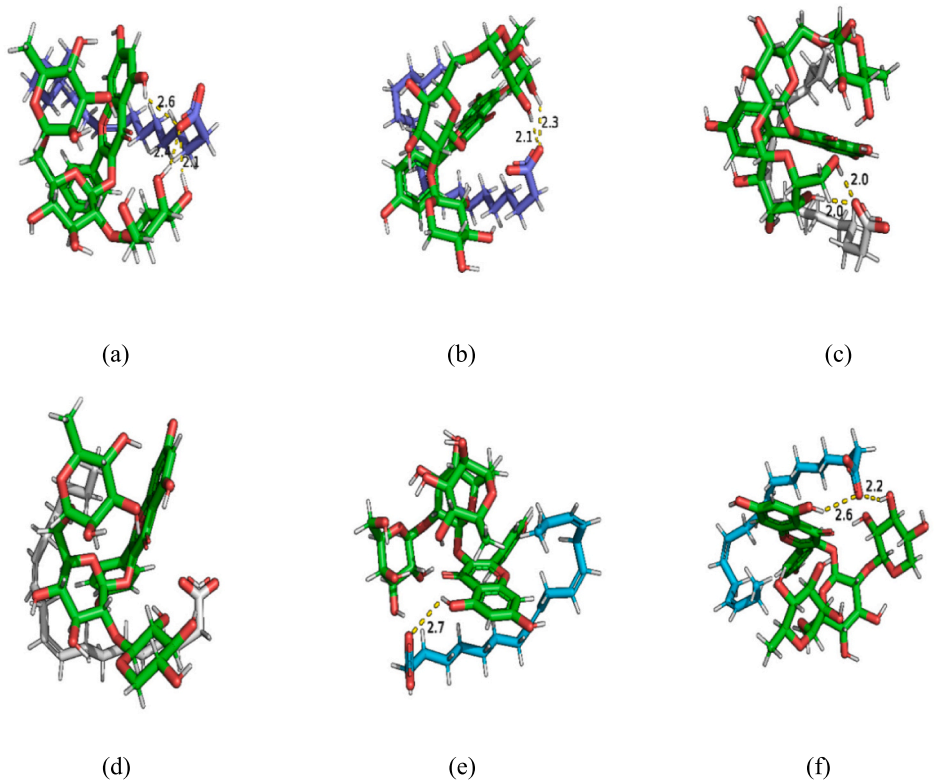

Fig. 5. Preliminary small-molecule complex conformations of Camelliaside A and Camelliaside B with cis-9-octadecenoic acid (a), (b), cis, cis-9,12-octadecadienoic acid (c), (d), and cis, cis, cis-9,12,15-octadecatrienoic acid (e), (f) by docking.

Table 4
The binding energy calculation by single point energy.

Complex energy	Compound1	Compound1 energy	Compound2	Compound3 energy	Binding energy
−1388.501809	9-octadecadienoic-acid	−851.5116468	TBHQ	−536.9699143	−12.70548848
−3622.08831	9-octadecadienoic-acid	−851.5166569	Camelliaside A	−2770.55528	−10.91037348
−3508.194101	9-octadecadienoic-acid	−851.5168923	Camelliaside B	−2526.661251	−10.01341212
−1387.266251	9,12-octadecadienoic-acid	−850.2833604	TBHQ	−536.9717147	−7.012977833
−3620.830491	9,12-octadecadienoic-acid	−850.282995	Camelliaside A	−2770.537456	−5.481918624
−3506.970982	9,12-octadecadienoic-acid	−850.2833224	Camelliaside B	−2526.677385	−6.447717726
−1386.037887	9,12,15-octadecatrienoic-acid	−849.0534606	TBHQ	−536.9722383	−7.648142443
−3619.630619	**9,12,15-octadecatrienoic-acid**	**−849.0517326**	**Camelliaside A**	**−2770.566396**	**−7.83758741**
−3505.733167	9,12,15-octadecatrienoic-acid	−849.0500575	Camelliaside B	−2656.674853	−5.180902556

binding energy of −10.01 kcal/mol. The interaction between cis-cis-cis-9-octadecatrienoic acid and TBHQ showed a binding energy of −7.01 kcal/mol.

Further analysis revealed that Camelliaside A formed two hydrogen bonds (2.0 Å) with cis, cis-9,12-octadecadienoic acid, with a binding energy of −5.48 kcal/mol, whereas Camelliaside B did not form any hydrogen or hydrophobic bonds with the same acid, resulting in a binding energy of −6.44 kcal/mol. The complex between cis, cis-9,12-octadecadienoic acid and TBHQ exhibited hydrophobic interactions, with a binding energy of −7.01 kcal/mol (Fig. 5 (c), (d) and Table 4).

In addition, Camelliaside A and cis, cis, cis-9,12,15-octadecatrienoic acid formed a hydrogen bond (2.7 Å) with a binding energy of −7.83 kcal/mol, while Camelliaside B formed two hydrogen bonds (2.2 and 2.6 Å) with the same acid, showing a binding energy of −5.18 kcal/mol. The interaction between cis, cis, cis-9,12,15-octadecatrienoic acid and TBHQ involved a hydrogen bond (2.1 Å) and hydrophobic interactions, resulting in a binding energy of −7.64 kcal/mol. These results highlight the varying binding affinities and interaction modes between these small molecules and the studied antioxidants (Fig. 5 (e), (b) and Table 4).

4. Discussion

The oxidative stability of edible oils is significantly influenced by factors such as temperature, oxygen availability, and the materials used in storage containers (Kodama et al., 1992; Zhang, Lyu, et al., 2019). High storage temperatures accelerate lipid oxidation, leading to the rapid degradation of oil quality. Even minimal oxygen exposure can initiate and propagate oxidative reactions, further compromising oil stability. Additionally, the type of material used for storage containers can greatly impact the rate of oxidation, with some materials potentially catalyzing these reactions and thereby reducing the oil's shelf life. While this study simulated accelerated aging at 60 °C to evaluate the antioxidant efficacy of AN extract, future experiments should consider the combined effects of these factors. Oils stored at lower temperatures, with reduced oxygen exposure, and in containers that minimize catalytic activity, would likely exhibit even greater stability when treated with AN extract.

This study highlights the significant antioxidant potential of *Adinandra nitida* (AN) extracts in inhibiting lipid oxidation across various edible oils, comparing its efficacy with that of tea polyphenols (TP) and tert-butylhydroquinone (TBHQ). Recent research has shown that *A. nitida* is rich in flavonoids and phenolic compounds (Zhang et al., 2005; Chen, Shen, et al., 2017), with a total flavonoid content dominated by camellia exceeding 20 % (Chen et al., 2022; Liu et al., 2010). The analysis of bioactive components and antioxidant activities revealed that AN extract contains high concentrations of total flavonoids and total phenolics, which contribute to its strong antioxidant properties. In vitro assays, including FRAP, DPPH, and ABTS, demonstrated that AN extract possesses significant antioxidant activity, although the efficacy varied among different tests. Specifically, AN showed superior iron-reducing antioxidant capacity (FRAP) compared to TBHQ, but lower free radical-scavenging activities (DPPH and ABTS) than both TP and TBHQ.

Previous studies have also found that leaf extracts of Adinandra nitida have good antioxidant and fat oxidation inhibition effects (Chen, Ma, et al., 2017; Yuan et al., 2019). This study investigated the antioxidant efficacy of AN extracts in six different edible oils, categorized based on their predominant fatty acids: oleic acid, linoleic acid, and linolenic acid. In camellia oil and olive oil, both rich in oleic acid, AN extracts significantly delayed lipid oxidation compared to TP and TBHQ, particularly in the early stages of oxidation. The peroxide values (POV) for AN were consistently lower than those for TP, indicating better protection against oxidative degradation. Similarly, in sunflower oil and sesame oil, which are high in linoleic acid, AN extract outperformed TP in inhibiting lipid oxidation, as evidenced by the lower POVs. In linseed oil and Perilla seed oil, rich in linolenic acid, AN extract also demonstrated effective antioxidant properties, with POVs comparable to or lower than those for TP at various time points.

Based on the POV data, which serve as indicators of lipid oxidation, the inclusion of AN extract in edible oils significantly delays the onset of oxidation. For oils rich in oleic and linoleic acids, such as camellia oil, olive oil, sunflower oil, and sesame oil, AN extract consistently maintained lower POVs compared to both TP and TBHQ, suggesting a prolonged shelf life. In linolenic acid-rich oils like linseed and Perilla seed oils, AN extract also exhibited robust antioxidant activity, further extending the shelf life by reducing the rate of oxidation.

Molecular docking studies and binding energy calculations provided further insights into the interactions between key bioactive compounds and fatty acids. Camelliaside A and B, along with TBHQ, exhibited different binding modes and affinities with fatty acids. Camelliaside A formed multiple hydrogen bonds and hydrophobic interactions with cis-9-octadecenoic acid, resulting in higher binding energy than Camelliaside B and TBHQ. Similar interactions were observed with cis-cis-9,12-octadecadienoic acid and cis-cis-cis-9,12,15-octadecatrienoic acid, where Camelliaside A consistently showed stronger binding energies compared to Camelliaside B and TBHQ. These molecular interactions underpin the observed antioxidant efficacy, reinforcing the potential of AN extracts in enhancing the stability and shelf-life of edible oils.

The molecular docking results align with the experimental data, indicating that the strong interactions between AN compounds and fatty acids contribute to the observed antioxidant effects. Especially in oils rich in linolenic acid, the ability of Camelliaside A to form multiple hydrogen bonds and hydrophobic interactions with fatty acids, resulting in higher binding energies, explains its superior performance compared to Camelliaside B and TBHQ.

The high content of flavonoids and phenolics in AN extract is directly correlated with its antioxidant efficacy. The molecular docking results align with the experimental data, indicating that the strong interactions between AN compounds and fatty acids contribute to the observed antioxidant effects. Especially in the two oils rich in linolenic acid,the ability of Camelliaside A to form multiple hydrogen bonds and hydrophobic interactions with fatty acids, resulting in higher binding energies, especially in the two oils rich in linolenic acid, explains its superior performance compared to Camelliaside B and TBHQ.

The analysis of fatty acids and endogenous antioxidants in the six types of edible oils before and after AN treatment provides essential

insights into the oxidative stability of these oils. The study revealed that the fatty acid composition plays a crucial role in the oxidative stability of oils, with oleic, linoleic, and linolenic acid-rich oils showing varying responses to antioxidant treatments. Gas chromatography and liquid chromatography analyses quantified the contents of these fatty acids and endogenous antioxidants, demonstrating that AN treatment significantly alters the antioxidant profile of the oils. Specifically, the increase in flavonoid and phenolic content in the oils after AN treatment is indicative of enhanced antioxidant capacity, which correlates with the improved inhibition of lipid oxidation observed in the study.

The findings suggest that the interaction between AN extract and the oils' fatty acids leads to the formation of stable complexes, thereby reducing the rate of peroxidation. This is particularly evident in oleic and linoleic acid-rich oils, where the AN treatment showed superior performance in maintaining lower peroxide values compared to TP and TBHQ. In linolenic acid-rich oils, while the AN extract's performance was comparable to TP, the formation of stronger binding energies with fatty acids, as supported by molecular docking studies, underscores its potential in enhancing oil stability.

These results indicate that the superficial analysis and discussion of the effects of AN treatment on fatty acids and antioxidants require further in-depth exploration. Future studies should focus on the detailed mechanisms through which AN extract enhances oil stability, including the role of individual flavonoids and phenolics in interacting with specific fatty acids. Additionally, the long-term effects of AN treatment on the shelf-life and sensory properties of these oils should be investigated to fully understand its potential as a natural antioxidant.

5. Conclusion

The findings of this study demonstrate that *Adinandra nitida* (AN) extract is a potent natural antioxidant, exhibiting superior performance in delaying lipid oxidation in various edible oils when compared to tea polyphenols (TP) and tert-butylhydroquinone (TBHQ). Particularly, AN extract proved highly effective in camellia oil, olive oil, sunflower oil, and sesame oil, especially during the initial stages of oxidation. This is evidenced by the consistently lower peroxide values (POV) observed for AN extract across different time points in these oils.

In linolenic acid-rich oils such as linseed oil and Perilla seed oil, AN extract also displayed significant antioxidant properties. The strong interactions between the key bioactive compounds in AN extract and fatty acids, as revealed by binding energy calculations, further support these findings. Notably, Camelliaside A consistently exhibited stronger binding energies compared to Camelliaside B and TBHQ, correlating with the observed superior antioxidant activity of AN extract.

These results suggest that AN extract has substantial potential as a natural alternative to synthetic antioxidants for enhancing the stability and shelf-life of edible oils. Future research should explore the synergistic effects of combining AN extract with other antioxidants to optimize efficacy across different oil systems and storage conditions. Additionally, further studies should delve into the specific roles of various flavonoids and phenolics in AN extract and their impact on the oxidative stability of oils under diverse storage conditions. The promising potential of AN extract to improve the shelf-life and quality of edible oils offers significant implications for food preservation and safety, paving the way for its broader application in the food industry.

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CRediT authorship contribution statement

Yuan Zeng: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zhengwen Yu:** Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Yubo Zhang:** Supervision, Project administration, Funding acquisition. **Chunyan Jiang:** Validation, Supervision, Methodology. **Xue Huang:** Visualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This research was funded by the National Natural Science Foundation of China [Grants No. 32060068 and No. U1812401], Natural science research project of Guizhou Provincial Department of Education [Qianjiaoji [2022] 009], and Guizhou Key Laboratory of Plant Protection Informatization for High-Efficiency Agriculture with Characteristics in Central Guizhou [Qianjiaoji [2022] 052].

Data availability

The authors do not have permission to share data.

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