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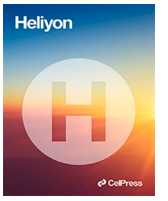

Research article

Phenolic compounds, antioxidant and antileishmanial activities of kombucha as affected by fermentation time

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A R T I C L E I N F O

Keys words:
Phenolic compounds
Anti-oxidant
Antileishmanial
Kombucha black tea
Fermentation time

A B S T R A C T

Objective: Study the impact of fermentation time on the phytochemical properties, antioxidant and antileishmanial activities.

Materials and methods: The preparation of Kombucha tea by fermentation was performed under aseptic conditions and symbiotic culture of bacteria and yeast (SCOBY) layer was maintained in culture for continuous growth in a water-sugar (4 L-500 g) mixture for 7, 14, 21, 28 and 35 days. The process of preparation was performed using a decoction. Phenolic compounds, flavonoids, and tannins was determined using standard method. The antioxidant activity was determined using three tests: DPPH•, ABTS• + and FRAP methods. Finally, the antileishmanial activity was performed *in vitro* on *Leishmania donovani* promastigote strains.

Results: The qualitative analysis of the constituents showed the kombucha drink was rich in saponins, terpenoids, quinones, phenolic compounds, catechins and coumarins depending on the fermentation times. Depending on the fermentation time (7 days, 14 days, 21 days, 28 days and 35 days), significant quantities of phenolic compounds were obtained in the tea with values ranging from 182.42 to 509.41 mg GAE/g dry extract; 15.83–53.05 mg QE/g dry extract and 6.16–51.82 mg TAE/g dry extract respectively for phenolic compounds, total flavonoids and total tannins. The SC_{50} values of DPPH• and $ABTS^{\bullet+}$, were 14.57 μg/mL; and 21.47 μg/mL after 14 and 21 days of fermentation respectively indicating a good antioxidant profile. The inhibition of the promastigote form of *Leishmania donovani* responsible for visceral leishmaniasis was observed with the samples obtained after 7 days, 14 days and 28 days with inhibitory concentrations 50 of: 131.2, 48.86 and 128.8 μg/mL respectively. The antileishmanial activity was more pronounced with the Kombucha tea after 14 days (KBT14) extract (48.86 μg/mL).

Conclusion: The Kombucha tea revealed the presence of phenolic compounds at different fermentation time. In addition, a good antioxidant profile was observed with the different radicals analyzed. Also, the inhibition of the Leishmania parasite was obtained. Therefore, the Kombucha tea constitutes a source bioactive molecules with antioxidant properties against *Leishmania* parasite.

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1. Introduction

Kombucha tea is traditional beverage that originated from Northeast China and Korea. It was made by mushroom Symbiotic Culture of Bacteria and Yeast (SCOBY). Kombucha is called kombu in Japanese meaning 'kombou tea' [1–3]. This beverage is obtained by fermentation from a symbiotic culture of acetic acid bacteria (AAB) (*Komagataeibacter*, *Gluconobacter*, and *Acetobacter* species) [4], lactic acid bacteria (LAB) (*Lactobacillus*, *Lactococcus*) [5], and yeasts *(Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*) [6] in a sweet medium in the presence of the main tea leaves (black green, red and white) from 7 to 14 days [7,8]. During kombucha fermentation in aerobic conditions, four phases occur: invertase production, ethanol fermentation, ethanol oxidation and acidification, and biofilm formation [9]. It is an enzymatic process that allows the cleavage and subsequent transformation of its monomeric components into ethanol and organic acids such as acetic acid, gluconic acid, glucuronic acid, citric acid, lactic acid, malic acid, succinic acid, saccharic acid and saccharic acid, cellulose, and carbon dioxide [10,11]. Indeed, yeast cells invertase hydrolyze sucrose into glucose and fructose Kombucha tea, a beverage with a low alcohol consumption rate can be produced at both home itself and commercial scales. It's consumption presents the important health benefits through its nutritional components (water-soluble vitamins, amino acids, and some minerals) and other metabolites such as phenolic compounds much abundant such as catechins, theaflavins, and flavanols [10, 12,13]. Regarding this huge of chemical compounds, some previous research revealed their effect on many pathogens. Kombucha showed a positive effect on digestion balances the gastrointestinal microbial flora, relieves arthritis, and possesses antimicrobial activity. This beverage also act against haemorrhoids, detoxifies the body, shows hepatoprotective activity, helps reduce insomnia, headaches, and positively influences mood [14–16]. It has been reported that Kombucha triggers good cholesterol levels, as it acts as a probiotic [10]. The phenols have also been demonstrated the inhibitory effects on protozoan parasites as antileishmanial agents [17, 18]. It is worth to noting that the antioxidant capacities are directly linked to the secondary metabolites found in an extract, and they rely on the antioxidant substances, their nature, quantity, structure, and any molecular interactions that may work in concert to enhance this activity [19,20]. Compounds containing one or more aromatic rings and one or more hydroxy groups that are plant-based antioxidants are known as phenolic compounds. The most common techniques are being thoroughly investigated to evaluate antiradical capability by using DPPH•, (using the diphenyl-p-picrylhydrazyl radical), ferric reducing antioxidant power (FRAP) and 2,20 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) techniques [20]. For every technique utilized, there is a strong correlation between the overall antioxidant activity and the quantity of phenolic compounds in the sets of samples [21,22].

However, to our knowledge in the literature, there is no investigation on the antileishmanial propriety of kombucha tea. Leishmaniasis is a health threat with almost 350 million people worldwide and approximatively 1.2–2 million new cases are reported annually. It is a vector-borne complex parasitic disease caused by the obligate intramacrophagic protozoan parasite genus *Leishmania* and transmited by the bite of phlebotomes sand fly [23]. A 95 % mortality rate is attributed to visceral leishamaniasis, the most severe form [24,25]. While parasite is progressing, macrophages rapidly induce an oxidative stress response whereby free radicals are released as a defense mechanism to activate pathogen clearance and trigger signaling pathways related to inflammation and immune response. The most common radical oxygen free radical is the superoxide, O2- [26]. The available drugs have limitations like high cost, low availability, toxicity, and painful route of administration [27]. Hence, there is an urgent need to develop novel therapeutic alternatives. Thereby, the objective of this research was to investigate the influence of fermentation time on phtytochemical properties and antileishmanial activities of kombucha tea.

2. Materials and methods

2.1. Kombucha tea preparation

The preparation of Kombucha tea was carried out through the fermentation process of black tea and sugar under aseptic conditionsin the Laboratory for Phytobiochemistry and Medicinal Plants Studies/Antimicrobial and Biocontrol Agents Unit [28]. Indeed, SCOBY was maintained in culture for continuous growth in a water (4 L)-sugar (500 g) mixture for 14 days. One hundred g of powder tea leaves bought at central market of Kousseri (North region of Cameroon) were introduced into a clean pot with 4 L water and heated for 30 min at 100 °C and cool (1 h). The mixture was filtered, 500g of sucrose was added to the filtrate and 505 g SCOBY pellicle kombucha was inoculated. The sweetened decoction was incubated in aeorobic conditions in a safe plastic bucket that was covered with white cheese cloth, and the fermentation at (28 ± 1) °C was monitored at different time (7, 14, 21, 28, and 35 days). The different beverages of kombucha tea were dried at room temperature to obtain dried matter in powder (extracts).

2.1.1. Preparation of extracts and reference drugs

Stock solutions of the extracts (100 mg/mL) were prepared by dissolving 100 mg of each extract in 1 mL of distilled water. Amphotericin B (Sigma Aldrich), Gallic acid (Sigma Aldrich), Quercetin (Sigma Aldrich), and Tannic acid (Sigma Aldrich) were used as reference drugs (1 mg/mL). They were 5-fold serially diluted in intermediate plates with the incomplete medium to yield concentration values ranging from 2000μg/mL-3.2 μg/mL for extracts and 10μM–0.016 μM for amphotericin B.

2.2. Phytochemical analysis of different kombucha black tea preparation

2.2.1. Phytochemical qualitative analysis

Phytochemichal screening was performed in order to determine the group of secondary metabolites (alkaloids, saponins, steroids, terpenoids, coumarins, catechins, quinones, phenolic compounds and flavonoids) present in different extract as described below.

2.2.1.1. Test for alkaloids. A test tube was filled with 1 mL of the extract. Dragendorff's reagent, 1 mL of potassium bismuth iodide solution, was then added and agitated. The presence of alkaloids is indicated by the formation of an orange-red precipitate [29].

2.2.1.2. Test for saponins. After being powdered, 2 g was heated in 20 mL of distilled water. 5 mL of distilled water and 10 mL of filtrate were vigorously shaken. There were saponins present because foaming appeared [30].

2.2.1.3. Test for steroids. A drop of concentrated H_2SO_4 was added after 1 g of Kombucha extract had been diluted in a few drops of acetic acid. The color becoming green signified the presence of steroids [31].

2.2.1.4. Test for terpenoids. Each sample weighed 0.2 g and 2 mL of chloroform and 3 mL of concentrated H_2SO_4 were combined. The presence of terpenoids was indicated by a reddish-brown coloring [30].

2.2.1.5. Test for coumarins. An extract (2 mL) was shaken in a 3 mL, 10 % aqueous NaOH solution. The yellow hue indicated a successful outcome [32].

2.2.1.6. Test for catechins. A few drops of 1 % lead acetate were mixed with 2 mL of the extract. The presence of tannins was indicated by a yellowish precipitate. The Stiasny reagent has demonstrated the presence of catechins (formalin 30 % in HCl extract: 2/1 v/v) [33].

2.2.1.7. Test for quinones. One milliliter of sodium hydroxide was applied for each milliliter of filtered beverage. The formation of blue color shows the presence of quinones [34].

2.2.1.8. Test for phenolic compounds. A few drops of ferric perchloride ($FeCl_3$) (10 %) were added to 2 mL of extract, and the formation of an intense black-green precipitate was the sign of the presence of phenolic compounds [35].

2.2.1.9. Test for flavonoids. In a tube 0.5 g of NaOH was dissolved in 5 mL of water (diluted NaOH), then mixed 1 mL of HCl in 2 mL of water (diluted HCl). Sodium hydroxide test for flavonoids: 2 mL extract +0.5 mL NaOH followed by the addition of 0.5 mL dilute HCl. The formation of a yellow solution with dilute NaOH which fades with dilute HCl is an indication of the presence of flavonoids [36].

2.2.2. Phenolic compounds quantification

Phenolic compounds: phenolic compounds was evaluated according to the spectrophotometric method [37]. Briefly, 0.2 mL of the sample solution and 0.8 mL of Folin-Ciocalteu reagent ($1/10^e$) were introduced into a test tubes. The test tubes were shaken for a few minutes (5 min) and 2 mL of sodium carbonate solution (7.5 % Na_2CO_3) was added to the mixture. The tubes were then incubated for 1 h in the dark and the absorbance was read against a blank at 765 nm. Gallic acid was used as standard compound (1 mg/mL). The results were expressed in milligram equivalent of GAE per gram of dry extract using the formula below.

$P=CxV/M$

P= Phenolic compounds (mg GAE/g dry extract).
C=Concentration of phenolic content extract (EG) obtained from the calibration cuves (mg/mL).
V=Volume (mL).
M = weight of phenolic compounds extract (g).

Determination of flavonoids content: A volume of 0.1 mL of the samples (100 μg/mL); 0.3 mL of distilled water and 0.03 mL of $NaNO_2$ 5 % solution were added into a test tubes. After stirring for 5 min, a volume of 2 mL of the sodium carbonate solution (7.5 % Na_2CO_3) was added to these tubes. After 5 min of incubation, 0.06 mL of an aluminium chloride reagent ($AlCl_3$ 10 %) was added, and the tests tubes were incubated for 1 min. Then, 0.4 mL of 1 mM NaOH and 2 mL of distilled water were added, the mixture was vortexed and the absorbance was read against a blank at 510 nm. The amount of flavonoids was calculated using a standard solution of quercetin equivalent (100 μg/mL) and the results were expressed in milligrams of QE per gram of dry extract [38].

Tannins Content: Estimation of Tannins was performed using the Folin Denis method (FDR) [39]. 1 mL of the sample solution, 5 mL of FDR reagent and 10 mL of 10 % sodium carbonate (in distilled water) were introduced into a test tubes. The test tubes were shaken for a few minutes (5 min) and blue color was measured at 700 nm after 30min. A standard graph was drawn by plotting the concentration versus the absorbance of the standard tannic acid and the amount of tannins present in the sample was calculated. The results were expressed in milligram equivalent of tannic acid per gram of dry extract.

2.3. Antioxidant assay of kombucha black tea extracts

Antioxidant activity was determined using three tests: DPPH•, ABTS●+ and FRAP methods.

2.3.1. DPPH free radical-scavenging activity

Briefly, 75 μL of a DPPH solution (0.02 %) was added to 25 μL of different concentrations of each extract (500–3.9 μg/mL) followed by incubation during 30 min protected from the light. After incubation, the absorbance at 517 nm was read using a multi-well plate reader (Magelan Infinite M2000 Tecan). DPPH without extract was used as negative control. Ascorbic acid was positive control and was prepared at the different concentrations ranging from 50 to 0.78 μg/mL. The experiment was performed in duplicate. Scavenging activity was then estimated based on the percentage of DPPH scavenging as calculated with the following formula: SC % = [(Anc-Asam)/Anc] × 100, where Anc: Absorbance of the negative control at t = 30 min; Asam: Absorbance of the sample containing the Kombucha black tea extracts at t = 30 min. From these values, the 50 % scavenging concentration was determined using the software GraphPad Prism 8.0.1 and defined as the concentration of the radical quenchers that neutralizes 50 % of the free radicals [40].

2.3.2. Antiradical activity of the kombucha black tea extracts by the 2, 2-azino-bis (3-ethylbenzo thiazoline-6-sulfonic acid) (ABTS) scavenging method

Indeed, 75 μL of ABTS●+ solution (reacting 7 mM ABTS in distilled water ($1/20^{e}$) + 4.9 mM aqueous solution of potassium persulfate in equal quantities) was added to 25 μL of different concentrations of each extract (500–3.9 μg/mL). Thereafter, the mixture was incubated in the dark for 30min. The measure of decrease in ABTS discoloration was performed at 734 nm using a Magelan Infinite M200 multi-well plate reader (Tecan). ABTS●+ solution without extract was considered as the negative control. Gallic acid was used as positive control and was prepared at the different concentrations ranging from 50 to 0.78 μg/mL. The experiment was performed in duplicate. Scavenging activity was then estimated based on the percentage of ABTS scavenging as calculated with the following formula: SC % = [(Anc-Asam)/Anc] × 100, where Anc: Absorbance of the negative control at t = 30 min; Asam: Absorbance of the sample containing the Kombucha black tea extracts at t = 30 min. From these values, the 50 % scavenging concentration was determined using the software GraphPad Prism 8.0.1 as described for DPPH method [41].

2.3.3. Antioxidant activity of the kombucha black tea by ferric ion reducing antioxidant power method (FRAP)

Experimentally, 25 μL of extracts at differences concentrations (500–3.9 μg/mL) were added to 25 μL of Fe^{3+}solution (1.2 mg/mL in distilled water). The plates were incubated for 15 min at room temperature and 50 μl of ortho-phenantroline (0.2 % in methanol) was added and the plates re-incubated for 15 min under the same conditions. The optical densities were determined at 505 nm by using the spectrophotometer (Tecan). The positive control for 100 % reduction was consisted by 25 μl Fe^{3+}, 25 μl gallic acid and 50 μl ortho-phenantroline. The test was run in duplicate and the reducing percentages were then calculated for each samples as followed: % Red = $[(A_c - A_e)/A_c] \times 100$ where Ac is the absorbance of the control, Ae the absorbance of the test samples. From these values, the 50 % reducing concentrations were deduced using the software GraphPad 8.0.1 [42].

2.4. Antileishmanial assay

2.4.1. Leishmania donovani maintenance

The cryopreserved promastigote form of *L. donovani* (1S (MHOM/SD/62/1S) was obtained from Bei Resources and continuously cultivated in Medium 199 supplemented with 10 % Heat-Inactivated fetal Bovine Serum (HIFBS) (Sigma, Darmstadt, Germany) and 100 IU/mL penicillin and 100 μg/mL streptomycin at the Laboratory of Phytobiochemistry and Medicinal Plants Studies/Antimicrobial and Biocontrol Agents Unit, University of Yaoundé I. Culture maintenance was done in microplates in 75 C m^2 cell culture flasks at 28 °C. The growth daily was checked and sub-cultured every 72 h.

2.4.2. Antileishmanial assay

The *in vitro* inhibitory activity of Kombucha black tea extract, against cultured *L. donovani* was evaluated using the resazurin colorimetric assay. Indeed, 10 μL of each tested sample (200–0.32 μg/mL) from intermediate plates were introduced in each well of the plate. Then, 90 μL of parasitic load at 4×10^5 cells/ml of *L. donovani promastigotes* were introduced in the wells for a final volume of 100 μL and the plates were incubated at 28 °C for 28 h. After incubation, 10 μL of rezazurin at 1 mg/mL were introduced in each well and the plates were incubated again for 44 h for a final incubation time of 72 h. Thereafter, the fluorescence was read with a spectrophotometer. The percentage of inhibition of each extract was calculated using Microsoft Excel Software. IC_{50} were calculated using Graphpad Prism Software 8.0.1. Amphotericin B (10–0.08 μg/mL) was used as a reference drug and 100 μL of parasitic load at de 4×10^5 cells/ml were without inhibitor were used as negative control [43].

2.5. Statistical analysis

All results of each sample were expressed as mean ± SD (standard deviation). Statistical analysis was performed by one-way ANOVA (analysis of variance) followed by Tukey and Dunnett's post hoc test for multiple comparisons test with 5 % of alpha value using GraphPad 8.0.1 software.

3. Results

3.1. Phenolic compounds of different extract of kombucha black tea

3.1.1. Qualitative phytochemical profile at various time of fermentation

The qualitative chemical composition of kombucha after different periods of fermentation is presented in Table 1.

It appears from the qualitative analysis that except alkaloids, and steroids, which were absent in the Kombucha black tea extracts, all other groups of metabolites were present in the extracts. However, a disappearance of terpenoids was noted in the drink after 21 days and also cathechins after 21 and 28 days of fermentation.

3.1.2. Quantitative phenolic compounds analysis

The quantification of phenolic compounds, flavonoids, and tannins was evaluated (Fig. 1A and B and C respectively) to determine the variation of these metabolites with fermentation time T7, T14, T28, T31 and T35 days.

The best content of phenolic compounds was recorded after 14 days of fermentation (350.05 mg Equivalent Gallic acid/grams of dry extract) and the lowest content after 21 and 28 days of fermentation. A significant difference was observed between the 7th, 14th, 21st, 28th and 35th day of fermentation respectively ($P < 0.005$) (Fig. 1A).

The highest flavonoids content was noticed after 35 days of fermentation, while the lowest is observed after 21 days. A significant difference was recorded between 7th, 14th, 21st, 28th, and 35th day of fermentation ($P < 0.005$) (Fig. 1B).

The highest tannins content was observed after 28 days of fermentation, while the lowest was observed after 7 and 14 days. No significant difference was observed between the 7th and 14th day. Contrary, a significant difference was observed between the 7th, 14 th 21st, 28th and 35th day of fermentation ($P < 0.005$) (Fig. 1C).

3.2. Antioxidant profile of kombucha black tea extracts at various time of fermentation

3.2.1. DPPH• scavenging activity

All tested Kombucha black tea extracts showed an antiradical activity with the values of SC_{50} ranging from 14.55 to 181.24 μg/mL. The good antiradical capacity for DPPH• was observed after 14 days ($SC_{50} = 14.55$ μg/mL). The value of SC_{50} of Gallic acid used as reference was 0.56 μg/mL. It has been noticed a significant difference between the 7th, 21st, 28th and 35th day of fermentation ($P < 0.0001$) (Fig. 2A).

3.2.2. ABTS●+ scavenging activity

A variable antiradical activity of different kombucha black tea extracts was noted depending on the fermentation time, with the best obtained after 21 days ($SC_{50} = 21.47$ μg/mL) for the ABTS $\bullet^{+}$ test. Gallic acid was used as reference molecule with SC_{50} value of 0.38 μg/mL.

A significant difference was observed between 7th, 14th, 21st, 28th, and 35th day of fermentation ($P < 0.005$) different (Fig. 2B).

3.2.3. Ferric iron reducing antioxidant power (FRAP) scavenging activity

The optimum of the reduction capacity of ferric ion (Fe_3^+) to ferrous ion (Fe_2^+) was observed after 21 days of fermentation with SC_{50} value of 65.55 μg/ml. A significant difference was observed between the 7th, 14th 21st, 28th and 35th day of fermentation respectively significant difference between the values ($P < 0.0001$) (Fig. 2C).

3.3. In vitro inhibitory potency of kombucha black tea according to time of fermentation against L. donovani promastigote

The different extracts were collected at different fermentation time were submitted *in vitro* against promastigote form of the *L. donovani* parasite. A significant difference was observed between 7th, 14th and 21st day of fermentation time ($P > 0.9999$) (Fig. 3). The good antileishmanial inhibition of Kombucha black tea was obtained after 14 days of fermentation with IC_{50} value of 48.86 μg/mL compared to the positive control Amphotericin B with IC_{50} value of 0.23 μg/mL. The extracts KBT28 and KBT35 showed no

Table 1
Chemical profile of different extracts of Kombucha black tea at five fermentation periods.

	KBT7	KBT14	KBT21	KBT28	KBT35
Alkaloids	–	–	–	–	–
Steroids	–	–	–	–	–
Saponins	+	+	+	+	+
Terpenoids	+	+	–	+	+
Phenolic Compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Quinones	+	+	+	+	+
Catechins	+	+	–	–	+
Coumarins	+	+	+	+	+

+ = presence; - = absence KBT: Kombucha Black Tea.

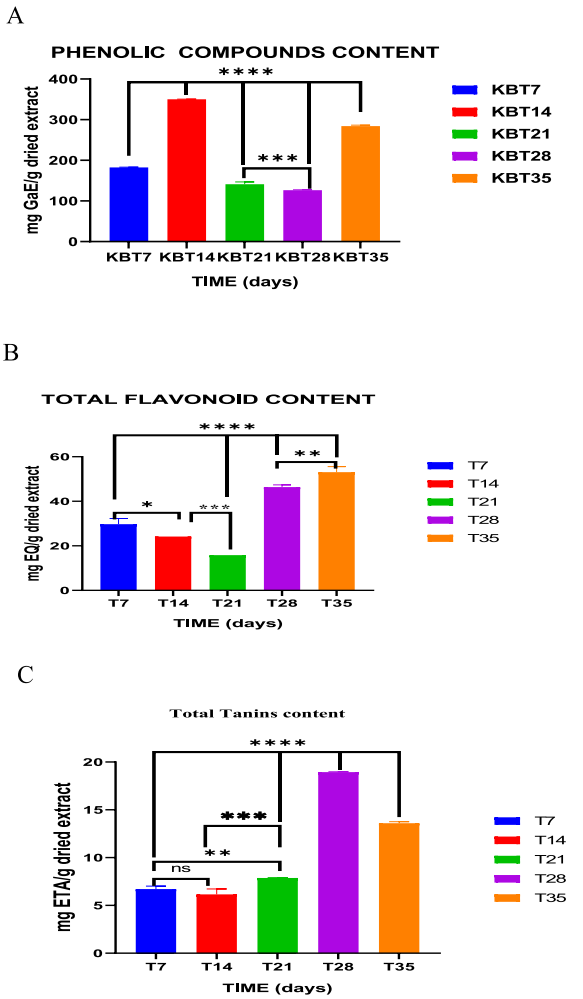

Fig. 1. Estimation of phenolic compounds in Kombucha black tea at five time of fermentation (7, 14, 21, 28 and 35 days).
ns: not significatively Different asterix ✱ in the same graph indicate significant difference ($P < 0.05$).
A: phenolic compounds content; B: flavonoid content; C: total tannins content.

antileishmanial inhibitory at the tested concentration ($IC_{50} > 200$ μg/mL).

4. Discussion

Many conditions may influence the chemical composition of Kombucha tea and therefore can change the final product: the kind of tea leaf variety, the sugar concentration, the fermentation time, the temperature and the composition of the tea fungus [44–46]. It is very relevant to know that the fermentation process has an essential impact on the content of metabolites and this parameter allow to better understand its variation. Among the components, tea phenolic compounds constitute the key metabolites [47]. Generally, tea is also known for its richness in tannins, phenolic compounds, flavonoids and terpenoids. These secondary metabolites were detected in Kombucha black tea tested. Particularly the bioactive compounds containing in tea leaves including flavan-3-ols, flavonols, organic acids, theaflavins, purine alkaloids and amino acid. The high level of bioactive compounds in fresh tea leaves include catechins, monomericflavonols, and (−)-epigallocatechin gallate (EGC) [48].

During kombucha fermentation by using black tea, the compounds present such as flavonoids, amino acids and phenolic acids [49] together with sucrose undergo a transformation by the action of yeasts and bacteria. It has been demonstrated that, the phenolic compounds ratio was highest on the 21st day of fermentation, it decreased significantly on the 35th day of fermentation [50]. Contrary to a previous research which evaluated the phenolic compounds and the antioxidant activity of Kombucha tea during the course of its fermentation (0, 7, 14, and 21 days). The authors observed a high tendency to increase especially after the 7th days [11]. These facts are in correlation with the results obtained which show a high concentration of phenolic compounds on 14 days. The same tendency has been found for total flavonoids and tannins content from 21 to 35 days.

Previously, *in vitro* determination of antioxidant capacity by DPPH• radical anion and ABTS●+ radical cation method exhibited

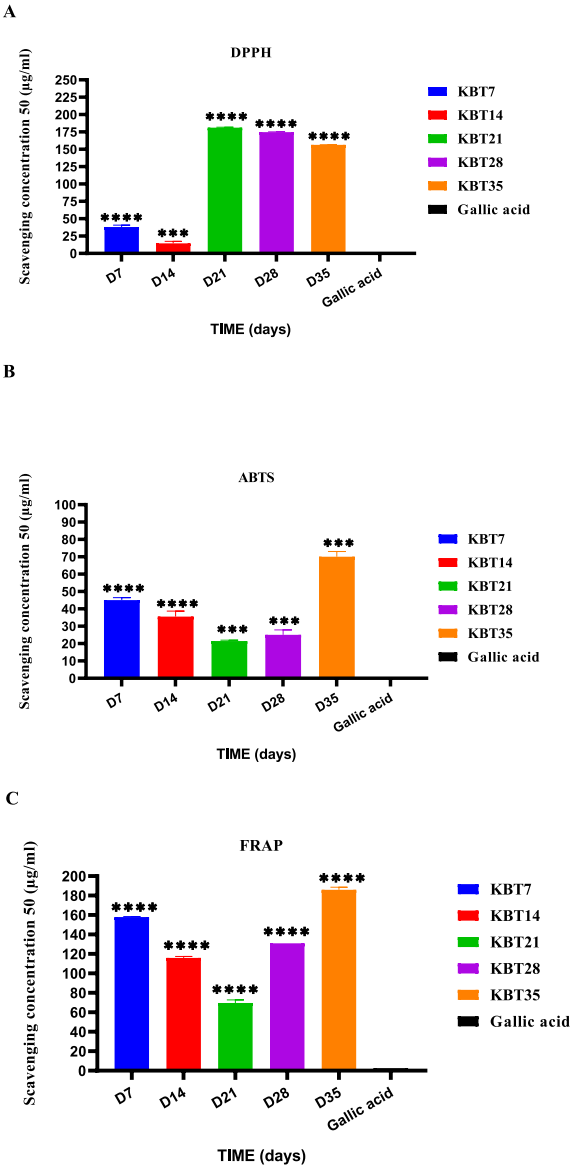

Fig. 2. Changes in the antiradical activity of kombucha black tea (KBT) fermented from five time of fermentation (7, 14, 21, 28 and 35 days). Different ✱ in the same graph indicate significant difference.
A: DPPH test; B: ABTS test; C: FRAP test.

that fermented black tea had about 70 % higher antioxidant activity than unfermented [51]. In addition, Kombucha tea fermentation normally ranges from 7 to 60 days and the biological activities may increase during this process; however, the best results have been obtained in an average of 15 days [52]. Indeed, it has been demonstrated that Kombucha prepared from green tea revealed the highest phenolic content and antiradical against DPPH• radical by 1.248 and 2.642 mg gallic acid equivalent/mL respectively [15]. This also corroborates with the results obtained concerning the anti-radical by DPPH• Free Radical-Scavenging Activity. The Kombucha black tea exhibited the best antiradical activity after 14 days with SC_{50} of 14.55 µg/mL. Some authors explained the high antioxidant ability by the fact of high amount of phenolic compounds which are among of active components produced during fermentation even during the metabolism of microorganisms in kombucha [53–55]. In this study, it is also observed that the antiradical potential was more pronounced after 21 days in ABTS ●+ assay than in the DPPH• test. This observation might be attributed to the fact that the test sample i.e. kombucha was prepared in a hydrophilic medium (water), thus favoring the scavenging potential against the hydrophilic radicals of ABTS●+. As the DPPH •free radicals are hydrophobic in nature, the presence of certain hydrophobic antiradical compounds in kombucha (lipids, organic acids, etc.) might have contributed to its scavenging activity against these radicals. Noteworthy, antiradical mechanisms of action of phenolic compounds -rich substances includes (i) hydrogen atom transfer, (ii) single-electron

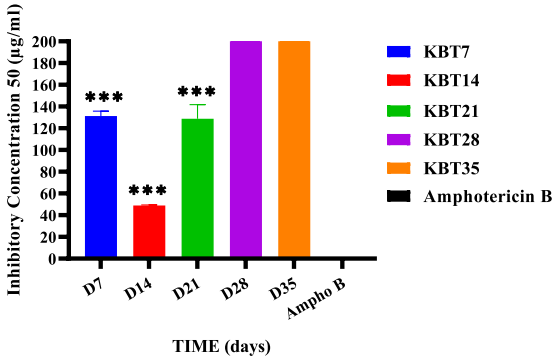

Fig. 3. Variation of inhibitory activity against *L. donovani* of Kombucha black tea fermented at five time (7, 14, 21, 28 and 35 days).

transfer followed by proton transfer, and (iii) sequential proton loss electron transfer [56]. Thus, it is not unreasonable to speculate that kombucha drink might have exerted antiradical activity through at least one among these mechanisms of actions.

There is no report on the investigation of antileishmanial activity of Kombucha tea in our best knowledge. Nevertheless in the literature, it has been proven that the consumption of Kombucha allow to gain some health benefits: prevention against some pathologies induced by the radical oxygen species (ROS); detoxifying activities, antimicrobial effects against some bacteria harmful such as *Shigellasonnei and, Salmonella enteritidis* and fungi: *Candida glabrata, and Candida albicans* [57–60]. As previously, it is known that a high level of biological activity is displayed after 15 days of fermentation [52]. In this present study, kombucha black tea leaves showed good inhibition against *L. donovani* after 14 days with IC_{50} of 48.86 µg/mL. Moreover, statistically there is no significant difference with positive control Amphotericin B. The antileishmanial activity could be attributed either to phenolic compounds or the combination of other secondary metabolites present in Kombucha tea. Phenolic compounds are the secondary plant metabolites found in the diet and have been reported amongst other natural compounds to have inhibitory effects against protozoan parasites particularly as potential compounds as leishmanicidal agents [18,61,and17]]. The most promising compounds from natural sources selected by their pharmacological activity against promastigotes in the anterior findings were flavonoids occurred as phenolic compounds. For instance, luteolin and quercetin isolated from *Vitex negundo* (Verbenaceae) and *Fagopyrum esculentum* (Polygonaceae) are potent antileishmanial compounds with IC_{50} values against *L. donovani* of 12.5 and 45.5 µM, respectively [62,63]. Both compounds are able to induce topoisomerase II-mediated kinetoplastid DNA minicircle cleavage in *L. donovani* promastigotes. The treatment of promastigotes with these compounds leads to cell cycle arrest in the G0/G1 phase followed by apoptotic cell death. Besides, it has been shown that the phenolic compounds (rosmarinic acid and apigenin) inhibited the growth of promastigotes with IC_{50} values of 16.34 ± 0.1 µM and 22.77 ± 0.01 µM respectively [64]. In fact, phenolic compounds such as flavonoids, and others [53] are well known to exhibit antileishmanial activity via (a) depolarization of the mitochondrial membrane potential of the parasite, (b) inhibition of pteridine reductase 1, an important enzyme for the parasite's folate pathway, and (c) inhibition of farnesyl diphosphate synthase, a crucial enzyme for ergosterol biosynthesis in *Leishmania* [65].

5. Conclusion

This research was to study the influence of fermentation time on phytochemical properties, antioxidant and antileishmanial activities of kombucha black tea.

The phytochemical profile showed high phytochemical diversity depending on fermentation time. The highest content of phenolic compounds was observed on the 14th day of fermentation.

Kombucha black tea exhibited antiradical activity on 14th and 21st days for DPPH •and ABTS ●+test respectively.

Kombucha black tea demonstrated a good inhibition of the growth of *Leishmania donovani* after 14 days.

Hence Kombucha black tea could be considered as promising agent against Leishmania.

CRediT authorship contribution statement

Alvine Ngoutane Mfopa: Writing – original draft, Supervision, Resources, Methodology, Investigation, Conceptualization. **Raoul Kemzeu:** Writing – review & editing, Software, Methodology. **Raymond Fokom:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Conceptualization. **Lauve Rachel T. Yamthe:** Resources, Methodology, Investigation, Conceptualization. **Darline Dize:** Resources, Methodology, Investigation. **Fabrice Fekam Boyom:** Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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