Austin Journal of Pharmacology and Therapeutics

Open Access

Austin Publishing Group

Research Article

Phytochemicals of Kombucha Extracts (Black and Green Tea) as Potential Therapeutic Agents Against Human Trypanosomiasis and Inflammation

Alvine NM[1]*, Raoul K[2], Canis Parfait DD[2], Raymond F[1], Lauve Rachel TY[3], Darline D[2] and Fabrice FB[2]

[1]Department of Processing and Quality Control, Yabassi, Institute of Fisheries and Aquatic Sciences University of Douala, Cameroon

[2]Department of Biochemistry, Faculty of Sciences, University of Yaounde I, Cameroon

[3]Laboratory of Pharmacology and Drugs Discovery, Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon

***Corresponding author:** Alvine NM, Department of Processing and Quality Control, Yabassi, Institute of Fisheries and Aquatic Sciences University of Douala P.O Box 7236 Douala, Cameroon

Tel: +237 699696176;

Email: ngmfalvine@yahoo.fr; rfokom@gmail.com

Received: August 16, 2025

Accepted: September 09, 2025

Published: September 12, 2025

Abstract

African trypanosomiasis, a neglected tropical disease caused by *Trypanosoma brucei brucei,* remains a public health challenge in sub-Saharan Africa. Current treatments are limited by toxicity, undesired side effects, long-term treatmen and the emergence of drug-resistant strains. This study evaluated the antitrypanosomal activity and anti-inflammatory potential of Kombucha extracts prepared using black tea, green tea, at varying fermentation periods.

Antitrypanosomal activity was assessed against bloodstream-form *T. b. brucei* and cytotoxicity was determined on Vero and RAW 264.7 cell lines. Subsequently, the anti-inflammatory was evaluated using the Bovine Serum Albumin (BSA) denaturation inhibition. Finally, the qualitative phytochemical screening and UHPLC-MS/MS was performed to identify bioactive compounds. Pharmacokinetic properties were predicted using the pkCSM predictor.

The results of green and black tea Kombucha extracts fermented for 7 and 14 days exhibited significant antitrypanosomal activity (IC_{50}< 200 µg/mL) and high selectivity (SI > 1). Furthermore, these extracts demonstrated notable anti-inflammatory potential through inhibition of protein denaturation. The phenolic compounds, flavonoids, and saponins were present except alkaloids. UHPLC-MS/MS analysis of the 14-day fermented green tea Kombucha extract identified compounds such as dicarboxylic acids, monosaccharides, xanthines, and phenolic derivatives. *In silico* (Absorption, Distribution, Metabolism, and Elimination (ADMET) predictions of these compounds indicated favorable gastrointestinal absorption and low toxicity in humans.

These findings suggest that Kombucha extracts, particularly with green tea after 14 days (KG14), hold promise as novel therapeutic candidates for human African trypanosomiasis.

Keywords: Kombucha ; Trypanosoma *brucei brucei ;* Antitrypanosomal activity ; Anti-inflammatory ; Phytochemistry ; ADMET : Absorption, Distribution, Metabolism, and Elimination

Abbreviations

HAT : Human African Trypanosomiasis ; NTD : Neglected Tropical Disease ; BSA : Bovine ; Serum Albumin ; ADMET : KG7 : Kombucha Green Tea After 7 Days ; KG14 : Kombucha Green Tea After 14 Days ; KG21 : Kombucha Green Tea After 21 Days ; KG28 : Kombucha Green Tea After 28 Days ; KB7 : Kombucha Black Tea After 7 Days ; KB14 : Kombucha Black Tea After 14 Days ; KB21 : Kombucha Black Tea After 21 Days ; KB28 : Kombucha Black Tea After 28 Days ; Sub-Saharan Africa : SSA ; Tbr : Trypanosoma Brucei Rhodesiense ; AAT : Animal African Trypanosomiasis ; WHO : World Health Organisation ; SCOBY : Symbiotic Culture Of Bacteria And Yeast ; NECT : Nifurtimox And Eflornithine Combination Therapy; LC-MS/MS : Liquid Chromatography Coupled With Tandem Mass Spectrometry ; UHPLC : Ultra High Perforance Liquid Chromatography.

Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, has posed a significant public health challenge in sub-Saharan Africa since the early 20th century. This parasitic disease, transmitted by the bite of *Glossina spp.* (tsetse flies) infected with protozoa of the genus *Trypanosoma*, persists as a neglected tropical disease (NTD) with profound socio-economic impacts. Recent estimates indicate approximately 977 annual cases across 37 endemic countries, with over 65 million individuals at risk as of 2017 [1]. By 2024, this risk extended to 60 million people in SSA [2]. *Trypanosoma brucei rhodesiense* (Tbr), the causative agent of the acute East African

Citation: Alvine NM, Raoul K, Canis Parfait DD, Raymond F, Lauve Rachel TY, et al. Phytochemicals of Kombucha Extracts (Black and Green Tea) as Potential Therapeutic Agents Against Human Trypanosomiasis and Inflammation. Austin J Pharmacol Ther. 2025; 12(3): 1196.

form, is associated with severe parasitemia, multi-organ damage, and rapid progression to fatal neurological complications if untreated [3]. Populations engaged in activities such as agriculture, fishing, or resource collection in tsetse habitats are disproportionately affected, with higher prevalence observed in adults [4].

Animal African trypanosomiasis (AAT) similarly devastates livestock and wildlife, with 50 million animals at risk and substantial economic losses due to reduced productivity and trade restrictions [2,5]. In Cameroon, recent studies confirm natural infections of *T. b. gambiense* (Tbg) in domestic animals (pigs, sheep, goats) and wildlife (pangolins, antelopes), highlighting their role as reservoir hosts in endemic regions such as Campo, Bipindi, and coastal areas [6,7]. These findings underscore the zoonotic potential of HAT and the challenges in achieving the WHO's 2030 elimination targets, particularly given inadequate diagnostic infrastructure and latent infections in both human and animal populations [4,8].

Current treatments for HAT (sleeping sickness) vary according to the disease stage and the infecting parasite species. In the haemolymphatic phase (early stage), pentamidine isethionate is used for *T. b. gambiense* infections, whereas suramin is administered for *T. b. rhodesiense*. These drugs require strict medical supervision and often involve complex administration protocols, such as intramuscular or intravenous injections. They are also associated with significant adverse drug reactions, including renal toxicity, hypersensitivity reactions to suramin, as well as cardiovascular disturbances and hypoglycemia [9-11]. In the advanced stage, when the central nervous system is affected, treatment relies on more toxic and challenging therapies such as melarsoprol. While effective, melarsoprol carries a substantial risk of fatal encephalopathy, occurring in 5 to 10% of cases. Combination therapy with nifurtimox and eflornithine (NECT), and more recently the oral drug fexinidazole, have enhanced the management of chronic forms of the disease. However, these options remain limited by their toxicity profiles and the requirement for prolonged treatment regimens [9-11].

Recent research explores probiotics as antiparasitic agents. For instance, *Saccharomyces cerevisiae* administration reduces parasitemia and enhances antibody responses in *T. brucei brucei*-infected models [12,13,14]. Similarly, natural products like Kombucha a fermented tea (*Camellia sinensis*) beverage containing a symbiotic culture of bacteria and yeast (SCOBY) exhibit bioactive properties modulated by fermentation parameters (time, pH, temperature), substrate type, and regional microbiomes [15,16]. Kombucha demonstrates antimicrobial, antioxidant, and anti-inflammatory activities, positioning it as a candidate for parasitic disease management [17,18]. A previous study conducted by our research team had already reported the impact of fermentation time on the phytochemical properties, antioxidant and antileishmanial activities of Kombucha extracts, with a stronger activity observed with tea leaves (*Camellia sinensis)* after 14 days with IC_{50} of 48.86 µg/Ml [19]. *Leishmania* and *Trypanosoma* are two parasites belonging to the same family, the Trypanosomatidae. They are intracellular parasites responsible respectively for leishmaniasis and trypanosomiasis. These parasites share similarities in their invasion strategies, intracellular survival, as well as in their ability to subvert the host immune response. They also present common molecular targets, which encourages the search for therapeutic inhibitors targeting similar biological mechanisms in both genera [20,21].

In silico studies in general, and the prediction of pharmacokinetic properties in particular, are increasingly used in the research of new drugs against trypanosomes. They offer significant advantages, notably for virtual screening of compounds, selection of safe molecules, structural optimization, and acceleration of drug development [22-24].

In Cameroon, where community health initiatives prioritize local resource valorization, this study evaluates the antitrypanosomal and anti-inflammatory potential of Kombucha extracts derived from indigenous teas at varying fermentation durations. By elucidating its therapeutic promise, this research aims to contribute to innovative strategies for HAT control and elimination.

Materials and Methods

Materials

Plant Materials : This study utilized two primary substrates : black tea or Ndjuittitsa (TOLE TEA), purchased from a supermarket in Yaoundé, Cameroon and green tea (*Camellia sinensis*), harvested in the North Cameroon region.

Microbiological and Cellular Materials :

Kombucha Master Strain (SCOBY) :

The kombucha fermentation process was initiated using a "SCOBY" biofilm provided by the Laboratory for Phytobiochemistry and Medicinal Plants Studies/Antimicrobial and Biocontrol Agents Unit. The SCOBY was maintained in culture within a sterile transparent container containing sweetened drinking water, covered with a clean, white, transparent cloth. The medium was renewed every 30 days to sustain microbial activity.

Parasite Strain :

The antitrypanosomal activity of kombucha extracts was evaluated *in vitro* against the bloodstream form of *Trypanosoma brucei brucei* Lister 427 VSG 221 (NR-42009), provided by BEI Resources (https://www.beiresources.org). The parasites were maintained in continuous culture at the Laboratory of Phytobiochemistry and Medicinal Plant Studies/Antimicrobial Agents and Biocontrol Unit, Faculty of Science, University of Yaoundé I.

Cell Lines :

The safety assessment of kombucha extracts was performed *in vitro* using RAW 264.7 macrophage cell lines obtained from the Department of Clinical Pathology, Noguchi Memorial Institute for Medical Research, University of Ghana, and Vero CRL1586 cells derived from African green monkey kidneys, provided by the Pasteur Center in Cameroon. Both cell lines were maintained in continuous culture at the Laboratory of Phytobiochemistry and Medicinal Plant Studies/Antimicrobial Agents and Biocontrol Unit, Faculty of Science, University of Yaoundé I.

Methods

Preparation of Kombucha Extracts : Kombucha was prepared by infusion using black tea (KB) and green tea (KG) substrates at varying

fermentation durations (7, 14, 21, and 28 days) [25]. Briefly one liter of tap water was heated to 80–90°C in a pot. Thereafter, 8 grams of tea leaves or lemongrass were added to the water along with 80 g of powdered brown sugar. The mixture was infused for 15 minutes before being cooled to room temperature and filtered to remove residues. Then, the filtered solution was transferred to a sterile plastic jar (3 L), and 3% (w/v) SCOBY biofilm was added. The container was covered with a clean, white cloth secured with string and left to ferment for the designated durations (7–28 days). Finally, the container was covered with a clean, white cloth secured with string and left to ferment for the designated durations (7–28 days).

At each fermentation time, 500 mL of the mixture was collected and dried in an oven at 45°C for 48 hours to obtain dried kombucha extracts. The same procedure was applied for all substrates (black tea and green tea). The dried extracts were stored at 4°C until further testing.

Evaluation of the Antitrypanosomal Activity of Kombucha Extracts :

Antitrypanosomal Activity Test :

Preparation of Stock Solutions : Stock solutions of the extracts were prepared at 100 mg/mL by dissolving 100 mg of each sample in 1 mL of a solvent consisting of 70% dimethyl sulfoxide (DMSO) and 30% sterile distilled water. The mixture was homogenized before use. Pentamidine isethionate and podophyllotoxin (P4405-25G) were used as positive controls for antiparasitic activity and cytotoxicity tests, respectively. They were prepared in 100% DMSO at 10 mM and 10 mg/mL.

***In Vitro* Culture and Maintenance of Bloodstream Forms of *T. brucei* :** The *T. brucei* strain was thawed from a cryotube stored at -80°C and preheated to 37°C for 2 minutes to facilitate thawing. The contents were transferred into sterile 25 cm^2 culture flasks containing 3 mL of complete HMI-9 culture medium. Then, cultures were incubated for 72 hours at 37°C in a humidified atmosphere with 5% CO_2. The medium was renewed every 3 days by adding fresh complete medium to ensure optimal parasite growth. Culture status and parasite growth were monitored using an Etaluma Lumascope 520 inverted fluorescence microscope. For the antitrypanosomal activity test, cultures predominantly containing the metacyclic trypomastigote form were selected.

Performance of the Inhibition Test : The antitrypanosomal test assessed the ability of bloodstream forms of T. *brucei. brucei* to multiply and survive in culture medium containing potential inhibitors. The evaluation of kombucha extracts antitrypanosomal activity on bloodstream trypomastigotes of *T. b. brucei* was conducted under aseptic conditions in a laminar flow hood using the resazurin colorimetric method [26].

Tests were performed in duplicate in 96-well microplates with a final volume of 100 µL per well. Briefly, 10 µL of extract and reference molecule (previously prepared in intermediate plates at concentrations ranging from 10 mg/mL to 0.016 mg/mL for extracts and from 10 mM to 0.016 µM for pentamidine ; dilution factor of 5 were added to the plates, followed by the addition of 90 µL of *T. b. brucei* inoculum at a load of 2×10^5 parasites/mL. Wells containing 100 µL of inoculum and 10 µM pentamidine served as negative and positive controls, respectively. Final concentrations ranged from 1000 µg/mL to 1.6 µg/mL for extracts and from 10 µM to 0.016 µM for pentamidine. The plates were incubated for 72 hours at 37°C, 5% CO_2. After a 68 hours incubation period, 10 µL of a resazurin solution (0.15 mg/mL) prepared in incomplete HMI-9 medium were added to all wells, and the plates were reincubated under the same conditions for 4 hours. Fluorescence was measured using the Infinite M200 microplate reader (Tecan) at an excitation and emission wavelength of 530 and 590 nm, respectively. The fluorescence values obtained were used to calculate the percentage inhibition (PI) according to the formula :

$$PI = ((Fluorescence_{Negative\ Control} - Fluorescence_{Test}) / Fluorescence_{Negative\ Control}) \times 100$$

Extracts showing good activity against *T. b. brucei* were selected for safety assessment on RAW and Vero cell lines.

Cytotoxicity Assay of Extracts on Normal Cell Lines : The evaluation of novel therapeutic candidates requires assessing both antitrypanosomal activity and non-cytotoxicity. Therefore, the cytotoxic profiles of the extracts were evaluated on RAW 264.7 macrophages and Vero cells.

***In Vitro* Culture of RAW 264.7 and Vero Cell Lines :** Cells stored in cryovials at -80°C were thawed and transferred to separate Falcon tubes containing 9 mL of complete Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS. The mixtures were centrifuged at 1800 rpm for 5 min to remove the cryopreservation solution. The resulting pellets were resuspended in 5 mL of complete culture medium in 25 cm^2 cell culture flasks (T-flasks). Cells were maintained in continuous culture under standard conditions (5% CO_2 at 37°C). The culture medium was refreshed every 72 hours, and cell status was monitored using an Etaluma Lumascope 520 inverted fluorescence microscope to ensure sterility and viability until a monolayer formed. Upon reaching 70-90% confluence, cells were detached via trypsinization. Briefly, cells were rinsed with Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium/magnesium and treated with 1 mL of 0.25% trypsin-EDTA for 5 min (RAW 264.7 cells) or 7 min (Vero cells). Trypsin activity was quenched by adding 9 mL of complete DMEM, and cells were centrifuged at 1800 rpm for 5 min. The pellets were resuspended in 1 mL of complete culture medium, and viability was assessed using trypan blue staining and a Neubauer hemocytometer for cell counting before subculture or cytotoxicity testing [26].

Performance of the Cytotoxicity Assay : The cytotoxicity assay assessed the ability of cells to survive and proliferate in the presence of potential inhibitors using the resazurin spectrophotometric method [26].

The assay was performed in duplicate in treated 96-well microplates. Indeed, 100 µL of cell suspension containing 1×10^4 cells/well were added to each well and incubated at 37°C, 5% CO_2 for 18 hours to allow cell attachment. Then, the existing culture medium was replaced with 90 µL of fresh culture medium, followed by 10 µL of extracts at various concentrations (5000 µg/mL to 1.6 µg/mL) or podophyllotoxin (500 µM to 0.8 µM) previously prepared in intermediate plates. Wells containing only cells served as negative controls, while wells containing complete culture medium supplemented with 10 µL of 10% DMSO or 50 µM podophyllotoxin

served as positive controls. The plates were incubated at 37°C, 5% CO_2 for 48 hours. After incubation, 10 µL of resazurin solution (0.15 mg/mL) were added to each well, and the plates were re-incubated for an additional 4 hours. Fluorescence was measured using the Infinite M200 microplate reader (Tecan) at excitation and emission wavelengths of 530 nm and 590 nm, respectively. The percentage inhibition (PI) was calculated using the formula:

$$PI = ((Fluorescence_{Negative\ Control} - Fluorescence_{Test}) / Fluorescence_{Negative\ Control}) \times 100$$

Selectivity indices (SI) were calculated using the formula :

$$SI = CC_{50}/IC_{50}$$

Following cytotoxicity testing, the anti-inflammatory potential of active and selective kombucha extracts was assessed.

Evaluation of Anti-inflammatory Potential by anti-BSA Denaturation : Given the involvement of inflammatory reactions mediated by cytokines in the pathogenesis of trypanosomiasis, the anti-inflammatory potential of the selected extracts was evaluated using the Bovine Serum Albumin (BSA) denaturation inhibition method with minor modifications [27].

Stock Solutions : Stock solutions of extracts were prepared at 5 mg/mL by dissolving 5 mg of each extract in 1 mL of Phosphate-Buffered Saline (PBS) at pH 7.4. For the standard, sodium diclofenac was prepared at 1 mg/mL in PBS (pH 7.4), followed by six serial 2-fold dilutions.

BSA Solution : The BSA solution was prepared at 0.1% w/v by dissolving 0.1 g of BSA in 100 mL of PBS at pH 7.4.

In microplates, 10 µL of BSA solution, 140 µL of PBS (pH 7.4), and 100 µL of the extract or sodium diclofenac were mixed to a final volume of 250 µL. Wells designated as blanks received 100 µL of methanol. Extracts and sodium diclofenac were tested at final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 µg/mL. After mixing, the plates were incubated at 37°C for 30 minutes, followed by heating in a 70°C water bath for 15 minutes. After cooling, optical densities were read at 660 nm. The tests were performed in duplicate.

Phytochemical Screening of Kombucha Extracts : Phytochemical screening was conducted to obtain a qualitative overview of the major secondary metabolite groups present in the kombucha extracts. This method involves identifying different chemical groups through precipitation or color reactions [28].

Alkaloid Detection : Each sample of the extract (5 mg) was dissolved in 1 mL of 50% HCl, followed by the addition of a few drops of Mayer's reagent (potassium mercuric tetraiodide). The formation of a yellow precipitate indicated the presence of alkaloids [29].

Terpenoid Detection : The extract (200 mg) of the extract were dissolved in 2 mL of chloroform, and then 3 mL of sulfuric acid were added to form two distinct phases. The appearance of a reddish-brown interface suggested the presence of terpenes [30].

Saponin Detection : The extract (25 mg) were mixed with 5 mL of distilled water in a test tube and vigorously shaken for 10 seconds using a vortex mixer. The persistence of a thick foam (approximately 1 cm) for more than a minute indicated the presence of saponins [28].

Identification of Phenolic Compounds : A few drops of ferric chloride were added to a 2 mL solution of concentrated extract (5 mg/mL). The observation of a blue-blackish color confirmed the presence of polyphenols [31].

Identification of Flavonoids : A 2 mL diluted ammonia solution was added to 1 mL of an aqueous solution of concentrated extract (5 mg/mL), followed by the addition of 1 mL of concentrated sulfuric acid. The appearance of a yellow color that faded over time characterized the presence of flavonoids [28].

Identification of Quinones : The extract (50 mg) were diluted in 4 mL of a chloroform-petroleum ether mixture (v/v), homogenized, and filtered. The addition of NaOH to 1 mL of the filtrate resulted in a red color, indicating the presence of quinones [32].

Detection of Coumarins : 1 mL of the extract was introduced into a test tube covered with filter paper soaked in a dilute NaOH solution. Under UV light at 254 nm, a fluorescent yellow color on the filter paper indicated the presence of coumarins [29].

Detection of Tannins : A few drops of ferric chloride were added to an alcoholic or aqueous solution of the extract. The presence of tannins was indicated by a color change: dark blue for gallic tannins or blackish-green for catechol tannins [28].

Identification of Chemical Constituents by LC-MS/MS : The identification of the compounds responsible for the activity of the promising extract was performed by using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Liquid chromatography (LC) separates compounds based on their differential affinity between a stationary phase and a mobile phase. Mass spectrometry (MS) identifies and quantifies molecules based on their mass-to-charge ratio, providing information on composition, structure, and abundance. In LC-MS/MS, separated LC compounds are ionized and undergo mass analysis, allowing precise identification and structural elucidation [33].

High-resolution mass spectra were obtained using a QTOF spectrometer (Bruker, Germany) equipped with an ESI source. The spectrometer was operated in positive and negative modes (mass range: 100-1500 m/z, scan rate: 1.00 Hz) with automatic gain control to provide high-precision mass measurements within 0.40 ppm using sodium formate as the standard.

CID was used for MS/MS analysis with a collision energy of 40 eV. The experiments used a spray voltage of 4.5 kV (positive mode) and 3.5 kV (negative mode), a capillary temperature of 220°C, and nitrogen as sheath gas (10 L/min). The spectrometer was coupled to an Ultimate 3000 UHPLC system (Thermo Fisher, USA), consisting of an LC pump, a diode array detector (DAD) (λ: 190-600 nm), an autosampler (injection volume: 5 µL), and a column oven (35°C). Separations were performed using a Synergi Max-RP 100A column (50 × 2 mm, 2.5 µm particle size) with a gradient of H_2O (+0.1% HCOOH) (A) and Acetonitrile (+0.1% HCOOH) (B) at a flow rate of 500 µL/min. The gradient program was as follows : 95% A isocratic for 1.5 min, linear gradient to 100% B over 6 min, 100% B isocratic for 2 min, returning to 95% A in 1 min and equilibrating for 1 min.

Prediction of Pharmacokinetic Properties (ADME): Pharmacokinetic properties (Absorption, Distribution, Metabolism, and Elimination; ADME) were predicted using the pkCSM predictor (https://biosig.lab.uq.edu.au/pkcsm/prediction) [34] and SwissADME (http://www.swissadme.ch/index.php) [35] software. This freely available software provides access to a robust, up-to-date, and high-quality database. Briefly, metabolite structures were drawn using ChemBio2D Draw, and their Simplified Molecular Input Line Entry System (SMILES) codes were generated. These codes were used by the pkCSM predictor software to estimate oral drug absorption, human intestinal absorption, skin permeability, and transdermal absorption. Plasma protein binding and blood-brain barrier (BBB) penetration models were used to estimate metabolite distribution [34].

Statistical Analysis

Microsoft Excel 2016 was used to calculate inhibition percentages, and GraphPad Prism 8.0.1 software was used to determine IC_{50} and CC_{50} values from dose-response curves. Data were expressed as mean ± standard deviation and analyzed by ANOVA using Turkey's test and Dunnett's test at the 5% probability threshold using GraphPad software. P-values ≤ 0.05 were considered significant.

Results

Antitrypanosomal Activity and Selectivity of Kombucha Extracts

The antitrypanosomal activity and selectivity of the extracts on normal cells were evaluated based on the following classification criteria and adjusted for a concentration of inhibitors tested at 1000 µg/Ml [36] : IC_{50} < 200 µg/mL (good activity), 200 µg/mL < IC_{50} < 1000 µg/mL (moderate activity), and IC_{50} > 1000 µg/mL (inactive).

Statistically, the data presented below illustrate the antitrypanosomal activity of kombucha extracts as a function of the fermented tea substrate.

The results of inhibitory activity of black tea kombucha extracts prepared after 7, 14, 21, and 28 days of fermentation are illustrated in (graph 1) below. A significant differences was observed between 7, 14, 21 and 28 days of fermentation time (P < 0.001). The highest inhibitory capacity was observed after 14 days of fermentation (IC_{50} = 133.45 µg/mL). However, the lowest inhibitory activity was observed after 28 days of fermentation (390.45 µg/ mL).

The inhibitory activity of KG extracts, prepared at the same fermentation times is represented in graph below (figure 2). A significant difference was exhibited between 7, 14, and 21 days of fermentation (P-value < 0.0001, at α = 5%). The highest inhibitory capacity was observed after 14 days of fermentation (IC_{50} = 47.38 µg/mL), and the lowest was observed after 21 days of fermentation (IC_{50} = 233±0,56 µg/mL).

Additionally, selectivity was assessed in Table 1 below. All active extracts showed CC_{50} > 500 µg/mL, indicating good selectivity indices (SI > 1) against Vero and RAW cell lines (37). In summary, the green tea kombucha extract obtained after 14 days of fermentation (KG14) not only showed the best antitrypanosomal activity but was also non-cytotoxic to normal cells.

Table 1 : Antitrypanosomal Activity and Selectivity Index (SI) of Kombucha Extracts.

Samples	Codes	IC_{50} (µg/ml) ±SD	CC_{50} (µg/ml) ±SD Vero & Raw	SI
Extraits	KB7	149.1±0,7	>500	>3.35
	KB14	133.45±2,19	>500	>3.74
	KB21	362.35±1,9	>500	>1.37
	KB28	390.45±1,9	>500	>1.28
	KG7	118.6±2,12	>500	>4.21
	KG14	47.38±0,26	>500	>10.55
	KG21	233±0,56	>500	>2.14
	KG28	>1000	ND	ND
	KL7	129.9±1,41	>500	>3.84
Reference Drug (µM)	Pentamidin Podophyllotoxin	0.015±0 .0009 ND	ND 0.4±0.12	ND

Legend : KB: kombucha Black tea; KG: kombucha Green tea; Fermentation Day: 7, 14, 21, 28 ; IC_{50}: Median inhibitory concentration ; CC_{50}: Median cytotoxic concentration ; ND: Not determined ; SD: Standard deviation.

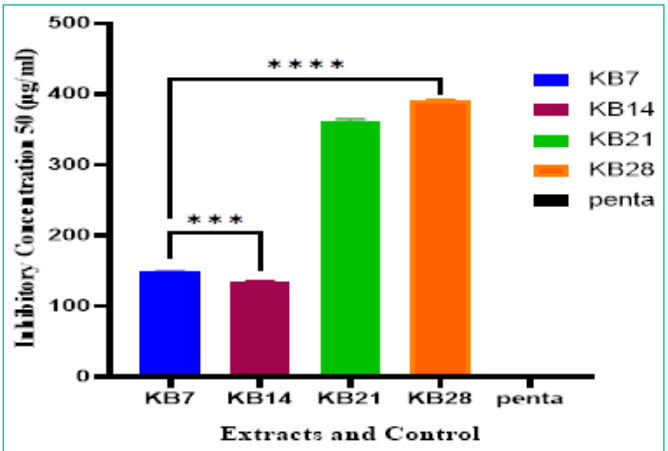

Figure 1 : Inhibitory Activity Against *T. b. brucei* of Black Tea Kombucha Extracts.
KB7: Black tea kombucha, day 7 ; KB14: Black tea kombucha, day 14 ; KB21: Black tea kombucha, day 21 ; KB28: Black tea kombucha, day 28 ; Pentamidine: Standard ; * (asterix indicates in the same figure, significant difference.

Anti-inflammatory Potential : inhibition of Protein Denaturation (BSA)

Inflammation plays a crucial role in the pathogenesis of trypanosomiasis. Therefore, the anti-inflammatory potential of the kombucha extracts of interest was assessed by evaluating their ability to inhibit protein denaturation.

Excessive protein denaturation can lead to inflammation ; thus, the capacity of extracts to inhibit protein denaturation reflects their anti-inflammatory potential. The graph (Figure 3) below illustrates the median inhibitory concentrations of the extracts of interest for the inhibition of protein denaturation.

The graph above illustrates the ability of kombucha extracts to prevent protein denaturation of bovine serum albumin (BSA), where the inhibitory concentration 50 (IC_{50}) is inversely proportional to the extract's capacity to prevent denaturation. Compared to diclofenac (IC_{50}= 2.91 µg/mL), used as the reference anti-inflammatory molecule, KG7 showed a significant difference with a two-star significance threshold (P < 0.001) and IC_{50} value of 2.45 µg/mL, respectively. This demonstrates the strong anti-inflammatory potential of the

Table 2 : Distribution of secondary metabolite classes in active kombucha extracts.

Extracts Metabolites	KB7	KB14	KB21	KB28	KG7	KG14	KG21
Alcaloïds	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+
Terpenoïds	+	+	-	+	-	-	-
Polyphenols	+	+	+	+	+	+	+
Flavonoïds	+	+	+	+	+	+	+
Quinones	+	+	+	+	+	+	+
Tannins	+	+	-	-	+	+	+
Coumarins	+	+	+	+	+	+	+

Legend: -: Absence; +: Present; KB: kombucha Black tea; KG: kombucha Green tea; The numbers 7; 14; 21 and 28 represent the different fermentation days.

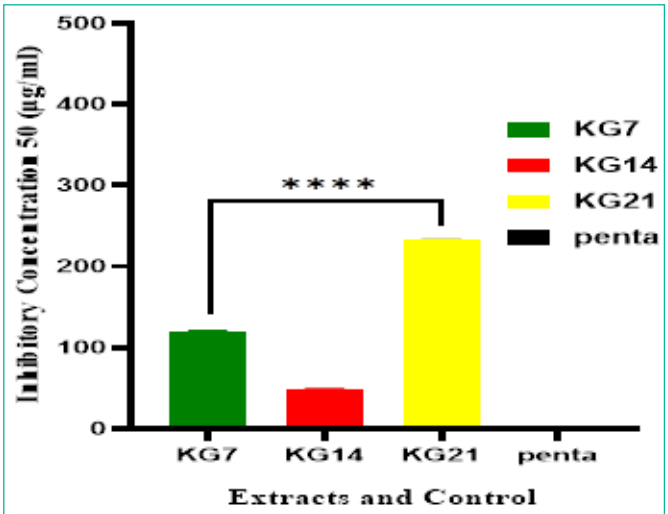

Figure 2 : Inhibitory Activity Against *T. b. brucei* of Green Tea Kombucha. KG7: Green tea kombucha, day 7 ; KG14: Green tea kombucha, day 14 ; KV21: Green tea kombucha, day 21 ; Pentamidine: Standard ; ****P < 0.0001.

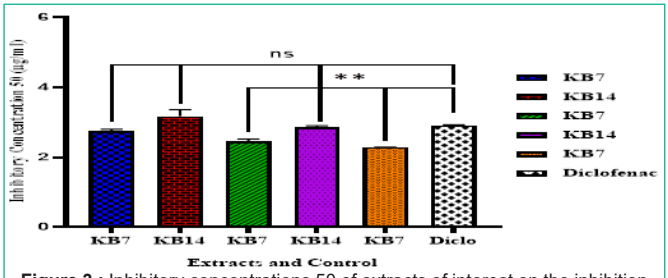

Figure 3 : Inhibitory concentrations 50 of extracts of interest on the inhibition of protein denaturation.
KB7: kombucha Black tea day 7 ; KB14: kombucha day Black tea 14 ; KG7: kombucha Green tea day7 ; KG14: kombucha Green tea day14 ; Standard: Sodium diclofenac, ns: not significant, **P=0.001 (Dunnett's multiple comparisons test).

kombucha extracts obtained after 7 days of fermentation. However, the anti-inflammatory potential of extracts obtained after 14 days of fermentation (KB14) was found to be non-significantly different from the standard.

Qualitative Phytochemical Profile of the Extracts

A qualitative phytochemical screening of the kombucha extracts was performed to identify the broad groups of secondary metabolites potentially responsible for the observed activities. The results of the qualitative test for detecting different classes of secondary metabolites are summarized in table 2 below.

Overall, the qualitative phytochemical screening results indicate an uneven distribution of secondary metabolic compounds across the extracts. Notably, alkaloids were absent while phenolic compounds, flavonoids, quinones, coumarins, and saponins were detected in all extracts. However, unlike the kombucha extracts prepared from green tea, only the terpenoid group was present in black tea kombucha.

Phytochemical Constituents of the Promising Extract

The kombucha extract exhibiting the best inhibitory activity against *T. b. brucei* was selected for in-depth phytochemical analysis using the high-performance chromatographic technique UHPLC-LC-MS/MS. Table 3 below summarizes the potential chemical constituents present in the KG14 extract.

The UHPLC-MS/MS profile of the KG14 extract, as presented in the table above, enabled the direct identification of nine chemical structures belonging to various families, with binding coefficients ≥ 0.90, from the utilized databases. These compounds include dicarboxylic acids and derivatives, monosaccharides, xanthines, medium-chain hydroxy acids, O-glycosyl compounds, alkyl phenyl ketones, and phenolic compounds (2-alkylphenyl ketones, phenol ether). Figure 4 illustrates the diverse chemical structures identified in the KG14 extract from the databases used. These chemical structures were selected based on a binding threshold ≥ 0.90 to ensure greater reliability.

Pharmacokinetic and Toxicity Properties (ADMET) of Identified Phytoconstituents

Pharmacokinetics, also referred to as ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), is a critical parameter in drug development and progression to clinical trials [38].

In this study, the pharmacokinetic properties of 13 chemical constituents were predicted using SwissADME and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) software.

The results presented in table 4 summarizes information related to the pharmacokinetic properties of the structures of interest,

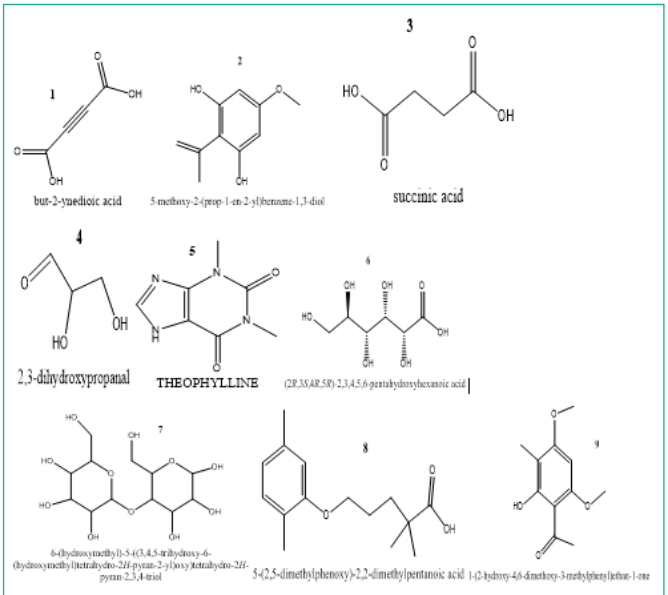

Figure 4 : Chemical structures present in the KG14 extract

Table 3: Enumeration of chemical constituents present in KG14 extract by UHPLC-MS/MS.

N	m/z	RT (min)	Type	Metabolite name	Ontology/Family	Database	Match score
1	112.986343	0.0787	[M-H]-	but-2-ynedioic acid/Acetylenedicarboxylic acid	Dicarboxylic acids and derivatives	BioMSMS-Neg-PlaSMA_2	0.98
2	180.975311	0.0787	[M-H]-	5-methoxy-2-(prop-1-en-2-yl)benzene-1,3-diol/ 4-O-Methylphloracetophenone	Alkyl-phenylketones	BioMSMS-Neg-PlaSMA_2	0.99
3	248.962814	0.0787	[M-H]-	succinic acid/ Succinate	Dicarboxylic acids and derivatives	BioMSMS-Neg-PlaSMA_2	0.96
4	89.0243835	0.39065	[M-H]-	2,3-dihydroxypropanal/ GLYCERALDEHYDE	Monosaccharides	MSMS-Neg-GNPS_1	0.99
5	179.057556	0.39065	[2M-H]-	THEOPHYLLINE	Xanthines	MSMS-Neg-GNPS_1	0.99
6	195.051636	0.39065	[M-H]-	(2R,3S,4R,5R)-2,3,4,5,6-pentahydroxyhexanoic acid/ GLUCONIC ACID	Medium-chain hydroxy acids and derivatives	MSMS-Neg-GNPS_1	0.99
7	341.111298	0.39065	[M-H]-	6-(hydroxymethyl)-5-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2,3,4-triol/ Maltose	O-glycosyl compounds	MSMS-Neg-GNPS_1	0.97
8	249.149048	5.82075	[M-H]-	5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid/ Gemfibrozil	Phenol ethers	BioMSMS-Neg-PlaSMA_2	0.99
9	248.964447	9.05515	[M-H]-	1-(2-hydroxy-4,6-dimethoxy-3-methylphenyl)ethan-1-one/Methylxanthoxylin	Alkyl-phenylketones	BioMSMS-Neg-PlaSMA_2	0.92

Table 4: Pharmacokinetic parameters of some chemical structures of KG14 extract.

Parameter	C1 : Méthyxanthoxylin	C2: 4-O-méthylphloracetophene	C3 : Gemfibrozil	Pentamidin	Unit/classification
Absorption					
Aqueous Solubility	-2.781	-2.097	-3.092	-3.728	Digital (log mol/L)
Permeability Caco2	1.245	1.23	1.356	0.087	Digital (log Papp en 10-6 cm/s)
Intestinal absorption	95.94	92.985	94.414	77.044	Digital (% absorbé)
Distribution					
VD (human)	0.005	-0.012	-0.683	0.207	Digital (log L/kg)
Permeability (BHE)	-0.238	-0.087	-0.166	-0.905	Digital (log BB)
Permeability of SNC	-2.872	-3.198	-2.298	-2.941	Catégorique (log PS)
Metabolism					
Substrate of the CYP2D6	No	No	No	No	Categorical (Yes/No)
Substrate of the CYP3A4	No	Yes	No	No	Categorical (Yes/No)
Inhibitor of the CYP1A2	Yes	Yes	Yes	Yes	Categorical (Yes/No)
Inhibitor of the CYP2C19	No	No	No	Yes	Categorical (Yes/No)
Inhibitor of the CYP2C9	No	No	Yes	Yes	Categorical (Yes/No)
Inhibitor of the CYP2D6	No	No	No	No	Categorical (Yes/No)
Inhibitor of the CYP3A4	No	No	No	No	Categorical (Yes/No)
Excretion					
Total clearance	0.75	0.423	0.294	0.85	Digital (log ml/min/kg)
Renal OCT2 substrate	No	No	No	Yes	Categorical (Yes/No)
Toxicity					
Toxicity	No	No	No	No	Categorical (Yes/No)
Max. tolerated dose (human)	1.39	0.883	1.027	0.522	Digital (log mg/kg/jour)
Inhibitor of the hERG I	No	No	No	No	Categorical (Yes/No)
Inhibitor of the hERG II	No	No	No	No	Categorical (Yes/No)
Acute oral toxicity in rats (LD50)	2.026	1.975	2.275	2.225	Digital (mol/kg)
Chronic oral toxicity in rats (LOAEL)	2.764	2.049	2.417	2.375	Digital (Log mg/ kgpc /jour)
Hepatotoxicity	No	No	No	No	Categorical (Yes/No)
Skin sensitization	No	No	No	No	Categorical (Yes/No)

Legend: VD: Rate of Distribution; BBB: Blood-Brain Barrier; CNS: Central Nervous System; CYP2D6: Cytochrome P2D6; OCT2: Octamer Transcription factor 2; hERG: human Ether-a-go-go-Related Gene; LD50: Lethal Dose 50; LOAEL: Lowest-Observed-Adverse-Effect-Level;

including molecular weight (MW), human oral bioavailability (OB), gastrointestinal absorption (GI), blood-brain barrier (BBB) permeability, drug-like properties (DL), interactions with cytochrome P450 isoforms, and toxicity. The results revealed that all three investigated structures adhered to Lipinski's "Rule of Five," which stipulates that a molecule must have: a molecular weight between 180 and 500Da ; ≤10 hydrogen bond acceptors ; ≤5 ; hydrogen bond donors ; log P value (partition coefficient) < 5 and ≤5 rotatable bonds [39]. Additionally, drug-likeness (DL) is a qualitative concept used in drug design to predict the likelihood of a compound being a potential drug candidate [40]. Among the structures analyzed, C2 exhibited superior properties as a potential drug candidate, including excellent solubility in aqueous media (-2.09), compared to C1 (-2.78) and C3 (-3.09). These compounds demonstrated the ability to cross the blood-brain barrier, suggesting their potential utility in treating phase II trypanosomiasis and other neurological disorders. Another crucial parameter is the interaction between compounds and cytochromes P450 (CYP), which play a central role in drug biotransformation and elimination. Five major isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) are commonly reported in the literature, with

their inhibition being a primary cause of pharmacokinetic-related drug interactions. As shown in Table VI, while C1 and C2 were identified as CYP1A2 inhibitors, C3 inhibited both CYP1A2 and CYP2C9 isoforms. This dual inhibition is unfavorable for drug metabolism and elimination or for the metabolism of co-administered drugs. It is noteworthy that all three compounds exhibited good gastrointestinal absorption and were predicted to be non-toxic in humans.

Discussion

To our knowledge, this study is the first to evaluate the antitrypanosomal activity of kombucha extracts. However, previous research has demonstrated that black tea kombucha may exhibit antiprotozoal effects against certain strains of Plasmodium and Leishmania donovani [19,41]. The observed antitrypanosomal activity of kombucha extracts appears to depend on the nature of the substrates (teas) used and the fermentation time. Notably, the most potent extract, KG14, with an IC_{50} of 47.38 µg/mL, aligns with findings on the antileishmanial activity of black tea kombucha against the promastigote form of *L. donovani,* which showed similar inhibition after 14 days of fermentation [19]. These results highlight kombucha's potential as an antiparasitic agent and suggest avenues for future research on its mechanisms of action and therapeutic potential. Although kombucha fermentation can last from 7 to 60 days, biological activities tend to increase throughout the process and are often optimal around 15 days [42].

The anti-inflammatory potential of kombucha was assessed *in vitro* using the protein denaturation inhibition method. This method evaluates kombucha's ability to inhibit protein denaturation, potentially attenuating inflammation and alleviating associated symptoms. Kombucha contains bioactive compounds like polyphenols, which have shown anti-inflammatory properties [43]. The presence of observed polyphenols could justify the anti-inflammatory potential of the extracts. Other studies have demonstrated that certain phenolic compounds, such as flavonoids in kombucha, have anti-inflammatory effects by targeting specific inflammatory pathways, including the inhibition of pro-inflammatory cytokines or enzymes involved in the inflammatory response [44,45]. Further exploration of kombucha's mechanisms of action using methods like *in vitro* protein denaturation could enhance understanding and validation of its anti-inflammatory activity, opening new therapeutic avenues. Phenolic compounds, such as flavonoids present in kombucha, may exert effects against *Trypanosoma brucei brucei* by inhibiting essential enzymes involved in the parasites' metabolic pathways and interfering with their cell signaling pathways. This interference compromises the parasites' ability to synthesize vital elements for survival and regulate cellular processes [46,47]. Additionally, flavonoids can disrupt the parasite cell membrane by altering membrane permeability, leading to the leakage of essential substances, chromatin condensation, DNA fragmentation, and loss of cellular integrity [48]. By combining these mechanisms, phenolic compounds could effectively target different aspects of *T. b. brucei* physiology and biology. The mechanisms may vary depending on the type of phenolic compounds present in kombucha, necessitating further research for a comprehensive understanding of their antiparasitic activity. Several factors influence the chemical composition and bioactive components of kombucha, including the substrates used (type of tea or plants), fermentation parameters (time, pH, temperature), preparation methods, and specific microbes (SCOBY) [15,16]. These factors may explain the variation in secondary metabolites among different kombucha extracts. Similar findings have been reported, which identified polyphenols, flavonoids, quinones, coumarins, saponins, and terpenoids in black tea kombucha extracts. Phenolic compounds are the major group of secondary metabolites in kombucha [25,42,49].

The structures identified in the KG14 extract, such as acetylenedicarboxylic acid, 4-O-Methylphloracetophenone, succinate, glyceraldehyde, gluconic acids, maltose, theophylline, gemfibrozil, and methylxanthoxylin, have been reported in previous kombucha studies [17]. The diversity of these chemical structures in the promising extract highlights its richness in secondary metabolites, which may be responsible for the observed activities. These compounds can interact through various mechanisms of action, and understanding how they act would be beneficial for a deeper comprehension of the antiparasitic activity and anti-inflammatory potential.

Phenolic compounds are the main class of secondary metabolites responsible for kombucha's bioactive properties [50]. The identified phenolic compounds, such as methylxanthoxyline, 4-O-methylphloracetophenone, and gemfibrozil, likely represent the primary phytochemical constituents responsible for the observed activities. In addition to antioxidant and anti-inflammatory effects, *in vivo* studies have shown that gemfibrozil is a hypolipidemic drug that reduces anxiety and enhances memory [51]. 4-O-methylphloracetophenone is a derivative of acetophenones, which are natural phenolic compounds found in numerous plant families and fungal strains. Compounds derived from this structure have exhibited antimicrobial, antimalarial, antioxidant, and anti-tyrosinase activitie. Methylxanthoxyline is a xanthoxyline derivative that acts as a plant growth inhibitor [52]. However, to our knowledge, none of the phytochemical constituents in the KG14 extract have been studied for antitrypanosomal activity, emphasizing the need to evaluate their pharmacokinetic properties for a better understanding of the observed activities.

All selected structures exhibited favorable pharmacokinetic profiles. The three predicted structures complied with Lipinski's "Rule of Five": 4-O-methylphloracetophenone (H-bond donor = 2; H-bond acceptor = 4; MW = 182.17 g/mol and LogP = 1.09); methylxanthoxyline (H-bond donor = 1; H-bond acceptor = 3; MW = 208.25 g/mol and LogP = 2.73); and gemfibrozil (H-bond donor = 1; H-bond acceptor = 3; MW = 250.33 g/mol and LogP = 3.35) [39]. These results provide pharmacological insights into the potential molecules present in the extract and require further in vitro and in vivo studies to confirm them.

Conclusion

This study demonstrated that kombucha extracts are rich in a variety of secondary metabolites such as saponins, polyphenols, flavonoids, quinones, tannins and coumarins where as alkaloids and terpenoids were absent, with notable antitrypanosomal potential observed for the Kombucha Green tea extract after 14 days of fermentation (KG14) with IC_{50} of 47.38±0,26 µg/mL and safe on normal vero and raw cell lines (SI>10.55). Furthermore, the selected compounds identified by UHPLC-LC-MS/MS exhibited favorable

predicted pharmacokinetic profiles. The promising antitrypanosomal activity and anti-inflammatory potential, along with the demonstrated phytochemical profile of these extracts, justify the need for further studies to evaluate their in vivo efficacy and safety, as well as to identify the precise mechanisms of action of the bioactive compounds involved.

Declaration of Interests

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.

References

1. WHO. Human African Trypanosomiasis (sleeping sickness). Memorandum. 2018.
2. Organization FAO. The disease | Programme Against African Trypanosomosis (PAAT) | Food and Agriculture Organization of the United Nations. 2024.
3. World Health Organization. Accelerating work to overcome the global impact of neglected Tropical diseases: a roadmap for implementation Updated 2012.
4. Büscher P, Cecchi G, Jamonneau V, Priotto G. Human African trypanosomiasis. The Lancet. 2017; 390: 2397-2340.
5. Angara T, Ismail A, Ibrahim A. Research Paper Veterinary An Overview on the Economic Impacts of Animal Trypanosomiasis. Global Journal for Research Analysis: Ahmedabad, India. 2014.
6. Magang EMK, Kamga RMN, Telleria J, Tichit M, Crouzols A, Kaboré J, et al. Prevalence of blood and skin trypanosomes in domestic and wild fauna from two sleeping sickness foci in Southern Cameroon. PLoS Neglected Tropical Diseases. 2023; 17: e0011528.
7. Mamia GF, Atangana BG, Kante ST, Fobasso TRM, Zebaze TAA, Awono-Ambene HP, et al. Human Africa Trypanosomiasis in Littoral Region of Cameroon: an Updated With First Evidence on the Circulation of Trypanosoma Brucei Gambiense in Manoka Island. International Journal of Scientific Research and Management (IJSRM). 2022; 10: 721-730.
8. Geerts S, Osaer S, Goossens B, Faye D. Trypanotolerance in small ruminants of sub-Saharan Africa. Trends in Parasitology. 2009; 25: 132-138.
9. Mouala C. Observance dans la prophylaxie primaire par le cotrimoxazole à Bangui.
10. Cattand P. Human African trypanosomiasis. Bull Soc Path Ex. 1994; 87: 307-310.
11. WHO. Human African trypanosomiasis (sleeping sickness). 2014.
12. Matovu E, Stewart ML, Geiser F, Brun R, Mäser P, Wallace LJ, et al. Mechanisms of arsenical and diamidine uptake and resistance in Trypanosoma brucei. Eukaryotic cell. 2003; 2: 1003-1008.
13. Mandal S, Mondal C, Lyndem LM. Probiotics: an alternative anti-parasite therapy. Journal of Parasitic Diseases. 2024; 48: 409-423.
14. Eze J, Orajaka L, Okonkwo N, Ezeh I, Ezema C, Anosa G. Effect of probiotic (Saccharomyces cerevisiae) supplementation on immune response in Trypanosoma brucei brucei infected rats. Experimental Parasitology. 2012; 132: 434-439.
15. Ivanišová E, Meňhartová K, Terentjeva M, Harangozo Ľ, Kántor A, Kačániová M. The evaluation of chemical, antioxidant, antimicrobial and sensory properties of kombucha tea beverage. Journal of food science and technology. 2020; 57: 1840-1846.
16. de Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez AGM, et al. Kombucha: A review of substrates, regulations, composition, and biological properties. Journal of Food Science. 2022; 87: 503-527.
17. Abaci N, Deniz FSS, Orhan IE. Kombucha–An ancient fermented beverage with desired bioactivities: A narrowed review. Food chemistry: X. 2022; 14: 100302.
18. Wang B, Rutherfurd-Markwick K, Zhang XX, Mutukumira AN. Kombucha: Production and microbiological research. Foods. 2022; 11: 3456.
19. Mfopa AN, Kemzeu R, Fokom R, Yamthe LRT, Dize D, Boyom FF. Phenolic compounds, antioxidant and antileishmanial activities of kombucha as affected by fermentation time. Heliyon. 2024; 10: 22.
20. de Morais CGV, Castro Lima AK, Terra R, dos Santos RF, Da-Silva SAG, Dutra PML. The Dialogue of the Host-Parasite Relationship: Leishmania spp. and Trypanosoma cruzi Infection. BioMed research international. 2015; 32: 4915.
21. Ouellette M, Olivier M, Sato S, Papadopoulou B. Le parasite Leishmania à l'ère de la post-génomique Studies on the parasite Leishmania in the post-genomic era. MEDECINE/SCIENCES. 2003; 19: 900-909.
22. Alkhaldi AA, Lamie PF, Abdelgawad MA. In silico study and in vitro antileishmanial and antitrypanosomal evaluation of azopyrazoles and azopyrimidines. Pakistan Journal of Pharmaceutical Sciences. 2024; 37.
23. Araujo SC, Sousa FS, Costa-Silva TA, Tempone AG, Lago JHG, Honorio KM. Discovery of new hits as antitrypanosomal agents by in silico and in vitro assays using neolignan-inspired natural products from Nectandra leucantha. Molecules. 2021; 26: 4116.
24. Ferreira LL, Andricopulo AD. ADMET modeling approaches in drug discovery. Drug discovery today. 2019; 24: 1157-1165.
25. da Silva Júnior JC, Mafaldo ÍM, de Lima Brito I, de Magalhães Cordeiro AMT. Kombucha: Formulation, chemical composition, and therapeutic potentialities. Current Research in Food Science. 2022; 5: 360-365.
26. Bowling T, Mercer L, Don R, Jacobs R, Nare B. Application of a resazurin-based high-throughput screening assay for the identification and progression of new treatments for human African trypanosomiasis. International Journal for Parasitology: Drugs and Drug Resistance. 2012; 2: 262-270.
27. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine. 2012; 2: S178-S180.
28. Harborne J. Methods of plant analysis. Phytochemical methods: a guide to modern techniques of plant analysis: Springer; 1984; 1-36.
29. Dohou N, Yamni K, Tahrouch S, Idrissi Hassani L, Badoc A, Gmira N, et al. Phytochemical screening of an Ibero-Moroccan endemic, thymelaea lythroides. Bulletin de la Société de pharmacie de Bordeaux. 2003; 142: 61-78.
30. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. BioMed research international. 2014; 2014: 497606.
31. Bekro Y-A, Mamyrbekova J, Boua BB, Bi FT, Ehile EE. Étude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. et Zarucchi (Caesalpiniaceae). Sciences & nature. 2007; 4: 217-225.
32. Odebiyi O, Sofowora E. Antimicrobial alkaloids from a Nigerian chewing stick (Fagara zanthoxyloides). Planta medica. 1979; 36: 204-207.
33. Yang M, Li J, Zhao C, Xiao H, Fang X, Zheng J. LC-Q-TOF-MS/MS detection of food flavonoids: Principle, methodology, and applications. Critical reviews in food science and nutrition. 2023; 63: 3750-3570.
34. Pires DE, Blundell TL, Ascher DB. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. Journal of medicinal chemistry. 2015; 58: 4066-4072.
35. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports. 2017; 7: 42717.
36. Ibrahim MA, Mohammed A, Isah MB, Aliyu AB. Anti-trypanosomal activity of African medicinal plants: A review update. Journal of ethnopharmacology. 2014; 154: 26-54.
37. Camacho M, Phillipson J, Croft S, Solis P, Marshall S, Ghazanfar S. Screening of plant extracts for antiprotozoal and cytotoxic activities. Journal of ethnopharmacology. 2003; 89: 185-191.

38. Lucas AJ, Sproston JL, Barton P, Riley RJ. Estimating human ADME properties, pharmacokinetic parameters and likely clinical dose in drug discovery. Expert Opinion on Drug Discovery. 2019; 14: 1313-1327.

39. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced drug delivery reviews. 2012; 64: 4-17.

40. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. Journal of cheminformatics. 2014; 6: 1-6.

41. de Noronha MC, Cardoso RR, dos Santos D'Almeida CT, do Carmo MAV, Azevedo L, Maltarollo VG, et al. Black tea kombucha: Physicochemical, microbiological and comprehensive phenolic profile changes during fermentation, and antimalarial activity. Food Chemistry. 2022; 384: 132515.

42. Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P. Understanding kombucha tea fermentation: a review. Journal of food science. 2018; 83: 580-588.

43. Su J, Tan Q, Wu S, Abbas B, Yang M. Application of Kombucha fermentation broth for antibacterial, antioxidant, and anti-inflammatory processes. International Journal of Molecular Sciences. 2023; 24: 13984.

44. Vázquez-Cabral BD, Larrosa-Pérez M, Gallegos-Infante JA, Moreno-Jiménez MR, González-Laredo RF, Rutiaga-Quiñones JG, et al. Oak kombucha protects against oxidative stress and inflammatory processes. Chemico-Biological Interactions. 2017; 272: 1-9.

45. Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard J-P, Renard T, Rollan S, et al. Impact of fermentation conditions on the production of bioactive compounds with anticancer, anti-inflammatory and antioxidant properties in kombucha tea extracts. Process Biochemistry. 2019; 83: 44-54.

46. Larit F, Elokely KM, Nael MA, Benyahia S, León F, Cutler SJ, et al. Proposed mechanism for the antitrypanosomal activity of quercetin and myricetin isolated from Hypericum afrum Lam.: Phytochemistry, in vitro testing and modeling studies. Molecules. 2021; 26: 1009.

47. Soto-Sánchez J. Bioactivity of natural polyphenols as antiparasitic agents and their biochemical targets. Mini Reviews in Medicinal Chemistry. 2022; 22: 2661-2677.

48. Gaggìa F, Baffoni L, Galiano M, Nielsen DS, Jakobsen RR, Castro-Mejía JL, et al. Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity. Nutrients. 2018; 11: 1.

49. Cardoso RR, Neto RO, dos Santos D'Almeida CT, do Nascimento TP, Pressete CG, Azevedo L, et al. Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. Food research international. 2020; 128: 108782.

50. Hakimizadeh E, Zamanian MY, Borisov VV, Giménez-Llort L, Ehsani V, Kaeidi A, et al. Gemfibrozil, a lipid-lowering drug, reduces anxiety, enhances memory, and improves brain oxidative stress in d-galactose-induced aging mice. Fundamental & clinical pharmacology. 2022; 36: 501-508.

51. Ahmadpourmir H, Attar H, Asili J, Soheili V, Taghizadeh SF, Shakeri A. Natural-derived acetophenones: Chemistry and pharmacological activities. Natural Products and Bioprospecting. 2024; 14: 28.

52. Chotpatiwetchkul W, Chotsaeng N, Laosinwattana C, Charoenying P. Structure–activity relationship study of xanthoxyline and related small methyl ketone herbicides. ACS omega. 2022; 7: 29002-29212.