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Antioxidant and Angiotensin-Converting Enzyme Inhibitory Activity of *Eucalyptus camaldulensis* and *Litsea glaucescens* Infusions Fermented with Kombucha Consortium

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Summary

Physicochemical and chemical properties, consumer acceptance, antioxidant, and angiotensin-converting enzyme (ACE) inhibitory activities of infusions and fermented beverages of *Eucalyptus camaldulensis* and *Litsea glaucescens* were compared. On physicochemical parameters, only the pH decreased as compared with the unfermented infusions. No relevant changes were reported in consumer preference between infusions and fermented beverages. Phenolic profile measured by UPLC MS/MS analysis demonstrated significant concentration changes of these compounds in plant infusions and fermented beverages. Fermentation process induced a decrease in the concentration required to stabilize 50 % of DPPH radical (*i.e.*, lower IC_{50}). Additionally, it enhanced the antioxidant activity measured by the nitric oxide scavenging assay (NO) (14 % for *E. camaldulensis* and 49 % for *L. glaucescens*); whereas, not relevant improvements were observed in the lipid oxidation assay compared with unfermented infusions. Same behavior was observed in the inhibitory activity of ACE; however, both infusions and fermented beverages resulted in lower IC_{50} than positive control (Captopril). The present study demonstrated that the fermentation process has an influence on the phenolic

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concentration and its potential bioactivity. *E. camaldulensis* and *L. glaucescens* can be considered as natural sources of bio compounds with antihypertensive potential either as infusions or fermented beverages.

Key words: herbal infusions, fermented beverages, Kombucha, antioxidant activity, polyphenols

Introduction

In recent years, the consumption of herbal infusions around the world has increased due to its beneficial health effects. These beverages are prepared by placing a small amount of the selected plant material in freshly boiled water, allowing the preparation to steep for a short period of time (*1*). Although, herbal infusions do not have any particular nutritional value, they represent an important source of bioactive compounds such as polyphenols. It has been shown that these compounds can act by diverse mechanisms providing significant protection against chronic diseases (*2*). For example, the consumption of some antioxidant herbal polyphenols may regulate hypertension through inhibition of the angiotensin-converting enzyme (ACE), a key component in the renin-angiotensin aldosterone system which regulates blood pressure (*3*). Recently, several research studies have focused on enhancing the beneficial health potential of herbal infusions. For example, Hoon *et al*. (*4*) reported that fermentation process with Kombucha fungus on five commonly consumed teas enhanced the phenolic content, the antioxidant activity, and the alpha-amylase inhibitory activity. Similar results were observed by Velićanski *et al*. (*5*) on fermented lemon balm (*Melissa officinalis* L.) infusion; they reported an enhancement of phenolic compounds such as rosmarinic acid (1.3-fold higher), caffeic acid (1.9-fold higher), and ferulic acid (4.6- fold higher fold), as well as major antioxidant activity against DPPH radicals compared to unfermented infusions.

The beneficial effects of these fermented beverages are attributed to the presence of restructured polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics and a variety of micronutrients produced during fermentation (*6*).

The fermentation process involves the activity of yeasts that ferments glucose and fructose to ethanol, which is then oxidized to acetic acid by acetic acid bacteria (AAB). The main source of carbon in this process is sucrose. The sugar is hydrolyzed by the enzyme invertase from

yeast present in the Kombucha consortium, producing ethanol via the metabolic pathway of glycolysis, with a preference for fructose as the substrate.

Subsequently, AAB convert glucose and ethanol into gluconic and acetic acids (*7*).

In occidental countries, the consumption of *Camellia sinensis* and analogues, such as fermented beverages is not that common, predominating the consumption of infusions made with plant materials typical of each region. In this same regard, in a previous study we analyzed phenolic composition and anti-inflammatory effect of different herbal infusions (*9*), including *Eucalyptus camaldulensis* and *Litsea glaucescens*. Results showed anti-inflammatory activity through the positive modulation of COX-2, TNFα, NFκB, and IL-8 in HT-29 cells, especially for *L. glaucescens*. Even though, both plants have different traditional uses (*e.g. Eucalyptus camaldulensis* is consumed for decongestant, antiseptic, anti-inflammatory, antibiotic, among other purposes; whereas, *Litsea glaucescens* infusion is traditionally consumed for its bactericide, antiseptic, expectorant and anti-inflammatory properties), both of them contain bioactive compounds such as polyphenols with antioxidant activity (*8-9*).

Although, these plant materials are commonly consumed as infusions, the fermentation process may or may not increase their positive effects. However, this is unknown and can be poorly related with black tea, despite the fact that fermentation conditions are similar. Therefore, the aim of this work was to compare chemical composition, consumer acceptance, antioxidant, and angiotensin-converting enzyme inhibitory activities of *E. camaldulensis* and *L. glaucescens* in traditional infusions and fermented beverages prepared with the Kombucha consortium.

Materials and Methods

Plant materials

Representative samples of *Eucalyptus camaldulensis* and *Litsea glaucescens* were collected in Durango, Dgo., Mexico and positively identified by botanist Socorro González-Elizondo. Voucher specimens (44008 and 44007, respectively) were deposited at the Herbarium of the Centro Interdisciplinario de Investigacion para el Desarrollo Integral Regional Unidad Durango (CIIDIR-IPN, Durango, Mexico).

Preparation of herbal infusions and fermented beverages

Both materials were dried at room temperature under shade, ground (1.13 mm) and stored in darkness for later determinations. Infusions (1 % by mass per volume) were prepared by adding 200 mL of freshly boiled water to 2 g of dried ground sample. Infusions were let to stand for 10 min and filtered.

Fermentation process was performed using Kombucha culture from a Mexican trading house (Healthy, Natural Life, Tlaquepaque, Jalisco, Mexico). A previous study reported that Kombucha consortium contained yeast strains such as: *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, *Saccharomyces bisporus*, *Torulopsis* sp., *Zygosaccharomyces* sp., *Dekkera*, *Kazachstania*, *Pichia*, among others. Whereas, bacteria such as *Acetobacter*, *Gluconacetobacter*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Bifidobacterium*, *Thermus*, *Allobaculum*, *Ruminococcaceae Incertae Sedis*, *Propionibacterium*, among others, were also reported (*10*).

The fermentation of infusions was performed using the conditions reported by Vázquez-Cabral *et al*. (*7*). The infusions were cooled at room temperature, and then sweetened by dissolving 10 g/L of sucrose. On small bioreactors, 1 L of sweetened infusions were inoculated and acidified with 2.5 g of inoculum (biofilm with a symbiotic culture of bacteria and yeasts) and 100 mL of black tea vinegar, respectively. The bioreactors were covered with cheesecloth, and the fermentation at 25±1 °C was monitored for seven days. On the last fermentation day, the biomass was removed from the beverage by filtration.

For preparation of black tea vinegar, inoculum was kept growing at 25 °C in sweetened (sucrose, 10 %) black tea for 20 days. Freshly cultured Kombucha was used for further subcultures or for fresh fermentation batches.

Physicochemical evaluation

The pH was measured using a digital pH-meter (pH meter Sens *Ion* 1, Hanna Instruments, Port Louis, Mauritius) calibrated at pH=4, pH=7, and pH=10 at 25 °C. Color characteristics of infusions were evaluated by measuring their color coordinates with a Konica Minolta colorimeter (Chroma Meter CR-400/410, Osaka, Japan). Measurements of color intensity were expressed as L^* (represents lightness), a^* (represents the red/green opponent colors), and b^* (represents yellow/blue opponent colors) values.

Consumer preference tests

The consumer preference evaluation of infusions was performed with 15 untrained panelists who declared drinking 1 cup of any herbal infusion at least once a week; they were included in the test using a Rank-Rating method. The scale used was: 1 = very unpleasant to 9 = very pleasant. The samples were coded with random numbers of three digits. Black tea was used as a positive control.

Sugar content in fermented beverages

Sample analysis was carried out with an Acquity UPLC system (Water Corp., Milford, MA, USA) coupled with an evaporative light scattering detector (ELSD) (Water Corp., Wexford, Ireland). The LC system consisted of a sample manager (5 °C) and a binary solvent manager. The column used to determine fructose, glucose and sucrose was an Acquity UPLC BEH Amide, 100 mm X 2.1 X 1.7 µm (Waters Corp., Wexford, Ireland) operated at 50 °C. The elution profile used two solvents (JT Baker, Mexico, Mexico) (A) acetonitrile/water with 0.1 % NH_4OH (80/20), and (B) acetonitrile/water with 0.1 % NH_4OH (30/70) and an initial gradient of 5-60 % B in 5 min, isocratic by 1 min, reset and equilibrated for 5.5 min. The flow rate was 0.25 mL/min. The UPLC control and data processing were performed using the Masslinx (Waters Corp., Milford, MA, USA) software.

Ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS) analysis

Sample analysis was carried out with an Acquity UPLC system (Water Corp., Milford, MA, USA) coupled with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Wexford, Ireland). The LC system consisted of a sample manager (20 °C) and a binary solvent manager. The column used to determine flavan-3-ols was an Acquity UPLC BEH C18 50 mm X 2.1 X 1.7 µm (Waters Corp., Wexford, Ireland) operated at 40 °C. The elution profile included two solvents (JT Baker, Mexico, Mexico), acidified water with 1 % formic acid (A) and methanol LC-MS (B): initial 98 % A; 0 - 2 min, 68 % A; 2 – 3.8 min, 55 % A; 3.8 – 4.5 min 45 % A, 4.5 – 6.0 min 5 % B (linear gradient) for column washing and subsequently 6.0 – 9.5 min, 98 % A for column stabilization. MRM data were collected from 0 to 9.5 min.

Negative ionization mode was used for MS assays. ESI conditions were as follows: capillary voltage, 2.85 kV; desolvation temperature, 500 °C; source temperature, 150 °C; desolvation and cone gas, 794 L/h and 151 L/h, respectively, and collision gas, 0.14 mL/min. For identification and quantification, a multiple reaction-monitoring mode with standards was used. Rutin (20 ng/μL) was employed to check stability of the ionization efficiency of mass spectrometer and a mixture of phenolic compounds standards (20 ng/μL, SIGMA Co., St. Louis, USA) for monitoring retention time and *m/z* values. The UPLC and tandem Xevo TQ-S triple quadrupole mass spectrometer control and data processing were operated using MassLinx (version 4.1, Waters Corp., Milford, MA, USA) software.

Radical scavenging and chain-breaking assay

Antioxidant capacity of herbal infusions and fermented beverages were evaluated using the stable radical *2,2-diphenyl-1-picrylhydrazyl* (DPPH•) (SIGMA Co., St. Louis, USA) method described by Brand-Williams *et al*. (*11*). The percentage of scavenging or quenching radicals Q was calculated using the following equation:

$$Q = 100\ (A_0 - A_c)/A_0 \quad /1/$$

Where A_0 is the absorbance of the blank; A_c is the absorbance of the mixture: radical and sample at different concentrations. For the calculation of the half maximal inhibitory concentration (IC_{50}: μg/μL), the logarithm of the concentration against the inhibition percentage was plotted.

For chain-breaking kinetics, 3 mL of 3×10^{-5} mol/L DPPH in methanol solution was used. The reaction began with the addition of 10 μL (previously determined the IC_{50} for each beverage). The DPPH bleaching was monitored at 515 nm with a spectrophotometer al 25 °C for at least 60 min (*12*). The following equation was used in order to obtain the reaction rate k:

$$\frac{1}{A^3} - \frac{1}{A_0^3} = 3kt \quad /2/$$

Where A is the absorbance at increasing time (t), and A_0 is the initial absorbance. Results were expressed as both: IC_{50} and chain-breaking activity k/mg dm (-1/min/mg dm). Catechin was used as positive control.

Lipid peroxidation assay

Lipid peroxidation was performed according to Jozwik *et al*. (*13*) with several modifications. Inhibition of the formation of thiobarbituric acid-reactive substances (TBARS), a lipid oxidation product was quantified spectrophotometrically (Varian Cay spectrophotometer model 50-BIO, Melbourne, Australia), using healthy human plasma donated by the blood bank of the Public General Hospital (Durango, Dgo., Mexico). Inhibited Fenton reaction (150 µL of 500 mM H_2O_2 and 150 µL of 100 mM $FeCl_3$ for 3 h at 37 °C) (Caledon Lab., Ontario, Canada); catalyzed plasma (600 µL) oxidation was evaluated for each herbal infusion and fermented beverage (100 µL) at several concentrations, using phosphate buffer (pH=7.4) to reach the final volume. Prior to oxidation phase, a thiobarbituric acid (TBA) reagent (MP bio, Illkirch, France) was prepared consisting of 40.5 mL 20 % acetic acid buffered at pH=3.5 with 1 M NaOH, 13.2 mL 8.2 % SDS (SIGMA Co., St. Louis, USA), 40.5 mL 0.8 % TBA, and made up with double distilled water to 100 mL. Samples (1 mL) were mixed with 4.0 mL of TBA reagent and incubated at 96 °C for 80 min, and cooled down on ice. Then, they were mixed with *N*-butanol (5 mL) and centrifuged at (3000xg) for 15 min. The ability of the samples to inhibit oxidation was determined by the decrease on absorbance at 532 nm. For the calculation of the IC_{50} (µg/µL), the logarithm of the concentration against the inhibition percentage was plotted. Catechin standard (SIGMA Co., St. Louis, USA) was included as positive control.

Nitric oxide inhibition and nitric oxide scavenging assay

Infusions and fermented beverages were screened for antioxidant capacity potential using the nitric oxide scavenging assay described by Balakrishnan *et al*. (*14*). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide, subsequently coupled with naphthylethylenediamine dichloride (Griess reagent, SIGMA Co., St. Louis, USA) was read at 546 nm. Catechin was used as a positive control and results were expressed as IC_{50}.

Angiotensin-converting enzyme (ACE) inhibitory activity

ACE inhibitory activity of infusions and fermented beverages were screened using the methodology described by Actis-Goretta *et al*. (*15*). ACE from rabbit lung (0.05 U/mg of protein, SIGMA Co., St. Louis, USA) activity was evaluated following the hydrolysis of

hippuryl-L-histidyl-L-leucine to hippuric acid. Hippuric acid formed, was separated and quantified by HPLC with UV detection. Chromatographic separations were performed with a column 150 mm x 4.6 mm i.d. 5 µm, Supelcosil LC-18-DB (Waters Corp., Wexford, Ireland), and a mobile phase composed of 0.1 % (by volume) trifluoroacetic acid in H_2O/acetonitrile (JT Baker, Mexico, Mexico) (75:25, by volume). The flow rate used to achieve a retention time of 2.9 min was 1 mL/min. The hippuric acid detection was carried out at 228 nm. Commercial hippuric acid was used as the standard (SIGMA Co., St. Louis, USA). For the inhibition of ACE activity by infusions and fermented beverages, a mixture of 10 µL of ACE (0.05 U/mg of protein) in 50 mM HCl-TRIS, 300 mM NaCl (pH=8.3), and different concentration of samples to reach 90 µL of volume was pre-incubated for 5 min at 37 °C. The above mixture was added with 10 µL of 3 mM hippuryl-L-histidyl-L-leucine (1 mM final concentration, SIGMA Co., St. Louis, USA), and incubated for 30 min at 37 °C. The reaction was stopped by placing the sample for 5 min at 100 °C in a water bath. For the calculation of the IC_{50} (µg/µL) the logarithm of the concentration against the inhibition activity percentage was plotted. Captopril was used as a positive control (SIGMA Co., St. Louis, USA).

Statistical analysis

Data were expressed as mean values ± standard error (SE). Statistical significance was determined by one-way variance analysis (ANOVA) ($p < 0.05$) followed by the Tukey´s test, where P-values < 0.05 were considered significant. Statistical analysis was made using JMP 5.0.1 software.

Results and Discussion

Results of physicochemical characterization are shown in Table 1. It was observed that the pH of fermented beverages decreased in 2 units compared with the unfermented infusions. The overall decrease in pH is attributable to the increased concentration of organic acids produced during the fermentation process by bacteria and yeasts from the tea fungus. In concordance, Velićanski *et al*. (*16*) reported decreased values of pH (between 2 and 3 units) in analogues of Kombucha at the same fermentation times.

On the other hand, several authors reported changes in color as a consequence of fermentation. For example, it has been reported a progressive lightening of color in fermented teas (*17-18*).

However, in this work we did not observe significant differences between infusions and fermented beverages (Table 1). This could be attributed to the nature of plant materials and their chemical composition. Contrary to the species studied in this work, epigallocatechin-3-gallate (ECGC) is the main polyphenol found in green tea (about 59% from total catechins)(*19*). Under fermentation process EGCG is the major precursor for other compounds such as theaflavins (*20-21*). These and others compounds, including bisflavanols and thearubigins are mainly responsible for the color change in Kombucha beverages (*22*).

In the fermentation process the sucrose is hydrolyzed to glucose and fructose by yeast. Glucose is mainly converted into gluconic acid via the pentose phosphate pathway by acetic acid bacteria, whereas, fructose is metabolized to acetic acid, and in minor proportion to gluconic acid (*23*). We can observe the results of sucrose consumption in Table 1. In both fermented beverages, the Kombucha consortium utilized more than 70 % of sucrose for fermentation process. The latter is consistent with results of residual glucose and fructose (Table 1), suggesting an active process of fermentation in these herbal infusions (Table 1).

Plant infusions such as *E. camaldulensis* is traditionally consumed by population due to its pleasant smell and taste; however, the changes produced in fermentation process can alter this consumer preference despite its nutraceutical potential. For this reason, we compared the acceptability between infusions and fermented beverages. For this evaluation black tea infusion and fermented beverage were included as positive controls, resulting in 5 and 4 points of score for consumer preference in hedonic scale, respectively. Results for *E. camaldulensis* and *L. glaucescens* infusions and fermented beverages are shown in Table 1. Both herbal infusions have similar preferences, but 1 point lower than the black tea infusion. For fermented beverages, *E. camaldulensis* had the lower acceptability, whereas *L. glaucescens* had similar results to the control. It has been reported that biotransformation of several compounds produced in the fermentation process, increments the preference by consumers compared with certain herbal infusions (*7*). Nevertheless, our results have not shown any differences on acceptability between herbal infusions and fermented beverages; even when a minor decrease on *E. camaldulensis* fermented beverage was observed (1 point in hedonic scale). This could be attributed to the formation of other compounds that can influence flavor; however, more chemical studies are needed.

Phenolic profile and concentrations of herbal infusions and fermented beverages

Results of phenolic profile of infusions and fermented beverages are shown in Fig. 1. Compounds such as gallocatechin, epigallocatechin, gallocatechin gallate, catechin, rutin, kaempferol, and quercetin were identified in both beverages. The most abundant detected compound was gallocatechin gallate (GCG), being higher in *L. glaucescens* infusions and in *E. camaldulensis* fermented beverages.

Chromatograms results (Fig. 1) and concentrations (Table 2) of each identified compound demonstrated that the fermentation process influenced both plants differently on the polyphenol present in their herbal infusions. For instance, in *L. glaucescens* fermented beverage, the concentration of GCG decreased significantly ($p < 0.05$) (1.8-fold), whereas in *E. camaldulensis* fermented beverage, the concentration increased (1.8-fold), both compared with their unfermented infusions. A similar trend was observed by Hoon *et al*. (*4*); they reported an increased concentration of compounds such epigallocatechin, epigallocatechin gallate, epicatechin gallate, among others in Chinese black tea, green tea, Sri Lankan black tea and Rooibos tea after 7 days of fermentation with Kombucha fungus. They attributed these results to the degradation of polyphenols complexes, as consequence of the increased acidic environment of the fermentation process and to the enzymes produced by the Kombucha consortium (bacteria and yeast).

On the other hand, the decreased concentration of phenolic compounds observed in *L. glaucescens* fermented beverage could be explained due to the partial oxidation of polyphenols to form polymerized compounds with higher molecular weights (*16*), and thus lower concentration of these compounds.

Antioxidant capacity of beverages

Both herbal infusions showed DPPH radical scavenging activity, being better for *E. camaldulensis*, and showing similar IC_{50} than the positive control (Table 3). Despite the chain-breaking DPPH activity results, the fermentation process induced a decrease in the concentration required to stabilize 50 % of DPPH radical (1.2-1.7 fold than unfermented infusions). In concordance with our results, Hoon *et al*. (*4*) reported an enhancement on DPPH scavenging activity in fermented beverages with respect to unfermented tea. Previous studies with Kombucha beverages, attributed the antioxidant activity to the polyphenols and catechins,

with a statistically significant correlation between the phenolic content and the DPPH scavenging capacity (*24*). However, in our work a lower correlation (by Spearman test $p<0.05$) was observed ($R = 0.3162$). This could be explained due to the existence of a partial oxidation of polyphenols, forming macromolecular compounds, and therefore decreasing their relative concentration. However, authors such as Hoon *et al*. (*4*) reported that these compounds still provide a noteworthy radical scavenging activity.

In order to complement the analysis of antioxidant activity of plant infusions and fermented beverages, we evaluated the inhibition of lipid peroxidation in pre-oxidized healthy human serum. Results showed that both herbal infusions have antioxidant capacity with IC_{50} values close to the positive control (catechin). For fermented beverages, no improvement on antioxidant capacity was observed, showing similar IC_{50} values to the unfermented infusions. Jayabalan *et al*. (*18*) reports that the antioxidant activity is extremely dependent on the conditions used and the substrates or products analyzed, therefore not all methods give the same results response for bioactivity.

Authors such as Arnao (*24*) reviewed limitations for antioxidant activity assays, suggesting that the activity must be measured using more than one method, primary and secondary oxidation products, and using tests that measure specific substrates or products. Therefore, we used a third method: the nitric oxide scavenging assay (NO). Nitric oxide reacts with superoxide ($O_2^{\cdot -}$) to form the peroxynitrite anion, which is a potential strong oxidant as the decomposition of this molecule produces hydroxyl radical and nitrogen dioxide; this contributes significantly to oxidative cell damage (*21*). The results of the NO assay showed lower effect of infusions compared with the positive control catechin (Table 3). However, the antioxidant activity increased with the fermentation process (49 % for *L. glaucescens* and 14 % for *E. camaldulensis*). Unlike the DPPH test results, where we did not find any statistical significant correlation between phenolic concentration and antioxidant activity. A high correlation (by Spearman test $p<0.05$) was observed on the NO Assay ($R=0.8000$). The same was observed by Jayabalan *et al*. (*25*), reporting that during fermentation, polyphenol structural modifications occurred such as glycosylations, resulting in better scavenging performance on nitrogen radicals.

Antihypertensive activity in vitro

Finally, we evaluated in vitro the antihypertensive potential of fermented beverages through the inhibition of the Angiotensin-converting enzyme (ACE) activity. ACE is a carboxypeptidase, and participates in regulating blood pressure by converting an inactive form of the deca-peptide angiotensin to a potent vasopressor octapeptide, angiotensin II. Therapeutic ACE inhibitors represent an important and common class of pharmaceuticals for hypertension control (*26*). Results on ACE inhibition activity demonstrate that herbal infusions inhibit the activity of this enzyme (Table 3). *L. glaucescens* infusion had the highest effect, being similar to the positive control (captopril, main pharmaceutical used to control blood pressure). On the other hand, fermented beverages did not enhance the potential of herbal infusions that inhibit ACE activity; on the contrary, fermentation process reduced the positive effect of infusions (1.8-fold for *E. camaldulensis* and 15.1-fold for *L. glaucescens*). However, the concentrations of fermented beverages were still lower than the positive control. Authors such Hur *et al*. (*27*) proposed that the increase or decrease on bioactivity of phenolic compounds produced by fermentation may be influenced by various factors, including microorganism species, pH, temperature, solvent, water content, fermentation time, kind of plant material and aerobic conditions. Thus, the variations on results could be attributed to these factors and not only to the final bioconversion of bioactive compounds.

Conclusions

The present study has demonstrated that fermentation with Kombucha consortium on *Litsea glaucescens* and *Eucalyptus camaldulensis* infusions modify their concentration of phenolic compounds, and their antioxidant and antihypertensive activities. Fermented beverages exhibited increased free-radical scavenging activities, but this response depends on the evaluation media, substrate and mainly on the plant material used for infusion preparation. Additionally, it is interesting to note that *E. camaldulensis* and *L. glaucescens* can be considered as natural sources of bio compounds with antihypertensive potential (especially the last one) either as infusions or fermented beverages. Therefore, they can be considered for further investigations, especially for in vivo assays.

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References

1. Astill C, Birch MR, Dacombe C, Humphrey PG, Martin PT. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J Agric Food Chem. 2001; 49:5340-47. http://dx.doi.org/10.1021/jf010759+
2. Annuzzi G, Bozzetto L, Costabile G, Giacco R, Mangione A, Anniballi G, *et al*. Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial. Am J Clin Nutr. 2014; 99:463-471. http://dx.doi.org/10.3945/ajcn.113.073445
3. Balasuriya BN, Rupasinghe HV. Plant flavonoids as angiotensin converting enzyme inhibitors in regulation of hypertension. Funct Food Health Dis. 2011; 5:172-188.
4. Hoon LY, Choo C, Watawana MI, Jayawardena N, Waisundara VY. Kombucha 'tea fungus' enhances the tea polyphenol contents, antioxidant activity and alpha-amylase inhibitory activity of five commonly consumed teas. J Funct Foods. 2014:in press. http://dx.doi.org/10.1016/j.jff.2014.07.010
5. Velićanski AS, Cvetković DD, Markov SL, Šaponjac T, Vesna T, Vulić JJ. Antioxidant and Antibacterial Activity of the Beverage Obtained by Fermentation of Sweetened Lemon Balm (*Melissa officinalis* L.) Tea with Symbiotic Consortium of Bacteria and Yeasts. Food Technol Biotech. 2014; 52:420-429. http://dx.doi.org/10.17113/ftb.52.04.14.3611
6. Vijayaraghavan R, Singh M, Rao PVL, Bhattacharya R, Kumar P, Sugendran K, et al. Subacute (90 days) oral toxicity studies of Kombucha Tea. Biomed Environ Sci. 2000; 13:293–299.

7. Vázquez-Cabral BD, Rocha-Guzmán NE, Gallegos-Infante JA, González-Herrera SM, González-Laredo RF, Moreno-Jiménez MR, *et al*. Chemical and sensory evaluation of a functional beverage obtained from infusions of oak leaves (*Quercus resinosa*) inoculated with the kombucha consortium under different processing conditions. Nutrafoods. 2014; 13:169-178. http://dx.doi.org/10.1007/s13749-014-0035-0

8. Singab AN, Ayoub N, Al-Sayed E, Martiskainen O, Sinkkonen J, Pihlaja K. Phenolic constituents of Eucalyptus camaldulensis Dehnh, with potential antioxidant and cytotoxic activities. Rec Nat Prod. 2011; 5:271-280.

9. Herrera-Carrera E, Moreno-Jiménez MR, Rocha-Guzmán NE, Gallegos-Infante JA, Díaz-Rivas JO, Gamboa-Gómez CI, *et al*. Phenolic composition of selected herbal infusions and their anti-inflammatory effect on a colonic model in vitro in HT-29 cells. Cogent Food Agric. 2015; 1:1059033. http://dx.doi.org/10.1080/23311932.2015.1059033

10. Marsh AJ, O'Sullivan O, Hill C, Ross RP, Cotter PD. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. Food Microbiol. 2014; 38:171-178. http://dx.doi.org/10.1016/j.fm.2013.09.003

11. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995; 28:25-30. http://dx.doi.org/10.1016/S0023-6438 (95) 80008-5

12. Manzocco L, Anese M, Nicoli MC. Antioxidant properties of tea extracts as affected by processing. LWT-Food Sci Technol. 1998; 31:694-698. http://dx.doi.org/10.1006/fstl.1998.0491

13. Jozwik M, Wolczynski S, Jozwik M, Szamatowicz M. Oxidative stress markers in preovulatory follicular fluid in humans. Mol Hum Reprod. 1999; 5:409-413. http://dx.doi.org/10.1093/molehr/5.5.409

14. Balakrishnan N, Panda AB, Raj NR, Shrivastava A, Prathani R. The evaluation of nitric oxide scavenging activity of Acalypha Indica Linn root. Asian J Res. Chem. 2009; 2:148-50.

15. Actis-Goretta L, Ottaviani JI, Fraga CG. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J Agric Food Chem. 2006; 54:229-234. http://dx.doi.org/10.1021/jf052263o

16. Velićanski A, Cvetković D, Markov S. Characteristics of Kombucha fermentation on medicinal herbs from Lamiaceae family. Rom Biotech Lett. 2013; 18:8034- 8042.

17. Chu SC, Chen C. Effects of origins and fermentation time on the antioxidant activities of kombucha. Food Chem. 2006; 98:502-507. http://dx.doi.org/10.1016/j.foodchem.2005.05.080

18. Jayabalan R, Marimuthu S, Swaminathan K. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. Food Chem. 2007; 102:392-398. http://dx.doi.org/10.1016/j.foodchem.2006.05.032

19. Mckay DL, Blumberg JB. The role of tea in human health: An update. J Am Coll Nutri. 2002; 21:1–13. http://dx.doi.org/10.1080/07315724.2002.10719187

20. Yang C, Li D, Wan X. Combination of HSCCC and Sephadex LH-20 methods: An approach to isolation and purification of the main individual theaflavins from black tea. J Chromatogr B. 2008; 861:140-144. http://dx.doi.org/10.1016/j.jchromb.2007.11.022

21. Xu Y, Zhao H, Zhang M, Li CJ, Lin XZ, Sheng J, *et al*. Variations of antioxidant properties and NO scavenging abilities during fermentation of tea. Int J Mol Sci. 2011; 12:4574-4590. http://dx.doi.org/10.3390/ijms12074574

22. Kim Y, Goodner KL, Park JD, Choi I, Talcott ST. Changes in antioxidant phytochemicals and volatile composition of Camellia sinensis by oxidation during tea fermentation. Food Chem. 2011; 129:1331–1342. http://dx.doi.org/10.1016/j.foodchem.2011.05.012

23. Chen C, Liu BY. Changes in major components of tea fungus metabolites during prolonged fermentation. J Appl Microbiol. 2000; 89:834-839. http://dx.doi.org/10.1046/j.1365-2672.2000.01188.x

24. Arnao MB. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. Trends Food Sci Tech. 2000; 11:419-421.

http://dx.doi.org/10.1016/S0924-2244(01)00027-9

25. Jayabalan R, Subathradevi P, Marimuthu S, Sathishkumar M, Swaminathan K. Changes in free-radical scavenging ability of kombucha tea during fermentation. Food Chem. 2008; 109:227-234.
http://dx.doi.org/10.1016/j.foodchem.2007.12.037

26. Heran BS, Wong MMY, Heran IK, Wright JM. Blood pressure lowering efficacy of angiotensin converting enzyme (ACE) inhibitors for primary hypertension. Cochrane DB Syst Rev. 2008; 4.
http://dx.doi.org/10.1002/14651858.CD003823.pub2

27. Hur SJ, Lee SY, Kim YC, Choi I, Kim GB. Effect of fermentation on the antioxidant activity in plant-based foods. Food Chem. 2014; 160:346-356.
http://dx.doi.org/10.1016/j.foodchem.2014.03.112

Table 1. Physicochemical evaluation and consumer acceptance of herbal infusions and fermented beverages of *Litsea glaucescens* and *Eucalyptus camaldulensis*.

	Litsea glaucescens		***Eucalyptus camaldulensis***	
	Infusion	Fermented beverage	Infusion	Fermented beverage
pH	$(5.1 \pm 0.05)^a$	$(3.0 \pm 0.01)^b$	$(5.0 \pm 0.05)^a$	$(3.0 \pm 0.05)^b$
Color parameters				
L*	$(22.0 \pm 0.05)^a$	$(22.1 \pm 0.01)^a$	$(22.0 \pm 0.05)^a$	$(22.1 \pm 0.02)^a$
a*	$(-0.13 \pm 0.01)^a$	$(-0.14 \pm 0.02)^a$	$(-0.14 \pm 0.05)^a$	$(-0.14 \pm 0.01)^a$
b*	$(0.34 \pm 0.01)^a$	$(0.40 \pm 0.07)^a$	$(0.35 \pm 0.01)^a$	$(0.33 \pm 0.02)^a$
***w* (consumed sucrose)/%**	----	$(70.46 \pm 0.06)^a$	----	$(75.59 \pm 0.29)^a$
γ(residual fructose)/(mg/mL)	----	$(37.47 \pm 0.02)^a$	----	$(37.19 \pm 0.02)^a$
γ(residual glucose)/(mg/mL)	----	$(43.90 \pm 0.18)^a$	----	$(40.09 \pm 0.32)^a$
Consumer preference/(Hedonic scale)	$(4.0 \pm 0.40)^a$	$(4.0 \pm 0.40)^a$	$(4.0 \pm 0.60)^a$	$(3.0 \pm 0.20)^b$

Values are means of duplicated determinations ± standard error. Different letters on each row indicate statistical difference ($P \leq 0.05$) by Tukey's test.

Table 2. Ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS). MS data and content (μg/mL) on major phenolic compound found in infusion and fermented products.

	Compound name	t_R/ (min)	$[M-H]^-$ *m/z*	MS/MS ions	*Litsea glaucescens*		*Eucalyptus camaldulensis*	
					Infusion	Fermented beverage	Infusion	Fermented beverage
1	Gallocatechin	1.19	305	164, 125	2.25 ± 0.24	1.78 ± 0.09	1.41 ± 0.08	2.59 ± 0.34
2	Epigallocatechin	1.85	305	164, 125	6.43 ± 0.30	4.58 ± 0.03	2.66 ± 0.07	5.57 ± 0.09
3	Catechin	1.91	289	203, 109	10.20 ± 0.18	5.60 ± 0.93	1.64 ± 0.21	3.29 ± 0.53
4	Gallocatechin gallate	2.26	457	305, 169, 125	32.67 ± 0.71	18.12 ± 2.41	14.37 ± 0.80	25.29 ± 0.63
5	Epicatechin	2.42	289	162, 125	23.54 ± 0.43	14.18 ± 1.37	1.30 ± 0.31	1.76 ± 0.14
6	Epigallocatechin gallate	2.46	457	305, 169, 125	ND	ND	1.92 ± 0.03	2.75 ± 0.13
7	Epicatechin gallate	2.80	441	289, 169	10.51 ± 0.69	6.65 ± 0.70	4.22 ± 0.08	8.43 ± 0.79
	Total content of analyzed flavan-3-ols				$(85.64 \pm 1.3)^a$	$(50.93 \pm 5.4)^b$	$(27.55 \pm 1.3)^c$	$(49.72 \pm 1.7)^d$
8	Rutin	3.45	609	301, 271	13.73 ± 1.54	7.41 ± 0.11	ND	ND
9	Kaempferol	4.48	285	187	0.34 ± 0.00	0.29 ± 0.00	ND	ND
10	Quercetin	4.62	301	179, 151	10.86 ± 0.48	7.55 ± 0.02	1.78 ± 0.22	3.62 ± 0.39

Total content of analyzed flavonols	$(24.95 \pm 1.0)^{a}$	$(15.26 \pm 1.0)^{b}$	$(1.78 \pm 0.2)^{c}$	$(3.62 \pm 0.3)^{c}$
Total content of analyzed flavonoids	$(110.59 \pm 2.4)^{a}$	$(66.20 \pm 4.4)^{b}$	$(29.34 \pm 1.6)^{c}$	$(53.35 \pm 1.3)^{d}$

Values are expressed as means ± standard error (n = 2). Values in a row followed by different letters for each infusion and fermented beverage are significantly different ($p < 0.05$) by Tukey's test. $\mathbf{t}_R$ = retention time, ND = not detected.

Table 3. Radical Scavenging and Chain-breaking activity (DPPH assay), Inhibition of Human serum lipid Oxidation (TBARS), and Nitric Oxide

	DPPH/ (IC_{50})	DPPH/ (-**1**/min/mg dm)	TBARS/ (IC_{50})	NO/ (IC_{50})	ACE inhibition/ (IC_{50})
Litsea glaucescens					
Infusion	$(4.6 \pm 0.13)^a$	$(5.11 \pm 0.40)^a$	$(1.91 \pm 0.10)^a$	$(22.44 \pm 6.3)^a$	$(0.70 \pm 0.02)^c$
Fermented beverage	$(2.7 \pm 0.01)^b$	$(5.21 \pm 0.13)^a$	$(1.13 \pm 0.13)^a$	$(11.40 \pm 1.3)^b$	$(1.08 \pm 0.01)^b$
Eucalyptus camaldulensis					
Infusion	$(3.4 \pm 0.05)^c$	$(3.74 \pm 0.18)^b$	$(0.36 \pm 0.01)^b$	$(6.73 \pm 0.9)^c$	$(1.40 \pm 0.05)^b$
Fermented beverage	$(2.7 \pm 0.12)^b$	$(3.01 \pm 0.10)^b$	$(1.01 \pm 0.30)^a$	$(5.75 \pm 0.8)^c$	$(2.61 \pm 0.01)^a$
Catechin	$(3.8 \pm 0.13)^c$	$(0.99 \pm 0.10)^c$	$(1.04 \pm 0.17)^a$	$(2.50 \pm 0.3)^d$	----
Captopril	----	----	----	----	$(2.75 \pm 0.15)^a$

Scavenging Assay (NO) of infusions and fermented beverages with Kombucha consortium.

Values are means of duplicate determinations ± standard error. Different letters on each column indicate statistical difference ($P \leq 0.05$) by Tukey's test. IC_{50} Results are expressed as μg of total content of analyzed flavonoids/μL.

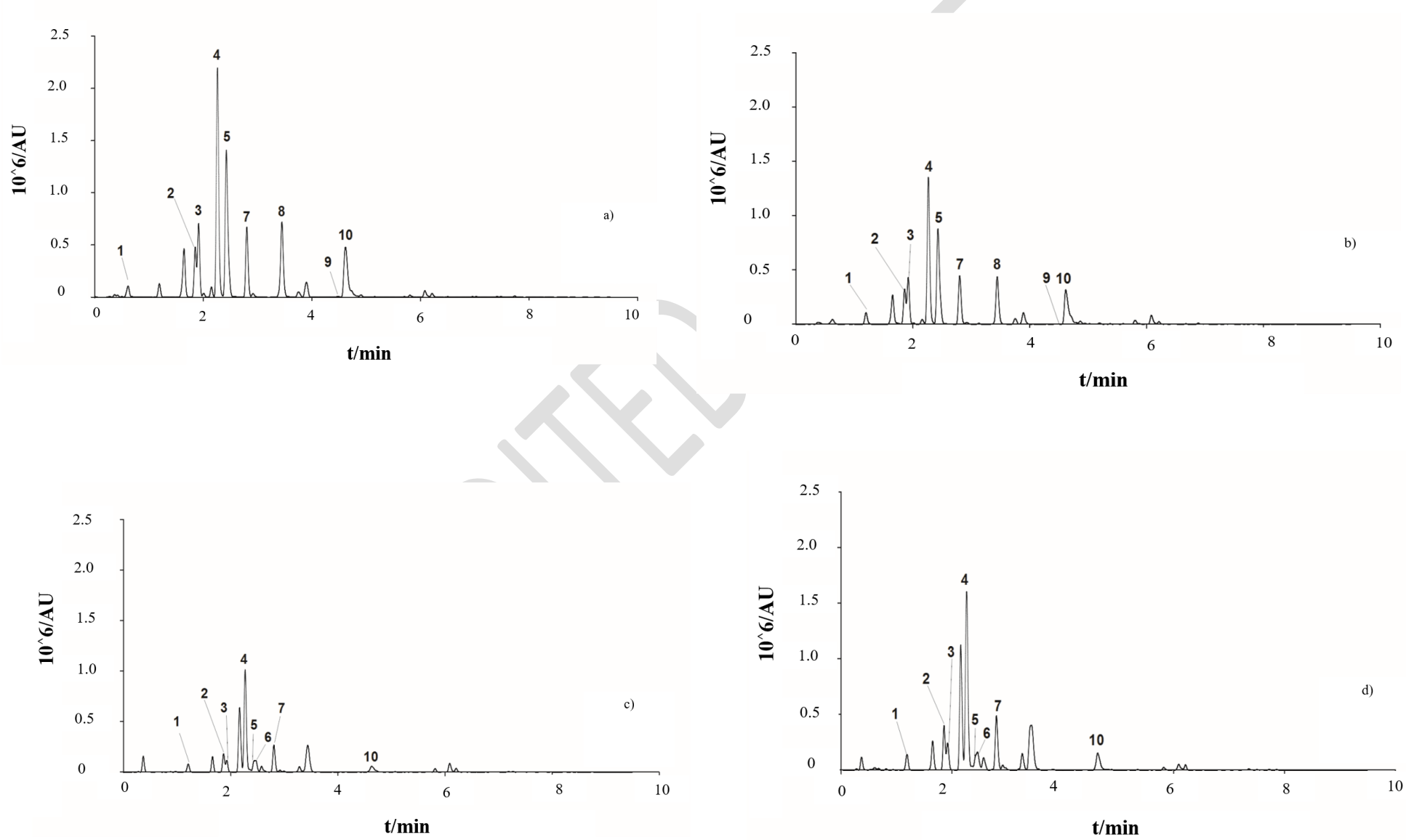

Fig. 1 LC-ESI-MS/MS profile of a) *Litsea glaucescens* infusion; b) *Litsea glaucescens* fermented beverage; c) *Eucalyptus camaldulensis* infusion and d) *Eucalyptus camaldulensis* fermented beverage. Identified compounds by retention time and MS/MS: 1, gallocatechin; 2, epigallocatechin; 3, catechin; 4, gallocatechin gallate; 5, epicatechin; 6, epigallocatechin gallate; 7, epicatechin gallate; 8, rutin; 9, kaempferol and 10, quercetin.