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ORIGINAL ARTICLE

Effects of lactic acid bacteria on antioxidant activity in vitro and aroma component of *Eucommia ulmoides* tea

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Abstract *Eucommia ulmoides* tea is a popular functional health drink in Asian countries, but its unique herbal aroma is difficult for consumers to accept. The effects of four lactic acid bacteria strains (*Lactobacillus plantarium*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*) fermentation on the physicochemical property, antioxidant activity in vitro and aroma component of *E. ulmoides* leaves were studied. Within the four strains, the sample by *L. bulgaricus* fermentation showed the higher concentrations of chlorogenic acid, geniposidic acid and stronger antioxidant activity in vitro. Moreover, the sample by *L. bulgaricus* fermentation produced a stronger fruity and floral flavor. These results suggested that *L. bulgaricus* was the best strain for fermentation *E. ulmoides* tea. The differences between different strains should be considered when selecting lactic acid bacteria for raw material fermentation of fruits and vegetables.

Keywords *Eucommia ulmoides* · Physicochemical property · Antioxidant activity in vitro · Relative odor activity value

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Abbreviations

LAB	Lactic acid bacteria
TSS	Total soluble solid
TA	Titratable acidity
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
ABTS	2,2′-Azino-di (3-ethylbenzthiazoline-6-sulfonic acid)
UHPLC	Ultra high pressure liquid chromatography
ROAV	Relative odor activity value

Introduction

Eucommia ulmoides belongs to a single species in the Eucommiaceae family, which is a rare and unique species found only in China (Le et al. 2022). The earliest understanding and utilization of *E. ulmoides* can be traced back to Shen Nong Ben Cao Jing, which was published 2000 years ago (Huang et al. 2021a). The bark, leaf, male flower and seed of *E. ulmoides* all have beneficial health care. *E. ulmoides* is used as a health food and food additive in China, Korea, Japan, Russia and other countries (Liu et al. 2022b). *E. ulmoides* leaves are used for medicinal purposes as well as food. It is rich in iridoid terpenes, flavonoids and phenolic compounds, and has the effects of antioxidation, lowering blood pressure, and nourishing the liver and kidney (Dai et al. 2013; Lee et al. 2018; Huang et al. 2021b). Among these, chlorogenic acid and geniposidic acid are the most important bioactive components of *E. ulmoides*, and chlorogenic acid is a natural antioxidant and geniposidic acid affects lowering blood pressure (Naveed et al. 2018; Akira et al. 2021).

As a kind of functional food, *E. ulmoides* natural resources and tea products have a good role in promoting health, and are popular functional health drinks in Asian

countries (Hosoo et al. 2017; Li et al. 2017; Shi et al. 2019; Song et al. 2020). At present, the problem of *E. ulmoides* tea is its unique herbal aroma, which is difficult for consumers to accept. Currently, LAB are extensively utilized in the fermentation process of fruits and vegetables to enhance the sensory quality and flavor of plant extracts while facilitating the conversion of bioactive constituents. Concurrently, it can also augment the antioxidant and antibacterial properties of plant extracts (de Godoy Alves Filho et al. 2017; Cagno et al. 2017; Feng et al. 2017b; Hashemi et al. 2017; Lao et al. 2020). For example, *Lactobacillus plantarum* fermentation changed the composition of lemon juice by reducing glucose, fructose, and citric acid, as well as increasing lactic acid, improving its antioxidant and antimicrobial properties (Hashemi et al. 2017). The fermentation of *L. plantarum i*ncreased the levels of γ-aminobutyric acid, short-chain fatty acids, conjugated fatty acids, total phenolic and flavonoid contents in jujube fruit puree while inducing the highest Trolox equivalent antioxidant activity (Cagno et al. 2017). However, the fermentation of melon and cashew apple juices by *Lactobacillus casei* altered volatile components, combined fruit and lactic fermentation volatiles, and slightly formed or degraded aroma compounds (de Godoy Alves Filho et al. 2017).

It can be seen that different strains of fermentation may have different effects on fruit and vegetable. However, few studies have been conducted to compare the differences between different strains. Therefore, the effects of LAB fermentation on the physicochemical properties, antioxidant activity in vitro and aroma components of extracts from *E. ulmoides* leaves were studied, which will provide technical support for improving *E. ulmoides* tea, and allow us to understand the differences in the fermentation of different LAB species.

Materials and methods

Materials

E. ulmoides leaves were collected in Aug. 2019 from the *E. ulmoides* research base of the Chinese Academy of Forestry in Yuanyang Experimental Base, Henan province. Leaves with uniform size and color, free from pests and diseases, were picked and transported to the laboratory for immediate drying. *Lactobacillus plantarium* (*L. plantarium*) GIM1.191 was purchased from the China Center of Industrial Culture Collection (Beijing, China), and *Lactobacillus bulgaricus* (*L. bulgaricus*) GIM1.155, *Lactobacillius acidophilus* (*L. acidophilus*) GIM1.412, *Streptococcus thermophilus* (*S. thermophilus*) GIM1.540 were purchased from the Guangdong Microbial Culture Collection Center (Guangdong, China).

Preparation of *E. ulmoides* fermented liquid

After drying in the oven at 50 °C, the fresh leaves were ultrasonically extracted with distilled water at 50 °C at a ratio of 1:20 for 30 min and filtered. The filtrate was added with 6% sucrose (carbon source) and 1% inulin (prebiotics) as carbon source, sterilized at 121 °C for 20 min, cooled to 37 °C, and used after checking sterility. Four kinds of LAB were inoculated into sterile MRS Liquid medium at 1% (v/v) and cultured at 37 °C for 48 h. After 2 generations of activation, the bacterial solution was centrifuged, washed in sterile water, and then transferred to sterile water for even shock. When the number of viable bacteria reached 10^8 CFU/mL, it was inoculated into the water extract of *E. ulmoides* leaves at 4% inoculation rate (v/v) for fermentation at 37 °C for 48 h. The technological process of *E. ulmoides* fermentation liquid preparation was shown in Fig. 1. All treatments were conducted in triplicate.

Physicochemical parameters

The TSS and pH value were measured using a digital handheld refractometer (PAL-1, ATAGO, Japan) and pH meter (FE28-Standard, Mettler Toledo, Switzerland), respectively. The TA was determined by titration with 0.05 M NaOH and calculated considering the conversion coefficient of lactic acid. The particle size was detected by a Zetasizer instrument (Nano-ZS90, Malvern, UK).

Color

Color measurements were measured using a color meter (SC-80C, JY kangguang, China). The color parameters was L^* (0 = black, 100 = white), a^* ($-a^*$ = greenness, $+a^*$ = redness) and b^* ($-b^*$ = blueness, $+b^*$ = yellowness),with light source type: tungsten lamp, angle: d/8 degree integrating sphere, instrument calibration: standard black and white plates (Feng et al. 2017b).

Active compounds detection

A UHPLC system (Agilent 1290 series, Agilent Technologies, U.S.) was used to detemine the content of bioactive compounds in the fermented extract of *E. ulmoides* leaves, including chlorogenic acid, geniposidic acid, geniposide, rutin and quercetin. The solution of five standard products with different concentrations (10, 20, 50, 80, 100 mg/L) was prepared, and the standard curve of a single standard product was drawn with the concentration of the standard product as the horizontal coordinate and the peak area of the standard product as the vertical coordinate. All the samples were filtered through a 0.22 μm nylon filter (Whatman, GE Healthcare, UK) and the injection volume was 3 μL with the detection wavelength at

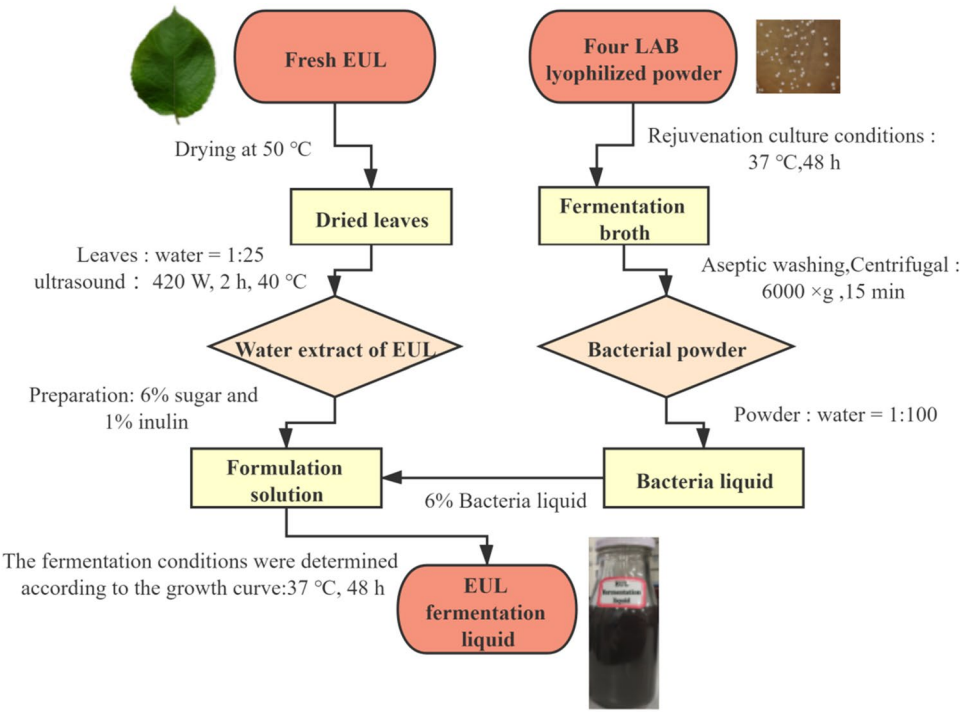

Fig. 1 Preparation of *E. ulmoides* leaves fermentation liquid. EUL: *E. ulmoides* leaves; LAB: Lactic aci

255 nm. Analyte separation was achieved on an Agilent ZORBAX×RRHD Eclipse Plus C18 column (100 mm×2.1 mm, 1.8 µm) at 30 ℃ with a thermos-tatted compartment (Gai et al. 2020). The mobile phase consisted of 0.1% (v/v) formic acid in water (solvent A) and 0.1% (v/v) formic acid in methanol (solvent B) using the following gradient elution at a constant flow rate of 0.2 mL/min: 0–8 min, 95%–5% A; 8–10 min, 5% A; 10–11 min, 5–95% A; 11–15 min, 95% A.

Antioxidant activity in vitro

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity assay was performed as described by Li et al. (Li et al. 2015) with some modifications. Briefly, 3.0 mL reaction mixtures included 1.0 mL $FeSO_4$ (6 mM), 1 mL salicylic acid solution (6 mM), 0.5 mL hydrogen peroxide (8.8 mM), and 0.5 mL different fermentation samples. The absorbance was then measured at 510 nm after the mixture was incubated at 37 °C for 1 h. The hydroxyl radical scavenging rate was expressed by the following equation (Eq. 1). Using Trolox as the reference material, the hydroxyl radical scavenging capacity of the sample was quantified in terms of water-soluble vitamin E content in the fermentation solution per liter (mmol/L).

$$\text{Scavenging activity (\%)} = \left[1 - \frac{A_s - A_b}{A_c - A_b}\right] \times 100 \quad (1)$$

where As, A_b and Ac represent absorbance measured for the sample, blank, and control, respectively.

ABTS-radical scavenging activity

The method used was according to the method of Thummajitsakul et al. (Thummajitsakul et al. 2020). In brief, the ABTS—radical cation solution was prepared by incubation of 7 mM ABTS (10 mL) and 2.45 mM potassium persulfate (5 mL) at room temperature under dark conditions for 12–16 h. The ABTS-radical cation solution was then diluted with absolute ethanol until to provide an absorbance of 0.70 ± 0.02 at 734 nm. After that, the ABTS-radical cation solution (3 mL) was used to react with a 0.01 mL aliquot of properly diluted *E. ulmoides* fermentation broth and then incubated for 6 min. The absorbance of the mixture was detested at 734 nm. The equation used for calculating the ABTS-radical scavenging capacity was the same as that for the hydroxyl radical scavenging activity. Using Trolox as the reference material, the ABTS—radical scavenging capacity of the sample was quantified in terms of water-soluble vitamin E content in the fermentation solution per liter (mmol/L).

DPPH-radical scavenging activity

The method used was based on the previous study of Chu & Chen (2006). 0.1 mL of fermentation sample (diluted 20 times with methanol) and 2 mL of 0.2 mM DPPH solution was prepared with methanol, and 2 mL methanol was added.

The mixture was then incubated at room temperature for 30 min and the absorbance at 517 nm was determined. The equation used for calculating the DPPH scavenging capacity was the same as that for the hydroxyl radical scavenging activity. Using Trolox as the reference material, the DPPH-radical scavenging capacity of the sample was quantified in terms of water-soluble vitamin E content in the fermentation solution per liter (mmol/L).

Gas chromatography electronic nose analysis

The volatile aroma was collected by Heracles II gas phase electronic nose (Alpha M.O.S., Toulouse, France), which consisted of an automatic sampling unit, and an ultra-fast gas chromatograph unit. The samples (5 mL) were placed into 20 mL headspace vial and sealed. After incubating at 90 °C for 5 min with an agitation speed of 500 rpm, 5000 µL of headspace gas was injected into the system at 200 °C. The volatile compound was absorbed by an embedded volatile concentrator at 20 °C for 30 s with a split mode of 10 mL/min and performed thermal desorption was performed at 240 °C for 30 s. Helium was employed as a carrier gas, with a flow speed of 1 mL/min. Two parallel chromatographic columns, including MXT-5 and MXT-1701 were used to separate the volatile compounds. The initial column temperature was held at 40 °C for 5 s, increased to 80 °C at a rate of 0.1 °C/s, then raised to 120 °C at 0.2 °C/s, and finally increased to 250 °C at 0.4 °C/s for 10 s. The temperature of both flame ionization detectors was set to 260 °C (Yang et al. 2022). GC results were retrieved from NIST 2011 and the relative contents of each chemical component were calculated by peak area normalization method for semi-quantitative analysis (Feng et al. 2017a).

ROAV was used to evaluate the contribution of volatile flavor components to the flavor of fermented extract of *E. ulmoides* leaves (Frauendorfer and Schieberle 2006; Wu et al. 2019). ROAV of each compound was calculated according to the following (Eq. 2). The component with a higher ROAV value had a greater contribution to the overall aroma of the sample, and substances with ROAV ≥ 1 were the key aroma components of the analyzed sample.

$$ROAV_i = \frac{C_i}{C_{\max}} \times \frac{T_{\max}}{T_i} \quad (2)$$

$ROAV_i$: the relative odor activity value of a volatile component.C_i: the concentration of a volatile component (µg/kg). T_{max}: the sensory threshold of a volatile component (µg/kg). C_{max}: the concentration of the component with maximum contribution to odors (µg/kg).T_{max}: the sensory threshold of the component with maximum contribution to odors. (µg/kg).

Statistical analysis

All the assays were carried out in triplicate. Statistical analysis was performed using the IBM SPSS statistic 22 (SPSS, USA). The data obtained was subjected to analysis of variance (ANOVA), and significance of means was evaluated by Duncan's multiple range test ($p < 0.05$). All graphs were drawn with origin 95 (Origin Lab Cooperation, Northampton, USA).

Result and discussion

Physicochemical characterization analysis

The physicochemical characterization of the fermentation broth of *E. ulmoides* leaves was shown in Table 1. The pH of the fermentation broth by *L. plantarium*, *L. bulgaricus*, *L. acidophilus*, and *S. thermophilus* was 3.82, 3.99, 3.93, and 4.10, respectively, and the TA of the fermentation broth was 0.17, 0.21, 0.24, and 0.19%, respectively. Compared with the control, it was observed that the pH decreased, but the TA increased after 48 h fermentation. The reason was that during the fermentation process, LAB produced many organic acids, which reduced the pH and increased TA in the fermentation broth of *E. ulmoides* leaves (Shang et al. 2022).

Table 1 The physicochemical parameters of *E. ulmoides* fermentation broth with different LAB

Treatment	pH	TSS (°Brix)	TA (%)	Color parameters		
				*L**	*a**	*b**
Control	5.89 ± 0.02^{a}	7.90 ± 0.10^{a}	0.05 ± 0.01^{d}	47.21 ± 0.50^{a}	11.88 ± 0.44^{a}	32.10 ± 4.75^{a}
L. plantarum	3.82 ± 0.06^{d}	7.77 ± 0.10^{a}	0.17 ± 0.02^{c}	31.67 ± 0.55^{d}	9.15 ± 0.29^{c}	24.02 ± 0.43^{b}
L. bulgaricus	3.99 ± 0.05^{bc}	7.87 ± 0.06^{a}	0.21 ± 0.01^{ab}	46.30 ± 0.41^{ab}	11.16 ± 0.12^{b}	32.28 ± 0.93^{a}
L. acidophilus	3.93 ± 0.03^{cd}	7.73 ± 0.06^{a}	0.24 ± 0.01^{a}	37.50 ± 0.15^{c}	9.62 ± 0.11^{c}	27.03 ± 0.02^{ab}
S. thermophilus	4.10 ± 0.04^{b}	7.87 ± 0.06^{a}	0.19 ± 0.01^{bc}	45.40 ± 0.18^{b}	11.64 ± 0.05^{ab}	32.07 ± 0.10^{a}

Values are the mean of three determinations ± SE (Standard error). Different letters indicate significant difference between different LAB by Tukey's test ($p < 0.05$)

The change in pH was the same with the oak leaf infusions and fermented beverages (Gamboa Gómez et al. 2017). The TSS of *E. ulmoides* fermentation broth varied from 7.7 to 7.8°Brix. No significant difference was detected between the control and fermentation broth and between strains, which was in agreement with the research on fermented blend beverages (de Oliveira Ribeiro et al. 2020).

Color analysis

According to the color parameter, the *L** value representing the lightness decreased after 48 h. The *L** value of the *L. bulgaricus* fermentation sample was the highest (46.30), and the *L. plantarium* fermentation sample was the lowest (31.67), which were lower than those of the controls (47.21). The reason was that the growth of LAB increased the turbidity, resulting in the decrease of *L** value (Isas et al. 2020). Meanwhile, the *a** and *b**value decreased, and only *L. bulgaricus* and *S. thermophilus* treated samples had similar values as the control after 48 h.

Bioactive compound analysis

Five peaks at the retention times of 5.4, 6.1, 6.9, 7.6, and 9.0 min were identified by UHPLC as chlorogenic acid, geniposidic acid, geniposide, rutin and quercetin. The quantification of the five compounds of four LAB strain fermentation samples was shown in Fig. 2A. The contents of chlorogenic acid and geniposidic acid of *E. ulmoides* leaves were higher, but the geniposide and quercetin contents were lower. No matter which LAB was used for fermentation, the chlorogenic acid content of fermentation broth was higher than the control ($p < 0.05$). The highest chlorogenic acid and geniposidic acid contents were observed in *L. bulgaricus* fermented samples with 0.24 and 0.19 mg/mL, respectively. Thus, fermentation could improve the number of phenolic compounds, which were the result of microbial hydrolysis reaction (Hussain et al. 2016). The same results were confirmed in Cagno 's study, which showed that *L. plantarum* affected the distribution of phenolic acids and flavonoids in jujube puree and enriched phenolic derivatives with high bioavailability (Cagno et al. 2017).

Antioxidant capacity analysis

The scavenging rates of hydroxyl, DPPH, and ABTS radicals of fermentation broth were presented in Fig. 2B. After 48 h of fermentation, the hydroxyl radical scavenging rates of *L. plantarium, L. bulgaricus, L. acidophilus* and *S. thermophilus* fermentation broth was 5.09, 5.39, 5.19, 5.24 mmol/L, respectively, *L. bulgaricus* and *S. thermophilus* fermentation broth were significantly higher than the control (4.92 mmol/L), whereas *L. plantarium* and *L. acidophilus* were not different from the control ($p < 0.0.5$). The DPPH-radical scavenging rate of *L. bulgaricus* fermentation broth was the highest (1.83 mmol/L), and *that L. plantarium* fermentation broth was the lowest (1.73 mmol/L). Meanwhile, *L. bulgaricus* and *S. thermophilus* fermentation broth were significantly higher than the control (1.70 mmol/L), whereas *L. plantarium* and *L. acidophilus* were not different from the control ($p < 0.05$). Meanwhile, the highest ABTS-radical scavenging rate was found in *L. bulgaricus* fermentation broth (0.38 mmol/L). Similarly, *L. bulgaricus* and *S. thermophilus* fermentation broth were significantly higher

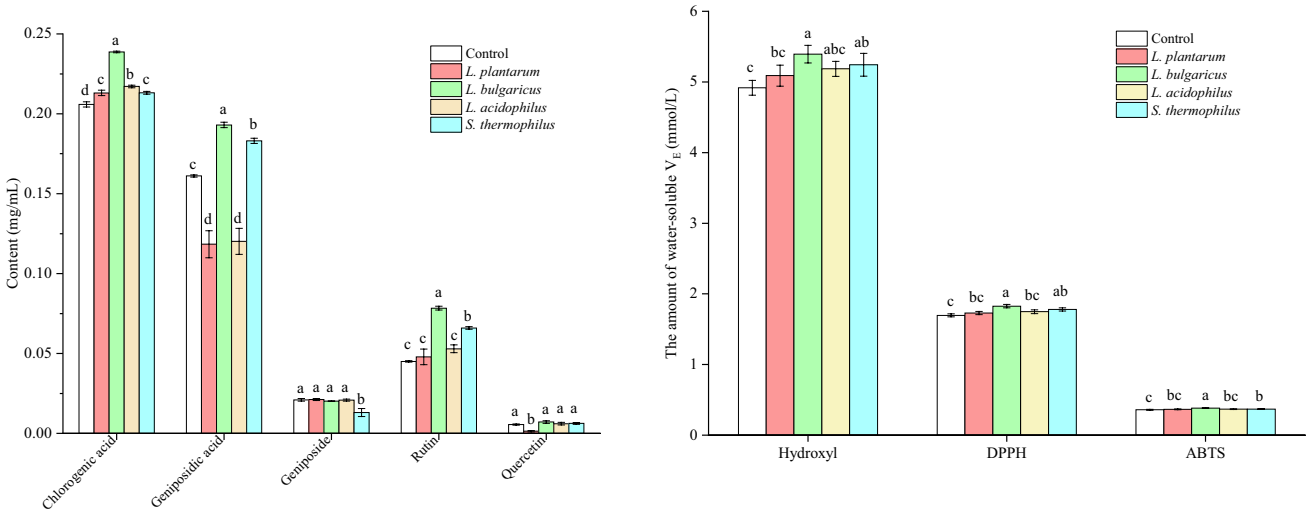

Fig. 2 Bioactive compounds and antioxidant activity in vitro of *E. ulmoides* fermentation broth by different LAB (**A**: bioactive compounds, **B**: antioxidant activity in vitro). Different letters indicate significant difference between different LAB by Tukey's test ($p < 0.05$). The standard error of the mean is denoted by a capped bar at the top of each column

than the control, whereas *L. plantarium* and *L. acidophilus* did not differ from the control ($p < 0.05$). Compared with the antioxidant activity of *E. ulmoides* leaves, the antioxidant activity of *E. ulmoides* leaves after fermentation by LAB was improved (Liu et al. 2022a).

LAB fermentation had positive effects on free radical scavenging components in *E. ulmoides* leaves. Our findings were consistent with Kwaw et al. (2018), which showed that LAB fermentation increased the antioxidant activity of mulberry juice. Furthermore, according to Cui et al. (2020), the increase in the antioxidant activity during fermentation may be due to some compounds, such as flavonoids, polyphenols and so on. According to Fig. 2A, the chlorogenic acid content in *E. ulmoides* fermentation broth was higher, which also confirmed the high free radical scavenging ability of fermentation broth.

Aroma component analysis

E. ulmoides tea is considered to contain more aroma components in most tea (Zhu et al. 2017). The volatile flavor compounds of different LAB fermentation samples were shown in Fig. 3. Totally 31 volatile compounds were identified in the control, with aldehydes being the most abundant constituents (14.36%). The volatile components identified by *L. plantarium, L. bulgaricus, L. acidophilus and S. thermophilus* after fermentation were 45, 38, 43, and 51, with ketones (18.83%), heterocycles (24.84%), alcohols (19.03%) and aldehydes (14.15%) as the highest relative contents, respectively.

The flavor features of fermented *E. ulmoides* leaves could not be accurately described by the content of volatile compounds alone, and its odor threshold should be considered.

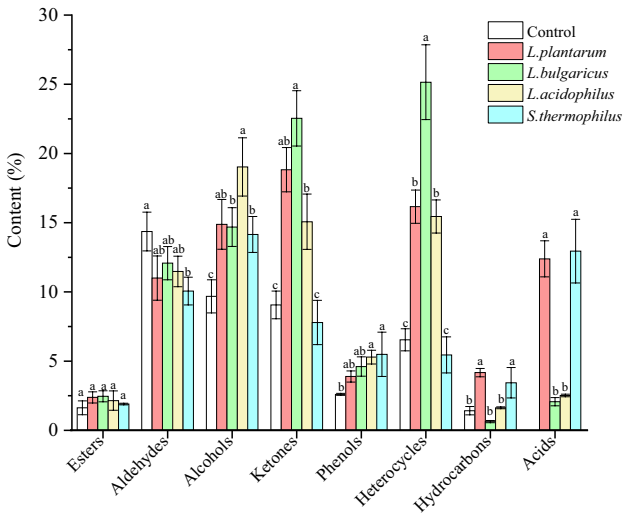

Fig. 3 Analysis of volatile components of water extract of water extract of EUL fermented by different LAB

The ROAV was used to evaluate the contribution of aroma components to the odor of fermented *E. ulmoides* leaves. According to the aroma threshold and relative content of some flavor substances, the ROAV of other flavor substances in the samples was calculated using the α-Damascenone, which contributed the most to the flavor in *L. bulgaricus* fermentation broth at $ROAV_{max} = 100$. The calculation results were shown in Table 2. Some research suggested that the components of ROAV > 1 were key flavor compounds, those of 0.1 < ROAV < 1 were modified flavor compounds, and those of ROAV < 0.1 were potential flavor compounds (Wu et al.2019). The results showed that there were 6 key aroma components in the control, and their contribution rates to the flavor were Phenylacetaldehyde, α-Damascenone, Linalool, (E, E)-2,4-Heptadienal, 3-Furfural, and (E)-2-Hexenal. There were 7 key aroma components in the fermentation broth of *L. plantarium*, and the order of their contribution rate to the flavor was α-Damascenone, 3-Furfural, Linalool, α-Ionone, (E)-Cinnamaldehyde, (E, E)-2,4-Heptadienal and (E)-2-Hexenal. Meanwhile, there were 7 key aroma components in the fermentation broth of *L. bulgaricus*, and their contributions were as follows: α-Damascenone, Linalool, α-Ionone, (E)-2-Hexenal, 3- Furfural, (E, E)-2,4-Heptadienal and (E)-Cinnamaldehyde. There were 8 key aroma components in *the L. bulgaricus* fermentation sample, including α-Damascenone, Phenylacetaldehyde, Linalool, α-Ionone, 3-Furfural, (E, E)-2,4-Heptadienal, (E)-2-Hexenal and 2,4-Di-tert-butylphenol. There were 9 key aroma components in the control, and their contribution rates to the flavor were α-Damascenone, Linalool, 3- Furfural, Phenylacetaldehyde, α-Ionone, 4-Vinylguaiacol, (E, E)-2,4-Heptadienal, (E)-Cinnamaldehyde and (E)-2-Hexenal. Therefore, the common aroma components of the fermentation broth and control were α-Damascenone, (E, E)-2,4-Heptadienal, Linalool, 3- Furfural and (E)-2-Hexenal. The contribution rate of (E, E)-2,4-Heptadienal represented green in the control was higher, and significantly decreased in the fermented samples. α-Damascenone and 3- Furfural, which represent floral and fruity, increased after fermentation. Meanwhile, α-Ionone represented floral produced after *E. ulmoides* leaves fermented. The fermentation process not only increased the main aroma but also reduced the original green flavor of *E. ulmoides* leaves, resulting in a stronger floral and fruity aroma.

7 odor types were confirmed on the basis of their odor descriptors, including woody, floral, fruity, green, roasted, chemical and fat (Table 2). To further understand the changes of the aroma components fermentation, the figure on aroma properties of sample volatiles was obtained and the coordinates were the sum of the ROAV of the aroma compounds in the same odor types with logarithmic computation. As shown in Fig. 4, the volatiles in *E. ulmoides* leaves mainly exhibited floral, woody, green, fruity odors followed

Table 2 The ROAV of *E. ulmoides* fermentation broth with different LAB

Treatment	Compounds																
	α-Damascenone	(E, E)-2,4-Heptadienal	Phenylacetaldehyde	Linalool	3- Furfural	(E)-2-Hexenal	α-Ionone	(E)-Cinnamal-dehyde	2,4-Di-tert-butylphenol	(E)-3-Hexenol	Benzal-dehyde	α-Terpineol	trans-2-Hexen-1-ol	Styrene	Phenylethyl alcohol	Benzyl alcohol	Hexanoic acid	4-Vinylguaiacol
Control	42.5	10.13	44.02	11.3	10.03	6.91	–	0.23	0.7	0.29	0.16	–	–	–	–	0.01	–	–
L. plantarum	70.85	2.02	–	21.54	30.78	1.58	19.38	2.44	0.36	0.17	–	0.14	–	0.01	0.08	0.02	0.01	–
L. bulgaricus	100	2.75	–	28.69	15.92	17.43	27.1	2.22	0.35	0.3	–	0.16	0.46	–	0.06	0.01	–	–
L. acidophilus	46.33	5.58	24.06	14.47	11.23	4.3	13.57	0.15	1.55	0.43	0.57	0.07	0.38	0.27	0.03	0.01	–	–
S. thermophilus	72.72	3.83	13.59	24.18	21.08	1.86	9.47	2.17	0.9	0.23	0.08	0.12	0.26	0.01	0.19	0.05	–	9.32
	2, 3 [a]	4	2	2, 3	2, 3, 5	4	2	1, 2, 3	3	4	3,5	2	4	3	2	2	7	1

[a]1 represents woody, 2 represents floral, 3 represents fruity, 4 represents green, 5 represents roasted, 6 represents chemical, 7 represents fat

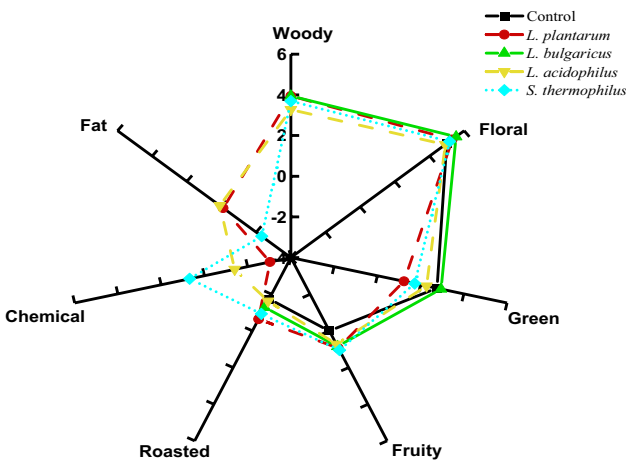

Fig. 4 The aromatic series of volatile compounds of unfermented and fermented EUL water extract by different LAB based on ROAV with logarithmic computations

by roasted, chemical and fat odors. The fermentation broth had higher floral and fruity odor than the control, especially those of *L. bulgaricus* and *S. thermophilus.* Moreover, *L. bulgaricus* fermented broth had less green odor than the control. LAB in *E. ulmoides* fermentation broth utilize different metabolic pathways for the synthesis of flavor substances, which may account for the variations in flavor composition (Li et al. 2020; Le et al. 2022a; Le and Yang 2022b). Based on these results, suitable selection of LAB strain may improve the flavor of *E. ulmoides* leaves and lead to a more appealing product with high acceptance, and *L. bulgaricus* was the best strain of *E. ulmoides* fermentation.

Conclusions

In this study, the fermentation effects of four LAB (*L. plantarium*, *L. bulgaricus*, *L. acidophilus* and *S. thermophilus*) on water extracts from *E. ulmoides* leaves were compared, including physical and chemical properties, antioxidant activity in vitro and aroma components. The results showed that proper fermentation could improve the content of active ingredients in the water extract of *E. ulmoides* leaves as well as its antioxidant and aroma properties. Among them, the content of chlorogenic acid and flavonoids increased, the antioxidant capacity was slightly improved, and the flavor was more intense. However, there were differences among different strains, and *L. bulgaricus* had the best fermentation effect on *E. ulmoides* leaves, which significantly enhanced the contents of chlorogenic acid, genipin acid, rutin and other substances as well as its antioxidant capacity. At the same time, the samples obtained after fermentation by *L. bulgaricus* had strong fruity and floral characteristics. In general, the differences between different strains should be

considered when selecting LAB for raw material fermentation of fruits and vegetables.

Author's contribution ML,WZ and LW: Methodology, Writing–review & editing, Project administration. LZ and JL: Data curation, Writing–original draft. GX: Methodology.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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