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Antimicrobial activity of kombucha fermented beverage (KFB) singly or in combination with some plant extracts .

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Abstract

Infectious diseases have increased in the last years and, therefore, search for diets or beverages rich with probiotics is necessary.In this regard kombucha is recommended as hygienic beverage . This is a classic black tea beverage that has been sweetened and fermented by a microbial alliance and consortium that consisted of yeasts and acetic acid bacteria. A potent mutual cooperation that can impede and inhibit the growth of potentially contaminating pathogens is formed by this mixed consortium. Because of the bacterial activity of specific strains of *Acetobacter sp*., the fermentation process also results in the formation of a polymeric cellulosic pellicle. Certain biological activities that have previously been studied increased during the microbial consortium's tea fermentation process.In that work, kombucha fermented beverage (KFB) appeared to include bioactive compounds such as organic acids including Formic,Lactic,Acetic, Citric,Succinic,Propionic and Butyric as elucidated by HPLC analysis. The fermented metabolic conistituents of kombucha fermented beverage seem actually to act in a mutually beneficial and collaporative action resulting in the antimicrobial activity. The agar well diffusion assay was used to demonstrate the antimicrobial activity of two types of kombucha preparations (fermented, neutralized) against a variety of pathogenic bacteria and fungi. In comparison to a neutralized sample, KFB exhibited the strongest and most effective antimicrobial activity. The organisms most vulnerable to the kombucha beverage preparations' antimicrobial efficacy were the strains of *Pseudomonas aeruginosa* ATCC (27853) and *Staphylococcus aureus* ATCC6538 *(S. aureus*). In addition to combined effect of combinations of both KFB and medicinal plants extracts - such as(garlic ,pomegranate,ginger and mint)- in vitro against both *S.aureus* and *P.aeruginosa* .These data reveal that kombucha singly or in combined is not only a prophylactic agent, offering protection against pathogenic bacteria and fungi, Owing to its many health advantages, kombucha is becoming more and more well-known globally. Traditionally, this beverage was made by fermenting tea from the Camellia sinensis plant and using cellulosic biofilm, which is made up of a mutually beneficial culture of yeast and bacteria (SCOBY). Further experiments in this study focused on controlling the contamination of *P. aeruginosa* and *S. aureus* in BHI broth. and medicinal plant extracts like that ginger, garlic, pomegranate and mint. The results demonstrated that in every trial, the KFB preparation greatly and dramatically inhibited both bacterial strains that either singly or in combination with medicinal plants. This indicates that using KFB not just alone can yield positive results but also in acombined beverage with medicinal plant due to its synergistic effect against pathogens for obtaining protective syrup .

Key words: kombucha fermented beverage (KFB), kombucha tea, HPLC analysis(High-Performance Liquid Chromatography), pathogenic fungi and bacteria, antimicrobial activity ,medicinal plant extract.

Introduction

Numerous nations experienced the prevalence of infectious diseases, which are still regarded as a serious issue. The process by which microorganisms like fungi, bacteria, and viruses invade their host is known as an infection [1]. Surprisingly, infectious diseases continue to be the leading cause of morbidity and death globally.Therefore ,direction for using natural antimicrobials that attributed to probiotics is the correct way as these substances with the ability to stop microorganisms from growing [2,3]. By identifying and using these compounds, the need for novel medications, microbial multidrug resistance, and the likelihood of organism toxicity can all be considerably reduced. The pharmaceutical and cosmetic industries are interested in these qualities [2,4–7].These different substances, which include peptides, bacteriocins, and organic acids, are produced during the fermentation process.

Live bacteria known as probiotics can aid in a variety of nutritional and digestive processes which are when taken in sufficient doses. [8,9]. The culinary sector and food industry has grown growing curiosity about those kinds of microbes subsequent to an extended period of safe probiotic usage in food items that have undergone fermentation and a growing understanding of their positive effects on human health in addition to their notable and unique potential use as remedial and therapeutic approaches for several diseases [10–17]; Multiple factors appear to be involved in the mechanisms pertaining to the advantageous impacts of probiotics. Several antimicrobial agents are produced, the gut microbiota is altered, competitive adhesion to the mucosa and epithelium occurs, the gut epithelial barrier is strengthened, and the immune system is regulated [8]. A variety of lactic and acetic acid bacteria genera in addition to yeasts are the most commonly used probiotics. These probiotics apply a variety of dietary supplements and functional foods. [8]. Additionally, it has been documented that probiotics perform a preventive effect by directly competing with gut pathogens by releasing antibacterial compounds such bacteriocins, that could eliminates and kill bacteria resistant to multiple drugs [11-20] or metabolic byproducts such as lactic acid and acetic acid [8,17]. Yeasts that are probiotic, like *S. boulardii* and *Saccharomyces cerevisiae* have also been shown to provide and be awarded wellness benefits [21,22].

In recent times, kombucha has become a popular beverage made from fermented tea leaves. Which aproduct that was called "kombucha," which is derived from the words "cha" and "kombu," which stand for tea and algae, respectively. Although it can be made at home, kombucha is also available for purchase in markets as a tea-like beverage. Five to ten percent(5-10%) sucrose is used in the fermentation process of black or green tea in the traditional method of preparation [23, 24]. Green or black tea is typically allowed to infuse with sucrose for 5 minutes before became cold and reached to temperature of room. The kombucha liquid from the earlier production is added once the cooled product has been filtered. "Mother" or "SCOBY" (Symbiotic Community of Bacteria and Yeast) is the name of the liquid biofilm, which is a mixture of osmophilic yeasts(*Candida* ,*Schizosaccharomyces*, *Brettanomyces*/*Dekkera*, *Saccharomyces*, *Starmerella* sp., *Torulopsis*, *Pichia* sp.,and *Zygosaccharomyces*) (25,26) as well as bacteria, especially from Family Acetobacteraceae (*Acetobacter aceti*, *A.estunensis*, *A.pasteurianus*, *Lactobacillus*, *Sp.*, *Komagataeibacter kombuchae, K. rhaeticus, and K. xylinus.* The mixture is covered, and after 10 to 14 days in a dark place to finish the fermentation process, the mixture is known as "tea fungus." These microbes use sucrose as a source of carbon. It is first hydrolyzed into glucose and fructose, and the microorganisms in the combination then use the glucose to produce ethanol and carbon dioxide. With the help of Acetobacter, the ethanol produced is converted to acetic acid, and the mixture's pH drops with increasing acidity.

Due to reports that fermented beverages have antioxidant and other health benefits,they have become more popular during the COVID-19 pandemic in many countries (27). Potential prophylactic effects of kombucha included weight loss, the treatment of metabolic disorders, AIDS, cancer, indigestion, arthritis, and other illnesses [28,29, 30]. Consuming kombucha beverage on a regular basis has been linked to both life elongation and the inhibition of weight gain [31]. Many active components among others, organic acids (primarily acetic, gluconic, and glucuronic acid), are produced during fermentation and are responsible for antioxidant and prophylactic qualities of kombucha.

According to reports, kombucha has antimicrobial properties against the following: *Staphylococcus epidermidis* CIP 106510, *Salmonella typhimurium* LT2, *Micrococcus luteus* NCIMB 8166, *S.aureus* ATCC 25923 , *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 19115, *Candida krusei* CCM 8271, , *Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Haemophilus influenzae* CCM 4454, *Escherichia coli* CCM 3954, and *C. tropicalis* CCM 8223[16]. Numerous by-products with activity against microbes, including organic acids—particularly acetic acid and catechins—have been discovered to be present in kombucha.For those who are lactose intolerant, kombucha and herbal drinks that have undergone fermentation may be suitable substitutes for dairy products with functional aspects [33]. The present work was undertaken to prepare Kombucha fermented beverage and to study its antimicrobial activities against some food-borne pathogens either singly or in combinations with some plant extracts.

Material and Methods

Kombucha Consortium: The traditionally Egyptian-made tea fungus starter culture used in this work was a symbiotic culture between acetic acid bacteria and yeast, which during the fermentation process of kombucha produces a layer of cellulosic pellicles floating on the fermented broth's surface [34].

Kombucha Fermented Beverage (KFB)Preparation: In order to make KFB, 1.2% black tea was added to boiling water, lefted to infuse for approximately five minutes, and then filtered via a sterile sieve. 10% sucrose was dissolved in hot tea, and the 200 mL solution was allowed to cool before being put into a sterile 500 mL glass vessel (22 × 20 × 15 cm). 10% (v/v) of previously KFB was asceptically added to the preparation, along with a 3% (w/v) inoculation of freshly harvested tea fungus that had been cultured in the same fermentation media for 14 days. For about 14 days, the glass vessel was kept in the dark at 30 °C and it was gently covered with a clean cloth [35]. Following a cellulose pellicle removal from KFB, the resulting supernatant was immediately subjected to microbiological and chemical analysis. The KFB aliquots that were obtained and used in the antimicrobial tests were refrigerated at 4 °C before use. Once the KFB aliquots had been stored for a week, they were surface-sterilized prior to conducting the tests for antimicrobial potency.

Determination of pH: An electronic pH meter from Denver Instruments, located in Bohemia, New York, United States of America, was used to measure the pH of KFB.

Analyzing KFB Instrumentally: Utilizing high performance liquid chromatography (HPLC), the main organic acids present in kombucha tea were identified. For HPLC systems (Germany using a conventional C18 column), the organic acids in kombucha tea were optimized for detection**.** Following asterile micofilter (Millex-GV filter, 0.22 µm pore size, Millipore, Burlington, MA, USA) filtering of the kombucha tea samples, HPLC analysis was performed using HPLC (Agilent Technologies, 1260 series). A 4.6 mm x 150 mm i.d., 3 µm AQ-C18 HP column was used to perform the separation. There was 0.005N sulfuric acid in the mobile phase. Following a linear gradient for flow rate, the mobile phase was programmed in this manner: 1-4.5 min (0.8

ml/min); 4-4.7 min (1 ml/min); 4-7.1 min (1 ml/min); 4-71-8.8 min (1.2 ml/min); 8.8-9 (1.3 ml/min); 9-23 (1.3 ml/min); 23-25 (0.8 ml/min). Monitored at 210 nm was the Diode Array detector (DAD). For every sample solution, there was a 5 µl injection volume. A constant 55 °C was kept in the column temperature.

Microbial Test Strains: Both bacterial and fungal pathogens were among the microbial strains employed in the antimicrobial tests. Among the strains of bacteria used were Gram-negative bacteria like *Salmonella typhimurium* ATCC14028 (*Sal. typhimurium*), *Klebsiella pneumonia* ATCC13883, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC11229 .Gram-positive bacteria included *Staphylococcus aureus* ATCC6538 .Glass beads were used to keep the subcultured test strains of bacteria, which were maintained at −20 °C in brain heart infusion broth (BHI broth, Oxoid). The fungal test organisms used were *Aspergillus flavus* ATCC16872 and *Aspergillus niger* ATCC20611, along with *Penicillium sp* (our strain collection) and *Candida albicans* ATCC 10231. After being kept at -20 °C, the fungal cultures were subsequently subcultured on sabouraud dextrose agar (Difco, Sparks, NV, USA).

Preparation of plant extracts:

Water Extraction:- The rhizomes, peels, and leaves of the following plants—ginger, pomegranate, cloves, and mint respectively—are carefully washed with tap water and then distilled water to remove any remaining dirt before being allowed to dry at room temperature. These plant parts were ground into apowder separately in a grinding machine and stored at room temperature pending extraction. Twenty grams of each dried powdered plant material from each plant were soaked in 100 milliliters of distilled water that had been sterilized for 48 hours while being constantly shaken in a sterile conical flask. Following 8 layers of muslin cloth filtration, the mixture was centrifuged for 10 minutes at 5000 rpm. To make the final volume half of the initial volume (stock solutions), the supernatant was collected and concentrated (in an oven at 45ºC) [49].

Antimicrobial Bioassays of Kombucha Beverage singly or in combinations with plant extracts:

The KFB was ready as previously mentioned. Additionally, By adjusting the pH with either 1 M NaOH or 1 M HCl it was neutralized (NKB) at pH 7.0. To get rid of cell debris, all of the prepared kombucha samples were centrifuged for 15 minutes at 15,000 rpm. Sterile supernatants were obtained by filtering the supernatants through a sterile microfilter (Millex-GV filter, 0.22 µm pore size, Millipore, Burlington, MA, USA). Using an agar well diffusion assay, the antimicrobial properties of each kombucha sample were investigated [36]. On Brain Heart Infusion Agar plates (BHI agar, Oxoid), the tested bacterial strains were prepared and inoculated. with7.3×10^{3}CFU/mL.Additionally,The tested fung al strains were prepared and inoculated with 10^{7} sp ores/mL on Sabouraud dextrose agar plates (SDA, Oxoid). Under completely aseptic conditions, sterile glass rods were used to spread microbial inocula onto the agar plates. A sterile cork borer was used to create wells 10 mm in diameter. Following the inoculation of the agar plates with the tested strains, sterile samples (100 µL) were added to their wells. The kombucha preparation was allowed to pre-diffusion into the agar by storing the plates at 4 °C for two hours.

Afterward, treated agar plates were incubated at 35 °C for 48 hours for bacterial strains and five days for fungal strains that were studied. Then, in compliance with the Clinical and Laboratory Standards Institute (CLSI) [37], the diameters of the inhibition zones were determined. To check the effect of combinations of both KFB and plant extracts such as(garlic ,ginger,pomegranate and mint).Aliquots of both KFB and plant extract (1:1) were mixed , sterilized and their antimicrobial activity against the indicator strains employed herein were tested as given previously[49].

Results

Black tea (1.2%), sweetened with sucrose (10%), was used to create kombucha fermented beverage (KFB) in an experimental setting. The mixture was then inoculated with 3% (w/v) of freshly grown tea fungus and incubated at 30 °C for approximately 14 days. The ultimate pH was discovered to have dropped to 3.0.KFB was subjected to HPLC analysis in the current work in order to identify its bioactive components(organic acids). The results shown in (Figure 1 and Table 1).

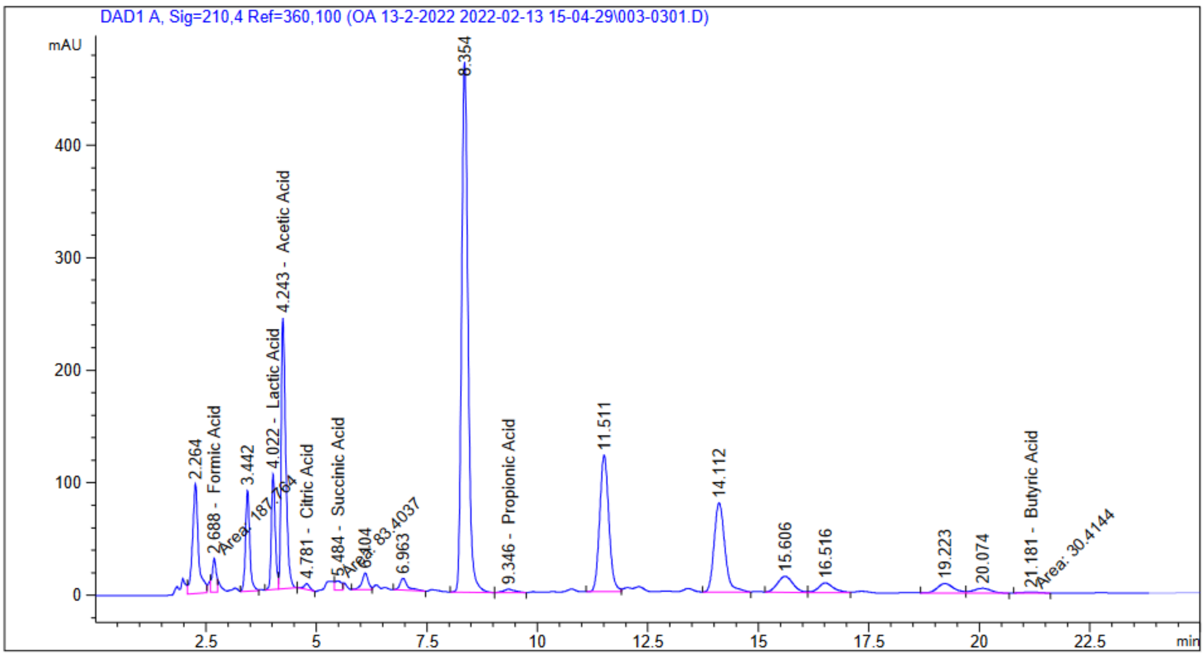

Figure (1): HPLC analysis of KFB

Table (1) Organic acids of KFB when subjected to HPLC (High Performance Liquid Chromatography).

Sample Kombucha		
	Area	Conc. (ug/ml)
Formic	187.76	598.31

acid		
Lactic acid	629.17	2834.29
Acetic acid	1732.56	8639.72
Citric acid	48.04	142.06
Succinic acid	83.40	530.04
Propionic acid	56.20	357.16
Butyric Acid	30.41	225.45

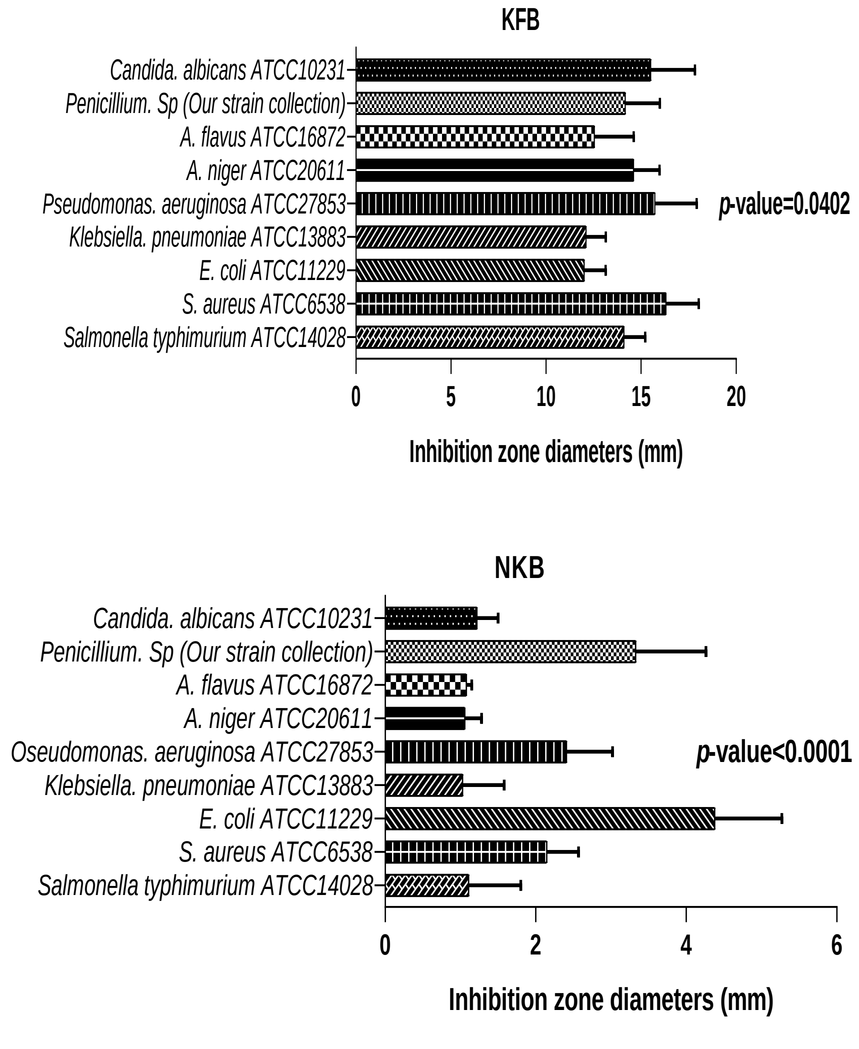

Figure 2: Antimicrobial activity of KFB and NKB.

Table (2): Antimicrobial activity of KFB and NKB .

Tested Organism	KFB	NKB
Sal. typhimurium	14.11±1.11	1.11±0.69
S. aureus	16.32±1.71	2.15±0.42

E. coli	12.01±1.12	4.38±0.89
K. Pneumoniae	12.11±1.03	1.03±0.55
P. aeruginosa	15.73±2.19	2.41±0.61
A. niger	14.61±1.36	1.06±0.22
A. flavus	12.55±2.06	1.08±0.07
Penicillium. Sp	14.17±1.82	3.33±0.93
C . albicans	15.51±2.32	1.22±0.28
p-value	0.0402	<0.0001

The antimicrobial activities of KFB and NKB are given in figure (2) and table(2) .The KFB showed broader antimicrobial activity than that showed by neutralized KB ,indicating on the inhibitory activity occurred by organic acids existed in KFB .The results indicated that the afore mentioned two antimicrobial agents had antimicrobial properties as they had significant effects on the inhibition of all tested organisms (*p*=0.0402, and <0.0001, respectively).The values of inhibition zone diameters maximized for *S. aureus and P. aeruginosa* when the kombucha fermented and neutralized kombucha were used as antimicrobial agents, respectively.

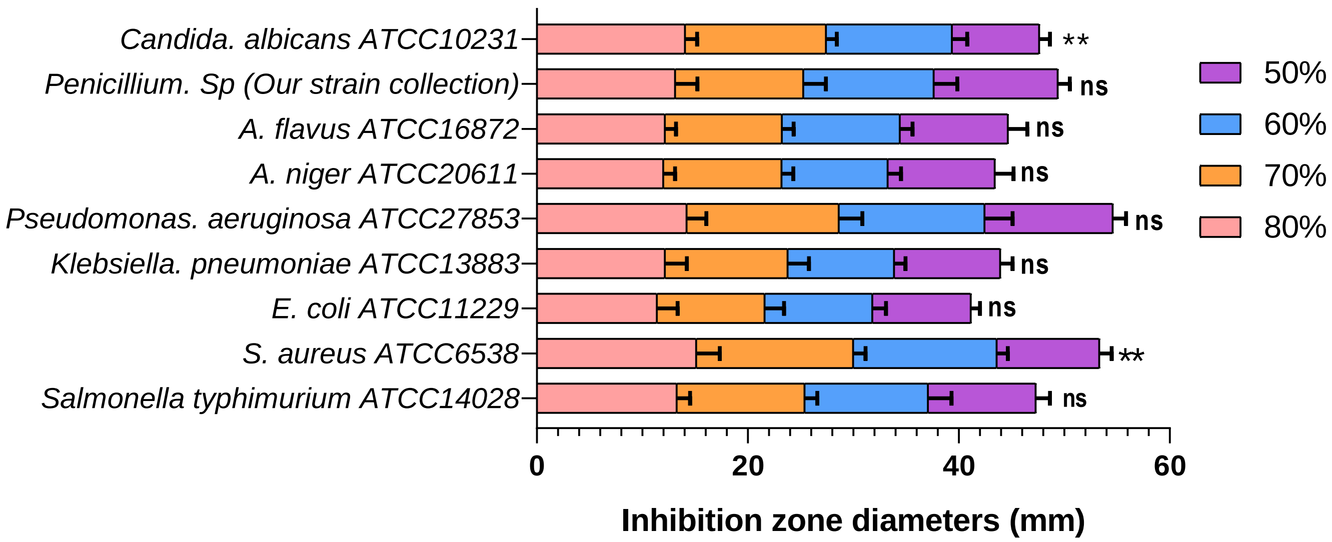

Figure 3: Antimicrobial activity of different concentration of Kombucha.

Table (3): Antimicrobial activity of different concentration of Kombucha

Tested Organism	80%	70%	60%	50%	*p*-value
Sal. typhimurium	13.29±1.25	12.11±1.18	11.71±2.22	10.22±1.33	0.1932
S. aureus	15.14±1.22	14.86±1.16	13.62±1.03	9.74±1.15	0.0172
E. coli	11.42±1.93	10.21±1.82	10.21±1.28	9.33±0.86	0.4656
K. pneumoniae	12.17±2.06	11.63±2.01	10.10±1.06	10.06±1.15	0.3426
P. aeruginosa	14.22±1.86	14.44±2.19	13.82±2.63	12.14±1.24	0.5376
A. niger	12.01±1.11	11.21±1.11	10.06±1.24	10.17±1.74	0.2972
A. flavus	12.17±1.03	11.09±1.09	11.18±1.18	10.24±1.86	0.4143
Penicillium. Sp	13.13±2.11	12.16±2.11	12.37±2.21	11.76±1.11	0.8492
C. albicans	14.08±1.12	13.36±1.01	11.93±1.44	8.29±1.00	0.0088

The treatment by Kombucha had not significant effects on the inhibition of all tested organism (p>0.05) except of its effect on *Sal. typhimurium* and *C. albicans* that was highly significant (*p*-value=0.0172 and 0.0088, respectively). There were significant difference in values of inhibition zone diameter between the level of 80% Kombucha and 50% being 13.29mm vs. 10.22 mm for *Sal. typhimurium* and 14.08mm vs. 8.29mm for *C. albicans* **(Figure 3 and Table 3).**

Table (4): Inhibition of food borne pathogens by kombucha with ginger each 24h during 96 h segment. (In vitro)

Time (h)	Bacterial count(CFU/mL)							
	S. aureus				*Pseudomonas aeruginosa*			
	Control	2 mL	4 mL	*p*-value	Control	2 mL	4 mL	*p*-value
0	3.914	3.914	3.914	1.000	3.914	3.914	3.914	1.000
6	5.978	3.845	3.491	0.0382	5.869	3.771	4.097	0.0479
18	7.919	3.613	2.845	0.0001	7.954	3.114	3.041	0.0001
24	10.041	3.013	2.083	0.0001	9.978	2.929	1.505	0.0001
48	11.362	2.699	1.968	0.0001	11.914	2.519	0.000	0.0001
72	12.176	2.230	0.000	0.0001	12.851	0.000	0.000	0.0001
96	12.462	0.000	0.000	0.0001	12.881	0.000	0.000	0.0001
b - reg	0.0819	-0.0361	-0.0419		0.0893	-0.0445	-0.0466	
***p*-value**	0.0069	0.0012	0.0005		0.0054	0.0005	0.0073	

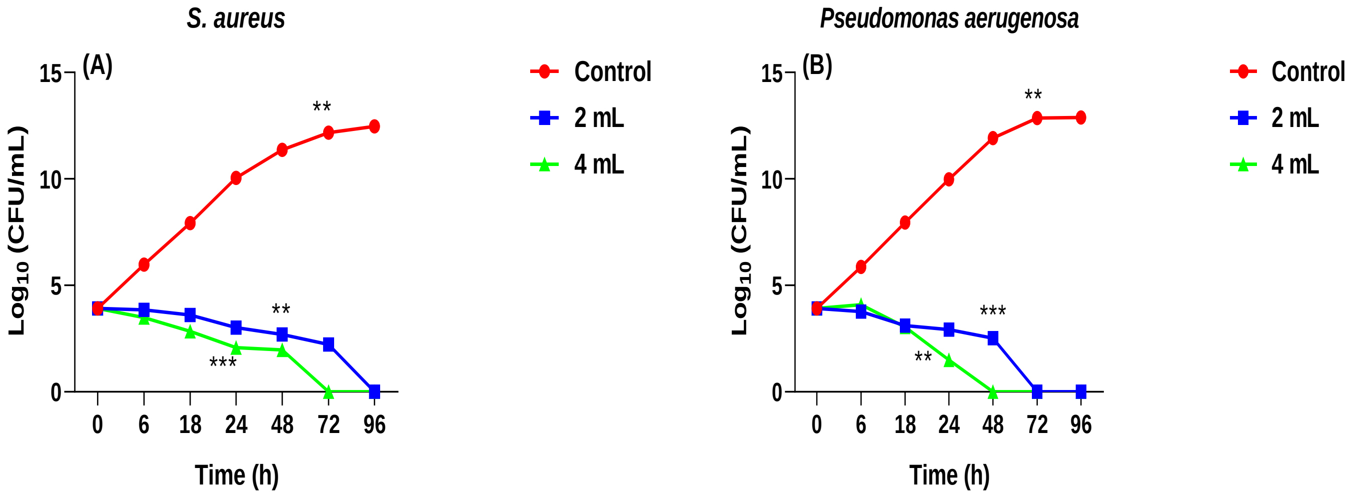

Figure (4): Combined effect of combinations of both KFB and ginger extract in vitro against both *S.aureus* and *P.aeruginosa*.

The study revealed a significant reduction in microbial counts of both *S. aureus* and *P. aeruginosa* with increasing doses of kombucha and ginger, with the most pronounced effect observed at a dose of 4 mL. Over the course of the time period, the control group exhibited a significant increase in microbial activity, while the treated groups demonstrated a significant inhibition in microbial activities (***p-value*** <0.001; **Figure** 4 and **Table** 4).

Table (5): Inhibition of food borne pathogens by kombucha with pomegranate each 24h during 96 h segment. (In vitro)

Time (h)	Bacterial count (CFU/mL)							
	S. aureus				*Pseudomonas aeruginosa*			
	Control	2 mL	4 mL	*p*-value	Control	2 mL	4 ml	*p*-value
0	3.914	3.914	3.914	1.000	3.914	3.914	3.914	1.000
6	5.978	3.845	3.462	0.0372	5.924	3.690	3.097	0.0217
18	7.919	3.505	2.903	0.0001	7.954	3.114	2.041	0.0001
24	10.041	3.017	2.083	0.0001	9.978	2.929	1.505	0.0001
48	11.362	2.699	1.724	0.0001	11.914	2.090	0	0.0001
72	12.176	2.431	0	0.0001	12.851	0	0	0.0001
96	12.462	1.041	0	0.0001	12.881	0	0	0.0001
b – reg	0.0819	0.0271	-0.0423		0.0891	-0.0448	-0.0399	
***p*-value**	0.0069	0.0003	0.0003		0.0054	0.0002	0.0070	

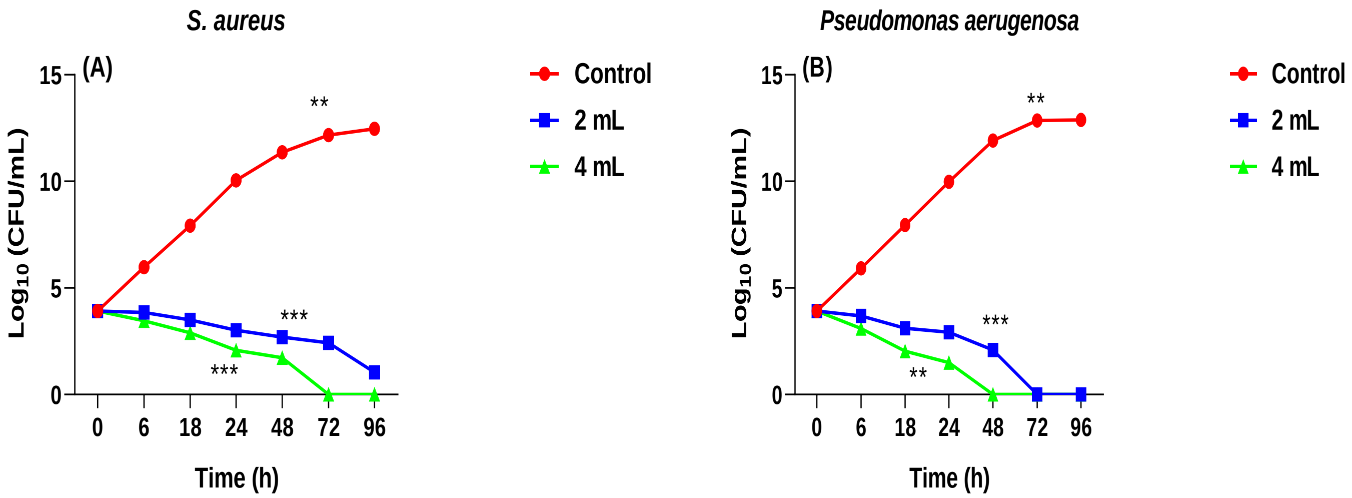

Figure (5): Combined effect of combinations of both KFB and pomegranate extract in vitro against both *S.aureus* and *P.aeruginosa*.

The synergistic effects of kombucha and pomegranate exhibited highly significant impacts on reducing the counts of *S. aureus* and *P. aeruginosa*, with the most pronounced decrease observed in the group treated with 4 mL of this mixture. Furthermore, the inhibition of foodborne pathogens showed a significant decrease in the treated group compared to the control as the time periods increased (***p-value*** <0.001; (**Figure** 5 and **Table** 5).

Table(6) Inhibition of food borne pathogens by kombucha with garlic each 24h during 96 h segment. (In vitro)

Time (h)	Bacterial count (CFU/mL)							
	S. aureus				*Pseudomonas aeruginosa*			
	control	2 mL	4 mL	*p*-value	Control	2 mL	4 mL	*p*-value
0	3.914	3.914	3.914	1.000	3.914	3.914	3.914	1.000
6	5.929	3.778	3.462	0.0371	5.924	3.690	3.130	510.01
18	7.919	3.322	2.903	0.0001	7.954	3.114	1.964	0.0001
24	10.041	2.968	2.004	0.0001	9.978	2.929	1.342	0.0001
48	11.362	2.602	1.724	0.0001	11.914	2.519	1.255	0.0001
72	12.176	2.230	1.415	0.0001	12.851	2.000	0.000	0.0001
96	12.462	0.000	0.000	0.0001	12.881	0.000	0.000	0.0001
b - reg	0.0821	-0.0351	-0.0358		0.0891	-0.0353	-0.0378	
***p*-value**	0.0069	0.0012	0.0006		0.0054	0.0006	0.0036	

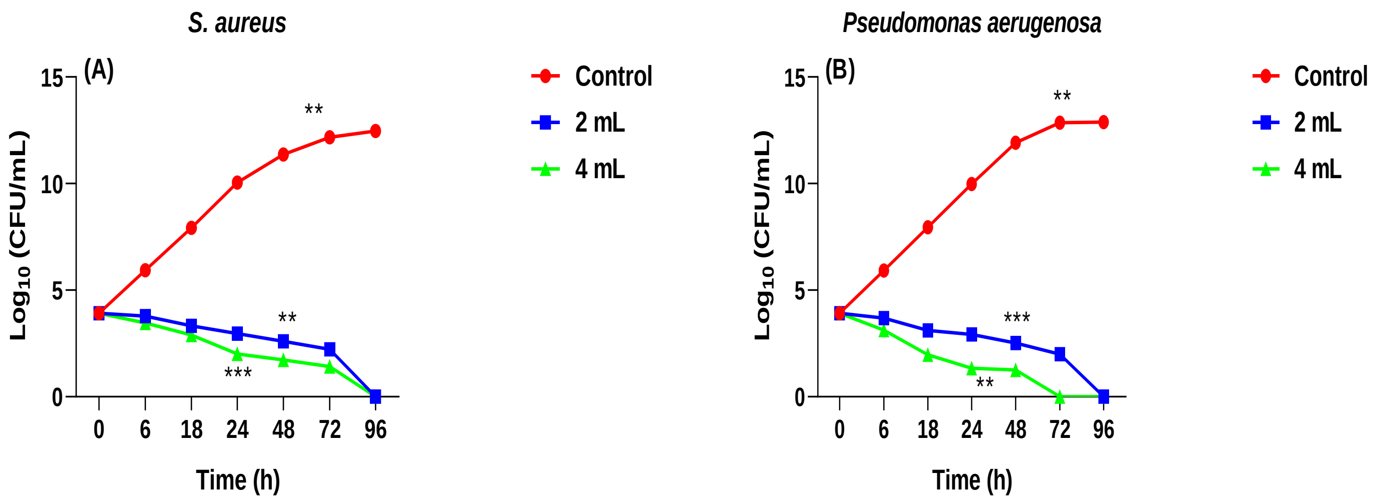

Figure (6): Combined effect of combinations of both KFB and garlic extract in vitro against both *S.aureus* and *P.aeruginosa*.

The combination of kombucha and garlic exhibited a highly significant decrease in the inhibition of foodborne pathogens (*S. aureus* and *P. aeruginosa*), with the most pronounced effect observed at a dose of 4 mL of this mixture, followed by 2 mL, and the control group. Additionally, over a 96-hour period, there were significant decreases in the total bacterial counts with the treatment, in contrast to a significant increase observed in the control group (***p-value*** <0.001; **Figure** 6 and **Table** 6).

Table (7): Inhibition of food borne pathogens by kombucha with mint each 24h during 96 h segment. (In vitro)

Time (h)	Bacterial count(CFU/mL)							
	S. aureus				*Pseudomonas aeruginosa*			
	Control	2 mL	4 mL	*p*-value	Control	2 mL	4 mL	*p*-value
0	3.914	3.914	3.914	1.000	3.914	3.914	3.914	1.000
6	5.978	3.778	3.462	280.03	5.924	3.690	3.152	0.0132
18	7.919	3.322	2.161	0.0001	7.954	3.114	1.978	0.0001
24	10.041	2.959	1.973	0.0001	9.978	2.929	1.623	0.0001
48	11.362	2.602	1.724	0.0001	11.914	2.519	1.301	0.0001
72	12.176	2.230	1.000	0.0001	12.851	2.000	0	0.0001
96	12.462	1.362	0	0.0001	12.881	0	0	0.0001
b – reg	0.0819	-0.0248	-0.0357		0.0891	-0.0353	-0.0384	
***p*-value**	0.0069	<0.0001	0.0010		0.0054	0.0006	0.0021	

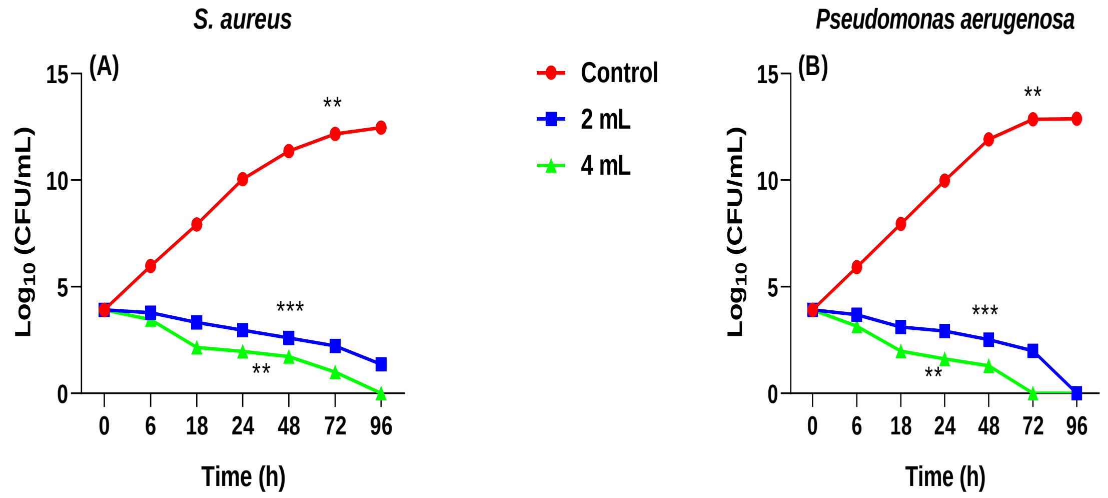

Figure (7): Combined effect of combinations of both KFB and mint extract in vitro against both *S.aureus* and *P.aeruginosa*.

The current findings demonstrated a significant decrease in microbial activities of both *S. aureus* and *P. aeruginosa* in the groups treated with a mixture of kombucha and mint. The regression analysis further confirmed a significant reduction in the total bacterial count as the time periods increased in both treatment groups (***p-value*** <0.001; **Figure** 7 and **Table** 7).

Discussion

Egyptian-made kombucha mat was used to ferment black tea to create the traditional culture of kombucha used in this work [34]. When kombucha fermentation process ocurred, a variety of fermentation byproducts are created.Seven organic acids that were intended to inhibit bacterial pathogens were found in this study when

KFB supjected to HPLC analysis. But research has shown that the bioactive metabolites of kombucha beverage have a collective effect that is both collaborative and synergistic [38, 39].

Mutually beneficial culture of microorganisms used for kombucha fermentation, in addition to the temperature and duration of the fermentation process, the type of tea used, the amount of sucrose present, and the analysis techniques employed for quantification, all affect the quantity and presence of kombucha's chemical constituents [39, 40].

The data used in this study demonstrated that the preparation of fermentation (KFB) from a 14-day kombucha starter incubation in black tea had the most powerful antimicrobial effects. Neutralized kombucha (NKB) preparation exhibited minimal antibacterial efficacy against the organisms under test.This is due to the organic acids found in KFB possessed the inhibitory activity of pathogenic bacteria[10]. To bolster the current investigation, Battikh et al. [32] provided evidence that the kombucha infusion had stronger antibacterial activity than the neutralized infusion .Kombucha made from fermented black tea has been shown in numerous other studies to have antibacterial activity against a wide range of bacteria[29,41,42, 43]. **Kaewkod et al**., **[44],** After 15 days of fermentation discovered that the kombucha made from various tea varieties (oolong, black and green) had effective antimicrobial activity on all pathogenic enteric bacteria tested, including *Shigella dysenteriae, Vibrio cholerae, Salmonella typhi DMST 22842* and. *E. coli* O157:H7 DMST 12743.

A limited number of researches has been published in the literature proving kombucha's antifungal properties. The statement from Sreeramulu et al. [30], kombucha tea with a 6-to 14-day fermentation period exhibited antifungal activity against *Candida albicans*. According to Battikh et al. [32], an infusion of fermented kombucha black tea for 21 days demonstrated antifungal activity against *Candida albicans*, *Candida tropicalis, Candida glabrata, and Candida dubliniensis*. In a prior study, 10% v/v kombucha supernatant made from black tea after 14 days of fermentation completely inhibited the growth of mycelia of *Acremonium implicatum* LC015097 and decreased the growth of mycelia of *Penicillium expansum* LC015096 and *Talaromyces purpureogenus* LC015095. Additionally, it prevented three fungal strains from producing the mycotoxin patulin. Organic acids acidity (gluconic acids, citric, and acetic) in kombucha beverages can be used to explain the antimicrobial activity of KFB [45]. The pH is shifted to final values of approximately 2.5–3.0 by these organic acids, which are created during fermentation from the sucrose conversion by yeasts and the kombucha consortium bacteria. Previous research showing that the pH dropped from 5 to 2.5 due to high concentration of organic acids that produced in fermentation of kombucha broth, [30,46] provides strong support for this. Furthermore, it was demonstrated by Greenwalt et al. [41] that acetic acid plays a major role in the ability of kombucha to combat harmful microbes through antimicrobial activity.

Bacterial cells could be destroyed by organic acid molecules that cause cytoplasmic acidification

[44]. The difference between the osmotic pressure of the solutes in the hypertonic medium and the outer aquatic medium can also be used to interpret the antibacterial activity of kombucha compounds. Bioactive materials from cell membranes can diffuse through selective permeability. Certain solutes are lipophilic, which makes it easier for them to attach to bacterial cell membranes and ultimately results in cell death [47, 48].

S. aureus, a Gram-positive bacterium, and *Pseudomonas aeruginosa*, a Gram-negative bacterium were the most susceptible bacteria for antimicrobial activity of kombucha beverage that prepared, the inhibition zones for these bacteria were measured at 16 and 15 mm, respectively. Battikh *et al.* [32] discovered via the use of the agar diffusion assay that the diameters of inhibition zone of the two bacterial species (14.5 and 11 mm) in kombucha tea were smaller than those that were obtained.

Ultimately, using kombucha beverage that arisk -free probiotic which has multiple antimicrobial components that act in a synergistic manner is preferable to using a single, potentially more costly component with diminished antimicrobial potency. This pattern is encouraging and has practical applications in The industry of functional food. All pathogenic bacteria tested in this study were effectively inhibited by the kombucha made from black tea, including Gram-positive bacteria like *Staphylococcus aureus* ATCC6538 (*S. aureus*) and Gram-negative bacteria like *Klebsiella pneumonia* ATCC13883, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC11229 (*E. coli*), and *Salmonella typhimurium* ATCC14028 (*Sal. typhimurium*). The pathogenic fungi *Penicillium sp* (our strain collection), *Candida albicans* ATCC 10231, and *Aspergillus flavus* ATCC16872 (*A. flavus*) are among them.

The control of *S. aureus* and *P.aeruginosa* contamination in BHI broth and medicinal plant extracts, including ginger, garlic, pomegranate, and mint, was the focus of additional experiments conducted in this study. The obtained results demonstrated that, in every trial, Significant inhibition of both bacterial strains was observed by the KFB preparation, either singly or in combination with medicinal plants. Because KFB has a synergistic effect against pathogens to produce protective syrup, this is a promising outcome when utilizing KFB singly as well as in combination with medicinal plants.

This study's scientific research demonstrated that kombucha tea greatly outperforms other health drinks in treating infections, which may encourage consumers to drink kombucha beverages.

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