

Full Length Research Paper

Antiproliferative and antimicrobial activities of kombucha tea

Monia DEGHRIGUE^{1*}, Jihene CHRIAA¹, Houda BATTIKH¹, Kawther ABID² and Amina BAKHROUF¹

¹Laboratoire d'Analyse, Traitement et Valorisation des Polluants de l'Environnement et des Produits, Faculté de Pharmacie, Rue Avicenne, 5000 Monastir, Tunisie.

²Laboratoire de Biochimie, Faculté de Médecine, Monastir, Tunisie.

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The present study was conducted to evaluate the antiproliferative and antimicrobial activities of kombucha tea. We investigated *in vitro* the efficacy of kombucha tea for its antiproliferative effects by its potential cytotoxic activity using the MTT colorimetric method against two human cancer cell lines (A549, lung cell carcinoma and Hep-2, epidermoid carcinoma). Treatment of human cancer cell lines A549 and Hep-2 with Kombucha tea showed a dose dependant inhibition of growth as a result of cytotoxicity. The antimicrobial activity of kombucha tea against a spectrum of Gram-positive and Gram-negative organisms is also reported. Kombucha tea demonstrated an antimicrobial activity against *Escherichi coli*, *Salmonella enterica serovar typhimurium*, *Micrococcus luteus*, and *Staphylococcus epidermidis* strains. Further investigations are needed to identify the compounds of kombucha tea associated with the antiproliferative and antimicrobial properties.

Key words: Kombucha, antiproliferative, antimicrobial.

INTRODUCTION

Kombucha tea is a traditional fermentation of sweetened tea, involving a symbiosis of yeast species (*Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Zygosaccharomyces rouxii*, *Candida* sp or *Pichia membranaefaciens*) and acetic acid bacteria (*Acetobacter xylinum*, *Acetobacter xylinoides*, *Bacterium gluconicum*) (Chen and Liu, 1997; Teoh et al., 2004). Kombucha tea is composed of two portions: a floating cellulose pellicle layer and the sour liquid broth (Chen and Liu, 2000). Presently, this beverage is popularly consumed around the world as a self-prescribed remedy for numerous ailments (Hartmann et al., 2000). Kombucha tea is claimed to help digestion, give relief from arthritis, prevent microbial infection, help in combating stress, cure cancer and AIDS, and enhance immunity (Ram et al., 2000). Some of these claims effects have been proven; the beverage

does exert antimicrobial activity against a range of bacteria (Greenwalt et al., 1998, 2000; Sreeramulu et al., 2000), inhibits the angiogenesis of prostate cancer cells (Srihali et al., 2013), prevents paracetamol induced hepatotoxicity (Pauline et al., 2001) and chromate (VI) induced oxidative stress in albino rats (Ram et al., 2000), inhibits pancreatic alpha-amylase in the small intestine (Kallel et al., 2012) and the activity of glucuronidase an enzyme indirectly related with cancers (Wang et al., 2010), has potent antioxidant properties such as decrease of the degree of lipid oxidation and DNA fragmentation (Dipti et al., 2003; Ram et al., 2000). 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 tea against two human cancer cell lines: A549 (lung cell

carcinoma) and Hep-2 (epidermoid carcinoma) and its antimicrobial activity against Gram-negative and Gram-positive pathogenic microorganisms.

MATERIALS AND METHODS

Preparation of kombucha tea

6 g of green or black tea was added to 1 L water that was just boiled for 15 min. Then, it was filtered through a sterile sieve and cooled to room temperature. 10% (w/v) sucrose was added to it. The cooled tea was poured into 2 L glass beaker that has been previously sterilized at 121°C for 20 min. It was then inoculated with freshly grown kombucha mat that had been cultured in the same medium for 14 days and 10% (v/v) of previously fermented liquid tea broth. The beaker was covered with clean cheese cloth and fixed with rubber bands. The fermentation was carried out under room temperature (25°C) for 12 days. New kombucha mat developed over the mother culture. Kombucha tea was centrifuged at 10,000 rpm for 15 min and the supernatant was filtered through 0.22 µm sterile microfilter.

Cell culture

The human tumor cell lines: A549 (lung cell carcinoma) and Hep-2 (epidermoid carcinoma) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were routinely grown with DMEM supplemented with 10% fetal calf serum and 1% penicillin/streptomycin; all obtained from Biochrom AG (Berlin, Germany). They were grown on Flasks (Nunc, Denmark) at 37°C in a humidified atmosphere containing 5% CO₂. Cells were replicated every 4-5 days and the medium changed once in-between.

Viability assay

The potential effects on cell viability were investigated according to previously reported conditions using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma-Aldrich Chimie, Saint-Quentin-Fallavier, France] as an indicator of metabolically active cells (Hu and Robert, 1995; Mossmann, 1983; Martine et al., 2008).

Known number of A549 or Hep-2 cells (10³) were transferred into 96-well plates (Nunc, Denmark) in a volume of 200 µl of culture medium and incubated for 24 h before addition of test compounds. Cells were then exposed for 24 h at 37°C to known concentrations of the kombucha tea supernatant to be tested. After drug exposure, the cells were washed with phosphate-buffered saline and then reincubated in fresh culture medium for a further 48 h, then the culture medium was removed and 200 µl of MTT reagent (diluted in culture medium, 0.5 mg/ml) was added. Following incubation for 4 h, the MTT/medium was removed and DMSO (200 µl) was added to dissolve the formazan crystals. Absorbance of the colored solution was measured on a microplate photometre (Bio-Tek Instruments) using a test wavelength of 570 nm and a reference wavelength of 630 nm. Results were evaluated by comparing the absorbance of the treated cells with the absorbance of wells containing cell treated by the solvent control. Conventionally, cell viability was estimated to be 100% in the solvent control. All experiments were performed at least twice in triplicate. The concentration of substance required for 50% growth inhibition (IC₅₀) was estimated as that giving a 50% decrease in absorbance as compared to controls incubated simultaneously without substances.

Antimicrobial activity

The antimicrobial activity of kombucha tea samples was assessed by agar diffusion assay (Mo et al., 2005) against eight human pathogenic bacteria: Gram-positive cocci including *Staphylococcus epidermidis* (Collection Institute Pasteur 106510), *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (NCIMB 8166), *Enterococcus faecalis* (ATCC 29212) and Gram-negative bacilli including *Escherichia coli* (ATCC 35218), *Salmonella enterica serovar typhimurium* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853) and Gram-positive bacilli including *Listeria monocytogene* ATCC 19115. Muller Hinton medium (20 ml) was poured into each Petri dish (90 mm diameter). Suspensions (100 µl) of target strain cultured for 24 h were spread on the plates uniformly, and wells of 9 mm diameter were made with a sterile metal tube by means of a vacuum pump. Kombucha tea samples were centrifuged at 10,000 rpm (Jouan centrifuge, ISO 9001) for 15 min to remove cell debris. Sterile supernatant was obtained by filtering the supernatant through a 0.22 µm sterile microfilter. Sterile samples (100 µl) were then transferred into the wells of agar plates inoculated with target strains. The plates were first put at 4°C for 2 h to make a prediffusion of kombucha tea samples into the agar. The plates were then incubated at 37°C. The diameter of the inhibition zone was measured after 12-15 h. For the purpose of control and comparison, unfermented tea samples at the same concentration as that of kombucha tea after 12 days (6 g/l) were prepared and sterile filtered for antimicrobial test as described above for kombucha tea samples. Standard discs of gentamycin (10 UI) served as positive antibiotic controls according to CASFM 2005 guidelines.

Minimum inhibitory concentration determination

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of kombucha tea (0 to 600 µg/ml) as recommended by the National Committee for Clinical Laboratory Standards Institute (CLSI, 2008).

An overnight culture (37°C) of the tested strains were diluted 10-fold in fresh tryptic soy broth (TSB) and incubated (37°C) until they reached exponential growth phase. Mueller Hinton (MH) Broth (Biorad, France) were prepared in a 96-wells plate (190 µl per well). The inocula (10 µl) containing 5.10⁶ cfu/ml of each reference strain were added to each well and the tested compound. A number of wells were reserved in each plate to test the sterility control of the medium (no inoculum added) and inoculum viability (no compound added).

After incubation for 24 h at 37°C, bacterial growth was evaluated by the presence of turbidity and a pellet on the well bottom. The MIC was defined as the concentration that completely inhibited visible cell growth during a 24 h incubation period at 37°C.

Statistical analysis

Data are presented as the mean ± standard error (SEM). Statistical analysis was assessed using Student's t-test. The significance of difference was considered to include values of P < 0.05.

RESULT

Evaluation of antiproliferative activity against tumor cell lines

Many tests for predicting the response of tumors to cytotoxic drugs have been developed in recent years. The

Table 1. *In vitro* growth inhibitory activity of the kombucha tea against the two human tumor cell lines: A549 (lung cell carcinoma) and Hep-2 (epidermoid carcinoma).

| Sample | IC ₅₀ (µg/ml) | |
|--------------------|--------------------------|---------|
| | A549 | Hep-2 |
| Kombucha green tea | 250±0.6 | 200±0.9 |
| Kombucha black tea | - | 386±0.4 |

IC₅₀, 50% inhibition of cell growth.

Table 2. Antimicrobial activity of Kombucha tea. Inhibition zone calculated in diameter around the disc (mm±SD).

| Bacterial strain | Control (-) unfermented tea | Control (+) gentamycin (10UI) | Kombucha green tea | MIC (µg/ml) | Kombucha black tea | MIC (µg/ml) |
|---|--------------------------------|----------------------------------|-----------------------|----------------|-----------------------|----------------|
| Gram negative bacilli | | | | | | |
| <i>Escherichia coli</i> ATCC 35218 | 0 | 27 | 15.33±0.00 | 150 | 15±0.66 | 150 |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | 0 | 16 | 13.33±0.66 | 228 | 13.33±0.82 | 228 |
| <i>Salmonella enterica</i> serovar typhimurium ATCC 14028 | 0 | 22 | 18±0.33 | 336 | 18.66±0.33 | 336 |
| Gram positive bacilli | | | | | | |
| <i>Listeria monocytogene</i> ATCC 19115 | 0 | 32 | 12.66±0.33 | 243 | 10.33±0.5 | 296 |
| Gram positive cocci | | | | | | |
| <i>Enterococcus faecalis</i> ATCC 29212 | 0 | 22 | 12±0.00 | 225 | 12.33±0.2 | 225 |
| <i>Micrococcus luteus</i> NCIMB 8166 | 0 | 26 | 14±0.66 | 216 | 11±0.7 | 282 |
| <i>Staphylococcus aureus</i> ATCC 25923 | 0 | 22 | 13.66±0.33 | 280 | 14±0.2 | 280 |
| <i>Staphylococcus epidermidis</i> CIP 106510 | 0 | 30 | 14±0.00 | 324 | 10.66±0.3 | 378 |

-, No antimicrobial activity; inhibition zone <1 mm. Standard deviation ± 0.5 mm. For all bacteria, the inhibition zone of the control (+) gentamycin (10 UI) was higher than 9 mm (++++). The diameter of well was 9 mm. MIC, minimum inhibitory concentration.

functional tests comprise the MTT reduction assay. Kombucha tea was tested for its effect on inhibition of cell growth against two human tumor cell lines (A549 and Hep-2) over a concentration range (50-400 µg/ml) to determine their potency (IC₅₀-50% inhibition of cell growth) and the results are shown in Table 1. Assay was performed *in vitro* on exponentially growing cells. The activity was evaluated by measuring the levels of surviving cell after incubation for 24 h with the test samples, using the MTT colorimetric assay (Martine et al., 2008). The results of this primary screening are reported in Figures 1 and 2. Kombucha green tea exhibited a cytotoxic effect. 50% inhibition of cell growth was obtained at concentrations of 250 and 200 µg/ml respectively against human tumor cell lines A549 and Hep-2 (Figure 1). However, kombucha black tea revealed a moderate cytotoxicity against tumor cell lines Hep-2 with IC₅₀ of 386µg/ml and had no effect against A549 cell lines (Figure 2).

Evaluation of antimicrobial activity of kombucha tea

In the present study, the antimicrobial activity of

kombucha tea was screened against various pathogens *in vitro* by agar diffusion method. The results are presented in Table 2. Gentamycin and unfermented teas were used as controls. Unfermented tea had no antimicrobial activity against target microorganisms. Gentamycin was inhibitory toward all of the bacteria. Both Kombucha tea had a higher antimicrobial effect against Gram-negative bacteria (*E.coli* at 150 µg/ml and *S.t yphimurium* at 336 µg/ml) and a moderate antimicrobial activity against Gram-negative bacteria (*P. aeruginosa* at 228 µg/ml). However, only kombucha green tea had an activity against Gram-positive bacteria (*M. luteus* at 216 µg/ml, *S.aureus* at 280 µg/ml and *S.epidermis* at 324 µg/ml).

DISCUSSION

The present study was conducted to evaluate the anti-proliferative and antimicrobial activities of kombucha tea. Many researchers have investigated the antiproliferative properties of kombucha tea. Several compounds were identified as polyphenols (Cushnie and Lamb, 2005;

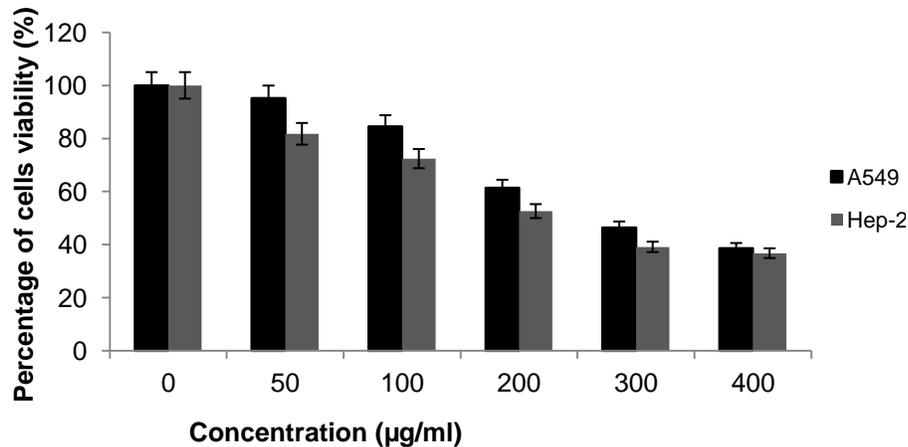


Figure 1. Effect of the kombucha green tea on cellular growth against the two human tumor cell lines (A549, lung cell carcinoma and Hep-2, epidermoid carcinoma).

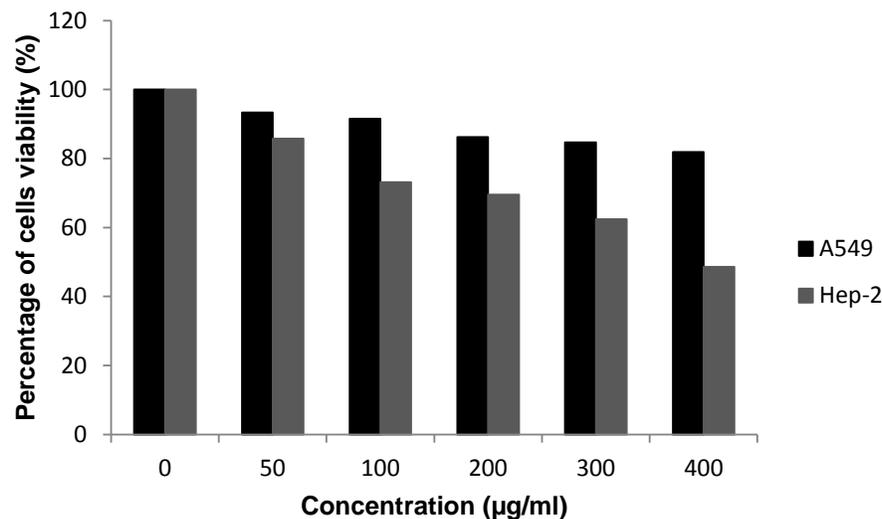


Figure 2. Effect of the kombucha black tea on cellular growth against two human tumor cell lines (A549, lung cell carcinoma; and Hep-2, epidermoid carcinoma).

Taguri et al., 2004), gluconic acid, glucuronic acid, lactic acid, vitamins (Bauer-Petrovska and Petrushevska-Tozi, 2000) as vitamin C which reduces stomach cancer (Hemila and Herman, 1995) and D-Saccharic acid-1,4-lactone (DSL), which inhibits the activity of glucuronidase, an enzyme indirectly related with cancers (Wang et al., 2010). Many reports demonstrate the antitumor properties of polyphenols which might act as cancer-blocking agents (Russo, 2007). In the current work, kombucha tea showed, *in vitro*, a significant antiproliferative activity against two human cancer cell lines A549 and Hep-2. The presence of polyphenols, D-Saccharic acid-1,4-lactone (DSL), vitamins, gluconic acid, glucuronic acid and lactic acid in the kombucha tea may be responsible for the antiproliferative activity. Other studies reported that kombucha tea have antiproliferative properties. Srihari et al. (2013) demon-

strate that Kombucha tea decreases the survival of prostate cancer cells by downregulating the expression of angiogenesis stimulators. The Kombucha tea showed also remarkable antimicrobial activity. There are numerous reports that antimicrobial activity of kombucha tea against pathogenic microorganisms is largely attributable to acetic acid (Greenwalt et al., 1998) and antibiotics present in the kombucha tea (Chen and Liu, 2000; Jayabalan et al., 2007). More, Sreeramulu et al. (2001) reported that the metabolites produced by the bacteria and/or yeasts during the fermentation of kombucha tea are responsible for its antimicrobial activity.

Conclusion

This study revealed that the kombucha tea possess in-

teresting antiproliferative properties associated with significant antimicrobial activity. These findings provide additional support for the traditional use of Kombucha tea in the treatment of metabolic diseases and various types of cancer.

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