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Measurement of catechin and gallic acid in tea wine with HPLC

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ABSTRACT

First a kind of fermented tea wine was prepared from Dancong tea. The content of four major catechins and gallic acid (EC, EGC, EGCG and ECG) in tea wine was measured with HPLC. The results showed that the content of EC, EGC, EGCG, ECG and catechins in tea wine decreased when compared with that before fermentation. The content of EC decreased the most, reaching 26.79%, while the content of GA changed the least with a decrease of only 13.56%. Nevertheless, tea wine still contains a relatively large amount of catechins, thus proper consumption of tea wine may be salubrious.

1. Introduction

Tea wine refers to wine made from tea, fermented or prepared. Fermented tea wine is made by adding sugar or juice to tea, which is then fermented with yeast or koji to produce tea wine with low alcoholic content (Zhang et al., 2008). Zhang et al. (2008) added pear juice to Tieguanyin tea to produce tea with light pear aroma through fermentation with active dry yeast; Qiu (2010) added apple juice to tea, then fermented special yeast for grape wine, using low-grade green and black tea as raw material; Ma (2014) fermented green tea containing sugarcane juice with grape wine yeast, producing tea wine with sugarcane aroma; He et al. (2015) produced Pu'er tea wine with the semi-solid fermentation process by adding xiaoqu and using Pu'er tea and rice as raw material; Joshi and Kumar (2017) produced high quality apple tea wine by fermenting apple juice tea with Saccharomyces cerevisiae and oval yeast.

Tea wine contains catechins, gallic acid (GA) and other strong active antioxidants, proper intake of which may be good for health. Catechins and GA are polyphenols contained in natural plants such as tea. Catechins in tea mainly consist of four kinds: (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG), the structure of which is shown in Fig. 1, Catechin has been reported to have a variety of physiological functions, such as anti-cancer, anti-virus and weight loss (Xu et al., 2017; Yang and Wang, 2016) functions. In addition, GA is anti-inflammatory, anti-tumor and liver protective, not to mention other physiological effects (Kim et al., 2018; Kylili et al., 2018; Liu, 2018; McClements, 2018; Sanae et al., 2003).

Dancong tea is a famous Oolong tea produced in Guangdong Province of China (Fakeeha et al., 2018; Jiao et al., 2018; More et al., 2017; Zong et al., 2018). It has both the fragrance of green tea and the thickness of black tea. It concentrates the fragrance of flowers, fruits and tea (Ahamed et al., 2017; Anzlovar et al., 2018; Camara et al., 2018; Girtler, 2018; Jiang et al., 2018; Liu and Liu, 2010). In this study, we first prepared fermented tea wine with Dancong tea as raw material and yeast as starter, and then measured the content of catechins and GA with high performance liquid chromatography (HPLC). This study is the first to determine the content of catechins and GA in Oolong tea wine by HPLC, which is of great significance for further understanding the health function of tea wine.

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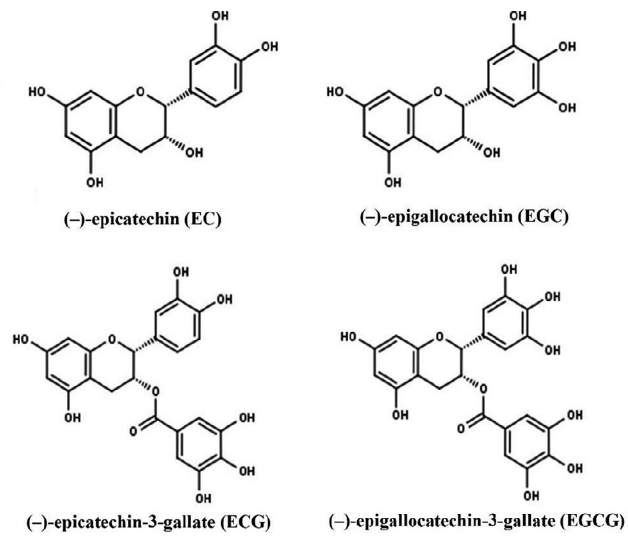


Fig. 1. Four main catechins in tea: EC, EGC, ECG, EGCG.

2. Materials and methods

2.1. Materials

Tea wine yeast T-5: Saccharomyces cerevisiae (ATCC 204508) was acquired from tea juice and kept as 4 °C slants.

Tea: Dancong tea (first grade), purchased from Chaozhou of Guangdong Province with a moisture content of 4.38%, sealed and reserved.

Acetonitrile (chromatographic pure); GA and four catechins EC, ECG, EGC, EGCG standard (analytical grade), both 20 mg/vial; wort medium, sucrose, ammonium sulfate, citric acid, Potassium sulfate, formic acid, etc. are all of domestic analytical grade.

Wort culture medium: Take 130.1 g of wort medium powder, add 1L of water, heat and stir the mixture until it is all dissolved, then store it in 250 mL flasks according to a volume percentage of 24% after, sterilization.

2.2. Equipments

ATC hand-held refractometer, FE20K desktop pH meter, precision alcohol meter (division value of 0.1% vol), ZY-F32 thermostatic fermentation chamber, JYD-900 ultrasonic cell disruption instrument and Agilent 1260 LC liquid chromatograph.

2.3. Tea wine production

The tea was first filtered with 85 °C water for 10 min, with a tea-to-water ratio of 1:40 (g/mL). Sucrose 15°Bx was then added to the juice, after which 30 g/L ammonium sulfate was added as a nitrogen source, and citric acid was added to adjust the pH to 4.0. Finally, 60 mg/L potassium metabisulfite was added to prevent oxidation of catechins and inhibit the growth of bacteria in tea juice. A portion of the chalcopyrite strains of tea and wine was placed into the wort medium at 28 °C, shaken for 48 h at a rate of 150 r/min. The 3% (V/V) Yeast seed liquid was added to the above tea juice, placed in the fermentation tank. Initially, the fermentation broth was kept at 28 °C under a certain amount of oxygen to ensure the normal growth and reproduction of yeast, and the process was maintained for 48 h. Then, the fermentation vessel was sealed with 8 layers of gauze and anaerobic fermentation at 20 °C until the surface of the fermentation broth had no more bubbles, indicating the end of the fermentation. Finally, the broth was clarified, filtered and sterilized to obtain tea wine with an alcohol content of 8.35% vol.

2.4. Tea wine catechins and GA measurement

2.4.1. Catechins and GA measurement

(1) Add 2 mL of water and 2 mL of methanol to each of the 5 standards (GA, EC, ECG, EGC, EGCG) vials. Since the vials are 20 mg each, the concentration of each standard solution is 5 mg/mL.
(2) Pipette 100 μL of each standard solution into a 25 mL volumetric flask and dilute with a solution of acetonitrile (0.2% formic acid = 5:95) to prepare a mixed standard solution, the single standard concentration of which is 20 mg/L.
(3) Pipette 0.5 mL, 1 mL, 2 mL, 4 mL, 5 mL and 10 mL of the above mixed standard solution respectively into a 10 mL volumetric flask, and dilute to volume with a solution of acetonitrile: 0.2% formic acid = 5: The mixed standard solutions were prepared at concentrations of 1 mg/L, 2 mg/L, 4 mg/L, 8 mg/L, 10 mg/L and 20 mg/L.
(4) First, we conduct separate HPLC analysis on the five kinds of standard solutions in (1) and the five kinds of standard mixed solutions in (3). The peak area and concentration of each standard in the mixed standard are respectively the abscissa and the vertical coordinate. Curves of the five standards were respectively drawn, and the curve equations of which were used to calculate the catechin and GA contents in the tea wine.
(5) The tea juice and tea wine were tested with HPLC, and the corresponding catechins and GA measured according to the retention time. Then the catechins and GA content in tea juice and tea wine were calculated according to the standard curve equation of each standard.

2.4.2. HPLC test conditions

Column: Agilent ZORBAX SB-C18 (4.6 × 250 mm, 5 μm). Mobile phase: Phase A is acetonitrile; Phase B is 0.2% formic acid solution. Flow rate: 1.2 mL/min. Column temperature: 25 °C. Injection volume: 10 μL. UV detection wavelength: 278 nm. Gradient elution, as shown in Table 1.

3. Results and analysis

3.1. Catechin standard curve

The results of HPLC analysis on GA, EC, ECG, EGC and EGCG standard solutions are shown in Fig. 2. The results of HPLC analysis of mixed standard solution with different concentration levels are shown in Fig. 3.

Table 1
Gradient elution process.

Time (min)	A: acetonitrile (%)	B: 0.2% formic acid solution (%)
0	5	95
3.5	5	95
5	10	90
10	10	90
30	28	72

Standard curves of 5 standards obtained from Figs. 2 and 3 as shown in Table 2:

3.2. Measurement results of tea catechins and GA in tea wine

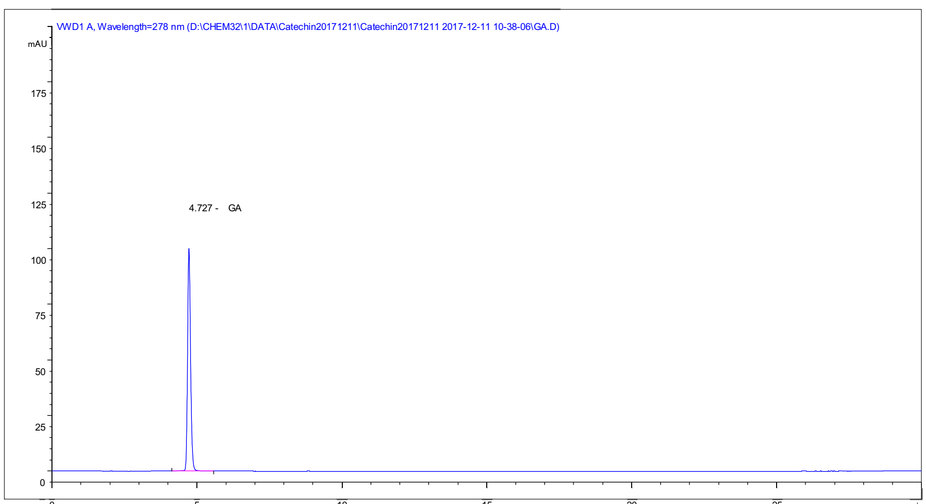
The measurement of catechins and GA by HPLC was carried out respectively on tea wine and tea juice before fermentation. The results are shown in Figs. 4 and 5:

Plug the catechins in tea juice and tea wine and GA peak area into the corresponding standard curve equation, and we can calculate the catechin and GA content, as shown in Table 3.

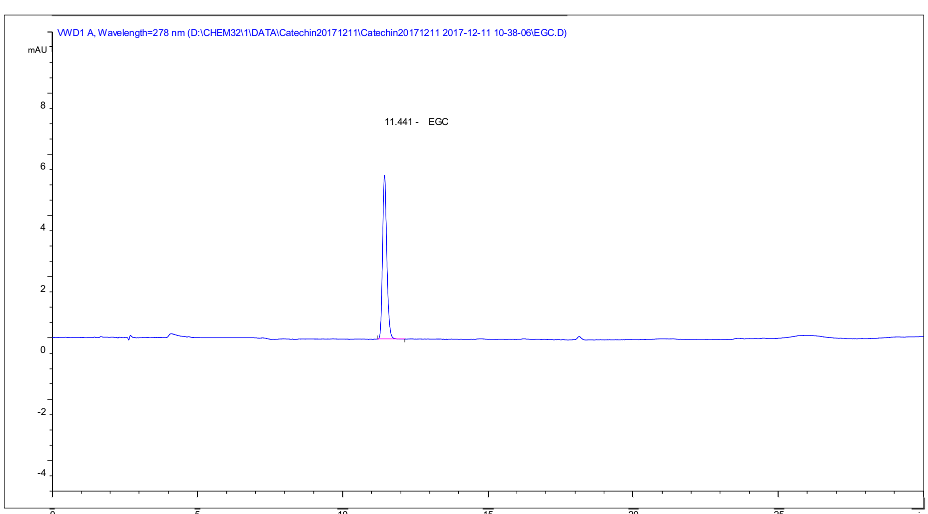
As can be seen from Table 3, the catechins and GA contents in the tea wine are high, the highest of which appears in the case of EGCG, followed by EGC. In addition, the catechin and GA content of tea juices decreased after fermentation, of which EC decreased most, reaching 26.79%, while GA changed the least, decreasing by only 13.56%. This may be due to the oxidization of some catechins and GA during tea brewing, which eventually led to the reduction of catechins and GA in tea wine.

4. Conclusion

Through HPLC analysis, we find in this study that fermented tea wine made from Dancong tea contains a relatively large amount of catechins (especially EGCG and EGC) and GA, despite the decrease in catechin and GA content after tea fermentation. Hence proper consumption of tea wine may be salubrious.

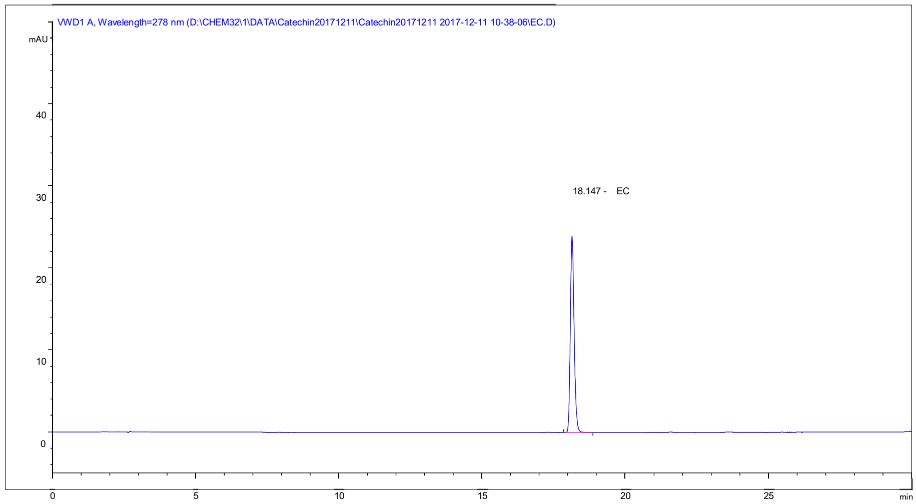


a. GA standard.

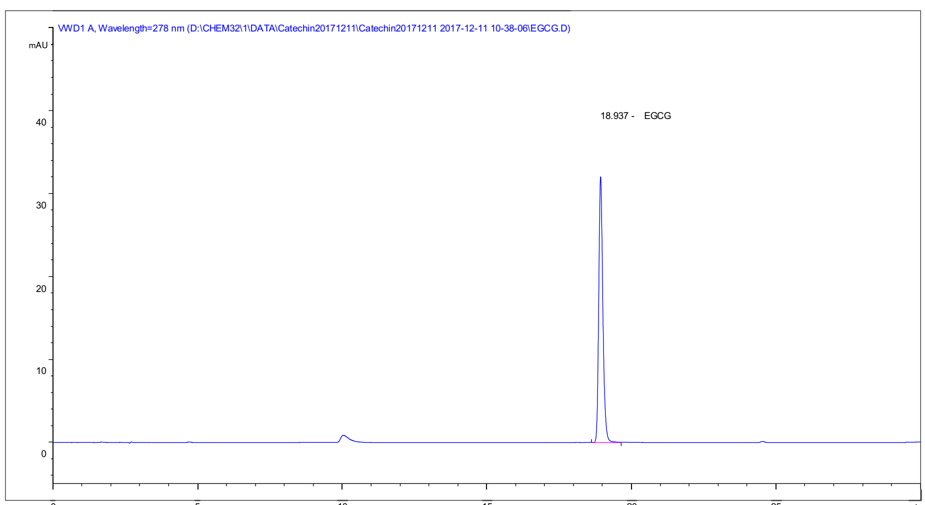


b. EGC standard.

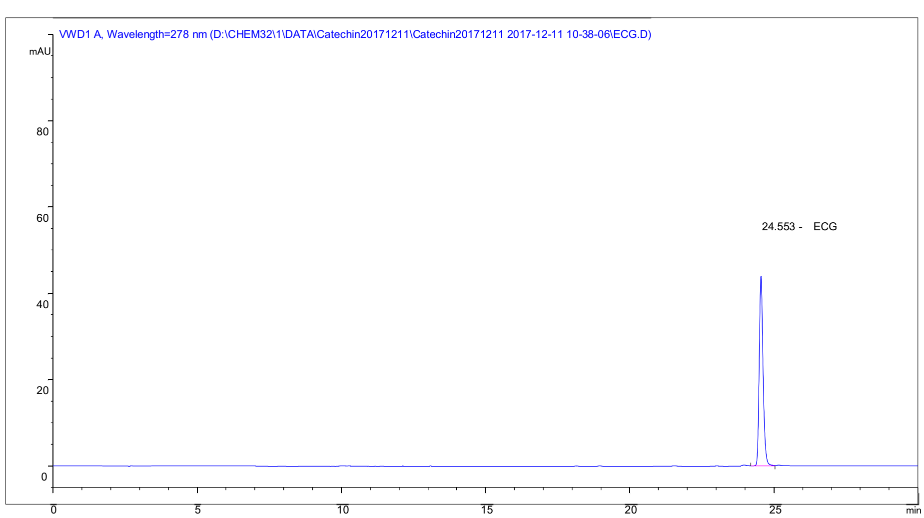
Fig. 2. HPLC chromatograms of 5 standards.



c. EC standard.

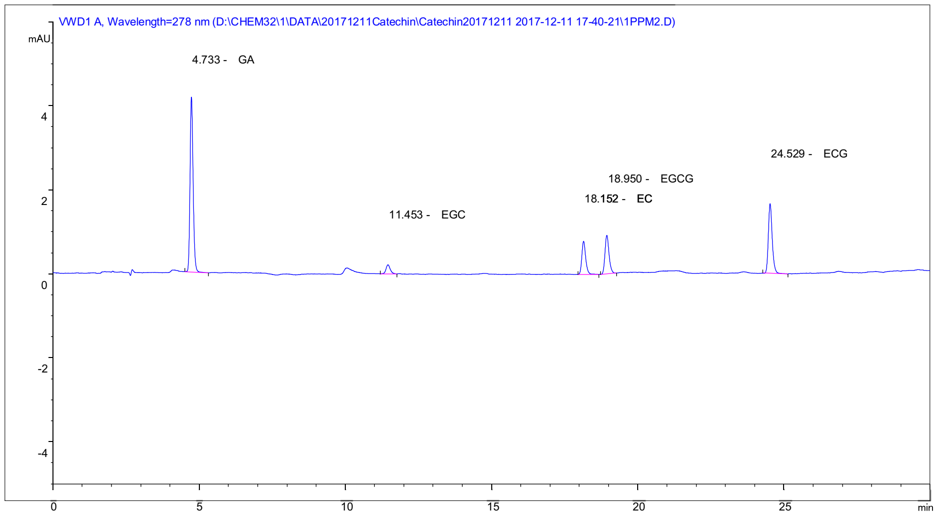


d. EGCG standard.

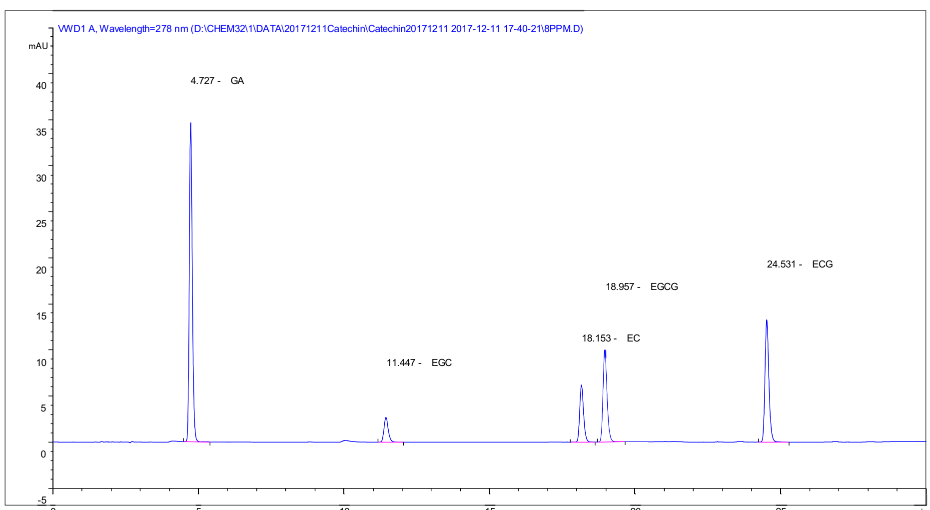


e. ECG standard.

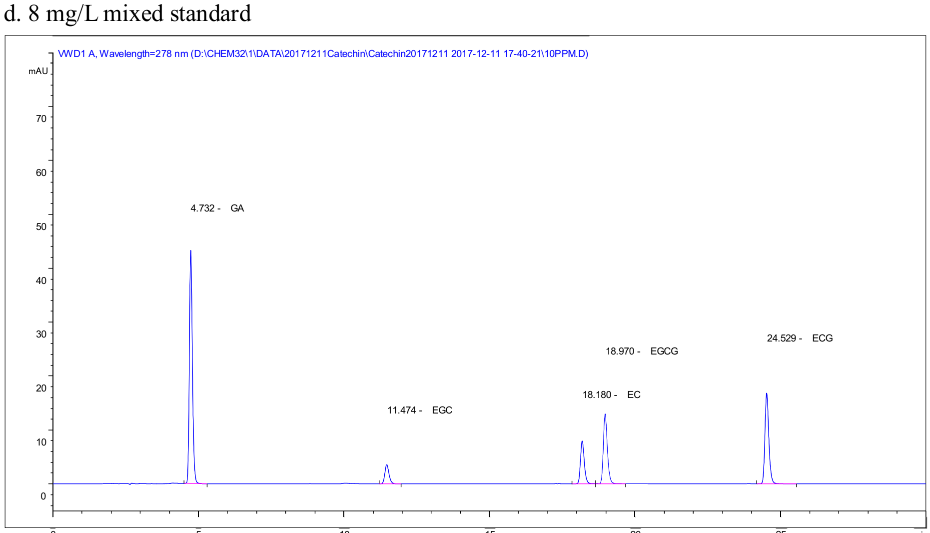
Fig. 2 (*continued*)



a. 1 mg/L mixed standard

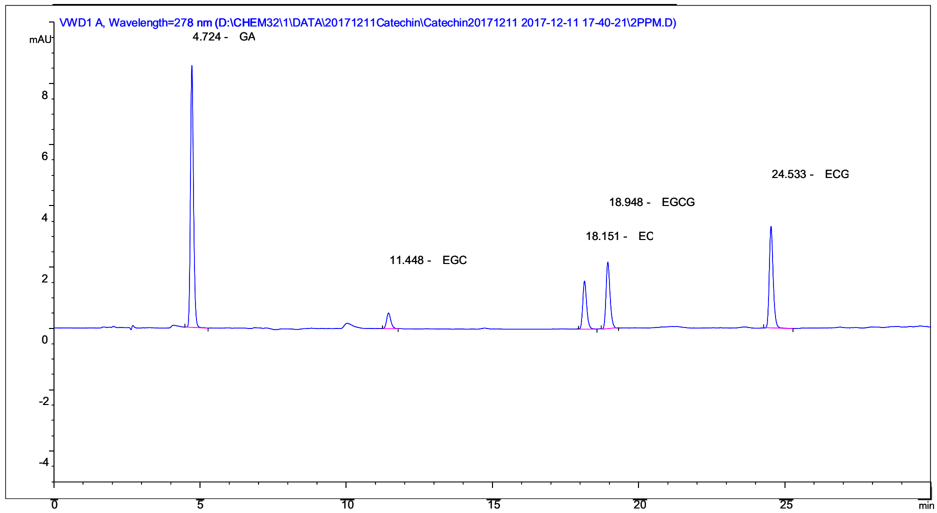


d. 8 mg/L mixed standard

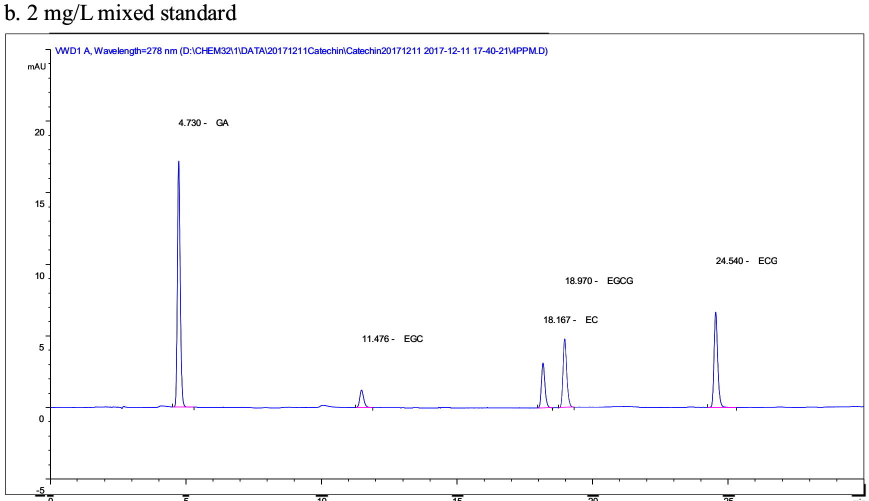


e. 10 mg/L mixed standard

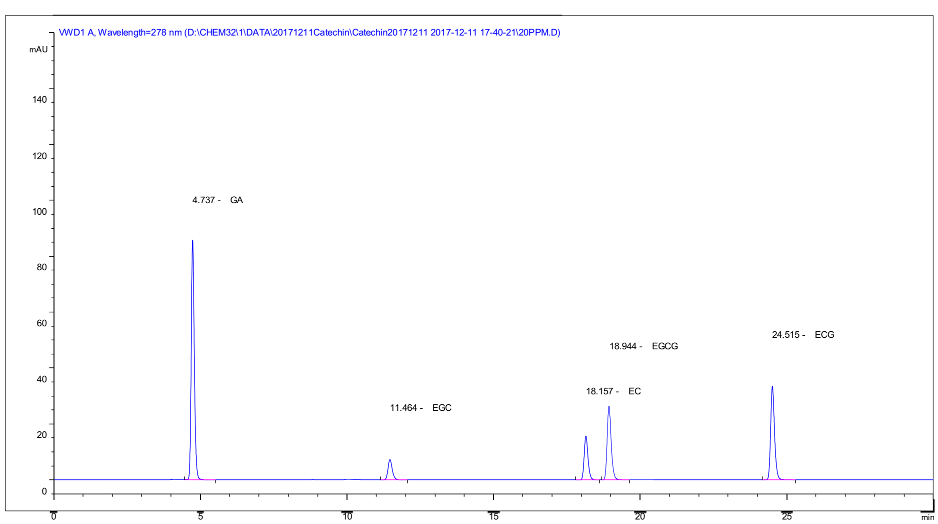
Fig. 3. HPLC analysis chromatogram of mixed standards with different concentration levels.



b. 2 mg/L mixed standard



c. 4 mg/L mixed standard



f. 20 mg/L mixed standard

Fig. 3 (*continued*)

Table 2
Standard curves of 5 standards.

Standard	Curve equation	R^2
GA	$y = 31.0977715x + 0.2595105$	0.99999
EGC	$y = 1.85341812x - 1.4555661$	0.99940
EC	$y = 7.10854605x + 0.1341142$	0.99999
EGCG	$y = 12.833587x - 3.0139447$	0.99981
ECG	$y = 15.5410578x + 0.1514005$	0.99999

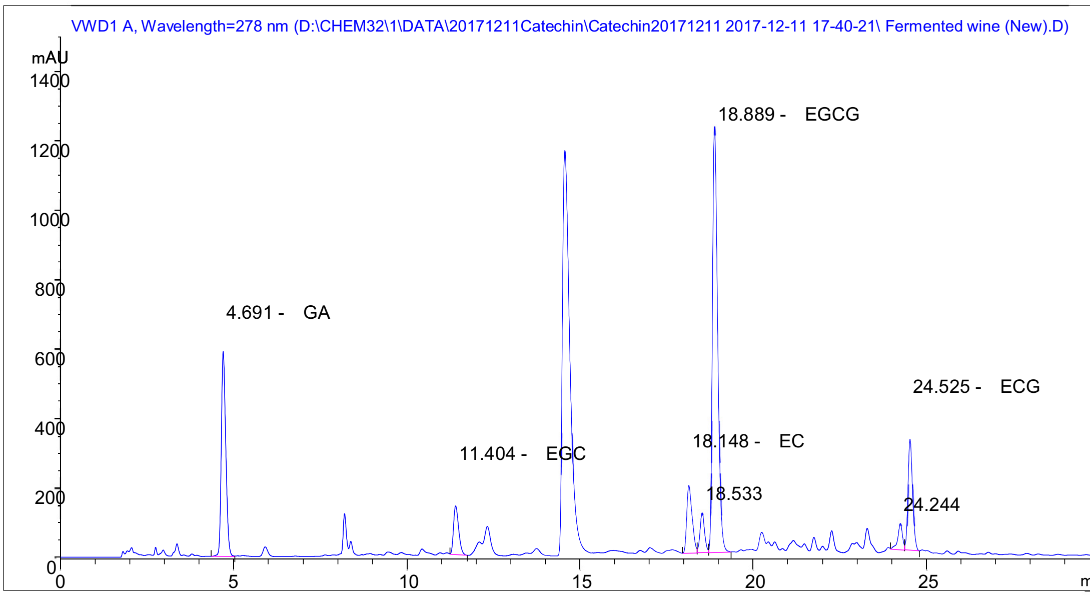


Fig. 4. HPLC measurement of catechins and GA in tea juice.

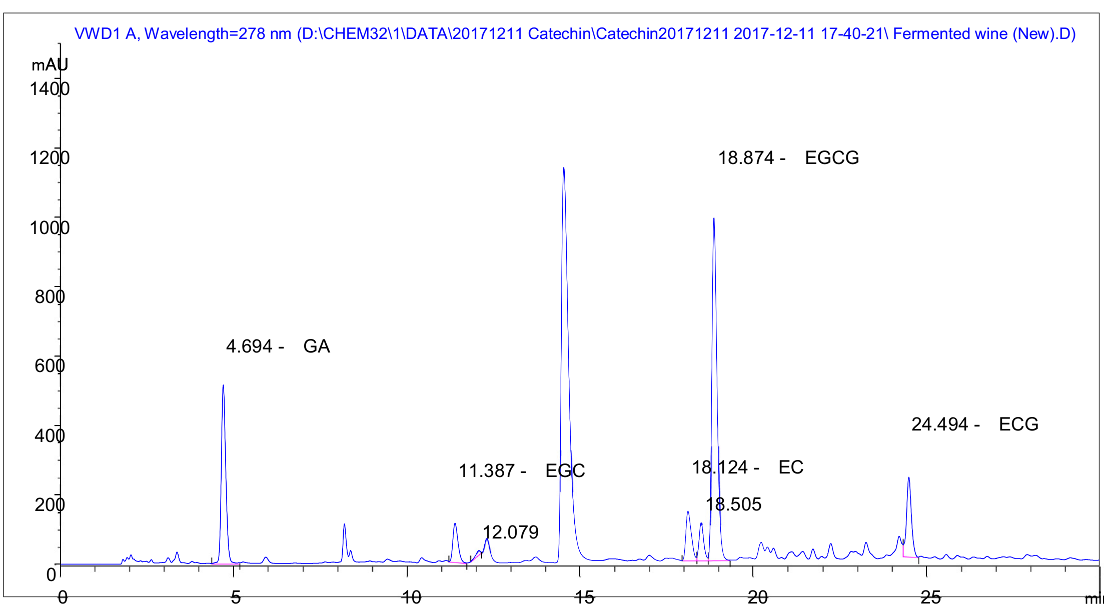


Fig. 5. HPLC measurement of catechins and GA in tea wine.

Table 3
Catechin and GA content in tea and tea wine.

Sample	Catechin content μg/mL				
	GA	EGC	EC	EGCG	ECG
Tea juice	171.02850	787.75397	294.37727	997.48563	189.97482
Tea wine	147.83345	616.58229	215.51305	787.52471	147.87288

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Author's contributions

Yong-jun Li and Shuai Zhang have produced Dancong tea wine and subsequently detected catechins and GA of the tea wine using HPLC method. Yuan-ming Sun has given detailed instructions for the design of the experimental protocol.

References

Ahamed, A.J., Loganathan, K., Ananthakrishnan, S., 2017. Evaluation of graphical and multivariate statistical methods for classification and evaluation of groundwater in Alathur Block, Perambalur District, India. Appl. Ecol. Env. Res. 15 (3), 105–116.

Anzlovar, A., Krzan, A., Zagar, E., 2018. Degradation of Pla/Zno and Phbv/Zno composites prepared by melt processing. Arab. J. Chem. 11 (3), 343–352.

Camara, E.M., Caramaschi, E.P., Di Dario, F., 2018. Short-term changes in two tropical coastal lagoons: effects of sandbar openings on fish assemblages. J. Coastal. Res. 34 (1), 90–105.

Fakeeha, A.H., Ibrahim, A.A., Khan, W.U., 2018. Hydrogen production via catalytic methane decomposition over alumina supported iron catalyst. Arab. J. Chem. 11 (3), 405–414.

Girtler, J., 2018. Features of load and wear of main propulsion devices on sea-going ships with piston combustion engines and their impact on changes in technical states of the systems. Pol. Marit. Res. 24 (4), 57–66.

He, T.T., Liu, F., Du, L.P., 2015. Development of Pu'er Tea Wine. Liquor-mak. Sci. Technol. 10, 103–106.

Jiang, M., Wu, H., Li, Z., 2018. Highly selective photoelectrochemical conversion of carbon dioxide to formic acid. ACS Sustain. Chem. Eng. 6 (1), 82–87.

Jiao, Y., Zheng, X., Chang, Y., 2018. Zein-derived peptides as nanocarriers to increase the water solubility and stability of lutein. Food Funct. 9 (1), 117–123.

Joshi, V.K., Kumar, V., 2017. Influence of different sugar sources, nitrogen sources and inocula on the quality characteristics of apple tea wine. J. I. Brew. 123 (2), 268–276.

Kim, N.Y., Jeon, E.J., Jung, S.H., 2018. Gene expression profiling and expression analysis of freshwater shrimp (Neocaridina Denticulata Denticulata) using expressed sequence tags and short-term exposure to copper. J. Environ. Biol. 39 (1), 51–57.

Kylili, A., Fokaides, P.A., Ioannides, A., 2018. Environmental assessment of solar thermal systems for the industrial sector. J. Clean. Prod. 176, 99–109.

Liu, H., Liu, Z., 2010. Recycling utilization patterns of coal mining waste in China. Resour. Conserv. Recy. 54 (12), 1331–1340.

Liu, Z., 2018. Economic analysis of energy production from coal/biomass upgrading; Part 1: hydrogen production. Energy Source. Part. B 13 (2), 132–136.

Ma, L., 2014. Production Technology of Fermented Sugarcane and Tea Wine. Anhui Agricultural University, Hefei.

McClements, D.J., 2018. Enhanced delivery of lipophilic bioactives using emulsions: a review of major factors affecting vitamin, nutraceutical, and lipid bioaccessibility. Food Funct. 9 (1), 22–41.

More, G., Raut, D., Aruna, K., 2017. Synthesis, spectroscopic characterization and antimicrobial activity evaluation of new tridentate schiff bases and their Co(Ii) complexes. J. Saudi. Chem. Soc. 21 (8), 954–964.

Qiu, X.P., 2010. Studies on the Fermentation Technology of Tea Wine. Anhui Agricultural University, Hefei.

Sanae, F., Miyaichi, Y., Hayashi, H., 2003. Endothelium-dependent contraction of rat thoracic aorta induced by gallic acid. Phytother. Res. 17 (2), 187–189.

Xu, J., Xu, Z., Zheng, W.M., 2017. A review of the antiviral role of green tea catechins. Molecules 22 (8), 1337.

Yang, C.S., Wang, H., 2016. Cancer preventive activities of tea. Molecules 21 (12), 1679.

Zhang, S., Dong, J., Chen, S.Y., 2008. Study on preparation of fermented TIKUANYIN tea wine. Sci. Technol. Food Ind. 29 (10), 159–161.

Zong, H., Cao, Y., Liu, Z., 2018. Energy security in group of seven (G7): a quantitative approach for renewable energy policy. Energy Sour. Part. B. 13 (3), 173–175.