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Antimicrobial activity of tea processing effluents collected from various Kenyan factories

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ABSTRACT

The escalating global challenge of antibiotic resistance demands exploration into alternative sources for antimicrobials. This study investigated the often-overlooked tea waste samples generated during tea processing from Kenyan processing factories to uncover novel resources containing potent and effective antimicrobial compounds. In this study, we collected tea waste samples from various tea processing factories in Kenya and assessed their antimicrobial activity against various microorganisms using the disk diffusion assay. To quantify the efficacy of each sample, we determined growth inhibition zones and minimum inhibitory and bactericidal concentrations (MICs and MBCs). The study unveiled diverse levels of antimicrobial activity in tea waste samples against specific microorganisms. Notably, the fluff sample from Gitambo factory demonstrated potent antibacterial effects against *Clostridium disporicum*. Various samples exhibited a moderate response to both *Streptococcus pyogenes* and *Escherichia coli*, yet they showed minimal to no activity, where applicable, towards *Staphylococcus aureus* and *Candida albicans*. This study showed that some tea effluents, which are often discarded during processing, show antimicrobial potential, as they demonstrated efficacy against certain pathogens. By further optimizing our handling and storage practices, we could enhance the isolation of potent antimicrobial compounds from these materials; this would provide valuable alternatives in combating antibiotic resistance.

Keywords: Microbial growth inhibition zone, Minimum Inhibitory Concentration, Minimum bacteriacidal Concentration, Tea processing waste, Disk diffusion Assay.

INTRODUCTION

The primary therapeutic approach for infectious diseases involves the use of antibiotics; however, recent years have seen a significant rise in antibiotic resistance, presenting an enormous challenge to infection control [1,2]. Some microorganisms can resist a single antimicrobial agent (Antimicrobial resistance (AMR), while others can resist numerous antimicrobials (multidrug-resistant (MDR). Infections resulting from MDR strains often resist standard treatment with detrimental effects and increase mortality risks by disseminating their own resistance among other microorganisms [3-5]. MDR microorganisms, or pan-resistant organisms in severe cases, exhibit resistance to all available antibiotics—making treatment with any single antibiotic futile [6-8]. This necessitates the exploration of alternative agents that can effectively eradicate these pathogenic microbes and foster health [9]. Among these innovative therapeutic strategies are natural antimicrobial compounds, especially the plant-derived products like spices and essential oils, as well as extracts or herbal teas.

Herbal teas boast global significance in treating human diseases, beyond their inherent beverage qualities [10]. More than two-thirds of the world's population favour green and black teas as beverages alongside water. The Republic of China, India, Kenya, Sri Lanka, and Turkey collectively produce and consume over 4.5 million tons annually [11-13]. Categorizing tea derived from the leaves of *Camellia sinensis* (L) Kuntze into white, green, black and oolong types is based on its harvesting and processing methods [12]. The typical preparation method involves infusing these leaves in hot water [12,14]. Around 2,000 diverse phytochemicals including phenolic compounds methyl-xanthines, carbohydrates, proteins - including free amino acids, L-ascorbic acid, and other organic acids, volatile compounds, carotenoids, as well as trace elements [15].

Previous research highlights that polyphenol phytocompounds, especially catechins emerge as the most crucial tea leaf components with diverse bioactive [11,16]. Of these catechins, epigallocatechin-3-gallate (EGCG), biologically active monomeric flavanol, predominates in fresh green tea leaves and possess health-promoting benefits. Additional derivatives of catechin include (−) epicatechin-3-gallate, (-)-epigallocatechin, (-) epicatechin; (+) – Catechin, (+) – gallocatechin, and (−) gallocatéchin- 3 gallate [17,18]. Research shows that tea and its components—through their diverse health-promoting properties—offer protection against cardiovascular diseases, aid in obesity and diabetes control, exhibit anticarcinogenic effects, serve as anti-aging agents, possess antihistaminic, antiarthritic,

anti-inflammatory properties, and possess antibacterial, antifungal, and even antiviral activities [18,19].

An ample body of literature exists, substantiating the health benefits of teas derived from *C. sinensis*; however, limited research is available on the tea effluents. The predominant attention has historically been directed towards processed leaves employed in the brewing of tea [20,21]. However, it is crucial to acknowledge that a significant quantity of biomass, comprising expended tea leaves, stems, flushes, fluffs, among others, is commonly disposed of during the manufacturing process. This byproduct poses an environmental challenge and frequently finds its way into landfills, thereby amplifying resource depletion and elevating greenhouse gas emissions [22]. The determination of the antimicrobial activity of the tea wastes may help appraise their potential benefits and could provide an alternative natural source of efficacious antimicrobial compounds for combating infections. This may also foster proper management of the tea processing wastes and their proper handling form beneficial uses. Therefore, this study aimed at determining the antimicrobial activity of the various tea effluents collected from various tea processing factories in Kenya to appraise their potential as alternative sources of antimicrobial agents.

MATERIALS AND METHODS

Sample Collection and Processing of Tea Waste

The tea waste specimens, encompassing fluffs, cyclones, cyclone fluffs, dry-offs, and fluff-dry mouths, were systematically gathered from diverse processing facilities in Kenya. The collection involved placing the samples into impeccably clean, brown-hued glass bottles, followed by their transportation to the Tea Research Institute (TRI, KARLO) laboratories situated at Timbilil Estate, Kericho (with geographical coordinates of latitude 0° 22'S and longitude 35° 21'E, at an altitude of 2180 meters above sea level) for subsequent analysis. To ensure uniformity, the collected samples underwent an oven-drying process (Menmert, 854 Schwab, Germany) at a consistent temperature of 103°C until reaching a constant weight. Subsequently, an electric blending device (Moulinex AR 1043, China) was employed to finely mill the dried samples, reducing their particle size. The powdered samples were stored in desiccators prior to antimicrobial assay.

In Vitro Assessment of Antimicrobial Activity

Test Microorganisms

The Tea Research Institute, through its Microbiology Laboratory, provided us with a panel of microbial strains for testing. The microbial strains used included *Clostridium disporicum* (43838), *Streptococcus pyogenes* (ATCC 19615), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25925), and *Candida albicans* (10231).

Preparation and standardization of Microbial Inoculums

Our preparation of inoculums followed the guidelines set by the Clinical Laboratory Standards Institute (CLSI) [23]. Briefly, we sub-cultured bacterial strains in Mueller Hinton Agar and fungal strain in Sabaroud Dextrose Agar and incubated them at a temperature of 37 °C for 24 hours. We then harvested the colonies using a sterilised wire loop and suspended them in 5 ml of normal saline. The absorbance of the inoculums was adjusted to between 0.11-0.14 at a wavelength of 530 nm using a spectrophotometer to achieve turbidity equivalent to the 0.5 McFarland scale. This yielded 1-5×10^6 colony forming units (cfu) for the fungal strain and 1-5×10^8 cfu for the bacterial strains.

The Disk Diffusion Assay

Using the disk diffusion technique, as per guidelines from the Clinical Laboratory Standards Institute (CLSI) [23,24], we determined the microbial strains' antimicrobial susceptibility to test samples. Each tea waste sample (0.2 g) was individually dissolved in 1.4 % dimethylsulphoxide (DMSO) and vigorously vortexed to achieve concentrations set at 200 µg/ml. We then serially diluted these stock concentrations two-fold to obtain a range of final concentrations: 200 µg/ml, 100 µg/ml, and 50 µg/ml. After that, 20 µl of each sample concentration was carefully aspirated using a P-20 micropipette and dispensed onto sterile 6 mm diameter disks, previously prepared from Whatman paper No. 1, equidistantly placed on petri dish plates containing 20ml of medium inoculated with 1 ml of the respective microbial strains. DMSO served as a negative control, while Ciprofloxacin (10 µg) or Nystatin (10 µg), acted as positive controls. After incubating at a temperature of 37 ºC for 24 hours, we measured and recorded the diameters of respective growth inhibition zones in millimetres (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The previously described method [25] was adopted with slight modifications to determine the MIC and MBC/MFC. We prepared two-fold serial dilutions of each tea waste sample in test tubes using Mueller-Hinton Broth (0.5 McFarland equivalent), to obtain concentrations ranging from 200 mg/ml to 3.125 µg/ml. Then, microbial inoculums at a density of 10^4 cfu were used to inoculate the test tubes and allowed to interact at room temperature for 30 minutes. The tubes were then incubated for 18 hours at 35 °C and then observed. We defined the lowest sample concentrations that inhibited microbial growth as MICs; furthermore, we considered those which completely suppressed microbial growth on freshly inoculated agar plates as MBC/MFC. Each experiment was conducted in triplicate with 1.4% dimethyl sulphoxide as negative control and Ciprofloxacin(10µg) or Nystatin (10µg) as positive control for bacterial and fungal strains, respectively.

Data management and statistical analysis

This study collected quantitative data which was tabulated on a spreadsheet (Microsoft 365), arranged, and then exported to Minitab version 21.4 for analysis. The data was analysed descriptively and expressed as mean± standard deviation (SD) of the triplicate experiments. Afterward, inferential statistics using One-Way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* were performed to determine the significance of differences among means and for pairwise comparisons and separation of means and means with $P<0.05$ were considered statistically significant.

Ethical approval

This study was ethically approved by the Jomo Kenyatta University of Agriculture and Technology Ethical review committee (JKUAT-ERC) (REF: BPS/HSB/411-1433/2020) before execution.

RESULTS

Growth inhibition zones

This study revealed no significant differences in each sample's generated growth inhibition zones, when tested against microorganisms at concentrations of 50 µg/ml and 100 µg/ml (P>0.05; Table 1). The fluff sample from Gitambo factory, produced a significantly larger growth inhibition zone compared to other samples when exposed to a concentration of 200 µg/ml against *Clostridium disporicum*, indicating a noteworthy antimicrobial effect (P<0.05; Table 1). Conversely, the positive control (ciprofloxacin) demonstrated a significantly superior growth inhibition zone against *C. disporicum* compared to all the test samples (P<0.05; Table 1).

Against *Streptococcus pyogenes*, most tested samples, except silvery needles at concentrations ≤100 µg/ml, did not exhibit significant differences in growth inhibition zones (P>0.05; Table 1). However, at a concentration of 200 µg/ml, silvery needles displayed a significantly larger growth inhibition zone on *S. pyogenes* compared to other samples, suggesting a distinctive antimicrobial impact (P<0.05; Table 1). Noteworthy findings also included the significantly larger growth inhibition zones of the fluff from Gitambo factory and cyclone from Itumbe against *S. pyogenes* compared to other tea effluents (P<0.05; Table 1). Nevertheless, ciprofloxacin, the positive control antibiotic, exhibited significantly greater effectiveness against *S. pyogenes* than all tested samples (P<0.05; 1).

Upon evaluating the antimicrobial activity against *Escherichia coli*, the fluff drier mouth sample from Tombe factory demonstrated a significantly larger growth inhibition zone compared to other samples (P<0.05; Table 1). Additionally, ciprofloxacin exhibited significantly superior efficacy against E. coli compared to all tested samples (P<0.05; Table 1).

The silvery needles sample at 200 µg/ml exhibited a significantly larger growth inhibition zone on *Staphylococcus aureus* than other tea samples (P<0.05; Table 1). The fluff sample from Mogogosiek factory also demonstrated a significantly larger inhibition zone compared to most samples, excluding silvery needles and the positive control antibiotic against *S. aureus* (P<0.05; Table 1). Nonetheless, Ciprofloxacin showcased a significantly superior antimicrobial efficacy against *S. aureus* compared to all other tested samples (P<0.05) as indicated in Table 1.

The studied tea waste samples did not exhibit any significant differences in the growth inhibition zones they produced on *Candida albicans* (P>0.05; Table 1). Nevertheless, the positive control drug (Nystatin) exhibited a significantly larger inhibition zone against C. albicans compared to all tested samples (P<0.05; Table 1).

Table 1: Antimicrobial activity of the tea effluents obtained from various tea processing factories

Sample	Concentration (µg/ml)	Growth inhibition zones diameter in mm				
		C. disporicum	*S. pyogenes*	*E. coli*	*S. aureus*	*C. albicans*
Gitambo fluff	50	6.00±0.00^{d}	6.50±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.50±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	9.00±0.00^{b}	8.10±0.00^{c}	6.10±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Gitambo Cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.10±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Itumbe Fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.10±0.00^{e}	6.40±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Itumbe cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.10±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	8.00±0.00^{c}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Kinoro cyclone Fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.20±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Kinoro drier fly off	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.10±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Kionyo next to fan	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	7.00±0.00^{c}	6.00±0.00^{e}	6.30±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Kionyo Fluff drier	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.10±0.00^{e}	6.10±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Kionyo fly off	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.20±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.50±0.00^{e}	6.40±0.00^{c}	6.20±0.00^{d}	6.00±0.00^{b}
Ogembo fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}

Sample	Concentration (µg/ml)	Growth inhibition zones diameter in mm				
		C. disporicum	*S. pyogenes*	*E. coli*	*S. aureus*	*C. albicans*
	100	6.00±0.00^{d}	6.40±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.80±0.00^{d}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Ogembo cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.40±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	7.00±0.00^{d}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Tombe tea factory	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.50±0.00^{e}	6.10±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Tombe fluff drier	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
mouth	100	6.00±0.00^{d}	6.20±0.00^{e}	6.20±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	7.20±0.00^{d}	8.00±0.00^{b}	6.00±0.00^{d}	6.00±0.00^{b}
Kaptumo cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
fluff	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Kaptumo fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	7.00±0.00^{d}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Mudete cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Momul cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
fluff	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	7.00±0.00^{d}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Momul fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Chelal cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
fluff	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Boito cyclone	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	7.00±0.00^{d}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Boito fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Mogogosiek	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
cyclone	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Mogogosiek fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.80±0.00^{c}	7.00±0.00^{c}	6.00±0.00^{b}
Gianchore fluff	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Green tea	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	7.00±0.00^{c}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	7.30±0.00^{c}	6.60±0.00^{d}	6.60±0.00^{c}	6.10±0.00^{d}	6.00±0.00^{b}
Purple tea	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	100	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
	200	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}
Silvery needles	50	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{c}	6.00±0.00^{d}	6.00±0.00^{b}

Sample	Concentration (µg/ml)	Growth inhibition zones diameter in mm				
		C. disporicum	*S. pyogenes*	*E. coli*	*S. aureus*	*C. albicans*
	100	6.00±0.00^{d}	7.40±0.00^{d}	6.00±0.00^{c}	6.10±0.00^{d}	6.00±0.00^{b}
	200	7.00±0.00^{c}	9.00±0.00^{b}	6.20±0.00^{c}	9.00±0.00^{b}	6.00±0.00^{b}
Positive control		14.50±0.00^{a}	18.20±0.00^{a}	21.10±0.00^{a}	21.40±0.00^{a}	24.50±0.00^{a}

Values are presented as $\bar{x}$±SD; Means with the same superscript lowercase alphabet within the same column are not significantly different (P>0.05; One-Way ANOVA with Tukey's post hoc). Positive control: Ciprofloxacin (10 µg) for *Clostridium disporicum*, *Streptococcus pyogenes*, *Escherichia coli*, and *Staphylococcus aureus*, and Nystatin (10µg) for *Candida albicans*.

Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs)

The results showed that among the four samples exhibiting activity against *C. disporicum*, the next to fan sample collected from Kionyo factory demonstrated the highest efficacy, with the lowest MIC (3.125 µg/ml) and MBC (6.25 µg/ml) values (Table 2). Other active samples, including the fluff from Gitambo factory, green tea, and silvery needles, showed MIC and MBC values of 6.25 µg/ml and 12.5 µg/ml, respectively (Table 2).

The cyclone fluff from Momul factory displayed notable effectiveness, revealing the lowest MIC of 1.5625 µg/ml and an MBC of 3.125 µg/ml against *S. pyogenes* as shown in Table 2. Similarly, other active samples, such as fluff from Gitambo factory, cyclone from Ogembo factory, and fluff drier mouth from Tombe factory, exhibited MIC values of 3.125 µg/ml and MBC values of 6.25 µg/ml against *S. pyogenes* (Table 2). The fluff from Kaptumo factory and the silvery needles, when tested against *S. pyogenes*, displayed MIC and MBC values of 6.25 µg/ml and 12.5 µg/ml, respectively (Table 2).

Upon evaluating the samples' effects on *E. coli*, the fluff drier mouth sample from Tombe factory showed an MIC of 3.125 µg/ml and an MBC of 6.25 µg/ml, while all other tested samples did not inhibit the growth of *E. coli* (Table 2). Additionally, the fluff sample from Mogogosiek factory had the lowest MIC (3.125 µg/ml) and MBC (6.25 µg/ml) values against *E. coli* (Table 2). Besides, the green tea showed an MIC of 12.5 µg/ml and an MBC of 25.0 µg/ml when tested against *S. aureus* (Table 2).

Table 2: Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs)

Sample Extract	*C. disporicum*		*S. pyogenes*		*E. coli*		*S. aureus*	
TEST	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Githambo fluff	6.25	12.5	3.125	6.25	ND	ND	ND	ND
Kionyo next to fan	3.125	6.25	ND	ND	ND	ND	ND	ND
Ogembo cyclone	ND	ND	3.125	6.25	ND	ND	ND	ND
Tombe fluff drier mouth	ND	ND	3.125	6.25	3.125	6.25	ND	ND
Kaptumo fluff	ND	ND	6.25	12.5	ND	ND	ND	ND
Momul cyclone fluff	ND	ND	3.125	6.25	ND	ND	ND	ND
Momul cyclone fluff	ND	ND	1.5625	3.125	ND	ND	ND	ND
Mogogosiek fluff	ND	ND	ND	ND	ND	ND	3.125	6.25
Green tea	6.25	12.5	ND	ND	ND	ND	12.5	25.0
Silvery needles	6.25	12.5	6.25	12.5	ND	ND	ND	ND

ND- Denotes samples whose MIC and MBC values were not determined.

DISCUSSION

The high rates of morbidity and mortality due to microbial infections, coupled with current antibiotics' limited efficacy, it is imperative to explore alternative strategies, especially utilising natural sources for potential leads for safe and efficacious antimicrobial chemotherapy [2,26,27]. Accordingly, this study delved into the antimicrobial activity of the tea waste samples collected from various tea processing factories in Kenya to determine their potential in providing lead antimicrobials.

We assessed the antimicrobial efficacy of the collected tea wastes based on the sizes of growth inhibition zones according to a previously established criterion [28,29]. In this study, a slight antimicrobial activity was denoted by growth inhibition zone diameters of 6-8 mm; moderate activity, by diameters between 9-12 mm; high with a range from13-15mm, very high indicated when it fell within16 –19mm and finally anything exceeding 20 mm was classified as remarkable [28].

Based on this criterion, we observed that the fluff sample from Gitambo factory at a concentration of 200 µg/ml showed moderative antibacterial activity against C. *disporicum*, while the next to fan sample from Kionyo factory (200 µg/ml), green tea sample (100 µg/ml and 200 µg/ml), and silvery needles (200 µg/ml) demonstrated slight activity. However, all the other samples were deemed inactive based on this appraisal criterion. Besides, the silvery needles sample at 200 µg/ml demonstrated moderate antibacterial activity against *S. pyogenes*, while at a concentration of 100 µg/ml, it showed slight activity. Also, the cylone from Boito factory (200 µg/ml), cyclone fluff from Momul factory (200 µg/ml), fluff from kaptumo (200 µg/ml), fluff drier mouth from Tombe factory (100 µg/ml and 200 µg/ml), cyclone and fluff samples from Ogembo factory (100 µg/ml and 200 µg/ml), fly off from Kionyo factory (200 µg/ml), cyclone from Itumbe factory (200 µg/ml), and fluff from Gitambo factory (50 µg/ml, 100 µg/ml, and 200 µg/ml) had slight antibacterial activity against *S. pyogenes*. Against *E. coli*, the silvery needles (200 µg/ml), fluff from Mogogosiek factory (200 µg/ml), fluff drier mouth from Tombe factory (100 µg/ml and 200 µg/ml), fly off and next to fan

samples from Kionyo factory (200 µg/ml), cyclone fluff from Kinoro factory (200 µg/ml), and fluff from Itumbe factory (200 µg/ml) had slight activity. The findings also showed that the fly off sample from Kionyo factory (200 µg/ml), fluff sample from Mogogosiek factory (200 µg/ml), green tea (200 µg/ml), and silvery needles (100 µg/ml) has slight activity against *S. aureus* based on the appraisal criterion. Notably, silvery needles sample showed moderate antibacterial activity against *S. aureus*. However, none of the studied tea waste samples showed activity antifungal activity against *C. albicans* in this study. These results differ from those obtained in purified tea samples probably due to the poor handling and storage of tea wastes, which may have facilitated leakage of active phytocompounds [30]. Nonetheless, the samples with slight to moderate antibacterial activity have potential to offer lead antimicrobial compounds upon further optimization. This may be enhanced through proper tea waste handling and storage as most phytochemicals are labile and easily degrade upon exposure to unfavourable environments.

Angiolella et al. [31] and Ramón-Sierra et al. [32], propose that plant extracts possessing MIC and MBC/MFC values below 1000 µg/ml might offer potential antimicrobial agents; particularly when these values fall ≤100 µg/ml. Therefore, the tea waste samples which showed lower MIC and MBC values (≤15 µg/ml) in this study may be a valuable resource for isolating efficacious antibacterial agents. The differences in antimicrobial activities may be attributed to the differences across the samples, collection sites, and differences in concentrations of bioactive components [33,34].

Various phytochemicals, recognized for their antibacterial properties in tea (*Camellia sinensis*), notably polyphenols like catechins (EGCG, ECG, EGC and EC) ultimately contributed to the observed efficacy in some samples [16,18]. Thus, the lack of antimicrobial activity of the other samples could be due to the low concentrations or lack of these phytochemicals. Numerous studies have established that either acting alone or in tandem these bioactive compounds inhibit microbial growth or survival [17,35,36]. For instance, theaflavins form during the oxidation process of tea leaves and caffeine exhibit antibacterial activity by impeding pathogenic bacteria growth [37-39]. Also, L-Theanine, a unique amino acid in green tea, has demonstrated antimicrobial effects, while flavonoids like quercetin, kaempferol, and myricetin contribute to tea's overall antibacterial properties [40]. Nevertheless, further investigations aimed at elucidating the mechanisms by which the studied plant extracts, and potentially tea-derived phytochemicals, exert their antimicrobial efficacy could offer valuable insights into discovering alternative antimicrobials to mitigate antimicrobial resistance.

CONCLUSION

This study sheds light on the underappreciated antimicrobial capacity of tea waste samples and accentuates a crucial necessity for adequate preservation measures, such as proper handling and storage to retain bioactive compounds. The observed fluctuations in antimicrobial activity underscore the need for optimizing management procedures related to tea waste to unearth potential lead compounds with powerful antimicrobial properties. Therefore, there is need to delve deeper into understanding mechanisms that drive antimicrobial efficacy and the development of methods for amplifying extraction processes of these vital components. This study suggests leveraging tea waste, a natural source, to develop alternative antimicrobial agents.

Availability of data and materials

All data is presented within the manuscript; however, the authors may provide any additional information upon reasonable request.

Competing interests

We, the authors, declare that we do not have any competing interests/conflicts of interest regarding this publication.

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Authors' contributions

Thaddeus Mangenya conceived the research idea, with contributions from Daniel Kariuki, Johnson Kinyua, Martin Obanda, and Simon Ochanda. Thaddeus Mangenya conducted the experiments, analyzed the data, and drafted the manuscript. Gervason Moriasi provided reagents and optimized the design and methods. Daniel Kariuki, Johnson Kinyua, Martin Obanda, and Simon Ochanda supervised the study. All authors reviewed and approved the final draft for submission and publication.

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