

Antioxidant and Antibacterial Activity of Kombucha Beverages Prepared using Banana Peel, Common Nettles and Black Tea Infusions

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Abstract

Backgrounds and Objective: Kombucha is a several thousand years old traditional fermented beverage originated from East. While black tea infusion is the common substrate for preparing kombucha, other herbal infusions can be applied for this reason too. Common medicinal herbs or even waste herbal materials, like banana peel, could be suitable substrates for preparing kombucha analogues. In this study, kombuchas were fermented using nettles leaf and banana peel infusions.

Materials and Methods: Herbal infusions were fermented by kombucha fungi. Folin-Ciocalteu assay was performed to evaluate total phenolic contents; Free radical scavenging activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl. Disk diffusion method was performed to measure inhibitory activity against testing bacteria. All data were statistically analyzed by ANOVA test at significant level of $p \leq 0.05$.

Results and Conclusion: Black tea contained highest amount of phenolics (530.5 ppm gallic acid equivalent) and fermentation decomposed approximately 50% of phenolic contents to 265.5 ppm while phenolic content of nettles infusion and fermented beverage were 173 gAE and 188 gAE respectively and for banana peel, 136.5 gAE and 155 gAE; it indicated increase of phenolic contents due to fermentation that may be cause of protein contents of nettles and banana peel gone under fermentation by lactic acid bacteria. Fermented beverage of three herbs had higher antioxidant potent than infusions. Kombucha from banana peel showed the highest antioxidant activity by inhibiting 94.62% of DPPH. While antioxidant activity of fermented beverages of black tea and nettles leaf were more related to their acetic acid content, it was found that a considerable part of antioxidant activity of banana peel kombucha was due to other acids and phenolics. No antibacterial activity was observed from either of samples. Banana peel, as a waste herbal material, and nettles leaf are good ingredients for being used as substrate to make antioxidant kombucha beverage.

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1. Introduction

Kombucha is a traditional fermented beverage originated from East and has a history of several thousand years but still remains quite popular in the West. Typical kombucha is a sweetened black tea (*Camellia sinensis*) fermented with a culture known as “tea fungus”, at room temperature for about two we-

eks [1]. “Tea fungus” used for fermenting kombucha is popularly called “kombucha fungi” in Iran. Kombucha fungi are actually a symbiosis of acetic bacteria and yeasts. The main acetic acid bacteria found in this fungus are: *Acetobacter xylinum*, *Acetobacter xylinoides*, *Bacterium gluconicum*, *Acetobacter aceti*

and *Acetobacter pasteurianus*; and identified yeasts are: *Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulasporea delbrueckii*, *Brettanomyces bruxellensis*, *Brettanomyces lambicus*, *Brettanomyces custersii*, *Candida stellate* [2].

Potential effects like weight loss, cancer and AIDS cure have increased the interest in Kombucha consumption. [3,4]. Regular Kombucha tea consumption contributes to weight gain inhibition and life elongation [5]. Also, kombucha was proved to have an antimicrobial activity against *Helicobacter pylori*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Agrobacterium tumefaciens*, *Bacillus cereus*, *Shigella sonnei*, *Salmonella enteritidis* and *Escherichia coli*. [6,7]. Tea leaves, the substrates for kombucha fermentation, contain antioxidants. These are mainly polyphenols. Antioxidants have many beneficial effects to the human body [8,9].

Some reports have been published on using different herbs tea as medium for preparing kombucha beverage; but as to author knowledge, no scientific report have been published on using banana (*Musa sapientum*) peel and nettles (*Urtica dioica*) leaf infusions as substrate for fermentation of kombucha. Several reports indicated antioxidant and antimicrobial activity of both herbal materials [10-13]. Therefore, in current study banana peel and nettles tea kombucha analogue were prepared, then total phenolic contents and free radical scavenging of fermented beverages was compared with common infusions. Also, antibacterial activities against food pathogens (*E. coli*, *S. typhimurium*, *S. aureus*, *S. saprophyticus*, *B. stearothermophilus* and *Pseudomonas aeruginosa*) were evaluated.

To investigate the impact of acids produced during the fermentation of beverages in antioxidant and antibacterial activities, two other samples were also prepared and tested. One was acidified infusion, which the pH of fresh infusion was adjusted to pH of fermented beverage using acetic acid; so that the impact of acetic acid, as the dominant acid produced during fermentation, in biological properties of fermented beverage could be understood. Another sample was neutralized fermented beverage which all acids were neutralized to investigate the impact of active compounds other than acids, in antioxidant and antibacterial properties of samples.

2. Materials and Methods

2.1. Herbal materials preparation

Dried nettles leaf and black tea no flavor added was bought from a local medicinal herbs store in Gorgan-Iran in February 2015. Fresh banana fruits were bought from a local market in Gorgan-Iran in Feb 2015. Fruit were washed with distilled water at room temperature and peeled. Peels were chopped and boiled in distilled water until its color changed dark; cooking process took 2 hours. Cooked peels rinsed off

and left at 50°C incubator for 24 h to dry. Dried peels were collected, grinded and mixed to be ready for use in further process.

2.2. Infusions and kombuchas preparation

The method used by Battikh et al. [2] was followed, with slight modification. To prepare infusions, 10 g of dry herbal material with 20 g sucrose were mixed in one liter of 5 min boiled distilled water and steeped for 15 min. The mixture was cooled to room temperature and then leaves were separated. The resulting filtrates were used as infusions. To prepare fermented beverages 300 ml of each infusion was poured into 500-ml glass jar; then, the preparation was inoculated with 10 g l⁻¹ of actively growing kombucha fungi which was bought from a local kombucha producer (Gorgan, Iran); and 30 ml of previously fermented kombucha was added to medium to stimulate the fermentation process. The inoculated jars lids were covered with a piece of cotton cloths and the body of containers were covered with newspapers; then incubated at room temperature. After 21 days, fermented liquids were passed through 0.45 µm sterile syringes filter (Jet-Biofil); filtered liquids were used in antibacterial and antioxidant tests. To understand the role of acids and pH in antioxidant and antibacterial activity of fermented beverages, acidified infusions were prepared using acetic acid 10% to adjust infusion pH to fermented beverage. Neutralized samples were also prepared using NaOH 1N to adjust fermented beverages' pH to 7.

2.3. Evaluation of antibacterial effect

Disk diffusion method, as described by the European Committee on Antimicrobial Susceptibility Testing, was followed for evaluation of antibacterial effects of infusions and fermented beverages [14]. 0.5 McFarland solution of selected strains of bacteria (*E. coli* ptcc 1395, *S. typhimurium* ptcc 1596, *S. aureus* ptcc 1436, *S. saprophyticus* ptcc 1440, *B. stearothermophilus* ptcc 1359 and *P. aeruginosa* ptcc 1430) was prepared in Mueller Hinton broth. Using a sterile cotton swab, the Mueller Hinton broth cultures were swabbed on the surface of sterile Mueller Hinton agar plates. 10 µl of each extract was inoculated on each 6 mm disk and disks were applied on cultured plates. Inhibition zones were measured and reported in mm after 18 h incubation at 37°C. Gentamycin and Amoxicillin discs were used as standard. All the samples were tested in triplicate.

2.4. Total phenolic content assay

2.5 ml of 10-fold diluted Folin-Ciocalteu reagent, 2 ml of a 7.5% solution of sodium carbonate, and 0.5 ml of phenolics solution were mixed well. The absorbance was measured at 765 nm after 15 min heating at 45°C; a mixture of water and reagents was used as a blank. Mean of triplicate measurements is reported. The content of phenolics was measured

before and after fermentation process and is expressed as gallic acid equivalents (gAE) [15,16].

2.5. Free radical scavenging activity

The electron donating ability of samples and standards (gallic acid and Vit C) were determined from bleaching of purple colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical 2, 2-diphenyl-1-picrylhydrazyl as a reagent. DPPH was prepared at a concentration of 0.002%. Different concentrations of extracts were taken in separate test tubes and volumes were made up to 2 ml using distilled water. Then, 2 ml of DPPH solution was added in each test tube and these solutions were kept in darkness for thirty minutes. The same procedure was followed for Vit C and gallic acid as well. Later optical density was recorded at 517 nm using spectrophotometer. All the samples were tested in triplicate. For infusions, test was performed just before incubation with kombucha fungi and for fermented beverages at the day 21. Distilled water with DPPH was used as control [15,17].

Inhibition of DPPH activity (%) = $(A-B/A) \times 100$

Where, A=optical density of control; B=optical density of sample.

IC₅₀ factor of samples were also evaluated; IC₅₀ factor indicates the concentration or amount of extract that can inhibit 50% of free radical agent. Samples with smaller needed amount to inhibit 50% of DPPH have higher radical scavenging activity [15].

2.6. pH

pH was measured using electronic pH meter (Denver, model 215).

2.7. Statistical analysis

ANOVA test followed by Duncan multiple range test were performed at the significance level of 0.05 using SPSS software ver. 20 to statistically analyze obtained data (n=3). Microsoft Excel 2013 was used to draw chart.

3. Results and discussion

3.1. pH

During fermentation process and acid production due to conversion of sugar to acids, pH of infusions dramatically changed. Table 1 presents pH value of infusions at initial time and after fermentation. It seems that pH change in black tea samples is bigger and final fermented beverage has lower pH in comparison with samples from two others. While initial sucrose content of samples was the same, it is supposed that the differences in final pH could be cause of natural herbal components inhibiting biochemical process of acid formation. Although, more investigations and observations on the kinetic of the fermentation process are needed to clearly declare

the reason.

3.2. Antioxidant properties

Total phenolic contents of all samples are presented in figure 1 as gallic acid equivalent. Obtained data indicates that black tea contains highest amount of phenolic contents (530.5 ppm) and fermentation decomposed approximately 50% of phenolic contents to 265.5 ppm. Malbasa et al. reported quercetin as dominant phenolic compound in black tea kombucha [18]. Also, according to Figure 2, IC₅₀ value for fermented beverage is 4.96 times larger than infusion. Acidified samples have a larger IC₅₀ value than fresh infusion but still smaller (2.8 time) than fermented sample; also neutralizing the fermented beverage increases IC₅₀. So, it indicates that acetic acid is effective in antioxidant activity of fresh and fermented black tea infusion. Belgheisi [19] suggested that decomposition of phenolics may be due to microbial enzymes and acids produced during fermentation.

While fresh infusion of nettles leaf contains 173 ppm gAE phenolic contents (approximately one third of black tea's), fermentation releases slightly higher amount of phenolics, 188 ppm gAE. Lactic acid bacteria and human colonic bacteria can produce some phenols [20,21]. The activity maintenance of protease of *A. aceti* over pH range of 3.0 to 5.0 was reported by Bossi et al. [22]; the increase in phenolic contents may be due to fermentation of protein content of nettles (approximately 2.7% protein content) while black tea has no protein content [23]. Phenolic content of banana peel fermented beverage (155 ppm) is higher than its infusion (136.5 ppm) too; the same reason may also apply here.

Regarding the percent of DPPH inhibition by samples (table 2) and IC₅₀ value of them, it seems that almost all of fermented beverages of three herbs has higher antioxidant potent than infusions. Although, ascorbic acid (30-50 ppm) could inhibit DPPH at maximum 64%, infusions and fermented beverages showed to have higher potent in inhibition of DPPH (69.93% to 94.62%). Neutralizing fermented beverages decreases antioxidant activity and acidifying infusions with acetic acid increases antioxidant activity. Comparing acidified infusions with fermented beverages and neutralized samples with fresh infusions showed that antioxidant activity of black tea sample is more due to acetic acid content. Fermented sample inhibited DPPH by 92.91% and acidified infusion 92.67%, fresh infusion 69.93% and neutralized sample 57.70%. Same result can be concluded for nettles samples. While for banana peel samples, fermented beverage has highest antioxidant activity among all tested samples that could inhibit DPPH by 94.62% while this value for acidified infusion obtained 79.95% so it can be concluded that a significant part of antioxidant activity of banana peel kombucha is due to other acids and phenolics. While acetic acid is the dominant acid in kombucha [24],

Table 1. pH of infusions at initial time and after 21 days fermentation.

Sample	Black tea	Nettles leaf	Banana peels
Fresh infusion	6.4	6.34	6.04
Fermented infusion (kombucha beverage)	1.95	2.89	2.63

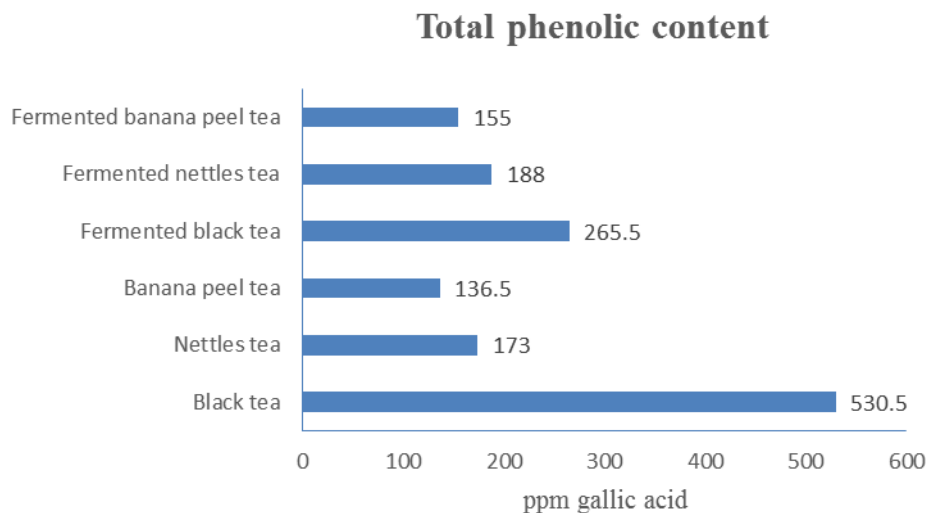


Figure 1. Total phenolic content of samples as gallic acid equivalent

Table 2. Percent of DPPH inhibited by different volume of samples

Sample concentrations (ml)	BT 0.5%	NT 5%	BPT 5%	FBT 5%	FNT 5%	FBPT 5%
0.25	15.65 ^{aA}	15.89 ^{aA}	16.14 ^{bA}	26.16 ^{cA}	19.56 ^{dA}	19.80 ^{dA}
1	27.63 ^{aB}	44.74 ^{bb}	50.61 ^{cB}	70.17 ^{dB}	40.83 ^{eB}	29.83 ^{fB}
2	69.9 ^{aC}	70.6 ^{aC}	73.59 ^{bC}	92.9 ^{cC}	92.4 ^{cC}	94.6 ^{cC}

Table 2- Continued

Sample concentration (ml)	NeFBT 5%	NeFNT 5%	NeFBPT 5%	ABT 5%	ANT 5%	ABPT 5%
0.25	6.60 ^{eA}	9.78 ^{fA}	1.96 ^{gA}	49.14 ^{hA}	52.92 ^{iA}	29.58 ^{jA}
1	25.67 ^{gB}	30.07 ^{fB}	19.80 ^{hB}	92.67 ^{iB}	61.12 ^{jB}	47.68 ^{kB}
2	57.70 ^{dC}	62.84 ^{eC}	39.12 ^{fC}	92.6 ^{cB}	90.2 ^{cC}	9.95 ^{gC}

BT=black tea infusion, NT=nettles leaf infusion, BPT=banana peel infusion, F=fermented, Ne=neutralized, A=acidified; Data with similar lowercase letters in each row have no significant difference ($p > 0.05$). Data with similar uppercase letters in each column have no significant difference ($p > 0.05$).

Jayabalan et al. also reported gluconic acid, glucuronic acid, vitamins and amino acids in kombucha [25].

3.3. Antibacterial effects

None of extracts (infusions and fermented samples) showed antibacterial activity against tested bacteria and inhibition zone for all samples obtained zero. Although, Gulcin et al. [10] reported low

antibacterial activity from water extract of nettles against *E. coli* and *S. aureus* and no effect against *P. aeruginosa*. Our finding about *P. aeruginosa* is similar to their report, but we also did not observed any inhibitory activity against other tested bacteria. The reason of the difference between our finding and their report might be cause of higher concentration of their extracts (20 g dry herb in 400 ml water).

Also, Battikh et al. [2], reported no inhibitory effect for black tea infusion against *E. coli*, *S. typhimurium*, *S. aureus* and *P. aeruginosa* which supports our findings. But they reported some inhibition activity from black tea kombucha on these bacteria. The difference between their report and ours might be due

to differences in methods; they performed well method and transferred 100 µl of sample into wells while we used disks incubated with 10 µl of samples.

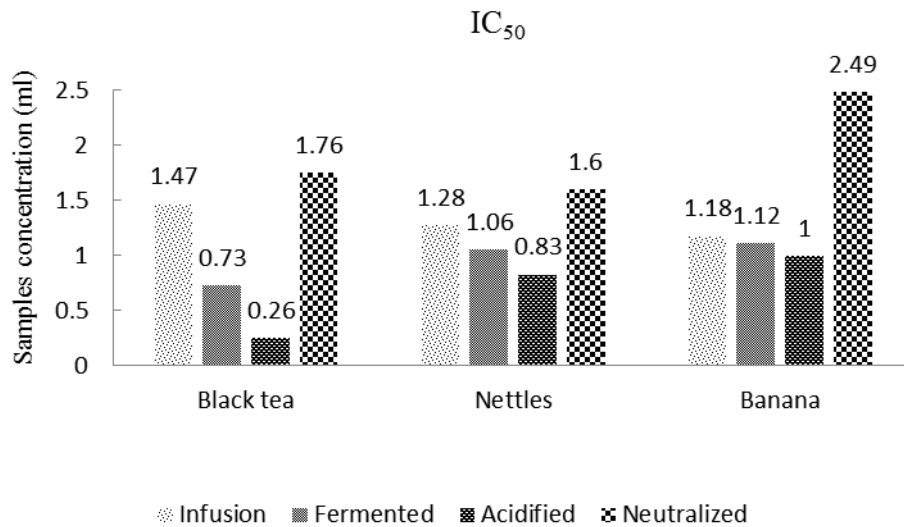


Figure 2. Needed amount of samples for inhibition of DPPH by 50%

4. Conclusion

While black tea is the most common herb to prepare kombucha with, this study introduced nettles leaf, a common medicinal herb, and banana peel, a waste herbal material, as good substrates for preparing fermented kombucha beverage. Final beverages prepared by these new substrates have higher pH in comparison with traditional kombucha; also each one of them presents a new taste, odor and color, different with common kombucha. Final beverages contain considerable phenolic contents and showed significant antioxidant activity. For further investigations we suggest sensory analysis and *in vivo* evaluation of antioxidant activity of these new kombucha analogues.

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6. Conflict of interest

There is no conflict of interests.

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