Article

Comparison of Chemical Compositions and Antioxidant Activity of Essential Oils from Litsea Cubeba, Cinnamon, Anise, and Eucalyptus

Shutian Liu, Chen Zhao [ID], Yuwei Cao, Yan Li [ID], Zhuo Zhang, Dechao Nie, Weixuan Tang and Yanling Li *

Animal Science and Technology College, Beijing University of Agriculture, No. 7 Beinong Road, Changping, Beijing 102206, China; liushutian622@163.com (S.L.); zhaochenunique@163.com (C.Z.); cyw2222021@126.com (Y.C.); liyan06122022@163.com (Y.L.); zzhuo0126@163.com (Z.Z.); niedechao0403@163.com (D.N.); winsometang@163.com (W.T.)
* Correspondence: yanl_li@163.com or 20137302@bua.edu.cn

Abstract: The purpose of this study was to compare the antioxidant activity of litsea cubeba oil (LCO), cinnamon oil (CO), anise oil (AO), and eucalyptus oil (EUC) in vitro. The chemical compositions of the essential oils (EOs) were analyzed using gas chromatography-mass spectrometry (GC-MS). The antioxidant activity of the four EOs was evaluated through scavenging DPPH free radicals, chelating Fe^{2+}, scavenging hydroxyl free radicals, and inhibiting yolk lipid peroxidation. The results showed that the major compounds found in LCO, CO, AO, and EUC are citral (64.29%), cinnamaldehyde (84.25%), anethole (78.51%), and 1,8-cineole (81.78%), respectively. The four EOs all had certain antioxidant activity. The ability to scavenge DPPH radical was ranked in the order of LCO > CO > AO > EUC. The hydroxyl radical scavenging ability was ranked in the order of EUC > CO > LCO > AO. The chelating Fe^{2+} capacity was ranked in the order of EUC > AO > CO > LCO. The yolk lipid peroxidation inhibition ability was ranked in the order of CO > AO > EUC > LCO. In different antioxidant activity assays, the antioxidant activity of the EOs was different. It was speculated that the total antioxidant activity of an EO may be the result of the joint action of different antioxidant capacities.

Keywords: litsea cubeba oil; cinnamon oil; eucalyptus oil; anise oil; antioxidant activity

1. Introduction

In livestock production, intensive farming patterns and harmful environmental factors (e.g., bacteria) can lead to oxidative stress in animals. The overproduction of reactive oxygen species and an imbalance of antioxidant systems can cause oxidative stress [1]. Oxidative stress can destroy a variety of cellular components via DNA hydroxylation, protein denaturation, lipid peroxidation, and membrane rupture, which results in cell apoptosis and other cell death patterns [2,3]. Oxidative stress causes oxidative damage to the digestive tract, which, in turn, induces an inflammatory response in the organism. This ultimately leads to stunted growth, reduced immunity, and even death in the animal, which can seriously affect economic efficiency [4]. Eliminating and slowing down oxidative stress is a key step in protecting animals from injury. Some synthetic antioxidants, such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA), may have carcinogenic potential and pose a risk to animal health and product quality. Therefore, it is important to find natural green antioxidants to reduce oxidative stress in animals.

Essential oils (EOs) are aromatic and volatile mixtures composed of dozens to hundreds of compounds, among which monoterpenes, sesquiterpenes, and their oxygen-containing derivatives are the main components [2,3,5]. The antioxidative and antibacterial activities of EOs are derived from these components. The toxicity and side effects of EOs are low, meaning they can be widely used in animal husbandry [6]. As a natural antioxidant,

EOs have been used in livestock and poultry production. Previous studies have reported that EOs reduce fat oxidation and improve the shelf-life of meat quality [7]. EOs can alleviate oxidative damage and inhibit the activation of NF-κB signaling pathways, thereby alleviating the damage of immune functions caused by oxidative stress [8,9]. Other studies have reported that EO supplementation increased the activity of antioxidant enzymes in the body and improved the growth performance and immune functions of animals [10]. Therefore, the antioxidant activity of EOs has important biological significance [11]. Some studies have indicated that *Litsea cubeba* (Lour.) Pers. oil (LCO), *Cinnamomum cassia* (L.) D. Don oil (CO), *Pimpinella anisum* L. oil (AO), and *Eucalyptus* spp. oil (EUC) have antibacterial, anti-inflammatory, and antioxidant activities, and these four EOs are widely used in the pharmaceutical and food industries in China [12–15]. Thus, the purpose of this study was to evaluate the chemical compositions of LCO, CO, AO, and EUC and compare their antioxidant activity via a DPPH radical scavenging assay, Fe^{2+} chelating assay, hydroxyl radical scavenging assay, and yolk lipid peroxidation assay. This study provides a theoretical basis for the application of EOs as a natural antioxidant in animal husbandry.

2. Results

2.1. Chemical Compositions of Essential Oils

The chemical compositions of the four EOs are shown in Table 1. There were 29, 20, 19, and 13 different active components that were detected in LCO, CO, AO, and EUC, respectively. The major components of LCO were citral (64.29%), d-limonene (12.39%), 4,5-epoxycarene (2.62%), β-caryophyllene (2.37%), and others (17.97%). CO contained cinnamaldehyde (84.25%), o-coumaric acid (6.43%), 3-(2-Methoxyphenyl)-2-propen (1.76%), cinnamyl acetate (1.46%), and others (6.1%). The major components of AO were anethole (78.51%), estragole (6.60%), 1-(3-methyl-2-butenyloxy)-4-(1-propenyl)benzene (3.47%), and d-limonene (2.0%). EUC contained 1,8-cineole (81.78%), 4-carene (5.35%), β-lauricene (4.58%), β-pinene (3.53%) and others (4.76%).

Table 1. Chemical constituents and contents of the four essential oils.

No.	Retention Indices	Retention Time RT/min	Compounds	Composition (% of Total)			
				LCO	CO	AO	EUC
1	847	2.015	Hydroperoxide, 1-methylhexyl	-	-	-	0.03
2	948	6.71	α-Pinene	1.46	-	0.98	-
3	943	7.251	Camphene	0.35	-	-	0.16
4	982	7.75	Benzaldehyde	-	1.06	-	-
5	897	8.075	Sabinene hydrate	1.08	-	-	-
6	943	8.194	β-Pinene	1.13	-	-	3.53
7	938	8.57	6-Methyl-5-hepten-2-one	1.80	-	-	-
8	958	8.695	β-Myrcene	0.71	-	-	4.58
9	969	9.162	α-Phellandrene	-	-	0.48	1.97
10	976	9.236	trans-β-Ocimene	-	-	0.37	0.30
11	919	9.53	4-Carene	-	-	-	5.35
12	1042	9.825	1-methyl-3-(1-methylethyl)-benzen	-	-	-	1.81
13	1018	9.95	D-Limonene	12.39	-	2.00	-
14	1059	10.092	1,8-cineole	-	-	0.7	81.79
15	998	10.818	Pinene	-	-	-	0.19
16	1082	12.229	3,7-Dimethyl-1,6-octadiene 3-ol	2.08	-	1.41	-
17	1174	13.294	3,3,5-Trimer-1,4-hexadiene	0.26	-	-	-
18	868	13.33	1,3-Hexadiene	-	-	-	0.25
19	1125	13.535	Citronellal	2.47	-	-	-

Table 1. *Cont.*

No.	Retention Indices	Retention Time RT/min	Compounds	Composition (% of Total)			
				LCO	CO	AO	EUC
20	1163	13.791	1,2,3,6-Tetrahydrobenzaldehyde	1.32	-	-	-
21	1181	13.793	Phenylpropyl aldehyde	-	0.74	-	-
22	1138	14.201	(1S)-endo)-(-)-borneol	0.10	-	-	-
23	1163	14.292	4,5-Epoxycarene	2.62	-	-	-
24	1137	14.303	4-Terpinenol	-	-	0.30	-
25	1172	14.746	Estragole	-	-	6.60	-
26	1143	14.767	α-Pinoresinol	0.78	-	-	0.05
27	1189	15.29	trans-Cinnamaldehyde	-	0.39	-	-
28	1174	15.891	β-Citral	28.71	-	-	-
29	1158	16.18	3-Methyl-2-cyclohexen-1-one	0.13	-	-	-
30	1171	16.256	4-Methoxybenzaldehyde	-	-	1.06	-
31	1228	16.322	Geraniol	1.93	-	-	-
32	1174	16.659	α-Citral	35.58	-	-	-
33	1189	16.884	Cinnamaldehyde	-	83.86	-	-
34	1031	17.086	7-Oxabicyclo [4.1.0] heptan-2-one	0.32	-	-	-
35	1190	17.16	Anethole	-	-	78.51	-
36	1199	18.101	2,7-Dimethyl-2,7-diol	0.24	-	-	-
37	1203	18.704	2,4,4,7-Tetramethyl-5,7-octadiene 3-ol	0.21	-	-	-
38	869	18.92	6-Hepten-3-ol	0.39	-	-	-
39	1221	18.961	α-cobalene	-	0.57	-	-
40	1424	19.094	2-methyl-epoxide	-	-	0.32	-
41	1398	19.284	β-Elemiene	0.33	-	-	-
42	1430	19.794	trans-α-bergamot	-	-	1.09	-
43	1494	19.925	Isoeugenol	-	0.18	-	-
44	1494	19.938	β-Caryophyllene	2.37	-	0.80	-
45	1430	20.221	trans-Bergamottin	-	0.12	-	-
46	1374	20.38	O-coumaric acid	-	6.43	-	-
47	1367	20.522	Cinnamyl acetate	-	1.46	-	-
48	1386	20.793	Balsamene	-	0.19	-	-
49	1435	21.111	γ-Ylang-ylangolene	-	0.16	-	-
50	1431	21.536	1,5-Dimethyl-8-(1-methylethylidene)-1,5-cyclododecene	-	-	0.24	-
51	1440	21.608	4,7-Dimethyl-1-(1-methylethyl)	-	0.4	-	-
52	1500	21.797	β-Red myrcene	-	0.38	0.2	-
53	1216	21.904	β-cobalene	-	0.17	-	-
54	1430	22.183	4,8,11,11-Tetramethyl-tricyclo[7.2.0.0(3,8)]undecylene-4-ene	-	0.16	-	-
55	1378	22.267	3-(2-Methoxyphenyl)-2-propenal	-	1.76	-	-
56	1564	22.924	trans-Nerolidol	-	0.34	0.24	-
57	1507	23.301	Graphene oxide	0.56	0.35	-	-
58	1580	24.757	alpha-Dauerol	-	-	0.23	-
59	1572	25.133	1-(3-Methyl-2-butenyloxy)-4-(1-propenyl)benzene	-	-	3.47	-
60	1490	30.762	β-Serinene	-	0.15	-	-
61	2192	31.513	trans-Geraniol	0.10	-	-	-
62	1281	31.632	Isododecane epoxide	0.2	-	-	-
63	1342	32.762	(2Z)-3,7-Dimethyl-2,6-octadienoic acid	0.22	-	-	-
64	1454	32.892	1-(2,2,5α-Trimethylperhydro-1-benzothiophen-1-yl)-2-buten-1-one	0.16	-	-	-
			Others	2.00	1.13	1.00	0.27
			Total	100	100	100	100

Note: "-" indicates that EO does not contain this component. LCO: litsea cubeba oil, CO: cinnamon oil, AO: anise oil, EUC: eucalyptus oil.

2.2. The Antioxidant Activity of Essential Oils

2.2.1. Effect of EOs on DPPH Radical Scavenging Rates

Figure 1 shows the effects of the EO type and the EO concentration on the scavenging rates of DPPH radicals. EO type (E) and EO concentration (C) had significant effects on DPPH radical scavenging rates ($p_E < 0.01$, $p_C < 0.01$), and the interactions between EO type and concentration (E × C) were significant ($p_E \times p_C < 0.01$). The DPPH radical scavenging rate quadratically increased ($p_Q < 0.01$) with an increase in the concentration of LCO, CO, AO, and EUC. In comparing rates among EOs, the DPPH radical scavenging rate differed ($p < 0.05$) at a concentration of 2.5 mg/mL (CO > LCO > AO, EUC), at a concentration of 10 and 20 mg/mL (CO, LCO > AO > EUC), at a concentration of 40 mg/mL (LCO > CO > AO > EUC), and at a concentration of 80 mg/mL (LCO > CO > AO > EUC). The BHA had consistently greater ($p < 0.05$) hydroxyl radical scavenging rates compared to all EOs, except there was no significant difference ($p > 0.05$) in DPPH radical scavenging between LCO and BHA at the concentration of 80 mg/mL.
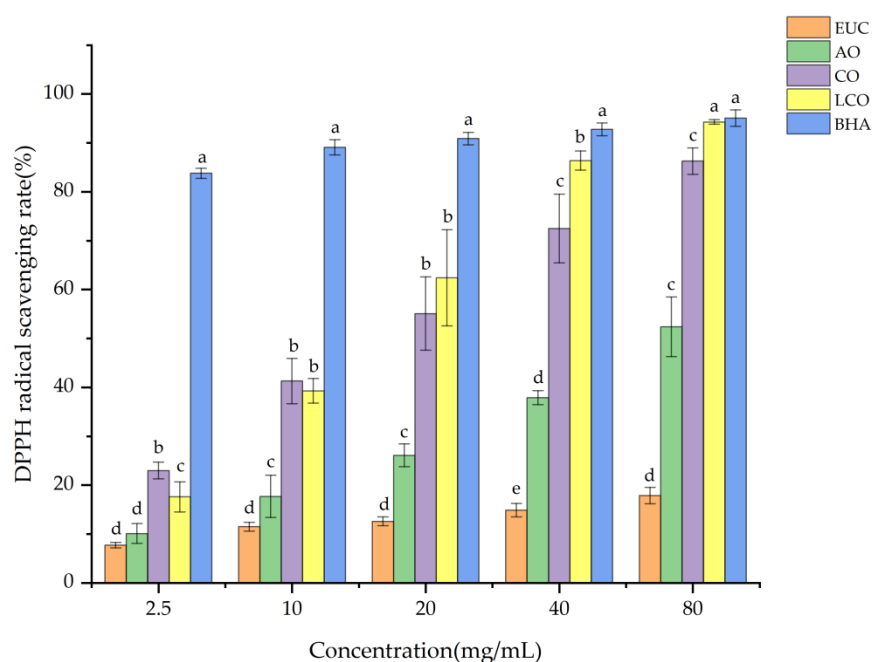

Figure 1. Effects of different concentrations of EOs on DPPH radical scavenging rates. EOs (E) and their different concentrations (C) had significant effects on DPPH radical scavenging rates ($p_E < 0.01$, $p_C < 0.01$), and their interaction (E × C) was significant ($p_E \times p_C < 0.01$). Different letters show differences ($p < 0.05$) among EOs. LCO: litsea cubeba oil, CO: cinnamon oil, AO: anise oil, EUC: eucalyptus oil; BHA: butylated hydroxyanisole.

2.2.2. Effect of EOs on Fe^{2+} Chelating Ability

Figure 2 shows the effects of the EO type and the EO concentration on Fe^{2+} chelating capacity. EO type (E) and EO concentration (C) had significant effects on the Fe^{2+} chelating ability ($p_E < 0.01$, $p_C < 0.01$), and the interactions between EO type and concentration (E × C) were significant ($p_E \times p_C < 0.01$). The Fe^{2+} chelating capacity quadratically increased ($p_Q < 0.01$) with an increase in the concentration of LCO, and linearly increased ($p_L < 0.01$) with an increase in the concentration of CO, AO, and EUC. In comparing capacity among EOs, the Fe^{2+} chelating capacity differed ($p < 0.05$) at five different EO concentration levels (2, 6, 8, 10, and 12 mg/mL) (EUC > AO > CO > LCO). The VC had a consistently greater ($p < 0.05$) Fe^{2+} chelating capacity compared to all EOs at all of the concentrations. At the concentration of 12 mg/mL, the chelation rate of VC to Fe^{2+} was 82.59%, and the chelation rates of AO and EUC to Fe^{2+} were 70.15% and 75.39%, respectively, reaching 84.94% and 91.28% of VC.
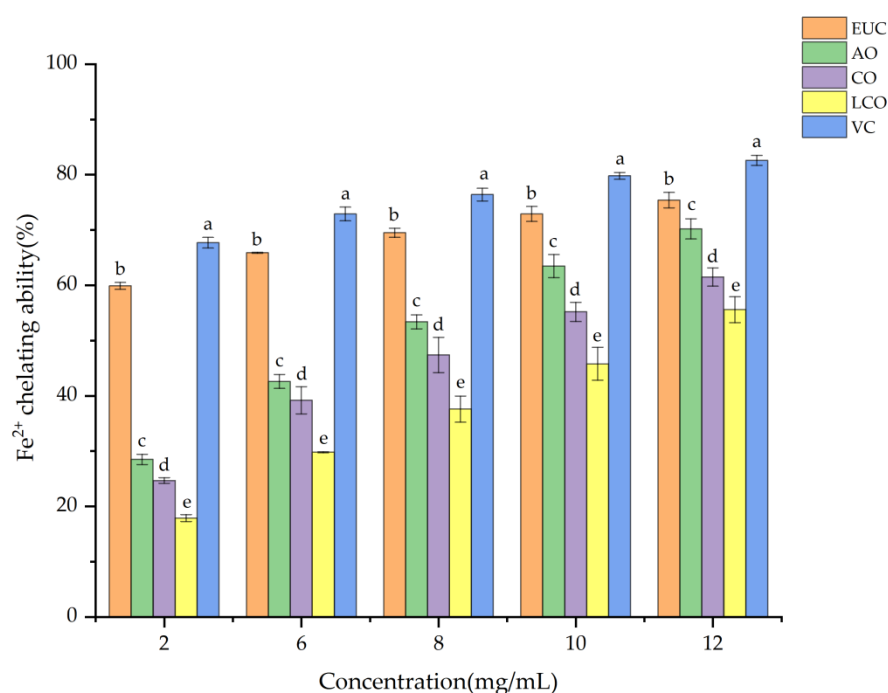
Figure 2. Effects of different concentrations of EOs on Fe^{2+} chelating ability. EOs (E) and their different concentrations (C) had significant effects on Fe^{2+} chelating capacity ($p_E < 0.01$, $p_C < 0.01$), and their interaction (E × C) was significant ($p_E × p_C < 0.01$). Different letters show differences ($p < 0.05$) among EOs. LCO: litsea cubeba oil, CO: cinnamon oil, AO: anise oil, EUC: eucalyptus oil; VC: Vitamin C.

2.2.3. Effect of EOs on Hydroxyl Radical Scavenging Rates

The effects of the EO type and the EO concentration on the scavenging rates of hydroxyl radicals are shown in Figure 3. EO type (E) and EO concentration (C) had significant effects on the hydroxyl radical scavenging rate ($p_E < 0.01$, $p_C < 0.01$), and the interactions between EO type and concentration (E × C) were significant ($p_E × p_C < 0.01$). The hydroxyl radical scavenging rate quadratically increased ($p_Q < 0.01$) with an increase in the concentration of LCO, CO, AO, and EUC. In comparing rates among EOs, the hydroxyl radical scavenging rate differed ($p < 0.05$) at five different EO concentration levels (1, 4, 8, 16, and 32 mg/mL) (EUC > CO > LCO > AO). The VC had a consistently greater ($p < 0.05$) hydroxyl radical scavenging rate compared to all EOs at all of the concentrations. At the concentration of 32 mg/mL, the hydroxyl radical scavenging rate of VC was 85.66%, and the hydroxyl radical scavenging rate of EUC was 75.05%, which reached more than 80% of the level of VC.

2.2.4. Effects of EOs on the Inhibition of Yolk Lipid Peroxidation

Figure 4 shows the effect of the EO type and the EO concentration on the lipid peroxidation inhibition rate of yolk. EO type (E) and EO concentration (C) had significant effects on the lipid peroxidation inhibition rate of yolk ($p_E < 0.01$, $p_C < 0.01$), and the interactions of EO type and concentration (E × C) were significant ($p_E × p_C < 0.01$). The lipid peroxidation inhibition rate of yolk quadratically increased ($p_Q < 0.01$) with increasing the concentration of LCO, CO, AO and EUC. In comparing among EOs, the lipid peroxidation inhibition rate of yolk differed ($p < 0.05$) at a concentration of 1.25, 5, 10, 20 mg/mL (PG > CO > AO > EUC > LCO) and at the concentration of 4 mg/mL (CO > AO, EUC > LCO). At the concentration of 2.5 mg/mL, the inhibition rate of PG on yolk lipid peroxidation was 90.41%, and that of CO was 86.42%, reaching 95.59% of PG.
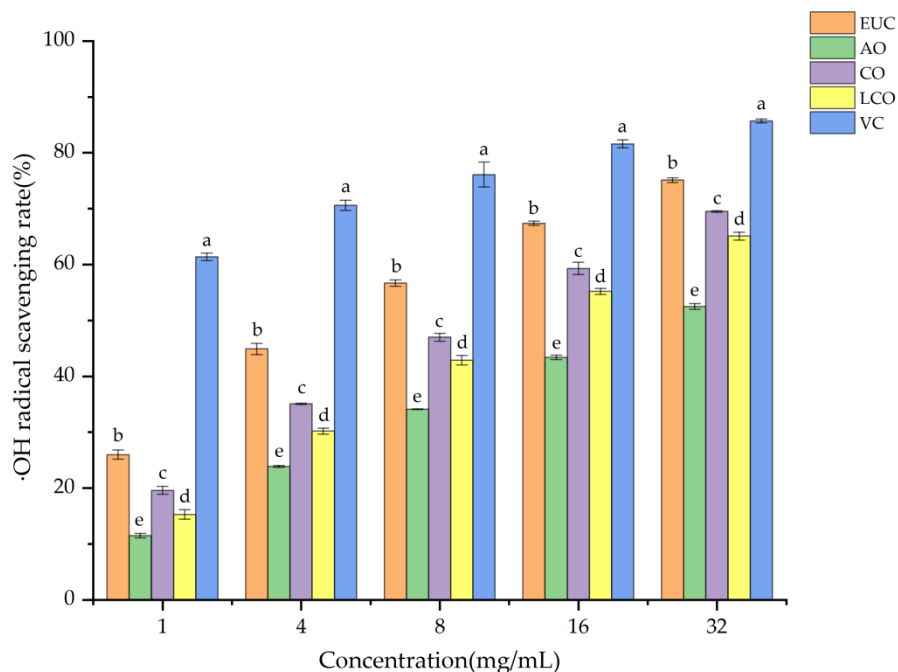
Figure 3. Effects of different concentrations of EOs on ·OH radical scavenging rates. EOs (E) and their different concentrations (C) had significant effects on ·OH radical scavenging rates ($p_E < 0.01$, $p_C < 0.01$), and their interaction (E × C) was significant ($p_E × p_C < 0.01$). Different letters show differences ($p < 0.05$) among EOs. LCO: litsea cubeba oil, CO: cinnamon oil, AO: anise oil, EUC: eucalyptus oil; VC: Vitamin C.
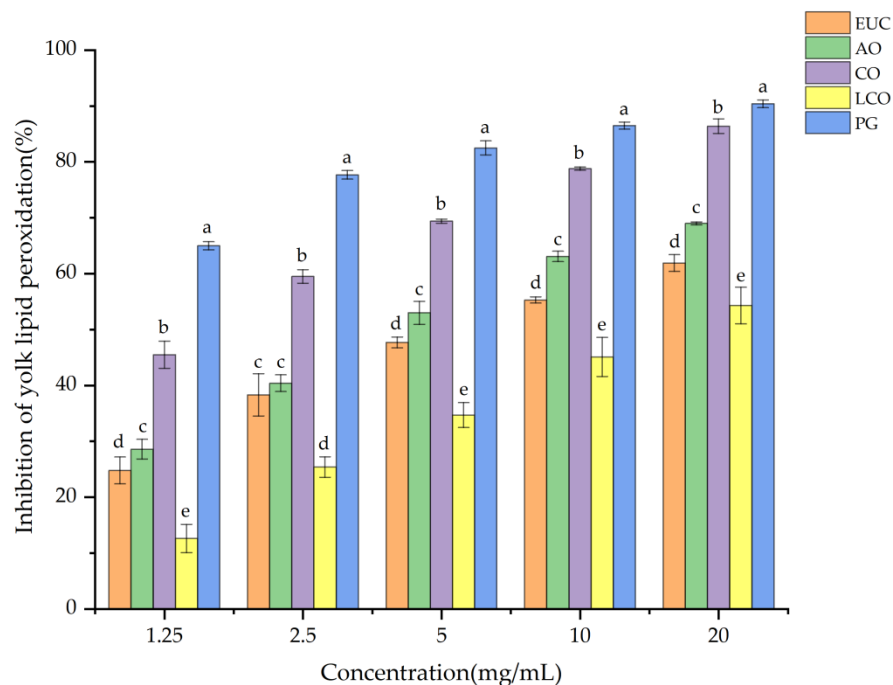

Figure 4. Effect of different concentrations of EOs on yolk lipid peroxidation inhibition. EOs (E) and their different concentrations (C) had significant effects on yolk lipid peroxidation inhibition ($p_E < 0.01$, $p_C < 0.01$), and their interaction (E × C) was significant ($p_E × p_C < 0.01$). Different letters show differences ($p < 0.05$) among EOs. LCO: litsea cubeba oil, CO: cinnamon oil, AO: anise oil, EUC: eucalyptus oil; PG: propyl gallate.

3. Discussion

A reduction in antioxidant defense mechanisms and the production of free radicals can lead to oxidative stress, which damages proteins, lipids, and enzymes [16]. Free radicals exist in the body widely, and they are the main cause of lipid peroxidation and the root of oxidative damage to macromolecular substances [17]. Some studies have shown that oxidative stress can cause inflammation and other diseases by producing excess free radicals to mediate oxidative damage to cells [18]. Antioxidants can scavenge free radicals and stop free radical reactions. As a result of the carcinogenicity and hepatotoxicity of synthetic antioxidants (such as BHA and BHT), EOs have attracted increasing attention as green natural antioxidants. Some studies have found that EOs can prevent free radical chain reactions and delay the lipid peroxidation process [6,19,20].

EOs are complex mixtures of different volatile and non-volatile components [21]. Different EOs have different chemical compositions. In our study, it was found that the active components of the four studied EOs were very complex and varied greatly. Citral, the main component of LCO, and cinnamic aldehyde, the main component of CO, are aldehydes. Anisole, the main component of AO, and 1, 8-eudinolein, the main component of EUC, are terpenoid compounds. The reasons for the differences in EO active components may be related to variety, heredity, maturity stage, culture conditions, etc. [22].

It has been reported that EOs can reduce or eliminate free radicals, block free radical chain reactions, and delay lipid peroxidation [23]. In our study, the antioxidant activity of four EOs was evaluated through scavenging DPPH free radicals, chelating Fe^{2+}, scavenging hydroxyl free radicals, and inhibiting yolk lipid peroxidation. Our study found that the antioxidant activity of EOs is related to the EO concentration and EO type. The DPPH and hydroxyl radical scavenging rate, the Fe^{2+} chelating ability, and the inhibition rate of the lipid peroxidation of yolk were dose-dependent, and they linearly or quadratically increased with an increase in EO concentration, which suggests that the antioxidant activity of EOs varies with concentration. Similarly, El amrani et al. [24] found that the DPPH radical inhibition percentages of cinnamon and clove essential oils presented a dose dependent effect. Other studies have found that the scavenging effect of AO was stronger than BHA and BHT and increased with the increase of concentration [25]. In DPPH radical scavenging experiments, the position of different EOs changes at different concentrations (cross effect). Similar results have been observed in our previous study, where it was found that the antioxidant activity of five citrus EOs increased with an increase in concentration. Additionally, the scavenging effect of bergamot EO was higher than that of lemon EO at low concentrations of 16–80 mg/mL, but the results were the opposite at higher concentrations of 112–144 mg/mL [26]. This is due to the interaction between the type of EO and its concentration. In addition, different EOs had different antioxidant activity. Wei et al. [27] measured the antioxidant activity of 25 EOs and found that cinnamon leaf oil, clove leaf oil, and thyme oil had good antioxidant activity, while sandalwood oil showed poor antioxidant activity. Similarly, Ghazghazi et al. [28] found significant differences in the antioxidant activity of two EOs (*Eucalyptus marginata* L. and *Eucalyptus pauciflora* L. EOs). Another study analyzed the antioxidant activity of LCO extracted in different months, and the results showed that LCO extracted in July had a better hydroxyl radical scavenging rate, and LCO extracted in August had a better DPPH scavenging activity and ferric reducing antioxidant power [29]. In this study, at the same concentration, EUC and CO had good radical hydroxyl scavenging ability, while AO and LCO had poor scavenging ability. This difference may be due to the different chemical compositions of different EOs, and the compositions and contents of EOs may affect their overall antioxidant activity [30].

In addition, the antioxidant activity of EOs is related to their chemical compositions [31]. It has been reported that phenols contribute greatly to the antioxidant capacity of EOs [32]. The phenolic hydroxyl can release hydrogen ions to bind with free radicals and block the oxidative chain reaction [33], and the antioxidant activity of EOs is significantly positively correlated with the phenol content [34]. Mechergui et al. [35] reported that the antioxidant activity of oregano oil mainly depended on the carvall and thymol contents.

In addition, fatty acids, esters, ketones, alcohols, and terpenoids have previously been reported to have antioxidant activity [36]. Adefegha et al. [37] showed that the free radical scavenging ability and metal chelating ability of Masson pine EO may be attributed to phenolic monoterpenes and oxygenated monoterpenes in EOs. Some studies have shown that the antioxidant activity of different antioxidants mainly depends on their stability after absorbing lone pair electrons, and phenols > aldehydes > alcohols [38]. The four EOs showed different antioxidant activity, which may have been caused by the different chemical components of different EOs. The active components of EOs are complex and diverse, and whether the antioxidant activity of different active components are based on synergistic, antagonistic, or additive effects needs to be further studied.

Various antioxidant activity assays have been used to evaluate the antioxidant capacity of EOs in vitro, and EOs showed different antioxidant activity using different assays. In our study, we evaluated the antioxidant activity of EOs using four methods: scavenging DPPH radical, chelating Fe^{2+}, scavenging hydroxyl radical, and inhibiting yolk lipid peroxidation. DPPH free radical is a stable radical that is widely used as a tool to evaluate the free radical scavenging activity of EOs [39]. The ability of EOs to scavenge DPPH free radicals may be related to their ability to provide hydrogen atoms. EOs can provide hydrogen atoms to DPPH radicals to form stable end products (DPPH-H), which will not further trigger or aggravate lipid oxidation [40]. It has been found that monoterpenes, such as citral, carvone, laurene, and γ-terpinene, have strong DPPH radical scavenging activity [41]. The scavenging ability of EOs against DPPH free radicals is related to their content of unsaturated hydrocarbon. LCO had the strongest scavenging ability against DPPH free radicals among the four EOs, which may be due to the effects of citral, the main component of LCO. The accumulation of Fe^{2+} in cells can induce the production of free radicals and malondialdehyde [37,42]. EOs can chelate with metal ions to form stable complexes that inhibit the formation of metal-induced free radicals. Some phenols in EOs can effectively chelate ferrous ions [43]. Singh et al. [44] found that the chelating activity of EOs of *Zanthoxylum armatum* DC was significantly higher than that of BHA and BHT, which may be attributed to the phenolic compounds contained in EOs that can chelate metal ions. In our study, EUC showed a good Fe^{2+} chelating ability and increased in a dose-dependent manner, which may be due to the large number of compounds in the oil and their possible affinity for Fe^{2+}. Hydroxyl free radical is a kind of highly active oxidant that can directly damage various biofilms and lead to many diseases [45]. Hydroxyl free radicals can extract electrons from polyunsaturated fatty acids and produce lipid radicals, which can trigger lipid peroxidation. In addition, hydroxyl free radicals are highly reactive products produced via the decomposition of hydrogen peroxide in the presence of Fe^{2+} and other metals, which can aggravate oxidative stress in the body, lead to immune system dysfunction, and affect the digestion, absorption, and transformation of nutrients [46]. Thus, a reduction in hydroxyl radicals can reduce lipid peroxidation and protect the immune system. In our study, all four EOs could remove hydroxyl radicals, among which EUC has the best effect. The scavenging effects of EOs on hydroxyl free radicals can not only act on the non-chain process before the oxidation reaction chain to remove the hydroxyl radicals, starting the chain reaction, but also remove the free radicals in the chain extension process, producing a dual-antioxidant effect. Lipid peroxidation is considered to be an important mechanism of cell damage under oxidative stress and is involved in the pathological processes of many diseases [47]. Previous studies have shown that EOs can effectively inhibit lipid peroxidation. Gargouri et al. [48] found that Henna EO showed very high potential for scavenging free radicals and inhibiting lipid peroxidation, which could be used to combat the oxidative stress generated by Raji cells. Alizadeh et al. [49] speculated that the EO of *Zeilan cinnamomum* L. had a strong free radical scavenging ability and the potential to inhibit lipid oxidation reactions, which was consistent with the results of our experiment. In our study, CO showed the best inhibition of lipid peroxidation of yolk, which may be attributed to the fact that its main component, 1,8 eucalyptol, is a monoterpenoid. It has been found that monoterpene hydrocarbon, γ-terpinene, retards

the peroxidation of linoleic acid because its chaincarrying peroxyls are HOO• radicals, which react rapidly with linoleylperoxyl radicals [50]. In our study, the four EOs showed different antioxidant activity in different antioxidant tests. EUC showed good hydroxyl radical scavenging ability and Fe^{2+} chelating ability, but poor DPPH radical scavenging ability and yolk lipid peroxidation inhibition. Similarly, Bi et al. [51] found that the EOs from Nandina domestica fruits exhibited significant scavenging activity against DPPH free radical and ATBS free radical, moderate scavenging activity against superoxide anion free radical, and low activity against metal chelating power. This difference may be due to the fact that the total antioxidant activity of EOs is the result of a combination of one or several pathways. In our study, EUC had the best hydroxyl radical scavenging ability and chelating Fe^{2+} ability, and CO had better yolk lipid peroxidation inhibition ability and DPPH and hydroxyl radical scavenging ability. Therefore, EUC and CO have good antioxidant activity and have the potential to be used in practical production as a new green antioxidant.

In summary, our results show that the four EOs possessed significant antioxidant activity, but their antioxidant capacity varied with EO type and concentration. In addition, the different antioxidant abilities of EOs may be due to the different chemical compositions of EOs. Combined with the results of the four antioxidant experiments, it is speculated that the total antioxidant activity of EOs may comprise different activities including scavenging free radicals, chelating Fe^{2+}, and inhibiting lipid peroxidation etc.

4. Materials and Methods

4.1. Chemicals and Reagents

Butylated hydroxyanisole was purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). DPPH was purchased from Sigma–Aldrich (Shanghai, China) and Sigma–Aldrich (St. Louis, MO, USA). Propyl gallat, vitamin C, Acido salicilico, and 2-thiobarbituric acid (TBA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

4.2. Essential Oils

LCO (purity 80%), CO (purity 80%), AO (purity 87%), and EUC (purity 80%) were purchased from Nanjing Vincero International Trade Co., Ltd. (Nanjing, China).

4.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The EO samples were diluted 1:100 with hexane and then injected into an Rxi-5Sil MS (Restek, Bellefonte, Newcastle, PA, USA, 30 m × 0.25 mm × 0.25 μm) column via the GC-MS injection system. The column temperature was initially set at 50 °C and held for 5 min, then increased up to 320 °C at a rate of 6 °C/min for 5 min and solvent delay for 3 min. Helium was used as a carrier with a shunt ratio of 10:1 and a flow rate of 1 mL/min. Total ion flow chromatography (TIC) was obtained by analyzing the samples. The data were collected using the GC-MS solution 2.6 software and screened and matched with NIST and other dedicated standard spectrum libraries to identify each component of the EOs. Finally, the relative content was calculated using area normalization.

4.4. Antioxidant Activity

4.4.1. Experimental Design

The antioxidant activity of the four EOs at different concentrations was evaluated by measuring DPPH free radical scavenging ability, Fe^{2+} chelating ability, hydroxyl radical scavenging ability, and yolk lipid peroxidation inhibition. The experiment was a completed randomized design with 4 EOs (LCO, CO, AO, and EUC) × 5 concentrations of factorial arrangements of treatments. The concentration of each EO was 2.5, 10, 20, 40, and 80 mg/mL in the DPPH measurements; 2, 6, 8, 10, and 12 mg/mL in the Fe^{2+} chelating ability assay; 1, 4, 8, 16, and 32 mg/mL in the hydroxyl radical scavenging ability assay; and 1.25, 2.5, 5, 10, and 20 mg/mL in the yolk lipid peroxidation inhibition assay. Butylated

hydroxyanisole (BHA), propyl gallate (PG), and vitamin C (VC), as strong antioxidants, were used as controls, respectively.

4.4.2. DPPH Radical Scavenging Ability Assay

The DPPH free radical scavenging activity was determined according to a previously described method [52]. LCO, CO, AO, EUC, and BHA were diluted with anhydrous ethanol to 2.5, 10, 20, 40, and 80 mg/mL, respectively. A 2 mL EOs diluent and 2 mL 0.2 mmol/L DPPH ethanol solution were taken to prepare 4 mL of the tested solution in a tube. After shaking, the solution was kept away from light at room temperature for 30 min. Next, 200 μL of the tested solution was added to a 96-well microtiter plate. The absorbance was measured using an automatic microplate reader at 517 nm, and the clearance rate was calculated. There were 3 parallel groups in each group. The DPPH free radical scavenging rate was calculated according to Formula (1). A_1 is the absorbance of the ethanol solution of DPPH mixed with the diluent of plant EOs, A_0 is the absorbance of the DPPH ethanol solution mixed with anhydrous ethanol, and A_2 is the absorbance of the plant EOs diluent mixed with absolute ethanol.

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

4.4.3. Fe^{2+} Chelating Ability Assay

The Fe^{2+} chelating ability was determined as described by Oboh et al. [53]. LCO, CO, AO, EUC, and strong antioxidant (VC) were diluted with anhydrous ethanol to 2, 6, 8, 10, and 12 mg/mL, respectively. A total of 1 mL of the diluted sample solution was taken, and 0.1 mL of 2 mmol/L $FeCl_2$ and 0.3 mL of 5 mmol/L phenanthrazine were successively added to the clean test tube. Then, 2.6 mL of distilled water was added to prepare 4 mL of the tested solution, and the solution was left at room temperature for 20 min. A total of 200 μL of the tested solution was added to a 96-well microtiter plate. The absorbance was measured using an automatic microplate reader at 517 nm, and the clearance rate was calculated. There were 3 parallel groups in each group. The chelating capacity of Fe^{2+} was calculated according to Formula (2). A_1 is the absorbance of the tested solution added to EOs, A_0 is the absorbance of absolute ethanol sample solution without EOs, and A_2 is the absorbance of distilled water without phenanthrazine.

$$Fe^{2+} \text{ chelating capacity (\%)} = [A_0 - (A_1 - A_2)/A_0] \times 100\% \tag{2}$$

4.4.4. Hydroxyl Radical Scavenging Ability Assay

The hydroxyl radical scavenging ability was measured according to the method of Radünz [54] with some modifications. LCO, CO, AO, EUC, and strong antioxidant (VC) were diluted with anhydrous ethanol to 1, 4, 8, 16, and 32 mg/mL, respectively. A total of 1 mL of essential oil diluent was taken from each, and 1 mL of a 2 mmol/L $FeSO_4$, 1 mL of a 6 mmol/L salicylic acid, and 1 mL of a 2 mmol/L H_2O_2 were added to prepare 4 mL of the tested sample solution. The solution was mixed well and left for 30 min at room temperature. A total of 200 μL of the tested solution was added to a 96-well microtiter plate. The absorbance was measured using an automatic microplate reader at 510 nm and the clearance rate was calculated. There were 3 parallel groups in each group. The hydroxyl radical scavenging rate was calculated according to Formula (3). A_1 is the absorbance of the tested solution added to EOs; A_0 is the absorbance of absolute ethanol sample solution without EOs; A_2 is the absorbance of distilled water without H_2O_2.

$$\text{The hydroxyl radical scavenging rate (\%)} = [A_0 - (A_1 - A_2)]/A_0 \times 100\% \tag{3}$$

4.4.5. Yolk Lipid Peroxidation Inhibition Assay

The yolk lipid peroxidation inhibition rate was determined according to a previously described method [55]. The yolk of fresh eggs was put into a 50 mL beaker, the same volume of phosphate buffer was added, the sealing film was fastened, and the solution

was stored at −4 °C. Four EOs and PG were diluted with absolute ethanol to 1.25, 2.5, 5, 10, and 20 mg/mL sample dilutions. A total of 0.5 mL of egg yolk stock solution was taken and 12 mL of phosphate buffer was added to prepare a 1:25 egg yolk suspension. A total of 200 μL of the yolk suspension was taken, a 100 μL EO sample diluent was added, and then 200 μL of a 25 mmol/L $FeSO_4$ was added and supplemented to 1 mL with phosphate buffer. After shaking at 37 °C for 30 min, 250 μL of a 20% trichloroacetic acid was added, 3500 r/min centrifugation was performed for 10 min until clarification, the supernatant was absorbed, and 250 μL of a 0.8% TBA was added. A total of 1.5 mL of the sample solution was prepared to be tested and reacted in a boiling water bath for 15 min. A total of 200 μL of the tested solution was added to a 96-well microtiter plate. The absorbance was measured using an automatic microplate reader at 510 nm, and the clearance rate was calculated. There were 3 parallel groups in each group. The yolk lipid peroxidation inhibition rate was calculated according to Formula (4). A_1 is the absorbance of the tested solution added to EOs; A_0 is the absorbance of absolute ethanol sample solution without EOs; A_2 is the absorbance of the tested solution without yolk.

$$\text{The yolk lipid peroxidation inhibition rate (\%)} = [A_0 - (A_1 - A_2)/A_0] \times 100\% \quad (4)$$

5. Statistical Analysis

Data for antioxidant activity were analyzed using the SAS 9.4 mixing procedure with a model including EO type, EO concentration, and their interactions as fixed effects and three replications of the experiment as random effects. The effects of increasing the EO concentration were examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. Differences were declared significant at $p \leq 0.05$. The graphs were drawn using the Origin 2021 software (Northampton, MA, USA).

6. Conclusions

The major compounds found in LCO, CO, AO, and EUC are citral, cinnamaldehyde, anethole, and 1,8-cineole, respectively. The four EOs effectively scavenged DPPH, chelated Fe^{2+}, scavenged hydroxyl radicals, and inhibited lipid peroxidation in a dose-dependent manner. Compared with that of the other EOs, the DPPH radical scavenging ability of LCO was the strongest; EUC had the greatest activity in its Fe^{2+} chelating ability and hydroxyl radical scavenging rate; and CO had the highest yolk lipid peroxidation ability. It is speculated that the total antioxidant activity of EOs might be the result of the joint actions of different antioxidant capacities.

Author Contributions: Y.L. (Yanling Li) covered the project. S.L., C.Z. and Y.C. designed and performed the experiments and analyzed the raw data. Z.Z., D.N., Y.L. (Yan Li) and W.T. assisted with the experiments. Y.L. (Yanling Li) supervised the project. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data present in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are available from the authors.

References

1. Zahra, K.F.; Lefter, R.; Ali, A.; Abdellah, E.C.; Trus, C.; Ciobica, A.; Timofte, D. The Involvement of the Oxidative Stress Status in Cancer Pathology: A Double View on the Role of the Antioxidants. *Oxid. Med. Cell Longev.* **2021**, *2021*, 9965916. [CrossRef]
2. Li, G.; Xiang, S.; Pan, Y.; Long, X.; Cheng, Y.; Han, L.; Zhao, X. Effects of Cold-Pressing and Hydrodistillation on the Active Non-volatile Components in Lemon Essential Oil and the Effects of the Resulting Oils on Aging-Related Oxidative Stress in Mice. *Front. Nutr.* **2021**, *8*, 689094. [CrossRef]
3. Gonzalez-Mas, M.C.; Rambla, J.L.; Lopez-Gresa, M.P.; Blazquez, M.A.; Granell, A. Volatile Compounds in Citrus Essential Oils: A Comprehensive Review. *Front. Plant Sci.* **2019**, *10*, 12. [CrossRef]
4. Zeng, Z.; Zhang, S.; Wang, H.; Piao, X. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. *J. Anim. Sci. Biotechnol.* **2015**, *6*, 7. [CrossRef]
5. Diaz-Sanchez, S.; D'Souza, D.; Biswas, D.; Hanning, I. Botanical alternatives to antibiotics for use in organic poultry production. *Poult. Sci.* **2015**, *94*, 1419–1430. [CrossRef]
6. Bączek, K.B.; Kosakowska, O.; Przybył, J.L.; Pióro-Jabrucka, E.; Costa, R.; Mondello, L.; Gniewosz, M.; Synowiec, A.; Węglarz, Z. Antibacterial and antioxidant activity of essential oils and extracts from costmary (*Tanacetum balsamita* L.) and tansy (*Tanacetum vulgare* L.). *Ind. Crops Prod.* **2017**, *102*, 154–163. [CrossRef]
7. Falowo, A.B.; Mukumbo, F.E.; Idamokoro, E.M.; Afolayan, A.J.; Muchenje, V. Phytochemical Constituents and Antioxidant Activity of Sweet Basil (*Ocimum basilicum* L.) Essential Oil on Ground Beef from Boran and Nguni Cattle. *Int. J. Food Sci.* **2019**, *2019*, 2628747. [CrossRef]
8. Younis, N.S.; Abduldaium, M.S.; Mohamed, M.E. Protective Effect of Geraniol on Oxidative, Inflammatory and Apoptotic Alterations in Isoproterenol-Induced Cardiotoxicity: Role of the Keap1/Nrf2/HO-1 and PI3K/Akt/mTOR Pathways. *Antioxidants* **2020**, *9*, 977. [CrossRef]
9. Younis, N.S.; Mohamed, M.E. Protective effects of myrrh essential oil on isoproterenol-induced myocardial infarction in rats through antioxidant, anti-inflammatory, Nrf2/HO-1 and apoptotic pathways. *J. Ethnopharmacol.* **2021**, *270*, 113793. [CrossRef]
10. Elbaz, A.M.; Ashmawy, E.S.; Salama, A.A.; Abdel-Moneim, A.E.; Badri, F.B.; Thabet, H.A. Effects of garlic and lemon essential oils on performance, digestibility, plasma metabolite, and intestinal health in broilers under environmental heat stress. *BMC Vet. Res.* **2022**, *18*, 430. [CrossRef]
11. Simitzis, P.E. Enrichment of Animal Diets with Essential Oils-A Great Perspective on Improving Animal Performance and Quality Characteristics of the Derived Products. *Medicines* **2017**, *4*, 35. [CrossRef]
12. Thielmann, J.; Muranyi, P. Review on the chemical composition of *Litsea cubebaessential* oils and the bioactivity of its major constituents citral and limonene. *J. Essent. Oil Res.* **2019**, *31*, 361–378. [CrossRef]
13. Abd El-Hack, M.E.; Alagawany, M.; Abdel-Moneim, A.E.; Mohammed, N.G.; Khafaga, A.F.; Bin-Jumah, M.; Othman, S.I.; Allam, A.A.; Elnesr, S.S. Cinnamon (*Cinnamomum zeylanicum*) Oil as a Potential Alternative to Antibiotics in Poultry. *Antibiotics* **2020**, *9*, 210. [CrossRef]
14. Adnan, M. Bioactive potential of essential oil extracted from the leaves of Eucalyptus globulus (*Myrtaceae*). *J. Pharmacogn. Phytochem.* **2019**, *8*, 213–216.
15. Koriem, K.M.M.; Fadl, N.N.; El-Zayat, S.R.; Hosny, E.N.; Abbas El-Shamy, K.; Arbid, M.S.; Morsy, F.A.; El-Azma, M.H. Geranium oil and anise oil inhibit brain cerebral cortex and hippocampus inflammation in depressed animal model. *Nutr. Food Sci.* **2020**, *51*, 439–456. [CrossRef]
16. Zhuang, S.; Yu, R.; Zhong, J.; Liu, P.; Liu, Z. Rhein from Rheum rhabarbarum Inhibits Hydrogen-Peroxide-Induced Oxidative Stress in Intestinal Epithelial Cells Partly through PI3K/Akt-Mediated Nrf2/HO-1 Pathways. *J. Agric. Food Chem.* **2019**, *67*, 2519–2529. [CrossRef]
17. Pisoschi, A.M.; Pop, A. The role of antioxidants in the chemistry of oxidative stress: A review. *Eur. J. Med. Chem.* **2015**, *97*, 55–74. [CrossRef]
18. Chatterjee, S. Oxidative stress, inflammation, and disease. In *Oxidative Stress and Biomaterials*; Academic Press: Cambridge, MA, USA, 2016; pp. 35–58.
19. Fancello, F.; Petretto, G.L.; Zara, S.; Sanna, M.L.; Addis, R.; Maldini, M.; Foddai, M.; Rourke, J.P.; Chessa, M.; Pintore, G. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of *Citrus limon* var. pompia leaf essential oil. *LWT Food Sci. Technol.* **2016**, *69*, 579–585. [CrossRef]
20. Lin, L.Y.; Chuang, C.H.; Chen, H.C.; Yang, K.M. Lime (*Citrus aurantifolia* (Christm.) Swingle) Essential Oils: Volatile Compounds, Antioxidant Capacity, and Hypolipidemic Effect. *Foods* **2019**, *8*, 398. [CrossRef]
21. Deng, W.; Liu, K.; Cao, S.; Sun, J.; Zhong, B.; Chun, J. Chemical Composition, Antimicrobial, Antioxidant, and Antiproliferative Properties of Grapefruit Essential Oil Prepared by Molecular Distillation. *Molecules* **2020**, *25*, 217. [CrossRef]
22. Lin, X.; Cao, S.; Sun, J.; Lu, D.; Zhong, B.; Chun, J. The Chemical Compositions, and Antibacterial and Antioxidant Activities of Four Types of Citrus Essential Oils. *Molecules* **2021**, *26*, 3412. [CrossRef]
23. Bhavaniramya, S.; Vishnupriya, S.; Al-Aboody, M.S.; Vijayakumar, R.; Baskaran, D. Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain Oil Sci. Technol.* **2019**, *2*, 49–55. [CrossRef]
24. Amrania, S.E.; Lalamib, A.E.O.; Zoubic, Y.E.; Moukhafia, K.; Bouslamtia, R.; Lairinia, S. Evaluation of antibacterial and antioxidant effects of cinnamon and clove essential oils from Madagascar. *Sci. Direct* **2019**, *13*, 762–770.

25. Singh, G.; Kapoor, I.P.S.; Singh, P.; Heluani, C.S.d.; Catalan, C.A.N. Chemical composition and antioxidant potential of essential oil and oleoresins from anise seeds (*Pimpinella anisum* L.). *Int. J. Essent. Oil Ther.* **2008**, *2*, 122–130.

26. Li, Y.; Liu, S.; Zhao, C.; Zhang, Z.; Nie, D.; Tang, W.; Li, Y. The Chemical Composition and Antibacterial and Antioxidant Activities of Five Citrus Essential Oils. *Molecules* **2022**, *27*, 7044. [CrossRef]

27. Wei, A.; Shibamoto, T. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *J. Agric. Food Chem.* **2010**, *58*, 7218–7225. [CrossRef]

28. Li, H.; Chu, X.; Li, D.; Zeng, Z.H.; Peng, X.X. Construction and immune protection evaluation of recombinant polyvalent OmpAs derived from genetically divergent ompA by DNA shuffling. *Fish Shellfish Immunol.* **2016**, *49*, 230–236. [CrossRef]

29. She, Q.H.; Li, W.S.; Jiang, Y.Y.; Wu, Y.C.; Zhou, Y.H.; Zhang, L. Chemical composition, antimicrobial activity and antioxidant activity of *Litsea cubeba* essential oils in different months. *Nat. Prod. Res.* **2020**, *34*, 3285–3288. [CrossRef]

30. Dhifi, W.; Bellili, S.; Jazi, S.; Bahloul, N.; Mnif, W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines* **2016**, *3*, 25. [CrossRef]

31. Peng, B.; Bai, R.F.; Li, P.; Han, X.Y.; Wang, H.; Zhu, C.C.; Zeng, Z.P.; Chai, X.Y. Two new glycosides from *Dryopteris fragrans* with anti-inflammatory activities. *J. Asian Nat. Prod. Res.* **2016**, *18*, 59–64. [CrossRef]

32. Li, Y.; Tan, B.; Cen, Z.; Fu, Y.; Zhu, X.; He, H.; Kong, D.; Wu, H. The variation in essential oils composition, phenolic acids and flavonoids is correlated with changes in antioxidant activity during *Cinnamomum loureirii* bark growth. *Arab. J. Chem.* **2021**, *14*, 3249. [CrossRef]

33. Tohidi, B.; Rahimmalek, M.; Arzani, A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chem.* **2017**, *220*, 153–161. [CrossRef]

34. Zhang, Q.A.; Fan, X.H.; Zhang, Z.Q.; Li, T.; Zhu, C.P.; Zhang, X.R.; Song, W. Extraction, antioxidant capacity and identification of Semen Astragali Complanati (*Astragalus complanatus* R. Br.) phenolics. *Food Chem.* **2013**, *141*, 1295–1300. [CrossRef]

35. Mechergui, K.; Coelho, J.A.; Serra, M.C.; Lamine, S.B.; Boukhchina, S.; Khouja, M.L. Essential oils of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) Ietswaart from Tunisia: Chemical composition and antioxidant activity. *J. Sci. Food Agric.* **2010**, *90*, 1745–1749. [CrossRef]

36. Liu, Y.; Sun, L.Y.; Zhu, Z.J.; Lin, W.; Qu, W.; Zeng, Z.G. Measles Virus Infection in Pediatric Liver Transplantation Recipients. *Transplant. Proc.* **2015**, *47*, 2715–2718. [CrossRef]

37. Adefegha, S.A.; Olasehinde, T.A.; Oboh, G. Essential Oil Composition, Antioxidant, Antidiabetic and Antihypertensive Properties of Two Afromomum Species. *J. Oleo Sci.* **2017**, *66*, 51–63. [CrossRef]

38. Gulcin, I. Antioxidants and antioxidant methods: An updated overview. *Arch. Toxicol.* **2020**, *94*, 651–715. [CrossRef]

39. Ma, Y.-L.; Zhu, D.-Y.; Thakur, K.; Wang, C.-H.; Wang, H.; Ren, Y.-F.; Zhang, J.-G.; Wei, Z.-J. Antioxidant and antibacterial evaluation of polysaccharides sequentially extracted from onion (*Allium cepa* L.). *Int. J. Biol. Macromol.* **2018**, *111*, 92–101. [CrossRef]

40. Ray, A.; Jena, S.; Dash, B.; Kar, B.; Halder, T.; Chatterjee, T.; Ghosh, B.; Panda, P.C.; Nayak, S.; Mahapatra, N. Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of *Hedychium coronarium* Koen. *Ind. Crops Prod.* **2018**, *112*, 353–362. [CrossRef]

41. Ciesla, L.; Kryszen, J.; Stochmal, A.; Oleszek, W.; Waksmundzka-Hajnos, M. Approach to develop a standardized TLC-DPPH* test for assessing free radical scavenging properties of selected phenolic compounds. *J. Pharm. Biomed. Anal.* **2012**, *70*, 126–135. [CrossRef]

42. Susanne, H.; Erich, F.E. Transition metal ion-catalyzed oxygen activation during pathogenic processes. *FEBS Lett.* **1999**, *443*, 1–7. [CrossRef]

43. Formisano, C.; Oliviero, F.; Rigano, D.; Saab, A.M.; Senatore, F. Chemical composition of essential oils and in vitro antioxidant properties of extracts and essential oils of *Calamintha origanifolia* and *Micromeria myrtifolia*, two Lamiaceae from the Lebanon flora. *Ind. Crops Prod.* **2014**, *62*, 405–411. [CrossRef]

44. Singh, G.; Kapoor, I.P.S.; Singh, P.; de Heluani, C.S.; de Lampasona, M.P.; Catalan, C.A.N. Chemistry and Antioxidant Properties of Essential Oil and Oleoresins Extracted from the Seeds of Tomer (*Zanthoxylum armatum* DC). *Int. J. Food Prop.* **2013**, *16*, 288–300. [CrossRef]

45. Zhang, P.; Yuan, S. Production of hydroxyl radicals from abiotic oxidation of pyrite by oxygen under circumneutral conditions in the presence of low-molecular-weight organic acids. *Geochim. Cosmochim. Acta* **2017**, *218*, 153–166. [CrossRef]

46. Wang, Y.; Jiang, F.; Cheng, H.; Tan, X.; Liu, Y.; Wei, C.; Song, E. Astragaloside IV Protects against Oxidative Stress in Calf Small Intestine Epithelial Cells via NFE2L2-Antioxidant Response Element Signaling. *Int. J. Mol. Sci.* **2019**, *20*, 6131. [CrossRef]

47. Al-Menhali, A.S.; Banu, S.; Angelova, P.R.; Barcaru, A.; Horvatovich, P.; Abramov, A.Y.; Jaganjac, M. Lipid peroxidation is involved in calcium dependent upregulation of mitochondrial metabolism in skeletal muscle. *Biochim. Biophys. Acta Gen. Subj.* **2020**, *1864*, 129487. [CrossRef]

48. Zeng, Z.; Wang, H.; Shang, F.; Zhou, L.; Little, P.J.; Quirion, R.; Zheng, W. Lithium ions attenuate serum-deprivation-induced apoptosis in PC12 cells through regulation of the Akt/FoxO1 signaling pathways. *Psychopharmacology* **2016**, *233*, 785–794. [CrossRef]

49. Alizadeh Behbahani, B.; Falah, F.; Lavi Arab, F.; Vasiee, M.; Tabatabaee Yazdi, F. Chemical Composition and Antioxidant, Antimicrobial, and Antiproliferative Activities of *Cinnamomum zeylanicum* Bark Essential Oil. *Evid. Based Complement Alternat. Med.* **2020**, *2020*, 5190603. [CrossRef]

50. Han, X.; Liu, Z.; Jo, M.C.; Zhang, K.; Li, Y.; Zeng, Z.; Li, N.; Zu, Y.; Qin, L. CRISPR-Cas9 delivery to hard-to-transfect cells via membrane deformation. *Sci. Adv.* **2015**, *1*, e1500454. [CrossRef]

51. Bi, S.F.; Zhu, G.Q.; Wu, J.; Li, Z.K.; Lv, Y.Z.; Fang, L. Chemical composition and antioxidant activities of the essential oil from *Nandina domestica* fruits. *Nat. Prod. Res.* **2016**, *30*, 362–365. [CrossRef]

52. Chen, Z.; Mei, X.; Jin, Y.; Kim, E.H.; Yang, Z.; Tu, Y. Optimisation of supercritical carbon dioxide extraction of essential oil of flowers of tea (*Camellia sinensis* L.) plants and its antioxidative activity. *J. Sci. Food Agric.* **2014**, *94*, 316–321. [CrossRef]

53. Oboh, G.; Akinbola, I.A.; Ademosun, A.O.; Sanni, D.M.; Odubanjo, O.V.; Olasehinde, T.A.; Oyeleye, S.I. Essential Oil from Clove Bud (*Eugenia aromatica* Kuntze) Inhibit Key Enzymes Relevant to the Management of Type-2 Diabetes and Some Pro-oxidant Induced Lipid Peroxidation in Rats Pancreas in vitro. *J. Oleo Sci.* **2015**, *64*, 775–782. [CrossRef]

54. Radünz, M.; Mota Camargo, T.; Santos Hackbart, H.C.d.; Inchauspe Correa Alves, P.; Radünz, A.L.; Avila Gandra, E.; da Rosa Zavareze, E. Chemical composition and in vitro antioxidant and antihyperglycemic activities of clove, thyme, oregano, and sweet orange essential oils. *LWT* **2021**, *138*, 632. [CrossRef]

55. Zeng, W.C.; Zhang, Z.; Gao, H.; Jia, L.R.; He, Q. Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (*Cedrus deodara*). *J. Food Sci.* **2012**, *77*, C824–C829. [CrossRef]