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ANTIMICROBIAL AND ANTIPROLIFERATIVE, PRO-APOPTOTIC ACTIONS OF KOMBUCHA FERMENTED SOLUTIONS AGAINST COLON AND HEPTAO CANCER CELL LINES

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ABSTRACT

Introduction: Kombucha fermented solutions (KFS_s) were produced from tea (KTFS), rice (KRFS) and barely (KBFS) after 8 and 10 days. These solutions tested as antimicrobial activity against microorganisms (Listeria monocytogenes, Shigelladysenteriae, Salmonella enteritidis, Staphylococcus aureus, Citrobacter sp., Klebsiella pneumoniae, Esherichia coli and Candida albicans) by agar well diffusion assay. KTFS was higher inhibitory activity than KRFS or KBFS, which gave the largest clear halo-zone diameter against S. enteritidis. Minimum lethal concentration: minimum inhibition concentration ratio wascalculated to known the KFS_s action. All tested KFS_s were micobicidal effect against S. aureus and C. albicans (≤2) and microbiostatic effect against other pathogenic bacteria (≥4). **Objective:** The study extends to examine the growth inhibitory effects and Apoptotic abilities of KFS_S, on human colon (HCT-116) and liver (HEpG-2) cancer cells. **Material and method:** using Neutral Red Uptake Assay and the AO/EB dual Staining assays to detect anti proliferative and apoptosis properties. **Results:** indicated that KTFS had more antitumor activity against different tumor cells than KRFS and KBFS. Although all tested KFS_s were found to reduce the cell viability in a concentration manner, but the magnitude of reduction was high in case of KTF_S with lower IC_{50} values, and induces a higher percentage of apoptotic cells after 24 h of exposure of HEpG-2 cell line. These results suggest that the KFS_s possess interesting antiproliferative properties and induce apoptosis on the HCT-116 and HEpG-2 cancer cells associated with significant antimicrobial activity. These findings provide additional support for the traditional use of KFS_s in the treatment of metabolic diseases and various types of cancer.

KEYWORDS: Kombucha fermented solutions; Minimum inhibitory concentration; Pathogenic microorganisms; Cytotoxicity, antiproliferative, apoptosis.

INTRODUCTION

Kombucha tea is called “tea fungus” composed of fermented different types of acetic acid bacteria and yeasts in symbiotic association with two portions, a floating cellulose pellicle layer and the sour liquid broth. Kombucha tea is a popular beverage across the world for its refreshing taste and beneficial effects on human health. The beneficial effects of Kombucha tea are attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics, and a variety of micronutrients produced during fermentation (Jayabalan et al, 2007). Kombucha tea is known to show a remarkable antimicrobial activity against a broad range of microorganisms. Many scientific studies have been done on this subject and the Kombucha broth has demonstrated inhibitory activity against many pathogenic microorganisms of both Gram positive and Gram negative origin (Dufresne and Farnworth, 2000). Kombucha tea has demonstrated the ability to inhibit the growth of pathogens such as Helicobacter pylori (the causative organism of peptic ulcers), Escherichia coli (the causative organism of common diarrhea), Entamoeba cloacae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermis, Agrobacterium tumefaciens, Bacillus cereus, Aeromonashydrophila, Salmonella typhimurium, Salmonella enteritidis, Shigellasonnei, Leuconostocmonocytogenes, Yersinia enterocolitica, Campylobacter jejuni, and Candida albicans (Sreeramulu et al, 2001; Sreeramulu et al, 2000; Dufresne and Farnworth, 2000). Some studies have demonstrated that Kombucha tea shows not only antibacterial activity but

also antifungal activity (Battikh et al, 2013). There are several testimonials available in the internet for the anticancer properties of Kombucha tea from Kombucha drinkers throughout the world, but there are no scientific reports to validate the facts. The chemoprevention of human cancers has long been the subject of research. The chemopreventive effects of tea polyphenols have been demonstrated in animal models of cancers of lung, skin, esophagus, colon, and mammary gland, but the anticancer property of Kombucha tea was not well characterized. Carcinogenesis is a multistage process, in which different genes are prime targets for chemopreventive agents because they regulate intracellular, cell-surface or extracellular functions. Acridine orange/ethidium bromide (AO/EB) staining is used to visualize nuclear changes and apoptotic body formation that are characteristic of apoptosis. Cells are viewed under a fluorescence microscope and counted to quantify apoptosis (Ribble et al, 2005). The ethidium bromide/acridine orange stain (EB/AO stain) is a viability stain that detects apoptotic cells. Viability stains determine the membrane integrity of a cell based on the uptake or exclusion of a dye from the cell (Park et al, 2000). Ethidium bromide is a dye that is only able to pass through the membrane of a dead or dying cell. Acridine orange is a membrane-permeable dye that will stain all cells in the sample. Each dye that is taken up by a cell fluoresces- AO makes a cell green, and EB makes a cell red (Baskíc et al, 2006). The possible cancer preventive activity of Kombucha tea has received much attention in recent years. So, the objective of this study was to evaluate the antiproliferative activity of Kombucha fermented solutions from tea (KTFS), rice (KRFS) and barely (KBFS) against two human cancer cell lines: human colon carcinoma cells (HCT-116) and human liver carcinoma (HEpG-2) by using Neutral Red Uptake Assay (NRU colorimetric Assay) and its antimicrobial activity against Gram-negative and Gram-positive pathogenic microorganisms.

MATERIALS AND METHODS

Preparation of Kombucha fermented solutions (KFS_s)

After boiling 1L of tap water for 15min, 4g of substrates (black tea, rice or barely) was added and infused for 15 min then removed by filtration. 70g/L of commercial sucrose was dissolved into the filtered infusion before it has cooled. After cooling to room temperature, pour the solution into a glass jar has been previously sterilized at 121°C for 20min. these solutions were inoculated with 10% of the fermentation broth from the previous fermentation of black tea obtained under the same conditions. The glass jars were covered with a clean piece of cloth and fixed with rubber bands. The fermentation was carried out under room temperature (25°C) for 10 days. The harvesting was done after 2, 4, 6, 8 and 10 days of fermentation. Kombucha tea, rice or barely was centrifuged at 10.000 rpm for 15 min and the supernatant was used as Kombucha fermented solutions (KFS_s).

Pathogenic strains

Seventeen pathogenic bacteria and one yeast strains used in this investigation were Listeria monocytogenes1,2& 3, Shigelladysenteriae1&2 and Salmonella enteritidis which collected from Department of Agric. Microbiology, Fac. of Agric., Ain Shams University and Staphylococcus aureus1&2, Citrobacter sp.1&2, Klebsiella pneumoniae1,2,3&4, Esherichia coli1,2&3and Candida albicans were obtained from Egyptian hospital. These strains were maintained on nutrient agar(Difco Manual, 1984) or Sabouraud dextroseagar (BAM, 1998) slants at 4°C for bacteria and yeast, respectively.

Inoculum preparation

From 4 to 5 colonies of a pure tested microbial culture were collected from agar plate after 24h of incubation period and subculture into tube containing 4 ml of Müller-Hinton broththen incubated at 37°C until it achieves the turbidity of 0.5 MacFarland standard after 24 h incubation according to NCCLS (1998). The inoculum was standardized by measurement the optical density using spectrophotometer at 625nm which ranged from 0.08-0.12. Standardized inoculum has a concentration of $1\text{-}2\times10^8$cfu/ml for bacteria and $1\text{-}5\times10^8$cfu/ml for yeast and diluted to1:10 in sterile saline solution to obtain the coveted concentration of 10^6cfu/ml.

Antimicrobial Activity

The antimicrobial activity of KFS_s was tested by agar well diffusion assay (Mo et al, 2005) against pathogenic strains. Müller-Hinton agar (MHA)medium was poured into Petri dishes. Suspensions (100µl) of target strain previously incubated for 24 h were spread on the plates, and wells of 7 mm in diameter were made with a sterile cork borer (ShahidiBonjar, 2004; Atata et al, 2003). KFS_s samples obtained after 2, 4, 6, 8, and 10 days from fermentation periods were centrifuged at 10,000 rpm for 10 min to remove cell debris and supernatant samples (100µL) were then transferred into the wells in the agar plates previously inoculated with the target strain. The plates were allowed to stand until KFSs samples were completely absorbed and after then incubated at 37°C for overnight (Sreeramulu et al, 2001). The growth inhibition was observed by the naked eye and the inhibition zone diameter (IZD) was measured using a ruler after 24 h of incubation. The antimicrobial activity was evaluated by measuring the growth inhibition zone surrounding the wells. Acetic acid sample was used as control at the same concentration as that of fermented solutions after 8 and 10 days were prepared and sterilized by filtration and then used for antimicrobial testing, as described previously. Antibiotic was used as standard. Each experiment was carried out in triplicate and the average diameter±standard deviation of the IZD was recorded.

Determination of minimum inhibitory concentration (MIC) by agar plate dilution method

The agar dilution method was carried out by Clinical and Laboratory Standards Institute (CLSI). Serial dilutions (1/2, 1/5, 1/10, 1/25, 1/50, 1/100 and 1/125) of the tested KFS_s were added to the melted MHAmedium at 50°C and mixed fully to get the desirable final concentrations of6.800 (control), 3.400, 1.360, 0.680, 0.272, 0.136, 0.068 and 0.054mg/ml for Kombucha tea fermented solution (KTFS), 5.14, 2.57, 1.03, 0.514, 0.2056, 0.1028, 0.0514 and 0.04112mg/ml for Kombucha rice fermented solution (KRFS) and 4.60 (control), 2.30, 0.92, 0.46, 0.184, 0.092, 0.046 and 0.0368mg/ml for Kombucha barely fermented solution (KBFS), respectively (Figure 1). The media containing KFS_s were poured immediately on plates,then leave till solidify. Suspensions (100 µl) of target strain previously incubated for 24h were spread on the plates with glass rods. The plates were allowed to stand until inoculum suspensions were completely absorbed (pre-diffusion) and after then incubated at 37°C for 24h. The lowest concentration (highest dilution) of tested agent preventing appearance of growth was examine by the naked eye and is described as MIC. Plates of MHA + inoculation were a positive control and MHA + KFS were negative control (Mazzola et al, 2003).

Time-kill test

After selected MIC of Kombucha fermented solutions, it's incubated with tested pathogenic microorganisms at different periods ranged from 1.5-24h.

Determination of the minimum lethal concentration (MLC)

Minimum bactericidal or fungicidal concentration (MBC or MFC) was recorded as a lowest KFS_s concentration killing 99.9% of the bacterial or fungal inoculate after 24h incubation at 37°C. The MBCs or MFCs were determined by selecting plates or dilutions that exhibited no growth during MIC determination; a portion of agar from each plate was sub-cultured on nutrient and Sabouraud dextrose agar media for bacterial or yeast strains, respectivelythen incubated at 37°C for 24h. The MLC was determined and the concentrations are ≥ MIC (Rabe et al, 2002).

Evaluation of KFS_saction

The action ofKFS_sas antimicrobial agent on the bacterial and fungal strains can be described with ratio of MLC: MIC. The action was microbicidaland microbiostatic effects when the ratio =1 or 2and ≥4, respectively(Berche et al, 1988).

Cytotoxicity and Antiproliferative of KFS_s

Cytotoxicity of KFSs were tested against human carcinoma HCT-116 (colon cancer) and HEpG-2 (liver cancer) cell lines.

Cell Culture

Human carcinoma cell lines were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were grown in complete culture medium (Dulbecco's Modified Eagles Medium (DMEM, SIGMA, USA) with 10% FBS in 5% CO_2 at 37°C under high humidity. The trypan blue dye exclusion test was used to assess cell viability (Siddiqui et al, 2008). Only cells that showed a viability of more than 98% were used in this study.

Neutral red uptake (NRU) assay

An NRU assay was performed according to Siddiqui et al(2010).Post treatment with KFSs, the cells were washed with phosphate-buffered saline (PBS;0.01 M; pH 7.4). Cells were then incubated for 3h in medium supplemented with neutral red (50µg/ml). The cells were then subjected to 3 h of incubation. The supernatant was removed, and the cells were washed with a solution of 0.5% CH_2O and 1% $CaCl_2$. Subsequently, a solution of 1% CH_3COOH and 50% EtOH was added, and the dye was extracted. The plates were then read at a wavelength of 550nm using UV–visible spectrophotometer multiplate reader (Synergy HT, Bio-Tek, USA).

Ethidium Bromide/Acridine Orange Staining (EB/AO staining)

Induction of apoptosis by the KFS_s were investigated with AO/EB staining(Ribble et al, 2005). The cells (2×10^5) were treated with concentration 45 µg/mlof the KFS_s and cultured overnight in a humidified CO_2 incubator at 37°C. The rest of the adherent cells were detached with Trypsin-EDTA (1ml) for 2min. The media and the detached cells from the same sample were pooled together and centrifuged at 1,000 rpm for 5 min. Cell pellets were then resuspended in 25µl cold PBS and 2µl EB/AO dye mix (EB/AO dye mix contained 100µg/ml of each dye). Stained cell suspensions (10µl) were visualized using an Olympus (1X70-S1F2) inverted fluorescence microscope at 100X magnification and photographed using Nikon D700 camera.

Statistical analysis

Data were expressed as means, standard deviations, Student's t-test and using IBM® SPSS® Statistics software (2011). The significance of difference was considered to include values of $P < 0.05$.

RESULTS AND DISCUSSION

Influence of KFS_s on pathogenic microorganism

Three types of Kombucha fermented solutions produced from tea (KTFS), rice (KRFS) and barely (KBFS) were tested as antimicrobial activity against genus Staphylococcus, Listeria and Candida (as $Gr^{+ve}$and Salmonella, Citrobacter, Klebsiella, Escherichia, andShigella (as Gr^{-ve}).

Table 1: Inhibition of pathogenic bacteria and yeast growth by Kombucha fermented solutions (KFS_s) produced from tea, rice and barley during different fermentation times.

Pathogenic bacteria and yeast	KTFS					KBFS					KRFS				
	(days)														
	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
Staph. aureus 1	++	+	±	-	-	++	++	+	-	-	++	++	+	-	-
Staph. aureus 2	++	+	±	-	-	++	++	+	-	-	++	++	+	-	-
S. enteritidis	++	+	+	-	-	++	+	+	-	-	++	+	±	-	-
Citrobactersp.1	+	+	+	-	-	++	+	+	-	-	++	+	±	-	-
Citrobactersp. 2	+	+	+	-	-	++	+	+	-	-	++	+	±	-	-
K. pneumoniae 1	++	+	+	-	-	++	+	±	-	-	++	+	+	-	-
K. pneumoniae 2	++	+	+	-	-	++	+	+	-	-	++	+	+	-	-
K. pneumoniae 3	++	+	+	-	-	++	+	+	-	-	++	+	+	-	-
K. pneumoniae 4	+	+	+	-	-	++	+	+	-	-	++	++	+	-	-
L.monocytogenes1	+	+	±	-	-	++	++	+	-	-	++	++	+	-	-
L. monocytogenes2	+	+	±	-	-	++	++	+	-	-	++	++	+	-	-
L. monocytogenes3	+	+	±	-	-	++	++	+	-	-	++	++	±	-	-
E. coli 1	+	±	±	-	-	++	+	+	-	-	++	++	±	-	-
E. coli 2	+	±	±	-	-	++	+	+	-	-	++	++	+	-	-
E. coli 3	+	±	±	-	-	++	+	+	-	-	++	++	+	-	-
S. dysenteriae1	+	±	±	-	-	++	+	±	-	-	++	+	+	-	-
S. dysenteriae2	+	+	+	-	-	++	++	+	-	-	++	++	+	-	-
C. albicans	+	+	+	-	-	++	++	+	-	-	++	++	+	-	-

-= No growth, ± = Very Scanty growth, + = Scanty growth, ++ = Moderate growth, KTFS= Kombusha tea fermented solution, KRFS = Kombusha rice fermented solution, KBFS= Kombusha barley fermented solution.Staph= Staphylococcus, S. enteritidis= Salmonella enteritidis, K. =Klebsiella, L.= Listeria, S. dysenteriae= Shigelladysenteriae & C.= Candida.

Table 2: Inhibition zone diameter (IZD) of pathogenic bacteria and yeast influence with kombusha fermented solutions after 8-10 days of fermentation period and compared with acetic acid concentrations and biotin as antibiotic.

Pathogenic bacterial and yeast	Antibiotic (mg/ml)	AA conc. (mg/ml) as presented in			Treatments					
		KTFS	KBFS	KRFS	KTFS		KBFS		KRFS	
		6.70	5.12	4.34	8	10	8	10	8	10
Staph. aureus 1	29^{cd}±0.08	29^{cd}±0.04	28^{cde}±0.04	20^{h}±0.11	32^{c}±0.13	30^{cd}±0.25	26^{e}±0.11	25^{ef}±0.14	25^{ef}±0.03	23^{fgh}±0.08
Staph. aureus 2	28^{cde}±0.15	29^{cd}±0.13	24^{f}±0.10	21^{gh}±0.09	30^{d}±0.20	28^{cde}±0.35	20^{h}±0.02	21^{gh}±0.17	25^{ef}±0.11	25^{ef}±0.08
S. enteritidis	30^{cd}±0.14	33^{c}±0.04	22^{fgh}±0.13	24^{f}±0.21	38^{a}±0.10	37^{a}±0.11	18^{i}±0.10	19^{hi}±0.09	23^{fgh}±0.10	22^{fgh}±0.02
Citrobactersp.1	30^{cd}±0.18	29^{cd}±0.01	28^{cde}±0.04	20^{h}±0.07	35^{b}±0.11	32^{c}±0.13	25^{ef}±0.08	25^{ef}±0.13	25^{ef}±0.04	23^{fgh}±0.16
Citrobactersp. 2	21^{gh}±0.11	21^{gh}±0.01	21^{gh}±0.01	16^{j}±0.03	21^{gh}±0.03	20^{h}±0.07	20^{h}±0.12	21^{gh}±0.22	19^{hi}±0.07	20^{h}±0.18
K. pneumoniae 1	19^{hi}±0.07	19^{hi}±0.05	18^{i}±0.08	14^{jk}±0.12	13^{jkl}±0.05	10^{l}±0.11	12^{k}±0.02	10^{l}±0.07	11^{kl}±0.11	10^{l}±0.23
K. pneumoniae 2	24^{f}±0.18	20^{h}±0.11	20^{h}±0.04	15^{j}±0.04	10^{l}±0.07	8^{m}±0.08	8^{m}±0.07	9^{lm}±0.12	9^{lm}±0.11	9^{lm}±0.17
K. pneumoniae 3	23^{fgh}±0.14	24^{f}±0.08	22^{fgh}±0.03	18^{i}±0.16	23^{fgh}±0.03	23^{fgh}±0.09	20^{h}±0.13	17^{i}±0.18	18^{i}±0.04	16^{j}±0.08
K. pneumoniae 4	28^{cde}±0.19	26^{e}±0.02	24^{f}±0.07	21^{gh}±0.04	24^{f}±0.13	24^{f}±0.18	20^{h}±0.08	21^{gh}±0.20	18^{i}±0.07	15^{j}±0.13
L. monocytogenes1	21^{gh}±0.07	27^{e}±0.06	20^{h}±0.02	23^{fgh}±0.03	25^{ef}±0.06	25^{ef}±0.16	12^{k}±0.06	13^{k}±0.11	19^{hi}±0.13	19^{i}±0.24
L. monocytogenes2	26^{e}±0.08	25^{ef}±0.05	21^{gh}±0.13	17^{i}±0.01	20^{h}±0.02	18^{i}±0.07	15^{j}±0.03	16^{j}±0.10	18^{i}±0.03	19^{hi}±0.17
L. monocytogenes3	26^{e}±0.18	26^{e}±0.03	22^{fgh}±0.08	21^{gh}±0.07	20^{h}±0.09	20^{h}±0.12	18^{i}±0.04	18^{i}±0.13	20^{h}±0.12	19^{i}±0.25
E. coli 1	13^{k}±0.11	17^{i}±0.01	18^{i}±0.03	16^{j}±0.12	24^{f}±0.11	22^{fgh}±0.14	20^{h}±0.03	21^{gh}±0.21	17^{i}±0.08	17^{ij}±0.22
E. coli 2	17^{i}±0.14	27^{e}±0.01	25^{ef}±0.05	11^{l}±0.08	28^{cde}±0.04	25^{ef}±0.07	23^{fgh}±0.08	21^{gh}±0.05	10^{l}±0.02	8^{m}±0.03
E. coli 3	19^{hi}±0.05	25^{f}±0.23	21^{gh}±0.13	14^{jk}±0.11	28^{cde}±0.03	26^{e}±0.08	14^{jk}±0.03	15^{j}±0.12	15^{j}±0.13	12^{k}±0.24
S. dysenteriae1	20^{h}±0.19	28^{cde}±0.09	20^{fgh}±0.02	17^{i}±0.09	20^{h}±0.11	19^{i}±0.20	15^{j}±0.03	13^{k}±0.25	17^{i}±0.08	15^{j}±0.03
S. dysenteriae2	12^{kl}±0.14	15^{j}±0.01	15^{j}±0.03	21^{gh}±0.12	12^{kl}±0.11	13^{jkl}±0.22	12^{kl}±0.03	10^{l}±0.18	20^{h}±0.13	20^{h}±0.06
C.albicans	17^{i}±0.15	26^{e}±0.07	14^{jk}±0.03	12^{kl}±0.14	10^{l}±0.13	8^{m}±0.25	11^{l}±0.03	10^{l}±0.11	9^{lm}±0.04	9^{lm}±0.16

Antibiotic=hibiotic (mg/ml); AA= acetic acid, Conc.= concentration, 8 and 10= fermented solution period; KTFS= Kombusha tea fermented solution, KRFS = Kombusha rice fermented solution, KBFS= Kombusha barley fermented solution. ± Standard deviation (SD). Values in the same column (followed by letters with aliphatic series) sharing the same letters do not differ significantly whereas the values followed letters in different alphabetic series are significantly different according to Duncan (1975) at 5% level.Staph= Staphylococcus, S. enteritidis= Salmonella enteritidis, K.=Klebsiella, L.= Listeria, S. dysenteriae= Shigelladysenteriae& C.= Candida.

Table 3: Minimum inhibition concentration (MIC) of Kombucha fermented solutions produced after 8 days' fermentation period on pathogenic bacteria and yeast.

Pathogenic bacteria and yeast	MIC (Dilution tested of Kombusha)																							
	KTFS								KBFS								KRFS							
	1/2	1/5	1/10	1/25	1/50	1/100	1/125	Cont.	1/2	1/5	1/10	1/25	1/50	1/100	1/125	Cont.	1/2	1/5	1/10	1/25	1/50	1/100	1/125	Cont.
Staph. aureus 1	-	-	-	-	-	-	+	-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	+	-
Staph. aureus 2	-	-	-	-	-	-	+	-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	+	-
S. enteritidis	-	-	-	-	-	-	+	-	-	-	-	-	-	++	++	-	-	-	-	-	-	-	++	-
Citrobactersp.1	-	-	-	-	-	-	+	-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	+	-
Citrobactersp. 2	-	-	-	-	-	-	+	-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	+	-
K. pneumoniae 1	-	-	-	-	±	+	+	-	-	-	-	-	+	+	++	-	-	-	-	-	+	+	++	-
K. pneumoniae 2	-	-	-	-	±	+	+	-	-	-	-	-	±	+	++	-	-	-	-	-	+	+	++	-
K. pneumoniae 3	-	-	-	-	±	+	+	-	-	-	-	-	±	+	++	-	-	-	-	-	+	+	++	-
K. pneumoniae 4	-	-	-	-	±	+	++	-	-	-	-	-	±	±	++	-	-	-	-	-	+	+	++	-
L. monocytogenes1	-	-	-	-	-	-	++	-	-	-	-	-	-	++	++	-	-	-	-	-	-	-	++	-
L. monocytogenes2	-	-	-	-	-	-	++	-	-	-	-	-	-	++	++	-	-	-	-	-	-	-	++	-
L. monocytogenes3	-	-	-	-	-	-	++	-	-	-	-	-	-	++	++	-	-	-	-	-	-	-	++	-
E. coli 1	-	-	-	-	±	++	++	-	-	-	-	-	±	±	+	-	-	-	-	-	±	++	++	-
E. coli 2	-	-	-	-	±	++	++	-	-	-	-	-	±	±	+	-	-	-	-	-	±	++	++	-
E. coli 3	-	-	-	-	±	++	++	-	-	-	-	-	±	±	+	-	-	-	-	-	±	++	++	-
S. dysenteriae1	-	-	-	-	±	++	++	-	-	-	-	-	±	+	+	-	-	-	-	-	±	++	++	-
S. dysenteriae2	-	-	-	-	±	++	+++	-	-	-	-	-	+	+	++	-	-	-	-	-	±	++	+++	-
C. albicans	-	-	-	+	++	++	++	-	-	-	-	+	+	++	++	-	-	-	-	+	++	++	++	-
Spectrum of activity (%)	18/18	18/18	18/18	17/18	8/18	8/18		18/18	18/18	18/18	18/18	17/18	8/18			18/18	18/18	18/18	18/18	17/18	8/18	8/18		18/18
	100	100	100	94	44.4	44.4		100	100	100	100	94	44.4			100	100	100	100	94	44.4	44.4		100

-= No growth, ± = Very Scanty growth, + = Scanty growth, ++ = Moderate growth, Cont.= Control (100%) Kombusha fermented solutions before diluted, KTFS= Kombusha tea fermented solution, KRFS = Kombusha rice fermented solution, KBFS= Kombucha barley fermented solution.Staph= Staphylococcus, S. enteritidis= Salmonella enteritidis, K.=Klebsiella, L.= Listeria, S. dysenteriae= Shigelladysenteriae& C.= Candida.

Table 4: Time-kill test of Kombucha fermented solutions on different pathogenic microorganisms after exposure of different periods during 24 h.

Pathogenic bacteria and yeast	Time of Exposure (h)																					
	KTFS							KBFS							KRFS							
	1.5	3	6	9	12	15	24	1.5	3	6	9	12	15	24	1.5	3	6	9	12	15	24	
Staph. aureus 1	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	
Staph. aureus 2	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	
S. enteritidis	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	
Citrobactersp.1	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
Citrobactersp. 2	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
K. pneumoniae 1	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
K. pneumoniae 2	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
K. pneumoniae 3	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
K. pneumoniae 4	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
L. monocytogenes1	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	
L. monocytogenes2	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	

L. monocytogenes3	+	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-
E. coli 1	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
E. coli 2	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
E. coli 3	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
S. dysenteriae1	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
S. dysenteriae2	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
C. albicans	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-

-= No growth, + = growth, KTFS= Kombusha tea fermented solution, KRFS = Kombusha rice fermented solution, KBFS= Kombusha barley fermented solution.Staph= Staphylococcus, S. enteritidis= Salmonella enteritidis, K.=Klebsiella, L.= Listeria, S. dysenteriae= Shigelladysenteriae& C.= Candida.

Table 5: Minimum lethal concentration (MLC) of Kombucha fermented solutions on various Gram positive and negative microorganisms.

Pathogenic bacteria and yeast		**Dilution tested of Kombusha**																	
		KTFS						**KBFS**						**KRFS**					
		1/2	**1/5**	**1/10**	**1/25**	**1/50**	**1/100**	**1/2**	**1/5**	**1/10**	**1/25**	**1/50**	**1/100**	**1/2**	**1/5**	**1/10**	**1/25**	**1/50**	**1/100**
Gr^{+ve}	Staph. aureus 1	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	Staph. aureus 2	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	L. monocytogenes1	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	L. monocytogenes2	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	L. monocytogenes3	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	C. albicans	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Gr^{-ve}	S. enteritidis	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	Citrobactersp.1	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	Citrobactersp. 2	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	K.pneumoniae 1	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	K. pneumoniae 2	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	K.pneumoniae 3	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	K.pneumoniae 4	-	-	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
	E. coli 1	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+
	E. coli 2	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+
	E. coli 3	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+
	S. dysenteriae1	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+
	S. dysenteriae2	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+

-= No growth, + = Growth. Staph= Staphylococcus, S. enteritidis = Salmonella enteritidis, K.=Klebsiella, L.= Listeria, S. dysenteriae= Shigelladysenteriae& C.= Candida.

Table 6: Bacterio/fungi-static (-) and Bacter/fung-icidal (+) effects of Kombucha fermented solutions.

Pathogenic bacteria and yeast	KTFS				KBFS				KRFS			
	MIC (mg/ml)	MLC (mg/ml)	MLC/ MIC	Effect	MIC (mg/ml)	MLC (mg/ml)	MLC/ MIC	Effect	MIC (mg/ml)	MLC (mg/ml)	MLC/ MIC	Effect
Staph. aureus 1	0.068	0.068	1	+	0.1028	0.2056	2	+	0.046	0.046	1	+
Staph. aureus 2	0.068	0.068	1	+	0.1028	0.2056	2	+	0.046	0.046	1	+
S. enteritidis	0.068	1.36	20	-	0.1028	2.57	25	-	0.046	0.92	20	-
Citrobactersp.1	0.068	1.36	20	-	0.1028	2.57	25	-	0.046	0.92	20	-
Citrobactersp. 2	0.068	1.36	20	-	0.1028	2.57	25	-	0.046	0.92	20	-
K. pneumoniae 1	0.272	1.36	5	-	0.2056	2.57	12.5	-	0.184	0.92	5	-
K. pneumoniae 2	0.272	1.36	5	-	0.2056	2.57	12.5	-	0.184	0.92	5	-
K. pneumoniae 3	0.272	1.36	5	-	0.2056	2.57	12.5	-	0.184	0.92	5	-
K. pneumoniae 4	0.272	1.36	5	-	0.2056	2.57	12.5	-	0.184	0.92	5	-
L. monocytogenes1	0.068	0.68	10	-	0.1028	0.514	5	-	0.046	0.46	10	-
L. monocytogenes2	0.068	0.68	10	-	0.1028	0.514	5	-	0.046	0.46	10	-
L. monocytogenes3	0.068	0.68	10	-	0.1028	0.514	5	-	0.046	0.46	10	-
E. coli 1	0.272	1.36	5	-	0.2056	1.028	5	-	0.184	2.3	12.5	-
E. coli 2	0.272	1.36	5	-	0.2056	1.028	5	-	0.184	2.3	12.5	-
E. coli 3	0.272	1.36	5	-	0.2056	1.028	5	-	0.184	2.3	12.5	-
S. dysenteriae1	0.272	1.36	5	-	0.2056	1.028	5	-	0.184	2.3	12.5	-
S. dysenteriae2	0.272	1.36	5	-	0.2056	1.028	5	-	0.184	2.3	12.5	-
C. albicans	0.68	1.36	2	+	0.514	1.028	2	+	0.46	0.92	2	+

Bactericidal (+)=≤2 and Bacteriostatic (-) effect= ≥4. Staph= Staphylococcus, S. enteritidis = Salmonella enteritidis, K.=Klebsiella, L.= Listeria, S. dysenteriae= Shigelladysenteriae& C.= Candida.

Table 7: In vitro growth inhibitory activity—IC_{50} values (µg/mL) of the KFS_s againstHCT-116 (human colon carcinoma cells) and HEpG-2G-2 (liver HEpG-2atocellular cells).

Sample	*IC_{50} (µg/ml)	
	HCT-116	HEpG-2G-2
Kombucha tea (KTFS)	46.3	46.7
Kombucha Rice (KRFS)	162.19	115.8
Kombucha barley (KBFS)	161.3	266.0

*IC_{50} (µg/ml) = 50% inhibition of cell growth.

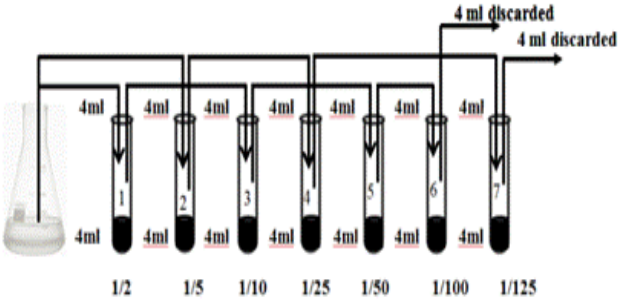

Figure 1: Illustrative scheme of successive dilutions method.

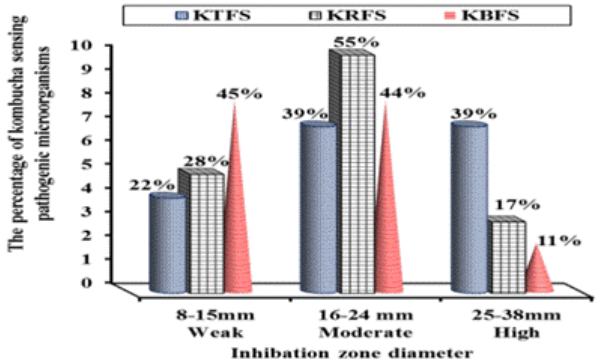

Figure 2: The percentage distribution of Kombucha sensing pathogenic microorganisms isolates into three categories that express as diameter of zone inhibition (mm). KTFS= Kombusha tea fermented solution, KRFS = Kombusha rice fermented solution, KBFS= Kombusha barley fermented solution.

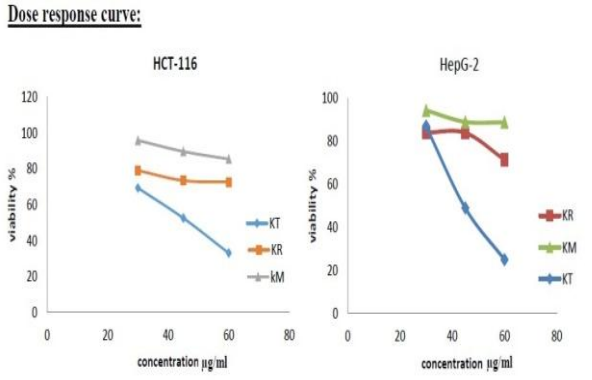

Figure 3: Cell viability from NRU cytotoxicity assay after 48h exposure of human colon carcinoma cells (HCT-116) and human liver carcinoma (HEpG-2G-2) cells exposed to KTFS, KRFS and KBFS.

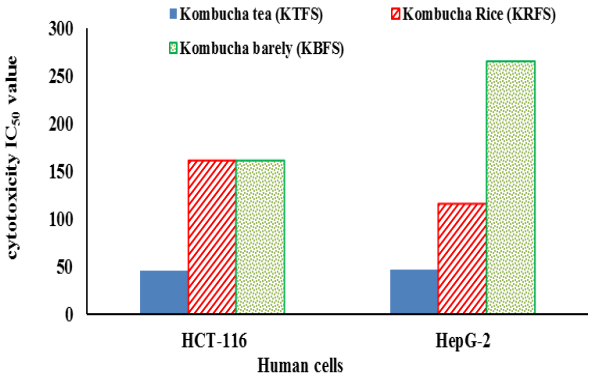

Figure 4: In vitro cytotoxicity IC_{50} value of KTFS, KRFS and KBFS exposed to HCT-116 and HEpG-2G-2 human cells line as determined by Neutral Red Uptake Assay.

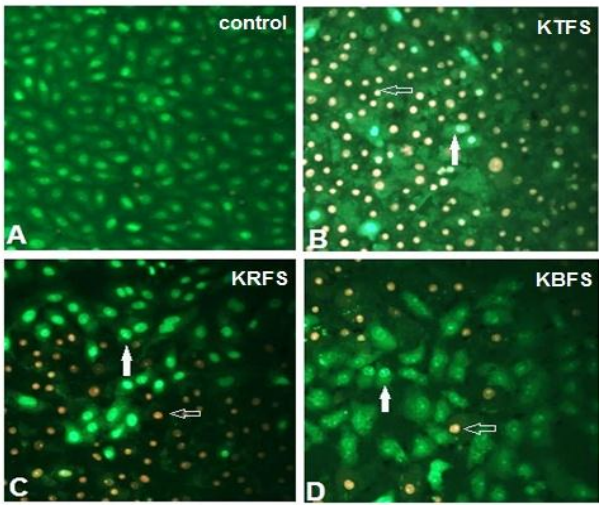

Figure 5: Fluorescent microphotograph of HEpG-2 cells treated with 45µg/mlKFS$_s$, stained with AO/EB. (a) control cells show a uniform green fluorescence and (B, C, and D) cells treated with KFS_s appeared green with bright green nuclei indicating nuclear fragmentation and early apoptotic cells; also, cells show late apoptotic cells by the orange red appearance due to the incorporation of both ethidium bromide and acridine orange (bold arrow) and (regular arrows) indicate early and late apoptotic cells respectively. (Magnification 400X).

Data in Table 1 showed that all tested pathogenic bacteria and yeast were resistant to all tested KFSs during fermentation times ranged from 2:6 days, which gave growth density on disc-agar diffusion plates ranged from moderate (++) to very scanty (±) growth. At fermentation times ranged from 8-10 days, all tested KFS_s were inhibited the growth of all tested pathogenic strains (which gave inhibition zone). So, KTFS, KRFS and KBFS during 8:10 days of fermentation period were selected as antimicrobial activity for further studies. In addition to, theseveral Kombucha teas inhibited the pathogenic bacteria in human and shrimp belong to species of Salmonella enteritidis, Pseudomonas aeruginosa, Vibriocholeraand V. vulnificus and the IZD

increased gradually with increase fermentation period of Kombucha from 0-14 days (Talawat et al, 2006). Moreover, both Kombucha green and black teas gave activities against all the $Gr^{+ve}$and Gr^{-ve} bacteria include; S. epidermidis, S. aureus, M.luteus, E. coli, S. typhimuriumand L.monocytogenes(Deghrigue et al, 2013; Battikh et al, 2012).

Results in Table 2 clearly show that tested 3 types of KFS_swere high inhibition activities for tested pathogenic microorganisms as compared with hibiotic and acetic acid. The IZD was ranged from 8 to 38, 25 or 26 mm of KTFS, KRFS or KBFS during 8-10 days fermentation periods, respectively. Whereas acetic acid (as chemical killer) and hibiotic (as antibiotic) were achieved the antimicrobial activities ranged from 15-33mm and 12-30mm, respectively. Also, results represented that KTFS was preferred than KRFS or KBFS, which gave the highest antimicrobial activity against of S.enteritidis(38mm) followed by Citrobactersp.1 (35 mm), S. aureus1 (32mm) and S. aureus2 (30 mm). While, KRFS gave the maximum activity against of S.aureus1, Citrobactersp.1 and S. aureus2 being 25 mm of IZD. In case of KBFS, the highest IZD was observed with S.aureus 1 (26 mm), then Citrobactersp.1 (25mm) and E. coli 2 (23mm).

No significant difference between KFS_sproduced after 8 days until 10 days, so it was selected the 8 days for reduce the time and cost of Kombucha production. The tested pathogenic microorganisms were classified into 3 categories (high, moderate and weak) according to IZD (sensibility of KTFS, KRFS and KBFS) ranged from 25-38 mm, from 16-24 mm and from 8-15 mm, respectively (Figure 2). In first category (high IZD), the number of pathogenic bacteria with high sensitivity to KTFS, KRFS and KBFS being 7, 3 and 2 represented about 39, 17 and 11% of all tested pathogenic organisms, respectively. While in second category consisted of a high number of these tested organisms were recorded a moderate sensitivity to all tested KFS_s. These results are accordance with Jayabalan et al (2014) they observed that Kombucha with high antimicrobial efficiency against pathogenic microorganisms of both Gram-positive and Gram-negative origin. Acetic acid and catechins are known to inhibit a number of $Gr^{+\&-ve}$ microorganisms (Sreeramulu et al, 2000). Moreover, the IZD of green fermented tea was ranged from 12-22mm, while it varied from 10.5-19mm for black fermented tea(Battikh et al, 2012). So, it was found that Kombucha exhibited its strongest antimicrobial effect against S. epidermidis, M. luteus, L. monocytogenes and P. aeruginosa (IZD $\geq$ 18 mm). From above results, it could be stated that the KFS_s produced after 8 days were selected for further investigation. Also, it was observed that the 4-pathogenic bacteria S. enteritidis, Citrobactersp.1, S. aureus1 and S. aureus2 were high susceptibility to KTFS more than acetic acid and hibiotic due to Kombucha tea is largely attributable to the presence of organic acids, particularly acetic acid, large proteins, catechins and antibiotics (Jayabalan et al, 2007; Veličanski et al, 2007; Chen and Liu, 2000). In addition, the fermented tea with antibacterial feature, it might due firstly to organic acids and ethanol formation and secondly to polyphenolic compounds within the tea (Talawat et al, 2006). Also, the metabolites produced by the bacteria and/or yeasts during the fermentation of Kombucha tea are responsible for its antimicrobial activity (Sreeramulu et al, 2001).

Evaluation of MIC of tested KFS_s

Results in Table 3 demonstrated that the antimicrobial spectra activity of tested KTFS, KRFS and KBFS at dilution ranged from 1/2- 1/10 were 100% and dilutions of 1/25, 1/50 and 1/100 were exhibited activity with 94.0%, 44.4% and 44.4% against the tested pathogenic microorganisms, respectively, except KBFS without spectrum activity at 1/100 dilution. Also, data recorded that the MIC values ranged from 0.0544~3.4 mg/ml, 0.04112~2.57 mg/ml and 00.0368~2.3mg/ml for tested KTFS, KRFS and KBFS, respectively. Both KTFS and KRFS at 1/100 dilution (with MIC 0.068 & 0.046mg/ml, respectively) and KBFS at 1/50 dilution (with MIC 0.1028mg/ml) had a bacteriostatic effect on genus of Staphylococcus, Salmonella, Citrobacter and Listeria. Meanwhile, Klebsiella, Eschericha and Shigellagenus (Gr^{-ve}) were inhibited at 1/25 dilution (with MIC 0.272, 0.2056&0.184mg/ml) of KTFS, KBFS and KRFS, respectively. While, Candida was inhibited at low dilution (1/10) with high concentrations of all KFSs (0.68, 0.514 and 0.46 mg/ml of KTFS, KBFS and KRFS, respectively) and stimulated in high dilutions. Similarly, MIC of both Kombucha green and black teas were 150, 228, 280&336 µg/ml against E. coli,P. aeruginosa, S. aureus and S. typhimurium, respectively (Deghrigue et al, 2013).The tested pathogenic microorganisms were incubated with the selected MIC of KFSs at different periods ranged from 1.5-24 h incubation period.

Results in Table 4 represented that the first exposure periods of each KFS_s (1.5 and 3h) were no bacteriostatic effect on all pathogenic bacteria and yeast. The tested KFS_swere bacteriostatic effect after 6 h exposure period for both genera of Escherichia and Shigella, 9h for both genus of Staphylococcus and Salmonella and 12 h for each genus of Citrobacter, Klebsiella and Listeria. While Candida albicans took a long time in inhibition was reached to 15 h of exposure period. Generally, it could be noticed that the tested pathogenic microorganisms were inhibited with minimum concentration of KFS_s ranged from 0.68:0.068, 0.514:0.0514 and 0.46:0.046mg/ml during 6-15 h of exposure for KTFS, KBFS and KRFS, respectively.

MLC of KFS_S

Results recorded in Table 5 demonstrated that all tested KFS_S had inhibitor effect on all tested microorganisms. Both strains of S. aureus were high sensitivity to KFS_S athigh dilutions (with low concentrations) and no growth appeared when re-subculture on agar medium. The MLC

was 0.068 and 0.046mg/ml (at dilution of 1/100) of KTFS and KRFS and 0.2056mg/ml (at dilution of 1/25) of KBFS. Whereas, in L. monocytogenes1- 3, the 100% kill were appeared with MLC ranged from 0.46-0.68mg/ml at 1/10 dilution of all tested KFS_S. However, in case of C. albicans (Gr^{+ve}), and other Gr^{-ve} bacteria (S. enteritidis, Citrobactersp.1&2, K. pneumonia 1-4, E. coli1-3 and S. dysenteriae1&2), the 100% kill were achieved at low dilution ranged from 1/2-1/5 (at high concentrations)of all tested KFS_S, which MLC recorded 1.36mg/ml of KTFS, ranged from 1.028mg/ml (against genera Escherichia and Shigella) to 2.57mg/ml (against genera Candida, Salmonella, Citrobacter and Klebsiella) of KBFS and ranged from 0.92mg/ml (against genera Salmonella, Citrobacter and Klebsiella) to 2.3mg/ml(against genera Candida, Escherichia and Shigella) of KRFS. So, these genera were stimulated when cultivated on agar medium without substance at high dilution with low concentrations.

Mode action of KFS_S

Results represented in Table 6 clearly showed that action effect of KFS_S against pathogenic strains. The actin effect was calculated as the ratio of MLC: MIC. It was found that all tested KFS_S had bacter/funal-icidal effect against both strains of S. aureus1&2and C. albicans (Gr^{+ve}) which the ratio MLC: MIC was ranged from 1:2. While KFS_S were bacteriostatic effects against other tested strains. Kombuchafrom lemon balm and black teas had higher bactericidal effects towardP. mirabilis,S. aureus and Bacillus sp. (Veličanski et al, 2007; Greenwalt et al, 1998).

Evaluation of cytotoxicity and antiproliferative activity against tumor cell lines

Anti-proliferation and induction of apoptosis by the tested KFS_s were studied using NRU and EB/AO staining assays against HCT-116 and HEpG-2over a concentration range (30-60µg/ml).

Results presented in Table 7 and Figure 3 displayed a marked decrease in metabolic activity in cell viability in a concentration dependent manner exposed to different KFS_S. The results clarify the different response in cytotoxicitywith different KFS_S where, KTFS found to be more cytotoxic than KRFS and KBFS either on HCT-116 or HEpG-2cell lines. Maximum reduction in cell viability at 60µg/mlwas found to be 25% for HEpG-2exposed to KTFS. Regarding to the cytotoxicity of KRFS and KBFS, both beverages exhibited nearly similar inhibitory effects on HCT-116 tumor cells but lower than KTFS. Meanwhile, treatment of HEpG-2tumor cells exhibited, KBF_S was less active than KTFS and KRFS. Comparing the cytotoxic efficacy of KTFS in HCT-116 colon cancer the result showed that the highest cytotoxic is observed with IC_{50} 46.3µg/ml Meanwhile, KRFS and KBFS exhibited nearly similar inhibitory effects less than KTFS with IC_{50} values of 162.19 and 161.3µg/ml, respectively. In liver cancer HEpG-2, the higher cytotoxic activity was observed with KTF_S with IC_{50} value, 46.7µg/ml while the lowest cytotoxic activity was observed with KBFS (with IC_{50} value 266µg/ml). Moreover, KRFS exhibited moderate inhibitory effects with IC_{50} value 115.8µg/ml. Data from dose–response curve indicated that, the concentrations 30, 45 and 60µg/ml of KTF_S could produce highest inhibitory effects against HCT-116; with value 30.7, 47.5 and 67%, respectively. Meanwhile, KRFS produced inhibitory values; 21, 26.61 and 27.4%, respectively. While the effect of the same concentrations of KBFS produced lower inhibitory effects; 4.25, 10.4 and 14.63%, respectively (Table 7 and figure 4).Determination of the antitumor activities of KFS_s against the different tumor cells indicated that KTFS has more antitumor activity than KRFS and KBFS. Although all tested KFS_s were found to reduce the cell viability in a concentration manner, but the magnitude of reduction was high in case of KTFS. Many researchers have investigated the antiproliferative properties of KFS_s because it might be contained several compounds were identified as polyphenols, organic acids and vitamins which reduces stomach cancer (Cushnie and Lamb, 2005; Taguri et al, 2004; Bauer-Petrovska and Petrushevska-Tozi, 2000) and D-saccharic acid-1, 4-lactone, which inhibits the activity of glucuronidase, an enzyme indirectly related with cancers (Wang et al, 2010). The antitumor properties of polyphenols which might act as cancer-blocking agents (Russo, 2007). In addition, KTFS decreased the survival of prostate cancer cells by down regulating the expression of angiogenesis stimulators (Srihari et al, 2013).

Fluorescence microscopic analysis of cell death by EB/AO staining

Treatment of KFS_S led to induction of apoptosis, where, KTFS (45µg/ml) exhibited apoptotic signal appears to be stronger than KRFS and KBFS against HEpG-2 cell line (Figure 5).KTFS showed increased percentages of late apoptotic cells than KRFS and KBF_Swhich had stained orange attributed to incorporation of both ethidium bromide and acridine orange (Figure 5B-C). From these result, it can conclude that the KFS_S fermented solution has cytocidal or cytostatic phenotype.Apoptotic cells were increased after treatment with KFS_S compared to control, suggesting that apoptosis could contribute to kill cancers cells via apoptosis. The figures show the potential of the KFS_S in inducing apoptosis in the HEpG-2cells by demonstrating the morphological changes characteristic to apoptosis such as cell shrinkage, nuclear and cytoplasmic condensation and formation of apoptotic bodies as compared to the negative control. Live cells displayed a uniformly green fluorescence which had regular, round-shaped nuclei in control (Figure 5A). The early apoptotic cells were observed at a concentration of 45µg/ml, which the cells were still green in color with bright green dots in their nuclei corresponding to nuclear fragmentation (Figure 5; bold arrow). The chemo preventive action of KTFS might be due to its ability to induce apoptosis. The AO/EB and the NRU assays showed similar effects on viability with different KFS_s.

According to obtained results it can concluded that KTFS has strong antiproliferative properties which increase with concentration. The KTFS exhibits best antiproliferative activity than the KRFS and KBFS extract with lower IC_{50} values, and induces a higher percentage of apoptotic cells after 24h of exposure. Scientific studies have claimed that Kombucha has anticancer effects as well (Dufresne and Farnworth, 2000) due to the presence of tea polyphenols and the secondary metabolites produced during the fermentation process (Jayabalan et al, 2014; Jayabalan et al, 2011).

CONCLUSIONS

Kombucha fermented solutions (KFS_S) had microbicidal impact against S. aureusand C. albicans and microbiostatic effect against other pathogenic bacteria. Also, it have capable of reducing cancer cell proliferation andinduced apoptotic cell death and may have a promising role to play in the development of new anticancer drugs in the future.

CONFLICTS OF INTEREST

All authors certify that this manuscript has not been published in whole or in part nor is it being considered for publication elsewhere. The authors have no conflicts of interest to declare.

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